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RESEARCH PAPER

Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*

Eric R. Larson[1] · Laura A. Twardochleb[2] · Julian D. Olden[3]

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Abstract Impacts of invasive species may manifest most strongly if these organisms are highly distinct functionally from the native species they often replace. Yet, should we expect functional differences between native and invasive species of generalist organisms like freshwater crayfish? Some existing evidence has pointed to native and invasive crayfish species as ecologically equivalent. We contribute to this literature by comparing the trophic niches of the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*, by applying carbon and nitrogen stable isotope analyses to replicated allopatric (alone) and sympatric (together) lake populations in western Washington State, USA, where *P. clarkii* has been recently introduced and *P. leniusculus* is presumed native. Our study corrected for potential inherent differences in lake food webs as a consequence of lake abiotic or biotic characteristics using random effects in linear mixed effects models. We found that although overall trophic niche size or area of these species was not significantly different, *P. leniusculus* was significantly higher in trophic position than *P. clarkii* when also accounting for the effects of body size, sex, and lakes as random effects. This pattern of increased trophic position of *P. leniusculus* over *P. clarkii* was conserved over time in one sympatric lake for which we had data over multiple years. Cumulatively, our findings point to trophic differences between the globally cosmopolitan crayfishes *P. leniusculus* and *P. clarkii*, particularly when accounting for the ways that ecosystem context can affect food web structure of communities and the trophic resources available to these consumers.

Keywords *Ambloplites rupestris* · Exotic species · *Bellamya chinensis* · Niche width · Non-native species · Red swamp crayfish · Signal crayfish · Trophic position · Urban lakes · Washington State

Introduction

Introduced crayfish have been attributed as among the most detrimental freshwater invasive species globally (Gallardo et al. 2016), negatively impacting a diversity of taxa across lotic and lentic food webs, including aquatic macrophytes, native crayfish species, aquatic invertebrates, and vertebrates including fish and amphibians (Matsuzaki et al. 2009; Lodge et al. 2012; Twardochleb et al. 2013). The breadth of these impacts primarily manifests as a consequence of the omnivorous or polytrophic feeding habits of crayfish (Olsen et al. 1991; Dorn and Wojdak 2004; Reynolds et al. 2013), although the effects of invasive crayfish can vary with habitat or ecosystem context (Peters and Lodge 2013; Magoulick 2014; Ruokonen et al. 2014). For example, the most severe whole-ecosystem impacts of invasive crayfish are often observed from regions that historically lacked native crayfish or analogous decapod

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✉ Eric R. Larson
erlarson@illinois.edu

[1] Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801, USA

[2] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA

[3] School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA

crustaceans altogether (Gamradt and Kats 1996; Geiger et al. 2005; Moore et al. 2012; Usio et al. 2013). Alternatively, in those ecosystems with native crayfish species, invasive crayfish have been observed to often achieve higher abundances or densities than native congeners (Hansen et al. 2013; Kreps et al. 2016), and these higher abundances should translate into greater invader impacts or interaction strengths (Kumschick et al. 2015). However, owing to the omnivorous nature of crayfish in general, should we anticipate ecological equivalence (i.e., functional redundancy) between crayfish species when invaders are not more abundant than native congeners?

Researchers have sought to answer this question by applying tools ranging from laboratory behavioral trials (e.g., Renai and Gherardi 2004), to mesocosm experiments (e.g., Usio et al. 2006), to field sampling of crayfish prey communities (e.g., Ercoli et al. 2015), to stable isotope analysis of crayfish trophic function or similarity (e.g., Olsson et al. 2009), to studies that have combined some of the above approaches (e.g., Jackson et al. 2014). To date, several meta-analyses of laboratory and field experiments comparing interactions of native and invasive crayfishes with their food webs have synthesized this literature, and generally found similar effect sizes between crayfish species irrespective of origin (Twardochleb et al. 2013; James et al. 2014). Efforts to allocate limited resources to prevent and manage biological invasions need reliable information on the particular species and habitat combinations where the most severe unwanted effects will manifest (Yokomizo et al. 2009; Hauser and McCarthy 2009). Accordingly, the apparent similarity in ecological function between many native and invasive crayfish species requires ongoing investigation and clarification in order to prioritize management activities.

One reason that the behavioral, enclosure, and mesocosm experiments synthesized in the above meta-analyses have largely failed to find consistent ecological differences between native and invasive crayfishes may be that they occur over too restricted spatial and temporal scales to accurately reflect actual ecological processes and associated subtle, persistent distinctions between these species (Lodge et al. 1998). Stable isotope analysis has emerged over recent decades as one of the primary tools used to infer ecological and trophic interactions between species under natural field conditions (Boecklen et al. 2011), with most tissue-derived stable isotope samples reflecting months to years of foraging behavior for focal organisms (Vander Zanden et al. 2015). For example, stable isotopes of carbon and nitrogen can be used to reflect energy source origins and trophic positions of organisms in freshwater lakes (Vander Zanden et al. 1999), and can be subsequently applied to evaluate and compare the trophic niche of species (Layman et al. 2007). When used to compare trophic function of native and invasive crayfish species, these stable isotope tools have produced inconsistent results, with some studies finding pronounced functional differences (e.g., Olsson et al. 2009; Jackson et al. 2014) and others a high degree of trophic similarity or niche overlap (e.g., Ercoli et al. 2014; Magoulick and Piercey 2016). Yet this emerging literature is small, and clearly needs further inquiry to evaluate whether native and invasive crayfishes are ecologically equivalent.

The two most globally invasive crayfish species are the signal crayfish *Pacifastacus leniusculus* and the red swamp crayfish *Procambarus clarkii* (Hobbs et al. 1989; Lodge et al. 2012). *Pacifastacus leniusculus* is native to the Columbia River drainage and some of the adjacent Pacific Northwest region of North America (Larson et al. 2012), and has been introduced elsewhere in the western United States, as well as to Japan and widely throughout Europe (Usio et al. 2007; Lodge et al. 2012). Impacts of *P. leniusculus* on recipient communities have included severe population declines and even one likely extinction of native crayfish species (Bouchard 1977; Light et al. 1995; Nakata and Goshima 2003), negative effects on other freshwater invertebrates and fish (Matsuzaki et al. 2012; Machida and Akiyama 2013; Wood et al. 2016), and changes to ecosystem processes owing to the burrowing and foraging behaviors of this species (Harvey et al. 2011). *Procambarus clarkii* is native to the southern United States and northeastern Mexico, and has been introduced to all continents except Antarctica and Australia, with particularly harmful invasions in the western United States, throughout Europe, and in Asia (Hobbs et al. 1989; Lodge et al. 2012). As a few examples, invasion by *P. clarkii* has transformed the food webs and ecosystem processes of Mediterranean wetlands in Europe (Geiger et al. 2005), and caused declines of vertebrate species like stream-dwelling newts (Gamradt and Kats 1996). Despite originating from disparate regions in North America, *P. leniusculus* and *P. clarkii* share some overlapping climate tolerances (Capinha et al. 2011; Larson and Olden 2012), and have been found occurring in sympatry or close proximity in some regions, like the Pacific Northwest region of North America (Hanshew and Garcia 2012; Pearl et al. 2013). These occurrences provide opportunities to compare the trophic function or niche overlap of the two most globally widespread invasive crayfish species under shared habitat circumstances, and evaluate whether these crayfish species are ecologically equivalent.

We used stable isotopes to compare the trophic niche of *P. leniusculus* and *P. clarkii* in a series of replicated allopatric (each species occurring in isolation) and sympatric (both species occurring together) lakes located in the Puget Sound lowlands of Washington State, USA (Larson and Olden 2013; Twardochleb and Olden 2016). Our analysis focused on comparing trophic niche size and similarity between *P. leniusculus* and *P. clarkii* using field, laboratory, and statistical approaches similar to a number of recent such studies on native and invasive crayfish species (e.g., Olsson et al. 2009; Ercoli et al. 2014; Jackson et al. 2014). Our comparisons were made between populations of a *P. clarkii* invasion initially discovered in 2000 (Mueller 2001) and locations in the putative native range of *P. leniusculus,* although a recent analysis of molecular and historical data suggests that *P. leniusculus* was introduced to this recently glaciated lake district by humans from a refugia roughly 100 km to the south sometime over the past century (Larson et al. 2012). Together, our study contributes to the developing literature on ecological equivalence between crayfish species, with a focus on the two most globally cosmopolitan invaders from this taxonomic group.

Methods

Study sites

The Puget Sound lowlands of Washington State were glaciated by a lobe of the Cordilleran ice sheet during the last glacial maxima, leaving behind hundreds of natural lakes that have recently experienced varying degrees of land development in association with urban and ex-urban growth (Alberti et al. 2007). These lakes have been invaded by a variety of non-native freshwater species owing to human trade in live organisms and historical ecosystem management practices (i.e., fish stocking), including at least four species of non-native crayfish as documented by Larson and Olden (2013). Larson and Olden (2013) conducted a systematic survey of 100 Puget Sound lowland lakes during the summers of 2007–2009 using a sampling protocol of 15–20 baited traps per lake to estimate crayfish relative abundance, measured as catch-per-unit effort (CPUE) or the average number of crayfish of each species per trap. We used data from the 2007 and 2008 sampling summers to identify eleven lakes for this stable isotope study, stratified as the only two lakes with known sympatric populations of *P. leniusculus* and *P. clarkii* at the time, three lakes with allopatric populations of each species, and three lakes with no previously detected crayfish populations (Fig. 1). Inclusion of these presumed no crayfish lakes was intended to allow for examination of how crayfish presence and crayfish species identity might affect trophic function of other members of the community like fish species (Nilsson et al. 2012; Kreps et al. 2016).

Effort was made to select study lakes according to similarities in physicochemical and watershed characteristics (Table 1). Relative abundance of both crayfish species was low relative to what has been observed for some invasive crayfish species in other systems (e.g., Kreps et al. 2016), ranging from CPUE of 0.05–0.33 for *P. leniusculus* and 0.15–0.75 for *P. clarkii*. These CPUE values were on the low end of what was observed in 48 other lakes where *P. leniusculus* was detected by Larson and Olden (2013), but higher than six other lakes where *P. clarkii* was collected by the same study (Fig. 1). Study lakes were generally relatively small, with shorelines that had experienced a high degree (30–95 %) of urbanization or human development, as visually estimated by the field sampling crew. We measured Secchi disk depth (m) as a metric of lake clarity during field sampling, used shoreline development index (SDI; ratio of lake perimeter to perimeter of a perfectly circular lake of the same area) values from Bortleson et al. (1976) to reflect littoral zone complexity, and compiled measures of water quality and chemistry (Table 1) from average epilimnetic values measured between 1996 and 2008 at each lake by government management agencies (Larson and Olden 2013).

Field sampling

The eleven study lakes were sampled between July 17 and September 10, 2009. We collected basal resources including benthic algae, aquatic macrophytes, and leaf litter from the littoral zone by hand while snorkeling, or with D-frame nets nearshore and an Ekman grab from a boat offshore that were primarily used to sample the aquatic macroinvertebrate community at multiple locations in each lake. We field sorted these collected organisms to morpho-species or coarse taxonomic categories (e.g., taxonomic level ‘order’), kept them in plastic bags on ice during sampling, and then immediately transported samples back to the laboratory for storage in a freezer in advance of laboratory stable isotope analysis. We collected crayfish and small fish using overnight sets of Gee minnow traps (0.42 m long by 0.21 m diameter) baited with a half cup of dry dog food, using 20–30 traps (dependent on lake size) with 6.0-cm openings to collect primarily crayfish and 9–10 traps (owing to trap theft) with 2.5-cm openings to collect primarily fish. Traps were set at depths between 0.5 and 6.0 m, and distributed around the entire lake perimeter, maintaining a minimum distance of 10 m between any two traps.

We collected larger fish using overnight sets of three hoop nets (7.9 m wing length, five hoops 0.8 m diameter) dispersed around lake perimeters and baited with punctured cans of wet cat food, and overnight sets of an experimental gill net (58.5 m length by 1.8 m height; six panels with 25, 32, 38, 51, 64, and 76 mm mesh). Collected fish were identified to species, measured to total length (mm), and a subset of individuals of each species across the available size range were euthanized and transported to the

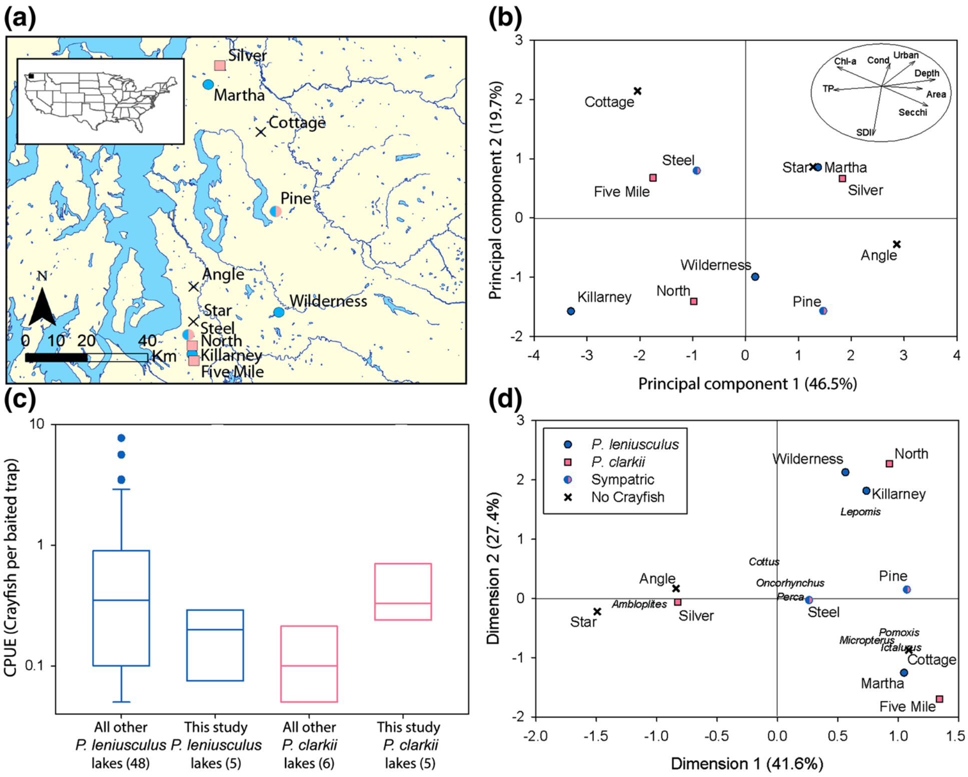

Fig. 1 Location of study lakes in the vicinity of the city of Seattle, Washington State, USA (**a**), with the first two axes of a principal component analysis (PCA) of lake physical attributes (Table 1) represented in the *inset* (**b**), the relative abundance of the crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii* in study lakes from preliminary data as catch per unit effort (CPUE) from baited traps (Electronic Supplementary Material) compared to all other lakes in the region where these crayfish were found (Larson and Olden 2013) (**c**), and the first two axes of a correspondence analysis (CA) of fish genus abundance (Electronic Supplementary Material) in study lakes from all sampling gears combined (**d**)

Table 1 Study lakes with date sampled for biota, geographic coordinates, and physical characteristics categorized as those with signal crayfish *Pacifastacus leniusculus* only, both crayfish in sympatry, red swamp crayfish *Procambarus clarkii* only, and no previously detected crayfish

Lake	Date sampled	Geographic coordinates			Depth (m)	SDI	Secchi (m)	Urban (%)	Conductivity (μS/cm)	TP (μg/l)	Chl-a (μg/l)
		Latitude	Longitude	Area (Ha)							
P. leniusculus											
Killarney	8/17/2009	47.2867	−122.2909	9.7	4.6	1.82	2.9	60	54	38.2	6.7
Martha	7/22/2009	47.8512	−122.2446	23.1	14.6	1.33	5.4	80	99	15.6	1.9
Wilderness	8/10/2009	47.3726	−122.0344	27.9	11.6	1.53	5.1	50	65	16.0	3.2
Sympatric											
Pine	8/24/2009	47.5866	−122.0448	34.8	11.9	1.84	6.5	80	72	9.6	3.1
Steel	8/13/2009	47.3269	−122.3029	18.6	7.3	1.41	2.3	80	76	14.8	5.2
P. clarkii											
Five mile	9/10/2009	47.2717	−122.2872	15.4	9.8	1.34	1.5	60	56	18.1	7.6
North	7/17/2009	47.3051	−122.2896	22.7	10.4	1.62	4.3	30	55	12.1	6.5
Silver	8/19/2009	47.8904	−122.2120	42.9	15.5	1.25	4.1	90	38	5.0	5.2
No crayfish											
Angle	7/23/2009	47.4261	−122.2883	41.7	15.8	1.53	8.2	95	72	11.0	3.0
Cottage	8/20/2009	47.7539	−122.0866	25.5	7.6	1.23	2.0	75	88	25.6	11.7
Star	8/12/2009	47.3547	−122.2889	14.2	15.2	1.33	5.2	85	84	9.6	2.7

Physical characteristics include area (Ha), maximum depth (m), shoreline development index (SDI), Secchi disk depth at time of sampling (m), shoreline urbanization as visually estimated by the field sampling crew (%), and the water chemistry variables conductivity (μS/cm), total phosphorus (μg/l), and chlorophyll-a (μg/l) as recent averages from governmental lake monitoring; see main text and Larson and Olden (2013) for additional detail on lake physical characteristics

laboratory, where they were frozen until stable isotope processing. All crayfish of each species were sexed, measured to total carapace length (mm) using vernier calipers, and subsequently euthanized, transported to the laboratory, and frozen as per fish. Collections of crayfish and fish were made under Washington Department of Fish and Wildlife permit 07-323 and 08-344, and University of Washington Institutional Animal Care and Use Committee permit 4172-04.

Stable isotope samples

We dissected fish muscle tissue from the lateral area posterior to the operculum, and muscle tissue from the abdomen of crayfish consistent with Stenroth et al. (2006), for use as stable isotope samples of these larger organisms. We used whole organisms for benthic macroinvertebrates and all collected biomass for basal resources; as an exception, we used a subsample of muscle tissue from the foot of larger snails (see below). Samples were dried at 60 °C for 24 h and then homogenized using a mortar and pestle. We weighed approximately 1 mg of animal tissue and 2–3 mg of plant tissue into tin capsules, and shipped samples to the University of California-Davis Stable Isotope Laboratory for dual carbon and nitrogen analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a 20–20 isotope ratio mass spectrometer. Long-term standard deviations of lab standards used at this facility have been 0.2 ‰ for 13C and 0.3 ‰ for ^{15}N. Stable isotope ratios are reported per convention in δ notation as ^{13}C/^{12}C relative to a Pee Dee belemnite standard and ^{15}N/^{14}N relative to an atmospheric nitrogen standard.

Statistical analyses

We first used multivariate statistics to explore the similarity of our sympatric, allopatric, and no crayfish lakes with respect to both their abiotic attributes (Table 1) and fish communities as observed in our field sampling (above; Electronic Supplementary Materials). Lake attributes like habitat area or productivity can affect trophic position and resource use of consumers (Post et al. 2000; Larson et al. 2011), whereas some predatory fish species may affect the distribution, abundance, and behavior of crayfish (Collins et al. 1983; Edwards et al. 2013), and invasive crayfish may in turn extirpate other fish species from lakes owing to competition or predation (Dorn and Mittelbach 2004; Kreps et al. 2016). We performed a principal component analysis (PCA) on the lake abiotic attributes and a correspondence analysis (CA) on total fish abundance from all sampling gears aggregated to the genus level, with both analyses conducted using the vegan library in R (R Development Core Team 2008; Oksanen et al. 2016). We visually evaluated the dispersion of our sympatric, allopatric, and no crayfish lake categories on the first two axes from each of these ordinations.

Calculations of trophic position using stable isotopes generally require comparison to a baseline organism; in freshwater lakes, this comparison is most often made to pelagic and/or littoral benthic primary consumers (trophic position of two), because the isotopic values of primary producers like phytoplankton or periphyton can be extremely variable in space and time (see Vander Zanden et al. 1999; Post et al. 2000). Long-lived, large bodied primary consumers average out this variability. For our study, we used the non-native Chinese mystery snail *Bellamya chinensis* to calculate trophic positions for other organisms including *P. leniusculus* and *P. clarkii.* We used *B. chinensis* because this large-bodied (up to 65 mm total length) snail is both a grazer and filter feeder (Olden et al. 2013), and consequently may reflect trophic baselines of both pelagic and littoral benthic food webs simultaneously. Further, both *P. leniusculus* and *P. clarkii* had been previously documented to feed on *B. chinensis* under laboratory conditions (Olden et al. 2009), and we collected *B. chinensis* from 10 of our 11 study lakes, therefore standardizing trophic position calculations between study sites. In the one lake where we did not collect *B. chinensis* (Martha), we calculated trophic position using the average of all other collected snails after correcting for their mean − 1.36 ‰ δ^{15}N depletion relative to *B. chinensis* observed in the other 10 lakes (Electronic Supplementary Material). We calculated trophic position for consumers as:

$$\text{Trophic position (TP)} = \frac{\delta^{15}\text{N}_{\text{Consumer}} - \delta^{15}\text{N}_{B.chinensis}}{3.4} + 2, \qquad (1)$$

where 3.4 is an average discrimination or fractionation factor (Δ) of δ^{15}N used between consumers of different trophic levels (Minagawa and Wada 1984).

We compared trophic niche size and overlap between *P. leniusculus* and *P. clarkii* by calculating small sample-size corrected standard ellipse areas (SEAc) of trophic position and a standardized δ^{13}C axis (below) using the siar library in R (Jackson et al. 2011; Parnell and Jackson 2013). The SEAc metric of isotopic niche area is analogous to standard deviations for univariate data (Jackson et al. 2011). Our stable isotope sample sizes were relatively low but generally consistent for both crayfish species. Allopatric sample sizes for *P. leniusculus* were 6 (Killarney), 7 (Wild), and 13 (Martha), sympatric sample sizes for this crayfish were 9 (Steel) and 10 (Pine), and the mean *P. leniusculus* stable isotope sample size across five lakes was 9. Allopatric sample sizes for *P. clarkii* were 4 (Five Mile), 8 (North), and 9 (Silver), whereas sympatric sample sizes for this crayfish were 10 (Steel) and 20 (Pine), and the mean *P. clarkii* stable isotope sample size across lakes was 10. See Discussion for some implications of these levels of replication for our comparison of SEAc between crayfish species.

Consistent with Olsson et al. (2009), we standardized δ^{13}C of crayfish samples relative to the δ^{13}C mean and range of all consumers collected from their lake in order to: (a) place all populations on an equivalent δ^{13}C axis relative to the breadth of available δ^{13}C sources from primary producers in their originating community, and (b) scale axes of trophic position and δ^{13}C over similar ranges with respect to influence in calculating trophic niche area (Larson et al. 2010). We standardized δ^{13}C using the formula:

$$\text{Standardized}\delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{Consumer}} - \delta^{13}\text{C}_{\text{LakeConsumerMean}}}{\delta^{13}\text{C}_{\text{LakeConsumerMax}} - \delta^{13}\text{C}_{\text{LakeConsumerMin}}}. \tag{2}$$

We compared trophic niche area as SEAc of *P. leniusculus* and *P. clarkii* with *t*-tests across all categories combined (allopatric and sympatric), in order to have enough degrees of freedom for statistical comparisons (i.e., there were only two sympatric lakes). We used the siar library in R to calculate percent overlap of SEAc between (and within; see below) species in some cases where sympatric (Parnell and Jackson 2013).

To further evaluate factors affecting isotope values of our individual crayfish, we performed linear mixed effects models in the nlme library of R (Pinheiro et al. 2016), in which we regressed crayfish trophic position and original (non-standardized) δ^{13}C against factors including whether or not the two crayfish species were occurring in sympatry as defined above (allopatric = 0, sympatric = 1), crayfish species (*P. clarkii* = 0, *P. leniusculus* = 1), sex (male = 0, female = 1), and size (continuous as mm carapace length). These models included individual lakes as unordered random effects, to account for ways that factors like ecosystem size, productivity, or disturbance (e.g., degree of urbanization) have been observed to influence the trophic ecology of freshwater consumers (Post et al. 2000; Larson et al. 2011). Given our relatively low level of replication at the lake level and the large number of both abiotic and biotic lake attributes that might affect crayfish trophic ecology, we used random effects to control for this heterogeneity between our study sites in general (Bolker et al. 2009). Finally, organism size can influence trophic function through ontogenetic niche shifts, a phenomenon that has been observed for crayfish (Larson et al. 2010), and sex has similarly been observed to affect crayfish behavior and trophic function under mesocosm conditions (Usio and Townsend 2002).

Isotopic consistency in time

To evaluate whether isotopic niche relationships were consistent between *P. leniusculus* and *P. clarkii* over time, we used additional crayfish specimens collected by baited trapping from one of our sympatric lakes (Pine) during summer 2012. Five *P. leniusculus* and 6 *P. clarkii* collected during this summer had stable isotope samples processed and analyzed as above, and we calculated SEAc on original δ^{15}N and δ^{13}C (rather than trophic position and standardized δ^{13}C) for these and specimens of both species collected from this lake in summer of 2009. We did not convert to trophic position and standardized δ^{13}C owing to differences in sampling protocols between years. We compared trophic niche area and overlap between these species within years, and within species between years, to evaluate the stability or consistency of our conclusions with respect to *P. leniusculus* and *P. clarkii* ecological equivalence.

Results

Lake abiotic characteristics

Lakes supporting crayfish populations of either one or both species demonstrated high environmental similarity. The PCA on lake abiotic attributes (Table 1) resulted in a first ordination axis that explained 46.5 % of the variation in the dataset reflecting a gradient of more productive lakes (negative) to larger and deeper lakes (positive), and 19.7 % of variation on a second axis that captured a gradient of more urban lakes with higher conductivity (positive) to lakes with more complex shorelines per SDI (negative). Lakes with *P. leniusculus* and *P. clarkii* both in allopatry and sympatry were well dispersed over these gradients on the PCA, although no crayfish lakes were generally more urban (positive on second axis).

Lake biotic communities

Owing to disparities in fish communities between the study lakes, we chose not to evaluate potential effects of crayfish or particular crayfish species on fish trophic function. The CA on fish abundance per genus collected from field sampling (Electronic Supplementary Material) resulted in a first dimension that explained 41.6 % of the variation in the dataset on a gradient of lakes dominated by rock bass *Ambloplites rupestris* (negative) relative to all other lakes (more positive), and a second dimension that explained 27.4 % on a gradient of lakes with more *Ictalurus, Micropterus*, and *Pomoxis* species (negative) to lakes dominated by sunfishes of the genus *Lepomis* (Fig. 1). The yellow perch *Perca flavescens* was common in many lakes (Electronic Supplementary Material). Two of three no crayfish lakes were characterized by *Ambloplites*, and none of the a priori allopatric *P. leniusculus* or sympatric lakes contained this species.

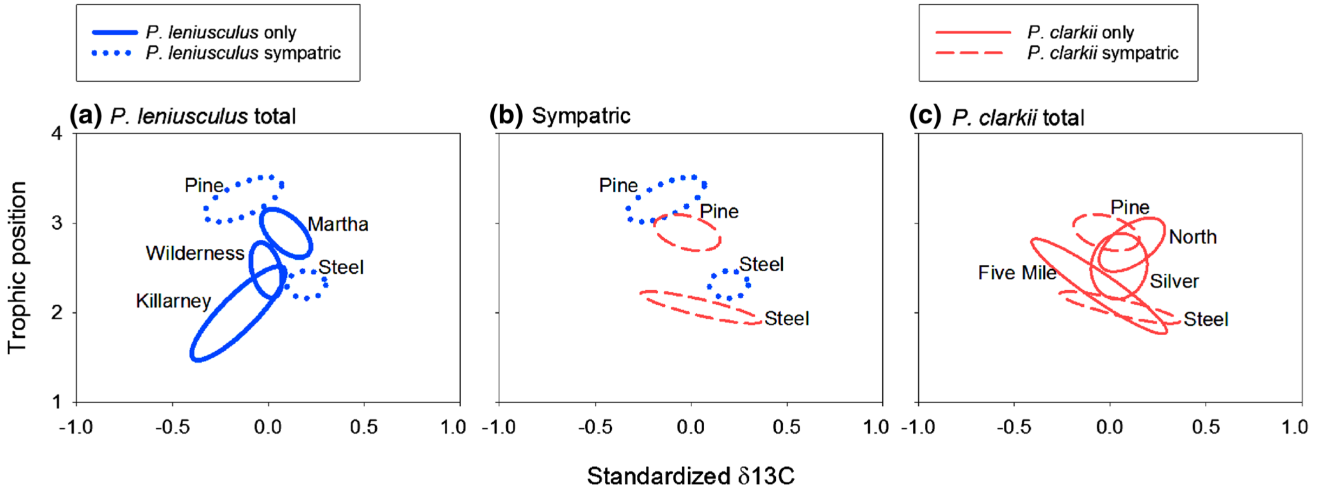

Fig. 2 Small sample size standard ellipse areas (SEAc) from the stable isotope analysis in R (siar) package, calculated on standardized δ^{13}C and trophic position for the signal crayfish *Pacifastacus leniusculus* (**a**), both crayfishes where sympatric (**b**), and the red swamp crayfish *Procambarus clarkii* (**c**), using only those lakes where more than three individuals were collected for a given species

In three cases we collected a single (Angle, Silver) or two (Cottage) *P. leniusculus* from lakes where this crayfish had not been detected in the previous 2007 and 2008 field sampling (Electronic Supplementary Material). Due to the overall rarity of *P. leniusculus* in these lakes, we retain our a priori sympatric, allopatric, and no crayfish designations in interpreting results. Similarly, as a consequence of the inadequate replication of *P. leniusculus* samples in these lakes, we excluded these four crayfish from our trophic niche comparisons.

Crayfish trophic niches

There was no significant difference in trophic niche area (as measured by SEAc) between all *P. leniusculus* and *P. clarkii* populations ($t_4 = -0.728$, $P = 0.507$; Fig. 2). Trophic position and niche width as standardized δ^{13}C varied widely among lake populations. We did observe significant effects of species, size, and sex on crayfish trophic position, and an effect of size on δ^{13}C; no significant effect of whether or not crayfish occurred in sympatry or allopatry was found for either measure of their trophic niche (Table 2). Trophic position was higher for *P. leniusculus* than *P. clarkii*, for female rather than male crayfish, and increased with increasing crayfish body size. Crayfish δ^{13}C depleted or decreased with increasing body size, indicating a shift from enriched δ^{13}C associated with littoral benthic primary producers (smaller crayfish) to depleted δ^{13}C associated with pelagic primary producers or terrestrial detritus (larger crayfish). We used sympatric crayfish populations in Pine and Steel lakes to illustrate increased trophic position of *P. leniusculus* over *P. clarkii* when accounting for the effect of body size and inherent food web differences between lakes (Fig. 3).

Table 2 Results of linear mixed effects models (nlme library in R) of crayfish trophic position (TP) and δ^{13}C values as predicted by crayfish occurrence in sympatry (allopatric = 0, sympatric = 1), species (*P. clarkii* = 0, *P. leniusculus* = 1), sex (male = 0, female = 1), and size (continuous as mm carapace length) given as regression coefficients with standard errors (SE) and significance as $P < 0.001$ (***), $P < 0.010$ (**), $P < 0.050$ (*)

	Trophic position (SE)	δ^{13}C (SE)
Intercept	1.774 (0.215)***	−24.677 (1.078)***
Sympatric	0.240 (0.352)	2.509 (1.337)
Species	0.348 (0.071)***	0.853 (0.474)
Sex	0.118 (0.052)*	−0.086 (0.365)
Size	0.011 (0.0002)***	−0.052 (0.015)**
Pseudo-R^2	0.760	0.570

Individual lakes (Table 1) are included in models as unordered random effects, and only included in cases where more than three individuals were collected of focal crayfish species. Models used 96 observations (crayfish) in eight groups (lakes). Pseudo-R^2 values of models are estimated as the fit of estimated versus observed isotope values for crayfish

Crayfish isotopic consistency in time

Higher trophic position or δ^{15}N enrichment of *P. leniusculus* relative to *P. clarkii* was consistent over time between 2009 and 2012 in Pine Lake (Fig. 4). Trophic position of *P. leniusculus* was higher than *P. clarkii* in 2009, with only 0.8 % overlap of *P. clarkii* into the *P. leniusculus* SEAc ellipse. Similarly, trophic position of *P. leniusculus* was higher than *P. clarkii* in 2012, with 19.8 % overlap of *P. clarkii* into the *P. leniusculus* SEAc ellipse. The 2012 *P. leniusculus* SEAc overlapped with 9.3 % of the 2009 *P. leniusculus* SEAc, whereas the 2012 *P. clarkii* SEAc overlapped with 54.1 % of the 2009 *P. clarkii* SEAc.

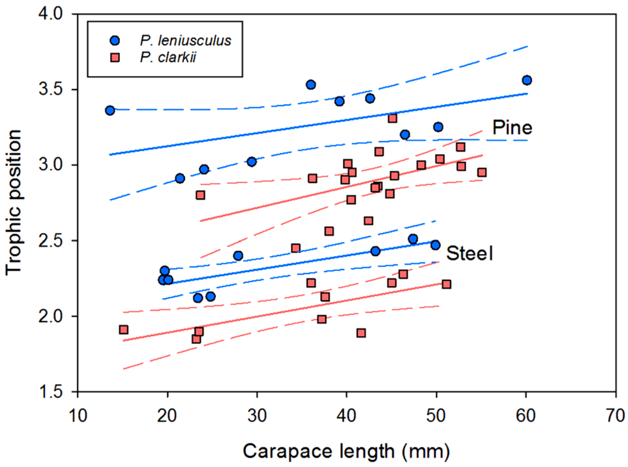

Fig. 3 Trophic position for *Pacifastacus leniusculus* and *Procambarus clarkii* in the sympatric Pine and Steel lakes regressed against body size, with 95 % confidence intervals

Procambarus clarkii trophic niche as SEAc was highly consistent between years, whereas the trophic niche as $\delta^{13}C$ seemingly narrowed for *P. leniusculus* in 2012 (Fig. 4).

Discussion

There are many instances where the introduction of an invasive crayfish species is clearly and unfailingly undesirable. Invasive crayfishes spread diseases including the crayfish plague *Aphanomyces astaci* that has decimated native European crayfishes (Jussila et al. 2015b), can have strong negative effects on native taxa that are naïve to interactions with crayfish (Gamradt and Kats 1996), may change the phenology of key ecosystem processes like decomposition of detritus (Kobayashi et al. 2011; Alp et al. 2016), and can reach hyper-abundance relative to native crayfish populations (Hansen et al. 2013; Kreps et al. 2016). Yet even outside of these particular examples, our study contributes further evidence that not all crayfish species are ecologically equivalent, despite their categorization as omnivores or polytrophic generalists. Across replicated lake ecosystems and irrespective of occurrence in allopatry or sympatry, *P. leniusculus* was significantly higher in trophic position than *P. clarkii*, and this distinction could have important implications for how these organisms interact with and affect freshwater food webs and communities. As one example, such differences in trophic position between *P. leniusculus* and *P. clarkii* could result in different roles as vectors of contaminants like mercury in freshwater food webs (Johnson et al. 2014).

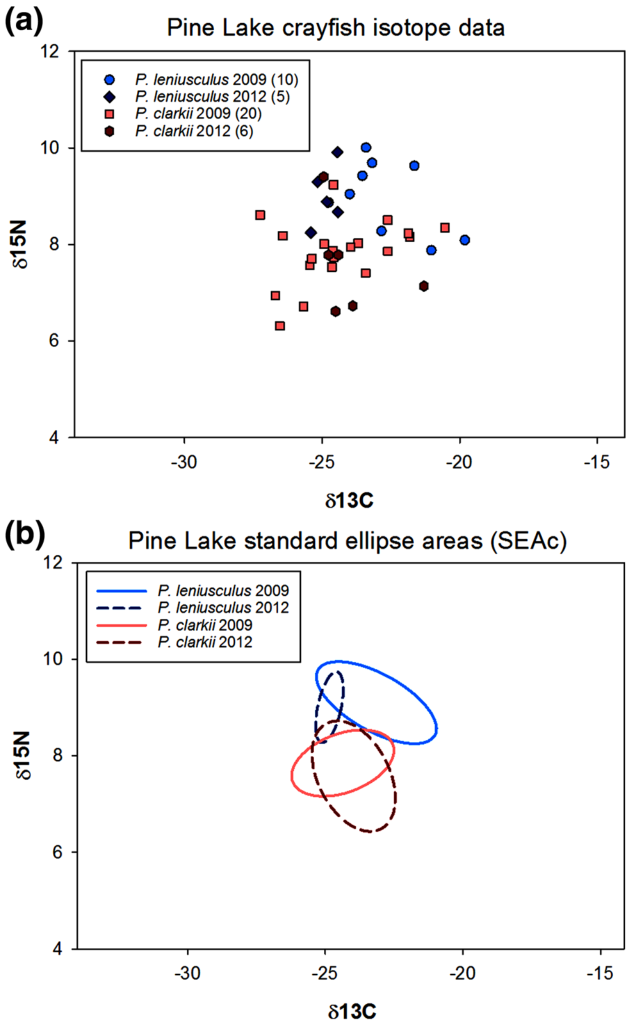

Fig. 4 Stable isotope biplots for *Pacifastacus leniusculus* and *Procambarus clarkii* in Pine Lake for the years 2009 and 2012 with sample sizes in parantheses in legend (**a**), and small sample size-corrected standard ellipse areas (SEAc) for these data calculated using the stable isotope analysis in R package (**b**)

We believe that findings of our study are likely transferable over space and time, owing to the general consistency in trophic niche position and size observed between *P. leniusculus* and *P. clarkii* from 2009 to 2012 in Pine Lake, as well as the previous results of Larson et al. (2010). That study compared the trophic niche between the native (Pacific Northwest) and invasive (Japan) range of *P. leniusculus,* and found that although trophic function of this crayfish could vary with ontogeny (size) and habitat context, this range and pattern of trophic niche variability were conserved between native and invasive regions. Similar to our current study, Larson et al. (2010) found increasing trophic position for larger crayfish, and $\delta^{13}C$ depletion with increasing crayfish size. This pattern in $\delta^{13}C$ may reflect a transition of larger crayfish towards more reliance on terrestrial or detrital food sources in these lakes (Larson et al. 2010, 2011). Furthermore, results of a recent mesocosm experiment also supported a higher trophic position of *P. leniusculus* relative to *P. clarkii.* Olden et al. (2009) compared consumption and handling times of the Chinese mystery snail *B. chinensis* by both *P. leniusculus* and *P. clarkii,* using specimens collected from the same lakes as our current study. Olden et al. (2009) found that *P. leniusculus* consumed more and larger *B. chinensis* relative to *P. clarkii*. Accordingly, several lines of evidence — feeding and mesocosm experiments as well as stable isotope analysis — suggest a higher trophic position of *P. leniusculus* than *P. clarkii* in lakes of the Puget Sound lowlands of Washington State, USA.

Our findings conflict with those of Jackson et al. (2014), who observed a higher trophic position of *P. clarkii* than *P. leniusculus* using stable isotopes on crayfish sampled from field sites in the United Kingdom. Notably, the comparison of Jackson et al. (2014) was made between a single allopatric site for each species, with no controls or corrections for potential ways that site differences may affect trophic resources available to each of these crayfish populations. Such differences may have been particularly severe, given that *P. clarkii* isotope samples were from a population in a lentic (pond) environment, whereas those for *P. leniusculus* were from a population in a separate lotic (navigation canal) environment. Our study is somewhat unique in being able to include comparisons of trophic niches of two invasive crayfish species in sympatry, as these studies are most often made only on allopatric populations, owing to the tendency of invasive crayfish to entirely displace native congeners through mechanisms including competition and disease transmission (Olsson et al. 2009; Ercoli et al. 2014).

Our results suggest that — where possible — these types of comparisons should include some sympatric populations, and when only allopatric contrasts are possible, study sites should be as closely matched by abiotic and biotic characteristics as feasible. Further, tools like linear mixed effects models can be deployed to accommodate site differences or heterogeneity. However, a suite of additional factors might contribute to variability in trophic function even between populations of the same crayfish species, ranging from behavioral syndromes associated with newly introduced or spreading populations of invaders (Pintor et al. 2008) to complicated effects of parasites or symbionts on crayfish foraging behaviors and ecological interactions (Reisinger et al. 2015; James et al. 2015). In particular, we propose that some trophic niche similarity (Magoulick and Piercey 2016) or distinctions (Jackson et al. 2014) observed between crayfish species might be attributable to phylogenetic similarity, with closely related crayfish (e.g., within the same genera) being more ecologically equivalent, and more distantly related crayfish (e.g., in different genera or families) being more dissimilar. Specific tests of this phylogenetic niche conservatism hypothesis (Webb et al. 2002) using crayfish stable isotope data might be a fruitful direction for future research (Comte et al. 2016).

Our results are dependent on consistent isotopic discrimination factors (e.g., 3.4 for δ^{15}N; Eq. 1) between species and diets, an assumption that has been called into question over recent years (Caut et al. 2009). Few studies have compared isotopic discrimination or fractionation factors for crayfish species, and those that have generally fail to run experiments on adult crayfish long enough for organisms to reach equilibrium with their diets (Carolan et al. 2012; Jussila et al. 2015a). As one exception, Glon et al. (2016) used fast-growing juvenile crayfish of two congeneric species that were fed both an invertebrate and algal diet. Over experimental durations generally long enough to reach isotopic equilibrium, Glon et al. (2016) found that δ^{13}C and δ^{15}N discrimination factors were largely indistinguishable between these species over both diets and in comparison to literature values (3.4 for δ^{15}N) in the majority of cases. However, more laboratory studies of isotopic discrimination or fractionation factors for crayfish species and diet combinations would be valuable for future applications of this tool. Furthermore, our relatively low sample sizes per crayfish species and lake (four to 20 individuals) could affect our results, particularly as the measure of trophic niche size or area (SEAc) that we used provides less precise estimates of the overall population trophic niche size at lower levels of replication (Syväranta et al. 2013). This may have contributed to our failure to find a significant difference in SEAc between our two crayfish species (i.e., a type II error), although we did have adequate statistical power to detect a suite of other trophic distinctions among these crayfishes (Table 2) and believe that both species do likely share similarly large trophic niches given what we know of their biology in general.

Pacifastacus leniusculus and *P. clarkii* are globally cosmopolitan freshwater invaders that have spread to multiple continents and may increasingly share sympatric regions or even specific habitats in the future (Capinha et al. 2011; Larson and Olden 2012). Our study provides guidance to researchers and managers in other regions on one type of ecological difference that should be anticipated between these two crayfish species, although there are certainly others (e.g., the superior burrowing ability of *P. clarkii;* Gherardi 2006). We re-emphasize that both species are highly plastic generalists or omnivores that can feed on a variety of potential diet items, from high reliance on low food-quality terrestrial detritus (Gutiérrez-Yurrita et al. 1998; Bondar et al. 2005) to predation on vertebrates (Gamradt and Kats 1996; Matsuzaki et al. 2012) dependent on community and ecosystem context, as well as crayfish ontogeny or life history (see also Larson et al. 2010). Yet, where all else is equal, our results predict that *P. leniusculus* will be higher in trophic position than *P. clarkii* under the same conditions, and we believe this distinction is likely consistent in time and space owing to our past work on niche conservatism for one of these crayfish species (Larson et al. 2010). More work is needed on ecological equivalence between native and invasive crayfish species in light of meta-analysis results (Twardochleb et al. 2013; James et al. 2014) and some stable isotope studies (e.g., Magoulick and Piercey 2016), but we lend support to the expectation that one crayfish species is not necessarily equal to another.

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