Connecting laboratory behavior to field function through stable isotope analysis

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Inherent difficulties of tracking and observing organisms in the field often leave researchers with no choice but to conduct behavioral experiments under laboratory settings. However, results of laboratory experiments do not always translate accurately to natural conditions. A fundamental challenge in ecology is therefore to scale up from small area and short-duration laboratory experiments to large areas and long durations over which ecological processes generally operate. In this study, we propose that stable isotope analysis may be a tool that can link laboratory behavioral observations to past field interactions or function of individual organisms. We conducted laboratory behavioral assays to measure dominance of invasive rusty crayfish, Orconectes rusticus[i], and used stable isotope analysis to hindcast trophic positions of these crayfish under preceding natural conditions. We hypothesized that more dominant crayfish in our assays would have higher trophic positions if dominance were related to competitive ability or willingness to pursue high-risk, high-reward prey. We did not find a relationship between crayfish dominance and trophic position, and therefore infer that laboratory dominance of crayfish may not necessarily relate to their ecology in the field. However, this is to our knowledge the first attempt to directly relate laboratory behavior to field performance via stable isotope analysis. We encourage future studies to continue to explore a possible link between laboratory and field behavior via stable isotope analysis, and propose several avenues to do so.
Connecting Laboratory Behavior to Field Function through Stable Isotope Analysis

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Abstract

Inherent difficulties of tracking and observing organisms in the field often leave researchers with no choice but to conduct behavioral experiments under laboratory settings. However, results of laboratory experiments do not always translate accurately to natural conditions. A fundamental challenge in ecology is therefore to scale up from small area and short-duration laboratory experiments to large areas and long durations over which ecological processes generally operate. In this study, we propose that stable isotope analysis may be a tool that can link laboratory behavioral observations to past field interactions or function of individual organisms. We conducted laboratory behavioral assays to measure dominance of invasive rusty crayfish, *Orconectes rusticus*, and used stable isotope analysis to hindcast trophic positions of these crayfish under preceding natural conditions. We hypothesized that more dominant crayfish in our assays would have higher trophic positions if dominance were related to competitive ability or willingness to pursue high-risk, high-reward prey. We did not find a relationship between crayfish dominance and trophic position, and therefore infer that laboratory dominance of crayfish may not necessarily relate to their ecology in the field. However, this is to our knowledge the first attempt to directly relate laboratory behavior to field performance via stable isotope analysis. We encourage future studies to continue to explore a possible link between laboratory and field behavior via stable isotope analysis, and propose several avenues to do so.

Keywords: mixing model; dominance; agonistic assays; *Orconectes rusticus*; individual variation; invasive species
Introduction

Animal behavior is inherently linked with the fields of ecology and evolution (Sih, Bell & Johnson, 2004; Réale, Reader & Sol, 2007), and informs applications such as management of biological invasions (Sih et al., 2010). Owing to logistical difficulties inherent to tracking and observing organisms without interference in the field, however, many behavioral studies are conducted ex situ in a laboratory setting, where it may be difficult to extrapolate findings to natural conditions (Niemelä & Dingemanse, 2014; Zavorka et al., 2015). For example, a suite of often-correlated behaviors including aggression, dominance, and boldness are believed to contribute to the success of some invasive over native species (Pintor, Sih & Kerby, 2009; Hudina, Hock & Žganec, 2014), but these same behaviors can be considerably muted in duration or intensity when observed in the field (Bergman & Moore, 2003; Larson & Magoulick, 2009).

One of ecology’s most fundamental challenges is scaling up from the type of small area and short duration experiments that are easy to conduct, to the larger areas and longer durations over which ecological processes often operate (Lodge et al., 1998). This same challenge applies when relating animal behaviors observed in the laboratory to ecological function and intra- or inter-specific interactions in situ.

We propose here that linking laboratory behavioral observations to past field interactions or function of specific, individual organisms may be an overlooked application of stable isotope analysis. Stable isotopes of elements such as carbon and nitrogen are assimilated into tissues of consumer organisms relative to their diets in predictable and quantifiable ways (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981). Importantly, stable isotopes of consumers equilibrate with those of their diets at different rates in different tissues, giving snapshots of ecological interactions that may scale from previous days to years (Buchheister & Latour, 2010). Analyzing
stable isotope ratios in organisms can provide ecological insights ranging from habitat use and movement (Hobson, 1999) to trophic position (Post, 2002). For example, stable isotope analysis of feathers has been used to make inferences about migration and habitat use of several species of seabirds that spend winter months far from land and are therefore difficult to study during this period (Phillips et al. 2009). In another example, Cherel et al. (2008) used stable isotope analysis to identify the trophic position and diet composition of southern elephant seals (Mirounga leonina) which forage at depths exceeding 1000 m and have largely digested their meals by the time they return to land, precluding them from being studied using traditional methods (e.g., direct observation, gut content analysis). Similarly to how these and other studies have applied stable isotope analysis to infer the influence of past behavior on current success of organisms, we propose that stable isotope analysis could permit researchers to link laboratory interactions with previous in situ habitat selection, movement, diet choice, or competitive interactions (Figure 1).

We conducted laboratory behavioral assays to measure individual dominance of invasive rusty crayfish, Orconectes rusticus, and used stable isotope analysis to hindcast trophic position of these crayfish under natural field conditions. We predicted that more dominant crayfish in the behavioral assays would have higher trophic positions if dominance were related to competitive ability in the field (e.g., ability to access high quality food such as macroinvertebrates; Roth, Hein & Vander Zanden, 2006) or willingness to pursue high-risk, high-reward prey such as fish or other crayfish (Taylor & Soucek, 2010). Alternatively, dominance and trophic position may not be associated if laboratory behaviors are ultimately uninformative with respect to past interactions of organisms. Numerous previous studies have used stable isotope analysis to infer various in situ behaviors of organisms, such as habitat use and diet preferences (e.g., Hildebrand et al., 1996; Rubenstein & Hobson, 2004); however, our study is the first to our knowledge to
seek a direct relationship for individual organisms between laboratory behaviors and field function as determined by stable isotope analysis, and proposes the linkage of laboratory behavioral assays and stable isotopes as a more common practice in the future.

Methods

*Orconectes rusticus* was introduced via the bait trade to the Laurentian Great Lakes circa 1960 and has negatively affected fish, macrophytes, and freshwater macroinvertebrates (McCarthy *et al.*, 2006; Peters *et al.* 2014). The invasion success of this crayfish has made it the focal point of a large number of laboratory and field studies (e.g., Olsen *et al.*, 1991; Wilson *et al.*, 2004) and hence, a useful organism to test for linkages between field and laboratory behavior. We collected adult form II (reproductively inactive) male *O. rusticus* (n = 40) by hand on 16 June 2015 in the Chippewa River, Michigan (43.5652°, -84.9183°), where this species is invasive. Because size influences the outcome of crayfish agonistic trials (Bergman & Moore, 2003), we used crayfish within a carapace length range of 23.41 to 27.53 mm, the smallest size range for which we could collect 40 crayfish (see supplementary material for additional morphometrics). *Orconectes rusticus* in this size range are small adults of the same age class (Momot, 1967) and are therefore unlikely to have diets that differ from one another due to ontogenetic shifts (Bondar *et al.*, 2005; Larson, Olden & Usio, 2010). Immediately following collection, we placed crayfish in individual 16 oz. plastic containers filled to a depth of 2 cm with river water and a rock for shelter.

Agnostic assays
Laboratory agonistic assays for crayfish are often conducted after isolating individuals for at least one week to remove possible previous social experience that could influence interactions (Seebacher & Wilson, 2007). We conducted our experiment directly following collection (17 June 2015 during daylight hours [07h19-18h59]), but believe that retaining any existing dominance hierarchies from the field would increase the likelihood of a relationship between laboratory behaviors and previous field function.

We conducted three rounds of twenty, randomized paired assays, with each crayfish fighting one opponent per round (no interactions between individuals were repeated). In order to track individual crayfish, we randomly assigned each crayfish a number from 1 to 40, which we wrote on the dorsal side of its carapace using a permanent marker. Prior to the start of each assay, crayfish were placed on opposite sides of a separator in a 19 l bucket and allowed to acclimate for 15 minutes. We then removed the separator and allowed the crayfish to interact for 10 minutes. During each assay, we scored each of the two crayfish individually based on the interactions that took place when they were within one body length of each other. All agonistic assays were watched and scored in real time by a single observer to ensure consistency in scoring. The agonistic assays within each of the three rounds were held in a random order, and the observer had no knowledge of totaled crayfish scores from previous rounds so as to avoid bias.

The scoring system we used has possible point values ranging from -2 (fast retreat) to 5 (unrestrained fighting) and is based on the ethogram modified from Bruski & Dunham (1987; Table 1). Following each assay, the participating crayfish were returned to their original holding container. We then rinsed buckets and refilled them to a depth of 5 cm with fresh water from the Chippewa River (18-20°C). At the conclusion of all assays, crayfish were placed in individual,
labelled bags and euthanized by freezing at -17.8°C. We calculated the dominance score of each crayfish by first summing its scores from each round, then taking the mean of the three resulting scores.

Stable isotope analysis

Stable isotope analysis is a technique based on the principle that the ratios of heavy to light isotopes in the tissues of consumers reflect those of their diets in a predictable way (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981). Stable isotope analysis generally entails drying and homogenizing tissue or whole-body samples of focal organisms, then using a mass spectrometer coupled with an elemental analyzer to determine their constituent ratios of heavy to light isotopes (i.e., $R_{\text{sample}}$). The isotope signatures of samples ($\delta^x$), expressed in per mille (‰), are then calculated as $\delta^x = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \times 1000$ where $R_{\text{standard}}$ is the isotopic ratio of a standard (e.g., Vienna PeeDee Belemnite for carbon; air for nitrogen). This technique is often used to study the roles and interactions of organisms in ecosystems, particularly as related to trophic position and diet composition (Vander Zanden & Rasmussen, 1999; Post, 2002), but patterns of stable isotope spatial structure can also be applied to study organismal movement and habitat use (Hobson, 1999; Seminoff et al., 2012).

In freshwater ecology, the most commonly used stable isotopes have been carbon and nitrogen (denoted $\delta^{13}C$ and $\delta^{15}N$, respectively). Specifically, $\delta^{13}C$ provides a tracer of energy source origin because it is fixed by primary producers at photosynthesis and is well-conserved up food chains with little change in value with each increasing trophic level (termed discrimination; generally 0-1 ‰; Fry & Sherr, 1984). Common sources of primary productivity in freshwater habitats that can often be distinguished by analyzing $\delta^{13}C$ include a benthic algal pathway, an
open water phytoplankton pathway, and an allochthonous terrestrial detrital pathway; the
importance of these pathways to consumers can vary depending on habitat attributes (Dekar,
Magoulick & Huxel, 2009; Francis et al., 2011). In contrast to $\delta^{13}$C, $\delta^{15}$N can be used to estimate
trophic position of organisms as it generally increases or discriminates at a predictable 3.4 ± 1.1
‰ with each increasing trophic level, from primary producers to primary, secondary, and tertiary
consumers (Minagawa & Wada, 1984). In some cases, $\delta^{15}$N can be used alone to infer trophic
position of organisms; however, this is not the case if different sources of primary productivity
used by a consumer are depleted or enriched in $\delta^{15}$N relative to each other (Vander Zanden &
Rasmussen, 1999; Post, 2002; Figure 2). Under these circumstances, mixing models can be used
to estimate contributions of different energy pathways to consumers, and subsequently correct
for differences in their $\delta^{15}$N enrichment while calculating trophic position of consumers (Post,
2002).

For this experiment, we collected snails ($Elinia livescens$; n=45) and mussels ($Elliptio
dilatata$; n=5) in the same stretch of the Chippewa River and on the same date as our crayfish
(see above), which we froze at -17.8°C, to be used as primary consumer endpoints in a two end-
member stable isotope mixing model related to calculating trophic position of crayfish. We chose
these specific organisms as they are reliable primary consumers (i.e., trophic position = 2) whose
relatively large size and long lives make their isotopic signatures more robust to spatial and
temporal variation than those of primary producers (Cabana & Rasmussen, 1996; Post, 2002).
Specifically, we used snails to represent the isotopic signature of algal-based primary production
and filter-feeding mussels as an additional endpoint to represent a broad range of other potential
sources of primary production in lotic systems (e.g., benthic algae, terrestrial detritus, and
phytoplankton from upstream lentic systems; Raikow & Hamilton, 2001; Cole & Solomon, 2002).

We dissected crayfish for abdominal muscle, snails for whole body without shell, and mussels for foot muscle. We dried samples at 60°C for 24 h, homogenized them in an ethanol-rinsed mortar and pestle, then weighed and encapsulated aliquots weighing 0.64 ± .04 mg of each sample into tin capsules. We sent these samples to the Stable Isotope Mass Spectrometry Lab at the University of Florida for analysis on a Micromass Prism II isotope ratio mass spectrometer coupled with an elemental analyzer. Two internationally recognized standards (L-glutamic acids), USGS40 (mean ± standard deviation $\delta^{13}$C, -26.39 ‰ ± 0.11; $\delta^{15}$N, -4.53 ‰ ± 0.12; measured repeatedly for calibration) and USGS41 ($\delta^{13}$C, 47.57 ‰; $\delta^{15}$N, 37.36 ‰; measured once as a check standard), were measured during the analysis to ensure precision.

We calculated the relative contribution of the primary productivity represented by snails (SPP) to our crayfish as $$\text{SPP} = \left( \frac{\delta^{13}\text{C}_{\text{crayfish}} - \delta^{13}\text{C}_{\text{mussel}}}{\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}}} \right) \times 100,$$

where $\delta^{13}\text{C}_{\text{crayfish}}$ is the $\delta^{13}\text{C}$ of each crayfish, $\delta^{13}\text{C}_{\text{mussel}}$ is the mean $\delta^{13}\text{C}$ of our mussel samples and $\delta^{13}\text{C}_{\text{snail}}$ is the mean $\delta^{13}\text{C}$ of our snail samples. We then calculated the relative contribution of the primary productivity represented by mussels (MPP) as $$\text{MPP} = 100 - \text{SPP}.$$ Lastly, we calculated the trophic position (TP) of our crayfish as $$\text{TP} = 2 + \frac{\delta^{15}\text{N}_{\text{crayfish}} - \left( \delta^{15}\text{N}_{\text{snail}} \times \frac{\text{SPP}}{100} + \delta^{15}\text{N}_{\text{mussel}} \times \frac{\text{MPP}}{100} \right) \right)}{\Delta^{15}\text{N}},$$

where $\delta^{15}\text{N}_{\text{crayfish}}$ is the $\delta^{15}\text{N}$ of each crayfish, $\delta^{15}\text{N}_{\text{snail}}$ is the mean $\delta^{15}\text{N}$ of the snails, $\delta^{15}\text{N}_{\text{mussel}}$ is the mean $\delta^{15}\text{N}$ of the mussels, and $\Delta^{15}\text{N}$ is a trophic discrimination factor of 3.4 (Minagawa & Wada, 1984).

**Statistical analysis**

We used linear regression to test for a relationship between the mean dominance scores
and calculated trophic positions of our crayfish. Additionally, we performed several linear
regressions controlling for the effect of body size on crayfish dominance by using residuals, as
well as a linear regression testing for a relationship between mean dominance score and
unaltered $\delta^{15}N$ signatures rather than calculated trophic position (supplementary material). All
analyses were conducted using the R statistical program (R Core Team, 2014).

Results

Snails were enriched in $\delta^{13}C$ (mean ± standard deviation; -27.9 ± 0.9 ‰; Figure 2) relative to mussels (-32.0 ± 0.2 ‰). The relatively depleted $\delta^{13}C$ signature of the mussels likely reflects utilization of either phytoplankton or allochthonous terrestrial detritus as food resources, relative to the generally more $^{13}C$ enriched benthic algal pathway (Raikow & Hamilton, 2001; Cole & Solomon, 2002). The percent reliance of crayfish (mean ± standard deviation) on the snail pathway was 67.6 ± 11.3 %, relative to 32.4 ± 11.3 % on the mussel pathway, indicating that most of these crayfish relied twice as much on the snail than mussel resource pathway (see supplementary material for analysis of the relationship between percent reliance of crayfish on the snail primary production pathway and dominance scores). Our mixing model calculations identified reliance of our crayfish on food resources from these two isotopically distinct pathways, allowing us to correct for the relatively depleted $\delta^{15}N$ signature of mussels with respect to the crayfish trophic position. Trophic positions of crayfish ranged from 2.1 to 2.6 with a mean of 2.3 ± 0.1, suggesting a range of foraging behaviors from high reliance on primary producers like benthic algae (i.e., trophic position = 2) to some predation on primary consumers like snails (i.e., trophic position = 3).
The mean crayfish dominance score from the agonistic assays was 29.93 (SD, 28.61; min, -23.33; max, 80.67). We did not find a significant relationship between dominance and trophic position ($y = 0.0005x + 2.32$, $R^2 = 0.013$, $F_{1,38} = 0.51$, $p = 0.48$; Figure 3). Our additional analyses accounting for the role of body size on both dominance and trophic position, as well as those using an alternative measure of trophic position, did not affect our conclusion that there is no association between dominance and trophic position (supplementary material).

Discussion

We failed to find a relationship between crayfish dominance and trophic position. We therefore infer that laboratory dominance among these organisms may not necessarily relate to their dietary preferences in the field, despite our prediction that more dominant crayfish should be more likely than subordinate crayfish to compete successfully for high quality food or to pursue high-risk, high-reward prey (Roth, Hein & Vander Zanden, 2006; Taylor & Soucek, 2010). However, this is to our knowledge the first attempt to relate laboratory behavior to field performance via stable isotope analysis; therefore, more studies are warranted to further explore linkages between these two techniques in light of possible sources of discord.

For example, other behaviors may correlate better with trophic position than dominance in paired agonistic assays. Dominance assays may instead be more informative with respect to acquisition of shelter to avoid fish predation or fitness via sexual selection (Garvey, Stein & Thomas, 1994; Bergman & Moore, 2003), whereas trophic position in the field might correlate better with other measures of laboratory behavior, such as boldness. However, dominance and boldness have been observed to correlate as “behavioral syndromes” in crayfish (Pintor, Sih & Kerby, 2009), and we would therefore expect boldness and dominance to both correlate with
trophic position. It is also possible that there is a temporal disconnect between our analysis of crayfish abdominal tissue, which has an isotopic half-life of approximately 20-30 days (Glon, Larson & Pangle, 2016), and the social memory of our crayfish, which is thought to last from 60 minutes to one week (Bergman et al., 2003). Use of a tissue with a faster turnover rate (e.g., haemolymph) may better reflect the most recent in situ behavior of crayfish. Further, male crayfish of the family Cambaridae cycle between a reproductively inactive form II and an active form I stage. We used form II male crayfish, which are typically less aggressive than crayfish in form I (Bergman et al., 2003). Replicating our experiment with form I individuals might alter the results of agonistic assays and their relationship to trophic position.

Lastly, a possible limitation of our study was our relatively small sample size (n = 40; Galván, Sweeting & Reid, 2010). In order to determine if our lack of a significant relationship stemmed from low power, we conducted power analyses using the pwr package in R (Champely, 2015). We found that for our observed effect size (0.013; calculated as \( \frac{R^2}{1-R^2} \) [Cohen, 1988]) and an alpha of 0.05 and conventional power of 0.80, we would have required 605 crayfish replicates to observe statistical significance. Conversely, for an alpha of 0.05 and power of 0.80, the smallest effect size we would have detected as significant with 40 crayfish replicates was 0.21 (R\(^2\) = 0.173). We therefore conclude that although the effect size observed here could only be detected as statistically significant with an uncommonly high level of replication (perhaps dismissed as statistical significance without biological significance; Nakagawa & Cuthill, 2007), our level of replication was adequate to find significant relatively weak effect sizes down to an R\(^2\) = 0.173.

Although our study failed to find an association between crayfish dominance and stable isotope-estimated trophic position, we believe that there are many unexplored and promising
avenues to combine behavioral and isotope ecology in order to learn more about how behavior observed in laboratories corresponds with movement and organismal interactions in the field. Laboratory experiments and stable isotope analyses have both separately been used to explore the “ecology of individuals” or variation within populations and species (Bolnick et al., 2003; Niemelä & Dingemanse, 2014; Zavorka et al., 2015), yet to our knowledge, researchers have not previously combined or compared these approaches for the same organisms. For example, stable isotope analysis and behavioral assays could be combined to together evaluate whether range expansion of invasive species is being driven by subordinate individuals with low trophic positions that are excluded from core habitats by dominant intraspecific competitors, or instead bold or aggressive individuals with high trophic positions that are inclined to disperse (Hudina, Hock & Žganec, 2014). Further, where distinct stable isotope signatures exist over habitat gradients (Hobson, 1999), researchers could infer whether individuals with or without dispersal-related behaviors observed in the laboratory were actually recent arrivals or longstanding residents of their collection locations. We encourage future studies to further explore the possible insights gained by linking laboratory behavior with field function through stable isotope analysis, as doing so could contribute meaningfully to an array of ecological and evolutionary questions.

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References

1. Bergman DA, Kozlowski CP, McIntyre JC, Huber R, Daws AG, Moore PA. 2003 Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. *Behavior* **140**, 805-825. (doi:10.2307/4536060?ref=no-x-route:f160d35c38bcbb64283778096994b59d)

2. Bergman DA, Moore PA. 2003 Field observations of intraspecific agonistic behavior of two crayfish species. *The Biological Bulletin* **205**, 26-35. (doi:10.2307/1543442)

3. Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003 The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist* **161**, 1-28. (doi: 10.1086/343878)

4. Bondar CA, Bottriell K, Zeron K, Richardson JS. 2005 Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2632-2639. (doi: 10.1139/f05-167)

5. Bruski CA, Dunham DW. 1987 The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I: An analysis of bout dynamics. *Behavior* **103**, 83-107. (doi:10.2307/4534636?ref=no-x-route:f30d21b77345a336a829ecff77a13880)

6. Buchheister A, Latour RJ. 2010 Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 445-461. (doi:10.1139/F09-196)
7. Cabana G, Rasmussen JB. 1996 Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America* 93, 10844-10847.

8. Champely S. 2015 pwr: basic functions for power analysis. R package version 1.1-3. http://CRAN.R-project.org/package=pwr.

9. Cherel Y, Kernaléguen L, Richard P, Guinet C. 2008 Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. *Biology Letters* 5, 830-832.

10. Cohen, J. 1988 Statistical power analysis for the behavioral sciences. 2nd edn. Hillsdale, New Jersey: Lawrence Erlbaum.

11. Cole JJ, Solomon CT. 2012 Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. *Limnology and Oceanography* 57, 1802-1815. (doi:10.4319/lo.2012.57.6.1802)

12. Dekar MP, Magoullick DD, Huxel GR. 2009 Shifts in the trophic base of intermittent stream food webs. *Hydrobiologia* 635, 263-277. (doi:10.1007/s10750-009-9919-1)

13. DeNiro MJ, Epstein S. 1978 Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42, 495-506. (doi:10.1016/0016-7037(78)90199-0)

14. DeNiro MJ, Epstein S. 1981 Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45, 341-351. (doi:10.1016/0016-7037(81)90244-1)
15. Francis TB, Schindler DE, Holtgrieve GW, Larson ER, Scheuerell MD, Semmens BX, Ward EJ. 2011 Habitat structure determines resource use by zooplankton in temperate lakes. *Ecology Letters* **14**, 364-372. (doi:10.1111/j.1461-0248.2011.01597.x.)

16. Fry B, Sherr EB. 1984 $\Delta^{13}$C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**, 13-47. (doi:10.1007%2F978-1-4612-3498-2_12)

17. Galván DE, Sweeting CJ, Reid WDK. 2010 Power of stable isotope techniques to detect size-based feeding in marine fishes. *Marine Ecology Progress Series* **407**, 271-278. (doi:10.3354/meps08528)

18. Garvey JE, Stein RA, Thomas HM. 1994 Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology* **75**, 532-547. (doi:10.2307/1939556)

19. Glon MG, Larson ER, Pangle KL. 2016 Comparison of $^{13}$C and $^{15}$N Discrimination Factors and Turnover Rates between Congeneric Crayfish *Orconectes rusticus* and *O. virilis* (Decapoda, Cambaridae). *Hydrobiologia* **768**, 51-61. (doi:10.1007/s10750-015-2527-3)

20. Hildebrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C. 1996. *Canadian Journal of Zoology* **74**, 2080-2088. (doi: 10.1139/z96-236)

21. Hobson KA. 1999 Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**, 314-326. (doi:10.2307/4222394?ref=no-x-route:da43d6d4b72fe6eabb7763bede305448)

22. Hudina S, Hock K, Žganec K. 2014 The role of aggression in range expansion and biological invasions. *Current Zoology* **60**, 401-409.
23. Larson ER, Magoulick DD. 2009 Does juvenile competition explain displacement of a native crayfish by an introduced crayfish? *Biological Invasions* **11**, 725-735. (doi: http://10.1007/s10530-008-9286-2)

24. Larson ER, Olden JD, Usio N. 2010 Decoupled conservatism of Grinnellian and Eltonian niches in an invasive arthropod. *Ecosphere* **1**, 1-13. (doi: http://10.1890/ES10-00053.1)

25. Lodge DM, Stein RA, Brown KM, Covich AP, Bronmark C, Garvey JE, Klosiewskt SP. 1998 Predicting impact of freshwater exotic species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology* **23**, 53-67. (doi:10.1111/j.1442-9993.1998.tb00705.x)

26. McCarthy JM, Hein CL, Olden JD, Vander Zander JM. 2006 Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology* **51**, 224-235. (doi:10.1111/j.1365-2427.2005.01485.x)

27. Minagawa M, Wada E. 1984 Stepwise enrichment of $^{15}$N along food chains: Further evidence and the relation between $\delta^{15}$N and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135-1140. (doi:10.1016/0016-7037(84)90204-7)

28. Momot WT. 1967 Population dynamics and productivity of the crayfish, *Orconectes virilis*, in a marl lake. *American Midland Naturalist* **78**, 55-81. (doi: 10.2307/2423370)

29. Nakagawa S, Cuthill IC. 2007 Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* **82**, 591-605. (doi: 10.1111/j.1469-185X.2007.00027.x)

30. Niemelä PT, Dingemanse NJ. 2014 Artificial environments and the study of ‘adaptive’ personalities. *Trends in Ecology and Evolution* **29**, 245-247. (doi:10.1016/j.tree.2014.02.007)
31. Olsen TM, Lodge DM, Capelli GM, Houlihan RJ. 1991 Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1853-1861. (doi: 10.1139/f91-219)

32. Phillips RA, Bearhop S, McGill RA, Dawson DA. 2009 Stable isotopes reveal individual variation in migration strategies and habitat preferences in a suite of seabirds during the nonbreeding period. *Oecologia* **160**, 795-806.

33. Peters JA, Cooper MJ, Creque SM, Kornis MS, Maxted JT, Perry WL, Schueler FW, Simon TP, Taylor CA, Thoma RF, Uzarski DG, Lodge DM. 2014 Historical changes and current status of crayfish diversity and distribution in the Laurentian Great Lakes. *Journal of Great Lakes Research* **40**, 35-46. (doi: 10.1016/j.jglr.2014.01.003)

34. Pintor LM, Sih A, Kerby JL. 2009 Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. *Ecology* **90**, 581-587. (doi:10.1890/08-0552.1)

35. Post DM. 2002 Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703-718. (doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)

36. R Core Team 2014 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL [http://www.R-project.org/](http://www.R-project.org/).

37. Raikow DF, Hamilton SK. 2001 Bivalve diets in a Midwestern U.S. stream: A stable isotope enrichment study. *Limnology and Oceanography* **46**, 514-422. (doi:10.4319/lo.2001.46.3.0514)
38. Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007 Integrating animal temperament within ecology and evolution. *Biological Review* **82**, 291-318. (doi:10.1111/j.1469-185X.2007.00010.x)

39. Roth BM, Hein CL, Vander Zanden MJ. 2006 Using bioenergetics and stable isotopes to assess the trophic role of rusty crayfish (*Orconectes rusticus*) in lake littoral zones. *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 335-344. (doi:10.1139/f05-217)

40. Rubenstein DR, Hobson KA. 2004 From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution* **5**, 256-263. (doi:10.1016/j.tree.2004.03.017)

41. Seebacher F, Wilson RS. 2007 Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. *Biology Letters* **3**, 471-474. (doi:10.1098/rsbl.2007.0289)

42. Seminoff JA, Benson SR, Arthur KE, Eguchi T, Dutton PH, Tapilatu RF, Popp BN. 2012 Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and δ15N analysis of amino acids. *PLOS One* **7**, e37403. (doi:10.1371/journal.pone.0037403)

43. Sih A, Bell A, Johnson JC. 2004 Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution* **19**, 372-378. (doi:10.1016/j.tree.2004.04.009)

44. Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR. 2010 Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos* **119**, 610-621. (doi:10.1111/j.1600-0706.2009.18039.x)

45. Taylor CA, Soucek DJ. 2010 Re-examining the Importance of Fish in the Diets of Stream-dwelling Crayfishes: Implications for Food Web Analyses and Conservation. *The
430 American Midland Naturalist 163, 280-293. (doi:10.2307/40730925?ref=no-x-
route:9c05d428ae4e32a102983467464740d)

432 46. Vander Zanden MJ, Rasmussen JB. 1999 Primary consumer $\delta^{13}$C and $\delta^{15}$N and the
trophic position of aquatic consumers. Ecology 80, 1395-1404. (doi:10.1890/0012-
9658(1999)080[1395:PCCANA]2.0.CO;2)

435 47. Wilson KA, Magnuson JJ, Lodge DM, Hill AM, Kratz TK, Perry WL, Willis TV. 2004 A
long-term rusty crayfish (Orconectes rusticus) invasion: dispersal patterns and
community change in a north temperate lake. Canadian Journal of Fisheries and Aquatic
Sciences 61, 2255-2266. (doi: 10.1139/f04-170)

439 48. Zavorka L, Aldven D, Naslund J, Hojesjo J, Johnsson JI. 2015 Linking lab activity with
growth and movement in the wild: explaining pace-of-life in a trout stream. Behavioral
Ecology 26, 877-884. (doi:10.1093/beheco/arv029)
Figure 1. Stable isotopes could permit researchers to hindcast the ecological interactions of organisms, linking behaviors observed in the laboratory with previous field function or behavior. Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/). Background image is of the study location where organisms were collected (Chippewa River).
Figure 2. Isotopic biplot of $\delta^{13}C$ and $\delta^{15}N$ for crayfish (red circles), mussels (green triangles), and snails (blue squares). All values are expressed in per mille (‰) relative to a standard of V-PDB (Vienna PeeDee Belemnite) for carbon and air for nitrogen.
Figure 3. Scatterplot (with 95% CI) of mean assay dominance score for each crayfish over three agonistic assays and in situ trophic position ($y = 0.0005x + 2.32$, $R^2 = 0.013$, $F_{1,38} = 0.51$, $p = 0.48$).
| Score | Description                                                                 |
|-------|-----------------------------------------------------------------------------|
| -2    | Tail flip or fast retreat                                                  |
| -1    | Slow retreat                                                               |
| 0     | Within one body length with no visible interaction                          |
| 1     | Approach without threat display                                             |
| 2     | Approach with threat display (e.g., meral spread, antennal whips)           |
| 3     | Boxing, pushing, or other agonistic interaction with closed chelae           |
| 4     | Grabbing, tearing, or other agonistic interaction with opened chelae        |
| 5     | Full out, unrestrained fighting, usually with interlocked chelae            |
Supplementary material for “Connecting Laboratory Behavior to Field Function through Stable Isotope Analysis”

Page Content

Table S1. Crayfish Morphometrics

Figure S1. Scatterplot of crayfish carapace length and dominance scores

Figure S2. Scatterplot of the residuals from dominance scores and carapace length regression and calculated trophic position

Figure S3. Scatterplot of crayfish dominance scores and $\delta^{15}$N signatures

Figure S4. Scatterplot of crayfish dominance scores and percent reliance on snail primary production

IV. References
I. Crayfish morphometrics

Size influences the outcome of crayfish agonistic trials (Rubenstein & Hazlett, 1974; Bergman & Moore, 2003); therefore, to better understand what intrinsic factors might be affecting the results of our agonistic assays, we used digital calipers to measure carapace length (CL; from the tip of the rostrum to the posterior edge of the carapace), chelae width (at the widest point of the palm), and chelae length (from the attachment of the carpus and the propodus to the most distal point of the fixed finger) to the nearest hundredth of a mm. We used a digital balance to measure mass to the nearest hundredth of a gram (Table S1). Prior to weighing, we dabbed all crayfish dry for 10 seconds with a paper towel.
| Measurement          | Mean   | Standard Deviation | Minimum | Maximum |
|----------------------|--------|--------------------|---------|---------|
| Carapace length (mm) | 25.38  | 1.26               | 23.41   | 27.53   |
| Chelae length (mm)   | 17.38  | 1.70               | 14.05   | 21.36   |
| Chelae width (mm)    | 7.20   | 0.85               | 5.02    | 8.31    |
| Mass (g)             | 5.06   | 0.78               | 3.7     | 6.5     |
II. Alternative comparisons of dominance and trophic position

Body size is a factor that strongly influences the outcome of agonistic encounters in crayfish, with larger individuals generally being more dominant (Bovbjerg, 1953; Rubenstein & Hazlett, 1974; Bergman & Moore, 2003). We used as small of a crayfish size range as logistically possible, but the difference between our largest and smallest study organisms was still 4.12 mm carapace length (Table S1). Despite this, most paired agonistic interaction trials were between more closely size-matched crayfish (mean ± standard deviation; 1.44 ± 1.15 mm carapace length). Regardless, we sought to determine if dominance scores might better correspond with the trophic positions of our crayfish if we corrected for the role of size differences in determining outcomes of agonistic interactions. We did not correct for potential ontogenetic effects of crayfish size on trophic position (Bondar et al., 2005; Larson, Olden & Usio, 2010), because we found no significant relationship between crayfish carapace length (Table S1) and trophic position ($y = 0.002x + 2.27$, $R^2 = 0.001$, $F_{1,38} = 0.02$, $p = 0.88$). However, as we anticipated, there was a significant relationship between crayfish carapace length and mean dominance score ($y = 11.503x - 261.971$, $R^2 = 0.26$, $F_{1,38} = 13.03$, $p < 0.001$; Figure S1). Yet, when we corrected for the effect of crayfish size on dominance by regressing residuals of the preceding analysis against trophic position, we still did not find a significant relationship, consistent with our main text conclusion ($y = 0.00x + 2.34$, $R^2 = 0.01$, $F_{1,38} = 0.54$, $p = 0.47$; Figure S2). The lack of a relationship between dominance and trophic position is therefore conserved even when accounting for the potential influence of crayfish size on dominance.

Carapace length is the most commonly used size metric for crayfish; however, chelae size has been shown to dictate success in agonistic encounters and may be a better measure of dominance in crayfish (Garvey & Stein, 1993). We therefore ran two additional iterations of the
analysis presented above, using chelae length and width instead of carapace length. We found significant relationships between mean dominance scores and both chelae length ($y = 9.125x – 128.686, R^2 = 0.29, F_{1,38} = 15.72, p < 0.001$) and chelae width ($y = 16.040x – 85.562, R^2 = 0.23, F_{1,38} = 11.07, p = 0.002$). Yet again, regressing residuals from the chelae length or width and dominance score analyses against trophic position did not change our main text conclusion that dominance and trophic position are unrelated (chelae length residuals vs trophic position: $y = 0.0002x – 2.34, R^2 = 0.001, F_{1,38} = 0.04, p = 0.85$; chelae width residuals vs trophic position: $y = -0.0001x – 2.34, R^2 = 0.01, F_{1,38} = 0.02, p = 0.89$).

The use of isotopic mixing models, applied here as a step in calculating trophic position (Post, 2002), is dependent on a number of assumptions. For example, stream and river ecosystems can have extremely high spatiotemporal variation in the $\delta^{13}C$ and $\delta^{15}N$ values of sources of primary production owing to a number of factors (Fry & Sherr, 1984; Finlay, 2001; Trudeau & Rasmussen, 2003). Accordingly, we followed convention in using primary consumers rather than primary producers in mixing model calculations of trophic position, as long-lived organisms like mussels or snails can integrate and correct for this variability (Post, 2002; Cabana & Rasmussen, 1996). However, we cannot exclude that our field sampling of primary consumer endpoints for our mixing model could have missed some such variability inherent to heterogeneous lotic ecosystems, and our collection of potential prey resources concurrent with crayfish consumers does not necessarily reflect isotopic values of prey items for *Orconectes rusticus* over preceding weeks or months (Moore & Semmens, 2008). Another assumption of mixing models is that constant discrimination factors can be used for each trophic step and between different taxonomic groups and diet items. However, discrimination factors can vary across taxa, diets, and tissues used (e.g., Stenroth *et al.*, 2006; Caut, Angulo & Courchamp,
2009; Phillips et al., 2014), and consequently may misrepresent trophic position of a focal organism (Bond & Diamond, 2011). Due to the potential vulnerability of our model to the preceding assumptions, we also conducted a simpler analysis using crayfish dominance scores and unaltered δ^{15}N values to determine if our results were dependent on our specific trophic position calculations. Doing so did not alter our overall nonsignificant result and conclusion (y = 0.002x + 11.04, R^2 = 0.03, F_{1,38} = 1.29, p = 0.26; Figure S3). We therefore conclude that our result of a lack of relationship between crayfish dominance in the laboratory and trophic position in the field is robust to our measures of both crayfish dominance and trophic position.
Figure S1. Scatterplot (with 95% CI) showing significant relationship between crayfish carapace length and dominance score from behavioral assays ($y = 11.503x - 261.971$, $R^2 = 0.26$, $F_{1,38} = 13.03$, $p < 0.001$).
Figure S2. Scatterplot (with 95% CI) of the residuals from crayfish dominance and carapace length regression against calculated trophic position ($y = 0.001x + 2.34$, $R^2 = 0.01$, $F_{1,38} = 0.54$, $p = 0.47$).

Figure S2. Scatterplot (with 95% CI) of the residuals from crayfish dominance and carapace length regression against calculated trophic position ($y = 0.001x + 2.34$, $R^2 = 0.01$, $F_{1,38} = 0.54$, $p = 0.47$).
Figure S3. Scatterplot (with 95% CI) of crayfish dominance scores and δ¹⁵N signatures ($y = 0.002x + 11.04$, $R^2 = 0.03$, $F_{1,38} = 1.29$, $p = 0.26$)
III. Analysis of the relationship between percent reliance of crayfish on the snail primary production pathway and dominance

Our mixing model calculations revealed variation in the percent reliance of *O. rusticus* from the Chippewa River on food resources represented by the two primary consumer endpoints used in our study. Specifically, crayfish relied more on the isotopically-enriched primary production represented by snails (mean ± standard deviation, 67.6 ± 11.3%) than on the isotopically-depleted primary production represented by mussels (32.4 ± 11.3 %; Figure 2). In lotic systems, the isotopic values of freshwater mussels generally reflect those of a broad range of potential sources of primary production including terrestrial detritus and phytoplankton from upstream lentic systems (Raikow & Hamilton, 2001; Cole & Solomon, 2002). Therefore, reliance of our crayfish on the mussel endpoint may reflect dependence of *O. rusticus* on terrestrial detritus, in part because we do not anticipate high reliance of crayfish on phytoplankton (Stenroth et al., 2006). Conversely, reliance of *O. rusticus* on snails likely represents use of benthic algae as a basal resource, particularly given the generally low trophic positions of the crayfish in our study. Benthic algae or other autochthonous production in freshwater ecosystems may be a higher quality diet item than terrestrial detritus (Finlay, 2001; Brett et al., 2009), and consequently, we hypothesized that more dominant crayfish in our behavioral assays might show greater dependence on algal or snail resources than the alternative resources represented by freshwater mussels. In order to test this hypothesis, we ran a regression between percent reliance on the snail endpoint of our mixing model and our dominance assay scores, as previously analyzed for trophic position (see main text and above). Again, we did not find a significant relationship between crayfish dominance and diet ($y = 0.02x + 66.98$, $R^2 = 0.002$, $F_{1,38} = 0.09$, $p = 0.76$; Figure S4), further supporting our conclusion that results of *ex situ*
laboratory behavioral trials do not necessarily translate to the hindcasted \textit{in situ} ecology of these same individual organisms.
Figure S4. Scatterplot (with 95% CI) of mean assay dominance score for each crayfish over three agonistic assays and percent reliance on the snail primary production pathway ($y = 0.02x + 66.98$, $R^2 = 0.002$, $F_{1,38} = 0.09$, $p = 0.76$).
IV. References

1. Bergman DA, Moore PA. 2003 Field observations of intraspecific agonistic behavior of two crayfish species. *The Biological Bulletin* **205**, 26-35. (doi:10.2307/1543442)

2. Bond AL, Diamond AW. 2011 Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications* **21**, 1017-1023.

3. Bondar CA, Bottriell K, Zeron K, Richardson JS. 2005 Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2632-2639. (doi: 10.1139/f05-167)

4. Bovbjerg RV. 1953 Dominance order in the Crayfish *Orconectes virilis* (Hagen). *Physiological Zoology* **26**, 173-178.

5. Brett MT, Kainz MJ, Taipale SJ, Seshan H. 2009 Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 21197-21201.

6. Cabana G, Rasmussen JB. 1996 Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 10844-10847.

7. Caut S, Angulo E, Courchamp F. 2009. Variation in discrimination factors (Δ¹⁵N and Δ¹³C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* **46**, 443-453. (doi:10.1111/j.1365-2664.2009.01620.x)

8. Cole JJ, Solomon CT. 2012 Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. *Limnology and Oceanography* **57**, 1802-1815. (doi:10.4319/lo.2012.57.6.1802)
9. Finlay JC. 2001 Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology* **82**, 1052-1064. (doi:10.1890/0012-9658(2001)082[1052:SCIROR]2.0.CO;2)

10. Fry B, Sherr EB. 1984 $\Delta^{13}$C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**, 13-47. (doi:10.1007%2F978-1-4612-3498-2_12)

11. Garvey JE, Stein RA. 1993 Evaluating how chela size influences the invasion potential of an introduced crayfish (Orconectes rusticus). *The American Midland Naturalist* **129**, 172-181. (doi:10.2307/2426446)

12. Larson ER, Olden JD, Usio N. 2010 Decoupled conservatism of Grinnellian and Eltonian niches in an invasive arthropod. *Ecosphere* **1**, 1-13. (doi: http://10.1890/ES10-00053.1)

13. Moore JW, Semmens BX. 2008 Incorporating uncertainty and prior information into stable isotope mixing models. *Nature* **11**, 470-480. (doi:10.1111/j.1461-0248.2008.01163.x)

14. Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ. 2014 Best practices for use of stable isotope mixing models in food web studies. *Canadian Journal of Zoology* **92**, 823-835. (doi: http://10.1139/cjz-2014-0127)

15. Post DM. 2002 Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703-718. (doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)

16. Raikow DF, Hamilton SK. 2001 Bivalve diets in a Midwestern U.S. stream: A stable isotope enrichment study. *Limnology and Oceanography* **46**, 514-422. (doi:10.4319/lo.2001.46.3.0514)
17. Roth BM, Hein CL, Vander Zanden MJ. 2006 Using bioenergetics and stable isotopes to assess the trophic role of rusty crayfish (Orconectes rusticus) in lake littoral zones. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 335-344. (doi:10.1139/f05-217)

18. Rubenstein DI, Hazlett BA. 1974 Examination of the agonistic behavior of the crayfish *Orconectes virilis* by character analysis. *Behavior* 50, 193-216. (doi:10.2307/4533609)

19. Stenroth P, Homqvist N, Nyström P, Berglund O, Larsson P, Granéli W. 2006 Stable isotopes as an indicator of diet in omnivorous crayfish (Pacifastacus leniusculus): the influence of tissue, sample treatment, and season. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 821-831. (doi:10.1139/f05-265)

20. Trudeau V, Rasmussen JB. 2003 The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology and Oceanography* 48, 2194-2199. (doi:10.4319/lo.2003.48.6.2194)