Connecting laboratory behavior to field function through stable isotope analysis

Mael G Glon, Eric R Larson, Kevin L Pangle

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.

1   **Connecting Laboratory Behavior to Field Function through Stable Isotope Analysis**

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24   **Abstract**

25   Inherent difficulties of tracking and observing organisms in the field often leave

26   researchers with no choice but to conduct behavioral experiments under laboratory settings.

27   However, results of laboratory experiments do not always translate accurately to natural

28   conditions. A fundamental challenge in ecology is therefore to scale up from small area and

29   short-duration laboratory experiments to large areas and long-durations over which ecological

30   processes generally operate. In this study, we propose that stable isotope analysis may be a tool

31   that can link laboratory behavioral observations to past field interactions or function of

32   individual organisms. We conducted laboratory behavioral assays to measure dominance of

33   invasive rusty crayfish, *Orconectes rusticus*, and used stable isotope analysis to hindcast trophic

34   positions of these crayfish under preceding natural conditions. We hypothesized that more

35   dominant crayfish in our assays would have higher trophic positions if dominance were related to

36   competitive ability or willingness to pursue high-risk, high-reward prey. We did not find a

37   relationship between crayfish dominance and trophic position, and therefore infer that laboratory

38   dominance of crayfish may not necessarily relate to their ecology in the field. However, this is to

39   our knowledge the first attempt to directly relate laboratory behavior to field performance via

40   stable isotope analysis. We encourage future studies to continue to explore a possible link

41   between laboratory and field behavior via stable isotope analysis, and propose several avenues to

42   do so.

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Introduction

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48   Animal behavior is inherently linked with the fields of ecology and evolution (Sih, Bell

49   & Johnson, 2004; Réale, Reader & Sol, 2007), and informs applications such as management of

50   biological invasions (Sih *et al*., 2010). Owing to logistical difficulties inherent to tracking and

51   observing organisms without interference in the field, however, many behavioral studies are

52   conducted *ex situ* in a laboratory setting, where it may be difficult to extrapolate findings to

53   natural conditions (Niemelä & Dingemanse, 2014; Zavorka *et al*., 2015). For example, a suite of

54   often-correlated behaviors including aggression, dominance, and boldness are believed to

55   contribute to the success of some invasive over native species (Pintor, Sih & Kerby, 2009;

56   Hudina, Hock & Žganec, 2014), but these same behaviors can be considerably muted in duration

57   or intensity when observed in the field (Bergman & Moore, 2003; Larson & Magoulick, 2009).

58   One of ecology's most fundamental challenges is scaling up from the type of small area and

59   short duration experiments that are easy to conduct, to the larger areas and longer durations over

60   which ecological processes often operate (Lodge *et al*., 1998). This same challenge applies when

61   relating animal behaviors observed in the laboratory to ecological function and intra- or inter-

62   specific interactions *in situ*.

63   We propose here that linking laboratory behavioral observations to past field interactions

64   or function of specific, individual organisms may be an overlooked application of stable isotope

65   analysis. Stable isotopes of elements such as carbon and nitrogen are assimilated into tissues of

66   consumer organisms relative to their diets in predictable and quantifiable ways (DeNiro &

67   Epstein, 1978; DeNiro & Epstein, 1981). Importantly, stable isotopes of consumers equilibrate

68   with those of their diets at different rates for different tissues, giving snapshots of ecological

69   interactions that may scale from previous days to years (Buchheister & Latour, 2010). Analyzing

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70   stable isotope ratios in organisms can provide ecological insights ranging from habitat use and

71   movement (Hobson, 1999) to trophic position (Post, 2002). For example, stable isotope analysis

72   of feathers has been used to make inferences about migration and habitat use of several species

73   of seabirds that spend winter months far from land and are therefore difficult to study during this

74   period (Phillips *et al.* 2009). In another example, Cherel *et al*. (2008) used stable isotope analysis

75   to identify the trophic position and diet composition of southern elephant seals (*Mirounga*

76   *leonina*) which forage at depths exceeding 1000 m and have largely digested their meals by the

77   time they return to land, precluding them from being studied using traditional methods (e.g.,

78   direct observation, gut content analysis). Similarly to how these and other studies have applied

79   stable isotope analysis to infer the influence of past behavior on current success of organisms, we

80   propose that stable isotope analysis could permit researchers to link laboratory interactions with

81   previous *in situ* habitat selection, movement, diet choice, or competitive interactions (Figure 1).

82   We conducted laboratory behavioral assays to measure individual dominance of invasive

83   rusty crayfish, *Orconectes rusticus*, and used stable isotope analysis to hindcast trophic position

84   of these crayfish under natural field conditions. We predicted that more dominant crayfish in the

85   behavioral assays would have higher trophic positions if dominance were related to competitive

86   ability in the field (e.g., ability to access high quality food such as macroinvertebrates; Roth,

87   Hein & Vander Zanden, 2006) or willingness to pursue high-risk, high-reward prey such as fish

88   or other crayfish (Taylor & Soucek, 2010). Alternatively, dominance and trophic position may

89   not be associated if laboratory behaviors are ultimately uninformative with respect to past

90   interactions of organisms. Numerous previous studies have used stable isotope analysis to infer

91   various *in situ* behaviors of organisms, such as habitat use and diet preferences (e.g., Hildebrand

92   *et al.*, 1996; Rubenstein & Hobson, 2004); however, our study is the first to our knowledge to

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93   seek a direct relationship for individual organisms between laboratory behaviors and field

94   function as determined by stable isotope analysis, and proposes the linkage of laboratory

95   behavioral assays and stable isotopes as a more common practice in the future.

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97   **Methods**

98   *Orconectes rusticus* was introduced via the bait trade to the Laurentian Great Lakes circa

99   1960 and has negatively affected fish, macrophytes, and freshwater macroinvertebrates

100   (McCarthy *et al.*, 2006; Peters *et al.* 2014). The invasion success of this crayfish has made it the

101   focal point of a large number of laboratory and field studies (e.g., Olsen *et al.*, 1991; Wilson *et

102   al.*, 2004) and hence, a useful organism to test for linkages between field and laboratory

103   behavior. We collected adult form II (reproductively inactive) male *O. rusticus* (n=40) by hand

104   on 16 June 2015 in the Chippewa River, Michigan (43.5652°, -84.9183°), where this species is

105   invasive. Because size influences the outcome of crayfish agonistic trials (Bergman & Moore,

106   2003), we used crayfish within a carapace length range of 23.41 to 27.53 mm, the smallest size

107   range for which we could collect 40 crayfish (see supplementary material for additional

108   morphometrics). Rusty crayfish in this size range are small adults of the same age class (Momot,

109   1967) and are therefore unlikely to have diets that differ from one another due to ontogenetic

110   shifts (Bondar *et al.*, 2005; Larson, Olden & Usio, 2010). Immediately following collection, we

111   placed crayfish in individual 16 oz. plastic containers filled to a depth of 2 cm with river water

112   and a rock for shelter.

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114   *Agonistic assays*

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115   Laboratory agonistic assays for crayfish are often conducted after isolating individuals

116   for at least one week to remove possible previous social experience that could influence

117   interactions (Seebacher & Wilson, 2007). We conducted our experiment directly following

118   collection (17 June 2015 during daylight hours [07h19-18h59]), but believe that retaining any

119   existing dominance hierarchies from the field would increase the likelihood of a relationship

120   between laboratory behaviors and previous field function.

121   We conducted three rounds of twenty, randomized paired assays, with each crayfish

122   fighting one opponent per round (no interactions between individuals were repeated). In order to

123   track individual crayfish, we randomly assigned each crayfish a number from 1 to 40, which we

124   wrote on the dorsal side of its carapace using a permanent marker. Prior to the start of each

125   assay, crayfish were placed on opposite sides of a separator in a 19 l bucket and allowed to

126   acclimate for 15 minutes. We then removed the separator and allowed the crayfish to interact for

127   10 minutes. During each assay, we scored each of the two crayfish individually based on the

128   interactions that took place when they were within one body length of each other. All agonistic

129   assays were watched and scored in real time by a single observer to ensure consistency in

130   scoring. The agonistic assays within each of the three rounds were held in a random order, and

131   the observer had no knowledge of totaled crayfish scores from previous rounds so as to avoid

132   bias.

133   The scoring system we used has possible point values ranging from -2 (fast retreat) to 5

134   (unrestrained fighting) and is based on the ethogram modified from Bruski & Dunham (1987;

135   Table 1). Following each assay, the participating crayfish were returned to their original holding

136   container. We then rinsed buckets and refilled them to a depth of 5 cm with fresh water from the

137   Chippewa River (18-20°C). At the conclusion of all assays, crayfish were placed in individual,

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138   labelled bags and euthanized by freezing at -17.8°C. We calculated the dominance score of each

139   crayfish by first summing its scores from each round, then taking the mean of the three resulting

140   scores.

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142   *Stable isotope analysis*

143   Stable isotope analysis is a technique based on the principle that the ratios of heavy to

144   light isotopes in the tissues of consumers reflect those of their diets in a predictable way (DeNiro

145   & Epstein, 1978; DeNiro & Epstein, 1981). Stable isotope analysis generally entails drying and

146   homogenizing tissue or whole-body samples of focal organisms, then using a mass spectrometer

147   coupled with an elemental analyzer to determine their constituent ratios of heavy to light isotopes

148   (i.e., R_{sample}). The isotope signatures of samples (δ^x), expressed in per mille (‰), are then

149   calculated as $\delta^x = ((\frac{R_{sample}}{R_{standard}}) - 1) * 1000$ where $R_{standard}$ is the isotopic ratio of a standard (e.g.,

150   Vienna PeeDee Belemnite for carbon; air for nitrogen). This technique is often used to study the

151   roles and interactions of organisms in ecosystems, particularly as related to trophic position and

152   diet composition (Vander Zanden & Rasmussen, 1999; Post, 2002), but patterns of stable isotope

153   spatial structure can also be applied to study organismal movement and habitat use (Hobson,

154   1999; Seminoff *et al.*, 2012).

155   In freshwater ecology, the most commonly used stable isotopes have been carbon and

156   nitrogen (denoted $\delta^{13}C$ and $\delta^{15}N$, respectively). Specifically, $\delta^{13}C$ provides a tracer of energy

157   source origin because it is fixed by primary producers at photosynthesis and is well-conserved up

158   food chains with little change in value with each increasing trophic level (termed discrimination;

159   generally 0-1 ‰; Fry & Sherr, 1984). Common sources of primary productivity in freshwater

160   habitats that can often be distinguished by analyzing $\delta^{13}C$ include a benthic algal pathway, an

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161   open water phytoplankton pathway, and an allochthonous terrestrial detrital pathway; the

162   importance of these pathways to consumers can vary depending on habitat attributes (Dekar,

163   Magoulick & Huxel, 2009; Francis *et al.*, 2011). In contrast to δ^{13}C, δ^{15}N can be used to estimate

164   trophic position of organisms as it generally increases or discriminates at a predictable 3.4 ± 1.1

165   ‰ with each increasing trophic level, from primary producers to primary, secondary, and tertiary

166   consumers (Minagawa & Wada, 1984). In some cases, δ^{15}N can be used alone to infer trophic

167   position of organisms; however, this is not the case if different sources of primary productivity

168   used by a consumer are depleted or enriched in δ^{15}N relative to each other (Vander Zanden &

169   Rasmussen, 1999; Post, 2002; Figure 2). Under these circumstances, mixing models can be used

170   to estimate contributions of different energy pathways to consumers, and subsequently correct

171   for differences in their δ^{15}N enrichment while calculating trophic position of consumers (Post,

172   2002).

173   For this experiment, we collected snails (*Elimia livescens*; n=45) and mussels (*Elliptio*

174   *dilatata*; n=5) in the same stretch of the Chippewa River and on the same date as our crayfish

175   (see above), which we froze at -17.8°C, to be used as primary consumer endpoints in a two end-

176   member stable isotope mixing model related to calculating trophic position of crayfish. We chose

177   these specific organisms as they are reliable primary consumers (i.e., trophic position = 2) whose

178   relatively large size and long lives make their isotopic signatures more robust to spatial and

179   temporal variation than those of primary producers (Cabana & Rasmussen, 1996; Post, 2002).

180   Specifically, we used snails to represent the isotopic signature of the algal food web, and filter-

181   feeding mussels as an additional endpoint to represent a broad range of other potential sources of

182   primary production in lotic systems (e.g., benthic algae, terrestrial detritus, and phytoplankton

183   from upstream lentic systems; Raikow & Hamilton, 2001; Cole & Solomon, 2002).

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184   We dissected crayfish for abdominal muscle, snails for whole body without shell, and

185   mussels for foot muscle. We dried samples at 60°C for 24 h, homogenized them in an ethanol-

186   rinsed mortar and pestle, then weighed and encapsulated aliquots weighing .64 ± .04 mg of each

187   sample into tin capsules. We sent these samples to the Stable Isotope Mass Spectrometry Lab at

188   the University of Florida for analysis on a Micromass Prism II isotope ratio mass spectrometer

189   coupled with an elemental analyzer. Two internationally recognized standards (l-glutamic acids),

190   USGS40 (mean ± standard deviation δ^{13}C, -26.39 ‰ ± 0.11; δ^{15}N, -4.53 ‰ ± 0.12; measured

191   repeatedly for calibration) and USGS41 (δ^{13}C, 47.57 ‰; δ^{15}N, 37.36 ‰; measured once as a

192   check standard), were measured during the analysis to ensure precision.

193   We calculated the relative contribution of the primary productivity represented by snails

194   (SPP) to our crayfish as $\text{SPP} = \frac{(\delta^{13}C_{crayfish} - \delta^{13}C_{mussel})}{(\delta^{13}C_{snail} - \delta^{13}C_{mussel})} * 100$, where $\delta^{13}C_{crayfish}$ is the δ^{13}C of

195   each crayfish, $\delta^{13}C_{mussel}$ is the mean δ^{13}C of our mussel samples and $\delta^{13}C_{snail}$ is the mean δ^{13}C of

196   our snail samples. We then calculated the relative contribution of the primary productivity

197   represented by mussels (MPP) as $\text{MPP} = 100 - \text{SPP}$. Lastly, we calculated the trophic position

198   (TP) of our crayfish as $\text{TP} = 2 + \frac{\delta^{15}N_{crayfish} - (\delta^{15}N_{snail} * SPP + \delta^{15}N_{mussel}(1 - SPP))}{\Delta^{15}N}$, where $\delta^{15}N_{crayfish}$ is

199   the δ^{15}N of each crayfish, $\delta^{15}N_{snail}$ is the mean δ^{15}N of the snails, $\delta^{15}N_{mussel}$ is the mean δ^{15}N of

200   the mussels, and Δ^{15}N is a trophic discrimination factor of 3.4 (Minagawa & Wada, 1984).

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202   *Statistical analysis*

203   We used linear regression to test for a relationship between the mean dominance scores

204   and calculated trophic positions of our crayfish. Additionally, we performed several linear

205   regressions controlling for the effect of body size on crayfish dominance by using residuals, as

206   well as a linear regression testing for a relationship between mean dominance score and

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207   unaltered δ^{15}N signatures rather than calculated trophic position (supplementary material). All

208   analyses were conducted using the R statistical program (R Core Team, 2014).

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210   **Results**

211   Snails were enriched in δ^{13}C (mean ± standard deviation; -27.9 ± 0.9 ‰; Figure 2)

212   relative to mussels (-32.0 ± 0.2 ‰). The relatively depleted δ^{13}C signature of the mussels likely

213   reflects utilization of either phytoplankton or allochthonous terrestrial detritus as food resources,

214   relative to the generally more ^{13}C enriched benthic algal pathway (Raikow & Hamilton, 2001;

215   Cole & Solomon, 2002). Our mixing model allowed us to correct for the relatively depleted δ^{15}N

216   signature of mussels with respect to the trophic positions of our crayfish. The percent reliance of

217   crayfish (mean ± standard deviation) on the algae/snail pathway was 67.6 ± 11.3 %, relative to

218   32.4 ± 11.3 % on the detritus or phytoplankton/mussel pathway, indicating that most of these

219   crayfish relied twice as much on the algal than mussel resource pathway. Trophic positions of

220   crayfish ranged from 2.1 to 2.6 with a mean of 2.3 ± 0.1, suggesting a range of foraging

221   behaviors from high reliance on primary producers like benthic algae (i.e., trophic position = 2)

222   to some predation on primary consumers like snails (i.e., trophic position = 3).

223   The mean crayfish dominance score from the agonistic assays was 29.93 (SD, 28.61;

224   min, -23.33; max, 80.67). We did not find a significant relationship between dominance and

225   trophic position ($y = 0.0005x + 2.32$, $R^2 = 0.0132$, $F_{1,38} = 0.5084$, $p = 0.4802$; Figure 3). Our

226   additional analyses accounting for the role of body size on both dominance and trophic position,

227   as well as those using an alternative measure of trophic position, did not affect our conclusion

228   that there is no association between dominance and trophic position (supplementary material).

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230   **Discussion**

231   We failed to find a relationship between crayfish dominance and trophic position. We

232   therefore infer that laboratory dominance among these organisms may not necessarily relate to

233   their dietary preferences in the field, despite our prediction that more dominant crayfish should

234   be more likely than subordinate crayfish to compete successfully for high quality food or to

235   pursue high-risk, high reward prey (Roth, Hein & Vander Zanden, 2006; Taylor & Soucek,

236   2010). However, this is to our knowledge the first attempt to relate laboratory behavior to field

237   performance via stable isotope analysis; therefore, more studies are warranted to further explore

238   linkages between these two techniques in light of possible sources of discord.

239   For example, other behaviors may correlate better with trophic position than dominance

240   in paired agonistic assays. Dominance assays may instead be more informative with respect to

241   acquisition of shelter to avoid fish predation or fitness via sexual selection (Garvey, Stein &

242   Thomas, 1994; Bergman & Moore, 2003), whereas trophic position in the field might correlate

243   better with other measures of laboratory behavior, such as boldness. However, dominance and

244   boldness have been observed to correlate as "behavioral syndromes" in crayfish (Pintor, Sih &

245   Kerby, 2009), and we would therefore expect boldness and dominance to both correlate with

246   trophic position. It is also possible that there is a temporal disconnect between our analysis of

247   crayfish abdominal tissue, which has an isotopic half-life of approximately 20-30 days (Glon,

248   Larson & Pangle, 2016), and the social memory of our crayfish, which is thought to last from 60

249   minutes to one week (Bergman *et al.*, 2003). Use of a tissue with a faster turnover rate (e.g.,

250   haemolymph) may better reflect the most recent *in situ* behavior of crayfish. Further, male

251   crayfish of the family Cambaridae cycle between a reproductively inactive form II and an active

252   form I stage. We used form II male crayfish, which are typically less aggressive than crayfish in

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253   form I (Bergman *et al.*, 2003). Replicating our experiment with form I individuals might alter the

254   results of agonistic assays and their relationship to trophic position.

255   Lastly, a possible limitation of our study was our relatively small sample size (n = 40;

256   Galván, Sweeting & Reid, 2010). In order to determine if our lack of a significant relationship

257   stemmed from low power, we conducted power analyses using the pwr package in R (Champely,

258   2015). We found that for our observed effect size (0.013; calculated as $\frac{R^2}{1-R^2}$ [Cohen, 1988]) and

259   an alpha of 0.05 and conventional power of 0.80, we would have required 605 crayfish replicates

260   to observe statistical significance. Conversely, for an alpha of 0.05 and power of 0.80, the

261   smallest effect size we would have detected as significant with 40 crayfish replicates was 0.21

262   ($R^2 = 0.173$). We therefore conclude that although the effect size observed here could only be

263   detected as statistically significant with an uncommonly high level of replication (perhaps

264   dismissed as statistical significance without biological significance; Nakagawa & Cuthill, 2007),

265   our level of replication was adequate to find significant relatively weak effect sizes down to an

266    $R^2 = 0.173$.

267   Although our study failed to find an association between crayfish dominance and stable

268   isotope-estimated trophic position, we believe that there are many unexplored and promising

269   avenues to combine behavioral and isotope ecology in order to learn more about how behavior

270   observed in laboratories corresponds with movement and organismal interactions in the field.

271   Laboratory experiments and stable isotope analyses have both separately been used to explore

272   the "ecology of individuals" or variation within populations and species (Bolnick *et al.*, 2003;

273   Niemelä & Dingemanse, 2014; Zavorka *et al.*, 2015), yet to our knowledge, researchers have not

274   previously combined or compared these approaches for the same organisms. For example, stable

275   isotope analysis and behavioral assays could be combined to together evaluate whether range

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276   expansion of invasive species is being driven by subordinate individuals with low trophic

277   positions that are excluded from core habitats by dominant intraspecific competitors, or instead

278   bold or aggressive individuals with high trophic positions that are inclined to disperse (Hudina,

279   Hock & Žganec, 2014). Further, where distinct stable isotope signatures exist over habitat

280   gradients (Hobson, 1999), researchers could infer whether individuals with or without dispersal-

281   related behaviors observed in the laboratory were actually recent arrivals or longstanding

282   residents of their collection locations. We encourage future studies to further explore the possible

283   insights gained by linking laboratory behavior with field function through stable isotope analysis,

284   as doing so could contribute meaningfully to an array of ecological and evolutionary questions.

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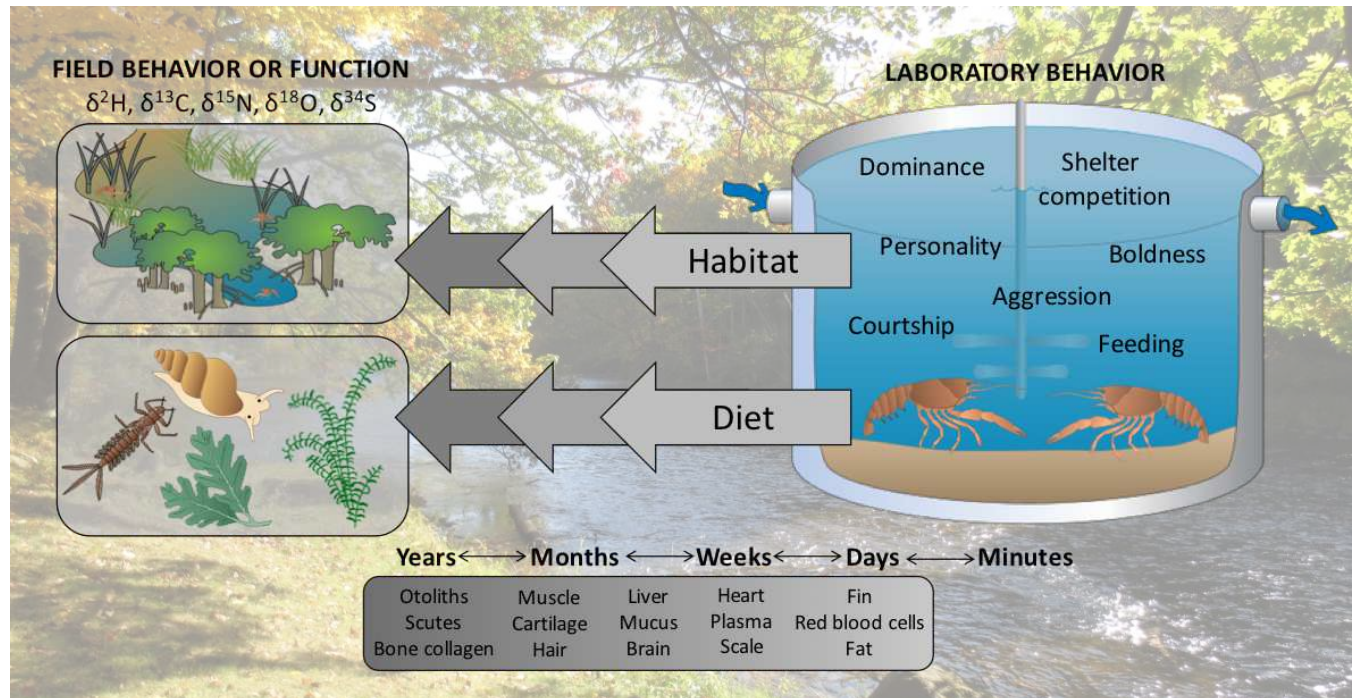436   *Ecology* **26**, 877-884. (doi:10.1093/beheco/arv029)

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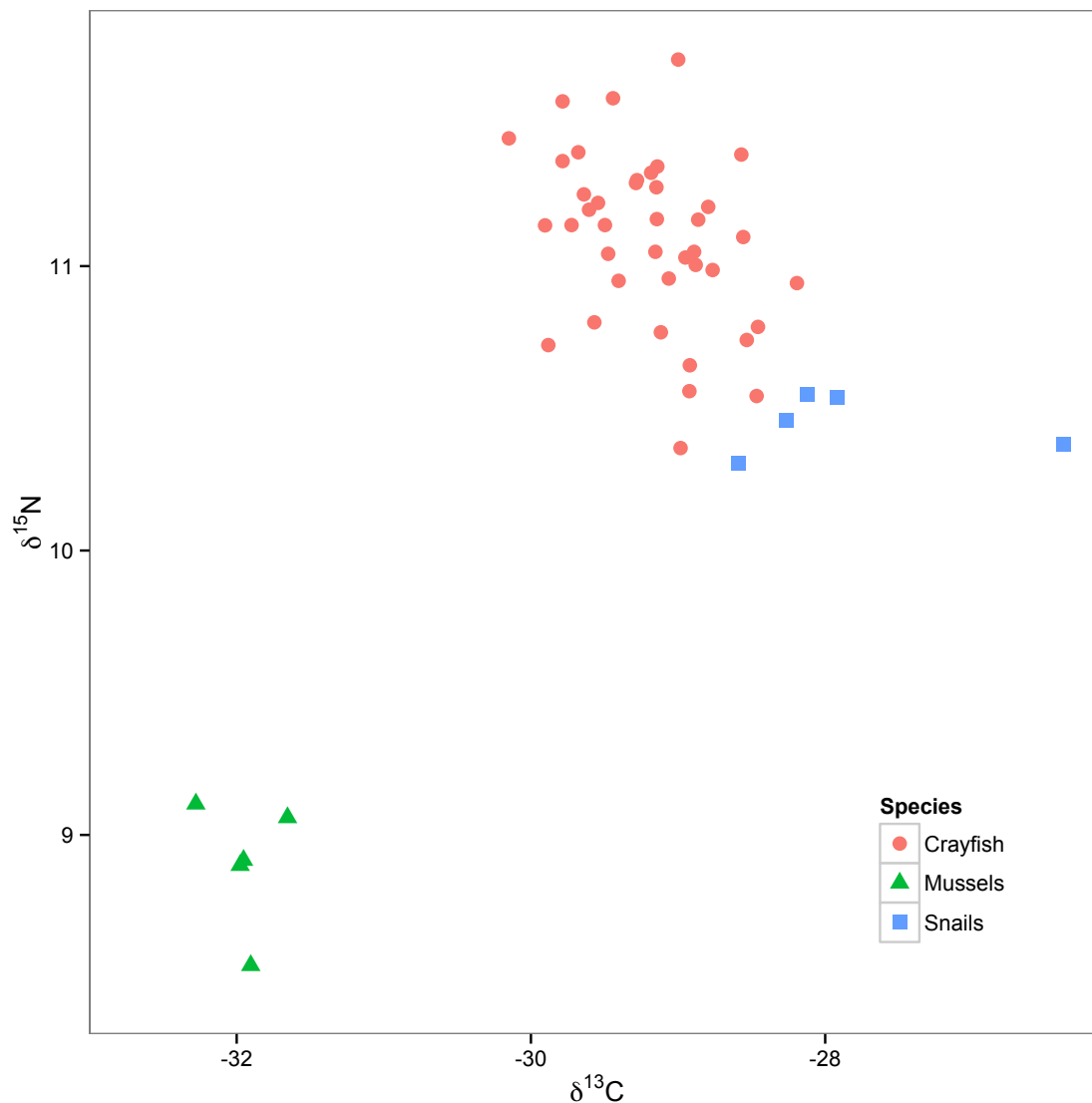454

455 **Figure 1.**

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457 **Figure 1.** Stable isotopes could permit researchers to hindcast the ecological interactions of

458 organisms, linking behaviors observed in the laboratory with previous field function or behavior.

459 Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/).

460 Background image is of the study location where organisms were collected (Chippewa River).

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469 **Figure 2.**

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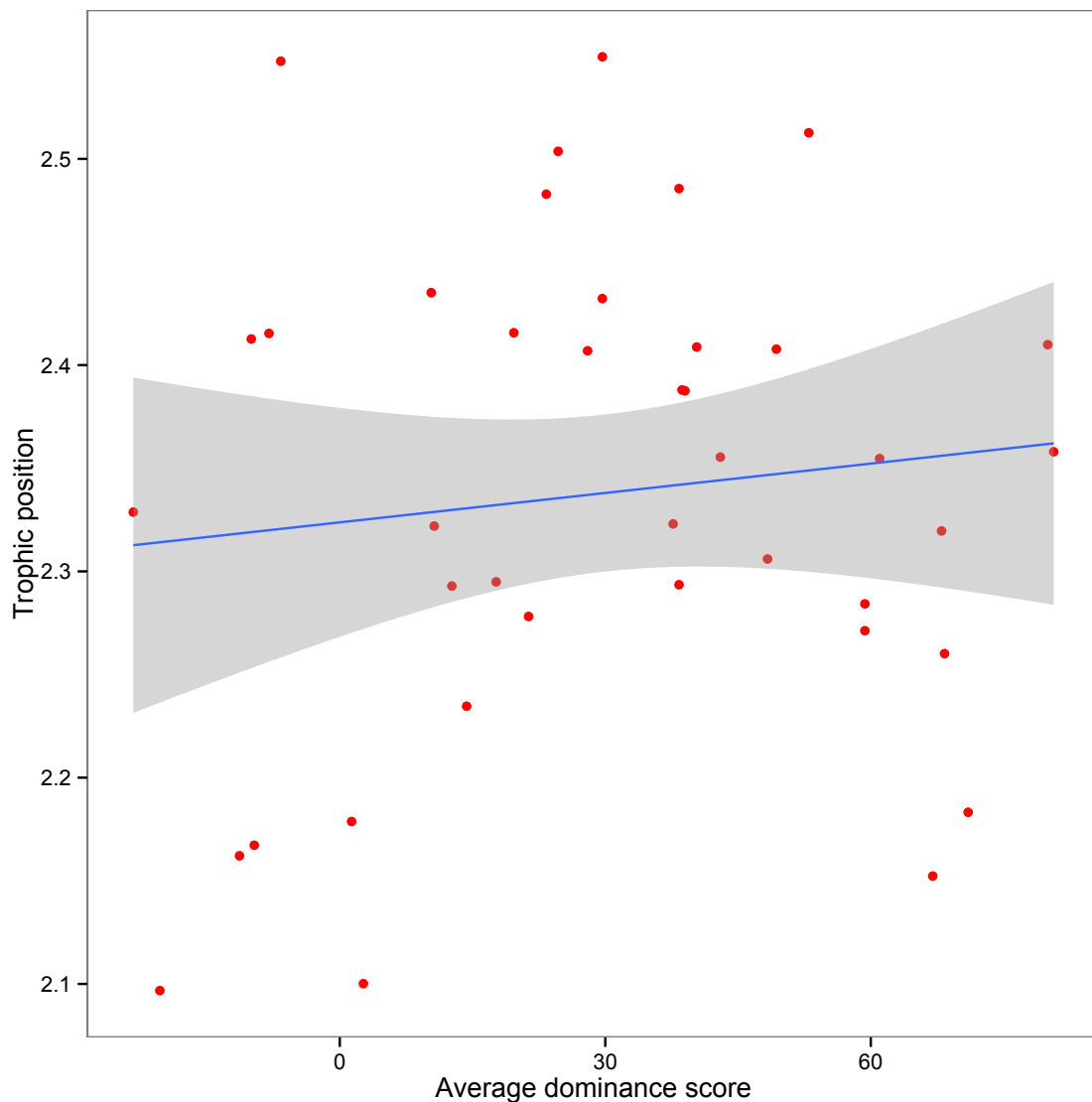
471 **Figure 2.** Isotopic biplot of δ^{13}C and δ^{15}N for crayfish (red circles), mussels (green triangles),

472 and snails (blue squares). All values are expressed in per mille (‰) relative to a standard of V-

473 PDB (Vienna PeeDee Belemnite) for carbon and air for nitrogen.

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477   **Figure 3.**

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479   **Figure 3.** Scatterplot (with 95% CI) of mean assay dominance score for each crayfish over three

480   agonistic assays and *in situ* trophic position (y =0.0005x + 2.32, R^2 = 0.0132, $F_{1,38}$ = 0.5084, *p* =

481   0.4802).

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Table 1. Ethogram modified from Bruski & Dunham (1987)

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

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503 **Supplementary material for "Connecting Laboratory Behavior to Field Function through**

504 **Stable Isotope Analysis"**

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I. Crayfish morphometrics

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527   Size influences the outcome of crayfish agonistic trials (Rubenstein & Hazlett, 1974;

528   Bergman & Moore, 2003); therefore, to better understand what intrinsic factors might be

529   affecting the results of our agonistic assays, we used digital calipers to measure carapace length

530   (CL; from the tip of the rostrum to the posterior edge of the carapace), chelae width (at the

531   widest point of the palm), and chelae length (from the attachment of the carpus and the propodus

532   to the most distal point of the fixed finger) to the nearest hundredth of a mm. We used a digital

533   balance to measure mass to the nearest hundredth of a gram (Table S1). Prior to weighing, we

534   dabbed all crayfish dry for 10 seconds with a paper towel.

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Table S1. Crayfish morphometrics.

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

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567   **II. Alternative comparisons of dominance and trophic position**

568   Body size is a factor that strongly influences the outcome of agonistic encounters in

569   crayfish, with larger individuals generally being more dominant (Bovbjerg, 1953; Rubenstein &

570   Hazlett, 1974; Bergman & Moore, 2003). We used as small of a crayfish size range as

571   logistically possible, but the difference between our largest and smallest study organisms was

572   still 4.12 mm carapace length (Table S1). Despite this, most paired agonistic interaction trials

573   were between more closely size-matched crayfish (mean ± standard deviation; 1.44 ± 1.15 mm

574   carapace length). Regardless, we sought to determine if dominance scores might better

575   correspond with the trophic positions of our crayfish if we corrected for the role of size

576   differences in determining outcomes of agonistic interactions. We did not correct for potential

577   ontogenetic effects of crayfish size on trophic position (Bondar *et al.*, 2005; Larson, Olden &

578   Usio, 2010), because we found no significant relationship between crayfish carapace length

579   (Table S1) and trophic position ($y = 0.002x + 2.27$, $R^2 = 0.001$, $F_{1,38} = 0.02$, $p = 0.88$). However,

580   as we anticipated, there was a significant relationship between crayfish carapace length and mean

581   dominance score ($y = 11.503x - 261.971$, $R^2 = 0.26$, $F_{1,38} = 13.03$, $p < 0.001$; Figure S1). Yet,

582   when we corrected for the effect of crayfish size on dominance by regressing residuals of the

583   preceding analysis against trophic position, we still did not find a significant relationship,

584   consistent with our main text conclusion ($y = 0.00x + 2.34$, $R^2 = 0.01$, $F_{1,38} = 0.54$, $p = 0.47$;

585   Figure S2). The lack of a relationship between dominance and trophic position is therefore

586   conserved even when accounting for the potential influence of crayfish size on dominance.

587   Carapace length is the most commonly used size metric for crayfish; however, chelae size

588   has been shown to dictate success in agonistic encounters and may be a better measure of

589   dominance in crayfish (Garvey & Stein, 1993). We therefore ran two additional iterations of the

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590   analysis presented above, using chelae length and width instead of carapace length. We found

591   significant relationships between mean dominance scores and both chelae length (y = 9.125x –

592   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,

593     $F_{1,38}$ = 11.07, p = 0.002). Yet again, regressing residuals from the chelae length or width and

594   dominance score analyses against trophic position did not change our main text conclusion that

595   that dominance and trophic position are unrelated (chelae length residuals vs trophic position; y

596   = 0.0002x – 2.34, R^2 = 0.001, $F_{1,38}$ = 0.04, p = 0.85; chelae width residuals vs trophic position; y

597   = -0.0001x – 2.34, R^2 = 0.01, $F_{1,38}$ = 0.02, p = 0.89).

598   The use of isotopic mixing models, applied here as a step in calculating trophic position

599   (Post, 2002), is dependent on a number of assumptions. For example, stream and river

600   ecosystems can have extremely high spatiotemporal variation in the $\delta^{13}C$ and $\delta^{15}N$ values of

601   sources of primary production owing to a number of factors (Fry & Sherr, 1984; Finlay, 2001;

602   Trudeau & Rasmussen, 2003). Accordingly, we followed convention in using primary

603   consumers rather than primary producers in mixing model calculations of trophic position (see

604   section IV), as long-lived organisms like mussels or snails can integrate and correct for this

605   variability (Post, 2002; Cabana & Rasmussen, 1996). However, we cannot exclude that our field

606   sampling of primary consumer endpoints for our mixing model could have missed some such

607   variability inherent to heterogeneous lotic ecosystems, and our collection of potential prey

608   resources concurrent with crayfish consumers does not necessarily reflect isotopic values of prey

609   items for *Orconectes rusticus* over preceding weeks or months (Moore & Semmens, 2008).

610   Another assumption of mixing models is that constant discrimination factors can be used for

611   each trophic step and between different taxonomic groups and diet items. However,

612   discrimination factors can vary across taxa, diets, and tissues used (e.g., Stenroth *et al.*, 2006;

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613   Caut, Angulo & Courchamp, 2009; Phillips *et al*., 2014), and consequently may misrepresent

614   trophic position of a focal organism (Bond & Diamond, 2011). Due to the potential vulnerability

615   of our model to the preceding assumptions, we also conducted a simpler analysis using crayfish

616   dominance scores and unaltered δ^{15}N values to determine if our results were dependent on our

617   specific trophic position calculations. Doing so did not alter our overall nonsignificant result and

618   conclusion (y = 0.002x – 11.04, R^2 = 0.03, $F_{1,38}$ = 1.292, p = 0.26; Figure S3). We therefore

619   conclude that our result of a lack of relationship between crayfish dominance in the laboratory

620   and trophic position in the field is robust to our measures of both crayfish dominance and trophic
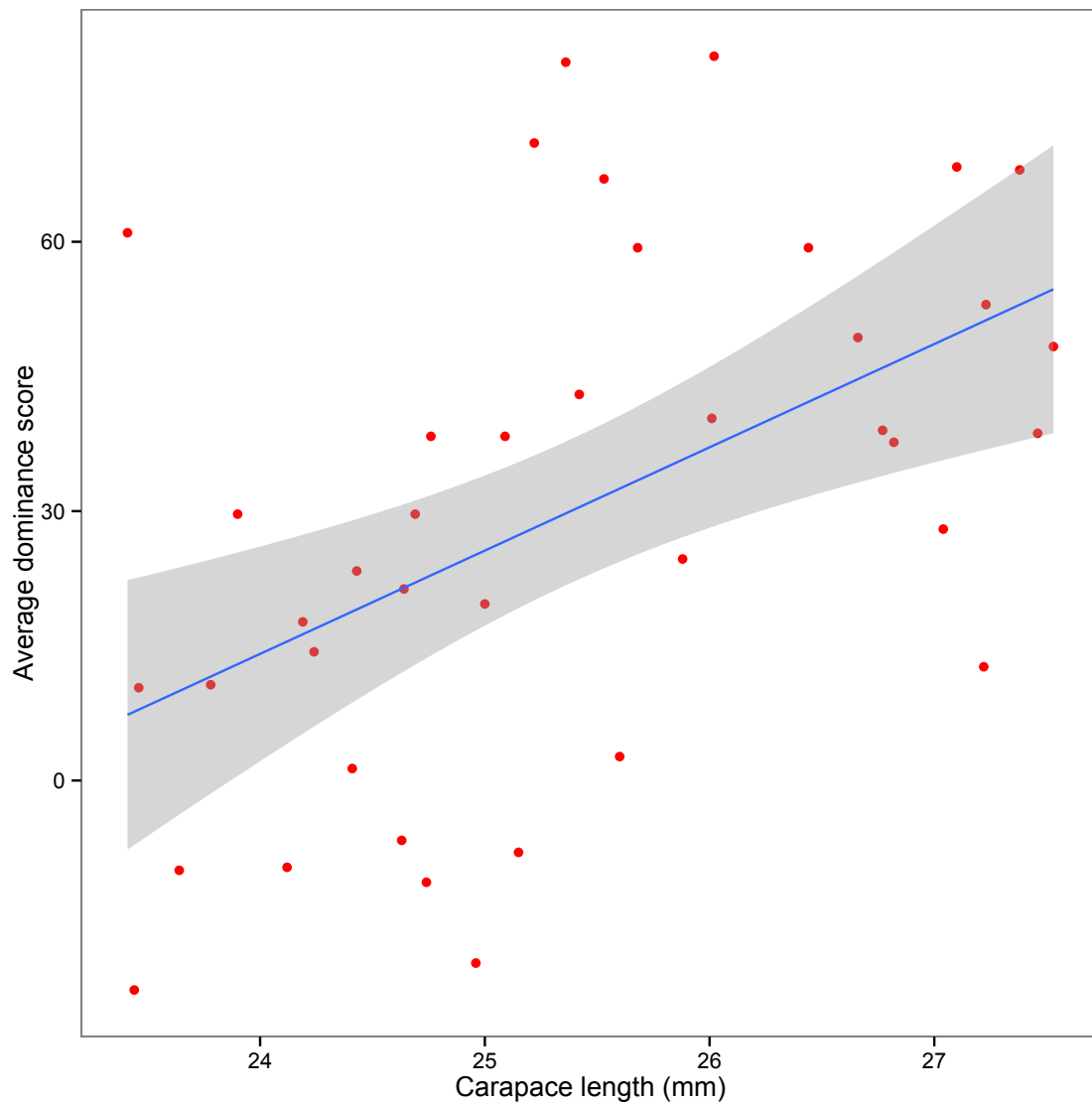
621   position.

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624   **Figure S1.** Scatterplot (with 95% CI) showing significant relationship between crayfish carapace

625   length and dominance score from behavioral assays (y = 11.503x - 261.971, R^2 = 0.26, $F_{1,38}$ =

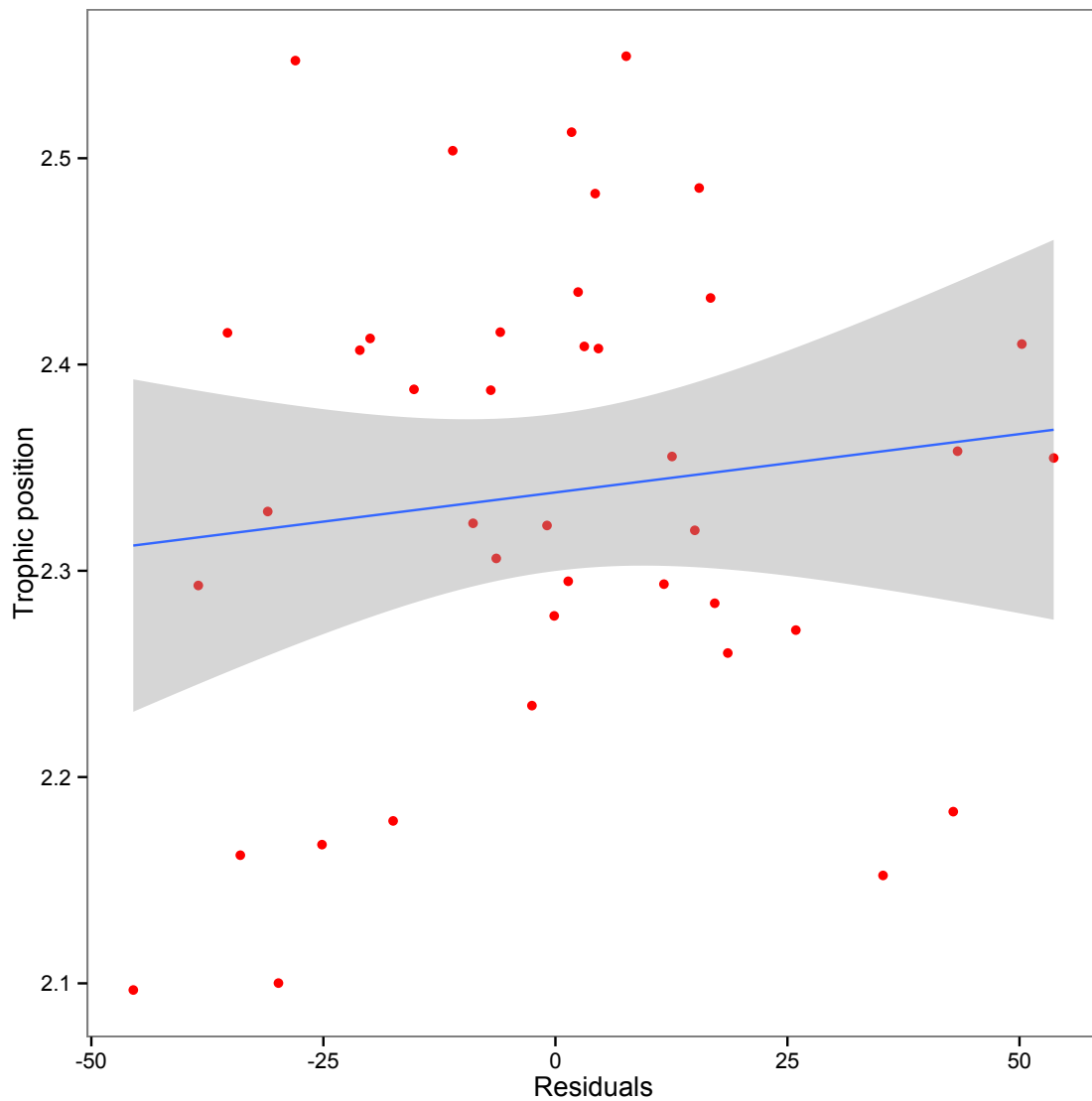626   13.03, p < 0.001).

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629   **Figure S2.** Scatterplot (with 95% CI) of the residuals from crayfish dominance and carapace

630   length regression against calculated trophic position (y = 0.00x + 2.34, R^2 = 0.01, $F_{1,38}$ = 0.54, p
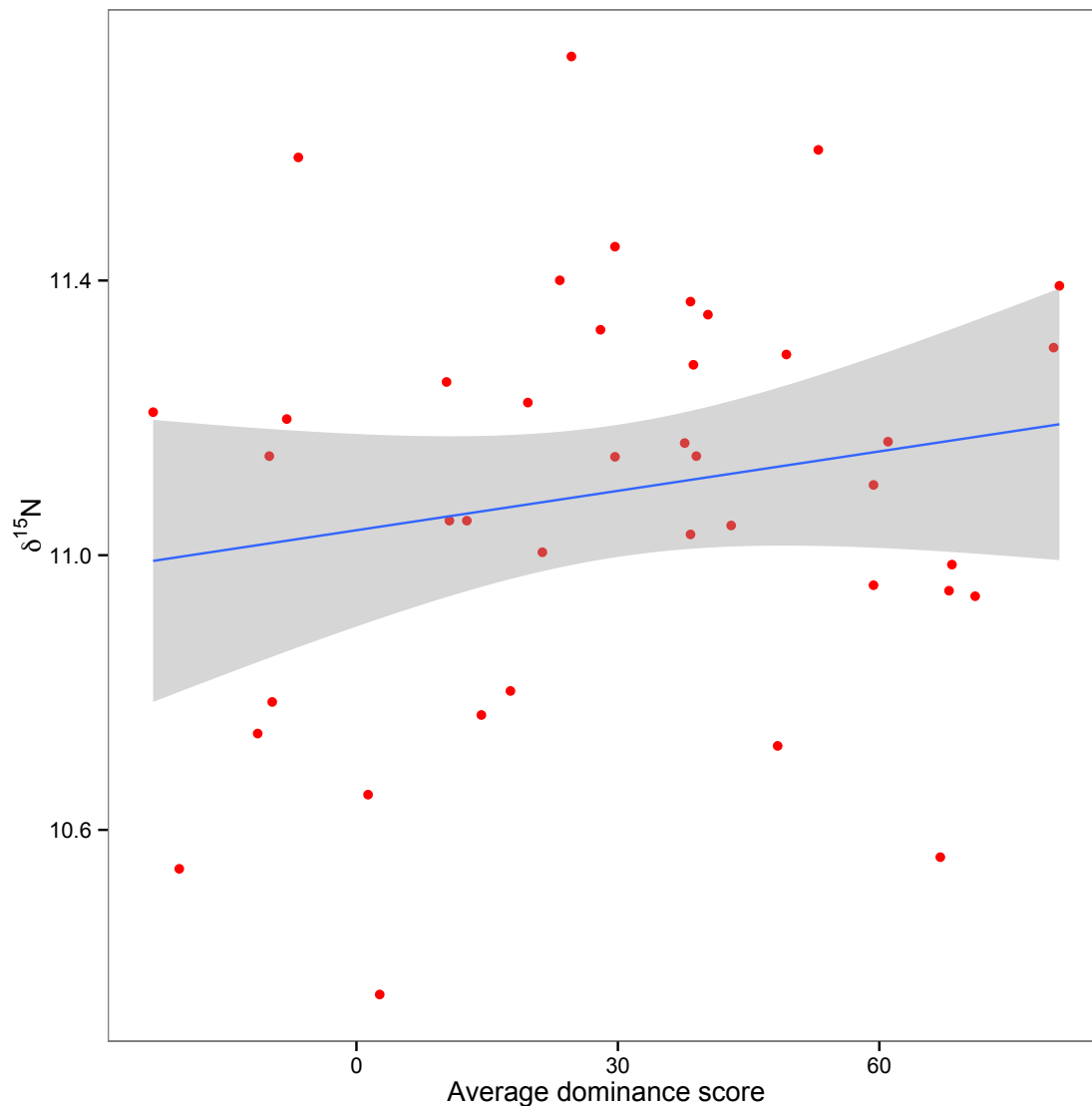
631   = 0.47).

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Figure S3. Scatterplot (with 95% CI) of crayfish dominance scores and $\delta^{15}N$ signatures (y =

0.002x − 11.04, R^2 = 0.03, $F_{1,38}$ = 1.292, p = 0.26)

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