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Flow discharge impacts competition for food and shelter between two overlapping species of crayfish

Sophia Adami-Sampson, Madison J. Wagner and Paul A. Moore

Bowdoin College, Brunswick, ME, USA; Laboratory for Sensory Ecology, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, USA; University of Michigan Biological Station, University of Michigan, Pellston, MI, USA

Abstract

Competition between aquatic organisms is heavily influenced by abiotic factors in the environment, specifically flow regime in aquatic systems. Flow regime has been shown to significantly affect the way in which a species uses the environmental resources and alterations in flow can exacerbate competitive advantages by congeners. However, little work has concentrated on the competitive outcome between native and invasive organisms as a function of flow regime. Here, we sought to uncover how competition between two crayfish species (the native virile crayfish [*Faxonius virilis*] and invasive rusty crayfish [*Faxonius rusticus*]) was affected by varying flow rates. To do this, we size-matched crayfish and quantified three separate behaviors (food use, shelter use, and fights) of crayfish in four different discharge levels (no discharge—0 cm$^3$/s; low discharge—116 cm$^3$/s; intermediate discharge—345 cm$^3$/s; high discharge—450 cm$^3$/s). We found that the number of foraging bouts was significantly ($p<0.001$) influenced by discharge levels for both species, where rusty crayfish foraged more often in no and discharge. The number of times crayfish sheltered also varied between species and was significantly ($p<0.001$) altered by discharge level, where rusty crayfish sheltered more often in no discharge and virile crayfish sheltered more in low and intermediate discharge levels. Similarly, the number of fights that crayfish engaged in also significantly ($p<0.001$) depended upon species and discharge levels. Rusty crayfish engaged in more agonistic activities in no and high discharge levels while virile crayfish participated most often in low and intermediate discharge levels.

Introduction

Competitive interactions greatly influence the structure of aquatic ecosystems (Quintana et al. 2015). Examples can range from the intense competition for space by different barnacle species found in rocky intertidal areas, competition for light and nutrients among benthic
macrophytes in lakes, to resource competition in benthic algal communities (Connell 1961; Ren et al. 2022; Fork et al. 2020). In each of the cases above, the competition centers around abiotic factors that are limiting in habitats (Chambers and Prepas 1990). While space is a particularly well researched factor, nutrients, refuges, light, temperature, and oxygen are other abiotic factors known to regulate or influence competition (Bornette and Puijalon 2011). The key factor in determining the intensity of this competition is the spatial heterogeneity of these abiotic variables as well as some aspects of limitation (Winemiller 1996). Another abiotic factor that is important for structuring aquatic communities is flow regimes.

Flow regime in aquatic ecosystems has been linked to changes in the geomorphology of habitats as well as species distributions, life history traits, behaviors that coincide with natural flow patterns, and overall habitat health (Lytle and Poff 2004; Rytwinski et al. 2017; Seebacher and Kazerouni-Ghanizadeh, 2021). As such, many organisms are adapted to living in and successfully reproducing, competing, or evading detection in specific flow parameters. Both lentic and lotic ecosystems are characterized by spatial heterogeneity of flow regimes (Buffagni 2021; Buffagni et al. 2009). Streams and rivers are structured by riffle, pool, and run sequences along a stretch of a stream (Wohl et al. 1993). Lakes can have large-scale circulation like turnover, mesoscale circulation like longshore currents, or even smaller changes in flow dynamics due to changing wind conditions (Boyce et al. 1989).

External climate changes or anthropogenic effects can alter the intensity of turbulence, daily and seasonal variances in discharge, or even water depth and velocity which may alter success in competition and ecosystem structure (Arnell and Gosling 2013). Some organisms rely on the flow of water for dispersal, and changes in the intensity of flow will alter dispersal dynamics (Palmer and Ruhi 2019; Gary et al. 2020). While the impact of flow and competition has been well researched within aquatic ecosystems, the interaction of the effects of discharge and other hydrological characteristics on competitive interactions remains elusive.

The impact of flow and changes in flow regimes on competitive interactions has received very little ecological attention. Brown trout (Salmo trutta) distribution has been shown to be heavily impacted by stream discharge when cutthroat trout (Oncorhynchus clarkia), an invasive species in this instance, are present in the environment in an Idaho, U.S. river (de la Hoz Franco and Budy 2005). Flow velocity alters food chain length in artificial streams where flow has a non-linear relationship to salmon (Salmo salar) growth but increases both chlorophyll a and invertebrate biomass (Blanchet et al. 2008). Flow has also been shown to impact grazing in macroinvertebrates (Poff et al. 2003). In coral systems, flow distributes organic matter which can alter competition in algal and microbial communities (Barott and Rohwer 2012). Changes in flow that alter the distribution of organic matter can alter competitive advantages and disadvantages of different species (Berger et al. 2003; Barott and Rohwer 2012). Differences in flow regimes also play a role in competitive interactions between native and invasive fish (Jermacz et al. 2015). Different flow velocities alters aggressive behavior and shelter behavior of invasive racer gobies (Babka gymnotrachelus) when in the presence of native bullheads (Cottus gobio) in central Poland (Jermacz et al. 2015). The changes in behavior as a result of flow dynamics may play a role in increasing the possibility of invasive species taking hold in a new habitat due to competitive advantages (Rahel and Olden 2008). At the intersection of flow dynamics, competition, and invasive species rests some interesting, but unanswered, questions that, if answered, may supply insight into changes in aquatic community dynamics. These questions are increasingly important as climate change will alter precipitation dynamics and further influence flow in streams and rivers (Milly et al. 2005).

Crayfish are recognized as one of the most potent invasive organisms in aquatic ecosystem, as well as one of the most threatened organisms often from other invasive crayfish
Because of their status as both ecosystem engineers and keystone species in aquatic habitats, any factors that either enhance or decrease the invasive potential of crayfish are of interest. Despite the wealth of knowledge about their behavior, ecological status, and ecological function, the direct impact of flow dynamics on competitive interactions between crayfish species remains understudied (Kreps et al. 2016). To begin to rectify this deficit, we examined competition between two different species of crayfish. Both the rusty crayfish (*Faxonius rusticus*) and virile crayfish (*Faxonius virilis*) are highly invasive species. The rusty crayfish has a native range within the Ohio River basin in southern Ohio, Kentucky, and Northern Tennessee in the United States and has extended its invasive range north to Ontario in Canada, west to Nebraska and east to Maryland in the United States. Conversely, the virile crayfish is native to the Great Lakes region of the United States and southern regions of Ontario, Canada and is considered invasive south of the Ohio River basin including areas as southern as Alabama and as far east as Massachusetts in (Guiasu and Labib 2021). Thus, these two species of crayfish overlap considerably across multiple habitats but switch between their native and invasive status. In addition, the ecological role that each crayfish plays within these aquatic ecosystems are identical (Reynolds et al. 2013). Their distributions seem to be influenced by water currents and the hydrodynamics of streams (Maude and Williams 1983). Given the wide range of aquatic habitats in these regions, it is quite likely that the two crayfish co-exist in vastly different flow regimes while competing for identical food and shelter resources. In addition, flow velocity has been shown to impact movement of crayfish across river-road crossings (Foster and Keller 2011) and movement to find food (Perry and Jones 2018). Differing water velocity has also been shown to impact the size and shape of crayfish (*F. rusticus*) (Perry et al. 2013). To investigate the role of flow regimes in altering competitive interactions, we created flow through mesocosms with limited shelter and food resources. We monitored competitive interactions for these resources via cameras in overnight trials under differing discharge levels. Our results will shed light on how different flow regimes may increase the invasive potential of some crayfish as well as create potential flow refuges for native crayfish. In particular, we wanted to increase discharge levels in a mesocosm and measure the impact of this abiotic change on the use of food and shelter resources by these two different species of crayfish.

**Materials and methods**

**Experimental design**

Flow-through artificial mesocosms were constructed to evaluate the impact of different discharge levels on the interspecific competition for limited resources in crayfish. Mesocosms held a single PVC shelter as well as limited food resources (*Chara* sp.) as the sources of competition. Four female form II crayfish (two *F. virilis* and two *F. rusticus*) were placed in the mesocosm along with resources. All crayfish were size-matched within 10% of carapace size since size differential is a predictor of both aggressive and competitive dominance (Moore 2007). Mesocosms were fed with unfiltered river water and animals were allowed to compete for approximately 23 h. After this time period, crayfish were removed from the mesocosms and the amount of food consumed was measured. During this time period, the mesocosm was allowed to flush to remove any potential biological chemicals left from the previous experiment. Four different flow velocities were used as the experimental treatment. Experiments started on July 6, 2022, and ended on July 30, 2022.
Mesocosm design

Flow-through artificial stream mesocosms (81.3 cm × 81.3 cm × 20.3 cm; l × w × h) were constructed using cinderblocks (40.64 cm × 20.3 cm × 20.3 cm; l × w × h) and 6-mil black polyethylene sheeting. At the downstream end of the mesocosm, a single cinderblock was turned 90° such that the holes in the cinderblock were facing into the mesocosm. In addition, this cinderblock was covered with mesh window screening and served as the outflow for each mesocosm. The bottom of each mesocosm was filled with approximately 2 cm of sand substrate and mesocosms were fed with unfiltered river water from the East Branch of the Maple River (latitude 45.5643, longitude −84.7514). To prevent detritus and other confounding aquatic organisms from entering the mesocosm, two knee high stockings (0.1 cm mesh size) were placed over the opening of the 7.6 cm (ID) PVC pipe that delivered the water into 209 L constant flow head tanks. Each head tank fed eight 1.9 cm (ID) garden hoses which were used to feed the mesocosms. Water flowed through the mesocosm at a rate of 0.1 ± 0.05 L/sec per garden hose, and alterations in discharge rates were achieved by altering the number of hoses feeding each mesocosm. A total of four mesocosms were constructed for the study and discharge treatments were randomized across the mesocosms. Mesocosms were exposed to natural light cycles (16 L: 8 D) and temperature fluctuations from June to August 2022. The downside of measuring discharge is that any detailed flow dynamics within the mesocosm are missing. Yet, with the movement of the crayfish and their adjustment of the position of both the food and shelter during the experiment, any measure of the details of flow within the mesocosm would not match the changing dynamics throughout any single experiment.

A wooden frame held an infrared DVR camera (Zosi ZR08ZN10, Zhongshan City, Guangdon Province, China) 1.5 m above the water’s surface of each mesocosm to record the crayfish’s nocturnal behaviors. Two low-intensity red light bulbs (Great Value, Model A19045 LED Lamp, 9 W, 145 mA, 120 V, 60 Hz) were used to illuminate each mesocosm from above. Previous studies have shown that red light does not affect crayfish nocturnal behaviors (Cronin and Goldsmith 1982).

Organism collection and holding

All experiments were conducted at the Stream Laboratory Research Facility (lat., long., 45.564, −84.7514) at the University of Michigan Biological Station, Pellston, MI, USA. Form II, female rusty crayfish F. rusticus and female virile crayfish F. virilis (n = 110 of each species, carapace length = 2.87 ± 0.01 cm for F. rusticus, 2.87 ± 0.05 cm for F. virilis) were collected using hand nets at Maple Bay State Park in Pellston, MI, USA (lat., long., 45.4868, −84.7073). Females were chosen to eliminate sex as a variable in the analysis. Crayfish were kept in flow through cattle troughs (237.5 × 86.4 × 60.1 cm; l × w × h) which were filled with the same unfiltered water from the East Branch of the Maple River that was used in the mesocosms. Muskgrass (Chara sp.) were collected using a homemade plant rake made with two 14-tined rakes (34 cm long) tied together with nylon rope (2.5 cm diameter). The macrophyte rake was deployed off of a pontoon boat, allowed to settle to the bottom, then brought back onboard the boat using the nylon rope. All muskgrass were collected continuously throughout the summer from near the East Branch of the Maple River (lat., long., 45.574, −84.727). Muskgrass were kept in a flow-through 100 L plastic drum under sunlit conditions.
**Mesocosm methodology**

Flow volume rate was calculated by measuring outflow of all of the garden hoses at the beginning and end of each experimental trial. Despite the filters in place, some garden hoses would occasionally be clogged with detritus during a trial. Any trials that showed a greater than 20% decrease in discharge rate over the length of the trial were rejected. Discharge was measured by filling a 2 l graduated cylinder for 10 s and repeating this measurement three times. The average of the three measurements was recorded as the flow rate.

Using previously published methods on measuring foraging activity (Wood and Moore 2020), muskgrass were removed from the storage tanks and spun in a salad spinner (Farberware 176 Basics; Farberware Cookware Division, Fairfield, CA, USA) for 20 rotations to remove excess water. After drying, 5 g of muskgrass was weighed on an Ohaus® scout scale (Model #H-5851; OHAUS Corporation, Parsippany, NJ, USA) to the nearest milligram. The plant stems were then attached to glass rods (255×6 mm; l×OD) with 26-gauge green painted floral wire. The loaded rods were then placed into a hardware cloth bracket (24×19 cm; l×w) which held the plant samples in position during the experimental trials. The bracket/muskgrass was placed in the center of the mesocosm alongside of a single PVC shelter constructed out of a 10 cm length of 5 cm diameter PVC cut in half lengthwise. Shelters were painted black and mounted on a 15×15 cm Plexiglas sheet that allowed the shelter to maintain its upright position. Previous work using this method shows that in control studies shear forces do not remove chara from the bracket (Wood and Moore 2020).

Once the mesocosms were fully stocked with resources, crayfish were randomly selected from the holding tanks and measured to the nearest 0.01 cm for their post-orbital carapace length using calipers. All animals chosen for the study had fully intact appendages and antennae. Four size-matched (within 10% variation) crayfish (two virile and two rusty) were selected and marked with a unique symbol using white correction fluid (Bic® Wite-out, Société Bic, Clichy, France) to aid in visibility. Marking crayfish carapaces does not alter crayfish behavior (Edwards et al. 2018; Wood et al. 2018; Jackson and Moore 2019).

Each experimental trial lasted 23 h, starting at 9:00 am and finishing the next day at 8:00 am. Because crayfish are nocturnal, red lights and video cameras were activated at midnight during the trial and recorded crayfish behavior for 210 min. Previous work has shown that this time period is when crayfish are most active, foraging is the highest, and aggression is the most greatest (Jackson and Moore 2019). Thus, this time is sufficient in capturing the diversity nocturnal behaviors displayed by crayfish.

The next morning, muskgrass was removed from the experimental tanks, spun again in the salad spinner, and weighed to the nearest 0.01 g. Discharge was remeasured and the mesocosm was reset for the next set of trials. A total of 55 trials were performed under all of the flow treatments. Crayfish were used only once within the entire data set and, per permitting regulations, *F. rusticus* were euthanized and *F. virilis* were released at their capture points. This total was divided among the individual discharge treatments in the following manner (mean ± SEM):

- **Control** \(0±0 \text{ mL/s},\ N = 15,\)
- **LowFlow** \(116±5 \text{ mL/s},\ N = 13,\)
- **IntermediateFlow** \(345±7 \text{ mL/s},\ N = 14,\)
- **HighFlow** \(450±10 \text{ mL/s},\ N = 13.\)

These discharge levels are at the lower end of what has been measured in the Maple River where both of these species coexist. Discharge measurements, over a 10-year period, have ranged from 0 L/s (beaver dam and low-level water effects) to a high of to over 4,000 L/s (Moore, per obs).
**Data Analysis**

Videos were retrieved from the DVR and analyzed for behavioral time budgets by a single observer (SAS) for time budgets and a different observer (PAM) for winning interactions to keep consistency of behavioral definitions across treatments and trials. Three separate behaviors were recorded for the crayfish: Shelter use, food use, and fights. A total of 1996 discrete sets of behaviors occurred across the 55 treatments. These were distributed among the treatments as follows: 510 behaviors in the high flow (139 fights, 332 foraging episodes, and 39 shelter use), 535 behaviors observed in the intermediate flow (165 fights, 320 foraging episodes, and 50 shelter use), 450 behaviors observed in the low flow (129 fights, 271 foraging episodes, and 50 shelter use), and 501 behaviors observed in the control flow (131 fights, 293 foraging episodes, and 77 shelter use). Shelter use was defined as the crayfish either on top of or inside of the shelter. At least 50% of the carapace had to be inside of the shelter for the crayfish to be considered inside of a shelter. A fight was defined as an agonistic interaction that occurred beyond two crayfish touching chelae or walking over or past each other (Wofford et al. 2015). When two crayfish exhibited agonistic behavior, the length and maximum intensity of the interaction was recorded along with the winner of the interaction. In addition, fights were placed into one of three categories depending on the participants: intra-specific between rusty, intra-specific between virile, and interspecific. Foraging was defined as a crayfish located on top of the muskgrass and visible motion of the first pair of walking legs.

**Data conditioning and statistical analysis**

For the number of behaviors displayed by the different species under the different discharge treatments, count data were analyzed in R using the chisq.test function after converting the counts to a contingency table. A Tukey multiple comparisons for proportions contingency table (Zar 1999) was used to compare whether there was a species and resource effect on the winners of fight interactions.

R was used for all data conditioning and statistical analysis (R Core Team 2022). Data conditioning followed those steps that are outlined in Zuur et al. (2009). The first step was to create Cleveland dot charts of the response variable to determine if the data contained any outliers and there were none, so, all of the behavioral data were included in the analysis. The normality of each of the behavioral response variables was checked through three different steps. Firstly, a histogram of the data was produced. This was followed by qqplots, and normality tests (Shapiro-Wilk) of the behavioral variable’s length of shelter use, length of agonistic interactions, and length of foraging bouts. If the data were not normally distributed, then the R package BestNormalized was used to select the transformation of the data and the transformed data was rechecked for normality (Peterson 2021). None of the three variables were normally distributed. Time spent foraging, time using the shelter, and length of fights were all normalized using the orderNorm transformation in R. Variances were evaluated for homogenous distributions with the Levene test and were not an issue. All significant interactions were set at $p < 0.05$.

Because multiple behavioral measures occurred within single trials and to control for possible mesocosm as random effects, a linear mixed model was used for all subsequent statistical analysis (Zuur et al. 2009). Models run in R used the lmer function from the lme4 package (Bates et al. 2015; R Core Team 2022) and were followed up with the anova function from the car package to extract the results (Fox and Weisberg 2019). For all models, the behavioral measure (foraging, shelter use, or fighting) served as the dependent variable and the flow speed treatment (as categorical: control, low, intermediate, and high) and the
species were the fixed measures with full interactions. Mesocosm number was used as the random effect to control for possible mesocosm effects. A separate set of models were performed using flow as a continuous variable as opposed to categorical. Model selection between using discharge as a continuous or categorical variable was based on Akaike’s information criterion (AIC) (Akaike 1974). The selection of best model was based on the lowest AIC value while removing all models with a ΔAIC greater than 2 from our selection process (Anderson and Burnham 2002). Post hoc analyses of comparisons were conducted on the categorical variables using the emmeans function (Lenth 2022). The statistical effect of each model was determined by extracting Cohen’s D (Cohen 1988) using the effectsize package cohens_d function (Ben-Shachar et al. 2020). Cohen’s D is a statistical measure that indicates the strength of the apparent relationship indicated by the statistical analysis and can be used to interpret the importance of statistical significance. Values for Cohen’s D can generally be interpreted as small (<0.2), medium (0.5), or large (0.8).

Results

Number of behaviors exhibited

Foraging
The number of instances that *F. rusticus* and *F. virilis* spent foraging over the recording time period was different depending on the discharge treatment and species under consideration ($\chi^2=116.68$, df = 3, and $p$-value <0.00001; Table 1; Figure 2). Under the high and zero discharge conditions, *F. rusticus* performed a significantly higher number of foraging bouts than *F. virilis*.

Shelter
Shelter use was significantly influenced by changes in the discharge treatment and this change was different across the two species of crayfish ($\chi^2=31.609$, df = 3, and $p$-value <0.00001; Table 1; Figure 1). *F. rusticus* used the shelter a larger number of times at zero discharge, but this was reversed for the low and intermediate discharge rates. Under these conditions, *F. virilis* used the shelter more often. There was no difference in shelter use at the high discharge rate.

Fights
The number of agonistic interactions was influenced by changes in the discharge treatment and this change was different across the two species of crayfish ($\chi^2=98.286$, df = 3, and $p$-value < 0.00001; Table 1; Figure 1). *F. rusticus* engaged in more agonistic interactions at the zero and high discharge rates, whereas *F. virilis* engaged in more fights at the low and intermediate discharge rates.

Table 1. Number of behaviors exhibited by crayfish divided among the two species of crayfish, the type of interaction (fight, competition for foraging, competition for shelter), and the treatment condition in which the interaction occurred.

<table>
<thead>
<tr>
<th>Foraging</th>
<th>Flow treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>Rusty</td>
<td>344</td>
</tr>
<tr>
<td>Virile</td>
<td>97</td>
</tr>
<tr>
<td>Shelter use</td>
<td>Flow treatment</td>
</tr>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>Rusty</td>
<td>18</td>
</tr>
<tr>
<td>Virile</td>
<td>21</td>
</tr>
<tr>
<td>Fighting</td>
<td>Flow treatment</td>
</tr>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>Rusty</td>
<td>123</td>
</tr>
<tr>
<td>Virile</td>
<td>16</td>
</tr>
</tbody>
</table>
Mixed model analysis of length of resource use

Foraging
The linear mixed model using flow as a categorical variable and the model using flow as continuous showed that foraging time was significantly influenced by the interaction of the flow treatment with species (\(F(3,1206,0.05) = 6.2052; p = 0.001\) and \(F(1,1208,0.05) = 5.21; p = 0.023\), respectively: Table 2). The AIC values indicated that the model which used flow as a categorical variable had more explanatory power, so this model was adopted as the best fit.

A post hoc analysis of the best fit model showed several significant differences across the species and treatment interaction. Foraging behavior within the rusty crayfish showed that rusty crayfish foraged significantly longer at the zero and high flow rates compared to the low and intermediate rates (emmeans post hoc, \(p < 0.05\); Figure 3). Foraging behavior for the virile crayfish only showed significant differences between foraging times at the high flow rate and low flow rate (emmeans post hoc, \(p < 0.05\); Figure 3). The only difference between the two species was found at the zero-flow rate (emmeans post hoc, \(p < 0.05\); Figure 3).

Shelter
Analysis of the time spent in the shelter by a mixed model showed a significant interaction of the flow treatment and species when flow was treated as a categorical variable (\(F(3,206,0.05) = 2.73; p = 0.045\) but not when flow was treated as a continuous variable (\(F(1,210,0.05) = 3.23; p = 0.074\); Table 2). The AIC values indicated that the model which used flow as a categorical variable had more explanatory power and the \(\Delta AIC\) was greater than two, so this model was adopted as the best fit.

A post hoc analysis of the best fit model showed that \(F.\ virilis\) used the shelter significantly more than \(F.\ rusticus\) only at the zero flow conditions (\(p = 0.018\)). None of the other comparisons were significant.

Fights
None of the linear models (either with flow as categorical or continuous) showed any significance across the treatments, participants of the fight, or interactions of those fights.

Winners of interactions—Tukey multiple proportions test
Food
A Tukey multiple proportions test indicated that the winners of interactions over the food resource were not randomly distributed (\(\chi^2 = 77.1, v = 6\); Table 3; Figure 5). A subsequent post hoc analysis of
Figure 2. Counts of the number of incidences of behavioral events within the mesocosm as a function of the species performing the behavior and the background flow rate. Foraging involves activity consuming the muskgrass (top figure), fighting is defined by the species that initiated the interaction (bottom figure), and shelter use is defined as entering the shelter (middle figure). The distribution of behavioral events is significantly different for all three behaviors. Chi-squared = 116.68, $p < 0.001$ for foraging; chi-squared = 98.286, $p < 0.001$ for fighting; and chi-squared = 31.609, $p < 0.001$ for shelter use.
the paired comparisons showed that \textit{F. rusticus} won the majority of interactions over food in the high flow food treatment. This was reversed in the low flow treatment where \textit{F. virilis} won the majority of interactions.

\textbf{Shelter}

A Tukey multiple proportions test indicated that the winners of interactions over the shelter resource were not randomly distributed ($\chi^2=33.1, v=6$: Table 3; Figure 5). A subsequent post hoc analysis of the paired comparisons showed that \textit{F. rusticus} won the majority of interactions over shelter in the high flow shelter treatment.

\begin{table}[h]
\centering
\begin{tabular}{lcccc}
\hline
\textbf{Behavior} & \textbf{Model} & \textbf{F} & \textbf{p} & \textbf{AIC} & \textbf{Effect size} \\
\hline
Foraging & Discharge as categorical & 6.2052 & 0.001 & 3369 & 0.43 \\
Foraging & Discharge as continuous & 5.21 & 0.022 & 3413 & 0.14 \\
\hline
Shelter use & Discharge as categorical & 2.72 & 0.045 & 601 & 0.40 \\
Shelter use & Discharge as continuous & 3.22 & 0.07 & 599 & 0.25 \\
\hline
Fight length & Flow as categorical & 1.51 & 0.22 & NS & NS \\
Fight length & Flow as continuous & 0.66 & 0.42 & NS & NS \\
\hline
\end{tabular}
\caption{Statistical output of the linear mixed models using discharge as both a categorical variable as well as a continuous variable.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
\textbf{Treatment} & \textbf{Rusty} & \textbf{Virile} & \textbf{Rusty} & \textbf{Virile} \\
\hline
Zero & 22 & 15 & 12 & 14 \\
Low & 21 & 33 & 14 & 20 \\
Intermediate & 27 & 32 & 20 & 17 \\
High & 54 & 8 & 18 & 1 \\
\hline
\end{tabular}
\caption{Number of interactions won in interspecific interactions based on treatment and resource type.}
\end{table}

Figure 3. Mean (± SEM) of the length of foraging bouts as a function of the flow treatment and species. Solid blue bars indicate foraging times for \textit{F. rusticus} and solid yellow bars for \textit{F. virilis}. Statistical analysis using a linear mixed model with flow as a categorical variable can be found in Table 1. Capital letters indicate a significant difference between foraging times for \textit{F. rusticus} using an emmeans post hoc analysis with different letters indicating a $p<0.05$. Lower case letters indicate a significant difference between foraging times for \textit{F. virilis} using an emmeans post hoc analysis with different letters indicating a $p<0.05$. Finally, the asterisk indicates a significant difference between species within a flow categorical using an emmeans post hoc analysis.
Discussion

The results of this study indicate that stream discharge rates have significant differential impacts on two species of crayfish (\textit{F. rusticus} and \textit{virilis}) for at least three different behaviors. First, the number of interactions exhibited by these two species is flow dependent where \textit{F. rusticus} demonstrates a higher number of interactions with no flow and the highest flow rates when compared to \textit{F. virilis} (Figure 2). Thus, competition between these two overlapping species may be altered depending on flow in a system. Specifically, \textit{F. rusticus} seemed to significantly outcompete \textit{F. virilis} for food and shelter resources in no and high flow treatments through agonistic behaviors (Figures 3 and 4), whereas \textit{F. virilis} was more successful in number of times sheltered and agonistic behaviors in low and intermediate flow rates (Figures 4 and 5). \textit{F. virilis} also won food in fights more often in low flow treatments (Figure 5). This shows that \textit{F. rusticus} may be a more successful competitor in areas of no or high flow rates while \textit{F. virilis} is more successful in low to intermediate flow rates. Thus, flow rate is a significant factor in determining the success of one species of crayfish over the other for various resources. Because these species are found to co-occur with one another in many different areas, these results could provide implications on the success and distribution of each species in various river systems.

\textit{F. rusticus} and \textit{F. virilis} are each native to North America and overlap in their native ranges (Lodge et al. 2000; Perales et al. 2021). However, \textit{F. rusticus} is an invasive species in many parts of the more northern USA (Smith et al. 2019), whereas \textit{F. virilis} is invasive in southern parts of the United States and Europe (Ahern et al. 2008; Rozansky et al. 2021). Both species have been known to outcompete and decimate native crayfish populations (Olden et al. 2006; James et al. 2016). It is possible that the competitive advantages demonstrated in the results presented here can provide explanations for the success or failure of these invasions. In northern Michigan, where this study was conducted, both species co-occur, with \textit{F. rusticus} being an introduced species. Past research has shown that \textit{F. virilis} are generally outcompeted in agonistic interactions for food and shelter.
resources by *F. rusticus* (Capelli and Munjal 1982; Hill and Lodge 1999). *F. rusticus* may also be at less risk of predation by fishes as they have larger body sizes and are more aggressive than their native counterparts (DiDonato and Lodge 1993; Kuhlmann et al. 2008). These factors could explain the decreases in native crayfish populations in the Midwest region of the United States. However, discharge levels of stream ecosystems seem to also impact the ability of these species to compete with one another, as evident in our results. We should also note that these experiments took place over the summer where changes in stream discharge are the highest and both species of crayfish are in a non-reproductive form. Within this form, these crayfish are concerned with foraging, shelter use, and predator avoidance as mating takes place during the fall and winter. Selecting this form and this season for the experiment promoted the greatest level of competition for resources and other seasons may have reduced competition outcomes. Thus,

Figure 5. Proportion of agonistic interactions won by *F. rusticus* (solid blue bars) and *F. virilis* (solid yellow bars) during interactions over food resources (top graph) and shelter resources (bottom graph). Bars with different capital letters are significantly different from each other using a Tukey multiple proportions test and post hoc analysis.
we believe that discharge is another factor that should be considered when understanding the dynamic interactions between *F. rusticus* and *F. virilis*.

Both lentic and lotic ecosystems have spatially heterogenous distributions of flow regimes (Buffagni 2021). Lotic ecosystems have sub-habitats of varying spatial scales with significantly different flow regimes such as riffles, runs, and pools (Buffagni et al. 2009). In those areas with the lowest (pools) and highest (riffles) flow velocities, *F. rusticus* would have advantages for foraging and fighting behaviors compared to *F. virilis* (Figures 2, 3, and 5). Within these habitats, *F. virilis* would spend more time in shelters rather than foraging and fighting for resources. Conversely, those habitats with flow velocities in between these two extremes would favor foraging, shelter use, and fighting by *F. virilis*. Interestingly, these results are applicable to two polar opposite ecological issues: invasion potential and refuges from invasion. Measuring the small scale flow dynamics within the mesocosm and how these microscale differences play a role in altering competition would be an interesting addition. Any object placed within the flow will create boundary layers and will have differing shear forces as water flows over them. In the current design, the movement of crayfish, their reworking of the sediment, redistribution of the feeding bracket, shelter, and even inflow tubes makes any meaningful measurement of microscale flow impossible.

Crayfish, in general, and these two particular species of crayfish, are highly invasive within the water ways of the North American continent (Gherardi 2007). If either *F. rusticus* or *F. virilis* demonstrate competitive advantages over native crayfish in differing flow regimes, this ability could provide pathways for successful invasion of new habitats. That is, the flow regime offers a mechanism that these invasive crayfish may be able to exploit in order to successfully inhabit a new area. As both species expand their invasive ranges, the fine scale locale of introductions and successful establishment should be noted with respect to local flow regimes. These competitive advantages, if they exist with other potential invasive crayfish, then may also provide significant refuges for native populations to resist invasion. For instance, our study showed that neither species was successful in all types of flow. This lends to the idea that native populations may be able to take advantage of the competitive differences of an invasive species in order to more successfully compete by localizing in areas of the habitat where they tend to have more success in necessary behaviors. In this way, flow regime could be important in understanding, not only invasion tactics, but refuge use in light of invasion as flow regime may be a main driver in population heterogeneity and competition throughout a habitat.

Flow rate has been shown to significantly alter interactions in various species of aquatic organisms, as many species tend to live in areas where the flow rates are optimal for them to perform feeding and breeding behaviors as well as evasion tactics (Blanchfield and Ridgway 2005; Fu 2015; Sun et al. 2018). The racer goby (*Babka gymnotrachelus*) out-competed the European bullhead (*Cottus gobio*) for shelters in high flow velocity areas, and racer gobies tended to stay in shelters for longer periods of time when flow rates were high (Jermacz et al. 2015). Changes in flow velocity not only effect growth in salmon (*Salmo salar*), but also influences food chain length (Blanchet et al. 2008). Situations where organisms have differing competitive advantages based on flow may put the disadvantaged organism at a higher likelihood of predation or parasitism because lack of refuge use. These competitive differences may be accentuated in sessile organisms. Coral reef mortality has also been shown to increase in areas where flow is more likely to bring algal pathogens and organic matter into contact with the reefs (Barott and Rohwer 2012). In this way, flow regime has a direct effect on the health of coral reefs and those animals that use reef ecosystems as habitats. Despite these documented effects of flow on competition and the importance of flow overall in aquatic systems, there is a dearth of studies that test the direct effects of flow and flow variation on competition.
The effects of flow on ecological processes like competition may become even more important to consider given the expected alterations to habitats as a result of climate change. Most models of precipitation change as a result of the climate crisis indicate alterations in baseline flow in aquatic ecosystems (Jha et al. 2004). In addition to changes in baseline flow, the variation in stream flow and lake levels is expected to increase as the intensity and periodicity of seasonal storms also increases (Jha and Gassman 2014). As a result of these changes, flow regimes in lotic and even circulation in more lentic ecosystems will see increases in variation that result in changes in flow regimes (Milly et al. 2005). If competitive advantages change as a result of flow differences, then competitive advantages will also change as a result of the climate crisis. Given the lack of studies on the direct impact of flow on competition in aquatic species, the ability to predict ecosystem changes as a result of changing flow regimes remains elusive.

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Author contributions


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Notes on contributors

Sophia Adami-Sampson was an REU student at the University of Michigan Biological Station in the summer of 2022.

Madison J. Wagner is a first year Ph.D. student in the Laboratory for Sensory Ecology at Bowling Green State University.

Paul A. Moore is a full professor and founder of the Laboratory for Sensory Ecology.

Data availability statement

Raw data will be available through the University of Michigan Biological Station’s research portal Mfield upon publication of the manuscript.
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