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# Interspecific competition affects resource use by three cryptic freshwater species of *Hyalella* Smith, 1874 (Amphipoda: Hyalellidae)

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## ABSTRACT

Species that use the same resources present a paradox for understanding their coexistence. This is especially true for cryptic species because they are phenotypically similar. We examined how competition affects food-resource use in three cryptic species of *Hyalella* Smith, 1874, a freshwater-amphipod genus. We hypothesized that competitively inferior species would use high-quality algae patches when alone and competitively superior species would displace inferior species to low-quality patches. We compared use of foraging patches varying in algal content (i.e., quality) when species were alone or with another species. Our results showed that the competitively inferior species spent more time on the low-quality patch in the presence of the competitively superior species, but the behavior of the competitively superior species was independent of heterospecifics. This study provides insight into the role of interspecific competition in shaping resource use and patterns of coexistence in nature.

**Key Words:** freshwater amphipods, interference competition, species displacement

Competition is an important mechanism that affects species coexistence in a community (Chesson & Kuang, 2008). While other mechanisms, like abiotic factors and predation, play a role in shaping community structure, interspecific competition structures each level of a community and the effect of competition can be similar in magnitude to that of predation (Hairston *et al.*, 1960). Competition and predation were previously thought to affect communities in different ways, but a recent ecological theory suggests that both types of interactions can equally cause either exclusion or coexistence. Exclusion occurs if there is no differentiation between species, whereas coexistence will occur if there is differentiation between species. Coexistence is dependent on the relationship between competition and predation when both competition and predation are present in a system (Chesson & Kuang, 2008).

Competition can manifest in different forms. Exploitative competition occurs when one species is able to depress resources to a lower level than competing species, which translates to higher relative fitness (Holt *et al.*, 1994). Interference competition occurs when one species uses aggression or other methods to exclude other species from the resource. Interference competition is common in nature but has received less attention in structuring communities than exploitative competition (Case & Gilpin, 1974; Amarasekare, 2002). Whether interference competition promotes or inhibits coexistence is expected to depend on whether interference is costly or beneficial (Amarasekare, 2002). If interference is beneficial (e.g., predation or parasitism on some life stages of a competitor), it can lead to coexistence through tradeoffs between interference ability and exploitative ability. On the other hand, if the interference is costly (e.g., territoriality, physical displacement, and allelopathy), coexistence is not achievable and the species that is better at exploiting the resource or the numerically superior species (i.e., priority effect) will exclude the other species.

We studied potential interference competition among three cryptic (i.e., species that are phenotypically similar making species assignment difficult without molecular markers) amphipod species in the genus *Hyalella* Smith, 1874. Two of the three species *H. spinicauda* (formerly Species A of Wellborn *et al.* 2005) and *H. wellborni* (formerly Species C of Wellborn *et al.* 2005) have recently been described and one, Species B, awaits formal description (Soucek *et al.*, 2015). These species, while phenotypically similar, are ecologically distinct (Cothran *et al.*, 2013a). Under close examination *H. spinicauda* and *H. wellborni* show differences in their telsons, second/posterior gnathopods, and third uropods (Soucek *et al.*, 2015). The species also exhibit differences in pigmentation patterns that allows sorting of species without molecular markers (Cothran *et al.* 2013b). It is unknown how this trait variation affects behavior and resource competition.

All three species can be found in all areas of the littoral zone of glacial lakes in the northern U.S. but their relative abundance varies both across a distance-from-shore and depth gradient (Wellborn & Cothran, 2007). The availability of food resources used by amphipods (e.g., algae and detritus) may vary spatially in a lake (Hargrave, 1970; Cothran *et al.*, 2014). Algal growth decreases as light penetration decreases with increasing depth and detritus has been found to be more abundant near the edge of some lakes (Doi, 2009). These studies suggest that food resources may be more plentiful in near shore areas of a lake. Species B, which is slightly larger in size than the other two species, is most abundant in this microhabitat and has been shown to be competitively superior in mesocosm studies (Wellborn *et al.* 2005; Cothran *et al.* 2013a, 2015). The mechanism, whether it exploits resources better and/or interferes with the smaller species causing shifts in habitat use, behind the competitive dominance of Species B, however, is unknown.

We explored how heterospecifics affect resource use patterns for the three cryptic amphipod species. Specifically, we tested whether use of high-quality and low-quality resource patches was sensitive to the presence of heterospecifics. We predicted that the larger size and better competitive ability of Species B would allow it to outcompete the other species (Cothran *et al.*, 2013a). We further predicted that this interaction would cause the displacement of the other two species to the low-quality resource patch.

Amphipods used in this experiment were collected from Crystal Lake (Crawford County, PA, USA; 41°32′59″ N, 80°21′59″ W) in July 2016, and housed at 21.58 °C ± 1.40 (mean ± SD) at Southwestern Oklahoma State University, Weatherford, OK. The species were kept in separate aerated bins and fed a combination of ground spirulina and fish flakes dissolved in an agar solution three times a week. The experiment was performed in November 2018, so the amphipod populations had been under laboratory conditions for two years and the populations consisted of individuals that were born and raised under laboratory conditions.

We used an additive design to explore potential interference competition between the species. We chose an additive design over a replacement/substitutive design for two reasons: 1) the latter confounds intra and interspecific competition (Snaydon, 1991) and 2) because there were two heterospecific competitors for each focal species, we were able to compare the magnitude of any behavioral shifts by the focal species to the other heterospecific specific competitor treatment (equal total densities) and the no heterospecific competitor control (half of the total density of the competitor treatments).

The experiment was performed under the same laboratory conditions as mentioned above. Test arenas were Y designs connecting three plastic containers each with a diameter of 4 cm. Each section was connected by a polypropylene Y connector with an inner diameter of 4 mm and each arm of the Y was 25 mm in length. The three sections contained either high-quality food, low-quality food or no food, where amphipods were initially released. Arenas were filled with artificial lake water (SAM-5S; Borgmann, 1996). In each section, we placed a nylon mesh grid (4 × 2.5 cm) to provide substrate for the amphipods. The high-quality food section received our standard recipe that we fed amphipods (4 g of spirulina fish flakes; (Pentair AES, Apopka, FL, USA) 1 g of pure spirulina (Nutrex Hawaii, Kailua-Kona, HI, USA), 1.44 g of granulated agar (Fisher, Fair Lawn, NJ, USA) in 50 ml of SAM-5S). The food was heated for 45 sec at 15 s intervals and allowed to cool. Amphipods were then fed plugs of the gelatinous food delivered by disposable pipets. The low-quality food was made by decreasing the nutritive component of the recipe (i.e., fish flakes and pure spirulina) by 75%. Using this artificial diet allowed us to keep resource supply constant across replicates and easily manipulate food-patch quality.

To begin a trial, six adult amphipods, chosen haphazardly without regard to sex, of each species were placed in the no-food section of the arena. These amphipods (six total in one-species arenas and 12 total in the two-species arenas) were left overnight to acclimate. The following day, the location of each amphipod in reference to food quality sections was recorded twice daily (between 0630–0730 and 1700–1815) for just over 3 d. Six replicates of each species alone and each heterospecific pairwise combination were performed except for *H. spinicauda* alone, with only four replicates, for a total of 34 experimental units.

For each replicate, we calculated the average difference in resources use (i.e., the number of amphipods in high quality section minus number of amphipods in low quality section) over the duration of the experiment. For heterospecific replicates, average difference scores were calculated for both of the species. We then plotted these average difference scores along with 88% confidence intervals to determine if resource patch use deviated from the null of equal use of each patch. Specifically, confidence intervals that do not overlap with 0 demonstrate a statistically significant preference for one of the two resource patches (Austin & Hux, 2002; Cumming, 2009).

Species B did not show a clear preference for either resource patch when alone or in the presence of heterospecifics (Fig. 1A). *Hyalella spinicauda* showed preference for the high- quality resource patch when alone and in the presence of *H. wellborni*. *Hyalella spinicauda*, however, decreased use of the high-quality resource patch by 123% in the presence of Species B (Fig. 1B). *Hyalella wellborni* showed a similar pattern to *H. spinicauda*. When alone and in the presence of *H. spinicauda*, it showed a clear preference for the high-quality resource patch, but decreased its use of the high-quality resource patch by 83% in the presence of Species B (Fig. 1C).

We discovered that the two smaller cryptic amphipod species (*H. spinicauda* and *H. wellborni*) shifted to using low quality resource patches in the presence of the larger species, Species B. Both of the smaller species showed a strong preference for the high-quality resource patch in the absence of Species B but showed no preference for either patch in the presence of the larger competitor. Species B, however, showed no preference for either resource patch regardless of whether alone or in the presence of heterospecifics.

Displacement of *H. spinicauda* and *H. wellborni* in the presence of Species B suggests that interference competition occurs in this group of amphipods. These results are consistent with previous experimental work that showed lower abundance of *H. spinicauda* and *H. wellborni* when in the presence of Species B (Cothran *et al.*, 2013a). The two smaller species also had higher incidences of wounding when in the presence of Species B. These studies together show that competition is occurring among the cryptic amphipod species and that Species B is a superior competitor. Moreover, it appears that interference competition explains, at least in part, the competitive dominance of Species B. Whether competition is occurring over food alone or other resources such as space and mates (i.e., reproductive interference) as well, is still unknown.

With one species alone, ideal free distribution theory predicts there would be a higher proportion of individuals on the higher quality food patch than on the low-quality food patch (Abrahams, 1986). Species B did not show a preference for the high-quality food even in the absence of the other two species, showing a deviation from the expected ideal free distribution. This could be due to antagonistic intraspecific competition. Similar studies have observed this deviation when studying the behavior of animal populations composed of individuals that vary in competitive ability (Abrahams, 1986). Variation in competitive ability could cause a relatively equal spread of individuals between patches that differ in quality with lower quality individuals being relegated to poor-quality patches (Abrahams, 1986). Such situation may have occurred in our experiment because the amphipods were

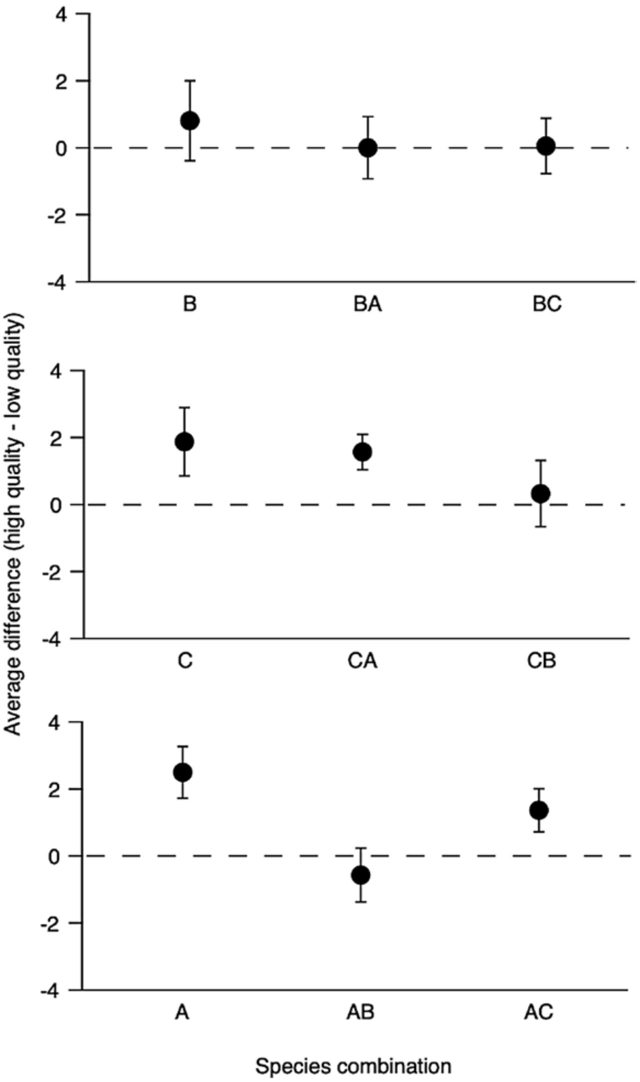


**Figure 1.** Results from the resource-patch-use experiment. Patch use by Species B (of xxx) by itself and interacting with other species (**A**); *H. spinicauda* (Species A) by itself and interacting with the other species (**B**); *H. wellborni* (Species C) by itself and with the other species (**C**). The markers are the average difference in the number of amphipods observed in the high-quality resource patch and low-quality resource patch. The zero line shows the null expectation if the amphipods show no preference for either of the two patches. The error bars are 88% confidence intervals and there is a statistically significant preference ($P < 0.05$) if they do not overlap with 0.

haphazardly chosen for the experiment. They were not separated based on factors that may affect competitive ability, such as size (Wellborn, 2002; Cothran *et al.*, 2013a). While we acknowledge that understanding sex- and size-dependent effects would provide further insights into the competitive dynamics between these amphipod species, this study shows strong effects of interference competition on food-resource use with this variation randomly distributed across treatments.

*Hyalella spinicauda* and *H. wellborni* did not affect each other's resource-patch use despite the fact that they show different vertical distributions in deeper areas of lakes (Wellborn & Cothran, 2007). Resources may not be a good predictor of the outcome of interactions between these two species. Our study, as well as results by Cothran *et al.* (2013a), suggests that competition is not occurring between these two species, so other factors must be explored to understand what is separating the species in lakes. Their vertical separation may be based on predator-prey relationships (i.e., one may be better at avoiding insect predators such as aquatic hemipterans that would attack near the surface of the water, whereas the other species may be better at avoiding fishes that attack deeper in lakes. Cothran *et al.* (2013a) found that *H. spinicauda*, which is more abundant near the surface, is as good if not better than *H. wellborni* at avoiding predation by fishes and larval dragonflies. Another possibility is that the slight differences in pigmentation, *H.* spinicauda being green whereas *H. wellborni* has a dark striped pattern (Cothran *et al.*, 2013b), and differences in ambient light and substrate may interact to affect predation risk. *Hyalella spinicauda* is most abundant near the water surface where green, submerged vegetation is the most common substrate, which matches the green phenotype of this species. The striping pattern may provide *H. wellborni* with enhanced crypsis in deeper areas where less light is available and more brown substrates (detritus and plant stems) are common. Future research should explore the relative risk of each species in both surface and deeper water in the littoral zone.

The ecology of food resources in the different zones of the lake could also be affecting amphipod spatial distributions based on the abundance or type of foods available. While the distribution of food resources has not been studied for the lake, we collected amphipods from, algal growth is expected to decrease with depth in lakes (Doi, 2009). Amphipods also consume microbes associated with detritus, which may be more abundant at the edge of the lake (Hargrave, 1970; Cothran *et al.*, 2014). Based on our study, it seems likely that Species B would occupy an area with higher food quality while the other two species would be displaced to areas with a lower food quality. Amphipod survival and growth is sensitive to fine-scale nutritional differences in food, so resource quality differences between areas of the lake could play a major role in structuring species distributions within a lake (Cothran *et al.*, 2014). Future research that explores amphipod performance on foods that are common to each major habitat of a lake (i.e., edge, offshore surface, and offshore deep) would provide insights into how food resource quality may structure amphipod species distributions.

Predation risk varies between the microhabitats inhabited by a species, which may be the reason for their separation. Species B is the worst of the three species at avoiding predation (Cothran *et al.*, 2013a). It is possible that *H. spinacauda* and *H. wellborni* are adapted to be better at avoiding predators but unable to compete with Species B for a higher quality food source. Future studies should explore the relative importance of food quality and protection from predation in shaping amphipod species distributions in lakes.

Interference competition may also affect species interactions over other resources as well. Another factor to consider is the possibility that *H. spinacauda* and *H. wellborni* are being displaced to avoid the effects of reproductive interference, interspecific mating interactions that cause lower fitness to at least one of the species involved. It is caused by incomplete species recognition (Gröning & Hochkirch, 2008; Burdfield-Steel & Shuker, 2011). Reproductive interference may contribute to the lower abundance of *H. spinacauda* and *H. wellborni* when males of Species B mistake females of the other two species for its own (Cothran *et al.*, 2013b). If females of the small-size *H. spinacauda* and *H. wellborni* are easier to mate with than conspecific females by species B males, or a subset of males (e.g., smaller individuals that are less competitive for mates), then reproductive interference could depress fitness in the small-size species. Evidence for reproductive interference being present in cryptic species is lacking, but the only study to date did not look at potential interference by competitively inferior, small-size Species B males (Cothran *et al.*, 2013b). While the specific resources over which the cryptic species compete for is unknown, our study provides evidence that Species B interferes with the two smaller amphipod species, causing their displacement to poorer quality environments.

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