Short-term predator avoidance behavior by invasive and native amphipods in the Great Lakes

Christopher Pennuto · Dawn Keppler

Abstract Understanding predator avoidance behavior by prey remains an important topic in community and invasion ecology. Recently, the Ponto-Caspian amphipod *Echinogammarus ischnus* (Stebbing 1898) was accidentally introduced into the Great Lakes. Since its introduction, it has displaced the native amphipod, *Gammarus fasciatus* (Say 1818), from several locations in the lower lakes. To assess whether behavioral differences in predator avoidance might be a causal mechanism increasing the success of the invasive amphipods, two experiments were conducted examining (1) native and invasive amphipod behavioral responses to five fish species with different foraging behaviors, and (2) amphipod responses to different densities of round gobies, a hyper-abundant benthic invertivore. *Echinogammarus* reduced its distance moved in the presence of all fish species tested, whereas *Gammarus* reduced its distance moved only after exposure to round gobies, black crappies, and rainbow darters. Both amphipod species increased the time spent motionless following exposure to round gobies, but not after encountering the scent of most of the remaining fish predators. The exception was that *Echinogammarus* also responded to black crappie scent whereas *Gammarus* did not. Although both amphipod species exhibited behavioral responses to many of the fish predators, the magnitude of their responses differed only after exposure to the brown bullhead. In the bullhead trials, *Echinogammarus* reduced its distance traveled significantly more than *Gammarus*. Both amphipod species increased their avoidance response to increasing goby density, however, the pattern of avoidance behavior was different. Invasive *E. ischnus* exhibited a consistently strong avoidance response to round gobies over the test duration. Native *G. fasciatus* initially avoided goby scent, but then either ceased their avoidance response or showed a hyper-avoidance response, depending on goby density. These results suggested (1) both species of amphipods were able to differentiate and react to a variety of fish predators, (2) invasive *Echinogammarus* amphipods avoided a larger range of fish predators than the native *Gammarus*, (3) increased avoidance behavior was associated with an increased density of fish, and (4) the avoidance response patterns of invasive *Echinogammarus* when faced with round goby predators might lead to increased predation on native *Gammarus* in habitats where they co-occur.

Keywords *Echinogammarus* · *Gammarus* · Invasive species

C. Pennuto (✉)
Biology Department & Great Lakes Research Center, Buffalo State College, 1300 Elmwood Avenue, Buffalo, NY 14222, USA
e-mail: pennutcm@buffalostate.edu

D. Keppler
Lake George Waterkeeper, 2199 Canada Street, P.O. Box 591, Lake George, NY 12845, USA

Introduction

Avoiding predators is an important activity performed by any potential prey organism. Multiple vertebrate and invertebrate prey species have been shown to alter their behavior in the face of predation risk (reviewed by Kats and Dill 1998), but studies especially focus on the foraging response of prey to the presence of predators (Fraser and Huntingford 1986; Culp and Scrimgeour 1993; Turner 1997; Heithaus and Dill 2002; Pennuto 2003). Every behavioral response performed by prey has an associated 'trade-off' (sensu Dill 1987). For example, when faced with increasing predation pressure prey may elect to reduce movement and remain in refuge. In doing so, it might avoid a predator, but potentially reduce its food intake, with negative growth consequences.

Amphipods represent an important prey item to a wide range of fish species in the Great Lakes of North America (Smith 1985). Recently, the Ponto-Caspian amphipod *Echinogammarus ischnus* (Stebbing 1898) was introduced into the Great Lakes, becoming the numerically dominant species in many locations, but especially within mussel beds (Ricciardi et al. 1997; Dermott et al. 1998: Gonzáles and Burkart 2004). Several studies have demonstrated the displacement of native *Gammarus fasciatus* by *E. ischnus* (Dermott et al. 1998; Van Overdijk et al. 2003), but few studies have been conducted on the actual mechanisms leading to the displacement of the native *Gammarus* (e.g., is the mechanism intraguild predation, food competition, differential predator avoidance, etc.).

Interestingly, life history and microhabitat preference differences between the two species of amphipods would seem to favor the native *G. fasciatus* over the invasive *E. ischnus*. Native *G. fasciatus* grow larger and produce more eggs than invading *E. ischnus*, and *G. fasciatus* eggs develop faster than *E. ischnus* eggs (Dermott et al. 1998). Palmer and Riccardi (2004) showed both *E. ischnus* and *G. fasciatus* increased in density in the presence of *Dreissena* sp. mussels. This finding was also reported by Gonzales and Burkart (2004), but they indicated *E. ischnus* were sometimes significantly more abundant in mussel beds than *Gammarus*. Further, the native *Gammarus* grew faster and survived better when fed food obtained from mussels

beds (i.e., feces and pseudofeces) than when fed macrophytes. Thus, mussel beds would seem to provide good habitat and food resources for both species, with a slight advantage to the native given life history features. However, Limén et al. (2005) used results of a stable isotope study to suggest these two amphipods might forage at slightly different trophic positions, with the invasive *E. ischnus* consuming more animal prey than the native *G. fasciatus*.

Several researchers have documented behavioral changes in amphipods induced by the presence of predators. An overall reduction in movement or drift rates is a common response employed by amphipods following exposure to the scent of fish predators (Williams and Moore 1985; Holomuzki and Hoyle 1990; Wudkevich et al. 1997). Recent studies have also shown amphipods distinguish between predatory and non-predatory fish. For example, Mathis and Hoback (1997) showed *Gammarus pseudolimnaeus* distinguish between chemical stimuli of rainbow trout and an herbivorous tropical fish and Baumgärtner et al. (2003) showed *Gammarus roeseli* behaved differently when exposed to chemical cues from fish with different feeding modes. Further, Baumgärtner et al. (2002) reported that the avoidance response of *G. roeseli* changed with predator density. Although these studies indicate amphipods are capable of determining the risk associated with different predators, few studies have examined the differences in predator avoidance behavior between invasive and native amphipod species.

Invasive prey species should, initially, be naïve to the scent of native predators and potentially respond inappropriately when faced with predation risk (e.g., Brown 2003; Hazlett 2000; Hazlett et al. 2002; Kristensen and Closs 2004), but invasive species also might learn quickly which predators should be avoided (e.g., Brown et al. 1997). Species-level differences in behavior could have important consequences for invasion success (e.g., Holway and Suarez 1999; Sih et al. 2004). In Europe, Dick and Platvoet (1996, 2000) and Kinzler and Maier (2006) suggest that differential susceptibility to intraguild predation and fish predators were important mechanisms leading to successful invasions of amphipods (e.g., *Gammarus tigrinus* and *Dikerogammarus villosus*). Gonzáles and Burkhart (2004) suggest the relative abundance of invasive *E. ischnus* and native

G. fasciatus in macrophyte and mussel beds of the Great Lakes could be explained by differences in predation vulnerability. Similarly, Barton et al. (2005) and Palmer and Ricciardi (2004) showed predation was an important force in determining relative abundances of these two species. Differences in predator avoidance behavior among amphipods ultimately may affect Great Lakes fisheries. If invasive amphipods somehow avoid fish predators better than native amphipods, while also displacing natives from dreissenid habitats, then fish may be forced to switch to other prey items of lesser abundance or caloric value.

Here we investigate the behavioral responses of native *Gammarus* and invasive *Echinogammarus* amphipods to fish predators having different predation modes. Additionally, we examine amphipod responses to different densities of round gobies, an extremely abundant and recent invasive benthic, invertivorous fish. We expect both amphipod species will respond to fish predators, but responses may differ depending on foraging mode of the predator and whether the predator is novel or not. Thus, we expect (1) both *E. ischnus* and *G. fasciatus* will respond to potentially threatening fish species, (2) *E. ischnus* will respond more strongly to the co-invasive round goby than will the native *G. fasciatus*, and (3) increased round goby density will heighten avoidance behavior from *E. ischnus*, but not from *G. fasciatus*.

Methods

Fish predators

We selected four fish species native to Lake Erie, plus the invasive round goby (*Apollonia melanostoma*, Pallas 1814) to assess predator avoidance behavior of amphipods. The round goby was formerly *Neogobius melanostomus* (Stepien and Tumeo 2006). Fish species represented a range of feeding strategies from visual-pelagic to chemosensory-benthic. The four native species included yellow perch (*Perca flavescens*, Mitchill 1814), black crappie (*Promoxis nigromaculatus*, Lesueur 1829), rainbow darter (*Etheostoma caeruleum*, Storer 1845), and brown bullhead (*Ameiurus nebulosus*, Lesueur 1819).

Like *E. ischnus*, the round goby is a successful Ponto-Caspian invader to the Great Lakes. Since its introduction in 1990, it has spread throughout all of the Great Lakes and its population was estimated to be about 9.9 billion in Western Lake Erie alone (Johnson et al. 2005). It is an obligate benthivore specialized for foraging on substrates. Adult gobies >7 cm in length are considered molluscivorous, feeding primarily on dreissenid mussels and snails, whereas smaller gobies are diet generalists, consuming a variety of benthic invertebrates (Ray and Corkum 1997; French and Jude 2001). Juvenile round gobies are reported to prefer amphipods over mussels (Diggens et al. 2002) and forage efficiently under low light conditions owing to a well-developed lateral line system (Jude et al. 1995). Thus, we considered gobies efficient predators of both amphipod species. Further, since the round goby co-occurs with *E. ischnus* in its native Ponto-Caspian range, we expected this amphipod to effectively avoid it and the native *G. fasciatus* to show less behavioral avoidance.

Yellow perch inhabit a variety of aquatic habitats and are found in all of the Great Lakes. They are benthopelagic, visual predators obtaining food from the entire water column (Smith 1985). Young-of-the-year perch rely primarily on plankton and smaller invertebrates for nutrition. As the gape of the fish increases, it is able to consume larger prey items, including benthic invertebrates and larger fish (Paszkowski and Tonn 1994; Richmond et al. 2004). Their foraging flexibility in response to the availability of suitable fish prey and their consumption of prey with a wide size range suggests they are highly opportunistic (Parrish and Margraf 1994). Thus, we considered yellow perch potentially effective predators of both species of amphipods. Since yellow perch and their close relative, the European perch (*Perca fluvaitilis*), have a Holarctic distribution, we did not expect any differences in the predator avoidance response between the two amphipods and we expected both amphipods to exhibit a strong avoidance of this predator.

Black crappie is benthopelagic, visual predators with a Nearctic distribution. Young fish tend to be plankton feeders, whereas older crappie isare piscivorous (Smith 1985). Recent studies suggest piscivory by black crappie is less predominant than previously reported and depends largely on responses to the availability of suitable fish prey and the relative size of predators and prey (Hodgson et al. 1997). Thus, black crappies were considered an intermediate

predatory threat to amphipods, and a predator, which would be novel to the invasive *E. ischnus*. We anticipated that both amphipods would respond to this predator, but that native *Gammarus* would exhibit a stronger response.

Rainbow darters are visually-feeding, invertivorous benthivores. Juveniles mainly feed on zooplankton, including fish larvae and midge larvae whereas larger darters are able to consume a variety of zoobenthos, including isopods, ostracods, gastropods and fish larvae and eggs (Smith 1985). Darters reside predominantly in stream ecosystems; thus, they are not likely to be encountered by either amphipod species. They were not considered a significant threat to large amphipods, though they readily consume small amphipods. Both amphipod species were expected to exhibit a low response to rainbow darters.

Brown bullheads are chemosensory and tactile benthic omnivores, consuming a variety of plant and animal resources. Juveniles feed mostly on chironomid larvae, cladocerans, ostracods, amphipods, and mayflies, whereas adults feed on benthic algae, weeds, mollusks, insects, leeches, crayfish, worms, and fish (Page and Burr 1981). Although juvenile brown bullheads may consume amphipods, adults do not actively forage on them and thus were not considered a major predatory threat. We did not expect either species of amphipod to respond significantly to brown bullhead predation threats.

Source and maintenance of animals

All amphipods were collected from dreissenid mussel beds in the Black Rock Canal along the southern shore of eastern Lake Erie (N 42.89781; W 78.90072) either by (1) scraping canal walls, or (2) retrieving mussel-covered rocks via snorkeling. Following either collection method, substrates were rinsed vigorously in a mesh-bottom bucket (0.5 mm^2) lakeside, emptied into white trays, and amphipods were sorted by species. We used the presence of orange-tinted antennae as our major sorting feature to distinguish *E. ischnus* from *G. fastiatus* (Witt et al. 1997). Samples were later verified under a dissecting microscope. The species were maintained separately in aquaria receiving once-through lake water and fed conditioned leaves and flaked fish food. They were

provided with rocks for shelter and were not held for more than one week. Multiple collections were performed to obtain enough amphipods for each fish trial. Since the size distribution of wild-caught amphipods changed through time, and size of prey can influence its response to predators, we analyzed each fish trial separately. Amphipod mean head capsule lengths ranged from 0.43 ± 0.04 to 0.95 ± 0.05 (SE) across all trials.

Round gobies were collected from the Black Rock Canal with minnow traps or angling throughout October 2004. The yellow perch and black crappies were collected from the Black Rock Canal in August 2004 using an electroshock boat. Darters were collected in August 2004 via kick-net from Ellicott Creek, a tributary to the Niagara River. Brown bullheads were caught using hook and line in Oak Orchard Creek within the Iroquois Wildlife Refuge in June 2005. All fish were kept separately by species in flow-through aquaria using raw lake water.

Previous studies have demonstrated that predator diet may influence the behavioral responses of amphipods (Mathis and Hoback 1997). To eliminate any potential prey response to amphipod alarm cues given off by predators, fish were not fed amphipods. Round gobies were maintained on a diet of worms and *Dreissena* mussels. Yellow perch and black crappie were fed diets of minnows and worms. Rainbow darters were fed worm pieces and brown bullheads were maintained on worms, corn, and commercial fish pellets. Fish were not fed 24 h prior to use in experiments.

To assess behavioral differences in predator avoidance, we performed two different experiments: (1) video-recorded, short-term prey behavioral responses to fish with different foraging behaviors, and (2) prey responses to different densities of round gobies.

Experiment 1: short-term prey behavioral responses to different fish predators

The total distance moved (cm) and time spent immobile (s) of *E. ischnus* and *G. fasciatus* was documented using the Videomex-V® digital recording system (Columbus Instruments, Columbus, Ohio) before and after exposure to raw lake water

conditioned with the scent of the target fish predator. We used raw lake water as our control water because kairomone and alarm substance concentrations in raw lake water (i.e., with no apparent fish activity) have been shown to be too low to induce predator avoidance behavior by amphipods (Baumgärtner et al. 2002). Additionally, lake water in our laboratory facility has a residence time in excess of 24 h. Hazlett (1997) indicated that kariomone and alarm cues for crayfish decay after six hours in laboratory studies. We are not aware of any studies for amphipod kariomone decay rates, but expect decay time to be similar to crayfish and thus any potential alarm substances in raw lake water should be ineffective

Experiments were carried out in a flow-through X-maze consisting of two, 40-L head tanks and two separate 7 × 7 cm arenas connected by 0.64 cm (O.D.) Tygon tubing (Fig. 1A). Water in each arena was 1 cm deep. The head-tanks either contained raw lake water or fish-conditioned lake water. To obtain fish-conditioned water, 150–500 g (1–10 adult fish, depending on species) of each fish species was placed in an aquarium for 24-h preceding a trial. The flow from each tank was maintained at 2.0 ml/s via control valves, giving each test arena a turnover time of 24.5 s. An outflow tube from each test arena was covered with a 1.0-mm mesh screen to prevent amphipods from exiting. Water flow through the arenas was verified prior to experiments by introducing a green dye. All trials were conducted in the dark with a single red fluorescent light.

Behavior trials lasted 1.5 min using a single, randomly drawn amphipod of comparable size of each species, one per test arena. Only non-mating adults were used in trials and no attempt was made to identify males or females. We ran 12–44 trials per fish species tested. Each trail was preceded by a 5-min period of raw lake water flow to ensure lines were clean of any residual kairomone from any previous trial. The trial duration for these short-term experiments was selected to measure the immediate reaction of prey to the odor of a predator and was dependent, in part, on the rate at which our experimental tanks completely filled with predator scented water (24.5 s).

Trials were divided into three sessions. Session I consisted of a 30-s flow of unconditioned lake water. This session served to establish baseline behavior. At
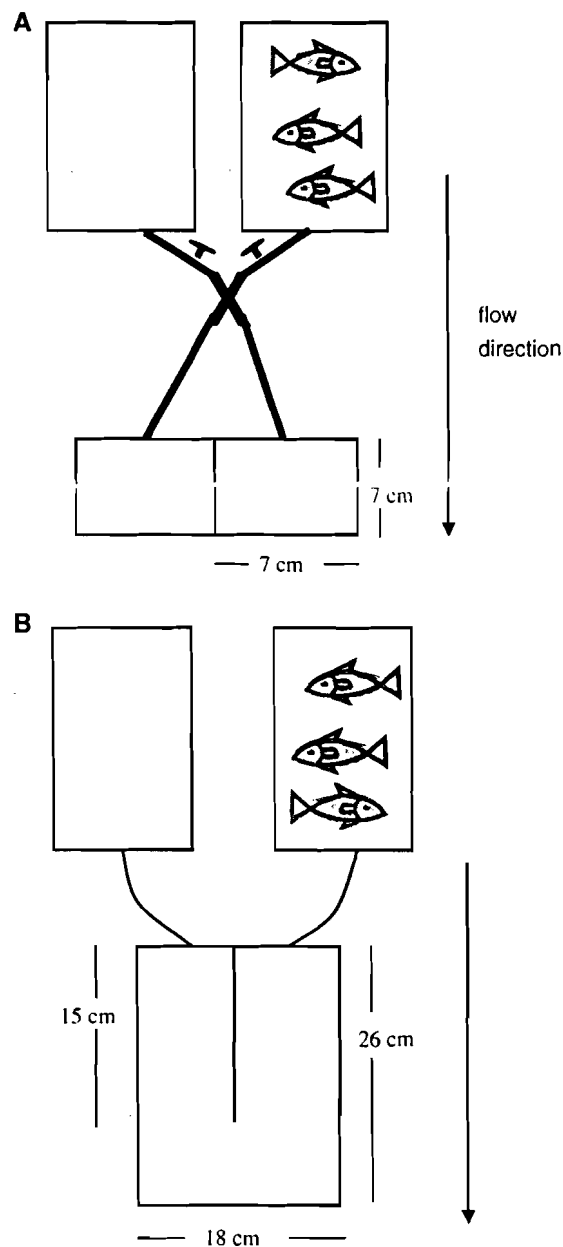
Fig. 1 Diagram of experimental X-maze (A) and Y-arena (B) used to determine amphipod behavioral changes following exposure to the scent of different fish species and amphipod avoidance behavior to increasing density of round gobies, respectively

30 s, the unconditioned lake water was turned off and fish-conditioned water was turned on using a series of valves and initiated Session II. Session II consisted of a 30-s flow of fish-conditioned water and represented the time it would take test arena water to be

completely replaced by fish-conditioned water. Session III began after the 30-s, fish-conditioned flow into the arenas and lasted another 30-s. This session represented water with full fish-scent concentrations. Thus, each trial consisted of a pair of amphipods, one of each species in each trial arena, exposed to 30-s unconditioned lake water, followed by a transitional low kariomone intensity 30-s water flow, and then a final concentration 30-s flow. After each trial, amphipods were preserved in 70% EtOH and their head length (mm) measured at a later date. Arenas were cleaned and flushed with lake water before the next trial.

Fish avoidance trials were conducted on different dates and sample sizes for each trial differed due to difficulty in obtaining sufficient numbers of adult amphipods. Because baseline activity levels differed between the amphipod species, data from each fish species trial were analyzed separately. Two analyses were performed. First, we examined each species response to the scent of a predator by comparing data from Session I (raw lake water) to data from Session III (fish-conditioned water) using a paired t-test $\ln(x + 1)$-transformed data, $\alpha = 0.05$. This analysis addressed the hypothesis that predator scent did not alter behavior. Second, we examined differences between amphipod species in their response to predator scent. For this analysis, data from Session I was subtracted from Session III to determine behavioral changes after exposure to predator scent. We used a t-test on the differences to compare species responses $\ln(x + 1)$-transformed data, $\alpha = 0.05$.

An additional treatment control (30-s raw lake water followed by 30-s of raw lake water followed by 30-s of raw lake water) was performed to account for any natural change in amphipod behavior over the 1.5-min trials. Twenty trials were performed as outlined above and the differences between the first and third sessions were compared using a paired t-test $\ln(x + 1)$-transformed data, $\alpha = 0.05$. In this control, there were no significant differences in distance moved nor time spent immobile for either amphipod species (*E. ischnus*: $t = 1.09$ and 0.87 for distance moved and time immobile, respectively; *G. fasciatus*: $t = 0.23$ and 0.76 for distance moved and time immobile, respectively; all $P > 0.05$). Thus, any differences in amphipod behavior before and after introduction of fish scent could be attributed to an avoidance response.

Experiment 2: goby density effects on predator avoidance behavior

Goby density effects on predator avoidance behavior of the two amphipod species were examined in a Y-arena. The Y-arena was made from an 18 × 26 cm (W×L) Plexiglas tank divided lengthwise with a 15-cm Plexiglas partition (Fig. 1B). Each branch of the arena received water (3.8-ml/s) from one of two 80-L head tanks. Water from the two branches of the Y-arena mixed with each other to form a mixing zone with an odor gradient (verified by test flows with green dye) prior to draining through a central outlet. One head tank contained raw lake water and the other contained goby-conditioned lake water representing three goby densities: zero (control), one, or eight gobies. The eight-goby density used in this experiment (44 fish/m^2) was comparable to low densities found by Vanderploeg et al. (2002) in Lake Michigan between 1993 and 1998 (40–100 fish/m^2). Water was conditioned with a single or eight gobies for 24 h prior to an experiment. The single outlet was covered with a 1.0-mm mesh screen to prevent amphipods from exiting the arena. All trials were conducted in the dark with a single red fluorescent light. Since head tanks could only hold enough water for three replicate trials, the species were tested and analyzed separately.

Each goby-density trial lasted 20-min. After an initial flow period of 5-min to establish a mixing zone, 20 randomly selected, adult amphipods of the test species were released in the downstream end of the Y-arena. Amphipods were collected from the Black Rock Canal as described above during the week each trial was conducted. The number of amphipods in each branch of the Y-arena was counted every 2-min ($n = 10$ counts per 20-min trial), as per methods of Buamgärtner et al. (2002). Amphipods located in the mixing zone, the area down-gradient near the outflow, were considered inactive and were not counted during each 2-min scan. Animals counted in the Y-branch receiving lake water were considered avoiding goby scent whereas animals in the Y-branch receiving fish-conditioned water were considered to prefer goby scent. After each trial, amphipods were removed, preserved according to their final location, and head lengths of each animal recorded. Tanks were cleaned between each trial.

Data for goby density effects on prey behavior were analyzed for each species separately with a two-

way, mixed-model repeated measures ANOVA (RM-ANOVA) examining treatment (i.e., kairomone intensity, $n = 3$), and trial ($n = 3$) as main effects and the 10, 2-min scan counts as repeated observations. Differences in the number of amphipods in each branch of the Y-arena was determined by subtracting the number of amphipods in the unconditioned branch from the number of amphipods in the goby-conditioned branch, for each 2-min interval. Thus, negative differences indicated avoidance behavior of goby-conditioned water and a positive difference indicated a preference for the goby-conditioned lake water. Following a significant RM-ANOVA, the trial was analyzed using Tukey's post-hoc tests to determine treatment differences.

Results

Behavioral responses to different fish predators

Both amphipod species moved significantly less following exposure to round gobies, black crappie, and rainbow darter, but *E. ischnus* also moved less after receiving scent from yellow perch and brown

Table 1 Results of paired *t*-tests examining the change in average distance traveled (cm) for *E. ischnus* and *G. fasciatus* following exposure to lake water conditioned with the scent of round goby, yellow perch, black crappie, rainbow darter, or brown bullhead

	df	*t*	*P*
E. ischnus			
Round goby	43	3.49	0.001
Yellow perch	11	2.53	0.014
Black crappie	17	4.53	<0.001
Rainbow darter	19	3.25	0.004
Brown bullhead	18	3.99	<0.001
G. fasciatus			
Round goby	41	4.81	<<0.001
Yellow perch	11	0.11	0.911
Black crappie	17	3.71	0.002
Rainbow darter	19	2.40	0.027
Brown bullhead	17	–0.87	0.398

Data were $\ln(x + 1)$-transformed to meet variance assumptions A positive *t*-value indicates a decrease in distance moved following exposure to fish scent whereas a negative *t*-value indicates an increase in distance moved

bullhead (Table 1). There was no apparent pattern in the differences in the baseline activity of the species. *Echinogammarus* exhibited more baseline activity than *Gammarus* during the yellow perch and black crappie trials, whereas *Gammarus* showed more activity during the round goby and rainbow darter trials (Fig. 2). The reduction in distance moved was significantly different between the species only after exposure to brown bullhead. In the bullhead trial, *E. ischnus* reduced movement distance more than *G. fasciatus* (Table 3, Fig. 2).

The invasive *E. ischnus* reduced travel distance most following exposure to black crappie scent ($\sim 12\%$ decline), whereas it reduced travel distance the least when exposed to round goby scent ($\sim 30.5\%$ decline). Native *G. fasciatus* reduced its distance moved most after exposure to rainbow darter ($\sim 52\%$ reduction), but increased distance moved following exposure to brown bullhead and yellow perch (~ 10 and 30% increase, respectively; Fig. 2). In all trials, at least one amphipod reduced movement distance to zero following exposure to fish scent. The greatest distance traveled by an individual of either species following introduction of fish scent was 266 cm (*G. fasciatus* after exposure to black crappie).
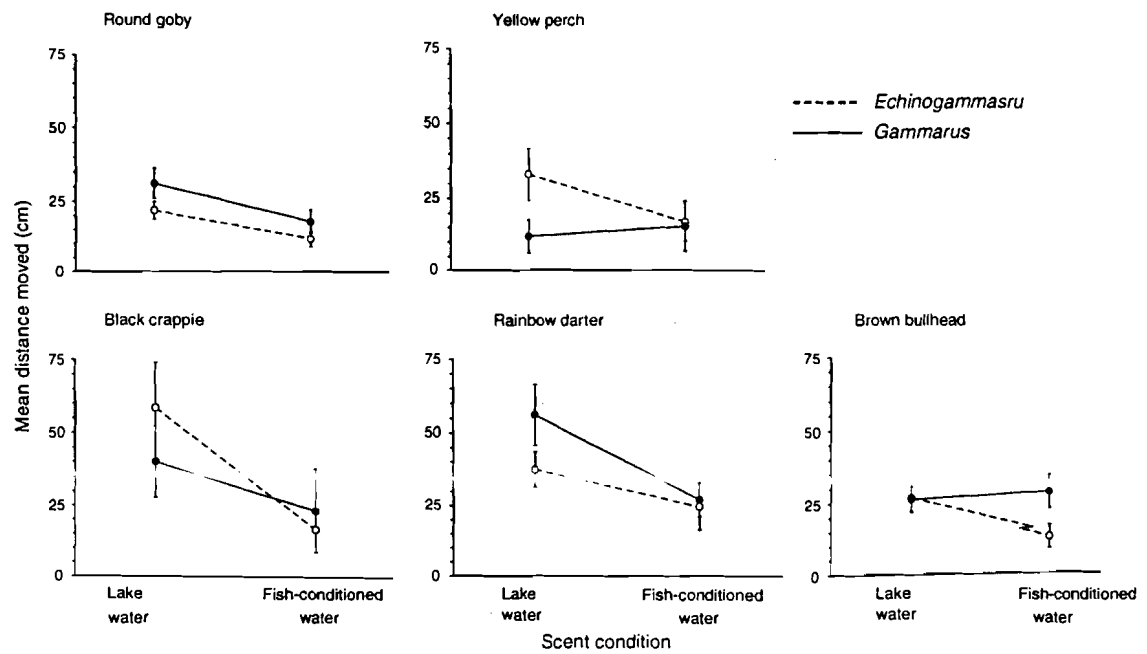
In general, amphipods increased time spent immobile following exposure to scent of the five fish species tested (Fig. 3). Both species spent significantly more time immobile after encountering scent from round gobies, but the invasive *Echinogammarus* also moved significantly less following exposure to yellow perch and black crappie (Table 2). During baseline sessions, the native *G. fasciatus* tended to remain motionless more than *E. ischnus* (Fig. 3), except during the round goby trials. There was no significant difference between the magnitudes of time spent motionless responses by amphipod species following exposure to fish predators (Table 3).

On average, *E. ischnus* increased time immobile by 5 s following exposure to fish scent whereas *G. fasciatus* increased their time by only 2.9 s. The invasive *E. ischnus* increased its time immobile the most when exposed to round goby scent (63% increase) and least when exposed to rainbow darter scent ($\sim 25\%$). The native *Gammarus* also increased time not moving most following exposure to round goby (41% increase) and least when exposed to yellow perch ($\sim 2\%$ decrease) (Fig. 3).

Fig. 2 Mean distance moved (cm) by *E. ischus* and *G. fasciatus* before and after exposure to the scent of different fish species in experimental arenas. Smaller values indicate animals moved less after exposure to fish scents. Error bars are 1 SE. The lake water represents Session I and the fish-conditioned water represents Session III (see Methods)
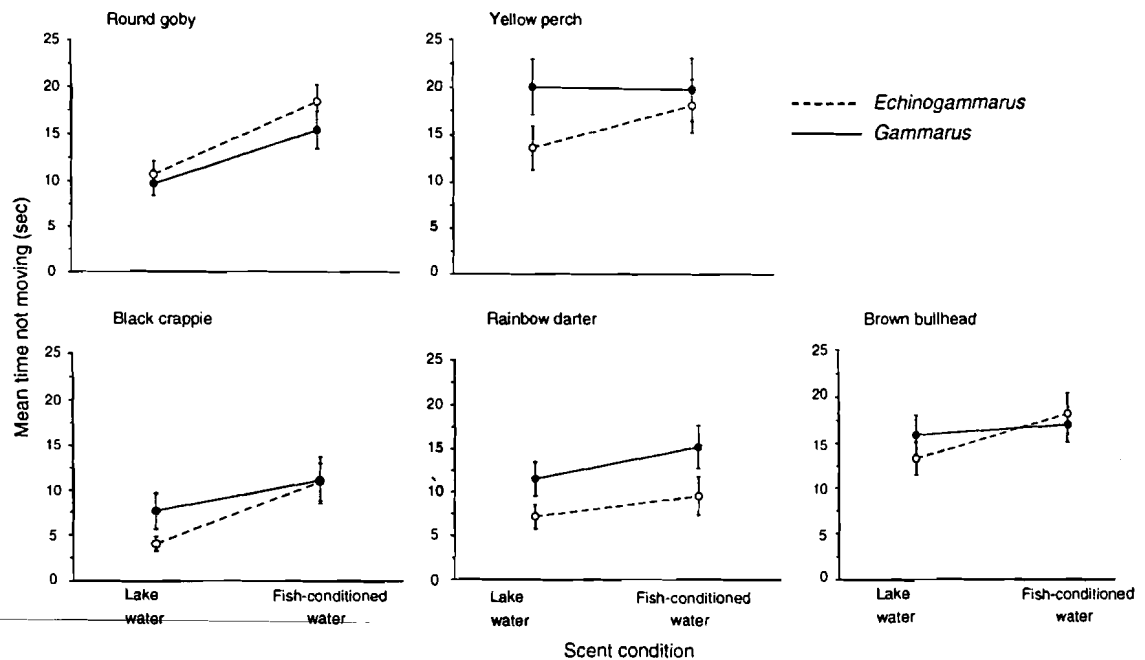
Fig. 3 Mean time spent immobile (s) by *E. ischus* and *G. fasciatus* before and after exposure to the scent of different fish species in experimental arenas. Larger values indicate animals remained motionless more following exposure to fish scents. Error bars are 1 SE. The lake water represents Session I and the fish-conditioned water represents Session III (see Methods)

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Table 2 Results of paired *t*-tests examining the change in average time spent immobile (s) by *E. ischnus* and *G. fasciatus* following exposure to lake water conditioned with the scent of round goby, yellow perch, black crappie, rainbow darter, or brown bullhead

	df	*t*	*P*
E. ischnus			
Round goby	43	–4.95	<<0.001
Yellow perch	11	–3.43	0.003
Black crappie	17	–3.11	0.003
Rainbow darter	19	–0.30	0.764
Brown bullhead	18	–1.77	0.094
G. fasciatus			
Round goby	41	–2.86	0.007
Yellow perch	11	0.19	0.850
Black crappie	17	–0.97	0.345
Rainbow darter	19	–0.52	0.609
Brown bullhead	17	–1.49	0.154

Data were ln(*x* + 1)-transformed to meet variance assumptions. A positive *t*-value indicates a decrease in time spent immobile following exposure to fish scent whereas a negative *t*-value indicates an increase in immobility

Table 3 Results of *t*-tests examining species differences in average distance traveled (cm) and average time spent immobile (s) following exposure to lake water conditioned with the scent of round goby, yellow perch, black crappie, rainbow darter, or brown bullhead

	df	*t*	*P*
Distance traveled			
Round goby	84	0.67	0.503
Yellow perch	22	1.09	0.287
Black crappie	34	1.28	0.211
Rainbow darter	38	1.12	0.268
Brown bullhead	35	2.05	0.047
Time spent immobile			
Round goby	84	0.01	0.993
Yellow perch	22	1.35	0.187
Black crappie	34	1.09	0.285
Rainbow darter	38	0.32	0.754
Brown bullhead	35	1.83	0.075

Data were ln(*x* + 1)-transformed to meet variance assumptions

Goby density effects on predator avoidance behavior

Both *G. fasciatus* and *E. ischnus* showed a significant increase in avoidance behavior to increasing goby density ($F_{2,27}$ = 29.39 and 163.04, respectively; both

Table 4 Results of a mixed model, two-way RM-ANOVA examining the effects of goby density on avoidance response by invasive and native amphipods

Source	df	MS	*F*	*P*
E. ischnus				
Goby density	2	858.700	163.04	<<0.001
Error	27	5.267		
Trial	2	22.533	2.89	0.095
Trial * goby density	4	40.183	10.35	<0.001
Error	54	3.881		
Total	89			
G. fasciatus				
Goby density	2	697.433	29.39	<0.001
Error	27	23.731		
Trial	2	48.633	2.69	0.082
Trial * goby density	4	482.817	30.30	<0.001
Error	54	15.934		
Total	89			

$P < 0.001$, Table 4) and there was no significant difference the response of either species among the replicate trials performed ($F_{2,18}$ = 2.69 and 2.89, respectively; both $P > 0.05$, Table 4). Significant trial by treatment interactions occurred for both species ($F_{4,36}$ = 30.30 and 10.35, respectively; both $P < < 0.001$, Table 4), indicating that there was variation in the pattern of response shown by groups of amphipods exposed to differing concentrations of fish kairomone. *Echinogammarus* tended to show a slight increase in avoidance intensity throughout the 20-min trials (Fig. 4), whereas *Gammarus* exhibited a rather different pattern of response. During the initial 12 min of trials, *Gammarus* showed a slight to steady decrease in avoidance of round goby scent followed by a dramatic and fluctuating change in avoidance across goby density (Fig. 4). After 15 min, *Gammarus* began to exhibit an attraction for the low-density, goby-scented water, but showed a strong avoidance of high-density, goby-scented water.

Discussion

Prey avoidance behavior in the face of predation risk can affect reproductive output, population growth, and distribution patterns in nature (Dill 1987). In this study, we saw differential predator avoidance responses by native and invasive amphipods, which
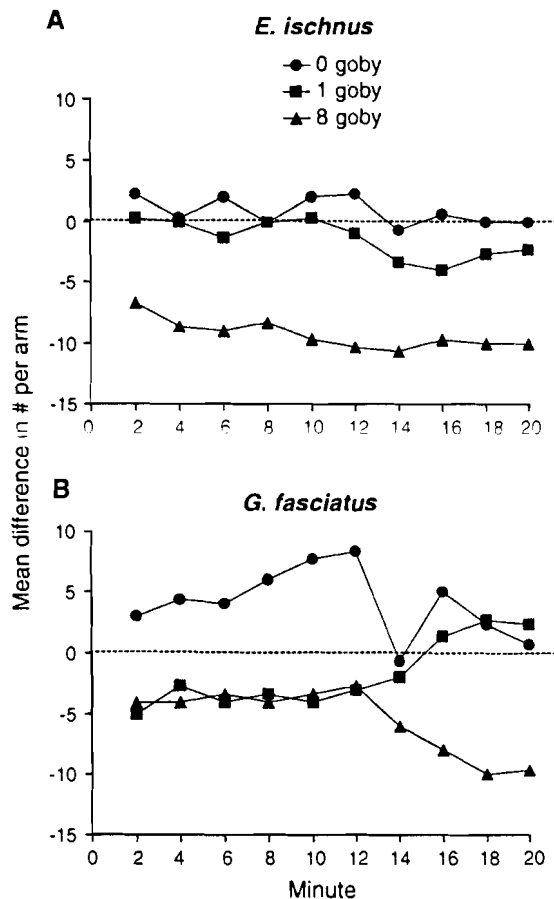
Fig. 4 Mean difference in the number of amphipods found in the unconditioned branch subtracted from the number of amphipods in the goby-conditioned branch under different treatment densities of round gobies. Negative differences indicate avoidance of goby-conditioned water. The dotted line at zero indicates neither attraction nor avoidance. (A) *E. ischnus*. (B) *G. fasciatus*

may support prior observations on their relative abundance and distribution in Great Lakes littoral zone habitats. The invasive amphipod, *E. ischnus*, reduced its distance moved following exposure to all tested fish predators, but the native *G. fasciatus* only responded to three species. Additionally, the invasive increased time spent immobile when exposed to three predator species whereas the native only responded to one. Both amphipod species responded to changes in predator density, but their pattern of response was different. Collectively, these results suggest short-term behavioral differences between invasive and native amphipods could affect invasion success.

Overall, there was good agreement between our predicted amphipod response to each predator and the observed response, but our predictions for the invasive *Echinogammarus* were correct more often than our predictions for the native *Gammarus*. We expected both amphipod species to avoid round gobies, yellow perch, black crappie, and to a lesser extent, rainbow darters. We did not expect a response by either species to brown bullheads. The invasive *E. ischnus* reduced distance traveled after exposure all fish predators, so the bullhead prediction was not supported by the data. Our predictions for the response of native *G. fasciatus*, on the other hand, were supported only for round gobies and brown bullheads. Curiously, yellow perch did not elicit either a reduction in distance moved or an increase in time immobile from *Gammarus*, even though amphipods are a diet mainstay for this fish predator. Potentially, the adult perch predators used here already had shifted to a fish prey diet and were thus not considered a potential predator by this amphipod species.

The invasive *Echinogammarus* reduced the distance moved significantly to more fish predators than did the native *Gammarus*. This significant reduction in distance moved by *Echinogammarus* following exposure to a larger array of predators than the native *Gammarus* might increase predation pressure on native amphipods where the two species overlap. If both species are detected by a visually feeding predator from the same distance away, the greater movements of *Gammarus* might be more attractive and result in a higher likelihood of attack. Similarly, greater sensitivity to a larger array of fish predators should be an advantageous characteristic of an invasive prey species, especially in environments dominated by invertivores.

Both amphipod species exhibited a statistically significant change in time immobile after introduction of scent from round gobies. We expected this response from *Echinogammarus* since it has a very long history of coexistence with the co-invasive round goby. It is unclear whether an increase in time immobile would actually increase avoidance of foraging gobies since these fish predators have an efficient lateral line system that might improve their detection of prey, even if immobile (e.g., Jude et al. 1995). Regardless of the predator avoidance value to *Echinogammarus*, they employ this strategy most

after exposure to the predator which they have the longest interaction history. Similarly, they exhibit a more consistent and stronger avoidance response to increasing density of round gobies compared to the native *Gammarus*.

Both amphipod species showed similar *statistical* results following exposure to increasing density of round gobies, but the patterns of response were quite different. Low goby density initially induced little response by invasive amphipods, which did not begin avoiding predator scent until after 10 min into the trials. However, native amphipods showed an immediate avoidance to predator scent and after twelve minutes, the native prey showed an attraction for predator scent. Clearly, attraction to the scent of a capable predator would be disadvantageous for *Gammarus*, but it is possible that the time elapsed since exposure to a predator's scent is as important as the overall response. If a prey remains in refuge too long following exposure to the scent of a predator, or seeks refuge from a predator too far away, the lost feeding opportunities are potentially great (e.g., Fraser and Huntingford 1986; Kats and Dill 1998). Thus, *Echinogammarus* might be able to better assess predation risk from a single goby, waiting until scent is very strong. *Gammarus*, on the other hand, responds immediately, but 'loses interest' more quickly. Under the highest goby density treatment, the invasive *Echinogammarus* showed a stronger and consistent avoidance of goby predators relative to low goby density. Again, the native *Gammarus* initially showed a lower response, but after 15 min dramatically increased its avoidance. Perhaps the native has learned that large numbers of goby predators should be avoided, at least for as long as these trials were conducted. Other research has shown equally dramatic differences in the behavior of invasive and native amphipods that help explain their distributions.

In Europe, *E. ischnus* has displaced native amphipods in several rivers throughout the British Isles and the central mainland (Kley and Maier 2003; MacNeil et al. 2004). The mechanisms behind their invasive success seem to be associated with both intraguild predation (Kinzler and Maier 2003) and differential predation by native fish predators (Kinzler and Maier 2006). Kinzler and Maier (2006) showed native fish in central Europe ate more native amphipods than invasive *Echinogammarus* and the invasive was less

active than the natives, in agreement with our findings in this study. Additionally, these authors report *Echinogammarus* is a stronger intraguild predator relative to native amphipods in Europe. In laboratory predation trials, they showed invasive amphipods consumed significantly more native *Gammarus fossarum* and *G. roeseli* from rivers in central Europe than visa versa. To our knowledge, intraguild predation between *Echinogammarus* and amphipods native to the Great Lakes has not been investigated. We suggest this is an important interaction for further study and may be, in addition to differential predator response, important in understanding the current displacement of native amphipods by this invader

Ecologists continue to pursue answers explaining invasion success of species around the world. Holway and Suarez (1999) make a compelling case that we need further understanding of species behaviors to predict the outcome of species introductions. However, species behavioral responses are often context-specific (e.g., Vet and Dicke 1992; Werner 1992; Keller and Moore 2000), taking one form under a given set of organism-specific (e.g., hunger state, age, size, physiological condition) and environmental conditions (e.g., temperature, water velocity, light intensity, substrate complexity), and another form under a different set of conditions. In the present study, presence of different fish species, and possibly, interaction history, resulted in different responses by the two-amphipod species examined. Each species showed some plasticity in response, which was dependent on the species of fish predator. Of further interest is whether the behavioral responses documented in this study would be the same under other environmental or species-specific conditions. For example, Dick and Platvoet (1996) showed that levels of water conductivity could modify species interactions between invading and native amphipods in the Netherlands. Intraguild predation between the species was reduced at higher conductivities, allowing some coexistence. Possibly, the observation of *Gammarus* displacement from some Great Lakes habitats by *Echinogammarus* represents this sort of context-dependency. Further work on behavioral responses of invasive and native species in diverse contexts is warranted to decipher if behavioral plasticity predicts invasion success.

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