Selective predation may be an important proximate cause of the success or failure of invader species. *Gammarus roeseli* is an invasive amphipod, for which the causes of establishment in rivers where the native species, *Gammarus pulex*, predominates remain unstudied. Freshwater amphipods are important prey for numerous fish predators, but empirical evidence of lower predation rates on exotic prey is scarce. In laboratory experiments, we compared the susceptibility of *G. pulex* and *G. roeseli* to fish predation, determined the mechanisms influencing prey selection, and studied the interaction between behavioural and morphological defences. Fish predators (brown trout, *Salmo trutta fario*) preyed selectively on *G. pulex*, but not because of differences in attack or capture probability. The presence of spines in *G. roeseli* appeared to contribute to its underpredation. Differential prey selection in this case might therefore have resulted from the trout’s reaction to an adverse stimulus. We found no significant difference in antipredator behaviour between *G. pulex* and *G. roeseli*. General behavioural differences were nevertheless found between species, with *G. roeseli* spending more time under shelters than *G. pulex*. However, microcosm experiments suggested that this difference was not important for differential predation. Antipredator behaviour may nevertheless be important for *G. roeseli* against other predators less sensitive to spines.

Predation risk is recognized as a major selective force in the evolution of numerous traits developed by many organisms to reduce their susceptibility to predation (Sih 1987; Abrams 2001). These traits include antipredator behaviours such as reduced activity and increased use of shelters to decrease detection, or chemical and morphological defences to diminish the probability of a successful capture (Kerfoot & Sih 1987; Laurila et al. 1997; Tollrian & Harvell 1999). Susceptibility to predation of exotic prey species introduced to novel areas is regarded as a potentially important proximate factor in their ability to become established (Lodge 1993), as natural predators contribute to biotic resistance by killing and eating exotic species (Robinson & Wellborn 1988; Reusch 1998). If natural enemies such as predators and parasites are rare or have little impact against new species, invaders may have an important advantage, which could be essential to their success (Sakai et al. 2001; Shea & Chesson 2002). Natural enemies may also drive community invasibility by altering competitive interactions between native and exotic prey species. This could be either through enemy-mediated apparent competition (Chaneton & Bonsall 2000), or when predators reduce the abundance of their prey, allowing potential competitors to coexist (Shurin 2001; Çelik et al. 2002). Understanding the mechanisms that influence prey susceptibility within new predator–prey interactions emerging after a recent colonization may be important for understanding the success of the invasion.

In Burgundy, eastern France, the invasive freshwater amphipod *Gammarus roeseli* is commonly found living in sympathy with the native species *Gammarus pulex*. Native to the Balkan area (Karaman & Pinkster 1977), *G. roeseli* has colonized several Western European countries over the past century, largely helped by human activities (Jazdzewski 1980; Jazdzewski & Roux 1988). Freshwater amphipods have a central position in the food web as prey of numerous invertebrates, fish and waterfowl (MacNeil et al. 1999a), and are known to have a large number of behavioural and morphological defences. For example, immobility, decreased activity and use of shelters between stones of bottom sediments, as well as morphological structures such as spines or carbonate deposits, have been reported in several species to offer protection against predation (Andersson et al. 1986; Holomuzki & Hoyle 1990; Friberg et al. 1994; Wellborn 1995; Starry et al. 1998; Ruff & Maier 2000). *Gammarus roeseli* individuals
differ morphologically from *G. pulex* in having a mid-dorsal carina on each of the metasomes, similar to three robust spines (*Karaman & Pinkster 1977; Fig. 1*). Spines are known to be morphological antipredatory adaptations in many organisms such as fish (*Hoogland et al. 1956*), gastropods (*West & Cohen 1996*) and crustaceans (*Morgan 1989; Tollrian 1995*). Variation among Gammarus species in the presence of dorsal spines might cause differential vulnerability to predation, but this remains to be demonstrated. A priori morphological defences such as spines may explain the establishment and persistence of *G. roeseli* in communities already saturated by *G. pulex* (*Jazdzewski & Roux 1988*). However, while antipredator defences have been documented in several gammarids, little is known about the relation between these different traits within a single species and the variability of such traits between species. Our objectives in this study were (1) to test whether the native *G. pulex* and the exotic species *G. roeseli* are differentially susceptible to fish predation, (2) to examine the role of *G. roeseli* dorsal spines against fish predation, (3) to compare the antipredator behaviour of *G. pulex* and *G. roeseli* and (4) to analyse the outcome of interactions between morphological and behavioural traits against fish predation in the two species.

**METHODS**

**General Methods**

We collected sympatric *G. pulex* and *G. roeseli* between February 2002 and March 2003 from the River Ouche, at Parc de la Colombière, Dijon, eastern France, using the kick-sampling method (*Hynes 1954*). Individuals were immediately transferred to the laboratory, in well-aerated holding tanks (50 x 40 cm and 15 cm deep) filled with dechlorinated tap water, with leaf material provided for food. Each species was maintained separately. The room temperature was kept constant at 15°C and the light:dark cycle was 12:12 h. Aquaria were lit with a solar spectrum fluorescent light with a 9000 K colour temperature.

To avoid a confounding effect of prey size in predation experiments, we used only *G. pulex* and *G. roeseli* males of similar size (mean total length ± SE of *G. pulex* individuals: 14.58 ± 0.17 mm; *G. roeseli*: 14.63 ± 0.22 mm, as measured according to *Bollache et al. 2000*; *t* sub 118 = 0.96, *P* = 0.34). To see whether a fish eating *G. pulex* or *G. roeseli* of the same size would acquire the same amount of food, we compared the relation between size and dry weight in the two gammarid species. The relation was highly significant in both *G. pulex* (N = 30; Y = −9.44 + 1.42X, *r*² = 0.69, *P* < 0.0001) and *G. roeseli* (N = 30; Y = −10.13 + 1.45X, *r*² = 0.74, *P* < 0.0001). An analysis of covariance revealed no significant difference between the slopes (*F* sub 1, 56 = 0.009, *P* = 0.92), and dry weight was the same in *G. pulex* and *G. roeseli* for the same size (comparison of intercepts: *F* sub 1, 56 = 1.26, *P* = 0.27).

**Microcosm Experiment**

We used brown trout, *Salmo trutta fario*, purchased from Corgoloin fish farm, Burgundy, eastern France, for the laboratory experiment. These fish had never experienced Gammarus as food before. They were less than 1 year old, with a mean fork length of 135 mm (range 120—152 mm). Before the experiment, trout were acclimated to laboratory conditions for at least 3 weeks in 500-litre aerated tanks filled with tap water, with a maximum density of 25 fish per tank, and fed during this period with frozen chironomid larvae.

We carried out the microcosm experiment (N = 10 replicates) in aquaria (40 x 21 cm and 25 cm deep) containing 50% trout tank water and 50% tap water, with washed river sand substrate on the bottom. A trout was placed into an aquarium 24 h before the experiment (*MacNeil et al. 1999b*). At the start of the experiment, 20 individuals of each of the two prey species, *G. pulex* and *G. roeseli*, were simultaneously offered to the trout. A plastic sheet separated the tank into a large compartment (30 cm) containing the substrate and the 40 gammarids and a smaller compartment (10 cm) containing the trout. After a prey acclimatization period (30 min), we carefully removed the partition to minimize the disturbance to both trout and prey. One hour later, the trout and all the surviving gammarids were removed and counted. To analyse differential predation between the two prey types, we used a two-tailed Wilcoxon signed-ranks test (*Siegel & Castellan 1988*).

**Responses to Predator Chemical Cues**

We tested the behavioural responses of *G. pulex* and *G. roeseli* to predator (brown trout) chemical stimuli in

![Figure 1. (a) Gammarus roeseli and (b) Gammarus pulex. Arrows highlight the ‘spines’ on the back of G. roeseli.](image-url)
the laboratory in two experiments. We obtained the predator stimuli by maintaining one trout for 24 h in a tank filled with dechlorinated tap water (scented water: 0.01 g of fish/ml of water, following Mathis & Hoback 1997). Dechlorinated tap water was used as a control. In the first experiment, the locomotor (i.e. swimming) activity of amphipods was investigated in Pyrex crystallizing dishes (11 cm diameter, 9 cm deep) filled with 100 ml of tap water, with a cylinder (5 cm diameter) placed in the middle to prevent gammarids crossing the dish. Under this apparatus we drew eight equidistant diameter lines. We estimated swimming activity by the number of lines crossed per unit of time. One gammarid was used per trial. Preliminary control experiments revealed no significant effect of gammarid size on locomotor activity (Pearson correlation; \( G. \ pulex: r_{23} = 0.23, P = 0.15; G. \ roeseli: r_{23} = 0.44, P = 0.92 \)), nor any effect of individual fatigue during a time compatible with the experimental procedure (three measures of 5 min each, made over 30 min, repeated measures ANOVA; \( G. \ pulex: F_{2, 25} = 0.02, P = 0.98; G. \ roeseli: F_{2, 25} = 0.35, P = 0.70 \)). During experimental series, individuals were allowed to acclimate in the dish for 5 min, and we recorded activity for 5 min. Then, 6 ml of control or scented water was added and homogenized with a spoon. We recorded activity after treatment for 5 min. This allowed us to compare activity before and after the treatment within a species and between the two species. We made 51 and 48 replicates with \( G. \ pulex \) and \( G. \ roeseli \), respectively, with both control and scented water.

In a second experiment, we investigated refuge usage in similar dishes, where the central cylinder was replaced by a black plastic cube (Duplo, Lego, Berkshire, U.K.; \( 5 \times 3 \times 2.5 \) cm) providing a refuge for the amphipods. The dish was filled with 100 ml of control or scented water. Again, one individual was tested per trial. After its introduction, we recorded the time spent under the refuge during 5 min. Data that did not meet normality (after a Shapiro–Wilk test) were analysed by Wilcoxon signed-ranks tests for intraspecific comparisons and Mann–Whitney \( U \) tests for interspecific comparisons (Siegel & Castellan 1988). Statistical tests were two tailed. Otherwise, parametric tests were used. We made 53 and 54 replicates for each species, with control and scented water, respectively.

**Trout Feeding Behaviour**

Feeding behaviour was observed in aerated tanks (40 × 21 cm and 25 cm deep) filled 24 h before each experimental replicate with 50% trout tank water and 50% tap water. To acclimatize the fish in the observation tanks and to standardize their feeding motivation, we placed all trout in the tank and deprived them of food for 48 h before the experiment (following MacNeil et al. 1999b). To avoid disturbance, we hid experimental tanks behind a curtain so that fish and observer were never in visual contact during the experiment. All tanks were also covered on three sides with black plastic, leaving one side clear for recording. The top of the tank was covered with a net, with a hole to introduce the prey. The experiment was carried out with a single prey type, \( G. \ pulex \) or \( G. \ roeseli \). Trout feeding behaviour was videorecorded behind the curtain, with a Panasonic RX 600 VHS-C camera recorder, and analysed frame by frame if necessary with a Toshiba V-362F VHS videorecorder and a Sony Trinitron KV A2520B colour TV set. One prey was introduced to the tank every 2 min during 10 min. The recording began when the first gammarid was introduced into the tank. In accordance with Gill & Hart (1994) and MacNeil et al. (1999b), we analysed five behaviours: (1) encounter (E): fish turns body and displays characteristic orientation movement towards the prey (sudden twitch of the head towards a particular prey item, see Metcalfe et al. 1987); (2) attack (A): fish suddenly lunges at prey and opens mouth to strike (Scott 1987); (3) capture (C): fish grasps prey in jaws; (4) mastication (M): prey is chewed inside the jaws before being swallowed and fish shows buccal movements that could be counted; and (5) ingestion (I): mastication ceases and prey does not reappear from mouth. If no encounter was detected 5 min after the introduction of the last prey, we excluded the fish from the analysis.

We calculated attack probability (proportion of encounters resulting in attack = \( A/E \)), capture success (proportion of attacks resulting in capture = \( C/A \)), attack efficiency (proportion of attacks resulting in ingestion = \( I/A \)) and capture efficiency (proportion of captures resulting in ingestion = \( I/C \)). We also measured handling time (the time from the first physical contact with the prey until the fish rejected the prey or the prey had been swallowed, Gill & Hart 1994), time spent by prey in the fish's mouth before ingestion and the number of chews by the trout for each gammarid ingested. Data were analysed with two-tailed Mann–Whitney \( U \) tests (Siegel & Castellan 1988).

**Dorsal Spines and Predation Probability**

Despite numerous attempts, we were unable to add artificial spines to \( G. \ pulex \). We therefore tested the effect of spines on the probability of being predated by removing spines from \( G. \ roeseli \) individuals (spineless individuals). Spines were cut with fine scissors. We obtained a control group by manipulating individuals for 1 min, the same time as for spineless \( G. \ roeseli \), and cutting the cuticle at the base of each spine with a scalpel three times. During both spine removal and cutting, no haemolymph escaped from individuals. The effect of treatment on predation rate was tested with the same protocol as the one described above for \( G. \ pulex \) versus \( G. \ roeseli \). We provided 20 individuals of control \( G. \ roeseli \) (i.e. not manipulated) to a single trout predator along with 20 individuals of spineless \( G. \ roeseli \) (\( \text{Gr}_{sp} \)) or 20 individuals of wounded \( G. \ roeseli \) (\( \text{Gr}_{w} \)). We counted surviving gammarids after 1 h. Ten replicates were made for each treatment.

**Refuge Usage and Predation Probability**

The effect of refuge usage on predation was tested with the same microcosm protocol as the one used for testing differential predation, but we placed a piece of air brick (21.5 × 5 × 5 cm) into the sand, where gammarids could
find refuge. We used 20 G. roeseli and 20 G. pulex per trial, and we counted the surviving gammarids after 1 h. Ten replicates were made.

RESULTS

Microcosm Experiment

Trout ate G. pulex significantly more than G. roeseli (Wilcoxon signed-ranks test: \( Z = 2.54, N = 20, P = 0.01 \); Fig. 2).

Responses to Predator Chemical Cues

Treatment with control water had no significant effect on locomotor activity (Wilcoxon signed-ranks test: G. pulex: \( T = -120.5, N = 51, P = 0.30 \); G. roeseli: \( T = 136, N = 48, P = 0.11 \)), whereas locomotor activity decreased after the introduction of scented water (G. pulex: \( T = 256.5, N = 51, P = 0.01 \); G. roeseli: \( T = 185, N = 48, P = 0.05 \); Fig. 3a). The decrease in activity (the activity before minus the activity after the introduction of the predatory signal) did not differ significantly between species (Mann–Whitney U test: \( U = 1354, N_1 = 51, N_2 = 48, P = 0.36 \)).

No significant difference in the time spent in the refuge was detected between control and scented water (Mann–Whitney U test: G. pulex: \( U = 1347, N_1 = 53, N_2 = 54, P = 0.61 \); G. roeseli: \( U = 1398, N_1 = 53, N_2 = 54, P = 0.85 \)), showing no effect of predator signal on this behaviour in the two species. However, the interspecific comparison showed that G. roeseli spent more time in the refuge than G. pulex did (\( U = 4605, N_1 = N_2 = 107, P = 0.01 \); Fig. 3b).

Trout Feeding Behaviour

There was no significant difference between prey species in attack probability and capture success (Table 1).

However, there was a significant effect of prey species on attack efficiency and capture efficiency. Significantly more G. pulex were successfully ingested when attacked compared to G. roeseli, and there was a significantly higher proportion of captures resulting in ingestion for G. pulex than for G. roeseli. Gammarus roeseli were more often rejected by the trout after their capture than G. pulex (Table 1). Among the prey that were ingested, we found no significant interspecific difference in mean time between the first contact and ingestion, nor in mean time spent in the mouth of the predator (Table 1). However, a significant species effect was found in the number of mastications before ingestion, G. roeseli being chewed for longer than G. pulex (Table 1).

Dorsal Spines and Predation Probability

Control G. roeseli (i.e. individuals with spines and not manipulated) were eaten significantly less than spineless G. roeseli (Wilcoxon signed-ranks test: \( Z = -3.01, N = 10, P = 0.002 \)).
Means are shown ± SEM. Sample sizes are given in parentheses. *Mann–Whitney U test.

$P = 0.002$; Fig. 4). However, we found no difference in predation between the control and wounded *G. roeseli* ($Z = 0$, $N = 10$, $P = 1.00$; Fig. 4), showing that the stress of manipulation and cutting was not the cause of the overpredation on spineless prey.

## Refuge Usage and Predation Probability

As in experiments without refuges, *G. pulex* were eaten significantly more than *G. roeseli* (Wilcoxon signed-ranks test: $Z = -2.48$, $N = 10$, $P = 0.01$; Fig. 2). Despite the lower number of individuals eaten in the two species in experiments with refuges (Fig. 2), there was no significant difference in predation according to refuge presence (Mann–Whitney $U$ test: $U = 65.5$, $N_1 = 10$, $N_2 = 10$, $P = 0.24$; Fig. 2).

## DISCUSSION

The results of the laboratory microcosm experiment indicated selective predation by trout on the two *Gammarus* species. Although the two prey species were represented in similar proportions in the microcosm experiment, *G. pulex* were eaten more often. The behaviour of the trout indicates that this selective predation was not due to differences in attack or capture probability. The higher number of *G. pulex* ingested seems directly related to a higher efficiency in capture, *G. roeseli* being more often rejected by the predator after their capture than *G. pulex*.

Prey handling time by fish predators varies with the morphological type of the prey, other things being equal (Hoyle & Keast 1987), such that a predator should consume prey of a morphological type that maximizes energy gain per foraging time (Pyke 1979). The two types of prey offered in our predation experiments differed mainly in the presence or absence of spines, and provided similar energetic rewards. Therefore, the observed differential predation could be based on a proximal reaction of the trout to an adverse stimulus. Experimental tests on the effects of the dorsal spines of *G. roeseli* on predation probability indicated that spineless individuals were more often ingested than individuals with spines. Spines are known to protect against predators by reducing capture success (e.g. Tollrian 1995), and, in our study, the presence of spines explains why *G. roeseli* was more frequently rejected by the trout than *G. pulex*. In addition, *G. roeseli* was chewed for longer before ingestion than *G. pulex*. Sneddon et al. (2003) found receptors in the head of rainbow trout, *Oncorhynchus mykiss*, capable of detecting noxious stimuli to which fish respond with profound behavioural and physiological changes. Thus, the differences in attack and capture efficiency between *G. roeseli* and *G. pulex* that we observed could have resulted from the fish reacting to spine-induced adverse stimuli.

Prey behavioural traits could also be implicated in the observed differential predation. A decrease in locomotor activity in the presence of a fish signal is an antipredator activity commonly reported in gammarid species (Williams & Moore 1985; Andersson et al. 1986). In our experiments, the two *Gammarus* species were both less active in the presence of a fish predator signal. However, *G. roeseli* always spent more time under a refuge than *G. pulex*.

The trait compensation hypothesis predicts that a species with an efficient morphological defence will show weak antipredator behaviour, whereas the cospecialization hypothesis predicts that a species with strong morphological defence will show marked antipredator behaviour (DeWitt et al. 1999; Mikolajewski & Johansson 2004). *Gammarus pulex* and *G. roeseli* showed a similar decrease
in their activity in reaction to the fish signal. This suggests no link (positive or negative) between the behavioural and morphological antipredator defences in *G. roeseli*. On the other hand, *G. roeseli* spent more time under refuges than *G. pulex* both in the presence and in the absence of the fish signal, which could be interpreted as a strong intrinsic antipredator behaviour. This finding supports the cospecialization hypothesis. However, the presence of refuges in the microcosm experiment did not increase the differential predation rate between *G. roeseli* and *G. pulex* compared to trials without refuges. This result suggests that longer shelter usage in *G. roeseli* does not increase the efficiency of spines against fish predation. Possibly, we had too few replicates, or the experiment was too short, for us to find evidence of a benefit of spending more time under a refuge. However, since gammarids are prey for a wide range of vertebrate and invertebrate predators (from fish and salamanders to dragonflies and crayfish, MacNeil et al. 1999a), behavioural defences could be efficient against predators other than fish, which are insensitive to spines (Wudkevich et al. 1997).

Our results may be important for understanding the success of the invasive species *G. roeseli*. While many studies have emphasized the importance of an ability to escape natural enemies in the successful establishment of exotic species (Crawley 1987; Shea & Chesson 2002), empirical evidence of lower predation rates on exotic prey as a component of invasion success is less clear (Byers 2002; Hazlett et al. 2003). Some studies have provided examples where community structures change when species differ in their sensitivity to a common enemy (e.g. Wanink & Witte 2000; Lingle 2002). In our case, direct evidence for the effect of differential predation on *G. roeseli*'s invasive success would require long-term experiments in seminatural habitats, or direct comparisons between rivers with different predator abundance, but we can tentatively propose that lower predation favours the installation of this species in communities saturated with competitors such as *G. pulex*.

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