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Influence of substrate preference and complexity on co-existence of two non-native gammarideans (Crustacea: Amphipoda)

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Abstract Substrate choice, swimming activity and risk to predation by burbot (*Lota lota*) of the well established *Gammarus roeselii* and the invader *Dikerogammarus villosus* were studied in mixed and single-species aquarium experiments. We used stones, gravel and aquatic weeds (*Elodea*, *Chara*) as substrates. We hypothesized that both species have different substrate preferences and that substrate affects the predation risk. We also assumed that presence of *D. villosus* influences substrate preference and predation risk of *G. roeselii* since the invader is known to affect the behavior of other gammarids. Adults of *D. villosus* in single species experiments and juveniles in mixed and single species experiments were evenly distributed over the different substrates but adults in mixed species experiments were more likely to prefer stone substrate. In contrast, adults and juveniles of *G. roeselii* clearly preferred aquatic weeds independent of the presence/absence of the invader. Both species preferred substrates with fissured surface over substrates with smooth surface. *Gammarus roeselii* was observed swimming more often than *D. villosus* in the open water but its swimming activity was lower when its preferred substrate was present compared with its swimming activity if non-preferred substrates were present. Predation rate of burbot on *D. villosus* was comparatively low and independent of the substrate. Burbot consumed many more *G. roeselii* than *D. villosus*, both in mixed and single species experiments. But when the preferred substrate of *G. roeselii* (weeds) was used in the experiments, predation rate of burbot on *G. roeselii* was somewhat lower than that when non-preferred substrates were present. The results of the experiments support our hypothesis that the gammarids studied here have different substrate preferences and that presence of the preferred substrate can affect predation risk. However, there is no evidence that presence of *D. villosus* affected substrate choice or predation risk in *G. roeselii*. We consider that differences in use of spatial niches permit co-existence of *G. roeselii* and *D. villosus* in the wild when substrates are diverse. The fact that *G. roeselii* than *D. villosus* is more often observed swimming in the open water may explain its higher risk of being captured by fish.

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Introduction

In the last decades amphipods mainly from the Ponto-Caspian region have invaded European waters and have replaced native amphipods in many river reaches. Understanding the causes and mechanisms behind these replacement scenarios is important for the prediction of the impact of future invasions on the native fauna. Intraguild predation is often regarded as a major force in structuring freshwater gammarid communities (e.g., MacNeil et al. 1997; Dick et al. 2002). *Dikerogammarus villosus* (Sowinsky), e.g., a species which invaded Central Europe in the 1990s, is known to prey heavily on native and invasive gammarids (Dick and Platvoet 2000; Kinzler and Maier 2003). Although recent morphological investigations show that the species is not a highly specialized carnivore (Platvoet et al. 2006; Mayer et al. 2008, 2009, its predatory impact can be serious. In several reaches of European waters, the arrival of *D. villosus* has been accompanied by the disappearance of native gammarids (Dick and Platvoet 2000; Kley and Maier 2003, 2006) most likely because of asymmetric mutual predation.

Another factor that may influence the composition of gammarid communities is predation by fish. Gammarids can sense fish and are able to react by anti predator behaviors. For example, Wudkevich et al. (1997) showed that *Gammarus lacustris* (Sars) reduced its time in the open water in the presence of chemical stimuli from pike (*Esox lucius* L.). Dahl and Greenberg (1996) reported that *Gammarus pulex* (L.) responded to the presence of sculpins (*Cottus gobio* L.) by change of habitat use. Kinzler and Maier (2006) showed that *D. villosus* was less eaten by trout than native species and attributed this difference to the greater activity of the native species. Their findings on activity were supported by van Riel et al. (2007) who studied interference competition among native and invasive gammarids and found that *G. roeselii* and *G. pulex* were more frequently present in the open water in the presence of the invader *D. villosus*. Pennuto and Keppler (2008) observed differences in the response to fish predators between *Echinogammarus ischnus* (Stebbing) and *Gammarus fasciatus* (Say). *Echinogammarus* reduced its distance moved in presence of all fish species tested while *Gammarus* did not respond to all fish species. Kaldonski et al. (2008) showed that presence of preferred substrates reduced the risk of predation by fish. All these investigations show that gammarids have developed strategies to reduce their risk to fish predation. However, anti predator strategies and their magnitude may differ from species to species and susceptibility of gammarids to fish predation may depend on the type of fish, associated conspecifics and the environment. Complete displacement scenarios have been observed in degraded river systems where shore substrate structures are simple, i.e., where substrates are often dominated by cobbles, or boulder or both. If many spatial niches are present, i.e., if structural complexity is high, predatory interactions between different gammarid species and impact of fish predation should be less severe.

D. villosus was first recorded in the north-western part of Lake Constance in 2002 (Mürle et al. 2004). In the following 2 years the species had spread over the entire shore of the Upper Lake and has now reached the Lower Lake (ANEBO Website). Before the invasion of *D. villosus* into Lake Constance, *G. roeselii* (Gervais) was the dominant species—a species of Balkan origin that had invaded Central Europe already in the 19th century; it is often named native to Central Europe or "well established" (e.g., Grabowski et al. 2007). Although presence of *D. villosus* can have negative effects on the established gammarid *G. roeselii* in Lake Constance (Mörtl et al. 2004), co-existence of the two seems likely by segregation of spatial niches. Lake Constance shores offer more complex substrates than the degraded shores of European waterways. *Dikerogammarus villosus* is often found in stone substrates (Kley and Maier 2005; van Riel et al. 2006, 2007; Hesselschwerdt et al. 2008; MacNeil et al. 2008) or substrates with *Dreissena* or *Corbicula* bivalves (Devin et al. 2003; van Overdijk et al. 2003; Kley and Maier 2005; Lods-Crozet and Reymond 2006; Kobak and Zytkowicz 2007). Substrate choice depends on the invaders size/age with smaller individuals preferring substrates with smaller particle size (Devin et al. 2003). In contrast to the upper mentioned studies, Guthruf-Seiler and Guthruf (unpublished monitoring report) found the highest densitiy of *D. villosus* in beds of *Myriophyllum spicatum* (L.) in the river Aare (Switzerland). Substrate choice of *Gammarus roeselii* is less known. In laboratory experiments with *Dreissena* shells, *G. roeselii* showed a preference for shells with biodeposited material and for shells with biodeposited material with chironomids whereas *D. villosus* showed only a preference for shells with biodeposited material with chironomids (Gergs and Rothhaupt 2008). In enclosure experiments conducted in Lake Constance, *G. roeselii* barely discriminated between stones, stones with *Dreissena*, *Chara* sp., shells of *Corbicula*, leaves and sand. In the river Ouche, Kaldonski et al. (2008) observed *G. roeselii* to be more frequent in the vegetation than in hard substrate. Thus, substrate choice of both gammarids may vary depending on ambient conditions and between populations.

In this paper, we tested substrate preference of *D. villosus* and *G. roeselii* in mixed and single species combinations in laboratory experiments, as well as the risk of both gammaridean species to predation by burbot (*Lota lota*, L.) in different substrates. Burbot, an important fish in the shore area of Lake Constance, is known to predate upon amphipods (Bailey 1972; Ryder and Pesendorfer 1992; Baumgärtner et al. 2003; Eckmann et al. 2008). Based on observations in the Danube River, where *D. villosus* was found under stones whereas *G. roeselii* preferred tree roots or grass bundles hanging into the water, we hypothesized that substrate preference differs considerably between the two species. We further hypothesized that presence of *D. villosus* can affect substrate choice of *G. roeselii* and that the risk of being eaten by burbot differs between the two gammarid species and depends on the substrate.

Methods

Maintenance

Gammarids were collected using a pond net (mesh size 250–400 μm) at the shore of Lake Constance near Langenargen (E 9°31′57″; N 47°36′07″). If necessary (low numbers of *G. roeselii*), additional samples were taken from a river near Ulm (E 10°2′; N 48°25′). Upon capture, gammarids were immediately transported to the laboratory, sorted by species and kept at natural densities (250–350 ind. l^{-1}) in plastic containers (50 × 40 × 40 cm) in a climate-controlled room at a temperature of 18°C and a 14:10 h light:dark cycle. Light was provided by Osram cool white lamps. All containers contained aged tap water and were equipped with substrates (pebbles, stones). Air stones provided a smooth water movement and sufficient oxygen. Leaves of alder and ash and chironomid larvae served as food for gammarids during their maintenance. Mortality of gammarids was low under these conditions.

Substrate preference

Experiments were conducted in aquaria (100 × 40 × 40 cm) provided with three equally sized areas of different substrates. One part contained gravel (grain size 2–3 cm; height 4 cm), one 10–12 large stones (ca. 15 × 15 × 3 cm) and one about 20 stems of aquatic weeds (*Elodea canadensis*, Michx.). Water height above the hard substrates was 25–30 cm. Two mixed-species series were run with *Chara* sp.; since the results were the same as for *Elodea* they were pooled. Substrates were cleaned (stones scraped and macrophytes washed) before they were used in an experiment. Thus attached benthos and periphyton communities were removed to exclude possible effects of food on substrate choice. Temperature and light conditions were kept the same as for the maintenance of the animals.

In the first mixed species trials, 40 adult individuals each of *D. villosus* and *G. roeselii* (size > 15 mm) were introduced randomly into one aquarium and their distribution over the three different substrates was monitored. A set of experiments were run for each 24, 48 and 72 h. After the experimental time, substrates (different parts of the aquarium) were separated from each other by the introduction of glass plates; then substrates were removed and gammarids present in each substrate were counted. Pre-experiments indicated that gammarids immediately occupied substrates and that the number of substrate changes decreased to about 30% after 1 h. We pooled the data for the three experimental sets since results did not significantly differ. In the single species trials with adults and the mixed and single species trials with juveniles, size of aquaria and substrates were nearly the same as in the mixed species ones with adults, but experiments were run for 24 h only and substrates were searched for gammarids after this 24 h exposure time only. Size of juveniles was 8–10 mm. Nineteen mixed species trials and 20 single species trials (10 with *G. roeselii* and 10 with *D. villosus*) were run with adults; and seven mixed species trials and 19 single species trials (ten with *G. roeselii* and nine with *D. villosus*) were run with juveniles.

Activity of prey organisms can be an important determinant of the predation risk. Active prey is more conspicuous than an inactive one and therefore easily captured by a predator. Activity of adult gammarids was investigated in mixed species trials with adults. Before substrates were searched for gammarids, number of gammarids swimming in the open water (defined as active ones) was noted during a 5 min observation period. Activity was monitored in 16 trials such that we had 48 observations for each gammarid (16 for each substrate).

In an other series of experiments we tested whether the surface of substrates affected substrate choice of gammarids. Substrates of similar size (10 × 20 × 3 cm) but with different surface (one stone with fissures, one with smooth surface and a piece of wood with fissures) were used in the experiments. In one set of experiments, two stones with different surfaces were placed into an aquarium (distance between the stones was ca.5 cm) and water was added to a height of 10 cm. Then 30–40 individuals of either *D. villosus* or *G. roeselii* were introduced and allowed to spread out over the substrates. After 2 h the number of gammarids under the two stones as well as those in the open water was noted. Again, the fraction in the open water was defined as active ones. In another set of experiments, the piece of wood was tested versus the stone with smooth surface. Experimental conditions and procedure were the same as in the stone versus stone experiments. Thirty experiments (10 with *G. roeselii* and 20 with *D. villosus*) were run with fissured stones versus smooth stones and 25 (10 with *G. roeselii* and 15 with *D. villosus*) with smooth stones versus fissured wood.

Experiments with fish predators

The experiments with fish were conducted in 100 × 35 × 35 cm (ca.120 l) aquaria. Temperature and light were as in the substrate preference experiments. The aquaria were stocked with either ten stones (size 10 × 10 × 3 cm), aquatic weeds (20 stems of *Elodea*) or a mixture of both. Substrates/weeds were thoroughly cleaned before they were used in an experiment to remove adhesive animals. In the mixed prey experiments, 30 adult individuals of which *D. villosus* and *G. roeselii* were taken from the stock cultures and introduced into each aquarium. The final density of gammarids of 180 ind. m^{-2} was within the range of densities observed in the field. We selected the largest individuals of G. *roeselii* and smaller adults of *D. villosus* (size range 10–15 mm) so that test animals were in about same size. After an acclimatization time of 15 min, which proved to be sufficient for gammarids to occupy substrates (e.g., MacNeil et al. 2000; this study), two fish (burbot; length 9–11 cm), which had been starved for 48 h, were introduced into the aquaria and allowed to feed on gammarids for 1 h. Burbot was obtained from the Limnological Institute at Lake Constance. Earlier experiments showed that between 10 and 50% of gammarids had been consumed after that time; longer exposure times proved to be unfavorable (Kinzler and Maier 2006). At the end of an experiment the fish was removed and the aquarium, in particular the substrate, was carefully searched for remaining gammarids. In the single-prey experiments 60 adult individuals of the same species (either *D. villosus* or *G. roeselii*) were introduced into the aquaria. Experimental conditions were the same as in the mixed-prey experiments. Twenty-nine mixed species and 53 single species, 24 with *G. roeselii* and 29 with *D. villosus*, were run.

Although we tried to use new specimens in each experiment, some gammarids were used more than one time in a type of experiment since we had not enough specimens to use each gammarid only once. However, in different experimental types we used different sets of gammarids, but only the ones that appeared healthy.

Statistics

Non-parametric paired tests (Friedman followed by Wilcoxon) were used to test for differences in substrate preference and for differences in activity between species. In the experiments with fish we used a paired test (Wilcoxon) to test for differences in predation risk between gammarids at a certain substrate. Then a Kruskal–Wallis ANOVA (followed by *U*-test, Mann–Whitney) was employed to test for differences in predation risk at different substrates. We selected non-parametric tests because, in some experiments (e.g., substrate preference of mixed species juveniles), we had only a few replicates and not all data sets were normally distributed.

Results

Substrate preference, activity

In the mixed species trials, adult *G. roeselii* preferred the plant substrate while adult *D. villosus* were most frequent in stone substrate (Fig. 1). The Friedman test showed significant differences in substrate choice both for adult *G. roeselii* and adult *D. villosus* (*G. roeselii*: $\chi^2 = 21.2$, $P < 0.0001$, $n = 19$; *D. villosus*: $\chi^2 = 25{,}6$, $P < 0.0001$, $n = 19$). The Wilcoxon test revealed significant differences in use of plant versus hard substrate in adult *G. roeselii* (Table 1). Adult *D. villosus* showed a preference for stones versus gravel and plants; plant and gravel substrates did not significantly differ (Table 1). In the single species trials again a much higher proportion of adult *G. roeselii* was found in plant compared with hard substrate whereas adult *D. villosus* was evenly distributed over all three substrates (Fig. 1). The Friedman test showed significant differences in the substrate choice for adult *G. roeselii* ($\chi^2 = 15.45$, $P < 0.0005$, $n = 10$) but not for adult *D. villosus* ($\chi^2 = 0.95$, $P = 0.62$ ns, $n = 10$). The Wilcoxon test revealed significant differences in use of plants versus hard substrate by adult *G. roeselii* but no difference in use between hard substrates (Table 1).

In mixed-species experiments (Fig. 2), juveniles of *G. roeselii* preferred the plants (Friedman: $\chi^2 = 10.5$, $P < 0.006$, $n = 7$; Table 1) while those of *D. villosus* were evenly distributed over the substrates (Friedman: $\chi^2 = 5.2$, $P = 0.073$ ns, $n = 7$;). In the single-species experiments (Fig. 2), juveniles of *G. roeselii* also preferred the plants over stony substrates (Friedman: $\chi^2 = 20.0$, $P < 0.0001$, $n = 10$; Table 1). Again, juveniles of *D. villosus* were evenly distributed over the substrates (Friedman: $\chi^2 = 2.39$, $P = 0.30$ ns, $n = 9$).

Activity of adult *G. roeselii* in the mixed species trials depended on the substrate (Friedman: $\chi^2 = 7.1$, $P < 0.03$, $n = 16$); it was lower in the plant substrate compared with stones (Fig. 3; Table 2). Activity of adult *D. villosus* did not depend on the substrate

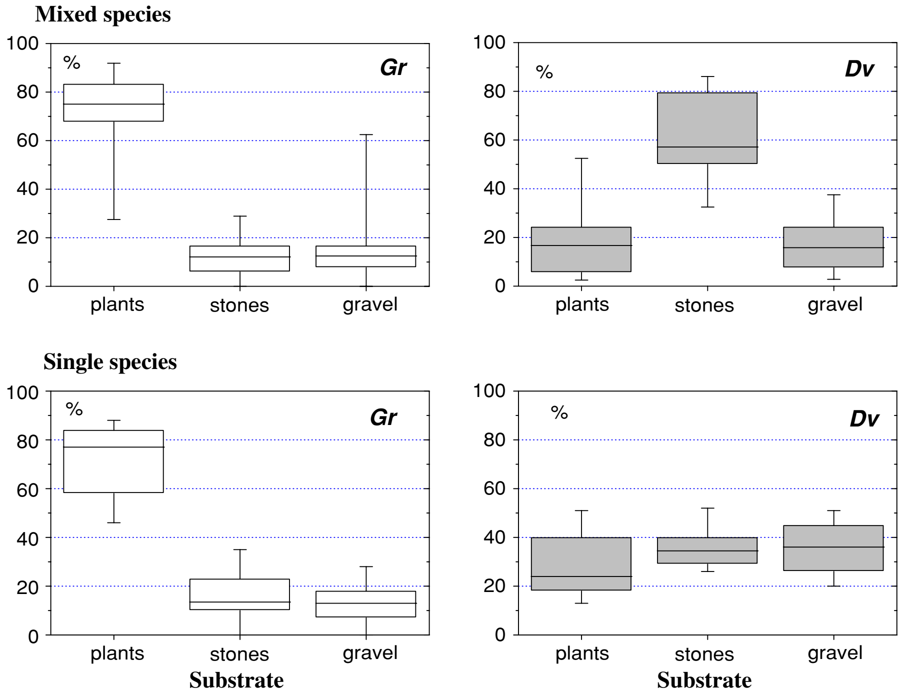

Fig. 1 Substrate choice patterns of adults of the established gammarid *Gammarus roeselii* and the invasive *Dikerogammarus villosus* in mixed and single species aquarium experiments with a combination of plants (*Elodea canadensis*, *Chara* sp.), large stones and gravel as substrates

Table 1 Results of the Wilcoxon-test concerning differences in substrate choice between adult and juvenile gammarid species in mixed and single species trials

Age	Experiment	Species	Substrates	*Z*	*P*
Adult	Mixed	*G. roeselii*	Plants vs. stones	−3.82	0.0002
			Plant vs. gravel	−3.60	0.0004
			Stones vs. gravel	−0.91	0.37 (NS)
		D. villosus	Plants vs. stones	−3.60	0.0003
			Plants vs. gravel	−0.09	0.93 (NS)
			Stones vs. gravel	−3.82	0.0002
	Single	*G. roeselii*	Plant vs. stones	−2.80	0.005
			Plants vs. gravel	−2.80	0.005
			Stones vs. gravel	−0.95	0.34(NS)
Juvenile	Mixed	*G. roeselii*	Plants vs. stones	−2.37	0.018
			Plants vs. gravel	−2.80	0.018
			Stones vs. gravel	−0.52	0.60 (NS)
	Single	*G. roeselii*	Plants vs. stones	−2.80	0.005
			Plants vs. gravel	−2.80	0.005
			Stones vs. gravel	−2.80	0.005

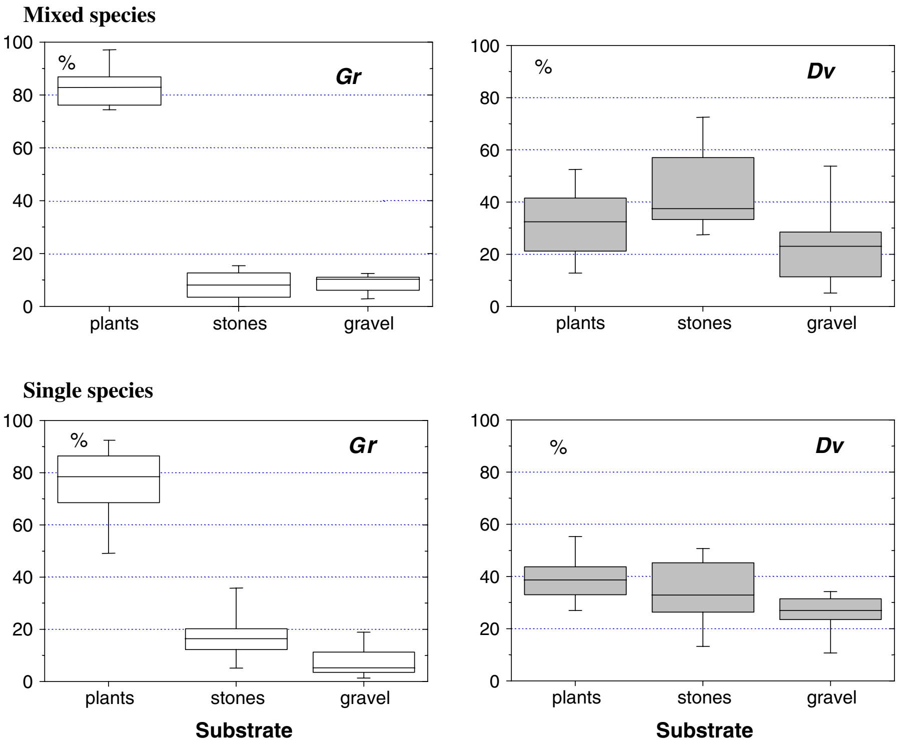

Fig. 2 Substrate choice of juveniles of the established gammarid *Gammarus roeselii* and the invasive *Dikerogammarus villosus* in mixed and single species aquarium experiments with a combination of plants (*Elodea canadensis*, *Chara* sp.), large stones and gravel as substrates

(Friedman: $\chi^2 = 0.4$, $P = 0.82$ ns, $n = 16$) and overall activity of *D. villosus* was significantly lower than overall activity of *G. roeselii* (Wilcoxon: $Z = -5.4$, $P < 0.0001$, $n = 48$).

In experiments with different substrate surfaces, both *Gammarus* species preferred the fissured substrate over that with smooth surface but activity in adults of *G. roeselii* and *D. villosus* differed (Fig. 4) The Friedman test showed significant differences between the three measured parameters in both species (smooth stones versus fissured stones versus outside: *G. roeselii*: $\chi^2 = 15.3$, $P < 0.0005$, $n = 10$; *D. villosus*: $\chi^2 = 29.8$, $P < 0.0001$, $n = 20$; smooth stones versus fissured wood versus outside:

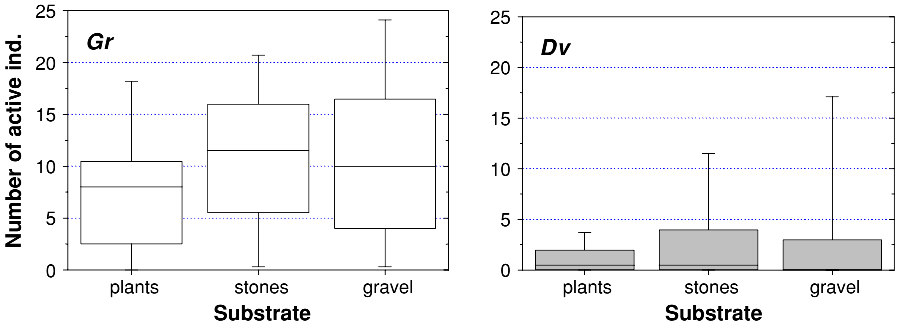

Fig. 3 Activity of adults of the established gammarid *Gammarus roeselii* and the invasive *Dikerogammarus villosus* in mixed species aquarium experiments with a combination of plants, stones and gravel

Table 2 Results of the Wilcoxon-test concerning differences in activity between gammarid species in mixed species trials

Experiment	Species	Substrates	*Z*	*P*
Mixed	*G. roeselii*	Plants vs. stones	−2.90	0.004
		Plants vs. gravel	−1.70	0.088 (NS)
		Stones vs. gravel	−0.85	0.39 (NS)

G. roeselii: $\chi^2 = 12.9$, $P < 0.002$, $n = 10$; D. villosus: $\chi^2 = 19.7$, $P < 0.0001$, $n = 15$). In *G. roeselii*, a higher or almost the same proportion of individuals was outside the substrates as in the fissured substrates (Fig. 4; Table 3) while in *D. villosus* much fewer individuals were observed outside the substrates than in the fissured substrate (Table 3).

Fish predation

In the mixed-prey experiments, burbot ate many more *G. roeselii* than *D. villosus* (Wilcoxon: $Z = -4.7$, $P < 0.0001$, $n = 29$). On average 12–19 specimens of *G. roeselii* were consumed by the fish while only 4–6 specimens of *D. villosus* were consumed during the one hour exposure period (Fig. 5). Consumption rate on *D. villosus* was independent of the substrate (Kruskal–Wallis ANOVA: $H = 3.13$, $P = 0.21$ ns, $n = 29$) whereas that on *G. roeselii* depended on the substrate (Kruskal–Wallis ANOVA: $H = 14.6$, $P < 0.0007$, $n = 29$). Burbot consumed least individuals of *G. roeselii* when plants were used as substrate;

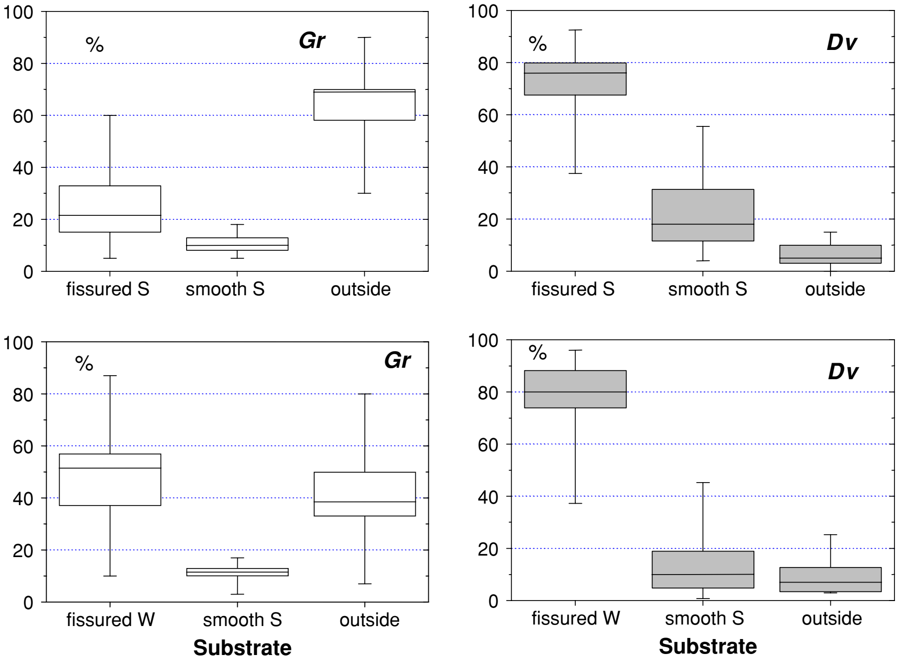

Fig. 4 Substrate surface choice and activity of adults of the established gammarid *Gammarus roeselii* (left column) and the invasive *Dikerogammarus villosus* (right column) in single species aquarium experiments with a combination of fissured stone and stone with smooth surface or a combination of fissured wood and stone with smooth surface

consumption rate of the fish did not differ between the experiments with stones and stones/plants (Table 4).

In the single prey experiments similar results were observed as in mixed prey experiments (Fig. 5).

Table 3 Results of the Wilcoxon-test concerning differences between gammarid species in experiments with different substrate surface

Species	Substrate	*Z*	*P*
G. roeselii	Outside vs. fissured stone	−2.5	0.013
	Outside vs. smooth stone	−2.8	0.005
	Fissured stone vs. smooth stone	−2.5	0.011
D. villosus	Outside vs. fissured stone	−3.9	0.0001
	Outside vs. smooth stone	−3.7	0.0004
	Fissured stone vs. smooth stone	−3.6	0.003
G. roeselii	Outside vs. fissured wood	−1.17	0.24 (NS)
	Outside vs. smooth stone	−2.67	0.008
	Fissured wood vs. smooth stone	−2.67	0.008
D. villosus	Outside vs. fissured wood	−3.41	0.007
	Outside vs. smooth stone	−0.76	0.44 (NS)
	Fissured wood vs. smooth stone	−3.30	0.001

Burbot ate many more *G. roeselii* than *D. villosus* (Wilcoxon: $Z = -4.3$, $P < 0.0001$, $n = 24$). Consumption rate on *D. villosus* was independent of the substrate (Kruskal–Wallis ANOVA: $H = 4.7$, $P = 0.096$ ns, $n = 29$). Consumption rate on *G. roeselii* depended on the substrate (Kruskal–Wallis ANOVA: $H = 15.70$; $P < 0.0004$, $n = 24$). Burbot consumed fewer individuals when plants were used as substrate than when stones were used and the same number of individuals when stones/plants were used compared with plants (Table 4).

The mean percentage of *G. roeselii* over all substrates relative to the initial density consumed by burbot in mixed species trials was 40.1 ± SD 18.3 and in the single species trials 49.3 ± SD 10.1. The mean percentage of *D. villosus* consumed by burbot in mixed and single species trials was 15.2 ± SD 7.7 and 16.1 ± SD 5.4, respectively. There was only a difference in predation rate of burbot on *G. roeselii* between mixed and single species trials when the stones/plant substrate was compared and there was no difference in percentage of *D. villosus* consumed by burbot between mixed and single species trials at all substrates (Table 5).

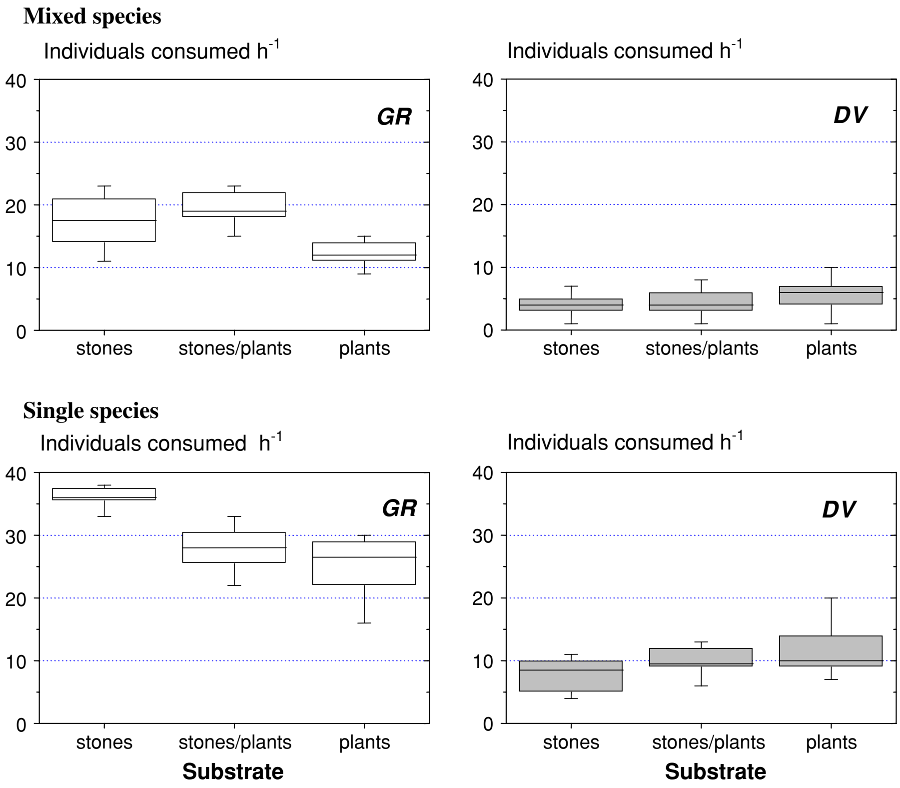

Fig. 5 Consumption rate of burbot in mixed and single species experiments on *Gammarus roeselii* and *Dikerogammarus villosus* at different substrates

Table 4 Results of the *U*-test (Mann–Whitney) concerning differences between gammarids in risk of being eaten by burbot at different substrates in mixed and single species trials

Experiment	Species	Substrates	*Z*	*P*
Mixed	*G. roeselii*	Stones vs. stones/plants	−1.11	0.27 (NS)
		Stones vs. plants	−2.81	0.005
		Stones/plants vs. plants	−3.54	0.0005
Single	*G. roeselii*	Stones vs. stones/plants	−3.32	0.0009
		Stones vs. plants	−3.37	0.008
		Stones/plants vs. plants	−1.05	0.29 (NS)

Table 5 Results of the *U*-test (Mann–Whitney) concerning differences in percentage burbot consumption rate between mixed and single species trials

Species	Substrate	*Z*	*P*
G. roeselii	Stones	0.40	0.69 (NS)
	Stones/plants	−3.47	0.001
	Plants	0.54	0.59 (NS)
D. villosus	Stones	−0.19	0.85 (NS)
	Stones/plants	−1.03	0.31 (NS)
	Plants	−0.17	0.87 (NS)

Discussion

Substrate choice and activity patterns of gammarids can be influenced by a number of factors such as age of the gammarid and time of day (Elliott 2005), structure of substrates and physical and chemical parameters in the environment (Wijnhoven et al. 2003; Palmer and Ricciardi 2004; Franken et al. 2006; McGrath et al. 2007), availability of food (van Dolah 1978; Dahl and Greenberg 1996; De Lange et al. 2005; McGrath et al. 2007), presence/absence of parasites in gammarids (Mazzi and Bakker 2003; Kaldonski et al. 2007), presence/absence/density of predators or predator odors and age of predators (Mathis and Hoback 1997; Dahl and Greenberg 1996; Wudkevich et al. 1997; Max Neil et al. 1999; Sudo and Azeta 2001; Baumgärtner et al. 2002, 2003; Pennuto and Keppler 2008) and presence/absence of conspecifics (van Riel et al. 2007; Piscart et al. 2007).

We conducted the substrate-choice experiments in the absence of predators, parasites and food (except macrophytes) and both test species faced the same physical and chemical conditions. Under these conditions *D. villosus* and *G. roeselii* show different substrate preferences and activities and substrate preference and activity differed between species independent of whether the two species were present together or singly. Our results further suggest that substrate preference is more pronounced in *G. roeselii* than in *D. villosus*. Adult *G. roeselii* always preferred the plant substrate whereas adult *D. villosus* in single species trials and juveniles in single and mixed species trials were evenly distributed over all substrates tested. That *D. villosus* did not necessarily prefer stones or gravel was unexpected because this species generally occurs in hard substrates.

One factor—in addition to those mentioned above—associated with morphology involved in food collection may also affect substrate choice and activity of gammarids. In some earlier works, Ponyi (1956, 1961) showed that differences in feeding modes exist between freshwater gammarids. He distinguished between “filter-feeding” gammarids (*D. villosus*) and “chewing” ones (*G. roeselii*). Platvoet et al. (2006) showed that *D. villosus* is able to collect suspended algae with the help of long setae on the antennae and gnathopods. Recently, Mayer et al. (2009) who compared mouthpart morphology of *G. roeselii* and *D. villosus* found the appendages involved in food acquisition to differ, in particular in the molar surfaces of the mandibles and the endites of the maxillulae, which are more suited for grinding plant material prior to ingestion and for scraping off adherent food in *G. roeselii* than in *D. villosus*. Furthermore, setae of antennae and gnathopods are shorter and more sparse in *G. roeselii* compared with *D. villosus*, implying that filter-feeding plays a minor role in food acquisition in *G. roeselii*. If filter-feeding is an important mode of food collection in *D. villosus*, this could explain its low activity since low activity or even sessile mode of life is common in filter-feeding animals. The ability of *G. roeselii* to feed on plant material and adherent food growing on it and grind them may contribute to its preference for macrophytes. That *G. roeselii* is able to feed on fresh leaf litter has already been reported by Pöckl (1995).

That the two gammarids studied preferred fissured substrate over substrate with smooth surface is expected. Fissured substrate offers more refuge, more possibilities for attachment and may also host more prey organisms than substrate with smooth surface. The fact that *G. roeselii* was present more frequently outside the substrate than *D. villosus* may partly be an effect of the substrates used in the "surface" experiments. Since we used no plant substrate there, this could have triggered search behavior in *G. roeselii*. This suggestion is supported by our third experiment where *G. roeselii* was least active in the presence of plants. On the other hand, this study as well as those of Krisp (2004) and Kinzler and Maier (2006) suggest that *G. roeselii* is generally more active than *D. villosus*.

Invasion of a new gammarid species can influence substrate choice of native and/or established gammarids. Van Riel et al. (2007) showed that in the presence of *D. villosus*, *G. pulex* shifted toward smaller stones and increased its activity while there was no change in the behavior of *D. villosus*. Also Krisp (2004) observed that *G. pulex* who generally preferred (similar to *D. villosus*) stone substrate in his experiments shifted to plant substrate in the presence of *D. villosus*. That we did not observe shifts in substrate choice in *G. roeselii* in the presence of *D. villosus* may be because differences in substrate preferences will prevent or reduce competition for substrates. We cannot exclude, however, that *D. villosus* may have accelerated the occupation of the preferred substrate in *G. roeselii*.

Fish predators can shape the structure of freshwater benthic communities (Newman and Waters 1984). Fishes generally prefer large and conspicuous prey (Dahl 1998; Mac Neil et al. 1999, 2000). Conspicuous color and large body mass increase the visibility of prey organisms. High activity results in frequent encounters between predator and prey. Gammarids seek to hide in refuges to reduce encounters with fish (Williams and Moore 1982; Hoyle and Holomuzki 1990; Wooster 1998). *Dikerogammarus villosus* is a large amphipod species and therefore an attractive prey for fish. However, hiding under stones and reduced activity may be an effective anti-predator strategy. The fact that *G. roeselii* is inclined to swim in the open water irrespective of if *D. villosus* is present or not has undoubtedly contributed to its comparatively high mortality observed in our experiments with burbot. We have no evidence that presence of the invader *D. villosus* increased the predation risk of the established *G. roeselii*. The percentage of *G. roeselii* consumed by burbot in mixed and single species experiments was quite similar.

Bollache et al. (2006) studied predation risk of *Gammarus pulex* and *G. roeselii* to fish predators and found that *G. roeselii* was more often rejected by predators than *G. pulex*. They attributed this to dorsal spines of *G. roeselii,* which are absent in *G. pulex*, thus interpreting the spines as morphological defence structures. Although spines can reduce predation risk, it is unlikely that they played an important role in our experiments. This because despite the presence of spines *G. roeselii* was more frequently eaten by burbot than *D. villosus*. Thus, differences in activity patterns (times spend in shelter) appears to be more important determinants of predation risks. The fact that *G. roeselii* is less frequently eaten by burbot in the presence of plant substrate compared with stone substrate may originate from its somewhat lower activity in its preferred substrate. Also Kaldonski et al. (2008) have shown that *G. roeselii* was less prone to predation by bullheads (*Cottus gobio*) in the presence of vegetation.

Pennuto and Keppler (2008) suggested that superiority in predator avoidance behavior in invasive gammarids when faced with fish may lead to increased predation on native gammarids. We also assume that effective anti-predator responses to a wide array of predators could be characteristic for invasive gammarids and could contribute to their success in their novel habitats.

The immigration of *D. villosus* into Lake Constance was accompanied by a decrease in numbers of *G. roeselii* (Mörtl et al. 2004; Eckmann et al. 2008). Meanwhile, 6 years after its immigration, *D. villosus* has become an important component of benthic food webs. But despite its predatory habit, *D. villosus* could not extirpate *G. roeselii* in Lake Constance until now. Most likely, differences in spatial niches and behaviors may permit co-existence of the two species. In places where sediments are diverse, i.e., composed of stones and macrophytes, both species may use different substrates and thus avoid competition and mutual predation. Co-existence of several gammarid species including *G. roeselii* and *D. villosus* has already been observed in a small brook near the Rhine River where sediments are composed of gravel, stones and different macrophytes (Kley and Maier 2005). In spite of its low activity and its hiding under stones, *D. villosus* has become an important food source for some littoral fish in Lake Constance (Eckmann et al. 2008). Possibly fish have adapted to these behaviors and have learned to capture this attractive prey.

Finally, we stress that we tested substrate choice using a limited number of substrates and predation risk with only one fish species of a certain age. Conditions in nature are much more diverse and complex. Grain size of hard substrates, interstitial pore space sizes and structure of plants, e.g., distance between leaves, may be important determinants of substrate choice. *Elodea* and *Chara* may be more suitable substrates for *G. roeselii* than other plants. Eckmann et al. (2008) analyzed stomach contents from 15 fish species sampled in Lake Constance and found that only four fish species consumed amphipods: burbot, European eel (*Anguilla anguilla* L., perch (*Perca fluviatilis* L.) and ruffe (*Gymnocephalus cernuus* L.). Thus, it is unlikely that the invasion of *D. villosus* into Lake Constance has a marked influence on the fish fauna. However, as *D. villosus* can be a predator, preying on various benthic species, it can affect fishes indirectly by reducing the available food. That *D. villosus* can impact on macroinvertebrate communities in particular in its preferred stone substrate has already been shown (Dick et al. 2002; van Riel et al. 2006).

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