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Preliminary study of the scars borne by Gammaridae (Amphipoda, Crustacea)

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ABSTRACT. Many gammarid shrimps from the rivers Meuse and Viroin bear scars. A recent invader in the Meuse, *Dikerogammarus villosus*, is claimed to exhibit a strong predatory behaviour against other Gammaridae. Therefore we tested the hypothesis that scarred Gammaridae should be more numerous in the Meuse where *D. villosus* is dominant than in a tributary, the Viroin, where it is absent. On the contrary there were significantly more scarred individuals in the Viroin (51%) than in the Meuse (32%). The most exposed appendages were the most frequently injured, and multiple scars on a single individual were not distributed randomly. These results are discussed.

KEY WORDS : invasive species, *Dikerogammarus*, *Echinogammarus*, *Gammarus*.

INTRODUCTION

Dikerogammarus villosus (Sowinsky 1874) (Amphipoda, Gammaridae) originated from the Ponto-Caspian region, which has been claimed to be an "invasion donor hot spot" (Ricciardi & MacIsaac, 2000; Dick & Platvoet, 2001). The penetration of *D. villosus* into Western Europe was facilitated by the reopening of the Main-Danube Canal in 1992 (Tittizer, 1996 in Dick & Platvoet, 2001, Bij de Vaate et al., 2002). The mechanism of this invasion was most probably passive transport in ballast water (Tittizer et al., 2000). *D. villosus* was first recorded in the Belgian Meuse in 1998 (Vanden Bossche, 2001) and has now become dominant, but it has not yet penetrated into the Meuse's tributaries.

Cannibalistic and predatory behaviours are well known and described in Gammaridae (Dick, 1995, Dick et al., 1999) and in the case of *D. villosus* intraguild predation seems to be even more frequent. Whereas other Gammaridae are restricted in their attacks to the few minutes following the moult of their victims (when their exoskeleton is still soft), very hard mouthparts allow *D. villosus* to prey upon intermoult individuals (with hard exoskeleton) (Dick & Platvoet, 2000).

When injured, amphipods, as do other invertebrates, trigger activation of the prophenoloxidase cascade (Johansson & Soderhall, 1996). This nonspecific immunoreaction results in black scars, which are easy to locate. To our knowledge these scars have never been studied as witnesses of agonistic relationships in amphipod communities. Knowing the predatory reputation of *D. villosus*, we tested the hypothesis that scarred Gammaridae should be less numerous in the River Viroin (*D. villosus* absent, *Echinogammarus berilloni* (Catta, 1878) dominant) than in the River Meuse (*E. berilloni* present, *D. villosus* dominant and the most abundant interstitial macroinvertebrate in this ecosystem).

MATERIAL AND METHODS

Amphipods were sampled in the river Meuse at Montigny-sur-Meuse, France (UTM co-ordinates U31 0623356 5545159, altitude 110 m), and in one of its tributaries, the river Viroin at Mazée, Belgium (UTM co-ordinates U31 0620956 5550380, altitude 115 m), from October 2001 to May 2002.

Interstitial macroinvertebrates were collected with artificial substrates. These consist of calcareous gravel (5.2 ± 3.4 cm^3 : av ± st dev) packed in a nylon net. Macroinvertebrates can enter and exit freely through the 1 cm-mesh. The volume of an artificial substratum is 1 litre and contains an interstitial space of about 0.5 litre. The substrates remained in the water and were replaced by new ones every two weeks (each month during winter). Samples were preserved in alcohol with picric acid, which stops the immunoreaction. In this way we could distinguish the natural scars (black) from the injuries caused by our sampling manipulations (not coloured). About 450 individuals from each river were examined : they were identified with the following keys : Carausu et al. (1955), Schellenberg (1942), Pinkster (1973), Karaman & Pinkster (1977) and Pinkster (1993). Every scar was counted, and its location on the body and the appendages noted.

The gammarid species encountered in the Meuse are (in decreasing order of numbers) : *Dikerogammarus villosus* (Sowinsky, 1874), *Echinogammarus berilloni* (Catta, 1878) and *Gammarus pulex* (L., 1758). In the Viroin, we found *E. berilloni*, *G. pulex* and a few *Gammarus fossarum* (Koch, 1835).

During the sampling period (October 2001-May 2002) all the adult and pre-adult individuals (i.e. 467 from the Viroin and 442 from the Meuse) were scrutinised for scars. Two kinds of scars were considered : (a) those borne on the gills and (b) those borne elsewhere, mainly on the appendages but also on the body. A possible seasonal effect was tested by distributing our samples into three periods : autumn 2001 (from October 27 until November 25), winter (from December 21 until March 15) and spring 2002 (from March 30 until May 24).

Unless otherwise stated the statistical analyses were performed with χ^2 contingency tests for independent samples; in case of 2x2 contingency tables, the correction for continuity was applied (SIEGEL & CASTELLAN, 1988).

RESULTS

The scars borne on the gills appear as black borders; sometimes a gill is pierced and the hole is black edged. These scars obviously cannot have been made by a predator but probably result from infectious micro-organisms. The results by river and species are figured in table 1. The following statistical comparisons were performed : (1) between rivers for the scars borne on the gills of all Gammaridae, (2) the same for *E. berilloni* alone, (3) the same for *Gammarus* spp., (4) between *E. berilloni* and *Gammarus* spp. in the Viroin, (5) between *D. villosus* and. *E. berilloni* in the Meuse and (6) between *D. villosus* and. *E. berilloni* + *G. pulex* in the Meuse. None of them showed any significant differences (χ^2 tests, $p>0.05$ in all cases). The numbers were, however, too small for testing a seasonal effect on the gill scars.

TABLE 1

Distribution between rivers and species of Gammaridae with scarred gills

River	Species	# individuals scrutinised	# individual scarred	% scarred
Viroin	*E. berilloni*	437	29	6.6
Viroin	*G. pulex**	30	2	6.7
Meuse	*E. berilloni*	92	5	5.4
Meuse	*D. villosus*	317	12	3.8
Meuse	*G. pulex*	27	1	3.7

* Including some *G. fossarum* (only in the Viroin)

The scars borne on the appendages and sometimes on the body that might be attributed to predator bites were not distributed randomly. As expected, the most exposed appendages were the most frequently injured. More than 47% of the scars were located on the antennae and almost 37% on the other appendages. Scars on the body (head, tergites) were less frequent. The percentages of injured parts are detailed on Figure 1 and the results by river and species are given in Table 2.

TABLE 2

Distribution of Gammaridae with scarred appendages between rivers and species

River	Species	# individuals scrutinised	# individual scarred	% scarred
Viroin	*E. berilloni*	437	205	46.9
Viroin	*G. pulex**	30	22	73.3
Meuse	*E. berilloni*	92	32	34.8
Meuse	*D. villosus*	317	91	28.7
Meuse	*G. pulex*	27	11	40.7

* Including some *G. fossarum* (only in the Viroin)

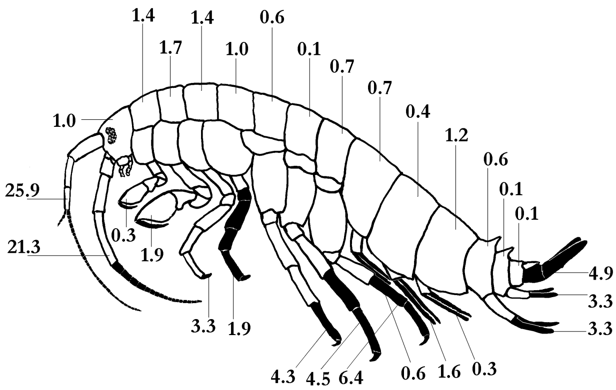

Fig. 1. – Distribution of scars on the body and the appendages : each number is the percentage occurrence of scars on the body and the appendages. The black parts are those that are most often lacking. Percentages on the coxae have been omitted for clarity.

There were very significantly more scarred individuals in the Viroin than in the Meuse (χ^2 tests, $p<0.001$ for all Gammaridae, $p<0.05$ for Gammarus spp. and $p<0.05$ for *E. berilloni* alone) : 50.7% of all the gammarid shrimps bore scars in the Viroin against 32.1% in the Meuse. There was no significant difference between species in the Meuse (χ^2 test, $p= 0.27$) but *Gammarus* spp. bore significantly more scars than *E. berilloni* in the Viroin (χ^2 test, $p<0.01$).

The results by river and season are figured in Table 3. The total catches followed a seasonal pattern (χ^2 test of homogeneity, $p<0.001$ in each river) and this pattern differed significantly between rivers (χ^2 test, $p<0.001$) with a drop in the winter period in the Meuse against a minimum in the spring in the Viroin.

TABLE 3

Distribution of Gammaridae with scarred appendages between rivers and seasons

River	Season	# individuals scrutinised	# individuals scarred	% scarred
Viroin	Autumn	166	51	30.7
Viroin	Winter	278	162	58.3
Viroin	Spring	23	14	60.9
Meuse	Autumn	204	68	33.3
Meuse	Winter	32	14	43.8
Meuse	Spring	200	52	26.0

The percentages of scarred individuals also followed a seasonal pattern in the Viroin (χ^2 test, $p=0.002$, with a lower percentage in the autumn) but the difference between seasons was not significant in the Meuse (χ^2 test, $p=0.23$)

The absolute frequency distributions of the number of scars borne by each individual are shown in Figure 2 and compared with random (Poisson's) distributions. In both rivers, the observed and theoretical frequency distributions differ very significantly (χ^2 test of goodness of fit, $p<0.001$). In both cases individuals without any scars were more numerous than expected in a random distribution, suggesting efficient hiding or escape from predators

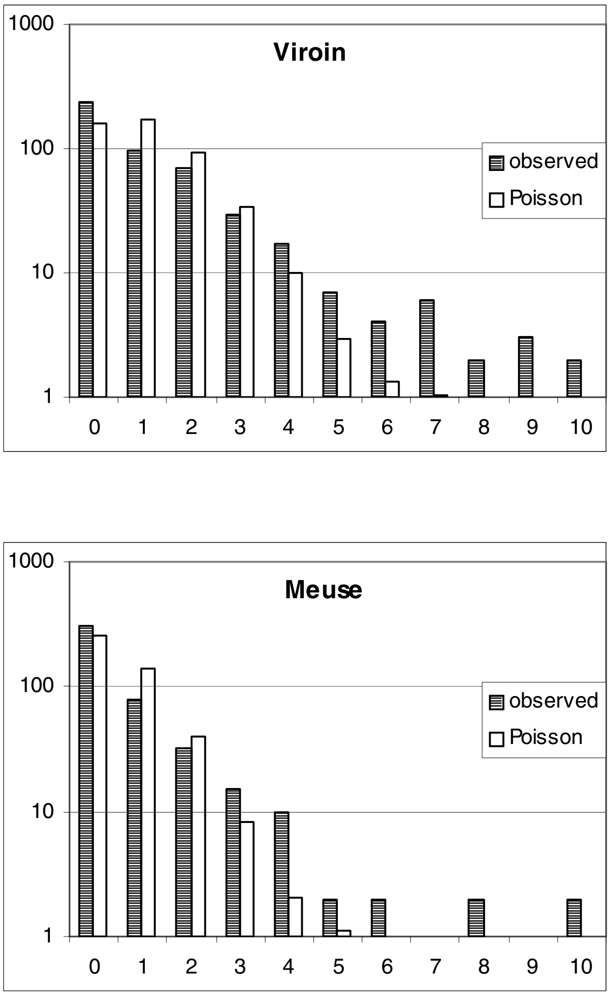

Fig. 2. – Observed and theoretical (Poisson) frequency distributions of scars among the Gammaridae (all species together) of the rivers Viroin and Meuse (semilog graphs of (x+1) transformed data).

following detection. Individuals bearing one or two scars were less numerous than expected and those with four or more scars were more numerous than expected.

Multiple scars on a single individual are not randomly spread on the body and the appendages : of 84 individuals bearing at least three scars, 36 individuals bore two to four scars on the antennae, 11 individuals bore two to five scars on successive tergites, seven individuals bore two to three scars on successive uropods of the same side, and six individuals bore two to five scars on successive pereiopods of the same side. Therefore 71% of those 84 individuals bore clumped scars.

DISCUSSION AND CONCLUSION

This study is part of a larger study on the population dynamics of Gammaridae. The scars that they bear, to our knowledge, have never been taken into consideration in the literature. These scars, however, could provide valuable clues for understanding some kinds of relationships occurring in river communities, as aggressive behaviours and failed attempts of predation.

The communities of invertebrate predators in the rivers Viroin and Meuse are not very different : they include leeches (Erpobdellidae and Glossiphoniidae), flatworms (Dugesiidae) and a variety of insect larvae : small numbers of damselflies (Calopterygidae and Lestidae) and beetles (Gyrinidae), and larger numbers of net building caddisflies (Hydropsychidae, Polycentropodidae and Rhyacophilidae) and snipeflies (Rhagionidae). There is, however, one recent invader, *Dikerogammarus villosus*, which is totally lacking in the Viroin and very abundant in the Meuse. From its known aggressive and predatory behaviour against other gammarids (Dick et al., 1999, Dick & Platvoet, 2000), more scarred shrimps might be expected in the Meuse but the opposite was observed. Of course it could be argued that *D. villosus* is such an efficient predator that it seldom lets its prey escape. On the other hand the other Gammaridae, which also exhibit agonistic behaviours but do not kill their prey unless it has moulted quite recently (Dick, 1995, Dick et al., 1999), could be the origin of the numerous scars observed in the Viroin. Obviously controlled experiments (Dick et al., 1999) should be carried out in order to appreciate to which extent intraspecific and interspecific aggressiveness can generate these scars.

Even if any of the invertebrate predators could be responsible for the isolated scars borne by the Gammaridae, none of them can account for the frequent clumped distribution of multiple scars. Fishes that forage on the bottom of the rivers are probably better candidates as predators that may explain these results. Among others, the bullhead, *Cottus gobio* Linnaeus, is common in both rivers, and one bite of such a young fish could produce clumped multiple wounds that were observed on individuals bearing at least three scars.

Why, finally, should the gammarids bear more scars in the river Viroin? This could be explained by different predator densities but also by differences in their efficiencies linked with visibility. The water of the Meuse is never transparent; its turbidity actually varies to a large extent with discharge, boat navigation and plankton development. On the contrary the water of the Viroin can be turbid after a rain shower, but from late spring to mid-autumn it is more often limpid. In the Meuse the visual perception of prey by predators is therefore always more or less hampered by the water turbidity whereas in the Viroin it can be fairly good at some periods. The gammarid catches dropped noticeably when the water of the Viroin became clear in April-May, whereas their activity was rising in the Meuse (Table 3). Either they were severely preyed upon or their spring activity was inhibited in the Viroin by the water transparency. This again should be tested experimentally.

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