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Dikerogammarus villosus

an amphipod with a bite



Dirk Platvoet

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Titel: *Dikerogammarus villosus* (Sowinsky, 1894), an amphipod with a bite

***Dikerogammarus villosus* (Sowinsky, 1894),
an amphipod with a bite**

The relation between morphology, behaviour, micro-distribution
and impact of this invading crustacean

ACADEMISCH PROEFSCHRIFT

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aan de Universiteit van Amsterdam
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ten overstaan van een door het college voor promoties
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Dit proefschrift is opgedragen aan

Sjouk Pinkster

(10 mei 1943 – 14 oktober 1996)



Pre-copula stage of *Dikerogammarus villosus* (Sowinsky, 1894): smaller female is held by large male (photograph Jan van Arkel/IBED).

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General introduction

Primarily as a result of human activities such as connecting formerly separated waterways, transferring species by ships or otherwise, many species have extended their distribution range. These species have a varying impact on the original fauna and flora. Species replacements can be the result, and ecosystems can be altered by large-scale invasions, sometimes leading to devastation of these ecosystems and economical damage. The best-known recent example is the invasion of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) in the Great Lakes of North America. By clogging up pipelines, waterways and fouling hulls of ships these molluscs cause a multi-billion dollar economical damage each year.

After opening of the Main-Danube Canal in 1992, the catchment basins of the Rivers Rhine and Danube were connected. With an average between one and two per year (Van der Velde et al., 2002) Ponto-Caspian aquatic species (originating from the tributaries of the Black and Caspian Seas) have moved into western European waters, sometimes replacing indigenous species. One of these high-impact species is *Dikerogammarus villosus* (Sowinsky, 1894), a large gammaridean amphipod. The arrival of this species has been well monitored and therefore a good opportunity presented itself to follow the invasion process in more detail. Producing a large portfolio of the species was considered a useful and a necessary step to be able to interpret this and other invasion events. In this thesis an attempt is made to combine a number of aspects of the biology of *Dikerogammarus villosus* with its recent range extension.

Structure of this thesis.

In the first four chapters several issues concerning the ecology and general biology of *Dikerogammarus villosus* are addressed: behaviour, competition, niche choice, food and feeding methods and invasive impact. In the process of interpreting the results of these studies it became clear that the mechanisms underlying the success of this invasion could not be explained without additional knowledge of the (functional) morphology of this and other species. Therefore, a large-scale inventory of the external morphology of *D. villosus* was started, the Amphipod Pilot Species Project (AMPIS), resulting in a number of new findings regarding its biology (chapters 6 to 10). The bridge between the ecological and morphological part of this thesis is formed by chapter 5, in which a major aspect of the invasive success of *D. villosus*, its predatory nature, is explained through a comparative morphometric study of the body parts involved in this predatory lifestyle.

First encounter.

The first living specimen of *Dikerogammarus villosus* (Sowinsky, 1984) I collected from the Gouwzee in the Netherlands in 1998 immediately made me realize that I had a species on my hand that plays in a totally different league than all other native and alien gammaridean amphipods. Through its mere body size (up to 30 mm) and sturdy composure it made a very strong first impression. Also the unparalleled force with which it tried to escape the grip of my hand sustained the developing notion that the local fauna was in for a nasty surprise, when facing this successful invader. And successful it is, as may become clear from the following short summary of its recent range extension.

Origin.

The geographical origin of the species is uncertain, but *D. villosus* most probably originates from the lagoons and estuaries of the Black and Caspian Sea (Dedju, 1967, 1980; Mordukhaj-Boltovskoj et al., 1969, Pöckl, 2007). Transplants of *D. villosus* within the former Soviet Union to some artificial Crimean lakes were mentioned by Dedju (1980). Jazdzewski (pers. comm.) pointed out that in the Atlas of the Caspian Sea Invertebrates (Birstein & Romanova, 1968) the species is not mentioned at all. Birstein was not convinced of the validity of *D. villosus* as a distinct taxon, but listed two other species, *D. aralensis* (Uljanov, 1875) and *D. oskari* Birstein, 1945, instead. Since the descriptions lacked much detail and the type material is most probably lost, we can never be certain of possible synonymy. Between 1945 and 1980 *D. villosus* was recorded from Black Sea drainages (Dniester, Dnieper, Danube), sometimes up to 1500 km upstream (Carausu et al., 1955, Ponyi, 1958; Straskraba; 1962, Dedju 1967, 1980).

Range extension in western Europe.

D. villosus took the southern corridor (Bij de Vaate et al., 2002) as its initial dispersal pathway. First it expanded its occurrence upstream in the Danube (Austria in 1989; southern Germany in 1992) (Nesemann et al., 1995). It passed the Main-Danube canal in 1993, the Main and Rhine in 1994 (Tittizer, 1996). In 1994 the species was first recorded from lower Rhine in the Netherlands (Bij de Vaate & Klink, 1995), in 1998 in the river Meuse in Belgium (Vanden Bossche, 2002), and from French river systems including the Rhône river and the Moselle river (Devin et al., 2001; Müller et al., 2002; Bollache et al., 2004). In Northern Germany the species was recorded from the large rivers (Grabow et al., 1998). Poland was invaded from the west through the Mittelland canal into the lower Oder river and Szczecin Lagoon and recently the Bug river (Müller et al., 2001; Jazdzewski et al., 2002; Grabowski et al., 2007). Between

2002 and 2006 the species was also found in Switzerland and Italy (Bollache et al., 2004; Mürle et al., 2004; Lods-Crozet and Reymond, 2006; Casellato et al., 2006).

Range extension in the Netherlands.

D. villosus was recorded for the first time from the Lower Rhine near the German border in 1994 (Bij de Vaate & Klink, 1995). It became very abundant in the lower Rhine and Meuse (Van der Velde et al., 2000, 2002; Bij de Vaate, 2004). In 1997 it had already spread throughout most of the IJsselmeer/Markermeer-complex, a Rhine fed lake system (Dick & Platvoet, 2000) and was making its way into other parts of the country in the following years. By 2006 the species was found in all major rivers and many of the larger water bodies in The Netherlands.

To get more understanding of the processes involved in the invasion of our waters by *D. villosus* I formulated the following research questions.

- a) Which factors, such as morphology, behaviour and physiology, determine the success of *D. villosus* as an invader?
- b) What is the impact of this species on the indigenous and alien aquatic fauna and will *D. villosus* be able to maintain its position in the coming decades?

To answer these questions the following topics were addressed

- 1) Interactions with a) other gammaridean species and b) other species (chapters 1, 2 and 4)
- 2) Feeding behaviour and food choice (chapters 2 and 3)
- 3) Habitat structure and niche competition (chapter 4)
- 4) Allometric growth of body parts involved in feeding (chapter 5)
- 5) General and functional morphology (chapters 6 – 10)

Chapter 1

Invading predatory crustacean eliminates both native
and exotic species

Proc. Roy. Soc. Lond. Ser. B Biol. Sci. 267: 977-983 (2000)

Jaimie T. A. Dick and Dirk Platvoet

Abstract

As the tempo of biological invasions increases, explanations and predictions of their impacts become more crucial. Particularly with regard to biodiversity, we require elucidation of interspecific behavioural interactions among invaders and natives. In freshwaters in the Netherlands, we show that the invasive Ponto-Caspian crustacean amphipod *Dikerogammarus villosus* is rapidly eliminating *Gammarus duebeni*, a native European amphipod, and *Gammarus tigrinus*, until now a spectacularly successful invader from North America. In the laboratory, survival of single (unguarded) female *G. duebeni* was significantly lower when male *D. villosus* were free to roam as compared to isolated within microcosms. In addition, survival of paired (guarded) female *G. duebeni* was significantly lower when male *D. villosus* as compared to male *G. duebeni* were present. *D. villosus* killed and consumed both recently moulted and, unusually, intermoult victims. Survival of *G. tigrinus* was significantly lower when *D. villosus* were free to roam as compared to isolated within microcosms and, again, both moulted and intermoult victims were preyed upon. Male *D. villosus* were significantly more predatory than were females, while female *G. tigrinus* were significantly more often preyed upon than were males. Predation by *D. villosus* on both species occurred over a range of water conductivities, an environmental feature previously shown to promote amphipod coexistence. This predatory invader is predicted to further reduce amphipod diversity in a range of freshwater habitats in Europe and North America.

Keywords: amphipods; interspecific interactions; invasions; microcosms; predation

1. Introduction

Invading species may interact with the fauna of new host locations in a variety of ways, for example, by competition (Human & Gordon 1986), predation (Zaret & Paine 1973), hybridization (Capelli & Capelli 1980) and disease transmission (Dogiel *et al.* 1970). Linking invasion patterns with interspecific processes is often difficult, but such information is crucial in the prediction of the impacts of invaders on the biodiversity of new host locations (Dick 1996a; Moyle & Light 1996; Holway & Suarez 1999; Williamson 1996; 1999). Successful invaders often show spectacular population increases and consequent eliminations of native faunas in short time spans (Elton 1958; Diamond & Case 1986; di Castri *et al.* 1990; Kinzelbach 1995; Williamson 1996; 1999). In this study, a species invasion was investigated in its phase of rapid geographical expansion and population explosion. In addition, there is growing realization that proximate causes of invasion success may be illuminated by considering behavioural characteristics of the species involved (Holway & Suarez 1999). In the present study, experimental laboratory assessment of individual interactions between the invader and natives was undertaken during the invasion, thus allowing details of the suspected mechanism of species interaction to be linked to the ongoing field displacement patterns.

Dikerogammarus villosus is an amphipod crustacean native to the Ponto-Caspian region of eastern Europe/Ukraine, particularly the Danube River system (Nesemann *et al.* 1996). This species has invaded western Europe via the Main-Danube canal (Tittizer 1996), appearing in the River Rhine at the German/Dutch border in 1994/5 (Bij de Vaate & Klink 1995). This is one of a suite of Ponto-Caspian organisms predicted to have an impact in Europe and North America (Ricciardi & Rasmussen 1998). We discovered *D. villosus* on the western shores of the Markermeer at Schardam, north of Amsterdam, in July 1998 (figure 1). This Dutch water body has received much attention as regards amphipod species diversity due to previous invasions by exotic amphipods and their interactions with native species (Pinkster *et al.* 1992; Dick & Platvoet 1996).

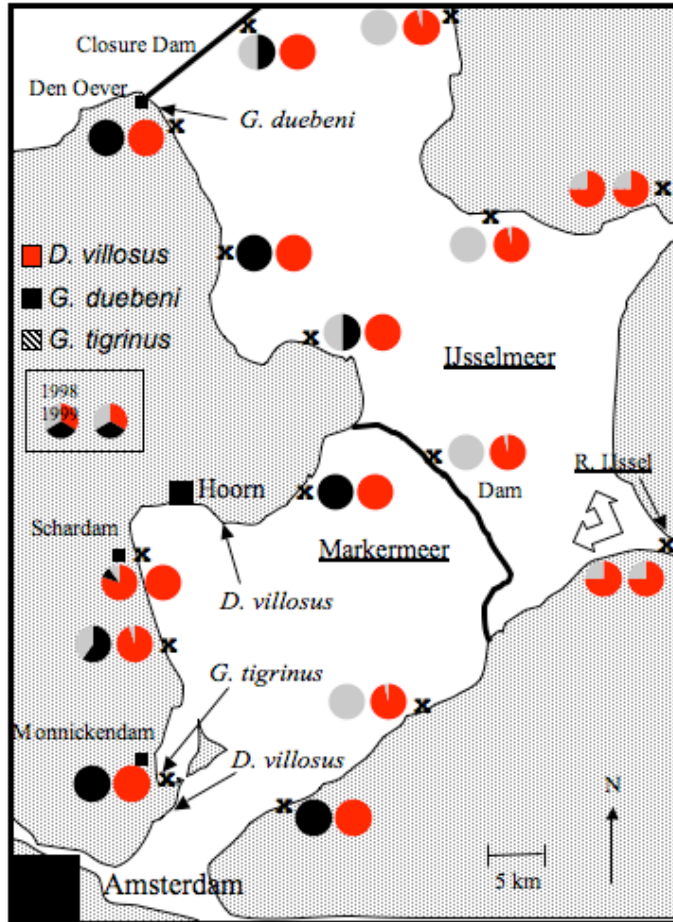


Figure 1. The Markermeer and IJsselmeer of Holland showing invasion and expansion by the Ponto-Caspian amphipod *D. villosus* in 1998 and 1999 and the elimination and replacement of the native, *G. duebeni*, and previous invader from North America, *G. tigrinus*. Pies show relative abundances based on at least 100 individuals collected per site (see paragraph 2). The double arrow shows the likely *D. villosus* invasion route from the River IJssel, which connects with the River Rhine. Sampling stations arrowed are those from which animals were collected for use in the 1998 microcosm experiments.

Gammarus duebeni is native and widespread in western Europe, although replaced over parts of its range by previous invaders such as the North American *Gammarus tigrinus* (Pinkster *et al.* 1992). Since 1984, the Dutch and German sections of the River Rhine have seen dramatic explosions in the distribution and abundance of *G. tigrinus*, intentionally or unintentionally introduced (Pinkster *et al.* 1992; Van der Velde *et al.* 2000). This invader is now, however, in sharp decline, coincidental with the invasion of *D. villosus* (Van der Velde *et al.* 2000). Both *G. duebeni* and *G. tigrinus* are found along rocky shorelines and sheltered vegetated bays of the Markermeer and IJsselmeer (figure 1), although *G. tigrinus* favours the latter habitat type and *G. duebeni* the former (J.T.A. Dick and D. Platvoet, unpublished data). Crucially, *D. villosus* can occupy a range of habitats and has wide environmental tolerances as regards temperature and salinity (Van der Velde *et al.* 2000) and is thus able to colonise the preferred habitats of both these native and exotic species, with resultant interaction.

Stable isotope analyses have shown that *D. villosus* is on the same trophic level as some predatory fish species (Marguillier 1998), while a review of the trophic ecology of *Gammarus* and related genera indicates that their traditionally designated role as detritivores and herbivores belies a predatory disposition (MacNeil *et al.* 1997). We thus suspected that the rapid expansion of *D. villosus* and its devastating impact on related genera may involve intraguild predation, or IGP (Polis *et al.* 1989), rather than interspecific competition as the likely mechanism operating within this ecological guild. In order to test this hypothesis, a sampling programme and three laboratory experiments were designed to establish any link between field patterns of invasion and elimination with any predatory disposition of *D. villosus* towards *G. duebeni* and *G. tigrinus*. Further, we argue that the resultant data, alongside other studies of the biological characteristics of *D. villosus*, may be used to make predictions regarding the future impacts of this invader on amphipod diversity.

2. Methods

(a) Study area and field sampling

Sampling of the Markermeer and IJsselmeer (figure 1) was conducted once in late June-early July 1998 and at the same sites again in late June 1999. Sampling entailed using kick nets to sweep vegetation, while large rocks or boulders and debris were disturbed and the net swept over the area. Sampling continued until approximately 100 individual amphipods were caught. Amphipod species presence and relative abundances were recorded on-site.

(b) *Laboratory microcosm experiments*

Three experiments, run concurrently, took place at the University of Amsterdam in September 1998. We collected *D. villosus* from near Hoorn and Monnickendam (figure 1). This is a relatively large amphipod, males in the present experiments ranging from 50 to 85 mg and females 35 to 65 mg (live weight). *G. duebeni* were collected from near Den Oever (figure 1), males ranging from 35 to 50 mg and females 15-25 mg. *G. tigrinus* were collected from near Monnickendam (figure 1). This is a considerably smaller amphipod, although size range is highly variable throughout the year. In the present study, male *G. tigrinus* ranged from 8 to 12 mg and females 3 to 5 mg.

In the laboratory, water temperatures were maintained at 15-17 °C and in a 6L:8D cycle, in accordance with field conditions. Experiments took place in aerated aquaria (“microcosms”) of 15 cm x 9 cm x 5 cm supplied with 4 cm depth of water from the Markermeer/IJsselmeer. Water from Hoorn was of relatively low conductivity, approximately 900 $\mu\text{S cm}^{-1}$, whereas water from Den Oever was of higher conductivity, approximately 1700 $\mu\text{S cm}^{-1}$. This difference reflects proximity to the sea. Habitat heterogeneity and food were supplied in the form of pieces of terra cotta plant pot (which provided numerous sizes of crevice), two pieces of 1x2 cm cuttings of reed and 6 pellets of food designed for catfish. Plastic vials of 3 cm diameter with 20 small holes in their sides and gauze mesh around the interior were constructed as the “isolators” for *D. villosus*. These “isolators” were present in all aquaria and *D. villosus* were either inside (i.e. “isolated” from the other species) or outside (i.e. “free” to interact with the other species).

Pilot trials indicated that adult *D. villosus* were predatory towards both adult *G. duebeni* and adult *G. tigrinus*, but the latter two species did not kill the former. In particular, *D. villosus* killed and consumed intermoult victims, whereas all previously reported instances of IGP in amphipods has involved the consumption of victims at the vulnerable time of moult, when the new exoskeleton is still soft (Dick 1996b; Dick & Platvoet 1996; MacNeil *et al.* 1997). The experiments were thus designed to characterize and quantify the degree of *D. villosus* predation on both species in replicated microcosms, simulating patches of habitat within which individuals may interact. Such experiments go some way to balance the scale at which interactions can be monitored yet be meaningful in extrapolation to field patterns (Dick *et al.* 1999).

(c) *D. villosus* versus *G. duebeni*

Experiments 1 and 2 aimed to assess predation by *D. villosus* on the native *G. duebeni*. Adult male *D. villosus* were used, as male amphipods may be both cannibalistic and predatory, particularly towards reproductive females (MacNeil *et al.* 1997). Adult, ovigerous female *G. duebeni* were used as the “target” of predation, as the killing of these individuals would have the greatest impact on populations. Females occur as “singles” and “pairs”, the latter occurring when conspecific males take hold of females in “precopulatory guarding” a few weeks prior to female moult and subsequent copulation (Dick 1989).

Experiment 1 was designed to assess predation in one-on-one inter-specific encounters, since individuals are likely to encounter one another in such a way in micro-habitats such as crevices in rocks. This design also allowed sufficient experimental replication with limited numbers of *G. duebeni*, which is increasingly rare (see below). Thus, twenty-eight aquaria were supplied with a 50:50 mix of Hoorn and Den Oever water (approx. 1300 $\mu\text{S cm}^{-1}$) and a single (i.e. unpaired) female *G. duebeni* carrying eggs in the early stage of development. Such females are several weeks from moult and have hardened exoskeletons (Dick 1989). In half the aquaria, a male *D. villosus* was isolated, whereas in the other half a male *D. villosus* was free to interact with the female as previously mentioned (see paragraph 2(b)). Each replicate was examined twice daily and the experiment allowed to run for five days. Comparison of frequencies of death of female *G. duebeni* between these groups allows for discrimination between active predation by male *D. villosus* and deaths of females followed by scavenging.

Experiment 2 was designed to assess any protection afforded to females by being in the precopulatory guarding phase and to compare levels of intraspecific killing (cannibalism) with inter specific killing (predation). In addition, the remaining populations of *G. duebeni* are mostly found in areas of high water conductivity areas such as Den Oever (figure 1), however, the availability of pairs is limited (see below). Thus, forty aquaria were supplied with high-conductivity water (Den Oever water, approx. 1700 $\mu\text{S cm}^{-1}$) and a pair of *G. duebeni*. Such females carry well-developed embryos and are closer to moult than single, unguarded females (Dick 1989). Half of these aquaria were supplied with a second male *G. duebeni* and the other half with a male *D. villosus*. Each replicate was examined twice daily and the experiment allowed to run for five days. Comparison of frequencies of death of female *G. duebeni* between these groups allows, first assessment of the strength of predation by male *D. villosus* compared to female deaths and/or cannibalism by male *G. duebeni* and, second assessment of whether mate-guarding protects female *G. duebeni* from male *D.*

villosus predation. In addition, the high-conductivity water used in this second experiment assessed the ability of *D. villosus* to both survive and prey upon *G. duebeni* in environmental conditions, which have favoured *G. duebeni* populations and may be its last refuge from invasion and replacement (Pinkster *et al.* 1992).

Ethical/practical note: although not protected under any legislation, *G. duebeni* populations are increasingly endangered in freshwaters due, principally, to replacement by invasive species (Pinkster *et al.* 1992; Dick *et al.* 1999). This raises an ethical issue with regards to destructive sampling of this species and practical problems in terms of sufficient replication for meaningful experiments. Thus, the present two experiments, which were designed individually to answer a range of questions and run concurrently, aimed to balance information gain with the number of replicates ethically justifiable and practically feasible with the dwindling availability of *G. duebeni* in these Dutch water bodies.

(d) *D. villosus* versus *G. tigrinus*

Experiment 3 was run concurrently with experiments 1 and 2 and aimed to assess predation by *D. villosus* on the North American invader *G. tigrinus*. The very dense field populations of the relatively small *G. tigrinus* led to an experimental design different from that with *G. duebeni* (and see the ethical/practical note above). We simulated the invasion scenario of limited numbers of *D. villosus* individuals encountering very abundant populations of *G. tigrinus* by placing four precopulatory guarding pairs of *G. tigrinus* in each of 38 aquaria (supplied with habitat, food and isolators) and monitoring their survival under all combinations of the following: (i) a *D. villosus* individual was either isolated within the aquarium or free to interact with the *G. tigrinus*; (ii) the *D. villosus* individual was either an adult male or adult female; and (iii) the water of the aquarium was either of low conductivity (approx. $900 \mu\text{S cm}^{-1}$, from Hoorn) or high-conductivity (approx. $1700 \mu\text{S cm}^{-1}$, from Den Oever). This experiment thus allows for assessment of the predatory impact of *D. villosus* (both males and females) on the reproductively active individuals of relatively abundant populations of *G. tigrinus*. As with experiments 1 and 2, the design allows for discrimination between active predation and deaths followed by scavenging. This experiment also assesses the ability of *D. villosus* to overcome the ability of *G. tigrinus* to avoid predation to a greater degree in high-conductivity water, as occurs when in the presence of *G. pulex* (Dick & Platvoet 1996). Each replicate was examined twice daily and full counts were made after five and ten days. Numbers of surviving male and female *G. tigrinus* after five days were arcsine transformed for statistical analyses (Sokal & Rohlf 1995). A four-factor ANOVA was conducted using

*SuperANOVA*TM (Abacus Concepts 1989) the factors being (i) to (iii) above, with the fourth factor sex of *G. tigrinus* entered as a repeated measure in the analysis due to the non-independence of these data, both sexes of *G. tigrinus* being present in each replicate.

In the experiments, female *G. duebeni* and male and female *G. tigrinus* in the '*D. villosus* isolated' replicates were monitored closely for moult, such that the moult frequency of animals in the '*D. villosus* free' groups could be estimated. Moulting is evidenced in amphipods by the shed exoskeleton and clean appearance of the new exoskeleton.

3. Results

In late June-early July 1998, *D. villosus* was found in the River IJssel (which links to the River Rhine) and in an inland pond north of that site (figure 1). A population with precopula pairs in abundance was also found at Schardam on the west bank of the Markermeer (figure 1). There was also a small proportion of *G. duebeni* and *G. tigrinus* at the Schardam site at this time, this site for the previous several years having an abundant and relatively stable mix of the latter two species (D. Platvoet and J.T.A. Dick, unpublished data). Thus, in 1998, *D. villosus* had invaded and perhaps begun replacing *G. duebeni* and *G. tigrinus*, although these latter species held most ground at this time (figure 1). In 1999, however, *D. villosus* had replaced both species almost completely at all sampling stations (figure 1). This process was extremely rapid; for example, at a site near Monnickendam, *G. duebeni* was the sole species present in June-July 1998 and was observed there again in September 1998 (J.T.A. Dick, unpublished data.), but this *G. duebeni* population had been entirely replaced by *D. villosus* by June 1999 (see figure 1).

In experiment 1, there was zero *D. villosus* mortality over the five day period. However, 86% of single female *G. duebeni* survived when the male *D. villosus* was isolated, whereas only 28% survived when the male *D. villosus* was free ($p < 0.005$, Fisher's exact test) (figure 2). Typically, female *G. duebeni* at first appeared to have escaped from aquaria where male *D. villosus* were free, however, the remains of those females were detected when the aquarium water was examined closely, often under binocular microscope. In one case, for example, the presence of the alimentary canal of the *G. duebeni*, plus assorted legs and pieces of exoskeleton, was proof that the female had been shredded and consumed. As expected with single females, there were no moults during the time span of this experiment and thus all females must have been killed in the hard intermoult state.

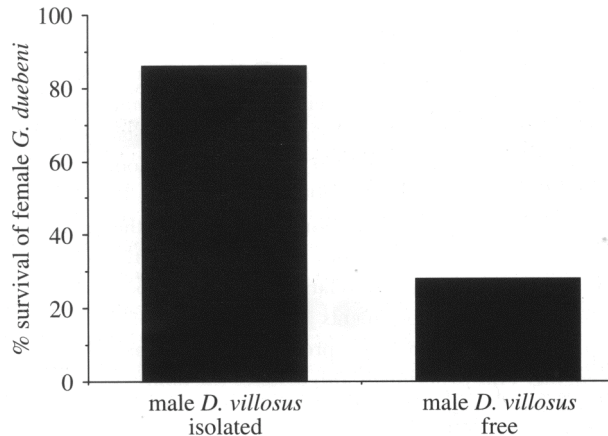


Fig. 2. Percentage survival of single intermoult female *G. duebeni* when male *D. villosus* were free to roam as opposed to isolated within experimental microcosms.

In experiment 2, there was again no *D. villosus* mortality over the five day period. No male *G. duebeni* were killed during this experiment. However, 80% of paired female *G. duebeni* survived when a second conspecific male was present, whereas only 45% of paired females survived when a male *D. villosus* was present ($p < 0.02$, Fisher's exact test) (figure 3). Thus, male *D. villosus* predation of female *G. duebeni* is significantly greater than female mortality and/or cannibalism and, further, mate guarding does not prevent significant killing of females. These females were in precopula and thus closer to moult than those in experiment 1. Thus, as expected, a small proportion (15%) of females in the 'conspecific male present' group moulted. Clearly, then, the male *D. villosus* in experiment 2 must have killed intermoult as well as recently moulted female *G. duebeni*. This experiment also confirms that *D. villosus* is capable of surviving in high-conductivity waters and killing *G. duebeni* females under such conditions, which are typical of the last refuges of the latter species (Pinkster *et al.* 1992).

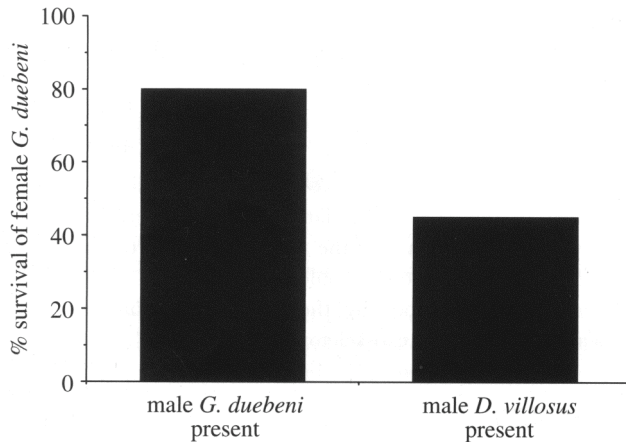


Figure 3. Percentage survival of precopula guarded female *G. duebeni* when male *D. villosus* were present as compared to when conspecific males were present.

In experiment 3, there was no *D. villosus* mortality over the ten days. When *D. villosus* were 'isolated' and thus unable to interact with *G. tigrinus*, survival of the latter after five days was high (figure 4); some cannibalism of females by males is likely to account for the slight drop in female survival in this group (figure 4). On the other hand, the presence of free *D. villosus* in the microcosms was characterized by significant reductions in survival of *G. tigrinus* ($F_{1,30}=47.9$, $p < 0.0001$) (figure 4), whose remains, which consisted of legs, antennae and assorted pieces of exoskeleton, littered the floors of the aquaria. Male *D. villosus* were significantly more predatory than were females ($F_{1,30}=4.7$, $p < 0.05$) (figure 4), while female *G. tigrinus* were significantly more often preyed upon than were males ($F_{1,30}=14.4$, $p < 0.001$) (figure 4). Indeed, after ten days, 27% of replicates with free *D. villosus* had total elimination of *G. tigrinus*, whereas 100% of replicates with isolated *D. villosus* had at least one male and one female *G. tigrinus* surviving. In those replicates with free male *D. villosus*, there were no surviving female *G. tigrinus* after ten days. We estimated that 30-40% of females in the '*D. villosus* isolated' replicates moulted within the five-day period. Males do not moult when in precopula (Dick 1989). Thus, male *D. villosus* predation of 80-90% of females and 60-75% of males (figure 4) indicates that intermoult as well as moulted individuals were killed and consumed. There was no significant difference in survival of *G. tigrinus* in low compared with high-conductivity waters ($F_{1,30}=0.8$, n.s.).

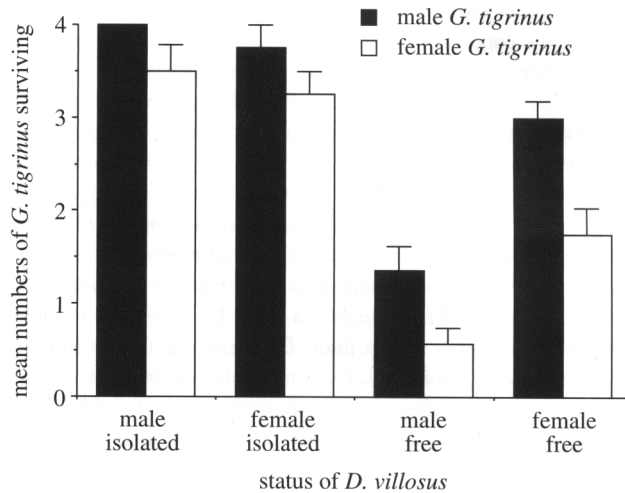


Figure 4. Mean numbers (\pm s.e.) surviving of *G. tigrinus* when *D. villosus* individuals were free to roam as compared to isolated within experimental microcosms (after five days).

(4) Discussion

Microcosms containing *D. villosus* and *G. duebeni* were characterized by high frequencies of predation by males of the former species on females of the latter. Male *D. villosus* were clearly able to overcome the hard intermoult exoskeleton of female *G. duebeni* and devour them, rather than being restricted to the vulnerable time of moult when most crustacean cannibalism and predation occurs (Dick 1996a; Dick & Platvoet 1996; MacNeil *et al.* 1997). Thus, the predatory impact of *D. villosus* is not restricted to the short (approximately 12hrs; Dick 1989) period of post-moult vulnerability, facilitating rapid eliminations of all stages of reproductive females. Both single females and those held in the precopulatory mate-guarding position were vulnerable to *D. villosus* predation, even though guarding male amphipods may attempt to aggressively repel intruding conspecifics and heterospecifics (Dick & Elwood 1990; Dick *et al.* 1995). Thus, female *G. duebeni* carrying embryos and the future of the population may be eliminated rapidly in the face of *D. villosus* invasion. Interestingly, no male *G. duebeni* were killed during experiment 2, indicating that males, being larger, are more able to fend off any predatory attack, although this may not be the

case at moult (see Dick 1996b). Indeed, the large size of *D. villosus* relative to other freshwater amphipods is in part likely to explain its successful predatory behaviour.

In addition, male *D. villosus* were able to survive and effectively prey upon female *G. duebeni* in the relatively high-conductivity water typical of the IJsselmeer at Den Oever (figure 1), a northern site not (by September 1998) colonised by *D. villosus*. Thus, we predict that *G. duebeni* populations will be unable to resist invasion by *D. villosus* even in traditionally 'safe' areas of raised conductivity to which the former species is optimally adapted (Pinkster *et al.* 1992). Indeed, the 1999 data from the Closure Dam at Den Oever show that *G. duebeni* is unable to resist replacement by *D. villosus* in high-conductivity waters (figure 1). We believe that the population of *G. duebeni* used in the present experiments (figure 1) has only persisted due to the physical enclosure of this site by dykes and a sluice gate at the seaward margin.

D. villosus also has the ability to eliminate large numbers of the North American *G. tigrinus* through predation in short time spans and, in particular, remove the reproductive females from the population. In addition, it is known that high-conductivities reduce predation by *G. pulex* on *G. tigrinus*, possibly due to the latter hardening its exoskeleton more effectively under such conditions (Dick & Platvoet 1996). Whatever the mechanism, this environmental modification of the strength of the predatory interaction facilitates the coexistence of these species (Dick & Platvoet 1996). However, the predatory impact of *D. villosus* on *G. tigrinus* is not alleviated in high-conductivity conditions, indicating that *D. villosus* will eliminate *G. tigrinus* regardless. *G. tigrinus* does, however, have a very high reproductive rate and forms extremely dense populations. Thus, the apparent persistence of a small proportion of *G. tigrinus* in some sites invaded by *D. villosus* (e.g. east coast of IJsselmeer) (figure 1) may reflect this ability of *G. tigrinus* to maintain itself in the face of predation pressures. However, the rapidity with which *D. villosus* eliminated *G. tigrinus* in microcosms, plus the rapidly diminishing sources of *G. tigrinus* to populate areas by immigration (figure 1), suggests that eventually *G. tigrinus* populations will succumb to *D. villosus* predation.

Prediction of the identity and ecological consequences of biological invaders has been a long-sought yet elusive goal of ecologists (Elton 1958; Diamond & Case 1986; Mooney & Drake 1986; Vitousek *et al.* 1986; di Castri *et al.* 1990; Kinzelbach 1995; Moyle & Light 1996; Holway & Suarez 1999; Williamson 1996; 1999). A major problem with prediction is that general features of successful invasions, for example, in terms of the intrinsic attributes of invading species (e.g. high dispersal rate) and community type (e.g. disturbed and/or low diversity) may be useful as general predictors, yet so many exceptions exist that prediction of the outcome of any specific invasion is difficult (Lodge 1993). However, the accumulation of case studies of

species attributes, together with linkage of processes of interspecific interaction with ecological pattern, particularly in laboratory microcosms (as proposed by Lodge (1993) and Holway & Suarez (1999)), may be major routes to refining explanations and predictions of the identity and impacts of invaders. Although the use of microcosms in ecological experimentation is contested (e.g. see debate among Drenner & Mazumder (1999), Carpenter (1999) and Huston (1999)), particularly in extrapolation from laboratory to field, microcosms have been recognised as fruitful routes to identifying and making tractable the proximate mechanisms behind successful invasions (Holway & Suarez 1999), particularly where different scales and approaches are combined (Huston 1999). In the present study, the rapid field pattern of exclusion of both native and previously successful invading species by *D. villosus* appears from microcosm studies to be linked with the predatory behaviour of this species. This link is supported by other types of study, such as the finding of Marguillier (1998) from isotope analyses that *D. villosus* is on the same trophic level as some predatory fish species. In addition, Mordukhai-Boltovskoi (1949) described the cannibalistic propensity of *D. villosus*, a characteristic which generally accompanies a propensity for IGP (Polis *et al.* 1989). Thus, accumulated evidence, and in the present study the bridging of scales of investigation by the use of microcosms, tends to support the link between the predatory behaviour of *D. villosus* and its exclusion of both native and exotic species.

While predation between member species of different ecological guilds has often been linked to the elimination of natives by invaders (Diamond & Case 1986), in many invasion scenarios mechanisms are often inferred incorrectly, such as the assumed role of interspecific competition within amphipod guilds (Dick & Platvoet 1996). With amphipods and many other arthropods, intraguild predation (IGP) has been increasingly recognised as a potent force in structuring communities (Polis *et al.* 1989; Holt & Polis 1997) and may over-ride interspecific competition (Dick *et al.* 1993; Dick & Platvoet 1996). In the present case, IGP appears to be the mechanism responsible for rapid species exclusions. In addition, however, the present study has illustrated important details of the nature of predation by *D. villosus*, such as the killing of intermoult victims, that may explain its rapid and decisive impact on populations of native and other exotic species. These predatory features of *D. villosus*, together with its wide habitat requirements and tolerances to temperature and salinity, high reproductive rate (Van der Velde *et al.* 2000) and ability to disperse by anthropogenic means, particularly boats (Nesemann *et al.* 1995), leads us to predict that this invader will further reduce amphipod diversity in Western Europe and elsewhere. Indeed, other European and Ponto-Caspian invaders such as the zebra mussel *Dreissena polymorpha*, the cladoceran *Bythotrephes cederstroemi* and the

amphipod *Echinogammarus ischnus* have reached and impacted upon the ecology of the North American Great Lakes (Morton 1997; Witt *et al.* 1997; Dermot *et al.* 1998). We predict that this trend will continue (see also Ricciardi & Rasmussen 1998), with *Dikerogammarus villosus* likely to impact severely on both native and previously successful invading species in such regions.

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Chapter 2

Flexible omnivory in
Dikerogammarus villosus (Sowinsky, 1894)
(Amphipoda)

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Abstract

Feeding in *Dikerogammarus villosus* (Sowinsky, 1894) males was observed in the field and recorded on video in the laboratory. The following feeding modes were recognized: detritus feeding, grazing, particle feeding, coprophagy, predation on benthic and free swimming invertebrates, predation on fish-eggs and larvae as well as feeding on byssal threads of the zebra mussel *Dreissena polymorpha* (Pallas, 1771). The feeding methods are described and illustrated with screenshots of video recordings. The very flexible feeding modes of *D. villosus*, which make diet switches possible, form a trait that must be an important factor in the invasion success of this Ponto-Caspian gammaridean species and explain for a great deal its high ecosystem impact.

Introduction

Gammaridean amphipods have long been considered purely as shredders of organic material. In a growing number of publications, however, the role of these amphipods in food-webs is put in a different perspective (Monakov, 2003 and literature therein). In Irish river systems 95% of the invertebrate biomass can consist of *Gammarus p. pulex* (Linnaeus, 1758), an invasive alien species in Ireland (MacNeil et al., 2000). Assuming that the absence of other invertebrates in these rivers is the result of interaction with this species, than a predatory role of this species becomes rational. Intraguild predation has been recognized as a common phenomenon in gammarideans (Dick et al., 1993).

In a stable isotope study of *D. villosus* from the river Rhine it was found that the Ponto-Caspian invasive gammaridean species *Dikerogammarus villosus* (Sowinsky, 1894) (Bij de Vaate et al., 2002) clustered with zoobenthivorous fish in contrast to *Gammarus tigrinus* Sexton, 1939 (Marguillier, 1998; Van der Velde et al., 2000; Van Riel et al., 2006b). The appearance of *D. villosus* in the river Rhine coincided with the decrease of the North American gammaridean *G. tigrinus* as well as other macroinvertebrates, in particular on the stones of groynes and river banks (Van der Velde et al., 2000; 2002; Van Riel et al., 2006a,b). In a Rhine-fed lake in the Netherlands, the IJsselmeer, it appeared that the appearance of *D. villosus* coincided with the total disappearance of the native *G. duebeni* Liljeborg, 1852 from the stony banks, its only habitat in the lake, whereas *G. tigrinus* disappeared also from the stones but maintained its population in deeper water in the sediment and zebra mussel beds. Predation on a wide range of other invertebrates was first demonstrated by

laboratory experiments (Dick et al., 2002), in which *Dikerogammarus villosus* proved to be a formidable predator, preying even on predaceous, hard skinned insects such as water bugs and damselfly larvae. On the other hand, in the absence of prey, this species is easily able to switch to particle feeding (Platvoet et al., 2006; this thesis, chapter 3). Being a food generalist is an important characteristic for an invading species (Van der Velde et al., 2000), and to determine how flexible *D. villosus* is with respect to feeding we studied its feeding related activities.

We also discuss the position of this species and gammaridean species in general in the classification of species as belonging to various feeding guilds or categories.

Materials and methods

In May 2001 male specimens of *Dikerogammarus villosus* were collected from Gouwzee, a Rhine-fed, slightly brackish lake that is part of the Markermeer/IJsselmeer complex. The specimens were immediately transferred to the laboratory and stored in a large tank with site water at site temperature (16°C).

Four male specimens of *D. villosus* were involved in the observations (body-length 21-22 mm). In gammaridean amphipods with a pre-copula stage, males are much bigger than females. Therefore males are far more active food collectors than females.

Recording on video was done with a JVC digital video camcorder GR-DVL100 on mini DV at the highest resolution, over a period of 6 weeks. Two types of cuvettes were used: a) two bolted together plexi-glass plates separated by a plastic hose bent in u-shape, leaving a 9 mm space between the plates with 25 ml of source water, and b) a U-shaped plexi-glass plate of 10 by 10 cm of 9 mm thickness lined with two 2 mm thick glass plates of 10 x 10 cm, with 16 ml of source water. Both cuvettes were aerated with a syringe needle connected to a hose and an air pump. Water temperature in the cuvettes was maintained at 19°C during the experiments.

The males were individually filmed in 20 recording sessions of one hour each. In four cases a mix of several food items was offered to give *D. villosus* a choice. Most offered invertebrates were collected from the Oosterpoel, a small inland water body bordering the Gouwzee with equal salinity and temperature as the lake, where *D. villosus* is absent. Water fleas (*Daphnia* sp.) were obtained from an aquarium shop. A pondweed (*Potamogeton pectinatus* L., 1753) was collected from the Gouwzee.

In most experiments the animals were given an abundant supply of vegetation or invertebrate specimens. Generally, potential prey or plants were already present in the cuvette for 15 minutes before a male *D. villosus* was added. The males of *D. villosus* were not starved before the experiments. Coarse sand and gravel served as substrate in most recordings. Observations were purely qualitatively.

Results

In all experiments a response to provided food was recorded. It became clear that the abundance of food lead to a continuous feeding activity, only interrupted by regular cleaning sessions at intervals of, on average, ten minutes. These cleaning sessions are described below in the particle feeding section.

During detritus feeding, grazing and some forms of predation the individuals actively browsed the substrate, while during predation of free-swimming animals attacks were made from a steady position, like from an ambush (sit and wait strategy).

We identified the following feeding-related activities:

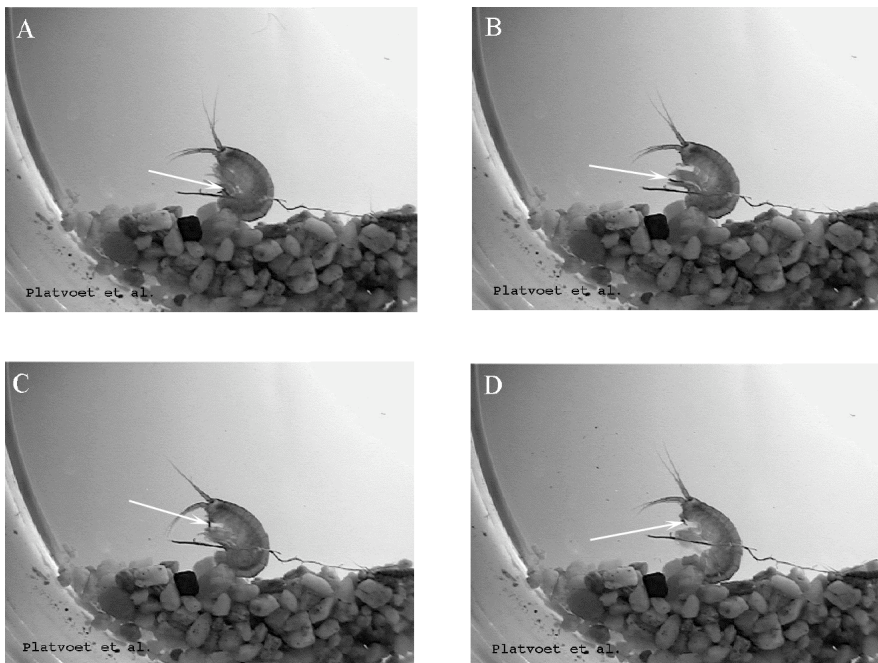
1. detritus feeding
2. coprophagy
3. grazing
4. particle feeding
5. predation of free-swimming animals
6. predation on benthic animals
7. feeding on byssus threads of Zebra mussels
8. predation on fish eggs

1. *Detritus feeding.*

In four experiments four different males were given a choice of decaying and non-decaying parts (ratio 50/50) of pondweed (*Potamogeton pectinatus*). Only feeding on decaying parts was observed, and the non-decaying parts were ignored. The first contact with the plant material was made by the first antennae carefully touching it. In a quick response the second antennae pulled the food towards the outstretched first and second gnathopods. With additional support from the antennae the food was directed toward the maxilliped to align it with the mouthparts. Finally the incisors of the mandibles cut off parts that were then moved to the molars for grinding. Fluids and particles were directed towards the oesophagus through a combined action of the inner rami of the maxilliped, the first maxillae and of both rami of the second maxillae. The feathered setae that line these parts assisted in maintaining a directed flow.

2. Coprophagy

The process of re-digestion of faeces in *D. villosus* started with bringing the urosome towards the mouthparts (Figs 1A-D). The third uropods were always in a horizontal position, ventral side up. The faeces were forced from the rectum in a quick action, lasting between two and four seconds, and then received by either the mouthparts directly or by the third uropods, after which the gnathopods lead the faeces to the mouthparts. The inside setae of the inner rami of the third uropods are lined with long, feathered setae, forming a network. The mouthparts apparently manipulated the faeces in such a way that digestible and indigestible fractions were separated, resulting in a cloud of rejected material. The whole process took about 30 seconds at 19°C. Coprophagy has been observed at least once in all experiments, in four experiments twice.



Figs 1A-D. Coprophagy by *Dikerogammarus villosus* (Sowinsky, 1894): A) the urosome is moved towards the cephalon; B) the faeces are forced from the rectum and received by the third uropods; C) the gnathopods bring the faeces to the mouthparts; D) the mouthparts check the faeces which are partly taken in and partly rejected (white arrows indicate position of faeces)

3. Grazing

In two experiments amphipods were found grazing the surface of the gravel. Such gravel may be colonized by bacteria, algae and protozoa forming a biofilm. These small gravel stones (diameter 2-5 mm) were first lifted from the substrate by the second antennae, taken over by the gnathopods and subsequently manipulated by the mouthparts (Fig. 2). To optimize the grazing process the gravel stone was turned around. Per gravel particle the grazing action lasted 11 seconds on average (S.D. 6.5 sec.; n=18). On one occasion the second antennae served as a safety net for a gravel stone that prematurely fell from the gnathopods and was immediately caught and returned to the gnathopods, after which grazing continued.



Fig. 2: Gravel particle is turned around by the gnathopods and simultaneously checked for food by the mouthparts of *Dikerogammarus villosus* (Sowinsky, 1894).

4. Particle feeding

Particle feeding, feeding on suspended organic matter including microscopic organisms, was observed throughout all sessions. In a separate study it was found that specimens of *D. villosus* removed the microscopic unicellular green alga *Monoraphidium griffithi* offered in containers. Large quantities of these algae were found in the guts of *D. villosus* (Platvoet et al., 2006).

The highly setose flagellae of the second antennae (distal whip-like parts) were regularly brought in contact with the mouthparts in a beating action, after which a raised activity of these mouthparts could be observed.

All feeding sessions were followed by a grooming session, where the body and especially the pleopods, the gnathopods and both pairs of antennae were cleaned of particles by the carpal setation of the gnathopods and by the maxilliped. Also here the grooming actions were always followed by a raised activity of the mouthparts. The body of *D. villosus* is covered by high numbers of micro-scales (tens of thousands per mm²). Between these scales micro algae, bacteria and other organisms can accumulate. The regularly observed cleaning action by the gnathopods of the body segments, followed by a raised activity of the mouthparts provided the amphipods with a continuous supply of micro-organisms, probably a welcome addition to their diet.

Predation.

Several forms of predation were observed: a) predation on benthic animals; b) predation on free-swimming organisms; c) predation on fish eggs; d) feeding on byssus threads of *D. polymorpha*.

5. Predation on free-swimming animals

Two free-swimming species were found preyed on by *D. villosus*: *Sigara* sp. and *Daphnia* sp., each in two one-hour experiments per species. Two specimens of *Sigara*, fast-swimming water bugs, were captured when getting too close to the second antennae of *D. villosus*. They were pulled in by the second antennae and eating started instantly. In one experiment a *Sigara* specimen was captured at its posterior end and brought towards the mouthparts of *D. villosus*. A sudden strong rejection of the prey could be observed, possibly the result of a chemical released by *Sigara*. Later the specimen was captured again and partly eaten.

In two experiments (with 20 specimens of *Daphnia* sp. each), predation on *Daphnia* sp. was recorded. During the first experiment eleven specimens of *Daphnia* sp. were consumed in the first thirty minutes. After one hour only three water fleas were left. In the second experiment, with another male of *D. villosus*, a total of twelve water fleas was eaten.

The free-swimming water fleas were captured by a high-speed motion of both antennae. In all cases, when the water fleas touched or came too close to the first antennae, an extremely rapid response of the antennae of *D. villosus* was induced. During the video recording with a frame speed of 20 frames/second it took four frames (fig.3A-D) from the first movement of the second antenna till securing the prey with the gnathopods. At 19 °C this action was taking a fraction of a second.

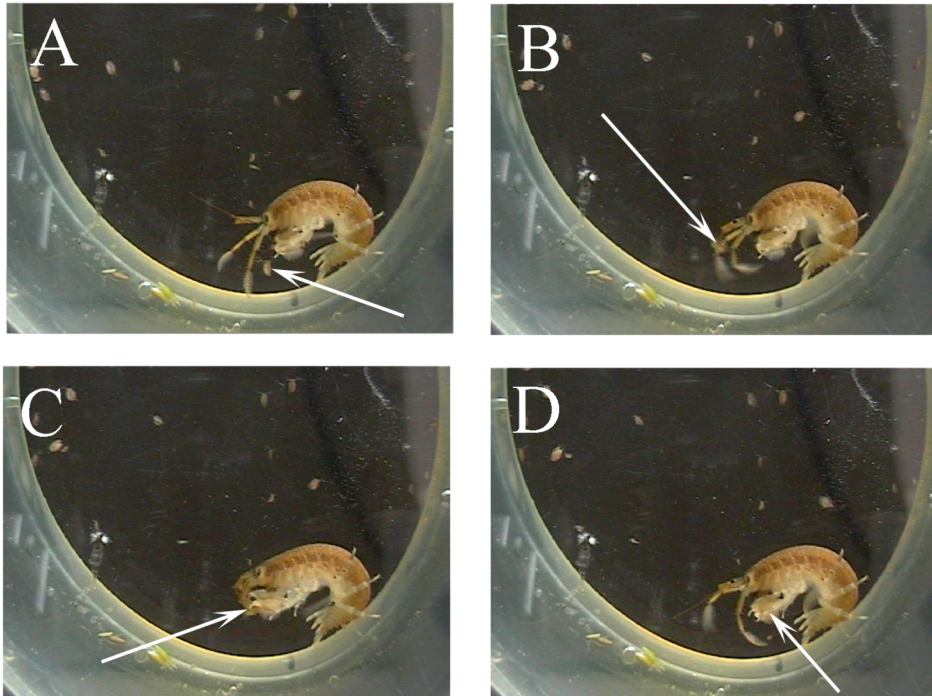


Fig. 3A-D. Preying of *Dikerogammarus villosus* (Sowinsky, 1894) on *Daphnia* sp. A) *Daphnia* sp. detected by *D. villosus*; B) flagella of the second antennae are moved towards the prey; C) second antennae bring prey to the gnathopods; D) prey secured by the gnathopods (white arrows indicate position of *Daphnia* sp.).

After the first contact of one or both of the second antennae the prey was forced into a space between the outreaching gnathopods, a space lined with the long, ornamented setae of the inner ventral edge of the propodal articles of both pairs of gnathopods. These setae form a receptive 'basket' for small prey like water fleas. It was very clear that the dactyls play no role in the initial capturing action. Only after the prey was secured in the 'basket', the subchelae of the gnathopods were used to bring the prey towards the mouthparts.

On twelve occasions *Daphnia* specimens escaped the first attack, after which a frantic search action of the second antennae followed, in seven cases leading to capture of prey. During these search movements the second antennae beat in turn, which probably increases the success rate by provoking a strong return flow in the direction of the predator, a flow that may be hard for small animals, like water fleas, to escape from.

6. Predation on benthic animals

In two experiments, each with one male and one female of *Gammarus tigrinus*, the females were preyed on by *D. villosus*. In one of the experiments *D. villosus* was able to pull a female from the substrate in which she had sought shelter. Presumably, she was discovered by the first antennae that were inserted in the interstitial spaces of the substrate. After their capture by the second antennae the two females were immediately consumed (Figs 4A-D). Both *G. tigrinus* males were ignored.

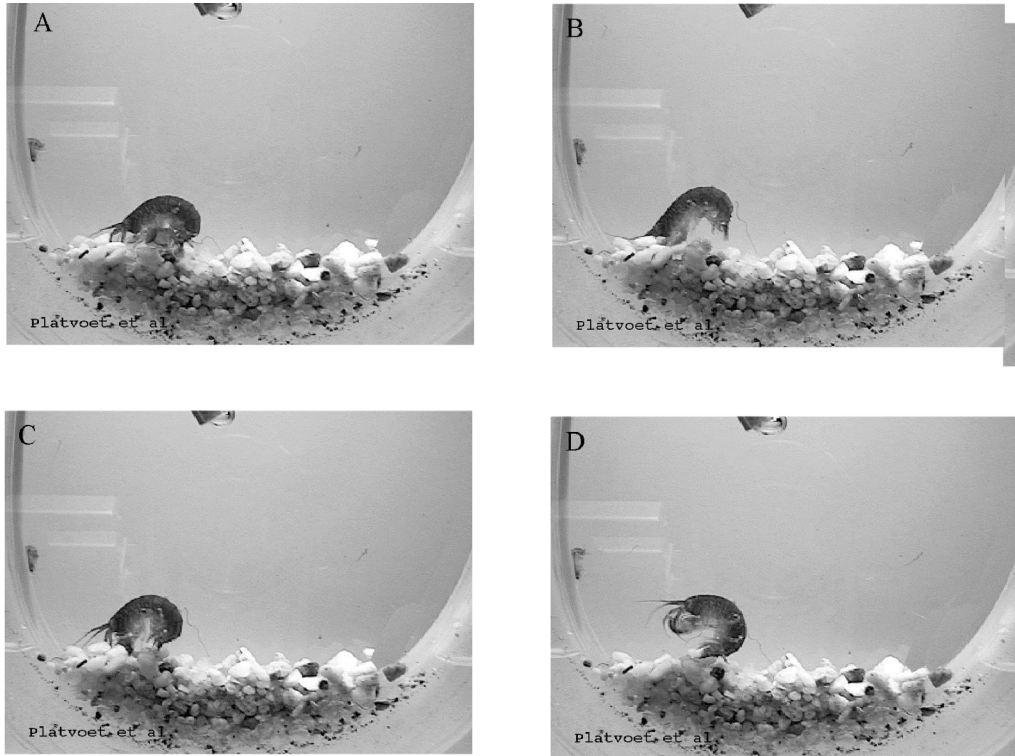


Fig. 4A-D Predation on *Gammarus tigrinus* Sexton, 1939 by *Dikerogammarus villosus* (Sowinsky, 1894): A) the substrate is checked by the antennae; B) the female *G. tigrinus*, hiding in the substrate, is detected; C) the female *G. tigrinus* is collected by the second antennae from the substrate; D) the female is immediately eaten by *D. villosus*.

In more or less similar way several specimens of *Tubifex* worms were collected from the substratum and quickly consumed. After each worm a cleaning session took place.

Also larvae of midges, *Chironomus* sp. (bloodworms), were easy prey and removed from the substrate by the digging action of the second antennae.

In three experiments, each with one specimen of *Asellus aquaticus* (Linnaeus, 1758) (2 males, 1 female, respectively), *D. villosus* rapidly captured the isopods. These slow moving animals were pulled by the second antennae of *D. villosus* towards the outreaching gnathopods. *D. villosus* started always eating that part that was closest to the mouthparts, thus without any selection with respect to body part. In the last of

these experiments, a female isopod was eaten till only one half of the body was left (Figs. 5A-D). The head with some anterior segments was released after four minutes and walked away to live on for another 15 minutes. At the end of all three experiments the specimens were almost completely eaten, and only some scattered body parts were found on the substrate. In two experiments, after capturing an isopod, the amphipod took the prey to the corner of the tank moving backwards to find shelter.

In a separate study (Dick et al., 2002) the species was found preying on zygopterans and ephemeropterans as well (Fig. 9).

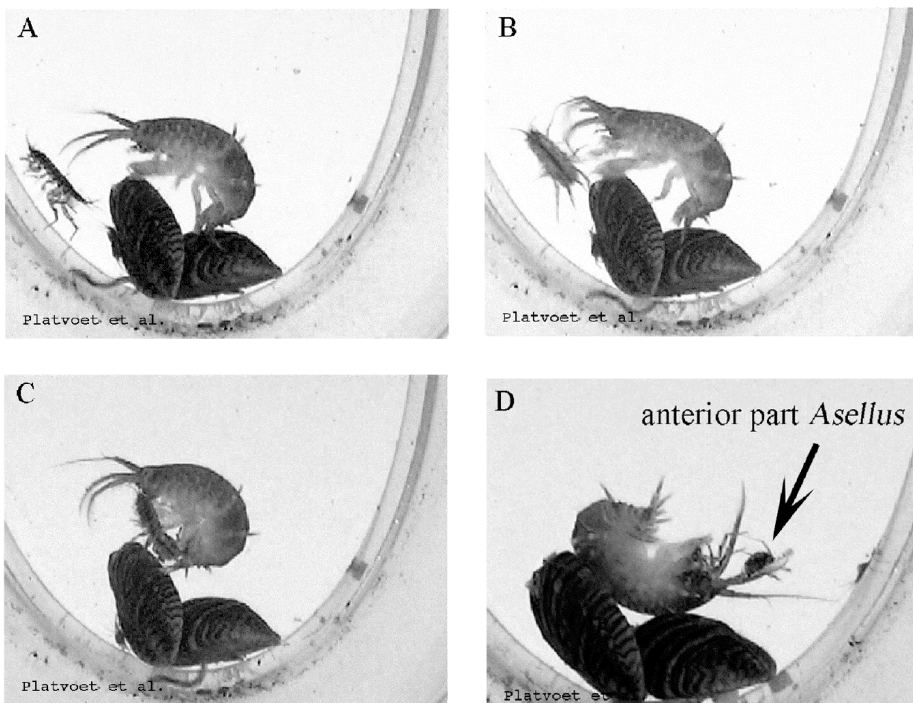


Fig. 5A-D *Asellus aquaticus* (Linnaeus, 1758) attacked and eaten by *Dikerogammarus villosus* (Sowinsky, 1894). A) *A. aquaticus* detected by *D. villosus*; B) the second antennae of the amphipod pull the isopod towards the gnathopods; C) the amphipod starts eating the isopod; D) the anterior part of the isopod body is released four minutes after capture.

Two freshly hatched fish larvae of the Bullhead (*Cottus gobio* Linnaeus, 1758 sensu lato) were immediately attacked and eaten after adding an adult male of *D. villosus* into the cuvette (fig. 8). One fish larva was eaten head-first, the other tail-first.

7. Feeding on byssus threads

In one experiment, where two small specimens of *Dreissena polymorpha* were combined with one male *D. villosus* it was found that the amphipod started to consume the byssus threads (fig. 6). These byssus threads were very translucent, indicating their recent production. For this experiment the bivalves were removed from the stock tanks and were in the process of reattachment when the experiment started and *D. villosus* was added.



Fig. 6. *Dikerogammarus villosus* (Sowinsky, 1894) feeding on freshly produced translucent byssus threads of *Dreissena polymorpha* (Pallas, 1771).

8. Predation on fish eggs

Field observation

In the field predation on eggs of *Cottus gobio* sensu lato, a bottom-dwelling fish that deposits yellow clumps of eggs on hard substrate, which are guarded by the male, was indirectly observed. After turning a boulder a clump of eggs was found covered with a number of male *D. villosus* and twelve empty eggshells.

Laboratory observation

Four eggs were taken into the laboratory and released in a cuvette. It took one minute and twelve seconds before the amphipod detected the eggs, probably by their odor, and started consuming them. The eggs were first opened by the incisors of the mandible after which the content was guided to the oesophagus through a combined action of the mouthparts as described in the detritus feeding section. Within 17 minutes all four eggs were eaten. The shells were only partly eaten, and fractions could be recognized on the substrate at the end of the experiment (Figs 7A – L).

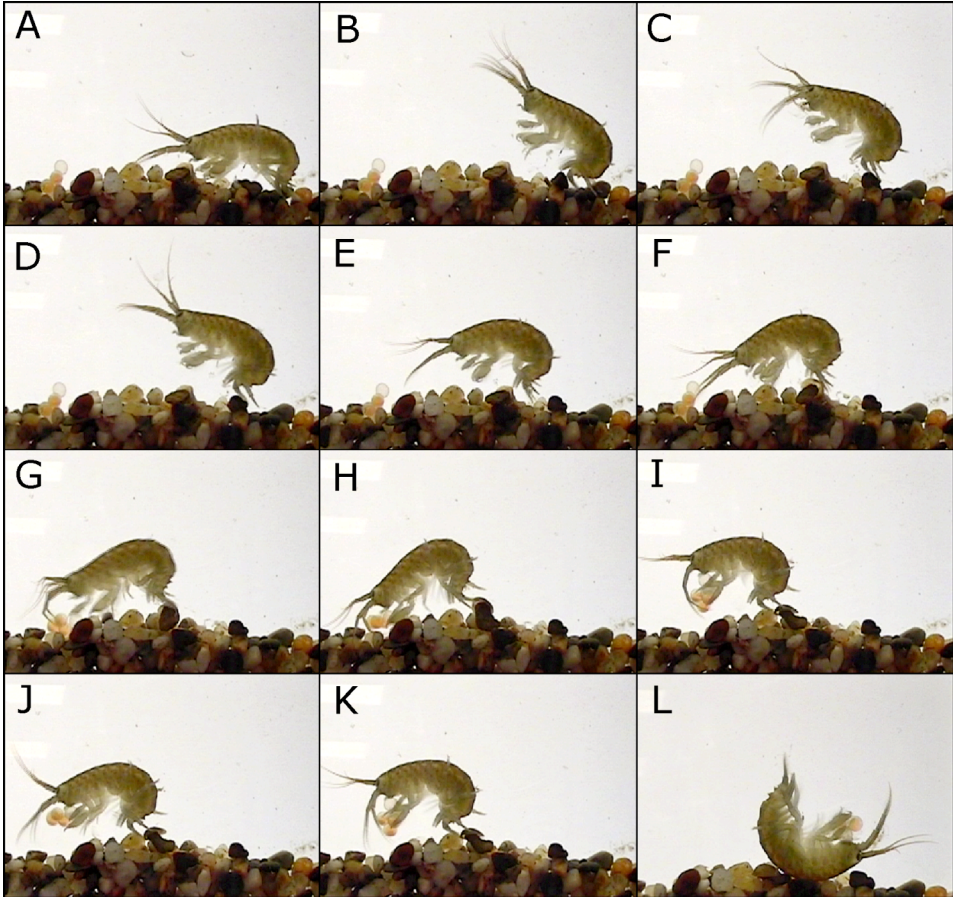


Figure 7 A-L. Male *Dikerogammarus villosus* (Sowinsky, 1894) detects (A-F), collects (G-I) and eats (J-L) four eggs of *Cottus gobio* Linnaeus, 1758.

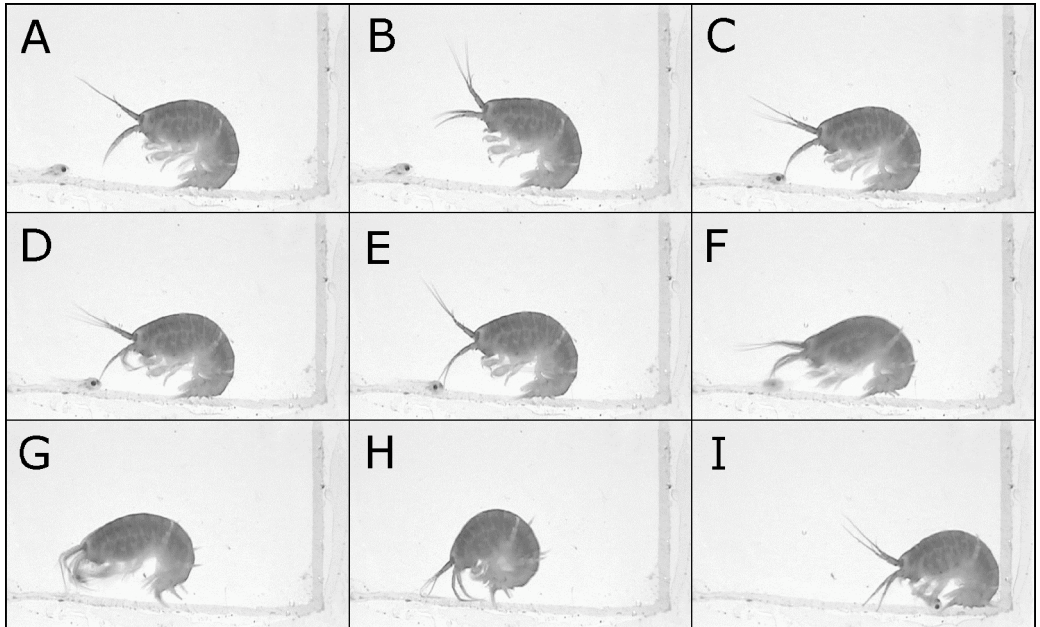


Figure 8 A-I. Male *Dikerogammarus villosus* (Sowinsky, 1894) detects (A-E), collects (F and G) and eats (H and I) a juvenile of *Cottus gobio* Linnaeus, 1758.

Discussion.

The laboratory experiments indicate many aspects of feeding in *D. villosus*. All observed feeding methods will be discussed below.

By offering both decaying and living plant material it became clear that there is a strong preference for decaying material. Detritus feeding is common amongst most freshwater gammarideans, and has labelled the members of this family as pure shredders for a long time. In fact, more and more information is emerging indicating that detritus feeding is only one of the feeding methods in gammarids.

In all sessions single or multiple coprophagy events were observed. It may be assumed that recycling of own faeces is very common in amphipods, and in fact in all animals that have to deal with limited food resources and food that is hard to digest, e.g. rabbits produce two types of faecal pellets, one of which is produced specially for recycling. The short digestive tract of amphipods is probably not able to break down

all ingested food and recycling is the answer to the potential loss of nutrition. Re-introduction of digestive agents, Vitamin K, and bacteria into the anterior gut may also play a role (Schram, pers. comm.). Coprophagy shows, as no other food-related activity, the efficiency developed in the exploitation of food and fits perfectly in an opportunistic feeding behaviour. Coprophagy is also known for isopods, such as *Asellus aquaticus* (Lammens & Van der Velde, 1978).

Grazing by *D. villosus* has been observed on many occasions and appears a normal part of the species' feeding pattern. Being able to scrape periphyton from the substrate provides the amphipod with a large food stock.

In all sessions periods of grooming by *D. villosus* followed feeding of particles. This grooming is done by specially designed setae on the carpal articles of the gnathopods that clean the antennae, pleopods and the body surface, always followed by increased activity of the mouthparts. This grooming attributes to the nutrition of the amphipods (Platvoet et al.: chapter 3, this thesis). Ponyi (1961) described filtration feeding for *Dikerogammarus haemobaphes* (Eichwald, 1841), a species living in a crevice. In the illustration of this process active participation of the antennae is suggested. This method probably is common in both *Dikerogammarus* species and, perhaps, many other gammaridean species from different families with setose antennae.

Grazing must affect the position of other settling organisms as well, and may form the steppingstone to predation of domicolous animals, like the amphipods *Chelicorophium curvispinum* (G.O. Sars, 1895) or tube building Oligochaeta and chironomids. In a study on the foodweb of the river Rhine it was found that the arrival of *D. villosus* coincided with a strong decline of *Chelicorophium curvispinum* from the surfaces of stones (Van Riel et al., 2006).

Before the occurrence of *D. villosus* in the Gouwzee in 1996, *Dreissena polymorpha*, the zebra mussel, abundantly covered most of the boulders that form the protection of the shores against wave erosion (Platvoet pers. obs.). In the years following the invasion of *D. villosus* the number of zebra mussels decreased rapidly, leaving a patchy distribution of these mussels on the rocks. The decline of zebra mussels was generally associated with major waterworks that took place during the development of a new suburb of the town of Amsterdam, named IJburg. The raised amount of silt in the water column would hinder the ventilation systems of the mussels and diminish chances for settlement of their larvae. When observing grazing in *D. villosus*, however, another explanation emerged: post-veliger larvae may be massively preyed on while settling to the substrate after their pelagic dispersal stage. There may be several factors involved in the decline of the mussels: (a) presence of *D. villosus* is abundant in the boulder zone, (b) virtually no other potential prey is present in this

zone as a result of the settlement of *D. villosus*, (c) the soft shells of the settling mussels form no match for the strong mouthparts of *D. villosus* and (d) *D. villosus* systematically grazes the surface for presence of attached organisms. After about one decade of presence of *D. villosus* in the lake, zebra mussels can still be found, but they are not as abundant as before the arrival of the amphipod (D. Platvoet, pers. obs.). The major waterworks were finished in 2001 and no major recovery of the zebra mussel populations has occurred in the Gouwzee.

During the experiments almost all offered benthic animals were attacked and eaten. This may explain the low species diversity in the boulder zone of Lake Gouwzee at presence of *D. villosus*.

At predation on free-swimming animals the second antennae are actively involved in food collecting. In contrast to the native gammaridean species the second antennae are extremely well developed. The relative diameter of the peduncle and the flagellar segments of the second antennae are much higher than in native species, indicating a strong musculature. The relative length of these antennae is also greater, giving the animal a much further reach during an attack. The rich setation of the flagellae and the similarity of these setae with Velcro can be considered to be adaptations for a predatory lifestyle.

Cottus gobio sensu lato, the bullhead is a bottom dwelling fish that prefers the same habitat as *D. villosus*. This fish species deposits clumps of yellow eggs in crevices on the substrate. In the laboratory, it was observed that in only a few seconds after adding *D. villosus* in a cuvette with a small cluster of eggs, these eggs were located and immediately eaten. In one field observation a large cluster of eggs of *Cottus gobio* sensu lato was found under a boulder, mixed with a number of *D. villosus*. Since some of the outside eggs at one side of the cluster were empty, these amphipods must have been feeding on these eggs, regardless the presence of a guarding male *Cottus*, and probably out of its view.

The observed consumption of byssal threads of *Dreissena polymorpha* may have consequences for the zebra mussel. On a local scale the mussel shows a strong mobility (Toomey et al., 2002). Every change of position is followed by the production of a new set of byssal threads, an energy consuming activity. By constant feeding on the freshly produced byssus by *D. villosus* the mussel may be hindered in its development. Furthermore, settlement of this mussel can be prevented by consumption of spat by *D. villosus*.

In most highly developed predators the prey is manipulated into a standard position before eating starts, a) to kill it quickly to avoid escape or problems during transport (e.g. birds of prey), or b) to accommodate easy ingestion (e.g. headfirst as in snakes), or c) to commence feeding on preferred body parts. No manipulation of the kind

described above was observed in *D. villosus*. Eating started immediately after capture and at any part of the body that was close to the mouthparts.

On several occasions during the predation experiments it was found that the prey was taken to the corner of the cuvette where *D. villosus* obviously sought shelter against food competitors or predators. During a habitat simulation experiment, where a grid of holes was offered to several size groupings of *D. villosus* it was found that there was a very strong correlation between specimen size and crevice size. As a result of this experiment it became clear that the animals positioned themselves in a way that both provides protection as well as accommodates a quick attack action best (Platvoet, pers. obs.). Even one of fingers of the first author was attacked and rejected, after which action the attacker returned to its 'den'. The combination of primitive predatory behaviour (no manipulation of prey) and derived behaviour (attacking from a 'den') makes it hard to determine whether the adaptation to its predatory lifestyle is a (relatively) recent development or not.

Observed morphological adaptation to a predatory lifestyle:

- a) the very well-developed second antennae play a role in virtually all feeding activities, but especially in predation (see predation section).
- b) the setae on the flagellar segments of the second antennae are hooked, probably to increase friction with a prey, analogous to Velcro (Platvoet et al., 2006).
- c) the steep allometric growth of the mouthparts allows the species to prey on intermoult invertebrates (Dick et al., 2002).
- d) the setae on the specially adapted gnathopods form a dense network that, in combination with the second antennae, facilitate capture of small free-swimming prey.
- e) the very large body-size of the males (up to 29 mm from tip of rostrum to base of telson) and the very large second gnathopods allow the species to attack large prey (Dick et al., 2002).

Like in *Dikerogammarus villosus*, gammaridean amphipods in general may have a much wider range in feeding habits than hitherto presumed, especially when becoming invasive. The large size of invaders (Stokstadt, 2001) in combination with specific allometric growth of body parts and selection can change the position of gammarids in food webs, and a harmless shredder may (temporarily or permanently) change in a formidable killer due to this 'size-effect'.

A virtually uninterrupted feeding activity of *D. villosus* was found during all experiments. As mentioned in the method section the amphipods were not starved

before the experiments, sustaining the assumption that feeding is an almost continuous process and only limited by food supply and temperature depending metabolism.

In his paper, Monakov (2003) mentions six categories of feeding according to food type: 1) phytophages, 2) phyto-detritophages, 3) detritophages, 4) phyto-zooprophages, 5) zoo-phytophages and 6) zoophages. The feeding methods and food choice found in *Dikerogammarus villosus* demonstrate that this species fits into all of these six categories, depending on the availability of food resources. Therefore, we hypothesize that such a classification is only applicable in stable biotopes, but not in situations where recent invaders form a large portion of the biomass. In this situation a shift in presence and niche occupation of species takes place, resulting in an alteration of the menu for most of the animals.

The observed feeding related activities of *Dikerogammarus villosus* in the laboratory are indicative for an opportunistic lifestyle in which virtually all available food resources contribute to the species' survival. Our observations are coherent with the present field distribution of *D. villosus* in Western Europe and reflect the strong interactions of this invader with indigenous and already established alien fauna, found in earlier studies. In dynamic ecosystems with recent invaders categorizing species according to food choice is not possible, due to (sometimes forced) shifts in menu.

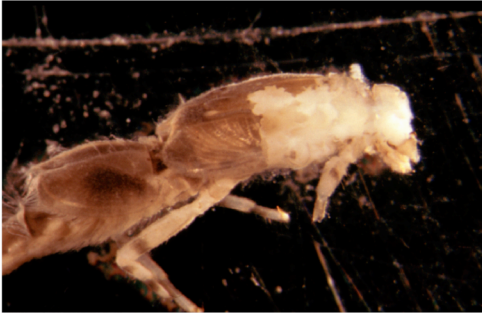
This is CWE publication nr. 496

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Caenis robusta



Asellus aquaticus



Sigara sp.



Ischnura elegans

Fig. 9. Several victims of *Dikerogammarus villosus* (Sowinsky, 1894) with bitewounds.

Chapter 3

Feeding on micro-algae in the invasive
Ponto-Caspian amphipod
Dikerogammarus villosus (Sowinsky,
1894).

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&
Gerard van der Velde.

Abstract

Feeding on micro-algae is shown in the invasive Ponto-Caspian amphipod *Dikerogammarus villosus*. Compared to controls, males, females and juveniles of this species significantly reduced the concentration in suspension of unicellular micro-algae. Juveniles had higher concentrations of algae in the cardiac gut than adults. The presence of these algae in the mid- and hindgut was also recorded. This feeding behaviour was filmed and the mechanisms involved are described and discussed. We comment on the use of the Functional Feeding Group (FFG) concept to classify feeding in amphipods. The role of being a feeding generalist in aiding the invasion process is also discussed.

Introduction

Most members of the amphipod family Gammaridae have long been considered, in regard to their feeding habits, primarily as shredders of detritus, with some recognition of omnivory (MacNeil et al. 1997). Although feeding on detritus is important, there is a growing number of observations of feeding on other types of food (Dick and Platvoet 2000), which has led to a reconsideration of the position of these amphipods in food webs (Kelly et al. 2002a,b, 2003). In the Ponto-Caspian amphipod *Dikerogammarus villosus* (Sowinsky 1894) which is invasive in western Europe (Dick and Platvoet 2000, Bij de Vaate et al. 2002), and is able to survive ballast water conditions (Bruijs et al. 2001), we observed the following feeding mechanisms: carnivory, detritus feeding, grazing and coprophagy. Thus far, particle feeding on micro-algae has not been described for this species. While there is evidence that amphipods ingest micro-fauna and micro-flora (Hargrave 1970; Gowing and Wishner 1992; Gladyshev et al. 1999, 2000; Sushchik et al. 2003), this has rarely been demonstrated (Blinn and Johnson 1982). Schram (1986) mentions several filter feeding methods in non-gammarid amphipods in which antennae, mouthparts, and pereopods are involved. Gruner (1993) described a 'passive' filtering method in which particles are collected by setose antennae (*Haploops* sp.), and an 'active' filtering method, in which the amphipod's ventilation current is used in combination with setose pereopods (*Leptocheirus* sp.). This ventilation current is the result of an almost constant beating action of the three pairs of pleopods (Fig. 1) as described by Dahl (1977). Crouau (1989) mentions a method of filter feeding for Mysidacea in which involvement of pereopods, maxillulae and maxillae is suggested. Holmquist (1982) describes grooming for talitroids and summarizes those activities of the

animals that are negatively influenced by lack of grooming: 1) respiration (epizoites hinder gas exchange); 2) locomotion (damage to the pleopodal setation inflicted by epizoites diminishes locomotory abilities); 3) sensation (chemo- and mechanosensitivity is affected by clogging up of pores and hindering movement of the sensillae); 4) water uptake in terrestrial species. In *Talitroides* sp. all the material 'gleaned from the body is eventually handled by the mouthparts', after which the animals either reject the material or ingest it (Holmquist, 1989)

Particle feeding is common in organisms that inhabit tubes, nests and other domiciles (Anderson, 1994). *D. villosus* is primarily found among zebra mussels or in crevices, where it keeps its antenna stretched out, thus showing a form of domicolous behaviour.

Determination of feeding on micro-algae in the family Gammaridae is important for recognising their true 'functional' role in food webs. In addition, because the present species is highly invasive, we require understanding of its basic biology and the reasons for being a successful invader. In this laboratory-based study, we focussed on determination of the rate of clearance of a known concentration of micro-algae and the resulting effect on gut contents. Several possible mechanisms involved in the ingestion of micro-algae are described

Materials and Methods

Specimens of *D. villosus* and water were collected in May 2003 from the Gouwzee, a part of the Markermeer (The Netherlands). The shallow lake has a soft bottom consisting of shell debris, coarse sand and clay. The shores are protected against wave erosion by basalt rocks, which form the primary habitat for *D. villosus*. The animals were sampled with hand nets and immediately transferred to the laboratory where they were kept in well-aerated containers of 65 x 90 x 15 cm with 5 cm of lake water at a temperature of 19°C. Substrate of the containers consisted of debris from the sampling site. The light/dark regime was synchronized with the prevailing day and night rhythm (17h:7h light:dark).

Algae feeding experiment.

Three size-groups of animals were used: 14 adult males (length, 15 - 20 mm), 14 adult females (length, 10 - 12 mm), and 14 juveniles (length, 3 - 5 mm). Single individuals were put in separate plastic containers (8 x 8 x 15 cm) with 70 ml of filtered water from the lake (filter paper: pore range 7-12µm), plus a control group of 14 containers without amphipods, all at 19°C. The chlorophyte micro-alga *Monoraphidium griffithii* (Berkeley) was added to a concentration of 5×10^7 cells ml⁻¹

in each of the 56 replicates. These unicellular algae are 30 - 150 μm long and abundant in the plankton and littoral zone of lakes (Streble and Krauter 2002) and in the Gouwzee (own observations). No additional algae were added during the seven days of the experiment. Strong aeration of the containers prevented settling of the algae. Evaporation of water was compensated for by adding demineralised water (to avoid salinity chances). After seven days, the concentration of alga cells was estimated in each replicate using a haematocytometer ($0.0625 \text{ mm}^2/0.2 \text{ mm}$ depth). Mean number of algal cells remaining was examined in a One-Factor ANOVA with respect to males, females, juveniles and control groups, with pair-wise comparisons of means with the Fisher Protected Least Significant Difference Test.

Gut contents.

Once the feeding experiment was completed, the gut content of 5 males, 5 females and 5 juveniles was determined by extracting samples from the cardiac stomach, mid- and hindgut from each animal. These samples were separately squashed between a slide and cover glass. For the cardiac stomach, the number of algal cells ml^{-1} was estimated by averaging counts of 5, randomly chosen, full frame microscope views per sample (diameter; 0.173 mm; sample depth: 0.013 mm; volume per frame $3 \times 10^{-4} \text{ mm}^3$). The depth of the samples was calculated for a known quantity of water ($1.5 \text{ mg} = 1.5 \text{ mm}^3$ at $20 \text{ }^\circ\text{C}$) and the resulting surface area under a cover glass (112 mm^2): $1.5/112 = 0.013 \text{ mm}$. The concentration was expressed in number of alga cells ml^{-1} .

Means were examined in a One-factor ANOVA with respect to males, females and juveniles, with FPLSD post-hoc tests. Since the other part of the gut contained only fractures of the algal cells, only presence or absence of these fractures was scored.

Photography and filming.

With a Scanning Electron Microscope (JEOL JSM 35C) the microstructure of relevant body parts was recorded. The specimens were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer and dehydrated in an ethanol series, critical point dried and covered with gold.

Five males of *D. villosus* were filmed with a digital video camera (JVC GR-DVL100) in order to determine feeding mechanisms. For this, a cuvette consisting of two glass plates separated by a plastic hose was used, containing 30 ml of water with micro-algae in a concentration as in the above feeding experiment.

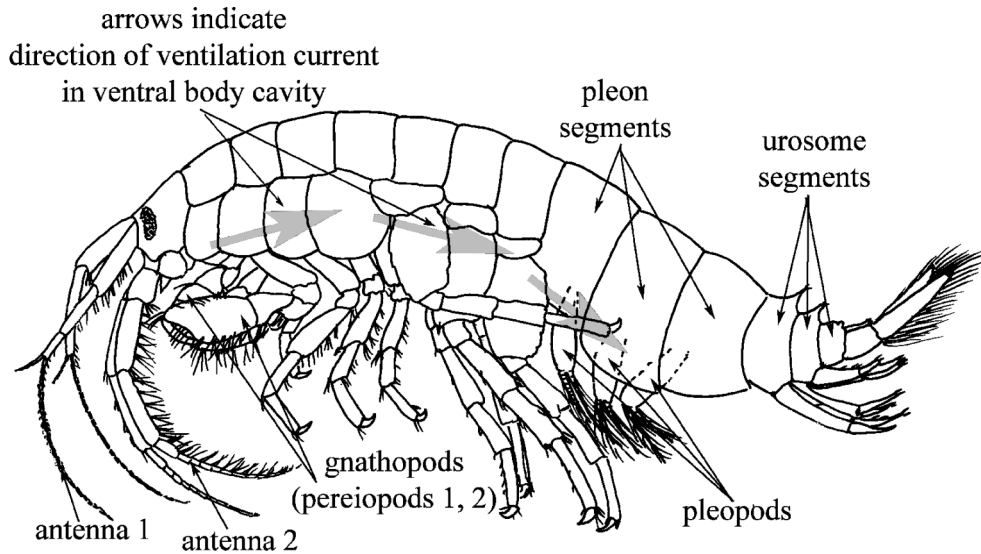


Fig. 1. Outline sketch of *D. villosus* showing the direction of the water current that results from the constant beating action of the three pairs of pleopods

Results

Alga feeding experiment

The mean number of micro-alga cells remaining in containers among the four groups ($F_{3,43} = 10.3$, $p < 0.001$) differed significantly, with all three *D. villosus* groups significantly reducing the algae remaining as compared to the control group ($p < 0.01$ in all cases; Figure 2). No significant differences between the replicates within each amphipod group were found.

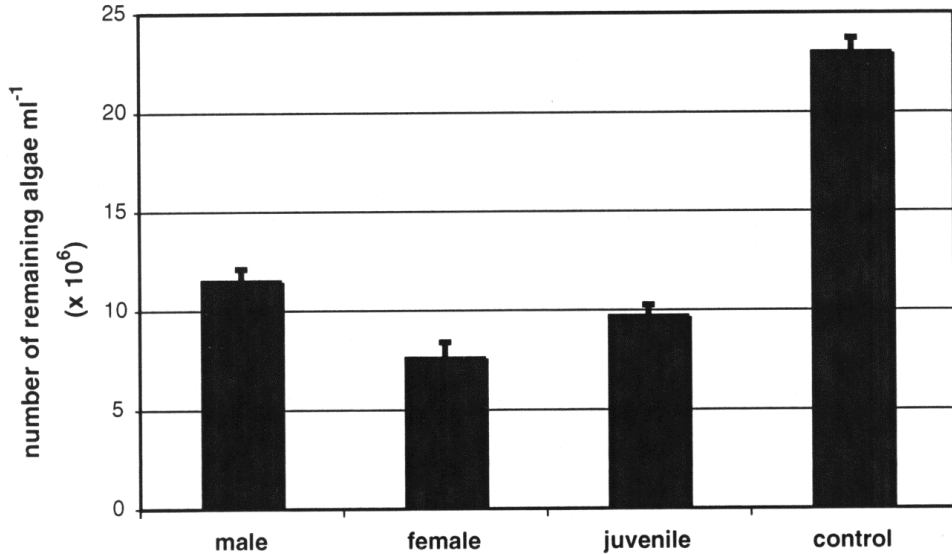


Fig. 2. Mean number (+SE) of micro algae remaining in containers after 7 days with, or without *D. villosus*

Gut contents

D. villosus clearly consumed the algae (Figures 3 and 4). There was a significant difference among males, females and juveniles in mean numbers of intact algae of cardiac stomach samples ($F_{2,12}=3.51$, $p<0.05$; Figure 3), with significantly more concentrated algae in juvenile than male ($p<0.03$) and female ($p<0.05$) stomachs (Figure 3). The mid- and hindgut of specimens contained only fragments of algae, a strong indication of digestion.

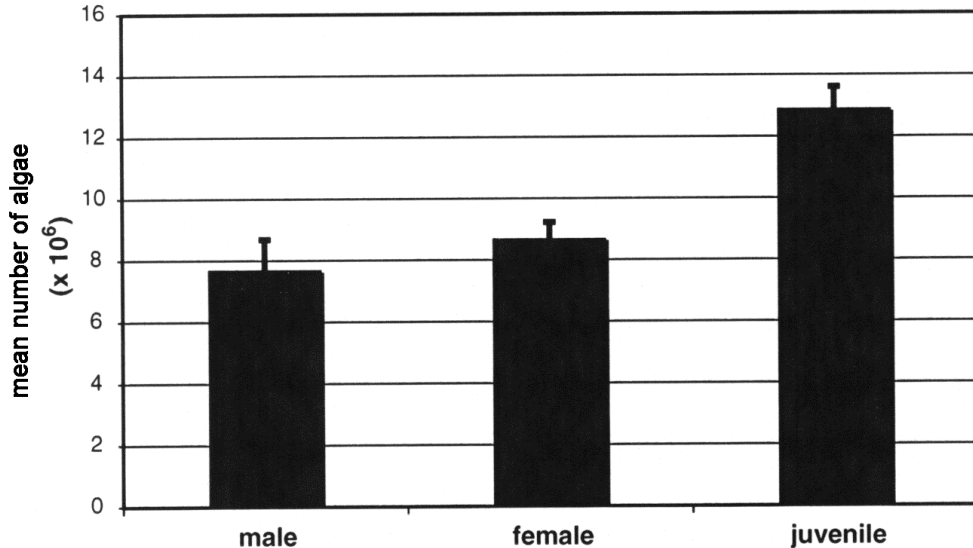


Fig. 3. Mean number (+SE) of algae/0.01 mm² of squashed cardiac stomach samples from *D. villosus*.

Photography and filming.

The SEM pictures revealed various setal structures. The long setae on the second antennae and on the propodi of gnathopods 1 and 2 have the irregularly placed lateral filaments in common. In the merus and carpus of the gnathopods, specialized grooming setae were found (Figure 7), matching those found in the third maxilliped of *Petrolisthus galathinus*, an anomuran decapod (Bauer, 1989).

The filming of specimens presented with algae shed light on the techniques involved in the uptake of micro-algae. The specimens were found grooming their antennae, gnathopods, pleopods, uropods and body surface on a regular basis. A summary of the found grooming activities in *D. villosus* is given below.

Antennae

The setation of the second antennae is more abundant than in males than in females, but in both sexes these long, thin setae are provided with small, irregularly placed lateral filaments. These flagellar setae (Figure 5) are of the same structure as

those found on the palms of the gnathopods. An exception is the distal part of the shortest inward directed setae, the apices of which have the form of a small hook, probably to increase friction between antennae and prey. The assemblage of setae on both antennae, and gnathopods may function as a particle-collecting device.

For the antennae two methods of grooming were observed:

Method 1: the left and right antennae were one by one held by the corresponding first gnathopods (Figure 6a-d), after which the special comb-like setae (Figure 7a-d) on the merus and carpus of the corresponding second gnathopod were used to clear the setae of particles. This grooming of the antennae was always followed by increased activity of the mouthparts, cleaning the gnathopods.

Method 2: the flagellae of the antennae were brought directly towards the mouthparts, resulting in increased activity of these appendages. The maxillae (especially the first) and the maxilliped have adaptations for grooming. The inner edges are provided with rows of strong setae or spines that can act as combs. The second maxillae, in combination with the upper and lower lip, sweep particles towards the oesophagus.

Gnathopods.

Grooming of the setae on the palms of the propodi of the gnathopods was observed regularly. These setae, forming a fine mesh, are located in the ventilation current, an ideal place to collect particles. Grooming is performed by a combined action of gnathopods in which the left gnathopods cleans the right and vice versa, as well as the mouthparts. Grooming setae on the meral and carpal segments (Figure 7) of the gnathopods play a major role in this.

Pleopods and uropods.

During a strong inward flexing of the body, the pleopods were bundled by the subchela of one of the gnathopods and then cleaned by stretching the body resulting in a proximal-distal pleopod movement through the palm of the gnathopod. This action was repeated 3 to 4 times within a few seconds. After this, the gnathopods were moved towards the mouthparts, resulting in an increase in activity of these appendages. Likewise the uropods were fixed and cleaned.

Body surface.

The animals were regularly observed moving the gnathopods along the sides of the head region and the anterior body segments (Figure 6). After a strong inward flexing of the body the gnathopods could also reach most of the posterior body surfaces. Grooming probably is performed by the specialized setae on the meral and carpal segments of the second gnathopods. All these activities were followed by an increased activity of the mouthparts.

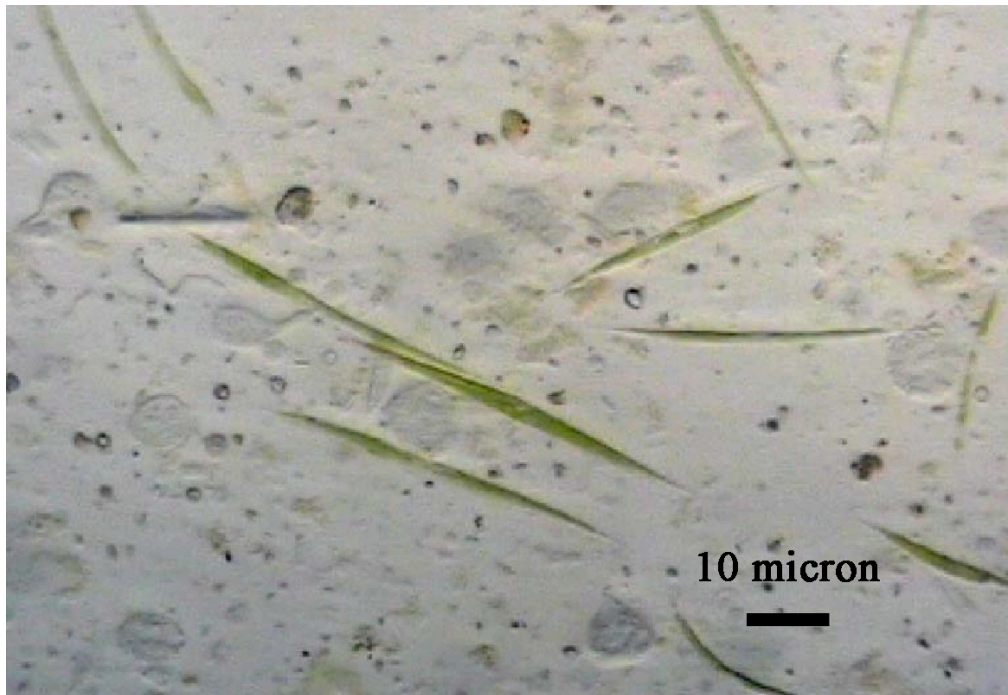


Fig. 4. Intact algae in squashed sample from cardiac stomach of *Dikerogammarus villosus* (photography Jan van Arkel/IBED).

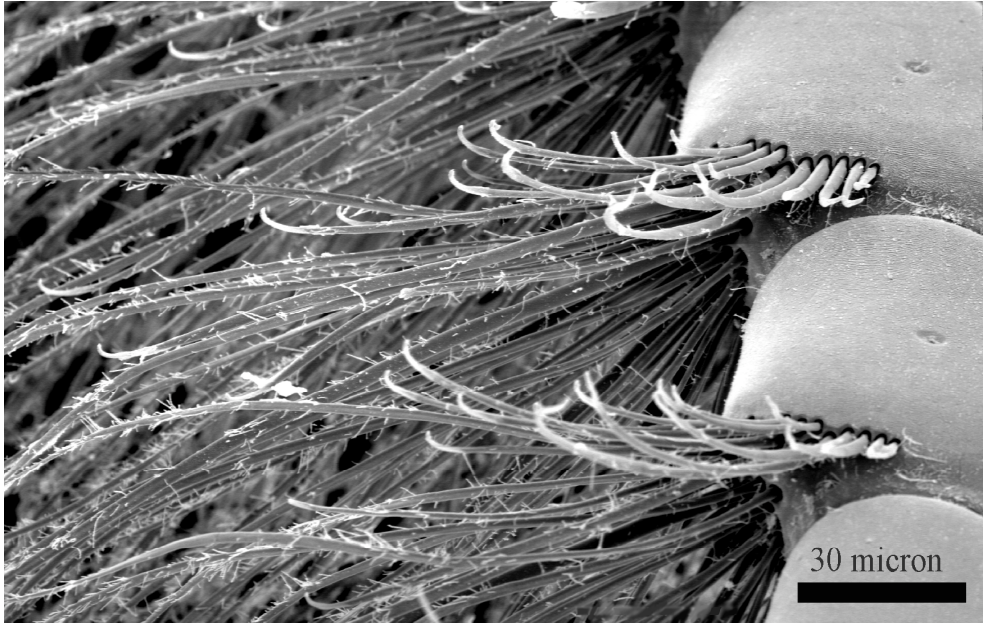
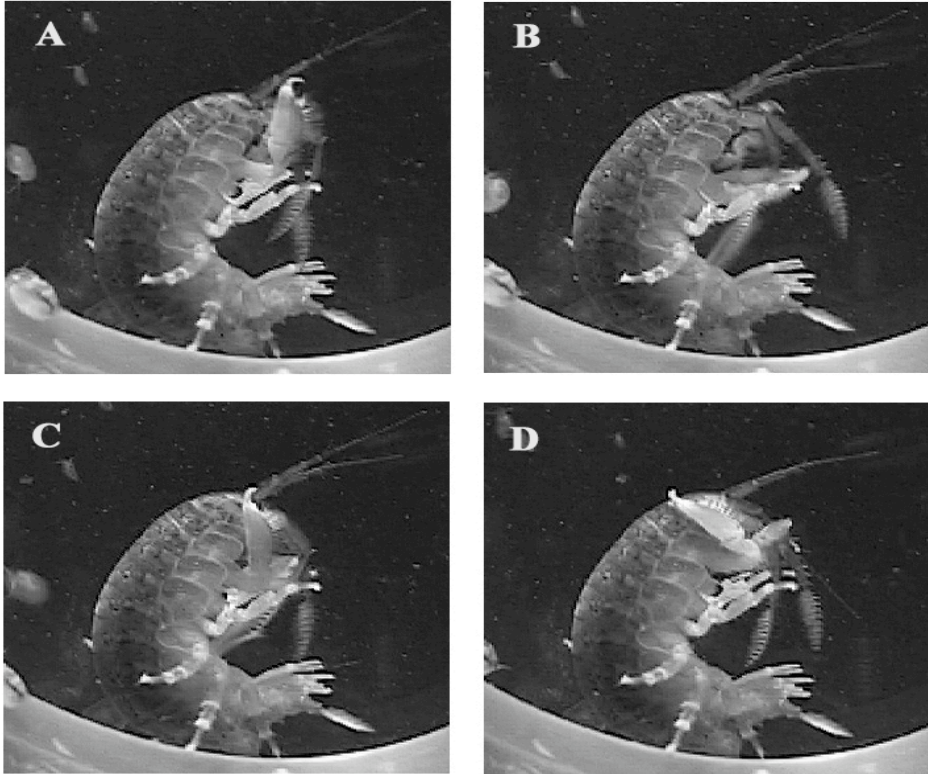


Fig. 5: Setae on the inner margins of the flagellum of antenna 2 of *Dikerogammarus villosus*.



Figs. 6: Grooming of antennae and head region by gnathopods in *Dikerogammarus villosus*.

(a) – (c): the right second antenna is fixed by left second gnathopod, and cleaned with the specialized setae of the merus of the right second gnathopod (see figure 1); (d) the head region and first body segments are cleaned by the second gnathopod

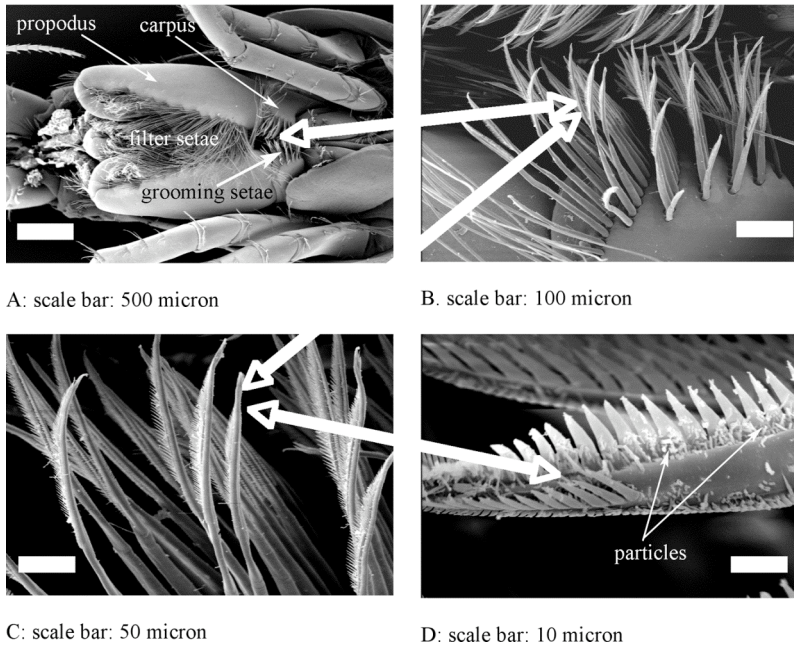


Fig. 7. Grooming setae on merus and carpus of gnathopods 2 of *D. villosus*: upper left, ventral view of gnathopods and mouthparts (scale bar = 500 μm); upper right, ventral view of setae on carpus of gnathopods 2 (scale bar = 100 μm); lower left, close up of setae (scale bar = 50 μm); lower right, detail of distal part of seta (scale bar = 10 μm , arrows indicate particles)

Discussion

After its first discovery in the Netherlands in 1994/95 (bij de Vaate and Klink 1995), *D. villosus* strongly extended its range in large parts of the country's fresh and brackish waters. It almost completely replaced at least one gammarid species, *Gammarus duebeni* Lilljeborg, and had a predatory impact on several other invertebrates (Dick and Platvoet 2000; Dick et al. 2002). *D. villosus* has now invaded throughout Europe (Devin et al. 2003; Müller et al. 2002; Kley and Maier 2005; Krisp and Maier 2005). Determination of the ability to feed on micro-algae in such an amphipod is important towards both recognising the true 'functional' role of Gammaridae in general in communities and assessing the success and impact of this particularly damaging invader.

This study provides indications of the ability of *Dikerogammarus villosus* to collect, consume and digest micro-algae. We found not only significantly decreased concentrations of alga cells in containers in the feeding experiment, but also clear signs of digestion (fractured cells in the mid- and hindgut). The juveniles that are considerably smaller in length than adults removed more algae and had significantly higher concentrations of alga cells in the gut. This indicates that juveniles may specialize in feeding on algae compared with adults which are predatory in nature (Dick et al. 2002). Such developmental shifts in diet are known in insects, which may move from herbivorous to carnivorous feeding as development proceeds (Cereghino 2002). Furthermore, although it is tempting to conclude that the consumption of the algae by *D. villosus* was suspension feeding, it is equally likely that much of the ingestion was a by-product of grooming of the body, especially by the antennae and the gnathopods that seem to be well fit to collect particles from the water. The existence of specifically directed and deliberate algae feeding still not clear.

D. villosus cannot be put into one Functional Feeding Group as a shredder (see Cummins 1973, 1974; Cummins and Klug 1979), but may fit several (filtering collector, gathering collector, scraper and grazer, shredder, scavenger, omnivore and predator). Placing a species into such functional groups may have little use if species can switch between FFGs seasonally, or due to their developmental state or food supply. Thus, FFGs can only be applied in combination with a thorough knowledge of the feeding habits of the organisms involved. Many other amphipod species are likely to have a much wider feeding range than assumed (MacNeil et al. 1997).

Cole and Watkins (1977) found structural differences between the planktonic amphipod *Hyaella montezuma* and other Talitroidea. Especially the maxillae of *Hyaella* are armed with feathered setae, a common feature in the family Gammaridae. If the presence of these setose maxillae indicates particle feeding, than such particle feeding exists in many, if not all members of the Gammaridae. We used only suspended micro-algae, but in some crustaceans the locomotory action of the thoracic limbs simultaneously creates the currents needed for suspension feeding (Brusca and Brusca 1990). The constant ventilation current produced by the pleopods, in combination with the presence of specialized filter setae on the first two pereopods in particular, and the frequent grooming of these setae, make filter feeding in *D. villosus* very likely. Also the action of the setose antennae must contribute. Important faunal elements like gammarids, forming up to 90% of the macrofaunal biomass (MacNeil et al. 1997) could never have become as successful as they are with only highly specialized feeding behaviour. This and other studies (Dick and Platvoet 2000; Dick et al. 2002) show the ability of *D. villosus* to exploit virtually all kinds of nutritional

sources present, allowing this extreme opportunist to invade practically any new fresh and brackish water system (Bruijs et al 2001). If the animal is limited in its distribution, it is probably not food that is the constraint. Of greater importance are factors, such as temperature, ionic content of the water (Wijnhoven et al. 2003a, 2003b), physical barriers, presence of controlling organisms and especially substrate structure.

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Chapter 4

Interactions between two invasive species,
Dikerogammarus villosus (Sowinsky) and
Gammarus tigrinus Sexton
(Crustacea, Amphipoda)

Dirk Platvoet, Jaimie T. A. Dick, Calum MacNeil, Mariëlle C. van Riel &
Gerard van der Velde

Summary

1. Invasive gammaridean amphipods have replaced native species on numerous occasions all over the world. These replacements have not been studied in much detail.
2. The invasion of the Ponto-Caspian amphipod *Dikerogammarus villosus* in Lake Gouwzee (The Netherlands) provided an opportunity to examine the replacement process and its mechanism. In this lake *Gammarus duebeni* and *Gammarus tigrinus* co-occurred in the littoral boulder zone, while *G. tigrinus* was also found in the soft sediments. The arrival of *D. villosus* in this lake coincided with the total disappearance of the indigenous *Gammarus duebeni* from the littoral boulder zone and with the presence of the earlier North American invader *Gammarus tigrinus* limited to the lake's soft sediments.
3. Various degrees of habitat complexity of the lake's shoreline, such as rock/crevice/hole presence and size, were simulated by means of containers with horizontal Plexiglas plates with a grid of holes of various sizes. This setup was used to examine how habitat preference, cannibalism and intraguild predation (IGP) between *G. tigrinus* and *D. villosus* may have influenced the changing dynamics and distribution of their respective populations.
4. Individual *D. villosus* and *G. tigrinus* positioned themselves in the smallest possible hole size that would accommodate their body size. The presence of holes decreased both cannibalism and mutual predation. These two species showed similar behaviour and resource preference, so competition for sheltered places in relation to body size plays a decisive role in their distribution. IGP is a way to reduce competition with *G. tigrinus* as the weaker party in the presence of *D. villosus*.
5. The minimum size of ovigerous females was similar for *D. villosus* and *G. duebeni*, while *G. tigrinus* matured at much smaller size. The small size at which *G. tigrinus* matured allowed its sustainability in the soft sediments, because it can also shelter there. For *D. villosus* and *G. duebeni* with their large minimum reproductive size, the small spaces of the sediments seem inaccessible, and it is therefore suggested that these species are totally restricted to the boulder zone, where *D. villosus* out-competed *G. duebeni* through IGP.

Key words: littoral zonation, habitat choice, simulation grid, invasive species, *Dikerogammarus villosus*, *Gammarus tigrinus*, Intra Guild Predation.

Introduction

Biological invasions occur all over the world and can have ecological impacts at all levels of ecological organization. Introduced species can impact on native species by competition, herbivory, predation, parasitism, vectoring of pathogens, and through physical or chemical modifications of habitats. When introduced species function as keystone species (or ecosystem engineers) they affect the functional diversity and food web structure of communities (Vitousek, 1990, Ricciardi, Whorisky & Rasmussen, 1997, Crooks, 2002, Van Riel *et al.*, 2006).

Crustaceans are among the most successful invaders, and often replace each other as a result of interspecific competition (Schoener, 1983). For example, several non-indigenous species of amphipods have replaced native species in various regions in the world (Dick & Platvoet, 2000, Van der Velde *et al.*, 2000, 2002, Van Overdijk *et al.*, 2003, Jazdzewski, Konopacka & Grabowski, 2004, Meyer, Kaschek & Meyer, 2004). The Irish *Gammarus duebeni celticus* was displaced over much of its range by the introduced congeneric species *G. pulex*, probably as a result of predation on moulted females of the former species by males of the latter (Dick, 1992).

The river Rhine is an example of a heavily invaded river that also shows amphipod replacements. Canals connecting rivers are important pathways for invaders, especially as they connect formerly separated biogeographical regions (Bij de Vaate *et al.*, 2002). The catchment basins of the rivers Danube and Rhine have been connected since the opening of the Main – Danube Canal in 1992. This has led to an increasing number of Ponto-Caspian species invading the Rhine drainage system (Schleuter *et al.*, 1994, Bij de Vaate *et al.*, 2002, Van der Velde 2002, Bij de Vaate, Breukel & Van der Velde, 2006). One of these species, *Dikerogammarus villosus*, is now widely distributed in Western Europe (Giesen, 1998, Reinhold, 1999, Devin *et al.*, 2003, Jøsen et al., 2005).

Understanding amphipod replacement processes requires a combination of field observations and experiments. In this study we focussed on behaviour and substrate structure in relation to the distribution of *D. villosus* and the North American *Gammarus tigrinus* along the littoral border of a Rhine-fed lake, Lake Gouwzee.

The following research questions were addressed:

- a) What is the distribution of the gammaridean species along the littoral border of the lake in relation to substratum structure and zonation?
- b) What is the role of microhabitat structural complexity in relation to the body size of the amphipods?
- c) Can the distribution be explained by interactions between the species?

Methods

Study site

The site we studied, Lake Gouwzee, is part of the Markermeer/IJsselmeer complex of lakes (Fig 1A). The borders of Lake Gouwzee are artificially protected from wave action by imported stone boulders. Before *D. villosus* entered the Gouwzee, the native *G. duebeni* and alien *G. tigrinus* coexisted in the boulder zone, while *G. tigrinus* was also found in the soft sediments and zebra mussel (*Dreissena polymorpha*) beds on the bottom of the lake. *D. villosus* has now replaced *G. duebeni*, which species completely disappeared, and *G. tigrinus* on hard substrates, probably through intraguild predation (Dick *et al.*, 2002). There are considerable differences in body size between these amphipod species, *D. villosus* being the largest.

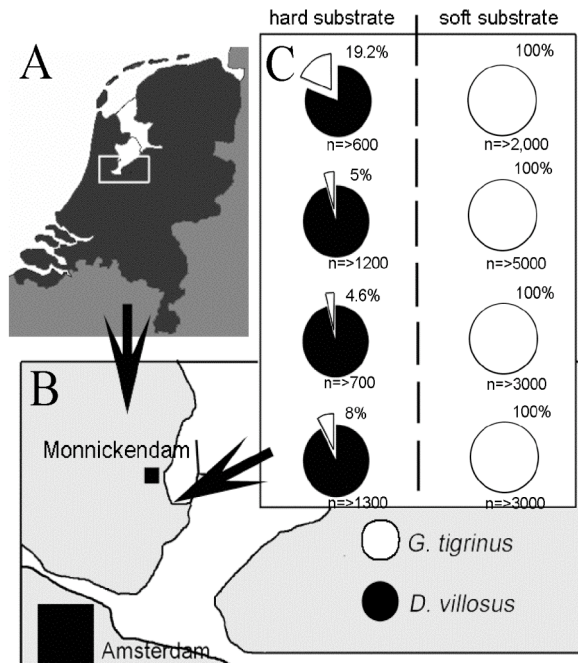


Figure 1. Distribution of *Gammarus tigrinus* and *Dikerogammarus villosus* at a 200 m stretch of shore of Lake Gouwzee, the Netherlands. Each pie diagram represents average percentage of 5 kick samples of 2 minutes each. Percentages for *G. tigrinus* only.

Field study

Dikerogammarus villosus and *Gammarus tigrinus* were collected by kick sampling the littoral shores of Lake Gouwee, part of the Markermeer/IJsselmeer complex, The Netherlands (Fig. 1), in June/July 2004. Ambient water temperature was 17°C and conductivity 850 $\mu\text{S}/\text{cm}^{-1}$.

Experiments

Specimens of the two species for experiments were maintained separately in large aquaria in the laboratory at 19°C with flora and fauna from Lake Gouwee and allowed 1 day to acclimate.

Experiment 1 – do amphipods optimize hole choice by size?

Experimental aquaria and grids

Plastic aquaria of 20 x 20 x 8cm (length x width x height) were supplied with lake water and, on the bottom, a plexi-glass grid of 20 x 20 x 2cm (length x width x height) with 81 (9x9) randomly distributed cylindrical holes in 7 diameters: 3, 4, 5, 6, 7, 8 and 9mm. The diameter/depth ratio was 0.5, so the respective depth of the holes was 6, 8, 10, 12, 14, 16 and 18mm. This ratio was derived from the dimensions of occupied spaces in and between rocks, between zebra mussels, as well as in the different sediments of the lake bottom. All experiments took place at a water temperature of 19°C.

Body size data

Dikerogammarus villosus were sorted into three size groups by body length, taken from the base of the antennae to the base of the telson: (1) 3-5mm; (2) 8-12mm; (3) 16-22mm. *Gammarus tigrinus* were sorted into two size groupings: (1) 3 – 5 mm; (2) 8 – 12 mm. The size groupings of both species represented the size groupings present in the lake at the time of the experiments. For 10 specimens of each species in each group we measured, with digital calipers, maximum lateral width, maximum dorso-ventral width and maximum ‘c-shape’ body height (animals curled) (see Table1). From this, we determined that size group 1 animals of both species had access to all holes, size group 2 animals to 4-9mm holes and size groep 3 animals to 6-9mm holes. We further confirmed this by placing 10 animals of each size group in separate tanks with grids that had their accessible holes blocked – no animal took up residence in holes deemed inaccessible as above.

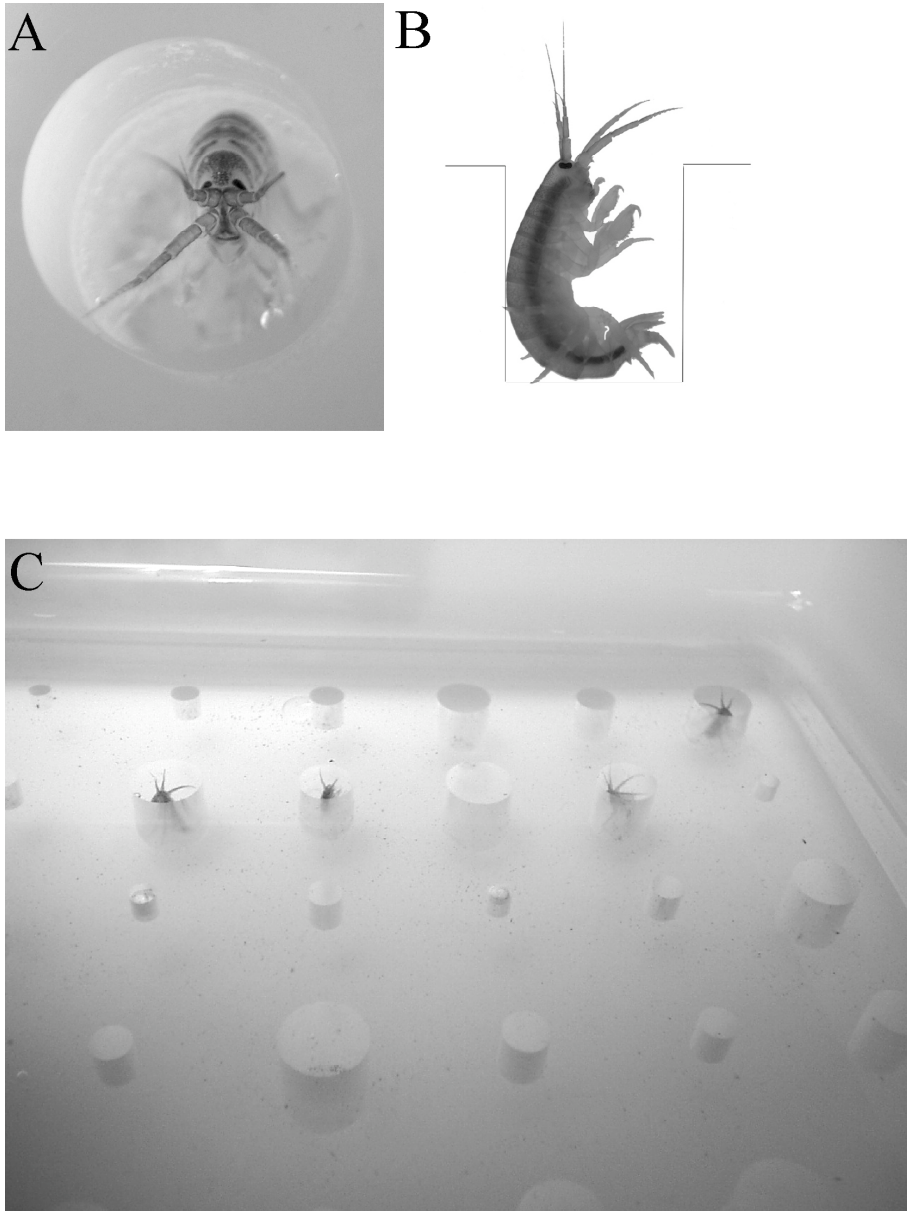


Figure 2. *Dikerogammarus villosus* inside holes during experiment: a) frontal view; b) lateral view; c) overview. The position of *Gammarus tigrinus* is similar.

Experimental Protocol

For each of the three size groups for *D. villosus* and the two size groups for *G. tigrinus*, 10 animals were placed in each of four replicate grid tanks ($n_{\text{total}} = 5 \times 4 (10) = 200$), and, 24hr later, we counted the numbers of animals for each hole size. Since the number of holes of accessible size in each tank exceeded the number of animals, we deemed each hole residency as an independent datum. We then used X^2 to determine if hole size residency deviated from random.

Experiment 2 – Intraguild predation and cannibalism

D. villosus and *G. tigrinus* were collected and sorted as above and frequencies of intraguild predation and cannibalism tested, with and without the experimental grid, in aquaria as above, in three protocols: (1) adult *D. villosus* versus adult *G. tigrinus*, juvenile *G. tigrinus* and juvenile *D. villosus*; (2) adult *G. tigrinus* versus juvenile *G. tigrinus* and juvenile *D. villosus*; (3) juvenile *D. villosus* versus juvenile *G. tigrinus*. In each protocol, 10 of each species/size class were simultaneously introduced to the aquarium with ($n=5$) and without ($n=5$) experimental grids, and left for 16hr (8hr light, 8hr dark) at 19°C and survivors counted. These data were converted to proportions and arcsine transformed for analyses (see Sokal & Rohlf 1995), but Figures show raw percentages for clarity. We analysed the data by ANOVA using Statview.

Minimum breeding size

To determine the minimum size for sexual maturity for *G. duebeni*, *G. tigrinus* and *D. villosus*, the collections of the Zoological Museum of the University of Amsterdam were checked for ovigerous females. For each species 10 samples from different localities in the Netherlands were checked for smallest ovigerous females (sample size $n > 10$ ovigerous females)

Results

Field study

At the time of sampling, *Gammarus duebeni* was completely absent from both hard substrates and soft lake sediments at the sampling site, and the only gammarideans found were *D. villosus* and *G. tigrinus*. The hard substrate zone was dominated by *D. villosus* with some *G. tigrinus* in the patches of soft substrate between the boulders. In the soft sediments of the lake bottom only *G. tigrinus* was present.

Behaviour

In the crevices in the field as well as in the grid holes in the laboratory, *D. villosus* and *G. tigrinus* both displayed their normal behaviour. They tend to wait in ambush with their antennae stretched out, holding themselves against the sides of the crevice with pereopods 5 to 7 in a distinctive position (Fig. 2A, B). The posterior part of the body, the urosome, and especially the last appendages (uropods), are in contact with the bottom of the crevice, and this hind part of the body acts as a spring. This arrangement enables the animals to strike quickly towards a food target. After detection of a nearby prey, the amphipod leaves its 'den' and uses its extended second antennae to make the first contact. In *D. villosus*, especially the males, these antennae are provided with a fringe of setae, the shortest and strongest of which end in a hook, very much similar to the 'active' side of Velcro. This probably increases friction with the prey, and may be a special adaptation to their predatory lifestyle. The second antennae pull the prey towards the forward-reaching gnathopods. After the prey has been secured, the amphipod returns to its den, where it starts to feed on whichever part of its prey is closest to its mandibles. Males of *G. tigrinus* are characterised by relatively dense setation of the second antennae, featuring long curled setae, presumably with the same function as those in *D. villosus* males.

In their dens and in frontal view, each antenna in both species extends over a separate quadrant (Fig. 2A, B), indicating the same detection function for all four antennae of individual specimens in this position.

Experiment 1 – Do amphipods optimize hole choice by size?

D. villosus of all three size groups distributed themselves with respect to hole size in a non-random manner ($X^2_6=42.1$, $P<0.001$, Fig. 3A; $X^2_5=40.21$, $P<0.001$, Fig. 3B; $X^2_3=12.6$, $P<0.01$, Fig. 3C). Clearly, individuals preferred to reside in hole diameters of around 1.5-2 times their 'C-shape' and indeed our observations suggest that animals position themselves within holes where they can take up this shape and touch the sides with their appendages (Fig. 2). *G. tigrinus* showed a similar non-random use of holes ($X^2_6=54.6$, $P<0.001$, Fig. 4A; $X^2_5=35.5$, $P<0.001$, Fig. 4B).

Experiment 2 – Intraguild predation and cannibalism.

In the first protocol, adult *D. villosus* as predator/cannibal, 99% of these adults survived. There was significantly higher overall survival of the other amphipods in the presence as compared to absence of grids ($F_{1,24}=177.8$, $P<0.001$; Fig 5A) and there was significantly lower survival of juveniles as compared to adults ($F_{2,24}=10.4$,

$P < 0.001$; Fig 4a), leading to a significant interaction effect ($F_{2,24} = 4.2$, $P < 0.03$; Fig 5A). In the second protocol, adult *G. tigrinus* as predator/cannibal, 96% of these adults survived. There was significantly higher overall survival of the other amphipods in the presence as compared to the absence of grids ($F_{1,16} = 69.2$, $P < 0.001$; Fig 5B), with no significant overall difference in survival between juvenile *D. villosus* and juvenile *G. tigrinus* ($F_{1,16} = 0.1$, NS; Fig 4b), but a significant interaction effect ($F_{1,16} = 4.6$, $P < 0.05$; Fig 5B) since some juvenile *D. villosus*, but no *G. tigrinus*, survived in the absence of grids. In the third protocol, where juveniles of the two species could be predators/cannibals, there was a significantly higher survival of amphipods in the presence as compared to the absence of grids ($F_{1,8} = 11.4$, $P < 0.05$, Fig 5C) and significantly higher survival of *D. villosus* juveniles as compared to *G. tigrinus* juveniles ($F_{1,8} = 11.7$, $P < 0.05$; Fig 5C).

Minimum breeding size

The minimum female reproduction sizes for the species were as follows: *G. tigrinus* 4.5 mm, *G. duebeni* 9.3 mm and *D. villosus* 9.5 mm.

Discussion

The presence of an animal in a particular habitat is a compromise between maximum protection against predators, e.g. by choosing the smallest possible hole, and optimal circumstances to perform its life functions, i.e. finding sufficient food and favourable physico-chemical conditions. Efforts to avoid being eaten overrule most other factors in the life of any species (except among some parasites or social insects). Availability of an adequate habitat is therefore of paramount importance to potential prey animals, and is a limiting factor for their distribution (Rees, 1972, Pringle, 1982, Hacker & Steneck, 1990, Olyslager & Williams, 1993, Moksnes et al., 1998, Phelan et al., 2001, Nykänen & Huusko, 2003, Kley & Maier, 2005).

In this paper, we emphasize habitat structure as a distribution parameter for gammaridean amphipods. These amphipods tend to choose the smallest possible refuge, a common phenomenon in many other animal groups. Hence the multitude of elongate interstitial juvenile and adult animals, all having one thing in common: predation pressure leading them to seek ever-smaller holes, while adapting the dimensions of their body.

In the case of an invasion by a related invader species, several interaction options may arise. Both species can coexist when resources are partitioned in space and time, when the species can adjust their niche, or when resources are not limiting. When

niches overlap, an invading species can reduce the niche of the related species by being competitively superior in a part of the niche of the species that was already present. When niches of species overlap completely, competition for limited resources can lead to replacement of one species by the other. The term niche is therefore mostly used in the case of interactions between species (Chase & Leibold, 2003). Intra Guild Predation (IGP) is a form of competitor elimination in which relative body size and degrees of trophic specialization are the most important factors that influence the frequency and direction of predation. It is mostly generalist predators that are involved in IGP, with predators that are larger than their prey also preying on smaller conspecifics (cannibalism) (Polis, Myers & Holt, 1989).

D. villosus and *G. tigrinus* were found in separate zones related to substrate structure (boulders vs. sediment) in Lake Gouwzee, so *D. villosus* has replaced *G. tigrinus* on the boulders. Experiments carried out provided insight in the possible mechanisms of this replacement process.

In the laboratory, the smallest size class (3-5 mm) of *D. villosus* was found in the smallest holes in the grid, even in the absence of predators. The medium size class (8-12 mm) preferred the holes best fitting their body size. The largest specimens (16 to 22 mm) were found in the three largest hole sizes of the grid, 7, 8 and 9 mm. *G. tigrinus* showed a similar pattern. This means that both species require a habitat with holes acting as refuges for their survival. The fact that the animals were not able to enter any of the smaller holes during our additional minimum hole size experiment indicates the lower limit of the hole sizes available to the various size classes: they simply do not fit into the smaller holes.

Predation on juveniles was tested using combinations of adults and juveniles of both species. We found that juveniles were subject to almost 100% predation in the no-grid control experiments, but considerably less so in the grid systems. No significant difference was found between cannibalism and interspecific predation rates, indicating non-specific predation of adults on juveniles. Tests with adults of *D. villosus* (16 – 18 mm body length) and *G. tigrinus* (8 – 11 mm body length) showed one-way predation by the former on the latter species. Their body size difference evidently played a role in this. The grid also provided protection in this combination experiment.

The results of both the cannibalism and interspecific predation experiments could explain the situation found in the boulder zone and sediments of Lake Gouwzee, and support the assumption that habitat structure is a highly important distribution parameter for these animals. We also found that *G. tigrinus* matures at small size and is able to take advantage of small holes in the sediment, where *D. villosus* was not found. It is remarkable that *G. duebeni*, which is being replaced on the boulders by *D.*

villosus, matures at a similar large size as the latter. This means that the body size at which they mature as well as the presence of suitable refuges play a role in establishing the present-day littoral zonation of gammaridean species.

Conclusions

(a) In the littoral border of Lake Gouwzee, the two gammaridean species *D. villosus* and *G. tigrinus* live in separate habitats.

(b) The results of our laboratory experiments with grids of holes provide evidence for the importance of substratum structure in the micro-distribution of *D. villosus* and *G. tigrinus*. The distribution over the grid holes is not random, but the result of specific behaviour e.g. taking refuge in the smallest hole.

(c) Because of the similar behaviour and resource preference, competition between *D. villosus* and *G. tigrinus* for sheltered places in relation to body size is very likely. The minimum size of ovigerous females was similar for *D. villosus* and *G. duebeni*. The latter species disappeared from the boulders through intraguild predation (Dick & Platvoet, 2000), and was unable to find refuge in the lake. *G. tigrinus* matured at a much smaller size than the other two species. Before the invasion of *D. villosus* *G. tigrinus* could be found on both soft and hard substrates. Its occurrence in the lake at the time of this study was, however, restricted to the soft lake bottom sediments, with small spaces providing refuge for reproductive males and females as well as for juveniles.

(d) The zonal distribution in the field is most likely the result of intraguild predation by adults on juveniles and interspecific predation between adults of both species, with *D. villosus* as the dominant predator as demonstrated through the experiments.

(e) In the experiments for neither of the species any difference was found between cannibalism and predation of adults on juveniles. This may lead to differential occurrences of adults and juveniles in the field too.

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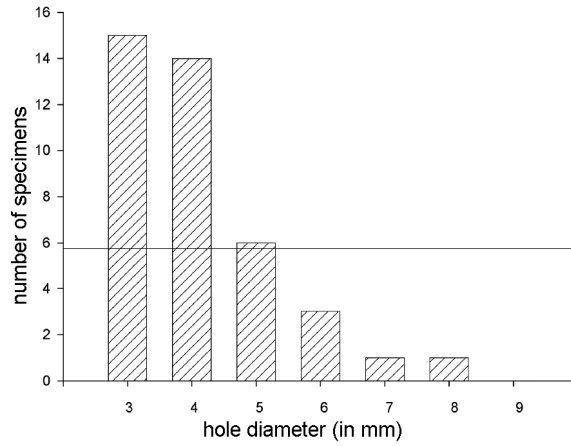


Figure 3A. Frequency distribution of *Dikerogammarus villosus* in the habitat grid, for size class(A) 3-5mm. Lines are expected frequencies of hole occupancy if animals distribute in holes at random

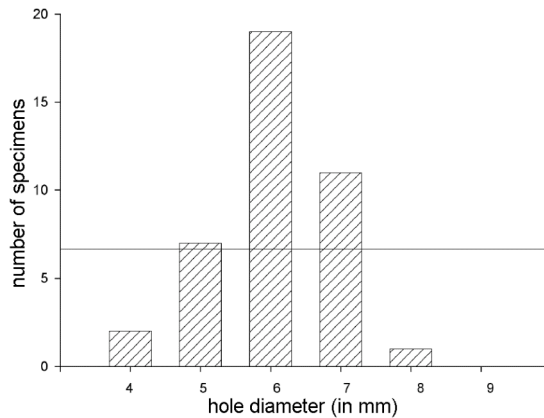


Figure 3B. Frequency distribution of *Dikerogammarus villosus* in the habitat grid, for size class B: 8-12mm. Lines are expected frequencies of hole occupancy if animals distribute in holes at random

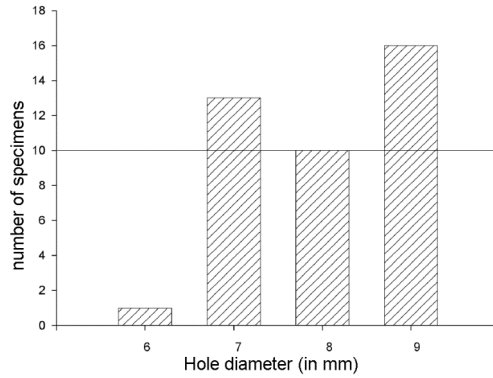


Figure 3C. Frequency distribution of *Dikerogammarus villosus* in the habitat grid, for size classes C: 16-22mm. Lines are expected frequencies of hole occupancy if animals distribute in holes at random

Fig. 4A

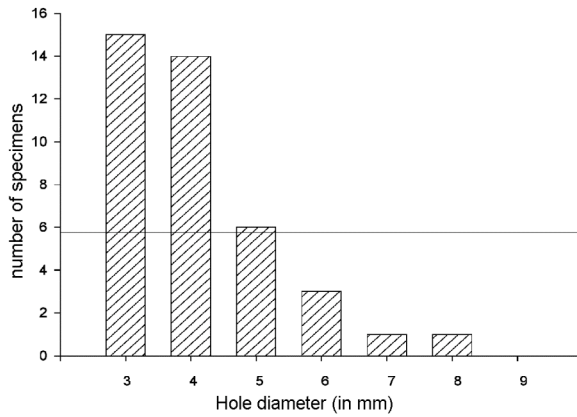


Fig. 4B

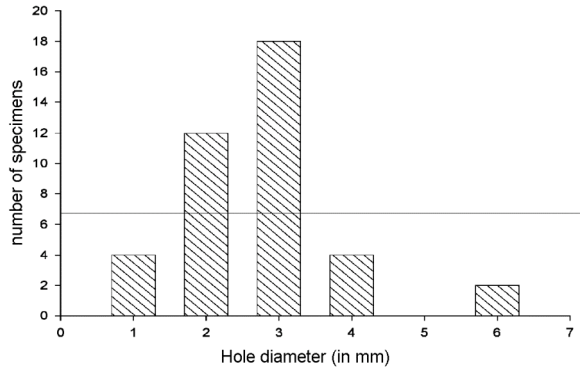


Figure 4. Frequency distribution of *Gammarus tigrinus* in the habitat grid, for the size classes: (A) 3-5mm and (B) 8-12mm. Lines are expected frequencies of hole occupancy if animals distribute in holes at random.

Fig. 5A

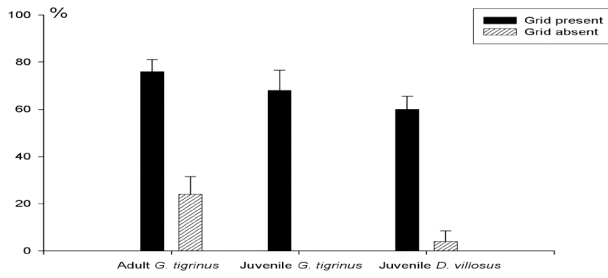


Fig. 5B

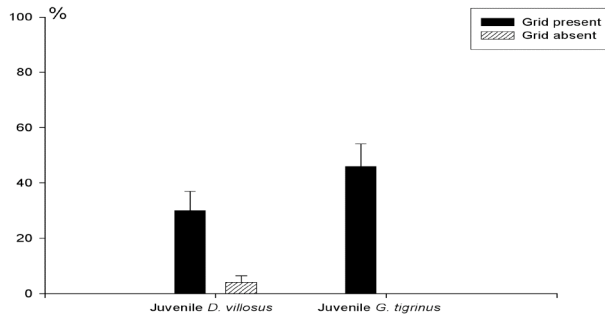


Fig. 5C

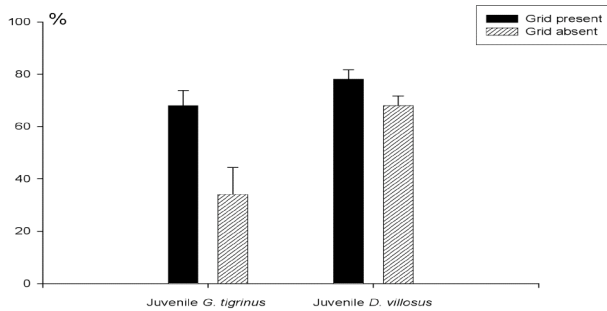


Figure 5. Mean (+S.E.) survival of amphipod specimens with and without experimental grids, in the presence of: (a) adult *D. villosus*; (b) adult *G. tigrinus*; and (c) where all individuals were juveniles of the two species

Table 1. Size ranges of body width, body height and body height when curled in three size-groups of *D. villosus* and two size-groups of *G. tigrinus*

Species/size groups (length)	Range of lateral widths (mm)	Range of dorso-ventral widths (mm)	Range of C-shape (curled) (mm)
<i>D. villosus</i> Group 1: 3-5mm	0.3-0.5	1.2-1.4	2-2.4
<i>G. tigrinus</i> Group 1: 3-5mm	0.2-0.4	1.0-1.2	1.8-2.4
<i>D. villosus</i> Group 2: 8-12mm	0.5-1.2	1.5-3	2.5-3.4
<i>G. tigrinus</i> Group 2: 8-12mm	0.4-0.9	1.3-2.6	2.1-3.2
<i>D. villosus</i> Group 3: 16-22mm	3-3.2	3.2-4	6-7

Chapter 5

Has allometric growth of body parts involved in predation a predictive value for the outcome of gammaridean interactions?

The case of the invasive *Dikerogammarus villosus* (Sowinsky, 1894) and the native *Gammarus duebeni* Liljeborg, 1852 (Amphipoda).

Dirk Platvoet, Jaimie T. A. Dick & Gerard van der Velde

Abstract

Gammarus duebeni Liljeborg, 1852, disappeared from the hard substrates of the littoral zone of a lake in the Netherlands after the invasion of *Dikerogammarus villosus*. In the Ponto-Caspian invasive amphipod *Dikerogammarus villosus* (Sowinsky, 1894), a steep allometric growth has been observed in the antennae, mandibles and maxilliped compared to *Gammarus duebeni* Liljeborg, 1852. These body parts are actively involved in predation in general including intraguild predation. The enlargement of these body-parts in the invader in combination with a very large body size may explain its aggressive and predatory nature, and therefore its impact on the indigenous aquatic fauna in the Netherlands.

Introduction

The Ponto-Caspian invasive amphipod *Dikerogammarus villosus* (Sowinsky, 1894) caused the disappearance of the native *Gammarus duebeni* Liljeborg, 1852 from the boulders along the littoral zone of lake Gouwee most likely through intraguild predation (IGP) (Dick & Platvoet, 2000). In this lake the largest males of *D. villosus* (29 mm) reached a more than 30% greater body length than the largest *G. duebeni* (22 mm) (unpublished results). A larger size in a generalist predator is a factor in intraguild predation (Polis et al., 1989)

D. villosus appeared to be a predatory omnivore (Dick & Platvoet, 2000; Van Riel et al., 2006). The antennae in combination with the mouthparts play a major role in food gathering and processing. This means that mouthparts as well as antennae may show morphological adaptation to a more predatory lifestyle in *D. villosus*. Therefore the focus of this study is on the allometric growth of these body parts.

The following research questions are addressed:

1. How are the antennae and mouthparts used in predation by *Dikerogammarus villosus*?
2. Is allometric growth of body, antennae and mouthparts stronger in *D. villosus* than in the native gammaridean *G. duebeni*?
3. Can allometric growth of body, antennae and mouthparts in *D. villosus* be related to a stronger predatory behaviour in the interaction with the native *G. duebeni*.

Methods.

The use of appendages in feeding related activities in cuvettes was studied by means of video recordings (JVC GR-DVL 100).

For the morphometric comparisons of the mouthparts and antennae of *D. villosus* and *G. duebeni* for each species two sets of 10 male specimens (13 mm (S.D 0.3 mm) and 20 mm (S.D. 0.3 mm) measured from the tip of the rostrum to the posterior edge of urosome 3, were studied. Males were chosen because predation increases with size, and males become much larger than females (Dick & Platvoet, 2000). *D. villosus* specimens were collected with a hand-net between littoral boulders at one site in the Gouwzee in early March 2003, the location where *G. duebeni* disappeared from in the previous years due *D. villosus*. Two months later males of *Gammarus duebeni* were collected in the same way from the Amstelmeer, in the North of the province of Noord-Holland, a small lake not yet occupied by *D. villosus* in 2003. Presence of two size classes at the sampling sites was the decisive factor for the moment of sampling. Sub-adult males of 13 mm and adult males of 20 mm of both species were separated. Wet weight (in mg), total body length and maximum width (in mm) of each individual were measured in the laboratory within two hours after collecting. The lengths of the second antennae, as well as five different measures of the right mandible and two of the maxilliped were made after dissection (Fig. 1). For testing the significance of differences between the size classes and species a Student's t test on a 5% significance level was used.

Results

Behaviour

The second antennae of *D. villosus* play a significant role in most feeding related activities. Especially in predation the setose flagellum of the antennae makes first contact with either a fast swimming or slow moving prey. The antennae pull the prey towards the outstretched gnathopods or produce a gnathopod directed flow from which the prey cannot escape (Fig. 2). The species has also been observed collecting fauna from the substrate through a digging action of the second antennae, such as hiding *Gammarus tigrinus* Sexton, 1939.

Measurements

Measurements on body, antennae and mouthparts are summarized in Table 1 and fig. 3.

In all measurements (except maxilliped measure 1) the allometric growth was significantly steeper in *D. villosus* than in *G. duebeni*. *D. villosus* had a 43% higher increase of wet weight than *G. duebeni*. The highest increase in allometric growth was found in the mandibles. The lowest increase in allometric growth was found in the body width.

In both 13 and 20 mm *D. villosus* the second antennae were about 50% longer than in *G. duebeni*.

The measures of the mandible showed a much stronger allometric growth in *D. villosus* than in *G. duebeni* (fig. 2). In *D. villosus* the allometric growth of the palp of the maxilliped is significantly stronger than in *G. duebeni*.

Discussion.

The mouthparts.

The larger mouthparts of the invader may explain why *D. villosus* is capable of preying on intermolt crustaceans and other invertebrates with a hard cuticle, a phenomenon unknown from the indigenous gammaridean amphipods (Dick & Platvoet, 2000) Especially the strong muscular mass of the mandible (fig. 1) indicates a strong incising and grinding ability. The longer incisor (measure 4, fig. 1) and greater diameter of the pars molaris (measure 5, fig. 1) than found in *G. duebeni* also point in that direction.

The second antennae.

The much longer second antennae in *D. villosus* (52% longer than in *G. duebeni*) play an important role in catching prey. When prey is approached these antennae make first contact. The antennae reach behind the prey and then pull the victim towards the out-stretched gnathopods. The rich setation of the flagella probably increases friction between antennae and prey. The fact that these second antennae in *D. villosus* are much longer than those of *G. duebeni* supports the assumption that this is an adaptation to the more predatory life-style of the first species.

In *D. villosus* allometric growth in nine out of ten measures is significantly steeper than in *G. duebeni*. Just after introduction the average body size in successfully invading populations often appears to be greater than in the populations of origin (Stokstad, 2001). This may be the result of factors such as a lower predation pressure, increased food supply caused by lack of competition, a lower chance of infectious diseases or parasites and different physico-chemical conditions.

In laboratory experiments the highest rate of predation in *D. villosus* was found for the largest male specimens (Dick et al., 2002). A gradual decrease of body size in this species and as a result, a considerable reduction of the relative size of the mouthparts and antennae can decrease its predatory impact. This may be expected in the future by size-selective predation on *D. villosus*. The assumption that its predatory impact on the indigenous organisms will diminish in time seems legitimate.

This short study demonstrates that biometric data are useful to study allometric growth in relation to behaviour and impact. This means that studies showing differences in allometric growth such as for *D. villosus* and *G. duebeni* can be used to predict which species will be the superior competitor, especially when environmental conditions allow growth to a larger size.

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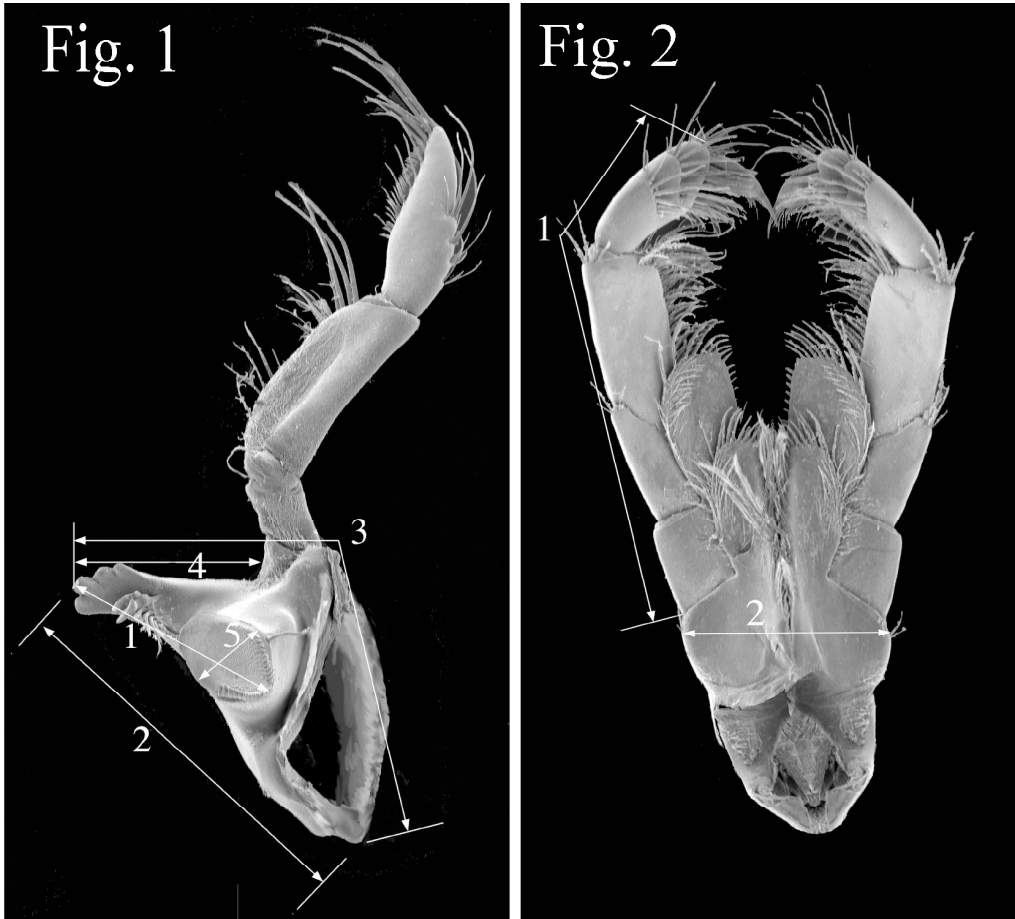


Figure 1: right mandible of male *Dikerogammarus villosus* with measured parts indicated; figure 2: maxilliped of male *D. villosus* with measured parts indicated.

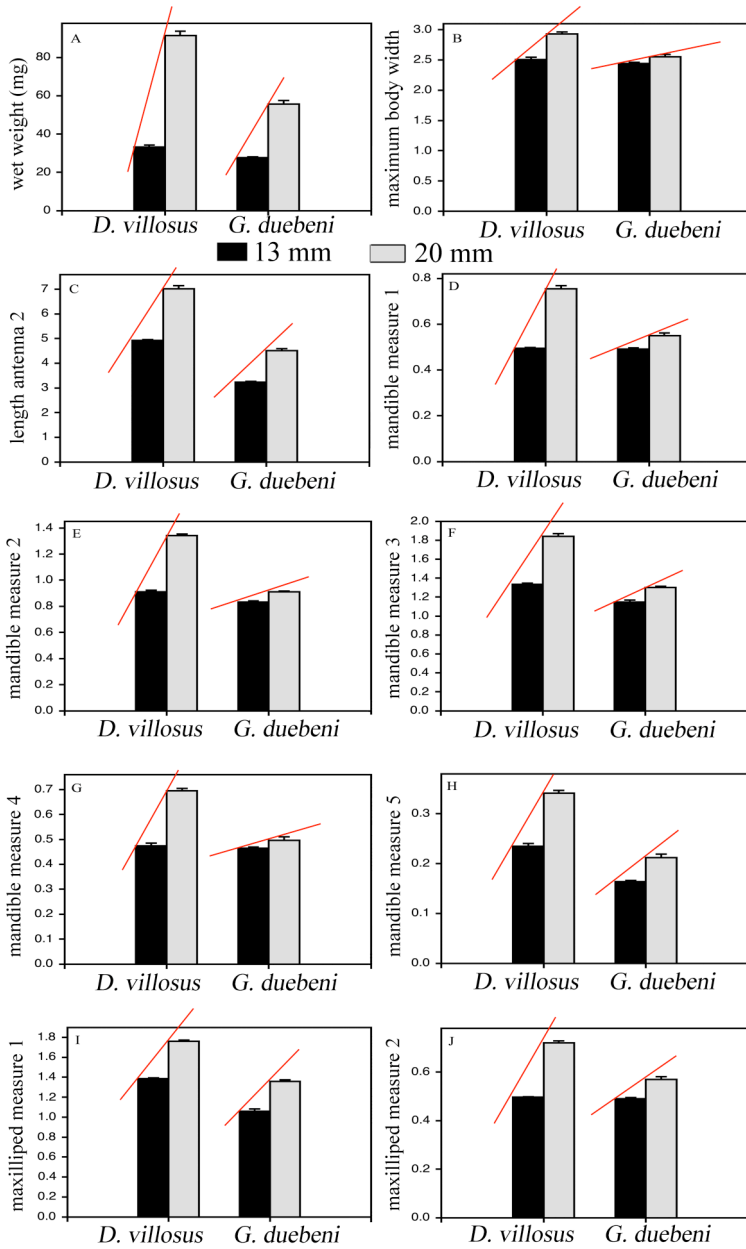


Figure 3. Mean values of measurements of body weight (mg) and length of antennae and mouthparts (mm) of *Dikerogammarus villosus* (Sowinsky) and *Gammarus duebeni* Liljeborg. Each column represents 10 specimens of either size class 13 (black) or 20 mm (grey). Red lines indicate allometric growth.

Table 1: Result of measurements in two size classes of males of the species *D. villosus* and *G. duebeni*. Values in mm except wet weight (mg). Standard deviations between brackets. Sample size for each size class: 10. Mand. = mandible, maxil. = maxilliped.

$$T\text{-statistic: } (x_{20,v} - x_{13,v}) - (x_{20,d} - x_{13,d}).$$

	<i>D. villosus</i>		<i>G. duebeni</i>		Student's
body size	13 mm n = 10	20 mm n = 10	13 mm n = 10	20mm n = 10	t-value (9 degrees of freedom)
wet weight	33,4 (1.35)	91,5 (7.30)	27.6 (1.35)	55,6 (5.92)	10.8
max. body width	2,51 (0.11)	2,93 (0.10)	2.44 (0.07)	2,55 (0.13)	5.6
length antenna 2	4.92 (0.11)	7.02 (0.40)	3.24 (0.10)	4.51 (0.28)	10.3
mand. measure 1	0.50 (0.01)	0.76 (0.04)	0.49 (0,02)	0.55 (0.04)	11.7
mand. measure 2	0.91 (0.04)	1.34 (0.05)	0.83 (0.02)	0.91 (0.02)	41.1
mand. measure 3	1.34 (0.04)	1.84 (0.10)	1.15 (0.05)	1.30 (0.05)	11.8
mand. measure 4	0.48 (0.04)	0.70 (0.03)	0.46 (0.02)	0.50 (0.05)	12.5
mand measure 5	0.24 (0.02)	0.34 (0.02)	0.16 (0.01)	0.21 (0.02)	8.28
maxil. measure 1	1.39 (0.03)	1.76 (0.04)	1.06 (0.07)	1.36 (0.05)	1.01 (not sign.)
maxil. measure 2	0.50 (0.01)	0.72 (0.03)	0.49 (0.01)	0.57 (0.03)	11.4

Chapter 6

*(presented at the 6th Int. Crustacean Congress, Glasgow:
17-22 July, 2005)*

The Amphipod Pilot Species Project (AMPIS), a
novel Dutch-Chinese Taxonomic Initiative
(Amphipoda, Crustacea):

Project Description

Dirk Platvoet, Zhong-e Hou, Shuqiang Li
&
Gerard van der Velde

Abstract

AMPIS is a comprehensive database of pictures and illustrations of the morphology of a collection of selected amphipod pilot species. All macro- and micro-characters of the entire body surface are systematically recorded. Each pilot species is represented in AMPIS by a large number of illustrations and scanning electron micrographs accessible via a series of increasing magnification steps. A wide range of techniques is applied to construct the database.

This unique vertical database, the first in its kind, will be complementary to the many overlapping horizontal database projects that have been developed or are under construction. AMPIS will meet a demand for a framework of more detailed information of the highly complex morphology of amphipods. It is an entirely new approach towards taxonomic research.

Keywords: morphology, database, amphipod, SEM.

Introduction

From the time Linnaeus started modern taxonomy by introducing the binominal system, species descriptions have one thing in common: they are all done differently. In the early days the reproduction of illustrations was a difficult and time-consuming process, resulting in primary emphasis on text. By the late nineteenth century printing techniques had improved so much that illustrations became more commonly used and their value for comparative research increasingly appreciated. However, illustrations in journals remain expensive, and therefore authors arbitrarily make selections, resulting in large differences in methods. This non-standardized approach has slowly undermined the status of taxonomy as a science. AMPIS tries to halt this process by systematically provide information of the entire morphology of species.

Horizontal taxonomic databases are in fashion (see website Fauna Europaea: http://www.faunaeur.org/external_databases.php). Most initiatives cover groundwork, providing collections of names. Morphological information, if provided at all, is often presented in an unsatisfactory manner. Promising picture databases, such as MorphBank (<http://morphbank.csit.fsu.edu/>) are underway, but they are random in purpose, content and form. AMPIS provides maps of the entire body surface. Buttons

give access to the smallest details (few μm). This systematic and detailed overall visualization of amphipod morphology will be explanatory to those studying these important biota. Also a series of publications will be produced concerning specific (functional) morphological subjects.

Description

- AMPIS is a unique initiative to get all available morphological information of amphipod pilot species ‘under one roof’.
- AMPIS facilitates for identifications and descriptions of related species.
- The accumulated knowledge developed by AMPIS, including genetic information, will form the basis of a highly sophisticated phylogenetic analysis of freshwater amphipoda.
- Our understanding of behaviour in amphipods (and crustaceans in general) will profit from this project. Behaviour and morphology go hand in hand.
- Our understanding of invasive processes will benefit from the knowledge of morphological pre-adaptations to new environments. AMPIS gives access to relevant detailed information.
- Nowadays scientists in technical and other sciences become increasingly aware of the fact that lessons can be learned from the millions of years of evolutionary development in organisms. AMPIS can produce a link between these scientists and the highly complex (functional) morphology of amphipods.

Structure of database

The database will have a layered setup, in which a user can navigate to higher levels of magnification, allowing an easy access to the smallest details (See Appendix 3). The database will be fully compatible with other database types and the internet.

During Phase I AMPIS will be developed and tested. First a selection of species will be entered. This process will be evaluated and the methodology optimized. After this the total number of species entered during Phase II will be decided.

Of each species an overview will be given consisting of a set of photographs and line drawings (lateral, anterior, posterior, dorsal and ventral views). Of all the larger body parts, like antennae or other appendages, line drawings will be made with buttons leading to sets of (SEM-) photographs and drawings of details of these parts. Each species will be represented in AMPIS by an estimated 2000 pictures and illustrations with a minimal amount of text.

Species choice

In fresh and brackish waters gammaridean amphipods can make up to 90% of the total macrofaunal biomass (MacNeil, 1997). The important role of these animals in food chains becomes increasingly recognized. Identification of the animals is difficult, and limited to a decreasing number of specialists. AMPIS will provide information for the development of new (pictorial) keys for non-specialists.

The large family Gammaridae is plesiomorphic in virtually all aspects compared to the other amphipod families (Barnard & Barnard, 1983), and therefore suitable as starting point for the project.

AMPIS will be an important tool for the phylogenetic analysis of amphipods. This will be a logical spin-off project for both counterparts.

Conclusion

AMPIS distinguishes itself from all other initiatives by the choice for few, but extremely detailed pictorial presentations of pilot species. It will be first in its kind and exemplary for students of many other groups of organisms. In contrast with the databases that summarize species, AMPIS will deliver new, highly sophisticated and detailed character information.

Publications:

- Platvoet, D., Z.-E. Hou, S. Li & G. van der Velde, 2005. Enigmatic pores in females of *Dikerogammarus villosus* (Sowinsky, 1894) (Amphipoda). Amphipod Pilot Species Project (AMPIS) report 1. *Crustaceana* 78: 1399-1404.
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Chapter 7

(published in Crustaceana 78: 1399-1404, 2005)

Enigmatic pores in females of *Dikerogammarus villosus* (Sowinsky, 1894) (Amphipoda)

Amphipod Pilot Species Project (AMPIS)

Report 1

Dirk Platvoet, Zhong-e Hou, Shuqiang Li
&
Gerard van der Velde

Introduction

During a SEM study that forms part of the production of the Amphipod Pilot Species Project (AMPIS) high-resolution database of external amphipod morphology, an enigmatic set of two pores was found in the females of the invasive *Dikerogammarus villosus* (Sowinsky, 1894). This species is a highly aggressive, Ponto-Caspian amphipod (Marguillier, 1998; Dick & Platvoet, 2000; Van der Velde et al., 2000, 2002). The two pores found in female *D. villosus* do not resemble any of the pores known in amphipods thus far.

Gammaridean amphipods possess a large number of small, cuticular pores with a diameter of less than 1 μm , arranged more or less regularly over the body surface in densities of up to 2.5 million/mm². Their presumed function is excretory (Halcrow, 1978, Read & Williams, 1990), and it is suggested that these pores play a role in anti-fouling of the body surface. Larger pore openings can also be found in the antennal glands, the male genital papillae and the female genital openings. In crustaceans in general, pores have also been associated with chemosensory organs (Oshel & Steele, 1988; Chaigneau, 1994).

In copepod crustaceans, glandular pores have been described that presumably are involved in sexual behaviour (Fleminger, 1973). For gammaridean amphipods, however, no references can be found for a role of glandular pores in reproduction, other than the genital pores,

The structure of the pores is described, they are illustrated and their possible function is discussed.

Material and methods

Specimens of *Dikerogammarus villosus* from Lake Gouwzee (the Netherlands) were collected and immediately fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in an ethanol series, critical point dried and coated with gold. Pictures were produced with a JEOL 6400 scanning electron microscope.

Description of the pores

On the first pereionite of *Dikerogammarus villosus* females, two large pores can be found that open dorso-laterally: one pore on each side, halfway the somite (Fig. 1). The diameter of these funnel-shaped pores is about 50 μm and including the surrounding, pore-related area, between 80 and 120 μm . The edge of the hole is formed by radial folding of the cuticle (Figs. 1-4). There is a transition zone between the surrounding surface structures and the pore proper, in which the regular row-pattern of the micro-scales changes into a circular pattern around the pore hole. In males, no trace of these, or even similar pores can be found.

Discussion and conclusions

As only females possess the pores, a role for these structures in the reproductive process of this highly aggressive species is suggested. Amphipods of the family Gammaridae show a pattern of reproductive behaviour that becomes apparent by the end of the intermoult stage of the female partner. When that stage is reached, a male grasps such a female in which moulting is imminent, and starts an amplexus (fig. 5). That pre-copula stage is probably induced by pheromones in the urine of the female (cf. Hammoud et al., 1975). This is in accordance with many observations in decapod Crustacea (cf. Ekerhalm & Hallberg, 2005). However, nothing is known of any mechanism that would inhibit cannibalism during the amplexus. In our opinion the newly described pores might play an important role in this respect.

We suggest four functions for the pores (combinations of more than one function are, of course, also possible):

- 1) release of pheromones for the initial attraction of males
- 2) release of an infochemical for proper placement of the male's claws
- 3) release of an infochemical providing information to the male of the moult stage of the female during amplexus

- 4) release of a substance that prevents predation of the female by the male, before and during amplexus

Future research

Next to a study of the anatomy of the organ to prove if the pores are really glandular, an experimental behavioural study should be undertaken to get insight in the actual function of these pores. Blocking of these pores seems an adequate procedure to find out to what extent they are actively involved in the reproduction process. The presence or absence of such pores in other species should also be investigated, to reveal possible differences in structure and/or function.

Acknowledgements

This work was made possible through a grant of the Royal Dutch Academy of Arts and Sciences/Koninklijke Nederlandse Akademie van Wetenschappen, project nr. 05CDP021. This is CWE publication no. 439.

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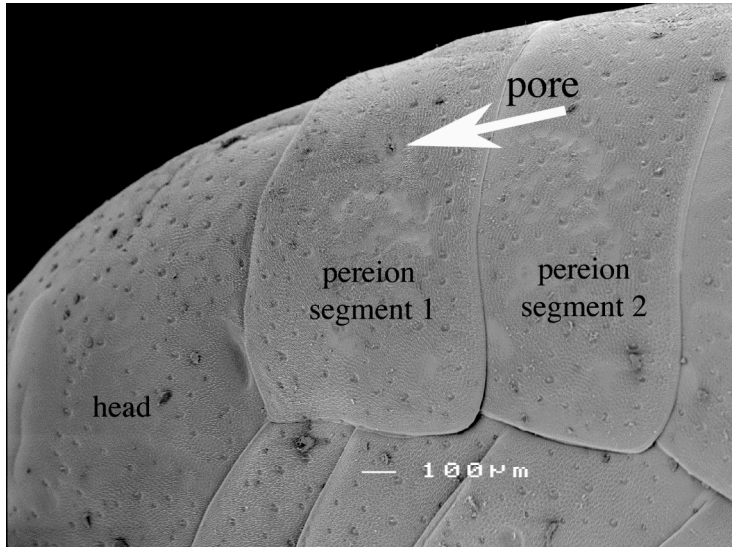


Figure 1: Overview of the anterior body part of a *Dikerogammarus villosus* (Sowinsky, 1894) female (arrow indicates position of a pore).

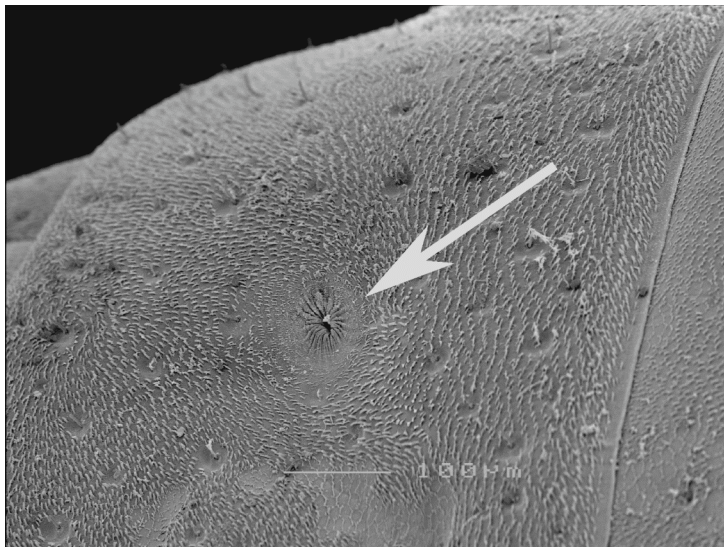


Figure 2: Pore on the left side of the first pereionite of a *D. villosus* (Sowinsky, 1894) female (arrow indicates position of a pore).

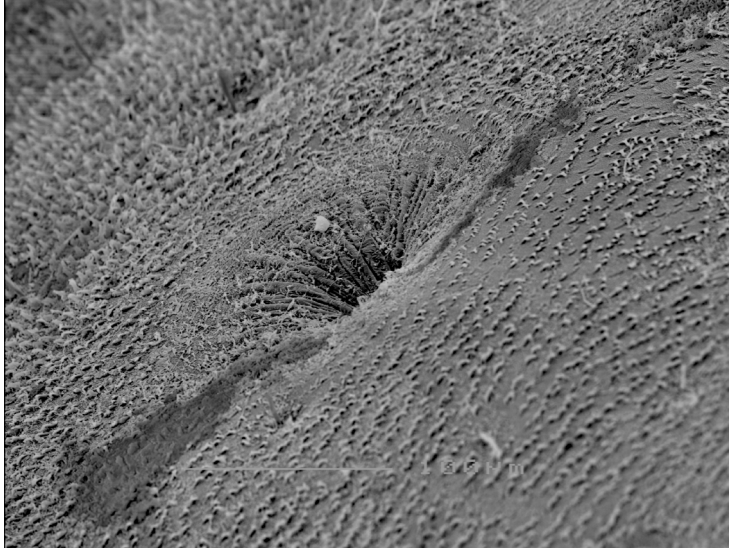


Figure 3: Lateral view of pore on the left side of the first pereonite of a *Dikerogammarus villosus* (Sowinsky, 1894) female.



Figure 4: Close-up of pore on the left side of the first pereonite of a *Dikerogammarus villosus* (Sowinsky, 1894) female.

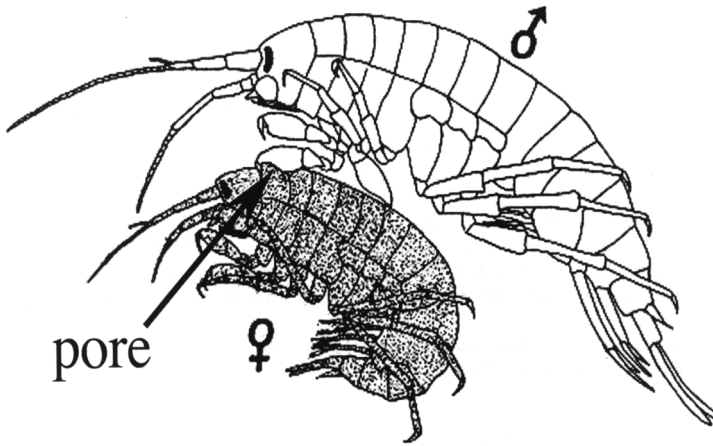


Figure 5: Gammarid male and female in amplexus.

Chapter 8

(published in *Crustaceana* 79: 993-1003, 2006)

A lock-on system in precopulae of *Dikerogammarus villosus* (Sowinsky, 1894), also present in *Gammarus pulex pulex* (Linnaeus, 1758) (Amphipoda).

Amphipod Pilot Species Project (Ampis)

Report 2

Dirk Platvoet, Yanjing Song, Shuqiang Li
&
Gerard van der Velde

Abstract

In *Dikerogammarus villosus* and *Gammarus p. pulex* a lock-on mechanism for amplexus was found. Large pores on the first and fifth female pereionites are involved, as well as the first pair of male gnathopods. On the anterior edge of pereionite 1, and the posterior edge of pereionite 5, specialized areas were found that allow penetration of the male's dactyli in the inter-segmental spaces of the female. Also a guidance system for male attachment to the female was found using palmar spines of the first pair of gnathopods. The mechanism is described and discussed. Morphometric analysis of this lock-on system explains the strong size-relationship between partners.

Chez *Dikerogammarus villosus* et *Gammarus pulex*, un mécanisme de verrouillage lors de la précopulation a été mis en évidence. Des pores de grande taille situés sur les premiers et cinquièmes péréionites de la femelle sont impliqués, ainsi que la première paire de gnathopodes mâles. Sur le bord antérieur du premier péréionite, et le bord postérieur du cinquième péréionite, des aires spécialisées ont été observées, qui permettent la pénétration des dactyles du mâle dans les espaces inter-segmentaires de la femelle. De même, un système de guidage pour l'attachement du mâle à la femelle a été trouvé, utilisant les épines palmaires de la première paire de gnathopodes. Le mécanisme est décrit et discuté. L'analyse morphométrique de ce système de verrouillage explique la forte relation liée à la taille entre partenaires.

Introduction

In many crustaceans such as gammaridean amphipods, often a much larger male takes a reproductive female in a characteristic amplexus to guard her against predators and competitive males. Larger males are better capable of guarding females than smaller ones, and are therefore more successful in reproduction (Brockerhof & McLay, 2005). Fitness in both males and females increases with size in *Gammarus duebeni* Liljeborg, 1852 (cf. McCabe & Dunn, 1997).

Initial amplexus in gammaridean amphipods is probably induced by pheromones produced by the female and released through the urine (Hammoud, 1975). In gammaridean amphipods the pre-copulatory stage can last between a few days and more than a week, depending on the water temperature (pers. obs.) and other factors, like predation pressure (Strong, 1973). At the end of this stage the female moults, after which she is taken in a specific belly-to-belly position, followed by copulation. Subsequently, the eggs are released from the ovaries into the brood-pouch and fertilized. Probably not sooner than after the female's cuticle has hardened, she is released by the male.

To date, not much is known about the mechanism of amplexus in gammaridean amphipods, whence we studied this phenomenon in *Dikerogammarus villosus* (Sowinsky, 1894) and *Gammarus pulex pulex* (L., 1758). During the development of the AMPIS high definition taxonomical database, two unusual pores were found in female pereonite 1 (Platvoet et al., 2006). Their function was unclear but new observations indicate that they are part of a lock-on system for males, as described and discussed below.

Material and methods

In January 2006, specimens of *Dikerogammarus villosus* from Lake Gouwzee (the Netherlands) were collected and transported to the laboratory. Precopulating pairs were isolated from the stock and immediately fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in an ethanol series, critical point dried, and sputter-coated with gold. The same dehydration procedure was followed for specimens of *Gammarus pulex* from the collection of the Zoological Museum of the University of Amsterdam. These specimens were collected from the Oosterpoel near Monnickendam, the Netherlands in March, 2005. Pictures were produced with a JEOL 6400 scanning electron microscope. The cuticle of three females was examined by light microscopy in addition, to study the subcuticular organization of the lock-on system.

In 10 females of *D. villosus*, coming from pairs, the shortest distance of the pores to the specialized and swollen body-segment edges was measured. In the corresponding males, the distance of the dactylus/propodus articulation to the largest palmar angle spine was measured (fig. 5). Live precopulae of *D. villosus* were cooled down to 1°C and then studied using a Wild M5 microscope (50x) to reveal the position of the large palmar angle spine during amplexus.

Results

On the first pereion somites of females of *Dikerogammarus villosus* two funnel shaped pores of about 50 μm can be found, dorso-laterally symmetrical, almost halfway the somite (fig. 1A), as earlier described and figured in Platvoet et al. (2005). A similar pore can be found in the mid-dorsal line, about halfway the fifth somite. Two sectors of the anterior edge of the first pereionite, each between 100 and 200 μm long, are specialized (fig. 1A-D): the edge is swollen (fig. 1D), leaving a space between the edge of the somite and the cuticle of the cephalon. These specialized sectors are precisely in line with the pores found on the same somite (fig. 1A, B). The rest of this anterior edge is flattened, and flush with the cuticle of the cephalon (fig. 1C). The same specialization can be found mid-dorsally at the posterior edge of the fifth pereionite. Likewise, this area is in line with the single pore on the somite. In all checked females of *D. villosus* two funnel-shaped invaginations of the cuticle can be observed, two beneath the anterior edge of the first pereionite and one at the posterior edge of the fifth pereionite (fig. 2). The narrow part of the funnel was close to, and perfectly in line with, the female pore in all cases.

The distal part of the male's propodal palmar ridge of the first gnathopods is covered with a dense field of ornaments (figs. 3 A, B, 4D). When clasping on to a female, these fields probably coincide with the dense ornamentation of the specialized edges of the pereionites, thus increasing friction between these body parts, analogous to Velcro.

In full-grown males of *D. villosus* the first gnathopods have a relatively short palm (fig. 4A). On the palmar angle, 2 or 3 spines can be found, the largest and conically shaped spine with a length of between 60 and 100 μm , and with a basis diameter of 20 to 30 μm (fig. 4C). When the dactylus is in its rest position (subchela closed), the largest spine is located at the exterior side of the palmar angle, flanking the unguis (distal part of the dactylus). A much smaller spine, accompanied by one or two very small spines, can be found flanking the other side of the unguis (fig. 4C).

During the light microscopy study on living precopulae, only in three out of eight observed precopulating pairs the palmar angle spines could be seen entered in the

pores in the relevant pereionites. However, observations, and especially photography, were severely hindered by a large number of long setae, present on the carpus and propodus of the male's gnathopods, and movements of the animals.

In the 10 pairs of *D. villosus* measured, the distance between the propodus/dactylus articulation and the large palmar angle spine of the male's first gnathopods was plotted against the distance of the segmental edge of the first pereionite and the corresponding female pore (fig. 5).

In females of the indigenous (sub-) species *Gammarus p. pulex* the same pores were found. Also the specialized claw-entry edges on pereionites 1 and 5 were similar to those found in *D. villosus*.

Discussion and conclusions

The specialized anterior margin of pereionite 1 and the posterior margin of pereionite 5 are perfectly in line with the large pores found on these segments. Since only in these specialized parts penetration of the dactylus into an inter-articular space seems possible, guidance of the dactylus during the start of amplexus is the most likely function of these specialized areas. The funnel-shaped invaginations of the cuticle in females probably guide the male dactylus in such a position that the palmar spines line up with the female pore during lock-on. The fact that the distance between the dactylus/propodus articulation to the largest palmar angle spine in males is strongly related to the distance of the female pores to the corresponding specialized segmental edges, is an indication for penetration of the palmar angle spines into the pores. This has been observed on three separate occasions, and it is most likely that this is the common position of these spines during amplexus. The distance of the articulation of the dactylus to the palmar angle spine was never smaller than the distance of the specialized edge of the female pereionites to the corresponding pore. This implies that sub-adult or small adult males will never be able to lock-on properly to a large female. Locking-on to a much too small female by a large male may be hindered by the large difference in the above-mentioned distances, possibly resulting in a decrease of grip for the male's gnathopods. The very high correlation found in the measurements of 10 pairs suggests that partner choice is strongly size-related and probably strongly regulated by the lock-on system.

We propose the following functions for the pore/spine-relationship (or combinations of functions):

- 1) size-selection in mate choice
- 2) species recognition (improper locking may lead to intraguild predation)

- 3) entrance of the palmar angle spine stimulates hormonal/behavioural processes in females (e.g. ecdysis, ovarian activity, activity in general)
- 4) entrance of the palmar angle spine stimulates hormonal/behavioural processes in males (e.g. preventing cannibalistic behaviour)
- 5) stabilization of the amplexus (optimal position for hydrodynamics and other conditions)
- 6) energy management (lock-on saves energy)
- 7) entrance of spines synchronizes behaviour of males and females

The fact that the anterior pores are symmetrically located on the left and right side of the first pereonite, in combination with the fact that only one of the first pair of gnathopods is hooked on to the corresponding left or right specialized areas of this segment, results in a position of the female's body in line with the male's body. If only one pore would have been present mid-dorsally on the female's first pereonite, her body would always make an angle with that of the male. This would have serious hydro-dynamical implications for swimming in water, as this is a highly viscous fluid at the scale of these animals. For the hind claw, this seems less important, and the female's body may even be used as a rudder.

We conclude that the female's large dorsal pores, in combination with the male's claws, form a sophisticated lock-on mechanism, with a range of possible functions. The fact that the mechanism was found in two, not very closely related, species may indicate its common occurrence in gammaridean amphipods.

Future research:

- 1) Presence of this lock-on mechanism in other amphipod species with and without precopulation in their reproductive stage must be further investigated.
- 2) A TEM study should reveal the actual anatomy of the pore system, and the presence of glands, innervation or musculature has to be determined.
- 3) With a SEM, entrance of a male palmar angle spine into a female pore must be shown. Speed freezing with liquid nitrogen is an option, electrocution another.
- 4) More morphometric work on this lock-on mechanism should be done in order to get more insight in size-related mate choice.

Acknowledgements.

The work was made possible through a grant of the Royal Dutch Academy of Arts and Sciences/Koninklijke Nederlandse Akademie van Wetenschappen, project nr. 05CDP021. This is CWE publication nr. 450.

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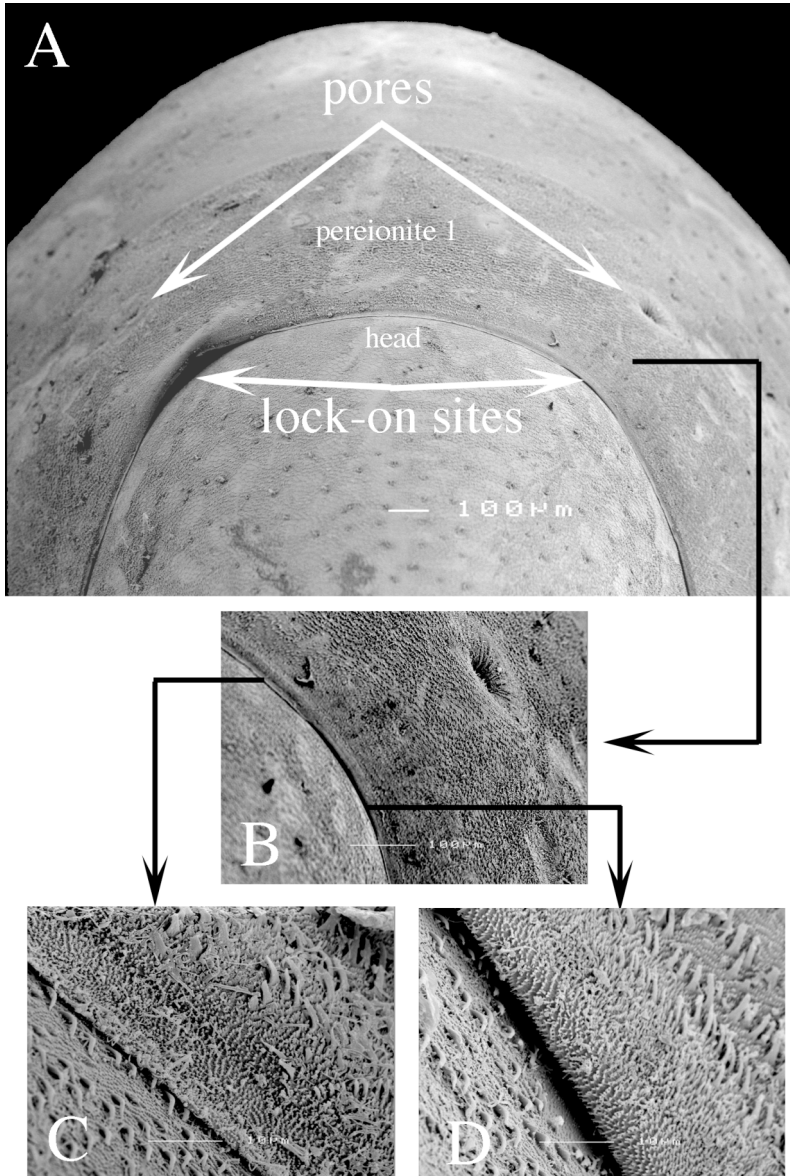


Fig. 1. Female *Dikerogammarus villosus* (Sowinsky, 1894) in frontal view: A, overview of head/pereonite 1 articulation; B, left pore with view on specialized edge of pereonite 1; C, non-specialized edge; D, specialized edge.

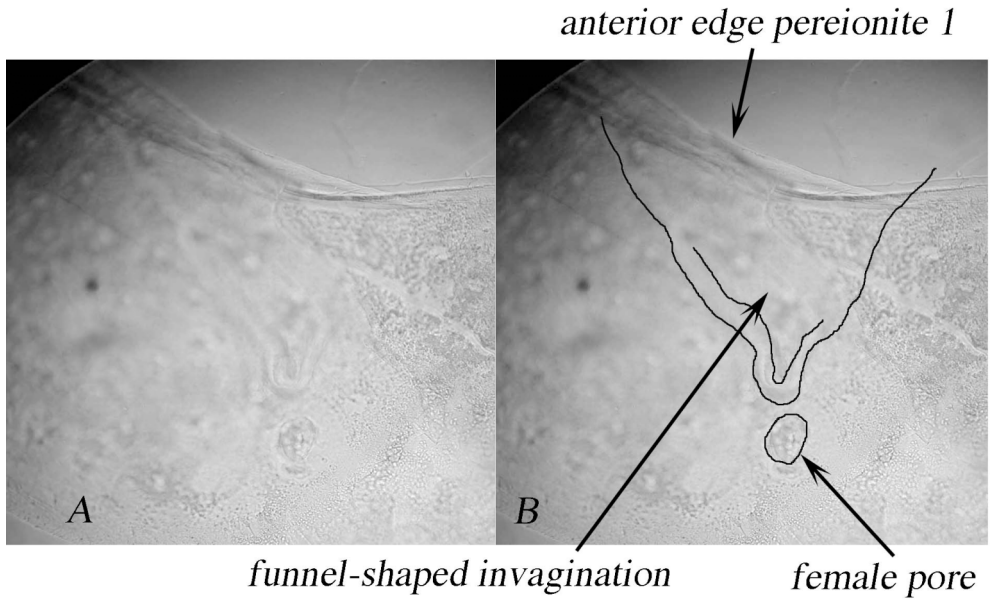


Fig. 2. Female *Dikerogammarus villosus* (Sowinsky, 1894) with A, funnel-shaped invagination of the cuticle beneath the anterior edge of pereionite 1; B, interpretation of same.

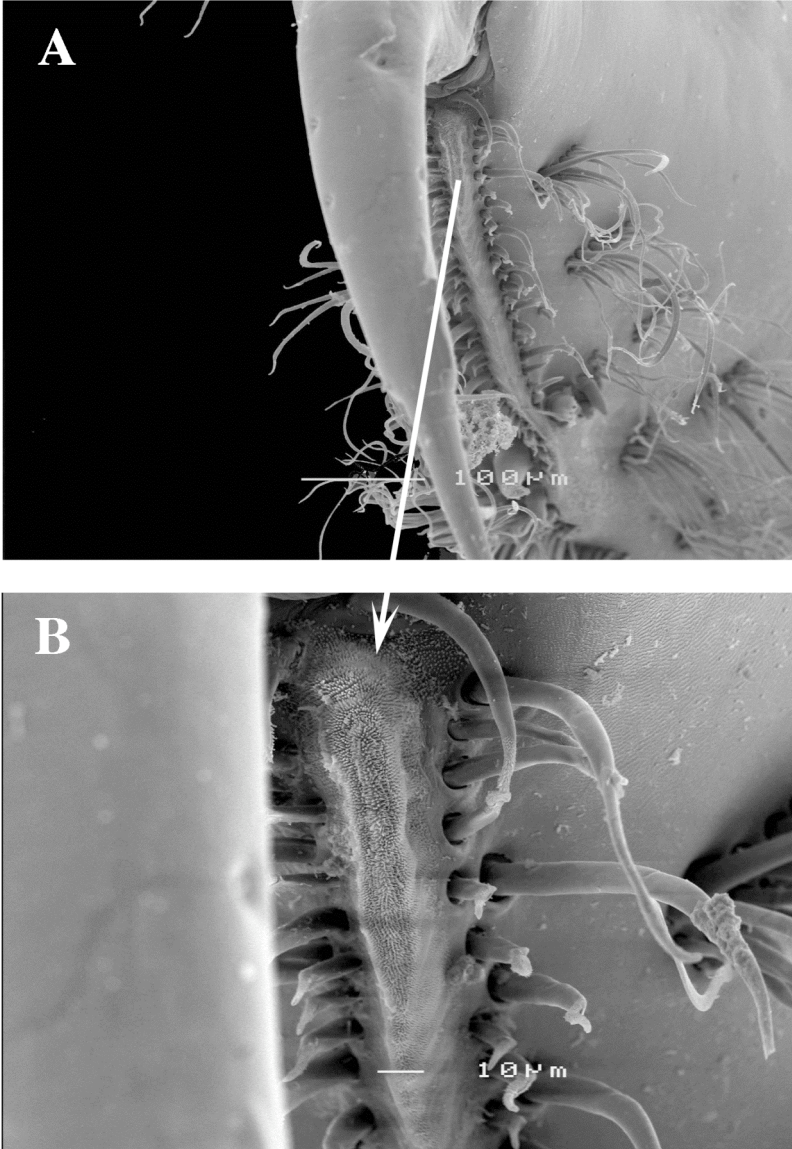


Fig. 3. Male *Dikerogammarus villosus* (Sowinsky, 1894): A, view on palmar ridge; B, detail of distal part of palmar ridge.

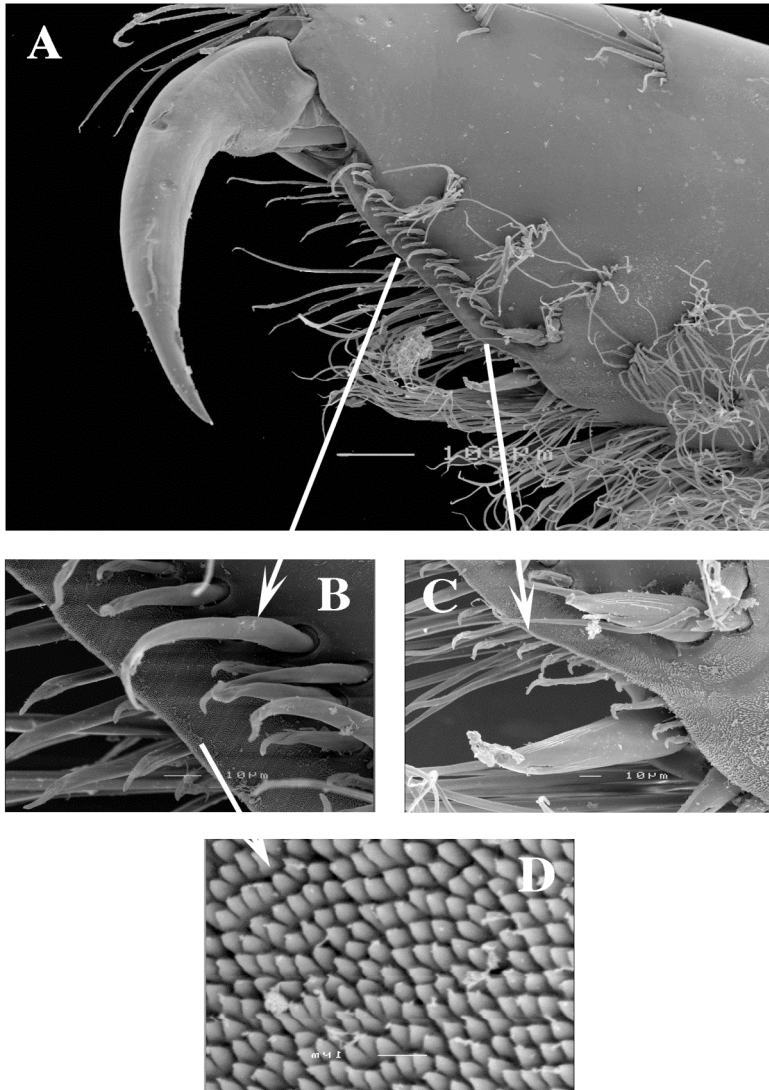


Fig. 4. Male *Dikerogammarus villosus* (Sowinsky, 1894): A, overview palm of propodus gnathopod 1; B, detail of palm; C, detail of palmar angle with spines; D, ornamentation on palmar ridge

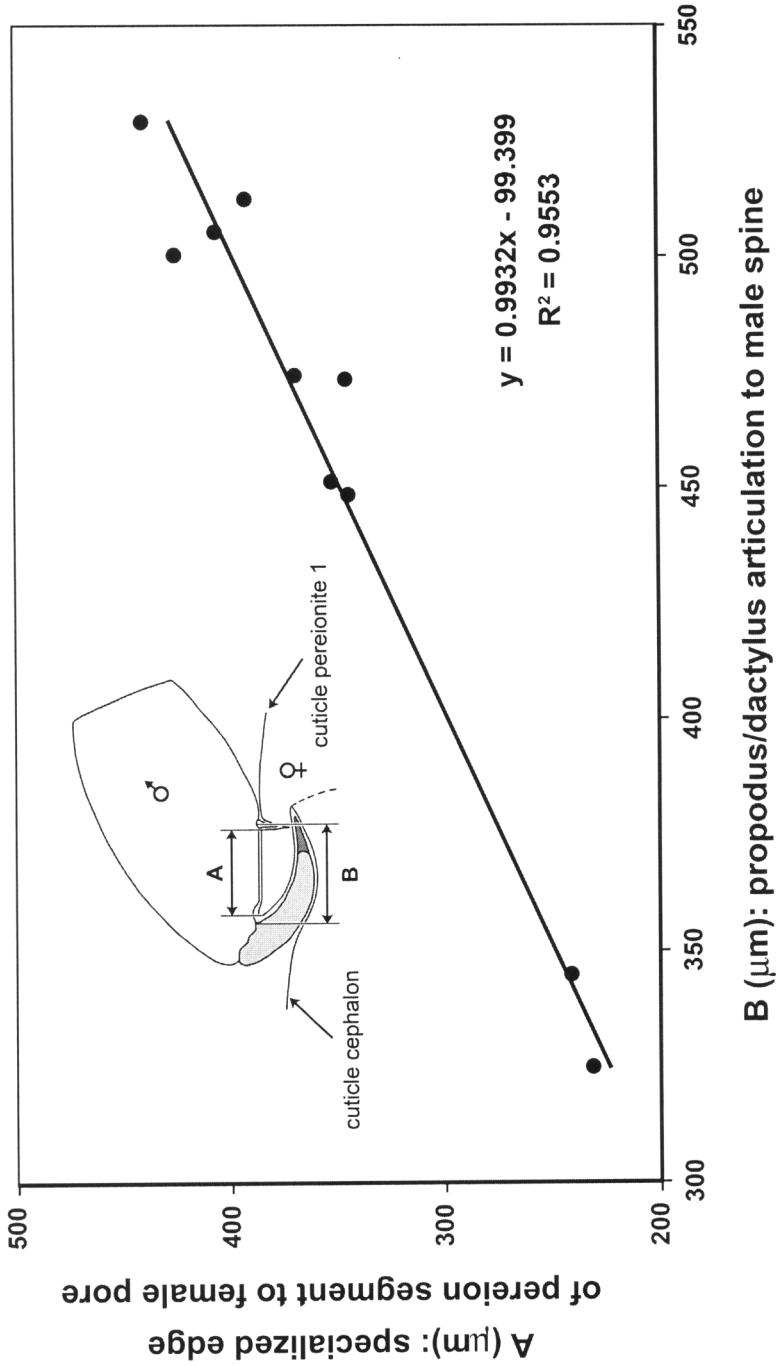


fig. 5. Graph representing correlation between Measure A (female pereionite edge to pore) and Measure B (male propodus/dactylus articulation to palmar angle spine) in *Dikerogammarus villosus* (Sowinsky, 1894), including schematic illustration of the lock-on mechanism.

Chapter 9

(published in Crustaceana 79: 1123-1127, 2006)

Presumed statocysts in the cephalon of
Dikerogammarus villosus (Sowinsky, 1894)
(Peracarida, Amphipoda).

Amphipod Pilot Species Project (AMPIS)

Report 3

Dirk Platvoet, Zhong-E Hou, Shu-Qiang Li
&
Gerard van der Velde

Abstract

In males and females of *Dikerogammarus villosus*, a depression in the integument was found at each side of the cephalon. These depressions have a specialized and, apparently, elastic cuticular lining. We assume that the depressions function as statocysts because of their backward location on the cephalon, just in front of the first pereonite, and also because of the remarkable resemblance with statocysts described from palinurid decapods, where such depressions form the outside expression of the statocysts present in the peduncles of the antennulae.

Résumé

Chez les mâles et les femelles de *Dikerogammarus villosus*, une dépression dans le tégument a été observée de chaque côte du céphalon. Ces dépressions sont bordées par une ride cuticulaire spécialisée et apparemment élastique. Nous supposons que ces dépressions fonctionnent comme des statocystes en raison de leur situation, en arrière sur le céphalon, juste devant le premier péréonite, et aussi par la ressemblance remarquable de ces structures avec les statocystes décrits chez les décapodes Palinuridae, chez lesquels de telles dépressions constituent l'expression externe des statocystes présents dans les pédoncules antennulaires.

Introduction

Statocysts are widely distributed throughout the invertebrates, and their function is generally associated with spatial orientation of the animal, or geosense. However, this is only one of the known functions of statocysts. Integrated in a neural network, a dual sensory-motor function for the organ has been described by Levi et al. (2003) in molluscs. Monitoring of hydrostatic pressure by statocysts has been observed in crabs (Fraser & Macdonald, 1997). Photic and rotational information is coordinated by statocyst neurons in the snail, *Lymnaea stagnalis* (L., 1758) (cf. Sakibara et al., 2005).

Statocysts in crustaceans have previously been found in the antennules of decapods and Anaspidacea (Syncarida), in the first segment of the peduncle. In peracarid crustaceans, statocysts have thus far been found in mysids (opossum shrimps), where they are located inside the bases of the internal rami of the uropods, and in isopods. A pair of statocysts is present in the telson of the isopod, *Cyathura polita* (Stimpson, 1855). The cuticular invagination forms a sac filled with water that communicates with the exterior to a greater or lesser extent (Halberg & Chaigneau, 2004, and literature cited therein).

During a SEM study that is part of the production of the AMPIS high-resolution database of amphipod external morphology (Amphipod Pilot Species Project), a depression on each side of the cephalic tagma was found that strongly resembles those of the statocysts described from the decapod, *Jasus edwardsii* (Hutton, 1875) (cf. Sekiguchi & Terazawa, 1997).

Material and methods

Male and female specimens of *Dikerogammarus villosus* (Sowinsky, 1894) collected in the Gouwzee, The Netherlands, were routinely prepared for SEM observations (JEOL JSM 6400).

Description

In both sexes, at each side of the head a depression is located (figs. 1, 2).

Maximum length and width of the depressions are approximately 150 and 70 μm , respectively. The cuticle of these crescent-shaped areas appears to be thin, deduced from wrinkles in SEM preparations. The architecture of the cuticle lining the depressions is different from that of the surrounding area, and it is apparently specialized: the size of the micro-scales inside the depressions is highly reduced.

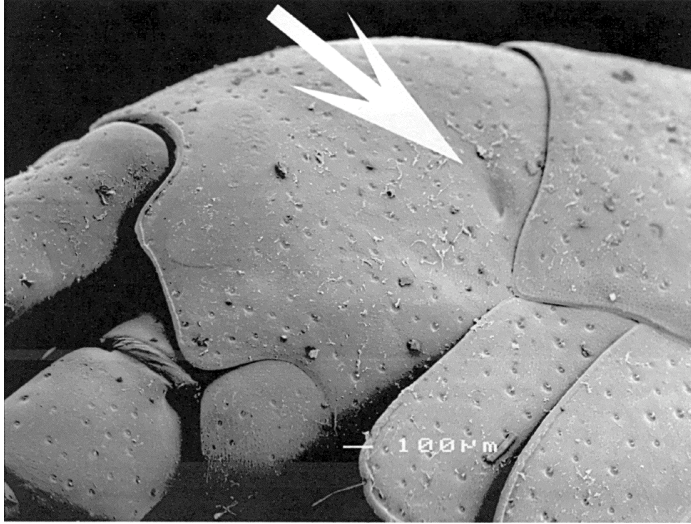


Fig. 1. Cephalon of *Dikerogammarus villosus* (Sowinsky, 1894) in lateral view (left side). The arrow indicates the position of the depression.

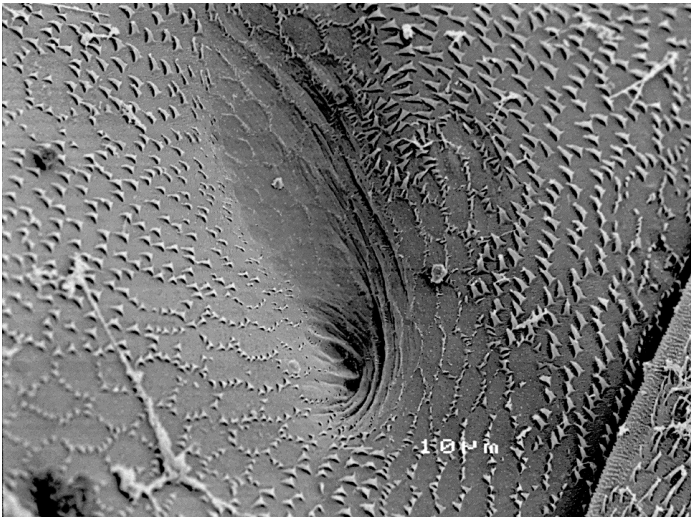


Fig. 2. Detail of cephalic depression in *Dikerogammarus villosus* (Sowinsky, 1894), presumed to be indicative of the presence of a statocyst.

Discussion and conclusions

These depressions constitute strong indications for the presence of statocysts. The resemblance with the external part of the palinurid statocyst is striking. The location on both sides of the cephalon as well as the fact that statocysts could play a role in monitoring hydrostatic pressure waves, may give support to the assumption that the organ plays a role in the detection of moving objects. Possibly, the specialized cuticle could act as a mammalian ear-drum.

Future research topics.

An anatomical study should reveal the actual nature of the observed structures. Other amphipod species as well as peracarids in general should be monitored for the presence of this organ. Morphometrical work should focus on the allometric growth of the structure to investigate a possibly wavelength-related type of perception.

By de-hydrating specimens for SEM-use, the volume of the organism decreases roughly 10%. For hard-bodied animals this reduction in size does not occur. Soft parts of their body, however, may be affected, and therefore, the depressions may not be easily visible in living animals. This could be checked with a low-vacuum scanning electron microscope.

Acknowledgements

This work was made possible through a grant of the Royal Dutch Academy of Arts and Sciences / Koninklijke Nederlandse Akademie van Wetenschappen, project nr. 05CDP021. This is CWE publication nr. 454.

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Chapter 10

(published in Crustaceana 80(11) 2007)

Description of the lateral line organ in
Dikerogammarus villosus (Sowinsky, 1894) with
discussion on its function
(Peracarida, Amphipoda)

Amphipod Pilot Species Project (AMPIS)

Report 4

Dirk Platvoet, Yanjing Song, Shuqiang Li
& Gerard van der Velde

Abstract

The lateral line organ of *Dikerogammarus villosus* (Sowinsky, 1894), consisting of two rows of sensory units along each side of the body, is described and its function discussed. All somites possess one lateral and one dorsal sensory unit on each side, except for the second urosomite, where only a lateral unit is present. At each side one lateral and one dorsal unit is present. Each receptor unit consists of a nearly straight or curved cuticular depression with a row of microtrichs. The flattened microtrichs are not varying with sex or size and are of equal length and width. The depressions from which the microtrichs arise are of constant depth. The distances between the individual microtrichs in all units are equal. However, the number of microtrichs per unit is body-size dependent with the highest number in the largest specimens. The orientation of the individual microtrichs in a unit varies from multi-angular (fan-, L- or question mark-shaped) in most anterior units to parallel or perpendicular to the mid-dorsal line in some posterior units. The microtrichs themselves are elliptical and hollow, with the outer mantle enclosing a central filament in its elliptical inner space, presumably of sensory function. A combined chemo-, mechano- and electro-sensory function with respect to their arrangement and their morphology is discussed.

Introduction

In fish and amphibians the lateral line system consists of a series of cutaneous receptors on the head and along the body. It subserves mechano-reception as well as electro-reception (Russel, 1999). In gammaridean amphipods a lateral line organ possibly with a similar function was demonstrated.

In 1975 structures, having a strong resemblance to a lateral line organ described in this paper, have been found in *Marinogammarus marinus* (Leach, 1815) (Mauchline & Ballantyne, 1975). Later on the lateral line organ was fully described for *Echinogammarus veneris* (Heller, 1865) and a mechano-sensory function suggested (Platvoet, 1985).

The lateral line organ consists in these amphipods of two rows of specialized receptor units (further referred to as 'units') at each side of the body. At each side of a somite two of these units are present, one situated dorsally and one laterally (figs. 1 and 2). Each unit consists of a mostly curved cuticular depression with a regular row of microtrichs arranged at a particular distance from each other.

In the subsequent years several papers provided overviews of microtrich sensillae in amphipods, including those of the lateral line organ. Based on the presence of an

apical pore in these microtrichs, a chemosensory function of the microtrichs involved in the lateral line organ was hypothesized (Steele & Oshel, 1987; Oshel & Steele, 1988; Steele & Steele, 1997, 1999; Diebel, 1992). However, Olyslager & Williams (1993) masked the microtrichs and concluded that the microtrichs in unison may act as a kinetic sensory organ. They did not find any proof for a chemo-sensory function.

In this paper we describe the lateral line organ in *Dikerogammarus villosus* (Sowinsky, 1894) and we hypothesize a near-field water displacement sensitivity for the organ system in combination with chemo- and/or electro-reception. The architecture of the microtrichs and their position in the units on the body is described. The number of microtrichs is calculated for juveniles, (sub-)adult males and females. In three specimens the number of microtrichs on the left and right body half was compared.

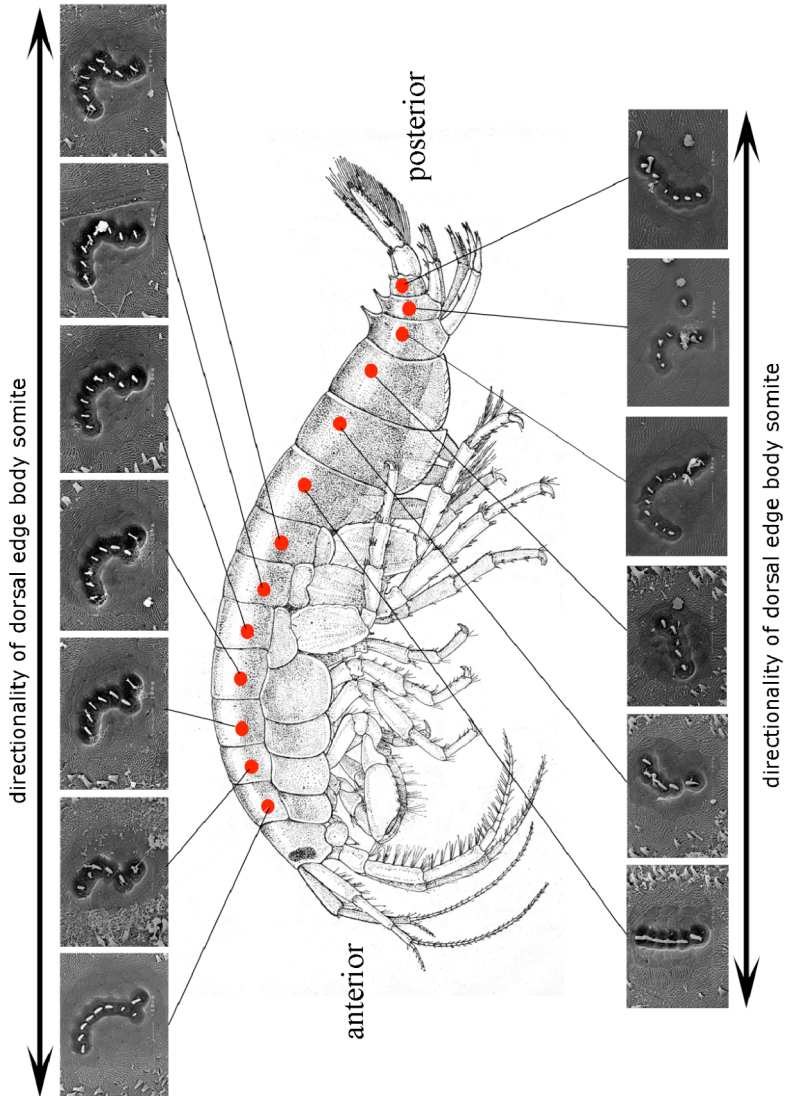


Fig. 1. Left lateral sensory units of the lateral line organ of *Dikerogammarus villosus* (Sowinsky, 1894) on pereonite, pleonite and urosomite, showing the position (dots indicated on the body) of the units with enlarged SEM pictures showing the arrangements of the microtrichia. Arrowed lines indicate the directionality of the mid-dorsal edge of each body somite.

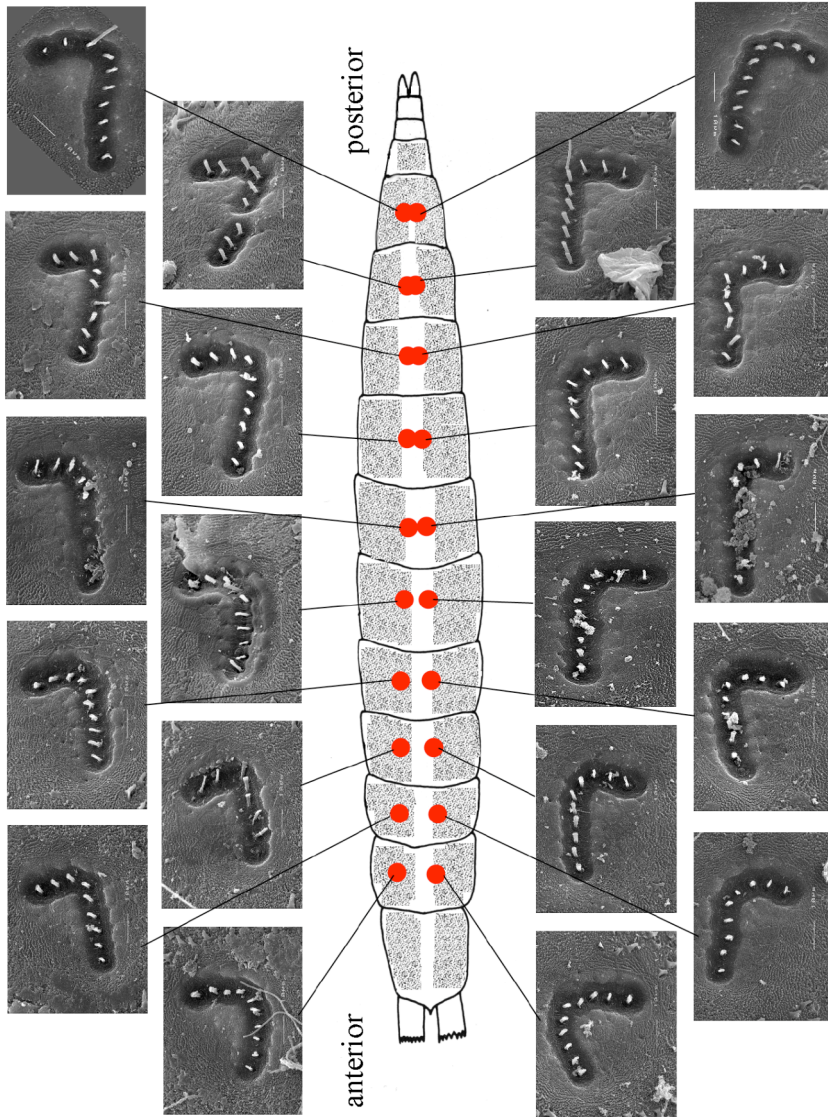


Fig. 2. Dorsal sensory units of the lateral line organ on pereionites and pleonites of *Dikerogammarus villosus* (Sowinsky, 1894). The position of the units on dorsum is indicated by dots on the body.

Material and methods

Specimens of *Dikerogammarus villosus* (Table 1) were routinely prepared and sputter-coated with gold for SEM (JEOL JSM 6400). In twelve specimens (4 males, 4 females, 4 juveniles) the number of microtrichs per unit was recorded and the dimensions of the microtrichs and the distances between them in a unit were measured using the program Semaphore[®].

Regression analysis was performed with StatView[®] (1988-1991), Abacus Concepts Inc.

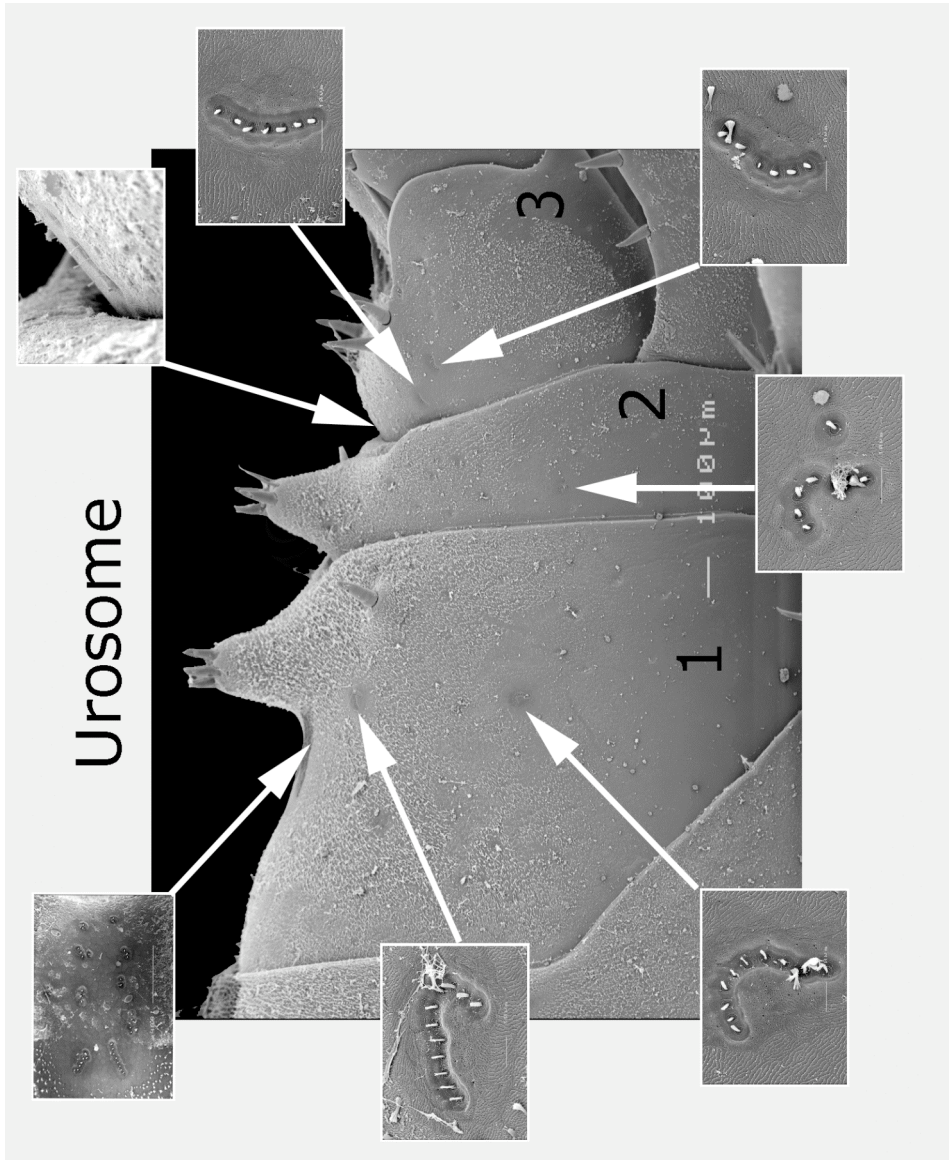


Fig. 3. Position of dorsal and lateral sensory units and the arrangement of microtrichs on urosomites 1 to 3 of *Dikerogammarus villosus* (Sowinsky, 1894).

Description.

Distribution of microtrichs

On the body somites, receptor units with a varying number of microtrichs can be found (figs 1 – 3, Table 1). Most somites (except the first and second urosomite and the telson) possess two lateral and two dorsal receptor units. These units are distributed over the body in four lines (2 dorsal and 2 lateral) and in a strong bilateral symmetry (Read & Williams, 1991; Olyslager & Williams, 1993), that is most obvious in the dorsal units, which are more or less L-shaped (fig. 4). Distance between dorsal units decreases posteriorly. In three specimens (juvenile, female and large male) the left and right body halves was compared. The difference in number of microtrichs between the left and right halves was less than 3% (111 versus 112; 229 versus 235; 258 versus 263).

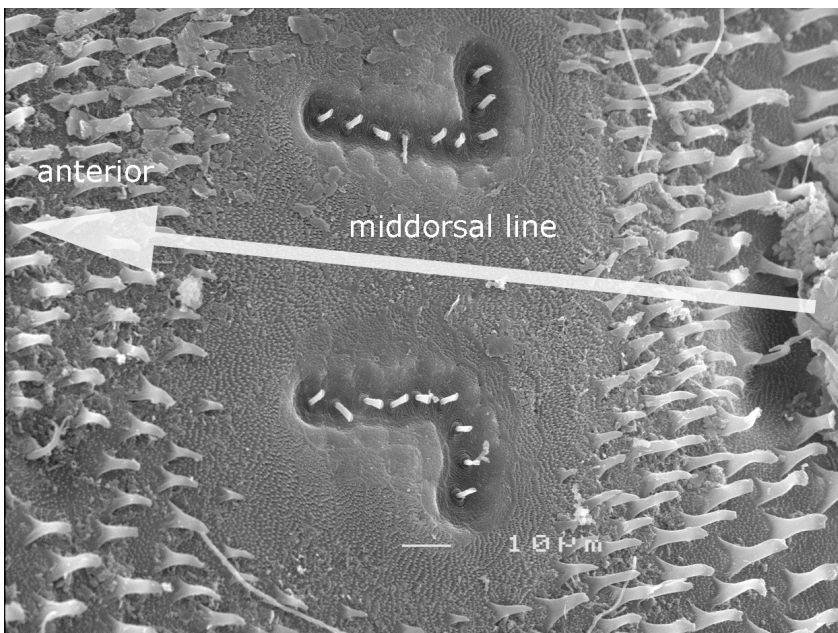


Fig. 4. Two dorsal sensory units on the first pleonite of *Dikerogammarus villosus* (Sowinsky, 1894) showing a distinct symmetry. The sensory units are surrounded by a specialized area lacking cuticular scales.

Most of the units present on the pereionites and pleonites show a fan- or L-shape. Measured from a microtrich from one end, the other microtrichs are positioned at an increasing angle. In dorsal units of the pereionite and pleonite (fig. 16) the greatest angle between two microtrichs in a unit is always larger than 90° ($90 - 105^\circ$; average 95.4 , $n = 18$, S.D. 7.6°). In the lateral units (fig. 17) the greatest angle is much more variable, between 18° in pleosomite 1 and 158° in pereionite 5, with an average of 107.9° (S.D. 41.7°). Sometimes one or two microtrichs stand separate from the main row at an angle lower than the largest angle found in that unit. In urosomite 1 the microtrichs are distributed over a varying number of small units: two fan-shaped (dorso-)lateral units on each side of the body and a number of small dorsal units close to the mid-dorsal line and anterior to the conical dorsal hump (fig. 5). The orientation of the posterior units of this dorsal cluster is more or less parallel to the mid-dorsal line. The anterior units are arranged at a $30-70^\circ$ angle to the mid-dorsal line. The total number of these dorsal subunits varies between 6 in juveniles to 12 in large adults.

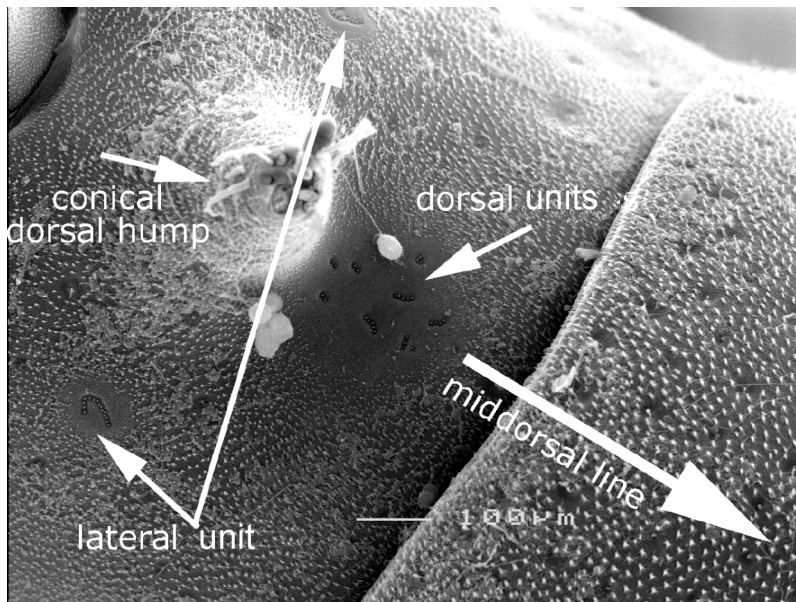


Fig. 5. Position of dorsal and lateral sensory units on urosomite 1 of *Dikerogammarus villosus* (Sowinsky, 1894). The large arrow indicates the mid-dorsal line and the anterior part of the body, smaller arrows indicate the lateral line organ units and a conical hump.

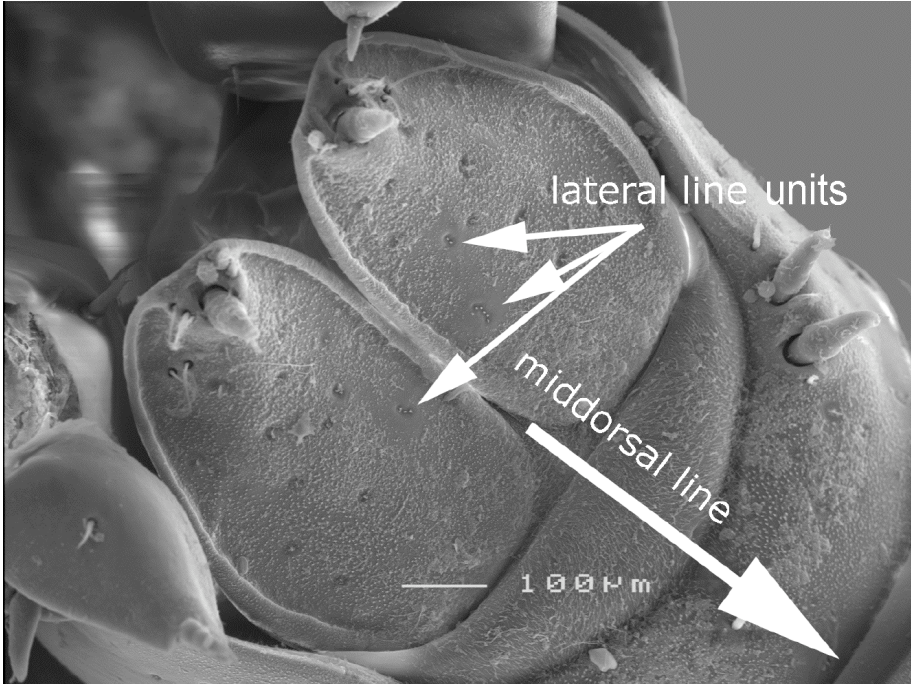


Fig. 6. Units of lateral line organ on telson of *Dikerogammarus villosus* (Sowinsky, 1894). The large arrow indicates the mid-dorsal line and anterior body part. Small arrows indicate three units.

On the telson of large specimens, at each lobe, two units with up to 5 microtrichs can be found, whereas in small animals only one unit, with up to 3 microtrichs.

The total number of microtrichs on one body-side (sum of microtrichs in dorsal and lateral units) in 12 specimens (4 juveniles, 4 females and 4 males) is related to body size and seems independent of sex (fig. 7, Table 1). The relationship seems non-linear. The equation of the curve is $y = 57.1 + 18.7x - 0.42x^2$; $r^2 = 0.98$; overall $F_{2,9} = 324.9$, $P < 0.001$. The regression equation is significant both for x (partial $F_{1,10} = 115.2$, $P < 0.001$) and x^2 (partial $F_{1,9} = 31.5$, $P < 0.001$).

Table 1: Number of microtrichs in the left lateral and dorsal body half of *Dikerogammarus villosus* (Sowinsky, 1894): pn = pereionite; pl = pleonite; ur = urosomite; tel = telson; lat./dor. = lateral/dorsal (only at urosomite 1). In urosomite 2 the dorsal unit is lacking. The units of telson are considered to be dorsal.

body length		pn1	pn2	pn3	pn4	pn5	pn6	pn7	pl1	pl2	pl3	ur1	ur2	ur3	tel	total
3.5mm	juvenile	10	10	9	8	8	7	7	8	8	7	13	5	8	4	112
	lateral	5	5	4	4	4	3	3	4	4	3	3	5	4	0	51
	lat./dor. dorsal	5	5	5	4	4	4	4	4	4	4	7	0	4	4	58
3.8mm	juvenile	10	10	10	9	9	8	8	8	8	7	14	6	9	5	121
	lateral	5	5	5	4	4	4	4	4	4	3	3	6	4	0	55
	lat./dor. dorsal	5	5	5	5	5	4	4	4	4	4	7	0	5	5	62
4.3mm	juvenile	11	12	11	10	10	9	9	8	8	8	16	7	8	6	133
	lateral	5	6	5	5	5	4	4	4	4	4	4	7	4	0	61
	lat./dor. dorsal	6	6	6	5	5	5	5	4	4	4	6	0	4	6	66
5.2mm	juvenile	11	12	12	11	12	11	9	10	9	8	16	8	9	7	145
	lateral	5	6	6	5	6	5	4	5	4	4	4	8	4		66
	lat./dor. dorsal	6	6	6	6	6	6	5	5	5	4	6		5	7	73
8.4mm	male	15	16	15	14	14	13	12	13	12	12	25	8	13	7	189
	lateral	7	7	7	6	7	6	5	6	5	5	7	8	6		82
	lat./dor. dorsal	8	9	8	8	7	7	7	7	7	7	12		7	7	101
11.5mm	female	18	18	19	19	18	16	14	15	15	14	26	9	16	7	224
	lateral	9	8	8	8	7	8	7	6	6	6	6	9	7		95
	lat./dor. dorsal	9	10	11	11	11	8	7	9	9	8	14		9	7	123
13.4mm	male	18	18	17	17	17	15	14	14	14	14	25	9	17	6	215
	lateral	9	8	8	7	8	7	6	7	6	7	6	9	8		96
	lat./dor. dorsal	9	10	9	10	9	8	8	7	8	7	14		9	6	114
13.6mm	female	20	19	20	20	18	16	15	15	15	16	26	10	18	7	235
	lateral	9	9	9	9	8	8	7	7	7	7	7	10	9		106
	lat./dor. dorsal	11	10	11	11	10	8	8	8	8	9	14		9	7	124
14.2mm	female	19	20	19	19	19	17	16	14	15	17	27	9	18	7	236
	lateral	9	9	9	8	8	7	8	7	7	8	8	9	9		106
	lat./dor. dorsal	10	11	10	11	11	10	8	7	8	9	15		9	7	126
17.3mm	female	22	21	22	21	21	16	18	16	15	17	29	10	18	8	254
	lateral	10	10	11	10	10	7	8	7	7	8	9	10	9		116
	lat./dor. dorsal	12	11	11	12	11	8	10	9	8	9	12		9	8	130
17.8mm	male	23	22	23	22	21	17	16	17	16	18	31	10	19	10	265
	lateral	11	11	11	10	10	8	7	8	7	7	9	10	9		118
	lat./dor. dorsal	12	11	12	12	11	9	9	9	9	11	14		10	10	139
21.2mm	male	24	21	22	21	22	18	17	16	15	17	29	11	21	9	263
	lateral	11	10	10	10	10	8	8	7	7	8	7	11	9		116
	lat./dor. dorsal	13	11	12	11	12	10	9	9	8	9	13		11	9	137

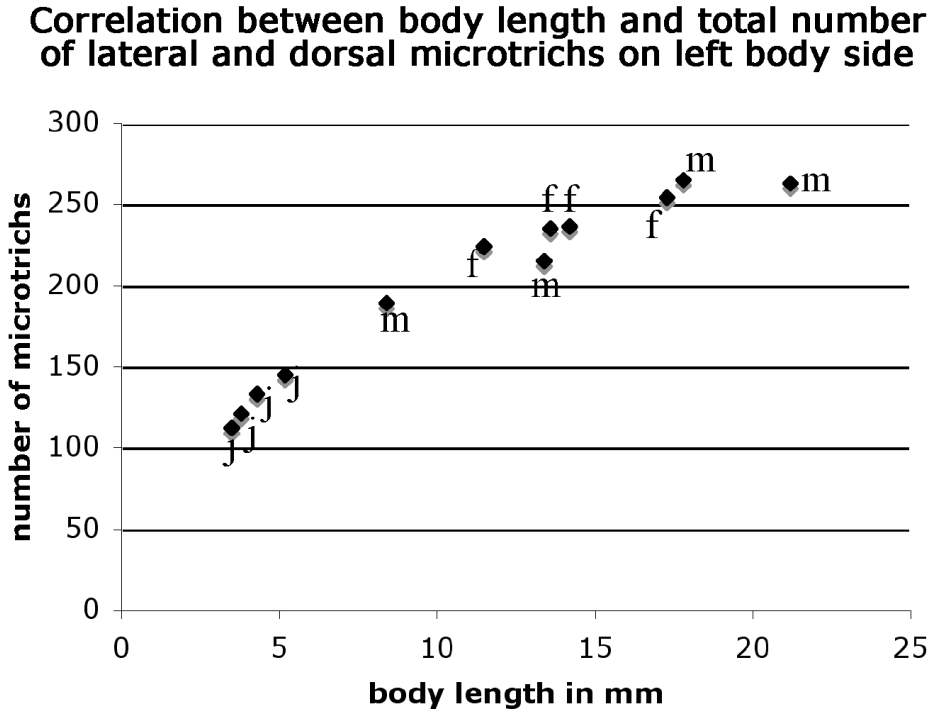


Fig. 7. Correlation of body length and summed number of microtrichs of left dorsal and lateral units (j = juvenile; f = female; m = male). Equation of curve is:
 $y = 57.1 + 18.7 - 0.42x^2$; $r^2 = 0.98$; overall $F_{2,9} = 324.9$, $P = <0.001$.

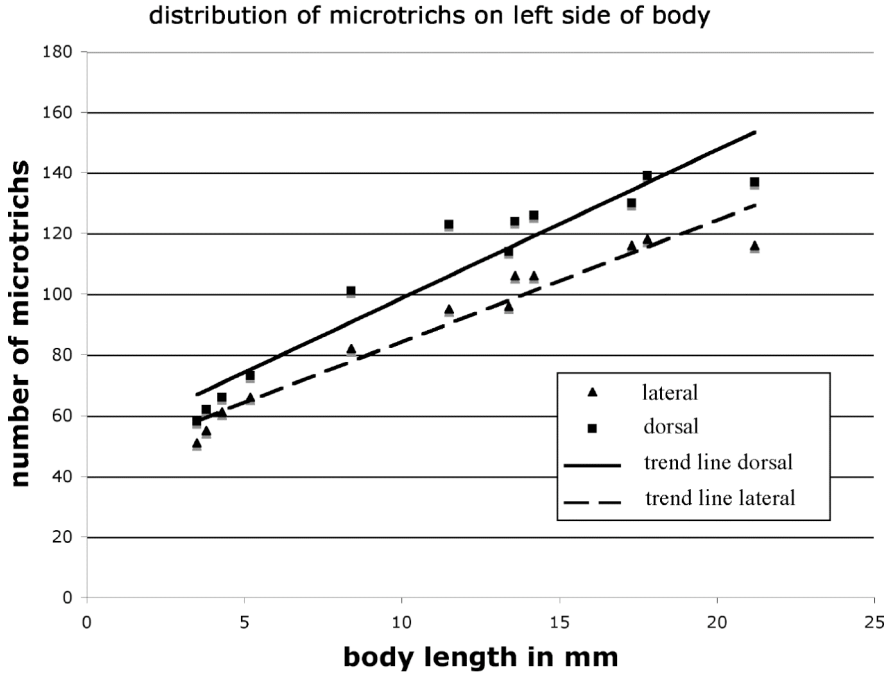


Fig. 8 The distribution of dorsal and lateral microtrichs on the left side of the body of *Dikerogammarus villosus* (Sowinsky) with trend lines

Microtrich structure

In three specimens (juvenile, female and male) the length of the individual microtrichs was compared in pereonite1, pleonite 1 and urosomite 1. The average length of a microtrich was $6.2 \mu\text{m}$ (S.D. $0.08\mu\text{m}$). The depth of the depression was approximately $1.5 \mu\text{m}$. In the three specimens the average distance between two microtrichs in a unit was $6.54\mu\text{m}$ (S.D. $0.77\mu\text{m}$).

A unit is surrounded by specialized cells with a very fine cuticular pore structure (pore diameter approximately 250 nm) intermixed with a few larger pores of approximately 800 nm . This specialized area is slightly elevated in relation to the surrounding cuticula and contains the groove in which the microtrichs occur.

Individual microtrichs are implanted in the cuticle within semi-circular concentric 'rings'. These 'rings' are not full circles, but the foldings reunite with the cuticle at two sides of the microtrich. At one side this reunion of the foldings with the cuticle is

more pronounced than at the other side, but always in line with the horizontal axis parallel to the oval shaped microtrich (fig. 9).

The cuticle area surrounding the microtrichs is not covered with the cuticular scales, which are abundantly present on the body. Depending on the number of microtrichs per unit this specialized area can be up to 180 μm long. Smallest distance of the scales to the microtrichs is approximately 25 μm (fig. 4).

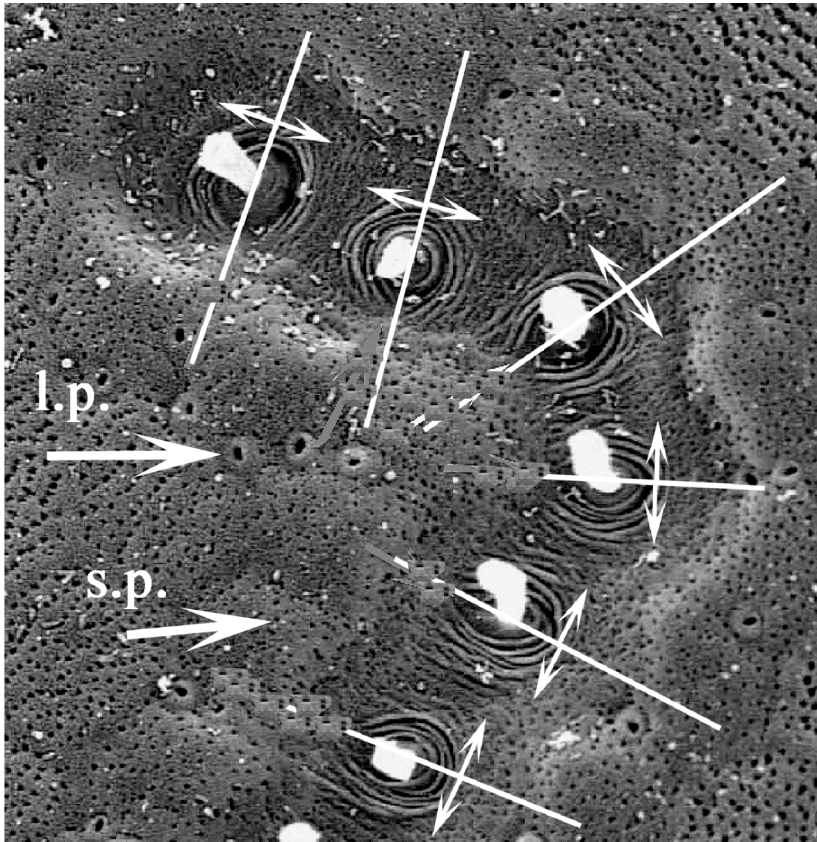


Fig. 9. Lateral line unit showing semi-circular patterns of the cuticle at the bases of the microtrichs. The white lines indicate the orientation of the microtrichs and the arrows their theoretical freedom of movement. Larger and smaller pores are indicated. Arrows indicate a small (s.p.) and a large pore (l.p.)

The microtrichs are strongly flattened and at cross section elliptical ($1.4\mu\text{m}/0.6\mu\text{m}$) and hollow. The inner space is narrowly oval. A long and slender central filament can be observed (figs 10, 12 and 13).

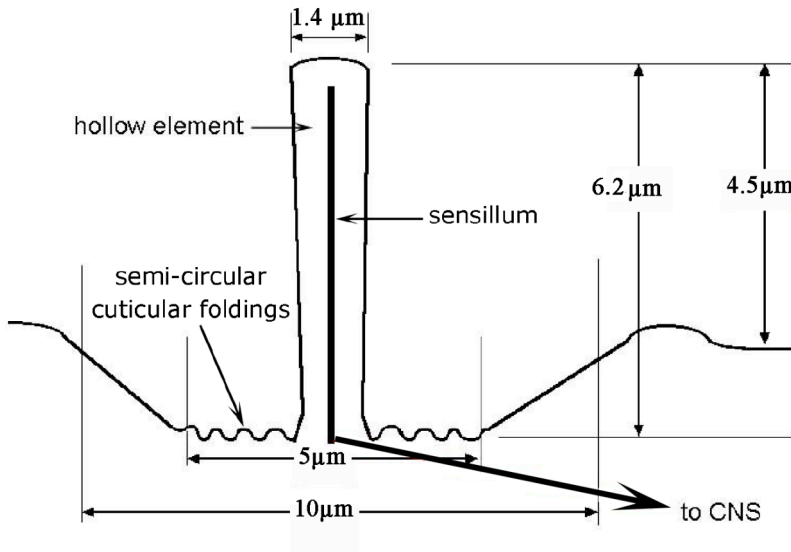


Fig. 10. Schematic representation of a lateral line microtrich in frontal view with dimensions. The arrow indicates connection to central nervous system (CNS)

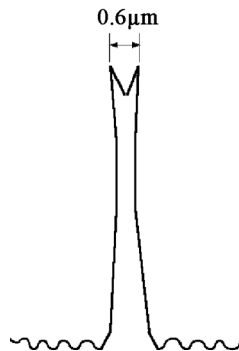


Fig. 11. Schematic representation of a lateral line microtrich in lateral view

Near the apex of the mantle the distance of the central filament to the mantle varies from 80 to 520 nm as a result of the elliptical shape of the inner space (figs 12 and 13).

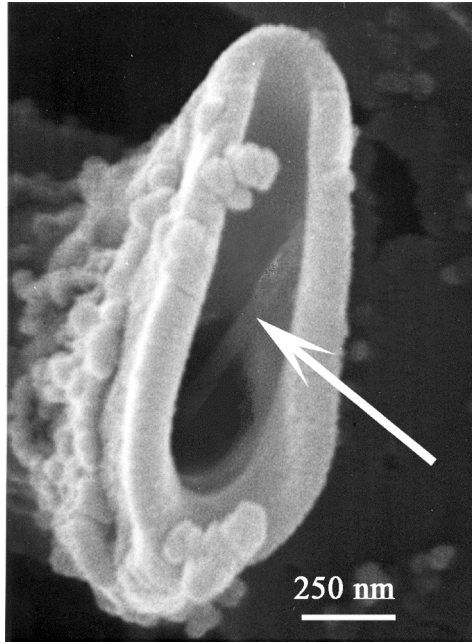


Fig. 12. Lateral line microtrich in apical view. The arrow indicates the central filament.

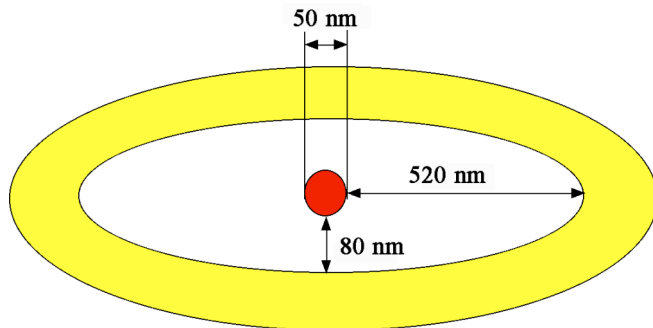


Fig. 13. Schematic representation of a microtrich in apical view with dimensions.

Discussion.

Hypothetical function of lateral line organ

Alongside the amphipod's body water will flow in different directions determined by the movement of the animal itself in stagnant or running water, the presence of nearby static objects or the movement of other organisms or objects approaching the amphipod (fig. 14).

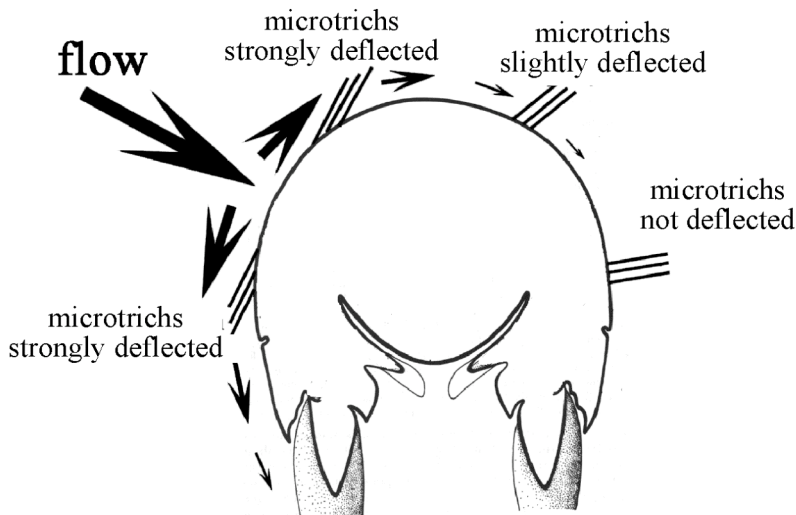


Fig. 14. Schematic representation of flexing of microtrichs resulting from flow along the amphipod's body, induced by a moving or static object. Arrows indicate flow direction. Arrow size is proportional to flow intensity (cross section after Charniaux-Cotton: Gruner, 1993).

The small size of the microtrichs reduces the sensitivity for water displacement by the microtrichs. Around every object in water a boundary layer builds up as a result of flow speed, time and other variables. This boundary layer is a layer of fluid in which the flow velocity changes from zero at the object's surface to the free stream velocity away from the object's surface. This boundary layer varies in thickness, depending on a number of variables, like flow velocity, water density and water viscosity. The fact

that the boundary layer builds up in time, limits the capacity to measure water displacement for very short microtrichs (4.5 μm), like those of the lateral line organ.

An individual microtrich may respond to flow by deflection of the mantle in the same direction as the flow, but deflection depending on the position and angularity of a microtrich in the unit, known as shear, seems more likely (fig. 15). The fact that the cuticular ring-structure at the basis of the microtrichs is not continuous (fig. 9), sustains a bi-directional rather than multi-directional freedom of movement for the microtrichs.

When the mantle of a microtrich deflects to such an extent that it makes contact with the central filament, this filament will deflect as well. This may excite nervous cells in the filament.

Deflection of a microtrich is a function of flow directionality and flow intensity (fig. 14). Not all the flattened microtrichs of a unit will respond equally to a unidirectional flow, due to their shape. A graphic representation of this is given in figure 14. In a unit there will be a number of microtrichs that do respond and others that do not, also depending on the response threshold described below (fig. 15 A,B). Given the shape and arrangement of the microtrichs the situation represented in figure 15B (the result of shear) appears more likely than in figure 15A, where the deflection is completely in line with the flow.

Each unit will provide the central nervous system with information on the directionality at the relevant part of the body. If each dendrite has two-directional sensitivity, a very common situation in arthropod mechano-reception (Wiese, 1976), a 360° angle is covered by a fan-shaped row of microtrichs. The central nervous system combines the information coming from the various responding units.

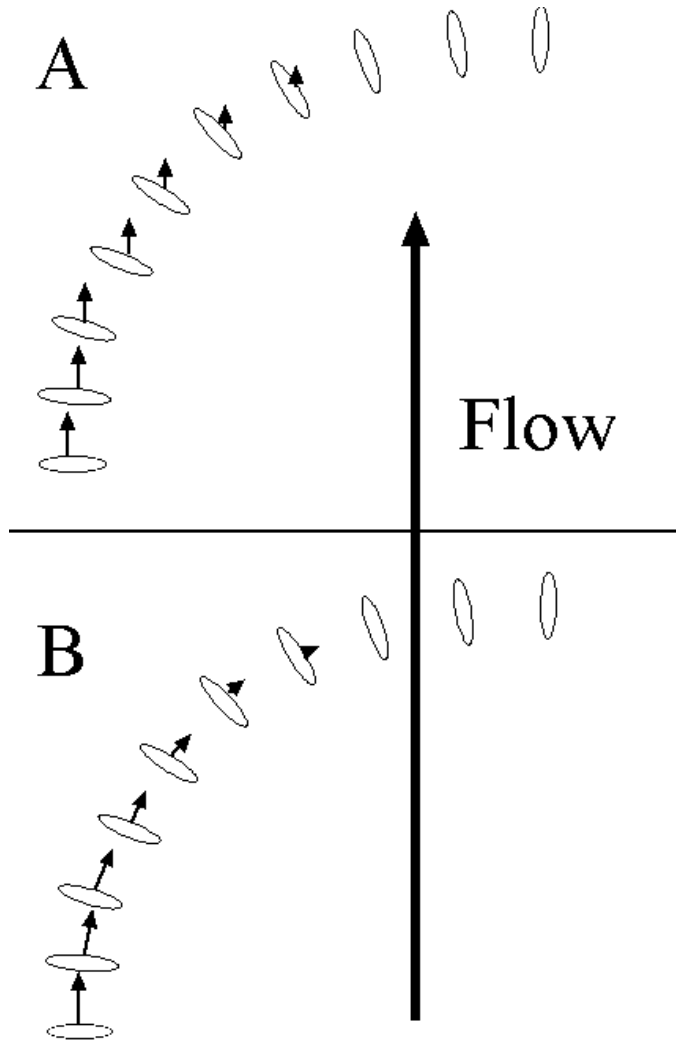


Fig. 15. Hypothetical representations of the influence of flow on elements of the lateral line organ. Ovals represent lateral line microtrichs in apical view, large arrow indicates flow direction, length of smaller arrows is proportional to the flow-induced deflection of the microtrichs: A) deflection of microtrichs in line with flow, B) deflection of microtrichs as a result of shear.

Threshold

If all microtrichs on the body would respond equally to flow, no useful information will reach the central nervous system. Therefore, a response threshold is necessary. In the lateral line organ the oval inner cavity may provide this threshold by the diverging distance from the mantle to the filament. In order to make contact with the central filament, the apical part of the mantle has to be moved over a more than 6 times greater distance when moved sideward as opposed to a 'front-back' movement: 520 nm instead of 80 nm (figs 11 and 12). This difference, in combination with the flow directionality depending deflection of the flat microtrichs, will provide the needed response threshold and the information of the sensilla to the CNS becomes unambiguous.

Chemo- or mechano-sense

Olyslager & Williams (1993) concluded that the lateral line system acts as a kinetic sensory organ, responsive to changing flow conditions. This conclusion was based on experiments, in which the microtrichs were masked with typewriter correction fluid. This is a harsh process for delicate animals like gammarids. Correction fluids in general contain solvents that are most probably toxic for small organisms like amphipods. The observation that non-masked controls were significantly more responsive to changing current conditions than masked specimens may very well be the result of a decreased general fitness of the latter specimens, caused by the masking of the microtrichs. On the other hand, the increasing thickness of the boundary layer that builds up in time, is very likely to hinder kinetic sensitivity as proposed by Olyslager & Williams (1993). The long plumose setae (> 50 μm) that can be found dispersed over the amphipod cuticle probably have this kinetic sensitivity (Wiese, 1976).

In the Olyslager & Williams paper vibration detection was declined as a possible function. However, the two frequencies that were measured in the experiment (57 and 133 Hz) may very well be outside the detectable frequency range of the microtrichs. Nothing is known about acoustic sensitivity in amphipods thus far, and therefore, vibration detection cannot be excluded as possible function for the lateral line organ.

In their study Steele & Steele (1999) found no evidence of a mechano-sensorial function for the microtrichs. They considered the sensilla 'poorly located' for detecting water movement, since the clusters occur in depressions, and the small size of the microtrichs would place them in the boundary layer. This is probably true for a continuous water flow, but for short-living pressure waves, the small size of the microtrichs is not necessarily a barrier. Near-field water displacement, resulting from pressure waves, that are produced by other organisms or objects, can fall within

measurable limits. Boundary layer thickness determines these limits, and, as stated above, this thickness is a function of time, water-viscosity and water-density.

Steele and Steele (1999) found that two sensory cells can be found at the basis of each microtrich. From one of the cells one ciliary process, from the other two ciliary processes project into the receptorlymph cavity. This may be indicative for a bimodal function of the microtrichs. This bimodality is common in invertebrates. In the evolutionary process reduction of numbers of pores in the cuticle is a logical step for species that have to maintain their physiological condition in environments with, for instance, a varying salinity. We therefore advocate near-field water displacement sense for the lateral line organ in amphipods, possibly in combination with chemo- and/or electro-sensitivity.

In general, epigean gammaridean amphipods live primarily in interstitial spaces, in full or semi-darkness conditions, to protect themselves against exposure to predators (this thesis, chapter 4). Their cryptic lifestyle is comparable to that of real hypogean amphipods like *Niphargus* and *Pseudoniphargus*. The blind species of these genera also possess the lateral line organ (pers. obs. first author). This organ may be essential for animals that are both able to swim freely and move about in narrow spaces.

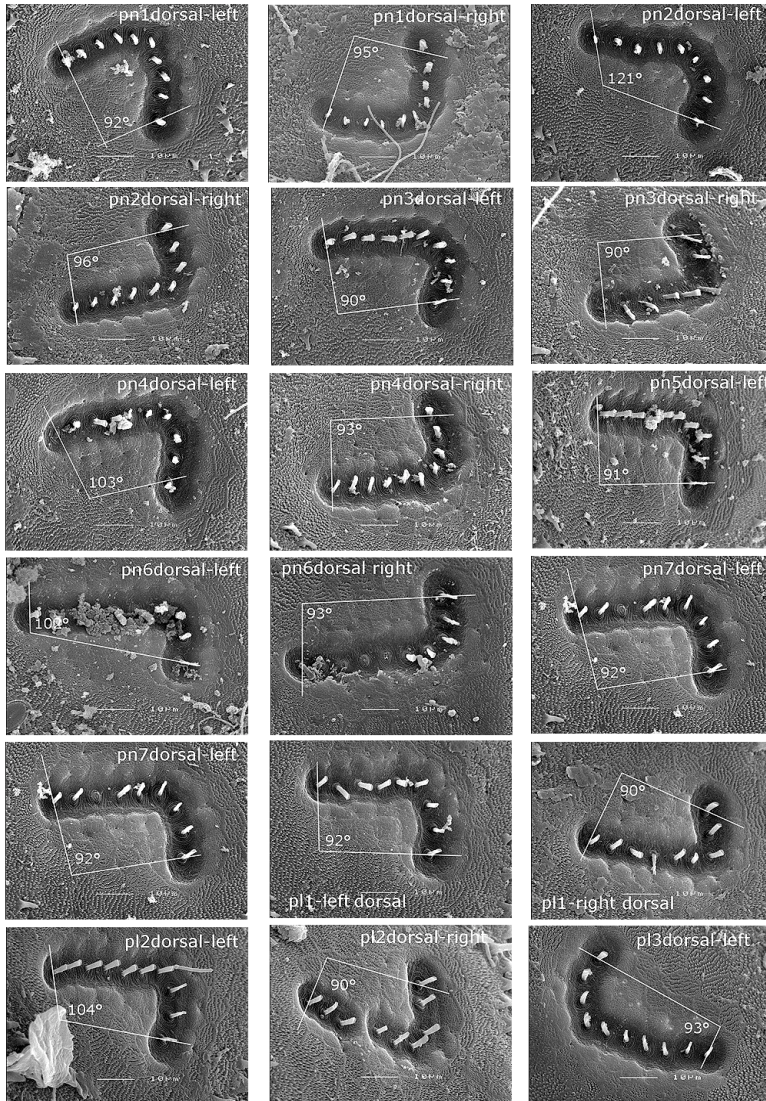


Fig. 16. Largest angle of two microtrichs per unit of the dorsal lateral line organ units of *Dikerogammarus villosus* (Sowinsky, 1894): pn = pereionite; pl = pleonite

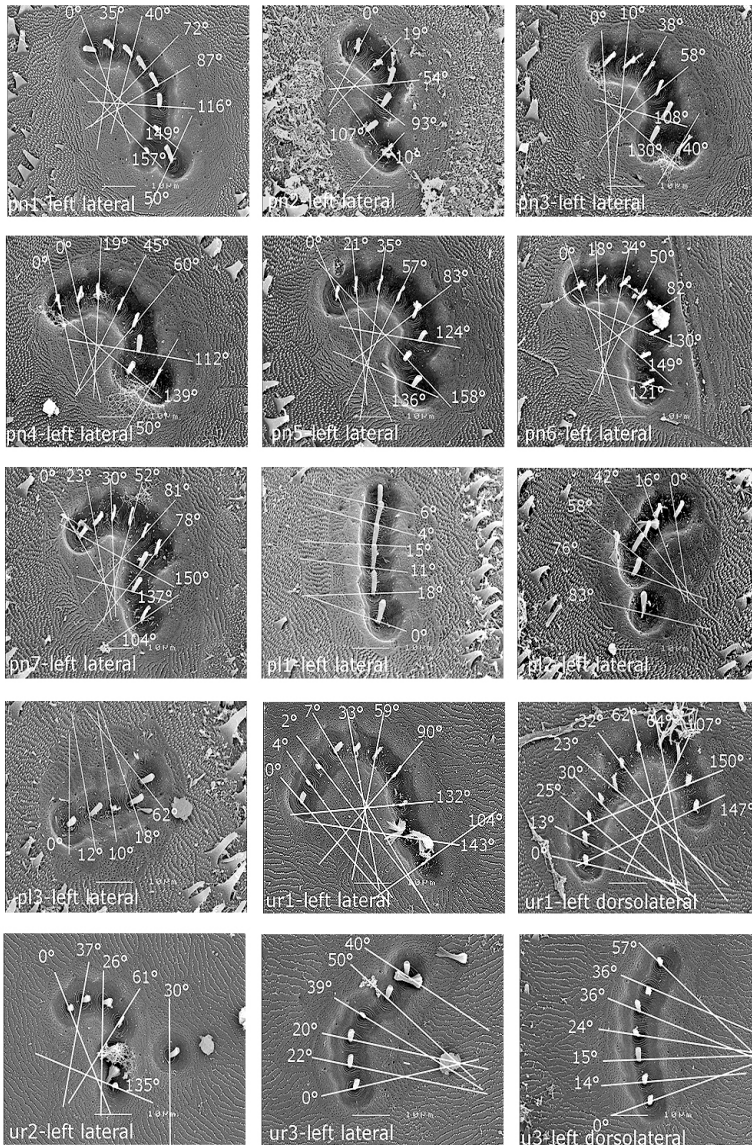


Fig. 17. Relative angularity of lateral microtrichs in *Dikerogammarus villosus* (Sowinsky, 1894): pn = pereonite, pl = pleonite, ur = urosomite

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Results

(research topics from introduction)

1a) *Interaction with other amphipod species (chapters 1 and 4)*

During 1998-1999 the range extension of *D. villosus* in the IJsselmeer/Markermeer-complex was monitored (Dick & Platvoet, 2000: this thesis, chapter 1). It became clear that the indigenous *Gammarus duebeni* was rapidly replaced by the invasive *D. villosus*. Both species prefer the stony banks of the lake. At the same time the successful North American invader *G. tigrinus*, a species well established in the lake for five decades, was forced from the hard substrates it preferred, but due to its small reproductive body size it was able to survive in the small spaces of the lake's softer substrates and zebra mussel beds (this thesis, chapter 4). Intraguild predation appears to be a major component of the interaction between these species, but habitat structure appeared to be an important factor too.

The interaction between the native *G. pulex* (Linnaeus, 1758) and *D. villosus* was also investigated in two laboratory studies. When contact occurs *D. villosus* easily out-competes *G. pulex* (MacNeil & Platvoet, 2005) as also found by Van Riel et al. (2007).

1b) *Interaction with other animals (chapter 2)*

In a laboratory study we found *D. villosus* preying on all offered invertebrates (Dick et al., 2002) and even vertebrates such as fish larvae (this thesis, chapter 2). Several methods of detecting and collecting prey were observed. *D. villosus* was observed digging in the substrate with their second antennae, while the first antennae were used as probes. In this way cryptic prey such as other amphipods and *Tubifex* worms were harvested. Cladocera and Heteroptera (*Sigara* sp.) were caught from the water column by a swift action of the second antennae. Large Zygoptera larvae, being fierce predators themselves, were attacked and killed. Consumption of freshly produced byssus threads of *Dreissena polymorpha* was observed, which may hinder the mussel's development. A relation between the decreased presence of the mussel on the stony banks in the Gouwzee and the arrival of *D. villosus* is suspected. Grazing of settling post-veliger larvae by *D. villosus* may be the cause of this decline.

2. *Feeding behaviour and food choice (chapters 2 and 3)*

D. villosus is an opportunistic species, a characteristic for successful invaders. Chapter 3 of this thesis describes how the species can easily survive on a diet of microscopic unicellular green algae suspended in the water column. Through cleaning

of its body while bringing the particles to its mouth it strongly reduces the concentration of algae as compared to controls.

In an overall study of its feeding techniques (this thesis, chapter 2) the animals were found grazing gravel and other particles by lifting and manipulating and cleaning these from organic matter.

Recycling of its own faeces was observed frequently. Perhaps this is a common phenomenon in omnivorous and herbivorous peracarids with a very short digestive tract.

A very important difference between the invasive *D. villosus* and indigenous species is the invader's ability to prey on hard-bodied animals, like intermoult crustaceans and insects with a sometimes very hard chitinous skin. They are able to prey on these animals due to a steeper allometric growth of the mandibles, especially their muscular mass (this thesis, chapter 5).

Apart from their sensory function the pair of second antennae plays a major role in most of the feeding in *D. villosus* (this thesis, chapter 5). Compared to the native gammarideans these antennae are much longer, thicker and stronger. The flagellum of these antennae is provided with a dense setation that contains hairs with a curved tip that act as Velcro, probably to increase friction with prey. The second antennae are used for a) the first contact with potential prey by pulling the victim towards the outstretched gnathopods, b) the collection of organic matter, c) the manipulation of gravel or other matter for grazing or d) the production of a strong water current from which escape is difficult for small waterborne organisms like Cladocera and Copepoda. Thus the second antennae perform a much wider range of activities than just being sensory, and their role in collecting food is generally underestimated, not only for this species, but also for gammaridean amphipods in general. To a much lower degree, due to their slender composure, the first antennae also have this dual sensory/food-manipulating role.

The palms of the propodi of the gnathopods are provided with a dense setation that was observed acting as a receiving net for the collection of small prey such as Cladocera. These animals are pushed between the outstretched gnathopods by the second antennae. Finally the dactyli secure the prey that is subsequently brought to the mouthparts (this thesis, chapter 2).

Probably as a function of its extreme size, *D. villosus* is different from all other gammaridean species in the Netherlands in especially the functionality and size of antennae, mandibles and gnathopods, which body parts are well adapted for a predatory life-style.

Temperature may play an important role in the dispersal process of *D. villosus*. In general, poikilotherm or cold-blooded animals have a level of activity depending on

the water temperature. As we found during our observations, catching prey can be a matter of tenths of seconds. We did our observations at 19°C. Rising average water temperatures (approximately +1°C over the last three decades; source: WaterStat, Rijkswaterstaat/RIZA/RIKZ) will favour those animals involved in high-speed activities like predation in *D. villosus* as described above and further in this thesis.

3) *Habitat structure and niche competition (chapter 4).*

Wave protected banks consisting of imported boulders border the Gouwzee, and many other waterways and lakes in the Netherlands. The spaces between these boulders form a habitat preferred by many invertebrates and small vertebrates because of the safe haven these boulders provide for these animals against predation by larger predators, such as fish and water birds. These predators are very well represented in the shallow Gouwzee. Before the arrival of *D. villosus* this boulder habitat was occupied by *Gammarus duebeni* and *Gammarus tigrinus*. Both species have been forced from the boulder zone by *D. villosus* through the effects of intraguild predation (Dick et al., 2000), whereby *G. tigrinus* (minimum reproductive size females 4.5 mm) was able to find refuge in the small spaces of the soft lake sediments, but *G. duebeni* (minimum reproductive size females 9.3mm), also being forced from the hard substrate, became caught between 'a rock and a soft place'. It completely vanished from the lake. Therefore, nowadays only *D. villosus* and *G. tigrinus* can be found in the littoral bank zones, although the species seem to live strictly separated.

Within the boulder zone a differential spatial occupation of the various size-classes of *D. villosus* could be observed: large adults between larger zebra mussels and in larger spaces, sub-adults between smaller mussels and in smaller spaces and juveniles on the photic side between the algae that cover the boulders. Size-class separation in *D. villosus* seems (apart from sexual encounters) to be complete.

To study the exclusion process of *G. tigrinus* due to *D. villosus* from the hard substrate a habitat simulation grid was developed that consisted of plexi-glass plates with a number of differently sized holes (chapter 4, this thesis). It was found that both species selected the smallest possible hole in relation to their body-size, and did so in a deliberate, non-random manner. In grid holes the small individuals of *G. tigrinus* found protection against predation by the much larger *D. villosus*, by selecting the smallest available holes. In controls without grids predation by adults of *D. villosus* on both juveniles and adult *G. tigrinus* was extremely high, in some cases almost complete. These results are completely in line with the situation found in the field, where one step from a boulder to the soft lake bottom sediment means a difference from a 100% *D. villosus* presence to a 100% *G. tigrinus* presence.

In the laboratory no difference was found between predation and cannibalism of juveniles of *D. villosus* and *G. tigrinus* by adult males of *D. villosus* in the experiments in which the juveniles were not protected by a grid with small holes.

4) *General and functional morphology (chapters 3 and 6 – 10).*

To study the success of an invader a thorough knowledge must be acquired of its morphology in relation to its behaviour. Classical descriptions are insufficient for in depth interpretation of behavioural adaptations to a certain life-style. Therefore, in 2005 the Amphipod Pilot Species Project (AMPIS) started, in which very detailed morphological information of a limited number of species is accumulated in a database. This database will form a reference point for students of many disciplines in the study of amphipods. *D. villosus* was, because of its exceptional characteristics, elected as first species in the program, and the inventory of its morphology is underway. A number of new findings resulting from this inventory are presented in the chapters 7 to 10. A lock-and-key system was described for precopulation (chapters 7 and 8) that possibly mediates in partner choice. Pores in the female's body accommodate the insertion of a palmar spine of a male during the precopulatory stage, a period of time in which the male guards the female prior to the copulation. For this the male hooks the first gnathopods onto the first and fifth body somite of the female at the start of the precopulation. Females may optimize their reproductive capacity by passively allowing only guarding males of preferred minimal body-size. This lock-and-key mechanism may also inhibit cannibalistic behaviour of the large predatory males towards the much smaller females.

A presumed statocyst, normally considered being a geo-sensitive organ was found in *D. villosus* (this thesis, chapter 9). In decapods such statocysts can be found in the basis of the antennae. In *D. villosus* the same type of depressions can be found near the posterior edge of the cephalon (the 'head'). The location of these depressions may be indicative for a function as vibration receptor, analogous to our ears. Although this may be considered another adaptation to the invader's predatory lifestyle, it is also found in other *Gammarus* species (Van der Velde, pers. comm.).

The lateral line organ in *D. villosus* is described in detail and its function discussed in chapter 10. It was found that the microtrichs (sensorial micro-setae) that built up the dorsal and lateral sensory units are hollow, with a centrally placed (presumably sensorial) filament. The organ may be sensitive to near-field water displacement, which allows detection of predators, prey, objects and possibly conspecifics, analogous to the lateral line organ in fish and amphibians. A combined chemo/mechano-sensory function is suggested.

Conclusions

a) Which factors determine the success of *D. villosus* as an invader?

- the species is a true omnivore which means that he can use all the resources available
- as a result of its extreme body size in invaded areas and the characteristic strong allometric growth of body parts like mandibles the species out-competes the indigenous and other invading amphipods
- through its relatively large jaws it can consume hard-bodied prey
- the preferred hard substrates are widely available in the Netherlands in the form of stony banks and groynes
- the species has a wide tolerance for changing salinities and temperatures.
- the Ponto-Caspian bivalve *Dreissena polymorpha*, already present in western Europe from the middle of the 19th century possibly facilitates for all invasive species of Ponto-Caspian origin, amongst those *D. villosus*.
- absence of specific predators gives the species a sort of ‘honeymoon’ time in the newly invaded area as does the absence of diseases

b) What is the impact of *D. villosus* on the indigenous and alien aquatic fauna and will the species be able to maintain its position in the coming decades?

- At least one amphipod species, *Gammarus duebeni* Liljeborg, 1852 was completely removed from the IJsselmeer/Markermeer complex.
- *Gammarus tigrinus* Sexton, 1939 was removed from the hard substrates, but found refuge in the soft sediments of the lake.
- In a laboratory study it was found that *Gammarus pulex* Linnaeus, 1758 is out-competed by *D. villosus*.
- Eggs of *Cottus gobio* Linnaeus, 1758, a bottom dwelling fish of hard substrates, were found preyed on. A negative correlation between presence of *D. villosus* and the reproduction rate of the fish is suspected.
- The disappearance of *Theodoxus fluviatilis* (Linnaeus, 1758), an egg-depositing snail, coincides with the arrival of *D. villosus*. Grazing of the snail’s eggs by the amphipod seems the most likely cause.
- The answer to the question whether this species can maintain its presence in the Netherlands can only be speculative. It is likely that the large size of the species is of a temporary nature. Arrival of specialized Ponto-Caspian predators, parasites,

pathogens and food-competitors may end the 'honeymoon' period the species experiences now, and its body size may decrease as a result. The advantages the species has acquired through the steep allometric growth of body-parts may diminish and a relatively modest position of the species may be the result. It even may be completely replaced by new invaders or indigenous species.

Samenvatting

Om inzicht te krijgen in de processen die leiden tot de verspreiding van soorten is het noodzakelijk kennis te verwerven van de biologie van die soorten. Louter kennis van verspreidingspatronen is niet voldoende. De vraag waarom een invasieve soort in Nederland zo succesvol is, het onderwerp van dit proefschrift, kan niet eenvoudig worden beantwoord. Alleen de optelsom van informatie over gedrag, concurrentie, beschikbaarheid van voedsel, (functionele) morfologie, allometrische groei, aan- of afwezigheid van pathogenen en parasieten, genetica, reproductie, ecofysiologie en abiotische factoren kan leiden tot enig begrip. In dit proefschrift is geprobeerd een groot aantal van de bovengenoemde factoren te onderzoeken. Hierbij is gekozen voor de agressieve Ponto-Caspische vlokreeft *Dikerogammarus villosus*, een soort die enige jaren geleden West Europa is binnengedrongen na de opening van het Main-Donau kanaal in 1993. Via deze verbinding hebben meerdere soorten vanuit de Donau de Rijn weten te bereiken. Over de gevolgen hiervan is nog maar weinig bekend. Eerder, in 1987, heeft de Ponto-Caspische slijkgarnaal *Chelicorophium curvispinum* via het Mittelland kanaal de Rijn bereikt en zich in het benedenstroomse deel van de Rijn gevestigd. Deze soort heeft een periode van grote bloei doorgemaakt op de harde substraten van de Rijn en andere wateren (>100.000 exemplaren per m²). Deze harde substraten bestaan voornamelijk uit stenen en rotsblokken die aangebracht zijn om de kribben en oevers te beschermen tegen erosie. Deze hoge dichtheden bleken echter tijdelijk te zijn en zijn mogelijk door de komst van andere Ponto-Caspische fauna-elementen (vissen, kreeftachtigen en platwormen) en parasieten omlaag gebracht.

Van de interactie van invaderende soorten onderling en van invaderende en inheemse soorten is zeer weinig bekend. Wel wordt langzamerhand duidelijk dat er een grote invloed van een invaderende soort kan uitgaan op inheemse soorten. In dit proefschrift wordt de verdwijning van de inheemse vlokreeft *Gammarus duebeni* in verband gebracht met de invasie van *Dikerogammarus villosus*, waarbij de laatste soort zou hebben gepredeert op de eerste, zgn. 'intraguild predation'. Tevens is een Noord Amerikaanse vlokreeft *Gammarus tigrinus*, die zich hier al vijftig jaar geleden heeft gevestigd en uitgebreid, eveneens van de harde substraten verdreven door de Ponto-Caspische vlokreeft. Dit laatste proces wordt in dit proefschrift beschreven. In dit proefschrift worden tevens de interacties tussen *D. villosus* en andere in- en uitheemse evertebraten en vertebraten bediscussieerd.

De belangrijke rol die substraat speelt bij interacties is onderzocht door middel van een proefopstelling. De in het veld gevonden strikte scheiding van individuen van *D. villosus* met verschillende lichaamsgrootte werd bevestigd door de uitkomsten van

experimenten met deze proefopstelling. De dieren kozen het kleinst mogelijke gaatje om contact met grotere soortgenoten en mogelijk andere predatoren te vermijden. Deze uitkomst van het experiment bleek significant te zijn. De soort bleek in controleproeven een sterk kannibalistisch gedrag te vertonen. Bij een proef waarbij adulten van *D. villosus* met juvenielen van de eigen soort en van die van *Gammarus tigrinus* in een container zonder schuilmogelijkheden samengebracht werden is geen verschil gevonden tussen predatie en kannibalisme. Een belangrijke conclusie uit dit onderzoek is dat gebiedsuitbreiding van een soort alleen mogelijk is als het juiste substraat aanwezig is. In de Gouwzee is een stap van de stenen naar de zachtere meerbodem een stap van 100% *D. villosus* naar 100% *G. tigrinus* aanwezigheid in het substraat. Deze soorten leven dus strikt gescheiden.

De plaats die *D. villosus* inneemt in het voedselweb en het trofische niveau waarop de soort zich bevindt, is door middel van stabiele isotopen vastgesteld. Hierbij is duidelijk geworden dat de soort zich in onze wateren op hetzelfde niveau bevindt als zoobenthivore vissen. Dit is geheel in overeenstemming met het door ons gevonden voedingsgedrag van de soort. Door de vlokreeft te filmen is inzicht gekregen in de wijze waarop de soort zijn voedsel verwerft. Hierbij is gebleken dat het dier morfologisch sterk aangepast is om een prooi te verschalken (zwarte kaken, zeer lange en sterke antennes en klauwen), maar niettemin ook als filtreerder en verzamelaar van micro-organismen kan overleven. Grazen van aangroeiende organismen op stenen is eveneens veelvuldig waargenomen. Recycling van voedsel is gedurende dit onderzoek veelvuldig waargenomen en is waarschijnlijk een normaal element van de spijsvertering. Het korte darmkanaal, waarbij heropname van enzymen en bacteriën belangrijk is, speelt hierbij mogelijk een rol.

Het is dus een echte opportunist, een nuttige eigenschap voor een soort die aan gebiedsuitbreiding doet. Het trofische niveau van een soort is een momentopname. Het is zeer wel mogelijk dat door verandering van de biotische samenstelling van een ecosysteem (concurrentie, predatie) dit trofische niveau op termijn zal veranderen. Vooral niet trofisch gespecialiseerde dieren kunnen zich deze flexibiliteit permitteren.

Invaderende soorten hebben vaak een groter lichaam dan de exemplaren in de populaties van het gebied van oorsprong. Hiervoor zijn diverse oorzaken aan te wijzen: gebrek aan natuurlijke vijanden (afwezigheid van specifieke predatoren), een overdadig voedselaanbod wegens gebrek aan concurrentie, afwezigheid van pathogenen en parasieten) en mogelijk abiotische factoren zoals substraatstructuur en de chemische samenstelling en temperatuur van het water. Deze lichaamsvergroting lijkt ook te gelden voor *Dikerogammarus villosus*, waarbij vooral in het voorjaar mannelijke exemplaren gevonden kunnen worden van 30 mm lang, een veel grotere lengte dan de max. 21 mm die bekend is uit het gebied van oorsprong. De mogelijke

gevolgen van deze reuzengroei op het gedrag van de vlokreeft wordt beschreven in het hoofdstuk over allometrische groei (groei van een afzonderlijk lichaamsdeel t.o.v. de groei van het totale lichaam). De sterkere allometrische groei van vooral de mandibels, de kaken, kan verklaren waarom *D. villosus*, in tegenstelling tot de inheemse vlokreeften, wel op andere vlokreeften en evertrebraten met harde pantsers zoals insecten kan prederen.

Naast onderwerpen die direct verband hebben met de potentie van de soort om nieuwe gebieden te koloniseren is in dit proefschrift tevens getracht de kennis van de morfologie van deze soort en die van amphipoden in het algemeen te verdiepen. Een aantal voorheen onbekende organen en orgaansystemen is aangetroffen tijdens de ontwikkeling van het AMPIS (Amphipod Pilot Species) project, een samenwerkingsverband tussen de Universiteit van Amsterdam, de Radboud Universiteit Nijmegen en het Zoölogisch Instituut van de Chinese Academie van Wetenschappen in Beijing:

- Een slot-sleutel systeem voor de paring van deze vlokreeft en een aantal andere soorten is gevonden. Dit heeft verregaande consequenties voor ons denken over soortsvorming binnen deze groep dieren, doordat speciatie mogelijk gestuurd wordt door dit systeem.
- Een op een statocyst gelijkend orgaan bevindt zich in het cephalon ('kop') van *D. villosus*. Een statocyst was tot op heden niet van amphipoden bekend. Statocysten (evenwichtsorganen) waren wel bekend van diverse andere kreeftachtigen. De overeenkomst tussen de uitwendige expressie van dit orgaan in decapoden en die, gevonden in *D. villosus*, is dermate groot, dat deze niet genegeerd kan worden. Deze vondst zal zeker consequenties hebben voor de fylogenie (afstammingsleer) van de peracariden.
- Zijlijnorganen zijn bekend van vissen en amphibiëen. Een dergelijk systeem, waarbij d.m.v. kleine zintuighaartjes informatie aan het centrale zenuwstelsel wordt doorgegeven over de beweging van water om het lichaam wordt in dit proefschrift uitgebreid voor *D. villosus* beschreven. Een dergelijk, uitermate geavanceerd, systeem bij ongewervelde dieren was niet bekend.

Summary

To understand the processes involved in species distribution, knowledge of the biology of these species is vital. Information of the distribution pattern is useful but only one of the factors. The question why a species is a successful invader, the subject

of this thesis, has no simple answer. Factors, such as behaviour, competition, availability of food, (functional) morphology, allometric growth, presence or absence of parasites and pathogens, genetics, ecophysiology, reproduction and abiotic factors can lead to some form of understanding. In this thesis an effort is made to investigate a number of these factors. For this purpose *Dikerogammarus villosus*, an aggressive Ponto-Caspian amphipod was selected. This species is one of a host of species that invaded Western Europe after the opening of the Main-Danube Canal in 1993.

Earlier, the Ponto-Caspian amphipod *Chelicorophium curvispinum*, a tube building amphipod of hard substrates, made its way into Dutch waters in the eighttees of the last century. The first few years it could be found in large densities (> 100.000 per m^2). Most of the hard substrates in the Netherlands consist of imported rocks that protect groynes and banks against wave erosion. The high densities appeared to be temporary and possibly as a result of the arrival of other Ponto-Caspian faunal elements (fish, crustacea and flatworms) the densities of these amphipods decreased.

Knowledge of the interaction of the invasive and indigenous species is limited. Slowly it becomes clear that an invasive species can have a high impact on ecosystems. In this thesis the replacement process of an indigenous amphipod species, *Gammarus duebeni* by *D. villosus* is described. Intraguild predation (IGP) may play an important role in this process. The former North American invader *Gammarus tigrinus* has also been removed from the hard substrates. The fact that, in contrast to *Gammarus duebeni*, reproductive specimens of *Gammarus tigrinus* are very small, provided the last species with a refuge in the soft sediments of the lake bottom. *Gammarus duebeni* simply is too large to find refuge in this substrate and disappeared completely from the lake. It became caught between a rock and a soft place. It can nowadays be found in high salinity refuges only.

The position of *D. villosus* in a foodweb as well as its trophic level, has been determined through stable isotope analysis. In Dutch waters the species proves to be at the same level as zoobenthivorous fish. During an experiment in which the amphipod was filmed while offered a variety of food this predatory behaviour was frequently observed and the techniques used in catching prey described. The results of the experiments show that although the species is morphologically adapted to a predatory lifestyle (large jaws, long and strong antennae and claws) the species can nevertheless survive with filterfeeding and collecting of micro-organisms and grazing. It is a true opportunist, which is a very useful characteristic for a species in the process of range extension. The trophic level of a species can change in time and space as a result of, for instance, a changing biotic composition of an ecosystem. Especially non-specialized feeders can permit themselves this flexibility.

Recycling of food by consuming its own faeces has been observed frequently during the research and is probably a normal component of the species feeding behaviour. The short digestive tract, and the re-uptake of enzymes and bacteria may play a role.

Invading species often grow a larger size body than specimens of populations from the area of origin. Several factors are of importance: absence of natural enemies, a wealthy food supply through lack of competitors, absence of pathogens and parasites and possibly abiotic factors like substrate structure and the chemical composition and temperature of the water. This enlargement of the body can also be found in the invasive *D. villosus*, who can grow up to 30 mm, especially in spring. The populations in the original distribution area have a maximum body size of 21 mm. The consequences of this extreme growth are described in the chapter concerning allometric growth (growth of particular body parts with respect to growth of the whole body). In *D. villosus*, especially the jaws, antennae and gnathopods show a much steeper allometric growth than in the other western European gammaridean amphipod species. This may explain that in contrast to the other species *D. villosus* can prey on hard-bodied invertebrates.

Next to the subjects that are directly related to the potency of the species to extend its range an effort was made to deepen our knowledge of the (functional) morphology of this species and gammaridean amphipods in general. A number of hitherto unknown organs and organ systems has been described as a result of a co-operative project (AMPIS: Amphipod Pilot Species Project) of the University of Amsterdam, the Radboud University of Nijmegen and the Zoological Institute of the Chinese Academy of Sciences in Beijing:

- A lock-and-key system was found that is involved in mating. This finding may alter our approach towards speciation in gammarids.
- The statocyst is a geo-sensitive organ that has been found in several groups of crustaceans but thus far not in amphipods. On the cephalon, the head, of *D. villosus* two structures can be found that show great resemblance to the external expression of statocysts in the antennae of decapods. In the phylogeny presence of statocysts may change the position of the amphipods within the peracarids.
- Lateral line organs are known from fish and amphibians. This system that consists of series of small hairs that are sensitive to water movement around the body may be present in amphipods as well. This system is described and is the first finding of such an elaborate sensory device outside the vertebrates.

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Appendix 1

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- Dikerogammarus villosus* and *Chelicorophium curvispinum* on bare stone substrate in the main channel of the River Rhine. Arch. Hydrobiol., 166: 23-39.
- Van Riel, M.C., G. Van der Velde, S. Rajagopal, S. Marguillier, F. Dehairs & A. Bij de Vaate, 2006. Trophic relationships in the Rhine food web during invasion and after establishment of Ponto-Caspian invader *Dikerogammarus villosus*. Hydrobiologia, 565: 39-58.
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- Zettler, M.L., 1998. Zur Verbreitung der Malacostraca (Crustacea) in den Binnen- und Küstengewässer von Mecklenburg-Vorpommern. Lauterbornia, 32: 49-65.

appendix 2

Curriculum Vitae Dirk Platvoet

Geboren: 10 december 1953, Amsterdam.

Openbare Lagere School Purmerend: 1959-1965

Middelbare School Purmerend: 1965 – 1970 diploma Mulo.

Colloquium Doctum Universiteit van Amsterdam: mei 1982.

Diverse betrekkingen 1970-1979: assistent-boekhouder (Rock Vaguero, Pardo), assistent-drukker (Henkes-Senefelder), beheerder theehuis (Ploeftia), medewerker plantsoendienst (Gemeente Purmerend), assistent-beheerder kinderboerderij (Gemeente Purmerend), metaal recycling (zelfstandig ondernemer), administratief medewerker (Day-Glo, RCA), assistent-werkvoorbereider (GTI-Groenpol), medewerker calculatie (GTI-Suyver en Roossen).

Universiteit van Amsterdam, Zoölogisch Museum 1979 – heden: het beheren van de collectie van de afdeling Crustacea, het verrichten van taxonomisch, functioneel morfologisch en gedrags-onderzoek aan gammariden in het algemeen en aan invasieve soorten in het bijzonder, alsmede het in kaart brengen van de externe morfologie van amphipoden door middel van o.a. de scanning electronen microscoop.

De Stolp, Deventer: productie van huizen op schaal: 1992 – heden. Dit zijn ‘poppenhuizen’ voor volwassenen en kunnen variëren van een klein winkeltje tot een huis dat overeenkomsten vertoont met kasteel Drakestein. Er staan in Nederland en de rest van de wereld enige duizenden poppenhuizen van onze hand. Dienke, mijn echtgenote, voert het bedrijf, ik ben hoofd werkplaats (zonder personeel).

Hobbies:

Drummer van UvA band ‘de Nasty Noises’

Macro-fotografie and macro-video.

List of publications Dirk Platvoet

- Platvoet, D. & S. Pinkster, 1980. A new species of *Echinogammarus* from northern Spain, *Echinogammarus pseudoaquilifer* nov. sp. (Crustacea, Amphipoda). Bull. Zool. Mus. Univ. Amsterdam 7(2): 13-19.
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- 1985 June 28 –July 3: VIth International Colloquium on Amphipod Crustaceans, Ambleteuse, France. Side-line organ in amphipods.
- 2002 February 25 - 28: The 11th International Conference on Aquatic Invasive Species, Alexandria, USA. Comparative morphometrics of mouthparts and antennae in the invasive *Dikerogammarus villosus* and the native *Gammarus duebeni* (Crustacea, Amphipoda).
- 2002 July 22 - 26: Fourth European Crustacean Conference, Lodz, Poland. Feeding behaviour of *Dikerogammarus villosus* (Sowinsky).
- 2003 June 9 - 12: The 12th International Conference on Aquatic Invasive Species, Windsor, Canada. *Dikerogammarus villosus*, a ferocious omnivore.
- 2004 September 19 – 23: The 13th International Conference on Aquatic Invasive Species, Ireland. If they can't meet you, they can't eat you: simulation grid experiment to investigate body-size related habitat choice in the freshwater amphipod *Dikerogammarus villosus* (Sowinsky) (Crustacea) and its introduction into a freshwater lake in the Netherlands.
- 2005 May 5 – 7: International Workshop on Biological Invasions in Inland Waters, Florence, Italy. Temperature dependent feeding in *Dikerogammarus villosus* Sowinsky.
- 2005 July 18 – 22: Sixth International Crustacean Congress, Glasgow. The Amphipod Pilot Species Project, a novel Dutch-Chinese taxonomic initiative.
- 2005 September 23: The Chinese Academy of Science. Institute for Zoology. Presentation of the Amphipod Pilot Species Project.
- 2007 May 20-25: 13th International Colloquium of Amphipoda, Tihany, Hungary. The Amphipod Pilot Species Project (AMPIS) after its first year.

Editorial activities:

Associate editor-in-chief of *Acta Zootaxonomica Sinica*.

Paper reviews for *Hydrobiologia*, *Crustaceana*, *J. Crust. Biol.* & *J. Aquatic. Ecol.*