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Predation by pioneer water beetles (Coleoptera, Dytiscidae) from sandpit ponds, based on crop-content analysis and laboratory experiments

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With 5 figures and 6 tables

Abstract: Crop-content analyses of 898 pioneer beetles, belonging to five dytiscid species of sandpit ponds, (Coleoptera: Dytiscidae) were performed to identify possible nutritionally-based niches and competitive effects. *Agabus nebulosus, Nebrioporus canaliculatus, Scarodytes halensis, Hygrotus confluens* and *Hydroglyphus geminus* mainly feed on chironomid larvae and planktonic crustacea, especially Copepoda. However, a large amount of filamentous algae was found in the crops of *A. nebulosus* and *H. confluens* fed on Rotatoria, especially *Keratella*, while the small hydroporine *H. geminus* fed on the small nauplius larvae of Copepoda. Experiments were used to test the relative feeding preference of *S. halensis* for Copepoda and Cladocera and for dead or living plankton. Dead plankton was preferred, whereas the size of the plankton was important in the selection of Copepoda or Cladocera. The crop clearance times for different food items were also ascertained: no differences in digestion times of chironomid larvae, *Chaoborus* larvae and plankton were found.

Key words: food-niche, competition, food selection, digestion, feeding preference.

Introduction

The study of invertebrate associations from sandpit ponds of different ages shows that there is a succession of invertebrates, especially beetles, progressing from pioneer species towards a climax stage (HEUSS 1975). Previous investigations of sandpit ponds of recent origin have revealed five characteristic dytiscid beetles (KEHL, unpubl.): *Scarodytes halensis* (FABRICIUS, 1787), *Nebrioporus canaliculatus* (LACORDAIRE, 1835), *Hygrotus confluens* (FABRICIUS, 1787), *Hydroglyphus geminus* (FABRICIUS, 1792) and *Agabus nebulosus* (FORS-

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TER, 1771). These pioneer beetles often occur together in the same pond and in such high individual numbers (up to 300 individuals of *S. halensis* and *N. canaliculatus* in 2 m^2 sampling areas), that we suspected intraspecific and interspecific competition to be of importance.

Studies at 30 ponds, and overall more than 42 sampling sites, showed that the five beetle species slightly differ in habitat and/or microhabitat selection. *Nebrioporus canaliculatus* was dominant in new ponds without any vegetation. *Scarodytes halensis* was also present in new ponds, but at a lower relative abundance. In addition, this species was found in ponds with sparse vegetation on the bank, but still on immature soil (i.e. with a sandy or silty bottom). In shallow, warm ponds and on banks, a high relative abundance of *H. geminus* was found. High relative abundance of *H. confluens* only appeared in ponds with a clay bottom (more than 15 % clay). *Agabus nebulosus* was found in all ponds with a clay or sand bottom and with no or sparse vegetation, but at a low frequency (KEHL & DETTNER, in press).

In order to study a possible competition between these species or resource-based niche occupation, we performed gut analyses to determine the dietary-composition of the beetles. In addition, laboratory feeding preference experiments were performed with adult *S. halensis* to test their predatory or scavenging nature, their feeding habits on the bottom and the free water body and their preference either for Copepoda or Cladocera.

Adult and immature Dytiscidae are well known as aquatic predators and some studies on the dytiscid nutrition have been published previously. DEDING (1988) gives a summary of the diet of 36 Danish Dytiscidae and Noteridae, DETTNER et al. (1986) dissected some rheophilic Dytiscidae, and BOSI (2001) described the diet of some Agabinae and Colymbetinae. Dytiscids are generally predators on microcrustacea, insect larvae, fish and amphibian larvae (e.g. BAY 1974, BISHT & DAS 1979, HENRIKSON 1990, NILSSON & SVENSSON 1994, NILSSON & SVENSSON 1995). No detailed information on the diet is available for the above-mentioned pioneer beetles from sandpit ponds.

Furthermore, little is known about the digestive process in dytiscids. Meat ingested by *Dytiscus circumcinctus* passes through the crop to enter the midgut only one hour after feeding (DEEGENER 1910). In the crops of *Platambus maculatus*, only a brown fluid with chitinious fragments was found after 3 hours of feeding (DETTNER et al. 1986). In order to examine how long prey from the crop could be recognised and whether there was a difference in digestion time between heavily sclerotised and less well-sclerotised food items in the crop, digestion experiments were carried out with adult specimens of *S. halensis*.

Study sites

Sampling was carried out in sandpit ponds situated around Bayreuth in Germany (49° 56′ N, 11° 34′ E), at an elevation of 400–450 m. In this area, sand is mined in many small pits from the Triassic Keuper. Pond area varied from 10 to 200 m^2 and ponds had a maximum depth less than 1.80 m. The ponds studied invariably possessed sandy or clay bottoms and had no or sparse vegetation (no submerged vegetation, only some species of Poaceae or few *Typha* spec. on the bank). All ponds possessed a similar macrozoobenthic fauna and fish were invariably absent.

Materials and methods

Crop analysis

The beetles for the gut analysis were collected from 10 different sandpit ponds. Samples were collected monthly, from May to September 2000. To collect samples, a circular aquatic net (diameter 30 cm, mesh width 1 mm) was used. Samples were then immediately transferred to boxes with filtered pond water and stored in the field in an insulated bag. The beetles were killed in the laboratory by freezing.

Whole foreguts of defrosted beetles were dissected and transferred to microscope slides: a foregut was opened under a binocular microscope and its contents spread over the microscope slide and covered with polyvinyllactophenol and a cover-slip.

Slides were systematically studied under a microscope at 100 × magnification, with details examined at 400 × magnification. Food fragments were assigned to 15 prey item groups: *Keratella* (Rotatoria); other Rotatoria; Copepoda (adults and copepodits); Nauplius larvae of Copepoda; Cladocera; Crustacea; Ephemeroptera larvae; Chironomidae larvae; *Chaoborus* larvae; Ceratopogonidae larvae; Diptera pupae/imagines (most of them appeared to be Chironomidae); Insecta (undeterminable fragments of legs and compound eyes); filamentous algae; other algae; vascular plant material (Fig. 1). The higher taxonomic groupings do not include records of the lower taxa identified.

For each food item type, the percentage of frequency of occurrence was defined as (the number of crops in which a food item was found / total numbers of filled crops) × 100.

Non-parametric Kruskal-Wallis tests, and Tukey-Kramer post-hoc tests were used to compare crop contents of each species for each sample (SACHS 1999).

In the case of ingested chironomids, the claws of parapods, abdominal procerci and head capsules (Fig. 1 f, g and h) remain in the crops, and the width of intact chironomid head capsules could be measured under the microscope. The quotient of chironomid records with a head capsule to all chironomid records helped to study feeding behaviour.

On each sampling date, plankton samples (plankton net 100 µm mesh width) and macrozoobenthos samples were taken from the ponds. The plankton composition was examined and estimated using a microscope. For each sample, three 1 ml subsamples were systematically studied and the abundance was estimated into one of four different

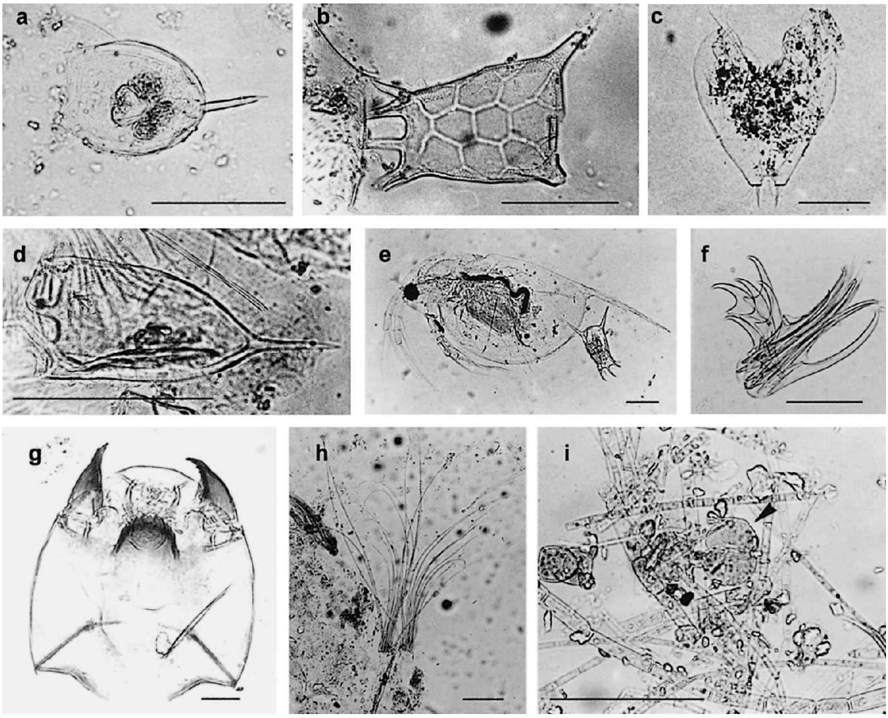

Fig. 1. Fragments of food items found in the crops of pioneer water beetles: **a** – Rotatoria, *Lecane* spec., **b** – Rotatoria, *Keratella* spec. 1, **c** – nauplius larvae of Copepoda, **d** – Rotatoria, *Keratella* spec. 2, **e** – Intact Cladocera and *Keratella* spec., **f** – claws of parapods of Chironomidae, **g** – head capsule of Chironomidae, **h** – Chironomidae abdominal procerci, **i** – filamentous and other algae. Scale bars: 0.1mm.

classes (1, 1–3 individuals per subsample; 2, 4–50 individuals per subsample; 3, 51–150 individuals per subsample; 4, more than 150 individuals per subsample). Two more subsamples were examined, if the abundance estimates differed in one subsample from the other two.

The abundance of macrozoobenthos (caught in 10 strokes, each 1m) was estimated in the field into one of five different classes (1, single individual; 2, up to 10 individuals per stroke; 3: up to 50 individuals per stroke; 4, up to 100 individuals per stroke; 5, more than 100 individuals per stroke). Some individuals of macrozoobenthos were fixed in 70% ethanol for preparation reference slides to assist with crop-content analysis.

In order to analyse the feeding preferences for Copepoda and Cladocera, the preference calculations formulated by Johnson (1980) were used. These calculations, based on ranking statistics, test the hypothesis that all components are equally preferred, using availability and usage data, and compares individual components using the multiple comparison procedure of Waller and Duncan. This procedure provides a measure of relationships between availability of food items and the frequencies at which they are consumed, which are expressed as T_{bar} values (average differences in ranks). Selected foods were those used more than they occurred in the habitat and preferred foods were those that differed significantly between ranks. For availability data, the estimated plankton composition from the sandpit ponds was used, while the usage data referred to the percent frequency of occurrence of Copepoda and Cladocera. These calculations were only done for *S. halensis, N. canaliculatus, H. confluens* and *H. geminus*. The larger *A. nebulosus* contained crustaceans in the gut only sporadically and was therefore excluded from the calculations.

Copepoda and Cladocera preference experiments

To test for feeding preferences, one 48 h-starved *S. halensis* was placed in a small glass tube (4 cm diameter) filled with water and 10 Cladocera and 10 Copepoda were provided as prey. For the Cladocera, two size classes were used: large and small (see Fig. 2). Different combinations of dead and living Cladocera (large and small) and Copepoda were tested.

The beetles were left in the tube for 30 min and then the remaining prey were counted. The beetles were removed earlier, if one prey resource reached zero. The Wilcoxon matched pairs test was used to test for differences.

The size of the plankton for the preference experiment was measured under a microscope and compared with the size of plankton from the field samples. Length was measured from head to the first abdominal segment in Copepoda and from head to the end of the carapace in Cladocera. The copepods (*Eudiaptomus vulgaris*) and cladocerans (small: *Ceriodaphnia quadrangula*, large *Daphnia* spec.) used in the feeding experiments were all taken from laboratory cultures. Plankton was killed by freezing it at –18 °C for one hour. All laboratory experiments were performed at constant room temperature (22 – 23 °C) in daylight.

Digeston time

Differently sclerotised food items were offered to *S. halensis* to test if there is a difference in the gut clearance time for different prey. Digestion time was defined as the duration of observability of food items in the foregut. Chironomid larvae were chosen as heavily sclerotised prey (for example, the head capsule, claws of parapods), while *Chaoborus* larvae were used as less well-sclerotised prey. The differential sclerotisation of Chironomidae and *Chaoborus* larvae was estimated 62 h after maceration of the larvae with 30 % KOH. While all important structures (head capsule, claws of posterior and anterior parapods, abdominal procerci) of chironomids could be easy identified after maceration, it was difficult or impossible to identify the structures (head capsule, mandible, prehensile antennae and setae of abdominal fan) of the *Chaoborus* larvae.

One prey item was offered to a 48 h starved *S. halensis*, using a pair of tweezers. The beetles were killed after variable periods of digestion by freezing, and the observability of the prey items was examined, using the previously described crop-analysis methods. The observability was tested hourly up to 21 h, with ten replicates for each hour.

Some beetles were also released in a small plastic box with a mixture of planktonic crustacea comprising small and intermediate sclerotised prey. After 30 min the crops of the beetles feeding on plankton were similarly filled than those of the beetles used in trials with Chironomidae and *Chaoborus*, and the beetles were transferred into boxes with filtered water for digestion. The observability of the food items was noted using a zero when nothing was observable and one when prey items were still observable. Logistic regression was used to test differences in digestion time.

Results

Gut analysis

All of the ten investigated ponds contained similar potential prey: Rotatoria, *Keratella* spec., Copepoda, Cladocera, Anisoptera, Zygoptera, Ephemeroptera, Chaoboridae, Culicidae, Chironomidae, Oligochaeta, *Notonecta* spec., Corixidae, amphibian tadpoles and filamentous algae. In this study, no obvious differences could be found in the qualitative and quantitative availability of prey except for Copepoda and Cladocera. For gut analyses, a total of 898 beetle specimens, of *A. nebulosus* (98), *N. canaliculatus* (313), *S. halensis* (281), *H. confluens* (123) and *H. geminus* (83), were dissected. The composition of the gut contents showed no obvious seasonal and locational differences, so data for all ponds and sampling dates are pooled in Table 1 (see also Table 2 for Cladocera and Copepoda).

A high number of beetles had empty guts (20 to 45 percent) and beetle guts also often contained unidentifiable material. *Agabus nebulosus* was notable in this respect as guts of this species often contained only a brown fluid.

The most common prey items of all studied species were chironomid larvae and planktonic Crustacea. *Nebrioporus canaliculatus* contained more Chironomidae than *A. nebulosus* and *H. geminus* (Kruskal-Wallis H-test, 4 degrees of freedom, $H = 19.50445$, $p = 0.006$). Copepods were significantly increased in *H. geminus* compared to *A. nebulosus* (Kruskal-Wallis H-Test, 4 degrees of freedom, $H = 11.50068$, $p = 0.0215$). Furthermore, *H. geminus* was the only species feeding on nauplius larvae of Copepoda, while rotatorian prey were preferred by *H. confluens* compared with *S. halensis, N. canaliculatus* and *A. nebulosus* (Kruskal-Wallis H-Test, 4 degrees of freedom, $H = 20.00524$, $p = 0.0005$). *Nebrioporus canaliculatus* and *S. halensis* exhibited the same prey spectrum: Chironomidae, Copepoda, Cladocera and Insecta. For the prey categories Cladocera, plant material (filamentous algae, other algae and higher vascular plants) and Insecta no significant differences between the beetle species investigated were found.

Within crustacean prey, the preference calculations of JOHNSON (1980) show significantly increased usage of Copepoda over Cladocera (Tables 2, 3) for *S. halensis, N. canaliculatus, H. confluens* and *H. geminus* in the field ($p < 0.05$). The preference experiments using copepod and cladoceran prey in

Table 1. Occurrence of food items in the foreguts of the five pioneer diving beetle species studied. The number of food items found in all beetles is indicated by n and the percent frequency of food items of beetles with filled foreguts is also given.

	Agabus nebulosus		*Nebrioporus canaliculatus*		*Scarodytes halensis*		*Hygrotus confluens*		*Hydroglyphus geminus*	
dissected individuals	98		313		281		123		83	
empty guts	23		68		128		31		29	
	n	%	n	%	n	%	n	%	n	%
unidentifiable material	63	84.0	58	23.7	45	29.4	37	40.2	26	48.1
Keratella	0	0.0	0	0.0	7	4.6	9	9.8	0	0.0
Rotatoria	0	0.0	1	0.4	4	2.6	18	19.6	1	1.9
Copepoda	8	10.7	39	15.9	35	22.9	13	14.1	21	38.9
Nauplius larvae	0	0.0	0	0.0	0	0.0	0	0.0	18	33.3
Cladocera	4	5.3	12	4.9	3	2.0	14	15.2	6	11.1
Crustacea	1	1.3	0	0.0	0	0.0	5	5.4	4	7.4
Chironomidae larvae	16	21.3	149	60.8	57	37.3	39	42.4	7	13.0
Chaoborus larvae	0	0.0	6	2.4	1	0.7	1	1.1	1	1.9
Ceratopogonidae larvae	0	0.0	16	6.5	2	1.3	1	1.1	0	0.0
Ephemeroptera larvae	0	0.0	0	0.0	1	0.7	0	0.0	0	0.0
Diptera pupae/imagines	2	2.7	28	11.4	3	2.0	0	0.0	0	0.0
Insecta	6	8.0	56	22.9	41	26.8	14	15.2	5	9.3
filamental algae	11	14.7	0	0.0	1	0.7	1	1.1	1	1.9
other algae	2	2.7	2	0.8	1	0.7	1	1.1	0	0.0
vascular plant material	1	1.3	3	1.2	1	0.7	1	1.1	0	0.0

Table 2. Usage and availability data of Copepoda (Cop) and Cladocera (Clad) for *N. canaliculatus, S. halensis, H. geminus* and *H. confluens* from different samples. Availability is given in four different classes (1, 1–3 individuals per subsample; 2, 4–50 individuals per subsample; 3, 51–150 individuals per subsample; and 4, more than 150 individuals per subsample) and usage in percent frequency of occurrence of Copepoda and Cladocera. For *N. canaliculatus* n = 313, for *S. halensis* n = 276, *H. geminus* n = 83 and for *H. confluens* n = 123.

	Availability		*N. canaliculatus* Usage		*S. halensis* Usage		*H. geminus* Usage		*H. confluens* Usage	
	Cop	Clad	Cop %	Clad %	Cop %	Clad %	Cop %	Clad %	Cop %	Clad %
Sample 1	2	2	0.00	0.00	25.00	6.25	–	–	–	–
Sample 2	2	4	14.29	14.29	–	–	–	–	–	–
Sample 3	3	4	20.83	0.00	9.84	1.64	–	–	–	–
Sample 4	3	3	56.25	25.00	30.43	0.00	35.71	0.00	–	–
Sample 5	3	3	–	–	–	–	16.67	0.00	–	–
Sample 6	3	3	–	–	13.64	0.00	–	–	–	–
Sample 7	2	3	0.00	0.00	11.11	0.00	–	–	–	–
Sample 8	2	3	0.00	0.00	11.76	0.00	–	–	–	–
Sample 9	2	4	3.70	0.00	2.56	0.00	–	–	–	–
Sample 10	4	2	25.00	0.00	–	–	–	–	–	–
Sample 11	3	2	–	–	–	–	–	–	8.33	0.00
Sample 12	3	3	10.71	0.00	75.00	0.00	–	–	–	–
Sample 13	3	3	0.00	8.33	6.67	6.67	–	–	–	–
Sample 14	3	3	30.77	7.69	–	–	–	–	–	–
Sample 15	3	3	23.81	4.76	–	–	28.57	7.14	–	–
Sample 16	3	3	5.26	42.11	14.29	0.00	7.69	0.00	–	–
Sample 17	3	3	25.00	0.00	100.00	0.00	40.00	0.00	7.69	53.85
Sample 18	3	3	0.00	0.00	0.00	0.00	27.27	18.18	–	–
Sample 19	3	3	10.71	3.57	–	–	–	–	3.85	0.00
Sample 20	1	4	–	–	–	–	–	–	11.11	0.00
Sample 21	1	4	–	–	–	–	22.22	33.33	32.00	28.00

Table 3. T_{bar} values for preference and selection for Copepoda and Cladocera. Negative T_{bar} indicates greater frequency of occurrence in the diet than at random feeding (selection) and preference is indicate with * (W statistic, $p < 0.05$).

	Copepoda	Cladocera
S. halensis	–0.433333*	0.433333
H. geminus	–0.428571*	0.428571
H. confluens	–0.5*	0.5
N. canaliculatus	–0.34375*	0.34375

the laboratory showed that the beetles prefer dead over living Crustacea (Table 4). Comparison of the usage of dead Copepoda and dead Cladocera, gives no significant preference for Copepoda over large Cladocera, but a significant preference for Copepoda over small Cladocera (Wilcoxon Matched Pairs Test,

Table 4. Results of the feeding experiment with dead and live Copepoda and Cladocera (large and small see Fig. 1) using 48 h-starved *S. halensis*. Significant differences were determined with Wilcoxon Matched Pair Test. The preferred prey is marked with * ($p < 0.05$) or ** ($p < 0.01$). N = Number of replicates.

Prey A	Prey B	N	Z	p-level
live Cladocera large	dead Cladocera large*	10	2.521	0.012
live Cladocera small	dead Cladocera small**	10	2.666	0.008
live Copepoda	dead Copepoda**	10	2.803	0.005
dead Copepoda	dead Cladocera large	13	0.622	0.534
dead Copepoda	live Cladocera large	10	1.244	0.214
dead Copepoda**	dead Cladocera small	10	2.803	0.005
dead Copepoda**	live Cladocera small	10	2.803	0.005
live Copepoda	dead Cladocera large**	10	2.803	0.005
live Copepoda	live Cladocera large	20	1.500	0.134
live Copepoda	dead Cladocera small*	10	2.201	0.028
live Copepoda	live Cladocera small	25	1.023	0.306

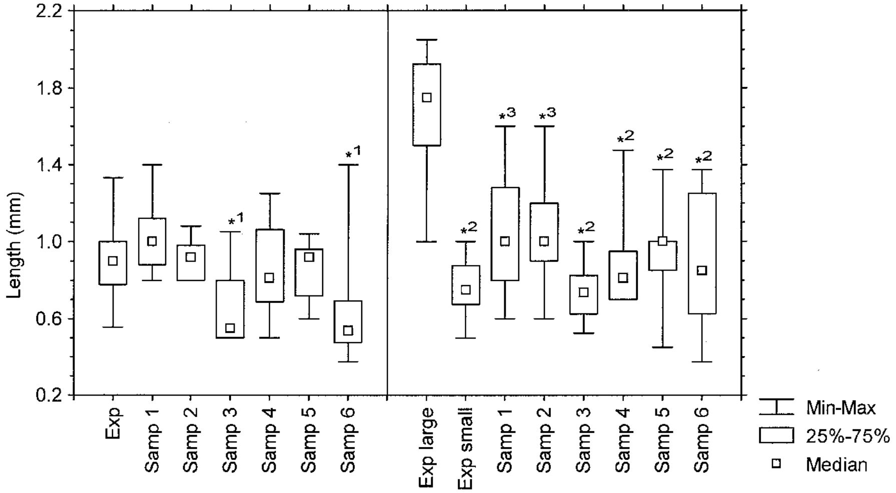

Fig. 2. Differences between the size of Cladocera and Copepoda used in the feeding experiment and caught in field. (EXP = Plankton used in the feeding experiment; SAMP = Sampling site number). Significant differences (Kruskal Wallace test $p < 0.05$ and Tukey Kramer post hoc procedure) are indicated with *1 = significantly different from Copepoda used in the feeding experiment, *2 = significantly different from large Cladocera used in the feeding experiment; *3 = significantly different from large and small Cladocera used in the feeding experiment. n = 25.

$p < 0.01$). On the basis of these results, we compared the size of the plankton from the field (6 samples from the two main ponds studied) with the size of the crustacean plankton in the laboratory feeding experiments (Fig. 2). The size of copepods used in feeding experiments does not significantly differ

Table 5. Percentage of filled crops and percentage of crops with only unidentifiable material at different times of the day for *H. geminus*. The numbers of dissected crops are indicated with n and the water temperature for each sampling time is given.

Time	filled crops in %	unidentifiable material in %	n	water temperature in ˚C
7:00 AM	40.0	83.3	15	19.1
10:00 AM	83.3	10.0	12	21.2
12:00 PM	70.0	0.0	10	25.7
3:00 PM	76.5	7.7	17	28.1

from copepods occurring in the field, except for field samples 3 and 6 in which copepods were significantly smaller than those used in the laboratory. The size of the large Cladocera in the feeding experiment differs significantly from the Cladocera found in the field whereas the size of the small Cladocera used in the feeding experiment is closer to the size of those found in the field. Thus, the results of the laboratory feeding experiments for small Cladocera are more comparable with the situation in the field than are those for large Cladocera.

Day activity of feeding of *Hydroglyphus geminus*

Four crop samples of *H. geminus* from the same pond at the same day at different daytimes were analysed, to test for a daily cycle of feeding activity (Table 5). In the morning 60 % of the beetles had empty crops, while gut contents of the beetles with the filled crops were mostly unidentifiable. During the day, more beetles possessed filled crops with less unidentifiable material. A Monte Carlo randomisation test (PATEFIELD 1981) showed no significant difference in filled crops for different times of day (number of random matrices = 25,000, T_{obs} = 171.63, T_{ran} = 171.61 ± 1.3, p = 0.40), but for unidentifiable material there was a significant difference (number of random matrices = 25,000, T_{obs} = 88.79, T_{ran} = 86.02 ± 1.3, p = 0.043).

Head capsule size of chironomid prey

There were significant differences in width of the chironomid head capsules consumed by different dytiscids (Fig. 3). The median for the chironomid head capsule for *A. nebulosus* (body length of beetle = 8.2–8.6 mm) was significantly higher than that of the smaller beetles *N. canaliculatus* (4.8–5.8 mm), *S. halensis* (3.8–4.3 mm) and *H. confluens* (3.4–3.7 mm). In the smallest beetle species, *H. geminus* (1.9–2.2 mm), chironomid head capsules were largely absent and were not measured (length of beetles according to NILSSON & HOLMEN 1995). Although in the guts of smaller beetles chironomid head

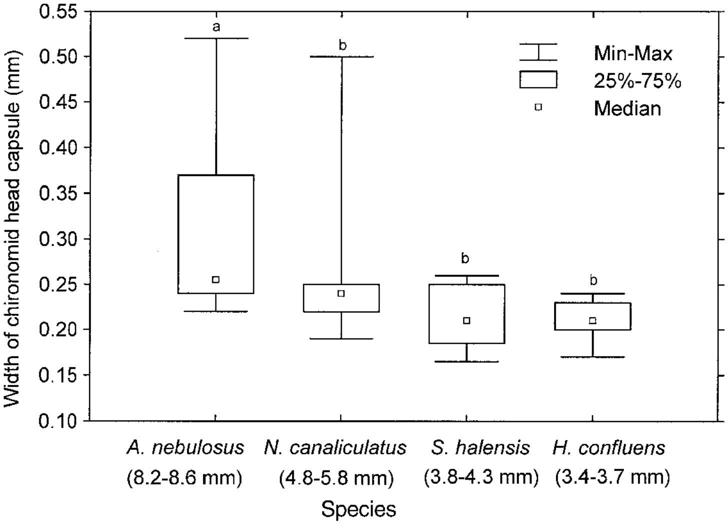

Fig. 3. Width of chironomid headcapsules found in the crops of *A. nebulosus, N. canaliculatus, S. halensis, H. confluens*. Size of the beetles decreases from left to right. Plots with identical letters are not significantly different (Kruskall Wallace test $p<0.05$ and Tukey Kramer post-hoc procedure). Body length of beetles given in brackets (according to Nilsson & Holmen 1995).

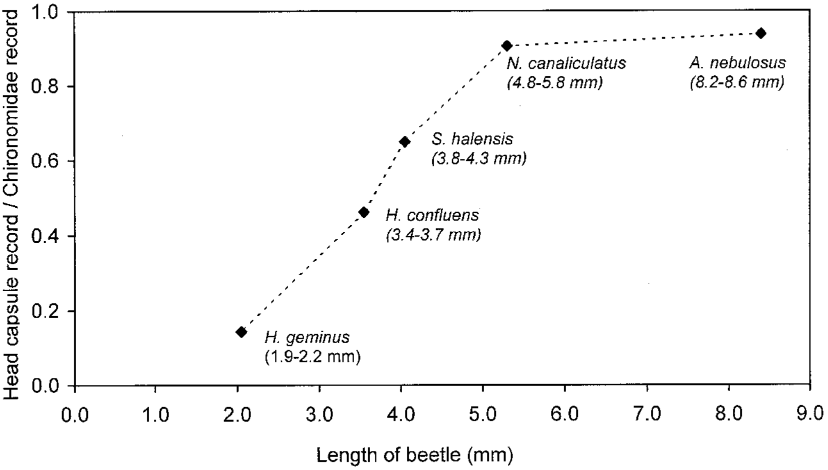

Fig. 4. The proportion of chironomid headcapsule records to total of Chironomidae for each beetle species, arranged according to body size (given in brackets). The smaller sized beetles often rejected the headcapsules of Chironomidae, but other chironomid pieces (e.g. claws of parapods or abdominal procerci) were found in the crops.

capsules were often absent, other chironomid pieces, such as the claws of parapods or abdominal procerci, could be found. In Fig. 4, the quotient of records of chironomid head capsules to records of Chironomidae is shown for

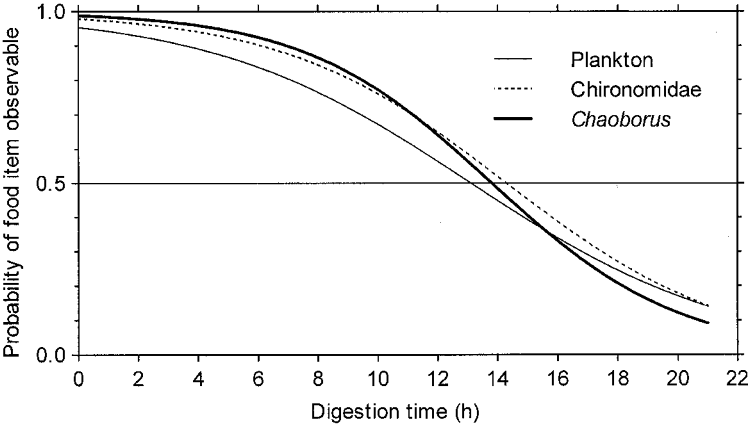

Fig. 5. Logistic regression lines of digestion times of *S. halensis* for heavily sclerotised larval Chironomidae, less well-sclerotised *Chaoborus* larvae and small and intermediate sclerotised planktonic Crustacea. 0 = no food item observable; 1 = food item still observable.

Table 6. Logistic regression models for digestion times of *S. halensis*.

Food item	Equation	Turning point
Chaoborus	$y = \exp(4.41919 + (-0.32008)*x)/(1 + \exp(4.41919 + (-0.32008)*x))$	13.81
Plankton	$y = \exp(3.01938 + (-0.23033)*x)/(1 + \exp(3.01938 + (-0.23033)*x))$	13.11
Chironomidae	$y = \exp(3.83678 + (-0.26837)*x)/(1 + \exp(3.83678 + (-0.26837)*x))$	14.30

each species. While the larger species ingested the Chironomidae completely, the smaller beetles apparently avoided the head capsule and fed on the soft structures alone.

Digestion time of sclerotised prey

Differences in digestion time of differentially sclerotised food items were analysed using logistic regression. The time food items were visible in the crops did not differ with sclerotisation of the prey items (Fig. 5): with a 50 % probability, *Chaoborus* larvae were not observable in the crops after 13.8 h, Chironomidae after 14.3 h and plankton (Cladocera and Copepoda) after 13.1 h (Table 6).

Discussion

Gut content analysis is a precise method to investigate the diet of an animal, but some methodological problems are apparent (DEDING 1988, PECKARSKY 1984).

Gut contents can be eaten directly or can enter the gut via the guts of other prey organisms. Additionally, some items could enter the gut accidentally (e.g. short pieces of green algae, vascular plant material, pollen). CUPPEN (2000), who dissected the crops of *Hygrobia hermanni* (Coleoptera: Hygrobiidae), was unable to determine whether the oligochaete chaetae he found were eaten as prey or entered the crops due to prior predation by consumed chironomids, which were also abundant in the crops of the *H. hermanni*. According to ARMITAGE et al. (1995), chironomids ingest a wide variety of foods, including algae, detritus, macrophytes, oligochaetes, zooplankton and aquatic insects, and it is possible that all of the prey of a chironomid eaten by a beetle is released inside the crop of the beetle. The size, condition and the number of food items in the gut of a beetle can give a reliable indication as to whether the gut contents were eaten directly as prey or entered via the guts of other prey animals. However, gut content analysis cannot indicate whether prey is caught alive or eaten when dead. Alternative methods, like laboratory feeding experiments and behavioural studies, may indicate whether particular consumers are predators or scavengers.

Heavily sclerotised prey may be overestimated compared with soft-bodied prey, because soft-bodied prey may be digested faster. The gut clearance time for different food items can help to interpret the results of the gut analysis. Data based on gut content analysis cannot be quantitative, because it is impossible to determine numbers of crushed prey. Using only one body part, for example head capsules, as an indicator of the number of a particular prey consumed assumes that beetles always feed on that particular body part.

Different daily activity patterns and prey searching periods may be responsible for the high percentage of empty crops. Other studies (DEDING 1988, DETTNER et al. 1986, BOSI 2001, THIELE 1977) also found a high proportion of beetles with empty crops. The Dytiscidae are believed to be primarily nocturnal (NILSSON 1978, DETTNER et al. 1986, BOSI 2001), but this study indicates that *H. geminus* feeds during daytime. In the morning only a few specimens showed filled crops and the ingested food was unidentifiable (at 7.00 am, 60 % empty crops and more than 80 % unidentifiable material). During the day, many more specimens had filled crops with less unidentifiable material. *H. geminus* is considered a thermophilous species (HEBAUER 1994 a and 1994 b) and may start feeding at higher water temperatures, which rose on the day of sampling from 19.1 °C in the morning to 25.7 °C at noon. The other investigated pioneer beetles did not show such a clear feeding activity pattern.

According to SEEGER (1971) and SÜSELBECK (1979), the crops of freshly emerged adults were more often filled than those of older beetles. In this study, not all beetles which were starved in the laboratory for two weeks were hungry: this also indicates a particular feeding period or age dependent feeding. Age related differences in quality of ingested food, as reported from SEEGER (1971) for the Haliplidae, were not observed.

Like most other Dytiscidae (Deding 1988, Dettner et al. 1986, Bosi 2001), the pioneer beetles studied here mainly feed on Chironomidae (Tanypodinae and Chironominae) and planktonic Crustacea, particularly Copepoda. Adult Diptera and other adult insects were, however, also present in the guts: these may fall on the water surface and drown, and will be eaten on the bottom of the pond: particularly during metamorphosis, many aquatic Diptera die and sink to the bottom (Deding 1988). The feeding of Rotatoria is so far not recorded for dytiscid beetles and, similarly, the usage of nauplius larvae is not described in literature. In 23 of 92 filled crops of *H. confluens* Rotatoria were found (including *Keratella* spec.). The fact that five of these crops only contained rotifers and no other prey items leads us to conclude that *H. confluens* feeds on rotifers directly, rather than rotifers simply occurring as remnants from the guts of other prey items. It remains questionable, whether the rotifers are eaten dead or alive, but uptake might be similar to that for crustacean plankton (see below).

Ephemeroptera and culicid larvae, which were highly abundant in the ponds, were not eaten at all, also only few *Chaoborus* larvae were found in the beetle crops. In aquarium feeding studies, the pioneer water beetles ate all of these prey. It is notable that Bellini et al. (2000) considered *H. geminus* an important predator of culicid larvae in the rice fields of northern Italy.

No obvious seasonal changes were recorded in the availability of prey in the ponds from May to September 2000. However, in November 2001 no chironomid larvae could be found in the ponds. At this time, a few beetles of *S. halensis* and *N. canaliculatus* were dissected: these beetles were all found to be feeding on planktonic crustaceans.

Deding (1988) first established the consumption of algae and plant material by Agabinae and Dytiscinae. However, Bosi (2001) was unable to confirm this. This study indicates that the agabine *A. nebulosus* supplements its diet with filamentous algae. Although the comparison of the gut contents among the investigated species showed no overall significant differences for plant material, some crops of *A. nebulosus* were only filled with a large clew of algae, while in the other species only small pieces of algae occurred. The latter could remain from the guts of other prey animals or might have been eaten accidentally. However, in the crops of *A. nebulosus*, the threads of algae were so long and so abundant that they could not be derived from other prey animals and were obviously eaten directly.

Cannibalism of adult beetles on their larvae, described in Deding (1988), Nilsson (1995) and Dettner et al. (1986), was not observed in this crop analysis, although it was expected because of the high densities of larvae and imagines in June/July. Possibly imagines did cannibalise their own larvae, but the remains could only be identified as Insecta. Cannibalistic behaviour has been observed for the larvae and adults of *S. halensis* and *N. canaliculatus* in the laboratory and additionally in the field, for the larvae. Hicks (1994) also reported cannibalism in the laboratory among adults of the dytiscid *Ilybius discedens*, even though all beetles were regularly fed.

In agreement with Deding (1988) and Dettner et al. (1986), there was a significant difference in the width of the chironomid head capsules eaten related to the body length of the dytiscid beetles. However, the conclusion of Deding, that, based on width of the head capsules, the larger the dytiscid beetles are the larger are the chironomid larvae eaten, was not confirmed. In feeding experiments, the smaller beetles also fed on large chironomids, but refused the larger sclerotised items, including the head capsule. This is also true for the field, because the quotient of chironomid head capsules recorded compared to the records of other parts of chironomids (claws of parapods or abdominal procerci) decreased with decreasing size of the beetle species. In the crops of *H. geminus*, the smallest dissected beetle, only one deformed chironomid head capsule was found at all, but in seven other crops of *H. geminus* chironomid pieces were identified. Dettner et al. (1986) observed adult Hydroporinae killing insect larvae in order to feed on soft larval structures alone, whereas *Platambus maculatus*, a larger agabine species, completely ingested the majority of its prey. Nevertheless, the small *H. geminus* preferred smaller prey, being the only species feeding on the small nauplius larvae of Copepoda. Smaller beetles are able to kill large prey: adult *S. halensis* attacked and killed earthworms (length approximately 6 cm) collectively in small groups of up to 10 beetles in aquarium feeding studies. According to Bellini et al. (2000), such collective attacks are peculiar to smaller predatory water beetles, while larger ones usually prey alone.

For *S. halensis*, no difference in digestion time of heavily sclerotised (Chironomidae) and less sclerotised food items (*Chaoborus* larvae, plankton) was observed, and the heavily sclerotised food items would not be overestimated. With 50 % probability *Chaoborus* larvae disappeared from the crops after 13.8 h, Chironomidae after 14.3 h and plankton (Cladocera and Copepoda) after 13.1 h. Undoubtedly, the observable time for algae or soft skinned prey (e.g. Oligochaeta) in the crops will be shorter and larger beetles will digest prey faster. Dettner et al. (1986) dissected *Platambus maculatus* (length of beetle 7–8.5 mm) and found after 30 min a complete set of chitinous fragments; after 3 h the crops only contained a brown fluid, together with chitinous fragments which were distinctly macerated. Crops were empty after 20 hours. The crop analysis shows a significant preference for Copepoda over Cladocera for *S. halensis, N. canaliculatus, H. geminus* and *H. confluens*. This preference might be explained by a higher energy content of Copepoda in comparison to Cladocera (Schindler et al. 1971, Wissing & Hasler 1971, Comita & Schindler 1963).

Sønnichsen (1977) observed that *Hydroporus morio* (as *melanocephalus*) in an arctic pond followed the migratory pattern of two planktonic crustaceans and DEDING (1988) supposed the smaller Dytiscidae to feed on living planktonic Crustacea in the free water body. In the field, the pioneer beetles were only observed on the bottom, at the water surface, where they renewed their air supply, or on the way from the water surface to the bottom or vice versa. The beetles should hunt pelagically if they feed on living plankton. Laboratory experiments showed that the beetles prefer dead planktonic Crustacea. Scavenging behaviour has been observed in other Dytiscidae, such as *Cybister fimbriolatus* (JOHNSON & JAKINOVICK 1970) and *Thermonectus marmoratus* (VELASCO & MILAN 1998).

Indeed the availability data used in the preference calculations remain questionable. The laboratory feeding experiments and observations in the field show that the beetles prefer dead plankton. The availability data are based on living plankton samples from the pelagic zone. The composition of the plankton on the bottom could be different to the pelagic zone. Also according to KRAUSE (1964), the decay of dead Cladocera is much faster than that of Copepoda.

RANTA & ESPO (1989) tested the food preference of the dytiscid *Stictotarsus griseostriatus* with benthic Chironomidae and planktonic *Daphnia*. The beetles fed markedly more on chironomid larvae than on *Daphnia*. The energy profit of feeding on Chironomidae is 2 to 3 orders of magnitude higher compared to feeding on *Daphnia*.

The size of the plankton seemed to be important, while in the laboratory feeding experiment living and dead large Cladocera were as attractive as dead Copepoda to *S. halensis*, small Cladocera were not. RANTA & ESPO (1989) tested the selection of different *Daphnia* size classes with *Stictotarsus griseostriatus*. The smallest *Daphnia* size class (1.7 mm) was selected, while the larger *Daphnia* individuals (3.8 mm) managed to escape. It is worth noting that the small size class described by RANTA & ESPO is equivalent to the large size class used in this investigation.

S. halensis and *N. canaliculatus* ate similar prey, while the other species investigated differ in some prey items. The two beetle species occur together in new ponds without any vegetation. However, *S. halensis* occurs at low abundances in these ponds. It is dominant in ponds at an advanced successional stage, but still on immature soil (sandy or silty bottom), where *N. canaliculatus* was absent (KEHL & DETTNER, in press). Therefore, we suspect competition occurs between these two species, particularly at densities of 150 beetles per m^2 as previously observed. Further studies must reveal if other biotic factors, such as oviposition sites, which remain unknown, and larval ecology may be also important in determining habitat selection.

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