SAMPLING METHODS FOR *GRAPHODERUS BILINEATUS* (COLEOPTERA: DYTISCIDAE)

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Two different sampling methods for the endangered carnivorous aquatic beetle *Graphoderus bilineatus* were investigated to obtain an optimal catching technique. The results for other middle-sized and large dytiscid beetles are included. Firstly, the effectiveness of bottle traps with and without bait (cat food or chicken liver) was examined. Secondly, we compared the effectiveness of the best trap from the previous experiment with macrofauna net sampling. Our results show that bait is indispensable for collecting beetles with traps. Of the two types of bait, cat food leads to equal or better scores than chicken liver, but mortality is higher. Comparing baited trap sampling versus net collecting, we obtained equal numbers of *G. bilineatus*. However, the success of the methods greatly depends on the habitat structure.

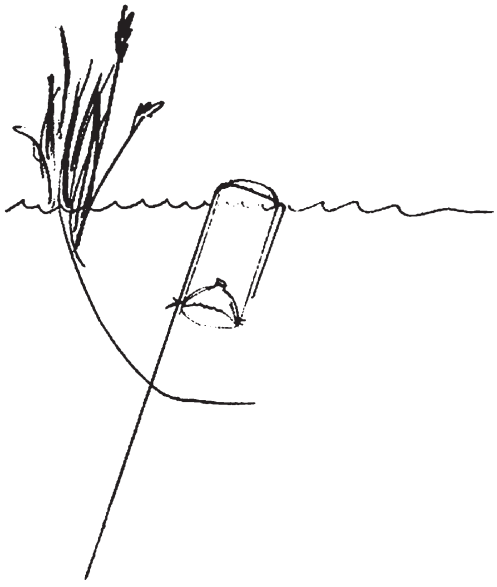

Figure 1. Bottle trap made from a plastic drink bottle. It is made by cutting the top end of the bottle and bring it in upside down. A rustproof metal rod (1 m) is attached to it, to facilitate the setting of traps in the bottom.
Figuur 1. Waterkeverfuik vervaardigd van een plastic frisdrankfles waarvan de top is afgezaagd en omgekeerd terug in de fles is gezet. De fuik wordt met een één meter lange ijzeren stok in de bodem bevestigd.

INTRODUCTION

As part of a study on *Graphoderus bilineatus* in the Netherlands (Cuppen et al. 2006), the aim of the present investigation was to compare two main sampling methods (trap versus net) and to develop an adequate sampling design. Aquatic beetles are generally sampled with a macrofauna net (or a sieve). A net is very effective for small and middle-sized species, but seems to be less effective for large species. Therefore baited bottle traps are often used to collect *Cybister* and *Dytiscus* species (Hilsenhoff 1987, Koese 1999). The best sampling method for *Graphoderus bilineatus*, a middle-sized to large species (14-16 mm), was not yet investigated in detail. A small comparative study in the Netherlands (18 traps involved) suggested an advantage of net sampling above traps (Cuppen & Koese 2005). In a recent Swedish study considerable numbers of *G. bilineatus* were caught with bottle traps in lakes (Nodmar 2002). However, no parallel study with net captures was made. Numerous papers present successful trap designs (with or without an additional attraction such as bait or light) (Faber 1981, Aiken & Roughly 1985, Mölle 1998), but to our knowledge, no comparative studies have been published. In this paper, we

Figure 2. Typical habitats for net-sampling, a. Dead end of a canal in Luinjeberd, Friesland. The animals concentrate in accumulated dead organic matter, b. Large, homogeneous waterbody in the Westbroekse zodden, Utrecht. The animals concentrate in the corners. Photo Bram Koese.
Figuur 2. Typische biotopen voor netbemonstering, a. Doodlopende vaart in Luinjeberd, Friesland. De dieren concentreren zich tussen de opeenhoping van grof organisch materiaal, b. Groot, homogeen petgat in de Westbroekse zodden, Utrecht. De dieren concentreren zich in de hoeken. Foto Bram Koese.

quantify the effectiveness of different types of bait (bait test) and compare net sampling with trap sampling (trap-net comparison). We will discuss the results of both experiments separately.

METHODS

A simple and effective trap can easily be made from plastic drink bottles of which the top end is cut off and brought in upside down thus shaping a funnel-like opening that prevents the escape of trapped beetles (fig. 1). We used bottles from the 'Spa' company and attached a rustproof metal rod to it, to facilitate the setting of traps in the field.

Bait test

The test consisted of a comparison of traps with and without bait (i.e. one bottle with cat food, one with chicken liver, and one empty bottle).

Figure 3. Typical habitats for trap-sampling, a. Continued canal in Luinjeberd, Friesland, b. Long ditch in the Westbroekse Zodden, Utrecht. Photo Bram Koese.
Figuur 3. Typische biotopen voor trapbemonstering, a. Doorgaande vaart in Luinjeberd, Friesland, b. Lange poldersloot in de Westbroekse Zodden, Utrecht. Foto Bram Koese.

Both cat food and chicken liver were regularly used to attract beetles (Cuppen 2005, Koese 1999). In total, 25 of these triplets (75 traps) were set on different locations in the Westbroekse Zodden (province of Utrecht), one of the most accessible peat bog areas with relatively high densities of *G. bilineatus*. The triplets were placed along the waterside, approximately one meter separate from each other, with the empty bottle in the middle. Traps were set in the evening of August 23, 2005 and picked up during the next day, after a period of 14 to 20 hours in the water. As cat food we used *Alexis* paté (4% beef).

Trap-net comparison

For this experiment, we compared series of trap samples (with chicken liver as bait) with series of net samples taken at exactly the same place. Sites that yielded *G. bilineatus* in the earlier bait test were chosen as starting points for four series of

Species	Cat food	Chicken liver	Without bait	Total
Cybister lateralimarginalis (Degeer, 1774)	73	34	0	107
Ilybius fenestratus (Fabricius, 1781)	32	27	0	59
Graphoderus cinereus (Linnaeus, 1758)	26	21	0	47
Hydaticus transversalis (Pontoppidan, 1763)	9	4	0	13
Graphoderus bilineatus (Degeer, 1774)	6	4	0	10
Ilybius ater (Degeer, 1774)	3	0	0	3
Dytiscus dimidiatus Bergsträsser, 1778	2	0	0	2
Dytiscus marginalis Linnaeus, 1758	2	0	0	2
Ilybius quadriguttatus (Lacordaire, 1835)	2	2	0	4
Hydaticus seminiger (Degeer, 1774)	1	2	0	3
Total	156	94	0	250

Table 1. Results of the bait test (75 traps).
Tabel 1. Resultaten van de aastest (75 fuiken).

trap-net comparisons. From each starting point, we placed 10 to 16 traps at every 10 meter in a direction that included the greatest diversity of habitats (such as corners, dead ends of ditches or inlets in the shoreline). In total we set 55 traps. Traps were set in the evening and picked up the next day. Two days later, we resampled the same locations with a macrofauna net (frame 35 x 23 cm, mesh width 1 mm). A net sample consisted of 'two quick' scoops of 1.5 meter through the bank vegetation. This sampling procedure was carried out between August, 24-27, 2005.

RESULTS

Bait test

Although the densities of *G. bilineatus* were low (ten specimens in five out of 25 locations) the preference for traps with bait was significant (table 1). No *G. bilineatus* (in fact no water beetles at all) were caught in the traps without bait. If we consider this sample as binominally distributed (*p* success (bait) = 2/3), the chance to get a distribution in which all ten individuals are caught in bait traps is equal to $2/3^{10}$= 0.02, which is highly significant. In total we captured 250 larger dytiscid beetles (>1 cm) of 10 species (table 1). The fact that not even one of them was found in the empty traps emphasizes the indispensability of bait once again.

No preference for cat food or chicken liver could be demonstrated for the smaller and middle-sized species in the genera *Ilybius*, *Hydaticus* and *Graphoderus,* although the total numbers of trapped specimens for each type of bait suggest a slightly better score for the cat food traps. Six specimens of *G. bilineatus* were found in cat food traps and four in the ones with chicken liver. Very large species, such as *C. lateralimarginalis* and probably also both *Dytiscus*-species, were more easily captured with cat food than with chicken liver. *Cybister lateralimarginalis* was the only diving beetle for which a significance preference for cat food could be revealed (Wilcoxon test: p<0,05).
All specimens of *G. bilineatus* in traps baited with cat food were dead when we picked up the traps, while all specimens in the chicken liver traps were still vital. For the other beetle species mortality was not documented. Although it is not proven that the cat food was responsible for the mortality of *G. bilineatus*, we continued further investigations with chicken liver as bait.

Species	Trap	Net	Total
Coleoptera			
Graphoderus bilineatus (Degeer, 1774)	10	8	18
Graphoderus cinereus (Linnaeus, 1758)	14	9	23
Cybister lateralimarginalis (Degeer 1774)	27	11	38
Ilybius fenestratus (Fabricius, 1781)	17	56	73
Hydaticus transversalis (Pontoppidan, 1763)	6	1	
Total	74	85	159
Heteroptera			
Ilyocoris cimicoides (Linnaeus, 1758)	0	243	243
Notonecta glauca Linnaeus, 1758	1	148	149
Total	1	391	392

Table 2. Comparison of trap sampling and net sampling (55 samples).
Tabel 2. Vergelijking van de fuikvangsten met de netvangsten (55 monsters).

	Small ditches (n = 25)		Wide ditches (n = 25)	
	Net	Trap	Net	Trap
Graphoderus bilineatus	2	4	2	4
Graphoderus cinereus	2	5	3	5

Table 3. The number of beetles caught with a net or a trap in small ditches (width <3 m) or large waters (width >10 m); n = number of sampling sites. 'Corners' are not included.
Tabel 3. Het aantal netvangsten van kevers in smalle sloten (breedte < 3 m) of brede sloten (breedte > 10 m); n = aantal monsterpunten. Overhoeken zijn niet inbegrepen.

Trap-net comparison

A summary of the results is shown in table 2. This reveals the following pattern:

- Very large beetles (*Cybister*) were more easily captured with a trap than a macrofauna net.
- Middle-sized to large beetles (*Graphoderus*) score equally well for both methods.
- Middle-sized beetles (*Ilybius*) were more easily caught with the net than with the trap.
- Traps were highly selective for dytiscid beetles. Heteroptera were rarely caught with a trap.

With net sampling, hardly any *Graphoderus* were caught outside the 'corners'. We define corner here as a 'distinct angle in a waterway or a dead end of a ditch, visible on a 1:25,000 topographical map'. Although only six out of 55 sampling sites (10%) overlapped such a corner, half of the net captures of both *G. bilineatus* and *G. cinereus* originate from these corners. A chi-square test showed that the association with corners is highly significant at the 1% level, p=0.0004 for *G. bilineatus* and p=0.0013 for *G. cinereus*. For trap samples, this is not significant for *G. bilineatus* (p=0.36) and *G. cinereus* (p=0.52). The 'corner-effect' may explain why the total amount of beetles was higher in larger water bodies (width >10 m) than in the small ditches (width <3 m). If we clip the corners out of the analysis, it seems that traps are more efficient than the net. Although the numbers are small, we caught twice as many specimens of both *G. bilineatus* and *G. cinereus* with traps. This pattern occurred in both small and wide ditches (table 3).

DISCUSSION

It appears that there is not one best method for sampling *Graphoderus*. The best strategy depends on the structure of the environment.

Very heterogeneous areas with winding ditches and a lot of 'corners', are more suitable for net samples (fig. 2). Relatively homogenous areas, such as large lakes or extended straight ditches are more eligible for trap samples (fig. 3). To a certain extent, the decision to use a net or a trap can be decided on the basis of a 1:25,000 topographical map. Travelling time is an important factor that should be taken into account. Although the total handling time in the field for trap samples (8 minutes/trap) was less than for net samples (11 minutes/sample), a double travelling time should be calculated for trap sampling, since traps have to be placed in the evening and picked up the following day.

Graphoderus bilineatus was caught in one out of eight net samples (1.5 hours of sampling) and one out of nine trap samples (1 hour of sampling). This gives an indication of the minimal effort that is needed for collecting *G. bilineatus.* Some comments on this are:

- The densities of *G. bilineatus* in other peat bog areas in the Netherlands were usually lower than in the Westbroekse Zodden. To claim the absence of this species in a particular area, it is recommended to sample at least 2-3 hours with a net or use at least 30 traps (Cuppen & Koese 2005). Obviously, the sampling should take place in the correct season, which is from the end of April to the beginning of June or from the end of August to the end of September.
- We deliberately used only one type of net, which reflects the 'standard' dimensions used for macrofauna sampling. A net with a bigger frame and/or mesh size could have lead to better results. However, it appears that very large nets become too cumbersome and heavy for quick scoops through the bank vegetation. A frame of 40x30 cm and a mesh size of 3 mm is probably optimal.
- We used only one type of trap but, as mentioned, there are many more types published in literature. Since bait is the main motivation to visit a trap, we don't expect many differences between different trap models. There are very few traps as cheap and simple as this bottle trap. In this respect, we consider the bottle trap as optimal.

Mortality of *Graphoderus* in traps remains a point of major concern. In this study 50% of the 20 specimens of *G. bilineatus* died. In most cases this could be assigned to oxygen deprivation in a sunken trap, possibly amplified by bacterial growth on cat food. For future experiments it is desirable to develop a less lethal design, for example, with an extended air chamber or a floating device.

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SAMENVATTING

Onderzoek naar vangmethodes voor *Graphoderus bilineatus* (Coleoptera: Dytiscidae)
Als onderdeel van een integrale studie naar de waterroofkever *Graphoderus bilineatus* in Nederland (in opdracht van het ministerie van LNV), werd een vergelijkend onderzoek uitgevoerd naar verschillende vangstmethodieken. Het doel was om tot een inventarisatieprotocol te komen. Er werden twee vergelijkingen uitgevoerd. Allereerst werden keverfuiken (gemaakt van frisdrankflessen) al dan niet voorzien van lokaas (kattenvoer, kippenlever of zonder aas) met elkaar vergeleken. Vervolgens werd op grond van deze resultaten de effectiviteit onderzocht van de beste fuik (met kippenlever), ten opzichte van het schepnet. Het blijkt dat aas onmisbaar is bij het gebruik van fuiken, daar in de lege fuiken geen enkele waterroofkever werd verzameld. Met kattenvoer werden gelijke aantallen van *G. bilineatus* verzameld als met kippenlever. Echter, omdat alle exemplaren dood bleken in de fuiken met kattenvoer werd het onderzoek voortgezet met kippenlever. *Cybister lateralimarginalis* komt beter op het kattenvoer af dan op lever, van de andere Dytiscidae werden ongeveer gelijke aantallen verzameld. De vergelijking tussen fuiken met kippenlever en het macrofaunanet leverde ongeveer dezelfde aantallen *G. bilineatus* op bij dezelfde tijdsinspanning in het veld. Het succes van beide methodes is echter afhankelijk van de biotoopstructuur: in overhoeken, kopeinden en inhammen lijkt het net succesvoller te zijn, de fuik in homogene biotopen, zoals rechte sloten. De reistijd naar het te bemonsteren gebied is een belangrijke post in de vergelijking, omdat bij fuikbemonstering tweemaal een bezoek aan het terrein gebracht moet worden.

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