Effect of two types and different quantities of bait on the efficiency of funnel traps for diving beetles (Coleoptera: Dytiscidae), with special emphasis on *Graphoderus bilineatus* DeGeer, 1774

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Abstract: The efficiency of funnel traps made of plastic bottles for collecting diving beetles (Coleoptera: Dytiscidae) was studied quantitatively using different types and amounts of bait in a small freshwater lake in Tver Oblast, European Russia. The efficiency of traps with bait was significantly higher than that of empty traps. Of the two studied types of bait, tinned stewed beef and frozen beef liver, neither proved more efficient when about 10 ml of bait were used. A greater amount of bait (20 ml, compared to 10 ml) did not increase the efficiency of the traps. The minimal effective amount of bait has been found to fall within the interval from 5 to 10 ml.

Key words: Funnel traps, bait efficiency, Dytiscidae, *Graphoderus bilineatus*, Tver Oblast, European Russia.

Introduction

Funnel traps made of plastic bottles (the bottle's neck is cut off and inserted into the bottle as a funnel, as shown e.g. in Golub et al. 2012), also called activity or bottle traps, are widely used in various studies all over the world for collecting aquatic macroinvertebrates. They appear to be effective for collecting fast moving macroinvertebrates (Becerra Jurado et al., 2008), largely represented by diving beetles (family Dytiscidae Leach 1815). These traps became widespread over the last few decades with the advent of plastic bottles, but similar traps made of other materials were already used in the early 20th century (Aiken, Roughley 1985). Funnel traps are rather efficient and easy to make, light and rather durable, and can easily be brought to any waterbody. The material for them (plastic bottles) is easily found almost everywhere. In addition to faunistic sampling, such traps can be used for collecting diving beetles and other aquatic macroinvertebrates for the purposes of other kinds of studies (e.g. ecological, physiological or behavioural). Several modifications of funnel traps are used, differing in shape, size, and way of attaching the reversed neck to the rest of the bottle, e.g. with adhesive tape or metal clips (Ryndevich 2004). The most common size of the bottles used for making the traps is probably 1.5 or 2 l, but 5 l bottles are also used (Kalniņš 2006).

It is believed that funnel trap efficiency for collecting diving beetles is increased by using bait, the smell of which attracts the beetles. Possible baits include fresh and tinned meat and other parts of vertebrates, whole or crushed fish or amphibians, as well as some invertebrates. Traps with bait of vertebrate origin seem to be especially efficient for collecting larger dytiscids, with average adult body length exceeding 7 mm. It can be explained by the fact that the food of these larger beetles tends to include vertebrate prey (Deding 1988), and they probably more actively search for food, using the sense of smell. These larger beetles are often more efficiently collected by funnel traps, compared to hand water nets.

Verdonschot (2010) tested the effects of trapping duration (48, 96 and 168 hours) and presence or absence of bait (20 g cat food: duck and poultry in jelly) on the number of macroinvertebrate taxa and individuals captured by traps made of 2 l glass jars with polyethylene funnels in drainage ditches and showed that the number of taxa and individuals captured increased with trapping duration and that after 48 hours (but not longer) more predaceous macroinvertebrates (which include Dytiscidae) were captured in traps with bait than in control traps. Only two species of Dyitscidae, *Cybister lateralimarginalis* (DeGeer, 1774) and *Graphoderus cinereus* (Linnaeus, 1758), are mentioned in that study; it is reported that after 48 hours each of these species occurred in the baited traps in higher numbers than could be expected by chance.

The dependence of funnel trap efficiency for collecting diving beetles on different types and amounts of bait has, to the best of our knowledge, never been assessed quantitatively.

Fibres of tinned stewed beef and pieces of uncooked beef liver are among the types of bait used in funnel traps. Reportedly, beef liver is one of the most efficient baits for such traps (Lars Hendirch, pers. comm.). In our experience, tinned stewed beef appeared to have comparable efficiency, but it remained unclear whether its efficiency was identical or somewhat higher or lower. Funnel traps are often used in the field where fresh or frozen liver is hard to preserve, unlike tinned stewed beef, which can also be used as food for the investigators. If its efficiency proved identical or higher than that of uncooked liver, tinned beef could be recommended as a bait of choice for funnel traps.

Therefore, the aims of this study were (1) to compare quantitatively the efficiency of tinned stewed beef and frozen beef liver as baits used in funnel traps for diving beetles and (2) to compare quantitatively the efficiency of different amounts of tinned stewed beef as a bait and thus to estimate threshold (minimal effective) quantity of bait and test whether bait is, indeed, essential in funnel traps for diving beetles.

Methods

Locality

The study was performed at Glukhoye Lake (European Russia, Tver Oblast, Udomlya Dist., N 57°46’28”, E 35°12’14”), a small eutrophic lake, with a surface area of around 10 000 m^2, overgrown on the periphery with 10–50 m wide plant mats secured by roots of shrubs and trees (Fig. 1). The maximum depth of the lake has not been measured, but at the edge of the plant mats off the southeastern bank of the lake, where the experiments were performed, it is around 1 m. In different years the traps were set off slightly different, but overlapping parts of the bank, within a range of 150 m.

Comparison of the efficiency of tinned stewed beef and uncooked beef liver as bait

In 2009 we compared the efficiency of two types of bait from June 30 to July 4 (in five 24-hour sessions).

The traps (10 replicates) were represented by pairs of plastic bottles connected with adhesive tape. We used 1.5 l bottles from mineral water, with an inner neck diameter of 20 mm at the top. The neck of each bottle was cut off, inserted into the bottle and attached with adhesive tape. The traps were put into water near a steep overgrown bank of the lake at intervals of about 5 m. Each trap was fastened to a tree or shrub by a cord (Fig. 2). The traps were immersed into water so that about 4/5 of their volume was filled with water and the rest of the volume (close to the bottom) was filled with air and protruded above the surface. We put 10 g of meat fibres (without fat) of tinned *Glavprodukt* stewed beef (GOST 5284-84) in one bottle in each pair and 10 g of frozen liver in the other. Each portion of bait was weighed using electronic scales.

Figure 1. Lake Glukhoye in Tver Oblast, European Russia, used as a model waterbody for studying funnel trap efficiency, photographed in July 2010.

The traps were set daily at around 11:30 (+/– 45 min), local (Moscow) time. At around 10:30 of the next day the trapped adult diving beetles were removed from each bottle separately for identification and preserved in 70% ethanol, and the traps were rinsed and wiped with paper, recharged and set at around 11:30 for another session.

Figure 2. Funnel trap set near lake bank and fastened to a shrub by cord (Lake Glukhoye, July 2010).

Comparison of the efficiency of different quantities of bait

For these experiments, performed in 2010, the method used in 2009 was slightly modified. Bottles were not attached together in pairs, and 20 one-bottle traps were used. Traps with different quantities of bait (10 traps for each quantity) alternated along the bank. The cut-off bottlenecks were attached to the bottles with metal clips, which simplified the cleaning procedure and removal of beetles. Also to simplify the procedure, the amount of bait was measured by volume, using a measuring cylinder filled with water, instead of by weight using scales.

This time only tinned stewed beef (of the same sort) was used as for bait, since the previous experiment revealed no differences in efficiency between this type of bait and liver (see Results), and tinned meat was easier to procure in the field. The volume of bait was measured as follows. The measuring cylinder was filled with 20 ml of water, then pieces of meat fibres (without fat) were added with pincers until the water rose to the level of $x + 20$ ml, depending on the required amount of bait in the trap (x ml, see below). Subsequent weighing showed that the mean density of stewed beef was 1.04 g/ml, so that 10 ml of bait effectively equalled 10 g used in 2009.

The study included four series of experiments:

(1) 11 sessions, 17–28 June 2010: ten traps with 10 ml of meat and ten traps without bait

were used;
(2) five sessions, 30 June – 5 July 2010: 10 ml and 20 ml of meat;
(3) ten sessions, 20–29 June 2011: 1 ml and 10 ml of meat;
(4) seven sessions, 30 June – 6 July 2011: 5 ml and 10 ml of meat.

Data analysis

All the diving beetles captured were identified to species by modern keys (Nilsson, Holmen 1995, Kirejtshuk 2001) and the sex of each captured individual was determined.

The total number of the only abundant species in the captured samples, *Graphoderus bilineatus* (DeGeer, 1774) (see last paragraph of Discussion) and the number of species in traps with different amounts or types of bait in each experimental session were compared by the non-parametric Wilcoxon test. The significance of difference from the expected 1:1, normally found in diving beetle populations (Ribera 1994), was tested for the ratio of males and females of each species by the test of proportions (Newcombe 1998). All statistical calculations and plotting were made in the statistical environment R (R Development Core Team 2004).

Three alternative hypotheses on the possible factors influencing the number of beetles trapped, other than the tested quantity of bait, have been verified by the following methods:

Hypothesis 1: The number of diving beetles trapped depended on the total quantity of bait in all traps (in all traps set simultaneously), i.e. the more bait was used, the more beetles were captured in the traps. To verify this hypothesis, we tested the significance of differences in the number of diving beetles (by the example of *G. bilineatus*) captured in traps with 10 ml of bait (stewed beef) between each pair of the experimental series.

Hypothesis 2: The number of beetles trapped depended on the order of the experiments, i.e. the longer the traps were set, the more beetles were trapped per 24-hour session. To verify this hypothesis, we tested the significance of the differences between the numbers of beetles captured in different sessions of one experimental series.

Hypothesis 3: The number of individuals trapped depended on the weather conditions. This hypothesis was not verified, because too many factors unaccounted for could be involved (see Discussion).

Results

A total of 1059 individuals of Dytiscidae representing 14 species were collected. The majority of the beetles belonged to *Graphoderus bilineatus* (DeGeer, 1774). Other species were represented by only a few individuals (Tab. 1), so that the efficiency of traps for collecting each species in particular could not be compared statistically, except for *G. bilineatus*, which was therefore used as a model species.

There were no significant differences between the number of *G. bilineatus* individuals collected in traps with stewed beef and in traps with liver in 2009 (Wilcoxon test: $p > 0.05$, Fig. 3). The same was true of the number of diving beetle species (Wilcoxon test: $p > 0.05$, Fig. 4).

The number of *G. bilineatus* individuals and the number of species collected in empty traps and in traps with bait in 2010 differed significantly (Wilcoxon test: $p < 2.2\times10^{-16}$). More diving beetle species (Fig. 4) and more *G. bilineatus* individuals (Fig. 3) were collected in traps with bait, compared to empty ones (Tab. 1).

No significant differences were found between the numbers of *G. bilineatus* individuals collected in traps with 10 ml and with 20 ml of bait (stewed beef) in 2010 (Wilcoxon test: p = 0.6, Fig. 3). This was also true of the number of species (Wilcoxon test: p = 0.75, Fig. 4).

The number of *G. bilineatus* individuals and the number of species collected in 2011 in traps with 10 ml of bait were significantly greater (Wilcoxon test: p < 0.004) than those collected in traps with 1 ml of bait (Fig. 3, Tab. 1). Traps with 1 ml of bait almost always yielded no more then one species (usually *G. bilineatus*) while in traps with 10 ml of bait sometimes 2–3 species were captured (Fig. 4, Tab. 1).

No significant differences were found between the numbers of *G. bilineatus* individuals collected in traps with 10 ml and with 5 ml of bait in 2011 (Wilcoxon test: p = 0.4, Fig. 3). At the same time, significantly more species of diving beetles (Wilcoxon test: p = 0.025) were collected in traps with 10 ml of bait. However, this effect was observed mainly because of the greater number of traps with 10 ml of bait that yielded at least one beetle species, compared to traps with 5

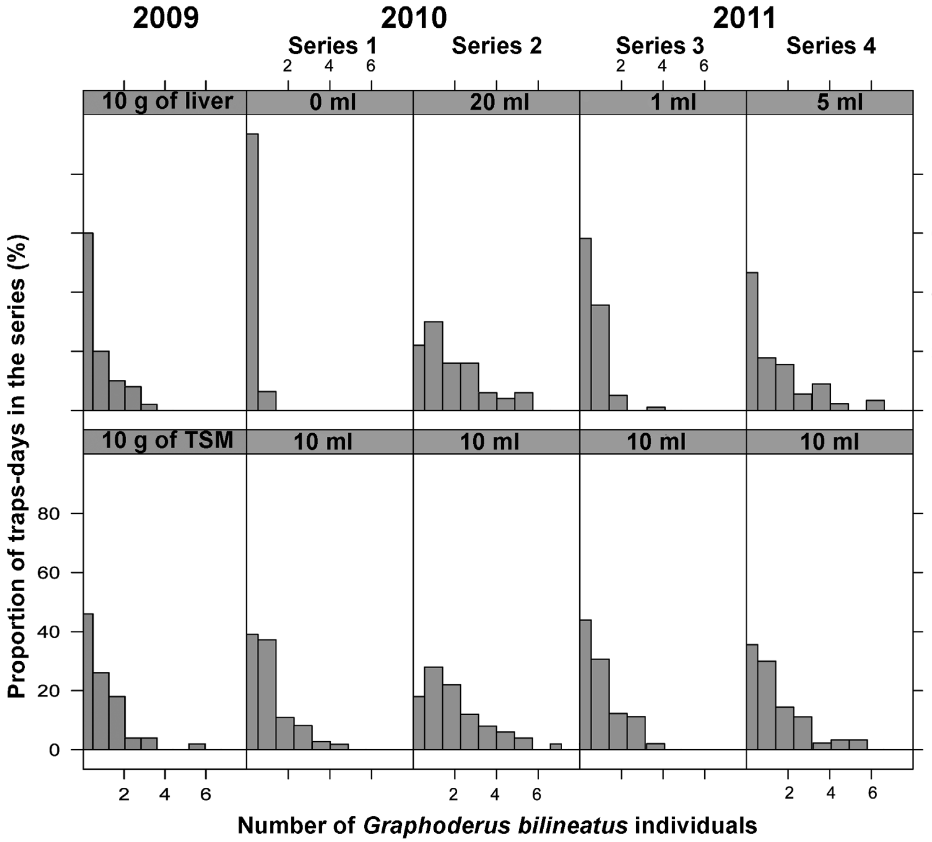

Figure 3. Number of *Graphoderus bilineatus* (model species) individuals collected in funnel traps with different types and different amounts of bait. Separate experimental series performed in 2009–2011 are shown, each vertical pair of histograms representing one series. For 2009, data for different types of bait (frozen liver = liver and tinned stewed meat = TSM) are shown. For 2010 and 2011 volumes of bait (tinned stewed beef) are given.

ml of bait (Fig. 4).

It was possible that the number of the trapped beetles (analysed by the example of the most abundant species, *G. bilineatus*) depended not only on the quantity of bait, but also on other factors. We verified two of the three alternative hypotheses proposed (see Methods: Data analysis).

Hypothesis 1. The greatest average daily number of trapped *G. bilineatus* individuals was recorded in 2010 in the experiment with 10 and 20 ml of bait and in 2011 in the experiment with 5 and 10 ml of bait; the differences in the number of beetles were significant, but rather small (Tab. 2, 3). The total volumes of bait used in these experimental series were also the greatest.

Hypothesis 2. The numbers of beetles trapped in different sessions of the second experimental series performed in 2011 were not significantly different (Tab. 4).

The sex ratio of species abundant enough for statistical analysis was also analysed (Tab. 1). In 2009 it was only *G. bilineatus* (37 males and 50 females), and

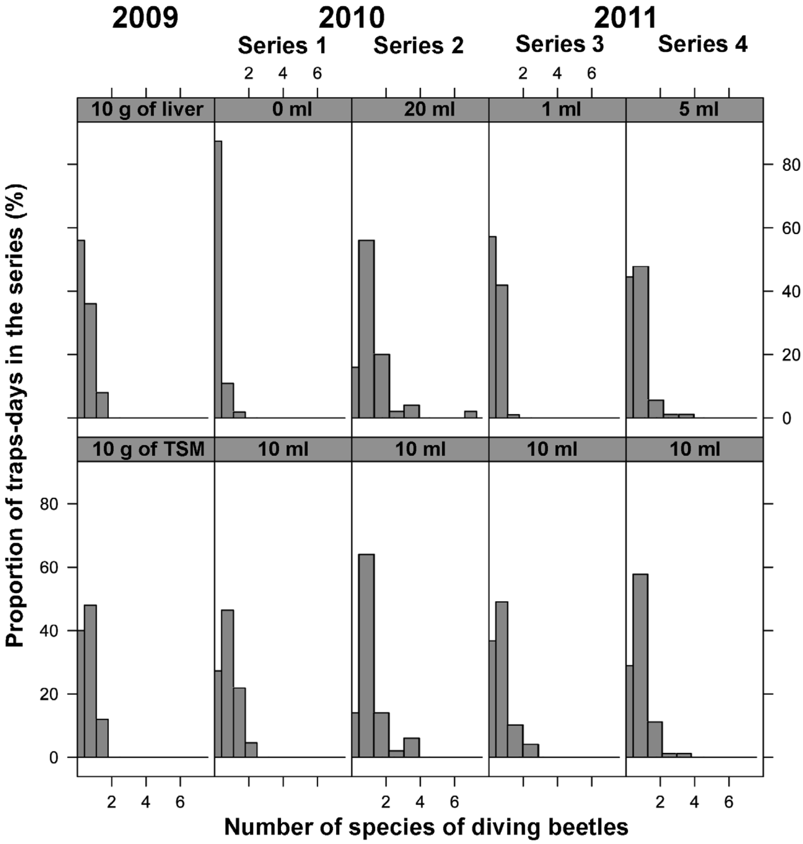

Figure 4. Number of species of diving beetles collected in funnel traps with different types and different amounts of bait. Separate experimental series performed in 2009–2011 are shown, each vertical pair of histograms representing one series. For 2009, data for different types of bait (frozen liver = liver and tinned stewed meat = TSM) are shown. For 2010 and 2011, volumes of bait (tinned stewed beef) are given.

in 2010 *G. bilineatus* (197 males and 169 females) and *G. cinereus* (27 males and 42 females). In all these cases there were no significant deviations from the normally expected (Ribera 1994) 1:1 proportion (test of proportions: $p > 0.05$). In 2011 we had enough material only for *G. bilineatus* (109 males and 281 females), and females were almost three times more abundant then males (the sex ratio significantly differed from 1:1, test of proportions: $p << 0.05$).

Table 1. Number of individuals of different diving beetle species captured in funnel traps in Glukhoye Lake (total number of each type of bait). Explanations: TSB, tinned stewed beef; L, liver; for 2010 and 2011 volumes of used TSB are given.

Species (in alphabetical order)	**2009**		**2010**				**2011**			
			Series 1		**Series 2**		**Series 3**		**Series 4**	
	L	TSB	0 ml	10 ml	10 ml	20 ml	1 ml	10 ml	5 ml	10 ml
Acilius canaliculatus (Nicolai, 1822)	1	2	0	2	3	3	0	6	2	3
A. sulcatus (Linnaeus, 1758)	0	0	0	0	1	0	0	0	0	0
Colymbetes paykulli (Erichson, 1837)	0	0	0	0	0	3	0	0	0	0
C. striatus (Linnaeus, 1758)	0	0	0	0	0	1	0	0	0	0
Dytiscus circumcinctus Ahrens, 1811	0	2	1	4	5	10	0	12	3	5
D. marginalis Linnaeus, 1758	0	1	0	5	1	0	0	2	2	2
Graphoderus bilineatus (DeGeer, 1774)	36	51	7	114	105	95	49	95	122	124
G. cinereus (Linnaeus, 1758)	3	3	4	32	10	17	2	9	5	14
Hydaticus seminiger (DeGeer, 1774)	1	0	0	0	1	0	0	0	0	1
Hyphydrus ovatus (Linnaeus, 1761)	0	0	2	4	0	1	0	0	0	0
Ilybius ater (DeGeer, 1774)	1	3	0	0	1	0	0	0	0	0
I. fenestratus (Fabricius, 1781)	1	1	0	0	0	0	0	2	0	0
I. fuliginosus (Fabricius, 1792)	1	0	0	1	2	0	0	0	0	0
I. subaeneus (Erichson, 1837)	0	0	0	0	0	1	0	0	0	0
Total	**44**	**63**	**14**	**162**	**129**	**131**	**51**	**126**	**134**	**149**

Discussion

As we expected, and in compliance with the results of Verdonschot (2010), significantly more individuals of the model species *Graphoderus bilineatus* and significantly more species of Dytiscidae were collected in traps with bait than in traps without it. Thus, using baited funnel traps is advisable for faunistic Dytiscidae studies, in which maximum representation is important, as well as for other kinds of studies in which maximum amount of material is required.

Our results did not show one of the two types of bait studied (tinned stewed beef or frozen liver) to be more efficient than the other. Thus, using tinned stewed beef, which is easier to procure and preserve, is probably recommendable.

The testing of alternative hypotheses on the factors that could have influenced the number of beetles trapped in our experiments (see Methods: Data analysis and Results) led us to the following conclusions.

Table 2. Significance of differences between numbers of individuals of *Graphoderus bilineatus* captured in traps with 10 ml of bait (stewed beef) in different experimental series (p-values of Wilcoxon test with Bonferroni adjustment are given). Average number of individuals per one 24-hour session was analyzed, as number of sessions per experimental series varied.

Experimental series: year (volumes of bait used in the series)	**2009**	**2010** (0 vs. 10 ml)	**2010** (10 vs. 20 ml)	**2011** (1 vs. 10 ml)
2010 (0 vs. 10 ml)	0.001	–	–	–
2010 (10 vs. 20 ml)	2.0×10^{-9}	0.001	–	–
2011 (1 vs. 10 ml)	0.009	1.0	0.0007	–
2011 (5 vs. 10 ml)	1.6×10^{-5}	1.0	0.16	0.8

Table 3. Number of *Graphoderus bilineatus* individuals captured in traps with 10 ml of bait (stewed beef) during one 24-hour session in different experimental series.

Experimental series: year and volumes of bait used in the series	**2009**	**2010**		**2011**	
		0 vs. 10 ml	**10 vs. 20 ml**	**1 vs. 10 ml**	**5 vs. 10 ml**
Average value ± standard deviation for number of individuals	0.5 ± 1.1	1.0 ± 1.0	2.1 ± 1.9	0.9 ± 1.0	1.4 ± 1.3

Table 4. Significance of differences between number of individuals of *Graphoderus bilineatus* captured in traps in successive 24-hour sessions in the second experimental series in 2011 (p-values of Wilcoxon test with Bonferroni adjustment are given).

Number of 24-hour session	**1st session**	**2nd session**	**3rd session**
2nd session	0.74	–	–
3rd session	1.00	0.39	–
4th session	1.00	1.00	1.00

Hypothesis 1: This hypothesis cannot be rejected, i.e. the total amount of bait can influence the number of beetles trapped. However, we can also offer two other explanations for the observed differences in the average daily number of beetles trapped between different experimental series: variation in the abundance of *G. bilineatus* adults in the lake and variation in the behaviour of the beetles; both could reflect, among other things, the variation of weather conditions (see Hypothesis 3 below).

Hypothesis 2: The experiment comparing 10 and 20 ml of bait was performed in 2010 after the experiment with 0 and 10 ml. The comparison of 10 and 5 ml of bait was performed in 2011 after the experiment with 1 and 10 ml. In both cases the experiment with a larger total amount of bait followed the experiment with a smaller total amount. One could expect that the longer the traps were set in the lake, the more beetles were caught per one 24-hour session, because the smell of bait attracted more and more beetles to the part of the lake where the experiment was performed. However, if this hypothesis is right, then the number of beetles captured in the 3rd and 4th sessions of one experimental series should be higher than in the 1st and 2nd ones, which was not true (Tab. 4). Therefore, this hypothesis should be rejected.

Hypothesis 3: We cannot reject this hypothesis, but it is difficult to verify, as we can only guess the principal factors determining the number of adult diving beetles in the lake and the frequency with which they are captured in the traps. The principal weather factors could include water temperature on the day of the experiment, the dates when the ice cover became fragmented and disappeared, and others. We have not noted any pronounced differences in the weather between the periods when different experimental series of this study were performed, and assume that the weather had no strong influence on the results.

The threshold (minimal effective) quantity of stewed beef as a bait in traps for diving beetles fell between 5 and 10 ml. Smaller quantities of bait were less effective for attracting diving beetles. Therefore, the optimal amount of bait should be 10 ml or slightly less.

The proportion of males and females of *Graphoderus bilineatus* and *G. cinereus* in 2009 and 2010 did not differ from 1:1, observed in natural populations in many species of the studied family (Ribera 1994). Because of female mortality associated with mating, male and female diving beetles use different mating strategies: essentially, males are selected for their ability to fertilize a greater number of females, while females are selected for their ability to avoid multiple mating (Bergsten et al. 2001; Bergsten, Miller 2007). In spite of these behavioural differences, in those two years diving beetles of both sexes were captured in funnel traps with equal probability, suggesting that bottle traps can also be used, among other things, also for representative sampling of the sexual structure of populations. However, in 2011 females of *G. bilineatus* were significantly more abundant in the traps. One possible explanation is that in this year our experiments could have coincided with a period of active mating, so that differences in mating behaviour affected the sex ratio of trapped individuals. But this explanation is difficult to verify; it can probably be tested only by similar experiments performed in aquariums or artificial pools in which the number of beetles of each sex is known and their behaviour can be observed. The question whether funnel traps are normally representative of the sex ratio of diving beetle populations remains to be settled.

Remarkably, *G. bilineatus*, by far the most abundant species in our samples, is increasingly rare in Western Europe (Nilsson, Holmen 1995) and protected in the majority of European countries (Kalniņš 2006). At least in some areas of European Russia it apparently remains common. We have noticed no signs of decline in the abundance of this species in the study area over the last few years. The population studied seems to be exceptionally abundant in comparison with populations of this species known in more western areas of its range. The species is present in at least two other lakes (Gniloye and Turishino) in the environs of Lake Glukhoye, and probably rather abundant in the region, but we know of no other waterbody in which this species appears to be dominant among the larger Dytiscidae.

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