A comparison of methods for sampling aquatic insects (Heteroptera and Coleoptera) of different body sizes, in different habitats using different baits

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Abstract. Although various methods exist for sampling aquatic Heteroptera and Coleoptera in standing water, there are very few comparisons of their performance in different types of habitat. In this study, we evaluated and compared the efficiency and selectivity of three sampling methods: hand netting, bottle traps baited with canned tuna and bottle traps baited with canned cat food. The methods were compared over the period 2010–2012 in two different habitats (temporarily flooded areas and canals) in the Nature Park Kopački rit, a floodplain on the banks of the River Danube. The results show that the effectiveness of the method differed in the two habitats. Overall, hand netting was the most successful method, mainly in canals. Tuna fish bait was more efficient than the commonly used cat food, especially for large and highly mobile species of Dytiscidae. These findings indicate that knowing the type of habitat and the habitat preferences of aquatic insects and their activity it is possible to predict which of these methods are the best for estimating species richness.

INTRODUCTION

Aquatic bugs and beetles (Heteroptera and Coleoptera) are groups of freshwater macroinvertebrates that includes predatory and scavenger species. They occur in almost any type of freshwater system, such as wetlands, snow pools, lakes, streams, rivers, wet rock surfaces, puddles and groundwater aquifers often with a high species richness (Franciscolo, 1979; Nilsson & Holmen, 1995; Balke, 2005; Ribera et al., 2007). Factors that positively influence their species richness are water permanence, rich vegetation and absence of shading by trees (Nilsson & Svensson, 1994; Nilsson & Holmen, 1995; Batzer & Wissinger, 1996; Lundkvist et al., 2001; Rundle et al., 2002; Bouchard, 2004; Schäfer et al., 2006). This makes them good bioindicators of habitat quality and recently they have been used for selecting habitats for conservation (Foster, 1987; Sánchez-Fernández et al., 2006; Lock et al., 2013). Large dytiscids and heteropterans are often top predators in fishless aquatic habitats. Dytiscids range in size from 1–50 mm, and are predators both as larvae and adults (Ribera et al., 2007). Dytiscids are excellent swimmers (Nilsson, 1996) that capture their prey while actively swimming (Ribera et al., 1997). Hydrophiloidea are poor swimmers compared to most species of hydradephagan water beetles (Hansen, 1987).

The most commonly used method for sampling aquatic insects in standing waters is hand netting, which includes simply sweeping the habitat with a hand net for a given period of time (Nilsson & Svensson, 1995; Boukal et al., 2007). Hand netting provides good information about species richness and community composition and is quick and cheap (García-Criado & Trigal, 2005; Florencio et al., 2012). In addition, various traps are used for collecting mobile swimming taxa of aquatic insects (Nilsson et al., 1994; Lundkvist et al., 2001; Boukal et al., 2007), with or without baits. The most commonly used baits are chicken, beef liver and cat food, and Koese & Cuppen (2006) report that cat food is the best bait. Activity traps constructed from plastic bottles (bottle traps) perform well in a variety of environments such as lakes (Nilsson et al., 1994; Hyvönen & Nummi, 2000), ponds (Jurado et al., 2008), wetlands (Lundkvist et al., 2001) and channels (Klečka & Boukal, 2011).

Previous comparisons of these sampling methods indicate that both methods can be used to sample most species as adults and larvae; however, they differ in their efficien-

cy, as well as the size and taxon of the insects captured (Hilsenhoff, 1987; Jurado et al., 2008; Klečka & Boukal, 2011). In particular, hand nets are commonly used for quantitative surveys and are more suitable for collecting small and less mobile taxa (Nilsson & Svensson, 1995; Nilsson & Söderberg, 1996; Klečka & Boukal, 2011), whereas bottle traps are better at capturing large and highly mobile species (Lundkvist et al., 2001; Nilsson et al., 1994; Klečka & Boukal, 2011). In addition, Klečka & Boukal (2011) emphasise the need for long-term studies using different methods in order to capture the full richness of water beetles in highly diverse aquatic habitats. Likewise, Jurado et al. (2008) also suggest that combining hand netting and bottle traps in heavily vegetated ponds will provide a more complete picture of the species richness. In analysing the results of the comparison of sampling methods, one must be aware of the ecological requirements of the species, which involves taking into consideration the constraints that the habitat impose, especially those associated with the morphology of the insects (Ribera et al., 1997). Nevertheless, comparisons of methods across different types of aquatic habitats are still rare [but see García-Criado & Trigal (2005) for pond macroinvertebrates and Tolonen & Hämäläinen (2010) for littoral macroinvertebrates].

In this paper we compare the efficiency of three different sampling methods in two different types of habitat: hand netting (HN), bottle traps with cat food as the bait (BCF) and bottle traps baited with tuna fish (BTF). Our specific aims were to: (1) determine which sampling method is the most efficient in each of the two habitats, (2) determine size selectivity of the different sampling methods and (3) determine the efficiency of a new type of bait (canned fish) for sampling aquatic insects. Based on previous studies, we hypothesized that the effectiveness of the methods would depend on the type of habitat. Furthermore, we assumed that the methods would be size selective and that of the two baits used, canned tuna fish would be the most effective.

MATERIALS AND METHODS

Study area

Nature Park Kopački rit is located in eastern Croatia (45°34′ latitude, 16°24′ longitude), near the town of Osijek (Fig. 1). It extends over an area of 100 km² and is one of the largest fluvial floodplains in Europe listed in the Ramsar Convention. The wider area of Kopački rit contains a variety of aquatic habitats that can be divided into three basic types: flooded areas, canals and ponds. Their appearance and structure depend on the specific water regimes in the Danube and Drava rivers.

The flooded areas are flowing or semi-flowing types of habitat, which directly depend on the water level and hydrological connectivity with the parent river. If the water level of the Danube is high, a large part of the floodplain area is flooded, creating a mosaic of permanent and temporarily flooded habitats. Canals are located in the wider area of Kopački rit, which collect water from the wider area of Baranya and spread it within the park. The complex of canals has a lower water flow capacity than the Drava and Danube rivers. The water quality in these habitats importantly depends on the proximity of agricultural fields.

Due to a great variety of aquatic microhabitats, Kopački rit includes numerous suitable habitats for aquatic insects. The

Fig. 1. Map of the study area showing the locations of the eight sites sampled (circles) in the Nature Park Kopački rit, Croatia (Canals: 1 – Podunavlje I, 2 – Podunavlje II, 3 – Čarna I, 4 – Čarna II; Periodically flooded areas: 5 – Čonakut I, 6 – Čonakut II, 7 – Kopačko Lake, 8 – Novi Canal).

changes in water levels and duration of the hydroperiod are the most important factors influencing the structure of the aquatic insect fauna (Schneider & Frost, 1996; Turić et al., 2015) because newly flooded wetlands are often nutrient-rich and may provide suitable feeding and reproduction possibilities for aquatic insects (Nilsson & Holmen, 1995; Batzer & Wissinger, 1996; Schneider, 1999; Larson et al., 2000; Wilcox, 2001). Research on aquatic insects in the Kopački rit area has increased during the last ten years. Recent studies on aquatic bugs and beetles (Heteroptera and Coleoptera) in this area have recorded 15 families, 40 genera and 71 species, and indicate that there is a considerable difference between the recorded species richness (71 species) and the number that is estimated could be collected with reasonable effort (between 83 and 111) (Turić et al., 2012). A full list of the species of aquatic insects in this area has yet to be published.

Sampling

The research was conducted in two types of habitat (flooded areas and canals) and sampling was carried out at eight sampling sites once a month over three years, from 2010 to 2012, in the period from April to October. Four sampling sites were canals situated in the forest area in the wider area of the Nature Park, which flow through the agricultural basin (Fig. 1). The other four sampling sites were located in periodically flooded areas within the most valuable section of the park, which has been given Special Zoological Reserve status. A description of each sampling site is provided in Table 1.

We used two sampling methods: a large hand net (mesh size 1 mm) and bottle traps with baits. Our hand net was 60 cm in diameter, with a 3-m long wooden handle. Together with the large

Table 1. Description of sampling sites.

Site	Site code	Habitat	Coordinates
Podunavlje I	Ch Pod I	Canal	45°41′N; 18°48′49″E
Podunavlje II	Ch Pod II	Canal	45°41′24″N; 18°48′49″E
Čarna I	Ch Car I	Canal	45°43′22″N; 18°50′28″E
Čarna II	Ch Car II	Canal	45°42′24″N; 18°52′14″E
Čonakut I	Fl Con I	Flooded area	45°36′34″N; 18°48′25″E
Čonakut II	Fl Con II	Flooded area	45°36′16″N; 18°48′60″E
Kopačko Lake	Fl KJ	Flooded area	45°36′27″N; 18°50′19″E
Novi Canal	Fl NC	Flooded area	45°36′23″N; 18°48′5″E

hand net a strainer (mesh size 0.5 mm) was used for collecting the smallest species along the shore of the water habitat. Each sampling session consisted of five sweeps just under the surface of the water at each site, including subaquatic and aquatic vegetation in the littoral zone, so that an area of 3 m² was sampled each time. On each occasion, the sampling time was 1 h, including time spent sweeping and removing adult insects from the sample so that the different vegetation units, substrates and open water areas were similarly sampled.

In addition to sampling by hand net, two bottle traps with bait (one with tinned tuna fish and other with cat food) were placed at each site on each sampling occasion (Koese & Cuppen, 2006). Traps were made from two 1.5 L plastic bottles with an inner neck diameter of 21 mm. The bottom of one bottle was cut off and the top of the other was placed upside down in first bottle, so the trap has funnel entrance and exit with a cap. After the traps were slowly removed from the water, the cap was removed above a strainer to enable the collection of all the specimens caught. Traps were placed along the water's edge in different microhabitats and immersed in water so that about 4/5 of their volume was filled with water and the rest with air and protruded above the surface. The minimum distance between neighbouring traps was 3 m. Considering the high water temperatures and competition between the trapped species (Boukal et al., 2007), traps were set in the afternoon and picked up during the next day, after a period of 20 h in the water (one trapping night). In total, 336 traps were set during the field survey.

We compared the efficiency of the methods used based on the samples collected, because we collected the same number of samples, at the same sampling sites over the same period of time. The sampling effort for each method was equal because we took samples at eight sampling sites once a month for seven months during three years. Thus, we had 168 samples collected by each method, which makes a total of 504 samples. Captured specimens were preserved in 70% ethanol, except for large specimens that we could be immediately identified after sampling, especially specimens of *Graphoderus bilineatus* De Geer, which were released after identification in the field. Only adult aquatic insects were collected; these were identified in the laboratory using the keys and descriptions of Macan (1976), Nilsson (1996), Csabai (2000) and Csabai et al. (2002). All species are stored in the Entomological collection of the Department of Biology at Josip Juraj Strossmayer University in Osijek.

Data analyses

To estimate and compare the species richness recorded by the different sampling methods, we computed sample-based rarefaction curves (accumulation curves) for each method and for the pooled methods in order to estimate the species richness obtained by all the methods combined. The observed species richness (Sobs) was compared with two commonly used estimators of species richness ICE (Incidence-based Coverage Estimator) and Chao2 to assess the sampling completeness of each method and all methods combined. Rarefaction curves with 100 randomisa-

tions without replacement and 95% confidence intervals were calculated using procedures and formulas implemented in the EstimateS 8.2.0 software (Colwell et al., 2004). Differences in the species richness and Shannon diversity index obtained using each method in two types of habitat were also tested by means of a generalized linear model (GLM) with Poisson distribution and log-link function, considering two categorical factors (method and habitat type). Tests for the significance of the effects in the model were performed using Wald statistics.

Finally, to evaluate the relative effect of the various sampling methods and habitats on the composition of aquatic insect assemblages, we performed a CCA (Canonical Correspondence Analysis) using CANOCO 4.5 (Ter Braak & Smilauer, 2002). For the CCA, we used only species for which at least 5 individuals were collected; thus, 61 out of 102 species were included in the analysis. For all our analyses, we used all the samples as our input data, where one sample represented all individuals collected by one method at one sampling site on one date.

To demonstrate the body size selectivity of the different methods, we compared the body size (mean length in mm) of collected species among the methods using a generalized linear model (GLM) in which body size was treated as a continuous dependent variable in the analyses. The body size of collected species was obtained from the identification keys of Csabai (2000), Csabai et al. (2002), Nilsson (1996) and Macan (1976). Generalized linear models and Wald statistics were calculated using STATISTICA 10 package.

RESULTS

Abundance and species richness

In total, 504 samples and 8082 adult individuals of 102 species of aquatic insects were collected using the three sampling methods. Of the 102 identified species, 77 belong to the order Coleoptera [suborder Adephaga (49): Dytiscidae 40, Haliplidae 6, Noteridae 2 and Gyrinidae 1; suborder Polyphaga (28): Spercheidae 1, Hydrochidae 1, Hydrophilidae 26] and 25 to the order Hemiptera (Suborder Heteroptera; Gerromorpha, Nepomorpha). The most abundant species were *Plea minutissima* (41.9%), *Ilyocoris cimicoides* (10.3%), *Laccophilus poecilus* (7.5%) and *Hygrotus versicolor* (4.7%) (Table 2). Most of the individuals were captured using HN [7051 (87.2%)]. Bottle traps with bait captured 1031 individuals in total (12.7%), of which 30.16% were captured by BCF and 69.8% by BTF traps.

Comparative analysis of sampling methods

Out of the 102 species recorded, only 25 were captured by all three sampling methods, while 53 species were captured by only one, indicating that the sampling methods used differed in the species composition of their captures. *Plea minutissima* was the most abundant species captured

Table 2. The list of species, their sizes and number of individuals caught using three sampling methods from April to October over a period of three years. Sampling method: HN – hand netting, BCF – bottle traps baited with canned cat food, BTF – bottle traps baited with canned tuna fish. Size: S – small 0.2–0.5 cm, M – medium 0.5–1.5 cm, L – large <1.5 cm).

Species (abbreviation)	Size	HN	BCF	BTF	Σ	Species (abbreviation)	Size	HN	BCF	BTF	Σ
Nepa cinerea Linnaeus, 1758 (*Nepa cin*)	L	14	0	1	15	*Ilybius fulinginosus* Fabricius, 1792 (*Ilyb ful*)	M	1	0	0	1
Ranatra linearis Linnaeus, 1758 (*Rana lin*)	L	42	3	6	51	*Colymbetes fuscus* Linnaeus, 1758 (*Coly fus*)	M	0	3	3	6
Hesperocorixa linnaei Fieber, 1848 (*Hesp linn*)	M	2	0	0	2	*Rhantus bistriatus* Bergsträsser, 1778 (*Rhan bis*)	M	1	4	0	5
Hesperocorixa sahlbergi Fieber, 1848 (*Hesp sah*)	M	1	0	0	1	*Rhantus exsoletus* Forster, 1771 (*Rhan exo*)	M	1	1	12	14
Sigara distincta Fieber, 1848 (*Siga dis*)	M	2	0	0	2	*Rhantus latitans* Sharp, 1882 (*Rhan lat*)	M	6	7	13	26
Sigara dorsalis Leach, 1817 (*Siga dor*)	M	134	3	2	139	*Rhantus notaticollis* Aubé, 1837 (*Rhan not*)	M	0	1	0	1
Sigara lateralis Leach, 1817 (*Siga lat*)	M	7	0	1	8	*Rhantus suturalis* MacLeay, 1825 (*Rhan sut*)	M	3	7	9	19
Sigara nigrolineata Fieber, 1848 (*Siga nig*)	M	60	0	1	61	*Acilius canaliculatus* Nicolai, 1822 (*Acil can*)	L	0	0	1	1
Sigara semistriata Fieber, 1848 (*Siga sem*)	M	21	0	0	21	*Acilius sulcatus* Linnaeus, 1758 (*Acil sul*)	L	0	1	9	10
Sigara striata Linnaeus, 1758 (*Siga stri*)	M	30	1	0	31	*Graphoderus austriacus* Strum, 1834 (*Grap aus*)	M	1	0	2	3
Sigara falleni Fieber, 1848 (*Siga fal*)	M	4	0	0	4	*Graphoderus bilineatus* De Geer, 1774 (*Grap bil*)	M	7	5	10	22
Cymatia coleoptrata Fabricius, 1777 (*Cyma col*)	S	3	0	0	3	*Graphoderus cinereus* Linnaeus, 1758 (*Grap cin*)	M	1	5	29	35
Ilyocoris cimicoides Linnaeus, 1758 (*Ilyo cim*)	M	731	25	83	839	*Cybister lateralimarginalis* De Geer, 1774 (*Cybi lat*)	L	21	43	170	234
Notonecta viridis Delcourt, 1909 (*Noto vir*)	M	3	0	0	3	*Dytiscus circumcinctus* Ahrens, 1811 (*Dyti cir*)	L	0	1	0	1
Notonecta maculata Fabricius, 1794 (*Noto mac*)	M	7	0	1	8	*Dytiscus dimidiatus* Bergsträsser, 1778 (*Dyti dim*)	L	0	3	2	5
Notonecta glauca Linnaeus, 1758 (*Noto gla*)	M	18	5	0	23	*Dytiscus marginalis* Linnaeus, 1758 (*Dyti mar*)	L	0	0	1	1
Plea minutissima Leach, 1817 (*Plea min*)	S	3344	5	45	3394	*Dytiscus semisulcatus* O.F. Müller, 1776 (*Dyti sem*)	L	0	0	1	1
Mesovelia furcata Mulsant & Rey, 1852 (*Meso fur*)	S	40	0	0	40	*Hydaticus grammicus* Germar, 1830 (*Hyda gra*)	M	6	4	4	14
Hydrometra gracilienta Horváth, 1899 (*Hydr gra*)	M	4	0	0	4	*Hydaticus seminiger* De Geer, 1774 (*Hyda sem*)	M	1	1	1	3
Hydrometra stagnorum Linnaeus, 1758 (*Hydr sta*)	M	45	0	0	45	*Hydaticus transversalis* Pontoppidan, 1763 (*Hyda tra*)	M	5	15	15	35
Aquarius paludum Fabricius, 1794 (*Aqua pal*)	M	82	0	0	82	*Noterus clavicornis* De Geer, 1774 (*Note cla*)	S	47	0	2	49
Gerris lacustris Linné, 1758 (*Gerr lac*)	M	305	0	0	305	*Noterus crassicornis* O.F. Müller, 1776 (*Not cras*)	S	38	2	3	43
Gerris odontogaster Zetterstedt, 1828 (*Gerr odo*)	M	38	0	0	38	*Gyrinus substriatus* Stephens, 1828 (*Gyri sub*)	M	2	0	0	2
Microvelia pygmaea Dufour, 1833 (*Micr pyg*)	S	3	0	0	3	*Spercheus emarginatus* Schaller, 1783 (*Sper ema*)	M	51	1	0	52
Microvelia reticulata Burmeister, 1835 (*Micr ret*)	S	15	0	0	15	*Hydrochus elongatus* Schaller 1783 (*Hydr elo*)	S	2	0	0	2
Haliplus fluviatilis Aubé, 1836 (*Hali flu*)	S	86	0	1	87	*Hydrochus flavipennis* Küster, 1852 (*Hydr fla*)	S	0	1	0	1
Haliplus furcatus Seidlitz, 1887 (*Hali fur*)	S	3	0	0	3	*Helophorus brevipalpis* Bedel, 1881 (*Helo bre*)	S	2	0	0	2
Haliplus immaculatus Gerhardt, 1877 (*Hali imm*)	S	8	0	0	8	*Helophorus obscurus* Mulsant, 1844 (*Helo obs*)	S	1	0	0	1
Haliplus ruficollis De Geer, 1774 (*Hali ruf*)	S	23	0	1	24	*Coelostoma orbiculare* Fabricius, 1775 (*Coel orb*)	S	1	0	0	1
Haliplus variegatus Strum, 1834 (*Hali var*)	S	5	1	0	6	*Cercyon pygmaeus* Illiger, 1801 (*Cerc pyg*)	M	1	0	0	1
Peltodytes caesus Duftschmid, 1805 (*Pelt cae*)	S	42	4	5	51	*Cercyon bifenestratus* Küster, 1851 (*Cerc bif*)	S	1	0	0	1
Copelatus haemorrhoidalis Fabricius, 1787 (*Cope hae*)	M	0	0	1	1	*Anacaena limbata* Fabricius, 1792 (*Anac lim*)	S	16	0	0	16
Bidessus unistriatus Goeze, 1777 (*Bide uni*)	S	1	0	0	1	*Anacaena lutescens* Stephens, 1829 (*Anac lut*)	S	1	0	0	1
Hydroglyphus confusus Klug, 1834 (*Hydr con*)	S	7	3	4	14	*Laccobius minutus* Linnaeus, 1758 (*Lacc min*)	S	9	0	0	9
Hydroglyphus geminus Fabricius, 1792 (*Hydr gem*)	S	149	11	4	164	*Enochrus melanocephalus* Olivier, 1792 (*Enoc mel*)	S	11	0	0	11
Hydroporus palustris Linnaeus, 1761 (*Hydr pal*)	S	6	0	0	6	*Enochrus bicolor* Fabricius, 1792 (*Enoc bic*)	M	4	0	0	4
Porhydrus lineatus Fabricius, 1775 (*Porh lin*)	S	1	0	1	2	*Enochrus fuscipennis* Thomson, 1884 (*Enoc fus*)	M	4	0	0	4
Hygrotus decoratus Gyllenhal, 1810 (*Hygr dec*)	S	1	0	0	1	*Enochrus ochropterus* Marsham, 1802 (*Enoc och*)	M	3	0	0	3
Hygrotus inaequalis Fabricius, 1776 (*Hygr ina*)	S	1	0	0	1	*Enochrus quadripunctatus* Herbst, 1797 (*Enoc qua*)	M	31	0	0	31
Hygrotus versicolor Schaller, 1783 (*Hygr ver*)	S	336	5	44	385	*Enochrus testaceus* Fabricius, 1801 (*Enoc tes*)	M	6	0	0	6
Hygrotus impressopunctatus Schaller, 1783 (*Hygr imp*)	S	20	1	0	21	*Enochrus nigritus* Sharp, 1872 (*Enoc nig*)	S	2	0	0	2
Hygrotus parallellogrammus Ahrens, 1812 (*Hygr par*)	S	4	0	0	4	*Helochares lividus* Forster, 1771 (*Helo liv*)	M	15	2	0	17
Hyphydrus anatolicus Guignot, 1957 (*Hyph ana*)	S	5	3	2	10	*Helochares obscurus* O.F. Müller, 1776 (*Helo obs*)	M	261	0	1	262
Hyphydrus ovatus Linnaeus, 1761 (*Hyph ova*)	S	11	1	2	14	*Hydrobius fuscipes* Linnaeus, 1758 (*Hydr fus*)	M	10	2	0	12
Laccophilus hyalinus De Geer, 1774 (*Lacc hya*)	S	10	0	0	10	*Hydrochara caraboides* Linnaeus, 1758 (*Hydr car*)	L	7	3	1	11
Laccophilus minutus Linnaeus, 1758 (*Lacc min*)	S	121	9	88	218	*Hydrophilus aterrimus* Eschscholtz, 1822 (*Hydr ate*)	L	1	1	0	2
Laccophilus poecilus Klug, 1834 (*Lacc poe*)	S	456	69	84	609	*Hydrophilus piceus* Linnaeus, 1792 (*Hydr pic*)	L	19	0	3	22
Agabus undulatus Schrank, 1776 (*Agab und*)	M	0	0	1	1	*Limnoxenus niger* Zschach, 1788 (*Limn nig*)	M	5	5	1	11
Agabus affinis Paykull, 1798 (*Agab aff*)	M	0	1	0	1	*Berosus geminus* Reiche & Saulcy, 1856 (*Bero gem*)	M	16	0	0	16
Ilybius ater De Geer, 1774 (*Ilyb ate*)	M	0	1	0	1	*Berosus signaticolis* Charpentier, 1825 (*Bero sig*)	M	116	1	0	117
Ilybius fenestratus Fabricius, 1781 (*Ilyb fen*)	M	61	41	49	151	*Berosus frontifoveatus* Kuwert, 1888 (*Bero fro*)	M	2	0	0	2

by HN (47.4%), *Laccophilus poecilus* (22.2%) by BCF and *Cybister lateralimarginalis* De Geer (23.6%) by BTF. If we examine the effectiveness of each method separately, the sample-based accumulation curves indicate that 89 species were collected by HN, 43 by BCF and 44 by BTF (Figs 2a–b). The accumulation curves for the different methods all displayed upward trends (Fig. 2a). Sample-based rarefaction curves were also used to compare the observed species richness (Sobs) with those estimated. We used two

estimators, Chao2 and ICE, known to be highly effective for freshwater invertebrate assemblages (Foggo et al., 2003). On the one hand, using BTF, there were smaller differences between the Sobs and species richness estimated by ICE and Chao2 (Fig. 2b). Comparisons of ICE to Sobs suggest little difference between the two baits, only Chao2 is different because BCF recorded fewer species than estimated. However, the accumulation curve for all three methods combined was close to plateauing and the estima-
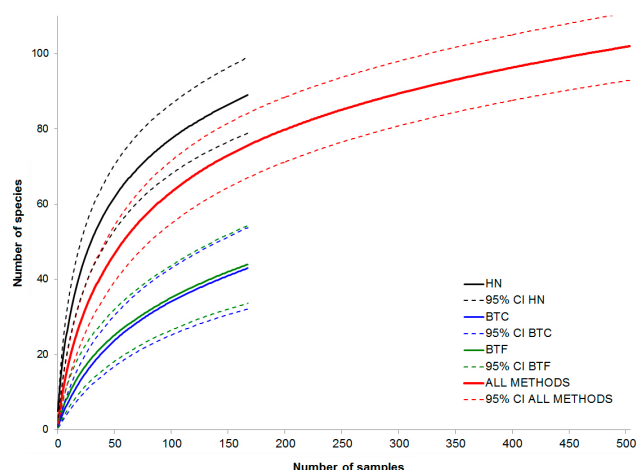
Fig. 2a. Comparison of sample-based accumulation curves (mean of 100 randomizations) for each method with 95% confidence intervals (dashed lines) and for all methods combined (red line) with 95% confidence interval (red dashed line).

tors used indicated almost complete sampling based on all of the data obtained by the different methods combined.

Comparison of the sampling methods in the different habitats

Our research was conducted in two different habitats (canals and flooded areas) and all three sampling methods were used in each habitat in order to compare their effectiveness. In the canals, we collected a total of 96 species, while in the periodically flooded area 65 species. We found

significant differences in the efficiency of the methods used in the two habitats.

A GLM analysis revealed significant differences in species richness and Shannon diversity index per sample both between methods and between habitats (Table 3). Interactions between factors were significant when the dependent variable was species richness, habitat type and a combination of these two variables. HN was by far the most effective method for collecting insects in both canals and flooded areas (Fig. 3). In canals, BTF was more effective than BCF, while they were equally effective in flooded areas. In the periodically flooded areas, where the abundance of individuals per sample was much lower due to it being a temporary habitat, differences between the methods were less pronounced; however, despite this, HN was still the most effective method.

To evaluate the composition of aquatic insect assemblages in relation to the sampling methods and habitats, we performed a CCA analysis (Fig. 4). The CCA confirmed that the sampling methods and habitats both influence the composition of aquatic insect assemblages recorded (a Monte Carlo test for significance of all canonical axes: $F = 7.105$, $P = 0.0001$). The first two CCA axes explained 70.3% of the variance, as follows: 40.5% was explained by the first axis, which was mainly correlated with the sampling method, and 29.8% was explained by the second axis, which was best related to habitat (Table 4, Fig. 4). The amount of the total variation that we can explain by habitat and method was 76.3% (Table 4).
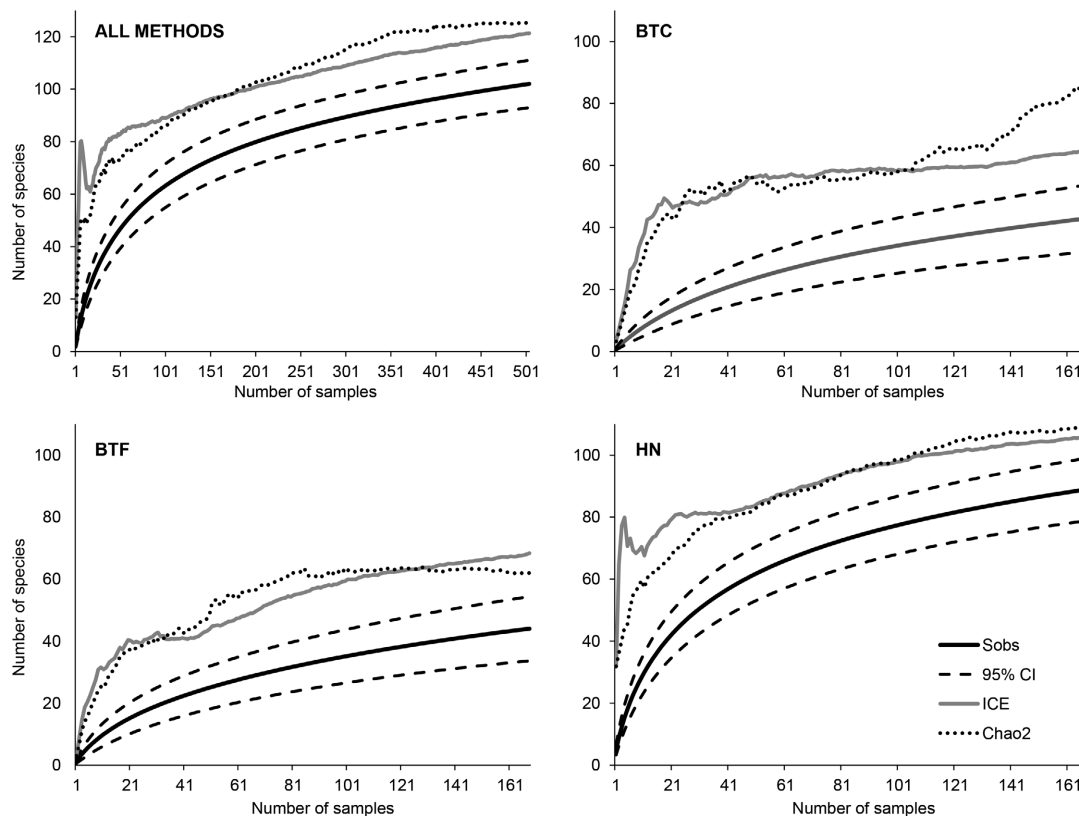

Fig. 2b. Randomized sample-based rarefaction curves for the three sampling methods and all methods combined in comparison with two nonparametric estimators of species richness (Chao2 and ICE – Incidence Coverage-based Estimator). Sobs = observed species richness with 95% confidence intervals (95% CI).
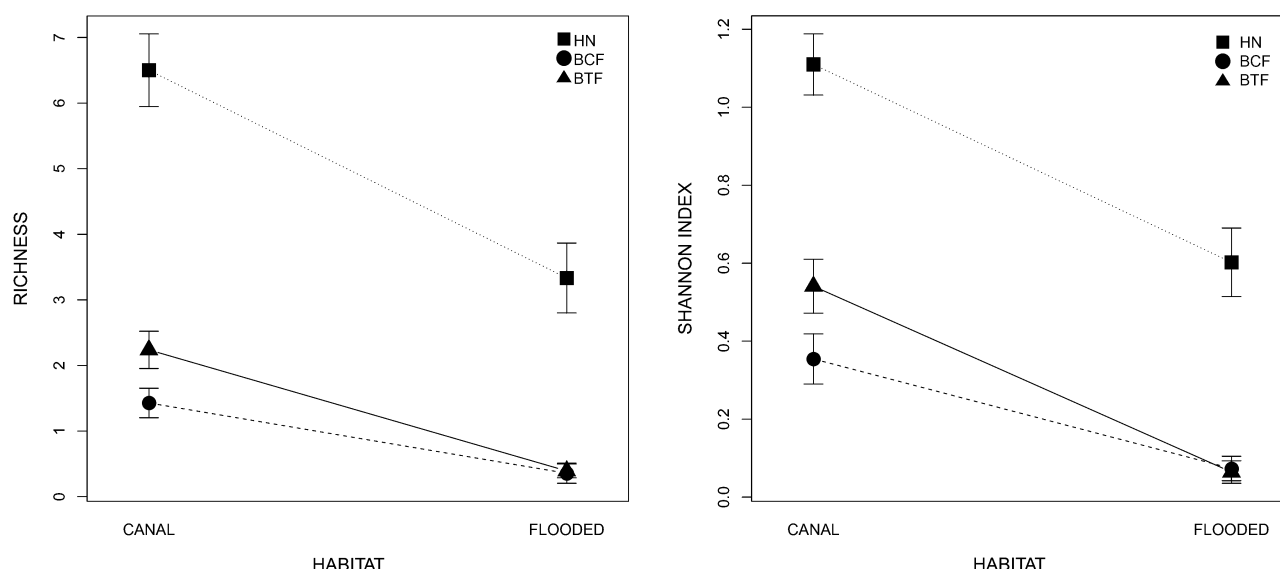
Fig. 3. Results of GLM analysis showing differences in the species richness (number of species) and Shannon diversity index per sample obtained using different sampling methods in two different habitats in the Nature Park Kopački rit. Values are means ± standard errors.

Canals and flooded areas had specific aquatic insect faunas. The correlation between species composition with habitat and sampling method revealed that each method yielded a different set of species. More species of Heteroptera and Hydrophiloidae were collected using HN, irrespective of the habitat. HN was also the most effective method for collecting Haliplidae, especially in canals, where all 6 species were collected. Only for some Dytiscide, was BTF and BCF more effective than HN in canals (Fig. 4).

Size of the insects collected depended on the sampling method. The difference in the body size of species that were caught by HN and bottle traps with bait was highly significant (GLM; $F = 4.088$, d.f. = 2, $P = 0.018$) (Fig. 5). More medium and large species of Dytiscidae were collected using BTF and BCF, and more small species using HN.

DISCUSSION

The wide range of different waterbodies in floodplains, from open water to densely vegetated water, makes them excellent environments for testing the effectiveness of sampling methods for evaluating aquatic insect richness and diversity. A rigorous sampling method is needed in order to avoid underestimating biodiversity, especially in wetland habitats (Becerra Jurado et al., 2008). In our study, we compared the efficiency and selectivity of three sampling methods (HN, BCF and BTF) in two different habitats based on the results of three years of sampling. We tested the efficiency of tuna fish as a bait, which has

not been used previously as bait in bottle traps. Our results reveal that tuna fish is more efficient than the commonly used cat food when the sampling interval is ± 20 h, especially for collecting large species of dytiscid. We also confirmed that habitat has a major effect on the effectiveness of the method, as HN was the most effective, primarily in canals with dense vegetation. It yielded the highest number of species (89 out of 102 collected in total). Previous comparative studies, (Klečka & Boukal, 2011; Florencio et al., 2012) report similar findings for netting and activity traps.

Furthermore, we compared methods based on species richness using accumulation curves. As suspected, the hand netting and activity traps caught different proportions of the aquatic insects. The difference in the numbers of swimming and non-swimming taxa was most pronounced in the catch of the hand net. Our results suggest that hand netting underestimated the number of actively mobile taxa and the higher percentage of taxa collected using traps with different baits were similar. Furthermore, Becerra Jurado et al. (2008) report that a combination of pond netting and activity traps yields a more complete estimate of taxon richness.

Twelve of the remaining 13 species that were not sampled using HN belong to the Dytiscidae. In general, only dyitiscids were sampled more effectively using bottle traps (of the 40 species collected, only seven were caught exclusively using HN). For the Hydrophilidae, HN was more effective than bottle traps in both habitats and especially in flooded areas. This is not surprising because these species usually reside in places with dense aquatic vegetation and are poor swimmers, which crawl more than swim (Nilsson, 1996).

There are two ways of optimizing the use of activity traps in aquatic biodiversity studies: use of attractants and trapping duration. It is reported that the number of taxa and individuals captured increases with use of baits and a longer trapping duration of 48 h (but not longer) (Verdonschot, 2010). Moreover, in our study canned tuna fish

Table 3. Tests for the significance of the effects (sampling method and habitat). GLM with Poisson distribution and log-link function was used. The significant *P*-values are in bold.

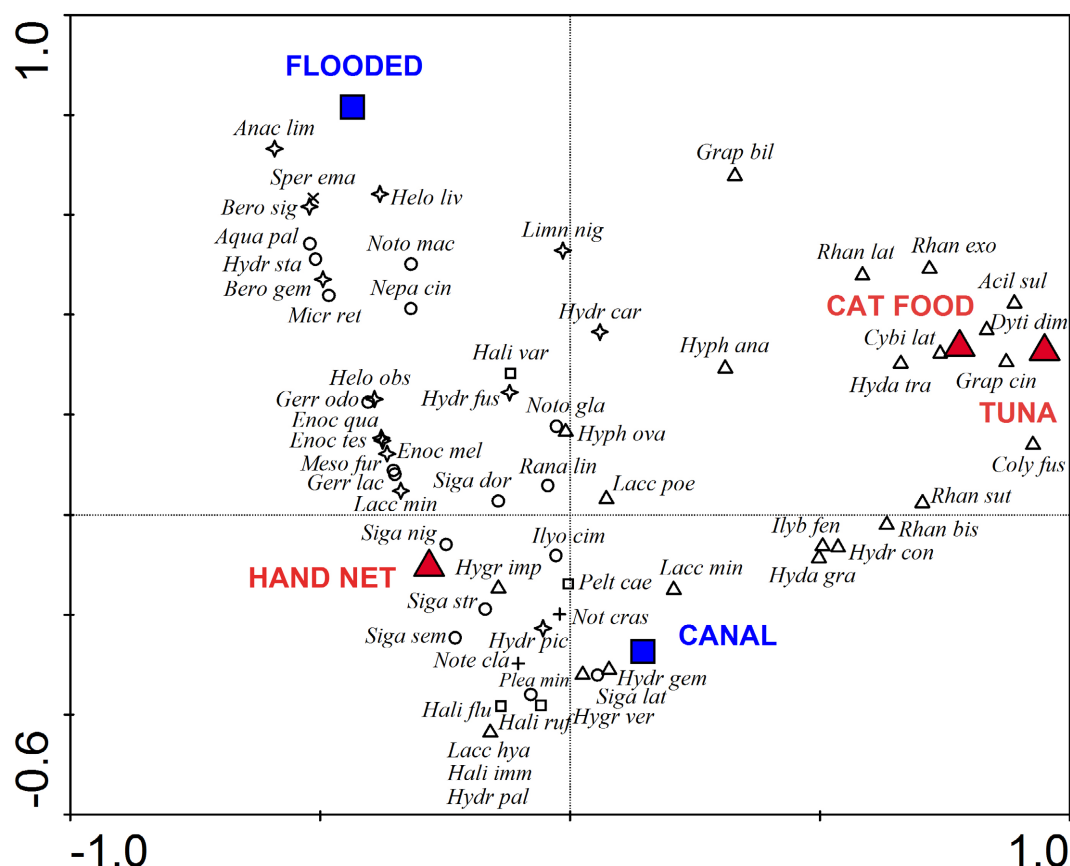|	Richness			Shannon		
	DF	Wald stat.	P	DF	Wald stat.	P
Sampling method	2	489.0	**<0.001**	2	73.7	**<0.001**
Habitat	1	174.1	**<0.001**	1	42.9	**<0.001**
Sampling method*habitat	2	35.2	**<0.001**	2	12.3	**<0.002**

Fig. 4. Ordination diagram of the first two axes of a Cannonical Correspondance analysis (CCA), showing the effect of sampling method and habitat on aquatic insect assemblages in the Nature Park Kopački rit. For abbreviations see Table 2. Different symbols denote different taxonomic groups of aquatic insects (○ Heteroptera; □ Haliplidae; + Noteridae; △ Dytiscidae; x Spercheidae; ◇ Hydrophilidae).

was generally more effective as a bait than cat food after ± 20 h, based on the differences in the species richness and Shannon diversity index. A previous study suggested that cat food is a better bait than chicken liver (Koese & Cuppen, 2006); however, our results demonstrate that canned tuna fish should be the first choice of bait. Differences in

the chemical cues released by different baits (tuna fish and cat food) could cause differences in efficiency. Nevertheless, in future studies other more common species of fish of lower conservation concern should be considered as bait instead of tuna fish, however, their effectiveness needs to be tested.

We are aware that we used only two methods from a large set of well-known methods that are used to survey aquatic insects, but using several methods, particularly hand netting and bottle traps is suggested to result in a more complete species richness for a specific area (Hilsenhoff, 1987; Becerra Jurado et al., 2008; Klečka & Boukal, 2011). Our results reiterate previous findings that using only bottle traps may not always produce representative data for all aquatic insect groups (Turner & Trexler, 1997). Bottle traps catch insects that actively move in the water; consequently, the more sluggish and immobile insects may be under-represented (Thomas et al., 2009). In addition, using several combinations of methods is necessary for long-term studies measuring diversity in species-rich freshwater habitats (Klečka & Boukal, 2011).

Performance of methods in different habitats

HN sampled the most species in the canals (80 out of 89). This finding was expected because species captured in canals are considered to be adapted to crawl among dense vegetation or detritus (Nilsson, 1996), and there is a rich
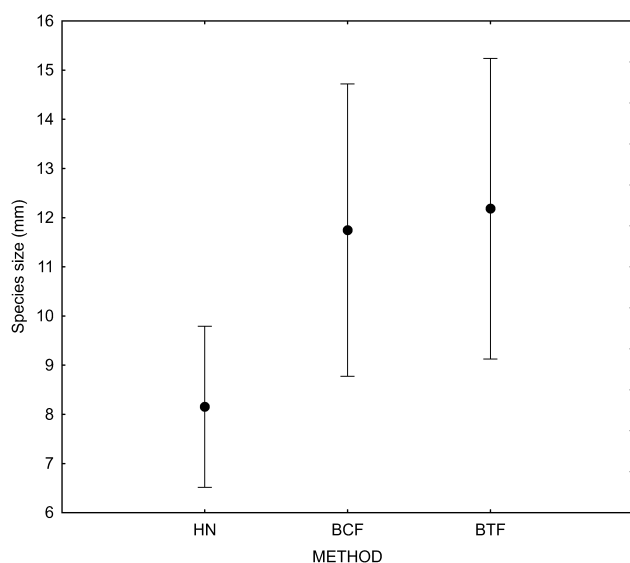

Fig. 5. Average size of the insects caught by the three sampling methods (GLM with Normal distribution and log-link function). Vertical bars denote 95% confidence intervals.

Table 4. Eigenvalues and percentage of the variability accounted for by the variables measured along the first four axes of the Cannonical Correspondance analysis (CCA). * The sum of the unconstrained eigenvalues represents the total inertia.

CCA Axes	1	2	3	4	Total inertia
Eigenvalues	0.405	0.298	0.060	0.480	9.284
Species environment correlations	0.837	0.831	0.489	0.000	
Cumulative % of variation (species data)	4.4	7.6	8.2	13.4	
Cumulative % of variation (species-environment relation)	53.1	92.1	100.0	100.0	
Sum of all eigenvalues*					9.284
Sum of all canonical eigenvalues					0.763

submerged vegetation along the banks of these canals. Previous studies demonstrate that the efficiency of hand netting may be less in the heavily vegetated areas, presumably because the vegetation interferes with the free movement of the hand net; thus, the probability of capturing highly mobile species decreases (Becerra Jurado et al., 2008). Contrary to this, our large hand net proved to be very useful because it easily passed through aquatic vegetation and brushed insects off the vegetation. Bottle traps with different baits were equally efficient in the flooded area, however BTF was more efficient in the canals than BCF. Habitat structure, biotic interactions and physicochemical factors can influence macroinvertebrate activity-density patterns (Verdonschot, 2010). Bottle traps catch mainly dytiscid species, i.e. insects that actively move in the water and that are normally the top predators in the trophic web of these ecosystems (Becerra Jurado et al., 2008; Thomas et al., 2009).

A lower number of species was collected in flooded areas than canals. Floodplains are unique habitats where sampling is dependent on the water level, which varies from month to month. Thus, food resources and the ability of species to find food and colonize different habitats changes with the constantly fluctuating water levels and is thus likely to influence the aquatic insect assemblages (Williams, 1996; Fairchild et al., 2003; Turić et al., 2015). In addition, in periodically flooded areas there is no dense vegetation and the absence of shading effects from trees affects the abundance of species and species composition of assemblages (Nilsson & Svensson, 1994; Nilsson & Söderberg, 1996; Lundkvist et al., 2001; Rundle et al., 2002; Schäfer et al., 2006).

The species composition depended on the habitat and sampling method. There were remarkable differences in species pools and abundances recorded in canals and flooded areas, most likely due to the highly specific nature of the flooded areas. We confirmed that adults of Heteroptera and Hydrophilidae are more efficiently sampled using a hand net (Ortmann-Ajkai & Kalman, 2011). Large species of Dytiscidae were under sampled by hand netting, because they were less abundant than small species and are also more efficient at avoiding being caught by hand netting (Klečka & Boukal, 2011). It is well known that the catches of activity traps better reflect the abundance of mobile aquatic insects, mainly dytiscids, corixids and notonectids (Elmberg et al., 1992; Verdonschot, 2010).

The methods used also differed in size selectivity. In this study, small species were mostly captured using a HN and medium and large species by the bottle traps, as previously shown by other authors (Hilsenhoff, 1987; Klečka & Boukal, 2011). In marked contrast, Nilsson & Söderberg (1996) conclude there is no significant difference in the body size of the species of dytiscids caught using bottle traps and hand netting; however, their samples were collected from different localities in the same area, which might have influenced their results (Klečka & Boukal, 2011). In this study, we collected samples from each habitat at the same locality using all three methods; therefore, we are confident that these methods are size selective.

The presence of a Red List species of water beetle in the study area (*Graphoderus bilineatus*) indicates it is necessary to improve the widely used bottle traps and use traps in which the beetles are more likely to survive in habitats that might harbour this or other endangered species. Our contribution to the ongoing debate about different sampling techniques (Nilsson & Söderberg, 1996; Turner & Trexler, 1997; Becerra Jurado et al., 2008; Klečka & Boukal, 2011; Florencio et al., 2012) is the testing of canned tuna fish as a bait in activity traps. Numerous papers report that the efficiency of traps used for collecting water beetles is increased by using bait, the smell of which attracts the beetles. Tuna fish is a good bait because it has a strong odour. The canned tuna fish we used was not of the endangered Bluefin tuna and we assume that bait of other species of fish could be similarly efficient.

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