

# Sampling macroinvertebrates in a temporary pond: comparing the suitability of two techniques to detect richness, spatial segregation and diel activity

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**Abstract** Recent and increasing interest in temporary ponds as biodiversity reservoirs fosters our need to test sampling techniques for characterising their biological communities. We compared the efficiency of dip-netting to that of fyke nets in sampling the macroinvertebrate assemblage of a temporary pond in Doñana National Park (SW Spain). We sampled the pond at four different times—morning, afternoon, evening and night—distinguishing between deep and shallow zones. In our sampling, dip-netting captured a higher number of taxa, and higher abundances of individuals than fyke nets. However, both techniques

captured exclusive taxa, not recorded with the other device. Fyke nets distinguished between nocturnal and diurnal macroinvertebrates, and hence are more appropriate to study macroinvertebrate diel activity. We detected nocturnal activity in *Gerris thoracicus* larvae, and adults of *Colymbetes fuscus*, *Rhantus suturalis*, *Rhantus hispanicus* and *Hydrochara flavipes*. Conversely, larvae of *Sympetrum fonscolombei* and *Notonecta* spp., and adults of *Notonecta glauca* were mainly diurnal. The overall diel activity pattern of the macroinvertebrate assemblage depended on the diel activities of their integrating taxa and stages. Although dip-netting was more appropriate to sample macroinvertebrate assemblages in different microhabitats, fyke nets better captured nocturnal and fast-swimming invertebrates. Consequently, the joint use of both sampling techniques would capture a better picture of the representative macrofauna of a temporary pond than either one on its own.

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## Introduction

Mediterranean temporary ponds, MTPs, included as priority habitats in the Habitats Directive (Natura code 3170, CEE, May 21st 1992), are drawing an increasing interest for conservation due to their high

vulnerability to disturbances (Grillas et al., 2004; Zacharias et al., 2007; Céréghino et al., 2008). These habitats are especially important for the conservation of rare or specialised taxa of aquatic macroinvertebrates (Collinson et al., 1995; Bilton et al., 2009), which may not survive or complete their life cycles in other types of aquatic habitats. To refine our knowledge of biological communities in temporary ponds, we need to test different sampling techniques for shallow and vegetated ponds (O'Connor et al., 2004; García-Criado & Trigal, 2005; Becerra Jurado et al., 2008).

The characterisation of macroinvertebrate assemblages in aquatic systems strongly depends on the sampling techniques used (Turner & Trexler, 1997; Hyvönen & Nummi, 2000; O'Connor et al., 2004). Macroinvertebrate surveys with different aims would require different sampling techniques. Although many studies have shown that the number of individuals and variety of taxa captured may vary with time of day (Rincón & Lobón-Cerviá, 1997; Céréghino & Lavadier, 1998; Hampton & Duggan, 2003), these differences are not considered in most studies concerning the representative species of macroinvertebrate assemblages. Dip-netting has been a technique frequently used in sampling macroinvertebrates in ponds (Maciolek, 1989; Collinson et al., 1995; Nicolet et al., 2004; Bilton et al., 2006; Florencio et al., 2009), but more specific traps have been used to describe diel activity in macroinvertebrates of different aquatic systems, such as nets (Waters, 1962), hand-made traps (Hampton & Duggan, 2003) and the so-called activity traps (Murkin et al., 1983; Hanson et al., 2000). Dip-netting efficiency for monitoring macroinvertebrates of temporary ponds has been stressed (Cheal et al., 1993; García-Criado & Trigal, 2005) but other studies found dip-netting inappropriate for sampling macroinvertebrates in shallow ponds (Muzaffar & Colbo, 2002; O'Connor et al., 2004). The efficiency of activity traps in capturing particular groups of fast swimming invertebrates has also been demonstrated (Murkin et al., 1983; Hanson et al., 2000).

Collecting techniques differ in their efficiency to capture active individuals and the whole pool of macroinvertebrates (Cellot, 1989; Rincón & Lobón-Cerviá, 1997). Many species present diel changes in spatial distribution or activity that are an essential part in the behavioural dynamism of a community, for which

day samples may not provide a complete picture (Elliott, 2005). Adequate sampling techniques have revealed activity cycles in macroinvertebrates, indicating that some species show high abundances at night (Johnson & Covich, 2000; Marklund et al., 2001; Hansen & Closs, 2007), especially just after sunset (Waters, 1962; Brittain & Eikeland, 1988) and before sunrise (Rincón & Lobón-Cerviá, 1997). These diel activity cycles can also be associated with differential uses of the microhabitats along the day for macroinvertebrate species (Elliott, 2002, 2005) and assemblages (Hamptom & Duggan, 2003). Macroinvertebrate diel activities can also differ among seasons, presenting activity peaks only in particular wet phases (Waters, 1962; Rincón & Lobón-Cerviá, 1997; Hansen & Closs, 2007).

In this study, we compare the suitability of two sampling techniques for describing the epibenthonic macroinvertebrate assemblage of a temporary pond: dip-netting, which requires the active researcher's movement; and setting fyke nets, which are static devices. We hypothesised that there would be variation in the taxon number, abundance of individuals and community composition detected by the two methods. Furthermore, abundance of individuals and community structure would vary across microhabitats within pond and at different times of day.

## Materials and methods

This study was conducted in Doñana National Park, (36° 59' N, 6° 27' W, Huelva province, SW Spain) in March and April of 2007. This Park is located between the Atlantic coast and the mouth of the Guadalquivir River, and its sandy area holds more than 3,000 water bodies. This pond network has a high conservation value and encompasses a wide range of hydroperiods, ranging from permanent to ephemeral (Díaz-Paniagua et al., 2010). A detailed description of these temporary aquatic habitats can be found in Espinar & Serrano (2009) and Gómez-Rodríguez et al. (2009). The climate is Mediterranean sub-humid, with hot and dry summers, mild winters, and rainfall mainly occurring in autumn and winter. Since the aim of this study was to compare two sampling techniques rather than study across-pond variation in macroinvertebrate assemblage composition, we focused on a single pond. The pond used has a maximum area of approx. 4,000 m<sup>2</sup>, and during our

study year it was flooded from November to May, with 54 cm maximum depth in February.

The dip-net used was rectangular (39 × 21 cm) with blunt ends and 1 mm mesh. We swept a stretch of water of ca. 1.5 m, in three successive thrusts in alternate directions. In turn, the fyke nets used had a semicircular aperture (68 × 34 cm) and were built with 5 mm mesh. These nets are commonly used in the study area to catch eels or crayfish. They have a conical structure with three internal inverted funnels and an external wing of net (length ca. 125 cm) flanking the entrance. The wing favours the entrance of animals, whereas the consecutive funnels prevent their exit once inside (Fig. 1). The sampling devices used differed in mesh sizes as fyke nets normally have a greater mesh size than dip-nets. Hence, we only compared abundances of trapped individuals with large body sizes (>5 mm). Fyke nets, however, retain a certain number of small individuals (<5 mm), so although their abundances are surely misrepresented, they are still suitable for detecting their presence in the pond.

Within the pond, we selected two zones, deep and shallow, with different vegetation and three random sampling areas within each depth zone. The shallow zone, with 11–26 cm depth, had a range of 6.7–8.6 mg l<sup>-1</sup> of dissolved oxygen and of 20–22°C water temperature and a dense cover of meadow plants (mainly *Mentha pulegium* L., *Illecebrum verticillatum* L., and *Hypericum elodes* L.). The deep zone, with 29–39 cm depth, ranged 0.2–1.2 mg l<sup>-1</sup> in dissolved oxygen, and 12–15°C in water temperature and had a low density cover of aquatic macrophytes (*Juncus heterophyllus* Dufour, *Myriophyllum alterniflorum* DC. in Lam and DC. and *Ranunculus peltatus* Schrank).

**Fig. 1** Fyke net collecting macroinvertebrates in a pond



In order to compare the efficiency of both techniques, we sampled the same sites in successive 24 h periods from March 16th to 18th (M1). We started with fyke nets (three fyke nets per pond zone), revised approx. every 6 h (referred to as “day periods”), between 6:00 and 12:00 h (morning), 12:00–18:00 (afternoon), 18:00–24:00 (evening) and 24:00–6:00 (night). After 1 day without sampling, we sampled at the same six sites using the dip-net, at approx. 9 h (morning), 15 h (afternoon), 20 h (evening) and 24 h (night). Time is shown in GMT hours. The same sampling procedure was repeated between March 24th and 26th (M2).

Most macroinvertebrates were identified and counted in situ, and then immediately released. The unidentified individuals were preserved in 70% alcohol for later identification in the laboratory. During nocturnal sampling, the inspection of fyke nets and dip-nets were made using a powerful head lamp. Whenever it was possible, we identified adults, larvae or nymphs to species level, or to genus for some larvae, except for dipterans for which we mainly identified only down to the family level.

In order to assess the consistency of diel cycle variations for species, we additionally sampled with fyke nets twice further, on April 16th (A1) and April 20th (A2).

#### Statistical analyses

We used the presence-absence data for comparisons of the macroinvertebrate assemblage depicted by each sampling technique. In addition, we tested for differences in small versus large individuals recorded to evaluate the importance of mesh size in our results.

We also compared the abundance of each taxon captured by either sampling technique, split by day period (morning, afternoon, evening and night) through nonparametric two-tailed Wilcoxon test. As multiple comparisons were required, we corrected the significance level using the Dunn-Sidak procedure (Sokal & Rohlf, 1997).

For analyses, we considered morning plus afternoon as daylight hours for diurnal activities; and evening plus night as night hours for nocturnal activities. For diel variation analyses, we considered the number of individuals recorded in fyke nets in March and April. For these analyses, we treated life-history stages separately, differentiating adults from larvae or nymphs of the same taxon (referred to as different taxa-stage), because we considered that adults and larvae differed considerably in their movements and preferred microhabitats.

To detect variations in the general composition of macroinvertebrates sampled with respect to day–night period and pond zone, we constructed a presence/absence data matrix of all macroinvertebrate taxa-stages per each sampling site and sampling day. Corresponding resemblance matrices were calculated through the Sorensen index (Legendre & Legendre, 1998). Per each sampling date and each sampling technique, we performed two-way crossed ANOSIM analyses (Primer v.6, 9,999 permutations, Clarke & Warwick, 2001) using day–night periods and pond zones (deep and shallow) as crossed grouping factors. Global Spearman coefficient (Global R) indicates the strength of the significant differences detected (range 0–1 in absolute value). Spearman R is close to 1 when the differences among levels of the factor are highest. In order to identify the taxa-stages with the largest contribution to the differences detected for these factors, we performed a two-way crossed SIMPER (Primer v.6; Clarke & Warwick, 2001), an exploratory analysis based on the original presence data. We indicated the three main contributed taxa-stages to these differences and their relative contribution.

To assess the diel variation of particular taxa-stages, we grouped data from both monthly sampling occasions. For those taxa-stages for which we obtained  $n > 10$  individuals in a sampling month, we illustrated the number of individuals captured

through different day periods. We compared whether the number of individuals of each taxa-stage was captured mainly during the daytime or at night in order to reveal their diurnal or nocturnal activity using chi-square tests.

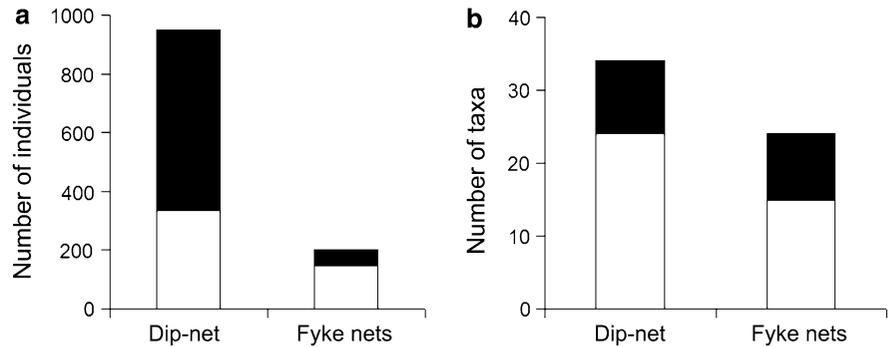
Using the most abundant taxa-stages, we tested for differences between techniques in detecting variations between day periods and pond zones using repeated measures ANOVA on number of individuals captured. We used linear contrasts in planned comparison analyses to detect whether taxa-stages with significant differences along day periods were more abundant during daylight hours, as diurnal taxa, or at night, as nocturnal taxa. Abundance for each taxon was transformed as  $\log(X + 1)$  or 4th root transformed to meet parametric assumptions.

## Results

### Abundance and taxon richness comparing both sampling techniques

We recorded 39 taxa in March, of which 17 were captured with both sampling techniques. Sixteen taxa were exclusively captured by dip-netting and five were exclusively found in fyke nets. Two of these five taxa were smaller than the fyke nets' mesh (*Bagous* spp., *Dryops* spp.) (see Appendix 1—supplementary material). The total number of individuals captured was 915 and 215 for dip-netting and fyke nets respectively. Dip-netting captured significantly higher number of individuals per day period than fyke nets even when we considered separately macroinvertebrates larger and smaller than 5 mm (Wilcoxon test, in all three cases  $Z = 2.52$ ,  $P = 0.01$ , Fig. 2a, Appendix 1—supplementary material). The total number of taxa per day period recorded through dip-nettings was also larger than that of fyke nets for all body sizes pooled together (Wilcoxon test,  $Z = 2.52$ ,  $P = 0.01$ ), restricting for body sizes  $>5$  mm (Wilcoxon test,  $Z = 2.37$ ,  $P = 0.02$ ) and marginally significantly so for body size  $<5$  mm (Wilcoxon test,  $Z = 1.96$ ,  $P = 0.05$ ). However, both techniques captured a similar total number of taxa with small body size when day period was not taken into account and all day samplings were pooled together (Fig. 2b).

**Fig. 2** Total number of macroinvertebrate individuals (a) and number of taxa (b) captured with the dip-net and fyke nets in the two sampling days of March. The number of individuals and taxa captured with body size shorter (filled fraction of bars) or larger (empty fraction) than 5 mm are shown



Variation across day periods: diurnal versus nocturnal activity

In March, macroinvertebrate assemblages detected through dip-netting did not significantly differ among daylight and night hours (M1 Global  $R = 0.055$ ,  $P = 0.215$ ; M2 Global  $R = 0.006$ ;  $P = 0.448$ ). On the contrary, we found significant differences among macroinvertebrate assemblages detected by the use of fyke nets (Table 1). Main taxa contributing to the diel activity of the macroinvertebrate assemblage were the larvae of *Sympetrum fonscolombei* (Selys, 1841), of *Cloeon dipterum* (Linnaeus, 1761) and of *Notonecta* spp. as diurnal taxa, and adults of *Hydrochara flavipes* (Steven, 1808) as nocturnal taxa (Table 1).

In Fig. 3, we show the day period variation of the number of individuals captured by fyke nets for the most abundant taxa-stages in March and April. We detected significant higher abundances in nocturnal periods for *Gerris thoracicus* Schummel, 1832 larvae, *Colymbetes fuscus* (Linnaeus, 1758) adults,

*Rhantus suturalis* (McLeay, 1825) adults, *Rhantus hispanicus* Sharp, 1882 adults, and *H. flavipes* adults which were only captured in nocturnal samplings (Fig. 3). Other taxa reached higher diurnal than nocturnal total abundances: *S. fonscolombei* larvae and *Notonecta* spp. larvae, and adults of *Notonecta glauca* Linnaeus, 1758 (Fig. 3). In other taxa, we did not capture significantly different total abundances in nocturnal or diurnal samplings, e.g. adults of *G. thoracicus* despite the larvae being mainly nocturnal in March (Fig. 3). All taxa-stages showed similar tendency in March and April towards nocturnal or diurnal activity although significant differences were only detected in the month when they were most abundant, e.g. *S. fonscolombei* larvae was only diurnal in March and *N. glauca* only in April (Fig. 3).

Spatial segregations: shallow versus deep zones

Dip-netting detected significant differences in the macroinvertebrate assemblages recorded between

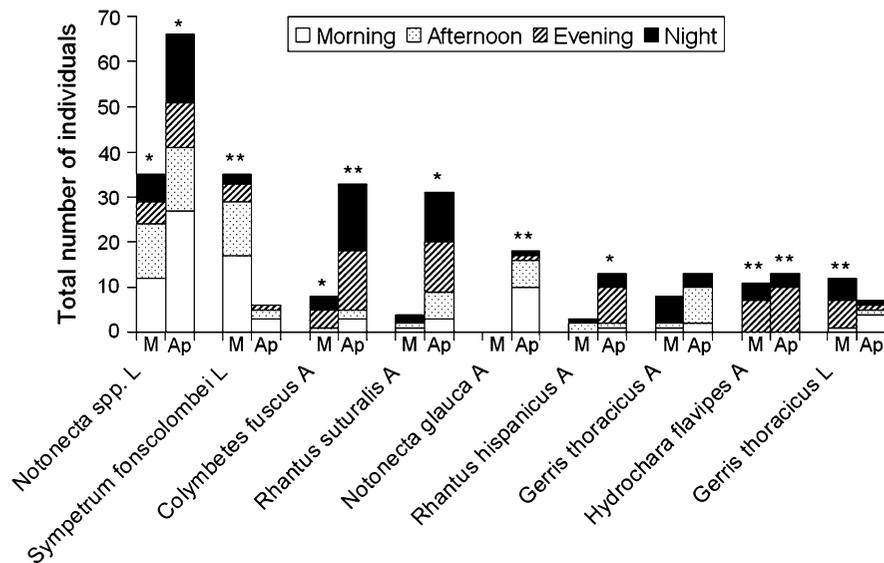
**Table 1** Results of a two-way crossed ANOSIM analyses ( $R =$  Spearman coefficient) showing significant differences in the macroinvertebrate composition at daylight and night hours determined by use of fyke nets (significant differences were not detected using dip-nets)

Dates	$R$	Main taxa	Hours	Contribution (%)
M1	0.240*	<i>Sympetrum fonscolombei</i> -L	Daylight	11.6
		<i>Cloeon dipterum</i> -L	Daylight	8.1
		<i>Notonecta</i> spp.-L	Daylight	8.0
M2	0.389**	<i>Notonecta</i> spp.-L	Daylight	19.7
		<i>Hydrochara flavipes</i> -A	Night	14.1
		<i>Sympetrum fonscolombei</i> -L	Daylight	11.5

The main taxa contributing to the significant differences, the percentage of contribution and the daylight–night hours when they were more frequent result from a two-way crossed SIMPER test. For each taxon, L indicates larvae and A indicates adults (M1 = March 16th; M2 = March 24th 2007)

\*\*  $P < 0.01$

\*  $P < 0.05$



**Fig. 3** Number of individuals of the most abundant taxa captured by fyke nets in the two sampling days in March (M1 + M2), M, and in April (A1 + A2), Ap, are shown. For each taxon, L indicates larvae and A indicates adults. Morning: 06:00–12:00 GMT hours, afternoon: 12:00–18:00, evening:

18:00–24:00 and night: 24:00–06:00. Significantly different abundances for diurnal (morning + afternoon) and nocturnal macroinvertebrates (evening + night) are shown ( $\chi^2$ , df = 1, \* $P < 0.05$ , \*\* $P < 0.01$ )

shallow and deep zones in the two sampling occasions of March (M1 and M2, Table 2). *Physa* spp., larvae of *Notonecta* spp., larvae of *Agabus* spp., and adults of *Berosus guttalis* Rey, 1883 were the main taxa-stages contributing to these differences (Table 2). Excluding the larvae of *Agabus* spp., all of them were more frequent in the deep zone. Using fyke nets, we did not find a significant differentiation between pond zones (M1 Global  $R = 0.085$ ,  $P = 0.146$ ; M2  $R = 0.137$ ,  $P = 0.075$ ).

Exploring the suitability of both techniques for most abundant taxa

We detected differences between techniques in their suitability for detecting spatial and temporal variation in species abundance using the most abundant taxa-stages captured in March (see Fig. 3). In dip-netted samples of *Sympetrum fonscolombei* larvae as well as of *Notonecta* spp. larvae, we did not find significant differences in the abundance captured at different day periods ( $F_{3,30} = 1.72$ ,  $P = 0.18$ ;  $F_{3,30} = 2.05$ ,  $P = 0.13$  respectively). However, we found significant differences between the abundances captured in the two sampling zones of the pond (*S. fonscolombei* larvae:  $F_{1,10} = 22.06$ ,  $P < 0.01$ ; *Notonecta* spp.

**Table 2** Results of a two-way crossed ANOSIM analyses ( $R$  = Spearman coefficient) showing significant differences in the macroinvertebrate composition at deep and shallow pond zones by means of dip-netting in March (significant differences were not detected using fyke nets)

Dates	$R$	Main taxa	Zones	Contribution (%)
M1	0.342**	<i>Physa</i> spp.	Deep	9.38
		<i>Notonecta</i> spp.-L	Deep	7.14
		<i>Agabus</i> spp.-L	Shallow	6.90
M2	0.359**	<i>Notonecta</i> spp.-L	Deep	10.72
		<i>Physa</i> spp.	Deep	10.48
		<i>Berosus guttalis</i> -A	Deep	8.24

The main taxa contributing to these differences, the percentage of contribution and the pond zone where they were more frequent were identified through two-way crossed SIMPER tests. For each taxon, L indicates larvae and A indicates adults (M1 = March 16th–18th; M2 = March 24th–26th 2007)

\*\*  $P < 0.01$

larvae:  $F_{1,10} = 23.27$ ,  $P < 0.01$ ) with higher number of individuals in the deep zone in both cases. On the contrary, in fyke net samples, we detected significant differences in the abundances at different day periods (*S. fonscolombei* larvae:  $F_{3,30} = 4.45$ ,  $P < 0.05$ ;

*Notonecta* spp. larvae:  $F_{3,30} = 5.55$ ,  $P < 0.01$ ), where both taxa exhibited higher abundance over daylight hours (planned comparisons:  $F_{1,10} = 13.60$ ,  $P < 0.01$ ;  $F_{1,10} = 9.86$ ,  $P < 0.05$  respectively). Fyke nets detected significant differences in species abundances between pond zones for *S. foncolombei* larvae ( $F_{1,10} = 17.82$ ,  $P < 0.01$ ) but not for *Notonecta* spp. larvae ( $F_{1,10} = 0.463$ ,  $P = 0.51$ ). No interactions between the two factors (day periods  $\times$  pond zones) were detected (all  $P > 0.319$ ).

## Discussion

### Comparing the efficiency of sampling techniques

Dip-netting ensured capturing a higher number of taxa and a higher number of individuals than fyke nets. We could expect that these differences were due to the smaller mesh size of the dip-nets used, as small macroinvertebrates could escape from fyke nets with mesh size larger than their body length or width. Nevertheless, mesh size did not seem to affect these results for various reasons. First, dip-netting captured higher abundances of taxa both smaller and bigger than 5 mm. Moreover, the number of total taxa with reduced body size (<5 mm) were equally captured by the dip-net (10 taxa) and fyke nets (9 taxa) despite the higher number of individuals captured per day period by dip-nettings. Sixteen taxa were exclusively captured by the dip-net, and only five of them had such reduced body size that individuals could escape from fyke nets [*Planorbis* spp., *Hydroporus gyllenhali* Schiödt, 1841, *Anacaena lutescens* (Stephens, 1829), *Microvelia pygmaea* (Dufour, 1833) and *Plea minutissima* Leach, 1817]. Furthermore, two of the five taxa exclusively captured by fyke nets were also smaller than 5 mm, indicating that fyke nets can also trap small species. The different suitability of both techniques for detecting macroinvertebrate assemblages' composition and its spatial and diel variation indicates that there are important differences among them, and hence complements each other. Dip-netting is fast and intensive, yielding a high number of individuals regardless of whether they were active or not at the moment. In contrast, fyke nets are more passive approach that captures fewer individuals but are more likely to detect fast swimming species (e.g. the diving beetles *Colymbetes fuscus* or *Dytiscus*

*circumflexus* Fabricius, 1801) and provide information on activity patterns.

An additional important difference between our sampling techniques is that dip-netting may be more strongly affected by among-researcher variation due to idiosyncratic differences in sweeping. In contrast, fyke nets allow for an easy standardisation of the sampling procedure, an advantage already mentioned for activity traps when they were compared to dip-netting (Murkin et al., 1983).

In our study, the sampling technique had a great influence on species abundances. Similar conclusions were obtained in studies comparing other techniques (Brinkman & Duffy, 1996; Muzaffar & Colbo, 2002; O'Connor et al., 2004), suggesting that evaluation and comparisons of biodiversity among macroinvertebrates assemblages should take into account the sampling technique used.

### Detection of diel activity cycles

Fyke nets exclusively capture active individuals, hence giving information about diel activity cycles. Indeed, although we sampled over 24-h periods, diel activity cycles were only detected with fyke nets, not through dip-netting. This dichotomy between catching techniques has been already demonstrated in lotic aquatic systems (Cellot, 1989; Rincón & Lobón-Cerviá, 1997) but not in lentic macroinvertebrates of temporary ponds.

Although dip-netting was more efficient for monitoring biodiversity, five taxa were only detected by fyke nets, including nocturnal taxa such as *Colymbetes fuscus* and *Hydrochara flavipes*. The nocturnal activity of predatory beetles has been previously reported (Holomuzki, 1985; Dolmen & Solem, 2002) and represents an example of resource partitioning within the food chains in aquatic environments, where some prey species shift their diel rhythms to nocturnal activity in order to avoid predation risk (Gilbert & Hampton, 2001).

Diel activity has been reported for particular aquatic macroinvertebrates taxa (Waters, 1962; Céréghino & Lavandier, 1998; Johnson & Covich, 2000), but very few studies have analysed diel variations for macroinvertebrate assemblages (Rincón & Lobón-Cerviá, 1997; Hampton & Duggan, 2003). Fyke nets detected different assemblages through day periods because of the differential

activity of nocturnal and diurnal species. We observed five taxa with higher abundances at night (*Gerris thoracicus* larvae, *Colymbetes fuscus* adults, *Rhantus suturalis* adults, *Rhantus hispanicus* adults and *Hydrochara flavipes* adults) whereas other three taxa were considered diurnal (*Sympetrum fonscolombeii* larvae, *Notonecta* spp. larvae, and adults of *Notonecta glauca*). In March, the strong macroinvertebrate assemblage variation detected at sampling day periods was favoured by the high contribution of diurnal taxa (*S. fonscolombeii* larvae and *Notonecta* spp. larvae), although nocturnal taxa also occurred but contributed with less abundance (*C. fuscus* adults, *H. flavipes* adults, and larvae of *G. thoracicus*). This indicates that it is important to examine the variation of particular taxa to determine whether apparent lack of diel cycles in macroinvertebrate assemblages can hide opposite diel cycles of particular taxa.

Fyke nets are therefore an important supplementary technique to complement biodiversity monitoring samples obtained through dip-netting, because the combination of techniques yields a more inclusive representation of the fauna (Turner & Trexler, 1997; Hyvönen & Nummi, 2000; Becerra Jurado et al., 2008). As macroinvertebrate sampling is usually conducted during the daytime, the use of fyke nets during prolonged sampling periods (i.e. 24-h periods) may offer a more complete picture of the macroinvertebrate community, as they sample both nocturnal and diurnal species. However, 24-h-sampling periods require regular visits (i.e. every 6 h) in order to minimise top-predator effects (e.g. Dytiscidae and Odonata larvae) on the sampled macroinvertebrate abundances.

#### Spatial segregations: shallow and deep

Although some studies found dip-netting inappropriate for sampling macroinvertebrates in shallow ponds (Muzaffar & Colbo, 2002; O'Connor et al., 2004), our results suggest that it is a good method to sample the macrofauna of different microhabitats within a pond. In contrast, the use of fyke nets did not capture differences in assemblage composition across pond zones and only detected different abundances per zone for *Sympetrum fonscolombeii*. Temporary ponds are characterised by a high diversity in microhabitats e.g., diverse vegetation covers and species (Grillas et al., 2004; Urban, 2004; Biggs et al., 2005),

rendering dip-netting highly suitable for monitoring, management and conservation programs.

Dip-netting can vary their efficiency capturing macroinvertebrates in vegetated or non vegetated zones of the pond (O'Connor et al., 2004; García-Criado & Trigal, 2005; Becerra Jurado et al., 2008). In this study, the main contributing taxa to different pond zones occurred mainly in the deep zone (*Physa* spp. exclusively occurred there), but both types of zones were vegetated. Although we cannot discard the influence of increased difficulty of dip-netting in shallow vegetated areas, it is certainly not the sole cause for zone differences because we observed other taxa with high abundance in the shallow zone [e.g. larvae of *Agabus* spp., larvae of *Gerris thoracicus*, larvae of *Laccophilus minutus* (Linnaeus, 1758)].

In conclusion, diel activity should be taken into account when sampling macroinvertebrate assemblages in temporary ponds. In comparison with fyke nets, dip-netting is a more appropriate technique to capture high number of individuals and taxa, and distinguish variations in assemblage composition according to microhabitat. In contrast, fyke nets are more efficient at capturing fast swimming species and provide information on diel patterns. Dip-netting supplemented by fyke nets would give the most representative picture of the macroinvertebrate community of a temporary pond.

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