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International Journal of Odonatology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tijo20

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Luigi Giugliano [a] , Sönke Hardersen [b] & Giacomo Santini [a c]

[a] Department of Evolutionary Biology, University of Florence, Firenze, Italy

[b] Corpo Forestale dello Stato, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale, "Bosco Fontana" di Verona, Marmirolo, MN, Italy

[c] CESPRO, University of Florence, Prato, Italy

Available online: 13 Mar 2012

To cite this article: Luigi Giugliano, Sönke Hardersen & Giacomo Santini (2012): Odonata communities in retrodunal ponds: a comparison of sampling methods, International Journal of Odonatology, 15:1, 13-23

To link to this article: http://dx.doi.org/10.1080/13887890.2012.660403

International Journal of Odonatology
Vol. 15, No. 1, March 2012, 13–23

Taylor & Francis
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Luigi Giugliano[a]*, Sönke Hardersen[b] and Giacomo Santini[a,c]

[a]*Department of Evolutionary Biology, University of Florence, Firenze, Italy;* [b]*Corpo Forestale dello Stato, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale, "Bosco Fontana" di Verona, Marmirolo (MN), Italy;* [c]*CESPRO, University of Florence, Prato, Italy*

(*Received 3 August 2011; final version received 19 January 2012*)

Dragonflies are commonly used as indicators of environmental quality and different methods have been employed to monitor odonate assemblages, such as surveys of all adults, evaluations based on breeding adults, sampling of larvae and collection of exuviae. Results obtained with different sampling methods may not be interchangeable, as the different life stages (e.g. larvae, adults) differ in mobility (aquatic, aerial) and as they are subjected to different ecological constraints. Therefore generalization about habitat quality based on only one survey method might be questionable. Additionally, detectability of species might vary when different methods are used. In this study, nine retrodunal ponds in the Migliarino, San Rossore, Massaciuccoli Regional Park (Tuscany, Italy) were repeatedly and contemporaneously sampled during May–September 2008 with the following methods: all adults, breeding adults, larvae and exuviae. In total, 22 species were detected and the results showed that the four methods were not interchangeable. First, some species were only found using certain methods. Second, univariate measures of diversity obtained with the four sampling methods were considerably different. Alpha diversity was maximal when computed on all adults and minimal with exuviae; breeding adults and larval collection had intermediate values. Beta diversity showed an inverse trend, with the lowest value for "all adults" surveys and higher values for all the others. Finally, congruence among the assemblages revealed by the four methods was generally low. The results show that the four survey techniques are not interchangeable and that monitoring of Odonata has to be based on a carefully chosen method, which should reflect the aim of the study.

Keywords: Odonata; dragonfly; sampling; retrodunal wetlands; monitoring; Italy

1. Introduction

Fresh water quality has considerably declined over the last few decades throughout the world and aquatic ecosystems are subjected to increasing threats (Strayer, 2006). Invasive species and chemical waste from agriculture, industry or urban areas are among the major causes of this decline (e.g. de Bono et al., 2004; Samways & Sharrat, 2009; Strayer, 2006). Abstraction and physical changes to channels and riparian habitats further contribute to loss of biodiversity. As a consequence, populations of many aquatic organisms are endangered, often far more than their

*Corresponding author. Email: jeggino@gmail.com

ISSN 1388-7890 print/ISSN 2159-6719 online

http://dx.doi.org/10.1080/13887890.2012.660403
http://www.tandfonline.com

terrestrial counterparts (Strayer & Dudgeon, 2010). Dragonflies (Insecta, Odonata), which depend on the availability of suitable water bodies to complete their life cycle, are no exception to this trend. This leads to rising concerns about the status of their populations throughout Europe, America, Asia and Africa (Bried & Mazzacano, 2010; Clausnitzer et al., 2009; Kalkman et al., 2010).

Odonata are commonly used as bioindicators of the quality of aquatic ecosystems, as they possess many of the defined features of bioindicators such as well-known and stable taxonomy, ease of identification, known biology, widespread distribution and sensitivity to chemical, physical and biological changes in water systems (Bried et al., 2007; Burger, 2006; Everard, 2008; Hodginson & Jackson, 2005). Consequently, dragonflies have been used as indicators of environmental health (Chang et al., 2007; Hardersen, 2000; Nummelin et al., 2007; Tollett et al., 2009) or to evaluate the impact of land management and human activities (Lee Foote & Rice Hornung, 2005; Muller et al., 2003; Twisk, 2000).

Sound conservation practices require well-tested and consistent survey methods for the monitoring of local populations. Odonata can be sampled in several different ways (e.g. Siedle, 1992; Raebel et al., 2010), basically involving the observation of flying adults, sampling of aquatic larvae or searching for exuviae. Most published studies on Odonata ecology have been carried out using only one or the other of these methods, under the implicit assumption that they were broadly equivalent. When more than one monitoring technique was applied, data from the different methods were often pooled (e.g. Lenz, 1991). However, it is also known that the various life forms have considerably different ecologies and different behaviours. Flying adults are more easily detected than immobile exuviae (e.g. Hardersen, 2008) and, due to their mobility and terrestrial life, are less likely to respond to characteristics of the aquatic habitat but should rely more on habitat features, such as tree cover on the banks or vegetation at the water margin (e.g. Butler & de Maynadier, 2008; Lee Foote & Rice Hornung, 2005; Rith-Najarian, 1998). Reproductive behaviour of adults has been observed in sites which were unsuitable for successful larval development (e.g. Hardersen, 2008; Horvàth et al., 2007; Raebel et al., 2010; Wildermuth & Horváth, 2005). In contrast, larval stages are more sensitive to local factors and their presence depends on "in water" variables such as water quality, drying out or aquatic predators (e.g. Corbet, 1999; Hardersen, 2008). Therefore, surveys based on different methods may give considerably different results, also leading to erroneous conclusions about population status, community composition and environmental quality (e.g. D'Amico et al., 2004; Hardersen, 2008; Hofman & Mason, 2005; Raebel et al., 2010). Despite these known facts, there is little literature on methodological aspects of sampling (e.g. Oertli, 2008). The only notable exception is the paper by Raebel et al. (2010), which compared the three most commonly used methods (surveys of adults, larvae and exuviae) for the monitoring of Odonata in farmland ponds in the UK.

In order to plan conservation strategies based on Odonata monitoring, it is important to understand the ecological requirements of each species. Several studies have examined the relationship between habitat features and species presence, but most of them suffer from the problem outlined above, since they relied on a single survey method (e.g. Butler & de Maynadier, 2008; Larson & House, 1990; Reece & McIntyre, 2009; Steytler & Samways, 1995).

This paper compares four different sampling methods (observation of adults, survey of breeding adults, sampling of larvae and collection of exuviae) to survey the odonate community of nine retrodunal ponds in central Italy. In particular, we wished to evaluate if the different survey methods, carried out concurrently in the same study sites, would result in similar communities being observed. The data were analysed by means of a set of uni- and multivariate techniques to answer the following questions: (i) Do different methods provide similar assemblages both in terms of species richness and species composition? (ii) Is there a method best suited to monitor local Odonata communities in standing waters?

2. Materials and methods

2.1. *Study area*

This study was carried out in the Migliarino, San Rossore, Massaciuccoli Regional Park in northern Tuscany (Italy). The area is a Site of Community Importance (SCI) "Selva Pisana" (IT 5160002) and is characterized by a rich variety of retrodunal wetlands interspersed in a matrix of Mediterranean-type vegetation composed of broad-leaved evergreen shrubs and small trees. Nine ponds were surveyed within three nature reserves: Lecciona (with seven ponds: Lec1 to Lec7), Vecchiano and Cornacchiaia reserves, with one pond each (Figure 1). The nine sites were chosen in order to represent maximum environmental heterogeneity and to cover the whole spectrum of wetland types present in the area.

2.2. *Adults, larvae and exuviae surveys*

All sites were sampled seven times between the beginning of May and the end of September 2008 on sunny calm days by L.G. All sampling methods (adults, larvae and exuviae) were carried out at each site on a single day and each method was applied for one hour; an attempt was made to standardize the sampling-effort for the different methods as described below. In each sampling round, the order of the single sites was randomly determined.

Adults were surveyed between 12:30 and 15:30 by slowly walking along the edge of the water body and with the aid of binoculars all observed species were noted (called "all adults").The number of individuals was estimated and individual behaviour was observed. Most individuals were identified without being captured. When necessary (e.g. for species of the genera *Sympetrum* and *Coenagrion*), a sweep-net was used to catch dragonflies for identification. Adults were identified following Dijkstra and Lewington (2006). The classification as breeding adults followed the criteria recommended by Lee Foote and Rice Hornung (2005): (i) presence of mature males at a site for two or more consecutive cycles; (ii) direct observation of tandem pairs and ovipositing females; (iii) presence of teneral individuals at the site. For breeding adults, only presence/absence data were used, as abundances could not be attributed.

Larvae were surveyed between 9:00 and 12:00. They were collected with a D-frame sweep net, by taking multiple samples from the different vegetation types present. The time allocated for sampling the different vegetation types was in proportion to their total area. The material collected was sorted in a white plastic tray and Odonata larvae were immediately preserved in 70% ethanol. Larvae of Zygoptera were stored in 1.5 ml Eppendorf Flex Tubes® to avoid the loss of caudal lamellae, which are essential for determination of species. Larvae were identified under a binocular microscope following the keys and recommendations described by Carchini (1983). All larvae which did not meet the criteria defined by Carchini (1983) were excluded.

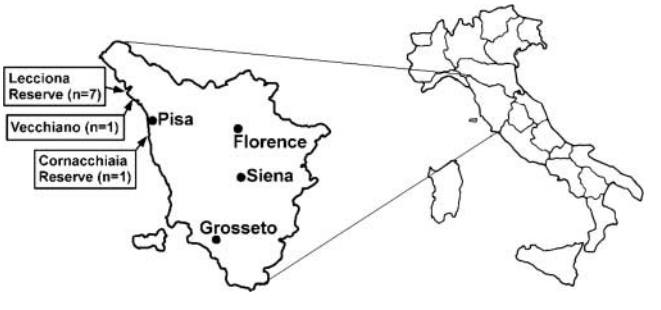

Figure 1. Map showing the three nature reserves in which the nine ponds investigated are located. The number of ponds in each reserve is given in brackets.

Exuviae were searched for visually on the vegetation, between 16:00 and 18:00. Care was taken always to search with a constant effort from the banks to the inner limits of the aquatic vegetation. The exuviae collected were stored in glass jars for subsequent laboratory identification using the keys provided by Gerken and Sternberg (1999).

Since the larvae and exuviae of *Sympetrum striolatum* (Charpentier, 1840) and *Sympetrum meridionale* (Selys, 1841) cannot be reliably discriminated (Carchini, 1983; Gerken & Sternberg, 1999), all samples were pooled and referred to as *Sympetrum str/mer*.

A complete list of the species observed at each site, separated by adults, larvae and exuviae, can be found in Giugliano and Terzani (2011).

2.3. *Data analysis*

Univariate and multivariate measures were computed for all four sampling methods (all adults, breeding adults, larvae, exuviae). Alpha diversity was computed as the mean number of species per pond (Magurran, 2000) while gamma diversity was computed as the total number of species recorded by each sampling method, with data from all sites pooled. Beta diversity across sampling sites, generally defined as variation in the identities of species among sites, was calculated using the index proposed by Harrison et al. (1992), which measures the amount by which regional (gamma) diversity exceeds the mean diversity of its constituent samples (see also Anderson et al., 2011). The formula:

$$\beta = \{[(S/\alpha m - 1]/(N - 1)\} \times 100 \quad (1)$$

was used, where S is the total number of species observed (i.e. gamma diversity), αm is the mean alpha diversity and N is the total number of sites examined. This measure ranges from 0 (complete similarity) to 100 (complete dissimilarity).

For alpha and beta diversity, 95% confidence intervals were computed from bootstrap samples, following Manly (1997). Since the detectability of the different life stages is different (flying adults are more easily detected than larvae or exuviae), an individual-based rarefaction analysis was performed by plotting the cumulative number of species observed (Mao Tau, see Colwell, 2009) against the total number of individuals observed (Gotelli & Colwell, 2001). "Expected" gamma diversity was also estimated using the Chao2 index, as described in Magurran (2000). Comparison of "observed" and "estimated" gamma diversity may provide information on the proportion of total species detected by each sampling method. Rarefaction curves and Chao2 index were computed with the EstimateS package ver. 8.2 (Colwell, 2009).

Multivariate similarity among assemblages was analysed by non-metric multidimensional scaling (nMDS), following Clarke and Warwick (2001). Both presence/absence data and abundance data were analyzed when possible (for breeding adults, only presence/absence data were available). Jaccard and Bray-Curtis distances were used for presence/absence and abundance data, respectively. The congruence among assemblages (adults, breeding adults, larvae and exuviae) was estimated using the PROTEST method (Jackson, 1995). In short, this method compares two similarity matrices using a procrustes rotation (Mardia et al., 1979) and significance of the fit is assessed through a permutation test using 50,000 random permutations. Multivariate analysis was carried out using R (ver 2.10.1) statistical software (R Development Core Team, 2009).

3. Results

In total, 2881 adults, 986 larvae and 612 exuviae were counted. All the adults were successfully identified to species, 93% of the exuviae could be attributed to a species, while only 58% of the larvae could be determined to the species level.

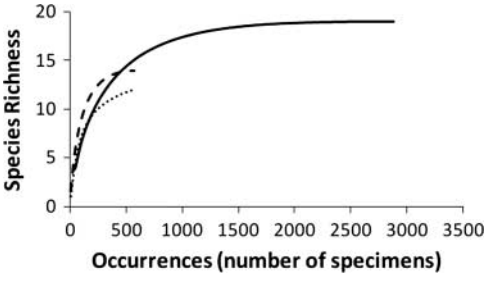

Figure 2. Individual-based accumulation curves for all adults (continuous line), nymphs (dashed line) and exuviae (dotted line). Occurrences indicates the total number of specimens sampled.

Species richness varied among the sampling methods. Nineteen species (gamma diversity) were observed by surveying all adults, and the same number was recorded when breeding adults were considered. In contrast, only 14 and 12 species were found via surveys of larvae and exuviae, respectively. The accumulation curve for all adults (Figure 2) clearly reached an asymptote, suggesting that the assemblage was adequately sampled and that very few (or no) species remained undetected. Similarly, the species richness of larvae reached an asymptote and thus very few additional species could have been found had the sampling effort been increased. In contrast, the accumulation curve for the surveys of exuviae did not reach an asymptote, suggesting that more species could have been detected if more exuviae had been sampled. The expected numbers of species computed using the Chao2 index generally agreed with this finding. Expected values were equal to observed ones for all the survey methods and had narrow confidence intervals. The only exception was the surveys of exuviae, for which the upper confidence limit was considerably higher than the expected number of species computed using the Chao2 index (Figure 3a).

The mean number of species per site (alpha diversity) was highest for the all adults survey (9.33) and lowest for the collection of exuviae (3.33), with surveys of breeding adults and larval sampling showing intermediate values (Figure 3b). The confidence limits of richness for the all adults data did not overlap with those of breeding adults, larvae and exuviae, indicating a statistically significant difference between the alpha value for all adults and that of the other sampling techniques.

A reverse pattern was observed for beta diversity (Figure 3c). The all adults data had the lowest value (14.32) and exuvial data the highest value (39.9), while the survey of breeding adults and larval sampling had intermediate values. Again, the overlap of confidence limits suggests that there were no significant differences among the values for the last three methods.

The nMDS ordination plots for presence/absence data and abundance data are shown in Figure 4a–d and Figure 4e–g, respectively. In all cases, the stress value of nMDS ordinations was low (≤ 0.1), indicating that the two-dimensional representation adequately depicted the compositional differences among assemblages (Clarke, 1993). Differences in species assemblages obtained with the different sampling techniques are evident when the graphs are compared. The plots obtained from surveying adults (all adults and breeding adults) were similar, with the points for the Vecchiano and Cornacchiaia sites fairly well separated from the Lec 1–7 sites. This arrangement was consistent in both plots, considering presence/absence data (Figure 4a, b) and abundance data (Figure 4e), respectively. Plots for the larval and exuvial survey data were different from the adult plots, the main difference being the lack of separation of the Vecchiano/Cornacchiaia sites from the Lec 1–7 sites. However, there was a general resemblance of the plots for the larval and exuvial data. Vecchiano and Cornacchiaia were in the middle of the plots, with Lec 2 and 4 on the left side and Lec 3, 6 and 7 on the right side. Furthermore, the arrangement of points in the plots was broadly consistent between the presence/absence data and abundance data.

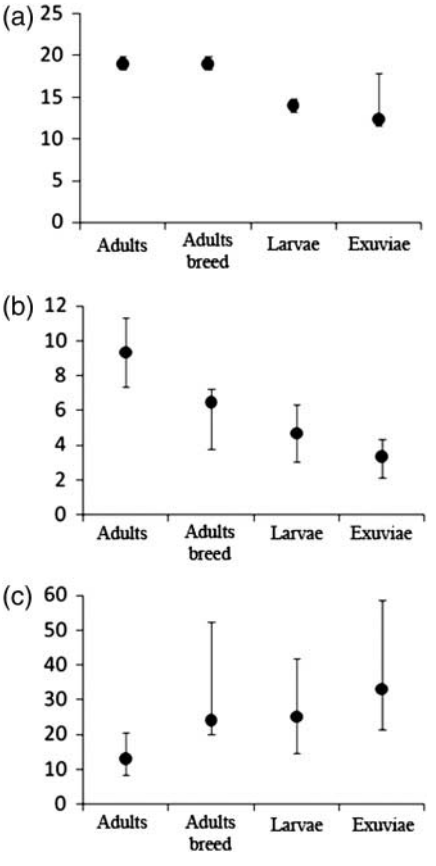

Figure 3. Species richness values obtained from the four sampling methods. (a) Estimated total number of species (Chao 2); (b) mean number of species per site (alpha diversity); (c) beta diversity. Mean values (dots) and 95% confidence intervals are shown.

The PROTEST analysis (Table 1) confirmed the low agreement among assemblages obtained from the different sampling methods. The presence/absence data showed significant associations between the surveys of all adults and breeding adults on the one hand and between survey data of larvae and exuviae on the other. No significant associations were detected using the abundance data.

4. Discussion

The results of this study show for Odonata that sampling methods which utilize different life stages are not interchangeable, as also found by Hardersen (2008) and Raebel et al. (2010) and that the four different methods employed led to considerable differences in the assessed odonate communities. To be more specific, both univariate and multivariate measures outlined differences between surveys of adults and sampling of larvae/exuviae. Gamma diversity was high for adults (both all adults and breeding adults) and low for larval and exuvial surveys. Similarly, alpha diversity showed extreme values for the data for all adults (maximum) and exuvial sampling (minimum), with breeding adults and larval collection showing intermediate values. Beta diversity showed an inverse trend, with the lowest value for "all adults" surveys and higher values for all

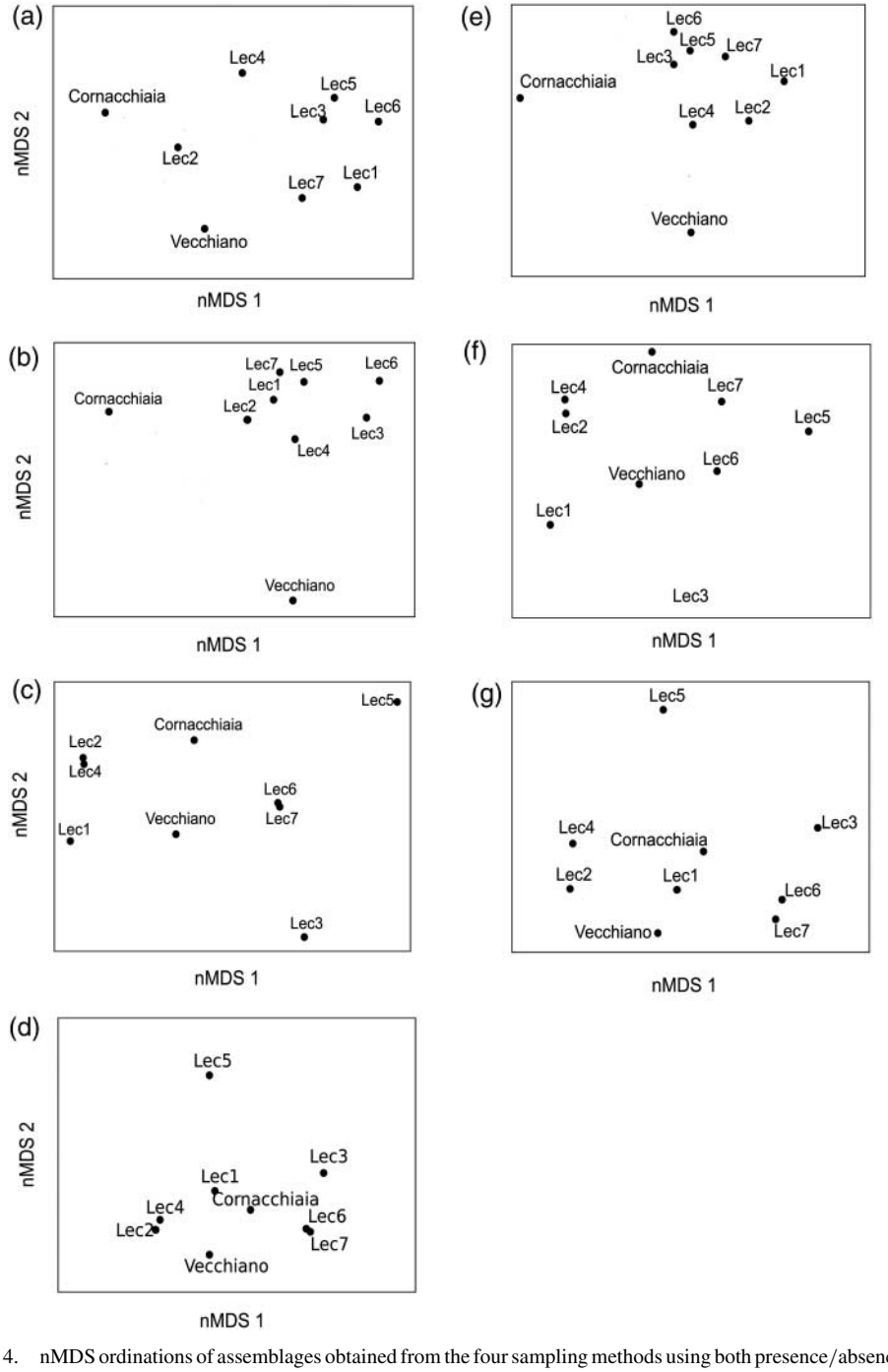

Figure 4. nMDS ordinations of assemblages obtained from the four sampling methods using both presence/absence (left column) and abundance data (right column). The seven plots represent the following data: (a) all adults, presence/absence; (b) breeding adults, presence/absence; (c) larvae, presence/absence; (d) exuviae, presence/absence; (e) all adults, abundance; (f) larvae, abundance; (g) exuviae, abundance. Each point represents a site.

Table 1. Congruence of the assemblages revealed by the different sampling methods, using the PROTEST procedure. Values shown are correlation coefficients between assemblages.

		Breeding adults	Larvae	Exuviae
Presence/absence	All adults	0.66*	0.44	0.39
	Breeding adults	—	0.47	0.5
	Larvae		—	0.74**
Abundance	All adults	NA	0.47	0.42
	Larvae	NA	—	0.59

Note: Dashes indicate the (meaningless) comparison of identical data sets; NA indicates that comparison is impossible because abundance data were not obtained for adults.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.

the other techniques. This means that assemblages of adults were more homogeneous across sites than the other datasets (Hardersen, 2008). Comparable results were obtained from the multivariate analysis. This paper is the first to clearly demonstrate this "cascade" of alpha and beta diversity when using sampling techniques which rely on life phases that differ in the degree to which they indicate successful *local* completion of development.

Several factors may interact to determine the observed patterns. Firstly, simple sampling-related effects may be involved. Three times more adults were surveyed than larvae, and even fewer exuviae were collected. Hardersen (2008) also observed many more adults than exuviae in a comparable study. Although these numbers may indeed reflect different abundances of the respective life forms, they are more likely a consequence of different "detectability" or "detection probabilities". Flying specimens are more easily detected than aquatic larvae or immobile exuviae. Sampling of exuviae is further hampered by their typically being lost in a short time period, as wind, rain, passing animals, etc., will cause them to fall to the ground, where they are often almost impossible to find and quickly degrade. In addition, some microhabitats where exuviae are generally found might be relatively inaccessible and these factors might further increase the differences. Moreover, whereas all adults were successfully identified to species, only 93% of exuviae and 58% of all larvae could be assigned to species. Thus, some species were likely "hidden" in the undetermined fractions, especially of the larval samples. The difficulties in determining larvae have been highlighted by various authors (e.g. Carle, 1979; Simaika & Samways, 2009; Smith et al., 2007), whereas we are aware of only one publication which contains the success rate of determining exuviae (Pollard & Berrill, 1992), which was above 99%, though they considered only Anisoptera species. Thus, utilizing exuviae seems to suffer less from problems of classification than when larvae are considered.

The accumulation curves obtained in this study suggest that adults, and probably larvae, were sampled adequately and only very few additional species could have been found if the sampling effort had been higher. In contrast, the curve for exuviae suggests that further species would have been found if the sampling effort had been increased. Any difference among sites, both in univariate and multivariate measures, may be in fact influenced by imperfect sampling of species at a local level, even for those components (e.g. adults) for which an adequate sampling at a broader level (all data pooled) was achieved.

Beyond these sampling effects, differences in species richness due to the methods employed can be expected because there are differences in ecology and behaviour among the different life stages investigated. Adults are known to be good fliers which disperse over large distances to colonize new water bodies or simply to forage, whereas larvae do not (e.g. Corbet, 1999; Hardersen, 2008). Because of the presence of dispersing or feeding specimens, adult assemblages can be expected to be more species-rich (higher alpha and gamma diversity) and more homogeneous

across sites (lower beta diversity) than assemblages obtained from larval and exuvial sampling (Hardersen, 2008; Raebel et al., 2010), a correlation found in the present study. It is known that adult dragonflies rely on proximate factors when choosing sites for oviposition, such as water flow or vegetation structure (Buchwald, 1992; Wildermuth & Horváth, 2005). Hence, it can be expected that assemblages of breeding adults will be less homogeneous across sites and more similar to those of larvae and exuviae than to those of all adults. However, local aquatic habitat conditions might not be suitable for larval development due to factors which do not affect adults to the same degree (presence of novel predators, drying out, etc.). Such factors could uncouple any close correlation between assemblages of breeding adults and larval communities (Hardersen, 2008; Raebel et al., 2010) and, in some cases, some wetlands may even constitute "ecological traps" (Hardersen, 2008; Raebel et al., 2010; Schlaepfer et al., 2002). For example, *Anax imperator*, *Aeshna isosceles*, *Coenagrion puella* and *Coenagrion scitulum* were all observed in tandem and while ovipositing. However, no larvae or exuviae of these species were found. One probable cause for this observation is the fact that the ponds were not suitable for larval survival (e.g. due to water salinity, presence of predators, etc.).

Thus it seems that the presence of adults, which may be partly unrelated to local water quality or other environmental features, may not be representative of larval and exuvial assemblages. However, Hawking and New (1999) found that larval and adult data corresponded closely along a river in Australia. These different results might be related to landscape characteristics. For a group of small ponds or streams with different habitat characteristics and which are home to diverse odonate communities (as in the present study), adult surveys might be inadequate to characterize the local community (e.g. a single pond) because dragonflies are good fliers and easily reach habitats, even if those habitats are not suitable for reproduction (e.g. Hardersen, 2008; Raebel et al., 2010). However, when a large river is surveyed and when sampling points are 10–15 km apart (e.g. Hawking & New, 1999), dispersing adults might influence the results to a much lesser degree and the larval survey (including exuviae) and adult survey should show similar results.

To conclude, is it possible to identify the "best" sampling method or at least make some suggestions for the planning of Odonate surveys? From a purely "efficiency" point of view, adult sampling is generally to be preferred over other sampling methods, being less time consuming and allowing a greater number of specimens to be counted per unit time (sampling & identification). However, adult assemblages are not always representative of larval assemblages (Hardersen, 2008; Raebel et al., 2010) and thus may not be closely related to local habitat quality. From this point of view, adult surveys could be misleading as they may overestimate the richness of the community that can successfully use these habitats for development.

In summary, it appears that methods relying on different life stages are not interchangeable and might describe different assemblages, as also found by Hardersen (2008) and Raebel et al. (2010). Therefore, an absolute "best" method for sampling Odonata does not exist, but sampling methods have to be carefully planned according to the aims and scope of the study and should not solely be chosen for their "efficiency". In general, adult sampling may give a better representation of diversity on a broader scale, where species which have emerged from a variety of habitats may merge due to dispersing individuals. Therefore, sampling of adults can be recommended for routine large-scale surveys when limited resources are available and when the aim of the study is to compile a complete species list. For the same reasons, adults may be less informative when specific factors acting at a very local level (e.g. presence of predators or water quality) are of interest. Sampling of larvae and exuviae may give better insights into site-specific effects and might help to identify conditions which limit survival of larvae (Pollard & Berrill, 1992; Hardersen, 2008). Ideally, the monitoring of Odonata should be based on different methods which include surveys of adults and the collection of either larvae or exuviae as independent data sources.

Acknowledgements

Many thanks are due to Luca Bartolozzi (Natural History Museum, University of Florence) for his assistance. We also express our gratitude to Andrea Fontanelli (LIPU preserve, Massaciuccoli) and to Antonio Perfetti ("Migliarino, San Rossore, Massaciuccoli" Regional Park) for logistic support and permission to work within the preserves. Thanks are also due to the Library of the Dutch Entomolological Society. Peter Christie provided linguistic revision of the final draft.

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