

Research articleSubmitted: November 20th, 2018 - Accepted: May 2nd, 2019 - Published: May 31st, 2019**Proposal for a time-based standard sampling method for the monitoring of *Gomphus flavipes* (Charpentier, 1825) and *Ophiogomphus cecilia* (Fourcroy, 1785) (Odonata: Gomphidae)**Sönke HARDERSEN¹, Ilaria TONI^{1,*}¹ Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale “Bosco Fontana” Carabinieri - Strada Mantova 29, I-46045 Marmirolo (MN), Italy - s.hardersen@gmail.com; ilariatoni2010@gmail.com

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Abstract

Monitoring of conservation status is an obligation arising from Article 11 of the Habitats Directive for all species of community interest. However, the development of monitoring methods for invertebrate species has received relatively little attention. *Gomphus flavipes* (Charpentier, 1825) and *Ophiogomphus cecilia* (Fourcroy, 1785) are two dragonfly species, listed in the annexes of the Habitats Directive, which suffered severe declines in the last century and have since recovered. Methods for the monitoring of these two gomphids have been proposed, but these have not been extensively tested and no abundance classes have been proposed for the evaluation of the conservation status of these species. A time-based standard sampling method is proposed for both species and results from numerous sites in Lombardy, northern Italy, are presented. Applying the standard method revealed that it is common for rivers that high water levels preclude sampling of exuviae through the summer and it is better to allow for two seasons when planning the monitoring. A further result is the fact that it was not always possible to sample the same stretches as the dynamic nature of the rivers and fluctuations in water level lead to some river banks becoming unsuitable for sampling during some visits. In these cases the time-based approach was advantageous, as the method did not need to be modified in response to the original bank section becoming unsuitable.

Key words: Dragonfly, monitoring protocol, exuviae, Habitats Directive, Gomphidae, Italy.**Introduction**

Halting the loss of biodiversity is probably the most important challenge in nature conservation. The recognition that the threat to species and ecosystems has never been so great as today has led to the convention on biological diversity (Rio de Janeiro, 5 June 1992). As a response many national authorities developed biodiversity action plans and improved the monitoring of biodiversity. However, invertebrates are mostly under-represented in international conservation measures and have largely been neglected in the literature on conservation (Clark & May 2002; Zamin et al. 2010; Cardoso 2012), even if they dominate among organisms in terms of richness, abundance and often biomass (Cardoso et al. 2011). This bias is also reflected in the Habitats Directive (European Union 1992), as its lists of the protected species contain few arthropod species (Haslett 2007; Cardoso 2012).

Monitoring of conservation status is an obligation arising from Article 11 of the Habitats Directive for all species of community interest and this provision is not restricted to Natura 2000 sites. Even though monitoring is compulsory,

the development of methods for invertebrate species has received little attention and currently well tested methods are available only for few species (e.g. Svensson & Larsson 2008; Campanaro et al. 2016; Hardersen et al. 2017). Monitoring is critically essential to determine the extent to which protected areas are effective in conserving biodiversity (Rao & Ginsberg 2010). As the overall objective of the Habitats Directive is to achieve and maintain favorable conservation status for all habitats and species of community interest, monitoring must lead to a clear picture of the actual conservation status and its trends on various levels and the information on biodiversity, collected locally, is needed to report trends at local, national or international scales and to support policies at all these scales (Mace et al. 2005).

Gomphus flavipes (Charpentier, 1825) and *Ophiogomphus cecilia* (Fourcroy, 1785), both listed in the annexes of the Habitats Directive, suffered a severe decline in the last century and have both recovered since the 1990s (Boudot & Kalkman 2015) and this also applies to Italy, where these species are currently categorized as “least concern” in terms of extinction risk (Riservato et al. 2014). The

knowledge on recovery and expansion of these two species is mainly based on distributional data, but has not been documented by monitoring single sites and it is often not clear if newly discovered populations have been founded recently or had been previously overlooked (Schiel & Hunger 2006; Ketelaar 2010; Westermann 2011; Boudot & Kalkman 2015). A number of methods for the monitoring of these two gomphids have been proposed (Schnitter et al. 2006; Trizzino et al. 2013; Janák et al. 2015) and all of these are based on the collection of exuviae, as it is well known that for gomphids exuviae are more observable than adults (Hardersen 2008; Hunt et al. 2010). Additionally, only exuviae in situ constitute completely dependable evidence that larval development has been completed successfully (Corbet 1993). However, none of the above methods proposed for the monitoring of *G. flavipes* and *O. cecilia* has been extensively tested. Such testing seems particularly important for river systems, where it is common that high water levels make sampling of exuviae during some parts of the summer impossible (Hunt et al. 2010; Farkas et al. 2012; Janák et al. 2015). Additionally, our experience has shown that the dynamic nature and large fluctuations in water level of the Italian river systems often leads to river sections, which were initially suitable for sampling of exuviae, can become unsuitable only some weeks later. A further problem is that there are currently no accepted criteria to class populations in the three levels of conservation status recognized by the Habitats Directive.

The aims of our study was to contribute to the development of a standard method for the monitoring of the two dragonflies *G. flavipes* and *O. cecilia*; in particular the aims of this study were: 1. to test the practicability of a time-based standard method in numerous sites. 2. to propose thresholds for three classes of abundance based on the data gathered.

Material and Methods

Study sites

The monitoring of the two species (*G. flavipes* and *O. cecilia*) by means of collecting exuviae was carried out at 13 sites located in the Lombardy region. For all locations we report identification number (id), site name, province, geographic coordinates and dates of monitoring (Tables 1 & 2).

Seven sites were located on the river Po, three sites on the river Oglio, and one site each on the river Terdoppio, the river Adda, and on the artificial channel “Naviglio Langosco” (Fig. 1).

The monitoring method for *G. flavipes* was applied in 11 sites (located on rivers: Po, Oglio, Adda, Terdoppio, Naviglio Langosco) and the monitoring method for *Ophiogomphus cecilia* was applied in 6 sites (located on rivers: Oglio, Adda, Terdoppio, Naviglio Langosco). The sites

were chosen on the basis of prior knowledge on the presence of the two species.

Sampling

In this study we applied a time-based sampling method, as the dynamic nature of Italian river systems can lead to river sections, initially suitable for sampling of exuviae, becoming unsuitable only some weeks later. Therefore the methods standardize the time to be employed in river sections suitable for sampling, rather than the length of the section (cf. Schnitter et al. 2006; Trizzino et al. 2013; Janák et al. 2015). A section suitable for sampling was defined as a river bank with an angle between 10° and 45° and with the bank substrate exposed (not covered by leaves, etc.). Shallow banks and sandbanks (< 10°) were excluded because exuviae tend not to accumulate here as any rise or fall in water level leads to large changes in the position of the shoreline (pers. obs.). Banks steeper than 45° were too steep to sample exuviae safely. This definition of suitable sites is not applicable to the artificial channel “Naviglio Langosco”, which has vertical concrete walls, and is always suitable for sampling on its entire length. Particular attention was paid to the hydrological situation of the rivers to be sampled and we selected days during which the water level was expected to be stable or falling and we avoided days directly after abundant rainfall.

Exuviae were collected in the years 2011 to 2015 (Tables 1 & 2) by searching and collecting all exuviae found in the first meters of riverbanks (about 2–4 meters from the shore), while walking slowly for 30 minutes in the same direction. If non-suitable areas (e.g. sandbanks, steep and slippery banks, overhanging or fallen trees, banks covered by leaves) (Fig. 2) were encountered, the timer was stopped and once a new stretch of riverbank suitable for sampling had been reached, the timer was re-started. Every site was visited at least 5 times, with intervals as evenly distributed as possible, between 23.V. to 03.VIII. (*G. flavipes*) and 12.VI. to 24.VIII. (*O. cecilia*).

All exuviae were collected in plastic jars, later air dried and determined using Carchini (1983) and Gerken & Sternberg (1999). All exuviae are deposited in the collection of the Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale Carabinieri “Bosco Fontana”, Marmirolo, Mantova – Italy.

Results

During the study we collected a total of 1176 exuviae of *G. flavipes* (11 sampling sites) and 79 exuviae of *O. cecilia* (6 sampling sites) (Tables 3 & 4) with average numbers per site being 106 exuviae for *G. flavipes* and 13 for *O. cecilia*. The rivers Adda, Oglio (Gazzuolo), Po, and Terdoppio flooded during the study and therefore it was impossible to respect the initial sampling scheme in one year and the samplings for these sites were completed during the fol-

Table 1 – Sites in the Lombardy region (Italy) where *Gomphus flavipes* was monitored by means of collecting exuviae (years 2011 to 2015).

ID	Site name	Province	UTM	Sessions
River: Adda				
1	Pizzighettone	Cremona	32T 560703 5005880	30.V.2012/19.VI.2012/29.VI.2012/20.VII.2012/03.VIII.2012
River: Oglio				
2	Gazzuolo	Mantova	32T 625782 4989516	24.V.2012/19.VI.2012/29.VI.2011/12.VII.2011/26.VII.2011
River: Terdoppio				
3	Pieve Albignola	Pavia	32T 497107 4996812	30.V.2012/19.VI.2012/29.VI.2012/20.VII.2012/03.VIII.2012
Artificial channel: Naviglio Langosco				
4	Tromello	Pavia	32T 488051 5005460	23.V.2013/07.VI.2013/21.VI.2013/05.VII.2013/22.VII.2013
River: Po				
5	Cava Manara	Pavia	32T 510714 4995736	04.VI.2014/05.VI.2015/24.VI.2014/14.VII.2015/29.VII.2015
6	Senna Lodigiana	Lodi	32T 543549 4997721	04.VI.2014/05.VI.2015/24.VI.2014/14.VII.2015/29.VII.2015
7	Stagno Lombardo	Cremona	32T 582256 4991506	04.VI.2014/05.VI.2015/24.VI.2014/14.VII.2015/29.VII.2015
8	Viadana	Mantova	32T 615616 4976989	04.VI.2014/08.VI.2015/23.VI.2014/13.VII.2015/28.VII.2015
9	Motteggiana	Mantova	32T 638616 4989328	05.VI.2014/08.VI.2015/23.VI.2014/13.VII.2015/28.VII.2015
10	Ostiglia	Mantova	32T 670427 4990419	05.VI.2014/08.VI.2015/23.VI.2014/13.VII.2015/28.VII.2015
11	Felonica	Mantova	32T 686314 4983398	05.VI.2014/08.VI.2015/23.VI.2014/13.VII.2015/28.VII.2015

Table 2 – Sites in the Lombardy region (Italy) where *Ophiogomphus cecilia* was monitored by means of collecting exuviae (years 2011 to 2013).

ID	Site name	Province	UTM	Sessions
River: Adda				
1	Pizzighettone	Cremona	32T 560703 5005880	19.VI.2012/29.VI.2011/12.VII.2011/20.VII.2011/12.VIII.2011
River: Oglio				
12	San Michele	Mantova	32T 623654 4996132	12.VI.2013/27.VI.2013/17.VII.2013/06.VIII.2013/24.VIII.2013
13	Canneto sull'Oglio	Mantova	32T 608993 4999915	12.VI.2013/27.VI.2013/17.VII.2013/06.VIII.2013/24.VIII.2013
2	Gazzuolo	Mantova	32T 625782 4989516	19.VI.2012/29.VI.2011/12.VII.2011/20.VII.2011/12.VIII.2011
River: Terdoppio				
3	Pieve Albignola	Pavia	32T 497107 4996812	19.VI.2012/29.VI.2011/12.VII.2011/20.VII.2011/12.VIII.2011
Artificial channel: Naviglio Langosco				
4	Tromello	Pavia	32T 488051 5005460	21.VI.2013/05.VII.2013/22.VII.2013/03.VIII.2013/17.VIII.2013

lowing year. The highest number of exuviae found in 30 minutes was 97 for *G. flavipes* (08.VI.2015, Po, Motteggiana) and 22 for *O. cecilia* (19.VI.2012, Terdoppio, Pieve Albignola).

The field work revealed that a method based on a pre-defined stretch of river bank would have led to practical problems, as in a number of sites the stretch initially sampled were not suitable later during the year. For example in the site Motteggiana (Po) on 28.VII.2015 approximately half of the river bank was occupied by a large sand bank, which had emerged due to low water level. On the same day the entire riverbank of the site Ostiglia (Po) was cov-

ered by a dense layer of fallen leaves (Figure 2), which were absent only some hundred meters downstream. In cases when the sampling could be effected without encountering unsuitable stretches we estimated that we sampled river bank sections between 130 and 190 m in length in 30 minutes.

The results of the fieldwork were used to calculate the abundances of the two species at the sites investigated. First the lowest count was removed, as proposed for other insect species (Trizzino et al. 2013; Hardersen et al. 2017). This should allow to eliminate eventual outlier and to reduce the variability of the final value. The remaining four

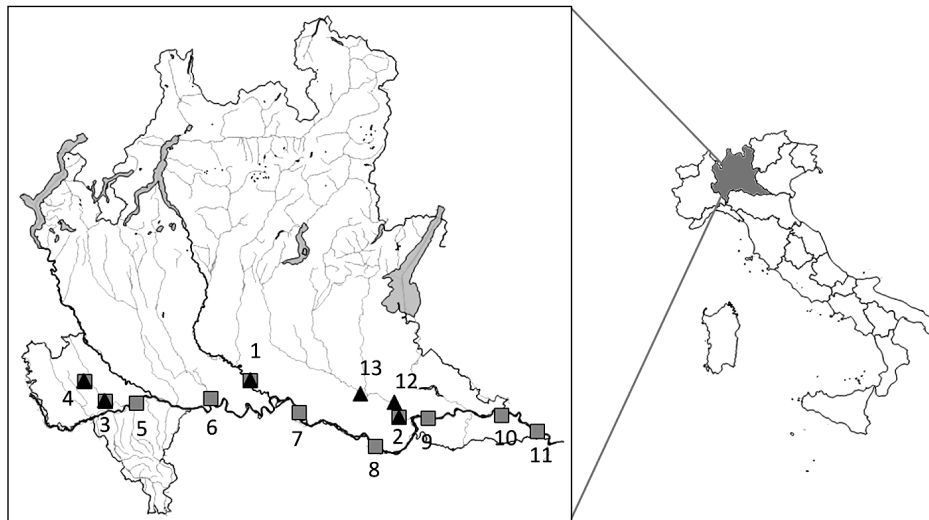


Fig. 1 – Sites in Lombardy region (Italy) where the monitoring of *Gomphus flavipes* (squares) and *Ophiogomphus cecilia* (triangles) was carried out between 2011 and 2015 (1 = Pizzighettone; 2 = Gazzuolo; 3 = Pieve Albignola; 4 = Tromello; 5 = Cava Manara; 6 = Senna Lodigiana; 7 = Stagno Lombardo; 8 = Viadana; 9 = Motteggiana; 10 = Ostiglia; 11 = Felonica).



Fig. 2 – On 28.VII.2015 the riverbank of the site Ostiglia (Po) was covered by a dense layer of fallen leaves and rendered this site unsuitable for sampling exuviae.

values were used to calculate the average number of exuviae collected in each session. Based on these values classes of abundance are proposed (Table 5).

Discussion

The time-based sampling of exuviae, repeated five times, allowed for the collection of a large number of exuviae of *G. flavipes* and *O. cecilia* in the selected sites. It is common for rivers that high water levels preclude sampling of exuviae during some parts of the summer (Hunt et al. 2010; Farkas et al. 2012; Janák et al. 2015). Also during this study some rivers flooded, which made it impossible to respect the initial sampling scheme. Thus, one important outcome of this study is that it was not possible to collect exuviae during five evenly spaced sampling sessions in 10-11 weeks in a single year in all sites. We therefore suggest to allow for two seasons when planning the standardized sampling of *G. flavipes* and *O. cecilia*. A further important result is the fact that it was not always possible to sample the same stretches of the single sites as the dynamic nature of the rivers and fluctuations in water level lead to some river banks becoming unsuitable for sampling during some visits. In these cases the time-based approach was advantageous, as the methods did not need to be modified in response to the original bank section becoming unsuitable. The above two points were revealed because we attempted to apply the standard methods over a number of years in numerous sites. Only this “testing” of the method allowed to evaluate its practicability, to identify shortcomings and to propose improvements. Similarly, Campanaro et al. (2017) found that not all monitoring methods tested for the cerambycid beetle *Rosalia alpina*

Table 3 – Number of *G. flavipes* exuviae collected during 5 monitoring sessions carried out in the Lombardy region (Italy) years 2011 to 2015.

	Session 1		Session 2		Session 3		Session 4		Session 4		
Site name	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	TOT
Cava Manara	04.VI.2014	13	05.VI.2015	36	24.VI.2014	14	14.VII.2015	52	29.VII.2015	7	122
Senna Lodigiana	04.VI.2014	13	05.VI.2015	8	24.VI.2014	30	14.VII.2015	49	29.VII.2015	14	114
Stagno Lombardo	04.VI.2014	8	05.VI.2015	15	24.VI.2014	18	14.VII.2015	72	29.VII.2015	29	142
Viadana	04.VI.2014	7	08.VI.2015	6	23.VI.2014	12	13.VII.2015	9	28.VII.2015	1	35
Motteggiana	05.VI.2014	20	08.VI.2015	97	23.VI.2014	34	13.VII.2015	90	28.VII.2015	17	258
Ostiglia	05.VI.2014	39	08.VI.2015	24	23.VI.2014	25	13.VII.2015	66	28.VII.2015	9	163
Felonica	05.VI.2014	9	08.VI.2015	41	23.VI.2014	29	13.VII.2015	28	28.VII.2015	11	118
Gazzuolo	24.V.2012	33	19.VI.2012	45	29.VI.2011	38	12.VII.2011	62	26.VII.2011	1	179
Pizzighettone	30.V.2012	0	19.VI.2012	1	29.VI.2012	13	20.VII.2012	14	03.VIII.2012	7	35
Pieve Albignola	30.V.2012	0	19.VI.2012	0	29.VI.2012	4	20.VII.2012	0	03.VIII.2012	4	8
Naviglio Langosco	23.V.2013	0	07.VI.2013	0	21.VI.2013	0	05.VII.2013	0	22.VII.2013	2	2
TOT		142		273		217		442		102	1176

Table 4 – Number of *O. cecilia* exuviae collected during 5 monitoring sessions carried out in the Lombardy region (Italy) years 2011 to 2013.

	Session 1		Session 2		Session 3		Session 4		Session 4		
Site name	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	Date	N. Exuviae	TOT
San Michele	12.VI.2013	0	27.VI.2013	1	17.VII.2013	0	06.VIII.2013	2	24.VIII.2013	0	3
Canneto Sull'Oglio	12.VI.2013	0	27.VI.2013	1	17.VII.2013	1	06.VIII.2013	2	24.VIII.2013	3	7
Naviglio Langosco	21.VI.2013	0	05.VII.2013	0	22.VII.2013	2	03.VIII.2013	2	17.VIII.2013	1	5
Pizzighettone	19.VI.2012	0	29.VI.2012	0	20.VII.2012	0	03.VIII.2012	0	16.VIII.2012	2	2
Pieve Albignola	19.VI.2012	1	29.VI.2012	22	20.VII.2012	13	03.VIII.2012	9	16.VIII.2012	4	49
Gazzuolo	19.VI.2012	1	29.VI.2011	6	12.VII.2011	3	20.VII.2011	2	12.VIII.2011	1	13
TOT		2		30		19		17		11	79

Table 5 – Proposed classes of abundance based on the average number of exuviae collected in 30 minutes during the four collections with the highest counts.

Population size	Abundant	Frequent	Rare
<i>Gomphus flavipes</i>	> 30	> 3-30	0,25-3
<i>Ophiogomphus cecilia</i>	> 6	> 1-6	0,25-1

were applicable in all sites. These two examples underline the importance of testing monitoring methods thoroughly before publishing them.

The periods during which the exuviae were sampled seem appropriate, as the lowest numbers of exuviae sampled were the sessions 1 and 5 for both species and peak

emergence is likely to occur in the center of the periods chosen. Farkas et al. (2012) and Horváth (2012) reported peak emergence for *G. flavipes* in June, whereas Müller (1995) reported a peak in June and July, with considerable variation between years. This is in keeping with our data, which showed the highest number of exuviae collected in

July. Müller (1995) and Farkas et al. (2012) reported peak emergence for *O. cecilia* in late May-early June, which is in contrast with our finding as we found high numbers only from late July onwards. Also Boudot & Kalkman (2015) indicated that the flight period for *O. cecilia* is later than that of *G. flavipes* and Torralba-Burrial et al. (2012) suggested for Spain that the best time period for sampling exuviae of *O. cecilia* is 23.05 to 30.07. When considering the best sampling period for *G. flavipes* and *O. cecilia* it is important to realize that the emergence period of these species can vary substantially between years (Müller 1995) and any standard method necessarily needs to allow for such variability. On the basis of our results we suggest for Northern Italy to carry out the monitoring for the two species during the following periods: *G. flavipes* from 30.05 to 30.07 and *O. cecilia* from 15.06 to 15.08. Our suggestion confirm the period advanced by Trizzino et al. (2013) for *O. cecilia*, and slightly changes the period for *G. flavipes*.

As the overall objective of the Habitats Directive is to achieve and maintain favorable conservation status for habitats and species of community interest, monitoring must lead to a clear picture of the actual conservation status. It is therefore necessary to repeat the monitoring at regular intervals and to evaluate population size of the species at numerous sites. The information on biodiversity, collected locally, is needed to report trends at local, national or international scales and to support policies at all these scales (Mace et al. 2005). To facilitate classification of population size and conservation status of local populations it is necessary to provide quantitative classes. The classes proposed here are based on the average number of exuviae sampled during the four collections with the highest numbers of exuviae (Table 5). The collection with the lowest number is not considered, in an attempt to reduce variability of the data, following Trizzino et al. (2013). We emphasize that the proposed abundance classes are a first proposal. It is clear that they are based on monitoring a limited number of sites during a few years and that they will probably modified in the future when more monitoring data become available. Data collected over a 6-year period at the river Oder (Germany) showed that abundances of exuviae of *G. flavipes* and *O. cecilia* varied up to 83% and 57% between consecutive years (Müller 1995). Similarly, Farkas et al. (2012) reported a 2.6 fold difference in the number of *G. flavipes* exuviae collected over a period of five years in Hungary. Thus it is important to allow for natural fluctuations in population sizes when evaluating the conservation status of a species. Moreover, it seems likely that abundance classes vary also in other geographical areas. Even given the above limitations, the abundance classes proposed here should be useful for future comparisons.

Even if the different methods employed do not allow for a direct comparison, it is clear that the highest average number of exuviae found in 30 minutes (*G. flavipes*: 60,25 (Po, Motteggiana) and *O. cecilia*: 12 (Terdoppio,

Pieve Albignola)) are indicative of abundant populations as often densities of exuviae of these two species are much lower (Schiel & Hunger 2006; Kolozsvári et al. 2015). Certainly, not all sites where these species reproduce can reasonably be expected to host abundant populations. One reason is that species also reproduce in sub-optimal habitats, where densities are bound to be lower. The other reason is that wider rivers offer more area for larval habitats than smaller, narrower rivers and therefore exuvial densities are expected to be higher along the shore (DuBois 2015). Thus, width of the river stretch sampled and habitat suitability need to be considered when evaluating the conservation status of local populations.

A further important point to consider is that densities of gomphid larvae vary locally in streams (Foidl et al. 1993, Müller 1995, Suhling & Müller 1996, DuBois & Smith 2016) and emergence occurs in the vicinity of larval habitats (DuBois & Smith 2016). Thus, also exuviae are unlikely to be distributed evenly along the river bank (cfr. Müller 1995). The time-based sampling of exuviae, proposed here, which often leaves some river-stretches unsampled, might result in bank-sections with exuviae to be excluded. However, we think that this is not an important shortcoming of the proposed method. One reason is that some of the excluded sites, such as very steep sections, are impossible to sample from the river bank and this also applies to the methods proposed by Schnitter et al. (2006) and Trizzino et al. (2013) even if the authors don't mention this fact. In contrast Janák et al. (2015) acknowledges the difficulties of sampling exuviae on rivers with steep, slippery banks. A further point to consider is, that normally the river sections excluded are small in comparison with the total stretch sampled (not more than the 10% of the total length of the transect) and in some cases no stretches were excluded. Additionally, larvae of gomphids, such as *G. flavipes* and *O. cecilia* live buried in the sediment, a behavior which reduces drift (Suhling and Müller 1996). This has been experimentally confirmed by DuBois & Smith (2016), who showed for *Ophiogomphus rupinsulensis* that 97% of exuviae moved less than 60 m from their larval habitat. It seems thus likely that a total length of 130 m to 190 m of river bank should provide a stretch long enough to represent the larvae present locally.

We advocate to apply the proposed monitoring to document long term trends in local population sizes, preferably investigating more sites in the selected river reaches. Combining the long term trends of many sites, it will be possible to evaluate changes in the conservation status of *G. flavipes* and *O. cecilia*, also at the regional and national level. Both species suffered a severe decline in the last century, recovered since the 1990s (Boudot & Kalkman 2015) and today are protected by the Habitats Directive. It is to be hoped that these species will not again suffer similar declines in the future. Monitoring populations, as imposed by the Habitats Directive, should enable us to detect early warning signs and to act quickly. The methods pro-

posed are intended to provide a methodological basis for successful long term monitoring.

Densities of exuviae of *O. cecilia* were generally lower than those of *G. flavipes* and the former species is less common in the Po Plains. It was thus a positive surprise that exuviae of *O. cecilia* were found (in low numbers) in six of the seven sites chosen for the monitoring of *G. flavipes* along the river Po. The highest number of exuviae of *O. cecilia* was found at Cava Manara (14) while the only site without exuviae of this species was Stagno Lombardo. It thus seems that *O. cecilia* is present in more rivers in northern Italy than currently acknowledged and it would be important to intensify surveys to map the current distribution of this protected species in Italy. This certainly, also applies to *G. flavipes*.

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