Hydrobiologia (2008) 607:11–16
DOI 10.1007/s10750-008-9359-3

PRIMARY RESEARCH PAPER

A comparison between local emergence patterns of *Perla grandis* and *Perla marginata* (Plecoptera, Perlidae)

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Received: 13 August 2007 / Revised: 22 January 2008 / Accepted: 20 February 2008 / Published online: 7 March 2008
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Abstract In this study we investigated the emergence patterns of two Systellognatha stoneflies: *Perla marginata* and *P. grandis* (Plecoptera: Perlidae) in a first order stream of the Northern Apennines (NW Italy). Exuviae of both species were collected in a selected area of the Curone creek by hand-picking, measuring the distance between river edge and substrate typology. Exuviae were found on different substrate types near the shoreline: stones in the stream bank were mainly used by both species, and gravel beach was the least-utilised substrate. No evident differences were found between the species in the typology and colour of emergence substrates. Interestingly, we found that *P. grandis* exuviae were significantly located at greater horizontal and vertical distances from the water edge than *P. marginata* ones. We discuss these findings on the basis of ecological and behavioural considerations.

Handling editor: R. Bailey

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Keywords Plecoptera · *Perla marginata* · *Perla grandis* · Exuviae · Emergence patterns

Introduction

Stonefly nymphal biology has attracted much interest for many years, but only in recent decades has adult behaviour, such as mating, drumming and feeding, received much attention (Rupprecht, 1968, 1990; Zeigler, 1990, 1991; Stewart, 1994, 1997, 2001; Tierno de Figueroa et al., 1998, 2000a, b; Tierno de Figueroa & Fochetti, 2001). In fact, despite the obvious importance in the life cycle, most ecological studies have focused primarily on the longer-lived and more easily studied nymphs, and therefore less is known about many aspects of the Plecoptera adult biology. In this context, emergence patterns represent an important and almost unknown aspect of the life cycle of most stonefly species. Emergence studies of aquatic insects have principally focused on flight period and its related environmental factors, such as temperature and photoperiod (Resh & Rosenberg, 1984; Watanabe et al., 1999), and this is particularly notable for Plecoptera (Flannagan & Cobb, 1991; Gregory et al., 2000).

Because adults of most stonefly species are short lived, and mating often occurs on the ground very soon after the emergence of the females (Hynes, 1976), it is likely that emergence patterns could be very important. In this context, an interesting aspect of Plecoptera emergence is the long permanence of exuviae on near-bank substrates: the common name of the order, stoneflies, undoubtedly derives from this fact. Especially in large-sized species, the distribution of exuviae can represent an important tool to investigate emergence patterns. Among the few studies on this topic, Hanada et al. (1997) and Alexander & Stewart (1996) underlined that emergence sites could play an important role in the reproductive success, representing possible encounter sites for mating. Recently, Jáimez-Cuéllar & Tierno de Figueroa (2005) supported the hypothesis that, in *Dinocras cephalotes* (Curtis, 1827), emergence site selection could represent an effective strategy to aggregate sexes in encounter sites.

Until now, studies on emergence place selection have been conducted on single species, and no comparative data are available, for example, about possible emergence site overlap among taxa. In this study, we investigate emergence patterns of two species of Perlidae: *Perla marginata* (Panzer, 1799) and *P. grandis* Rambur, 1842. The two species show an approximately similar distribution and are dispersed throughout central and southern Europe, the former also present in northern Africa and Iran (Tierno de Figueroa et al., 2003). The aim of this study is to describe emergence patterns of *P. marginata* and *P. grandis*, investigating the existence of possible differences between the two species.

Materials and methods

The study was conducted in the Curone stream (44°44′28″ N; 9°09′49″ E, 750 m a.s.l.) in NW Italy. The sampling station, the stream, had a typical mountain Apennine lotic environment, 4.0–4.5 m wide, with coarse substrate and riparian vegetation composed mainly of *Salix* spp., *Populus* spp. and *Robinia pseudoacacia*. Riparian herbaceous vegetation was composed mainly of *Conium maculatum*, *Epilobium hirsutum* and Asteraceae.

This lotic system shows a good environmental quality, reaching First Class in the Italian Extended Biotic Index (I.B.E., Ghetti, 1997), corresponding to an environment without trace of human-induced alteration.

On May 10th and 17th 2007, *P. marginata* and *P. grandis* exuviae were collected in a stream reach of 20 m, by hand-picking in the in-stream stones and in the left bank of the stream (4 m from the water edge) (Fig. 1). The sampling date coincides with the first part of the flight period of both species (Consiglio, 1980).

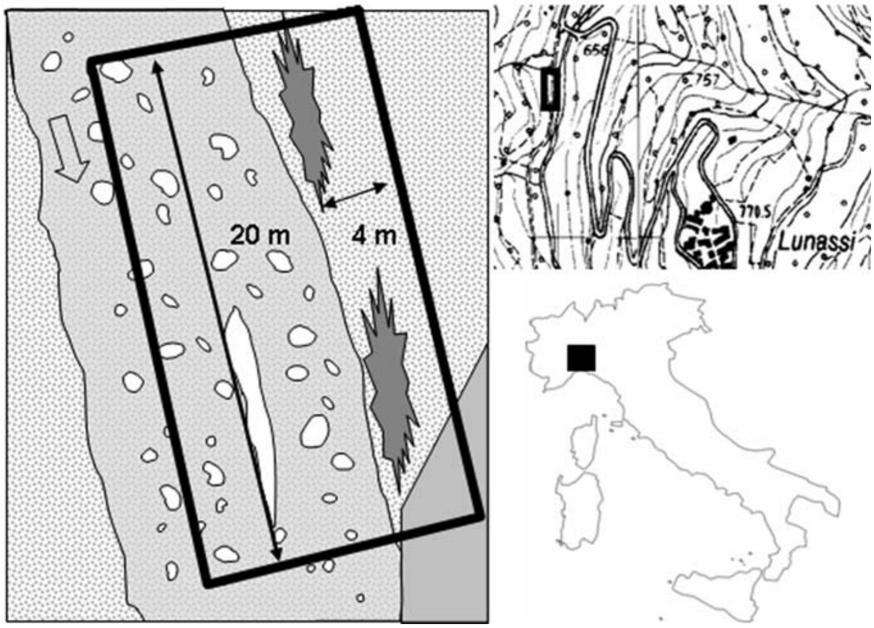
In the sampled reach, an accurate observation of all available substrates was performed, and all visible exuviae were collected, with the following data and measurements: substrate type (in-stream stones, riparian stones, riparian vegetation or gravel beach), substrate colour (only for stones; we classified colours into three classes: dark, light or neutral-same exuviae colour), substrate position (lotic zones or pools), substrate measurement (height and perimeter of water contact), number of exuviae around each collected exuvia, existence or not of flow under the emergence point, vertical distance from the water surface to exuvia and exuvia orientation (vertical or horizontal, head up or down). Horizontal and vertical distances from each exuvia to the creek edge were also measured. After recording these data, each exuvia was collected and put in a separate numbered vial. In the laboratory, right metathoracic wingpad (from the basal part of the suture line to the apex), right third femur (in its major length) and total length (from the labrum to the last tergum) were measured (0.1 mm accuracy) with a NIKON 1500 SZM binocular microscope micrometer.

Statistical analyses were performed with Statistica 7.1 (StatSoft, 2005). Nonparametric statistic was employed because Normality assumptions were not achieved. When analysing data we used a Mann–Whitney *U* test in order to detect differences between species, considering the relatively high number of cases and the similarities in their distribution, and a Gamma correlation test for assessing the relation between the three body measures (total length, femur length and wingpad length). This test was also used for analysing the existence of a correlation between body size and vertical and horizontal distances from the exuvia to the creek edge.

Results

In total, we collected 48 exuviae of *P. marginata* and 256 exuviae of *P. grandis*. We detected no significant

Fig. 1 Sampling station scheme



differences between the species for emergence substrate types (M–W $U = 5{,}927$; $P > 0.05$). For both species, the substrate where a higher number of exuviae were found was large riparian stones, followed by in-stream stones, riparian vegetation and finally gravel beach (Fig. 2).

Regarding the exuviae position, most specimens were found in a vertical position, with head up (87.9% of *P. grandis* and 87.5% of *P. marginata* exuviae). No differences were detected among substrate colour selection between species (M–W $U = 6{,}103$; $P > 0.05$). Nymphs of both species were mixed in assemblages in the same substrates, i.e. on the same in-stream stones. We noticed that 18.4% of total exuviae were found alone, 32.9% in groups with less than 5 exuviae, 26.0% in groups with 5–10 specimens, 7.6% in groups with 10–20 specimens and 15.1% in groups with more than 20 specimens. The largest assemblage of exuviae was composed of 27 exuviae, found in an area (about 2 m^2) of an enormous stone near the Curone bridge.

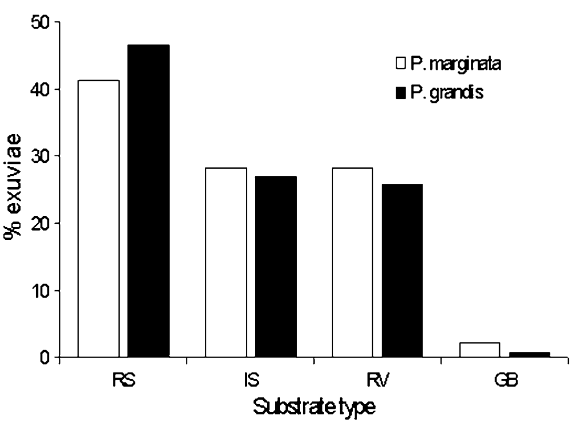


Fig. 2 Emergence substrates preference in *P. marginata* and *P. grandis* (RS = riparian stones, IS = in-stream stones; RV = riparian vegetation; GB = gravel beach)

Interestingly, we detected a significant difference between the species in both vertical (M–W $U = 4{,}137$; $P < 0.05$) and horizontal (M–W $U = 4{,}101$; $P < 0.05$) distances travelled by nymphs from the creek's edge (Figs. 3, 4). *P. marginata* exuviae were found at a mean vertical distance of 22.6 cm (max: 91.0 cm; min: 1.0 cm), while *P. grandis* were found at a mean vertical distance of 32.8 cm (max: 170.0 cm; min: 0.0 cm). Regarding the horizontal distance, *P. marginata* travelled a mean distance of 7.9 cm from the water edge (max: 13.7 cm; min: 0.0 cm), while *P. grandis* reached a mean distance of 23.5 cm (max: 298.0 cm; min: 0.0 cm). We also detected a significant dimensional difference between the species: *P. grandis* exuviae were always larger than *P. marginata* ones, considering femur length (M–W $U = 2{,}517$; $P < 0.05$), metathorax wingpad length (M–W $U = 1{,}462$; $P < 0.05$) and total length (M–W $U = 2{,}143$; $P < 0.05$). The three

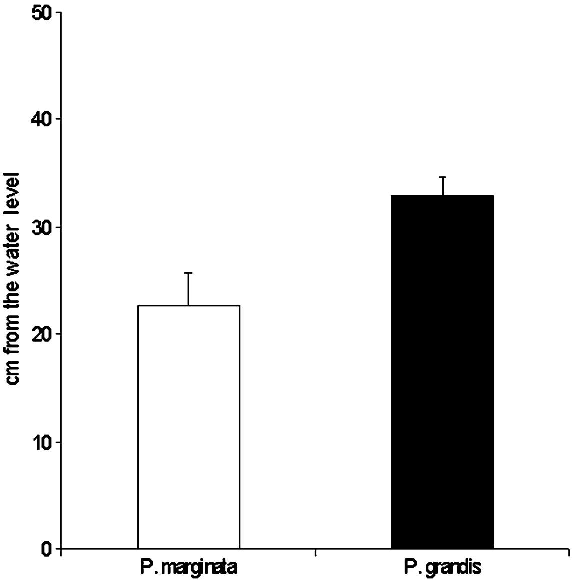


Fig. 3 Vertical distance travelled by nymphs of the two species to metamorphose (mean ± SE)

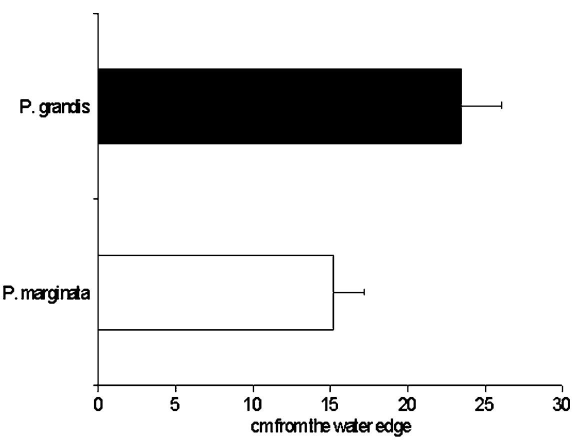


Fig. 4 Horizontal distance travelled by nymphs of the two species to metamorphose (mean ± SE)

measures were significantly correlated for each species (*P. marginata*: Gamma correlation = 0.58 for total length vs. femur length; Gamma correlation = 0.61 for femur length vs. wingpad length; Gamma correlation = 0.75 for total length vs. wingpad length; *P. grandis*: Gamma correlation = 0.41 for total length vs. femur length; Gamma correlation = 0.41 for femur length vs. wingpad length; Gamma correlation = 0.63 for total length vs. wingpad length; $P < 0.05$ in all cases). This allowed us to use them indiscriminately as size indicator. No correlations were detected within each species when comparing vertical and horizontal distances with total length (*P. marginata*: Gamma correlation = −0.09 for total length vs. vertical distance; Gamma correlation = 0.16 for total length vs. horizontal distance; *P. grandis*: Gamma correlation = 0.006 for total length vs. vertical distance; Gamma correlation = 0.04 for total length vs. horizontal distance; $P > 0.05$ in all cases). As males are smaller than females in both species, these data could also suggest that there are probably no differences between sexes regarding the horizontal and vertical distances reached.

Discussion

The existence of patterns in the emergence site selection has been underlined by recent studies (Jáimez-Cuéllar & Tierno de Figueroa, 2005). Among Plecoptera, an accurate site selection could be particularly important for large-sized Systellognatha, such as Perlidae and some Perlodidae. These taxa base their entire energetic demand on the pre-imaginal carnivorous diet and do not ingest solid food in the adult life (Tierno de Figueroa & Sánchez-Ortega, 1999; Tierno de Figueroa & Fochetti, 2001; Fenoglio & Tierno de Figueroa, 2003). Short living adults of *Perla* could be advantaged by starting imaginal life in a substrate that, besides protection and stability during last moult, also offers the possibility of rapid mate encounters. In fact, it has been pointed out that for some *Perla* species mating happens within 3 days after the emergence (Elliott, 1991) or even on the same day (Zwick, 1980). *P. marginata* and *P. grandis* are very similar in many aspects of their behaviour: nymphs of both species are carnivorous (Bo et al., 2007; Fenoglio et al., 2007), inhabiting oligotrophic, fast flowing and well-oxygenated environments, with coarse substrates. For both species flight period is from May to July (Consiglio, 1980). Furthermore, both species produce intersexual communication duets by drumming: *P. marginata* call-response duet is characterized by a low frequency (around 5.1 Hz for males and 5.4 Hz for females) and a high duration (around 4 s for males and 1.3 min for females) while that for *P. grandis* is shorter (around 0.36 s for males and 0.3 s for females) and presents a higher frequency (around 20.1 Hz for males and 14.6 Hz for females) (Rupprecht, 1969).

In our comparative analysis, we detected no significant differences between both species in the selection of emergence sites, considering substrate type and colour. Moreover, in our study most exuviae were found in a vertical position (head up), similar to the description by Zwick (1980) for Perlidae and *Isoperla*, and by Jáimez-Cuéllar & Tierno de Figueroa (2005) for *Dinocras cephalotes*.

Bukantis & Peckarsky (1985), analysing emergence patterns of two Perlidae species in the Six-Mile Creek, New York, reported partially similar results: *Agnetina capitata* (Pictet, 1841) and *Acroneuria carolinensis* (Bank, 1905) exuviae were found on living and dead plants, exposed roots, rocks and bare soil, with no apparent preferred emergence substrate. But, in contrast with our findings, they noticed that the location of exuviae with respect to distance from the stream edge did not differ between the species.

We can hypothesize that: (i) substrate selection could be related to many factors (ecological and behavioural) that may be similar for different Systellognatha species; this could explain, for example, why exuviae of different taxa were found mixed in assemblages in the same stone; (ii) emergence places could be important as encounter sites to aggregate sexes; but, the presence of different species in the same place could represent an element confusing mate encounters; (iii) when phylogenetic distance between species is high (*Agnetina capitata*–*Acroneuria carolinensis*), there is no need to separate emergence sites; for this reason, moult places were selected by their characteristics, independent of the river edge distance; (iv) when species are in close phylogenetical proximity (*Perla marginata*–*P. grandis*), the risk of mate confusion and outbreeding could be higher; so, we can hypothesize that in these cases it could be important to have some strategies to separate emergence sites. Despite the existence of differences between the two studied species in the intersexual communication duets (Rupprecht, 1969), its effectiveness as reproductive isolating mechanism is incomplete: it is known that drumming duets, that can occur when both sexes contact, are not a prerequisite for mating (Berthélemy, 1979; Tierno de Figueroa et al., 1998, 2000a), and particularly drumming calls cannot be transmitted through stones (Stewart, 1997), the more-used substrate by the studied species.

In our study, we found that large-sized *P. grandis* nymphs travelled longer distance, both vertically and horizontally, than smaller *P. marginata* nymphs. Considering that the displacement of emerging nymphs increases the exposition to predators, we suggest that the species with larger nymphs would have some advantages in selecting emergence sites more far away from the water body. Thus, they would avoid competition for both emergence and post-emergence needs.

Acknowledgements We are grateful to M. Grenna, S. Giordanengo, M. Cammarata and D. Vigani for the help in field work, and to G. Lingua for the useful suggestions and floristic help.

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