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Mud sediments on anal pyramids of *Libellula quadrimaculata* larvae – accidental phenomenon or bioindicator of heavy metal pollution? (Odonata: Libellulidae)

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Abstract. The morphology and morphometry of 1 089 *Libellula quadrimaculata* exuviae, collected at three selected sites in Upper Silesia, were examined with regard to their potential use as bioindicators of habitat quality. The biometric parameters of exuviae were only weakly linked to water quality, i.e., to heavy metal contamination. However, terminal thickenings on the anal pyramid, which were originally thought to be teratogenic, were finally identified as sediments that had been deposited on the anal appendages. These modifications were observed in *ca* 12 % of the exuviae that had been collected from a site on which river contamination by metals had reached extreme values, thus probably having caused a long-lasting and complex change of the water habitat.

Further key words. Dragonfly, Anisoptera, cyanobacteria, Upper Silesia, Poland

Introduction

Because many Odonata species of temperate regions with a few exceptions spend the main part of their life as larvae, they can exhibit traits which reflect the condition of water. At the end of the larval stage, after emergence, a record of their aquatic life stage is preserved in the form of exuviae. The presence of exuviae proves successful completion of larval development at a particular site (e.g., Hardersen 2008; Raebel et al. 2010). In routine long-term studies, they are used to evaluate the species composition and numbers of emerged individuals of Odonata in particular areas, which estimate local biodiversity and its possible changes (e.g., Kalkman et al. 2008; Harabiš & Dolnỳ 2010).

Odonata have rarely been used for direct examinations of pollutant content in their bodies (larvae, exuviae or adults). Corbi et al. (2008, 2011) examined the metal content in Odonata larvae inhabiting Neotropical streams that were contaminated with fertilizers. Tollet et al. (2009) studied the toxicity of selected metals in dragonfly larvae. The extent of the excretion of cadmium was investigated by Martin et al. (1990) using larval *Aeshna canadensis* Walker, 1908. They found accumulation of this element in the faecal pellets. Their data do not enable a clear understanding of the type of the adaptation to increased metal content of dragonflies in their habitat, which is probably similar to that of other aquatic invertebrates (Rainbow 2002) that combine balancing uptake and excretion with the storage of metals depending on the type of metal and its concentration.

Little information is available on using the exuviae for similar purposes. Kormondy (1965) found in *Plathemis lydia* (Drury, 1773) that Zn is adsorbed in the exoskeleton and to a great extent left in the exuviae after molting and emergence, but not metabolically incorporated during larval life.

The starting point of our research was the fact that during a routine dragonfly inventory within the Silesian Province of Poland – an ongoing data collection and monitoring of databases maintained by the Centre for Natural Heritage of Upper Silesia – some of the exuviae of *Libellula quadrimaculata* Linnaeus, 1758 collected were found to have a posterior thickening. At first glance, we supposed that this was a teratological abnormality, probably caused by habitat contamination. Further examination showed they resulted from deposits of mud. Therefore, the aim of our study was to determine whether the morphometry of dragonfly exuviae as well as the terminal thickenings caused by mud deposits could be used as bioindicators of the quality of the water habitat.

Material and methods

Study sites

Exuviae of *Libellula quadrimaculata*, a common dragonfly species in Poland, were collected during May and June of 2010 and 2011 in three areas of Upper Silesia, an industrial region located in the South of Poland. The three study sites represented different grades of environment degradation, ranging from heavily polluted waters (Pniowiec) to a recreation area with low pollution (Goczałkowice reservoir).

Pniowiec (56°29'39"N, 18°48'54"E): 'Graniczna Woda' is a stream with a sandy and muddy bed and banks covered with metallophytes. In the vicinity of the village of Pniowiec it forms a floodplain, including a peat bog, and runs through the Forest of Lubliniec into Stoła River. At the stream head, non-ferrous smelter sedimentation tanks are located as a permanent source of pollution.

Borowa Wieś (50°14'9.95"N, 18°49'24.56"E): There are two types of habitat within this site; fish ponds and a peat bog that sustains two different dragonfly fauna that are typical for such habitats (T = 35; R = 23). The 'Korytnioki' fish ponds and a peat bog, both situated in pine and mixed forests near the village of Borowa Wieś in the Halemba district of Ruda Śląska.

Goczałkowice (50°30'48.52"N, 18°49'55.92"E): The water-dam reservoir Goczałkowice is the largest reservoir in the Silesian Province and one of the largest in Poland. It was constructed in 1956 on the Vistula River as a potable water reservoir and for recreation and flood protection. To the South of the reservoir the Rotuz Nature Reserve is situated, which includes a peat bog.

One year after our investigations in 2012, Augustynowicz et al. (2014) data collected from the 'Graniczna Woda' stream, showing that the concentrations [mg/dm^3] of Zn (1.06), Cd (0.06), Pb (0.05), and Tl (0.24) were more than one hundred times higher than the control values and surpassed the European upper limits for surface waters (annual averages: Cd – 0.00008; Pb – 0.0072 mg/dm^3), published as water quality standards (EP 2008).

Examination of specimens

After species identification, the morphology of the exuviae was analysed more thoroughly in order to detect any visible posterior malformations by screening 306 (Pniowiec), 366 (Borowa Wieś), and 417 (Goczałkowice) exuviae from their respective sites. For precise morphometry 20 exuviae (10 male and 10 female) were selected from each group. Biometric parameters, i.e., external and internal epiproct length and width as well as the cerci length (Fig. 1), were measured using binocular Nikon SMZ1500 equipped with a Microscopic photographic system (NIS – Elements D) and a Nikon digital DS Fi1 camera. Data analysis used the nonparametric Kruskal-Wallis test. Some of the exuviae with an unusual shape, which had previously been considered as teratogenic abnormalities, were additionally analysed using scanning electron microscopy (SEM). The abnormalities and the structures, which covered the surface of exuviae, were photographed. The sediments that covered the exuviae were treated with a solution containing 7.5% $NaOH$ and 2.5% $NaClO$ for 15 minutes, then washed with distilled water, decanted, and dried prior to the SEM study.

For the SEM study, dried material of the exuviae and sediments was mounted on aluminium stubs with double-sided adhesive carbon tape and sputter coated with gold for 50 seconds using a Pelco SC-6 sputter coater. The specimens were then scrutinised and photographed with a Tesla BS 340 SEM operating at 20 kV.

Results

Exuviae with thickenings on the terminal areas (Fig. 2) were observed in 36 (*ca* 12%) of the 306 examined exuviae of *Libellula quadrimaculata* originating from Pniowiec, where the contamination of the river and shore sediments by metals was highest. However, during a more thorough analysis, these thickenings, primarily considered malformations, turned out to be mud sediments that had stuck to the posterior end of the exuviae most probably during the larval stage. This mud could easily be removed mechanically or with the method described above to uncover the appendages, which subsequently could be measured correctly. The presence of thickenings that had been formed from mud, only found in Pniowiec, changed the biometric parameters of the exuviae – the epiprocts were significantly wider

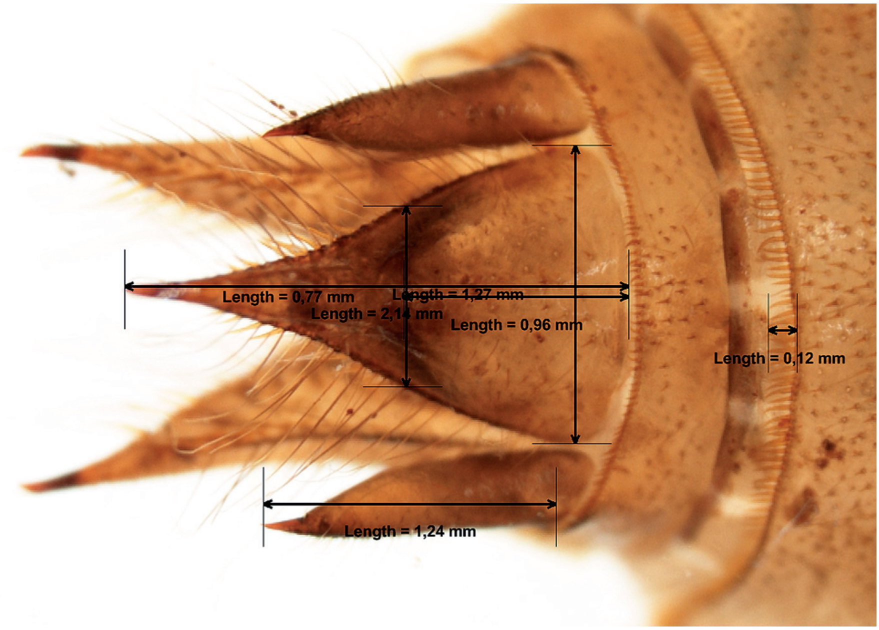

Figure 1. Example of biometric parameter measurement on the abdomen tip of exuviae of *Libellula quadrimaculata* using light microscopy

Figure 2. Exuviae of *Libellula quadrimaculata*, collected at the 'Graniczna Woda' stream near Pniowiec, Upper Silesia, Poland, with (right) and without (left) terminal thickening.

but there was no significant change in their length, while cerci were significantly longer (Fig. 3).

The SEM images of the anal pyramid of exuviae from the sites that were examined and the surface features of the sediments that covered this part of the exuviae are shown in Figure 4. The presence of thickenings that had been formed on the terminal part of exuviae was only observed in the samples from Pniowiec (Figs 4b, c). Additionally, SEM analysis of the sediments revealed that exuviae from Pniowiec were covered with a specific type of sediment, which was different from the analogous samples that had been taken at the other sites. The sediments from Pniowiec contained plenty of cyanobacteria (Fig. 4d), which were absent in the mud samples from the other study sites. Instead, many diatoms (Bacillariophyta) were involved there (Figs 4f, h).

Discussion

Exuviae of *Libellula quadrimaculata* can be collected chiefly in spring (e.g., Bernard et al. 2009), which means that this potential bioindicator is largely seasonal. On the other hand, due to the species' holarctic distribution (Boudot & Kalkman 2015), exuviae can be used for chemical and morphometric analyses from many sites in almost the entire Northern Hemisphere simultaneously. Dragonfly larvae have the potential to change their morphology in response to the state of the environment that they exist in. There is one observation on *Aeshna cyanea* (O.F. Müller, 1764) showing a spontaneous teratological change in the distal part of the exuvia (Rodríguez-Martínez & Torralba-Burrial 2012). Arnqvist & Johansson (1998) measured some morphometrical parameters, e.g., the shape of the dorsal and abdominal spines of *Leucorrhinia dubia* Vander Linden, 1825 and found a wide range of ontogenetic allometry as a result of the presence or absence of fish. Besides the adaptability mentioned above, there are also possible nonspecific changes of a teratogenic nature caused by pollutants (Chang et al. 2009). We expected some morphometric changes in the exuviae from the most polluted area in our investigations. However, the total length of the exuviae from all of the sites that were examined did not exceed the range of 18–28 mm for *L. quadrimaculata* larvae and exuviae (for larvae cf. the database accessible via Powney et al. 2014; for exuviae Heidemann

& Seidenbusch 1993; Doucet 2010). The morphometric parameters that were measured were mutually correlated, which is also recorded, e.g., in the exuviae of *A. cyanea* (Ábelová & David 2014). The epiproct was significantly wider in the exuviae from the polluted area than from the rest of sites that were examined. We did not find any asymmetry in their morphology, and therefore most probably the observed abnormalities, which were finally identified as algae and mud deposits, were unimportant for moulting and the future life of dragonflies. On the other hand, we have no data about whether, and if yes, how the described changes influenced the aquatic part of their life cycle.

The presence of mud thickenings at the posterior end of the exuviae might have been related to the physical features of the sediments, only indirectly

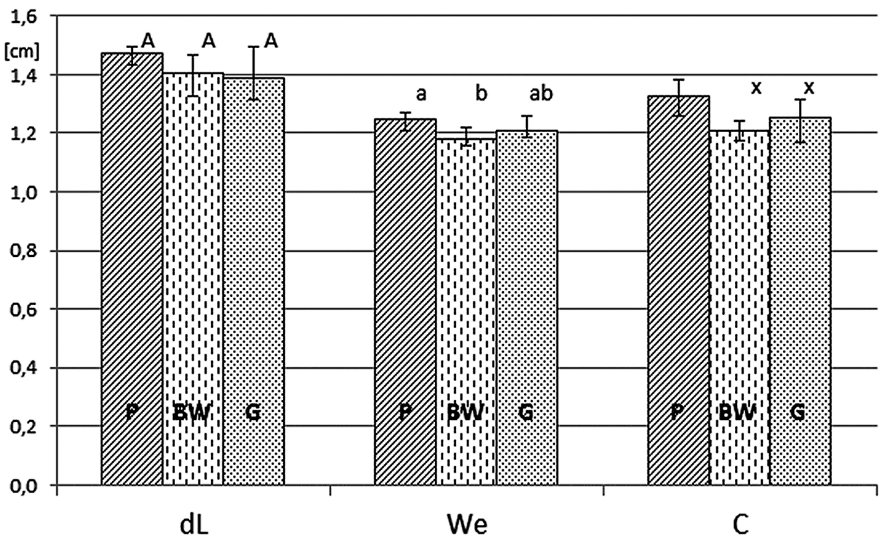

Figure 3. Biometric data of exuviae of *Libellula quadrimaculata* collected at Goczałkowice (G), Borowa Wieś (BW), and Pniowiec (P), Upper Silesia, Poland. Values representing dL – difference in the external and internal length of the epiproct (exuviae); We – external width of the epiproct (exuviae); C – length of cerci [cm] are presented as the median ± quartile whiskers. The same letters: A for dL, a, b for We and x for C denote the homogeneity of the compared bar/values. Bars/values lacking a letter differ significantly from others (Kruskal-Wallis test, $p < 0.05$, n varies from 14 to 44).

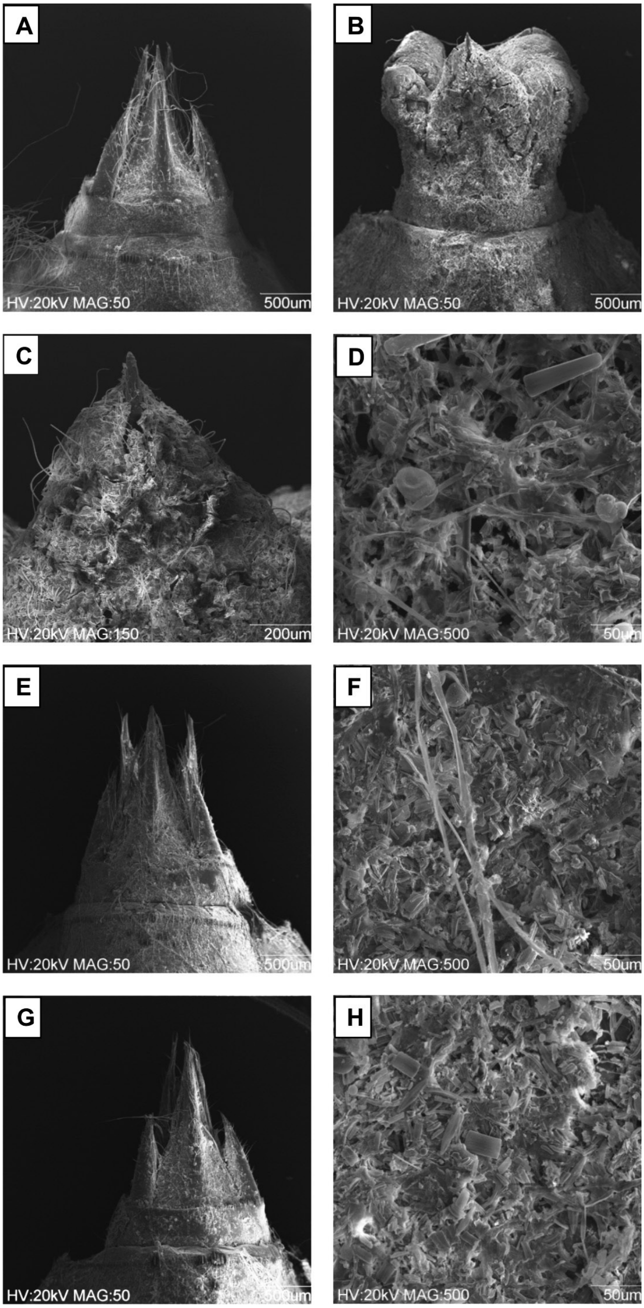

Figure 4. SEM images of *Libellula quadrimaculata* exuviae showing the posterior end (anal cone) and sediments, collected in Pniowiec (A–D), Borowa Wieś (E, F) and Goczałkowice (G, H), Upper Silesia, Poland. A–D – Exuviae without (left) and with (right) terminal distension and the structure of sediments covering this terminal thickening from Pniowiec; E–F – Exuvia with a normally shaped anal cone and sediment cover from Borowa Wieś; G–H – Posterior end of exuvia from Goczałkowice and sediment from the same site.

linked to the pollution by metals. The presence of numerous cyanobacteria in the sediments from Pniowiec might have increased water viscosity, because of the large amount of mucus that is produced by these organisms (Pliński & Komárek 2007). Aboal et al. (2002) described indirect and direct effects of cyanobacteria that were evaluated by changes in the macroinvertebrate water quality indices – neurotoxic and hepatotoxic – in some taxa of Ephemeroptera, Plecoptera, and Trichoptera. With regard to other invertebrates the picture is heterogenic: zooplankton may be affected, but mussels and crayfish appear to be tolerant, only playing a role of intoxication along food chains (Vasconcelos 2001). There is no available data about the impact of cyanobacteria on Odonata. Like all Anisoptera, larvae of *L. quadrimaculata* breathe using internal rectal gills. The water is pumped in and out of the rectum through the anus in order to ventilate the gills. As the larva of this species inhabits mud near the surface, it sometimes sinks into it; according to Corbet (1999: 149, 155) the larva of *L. quadrimaculata* is categorized as »shallow burrower« dwelling on or partly burrowed in muddy sediment. The highly viscous mud, such as the one from Pniowiec, covers the anal pyramid during ventilation. This is probably the main factor causing the formation of thickenings, which may subsequently influence the morphometrical parameters during the ontogenesis of the larvae – the epiprocts are shorter but there is no change in their width and additionally they are drawn slightly to the side in order to preserve proper gill ventilation. Exuviae indicate completed ecdysis, thus the last and successful moulting at the end of the larval life. We do not know whether and how these abnormalities influence the moulting process prior to the final stage or the larval survival ratio. It would be necessary to collect larvae in earlier stages and to determine how many of them have such mud thickenings and how many of them survive until the final moult. Despite this, the observed abnormalities are probably unimportant for the future life of adult dragonflies as we did not observe any deformed imagines. However, the presence of such mud thickenings may be interpreted as a biological sign indicating a non-optimal quality of the wetlands; and if treated as a bioindicator they should assist more detailed environmental investigations and also be of use in historical analysis of water quality based on existing collections of exuviae.

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