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Biological notes on *Isoptena serricornis*, an exceptional stonefly in shifting river sand (Plecoptera: Chloroperlidae)

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With 11 figures and 2 tables

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Larvae of the European stonefly *Isoptena serricornis* (Pictet) live buried in shifting sand and prefer erosional over depositional habitats. Specimens from Germany, Sachsen-Anhalt, were studied in laboratory streams. The species is univoltine. Eggs develop directly between ca 12 and 21 °C; at 7 °C development is delayed, at lower temperatures it fails. Setal screens on the head are used to shovel sand away; screens also protect articulations. First instar larvae live on yolk reserves. Later instar larvae swallow entire Oligochaeta, larval Chironomidae and sediment-dwelling *Leuctra nigra* (Olivier) (Plecoptera, Leuctridae) were not eaten. Mode of hunting is unknown. Feeding larvae contain much sand in the fore-gut which may extend far into the abdomen. Individual sand grains may be half as wide as the head. Amount of sand in the gut decreases with body size; between 40 and 75 % of larval dry weight are ash. In view of gut structure, sand cannot be used to grind food; probably, sand is ballast permitting the larvae to live in erosional habitats. In the laboratory, adults emerged at dusk as soon as larvae were full grown. Adults ate little, and indiscriminately. Signalling was not observed, mating occurred upon encounter, oviposition followed at the age of 8-10 days. Potential fecundity was 75 eggs or less. Eggs are large, compared with other Perloidea.

1 Introduction

The larva of *Isoptena serricornis* (Pictet, 1841) was first described by Esben Petersen (in Ussing 1910) but only Kittel's illustrations (1976) gave a clearer idea of its exceptional habitus and extraordinary pilosity. Kaiser reported collections in 1-1.5 m deep river sand. Larval guts were filled with sand grains about 100-450 μm long, with rounded contours, as if they had been specifically selected (Kaiser 1977). When we found larvae also exclusively in shifting sand in the river bed, with their guts full of sand which was large in comparison with the animal we were thrilled to learn more about these stoneflies and kept them in the laboratory, for study. Maintenance and rearing over months, to adult emergence, was easy. However, because the larvae are permanently hidden in sand not all of their habits were accessible to study. We publish our findings, also to encourage further work.

2 Methods

2.1 Study site and field samples

Specimens were collected from the stream Rossel, near Hundeluft (51°58'N, 12°20'E), 90 m a.s.l., upstream from Rosslau, tributary of.River Elbe, District Anhalt-Zerbst, Saxony-Anhalt, Germany. Random kick samples in the *Isoptena* habitat were taken using a 1 mm mesh net each month from 10 July 2002 to 11 April 2003. Live material for laboratory breeding was collected on 21 May 2002 and again on 12 October 2003.

At the collection site the Rossel is approximately 4 m wide and 0.2-0.8 m deep, the water is clear to slightly turbid. Speed of flow approximates 0.6 m/s. Selected abiotic factors recorded at seven occasions between October 2001 and April 2004 (M. Hohmann, unpublished data) were: oxygen saturation, mean 83 %, range 63-105 %; pH 6.7-7.1; conductivity 274-325 (μS/cm) at 20 °C; temperature 6.6-14.1 °C.

Large alder (*Alnus glutinosa*), willows (*Salix* spp.) and other riparian trees fringe the stream which meanders through grassy plains. Iron-rich water enters from some drainage ditches. The type of herbaceous riparian vegetation at several sites suggests influx of groundwater from the meadows, in which there are several groundwater collecting installations. Stream surface is ca. 0.75 m below the grassland, banks are steep. Near the banks are remains of wooden fascines between which there are shallow pockets and areas with stands of emergent vegation including *Caltha palustris, Scirpus silvaticus, Phalaris arundinacea, Phragmites australis, Iris pseudacorus* and floating *Glyceria fluitans.*

Near the banks the stream bottom is soft and muddy while the central part of the stream bed is covered with a firm layer of quartz sand. Ripples on the surface indicate continuous movement. Occasional stands of floating *Berula erecta, Ranunculus* sp. and *Elodea canadensis* are deeply rooted in the sand, or are perhaps being covered by it, like by a wandering dune. Buried in the sand are many twigs and pieces of bark from riparian trees.

About 3 litres of shifting sand were analysed for grain size (Tab. 1). A sond with integrated thermosensors (Thermochron I-Buttons) revealed sand depths between 40 and over 75 cm. During the day, temperature at considerable depths noticeably followed air and surface temperature, suggesting some exchange between layers (Tab. 2). However, in two other nearby spots there was no significant flow of water through the sand layer because when water was removed from or added to, respectively, a 20 cm wide pipe pushed into the sand to a depth of about 20 cm, differences in water level between the stream and the pipe remained unchanged for 30 mins or longer. The actual situation may differ from one spot to the next.

Tab. 1: Abiotic parameters of the *Isoptena* habitat in the stream Rossel on 20 May 2002. Left: Temperature in air, water and at several depths in the sandbed, at two times of day. Right: Grain-size distribution in a 2.5 kg sample of sand

	12:00 hrs	14:45 hrs	Sand grain size (mm)	% of total weight
Air	15.0	19.8		
Water	12.1	13.8	< 0.063	0
-10cm	11.0		0.063-0.199	6
-20cm	10.7		0.200-0.399	20
-30cm	10.5		0.400-0.629	30
-40cm	10.5		0.630-0.799	25
-50cm	10.4	11.5	0.800-0.999	7
-60cm	10.7		1.000-1.600	12
-70cm	10.6		> 1.600	1

2.2 Laboratory methods

The May 2002 sample consisted of some adults netted along the Rossel and of about 30 last instar larvae which were transported in sand and aerated water from the collecting site. Subsequently, specimens were kept in water from the nutrient-poor stream Breitenbach near Schlitz (50°40'N, 09°33'E) or in untreated ground water from the Schlitz water supply system. After about a week, all specimens had emerged as adults which were kept in a 1 mm mesh gauze cage (25 x 25 x 40 cm). The cage contained a bunch of garden weeds including flowering *Ranunculus* and grasses in a vase and was powdered daily with *Pinus* pollen. It was sprayed with water at least twice a day. Initially, the cage was kept at an open basement window, later in the open, in the shade. After 2 weeks, study of adults had to be discontinued; by that time, about half the animals had died, several females had oviposited and the two last pairs were preserved in copula after spraying and freezing them with ethyl chloride.

Recovered egg masses were incubated in a little water in 15 ml vials (Zwick 1996). Several batches were subdivided and studied at several temperatures. The following constant temperatures were used (mean °C ± s.d.): 4.8 ± 0.5; 6.7 ± 0.5; 7.3 ± 0.6; 11.6 ± 0.2; 14.4 ± 0.9; 15.2 ± 1.0; 21.5 ± 0.7. When eye-spots became visible some fine sand was added to provide a foothold and facilitate hatching. Hatching larvae were kept in the same vials.

The October 2003 sample included ca 60 5-7 mm long larvae. They were kept in water recirculated by an aquarium filter pump in several kinds of cages (see below) from which water returned to the reservoir through 0.5 mm screen windows. Laboratory water temperature varied between 9.5 and 14.5 °C (Fig. 1) and followed no daily cycle or uniform trend. Illumination with a fluorescent tube was 12 hrs per day.

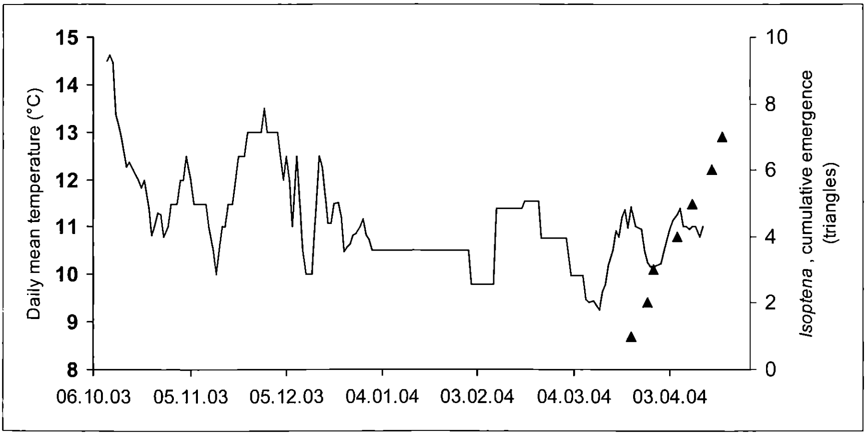

Fig. 1: Temperature regime (daily mean °C) during laboratory maintenance of *Isoptena* larvae. Triangles: cumulative adult emergence until 19 April 2004

Polyethylene boxes containing 5 cm sand percolated with water from below, through screen windows in the bottom were initially used as cages. However, preferred percolation channels immediately formed and sand was not well mixed by the current. Also, the vertical direction of flow was unnatural.

Boxes were therefore replaced by circular arenas made of PVC drainage pipes or plexiglass tubes, respectively, in which a horizontal jet of water better moved sand at the bottom. Water overflow was through windows and sieves in the inner pipe and eventually through a hole in the bottom of the central space (Fig. 2). Speed of flow near the surface was up to 29 cm/s (Schiltknecht MiniAir2 propeller meter). However, introducing the instrument instantly altered flow patterns, leading to strong movement of sand. Speed of flow and sand depth in the arena therefore could not be mapped.

The larger PVC arena was eccentrical, providing a spectrum of different conditions; it contained ca 550 ml sand from the collecting site. Seven experiments were run in which 29-33 larvae were left in the arena for 48 hours after which time current was turned off and arena space divided into 4 compartments by tightly fitting vertical PVC plates (Fig. 2). The content of each compartment was recovered separately. Larvae were counted, wet weight of sand determined to the nearest 0.5 g and converted to volume, after calibration. Because numbers or larvae differed between experiments, larval density was expressed as percentage of total number studied each time.

The transparent concentric arena was smaller (Fig. 2). The experimental channel was provided with commercially available colourless glass beads 0.4-0.6 mm in diameter.

Several times larvae were observed with a dissecting microscope at magnifications of 6–15x, using a mirror placed below a plexiglass trough containing recirculating water. The bottom of the trough was divided into three chambers, each 8 x 8 cm. Each chamber contained 2 larvae and a few Oligochaeta (10-20 mm long when extended) in sand less than 2 mm deep. The trough was kept dark except during observations.

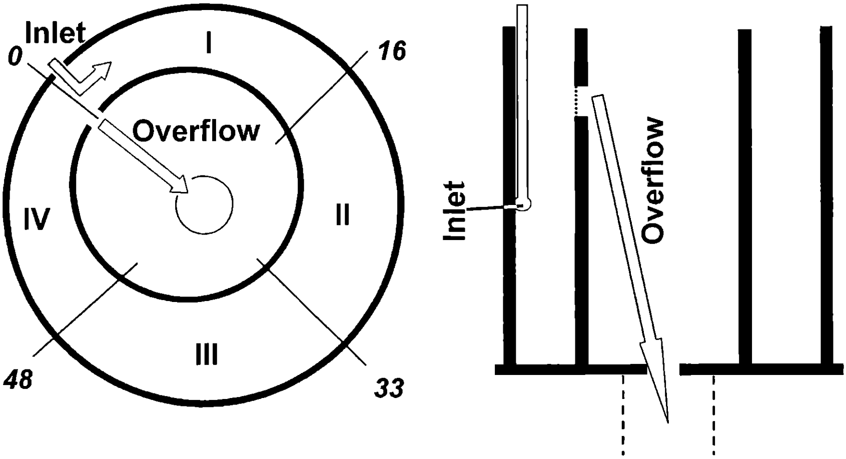

Fig. 2: Through-flow arenas to maintain and experiment with *Isoptena*-larvae. Left, top view of eccentrical arena with removable separators to divide arena space into 4 compartments (Roman figures) of different flow regime and sand depth. Arabic figures are distances (in cm) from inflow along periphery. Diameter of outer and inner cylinders is 20 and 11.5 cm, respectively. Right, longitudinal section through concentric transparent arena, outlet sieve in base plate only partly shown. Diameter of outer and inner cylinders is 15 and 11 cm, respectively

Dry weight of *Isoptena* (and of several other Plecoptera collected from the River Fulda near Schlitz, for comparison) was determined from specimens killed by freezing and dried in aluminium cups at 105 °C for at least 48 hours. They were weighed on a Sartorius Ultramicro Balance, to the closest 0.001 mg. Larvae were subsequently burnt in a Muffle furnace at 510 °C for 4 hours; ash was weighed after cooling. The ash of 10 specimens was spread on microscopic slides and mounted in Euparal, for analysis of sand grains.

Larvae are close to colourless and rather transparent, and live specimens were often observed under a dissecting microscope. A number of intact larvae were also preserved, dehydrated, made transparent with clove oil and permanently mounted in Euparal or Caedax. A few more larvae were dissected and their gut contents studied at 40x magnification.

Oligochaeta (from a pet shop) were readily eaten and were provided most of the time. However, field collected larvae of Chironomidae and *Leuctra nigra* were also offered, each time after a week of fasting. Two days after introducing insect larvae, 5 to 7 *Isoptena* that looked full were preserved and examined microscopically.

3 Results

3.1. Accompanying fauna, potential food organisms

The fauna of the stream Rossel is diverse for a lowland area, with 116 species of Ephemeroptera, Plecoptera, Odonata, Trichoptera and Coleoptera having been recorded (Hohmann 2000). Taxa abundant to very abundant at the *Isoptena* study site are listed (Tab. 2). However, most of the fauna occurs in marginal stream areas. In the sand beds we encountered only *Isoptena, Ephemera danica,* occasional Limoniidae, and a few Oligochaeta. *Isoptena* larvae were found exclusively in ripply sand areas. In May, they were the only abundant animals in sand.

Tab. 2: The dominant Ephemeroptera, Plecoptera and Trichoptera at the *Isoptena* study site of the stream Rossel. Pooled data, all seasons (Hohmann 2000, and unpublished data)

EPHEMEROPTERA	PLECOPTERA	TRICHOPTERA
Baetis rhodani (Pictet)	*Taeniopteryx nebulosa* (Linné)	*Hydroptila* spp.
Baetis vernus Curtis	*Amphinemura standfussi* (Ris)	*Polycentropus flavomaculatus* (Pictet)
Baetis niger Linné	*Nemoura flexuosa* Aubert	*Hydropsyche saxonica* McLachlan
Paraleptophlebia submarginata (Stephens)	*Nemurella pictetii* Klapálek	*Hydropsyche siitalai* Döhler
Ephemera danica Müller	*Leuctra fusca* (Linné)	*Lasiocephala basalis* (Kolenati)
Serratella ignita (Poda)	*Isoptena serricornis* (Pictet)	*Adicelia reducta* (McLachlan)
		Chaetopteryx villosa (Fabricius)
		Sericostoma personatum Kirby et Spence

3.2 Oviposition, fecundity, eggs, and temperature dependence of incubation

The few egg masses obtained in captivity contained 7-54 eggs. Females were dissected; some still contained up to 33 large eggs or over 30 early egg rudiments, respectively. Potential fecundity was 40-75 eggs.

The oval eggs are typical of Chloroperlidae and lack striking characteristics; they were described at length by Tierno de Figueroa & Derka (2003). Egg size is 332 (±22) x 255 (±15) μm (mean ± SD; n=17), including the short collar with a serrate edge. The chorion is finely and sparsely punctate. There is no opercular suture, micropyles were not observed. The anchor is of the usual mushroom-shape.

Mean incubation period rose from 11 or 15 days at 21.5 °C to 54 days at 11.6 °C. Incubation periods between 86 and 131 days were therefore expected between 7.3 and 4.8 °C. However, at 7.3 °C mean incubation actually took on average 282 days, and incubation at lower temperatures was unsuccessful (Fig. 3).

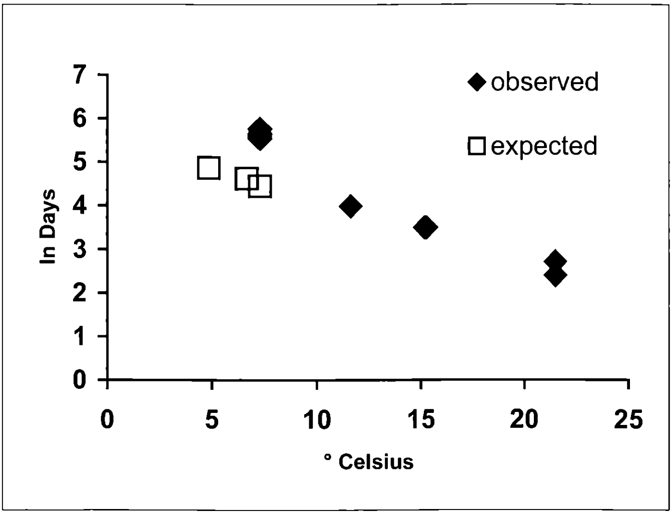

Fig. 3: ***Isoptena serricornis*, mean incubation period (i.e., until 50 % of all larvae that eventually hatched had hatched) at several constant laboratory temperatures**

Hatching success between 83 and 76 % was recorded between 11.6 and 15.2 °C, but only 50 % at 21.5 °C and 25 % at 7.3 °C. However, hatching success was not determined in several batches which were transferred *in toto* to breeding cages after mean larval hatch had been attained.

3.3 Life cycle

Isoptena serricornis is univoltine (Fig. 4). Egg development at summer temperature is direct and fast, so first instar larvae must be abundant in the stream about a month after adult emergence which ended in May. In the laboratory, first instar larvae moulted after only 3 days but very few survived for another day. Because random samples were taken with a 1 mm mesh net, early instars were missed in the field. Single last instar specimens first occurred in early March but even on 11 April they were less common than penultimate and even antepenultimate instar larvae.

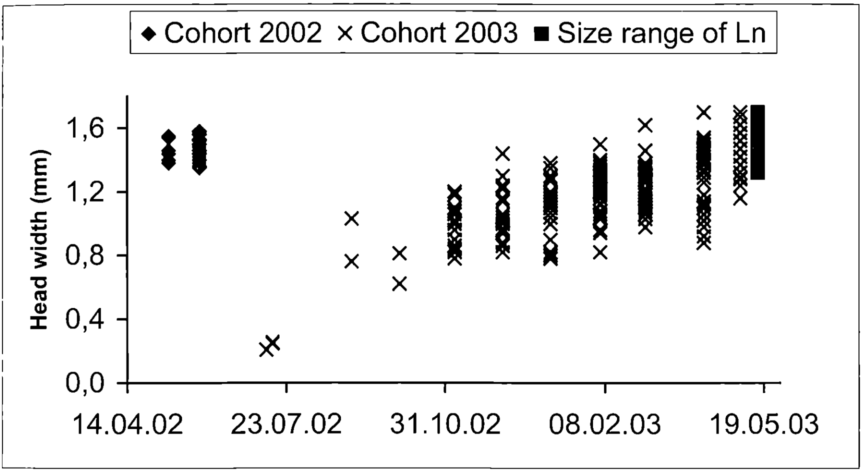

Fig. 4: *Isoptena serricornis*, larval head width in the stream Rossel, in 2002/2003. Ln refers to last instar larvae. Size and date of occurrence of first and second instar larvae from laboratory data; small larvae were missed in the field. Each symbol represents one individual

3.3.1 Larvae

Morphology of larval head and legs unique. The head is from the first instar on short and roughly triangular, with prominent posterior corners. Dorsal setation stronger than is usual, already in first instar (Fig. 5), especially on the maxillary palpus. Large larvae have huge setal screens protecting eyes and articulations, especially antennal insertion, maxillar joints and intersegmental membrane between head and prothorax (Fig. 6). Setae are backwardly directed, allowing only forward movement through sand. Distal half of antennal flagellum short, weak, hanging back from normal base. Tibiae with fossorial setal combs of strong setae, tarsus inserted subapically and folded sideways.

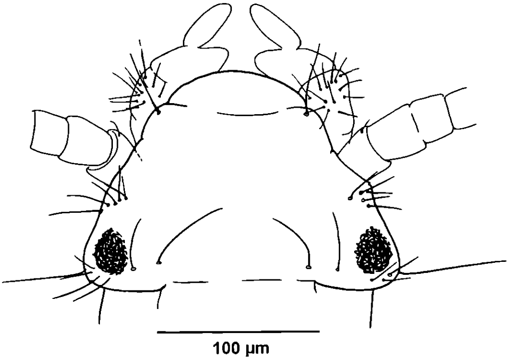

Fig. 5: First instar larva of *Isoptena serricornis*, dorsal view of head

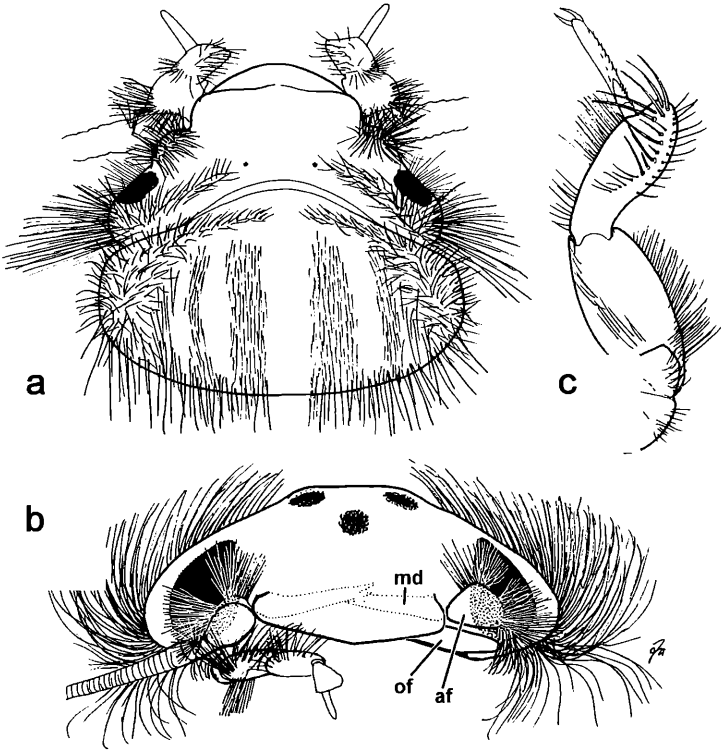

Fig. 6: Late instar larva of *Isoptena serricornis*. a, head and pronotum dorsally; b, head in frontal view, mandibles (md) seen by transparency. Left antenna and maxilla removed, exposing antennal (af) and occipital foramen (of), labium not shown. c, ventral view of fore-leg. Figures a and c to same scale

Thorax, abdomen and cerci normally structured. On dorsal side of hind body, procumbent pilosity is unusally dense and forms 4 longitudinal bands. Ventral face without obvious vestiture.

Biometry

First and second instar mean head widths are 202 and 249 μm, respectively; there is no overlap (ranges 198-208 and 245-255 μm, respectively). Number of antennal and cercal segments were 9 and 3, respectively, in both the first and 2nd instars, but number of setae rose at the moult. Distinction of later instars was not attempted. Maximum head width observed was 1.7 mm. Head width (HW) and body length (BL) are related as follows:
$BL = 8.27\ HW - 1.97\ (r^2 = 0.92, P < 0.001, n = 21)$

Behaviour

Larvae are constantly hidden in sand. Disturbed floating larvae immediately swam down with the sideways-wiggling movements typical of Plecoptera. Amplitude of movement is low but frequency unusually high, leading to hasty and much faster movements than in other Plecoptera. Upon contact with sand, wiggling movements continued, including sideward shaking movements of head. Mainly the large setae at the rear of the head pushed sand sidewards, legs aided often little. Normally, larvae disappeared in sand within a second.

Individuals severely disturbed by holding them briefly with forceps, poking them with a pipette or the like often exhibited thanatose. They bent their prothorax and head ventrally, resting motionless for a few to about 30 seconds. Sometimes, they would then spin around their longitudinal axes extremely rapidly, for up to two seconds. In the end, larvae escaped by running and swimming rapidly.

No parts of larvae were ever seen to project into the water from between the sand grains. During occasional nocturnal inspections, larvae were also not noticed on top of sand. Two different attempts to elucidate behaviour within the substratum were made.

Even in only a few millimeters of sand larvae kept their dorsal sides covered but also could not be seen from below with a mirror. In about 2 mm depth of sand larvae remained dorsally covered but their ventral sides lay directly on the glass bottom of the observation chamber. They were usually inactive except when the microscope light hit them via the mirror. Then, they moved away hastily using their legs in normal gait. They simultaneously performed undulating sideways movements of the entire body, pushing individual sand grains away with the head setae. The base of each antenna was directed forward, the narrow tip of antenna was hanging back, like a whip. Activity never lasted long, larvae sat still again after a minute or so and moved no more, often for hours. Single Oligochaeta added to the observation chambers moved constantly between the sand grains. Several encounters with resting *Isoptena* larvae were observed; each time, *Isoptena* hurriedly moved away.

In a circular arena with glass beads instead of sand larvae were visible as dark shadows. However, even last instar larvae had great difficulty burrowing into the artificial substratum and succeeded only when the current was minimal. Some rested as soon as the body was covered by substratum to the base of the cerci. However, some were also seen at a depth of about 2 cm. Larvae were not oriented with regard to current direction, facing either way. Some stood almost vertical, others lay horizontally just beneath the substratum surface. The arena was covered to admit only very dim light, repeated inspections during the day revealed no movement or change of position within the substratum. By night, larvae left the apparently unsuitable substratum and every morning almost all had drifted into the outflow sieve.

Distribution across different microhabitats

In the eccentrical arena current moved the sand continuously. At the water inlet in the narrow part of channel sand was swept away exposing the bottom. Further on, in the wide part of arena, sand piled up and rolled over until it slowly approached the inlet again, from where it re-circulated.

Distribution of larvae across 4 compartments (Fig. 1) varied. Generally the greatest larval density was in areas with strong sand movement, on either side of the deep hole created in the sand layer by the water jet. Larval density was less in the large areas with a thick stable layer of sand. Data for Compartments I+II were pooled as erosional and for Compartments III+IV as depositional, between which larval density differed significantly (T-test; $p < 0.05$) (Fig. 7).

Moulting and growth

More than 60 exuviae were recovered from the sand surface on the percolated boxes, or on the outlet screens of the arenas. During transfer from field to laboratory water temperature rose abruptly from 9 to about 16 °C. In the following week, 15 exuviae were found. Later moults occurred at irregular intervals. By March 2004, most of the 21 surviving specimens were in the last instar. The first adult emerged on 22 March.

Isoptena exuviae differ from average plecopteran exuviae in that mouthparts and cephalic sclerites are not inverted. Apparently, the short head is simply pulled back through the open Y-shaped ecdysial suture. The cuticular lining of the fore-gut was still attached in less than 5 exuviae but was missing in the others. Several freshly moulted larvae found resting on the outlet screen, besides their exuviae, were inspected alive under a dissecting microscope, in transmitted light; each had only 1-4 sand grains in the fore-gut.

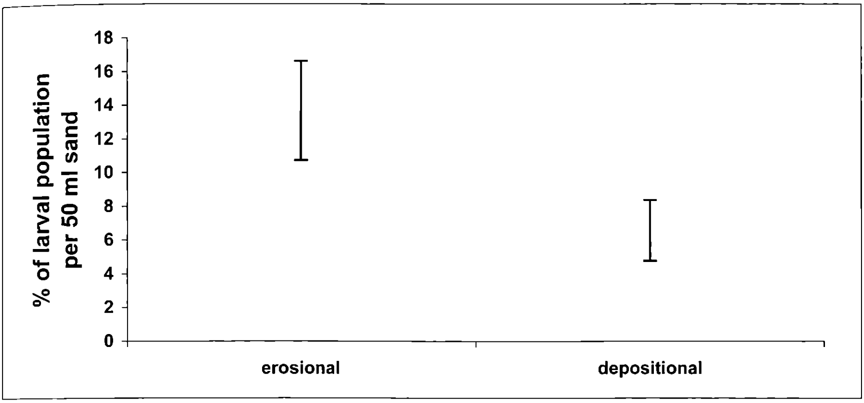

Fig. 7 ***Isoptena serricornis*****, larval density (% of total population per 50 ml sand; mean ± SD) across habitat types in the eccentrical arena**

Food and gut content

First instar larvae do not feed and their guts contain yolk droplets (Fig. 8a). Except for the flat setose palpus, mouthparts of *Isoptena* resemble those of other Chloroperlinae, strongly suggesting larvae are carnivorous engulfers. Because small Chloroperlidae are said to be omnivorous (see references in Zwick 1980), the freshly collected relatively small larvae were offered both fine detritus (*Gammarus* faeces) and buried pieces of conditioned alder (*Alnus glutinosa*) leaves. However, even after 2 weeks, none of this was observed in guts, except once a single large apparently woody piece. Larvae were never noticed on or near the buried leaves.

After 2 weeks, 15-20 mm long Oligochaeta were first provided. Two days later, many *Isoptena* appeared pink and dissection of 6 of them revealed one entire folded oligochaete each in the fore-gut of 4 specimens. Experiments and inspections continued over many weeks during which time larvae were removed from sand many times by sieving or flushing with water. Almost every time, a larva was noticed eating an oligochaete, which was still largely hanging out of its mouth. By seezing the worm with forceps *Isoptena* could be lifted and transported to another container. Larvae did not bite off, or let go. Specimens with partly swallowed Oligochaeta were also observed in two of the random field samples.

Larvae of Chironomidae and of *Leuctra nigra* offered after a week of fasting lived with *Isoptena* in the experimental arenas for up to 3 weeks. Head capsules or appendages of potential prey were never noticed in *Isoptena* guts. There was also no other evidence these insects were ever eaten, and no dead or damaged Chironomidae or *Leuctra* ever drifted onto the outlet screens. However, fore-guts sometimes contained an amorphous chylus coagulating instantaneously when occasionally regurgitated during preservation. This amorphous mass contained no identifiable material.

Fore-gut, inorganic material

Semi-transparent larvae mounted on slides and all dissected larvae had much sand in the fore-gut (Fig. 8b-f), but none or very little in the mid gut (Fig. 8c, e). Three of 4 specimens in instar 2 surviving for a day ingested nothing but sand that was large in relation to the body (Fig. 8b); the 4th individual additionally contained some detritus.

The sand-filled fore-gut forms a solid, tightly packed tube in preserved specimens (Fig. 8b-f) but in living specimens the fore-gut is wide and occupies the entire space between thoracic muscles. Sand grains lay loosely in this wide, largely empty tube which lacks strong musculature. At irregular intervals, the delicate protractor muscles connecting the border between fore- and middle gut with the thorax pulled the gut forward so that it changed shape temporarily. The sand grains then moved freely in the gut lumen, and small sand grains "flew" across the entire gut diameter.

Sand in the gut is of fairly uniformly oval shape, mean length/width ratio is 1.42 (s.d. = 0.31; n = 153). Size of the largest sand grains in the gut increases with larval body length, but even the largest specimens had also ingested very small sand. In contrast, relative size of the largest sand grains drops with larval length (Fig. 9). Width of the largest sand grains in the gut is 47-32 % of head width in specimens of ca 5 mm body length but only 31-21 % in 12 mm long larvae.

Dry weight (DW) of *Isoptena* does not strikingly differ from the DW of other Plecoptera of similar size, but the share of ash which is mainly sand in the gut is exceptional (Fig. 10).

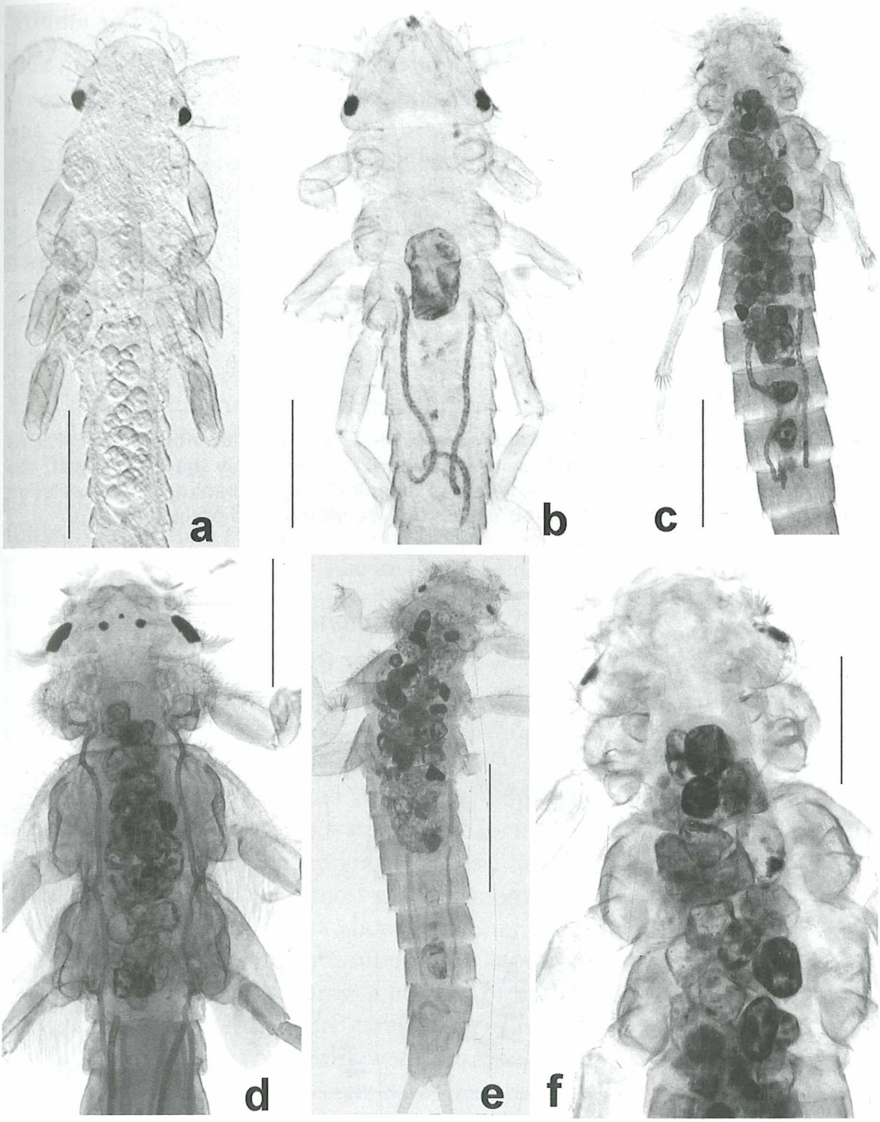

Fig. 8 Gut filling of *Isoptena*-larvae (from permanent whole mounts). a, first instar specimen with yolk droplets; b, larva containing single large sand grain a few hours after moult to second instar; c-f, larger specimens with sand filled fore-gut; c and e have few smaller stones in the midgut. Scale is 200 µm, in all figures

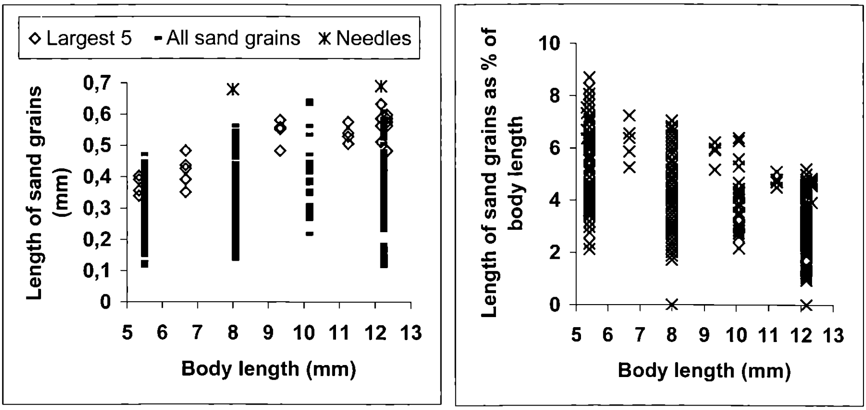

Fig. 9 *Isoptena serricornis*, sand in the fore-gut of larvae of different body lengths. In some specimens, all sand grains were measured, in others only the five largest; exceptionally slender stones were identified as "needles". Left = absolute, right = relative size of sand grains. Each symbol represents one individual sand grain

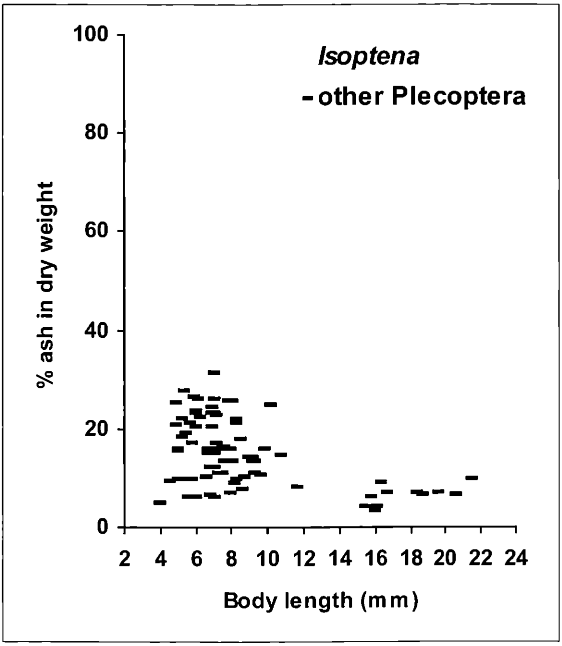

Fig. 10 *Isoptena serricornis* compared for dry weight and ash with various other Plecoptera: *Brachyptera risi* (Morton), *Protonemura meyeri* (Pictet), *Nemoura* sp., *Leuctra nigra* (Olivier), *Siphonoperla torrentium* (Pictet), *Isoperla grammatica* (Poda)

Middle and hind-gut

The middle gut never contained much material; single sand grains of small size were occasionally observed in mounted individuals. The content of the hind-gut consisted of some amorphous brownish material. Because two dark Malpighian tubules lying parallel to the gut were always easily seen, the observed relative emptiness of the gut was real, and not a matter of poor visibility. A single ready-to-emerge pharate adult that was sacrificed still had 3 stones in the fore-gut. However, several sand grains were contained in the cuticular hind-gut lining of two exuviae, after adult eclosion.

3.3.2 Adults and emergence

Field population structure suggests an emergence season beginning in late April to early May when daylength in central Germany is ca 15.5 hrs. On 21 May, we found only last instar larvae. In contrast, adults emerged from larvae raised in the laboratory at a constant 12 hr artificial daylength as soon as larvae were fully grown, in late March.

Emergence occurred after sunrise in the field and after light-on in the laboratory, respectively. On the sunny late morning of 21 May 2002 no adults were found in the field but in the early afternoon, numbers were netted from riparian vegatation. Flight or other activity were not observed. From the laboratory stock of field collected last instar larvae, adults emerged mainly in the early morning before 07.00 h. Most exuviae rested on the container wall, only few on emergence substrates provided. Two specimens were watched swimming up in their typical hasty way and swiftly creeping up the plastic wall of the container to about 10 cm above water level. The insect then stopped and immediately emerged in the conventional manner, head up. Specimens were apparently held in place on the almost vertical smooth wall by adhesion of their wet body. Actual emergence took about 5 minutes, spreading and folding of wings was complete about 15 minutes after larvae left the sand.

Despite frequent water sprays a number of adults died, seemingly of desiccation. Survivors were therefore carefully kept in the shade. They were largely inactive during the day; females were occasionally seen to browse on leaf surfaces dusted with pollen. Search behaviour or signalling were not observed, specimens mated upon accidental contact. When specimens were between 8 and 10 days old the cage was inadvertently exposed to afternoon sun on a warm and humid day. Several females were unusually active at dusk and each carried an egg mass.

The guts of eight females that had oviposited were dissected and examined for contents; none was well filled. Four were empty, or almost so. Two had ingested some fine detritus with some pollen and another specimen contained coarse detritus but no pollen. The last female had eaten mainly *Pinus* pollen.

4 Discussion

Distribution, habitat and morphology

Distribution, habitat and morphology of *Isoptena serricornis* are intimately linked. The species is restricted to shifting sand at the bottom of streams. *I. serricornis* is widespread in North and Central Europe from North Sweden and Finland in the north (Illies 1953) to Rhineland-Palatinate in the South West (Otto & Westermann 2003) and Slovakia (Krno, 2003) and NW Romania (Kis 1974) in the East. Pleistocene moraines to the west and south of the Baltic Sea provide a large continuous area with suitable habitats; the Rossel is one of these.

Free flowing water of the stream Rossel is reasonably well oxygenated (Tab. 1) but interstitial water probably contains less oxygen, given the large amount of detritus in the sand and the at least locally clogged interstices at greater depth. However, *Isoptena* larvae do not appear to be very sensitive with regard to oxygen or temperature, as all specimens survived 4 hours of transport in deep sand without aeration at temperatures near 20 °C, and no damage occurred when larvae were twice inadvertently left in 500 ml of sand in a little stagnant water for a day and a half.

Constant 21 °C are certainly above long term field maxima, but many eggs incubated at such high temperatures hatched. In contrast, at temperatures only a little below 8 °C (the annual mean temperature in Central Europe) eggs developed very slowly and already at approximately 7 °C no development occurred. However, several populations in the north of Europe live outside the annual 8°-isotherm, so there are likely to be regional differences in response to thermal conditions.

Quartz glass beads offered as transparent substrate in the laboaratory agreed in density with sand in which larvae normally live, and their 0.4-0.6 mm diameter agreed with the dominant size class of sand in the field. However, the uniformly spherical shape of the smooth beads lead to them being densely packed. Water flow through this densely packed material must have been low compared with flow through irregularly shaped and sized sand. Larvae were barely able to penetrate into the substratum of glass beads, despite attempts to push the head in and shovel particles sideways which were aided by hasty digging movements of the fore- and middle legs. At night, larvae left the artificial substratum and drifted into the outflow. In contrast, no significant larval drift was noticed in sand, except at moults. Larvae that had left the sand could readily burrow back into it; some nocturnal activity on the sand surface can therefore not be excluded.

Isoptena as well as other Plecoptera and Ephemeroptera larvae living buried in substrata are dorsally remarkably hairy, for example the European stoneflies *Leuctra nigra* (Olivier), *L. braueri* Kempny, and *Capnopsis schilleri* (Rostock) (Zwick 2004). The dorsal hair cover certainly reduces friction and facilitates movement in the substrate. In contrast, ventral faces and lower sides of legs which need to gain a foothold on substrata are void of fine pilosity. Both *L. nigra* and *I. serricornis* use stiff setae on the head to shovel sand away by swinging movements. The huge setal screens of *I. serricornis* protect the eyes and important articular membranes.

Mayflies of family Behningiidae, of which *Dolania americana* Edmunds & Traver is best studied (for example Fink & al. 1991, Tsui & Hubbard 1979, Peters & al. 1986), represent an in some ways similar life form, even though they look quite different. Some of their remarkable pilosity also has protective function, especially of the gills (Dolan, in Edmunds & Traver 1959).

Larval growth and development

Numbers of antennal and cercal segments of the 1st instar of *Isoptena* are standard for those Plecoptera whose adults have plurisegmented cerci (Zwick 1980). The number of segments normally increases at moults, but not necessarily at the moult from the non-feeding 1st to the feeding 2nd instar of Systellognatha (Zwick, unpublished data on several Perloidea; Zwick & Teslenko 2002 on *Pteronarcys*). Body pilosity and setal combs on tibiae quickly attain the condition shown in figure 6. There are no significant allometric changes of body shape during growth after the 2nd instar.

Moulting apparently occurs at or very near the sediment surface because numerous exuviae were found on it in the percolated boxes, or they had drifted onto the outlet screens of arenas. The large sand-laden fore-gut lining was mostly torn off. Perhaps this piece of cuticle and the sand within it are regurgitated only after the body is free of the main part of the exuviae. Clearly, the bulk of sand in the gut is not transferred between instars. However, occasional sand grains seen in freshly moulted larvae as well as others in the hind-gut lining of exuviae remaining after adult eclosion suggest that the gut is not rigidly evacuated in preparation of moults. Certainly, the valve at the beginning of the midgut prevents material in it from being regurgitated.

Larval food

Isoptena larval mouthparts are typical of the generally carnivorous Perloidea. The few huge sharp spines on the maxillae grasp and hold prey, and push it into the wide mouth opening. Other Chlorperlidae have similar mouthparts but small larvae in particular may contain a mixture of items, including detritus. However, detritus was never a significant component of *Isoptena* gut contents. However, large larvae of Chloroperlidae (all European genera; Zwick, unpublished data) are predators and as a rule their guts contain one or several entire larvae of Chironomidae, Simuliidae, or Ephemeroptera.

Presently Oligochaeta are the only proven prey of *Isoptena* in the field and in the laboratory. Larvae were observed eating Oligochaeta, and freshly ingested worms were found in the fore-gut but not in the mid gut. Among the little material in the hind gut oligochaete setae were not noticed but may have been overlooked. The cuticle-lined fore-gut in which Oligochaeta lay lacks digestive enzymes of its own. Prey may nevertheless be digested in it, because Plecoptera belong to those Neoptera in which digestive enzymes circulate freely in the whole gut (Terra & Ferreira 2003).

No other recognizable prey items were found in the guts of *Isoptena* larvae. The most common insect sharing the habitat with *Isoptena* is *Ephemera danica* which is most of the time too large to be eaten, except perhaps when hatching from eggs. Life cycle timing of *Ephemera* in the Rossel is not known. At the time of our collections, *Isoptena* larvae were smaller than *Ephemera*.

In the laboratory, *Leuctra nigra* larvae were offered because more than many other Plecoptera they tend to live hidden in substrata, also sand (Zwick, unpublished field observations). However, *L. nigra* does not occur in the stream Rossel. In our experiments, many chironomid larvae and also *L. nigra* were clinging to sand on the surface or were only shallowly covered with sand. The absence of Chironomidae and *Leuctra nigra* from *Isoptena* guts may simply have resulted from low encounter frequency.

Whether or how *Isoptena* hunts for prey or instead relies on current action to expose potential prey is not known. Certainly, *Isoptena* does not create a digging vortex, like the carnivorous mayfly *Pseudiron*, which dwells on top of fine drifting sand (Soluk & Craig 1990). *Dolania* eats mainly Chironomidae (Tsui & Hubbard 1979) which abound where *Dolania* feeds (Peters & Soponis 1983), but *Dolania* also ingests many other invertebrates; however, the way in which it catches prey is not known. Keffermüller (1959) found guts of the closley related *Behningia* empty, except for spicules which she thought belonged to sponges. More probably these were setae of Oligochaeta (J. Peters, in a letter).

Sand in gut

When we noticed that 2nd instar larvae had swallowed sand before any food had been ingested, and also that freshly moulted soft larvae immediately ingest sand we considered a possible role of a digestible biofilm coating the sand in the nutrition of *Isoptena*. However, biofilm on permanently rolling sand constitutes probably no adequate resource (J. Marxsen, personal communication) and to gather enough food, *Isoptena* would have to ingest and regurgitate very large amounts of sand.

Sand in the gut of *Isoptena* cannot have a food grinding function as significant gut musculature is lacking. Furthermore, the gut has no strong protective cuticular lining which would be required in a gastric mill. Lastly, sand definitely does not originate from prey guts, as potential prey is smaller and none contained sand of the size seen in *Isoptena*. Unintentionally ingested sand was much smaller than sand in *Isoptena* guts as evidenced by Kaiser's (1977) comparison of stones in the guts of *Isoptena* and the large *Ephemera*, respectively, from the same habitat. Indeed, no comparably large stones were ever reported from the gut of any aquatic insect and many stones are very large and require specific handling to be swallowed, which excludes inadvertent ingestion.

Field and laboratory observations indicate that *Isoptena* prefers erosional habitats. We assume ingested sand serves as ballast helping to keep the larvae in place. The large share of ash, almost entirely sand (Fig. 10), must increase overall larval density distinctly beyond average for insect larvae of their size, thereby reducing risk of being swept away. Even the largest larvae ingested some very fine sand. Maximum ingested grain size increased with larval body size, over a certain range. Apparently, mouth gape limits size of stones that are swallowed. However, the relation of sand size to body size (Fig. 9) and the proportion of ash in total dry weight (Fig. 10) are negatively related to *Isoptena* body length. Clearly, small larvae ingest as much large sand as they can, larger larvae less. This is in line with the supposed ballast function of sand: tiny light larvae are at greater risk of being swept away than strong late instar larvae.

Adults

The natural flight period of *Isoptena* is not well known; Illies (1955) recorded it in May. Our field samples suggest only exceptional individuals might emerge earlier. When we collected it in May, all larvae were in the last instar and emerged within a week.

Non-functional adult mouth parts are a groundplan characteristic of infraorder Systellognatha. However, some small members of superfamily Perloidea secondarily resume adult feeding (Zwick 2000), for example Isoperlinae (Rupprecht 1990) and Chloroperlidae. Adult Chloroperlinae usually fill their guts with conifer and other pollen (Zwick 1973, 1980; Tierno de Figueroa & Sánchez-Ortega 1999) and the weight of *Siphonoperla torrentium* increases considerably within a short period of time (Zwick 1991). Therefore, mainly *Pinus* pollen was offered as potential food. However, captive adults indiscriminately nibbled on cage materials, browsed on leaves, or the cage floor. Nevertheless, upon dissection no gut was well filled.

Oviposition in captivity occurred at dusk and may have been released by special environmental conditions on that day, but the adults may also be generally night active. The variable number of small egg rudiments found in females after oviposition suggests that some individuals were fully mature, others might have developed and laid more eggs, had they lived longer. In any case, total potential fecundity was low; Tierno de Figueroa & Derka (2003) report even lower numbers. Low fecundity is typical of Chloroperlidae (Zwick 1980) and appears to be related to larval life in deep layers of stream substrata (Stewart & Stark 2002) which presumably provides protection against many predators.

One would expect lower fecundity also if individual eggs were relatively large. The small *Cosumnoperla hypocrena* Szczytko & Bottorff (Plecoptera: Perlodidae; C in figure 11; data from the original description) uses this strategy to survive in intermittent water courses; hatching larvae are exceptionally large and can complete development before the stream dries up (R. Bottorff, in a letter).

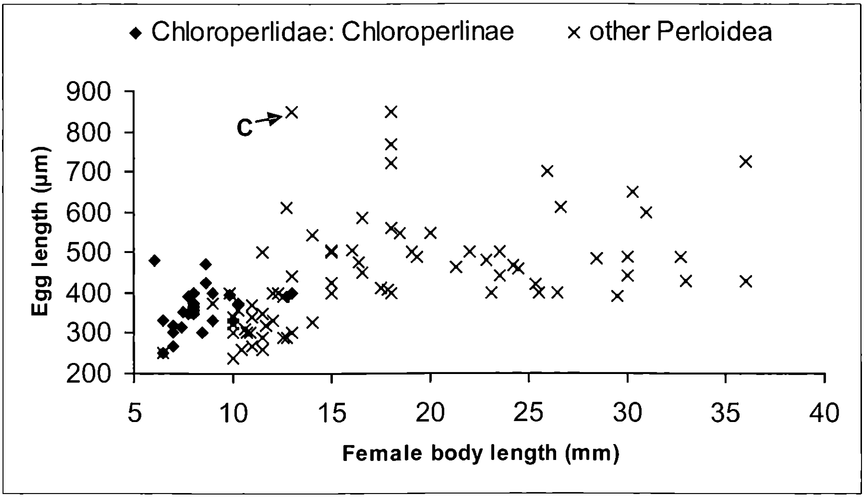

Fig. 11 Female body length and egg length for various Chloroperlidae: Chloroperlinae and other Perloidea; C identifies the *Cosumnoperla*-egg, *Isoptena* is not distinctive. Taxa names and data sources available on request

A different reason apparently determines large egg size in *Dolania* which has the largest of all mayfly eggs: when hatching its carnivorous larva is large and at a size advantage over many potential prey items (Fink & al. 1991). In Chloroperlidae, the same point deserves future attention. Members of superfamily Perloidea agree in distinctive egg structure, and length of the oval eggs is an adequate parameter of egg size. Eggs of the small Chloroperlinae, including *Isoptena*, are relatively large and lie clearly outside the general perloidean trend.Chloroperlid eggs are of similar size as eggs of the distinctly larger Perlodidae: Isoperlinae which are the next smallest Perloidea. In contrast, eggs of the larger Chloroperlidae: Paraperlinae fall into the average Perloidea range (Fig. 11).

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