22 Food-filtering mechanism of the larvae of *Oligoneuriella rhenana* Imhoff (Ephemeroptera: Oligoneuriidae)

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The larvae of Oligoneuriella rhenana *belong to the functional feeding group of filter-feeders. The structure of the mouthparts and the filtering mechanism of the larvae were investigated by scanning electron microscopy and videomacroscopy, respectively. The larvae filter food by exposing setae on the prothoracic legs to the current. The filtered particles are then removed by the setae of the maxillary and labial palps.*

Introduction

In running waters, there are different types of food resources that are available to aquatic insects (e.g., particulate organic matter (POM), attached or drifting algae, leaves etc.). Larvae of Ephemeroptera have evolved several adaptations to use these food resources; the food gathering parts of the larvae are often highly specialized. Using these structures, feeding habits and diet, larvae can be classified into different types of functional feeding groups. Merritt et al. (1984) provide a general classification system of such functional feeding groups for aquatic insects, while Edmunds (1978) gives a functional feeding group classification for the larvae of the North American Ephemeroptera. Information on the type of food and the feeding mechanism of the larvae is the basis of the classification into functional feeding groups. Limited information on feeding ecology prompted our investigations of the feeding habits and food of the larvae of *Oligoneuriella rhenana* Imhoff.

Materials and Methods

Larvae of *O. rhenana* were sampled from May-August 1991 near Flamatt, River Sense, Kanton Fribourg in Switzerland (cf. Hefti and Tomka 1991). The larvae were held in an artificial stream in the laboratory at a water temperature of 17±1°C. The artificial stream has a length of 450 cm, width and height are 30 cm each. Larvae can be observed from underneath through the glass-bottom of the artificial stream. A pump circulates water; the current velocity can be manipulated by changing the slope of the artificial stream. Current velocity was adjusted to 0.2-0.3 m/s, measured above the substrate (consisting of stones of the River Sense). Light was provided by infinitely variable controlled fluorescent lamps and lasted for 12 hours. Dawn and dusk were simulated for 30 minutes each; darkness lasted for 11 hours.

For examining filter-feeding behaviour of the larvae, a Panasonic NV-180 VHS video recorder was used with two observation systems:

1. A Sony high-resolution AVD-C7 video camera in combination with video lenses and extension tubes of various lengths. The larvae were filmed through the glass bottom of the artificial stream. General observations on the feeding habits of the larvae were made using this system.
2. The video camera in combination with a binocular microscope. This system was applied in order to get more detailed information on the feeding behaviour, especially movements of the mouthparts. In this system, the larvae were placed in a special observation cell (Appendix 1).

The bottom of the cell is built using a microscope slide, which is glued to the walls (made of Perspex, 1 cm thick); the lid, also made of Perspex, is fixed to the observation cell by four screws. The cell is made watertight by placing a rubber seal between lid and walls. The so formed free space of the cell is 3 cm in length and 1.4 cm in width and height. The height can be reduced by placing a Perspex cube into the free space of the cell. This cube is held at a chosen height by three thin "feet" made of wire. Through two tubes (cemented into holes in the walls, oriented face to face), water can be circulated through the cell by a pump. The current velocity can be adjusted by two valves, situated at the tubes of the in- and outlet; the chosen current velocity ranged from 0.0-0.4 m/s, all feeding movements took place at a current velocity of about 0.2 m/s.

White light was provided by a cold light source; red light and infrared light were used as well. The source of light made no difference in the movements or habits of the larvae.

For scanning electron microscopy (SEM), the mouthparts of the larvae were critical point dried and platinum coated. The structures were then examined with a Hitachi S-700 SEM.

A total of 37 larvae was observed by the above mentioned methods, and about 30 hours of feeding behaviour were documented on videotape. The feeding behaviour of 20 of these larvae was studied under microscope to obtain detailed information on the movements of the mouthparts during feeding. About 200 feeding cycles were analyzed by single frame advance feature and slow motion, additionally to direct observation.

The observed larvae had a head capsule width from 1.6 mm to 3.2 mm, measured by microscope. Feeding behaviour did not differ among the observed larvae; therefore, no differentiation according to the size of larvae was made.

Results

Habitat of the Larvae

The larvae were found in riffles on the underside or between loosely packed stones; the stones had an average size of about 10 cm. Usually, most of the larvae were found at the river margins, at a water depth of about 20-50 cm and a current velocity of 0.4-0.7 m/s, measured above the substrate.

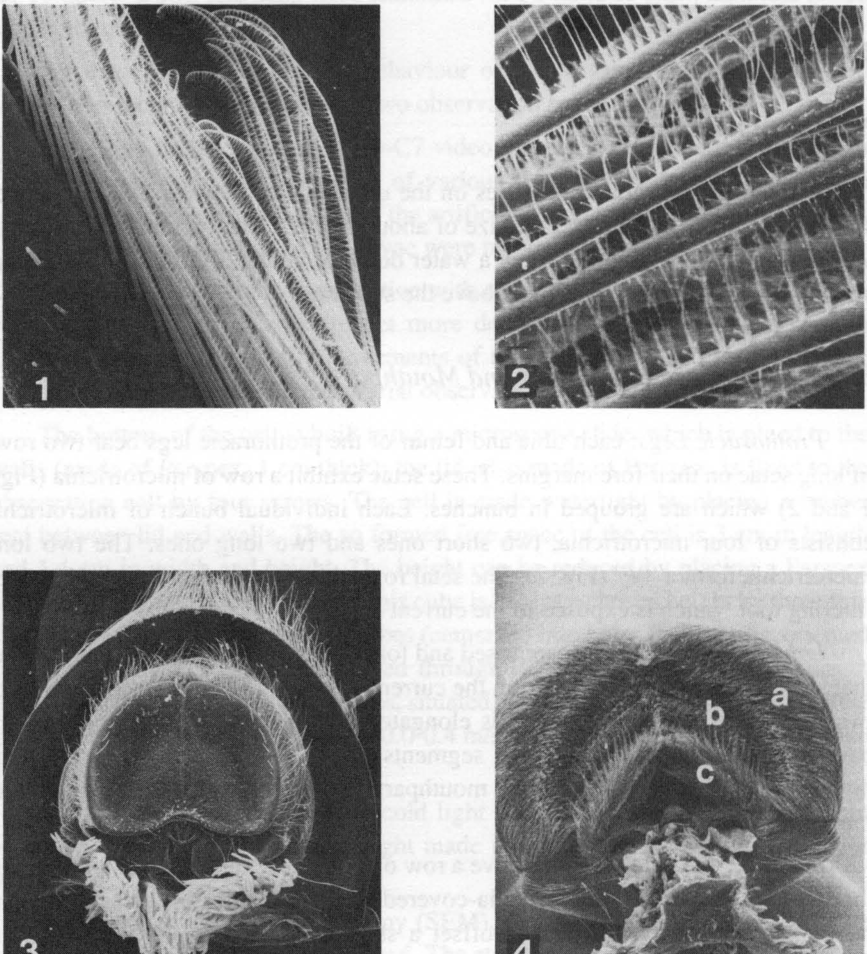
Structure of Prothoracic Legs and Mouthparts

Prothoracic Legs: each tibia and femur of the prothoracic legs bear two rows of long setae on their fore margins. These setae exhibit a row of microtrichia (Figs. 1 and 2) which are grouped in bunches. Each individual bunch of microtrichia consists of four microtrichia, two short ones and two long ones. The two long microtrichia form a "V" (Fig. 2). The setal rows themselves also form a V-shaped filtering tool, which is exposed to the current (Fig. 9).

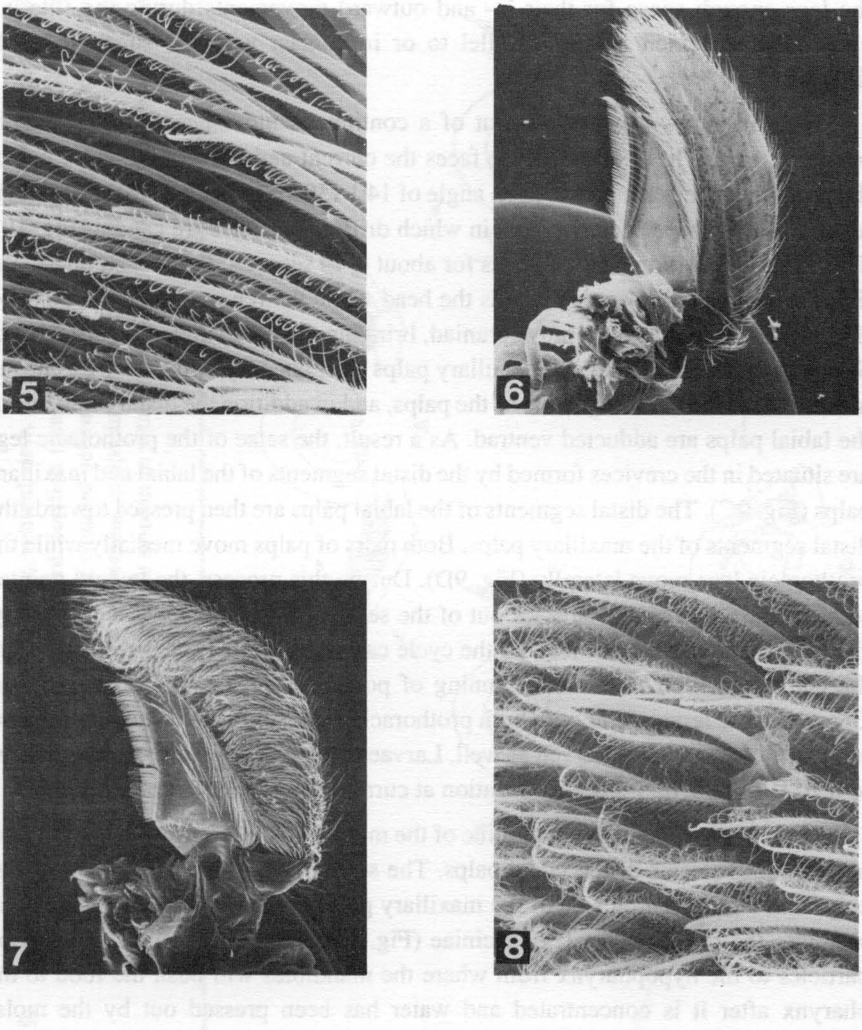
Labium: the paraglossae are fused and form a plate-like structure (labial plate) that shelters the preoral cavity from the current (Fig. 3). The labial palp consists of two segments; the distal segment is elongated and about twice the length of the basal segment (Fig. 4). The distal segments of the labial palps bear long setae directed towards the midline of the mouthparts. These setae also are covered with microtrichia (Fig. 5).

Maxillae: the galea-laciniae have a row of stout setae on their medial margins; there is a second row of microtrichia-covered setae on the dorsal surface that runs parallel to the medial row but is offset a short distance laterally (Fig. 6). The maxillary palp consists of two segments; the distal segment is approximately six to seven times as long as the basal segment (Figs. 6 and 7). The ventral side of the distal segment is covered with long, microtrichia-bearing setae (Figs. 7 and 8); these setae point to the midline of the mouthparts.

Figures 1-4. *Oligoneuriella rhenana*. 1, 2: setae on prothoracic leg; 3: ventral view of the head, labial plate; 4: dorsal view of the labium: labial palps (a), fused paraglossae (labial plate) (b), glossae (c) (scale: 1, 2, 4: 50 µm, 3: 500 µm).



Figures 5-8. ***Oligoneuriella rhenana.*** **5: Setae on the dorsal side of the distal segment of the labial palp; 6: maxillae, dorsal view; 7: maxillae, ventral view; 8: setae on the ventral side of the distal segment of the maxillary palp (scale: 5: 5 µm, 6, 7: 500 µm, 8: 50 µm).**



Filtering Cycle

The larvae filter food particles from the current with the setae on the femora and tibiae of their prothoracic legs. They use the meso- and metathoracic legs to fix themselves to the substrate while filter-feeding. During the filtering process the larvae do not press their body to the substrate. They hold the head and the front of the thorax about 1.5-2 times the height of their head above the substrate; this allows the legs enough space for their in- and outward movements during the filtering cycle. The abdomen is held parallel to or in contact with the substrate while filtering.

Figure 9 shows five points out of a continuous movement. In the filtering position (A), the head of the larvae faces the current and the femora and tibiae of the prothoracic legs are spread at an angle of 140-150°. The setae of the prothoracic legs form a V-shaped filtering tool in which drifting particles are caught (Fig. 10). This filtering position (Fig. 9a) lasts for about 4-10 seconds. In the next phase, the tibiae are adducted inwards towards the head while the femora remain in position (Fig. 9B). The femora then move craniad, bringing the tibiae under the headshield. Simultaneously, the labial and maxillary palps shift laterally outwards by a caudad movement of the basal segments of the palps, and in addition the distal segments of the labial palps are adducted ventrad. As a result, the setae of the prothoracic legs are situated in the crevices formed by the distal segments of the labial and maxillary palps (Fig. 9C). The distal segments of the labial palps are then pressed towards the distal segments of the maxillary palps. Both pairs of palps move medially while the prothoracic legs move laterally (Fig. 9D). During this process, the inward pointed setae of the palps comb particles out of the setae of the prothoracic legs. Finally, filtering position A is reached and the cycle can start again. This movement, from the end of position A to the beginning of position A, takes about 0.5 seconds. Generally, the larvae filter with both prothoracic legs simultaneously, but filtration with only one leg was observed as well. Larvae filtered only at current velocities up to 0.20-0.25 m/s; they stopped filtration at current velocities >0.3 m/s.

Food is transported to the midline of the mouthparts by alternate lateral in- and outward movements of the labial palps. The setae on the dorsal side of the labial palps and on the ventral side of the maxillary palps comb particles by a reciprocal way into the reach of the galea-laciniae (Fig. 11). The galea-laciniae bring food particles to the hypopharynx from where the mandibles will push the food to the pharynx after it is concentrated and water has been pressed out by the molar surfaces (cf. Elpers and Tomka 1992).

Figure 9. ***Oligoneuriella rhenana*, filtering cycle, ventral view. A: filtering position, B: inward movement of prothoracic legs, C: labial and maxillary palps in outward position (maxillary palps not visible), prothoracic legs in maximum inward position: setae of prothoracic legs are situated between dorsal side of labial palps and ventral side of maxillary palps, D: prothoracic legs in outward movement, palps in inward movement: particles, which are caught in the setae of prothoracic legs during filtering cycle remain in setae of palps. B: outward movement; arrows indicate the following movements.**

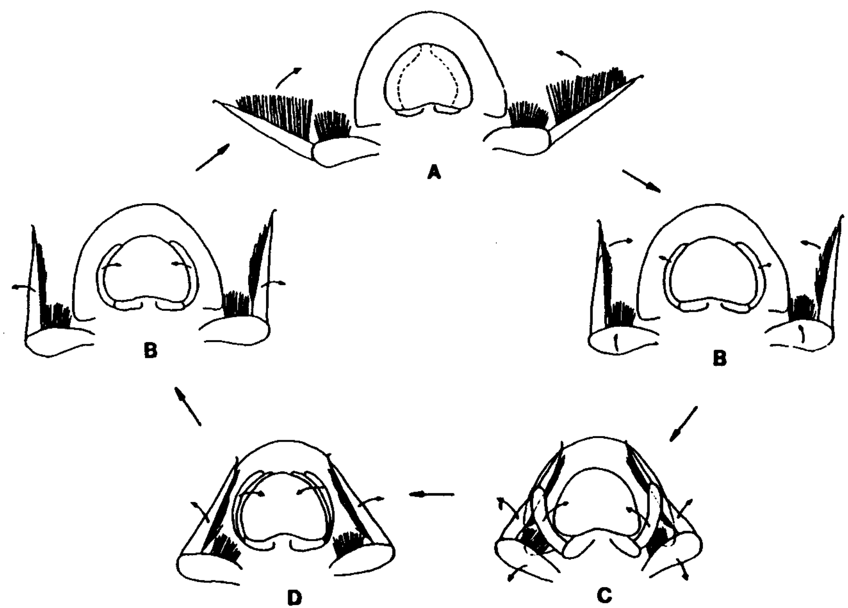
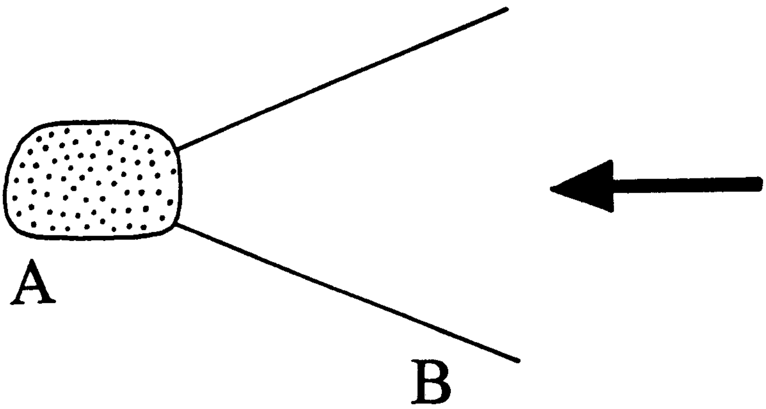


Figure 10. Cross-section of prothoracic tibia of *Oligoneuriella rhenana*. A: tibia, B: setae of tibia (arrow indicates direction of current).



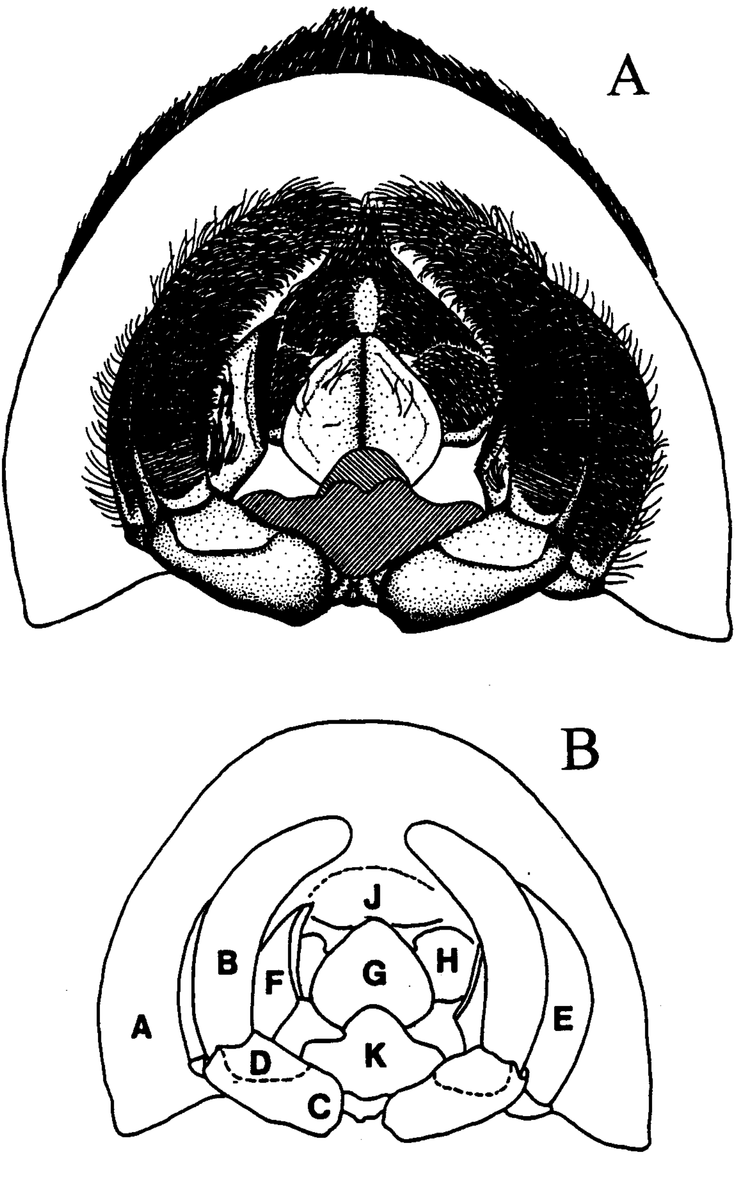
Discussion

The larvae of *Oligoneuriella rhenana* belong to the functional feeding group of passive filterers (Elpers and Tomka 1992). They gather food by using the setae on the prothoracic legs as a filter.

Life underwater presents several problems for feeding in aquatic insects. Arens (1989) mentioned the shielding of the preoral cavity to be one of the problems occurring with feeding in rheophilic insects. The preoral cavity of *O. rhenana* is shielded dorsally by the bell-shaped head capsule and anteriorly by the labrum and the setae on the labrum. The ventral side of the preoral cavity is shielded by the labial plate (Fig. 3). During the filtering cycle (Fig. 10), the head is held at a distance from the substrate. The substrate, therefore, cannot act as a ventral shield during filtration as it does in the feeding of other mayflies such as *Stenacron interpunctatum* or *Rhithrogena pellicuda* (Heptageniidae) (McShaffrey and McCafferty 1986, 1988).

Steinmann (1919) and Sowa (1961) gave another interpretation of the function of the labial plate. They describe the labial plate and the setae of the prothoracic legs as a structure with which the larvae fix themselves to the substrate in fast currents. In their scheme, the setae of the prothoracic legs would first clean the substrate and then the larvae would fix themselves to the cleaned surface by means

Figure 11. A. Ventral view of head of *Oligoneuriella rhenana*, revealing mouthparts. Labial plate has been removed. B. Schematic drawing of A. A: head capsule, B: distal segment of labial palp, C: basal segment of labial palp, D: flattened part of the basal segment of labial palp as recess of the labial plate, E: maxillary palp, F: galea-lacinea, G: lingua of hypopharynx, H: superlingua of hypopharynx, J: labrum, K: base of the labial plate.



of the labial plate. We could find no evidence for this assumption. Our observations indicate that the function of the labial plate and the setae of the prothoracic legs is only associated with the filter feeding habits of the larvae.

There is a remarkable correspondence in the structure of the prothoracic legs and the feeding behaviour of larvae of *Isonychia* spp. (Wallace and O'Hop 1979; Grant and Stewart 1980; Braimah 1987a) and *O. rhenana*. Larvae of both genera take up food as filter-feeders. *Isonychia* sp. faces the current and spreads the prothoracic legs to the current in the same way as *O. rhenana*. The current separates the two rows of long setae on prothoracic femora and tibia in a V-shaped manner (Grant and Stewart 1980). A difference is seen in the way the legs are flexed towards the mouthparts in order to comb out filtered particles. Larvae of *Isonychia sicca* always flex their legs alternately (Grant and Stewart 1980), whereas larvae of *O. rhenana* usually flex the legs simultaneously; but movements and filtration with only one leg are possible as well.

In a detailed study, Braimah (1987b) discussed the mechanism of filter-feeding of *Isonychia campestris*. He observed a stagnant zone of no flow between the first and second row of rays of the prothoracic legs at low current velocities (0.2 m/s). He also showed ways in which *Isonychia campestris* can filter particles even smaller than the mesh size formed by the setae and microtrichia of the prothoracic legs. *Oligoneuriella rhenana* filters particles only at current velocities less than 0.25 m/s. According to the similarity of the structure of prothoracic legs, feeding behaviour and preferred current velocity during filtration, we can assume that the filtering mechanisms that Braimah (1987b) postulated for *Isonychia campestris* are applicable to *O. rhenana* as well. The filtering mechanisms include direct interception, inertial impaction, gravitational deposition, diffusion or motile-particle deposition and adhesion of particles to filters. Whether *O. rhenana* takes up food particles smaller than the mesh size of the prothoracic filter indicates will be investigated.

Stenacron interpunctatum (Heptageniidae) may change its feeding behaviour to gatherer or filterer depending upon the predominant food resources (McShaffrey and McCafferty 1986). The observations on feeding habits of *O. rhenana* were made on older larval stages. These stages feed exclusively by the filter-feeding methods described above. The first larval stages of *Isonychia sicca* (Oligoneuriidae) feed as scrapers, whereas the older stages take up food as filter-feeders (Grant and Stewart 1980). The structure of mouthparts and prothoracic legs of first, second and older larval stages of *O. rhenana* differ only slightly. In the older stages, the labial plate is larger whereas in the first stages the labial palps are the dominant structure (Grandi 1947). We could not observe feeding habits of the first stages, so it remains unknown whether the difference in size of the mentioned mouthparts affects the way the larvae gather food.

Acknowledgements

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References

Arens, W. 1989. Comparative functional morphology of the mouthparts of stream animals feeding on epilithic algae. *Arch. Hydrobiol./Suppl.* 83: 253-354.

Braimah, S.A. 1987a. Pattern of flow around filter-feeding structures of immature *Simulium bivittatum* Malloch (Diptera: Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera: Oligoneuriidae). *Can. J. Zool.* 65: 514-521

Braimah, S.A. 1987b. Mechanisms of filter-feeding in immature *Simulium bivittatum* Malloch (Diptera: Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera: Oligoneuriidae). *Can. J. Zool.* 65: 504-513.

Edmunds, G.F., Jr. 1978. Ephemeroptera. P. 67-76 in R.W. Merritt and K.W. Cummins (Eds.), *An Introduction to the Aquatic Insects of North America.* Dubuque: Kendall/Hunt.

Elpers, Ch. and I. Tomka. 1992. Struktur der Mundwerkzeuge und Nahrungsaufnahme bei den Larven von Oligoneuriella rhenana Imhoff (Ephemeroptera: Oligoneuriidae). *Bull. Soc. Entomol. Suisse.* 65: 119-139.

Grandi, M. 1947. Contributi allo studio degli efemeroidei italiani 9. *Oligoneuriella rhenana* IMH. *Boll. Ist. Entomol. Univ. Bologna* 16: 176-218.

Grant, P.M. and K.W. Stewart. 1980. The life history of *Isonychia sicca* (Ephemeroptera: Oligoneuriidae) in an intermittent stream in North Central Texas. *Ann. Entomol. Soc. Am.* 73: 747-755.

Hefti, D. and I. Tomka. 1991. Mayfly communities in a prealpine stream system of Switzerland. *Aquatic Sciences* 53: 20-38.

McShaffrey, D. and W.P. McCafferty. 1986. Feeding behavior of *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). *J. N. Am. Benthol. Soc.* 5: 200-210.

McShaffrey, D. and W.P. McCafferty. 1988. Feeding behavior of *Rhithrogena pellicuda* (Ephemeroptera: Heptageniidae). *J. N. Am. Benthol. Soc.* 7: 87-99.

Merritt, R.W., K.W. Cummins and T.M. Burton. 1984. The role of aquatic insects in the processing and cycling of nutrients. P. 134-163 in V.H. Resh and D.M. Rosenberg (Eds.), *The Ecology of Aquatic Insects.* New York: Praeger.

Sowa, R. 1961. *Oligoneuriella mikulskii* n. sp. (Ephemeroptera). *Acta Hydrobiol.* 3: 287-294.

Steinmann, P. 1919. Zur Kentnis der Eintagsfliege *Oligoneuria rhenana. Mitt. Aarg. Nat. Ges.* 60-66.

Wallace, J. B. and J. O'Hop. 1979. Fine particle suspension-feeding capabilities of *Isonychia* spp. (Ephemeroptera: Siphlonuridae). *Ann. Entomol. Soc. Am.* 72: 353-357.

Appendix 1. Observation cell (A: top view; B: lateral view; C: front view). 1 and 4: Perspex walls, 2: screw thread, 3: water tubing 5: lid, 6: rubber seal, 7: microscope slide (scale 1 cm).