Fine Particle Suspension-Feeding Capabilities of *Isonychia* spp. (Ephemeroptera: Siphlonuridae)[1]

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ABSTRACT

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Stream-inhabiting mayflies belonging to the genus *Isonychia* possess a well-developed fringe of long setae on their forelegs which is used to filter particulate materials suspended in the water column. In this paper we report on a mechanism, composed of 2 different types of microtrichia, that latches the long filtering setae to adjacent setae. This coupling mechanism results in an effective pore size of 0.1–0.7 μm for portions of the filtration device. Both ultrastructure of the filtering mechanism and foregut particle size analysis indicate that *Isonychia* are capable of feeding on much smaller particles than previously suspected.

Isonychia spp. are 1 of 4 ephemeropteran genera in North and Central America that are generally regarded as suspension or filter feeders (Edmunds et al. 1976). Nymphs inhabit flowing waters of streams of various sizes and, while strong swimmers, they are usually found on various stable debris and substrate in streams (Clemens 1917, Berner 1959).

Clemens (1917) has, by far, the most extensive treatment of *Isonychia* biology (as *Chirotenetes*). Nymphs have a well-developed fringe of elongate setae on their forelegs which is used to filter particulate materials from flowing waters (Clemens 1917, Berner 1959, Leonard and Leonard 1962, Edmunds et al. 1976). Food habits of *Isonychia* reported in the literature indicate that the nymphs are omnivorous (e.g., Clemens 1917 and Coffman et al. 1971) or primarily detritivores (Shapas and Hilsenhoff 1976). A number of authors (e.g., Cummins 1973) have suggested that food particle size may be an important means of delineating feeding habits of aquatic insects.

In this paper we describe several morphological features which indicate that *Isonychia* nymphs have the capability of both sieving and feeding upon much smaller particles than previously considered. We also present evidence, from particle sizes measured in the foregut, that these animals ingest primarily very fine particulate detritus.

Materials and Methods

Nymphs were examined from three different drainage basins in the southeastern United States. These were as follows: the Chattahoochee River at Helen, White Co., Ga. (Chattahoochee-Apalachicola Basin); Shope Creek, Coweeta Hydrologic Laboratory, Macon Co., N. C. (Tennessee-Mississippi Basin); and Barton Creek, Oconee Co., S. C. (Savannah River Basin). No specific identification was possible on the nymphs used in this study since satisfactory specific keys for nymphs of *Isonychia* spp. do not exist (Edmunds et al. 1976). However, some morphological evidence, based on ultrastructure of the filtering device is presented later that suggests at least 3 species were examined.

The external structures used in feeding were examined by scanning electron microscopy (SEM). Specimens were subjected to critical point drying procedures described by Anderson (1951) using 100% ethanol as the initial fluid and liquid CO_2 as the transition fluid. Specimens were then mounted on aluminum stubs and vacuum coated with palladium gold (40:60) alloy prior to SEM examination.

Foregut content analyses were conducted on specimens from 2 localities. Nymphs were preserved in Kahle's Fluid and gut contents were extracted within 3 days of collecting to avoid deterioration resulting from storage. Gut contents were analyzed using 2 different methods. The 1st method involved a membrane filtration and sketching technique using a compound light microscope and described previously (Wallace 1975). Since many of the particles contained in *Isonychia* guts were extremely small, a 2nd method, employing the SEM and a Ladd Digitizer was also used. This latter procedure was identical to that described by Malas and Wallace (1977) except a Nucleopore filter (0.45 μm pore size) was used for the filtration device.

Results and Discussuion

Filtration Device

The filtering device used by *Isonychia* spp. can be seen in Fig. 1 and 2. As the animals orient facing into the current the setiferous forelegs are extended to sieve particles from flowing waters (Clemens 1917). The foretibiae and -femora of *Isonychia* possess a double row of long setae on their inner margin (Fig. 1 and 2). The very long setae which comprise the anterior row, either touch or extensively overlap adjacent setae apically. SEM examination revealed a dense row of minute, elongate setae (microtrichia) projecting from the margin of each of the large anterior filtering setae (Fig. 3 and 4, mtr). Microtrichia are absent for ca. the basal ⅕ of these setae. On each large filtering seta there is a 2nd series of smaller, hooked or slightly curved microtrichia (Fig. 3, 4, mtr h). This 2nd series is located on the margin of the large filtering seta opposite that occupied by the elongate microtrichia (Fig. 3, 4). In many areas (e.g., Fig. 5–7) the elongate microtrichia of one large filtering seta can be observed latched to the smaller, curved microtrichia of the adjacent large seta. Frequently, series of large filtering setae can be seen latched together by this mechanism (Fig. 5). This suggests that the larger setae are actually secured to adjacent setae by an effective coupling mechanism composed of the 2 different types of microtrichia. This coupling mechanism

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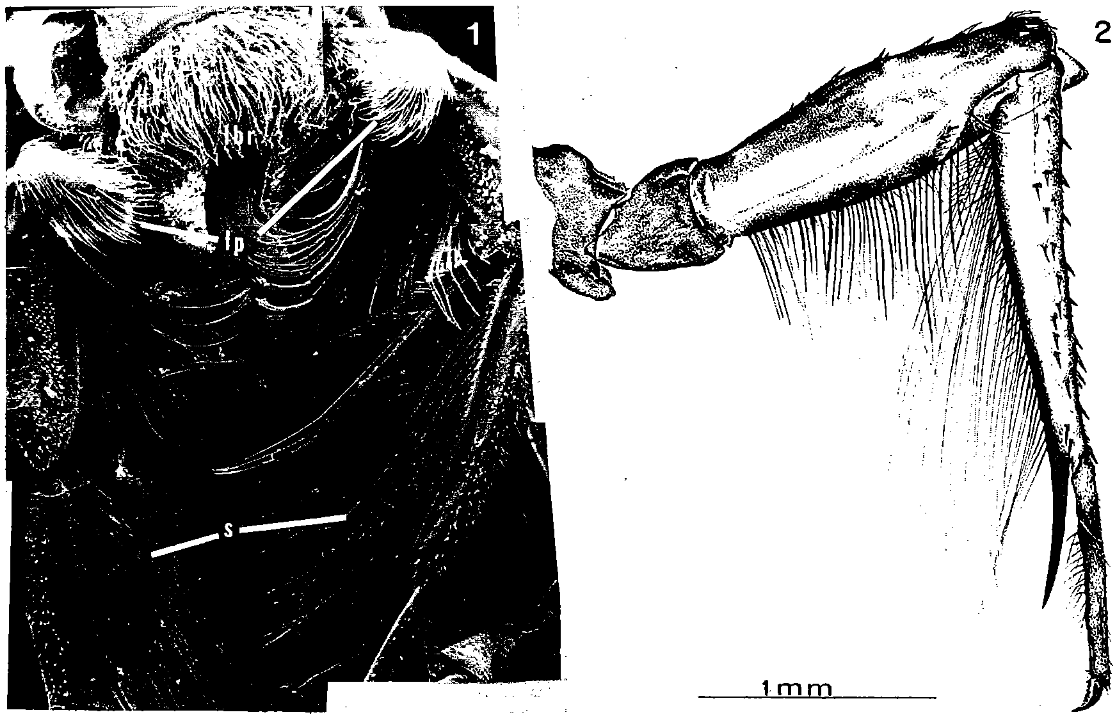

FIG. 1–2.—1. Composite photomicrograph, frontal view, of *Isonychia* (SEM at ca. 30X); filtering setae of forelegs (s); labial palps (lp) and labrum (lb). 2. Left foreleg of same showing the long filtering setae on the inner margin.

results in a much smaller pore size for the filtration device in *Isonychia* than previously suspected. The largest opening found in areas where the large setae were interlocked was less than 0.7 μm. This also suggests that *Isonychia* spp. have the capability of sieving much smaller particles from flowing waters than previously realized.

Unlike the anterior row of long setae on the fore-tibiae and -femora, the 2nd and shorter row of setae possesses no interlocking microtrichia. Their structure and arrangement suggests that this 2nd row of setae possibly serves 2 functions: as supports for the anterior, interlocking setae as the forelegs are extended into the current; and to keep the elongate, anterior row of setae in alignment with adjacent anterior row setae and therefore allow the 2 types of foresetal microtrichia to latch with those of adjacent setae (Fig. 5–7).

Both Clemens (1917) and Leonard and Leonard (1962) report that when feeding, the forelegs of *Isonychia* are periodically brought within reach of the labial and maxillary palps. Their labrum, maxillae, hypopharynx and portions of the labium bear numerous setae which undoubtedly aid in the removal of particles filtered onto the foreleg setae. Some setae on the fused galea-lacinia and palps of both the maxillae and labium bear microtrichia. Those of the latter have both microtrichia and microtrichial hooks (Fig. 8). Possession of both types of microtrichia indicate that these setae reduce the loss of fine particles from the mouthparts. The mandibles contain extensive molar areas which undoubtedly generate some of the small particles in the foregut; however, the sieving capabilities and the numerous setae associated with the mouthparts cannot be ignored.

Variations in Ultrastructure

Examination of the foreleg setal fringe of several *Isonychia* from three different drainage basins shows that each of the localities has specimens that have slight differences in the ultrastructure of microtrichia (cf. Fig. 5–7). However, from each locality, specimens have large filtering setae that are capable of interlocking with those of adjacent setae. Although we are not certain of the species involved from the various locations, these preliminary observations may indicate that microtrichial arrangements may have some value as a taxonomic tool.

Gut Analyses

Results of analyses of over 8000 individual particles contained in the foreguts of 2 mature nymphs are shown in Table 1. These results were obtained using the compound light microscope (at 100X magnification) sketching-cutting technique. Fine detritus was by far the most abundant material encountered in terms of both numbers of particles and on a projected area basis. Recognizable vascular plant detritus, diatoms and animal material composed less than 12% of the foregut contents (Table 1). Algae and animal material were less abundant than that reported by some authors (e.g., Clemens 1917, Coffman et al. 1971). Shapas and Hilsenhoff (1976) measured gut contents, using a projected area method, of 22 specimens of *Isoncyhia* spp. from Wisconsin and reported a composition of 97% detritus and 3% algae. These latter results are remarkably consistent with those data in Table 1 from the Tallulah River specimens.

Gut particle sizes measured with the SEM at 1000X resulted in much smaller mean particles sizes than those measured with the compound light microscope at 100X.

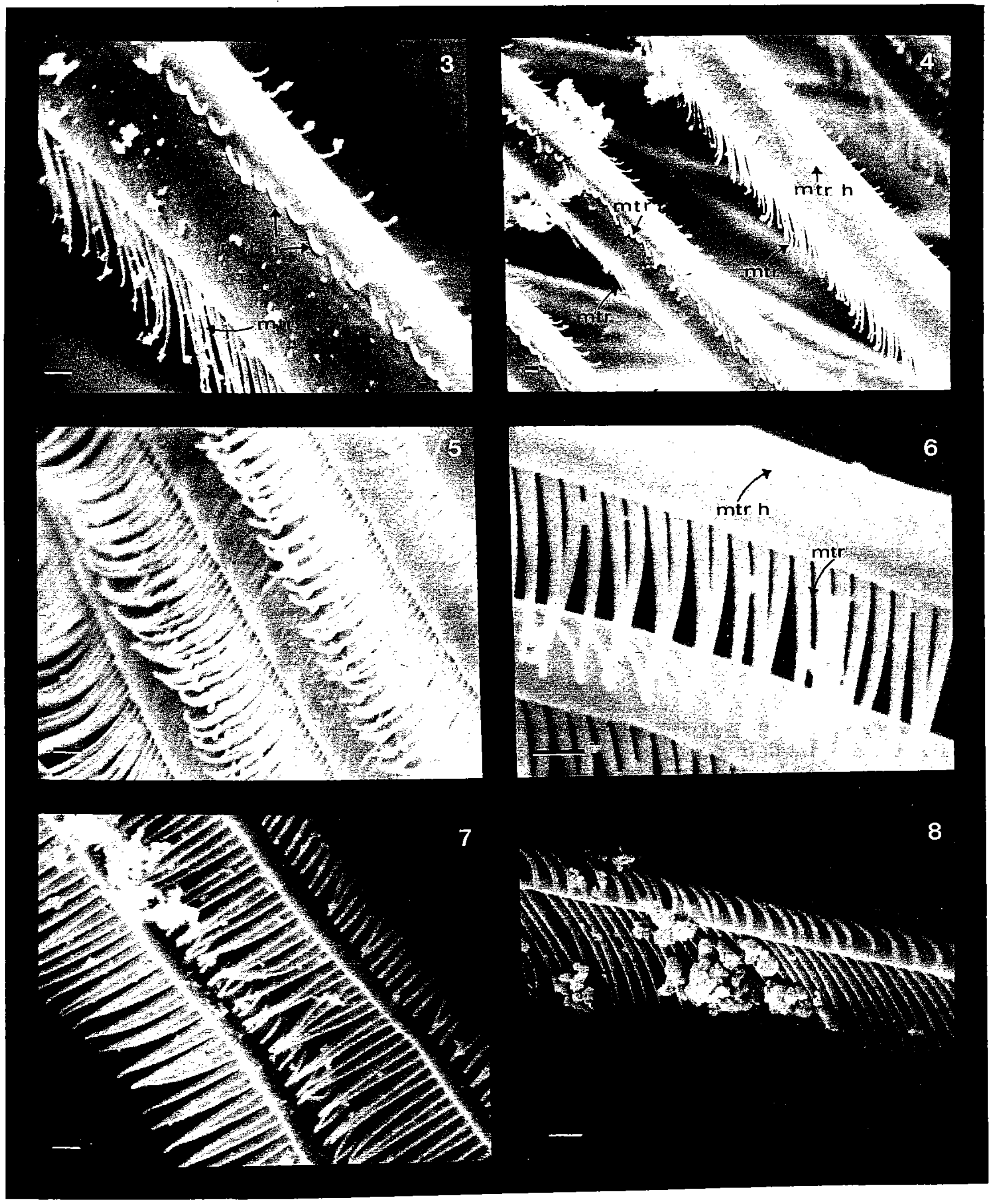

FIG. 3–8.—SEM photomicrographs of *Isonychia* seta. 3. Foreleg filtering seta of specimen from Chattahoochee River at Helen, Ga. (5500X). Note 2 types of microtrichial arrangement, microtrichia (mtr) and hook-like microtrichia (mtr h); 4. Same showing several large filtering setae (2200X); 5. Same showing several large adjacent filtering setae coupled by microtrichia (5600X); 6. Same of *Isonychia* sp. from Oconee Co., S.C. (10,250X); 7. Same of specimen from Coweeta Hydrologic Lab., Macon Co., N.C. (5500X); and 8. Seta from the labial palp of *Isonychia* sp. from Coweeta Hydrologic Lab. Note the latter also has 2 distinct types of microtrichia (6500X). Scale line at lower left margins = 1 μm.

About 67% of the particles by number were smaller than 50 μm^2 (Fig. 9). A number of particles smaller than 1 μm^2 were seen on the membrane filter during SEM examination. These were not included in the above figures since they could not be measured using the digitizer procedure.

Rubenstein and Koehl (1977) suggested that a number of filter-feeding animals may rely on mechanisms other than direct particle interception (or sieving) as a means of food capture. LaBarbera (1978) presented evidence that the brittle star, *Ophiopholis aculeata* (L.), may capture particles much smaller than the space between the

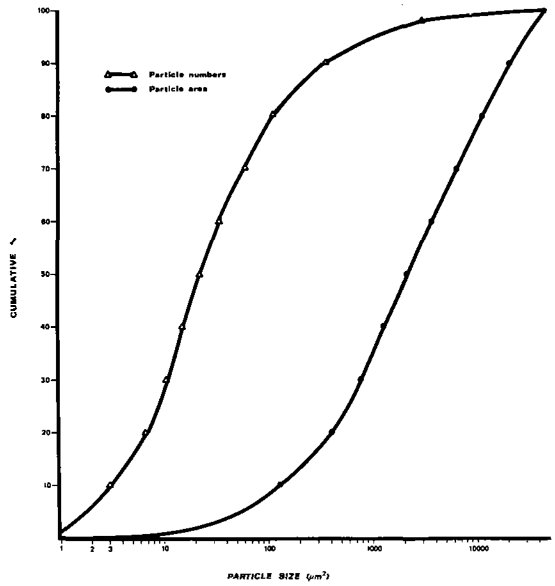

FIG. 9.—Foregut content particle sizes of *Isonychia* sp. from Coweeta Hydrologic Lab., Macon Co., N.C. Curve delineated by triangles represents cumulative percentage of particles by numbers in the various size categories. Line denoted by circles represents cumulative percentage of particles by area. Number of particles measured = 178. Note: particle sizes below 1 μm^2 are not included in the above figure.

filtering structures. Regardless of other mechanisms, the remarkable situation with *Isonychia* spp. is that direct sieving alone may afford these animals great latitude in particle size capture capabilities. Evidence from gut analyses obtained here and elsewhere (e.g., Clemens 1917, Coffman et al. 1971, Shapas and Hilsenhoff 1976) supports this contention. The filtration device in *Isonychia* spp. suggests that these animals are capable of capturing a wide range of particle sizes since, in addition to the minute pores formed by the microtrichia (Fig. 5–7), there are also many areas where the microtrichia are not interlocked between the long filtering setae (Fig. 4). This latter condition would produce a much larger pore size in the filtration device. Furthermore, there is the intriguing unanswered question: Can *Isonychia* spp. actively alter their pore size?

Conclusions

Edmunds et al. (1976) suggest that *Isonychia* evolved early in geological time. The results presented here suggest that one reason for the success of this group may be their ability to utilize the most abundant food resource (in quantity, not necessarily quality) in streams. Wallace and Malas (1976) suggest that a similar evolutionary pattern existed in the "primitive" Philopotamidae of the Trichoptera. They also suggest that few groups, excluding the Simuliidae (Diptera), could exploit such small particles suspended in the water column. Analysis of filtering mechanisms, ultrastructure and foregut particle size, supports the contention that *Isonychia* are capable of capturing very fine particulate organic matter (VFPOM). Although little information exists on the quality of VFPOM as a food source in streams, J. Brock (pers. comm.) found much higher respiration rates on the smallest size fraction (0.5–52 μm) of particulate organic matter in Amazon River transport.

Sweeney (1978) found a direct influence of temperature on the development rate of *Isonychia bicolor* (Walker) nymphs. In his study, maximal development rate was associated with increased diel temperature pulses. The unassessed possibility exists that temperature may influence microbial activity, and thus affect the quality of food available to *Isonychia*. Based on results suggested by this study, *Isonychia* may readily harvest microbial materials from the water column.

The filtering mechanism of *Isonychia* may also be capable of intercepting colloidal particles. In this study we have observed the pore size may have up to 1.0-μm openings in places where the microtrichia are latched. However, most of these openings are considerably smaller (0.1–0.7 μm). As the colloidal size range can include particles with diameters between 0.021–1.0 μm (Lock et al. 1977), *Isonychia* may be able to harvest colloidal particles from suspension. Wotton (1976) provides evidence that a blackfly larva (Diptera: Simuliidae) is able to feed upon colloidal materials. However, the extent to which *Isonychia*, or any other filter-feeding insect, harvest colloidal materials in nature is unknown.

Acknowledgment

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REFERENCES CITED

Anderson, T. F. 1951. *Techniques for the preservation of three dimensional structures in preparing specimens for electron microscope.* Trans. N. Y. Acad. Sci. 13: 13–134.

Table 1.—Distribution of foregut contents by food category of 2 mature *Isonychia* sp. nymphs from the Tallulah River, White Co., Ga., on June 24, 1976. Percentages are based on a projected area basis of individual particles.

Food type	No. of pieces measured	% by no. of particles	Total area of particles (μm^2)	% by projected area
Animal particles	4	0.05	19,959	0.48
Vascular plant detritus	34	0.41	372,943	9.05
Fine detritus	7,826	95.14	3,688,241	89.48
Diatoms	362	4.40	40,726	0.99
Total	8,226	100.00	4,121,869	100.00

Berner, L. 1959. A tabular summary of the biology of North American mayfly nymphs (Ephemeroptera). Bull. Fla. St. Mus. Bio. Sci. 4: 58 pp.

Clemens, W. A. 1917. An ecological study of the mayfly *Chirotenetes*. Univ. Toronto Stud. Biol. Ser. 17: 1–43.

Coffman, W. P., K. W. Cummins, and J. C. Wuycheck. 1971. Energy flow in a woodland stream ecosystem. I. Tissue support trophic structure of the autumnal community. Arch. Hydrobiol. 68: 232–76.

Cummins, K. W. 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. 18: 183–206.

Edmunds, G. F. Jr., S. L. Jensen, and L. Berner. 1976. The Mayflies of North and Central America. Univ. Minn. Press, Minneapolis. 330 pp.

LaBarbera, M. 1978. Particle capture by a Pacific brittle star: experimental test of aerosol suspension feeding model. Sci. 201: 1147–9.

Leonard, J. W., and F. A. Leonard. 1962. Mayflies of Michigan trout streams. Cranbrook Inst. Sci. Bull. 43: 139 pp.

Lock, M. A., P. M. Wallis, and H. B. N. Hynes. 1977. Colloidal organic carbon in running waters. Oikos 29: 1–4.

Malas, D., and J. B. Wallace. 1977. Strategies for coexistence in three species of net-spinning caddisflies (Trichoptera) in second order southern Appalachian streams. Can. J. Zool. 55: 1829–40.

Rubenstein, D. I., and M. A. Koehl. 1977. The mechanisms of filter-feeding: some theoretical considerations. Am. Nat. 111: 981–94.

Shapas, T. J., and W. L. Hilsenhoff. 1976. Feeding habits of Wisconsin's predominant lotic Plecoptera, Ephemeroptera, and Trichoptera. Great Lakes Entomol. 9: 175–88.

Sweeney, B. W. 1978. Bioenergetic and developmental response of a mayfly to thermal variation. Limnol. Oceanogr. 23: 461–77.

Wallace, J. B. 1975. Food partitioning in net-spinning Trichoptera larvae: *Hydropsyche venularis, Cheumatopsyche etrona* and *Macronema zebratum* (Hydropsychidae). Ann. Entomol. Soc. Am. 68: 463–72.

Wallace, J. B., and D. Malas. 1976. The fine structure of capture nets of larval Philopotamidae (Trichoptera): with special emphasis on *Dolophilodes distinctus*. Can. J. Zool. 54: 1788–802.

Wotton, R. S. 1976. Evidence that blackfly larvae can feed on particles of colloidal size. Nature (London) 261: 697.

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