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FOOD AQUISITION BEHAVIOR OF PYCNOPSYCHE GUTTIFER (LIMNEPHILIDAE: TRICHOPTERA) PTERONARCYS PICTETTI (PTERONARCIDAE: PLECOPTERA) AND ORCONECTES PROPINQUIS (DECAPODA: CRUSTACEA).

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

GAIL L. MOTYKA

1983

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ABSTRACT

FOOD AQUISITION BEHAVIOR OF PYCNOPSYCHE GUTTIFER (LIMNEPHILIDAE: TRICHOPTERA) PTERONARCYS PICTETTI (PTERONARCIDAE: PLECOPTERA) AND ORCONECTES PROPINQUIS (DECAPODA: CRUSTACEA).

By

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Laboratory experiments were designed to discern whether two aquatic detritivores Pycnopsyche guttifer and Pteronarcys pictetti are capable of using chemical cues alone to find their food. These insects were given sterile and microbially colonized leaf discs and allowed to feed for 2-4 days. Both P. guttifer and P. pictetti fed significantly more on the microbially colonized leaf discs. Flow through chamber experiments required the insects to find the more acceptable leaf discs without contacting or seeing the discs. Behaviors were quantified as average percent time spent per port. No significant differences were found between time spent at stimulus ports (microbially colonized discs, leaf extracts or fungal extracts) than at control ports (sterile discs or distilled water respectively). Immature Oronectes propinquis were used as a positive control since crayfish are known to respond to chemicals from food sources. O. propinquis spent significantly more time at stimulus (crayfish juice) than control ports (distilled water). Preliminary experiments concerning the effects of light, starvation periods, acclimation periods, and stimulus strength are discussed.

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By

Gail L. Motyka

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To those who promote the quality of education.

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INTRODUCTION

Deciduous woodland stream ecosystems rely primarily on autumnal leaf input as an energy base (Hynes 1970, Hall 1971, Fisher & Likens 1973, Cummins 1974). Before man's alteration of the environment, most streams were heavily shaded by riparian vegetation, favoring the evolution of communities capable of subsisting on allochthonous input (Hynes 1963).

Autumn shed leaves undergo a series of biological and abiological transformations after entering a stream; as a result, the leaves are more readily eaten (or more acceptable, see Miller & Strickler (1983) for definition of behavioral terms) and nutritious to benthic invertebrates (Barlocher & Kendrick 1981, Cummins & Klug 1979). After the initial leaching of the leaf, a succession of bacteria and fungi colonize and gradually decompose the leaf. The microbes are presumed to be nutritionally important since most detritivores are unable to survive on uncolonized leaves (Kostalos & Seymour 1976, Kaushik & Hynes 1971, Rossi & Fano 1979). This inability to survive might be attributed to benthic invertebrates' lack of digestive enzymes effective in the degradation of structural carbohydrates (Monk 1977).

Few aquatic invertebrates have been found to produce enzymes active against native crystalline cellulose; however, all could degrade basic subunits of cellulose such as cellobiose (Monk 1977, Bjarnov 1972, Kristensen 1972). Barlocher (1982) proposed that fungal enzymes capable of degrading recalcitrant substances such as cellulose, may supply subunits of these compounds which are readily degraded by invertebrate enzymes, allowing assimilation of otherwise undigestable leaf compounds. Thus, the extent of colonization by microbes would control the availability of assimilable compounds.

Several laboratory studies have correlated leaf species and the degree of microbial colonization of leaf litter with food acceptance by selected shredders (detritivores that feed on coarse particulate organic matter) (Table 1).

Also leaf species without microflora, have been ranked according to their acceptability to various shredders. Kaushik and Hynes (1971) offered elm, maple, alder, oak and beech leaves to the amphipods, Hyallela, Gammarus and the isopod, Asellus (an isopod). Elm was the most acceptable species and was subsequently omitted from the next preference experiment. This procedure was repeated until an ordered array of leaf acceptabilities was completed. Wallace et al (1970) presented 3 sets of 5 different species of leaves to Peltoperla maria NEEDHAM and SMITH (Plecoptera) and recorded the amount of leaf material eaten. Alder, dogwood, sourwood and elm were the most acceptable species (Table 1). Unfortunately, these investigations did not consider the fungal biomass which probably varied over the range of species used. The tested invertebrates may have been responding to differences in biomass and not to chemical or physical differences in the fungal species.

Other investigations concerned shredder acceptance of leaves colonized with variable amounts or types of microbiota. Kostalos and Seymour (1976) treated leaves with antibiotics to discriminate between the effects of bacteria and the effects of fungi on leaf acceptability. Gammarus minus SAY (Amphipoda) consumed more fungus enriched leaves and microbially colonized leaves with reduced bacteria than leaves with a natural milieu of microbes. In a subsequent test, G. minus spent much more time feeding on naturally colonized leaves than sterile or bacteria enriched leaves. Kaushik and Hynes (1971) allowed G. lacustris limnaeus SMITH to feed on autoclaved, antibiotic treated,

TABLE 1. Synopsis of food acceptability experiments.

SPECIES	ORDER	DEGREE OF ACCEPTABILITY	MICROBIAL COLONIZATION	REFERENCE
Asellus communnis	Isopoda	elm > maple > alder > oak > beech	no	Kaushik & Hynes 1971
Hyallela azteca	Amphipoda	conditioned > sterile > leaves	yes	
Gammarus lacustris limnaeus	Amphipoda			
Peltoperla maria NEEDHAM & SMITH	Plecoptera	elm, sourwood & alder most preferred, rhododendron, white pine & white oak least	no	Wallace et al 1970
Gammarus minus SAY	Amphipoda	conditioned > sterile leaves	yes	Kostalos & Seymour 1976
Pycnopsyche gentilis	Trichoptera	conditioned > bacterial > sterile	yes	Makay & Kalff 1973
Pycnopsyche luculenta	Trichoptera			
Tipula abdominalis	Diptera	surface > leaves > buried (terr.)	yes	Herbst 1980, 1982
Pycnopsyche guttifer WALKER	Trichoptera	silver maple > cottonwood		
Gammarus pseudolimnaeus BOUSFIELD	Amphipoda	ash > maple > oak	no	Barlocher & Kendrick 1973
		fungal colonies > uncolonized maple		

and microbially colonized leaves. The colonized leaves were most acceptable, but the meaning of this result was unclear since the effect of autoclaving and antibiotic treatment on the leaves was unknown. In order to avoid these complications, they set up an acceptability experiment using leaves grown without nutrients at 10°C and leaves supplemented with nitrogen and phosphorous at 20-22°C. Nitrogen-and phosphorous-enriched leaves were most acceptable since they sustained more abundant microflora relative to the unenriched leaves. Another study related the degree of colonization to leaf consumption by comparing leaves incubated on the surface of the stream bottom and leaves buried in the stream substrate (Herbst 1980). Surface incubated leaves were more acceptable than buried leaves since they decompose much more rapidly and therefore supported a greater population of microbes than did the buried leaves. Other investigators found that shredders fed upon microbially colonized leaves more than sterile or uncolonized leaves (Kostalos & Seymour 1976, Mackay & Kalff 1973, Kaushik & Hynes 1971, Herbst 1980). Barlocher and Kendrick (1973) reduced the question further by testing fungal species. They presented 11 ash leaf discs, each inoculated with a different fungal species, and one sterile control disc to 10 Gammarus which fed on the discs for one day. Leaf discs colonized at 17°C by terrestrial hyphomycetes were more acceptable than those with aquatic hyphomycetes; although, when discs were inoculated and incubated at 0°C, the results were reversed. They concluded that different fungal species affect feeding differentially. All tested shredders were somehow able to distinguish between the different food substrates. None of the studies suggested any behavioral mechanism for the detection of differences between leaves and their state of colonization. Barlocher and Kendrick (1973) suggested that some

chemical or physical differences may exist between leaves which directs their preferential feeding habits.

It is plausible that compounds released from leaves during decomposition could act as cues, leading shredders to microbially colonized leaves. Suberkropp et al (1976) followed the loss of soluble reducing sugars, polyphenols, hemicellulose, lignin, lipid, and nitrogen from leaves during decomposition in a stream. They later demonstrated differential enzyme activity and microbial biomass during leaf decomposition (Suberkropp & Klug 1979).

Electrophysiological and behavioral studies have demonstrated the olfactory and gustatory capabilities of numerous aquatic organisms. Lobsters have aesthetascs (typical decapod chemosensilla) on their antennules which function primarily as olfactory chemosensilla while receptors on mouthparts respond largely to gustatory stimuli. The aesthetascs of spiny and clawed lobsters are highly sensitive to amino acids, particularly taurine, but are much less responsive to proteins, carbohydrates and fatty acids (Phillips et al 1980). Food finding behavior in crabs can be elicited by stimulating the unbranched antenullar aesthetascs with fish juice (Warner 1977). The fish juice concentration had to be significantly increased to invoke grasping behavior during simultaneous squirtings of juice on dactyli and touching of dactyli with a glass rod. The inner flagellum and dactyls of blinded crayfish Cambarus bartoni sciotensis RHOADES, exhibited changes in spike potential when stimulated by glutamic acid (Hodgson 1958). Also, a typical series of feeding behaviors was exhibited by the crayfish when presented with muscle extracts. Planktonic shrimp, Acetes sibogae australis COLEFAX are able to follow scent trails of food or paper soaked in meat extract, (L-alanine, L-leucine, and L-methionine) (Hamner & Hamner 1976). In

the event of crossing a trail, the shrimp either move in rapid horizontal circular paths along the trail, or sharply reorient the body axis to the trail and follow it at three times their normal speed. Rittschof (1980) found that the flesh consumers Fundulus similis, Callinectes sapidus RATHBUN (blue crab), and Melongena corona GMELIN (predatory gastropod), were attracted to small molecules from the flesh of bivalves, gastropods and crabs, while shell users Clibanarius vittatus (Bosc) and Pagurus longicarpus SAY (hermit crabs) were only attracted to small molecules from gastropods. Mosquito larvae Culex pipiens quinquefasciatus SAY respond to gradients of RNA and particular nucleoside monophosphates in an aquarium by congregating in regions of high concentration (Barber et al 1982). Chemotaxis was concluded to be the behavioral mechanism employed by the larvae; however, the actual movements of the larvae from original release position to the area of chemical stimulus were never observed.

Ultrastructural characteristics of the receptors thought to be involved in olfaction and gustation, have been described for various crustaceans and numerous terrestrial insects (Slifer 1970) while few aquatic insects have been studied (Pritchard 1965, Bassemir & Hansen 1981). Zacharuk (1980) recently reviewed insect chemosensilla classification, presenting a simplified version of Altner's (1977) system. Most sensilla are some type of hair or bristle-like structures. The terms multiporous and uniporous chemosensilla crudely distinguish olfactory or non-contact chemosensilla from gustatory or contact chemosensilla. Multiporous sensilla typically have thin cuticle ($\simeq 0.1\mu$ thick) relative to that of the uniporous sensilla. The decapodan counterpart to the multiporous sensilla is the aesthetasc, a long and extremely thin walled receptor with no pores. The walls may be entirely or partially permeable to environment-

al compounds, a luxury aquatic animals can afford. The pores of terrestrial insects allow contact with the external environment without risking dessication.

The primary objective of this study was to determine if shredders respond to compounds released from the leaf matrix during decomposition and ultimately use these compounds to find microbially colonized leaves. In support of this objective, the ultrastructural characteristics of Pycnopsyche guttifer and Pteronarcys pictetti were studied to determine if these insects actually possess the apparatus necessary for olfactory chemoreception.

MATERIALS AND METHODS

Immatures of Pteronarcys pictetti HAGEN were collected from the Escanaba River, Dickinson Co., MI. Those of Pycnopsyche guttifer WALKER, Gammarus pseudolimnaeus BOUSFIELD, and Orconectes propinquis GIRARD were collected from from Augusta Creek and its tributaries, Kalamazoo Co., Michigan.

LEAF DISCS

Pignut hickory (Caryaya glabra (Sargent)) and ash (Fraxinus pennsylavanica Marsh)) leaves were collected in nets, as shed, and stored dry. Hickory leaves were soaked in water, cut into discs, dried at 50^{o}C, weighed, and sterilized by treatment with ethylene oxide. Leaves for preference experiments were confined in a screen container within an artificial stream containing natural detritus and benthic organisms. Concurrently, sterile leaf discs were asceptically transferred to flasks of autoclaved water and incubated in a 15^{o}C shaker bath; this treatment served as a sterile control.

Pure cultures of Tetracladium marchalianum deWILD were grown in 150 ml of an autoclaved mineral medium containing 10mM KNO_3, 2.5mM KH_2PO_4, 3mM NaCl, 1mM $MgSO_4$, .01% yeast extract, and .5% glucose. The culture was incubated at 15°C in 500ml Erlenmeyer flasks on a reciprocating water bath shaker for at least 3 weeks before use or until fungal colonies were 5-10mm in diameter.

FOOD ACCEPTABILITY EXPERIMENTS

Experiments were performed to determine if P. guttifer and P. pictetti would feed more on microbially colonized leaves than on non-colonized leaves as other shredders do (Barlocher & Kendrick 1973, Mackay et al 1977). The results also helped to ascertain the propriety of the leaves chosen as food stimuli for behavioral experiments.

Animals were placed in 300 ml pyrex dishes (10 cm diameter x 5cm high) with step tread (no slip surface) affixed to the bottom to provide a consistent friction surface for locomotion. Two #2 pins were glued, upside down, to the bottom, and each dish was aerated by passing air through a hypodermic needle rather than through an airstone which disturbs the animals with air bubbles. Animals were starved for 2 days before introduction into a dish. Three unconditioned leaf discs were placed on one pin and 3 conditioned leaf discs on the other pin. P. pictetti was allowed to feed for 2 days and P. guttifer was left for 3-4 days as they are slower feeders. Ten replicates were run for each species.

Since it was imperative that all leaf disc tissue remain unchanged by quantification methods, a leaf area meter was used to assess leaf loss by

comparing change in leaf area over time. Excess moisture was drawn from each disc using a paper towel before it was placed in the area meter. Discs were immediately rewetted and placed in the feeding dishes. After the appropriate feeding period, was complete, leaf areas were again determined and % change calculated.

FEEDING BEHAVIOR

Behavioral experiments were designed to discern whether shredders can find palatable food substrates using non-contact chemical cues. Initially, tests were run in a static water arena (Fig. 1) to facilitate comparison with other behavioral studies (Fraenkel & Gunn 1963). Microbially colonized leaves were placed carefully in the middle of the arena. After currents had dissipated, the insects were introduced; their movements, time, and number of animals reaching the leaves were quantified by video tape analyses. To determine if successful contact with the stimulus was accidental, control tests were run using pieces of plastic screen (similar in size to treatment leaves) in place of leaves.

Tests of response to extracts were also conducted in the static chamber. Organisms were allowed to acclimate to the chamber for 15 minutes, after which time, leaf or fungal extract, or crayfish water, was poured gently into the center of the arena. Paths of movement were traced from the video screen and analyzed to quantify the speed of movement and rate of turning using a computer program (see Appendix 1) in conjunction with a Tektronix® digitizer. These two variables plus the amount of time spent in center v.s. sides, and unusual, behaviors, were compared with control experiments using water as a stimulus.

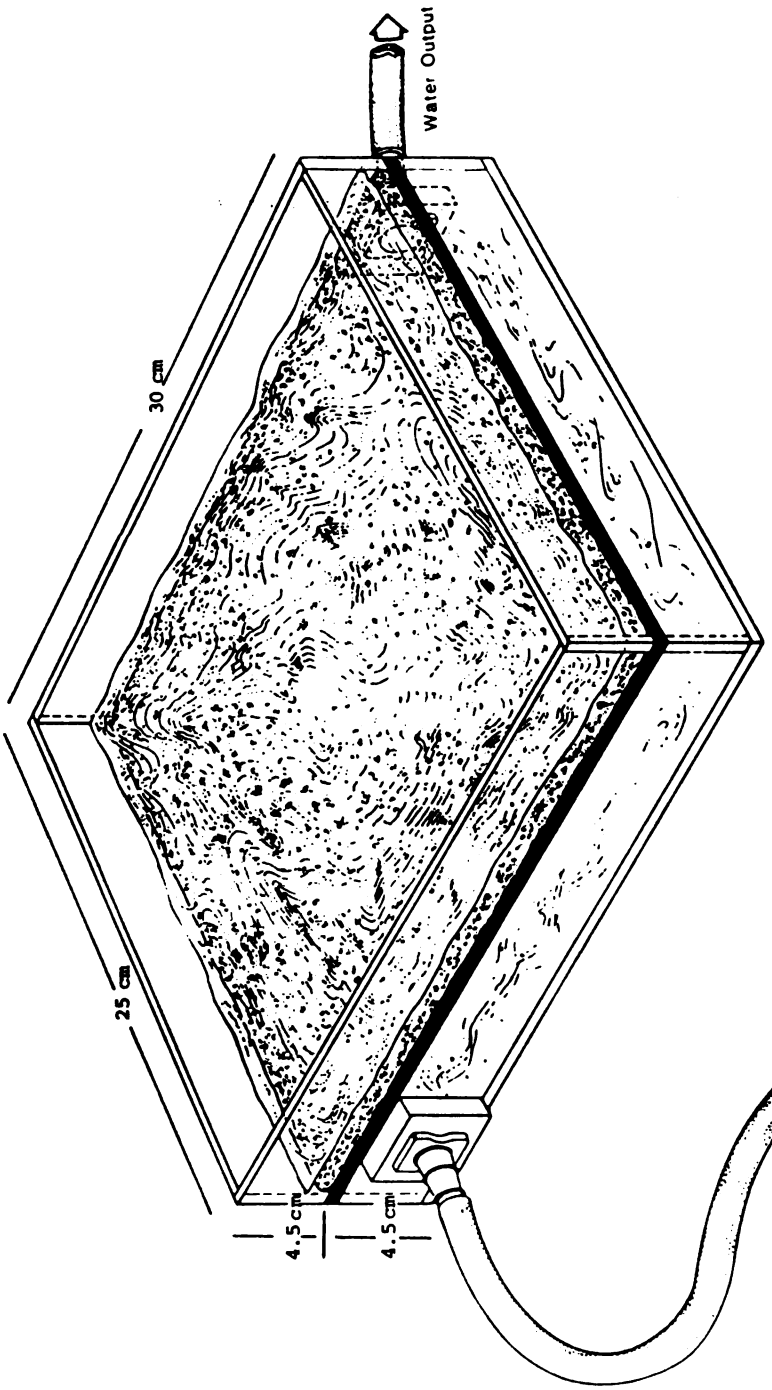


FIGURE 1. Plexiglas static chamber. Water is input at 10°C through the resevoir in order to maintain the upper behavioral arena water temperature at 15°C.

A flow through chamber (Fig. 2) was used for further behavioral testing. Individual P. guttifer and P. pictetti were placed in this chamber which consisted of 8 ports opening into a common center area with drain. Water was pumped into a reservoir below the main arena level, up through uniform holes around the outer rim, and finally in to the drain. A 5cm high piece of screen was placed across the ports so that test animals could not enter the ports. Water for the arena was pumped from a 15°C living stream unit with a Teel• submersible pump.

Test animals were allowed to acclimate in the center area for 15 minutes on the average. After acclimation, stimulus and control were carefully placed in randomly chosen ports without creating shadows or vibrations which might disturb test animals. The chamber was kept in an undisturbed room with natural light averaging 4-6 foot candles. Animal movements were videotaped using a Sony 340 Betamax• video cassette recorder, and a Sony AVC-3450 black and white video camera containing a Sony nuvicon• tube which can record at light intensities as low as 2 foot candles. The Sony 323 VCR, used in conjunction with a 303 Auto Search Control, facilitated tape analysis through its slow/fast motion and time display features. Time spent in front of each port and unusual behaviors were determined from taped sequences.

Conditioned leaves and extracts were used as stimuli in behavioral experiments (Table 2). Extracts were made by macerating 3g of leaf or fungal material in a tissue grinder with 50 ml of distilled water until homogenous. The homogenate was then filtered through Whatman #4 qualitative filters. Crayfish food stimulus (crayfish water) was prepared by cutting crayfish into pieces, leaving the pieces in distilled water overnight, and filtering the water. Stimuli

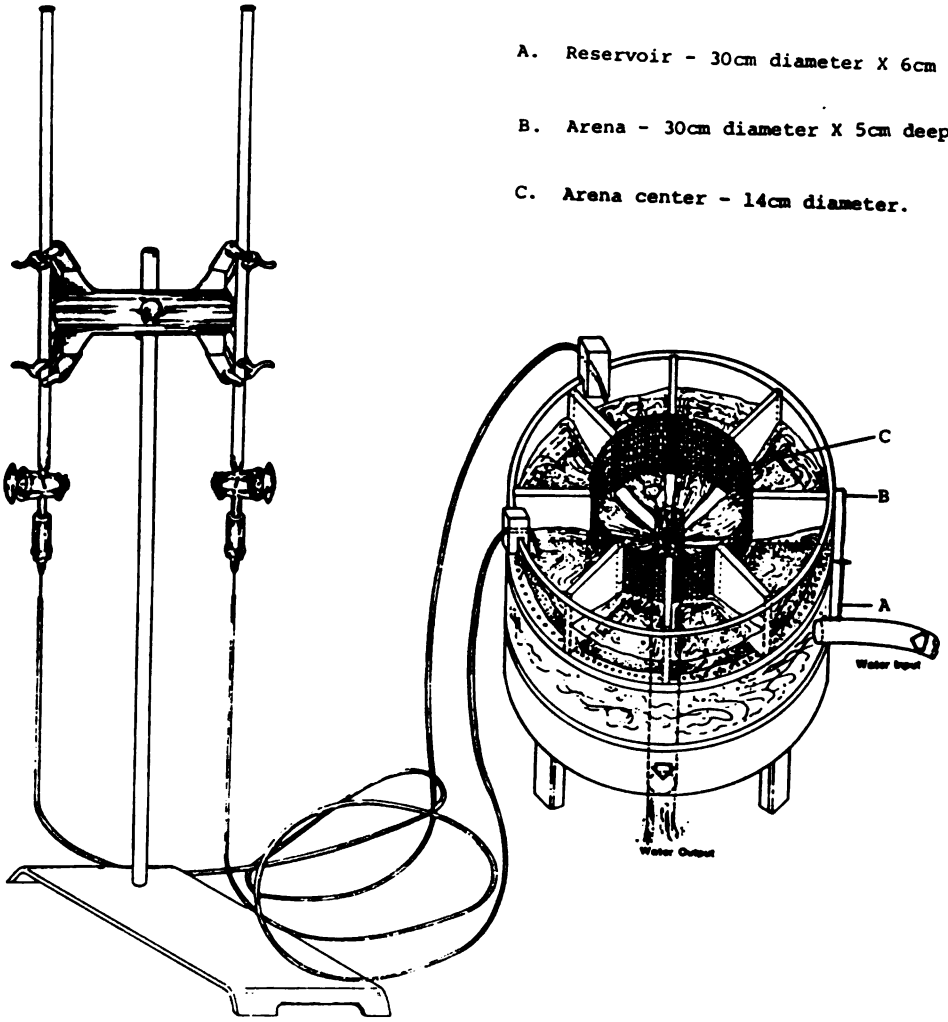


FIGURE 2. Plexiglas flow through chamber. Water flow is in the direction of the arrows. Extracts were introduced into individual ports via burets and Teflon tubing.

TABLE 2. Flow through chamber behavioral experiments.

EXP.	TREATMENT	CONTROL
Pycnopsyche guttifer		
1	conditioned ash leaf discs	sterile hickory leaf discs
2	*Tetracladium marchalianum* extract	distilled water
3	conditioned leaf extract	distilled water
Pteronarcys pictetti		
4	conditioned ash leaf discs	sterile hickory leaf discs
Orconectes propinquis		
5	crayfish water	distilled water

TABLE 3. Results of food acceptability experiments.

SPECIES	% CHANGE IN DISC AREA	
	CONDITIONED	STERILE
P. pictetti	79.68 *	.91
P. guttifer	81.52*	.85

* stimulus different from control at =.001 using t test of diffs. between means.

were introduced into the arena through a buret with a hypodermic needle attached to the tip and 1 m of .01cm diameter Teflon tubing running from the needle to the appropriate port (Fig. 2). The extra tubing permitted the maintenance of a relatively constant flow rate of extract into the arena.

SCANNING ELECTRON MICROSCOPY

Heads, mouthparts and antennae of P. pictetti and P. guttifer were fixed in 4% glutaraldehyde and rinsed in .1M buffer, fixed in 1% O_5O_4 .1M buffer and dehydrated in a graded ethanol series (25%-100%). Specimens were then critical point dried, (with CO_2 as the transitional fluid), mounted, coated, and examined in a JEOL JSM-356 SEM.

RESULTS

FOOD ACCEPTABILITY

Both P. pictetti and P. guttifer fed almost exclusively on the conditioned ash leaves. At the end of each feeding trial, only the veins of the ash leaves remained, while sterile leaf discs were virtually untouched (Table 3).

FEEDING BEHAVIOR

In preliminary tests using the static chamber, the number of animals contacting the stimulus and observed behaviors were the same for leaves and control screens, except that the shredders did not remain on the screen. Analysis of differences in the speed of movement and rate of turning in response to fungal extracts was not informative for G. pseudolimnaeus because its pattern of locomotion was so erratic (Fig. 3a). Similar analyses of P. guttifer and P.

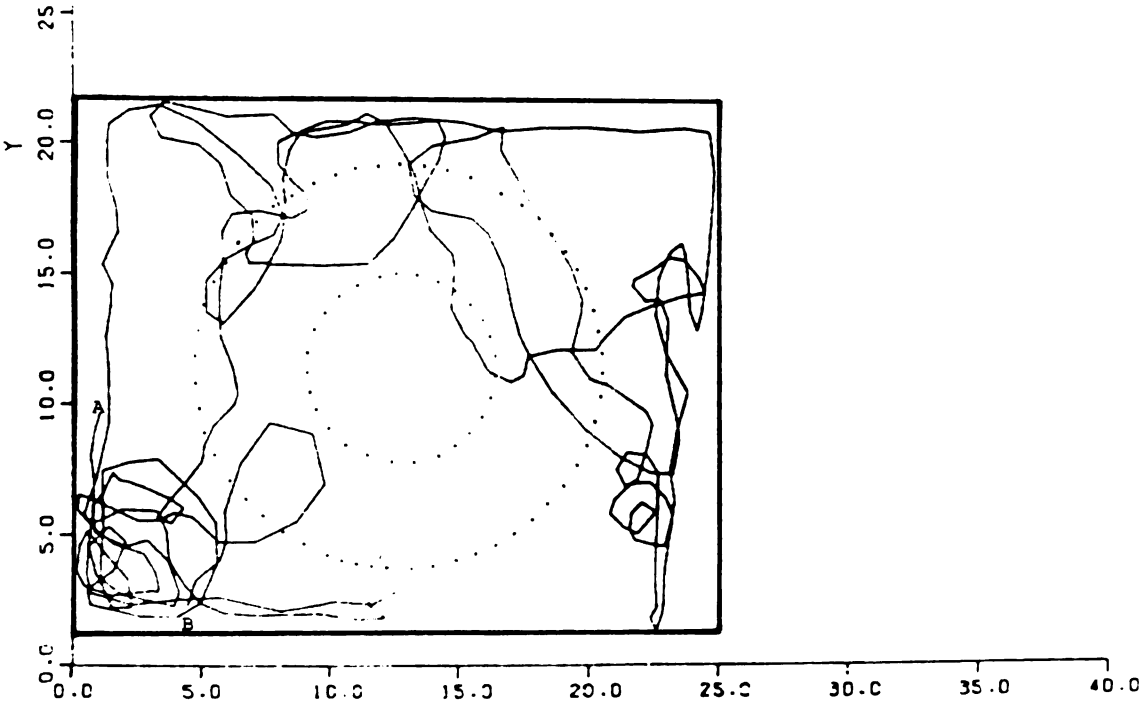


FIGURE 3a. *A typical path of movement for G. pseudolimnaeus in the static water chamber. Observation period = 7 mins.

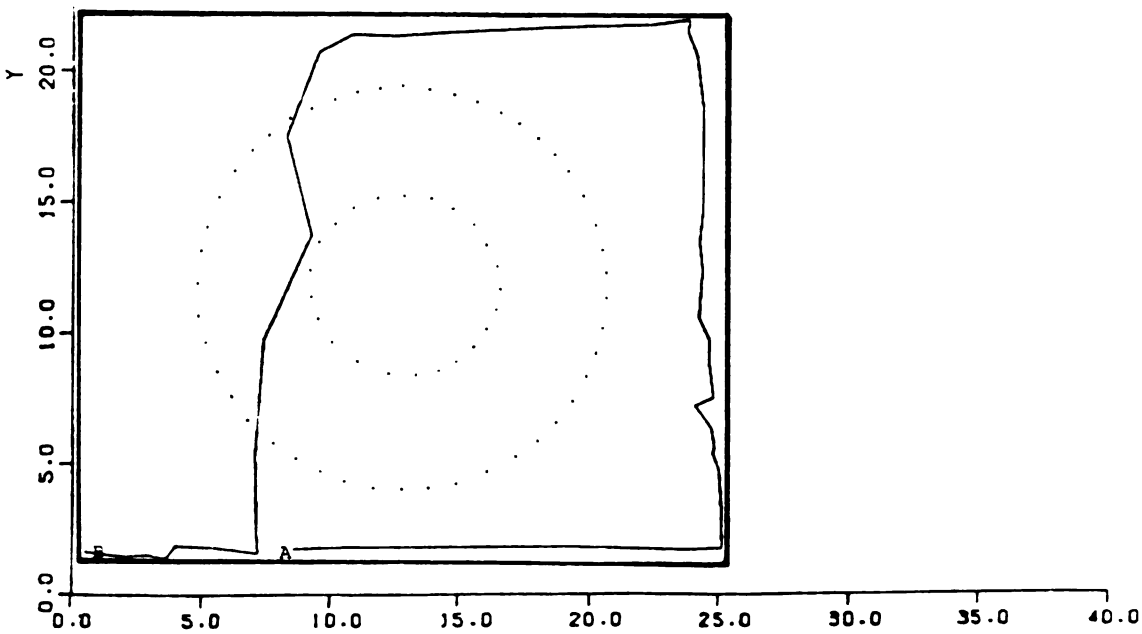


FIGURE 3b. *A typical path of movement for P. guttifer in the static water chamber. Observation period = 12.5 minutes.

*A = start, B = end. Concentric circles represent the diffusion of stimulus from the point of introduction over time. The path was traced from the monitor screen, reduced, and digitized on a Tektronix tablet. The data points were then plotted and output using the MSU SPOCS program.

pictetti revealed no differences between treatment and control; these insects spent the majority of their time along the edge of the arena (Fig. 3b). Control experiments were conducted using the crayfish which is known to respond to chemicals from food sources. In these experiments, O. propinquis spent significantly more time in the center of the arena after introduction of crayfish water (Fig. 4a and b, Table 4).

In the flow-through chamber P. guttifer, P. pictetti and O. propinquis exhibited thigmotaxis since they walked consistently around the edge of the screen which delimited the ports from the central arena (Fig. 2). This behavior caused them to enter the flow from each port. Individuals rarely left the screen, but when this occurred, they typically crossed through the center and returned to the screen, or simply turned 180^{o} and began moving in the opposite direction. In these experiments, there were no changes in the insects' behavior when entering the stimulus plume, and the time spent per port was never significantly different (Table 4). All t values were checked against larger values of α in order to assure detection of any possible differences (Table 5). The crayfish, O. propinquis, spent significantly more time at the stimulus port in response to crayfish extract (Table 5). Feeding behaviors similar to those described for the crayfish Procambarus clarkii (Girard) (Ameyaw-Akumfi 1977) were observed for O. propinquis when it entered the plume of the stimulus port. O. propinquis made brief movements in various directions which resulted in the maintenance of its original position. The mouthparts and chelate walking legs made scooping movements toward the mouth as though grasping something and placing it in the mouth. In addition, the chelate walking legs picked at the substrate and the first enlarged chelae appeared to clean the first antennae periodically.

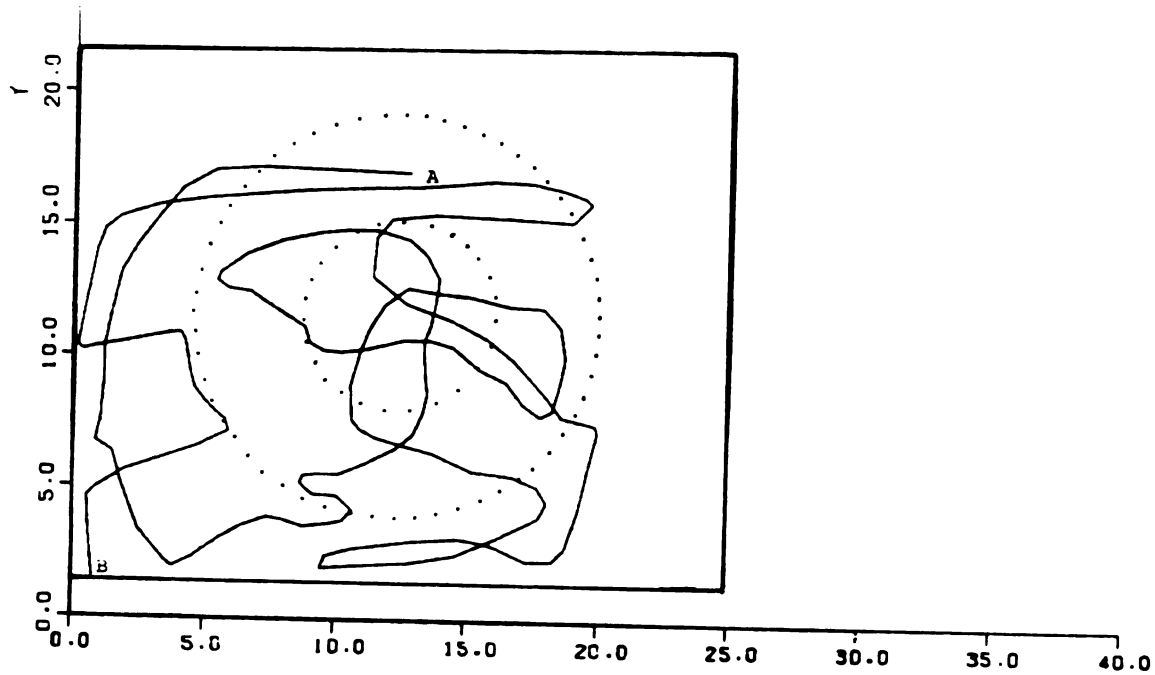


FIGURE 4a. *A typical path of movement for O. propinquis in the static water chamber with stimulus.

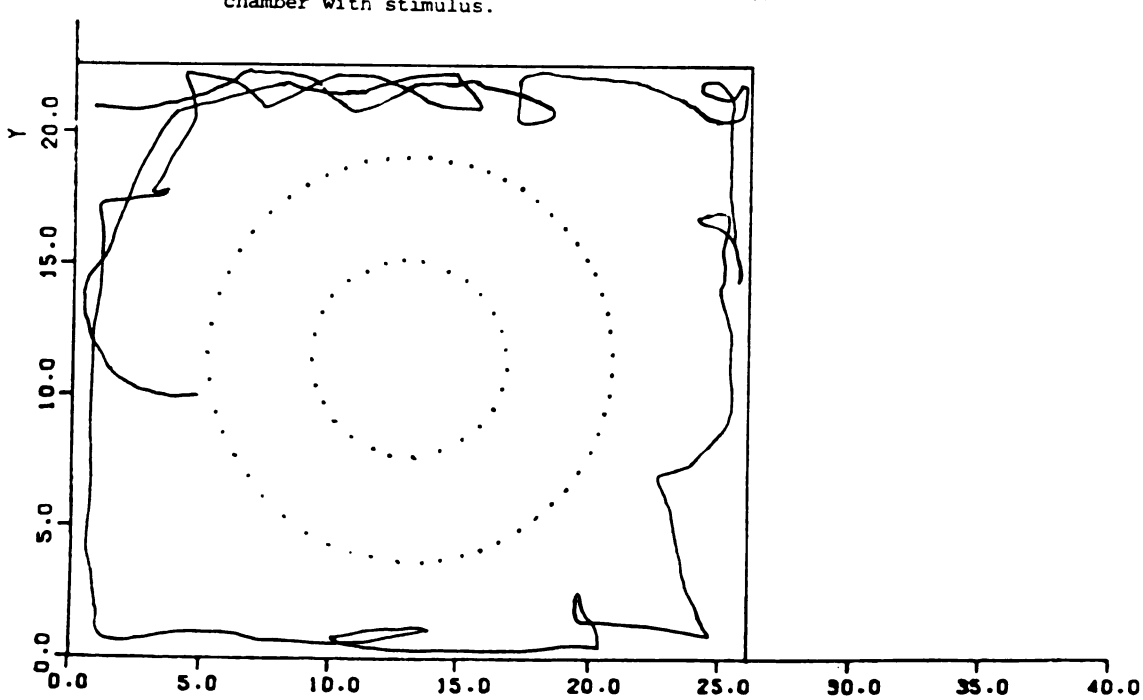


FIGURE 4b. *A typical path of movement for O. propinquis in the static water chamber without stimulus.

*A = start, B = end. Concentric circles represent the diffusion of stimulus from the point of introduction over time.

TABLE 4. Results of static water arena experiments with O. propinquis.

BEHAVIOR	TREATMENTS: CONTROL - NO STIM. (% time)	STIMULUS (% time)
locomotion away from arena edge	4.266	13.7
picking at substrate with chelae	—	4.5
cleaning antenn.	—	4.0

TABLE 5. Results of flow through chamber behavioral experiments.

SPECIES	# of REPLICATES	TREATMENT	AVE. % TIME/PORT: STIMULUS	CONTROL
P. guttifer	10	condit. ash leaves	14.13ns	14.67
	4	T. mar extract	15.36ns	15.12
	5	condit. leaf extract	15.38ns	16.40
P. pictetti	9	condit. ash leaves	13.70ns	14.74
O. propinquis	7	crayfish water	33.19*	10.91

ns no significant difference between stimulus and control

* significantly different at =.001

SCANNING ELECTRON MICROSCOPY

Several structures similar to sensilla found in other animals, were observed using SEM. The antennae of P. pictetti, have an orderly arrangement of sensilla around the upper edge of each antennal segment (Fig. 5). The short stout sensilla are uniporous sensilla (Fig. 6). The long thin sensilla may be multiporous sensilla or some type of mechanoreceptor (Fig. 5). Those of most interest are the grouped sensilla which appear to be thin walled or multiporous sensilla (Fig. 7) and are somewhat reminiscent of the decapod aesthetascs discussed earlier. The head (Fig. 8) and antennal base have uniporous sculptured sensilla (Fig. 9) scattered over the entire surface, while the glossae and palps have typical socketed mechanoreceptors (Fig. 10). Peculiar plate-like cuticular areas and campaniform sensilla (Fig. 11) were found on the paraglossae. The only apparent sensilla on P. guttifer were tiny pegs on the head (Fig. 12), and contact chemosensilla on mouthparts (Fig. 13).

DISCUSSION

Data from these experiments failed to show that non-contact chemical stimuli significantly influenced the food finding behavior of aquatic detritivores. Such results often imply deficiencies in experimental design or errors in apparatus design. The suitability of the design and execution of these behaviorial experiments was supported by the positive results from experiments using the crayfish. O. propinquis was tested because it is known to use olfactory chemoreception in finding its food and it is similar to our test insects in size and locomotory capabilities. Crayfish did respond as expected; they spent more time in the middle of the static water arena where crayfish water was introduced and

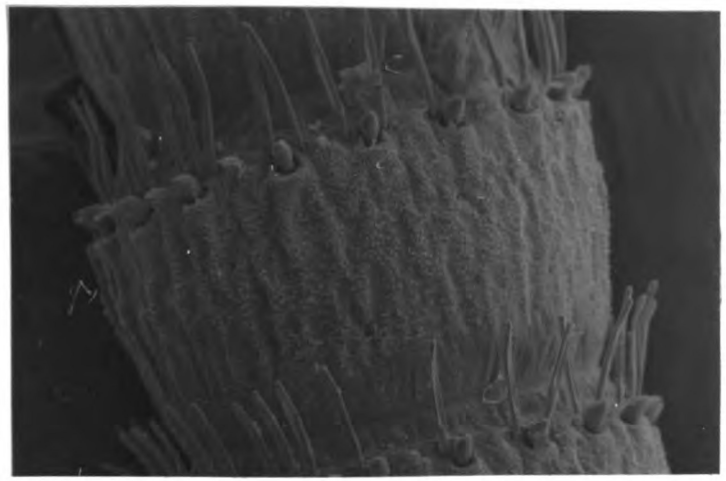
PTERONARCYS PICTETTI - SEM

FIGURE 5a. Sensilla of one segment of antenna. X20000

FIGURE 5b. Oblique view of antenna showing arrangement of sensilla at upper edge of each antennal segment. X18000

FIGURE 6a. Top view of antennal uniporous sensilla. X4400

FIGURE 6b. Side view of antennal uniporous sensilla. X3200



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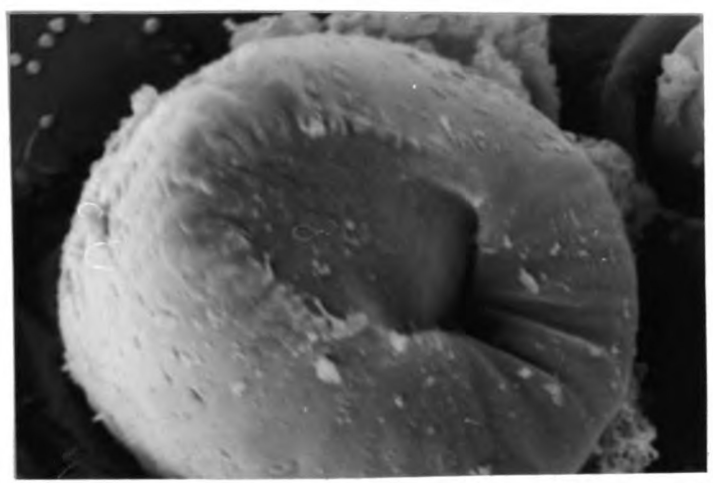

6a

6b

PTERONARCYS PICTETTI - SEM

FIGURE 7. Grouped sensilla of antenna. Probably thin walled. X2000

FIGURE 8. Mouthparts, general view. X26

FIGURE 9. Sculptured uniporous sensilla. X2000

FIGURE 10. Socketed receptors of paraglossa. X2000

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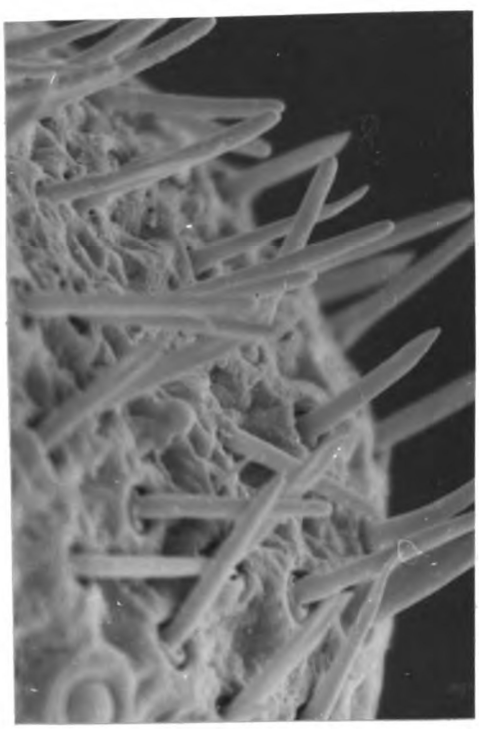

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PTERONARCYS PICTETTI - SEM

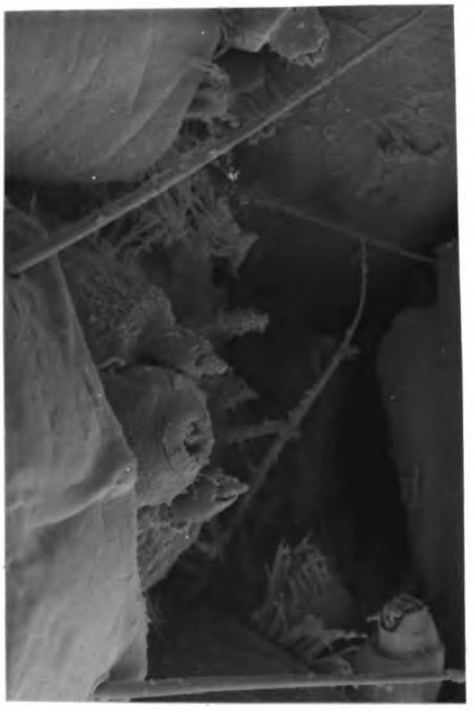
FIGURE 11. Cuticular plates and campaniform sensilla of paraglossa. X3000

PYCNOPSYCHE GUTTIFER - SEM

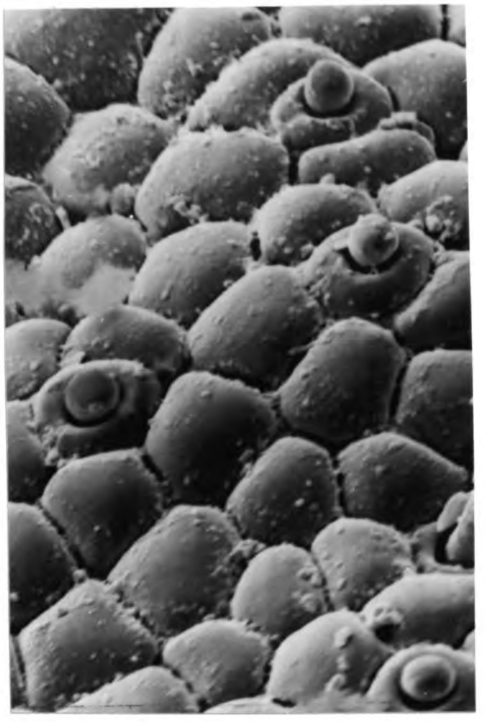
FIGURE 12. Head and mouth. X60

FIGURE 13. Sensilla of mouthparts. X260

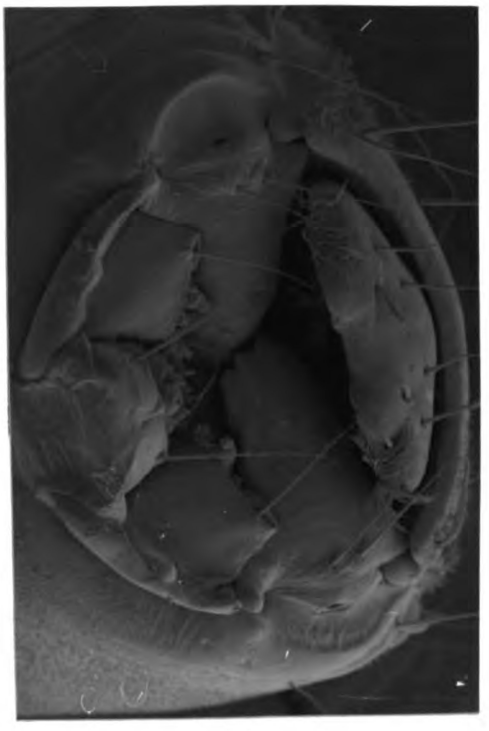
13



11



12



spent more time at stimulus than at control ports in the flow-through chamber experiments. Thus, we believe that the general experimental design was appropriate. Numerous questions remained unanswered: 1) were the insects hungry; 2) was the light affecting their behavior; 3) were insects given enough time to acclimate properly to their new environment; and 4) was the stimulus strong enough? To answer these questions, starvation periods of 0-10 days were tried and penultimate instar nymphs and larvae were used as they are highly involved in feeding. Flow-through and static chamber experiments were repeated in complete darkness and insects were allowed acclimation periods of 0-60 minutes and 1 day, to decrease the effects of the unnatural environment. Concentrations of extracts were varied before experemental techniques were finalized. No changes in behavior were apparent with any of these alterations in experimental design.

It appears that P. pictetti and P. guttifer find their food through random movement without the information derived from non-contact chemostimuli. Considering their environment, it may not be particularly advantageous for these shredders to use a long distance chemical cueing mechanism since their life cycle is synchronized with the yearly flux of organic matter. Reice (1981) found that competition for food was relatively unimportant among stream benthos in New Hope Creek even though species microhabitats, feeding habits, and body sizes were similar. Another consideration is that many shredders are actually opportunists, switching to periphyton, macrophytes, or autochthonous detritus when necessary (Minshall 1978, Hynes 1975, Anderson & Sedell 1979, Peckarsky 1980). Williams & Williams (1982) and Cummins (1964) supported this observation in their studies of P. guttifer. Guts of P. guttifer contained large

percentages of leaf fragments and algae; chiefly diatoms and several filamentous forms. Unfortunately, these investigations did not consider the suitability of diatoms and periphyton as food since the ability of the shredders to assimilate them is unknown. Shredders may not require a special mechanism for food finding if they are essentially opportunists that have large quantities of suitable food available throughout their larval and nymphal growth periods. Crayfish, on the other hand, are scavengers that take advantage of ephemeral and spatially restricted food resources such as recently killed animals. Therefore, it is advantageous for them to have effective mechanisms for locating these resources.

The fact remains that many shredders do feed on certain leaves more than others and are not randomly distributed on leaves in natural stream systems. Shredders typically exhibit a clumped distribution since they are found in detritus, a food resource which is clumped in distribution (Egglishaw 1964). They may use taste or tactile cues to recognize appropriate food; although, in natural stream systems, this clumped distribution could occur in response to stream microhabitats rather than food. Interactions between current velocity, substrate size and food availability affect the distribution of invertebrates in a stream (Cummins & Lauff 1969) (Fig. 14). A specific heirarchical arrangement of these factors exists for each species. In the case of P. guttifer, different instars have different substrate size preferences depending upon the requirements of case building, feeding, and pupation (Fig. 15). Generally, P. guttifer inhabits slow water areas of a stream, particularly stream margins, although no current velocity preferences were found in laboratory studies (Cummins 1964).

Minshall and Minshall (1976) observed that small particle substrates (1.5cm) accumulated more detritus and supported an abundance of invertebrates relative

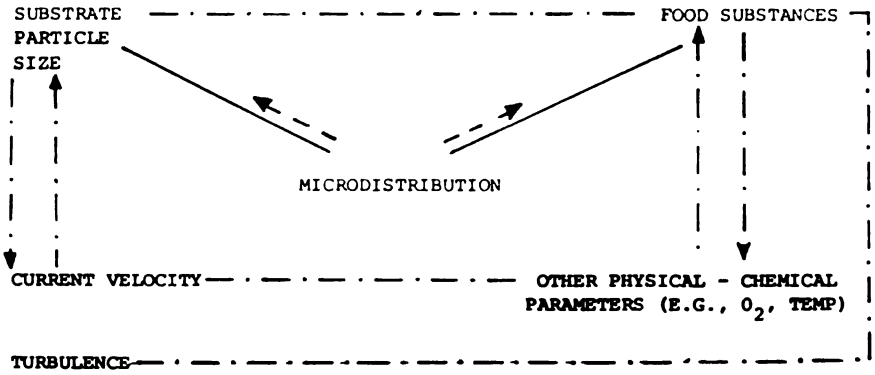


FIGURE 14. General relationships between environmental parameters and the microdistribution of a species of benthic stream macroinvertebrate. (Cummins & Lauff 1969).

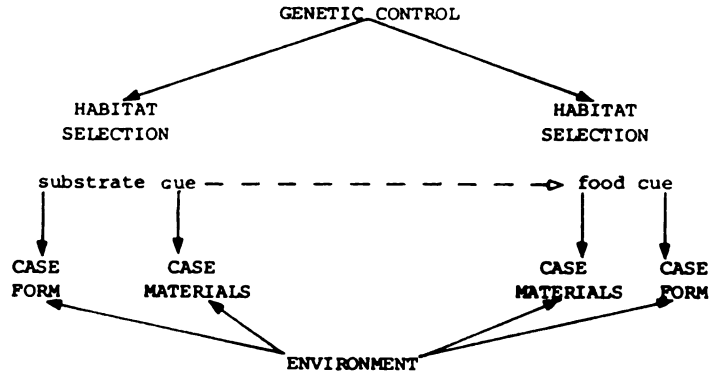


FIGURE 15. Genetic and environmental influences on microhabitat selection by Pycnopsyche larvae (Cummins 1964).

to larger substrates (4.5-7cm). When similar amounts of detritus collected on larger substrates, invertebrate colonization was then similar on both substrates. Correlations between the amount of detritus and invertebrate numbers may have been coincidental since the accumulation of detritus is the result of the combined effects of current and substrate.

Other studies have shown substrate preferences when detritus was not a confounding factor. Limnephilus rhombicus (Linnaeus) and Potamophylax rotundipennis, both shredders, spent more time on pebbles than on sand and more time on coarse pebbles than on crushed brick (Higler 1975). If shredders respond to substrate differences as described by Higler, then chemical cues may be of secondary importance or may supply additional feeding information at best.

Peckarsky (1980) found more shredders in cages with conditioned and unconditioned coarse particulate organic matter than in control cages. The animals located a food substrate typically considered unacceptable and not conducive to proper growth. The large numbers of shredders in control and unconditioned leaf cages suggest a random search mechanism. Once appropriate or inappropriate food is encountered, the decision can be made whether or not to move to another area.

SCANNING ELECTRON MICROSCOPY

The purpose of studying the morphology of P. pictetti and P. guttifer was to document the potential chemosensory apparatus available for facilitation of food finding. P. pictetti does have sensilla which morphologically appear to have olfactory capabilities, but their effectiveness in this capacity is unknown.

It is believed that insects evolved as terrestrial organisms and only secondarily invaded aquatic habitats (Boudreaux 1979, Ross 1967, Edmunds 1972);

thus, as one might expect, the sensilla of P. pictetti are structurally similar to the sensilla of terrestrial insects. The degree of adaptation of these sensilla to the aquatic environment is an intriguing question.

What characteristics exemplify aquatically adapted chemosensilla? Ghiradella et al (1968b) identified distinct differences in structure between the aesthetasc hairs of Coenobita compressus, a terrestrial hermit crab, and Pagurus hirsutiusculus (Dana) an aquatic hermit crab (Ghiradella et al 1968b). They related the divergence in sensilla morphology to the "newly" aquired terrestrial habits of Coenobita.

Several characters of Coenobita sensilla are convergent with terrestrial insects while typical aquatic decapod characteristics are also present. Differences relate primarily to the problem of water conservation, encountered in the switch from aquatic to terrestrial life. Both terrestrial insects and Coenobita have blunt pegs which expose less surface area to evaporation. The ciliary apparatus is set below the surface of the flagellum, effectively isolating all structures except receptor elements of the cells from the permeable surface. Vacuoles are found throughout the flagellum which insure a supply of moisture to cilia and more distal elements. Aquatic decapods such as Pagurus have completely permeable flagella (Snow 1974, Ghiradella et al 1968b). Terrestrial insect olfactory sensilla are not permeable to most compounds but have pores which connect to the outside and liquor bathing the dendrites, thereby preventing dessication of the sensilla. Coenobita aesthetascs are intermediate in form in that only one side is permeable.

In addition to water conservation, there are problems of structural support. Short stout pegs of terrestrial insects and Coenobita can maintain their integrity

without additional support, whereas long thin aesthetascs of aquatic decapods require the pressure of an aquatic medium to prevent folding and matting of the sensilla.

P. pictetti sensilla (50um x 10um) are proportional to the reduced Coenobita aesthetascs (100um x 20um). Pagurus aesthetascs are 200-1400um in length and only 18-25um in diameter (Snow 1974). They typically occur in populations of 400-600/outer flagellum and are densely packed. Thin walled sensilla of P. pictetti are sparsely distributed on the antennae relative to Pagurus and terrestrial insects in which olfaction is important such as Hymenoptera (Agren 1978) and Lepidoptera (Slifer 1979).

In conclusion, the evidence presented here, supported by evolutionary and ecological studies, suggests that these shredders do not use long distance chemical cues to facilitate food finding. They may drift in the stream current and settle out in pools with relatively fine particle organic matter, or become snagged in rocks and other stream debris along with leaves. The chemosensory apparati do not seem appropriate for effective long distance cuing in aquatic habitats since the sensilla generally resemble those of terrestrial arthropods in structure and are sparsely distributed. Electrophysiological and TEM studies of the sensilla must be done before any firm conclusions can be made concerning their function.

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100=      PROGRAM OUT(OUTPUT,TAPE1,TAPE2,TAPE4=OUTPUT,TAPE3=OUTPUT,TAPE5)
110=      REWIND 1
120=      REWIND 3
130=      REWIND 2
140=      REWIND 5
145=      S=0
150=      I=0
160=      SDANG=0
170=      READ(1,50)X1,Y1
180=4     READ(1,50)X2,Y2
200=5     A=(X1-X2)
210=      B=(Y1-Y2)
220=      F=(A**2+B**2)
230=      ROOT=SQRT(F)
240=      WRITE(5,51)S,ROOT
250=6     CONTINUE
260=      HYP=0
270=      S=0
280=10    SDANG=0
290=      IF(I.GT.0)GO TO 63
300=      HYP=HYP+ROOT
310=60    CONTINUE
320=      X1=X2
330=      Y1=Y2
340=      S=S+1
350=63    READ(1,50)X2,Y2
360=      IF(EOF(1))30,61
370=61    IF(Y2.LT.0.0)21,8
390=8     C=(X1-X2)
400=      D=(Y1-Y2)
410=      O=(A**2+B**2)
420=      R=(C**2+D**2)
430=      SO=SQRT(O)
440=      SR=SQRT(R)
450=      IF(SR.LT..3)GO TO 63
460=      T=(A/SO)*(C/SR)+(B/SO)*(D/SR)
470=      ANG=ACOS(T)
480=      DANG=ANG*57.3
490=      HYP=HYP+SR
500=      WRITE(5,51)S,SR
510=      A=C
520=      B=D
530=      WRITE(2,51)S,DANG
540=      SDANG=SDANG+DANG
550=      XA=SDANG/S
552=      IF(I.EQ.0)70,72
553=70    XH=HYP/(S+1)
554=      GO TO 20
560=72    XH=HYP/S
570=20    GO TO 60
580=21    I=I+1
590=      WRITE(3,54)XH,XA
600=      WRITE(4,54)HYP,SDANG
610=      GO TO 6
620=30    WRITE(4,54)HYP,SDANG
630=      WRITE(3,54)XH,XA
640=50    FORMAT(1X,G12.4,3X,G12.4)
650=51    FORMAT(F5.0,3X,F12.4)
660=53    FORMAT(F12.4)
670=54    FORMAT(F12.4,3X,F12.4)
680=55    CONTINUE
690=      END
```

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