THE EFFECTS OF SUBSTRATE AND THE HYPORHEOS ON THE RECOLONIZATION OF STREAM INSECTS IN A RIFFLE AREA

Margaret Taylor
UNDERC Research Project
Dr. Ronald Hellenthal
November, 1989

ABSTRACT

The recolonization of aquatic insects in a riffle area was studied over a 34 day period at three sampling dates over one summer. The entire progression of the recolonization was very rapid. Significant changes in species numbers over time were recorded among the insects *Hydropsyche bifida* and *Hydropsyche morosa* while *Cheumatopsyche sp.* and *Baetis sp.* populations remained in consistent numbers over the 34 days. Comparing control trays to trays which prevented recolonizers from the hyporheos, significant effects of vertical movement from the substrate were not shown. All trays to be recolonized were exposed to normal downstream water flow and therefore to invertebrate drift. Relative to the rapid recolonization of all trays due to drift and including the high variability of the stream, the hyporheos proved to be insignificant as a source of recolonizers. Examining a second aspect of recolonization, trays with nonstream rocks versus stream conditioned rock substrates were also found to be nonpreferentially recolonized. This result can possibly be attributed to standard deviations due to stream variability too large to detect the subtle changes which may have occurred.

INTRODUCTION

Colonization is a series or sequence of occurrances leading to the establishment of populations in areas from which they were, over any time period, absent (Sheldon, 1984). In the rapidly changing environment of the stream, movement and recolonization is very frequent. This frequency is easily demonstrated by stream disturbance and observation of the subsequent rapid recolonization of aquatic insect populations. These populations of insects are vital to the entire stream habitat. To understand the subtleties of recolonization, two aspects worth examination are sources of insects and substrates for recolonization.

Most past studies have assumed four major sources of recolonizers: invertebrate drift, upstream movement, oviposition from aerial sources, and movement from the hyporheic zone (Williams and Hynes, 1976). Invertebrate drift has shown to be the most dominant source of recolonizers from within the stream (Townshend and Hildrew, 1976). Most studies also have found that downstream movement was dominant and vertical movement between the stream and hyporheos was insignificant (Delucchi, 1989). Neither the extent nor the purpose of hyporheic movement and, likewise, the contribution of hyporheic populations to recolonization is understood.

Substratum type is a second variable which directly acts on insects by controlling abilities to adhere, burrow, construct cases, feed, and grow (Minshall,1984). Preference for a substrate is contingent on many aspects: size, surface area, heterogeneity, texture, and abundance of organic material. All of these aspects are highly dependent on the particular species involved and what that species' needs may be.

Recolonization as a whole, its progression, variation, and the succession of insect species has also been widely studied. The normal life cycles of colonizers, outside environmental changes, and interaction among recolonizers are not easily separable for studies (Sheldon, 1984). Some studies have suggested differential colonization rate among trophic groups: collector-detrivores first, scraper-grazers second, and predators appearing last (Gore, 1982). In the past, experimenters have found difficulty in demonstrating the true interaction among recolonizers in a stream's succession because of the impact of external changes.

MATERIALS AND METHODS

All experimental data was taken from a stream in the University of Notre Dame Environmental Research Center, a property located in the upper peninsula of Michigan. A riffle area of Tenderfoot Creek was sampled over a period from June 29 to July 17, 1989. Preliminary samples were taken at the designated riffle area on June 29. Five samples were taken using a Surber sampler. All insects clinging to the rocks and those in the shallow sediments in the area covered by the Surber were hand brushed and swept by the current into the Surber net. The net contents were put in 80% ethanol, detritus was removed in the laboratory, and all insects were preserved in four dram vials of ethanol. In these initial samples, all insects found were identified and counted using the keys found in Hilsenhoff (1981).

After tabulating the results of these initial five samples, the coeffecients of variations were found for each species. Based on this, the estimated necessary sample size, and the general abundance of each species, several species on which to concentrate were chosen. After all sampling was complete for the summer, the data for the following species was analyzed: *Hydropsyche morosa* Ross, *Hydropsyche bifida* Banks, *Cheumatopsyche sp.*, and *Baetis sp.*. Many other species of insects, especially trichopterans, were identified and counted, but had population densities too low to show differences given the numbers of samples taken.

Experimental samples were taken using specially designed trays. Trays 500 square centimeters in area were constructed using 2 by 2 inch pieces of wood nailed in a square with a screen of .25 inch holes stapled across one side. This screen would face down toward the stream bed. Two of these trays were places in the stream on June 29 and filled with stream rocks that had been removed of insects. Upon collecting them two weeks later, the trays had remained intact and had been substantially recolonized. Insects were removed from these trays by brushing the rocks that had been in the tray bottom. Insects were brushed off rocks taken from each recolonization tray into a Surber sampler net. No insects that had colonized on the sides or bottom of the tray were included in the sample. The Surber sampler contents were then placed in 80% ethanol and sorted at the laboratory.

Two variables were manipulated in the recolonization trays. First, certain trays were filled with rocks removed from the stream and cleaned off so they were free of all insects. Other trays contained rocks which were found on land. These were not covered with periphyton like the stream rocks. Rocks of medium size were distributed evenly to cover the entire tray bottom. Second, some trays were lined with a very fine mesh on top of the screen. These meshed-lined trays were designed to eliminate hyporheic recolonizers while allowing normal water flow patterns to remain undisturbed. Unlined trays containing rocks that had not been cleaned of organisms served as controls.

On June 13, five groups of five trays were put in the designated riffle area. Each group contained a control, a nonstream rock open bottomed tray, a nonstream rock closed bottomed tray, a stream rock open bottomed tray, and a stream rock closed bottomed tray. These stream rocks had been cleaned of all insects. A map was made and the position of each tray was recorded. Three sets of five trays were removed from the stream on June 25 and the remainder were removed on July 10. One set of five trays was put in place on June 25 and removed on July 17. Several trays from the first and second dates could not be used because of the water level of the stream dropped and exposed the rocks to the air. Each set of five trays was numbered according to its position in the stream. If a full group of five could not be pulled from a reasonably small area, as close to five as possible were pulled. A total of thirty out of the thirty five trays originally placed in the stream were counted.

Insects were separated from rocks and detritus using a forceps and dissecting scope. Species were identified using keys in Hilsenhoff(1981), Wiggins (1977), and Schuster and Etnier (1978). Other insects from each recolonization tray were placed in separate four dram vials, but were not identified or counted.

All data was analyzed using the computer program SYSTAT. Two one way analyses of variance were run on each species' data set. The first ANOVA tested density related to the five treatments. The second ANOVA tested density related to date collected.

RESULTS

A total of six sets of trays were collected and analyzed (table 1). Date two refers to both the trays collected on July 10 and those collected on July 17. Date one refers to trays collected on June 25. In general, highest insect densities occurred in the swifter, but still relatively deep water. The lowest insect densities were collected from a set of trays in a fairly swift but shallow area of the stream that later dried up substantially.

A one way analysis of variance of density according to the five treatments showed no significant results (tables 2 and 3). *Cheumatopsyche sp.* (figure 1), *Hydropsyche morosa* (figure 2), *Hydropsyche bifida* (figure 3), and *Baetis sp.* (figure 4) densities all resulted in p-values greater than .05; thus no correlation between treatment and density was found.

One obvious change not occurring within a key insect group involved the presence of extremely dense populations of *Simulium* larvae in the 12 day (date one) trays. These larvae were present in huge numbers and seemed to nonselectively colonize all substrates that were placed in the water. By date two, virtually all had disappeared.

A one way analysis of variance of density according to date showed significant results (tables 4 and 5). Changes in species composition and total insect densities over time were found (figure 5). *Cheumatopsyche sp.* remained throughout the two months in consistent numbers (p=0.90). Standard deviations relative to total density for *Cheumatopsyche sp.* indicate high variability across the stream. *Hydropsyche bifida* (p=0.0) and *Hydropsyche morosa* (p=0.0) both increased significantly from the first to the second date. Standard deviations, and therefore variabilities, were still quite high, but the density difference over time was highly significant. *Baetis sp.* also changed significantly in density over time (p=0.01), although this was the least abundant species collected and the results are therefore less conclusive.

DISCUSSION

In terms of recolonization sources, results pointing to significant recolonization from the hyporheos were not found. The explanation for this lack of significant results can take two approaches: a true lack of hyporheic recolonizers or a lack of results due to external error.

The first possible approach is that, for the key species studied, the hyporheos is not a significant recolonization source. None of the key genera, *Baetis, Hydropsyche,* nor *Cheumatopsyche*, showed significant differences between the closed and open bottomed trays. It is possible that the hyporheos does not significantly contribute to the recolonization of any of these species. In a 24 hour drift study performed on Tenderfoot Creek in late July, all of the key insect species were found to appear in the drift. This supports the assertion that the drift is such a common method of stream migration by insects. It is questionable, however, if recolonization is so largely contingent on the invertebrate drift phenomenon. Moreover, the drift study was only done once near the end of the summer. The composition of the drift will change over the summer and one drift study is therefore not sufficient for significant conclusions. It has been found, however, that more than 80% of moving insects do so in the drift (Townshend and Hildrew, 1976). One study recorded that *Hydropsyche* was found exclusively on the surface and not in the hyporheos (Townshend, 1979). If the species are not present there, the hyporheos would not be a recolonizing source. *Cheumatopsyche*, on the other hand, has been reported to be found up to depths of 20 cm in the hyporheos (Williams and Hynes, 1974). Results are not conclusive enough to completely eliminate hyporheic recolonization of any of the key species.

A second approach to explaining the lack of significant results that would indicate the hyporheos as a recolonization source involves procedural drawbacks. Aside from not ruling out recolonization from the hyporheos for the key insects studied, perhaps other insect families or genera besides ephemeroptera and trichoptera are more prevalent in the hyporheic zone and more obviously recolonize from the hyporheos. A more delicate and tightly controlled sampling method may have been necessary to detect subtle changes that may have occurred. Surface area in each tray of rocks was not strictly monitered and this is a variability that may have masked other density changes. As indicated by the large standard deviations, variability was very high among group sets in the stream. This error was compensated for because almost each set had one of each tray type and trays within sets would then have had similar environmental exposure. Delicate differences between trays, however, could not be detected because of such large standard deviations. High within group variability was also present especially in the second date trays as some of the stream began to dry. Fewer full groups with all five tray types could be found.

Ideally, more samples were also needed. With more groups of similar depth and flow conditions, more viable data could have been obtained. Space and time were both limiting. The riffle area of

Tenderfoot Creek was not very large and riffles ranged in depths from a few inches to over a foot. Samples had to be large enough to yield a fairly consistent number of each species uniformly distributed in a group area. One problem with the *Baetis sp.* was a sheer lack of organism numbers.

In reference to recolonization substrates, a significant preference for stream conditioned or nonstream rocks was not found. Again, perhaps there is no preference shown among the species studied. All trichopterans studied were of the family Hydropsychidae. They are also most accurately termed as collectors and omnivores, collecting detritus, vegetation, and other invertebrates in their nets. As such, they would not need a rock covered with periphyton as long as there was sufficient water flow through the nets. *Baetis*, on the other hand, are grazers which do eat vegetation on rocks. This lack of significant results could be due to a lack of numbers of *Baetis* sampled. The *Baetis* seemed to be present consistently, but too sparsely, throughout the stream and over time. With additional samples taken from more similar stream areas, it seems a difference between nonstream substrate and cleaned stream rock substrate may have been demonstrated.

Overall, recolonization progress over time was monitered and significant results were found. Not only did the density of key insects change significantly over time, but other changes in insect and stream composition were observed. Early in the summer, after 12 days in the water, the very dense *Simulium* larvae colonized all surfaces indiscriminantly. There were large populations even on the wood of the trays. This observation is in keeping with past studies that have demonstrated that overpopulation of Simuliidae on newly exposed surfaces (Disney, 1972). Few had been found in either of the preliminary samples of May 29 or June 15. Their almost complete disappearance by 25 days indicates an emergence.

Both *Hydropsyche morosa* and *Hydropsyche bifida* populations showed overwhelmingly significant density increases over the two dates of sampling. At date two, these comprised the vast majority of insects collected in all trays, and both species were present in roughly equal numbers. This large density increase is most probably due to a group of eggs hatching into larve during the period between dates one and two.

The *Cheumatopsyche sp.* population was the most consistent of any of the key species over the 35 days of the experiment. The majority of the organisms collected on date one were very small, indicating early instars. Those from July 10 and July 17 were noticibly larger and therefore older instars. The most likely explanation for these patterns is that the *Cheumatopsyche* did not emerge, nor did a new larval population appear. The *Baetis* population also did not significantly change in density over time although more fluctuation was recorded. This could be attributable to sampling difficulties and a lack of total sample numbers.

The overall progression of colonization was very fast in this riffle of Tenderfoot Creek. In only twelve days, all tray types were recolonized enough such that no highly significant differences were found between the control and experimental trays. Twelve days proved to be a long enough time period for near full recolonization to occur. Moreover, with such large standard deviations due to high group variance in the stream, true differences may have been masked. By twenty and twenty seven days, colonization had definitely reached the densities found in the control trays. In the rapidly changing and highly variable environment of the stream, species density can undergo subtle to drastic changes that are contingent on many factors. Recolonization is a very rapid process and subsequently requiring delicate and frequent sampling.

There are many areas which would be of interest for future studies. Studies making use of similar types of recolonization trays would be interesting. More variables hypothesized to influence recolonization could be manipulated. For example, substrate type or size could alter the patterns of recolonization. Relative to hyporheic recolonization, it would be worthwhile to simultaneously sample the insects present in the hyporheos over the experimental period and compare this to the insects which recolonize the trays. Frequent drift studies would also help to clarify the relationship between the drift composition to the types of recolonizers. Perhaps of greatest value, methods could allow more precise sampling of the stream should be investigated. It is impossible to eliminate the great dependence of aquatic insects on the many variables of the stream, thus making the isolation of the effects of individual variables very difficult.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Bernard J. Hank family who provided the funding for the UNDERC course which made this project possible. I would also like to especially thank Dr. Ronald Hellenthal for his advising and help in this project. Special thanks to Dr. Marty Berg, Dr. Michael Dini, and Kathleen McTigue for their contributions throughout the summer. Finally, I would like to thank the 1989 UNDERC class, especially Tom Stahl and Christy Gurnett, for their time and help.

LITERATURE CITED

Delucchi, C.M. 1989. Movement patterns of invertbrates in temporary and permanent streams. Oecologia 78:199-207.

Disney, R.H.L. 1972. Observations on sampling pre-imaginal populations of blackflies (Dipt., Simullidae) in West Cameroon. Bulletin of Entomological Research 61: 485-503.

Gore, J.A. 1982. Benthic Invertebrate Colonization: Source distance effects on community composition. Hydrobiologia 94: 183-193.

Hilsenhoff, W.L. 1981. Aquatic Insects of Wisconsin. Geological and Natural History Survey, Madison, WI.

Hynes, H.B.N. 1970. The Ecology of Stream Insects. Department of Biology, University of Waterloo, Ontario.

Minshall, G.W. 1984. Aquatic Insect-Substratum Relationships. In: The Ecology of Aquatic Insects (Ed. V. Resh, D. Rosenberg). Praeger Scientific, New York.

Schuster, G.A. and D.A. Etnier. 1978. A Manual for the Identification of the Larvae of the Caddisfly Genera *Hydropsyche* Pictet and *Symphitopsyche* Ulmer in Eastern and Central North America (Trichoptera, Hydropsychidae). USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Sheldon, A.L. 1984. Colonization Dynamics of Aquatic Insects. In: The Ecology of Aquatic Insects (Ed. V. Resh, D. Rosenberg). Praeger Scientific, New York.

Townshend, C.R. and A.G. Hildrew. 1976. Field Esperiments on the drifting, colonization and continuous redistribution of stream benthos. J. Animal Ecology, 45, 759-772.

Townshend, C.R. 1979. The Ecology of Streams and Rivers. The Institute of Biology's Studies in Biology no. 122, pp. 51-66.

Wiggins, G.B. 1977. Larvae of the North American Caddisfly Genera. University of Toronto Press, Toronto Canada.

Williams, D.D. and H.B.N. Hynes. 1974. The occurrence of benthos deep in the substrature of a stream. Freshwater Biology 4 (3): 233-256.

Williams, D.D. and H.B.N. Hynes. 1976. The recolonization mechanisms of stream benthos. Oikos, 27, 265-272.

TABLE 1A. RAW DATA TABLE, DATE 1 (June 13-July 25).

Tray # , Treatment	Che.	H.mo	H.bl	H.br	H.sp.	H.sl.	H.be.	Bae.	Chl.
1, cntrl	154	121	32	8	0	1	10	5	2
1, ns op	64	45	28	1	2	0	0	6	4
1, ns cl	18	8	3	0	0	1	3	1	1
1, cln op	18	3	4	0	0	0	0	3	0
1, cln cl	43	19	13	0	0	2	4	8	3
2, cntrl	35	17	18	0	0	0	2	8	0
2, ns op	33	14	9	0	0	0	0	3	0
2, ns cl	17	4	7	1	0	0	0	3	2
2, cln op	57	20	15	1	0	1	1	16	1
2, cln cl	35	23	8	2	0	0	0	15	1
3, cntrl	79	198	62	4	1	2	15	7	1
3, ns op	80	47	21	2	0	0	1	5	0
3, ns cl	17	7	4	0	0	0	1	2	0
3, cln op	45	40	14	1	0	0	0	0	0
3, cln cl	51	197	22	0	0	0	0	9	0

Che.= *Cheumatopsyche sp.*
H. mo.= *H. morosa*
H. bi. = *H. bifida*
H. br.= *H. bronta*
H. sp. = *H. sparna*
H. sl. = *H. slossonae*
H. be. = *H. bettini*

Bae. = *Baetis sp.*
Chi. = *Chimarra sp.*
Tray # = grouping according to stream area
cntrl = control
op = unlined tray bottom
ns = nonstream rock substrate
cln = cleaned rock substrate cleaned of insects
cl = mesh-lined tray bottom

TABLE 1B. RAW DATA TABLE, DATE 2 (June 13-July 10).

tray #, treatment	Che.	H.mo.	H.bl.	H.br.	H.sp.	H.sl.	H.be.	Bae.	Chl.
5, cntrl	56	270	250	4	0	0	0	27	0
5, ns op	24	196	318	2	0	4	4	6	0
5, ns cl	114	166	160	2	4	0	0	10	0
5, cln op	34	142	144	0	0	0	2	18	0
5, cln cl	46	156	156	0	0	0	0	25	0
6, cntrl	40	262	244	0	0	0	0	16	0
6, ns op	26	192	256	6	0	0	2	2	0
6, ns cl	76	228	256	0	8	0	0	32	0
6, cln op	28	196	240	0	0	0	0	12	0
6, cln cl	no data								

TABLE 1C. RAW DATA TABLE, DATE 3 . (June 25-July 17).

Tray #, treatment	Che.	H.mo.	H.bl.	H.br.	H.sp.	H. sl.	H. be.	Bae.	Chl.
8, ctrl	18	104	158	2	0	0	0	5	0
8, ns**op	26	72	120	0	0	0	2	7	0
8, ns**cl	56	124	156	0	4	0	12	16	0
8, cln op	8	42	113	3	0	0	5	2	1
8, cln cl	52	232	244	0	0	0	0	9	3

**These nonstream rocks had been in the stream for two weeks.

TABLE 2. SPECIES DENSITIES AND STANDARD DEVIATIONS.

(according to treatment and date)

Species	Treatment	Date 1		Date 2	
		density	std. dev.	density	std. dev.
Cheum.	control	89	60	38	19
	ns, open	59	24	25	1
	ns, closed	17	.5	95	27
	cln, open	51	8	23	14
	cln, closed	43	8	77	48
H. bifida	control	37	22	217	51
	ns, open	19	10	287	44
	ns, closed	5	2	208	68
	cln, open	15	1	166	66
	cln, closed	14	7	203	44
H. morosa	control	112	91	212	94
	ns, open	35	19	194	3
	ns, closed	6	2	197	44
	cln, open	30	14	127	78
	cln, closed	46	44	197	38
Baetis sp.	control	7	2	16	11
	ns, open	5	2	4	3
	ns, closed	2	1	21	16
	cln, open	8	11	11	8
	cln, closed	11	4	18	8

Cheum = *Cheumatopsyche sp.*
ns = nonstream rock substrate
cln = stream rock substrate cleaned of insects
open = unlined tray bottom
closed = mesh-lined tray bottom

TABLE 3. ANOVA RESULTS OF SPECIES DENSITIES BY DATE.

Species	**Degrees of Freedom**	**p-value**
Cheum.	4	0.704
H. bifida	4	0.976
H. morosa	4	0.611
Baetis sp.	4	0.438

Cheum. = Cheumatopsyche sp.

TABLE 4. SPECIES DENSITIES AND STANDARD DEVIATIONS.

(all treatments, according to date)

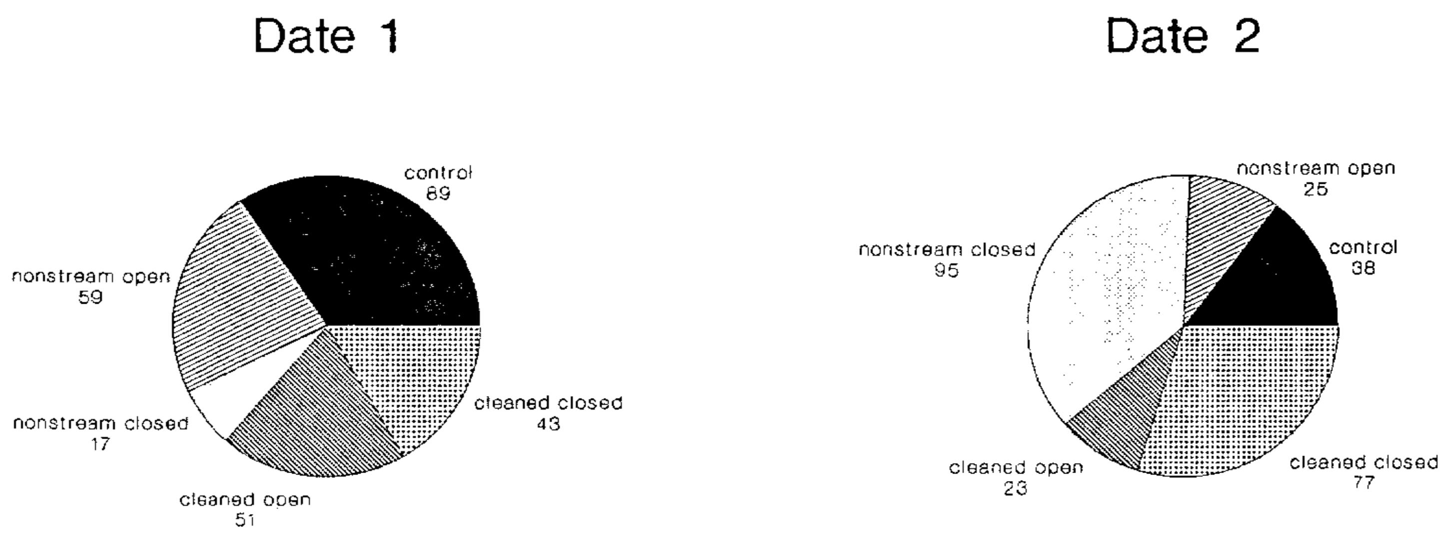
Species	Date 1 density	std. dev.	Date 2 density	std. dev.
Cheum.	52	34	50	37
H. bifida	18	15	211	60
H. morosa	47	55	183	63
Baetis sp.	6	5	14	10

TABLE 5. ANOVA RESULTS OF SPECIES DENSITIES BY TREATMENTS.

Species	Degrees of Freedom	p-value
Cheum.	1	0.905
H. bifida	1	0.000
H. morosa	1	0.000
Baetis sp.	1	0.438

Cheum. = Cheumatopsyche sp.

Figure 1. <u>Cheum</u>. Densities by Treatments



Date 1

Date 2

Figure 2. *H. morosa* Densities by Treatment

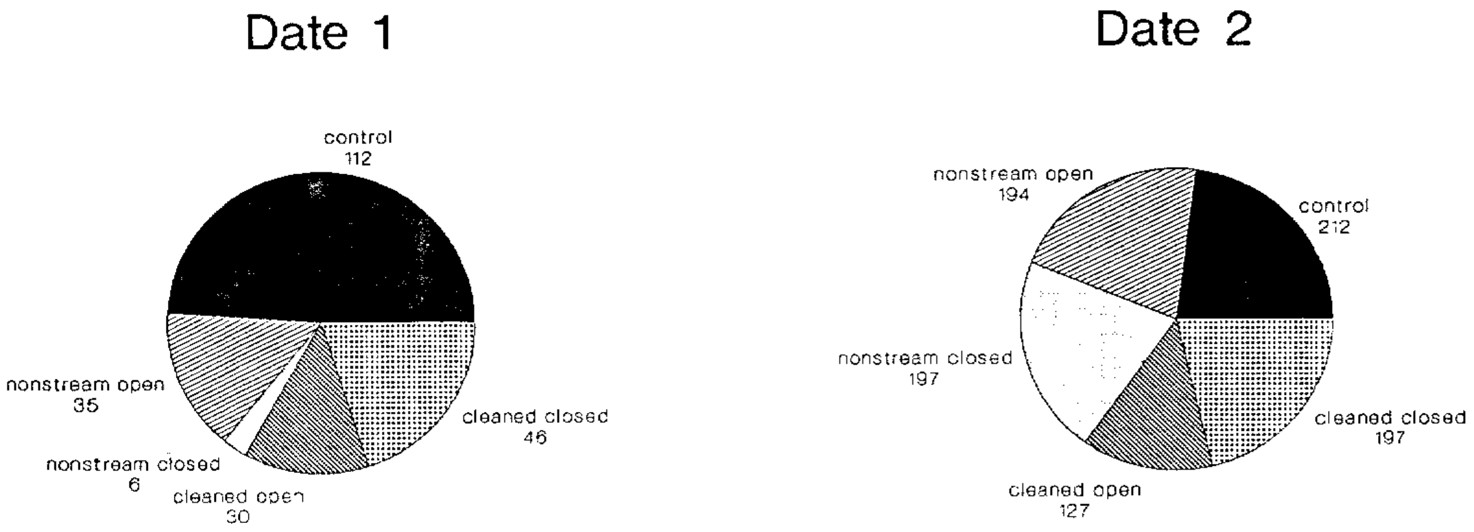


Figure 3. *H*. *bifida* Densities by Treatment

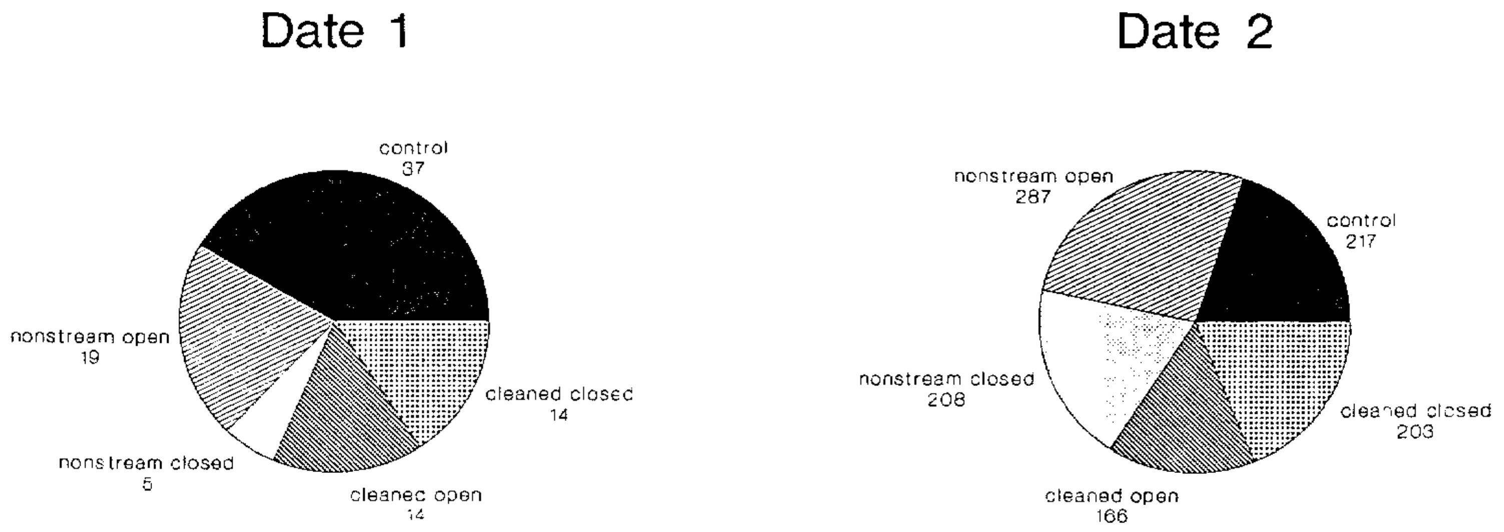
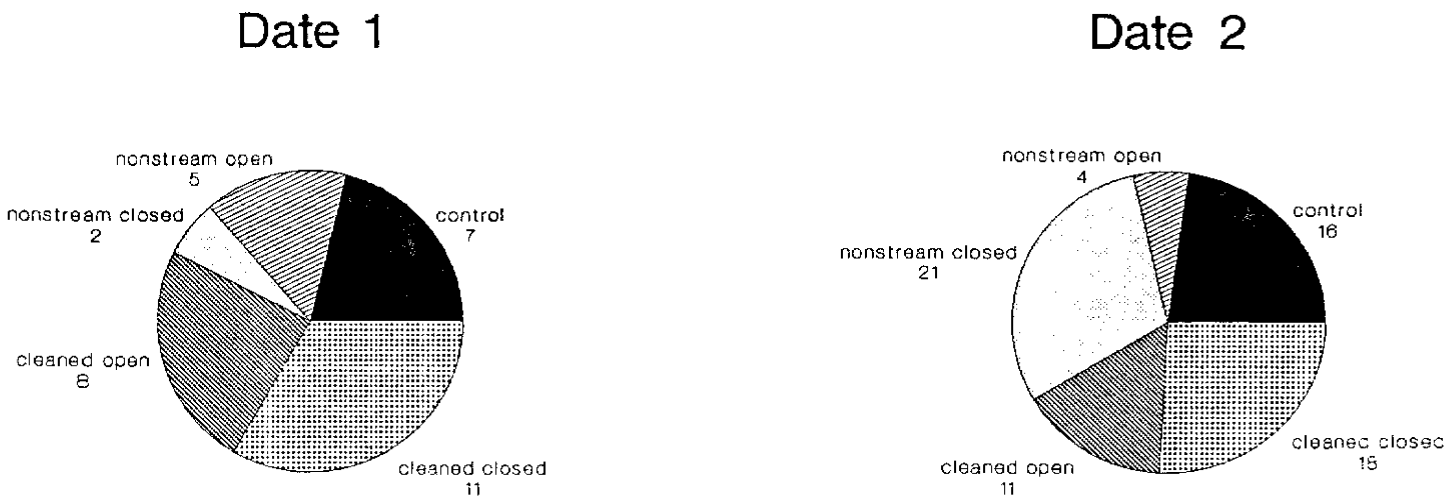


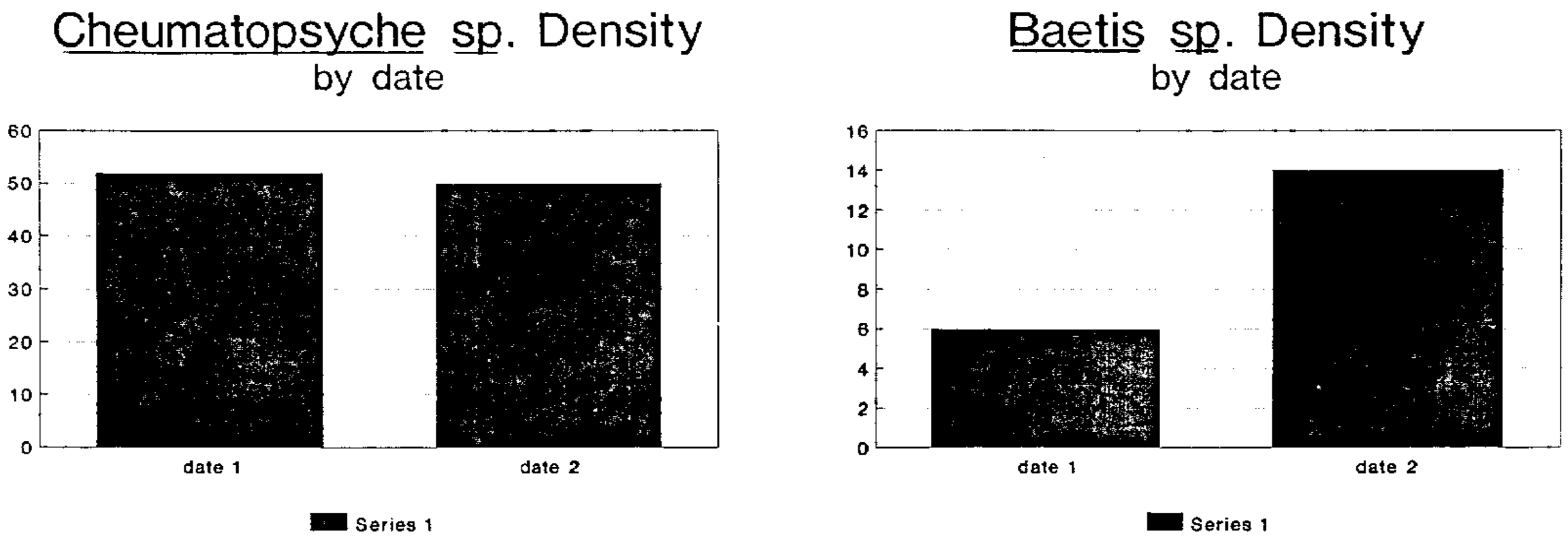
Figure 4. _Baetis_ Densities by Treatments



Date 1

Date 2

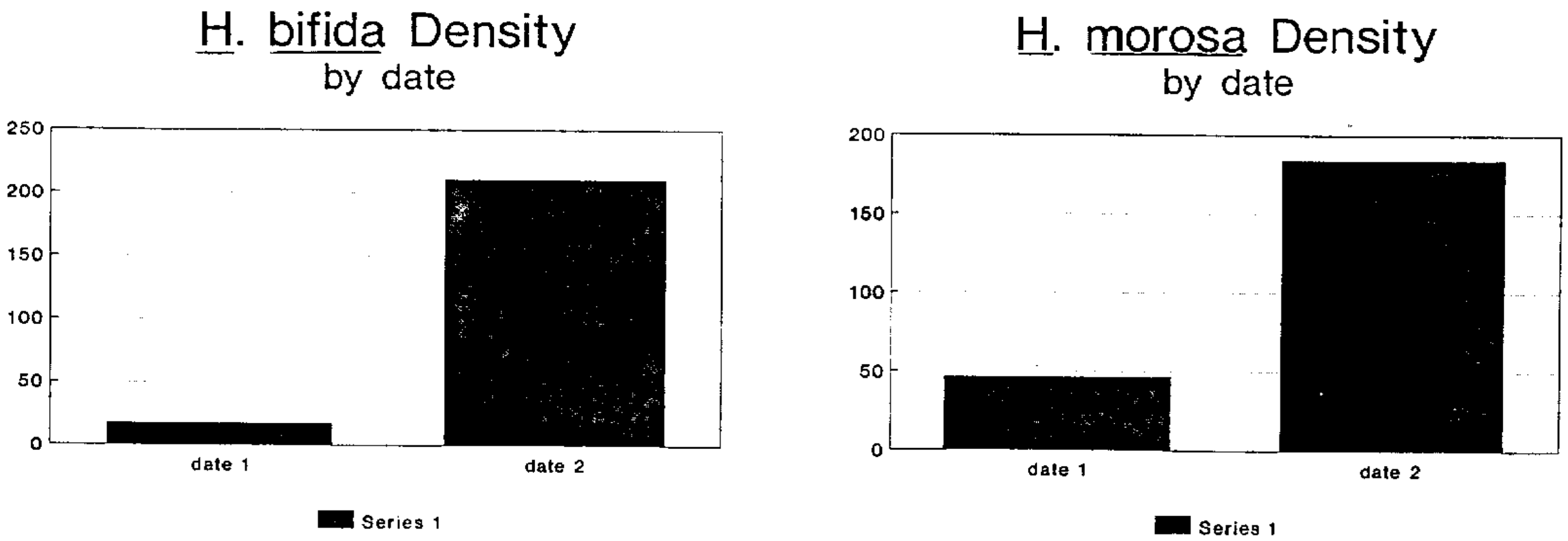
Figure 5a. Species Densities by Date



Cheumatopsyche sp. Density
by date

Baetis sp. Density
by date

Figure 5b. Species Densities by Date



H. bifida Density
by date

H. morosa Density
by date