An Enclosure for Experimental Manipulation of Lentic Littoral and Benthic Communities

Philip H. Crowley and Clay L. Pierce
T. H. Morgan School of Biological Sciences
University of Kentucky
Lexington, KY 40506

Dan M. Johnson and Robert E. Bohanan
Department of Biological Sciences
East Tennessee State University
Johnson City, TN 37614

ABSTRACT

We describe an experimental enclosure for use in lentic habitats which is relatively inexpensive, easily constructed, and conveniently stored between experiments. The basic design is quite versatile. Enclosures can be built in sizes ranging from 4 inches (10 cm) to 17 inches (43 cm) in diameter (area of bottom = 81 to 1444 square centimeters); the mesh size of the sides may be varied; and the tops may be open or closed by a clear plastic lid. Results of independent one-month-duration experiments suggest no "enclosure effects" on water temperature or dissolved oxygen concentrations. Comparison of benthos densities within enclosures and in open sites located nearby in the same habitats indicate that most taxa exhibited similar changes in abundance under the two conditions; some exceptions are attributable to exclusion of certain predators from the enclosures; and a few cases suggest that certain insect taxa may have experienced reduced recruitment when enclosure tops were closed.

We are currently performing a variety of competition experiments involving odonate larvae. Our experience suggests that this enclosure design might be appropriate for many kinds of in situ experiments in lentic littoral habitats.

INTRODUCTION

In recent years, attempts to document and understand the complex interactions among coexisting populations in freshwater communities have become increasingly experimental. One particularly useful sort of experiment involves the use of replicated enclosures subjected to experimental treatment and placed into the natural habitat. Such experiments attempt to reduce the number of assumptions needed for extrapolating experimental results to the unfettered "real world", obtaining the statistical power to deal with realistically high variance via replication. Some of these experiments have simply excluded predators from representative parts of the habitat (Thorpe and Bergey 1981a & b; Bohanan 1981), while others have involved manipulating predator densities (Peckarsky and Dodson 1980a; Thorp and Cofman 1982), food supply (Kajak and Kajak 1975; Neill and Peacock 1980; Polson 1980), habitat structure (Gliński 1981; Rabinow 1981), or community composition (Benke 1978; Kerfoot and Demott 1980; Peckarsky and Dodson 1980b; Benke et al. 1982) within the enclosures.

Most of the in situ enclosure experiments reported to date have not involved the sort of enclosure that could easily be removed from the habitat.
after the experiment, stored conveniently, and then re-used for additional experiments. Notable exceptions include Kajak's plastic cylinders for creating core microcosms (Kajak 1966, Kajak and Kajak 1975) and cubic cages of perlone bolster cloth (Kajak et al. 1965), both intended for muddy substrate, Thorp and Covich's floating microcosms, and Peckarsky's steel cages for use in stream systems (Peckarsky 1979). Renken, Crowley and Johnson (1982) described enclosures intended to meet these requirements, but reported relatively severe "enclosure effects" limiting their usefulness. In this paper we describe a convenient, reusable enclosure that is conceptually similar to Kajak's cubic cages but somewhat more versatile and easier to construct; it is considerably larger than Kajak's plastic cylinders or Thorp and Covich's microcosms and less expensive than Peckarsky's cages. These advantages have facilitated a series of replicated enclosure experiments on competition among larval odonates in lentic littoral-zone habitats of Bays Mountain Lake (City of Kingsport, Sullivan County, Tennessee). The primary goal of these experiments was to determine the intensity of competition within and among populations of odonate larvae, the dominant macroinvertebrate predators in this littoral community. The details of these experiments will be reported elsewhere (Pierce 1982; Pierce, Crowley and Johnson, in preparation; Johnson, Crowley, Bohanan, Watson and Martin, in preparation); but the collective evidence on enclosure effects derived from these experiments will be reported here.

One of the problems associated with most in situ enclosure experiments is the presence of "enclosure effects", influences on the response variables resulting from the container rather than the experimental treatment. If such effects are not eliminated or accounted for, results of the experimental treatment may be masked or distorted. We have attempted to minimize "enclosure effects" by: 1) utilizing shapes, sizes and materials that allow for adequate exchange of water and gases, 2) initiating experiments with natural substrate and natural densities of benthos rather than relying on colonization, 3) limiting the duration of experiments to one month to minimize divergence due to complicating historical events, and 4) avoiding the midsummer months, when water temperature and dissolved oxygen might be especially sensitive to the reduced exchange rates that accompany enclosure. Nevertheless, as an initial evaluation of the design, it is important to attempt to detect any remaining enclosure effects. This can be done by comparing certain characteristics of the environment or the benthic assemblage within enclosures to those nearby in the lake at the end of each experiment. Differences found in water temperature and in dissolved oxygen concentration should reflect any serious problems related to reduced mixing caused by the nylon netting barrier. Differences in population densities of benthic organisms should indicate problems related to reduced oviposition or migration, changes in productivity or food quality, and the effects of excluding certain predators. Interpretation of such differences in population density is facilitated if initial densities are also available for comparison.

METHODS AND MATERIALS

The enclosure is illustrated in Figure 1. It consists of a cylinder of chicken-wire supporting an inner cylinder of nylon netting with a mesh-size chosen to be large enough to permit a free exchange of water but small enough to prevent benthic organisms from moving through it. For the experiments de-
scribed here the mesh openings were 0.5 mm (Nitec NC 3-500), but this feature could be varied to suit the purposes of particular experiments. The bottom of the enclosure is formed by two circular clear plastic dishes — one within the other — with sides high enough to hold the bottom edge of the nylon netting firmly between them. These dishes, intended for use beneath flower pots and sold in many "Farm & Garden" stores, are manufactured by Curtis Wagner Co. (P.O. Box 55753, Houston, TX 77055) and are available in the following diameters (inches): 4, 5, 6, 7, 8, 9, 10, 12, 14 and 17. The sides of the dishes are manufactured with a slight rim which must be trimmed off to assure a tight grip on the netting. The bottom dishes are attached to the wire cylinder by a network of nylon cord underneath. It is possible to build enclosures of this design varying in bottom area from 81 to 1464 square centimeters. They are quite stable resting on the bottom in the absence of strong currents, wave action, or large inquisitive animals, but tying them to wooden stakes provides both markers and additional support.

Figure 1. Enclosure designed for in situ experimentation with lentic littoral benthos.

The experiments described here involve enclosures of two sizes: "small" enclosures, 30.5 cm high and 324 cm² in bottom area, made from 8 inch (20.3 cm) dishes which are 3 cm deep; and "large" enclosures, 45.7 cm high, 1464 cm²
in bottom area, made from 17 inch (43 cm) dishes which are 5 cm deep. The sturdy sides of these dishes and the tight sealing of the nylon netting at the bottom permit substrate of several centimeters thickness to be securely retained within the enclosure during handling and during the experimental period.

Depending on the purpose and particular of the experiment, a top on the enclosure may be desirable, if so, another plastic dish, with rim left on, can be inserted into the top of the cylinder and held in place with flexible metal strips attached to the chicken-wire cylinder (see Figure 1). Three of the experiments described here used enclosures equipped with tops; the other was conducted without tops.

The first experiment was a trial run that resulted in alteration of some procedures. Thus only the water temperature and dissolved oxygen comparisons are considered here. This experiment was conducted between 4 April and 1 May 1981 using 12 replicate large enclosures (see above) with tops. Each enclosure contained a layer of allochthonous detritus substrate and associated benthos removed from the lake with an Ekman grab, washed through a #35 (0.5 mm mesh) brass sieve, and sorted to remove odonate larvae. Both the large particles retained by the sieve and the small particles that passed through the sieve were placed into the enclosures after they were positioned in the lake along the 0.5 m depth contour; the clear plastic tops were then inserted and secured. Most enclosure tops were a few cm below the surface of the water. When the enclosures had been in place in the lake for one month, the top of each was carefully lifted, and water temperature and dissolved oxygen concentration were measured by inserting probes (Beckman Alton Monitor II, Model 531162). Similar readings were also made in the lake immediately adjacent to each enclosure.

Difficulty in washing and sorting the contents of Ekman grab samples to set up the first experiment led to alteration of that procedure in subsequent studies. Clear plastic dishes, identical to those used as enclosure tops, were buried approximately 6 cm deep under a layer of substrate in appropriate habitats at least two weeks before initiating each experiment. Since they were buried in existing natural substrate colonization was not an important consideration. A cylinder of chicken-wire and nylon netting identical to those used in the enclosures was used to secure the contents of each dish before it was lifted and placed into a large metal tub. Each sample was washed through sieves and sorted as before. This procedure resulted in a representative sample of all benthic populations but avoided the troublesome deeper layers of mud and fine organic material retained by the Ekman grab.

Complementary sampling techniques were used to estimate the density of all important populations in the benthic invertebrate assemblage. The densities of microcrustaceans (principally copepods and cladocerans) were estimated with an "inverted funnel sampler" (cf. Brarke 1976; see also Whiteside and Lindroth 1980) composed of two (for small enclosures) or three (for large enclosures) 7.5 cm diameter long-neck pyrex funnels attached to 60 ml collecting bottles with rubber stoppers. The two or three funnels comprising each trap were held together and stabilized by a small piece of "hardware cloth" between stoppers and funnels. These traps were placed on the substrate over night; individuals which migrated into them were concentrated with a Wisconsin-style plankton bucket, preserved in 70% alcohol, and then identified and counted under a dissecting microscope. Data for all funnels within each enclosure were pooled for statistical analysis, and mean numbers per funnel for each taxon were multiplied by an appropriate factor (226.35) to convert
them to estimates of numbers per square meter.

Macrowebrator densities were estimated by lifting each enclosure or "open" dish from the lake and preserving the contents in a plastic bucket with either formalin or alcohol. Benthic organisms were separated from the substrate by sugar flotation (Anderson 1959), preserved in 70% alcohol, and then identified and counted under a dissecting microscope. Any macroinvertebrates captured in the inverted funnel samples were added to make this a census of each benthic taxon in each enclosure or "open" dish. Mean numbers per enclosure (or "open" dish) were multiplied by an appropriate factor (6.83 for large, 30.84 for small enclosures) to obtain estimates of numbers per square meter.

The second experiment was conducted from 5 September until 7 October 1981 using large enclosures with tops on. The treatments relevant to this paper were: 1) initial samples taken from randomly selected dishes during initiation of the experiment; 2) open dishes left unenclosed and sampled during termination of the experiment; and 3) control enclosures which excluded both fish and larval odonates (Johnson, Crowley, Bohanan, Watson and Martin, in preparation). There were three replicates of each treatment assigned to randomly selected sites along one part of the 0.5 m depth contour. Initial and final benthos densities were estimated using the procedures described above. Water temperature was measured both inside enclosures and nearby in the lake. Dissolved oxygen concentrations were estimated using the aside modification of the Winkler method on water samples taken near the bottom of each control enclosure or open dish immediately before termination of the experiment.

The third experiment was conducted from 24 October until 21 November 1981 using small enclosures with tops on. There were two replicates in each of two blocks at different locations along the 0.5 m depth contour. The control enclosures in this experiment contained known densities of odonate larvae (Pierce 1982; Pierce, Crowley and Johnson, in preparation). Benthos densities were estimated as before. Temperature and dissolved oxygen were not measured.

The fourth experiment was conducted from 22 March until 30 April 1982 using three replicates in each of three blocks (located at different sites). Temperature and dissolved oxygen measurements were made at the conclusion of the first two experiments detected no apparent "enclosure effect" related to reduced exchange of water or gases through the enclosure surfaces. Water temperatures within enclosures were identical to those in the lake nearby on both occasions: 19(±0.5) C on 1 May 1981, and 16(±0.5) C on 6-7 October 1981. Dissolved oxygen concentrations (mg/l) on 1 May 1981 averaged 9.07 (S.E. = 0.14; N = 12) inside enclosures and 9.12 (S.E. = 0.12; N = 12) outside. On 6-7 October 1981, dissolved oxygen concentrations averaged 7.30 (S.E. = 0.18; N = 6) inside enclosures and 7.49 (S.E. = 0.4; N = 6).

RESULTS AND DISCUSSION

Analysis of water temperature and dissolved oxygen measurements made at the conclusion of the first two experiments detected no apparent "enclosure effect" related to reduced exchange of water or gases through the enclosure surfaces. Water temperatures within enclosures were identical to those in the lake nearby on both occasions: 19(±0.5) C on 1 May 1981, and 16(±0.5) C on 6-7 October 1981. Dissolved oxygen concentrations (mg/l) on 1 May 1981 averaged 9.07 (S.E. = 0.14; N = 12) inside enclosures and 9.12 (S.E. = 0.12; N = 12) outside. On 6-7 October 1981, dissolved oxygen concentrations averaged 7.30 (S.E. = 0.18; N = 6) inside enclosures and 7.49 (S.E. = 0.4; N = 6).
outside. Because of these results, we were not concerned about such effects in subsequent experiments. Visual inspection of the nylon netting after each of the four experiments indicated only minor epiphyte growth that might have impeded gas or water exchange, and benthic data suggests that no such effects existed. We conclude that there is no convincing evidence of an enclosure effect on temperature or dissolved oxygen concentration in the experiments conducted to date.

Comparisons of benthic population densities of the more abundant taxa for each of the last three experiments are summarized in Figure 2. Detrimental enclosure effects should be apparent as cases where the control enclosure means were less than those for the open dishes. There are only two such cases which are statistically significant (September 1981, Cladocera and Ostracoda). These effects might be attributed to reduced epiphyte and/or colonization, since the enclosure tops were sealed throughout the experiment. Two others are of sufficient magnitude to merit mention (October 1981, Rotifera; and April 1982, Chydoridae). There are a few cases suggesting positive enclosure effects, where mean densities within the enclosures exceed those in open dishes at the end of the experiment. None of these are statistically significant, but three of considerable magnitude are: September 1981, Simocephalus; April 1982, Simocephalus and Trichoptera. Since both fish and odonate larvae were excluded from the control enclosures in these two experiments, it seems reasonable to attribute these effects to release from predation pressure.

In contrast to the relatively few enclosure effects indicated in Figure 2, it is interesting to note the frequency with which both control enclosure and open dish means diverged similarly from initial densities. Seven such cases are statistically significant, and another five are suggestive. This indicates that populations within the enclosures exhibited temporal changes in density that were very similar to those experienced by populations above unenclosed dishes within the lake.

Based on our experience using these enclosures we are confident that others interested in experimental manipulation of lentic benthos communities will find them useful. We should caution, however, that our failure to detect significant enclosure effects is partially attributable to our conscious effort to avoid attempting such experiments during the warmest months when fouling by epiphytes and reduced dissolved oxygen levels might present greater problems. Also, we have not evaluated the influence of buried plastic dishes on the composition of benthic communities — an effect that might exist if vertical migration within the sediments were important. We encourage those who might adopt this enclosure design to investigate "enclosure effects" carefully for the conditions under which they conduct their experiments.

ACKNOWLEDGEMENTS

Removal of odonate larvae from the substrate placed into enclosures during these experiments required considerable effort, much of it provided by the following people: Flora Bohanan, Patricia Brown, Pam Martin, Tom Martin, Ann Pierce, and Charles Watson. We thank Marian Cochran, Jim Tharp and anonymous reviewers for commenting on an earlier draft of this manuscript. We also acknowledge the cooperation and assistance of Tom Bowman, director of Bays Mtn. Park, and his staff. This research was funded by National Science Foundation Grants DEB-8104425 (to DNU) and DEB-8104424 (to PHC).
Figure 2. Mean population densities (number per square meter) for the more abundant benthic taxa in three experiments described in the text shown on a logarithmic ordinate. Initial density estimates for each experiment are arranged in descending order and connected by a solid line. Dotted ovals surround means that are not significantly different (P > 0.05) as inferred from either designed orthogonal contrasts (enclosure effect; open dish vs. control enclosure; temporal trend; initial vs. open dish and control enclosure) or a posteriori tests (Duncan's Multiple Range). Benthic taxa are identified by the following abbreviations: CANIdae, CERAtogobiosidae, CHIRonidae (or CHIRoninae if TANypodinae are presented separately), CANTeridae, CYClopolydoidea, ALlOechnata, GSTRacida, POTRita, SIDA, SINDephalus, VICTebrata.

LITERATURE CITED


Bohanan, R.C. 1981. The effects of fish predation upon the littoral invertebrates of Bay Mountain Lake: a replicated field exclusion experiment. M.S. Thesis, East Tennessee State University, Johnson City, Tennessee, USA.


65


Peckarsky, B.L. 1979. Biological interactions as determinants of distributions of benthic invertebrates within the substrate of storm streams. Limnology and Oceanography 24:59-68.


Pierce, C.L. 1982. The relationship of behavior to competition and predation in two larval odonate populations. M.S. Thesis, University of Kentucky, Lexington, KY., USA.


