

ARTIFICIAL SUBSTRATES FOR *HEXAGENIA* MAYFLY NYMPHS¹

CALVIN R. FREMLING

Winona State College, Winona, Minnesota 55987, U.S.A.

and

GARY L. SCHOENING

Ashland Chemical Co., Box 340, Grassilli Station, Linden, New Jersey 07036, U.S.A.

Attempts have been made to study the behavior of *Hexagenia* nymphs within their burrows by utilizing thin aquaria filled with mud and water. Because the nymphs avoid light, however, they usually construct their burrows so that little of them is exposed to the viewer. *Hexagenia* nymphs are also used in bioassay experiments to determine their tolerance to various toxicants. When test nymphs are contained in clean, water-filled vessels, however, they swim constantly as they attempt to burrow into the bottoms of the vessels. As a consequence of this activity they become fatigued and their susceptibility to toxicants is increased. If the bioassay vessels are provided with mud bottoms, the water in the vessels becomes turbid and it becomes difficult to observe the nymphs. Also, the mud makes accurate toxicant monitoring virtually impossible. We have succeeded in fabricating inexpensive, inert, artificial substrates which are suitable for nymphal behavior studies and for bioassay work.

The first substrate was made by imbedding 10 curled pretzels at uniform intervals around the interior of a 9 in. × 2 in. wooden mold which had been filled to a depth of 2 in. with epoxy resin and its hardening catalyst. The resin had been previously mixed with equal parts of dry sawdust to economize on resin. After the resin block had hardened it was removed from its mold and allowed to soak until the pretzels became soft enough to flush out. The edges of the burrows were then trimmed with a small chisel.

Epoxy resin substrates are quite expensive and it requires considerable time to construct them. Therefore, a master mold was made of epoxy resin so that subsequent substrates could be easily manufactured from a resilient polyvinyl acetate plastic (Plate Ia.).

Observation aquaria and bioassay vessels are constructed by building a tight-fitting glass aquarium around each substrate with glass glue. When used as bioassay vessels in conjunction with a MOUNT-BRUNGS proportional diluter (MOUNT and BRUNGS, 1967), the aquaria are fitted with intermittent siphon drains and the vessels are arranged so that their sides touch, thus making the burrows dark. Blocks of wood are placed around the perimeter of the resultant block of vessels so as to darken the outer burrows. At prescribed intervals during the bioassay, the wooden blocks are removed and the vessels are separated so as to view the nymphs in their burrows (Plate Ib.). Nymphs almost always leave their burrows before they die. Thus, it is possible not only to see which are dead but also those which are "ecologically dead" (those which may be still feebly alive but which have abandoned their burrows and are thus exposed to predators). Behavior studies of nymphs within their artificial burrows are best made under red

¹ Supported in part by Federal Water Pollution Control Administration Grant No. WP-009871.

or yellow light. The nymphs are relatively insensitive to long wave lengths of the visible spectrum.

When the polyvinyl acetate substrates are newly-constructed they apparently contain excess plasticizer and volatiles which are toxic. Therefore, they are routinely stored in a well-aerated area until their smell is gone. They are also washed thoroughly in detergent water prior to use. Polyvinyl acetate mayfly substrates can now be obtained from the Nasco Company, Fort Atkinson, Wisconsin 53538, U.S.A. ¹

RÉSUMÉ

Substrats artificiels pour les larves de Hexagenia

Au cours de nos recherches sur les larves d'*Hexagenia*, nous avons mis au point la construction de substrats artificiels bon marché et inertes permettant la réalisation de bio-essais et l'observation du comportement de ces nymphes. Les substrats sont faits d'une matière plastique flexible, à base de polyvinyl acétate. Les aquariums d'observation et les cuvettes de bio-essai sont obtenus par la confection d'une enceinte de verre étanche autour de chaque substrat avec une colle spéciale pour verre.

ZUSAMMENFASSUNG

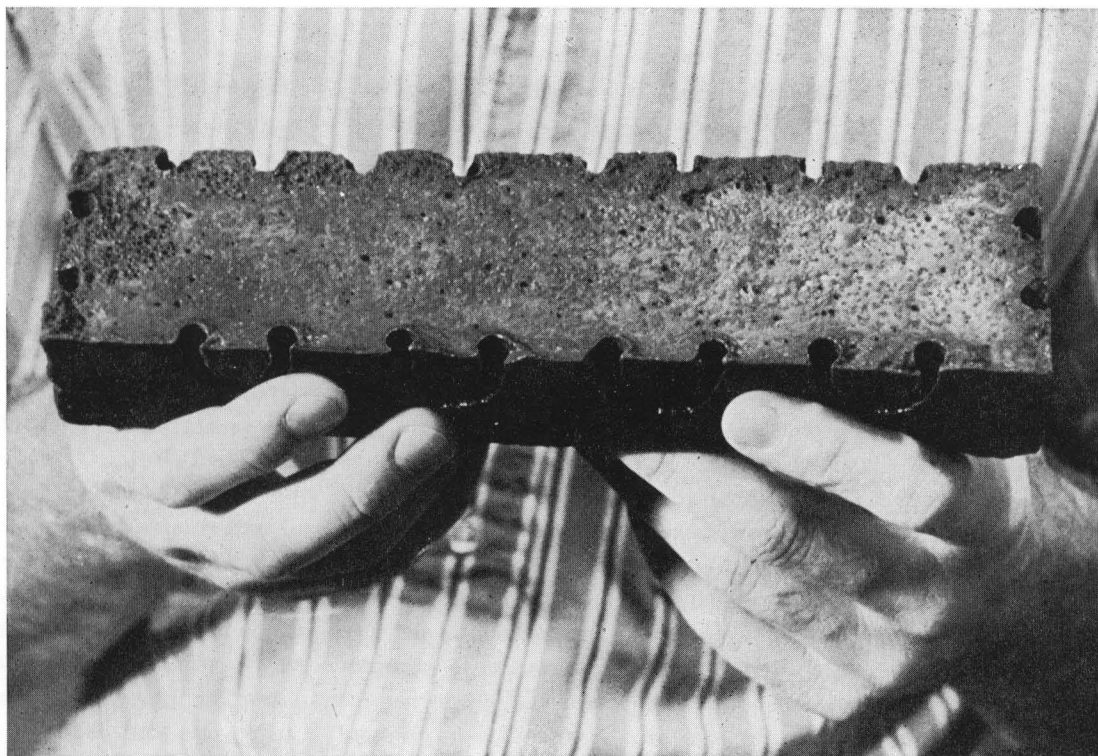
Substrate für Hexagenia Eintagsfliegen Nymphen

Während Studien mit *Hexagenia* Nymphen ist es uns gelungen billige, leblose künstliche Unterlagen herzustellen, die passend für das Studium des Nymphenbetragens und Bioproben sind. Diese Substrate sind aus elastischen Polyvinyl-azetat-Plastik gemacht. Beobachtungsaquarien und Bioproben-Gefäße sind konstruiert, durch bauen eines engpassenden Glas-aquariums um jedes Substrate mit Glassklebe.

REFERENCES

MOUNT, D.I., and W.A. BRUNGS. (1967). A simplified dosing apparatus for fish toxicology studies. *Water Res.* 1 : 21-29.

¹ Subsequent to the presentation of this paper, increased attention has been given to the extreme sensitivity of aquatic insects to polychlorinated biphenyls. It is recommended, therefore, that epoxy resin substrates be used in lieu of polyvinyl acetate substrates for bioassay experiments.



a



b

PLATE I

- a. Artificial substrate for *Hexagenia* mayfly nymphs. Ten burrows are arranged around the periphery of the block.
b. *Hexagenia* mayfly nymph residing in an artificial burrow.