

200000-2

SOME PROBLEMS AND TECHNIQUES IN REARING BIVALVE LARVAE

RUTH D. TURNER
Museum of Comparative Zoology
Harvard University

AND

ARTHUR CHRISTIAN JOHNSON
Marine Science Institute, Nahant, Massachusetts
Northeastern University

The successful rearing of bivalve larvae is a relatively recent accomplishment. The reasons are that bivalve eggs and larvae are small, the free swimming period relatively long, and for most of this period the larvae must be fed. The difficulty of handling such small larvae (early straight-hinge average 75-85 μ in length), the lack of good algal cultures for feeding, and the frequent infestations of the cultures by bacteria and fungus were the major causes of failure. Much of the early work of developing algal cultures and controlling infestations was done by Loosanoff and his co-workers at the USBCF laboratory, Milford, Connecticut.

The ultimate objective of our interest in rearing boring and fouling bivalves is to understand the factors controlling the settlement and successful attachment or penetration of the larvae. As we wanted to study larvae in the field, an inexpensive 'traveling laboratory' was developed that could be easily packed, shipped and assembled in any laboratory no matter how small, so long as there was a seawater system and electricity. The system has proved worthwhile and economically feasible for working periods of 2½ to 3 months or more. The specialized equipment shipped for our first work in the tropics included: 1) 2 Fulflo Filter Assemblies (Model F1 50-10) and a carton each of 1, 15 and 30 μ replacement cartridges to remove particulate matter and biological contaminants from the water; 2) an ultra-violet water treatment unit obtained through the USBCF laboratory at Milford, Connecticut with 2 extra General Electric U-V germicidal light tubes (model G36T6) for controlling bacteria and fungus; 3) a set of stainless steel sieves with meshes ranging from 23 to 300 μ for separating and washing the eggs and the larvae; 4) an abundance of glass, flexible tygon and gum rubber tubing; 5) a Marco air pump; 6) two 5 gal. glass carboys; 7) 4 each of 1000, 400 and 250 ml. beakers; 8) a quantity of disposable pipettes; 9) a Leitz Labolux-D microscope with standard base and pillar stand, an ultra pak illuminator, dipping cones and a Leica camera attachment with exposure meter. Dissecting microscopes were available at the laboratory. In place of an autoclave we used a large pressure cooker.

During this first working period we followed the general procedures for rearing larvae. The adults of all species were dissected from the wood so that we could identify the species and be sure of the source of the eggs and sperm. Ripe adults of oviparous species were placed in individual petri dishes until they spawned. The eggs were removed by pipetting, washed and placed in large beakers of fresh millipore filtered seawater and a small

suspension of sperm was added. When fertilization was completed the eggs were again washed to remove excess sperm and the dividing eggs were put in large carboys $\frac{3}{4}$ filled with sea water which had been passed through the 15 and 1μ filters and the U-V treatment unit. Air was bubbled through the culture and the water was changed every two days. We found, however, that the carboys were difficult to handle and that keeping all the larvae in one large container could result in the loss of the entire culture. Consequently we now keep our cultures in several 1 or 2 quart plastic refrigerator boxes, which are easier to handle, and an infestation in one subculture will not endanger the entire crop of larvae.

In our early experiments the pediveligers were held in beakers and small pieces of wood were suspended in the water. This procedure did not allow continuous observations of the larvae under the microscope and made repeated observations of the same specimen difficult. Changing the water and removing the wood for examination and photographing disturbed the larvae and reduced the number that penetrated the wood. To alleviate this, micro-aquaria were made by inserting the tip of a pipette through a small hole made near the rim of the bottom portion of a 20×100 mm disposable plastic petri dish. The pipette was sealed in place with silicone cement and then connected with tygon tubing to the sea water system. A series of these were set in a shallow water table, and the flow of the water to each was controlled by a valve or clamp. The substrate glued in the bottom of the petri dish can vary with requirements of the larvae being observed. For wood borers we used discs of white pine 3-4 mm thick. This procedure allows larvae to settle on only one surface and they can be observed continuously as they explore and penetrate the wood. It eliminates the necessity of changing the water or feeding the developing young and, when the borers have grown to adults, they are easily removed for spawning. Increase in burrow length can be observed by placing a strong light below the petri dish, and, by placing the microscope on the pillar stand, using the ultrapak illuminator and dipping cones, it is possible to observe all of the cultures with a minimum of effort without disturbing the animals.

For the past year, through the kindness of Dr. N. W. Riser, we have had laboratory space at the Marine Science Institute of Northeastern University at Nahant, Massachusetts. The laboratory has a fine seawater system but, unlike the tropics, the temperature of the water drops to 0° to 2°C in the winter. Consequently, without a heating system it is impossible to breed even the local species during the winter, and the adults of tropical and temperate species cannot be maintained. A two stage heating system was designed and

Plate 1

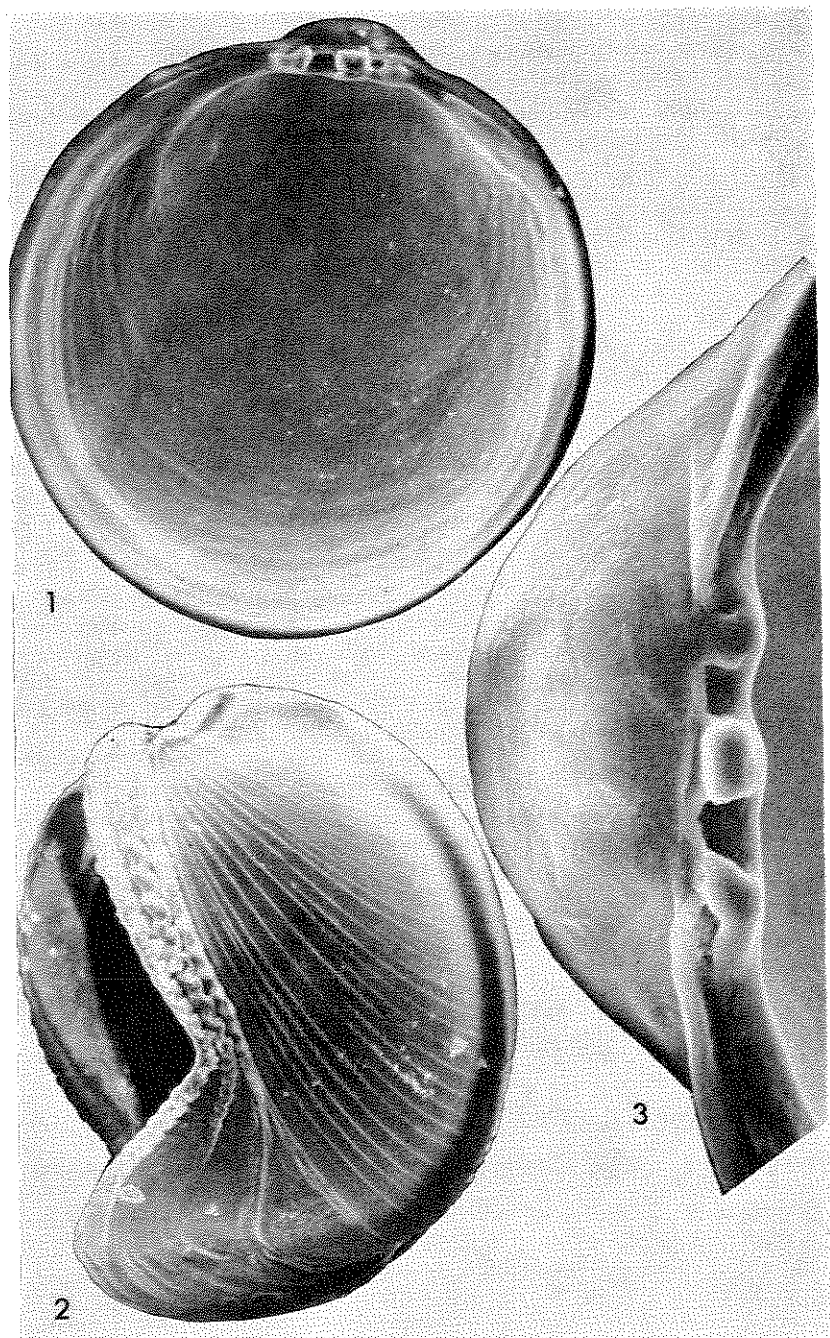
→

Young laboratory reared specimens of *Lyrodus pedicellatus* Quatrefages. Adult specimens from Alamitos Bay, California. Scanning electron microscope pictures show details of the hinge and sculpture impossible to obtain with the light microscope.

Fig. 1. Inner view of right valve of pediveliger ($300\times$)

Fig. 2. Hinge line of same specimen ($1100\times$).

Fig. 3. Side view of newly settled specimen showing sculpture of the larval shell and the beginning of the development of the adult shell ($240\times$).



built in cooperation with Mr. Thomas Wait of Northeastern University. The water, after passing through three 120 μ filters, enters a 54 cubic foot wooden, fiber glass lined tank set as high as possible so that the water is fed to the culture tanks by gravity. Here the temperature of the water is raised to 10° to 15°C by means of three 5,000 watt quartz heating units. Most of this water is used for work with temperate species or for maintaining summer conditions for northern species. It can be passed through 30, 15 and 1 μ filters for work with larval cultures. Some of the water goes into a small plexiglass heating tank where the temperature is further raised by using 1 to 3 vycor 1000 watt immersion heaters, depending on the amount of increase needed to reach the desired temperature. This tank is usually maintained at 27°C and most of the water feeds into the tropical holding tank which is held at 27°C but can be raised to 30° to 34°C when inducing animals to spawn. It is mixed with 10° to 15°C water from the main tank to maintain the temperate water tank at 18° to 20°C. Except for the large heating tank the system at Nahant is portable and can easily be moved.

The purchase of a window air conditioner for the smallest room in the laboratory has allowed us to maintain algal cultures for feeding purposes. Stock cultures were obtained from Dr. Guillard of the Woods Hole Oceanographic Institute, and we follow his technique in maintaining them.

Problems of bacterial, fungal or ciliate infestations in the larval cultures are best controlled by employing good laboratory techniques. Frequent thorough washing of the eggs and larvae with 1 μ filtered water drawn slowly through the U-V tube greatly reduces bacteria and virtually eliminates all other contaminants. All specimens, wood or otherwise, must be carefully cleaned on the outside as soon as they arrive at the laboratory in order to prevent contamination of the holding tanks.

REFERENCES

- Davis, H. C. (1961). Effects of some pesticides on eggs and larvae of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*). *Comm. Fish. Rev.* 23, 8-23.
- Davis, H. C., and P. E. Chanley. (1956b). Effects of some dissolved substances on bivalve larvae. *Proc. nat. Shellfish Ass.* 46, 59-74.
- Davis, H. C., and R. R. Guillard. (1958). Relative value of ten genera of microorganisms as foods for oyster and clam larvae. *Fish. Bull., U. S. No.* 136, 58, 293-304.
- Davis, H. C., V. L. Loosanoff, W. H. Weston, and C. Martin. (1954). A fungus disease in clam and oyster larvae. *Science*, 120, 36-38.
- Guillard, R. R. (1959). Further evidence of the destruction of bivalve larvae by bacteria. *Biol. Bull., Woods Hole*, 117, 258-266.
- Imai, T., M. Hatanaka, and R. Sato. (1950a). Breeding of marine timber-borer, *Teredo navalis* L., in tanks and its use for anti-boring test. *Tohoku J. agric. Res.* 1, 199-208.
- Loosanoff, V. L. (1951). Culturing Phytoplankton on a large scale. *Ecology* 32: 748-750.
- Loosanoff, V. L., and H. C. Davis. (1963). Rearing of bivalve mollusks. *Advances Mar. Bio.* 1, 1-136.
- Loosanoff, V. L., J. E. Hanks, and A. E. Ganaros. (1957). Control of certain forms of zooplankton in mass algal cultures. *Science*, 125, 1092-1093.
- Prytherch, H. F. (1934). The role of copper in the setting, metamorphosis, and distribution of the American oyster, *Ostrea virginica*. *Ecol. Monogr.* 4, 47-107.

- Ukeles, R. (1961). The effect of temperature on the growth and survival of several marine algal species. *Biol. Bull., Woods Hole*, 120, 255-264.
- Walne, P. R. (1958). The importance of bacteria in laboratory experiments on rearing the larvae of *Ostrea edulis* (L.). *J. Mar. biol. Ass. U. K.* 37, 415-425.
- Waugh, G. D. (1958). Ultra-violet sterilization of water for rearing oyster larvae. *Nature, London* 181, 1747.
- Wilson, D. P. (1951). A biological difference between natural sea waters. *J. Mar. Biol. Ass. U. K.* 30, 1-26.
- Wood, P. C. (1961). The principles of water sterilization by ultra-violet light, and their application in the purification of oysters. *Fish. Invest. Lond., Ser. II*, 23, 1-48.

CABEZA DE VACA, DEALER IN SHELLS

JAMES X. CORGAN

Austin Peay State University, Clarksville, Tennessee

ABSTRACT

Every American school child learns that Alvar Núñez Cabeza de Vaca led the first European exploration of the American Gulf Coast. Few people realize that Cabeza de Vaca was the first Caucasian merchant in the south-western United States. Few conchologists realize that he was a dealer in shells. During the years 1530-1534 he operated out of the Galveston area and traded widely in the central Gulf Coast. About twenty-two months were spent in the "field," on extended business trips into the then unknown interior of the continent.

One result of Cabeza de Vaca's mercantile activities was an increased knowledge of American geography. Another result was the distribution of marine molluscan shells to localities in northern Texas, northern Louisiana, and perhaps southern Oklahoma. For at least five centuries before Cabeza de Vaca's time, marine shells had been used by inland aborigines. They are common in scores of archaeological sites and they pose a major problem in historical interpretation. How did thousands of marine shells reach the interior of the continent? Do pillage and occasional barter between tribes or individuals account for the movement of vast numbers of shells? Why do similarly carved marine shells occur at widely separated, but roughly contemporaneous, sites? Were there professional traders? Did the traveling salesman play a significant role in the aboriginal American economy?

Cabeza de Vaca, dealer in shells, provides us with the autobiography of one 16th Century American traveling salesman. It is a unique source of information on commerce in aboriginal America.

A detailed reconstruction of Cabeza de Vaca's trade is not possible. He was generally lost as he traveled and recorded his experiences years after his adventure ended. His literary style de-emphasized objective description and the species he traded in are unknown. In writing his memoirs, Cabeza de Vaca de-emphasized his mercantile career. It was lost time, it did not directly contribute to an escape to New Spain. Despite these severe limitations, translations of the original manuscripts (Bandelier, 1905; Smith, 1851) and scholarly interpretations (cited in Covey, 1961; Terrell, 1962) can be used to establish the chronologic and geographic limits of his trade. Writings

can also be examined for lists of trade goods, evidence of business competition, and buyer-seller relationships.

Cabeza de Vaca, a slave of coastal Indians, became a merchant when he convinced his masters that a merchant might bring peace. Raiding parties from the interior regularly pillaged the coast. They were drawn, at least in part, by a cultural need for shells. A neutral trade, an alien trader, obviated the need for war. Trade brought peace. Wherever he journeyed, Cabeza de Vaca was warmly received. Undoubtedly a fair skin and an alien appearance were great assets; still his customers had clear concepts of trade and experience in trade. Inland customers needed marine shells. Coastal peoples wanted ocre and other products of the interior.

In 4 short years his shell trade carried Cabeza de Vaca from abject slavery to honor and wealth. He became a celebrity and eventually a great medicine man. Other things came to overshadow the shell trade but the business itself was a commercial success. Cabeza de Vaca's manuscripts do not allude to competitors. His concept of a trader's role in commerce was apparently new to those who had held him slave. His commercial relationships suggest that he was the only trader. Apparently plunder and disorganized trade between individuals were the normal methods through which marine shells moved inland on the 16th century Gulf Coast.

It is unfortunate that Cabeza de Vaca was a 16th century shell dealer and that his base of operations was an economically deprived region where the cultural level was presumably low. Most archaeological sites that yield abundant shells predate the 16th century. They may have been supplied by direct or indirect contact with more advanced coastal peoples. Still, this is conjecture, and Cabeza de Vaca's recollections remain the only objective description of aboriginal commerce in the American southwest.

REFERENCES

- Bandelier, F., 1905. The journey of Alvar Núñez Cabeza de Vaca. . . . Allerton Book Co.
Covey, C., 1961. Cabeza de Vaca's adventures in the unknown interior of America. Collier Books.
Smith, B., 1851. Narrative of Alvar Núñez Cabeza de Vaca. G. W. Riggs.
Terrell, J. U., 1962. Journey into darkness. William Morrow & Co.

SOME MOLLUSCA OF CEDAR BOG, CHAMPAIGN COUNTY, OHIO

EUGENE P. KEFERL
Columbus, Ohio

Cedar Bog is a nature sanctuary maintained by the Ohio Historical Society. It is located about five miles southwest of Urbana in Champaign County, Ohio. This sanctuary has been of great interest to the botanists for some time because within its boundaries is an arbor vitae or northern white cedar swamp, a bog meadow and a marl meadow. Each of these plant associations is more typical of northern Michigan, Wisconsin, and Canada.

The sanctuary is situated over 460 feet of glacial deposits from three, possibly four, glaciers. The last glacier, the Wisconsin, covered Champaign County about 19,000 years ago. When the Wisconsin glacier advanced, it