

*Advances in*

**MARINE BIOLOGY**

**VOLUME 1**

*Edited by* **F. S. RUSSELL**

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**MARINE  
BIOLOGY**

VOLUME 1

*Edited by*  
**F. S. RUSSELL**  
*Plymouth, England*



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## PREFACE

The very great expansion of marine research in recent years has resulted in a mass of published results scattered through a very wide range of periodicals. In consequence it is becoming increasingly difficult to obtain a general picture of the overall advance that is being made in our knowledge of the many aspects of life in the sea.

It is hoped that the production of this new serial publication will help biologists to keep abreast of knowledge in the different lines of research on the biology of marine organisms. It is intended that each annual volume shall contain comprehensive review articles summarizing the general position of our knowledge in individual fields. Attention will be given to recent advances in fisheries biology, the results of research in which are often published in periodicals that may not normally be available in the libraries of university biology departments. These investigations are, however, of very general interest since they usually concentrate on the biology and ecology of a few individual species in greater detail than for other marine organisms.

When possible shorter review articles may also be published drawing attention to new developments and growing points in marine biology.

General articles on the biology of marine organisms will include information on the environment only in so far as it is necessary for an understanding of their habits. Articles will not be published which relate only to the physical and chemical conditions in the sea in relation to water movements and deep-sea oceanography.

Any suggestions from readers on fields of research that need reviewing and might form subject matter for future volumes will be welcomed. Editorial correspondence should be addressed to me at Wardour, Derriford, Crownhill, Plymouth, Devon.

*April, 1963*

F. S. R.

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# REARING OF BIVALVE MOLLUSKS

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## I. INTRODUCTION

Until recently rearing of larvae and juveniles of marine bivalves, on a basis where repeatable results could be expected, was virtually impossible because of the lack of satisfactory, reliable methods. Thus, although culturing of larvae of bivalves was first attempted in the last century, few workers succeeded in rearing them to metamorphosis and, as a rule, they were rarely grown beyond early straight-hinge stage. Even though, in the twenties, Wells (1927) was able to rear the American oyster, *Crassostrea virginica*, from artificially-fertilized eggs to spat, and Prytherch (1924) raised larvae of the same species in large numbers, their results could not be consistently repeated by other investigators. The failures were usually due to poor culture methods and want of good food for the larvae, especially when they were grown in heavy concentrations. It is also possible that diseases, including those caused by fungi, were responsible for the persistent failures.

Attempts to rear larvae of bivalves were not confined, of course, to *C. virginica*. Cultivation of larvae of several other species was also tried by early workers. For example, Belding (1912) attempted to raise larvae of clams, *Mercenaria mercenaria* (formerly *Venus mercenaria*), but without success. He concluded that there was no practical method for raising clam larvae to straight-hinge stage because of the small size and delicate nature of the egg. Wells (1927), however, was more successful and carried the clam larvae in his cultures until they metamorphosed.

Even in more recent years the situation remained practically the same. This is well demonstrated by the work of Yoshida (1953) who, in his attempts to identify larvae of Japanese bivalves, had to depend upon obtaining the larvae from plankton, instead of trying to grow them from fertilized eggs under controlled laboratory conditions where their identity would be assured. The difficulties experienced as recently as 1953 by Nikitin and Turpaeva (1959), in their attempts to raise larvae of some bivalves of the Black Sea by using old methods, vouch for the inefficiency of these now obsolete approaches.

Obviously, as the general studies of marine organisms progress, the necessity for methods by means of which bivalve larvae can be reared successfully becomes more and more urgent. The availability of such methods would immediately offer the opportunity to study the effects of numerous environmental factors, singly and in combination, upon the growth of larvae, thus helping to determine the physiological requirements of these organisms. It would also offer the means for studying the genetics of bivalves and initiating properly controlled experiments on selective breeding of these mollusks. Moreover, by

growing larvae under different conditions their diseases and parasites could be studied and methods for their control developed. Finally, because the larvae of many species of bivalves are much alike in size and appearance, it was virtually impossible to identify them, with any degree of accuracy, in plankton collections. With the recent development of methods of rearing larvae in the laboratory, however, this difficulty should soon disappear because larvae found in plankton can now be easily and accurately compared with preserved samples and photomicrographs of larvae grown from known parents under controlled conditions.

By using successfully conditioning and rearing methods, many aspects of which were developed at Milford Laboratory (Loosanoff and Davis, 1950; Loosanoff, 1954) and are described in this article, larvae of approximately twenty species of bivalves have been cultured at Milford. Not all of these species are indigenous to New England waters or even to our Atlantic coast. Several are native to the Pacific and one species came from Europe. The non-indigenous forms were representatives of commercially important species in which we were interested. The bivalves, the larvae of which have been reared from fertilization to metamorphosis, included the transverse arc clam, *Arca transversa*; the ribbed mussel, *Modiolus demissus*; the common mussel, *Mytilus edulis*; the bay scallop *Pecten irradians*; the jingle shell, *Anomia simplex*; the European cyster, *Ostrea edulis*; the native Pacific coast oyster, *Ostrea lurida*; the American oyster, *Crassostrea virginica*; the Japanese oyster, *Crassostrea gigas*; Morton's cockle, *Laevicardium mortoni*; the hard shell clam, *Mercenaria* (—*Venus*) *mercenaria*, and its relative, *Mercenaria* (—*Venus*) *campechiensis*; hybrids of these two species; the Japanese clam, *Tapes semidecussata*; the small clam, *Pitar* (—*Callocardia*) *morrhua*; the rock borer, *Petricola pholadiformis*; the razor clam, *Ensis directus*; the surf clam, *Mactra* (—*Spisula*) *solidissima*; the soft shell clam, *Mya arenaria*; and the common shipworm, *Teredo navalis*.

Of the above species the larvae of *Crassostrea virginica* and *Mercenaria mercenaria* have been studied most intensively and, as a result, we have accumulated an extensive knowledge of their physiological and ecological requirements (Loosanoff and Davis, 1950; Loosanoff *et al.*, 1951; Loosanoff and Davis, 1952a; Loosanoff and Davis, 1952b; Davis, 1953; Loosanoff, 1954; Loosanoff *et al.*, 1955; Davis and Chanley, 1956a; Davis, 1958; Davis and Guillard, 1958; Loosanoff, 1958a; Loosanoff, 1958b; Loosanoff, 1959; and Davis, 1960). Several other species, such as the European oyster, *Ostrea edulis*, and the Olympia oyster, *Ostrea lurida*, have also received much attention.

Most of the other species, however, were studied less intensively, work on them being confined to culturing their larvae and observing the appearance and general behavior of the latter. Naturally, our knowledge of the requirements of larvae of such species is still fragmentary but, nevertheless, we shall present the information already available even though it is admittedly incomplete.

## II. EQUIPMENT

The rearing of larval and juvenile bivalves requires an adequate supply of sea water of proper salinity and free of substances that may interfere with their normal development. The water used at Milford Laboratory is pumped from the Wepawaug River at a point about 100 yd from its entrance into Long Island Sound. Because the tidal rise and fall in this area is from 6 to 10 ft, the flushing rate of this comparatively narrow and shallow inlet is relatively high.

The sea water is pumped into a 6 000-gal wooden storage tank located in the laboratory attic. Because pumping normally takes place  $1\frac{1}{2}$  hr before and after the high tide stage, the salinity of the water is usually near 27 parts per thousand, which is virtually the same as in Long Island Sound, where the majority of the forms, the larvae of which are described in this article, exists. To assure a supply of water of high salinity the intake of the salt water system is located approximately 4 ft below the mean low water mark; therefore, it is at a safe distance from the surface layers which, after periods of heavy rains, may be greatly diluted.

The main pump providing the laboratory with salt water is rubber-lined. The intake and distribution lines, as well as the check and cutoff valves, are made of lead. The faucets, however, are of hard rubber. The storage tank is of cypress wood and is painted inside with asphalt paint.

We prefer lead pipes because, although pipes made of several new plastics are nontoxic, light and inexpensive, they possess several important disadvantages. One of them is that since it is often necessary to reduce fouling inside of the pipes by treating them with hot water or steam, this treatment, commonly used with lead pipes, cannot be employed in systems containing plastic parts as it may cause damage, especially at the joints of the pipeline.

Another serious disadvantage in using plastics is that they adsorb and absorb many chemicals, including insecticides, and once contaminated can themselves become a source of later contamination of the sea water. Moreover, since some plastics are permeable to insecticides and other compounds, these materials might enter from the sur-

rounding soil into pipes carrying sea water. Finally, some laboratories that have plastic sea water systems have complained that since these pipes are not electrically self-grounded, they present a serious element of danger in laboratories with wet floors.

We are finding an increasing usage for plastic pumps and pipes, especially in our temporary installations. We have also found that tanks made of Fiberglas, instead of wood, can be advantageously used, especially in areas where wood-boring organisms, such as *Teredo*, are common.

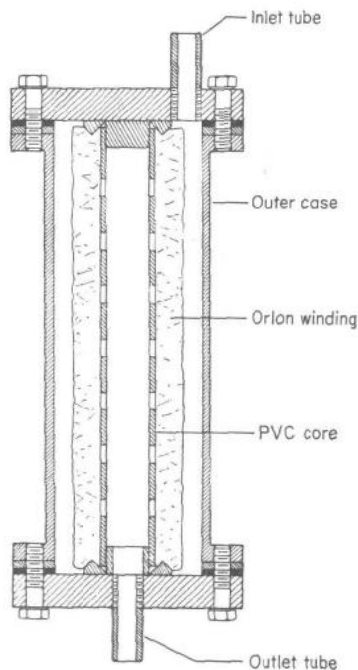


FIG. 1. Diagram of water filter designed to remove all particulate matter larger than  $15\ \mu$  in diameter. Description in text.

Normally, in addition to small algae on which larval and juvenile mollusks feed, sea water contains many large diatoms, free-swimming crustaceans, gastropods, worms, etc., and their eggs and larvae. Many of these forms compete with bivalve larvae for food, prey on them or may even harbor diseases or parasites that could be transmitted to larvae. We prevent undesirable organisms of larger sizes from entering our larval cultures by filtering the water and later killing the smaller forms with ultraviolet light.

The filter element consists of a polyvinylchloride (PVC) core wound with Orlon. The complete unit (Fig. 1) is manufactured by Commercial Filters Corporation, Melrose, Massachusetts (filter no. CFXI-10-5

with an O15-R10X filter element). These filters, designed to remove all particulate matter larger than  $15\mu$  in diameter, are made with a variety of core and winding materials. We chose the PVC core because it is nontoxic, and the Orlon winding because it is inexpensive, nontoxic and does not support bacterial growth.

To prevent fungus diseases in clam larvae and juveniles we began treating sea water with ultraviolet light in 1954 and, within a short time, had some evidence that such treatment, even of running, unfiltered sea

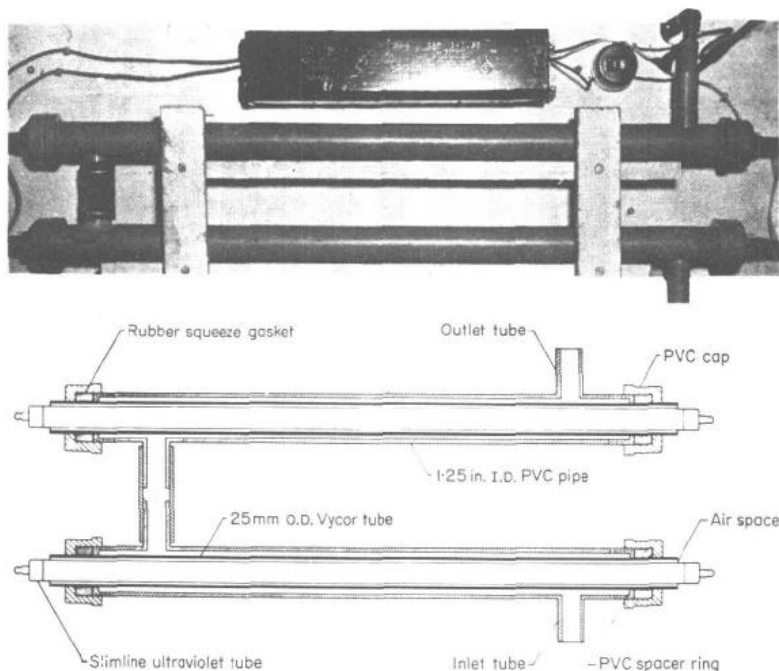


FIG. 2. Photograph (*above*) and drawing (*below*) of ultraviolet water treatment unit used at Milford Biological Laboratory. Description in text.

water, was helpful in preventing mortality of juvenile clams. In the summer of 1955 it was definitely demonstrated that larval cultures, receiving treated water and untreated phytoplankton from the outdoor mass culture, developed fungus, whereas larval cultures in which phytoplankton and sea water were both treated did not. Since that time, it has become a routine practice to treat with ultraviolet light all sea water used for our larval cultures and for keeping recently-set clams and oysters. Moreover, we are attempting to supply ultraviolet-treated running sea water to all containers in which later stages of juvenile clams are grown.

Ultraviolet treatment of sea water for purification of shellfish has been described by several workers in Japan (Sato, 1954; Satoh, 1960) and Wood (1961) in England. As is the practice in our laboratory, Waugh (1958) also used ultraviolet-treated sea water for rearing larvae of the European oyster, *O. edulis*. Several of these authors have described the equipment used but, because of certain considerations, we constructed our own units, a description of which is offered here.

The ultraviolet water treatment unit consists of a  $1\frac{1}{4}$ -in inside diameter PVC pipe, 30 in long, threaded at each end for caps (Fig. 2). A small ring of PVC is cut to fit inside of each end of this pipe and reamed to act as a spacer for a 25-mm Vycor tube. A squeeze gasket is used to make a water-tight seal between the Vycor tube and the end of the PVC pipe. An inlet tube is located on the side at one end of the PVC pipe and an outlet tube is located on the opposite side at the other end. The 33-in-long, slimline ultraviolet tube lays free in the  $32\frac{1}{4}$ -in-long Vycor tube and extends slightly beyond at each end.

In practice we use two such units connected in a series so that the water passes the length of both tubes. Since there is only about a  $\frac{1}{8}$ -in layer of water surrounding the Vycor tube, this apparatus, when used with filtered sea water, should give practically sterile water at the rate of flow of about 10 gal per min. With unfiltered sea water the efficiency is not expected to be as great, but our experience has shown that even then the treatment is of considerable help in reducing mortality of juvenile clams and in preventing fouling by tunicates, worms and bryozoa.

To condition mollusks for out-of-season spawning it is necessary to keep them in running sea water at temperatures of 18° to 20°C or sometimes higher. Warm sea water is also needed for rearing larvae and juveniles during the cold season. Since the water must not contact toxic metals, conventional water heaters cannot be used. Therefore, to heat the water we use a type of heat exchanger (Loosanoff, 1949). The sea water is heated as it passes through a coil of lead pipe immersed in hot fresh water, which fills the tank of a conventional gas water heater that has had the top removed to permit insertion of the lead coil (Fig. 3). However, because the thermostatic controls of a conventional water heater are not sufficiently accurate, the gas flame is controlled through a solenoid gas valve by a Minneapolis-Honeywell thermostat (T415A323XA3). The thermostat-sensing bulb is encased in a lead well in the warm sea water line and maintains the temperature at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ .

By mixing varied amounts of cold and heated sea water any temperature between that of the unheated water and 37°C can be main-