

SELECTIVE FEEDING ON ALGAL CELLS BY THE VELIGER  
LARVAE OF NASSARIUS OBSOLETUS (GASTRO-  
PODA, PROSOBRANCHIA)<sup>1, 2</sup>

THEODORA C. PAULSON AND RUDOLF S. SCHELTEMA

*Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543*

Food is an important factor determining the survival and developmental rate of planktotrophic larvae. Both the quality and quantity of food available to molluscan veligers have been related to their successful completion of pelagic life. The nutritional value of various species of algae to certain bivalve larvae (*viz.*, *Venus mercenaria*, *Crassostrea virginica*, *Ostrea edulis*, and *Mytilus edulis*) has been studied by Davis (1953), Davis and Guillard (1958), Walne (1963), Davis and Calabrese (1964), and Bayne (1965). However, while it is known from these studies that some species of algae are apparently more favorable as food than others, no experiments have yet been made that test the ability of molluscan larvae to discriminate between phytoplanktonic forms or that demonstrate that veligers can feed selectively upon those microorganisms that result in faster growth. Only the single experiment described in a short note by Loosanoff *et al.* (1953, pp. 586-587) declares that ". . . the larvae [of *Venus mercenaria*] showed both quantitative and qualitative selectivity in feeding . . . qualitative selectivity of food was demonstrated when the larvae were fed a mixture of several food organisms; given a mixture of *Porphyridium* (3  $\mu$ ) and *Chlamydomonas* (10  $\mu$ ), the larvae rejected *Porphyridium* while ingesting the much larger *Chlamydomonas*." No further details of this experiment are given. The question as to whether a gastropod larva can choose or select from a mixture of microorganisms remains to be answered.

The method of feeding by gastropod veligers has been described by Werner (1955) for the larvae of *Crepidula fornicata* and by Thompson (1959) for those of the nudibranch *Adalaria proxima*. Feeding is accomplished by the velum. According to Thompson (p. 241), "the long velar cilia serve a dual purpose in both imparting a forward motion to the larva and bringing a constantly renewed supply of seawater within the influence of the feeding apparatus." A second row of cilia on the hind side of the velum serves in the actual collection and propulsion of the food particles. The direction of beat of these cilia is such that food from either velar lobe is directed toward the mouth (*vide* Werner, 1955, Fig. 9, p. 196). The border of the mouth is ciliated and particles that are sufficiently small are carried into the foregut. Material which is too large or rejected for other reasons is carried forward along the foot to its border where it is cast off. This pattern of feeding is common to all gastropod veliger larvae where it has been observed. The differences

<sup>1</sup> Contribution No. 2058 from the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

<sup>2</sup> This research was supported by a summer-fellowship to the first author and by National Science Foundation Grant GB-2207.

in detail among various species are mainly concerned with the rejection tracts along the foot. Our studies have confirmed this mode of feeding in *Nassarius obsoletus*.

If veliger larvae are allowed to feed on a mixture of several species of algae, the total or absolute amount consumed at the end of a given time may be determined by finding the difference between concentration of the resulting mixture and that of a control identical in all ways but not containing larvae. If the veligers feed at random, the relative proportions of the different species of algae should be the same in both mixtures at the end of the experiment. However, if larvae feed preferentially on any one species, this will be indicated by a shift in the relative proportions of the different algae compared with that found in the control mixture. Each species of alga can be assigned a rank preference according to the proportion eaten. We will present here evidence that the veliger larvae of the prosobranch gastropod *Nassarius obsoletus* can feed selectively when offered a mixture of three species of algae.

#### MATERIALS AND METHODS

The larval development of *Nassarius obsoletus* and the methods for rearing the veligers have already been described (Scheltema, 1962). Larvae were "hatched" in the laboratory from egg capsules collected from Barnstable Harbor (Cape Cod) during July and August. The eggs were stored at around 12° C. to inhibit development until needed in the experiments (*vide* Scheltema, 1967). When transferred from a storage temperature of 12° to a room temperature of approximately 25° C., the egg capsules released larvae in from several hours to three or four days. The veligers were raised in 10-liter culture jars at room temperature. Following a change in sea water every third or fourth day, *Phaeodactylum tricornutum* was added to each culture so that the resulting concentration of algal cells was approximately  $2 \times 10^5$  cells/ml. Each 10-liter culture contained several thousand larvae. Within two weeks from the time of "hatching" the veligers reached the settling stage, at which time some metamorphosed larvae were observed on the sides of the culture jars. The veligers grew from approximately 250  $\mu$  to 600  $\mu$  during the free-swimming larval period.

Two large uni-algal cultures of *Phaeodactylum* in 15-liter carboys were maintained on an autoclaved medium of a commercial chemical garden fertilizer. These cultures served as the source of food for both feeding experiments and rearing the larvae. Cultures of *Cyclotella nana* and *Dunaliella tertiolecta*, used for the feeding experiments, were grown axenically in 1- and 2-liter flasks on "f/2" medium (Guillard and Ryther, 1962). All cultures were illuminated continuously in a constant temperature room at 16° C. The density of the cultures before use for experimentation was from  $4 \times 10^5$  to  $10 \times 10^5$  cells/ml. of *Cyclotella*, from  $8 \times 10^5$  to  $20 \times 10^5$  cells/ml. of *Dunaliella*, and from  $15 \times 10^5$  to  $40 \times 10^5$  cells/ml. of *Phaeodactylum*.

Feeding experiments were conducted in 3-liter jars at room temperature. Suspensions of phytoplankton to be used were made up by the addition of known amounts of the culture described above to filtered sea water so that the resulting concentration was about  $3 \times 10^5$  cells/ml. One to 1½ liters of cell suspension were added to each of several 3-liter jars. Larvae which had been starved for six hours prior to the experiments were added to each jar except that which was to serve

as a control. Each experimental jar contained from several hundred to several thousand veligers of the same age. The mixtures were stirred and aerated continuously for a period of from 10 to 16 hours. At the end of this period the concentrations of each species of algae in both the control and the experimental jars were determined. This was done by obtaining a sample of each with a wire loop having an 0.5-mm. diameter, fixing the sample with an iodine-acetic acid solution, and making counts on a Levy phase hemacytometer using a phase microscope at a magnification of 250  $\times$ . The entire grid of the hemacytometer was used. Each mixture was counted several times; the total number of cells counted from each control was *ca.* 1500 cells. From each of the experimental mixtures, between 500 and 1500 cells were enumerated. It required 4 to 7 counts to achieve this total. The standard errors of the estimates ranged between 4 and 12% of the mean.

TABLE I

*Preference among three different species of algal food by veliger larvae of Nassarius obsoletus (Say) (based on differences in numbers of cells ingested)\**

Species of algae	Percentage of experiments		
	1st choice	2nd choice	3rd choice
<i>Cyclotella</i>	81.3	18.8	0.0
<i>Phaeodactylum</i>	12.5	62.5	25.0
<i>Dunaliella</i>	6.3	18.8	75.0

\* Results from sixteen experiments.

## RESULTS

The relative proportions of the three species of algae in the experimental mixtures differed significantly from those of the controls. Each of the three species of algae could be assigned a rank of preference according to the percentage of cells eaten. If all the data are pooled and summarized, *Cyclotella* was the first choice in 81.3% of all the experiments. In most instances *Phaeodactylum* was second choice while *Dunaliella* most commonly was last to be selected (Table I).

A triangular graph with three coordinate axes can be used to compare control and experimental concentrations in the experiments (Figs. 1, 2, 3). The coordinate axes are the three perpendicular bisectors, and are labelled at the vertices, "P", "D", and "C" for *Phaeodactylum*, *Dunaliella*, and *Cyclotella*, respectively. The scale runs from 100% at the vertex to 0% at the side opposite the vertex. A point at the intersection of the three bisectors represents a mixture in which each of the three species make up 33.3% of the total, that is, each is present in equal amounts. Points indicated by squares represent the compositions of the control while points shown by circles represent the compositions of the experimental mixtures. Lines connect experiments with the controls to which they are compared; control points are lettered and experimental points numbered so that each line is labelled with a letter and number. The experiments were distributed among three veliger size-categories so that it is possible for experiments having a common control to be represented on different graphs if the experiments involve veligers of

different ages. Each graph represents veligers in the same size-category. The size-categories represent different ages that roughly correspond with the three developmental stages that have been described previously (Scheltema, 1962, p. 4, Pl. I) except that the category in Figure 2 here overlaps the "early" and "intermediate" stages. Newly "hatched" veligers, those that had not fed on plankton prior to the experiment, are grouped separately (Fig. 1).

*Dunaliella* is rarely the first choice of veligers of any size. In all but one case (D-1, Fig. 1), the percentage composition of *Dunaliella* is greater in the experi-

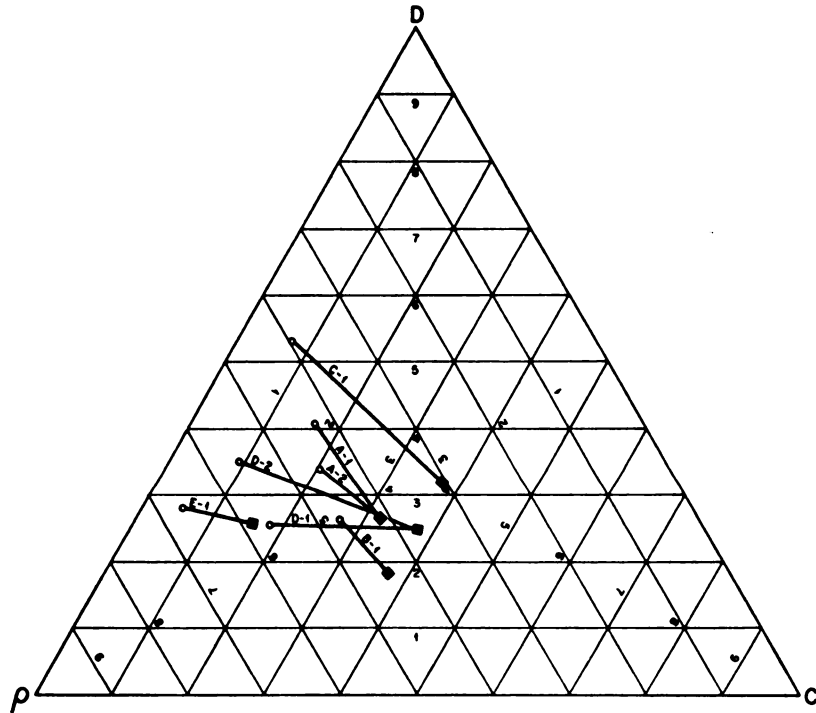


FIGURE 1. Choice among three different species of algal food by newly emerged veliger larvae of the gastropod *Nassarius obsoletus* (Say). For explanation see text.

mental mixture than in the control. On the graphs this is indicated by the fact that experimental points are closer to the *Dunaliella* vertex ("D") than the control points. This becomes more marked with increasing size of the larvae.

Experimental points are always further from the *Cyclotella* vertex ("C") than points representing controls. This shows that the percentage of *Cyclotella* remaining in the mixture always decreases and consequently that *Cyclotella* has been consumed by the larvae. *Cyclotella* is never the last choice of veligers of any size.

Preference for *Phaeodactylum* is different for newly "hatched" than for older larvae. Figure 1 shows that the percentage composition of *Phaeodactylum* is greater in the experimental mixture than in the controls, while Figures 2 and 3 show the experimental composition of *Phaeodactylum* in per cent to be less than

that of the control. Older larvae have a greater preference for *Phaeodactylum* than do newly hatched larvae.

Although no marked difference in the outcome of the experiments can be related to the original proportions of the three algal species present, future experiments should use initial mixtures of varied composition.

The results shown on the triangular graphs (Figs. 1, 2, 3) can be compiled and summarized in a somewhat different way. The relative amount of each algal species removed in the experiments can be computed. Hence:

$$\% \text{ removed} = \frac{\text{control concentration} - \text{experiment concentration}}{\text{control concentration}} \times 100$$

When the data from all the experiments in each category are averaged the results (Table II) show that *Cyclotella* is preferred over *Dunaliella* by all size categories of larvae ( $P \cong 0.05$ ).

TABLE II

*Average percentage of cells removed from suspension by veliger larvae of Nassarius obsoletus at different stages of development when given a choice of three algal species as food*

Stage of larval development	Species of algae		
	<i>Cyclotella</i>	<i>Phaeodactylum</i>	<i>Dunaliella</i>
	Average per cent removed*		
Newly emerged veliger larvae (6 experiments)	72.9 $\pm$ 6.6	39.2 $\pm$ 8.1	33.1 $\pm$ 8.4
Veliger larvae of 200–400 $\mu$ length (7 experiments)	83.9 $\pm$ 3.8	69.1 $\pm$ 8.8	50.1 $\pm$ 11.5
Veliger larvae of 400–600 $\mu$ length (3 experiments)	70.2 $\pm$ 11.9	71.9 $\pm$ 4.0	24.5 $\pm$ 3.6

\* One standard error of the mean indicated.

In order to check the possibility that rearing larvae upon *Phaeodactylum* might influence the veliger's behavior in the feeding experiments, two choice experiments were made using two-day-old veligers that had been exclusively reared on either *Dunaliella* or *Cyclotella*. The outcome of the experiments was conclusive. Veligers reared on *Dunaliella* consumed 60% *Cyclotella*, 50% *Phaeodactylum* and 21% *Dunaliella*. Veligers reared on *Cyclotella* ate 66% *Cyclotella*, 67% *Phaeodactylum*, and 31% *Dunaliella*. Selection against *Dunaliella* occurred regardless of the species of microorganisms upon which the larvae had been previously reared.

Newly hatched larvae do not discriminate between *Phaeodactylum* and *Dunaliella*; intermediate stage larvae show no clear-cut preference for *Phaeodactylum* over either *Dunaliella* or *Cyclotella*. The largest size class of veligers show equal preference for *Phaeodactylum* and *Cyclotella*. The net result is an increasing preference for *Phaeodactylum* with increasing veliger size.

The relative volumes consumed in each of the experiments may be computed if the cell volume for each species of alga is known. The average volume for *Cyclotella* may be regarded as approximately  $203 \mu^3$  (Guillard and Cassie, 1963, p. 62), that of *Phaeodactylum* and *Dunaliella* as  $120 \mu^3$  and  $400 \mu^3$ , respectively (Parsons *et al.*, 1961, p. 1004). If the volume of *Cyclotella* cells consumed is arbitrarily given a value of one, then the relative volume for the other two algal species for each size range of veliger larvae may be computed from the data in Table III. These values show that although *Dunaliella* cells are removed from

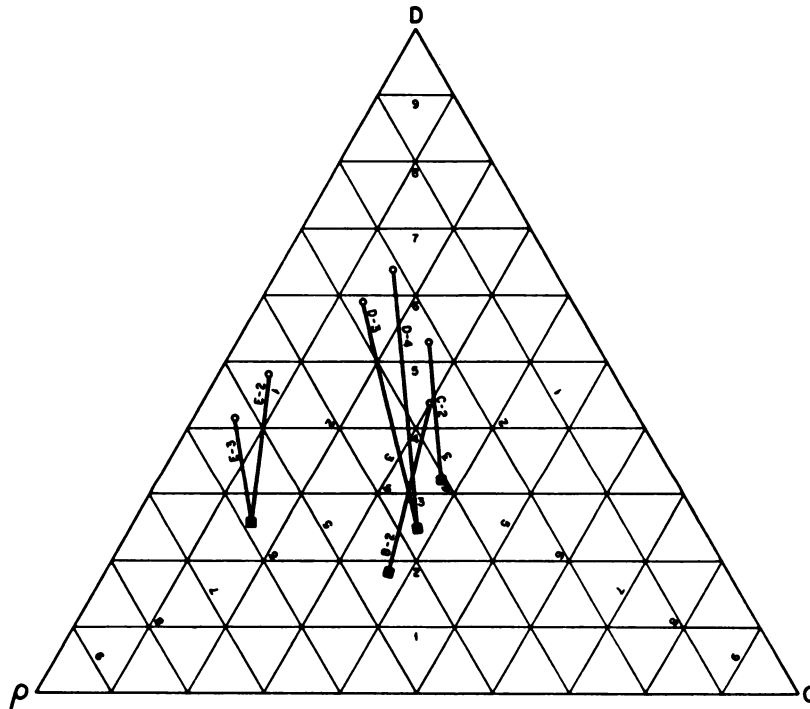


FIGURE 2. Choice among three different species of algal food by veliger larvae of the gastropod *Nassarius obsoletus* with a shell length between  $250 \mu$  and  $400 \mu$ . For explanation see text.

suspension at a significantly slower rate, the volumes of cells removed by all veligers up to  $400 \mu$  in length are nearly identical to that of *Cyclotella*. Only in larvae over  $400 \mu$  does the relative volume of *Dunaliella* removed become somewhat less. At all ages the relative volume of *Dunaliella* removed by veligers seems to equal or exceed that of *Phaeodactylum*.

#### DISCUSSION

Three qualities of microorganisms in the natural environment are likely to make possible their selection as food by larvae. These are their (1) size, (2) availability or concentration, and (3) chemotactile attractiveness or "taste."

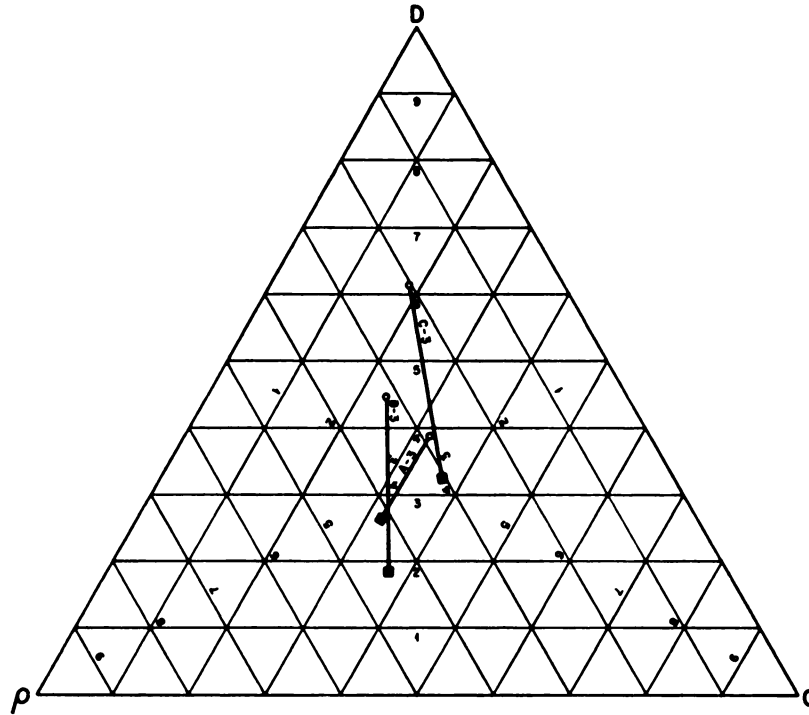


FIGURE 3. Choice among three different species of algal food by veliger larvae of the gastropod *Nassarius obsoletus* with a shell length between  $400\ \mu$  and  $600\ \mu$  (creeping-swimming stage). For explanation see text.

The size of the microorganisms which may be ingested is limited by the diameter of the mouth and intestine of the larvae. Thorson (1946, p. 447) summarizes data on the diameter of the esophagus and anterior portion of the intestine

TABLE III

*Relative volume of cells consumed by veliger larvae of Nassarius obsoletus when given a choice of three algal species as food*

Stage of larval development	Species of algae		
	<i>Cyclotella</i>	<i>Phaeodactylum</i>	<i>Dunaliella</i>
	Relative volume removed		
Newly emerged veliger larvae (6 experiments)	1.0	0.3	0.9
Veliger larvae of $200\text{--}400\ \mu$ length (7 experiments)	1.0	0.5	1.2
Veliger larvae of $400\text{--}600\ \mu$ length (3 experiments)	1.0	0.6	0.7

of prosobranch veligers and concludes that the theoretical size range of food must be from 5 to 45  $\mu$ . The size range of food actually accepted falls within these dimensions but is usually less than 30  $\mu$ . Hence, prosobranch veliger larvae are largely restricted to nannoplanktonic microorganisms (< 25  $\mu$  diameter) as food and in nature consequently must be restricted to dinoflagellates, minute species of diatoms and possibly bacteria. It may be that the shape of some food organisms affects their acceptability.

It is rather well known that when larvae of Mollusca are placed in very dense concentrations of algae, their ability to feed becomes impaired. For example, Loosanoff *et al.* (1953) showed that *Venus mercenaria* larvae held in heavier than optimum concentrations of cells contained less food in their stomachs than those held in lighter food concentrations. Veligers of *Nassarius obsoletus* when placed in very heavy concentrations of algal cells are unable to feed normally and reject cells along the foot. The concentrations used in the experiments here described were within the range at which cells were readily accepted and near those concentrations observed in regions where the larvae regularly occur naturally. Hence Hulburt (1956) showed  $10^6$  to  $10^7$  cells/l. total phytoplankton in Great Pond, Massachusetts, values that compare reasonably well with *ca.*  $10^5$  cells/ml. used by us.

The experiments do not reveal how the larvae select their choice of food, whether it be by size, concentration or chemotactile sense. However, they do show that a direct comparison of the "food value" of different algal species must take into account the volume or biomass of cells consumed per unit time, and that this varies according to the phytoplankton species encountered.

#### SUMMARY

Planktotrophic veliger larvae of the prosobranch gastropod *Nassarius obsoletus* (Say) are able to feed selectively. Presented with a mixture of *Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, and *Cyclotella nana*, they exhibit a definite order of preference in the number of cells of each algal species they ingest. *Dunaliella* is clearly the last choice for veligers of all sizes. Early larvae choose *Cyclotella* in preference to the other two species, but with increasing veliger size and age, ability to discriminate between *Phaeodactylum* and *Cyclotella* diminishes while the selection of *Phaeodactylum* in preference to *Dunaliella* increases. However, in recently emerged and intermediate stage veliger larvae the relative cell volume of *Dunaliella* consumed is approximately equal to that of *Cyclotella*, whereas that of *Phaeodactylum* is substantially less than that of either of the other two algal species. In older larvae the relative volume of *Cyclotella* eaten exceeds the volumes of both other species, whereas the volumes of *Phaeodactylum* and *Dunaliella* become approximately equal.

#### LITERATURE CITED

- BAYNE, B. L., 1965. Growth and the delay of metamorphosis of larvae of *Mytilus edulis* (L.). *Ophelia* (Copenhagen), 2: 1-47.
- DAVIS, H. C., 1953. On food and feeding of larvae of the American oyster *C. virginica*. *Biol. Bull.*, 104: 334-350.



- DAVIS, H. C., AND A. CALABRESE, 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fishery Bull. (U. S. Fish and Wildl. Ser.)*, 63: 643-655.
- DAVIS, H. C., AND R. R. GUILLARD, 1958. Relative value of ten genera of microorganisms as food for oyster and clam larvae. *Fishery Bull.* 136 (*U. S. Fish and Wildl. Ser.*), 58: 293-304.
- GUILLARD, R. R. L., AND J. H. RYTHER, 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, 8: 229-239.
- GUILLARD, R. R. L., AND V. CASSIE, 1963. Minimum cyanocobalamin requirements of some marine centric diatoms. *Limnol. Oceanog.*, 8: 161-165.
- HULBURT, E. M., 1956. The phytoplankton of Great Pond, Massachusetts. *Biol. Bull.*, 110: 157-168.
- LOOSANOFF, V. L., H. C. DAVIS AND P. E. CHANLEY, 1953. Behavior of clam larvae in different concentrations of food organisms. *Anat. Record*, 117: 586-587.
- PARSONS, T. R., K. STEPHENS AND J. D. H. STRICKLAND, 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Canada*, 18: 1001-1016.
- SCHELTEMA, R. S., 1962. Pelagic larvae of New England intertidal gastropods. I. *Nassarius obsoletus* Say and *Nassarius vibex* Say. *Trans. Amer. Microsc. Soc.*, 81: 1-11.
- SCHELTEMA, R. S., 1967. The relationship of temperature to the larval development of *Nassarius obsoletus* (Gastropoda). *Biol. Bull.*, 132: 253-265.
- THOMPSON, T. E., 1959. Feeding in nudibranch larvae. *J. Mar. Biol. Assoc.*, 38: 239-248.
- THORSON, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates with special reference to the planktonic larvae in the sound (Øresund). *Medd. Komm. Danm. Fiskeriog Havunders.*, Ser. Plankton, 4: 1-523.
- WALNE, P. R., 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. I. Feeding experiments. *J. Mar. Biol. Assoc.*, 43: 767-784.
- WERNER, B., 1955. Über die Anatomie, die Entwicklung und Biologie der Veligers und der Veliconcha von *Crepidula fornicata* L. (Gastropoda Prosobranchia). *Helgol. Wiss. Meeresunter.*, 5: 169-217.