EXPERIMENTAL CULTURE OF THE ESTUARINE ECTOPROCT *CONOPEUM TENUISSIMUM* FROM CHESAPEAKE BAY

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EXPERIMENTAL CULTURE OF THE ESTUARINE ECTOPROCT *CONOPEUM TENUISSIMUM* FROM CHESAPEAKE BAY[1]

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Virtually all of the 3500 extant species of ectoprocts feed by filtering plankton. But in spite of the fact that they are common components of both marine and estuarine suspension-feeding communities, little is known about the kinds of particles which are utilized by ectoprocts as food.

Gut contents of ectoprocts were described by Hentschel (1922) for *Sargassum*-encrusting *Membranipora tuberculata*, and by Hunt (1925) for two species from the Plymouth fishing grounds. The gut of *Membranipora tuberculata* held diatoms, coccolithophores, peridinian dinoflagellates, and *Physalia* nematocysts. The two Plymouth area ectoprocts contained small diatoms, silicoflagellates, peridinians, coccolithophores, algal cysts and detritus. Hunt also observed the capture of small flagellates by ectoprocts and speculated that they might serve as food sources.

Literature on the culture of marine ectoprocts has been scarce until recently. Hasper (1912) fed young colonies of *Bowerbankia pustulosa* on the diatoms *Nitzschia closterium forma minuta* (= *Phaedactylum tricornutum?*) and the cyanophyte *Pleurococcus mucosus*. Schneider (1959, 1963) cultivated *Bugula avicularia* on the colorless dinoflagellate *Oxyrrhis marina*.

Bullivant (1967, 1968) found that colonies of the ctenostome *Zoobotryon verticillatum* grew well on the chrysophyte flagellate *Monochrysis lutheri* and the aberrant diatom *Cyclotella nana* and the coccolithophore *Criocosphaera carterae*, but could not grow on the dinoflagellate *Amphidinium carterae*, the green flagellate *Dunaliella tertiolecta*, or the diatom *Thalassiosira fluviatilis*. Further experiments showed that colonies of the cheilostome *Bugula neritina* grew well on *Monochrysis*, but not on *Phaeodactylum*.

Recently Jebram (1968) has used *Oxyrrhis marina* (fed upon *Dunaliella*) and *Cryptomonas* sp. to culture several ectoproct species including *Alcyonidium* sp., *Bowerbankia gracilis*, *Farella repens*, *Electra crustulenta*, *E. monostachys*, *Conopeum seurati*, *C. reticulum* and *Bugula stolonifera*. Menon (1972) has also used *Cryptomonas* sp. to maintain colonies of *Electra pilosa*, *Conopeum reticulum* and *Membranipora membranacea* for experimental work.

The existing literature seems to suggest then, that each ectoproct species may react differently to each of a variety of food species. The factors responsible for such differential growth responses are unknown and require examination.

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This report examines the growth reactions of one species of ectoproct fed a variety of foods. The purpose of this experimentation is to identify some of the factors which may be responsible for differential growth responses and to examine the role of nutrition as it affects colony morphology.

The estuarine cheilostome *Conopeum tenuissimum* was chosen as the ectoproct to be used for the series of culture experiments. This species was common in the research area and is an important component of estuarine fouling communities along the east coast of the United States from Maine to Florida.

Methods

Conopeum colonies used in the culture experiments were collected in the York River at the Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia. Artificial substrates, *i.e.*, bryozoan traps, were suspended from the VIMS pier for several days. The glass slide substrates were then removed and examined for *Conopeum* colonies. When one was located near the center of a slide it was isolated by careful cleaning and removal of all other organisms on the slide.

The size of the experimental colonies at the start of the culturing period ranged from 2–28 zooids. Six to eight colonies were grown on each experimental food medium.

The slides bearing the colonies were placed in rectangular pyrex dishes capable of holding one liter of water. Slides rested on the bottom or against the sides of the dishes so that expanded polypides were in either an upright or a horizontal position. The colonies were cleaned and rotated in position in the dish each time the feeding medium was changed. It was assumed that the position of colonies had no effect on growth as long as there was no build up of debris. This is indicated in nature by the occurrence of colonies on all surfaces of eelgrass and other substrates.

The experiments had been planned to begin in late June, 1972. At this time the Virginia coast and Chesapeake Bay were struck by the rains and flooding associated with tropical storm Agnes. This flooding caused salinities over the entire Bay to drop drastically. At Gloucester Point, Virginia where salinities in late June are usually around 16–17‰, the salinity dropped from 15.8‰ on the 23rd of June to 8.8‰ on the 28th.

It appeared that it might be several months before the York River salinity reached its pre-Agnes level, and it was feared that this low salinity might affect growth. Thus it was decided to raise the colonies on York River water mixed with enough water obtained from the Eastern Shore of Virginia (marine) to reach a salinity of about 16‰. This mixture was filtered through the laboratory filter system. Water was changed three times weekly, and tanks were cleaned and colonies fed at the same time. Temperatures for the 1972 experimental period ranged from 23.0–26.5° C and salinities from 15.8–17.5‰. Each of the media used to culture the *Conopeum* colonies is described below.

The chrysophyte flagellate *Monochrysis lutheri* (5.4 μm) was chosen as the first experimental food because it had been found to promote growth in numerous other invertebrate species including oyster larvae.

Dunaliella tertiolecta (6 × 9 μm), a chlorophyte flagellate, has also been utilized in culturing experiments, but reports of its food value have varied.

Va-12 is an unidentified chrysophate flagellate isolated from a "red tide" in the York River. Cells of this species average 4.5 × 4.2 μm in size; the shape is slightly longer than broad, the apical end somewhat smaller than the posterior end; some cells appear slightly concave on one side. This species was tried because oyster larvae being cultured at VIMS had shown good growth when Va-12 was used in combination with other foods.

Nannochloris occulata is a nonmotile chlorophyte only 2–3 μm size. This small species has also been used successfully in oyster larviculture at VIMS.

Cyclotella nana (6.4 μm), a small centric diatom with a siliceous cell wall, was chosen because of its size and because it had been utilized in the culture of other invertebrates.

Gymnodinium simplex is an unarmored dinoflagellate 6 × 8 μm in size. Because dinoflagellates are often an important component of nearshore and estuarine phytoplankton, I decided to try to culture *Conopeum* on species that would be found in its natural habitat. *Gymnodinium simplex*, while never a dominant in the York River waters, occurs with some frequency (MacKiernan, 1968) throughout the year.

Anacystis marinus is a small (1–2 μm) rounded species of cyanophyte. In the inner parts of the bay, blue-green algae are often of considerable importance. Therefore, it seemed desirable to test the value of a member of this group as a food for *Conopeum* colonies. While various workers have attempted to culture freshwater organisms on blue-green algae, there is no information on their use as a food for marine or estuarine invertebrates.

Because other invertebrates had been shown to grow better on a combination of foods than on a single algal food, two mixtures of foods were tried also: *Monochrysis-Dunaliella*, and *Monochrysis-Dunaliella-Cyclotella*.

In preliminary experiments during the previous year, oyster tank detritus had failed to support growth of *Conopeum* colonies, so it was decided to try feeding colonies directly with a culture of bacteria which had been isolated from the sides of the oyster tanks at VIMS. This bacterium has not been positively identified but is probably a pseudomonad or *Vibrio* species (S. Rivkin, VIMS, personal communication).

In addition to the culture of *Conopeum* on the different diets listed above, colonies were also cultured on five different concentrations of *Monochrysis* and also five concentrations of *Dunaliella*.

The algal cultures used were obtained from Dr. Franklyn D. Ott of the VIMS algal culture lab. The cultures were grown in large aerated glass vessels with constant illumination provided. *Nannochloris* was cultured at room temperature; the other species, at 16° C ± 4° C. The medium used in culturing the algae consisted of filtered York River sea water enriched by a stock nutrient mineral medium.

The algae were filtered through a 50 μm filter to remove any undissolved particles of medium, etc., and added to one liter of York River water which had been passed through a sand filter and then through two 1.0 μm Cuno cotton filters. At the time the medium was renewed, the colonies were carefully cleaned with a soft brush and the dishes were scrubbed clean of any detritus, dead algae, and fecal material that might have settled.

At each feeding the following quantities of algal medium were used: (1) for single foods, 60 ml of algal suspension; (2) for the two-food mixture, 30 ml of each algal suspension; (3) for the three-food mixture, 20 ml of each algal suspension; (4) for the first control, 60 ml of algal medium only; (5) for the bacteria, one pipetteful of bacteria; and (6) for colonies being given the various concentrations of *Monochrysis* or *Dunaliella,* 15, 30, 60, 90, or 120 ml/liters of sea water at each feeding.

Although the numbers of algae present per ml of culture suspension varied both according to species and according to stage in the growth of the algal population cycle, it was desirable to have an estimate of the quantities being fed to the ectoprocts. To facilitate estimation of food concentration each time the algal food suspensions were obtained a few ml were removed, the cells killed with a drop of formalin and immediately counted under the microscope using a hemocytometer.

The experiments ran for about 40 to 42 days for two reasons. First, by this time reproduction would have occurred in nature, and it was desired to see if some foods supported reproductive activity; and secondly, by this time the colonies could grow to the edges of the slides and thus nullify further quantitative growth records.

The growth of colonies was recorded by photography at least five times during the experimental period: at the start, at the finish and at three other times during the six week growth period. The few exceptions were due to photographs lost in processing, and the reduced photography of series exhibiting very little growth.

The photographs were arranged to prove a sequential picture of the changes in size and shape of each colony as it grew. Measurements were made of the number of zooids and number of generations (zooids in a direct line out from the ancestrula). These data were used to construct both growth curves for individual colonies and mean growth curves for all colonies receiving a particular diet.

Results

General patterns of colony growth

In the initial four to six days of culture all young colonies of *Conopeum* increased zooid numbers at an exponential rate. This response is independent of diet as even starved colonies exhibited such initial growth. After this initial growth period both zooid number and colony growth pattern were found to be diet dependent. Each food treatment produced consistent characteristics of colony growth. The best single species food, *Dunaliella,* produced a healthy roundish colony similar in shape to colonies observed in nature (Fig. 1A, B). In contrast, a poor food such as *Cyclotella* produced a colony response typified by radiating biserial chains with few zooids (Fig. 1D). Most of the zooids present were heavily calcified and lacked functional polypides.

Growth responses to single species foods

Growth of *Conopeum* colonies was quantified by direct zooid counts. Table I and Figures 2 and 4 summarize and contrast the responses of the colonies to various foods. From Table I and Figure 2A it is evident that *Dunaliella* and *Gymnodinium* produced the best growth of all single foods tested. *Dunaliella-*

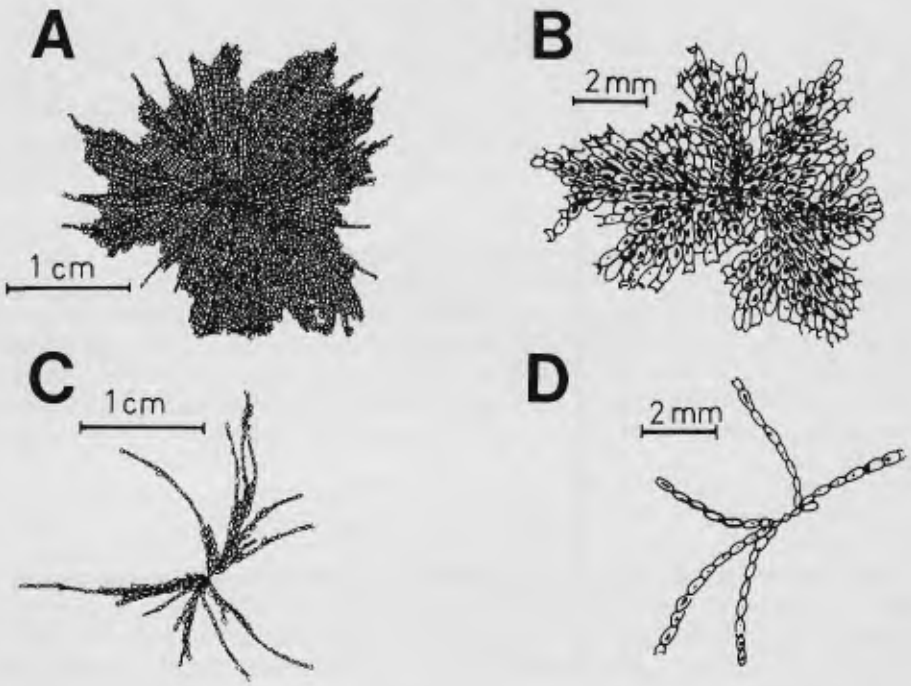

FIGURE 1. Examples of variation in growth form among *Conopeum tenuissimum* colonies raised on different foods. (A) Example of a colony raised on a good food, the chlorophyte flagellate *Dunaliella tertiolecta*, after 40 days of growth. Note the large number of zooids and almost circular shape of the colony due to distolateral budding to fill in the space between the major branches. (B) Morphology of a colony grown in the natural York River environment, after 20 days of growth. Dark coloration is due to food in the gut of polypides. Polypides in the outermost rows of zooids are still developing and have not yet begun to feed. (C) Example of a colony raised on a fair food, the chrysophyte flagellate *Monochrysis lutheri*, after 42 days of growth. Colony shows typical biradiate, branching shape, due to budding along major growth axes only. (D) Example of a colony raised on a poor food, the diatom *Cyclotella nana*, after 41 days of growth. Colony has small number of zooids, arranged in six branches. Most zooids are lacking polypides and are heavily calcified.

fed colonies (7/15–8/26; 42 days) exhibited the healthy circular form (Fig. 1A). At the end of the 42-day culture period all colonies appeared healthy. Observation of polypides showed some with intertentacular organs for release of eggs; these zooids contained ovaries with developing eggs. Other zooids had regenerating polypides and developing ovaries.

Gymnodinium was also a good food for *Conopeum* (Fig. 2A). The *Gymnodinium*-fed colonies (7/15–8/19; 8/21–10/2; 42 days) always had a pinkish or orangeish tinge due to the dinoflagellates in their guts. This unique coloration was the same as that observed in colonies taken from the York River, substantiating the idea that this species might naturally feed upon suitably sized dinoflagellates. It was not possible to examine the total growth response of *Conopeum* to *Gymnodinium*, as a failure in the VIMS algal culture system eleven

TABLE I

Ratio of mean number of zooids to mean number of generations in Conopeum colonies after six weeks of culture. A generation is the longest chain of zooids in a direct line outward from the ancestrula, usually measured on primary (distal) chain.

Food used	Number of colonies	Mean number of generations per colony	Mean number of zooids per colony	Ratio of zooid number to generation number
Mono-Dun-Cyclo	6	36	1629	45:1
Dun-Mono	8	29	1291	45:1
Dunaliella	7	33	2484	75:1
Gymnodinium	4	22	1409	64:1
Monochrysis	5	40	370	9.3:1
Va-12	7	31	201	6.5:1
Anacystis	7	12	48	4:1
Cyclotella	8	10	35	3.5:1
Nannochloris	5	15	42	2.8:1
Bacteria	7	7	16	2.3:1
Control (algal medium)	6	11	34	3.1:1
Control (sea water only)	5	8	21	2.6:1

days after commencement of the culture run killed the *Gymnodinium* culture. The colonies initially responded with rapid growth similar to that of *Dunaliella*-fed colonies. As the quality of the *Gymnodinium* decreased, the more or less even outward growth stopped and colonies begun producing radiating biserial chains. Once the algal culture was dead colony growth gradually ceased. This culture was repeated (8/21 to 10/2) and again the algal culture fluctuated in concentration and vigor. While the food supply was declining, the growth rate of the ectoprocts decreased, with many of the polypides in the central portions of the colonies degenerating. Once the food started to increase in density, the rate of growth of the colonies also increased.

At the end of the culture period two of the four cultured colonies contained some zooids full of sperm as well as some with developing eggs and polypides with intertentacular organs. Two other colonies, both smaller than those above, showed no evidence of reproductive activity.

Foods producing fair growth of *Conopeum* were *Monochrysis* (7/15–8/26; 42 days) and Va-12 (Fig. 2C). Both foods produced colonies of intermediate form (Fig. 1C). Zooid production was considerably less than for *Dunaliella*-fed colonies (Table I) and neither food supported production of polypides with intertentacular organs or other reproductive structures.

The foods producing poor *Conopeum* growth were *Nannochloris, Cyclotella, Anacystis* and bacteria. The *Nannochloris*-fed culture (7/15–8/25; 41 days) produced a mean growth curve (Fig. 2D) very different from the growth on the other chlorophyte species *Dunaliella*. At the end of the culture period one colony was broken and appeared to be decaying. Among the other colonies, some had broken zooids while a few appeared to have functional polypides and were producing occasional new buds.

Conopeum cultured on *Cyclotella nana* (7/15–8/25; 41 days) appeared in poor condition. At the end of the culture period there were functioning polypides only

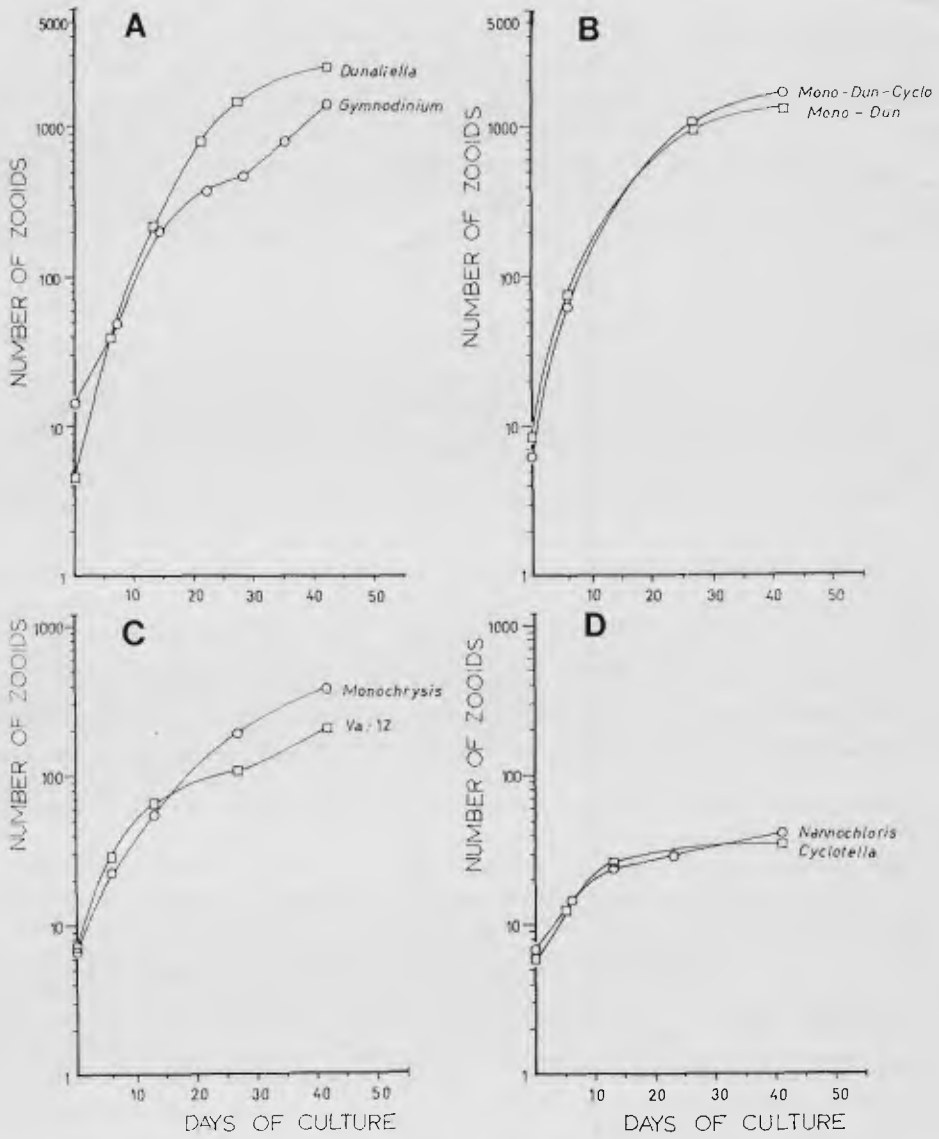

FIGURE 2. Mean growth curves for *Conopeum tenuissimum* colonies cultured on various algal foods. (A) Colonies raised on two good foods: *Dunaliella,* based on growth of seven colonies; *Gymnodinium,* based on growth of four colonies. (B) Colonies raised on algal food mixtures: *Monochrysis-Dunaliella-Cyclotella,* based on growth of six colonies; *Monochrysis-Dunaliella,* based on growth of eight colonies. (C) Colonies raised on two fair foods; *Monochrysis,* based on growth of five colonies, Va-12, based on growth of seven colonies. (D) Colonies raised on two poor foods: *Nannochloris,* based on growth of five colonies; *Cyclotella,* based on growth of eight colonies.

in the outermost zooids. In these colonies the gymnocyst appeared to be more heavily developed than in those fed on any other food and only inner portions of some colonies had any lateral spines (Fig. 1D).

Conopeum-fed *Anacystis marinus* (7/15–8/19; 35 days) grew poorly, but initial growth was greater than exhibited by colonies fed *Nannochloris* or *Cyclotella*. Unfortunately the stock culture of *Anacystis* was among the stock cultures affected by the VIMS culture failure (July 26, 1972), and it could not be regrown in time to start a new experiment. For the first eleven days of the culture period, the mean growth rate exceeded that of other poor-food cultures; but after the loss of the *Anacystis* culture, the growth rate was greatly retarded and lagged behind all other algal foods. There were, however, some live zooids present in all colonies at the end of the 35-day period.

Bacterial food (7/15–8/25; 41 days) appeared to be the worst of all foods tested for *Conopeum*. Growth was less than that exhibited by colonies given no food at all. At the end of the culture period, one colony had decayed completely. All other colonies had at least one functional polypide, but were obviously in a state of decay with many broken zooids.

Growth responses to food combinations

Conopeum was cultured on a bi-algal mixture of *Monochrysis* and *Dunaliella* (7/15–8/26; 42 days). This mixture proved to be a good food, giving better growth results than any single foods except *Dunaliella* and *Gymnodinium* (Fig. 2B). Post-culture examination showed one zooid with an ovary and several polypides with intertentacular organs. The shape of the colonies grown on this mixture was intermediate between that of colonies on a *Monochrysis*-diet and that of those on a *Dunaliella*-diet.

Conopeum colonies were also cultured on a tri-algal diet of *Monochrysis*, *Dunaliella*, and *Cyclotella* (7/15–8/26; 42 days). This tri-algal mixture also proved to be a good food. The mean growth rate was similar to the growth rate of colonies fed the *Monochrysis-Dunaliella* mixture, the only difference being that colonies given the three food combination had a slightly higher final mean zooid count (Table I). The tri-algal mixture also supported colony production of reproductive structures and an intermediate colony shape.

Growth of Conopeum *in the natural environment*

Attempts were made to assess growth of *Conopeum* in nature by placing colonies isolated on slides in a holder suspended from the VIMS pier into the York River. Unfortunately after about 20 days, overgrowth by other organisms was so intense that accurate zooid counts were impossible. A second attempt to assess natural growth was made by placing isolated colonies in an oyster culture tank receiving a continuous flow of York River water. This system eliminated the overgrowth problem. It was felt that this simulated natural conditions, except for lack of predators and a slightly lower water temperature. A comparison of the growth rate in the oyster tanks to cultured growth rates substantiated the belief that laboratory diets and cultures do serve to approximate growth in nature and can be used and interpreted in that light.

Control cultures

It was also deemed important to examine whether or not the algal medium itself could cause significant growth in *Conopeum* colonies. Colonies were cultured 41 days (7/15–8/25) on filtered sea water, using 60 ml of algal medium per feeding. Some initial colony growth occurred, but this was thought to be a result of polypide feeding on tiny diatoms which entered the culture *via* the glass slides used to isolate the colonies. At the conclusion of the culture, zooids of most colonies were broken or empty. The growth response was similar to that produced in colonies fed the diatom *Cyclotella.*

A second control experiment examined the growth potential of a colony given only its own food reserves and cultured in filtered sea water. Initial growth response produced a growth curve with a slope similar to those of *Cyclotella, Nannochloris,* and the algal medium. However, between two and three weeks after the start of the cultures the colonies started to decay. One colony had died by the 22nd day of culture. This colony had only two initial zooids at the start of

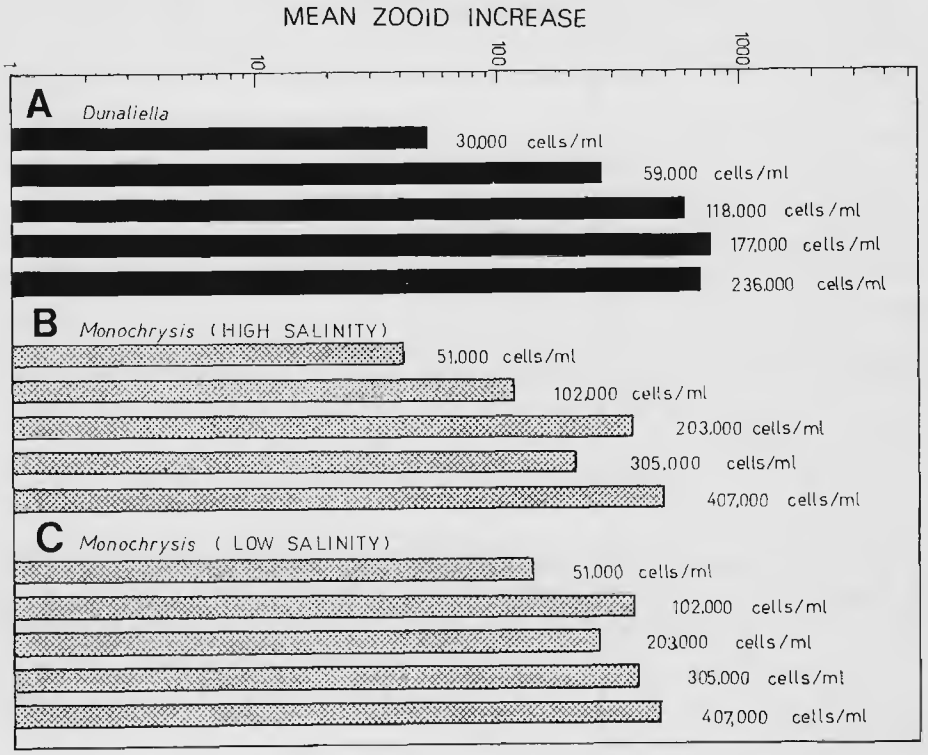

FIGURE 3. Growth of *Conopeum tenuissimum* colonies on various concentrations of algal foods (given as mean zooid increase): (A) *Dunaliella,* 15, 30, 60, 90, 120 ml per feeding; (B) *Monochrysis* (16‰ salinity), 15, 30, 60, 90, 120 ml per feeding; (C) *Monochrysis* (12‰ salinity), 15, 30, 60, 90, 120 ml per feeding. Figures indicate average concentration of the food (in cells/ml/l sea water) for each of the treatments.

culture and therefore a low nutrient reserve. However, another two-zooid colony survived 41 days, produced a total of six zooids and had one zooid with a polypide at the end of the culture. Of the other six colonies, four appeared to have at least one live zooid; the other two colonies were in a definite state of decay.

Growth response to various concentrations of food

Since food concentrations varied with both species and time, it was desired to test the effects of food concentration on colony growth. The results of three culture experiments utilizing similar concentrations of foods indicate that *Conopeum* growth was a direct function of food concentration up to about 60 ml. Above this amount there was no significant increase in growth. Figure 3 summarizes the results of the concentration of experiments.

The various concentrations of *Monochrysis* used to culture *Conopeum* generally supported moderate colony growth and a colony shape indicative of foods of moderate value (Fig. 1C).

Due to a change in laboratory water conditions, colony cultures using *Monochrysis* were carried out at two salinities. For the first series, salinity was 12‰ (7/17–8/26; 40 days).

Colonies fed various concentrations of *Monochrysis* at the low salinity supported growth, but there was frequent colony death. There were no colony deaths at the higher salinity, but there was evidence of zooid breakage. Other differences believed related to salinity were: (1) growth rates for the colonies fed small amounts of *Monochrysis* were lower for the colonies raised at the higher salinity than for those raised at the lower salinity; and (2) those colonies fed at lower salinities had more closely-grouped growth curves than those raised in the higher

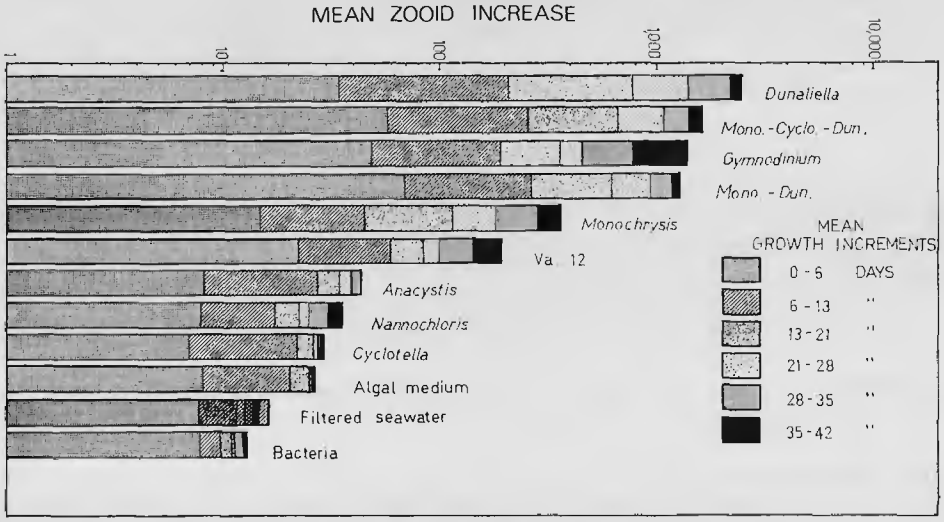

FIGURE 4. Growth of *Conopeum tenuissimum* colonies on all foods investigated (given as mean zooid increase).

salinity. The reasons for these differences are unknown but suggest further research.

Conopeum cultures on various concentrations of *Dunaliella* (8/21–10/2, 42 days) were compared to *Monochrysis*-cultured colonies. The *Dunaliella*-fed cultures had to be moved from VIMS to Woods Hole, Massachusetts (9/7/72), and the subsequent growth of these colonies reflected some degree of stress. The colonies did recover however.

From Figure 3 it is obvious that those colonies fed the smallest amount (15 ml) of *Dunaliella* did not grow appreciably more than those fed the smallest amount of *Monochrysis* at a similar salinity. Those fed 30 ml, however, showed slightly more growth than those fed 30 ml of *Monochrysis*. It is interesting that even in colonies fed the smaller amounts of *Dunaliella,* some showed the growth pattern characteristic of those on good diets, with zooids filling in between the main branches, rather than just growing outward in uniserial chains from the ancestrula.

The colonies fed 60, 90, and 120 ml of *Dunaliella* had very similar and closely spaced growth curves. Those fed 120 ml showed the least individual variation between individual colonies.

Colonies fed 60 90, and 120 ml of *Dunaliella* achieved better growth than those fed 60, 90, and 120 ml of *Monochrysis*. The *Dunaliella*-fed colonies showed quite circular growth patterns compared with those fed *Monochrysis*. When the colonies were examined at the end of the experimental period, none of the colonies showed any signs of reproduction except for the colonies fed 120 ml, which had polypides with intertentacular organs and ovaries in the zooids. It was observed that many of the polypides in the colonies fed 90 and 120 ml amounts of *Dunaliella* had lophophores that were shorter than normal and bodies that were like large sacks, possibly an adaptation to bloom conditions.

Discussion

To aid in the interpretation of results, bar graphs showing the amount of growth (as mean zooid increase) for *Conopeum* colonies on all diets were constructed (Figs. 3 and 4). For the food concentration experiments, Figure 3 shows only total mean zooid increase. The more detailed graph (Fig. 4) shows the amount of growth that occurred on each diet after 6, 13, 21, 27, 35, and 42 days. Growth at each of these points was measured directly from colony photographs when possible, and in a few cases interpolated from the mean growth curve. Examination of these figures and Figure 1 leads one to conclude that different species of algae affect both pattern and amount of colony growth.

The effect of food on colony growth patterns is of interest with respect to life strategy of this estuarine species. Colonies on a good diet (Fig. 1A) developed buds not only along the major growth axes, but also distally and laterally to fill in the spaces between the branches, giving the colony a circular rather than double-fan-like form. Colonies fed fair foods (*Monochrysis* and Va-12) generally grew in a biradiate pattern, in chains only one to two zooids wide (Fig. 1C). Though the chains leading out from the ancestrula in the distal and proximal directions were often the longest, in older colonies this was only on the order of a few generations of zooids compared with the length of the secondary chains (which generally developed from distal-lateral buds produced from the young colony,

either before the experiment started or during the first week of growth). The secondary chains were usually at about a 45° angle (the angle at which distal-lateral buds are produced) to the primary chains; thus, colonies commonly consisted of six main chains or branches (three to either side of the ancestrula) in a bi-radiate pattern. Shorter tertiary chains developed by distal-lateral budding of the zooids of the primary and secondary chains.

This development in all directions outward from the young colony suggests that colony energy is being put toward the location of a more nutritionally favorable microenvironment. It is worth noting that colonies fed good foods did not have a larger number of generations (measured as number of zooids in a chain outward from the ancestrula). For colonies fed *Dunaliella* the mean number of generations was 33; for those fed *Gymnodinium,* 22; for the two and three-food mixtures, 29 and 36 respectively. For colonies given Va-12 it was 31; for those fed *Monochrysis,* it was 40. The poorly nourished colonies had mean generation numbers ranging from seven (bacterial-fed) to 15 (*Nannochloris*-fed).

What this means in terms of colony strategy can perhaps be roughly quantified by a simple ratio between final mean zooid number and final mean generation number (Table I). In well-nourished colonies, the amount of energy put into total colony growth including storage for maintenance and reproduction (as measured by final mean zooid number) versus the amount put into outward expansion (as measured by final mean generation number) ranges from 45 : 1 to 75 : 1, while ratios for those on fair foods ranged from 6.5 : 1 to 9.3 : 1. For colonies on poor diets, the range was from 2.3 : 1 (bacteria) to 4 : 1 (*Anacystis*). Thus colonies on a poor or fair diet were putting a proportionately much greater amount of energy into a "search" for a more favorable microhabitat, while well-nourished colonies were expanding as much as possible within their favorable habitat and were probably accumulating excess food energy (as shown by the onset of reproductive processes in these colonies).

What this could mean to colonies in their natural estuarine environment is apparent from a discussion of the life cycle (Dudley, 1973). If the colonies are not able to grow, store some energy and reproduce within about four weeks, colonies on hard substrates will become covered by other fouling organisms, such as colonies of the faster-growing, but less numerous ectoproct *Membranipora tenuis,* as well as by tunicates and barnacles. Their other primary substrate, the eel-grass, is short-lived in summer and may come detached and decay; therefore rapid completion of the life cycle is favored. Moreover, colonies in nature which find themselves in a nutrient-poor microenvironment due to current conditions probably also put all colony reserves into single chains of zooids, favoring expansion into an area where zooids can feed.

Examination of colony growth curves (Fig. 2) shows that the response does not differ significantly from the sigmoid growth curves characteristic of many animals (Odum, 1971). Growth curves of young colonies taken from the natural environment had an exponential form (Dudley, 1973). This type of curve also appeared in the early growth of cultured colonies. As the cultured colonies increased in size, the growth curves began to approach the sigmoid form. Exponential population growth can continue only as long as environmental factors are not limiting. As the number of zooids increase and factors such as space for colony

expansion decrease, the growth rate slows until it reaches the carrying capacity of the environment. In the case of the cultured *Conopeum* colonies the chief constraint on growth was probably food supply. To simplify the experiments, colonies were fed a constant amount of food, and the food was not increased as the colonies grew; thus food supply might have been adequate for young colonies, but inadequate for the 1000–2000 zooids present in colonies at the end of the experimental period.

Other limiting factors may also be operating. It has been found true for many organisms that somatic growth diminishes or stops before sexual maturation, as energy is needed for the development of reproductive structures, eggs, brooding of larvae, etc. However, few studies are available, concerning the effects of the reproductive process upon the growth of colonial organisms.

As has been shown to be true for other organisms from protozoans to crustaceans, different species of algae were found to be of varying food value to *Conopeum* (see Fig. 4). Two single foods, *Dunaliella tertiolecta* and *Gymnodinium simplex*, supported good growth of *Conopeum*. The results with *Dunaliella* are interesting since species of this genus have been shown to be of quite variable value to other organisms (Walne, 1963, 1970; Gibor, 1956; Pilkington and Fretter, 1970). In addition Bullivant (1967) found no growth in *Zoobotryon* colonies fed *Dunaliella* except for an elongation of the stolons which seemed to be a reaction to adverse food conditions and Schneider (1959) stated that *Dunaliella* sp. passed intact through the gut of *Bugula avicularia*.

The results with *Gymnodinium* are perhaps even more interesting. Very few dinoflagellates have been used in culturing experiments, and the few species tried (which were generally species known to produce toxic metabolites) have usually given poor results. Buillivant (1967) found no growth and some disintegration in colonies of *Zoobotryon*-fed *Amphidinium carterae*.

Some dinoflagellates may have food value, as is suggested by the literature. Pilkington and Fretter (1970) reported that *Exuviella baltica* was a good food for *Crepidula* veligers. Moyse (1963) found that the small dinoflagellate *Prorocentrum micans* was able to support growth of the barnacle *Chthamalus stellatus* to the settlement stage.

It was probably significant that only the *Conopeum* colonies grown on *Gymnodinium* showed the natural reddish-brown coloration of the polypides, apparently due to the pigment of the dinoflagellate cells. One organism which has been cultured on dinoflagellates is a tintinnid (Gold, 1970). The author remarked that after overnight growth on the dinoflagellates *Glenodinium foliaceum* and *Amphidinium* spp., the tintinnids showed a characteristic coloration resulting from the ingestion of dinoflagellates. Since tintinnids are typical of estuarine zooplankton it is not so surprising that they, like the estuarine ectoproct *Conopeum*, appear to thrive on dinoflagellates (also common in estuaries).

Gymnodinium simplex had been chosen as a food organism because it was known to occur in the York River and could thus be considered a "natural" food organism for *Conopeum*. Furthermore, information available on the dinoflagellates of the York River (data in MacKiernan, 1968) indicated that about 50% were of a size (diameter 50 μm) that would make them available as food for *Conopeum*. In the warm temperate York River estuarine environment, dinoflagellates

occurred year-round and some suitably-sized species were always present. In addition, various nannoflagellates were common especially in summer months, while diatoms did not appear to be significant as food for *Conopeum* because most of the species reported were too large for the ectoproct to ingest. Thus it appears that the value of *Gymnodinium* as food for *Conopeum* might actually reflect the natural food of this organism. Whether this finding can be extrapolated to other estuarine ectoprocts remains to be seen.

The two foods found to support fair growth in *Conopeum* were *Monochrysis lutheri* and Va-12. On those diets, the colonies were able to grow moderately well, but did not achieve reproductive conditions by the end of the experimental period. Though both species were chrysophyte flagellates of similar size and volume, *Monochrysis* proved to be a better food than Va-12.

Monochrysis has been used in many culture experiments and has been shown to be one of the best foods for oyster larvae (Davis and Guillard, 1958; Walne, 1963). Va-12 has been used only in the VIMS larval culture work where it was found to increase the growth rate of oyster larvae when mixed with other foods, but was unable to support growth in monoculture (M. Bolus, VIMS, unpublished manuscript).

Several foods supported only poor growth of *Conopeum* colonies. The cyanophyte *Anacystis marinus* was the best of the poorer foods and might support moderate growth. Schindler (1971) pointed out the ingestion and assimilation of blue-green algae indicated that no generalization could be made about the food value of those algae on taxonomic grounds, although complex or filamentous forms were unsuitable. His experiments suggested that members of this group could be potential food sources at least for freshwater suspension feeders. No attempts appear to have been made to culture estuarine organisms on blue-green algae, but as members of this group can be of considerable importance, particularly in the upper reaches of estuaries, their food value to suspension-feeders should be more thoroughly investigated.

The very small chlorophyte *Nannochloris occulata* was a poor food for *Conopeum*. This may have been partly because of its size (perhaps the cells pass between the cilia and cannot be captured by the lophophore) although the *Anacystis*, with cells in the same size range, was apparently more efficiently utilized.

The small centric diatom *Cyclotella nana* (= *Thallassiosira pseudonana*) also appeared to be of little food value to *Conopeum*. Bullivant (1967) found that *Cyclotella* produced some growth of *Zoobotryon*, a gizzard bearing ctenostome, but not of *Bugula*, a species lacking a gizzard. I have also found that *Cyclotella* does not support growth of *Bugula stolonifera*.

The colonies cultured on bacteria showed even less growth than those receiving only filtered sea water. While there has been much speculation in the literature concerning the role of bacteria in the nutrition of suspension-feeding organisms, most attempts to culture invertebrates on bacteria have ended in failure. Bacteria in combination with phytoplankton may play some role in bivalve nutrition, but it is still unclear whether they are generally useful or harmful (Ukeles, 1971). It appears that certain species of bacteria produce toxic metabolites and others do not (Calabrese and Davis, 1970). The bacteria utilized in the *Conopeum* experiments could not have produced extremely toxic products as the colonies did not

die immediately. The reason for poor growth may be one or a combination of the following: the cells were present in too low a concentration to have nutritional value; or, as has been shown to be the case for many marine bacteria (Provasoli, Conklin and D'Agostino, 1970), they created an unfavorable environment for the ectoprocts by acidifying the sea water and reducing the oxygen concentration. The nutritive value of algae appears important in that not all foods that supported growth were adequate for reproductive activity. The fact that not all the foods tested were able to support reproductive activity of *Conopeum* may be accounted for either by assuming that: (1) not all algae are alike in food value, especially in the amounts of trace elements, vitamins or other factors necessary for reproduction; or (2) colonies cannot undergo reproduction until they reach a certain size, as measured in the number of zooids. Actually both factors may be operating, but in the absence of any knowledge of a minimum-size requirement (and such a requirement would seem to be less likely in an r-strategist like *Conopeum*, than in a generally long-lived species which puts its energies first into obtaining as much substrate as possible by asexual growth), it is most probable that the food is lacking in some element necessary for the initiation of the reproductive processes.

The survival of some colonies for as long as six weeks, during which time they received only filtered sea water, suggests that colony reserves are important in insuring the survival of the species in the event of unfavorable conditions. As is apparent from Figure 4, growth of the colonies fed good, fair and poor foods is distinguishable even after only six days of culture. The control colonies (filtered sea water) grew as much as the colonies fed poor foods for the first six days. Between six and thirteen days the controls showed more growth than the colonies fed bacteria, but were apparently becoming more and more unhealthy. By the time they were photographed again at 21 days, zooids had degenerated and decayed so that the total mean zooid number was less than it had been after only 13 days. From 21 days to 42 days the colonies made only a tiny increase in growth (from $\bar{x} = 12$ to $\bar{x} = 15$ zooids/colony). This very small increase may have been due to remaining food reserves or to ingestion of benthic diatoms or bacteria that could not be completely removed from the dishes or the surfaces of the colonies.

For colonies receiving 60 ml of sterilized algal medium per feeding, growth continued up until the third week (Fig. 4), and even after six weeks was almost equivalent to the growth of colonies receiving *Cyclotella*. As was mentioned in the results section, continued growth may have been due to the fact that numbers of small benthic diatoms were encouraged by the algal medium and might provide some nutrition for the *Conopeum* colonies.

Conopeum growth response to a combination of algal foods was better than to most single foods. Colonies fed a mixture of two foods, *Monochrysis* and *Dunaliella*, showed better growth than colonies fed all but two single foods (Fig. 4). Moreover, the growth curves for all of the colonies grown on the two-food mixture were remarkably similar. The results are not surprising since a mixture of foods has been found to support better growth than a single algal food for many other suspension feeders (Davis and Guillard, 1958; Ukeles, 1971; Provasoli, Conklin and D'Agostino, 1970).

Growth of *Conopeum* on a mixture of three foods (*Monochrysis-Dunaliella-Cyclotella*) was slightly better than on a mixture of two foods and better than on

any single food except *Dunaliella.* Calabrese and Davis (1970) stated that a mixture of several species of algae caused larvae of *Crassostrea* and *Venus* to grow more rapidly. They found that a mixture of chrysomonads and green algae (no species given) appeared to provide a more balanced diet than any single food. The fact that, in a 2–3 food mixture, the *Conopeum* colonies were receiving only one-half or one-third the amount of the "good" food suggests that the important factor lies in some form of nutrient balance rather than in the quantity of nutrient offered. The culture experiments using various concentrations of algae showed that food value increased with increasing amounts of food only up to a certain concentration. Figure 3 shows the effect of increasing food concentrations on colonies fed *Dunaliella* and *Monochrysis* (at high and low salinities). In the colonies fed *Dunaliella* (Fig. 3A), growth increased at food concentrations up to 177,000 cells/ml (average quantity), but seemed to be slightly decreased in colonies receiving an average of 236,000 cells/ml. This decrease could be accounted for by two factors. First, as several workers have noted, suspension feeders will ingest food in increasing quantities only up to a certain "satiation concentration" related to the maximum amount of food the individual can process in a certain time period (Bullivant, 1967). If the highest concentration tested was above the maximum that the colonies could consume during the time between feedings, then there could be no increase in growth due to increased concentration. Secondly, it is also possible that *Dunaliella* produces a metabolite that at high concentrations has a negative effect on growth (which might explain the varied results noticed by other workers in culturing experiments with members of this genus).

Colonies grown on *Monochrysis* in general showed an increase in size with increasing amounts of nutrient, but there was more irregularity in the pattern. Colonies grown at the lower salinity (Fig. 3C) appeared to grow better at the two lower levels of food than did those grown at the higher salinity (Fig. 3B). At the highest concentration (406,800 cells/ml) offered, both series achieved approximately equal growth.

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Summary

1. The pattern and form of colony growth in the ectoproct *Conopeum tenuissimum* was found to be diet-dependent.

2. Poorly nourished colonies were characterized by their straggling shape and low zooid number to generation number ratios. These colonies apparently attempted to maximize substrate covered, facilitating location of a more favorable nutrient regime.

3. Well fed colonies were rounded in shape and characterized by high zooid number to generation number ratios. These colonies maximized the number of zooids produced in their already favorable area, creating colony reserves, and preparing for reproduction.

4. Initial growth in all colonies was exponential, but after a few days of culture growth, rates varied with food used. The chlorophyte flagellate *Dunaliella tertiolecta* and the dinoflagellate *Gymnodinium simplex* were good foods, supporting growth to more than 1,000 zooids and sexual maturation within the 42 day culture period. The chrysophyte flagellate *Monochrysis lutheri* and Va-12 (an un-named chrysophyte flagellate) proved to be fair foods, supporting moderate growth, but no sexual maturation. The blue-green alga *Anacystis marinus,* the chlorophyte *Nannochloris occulata,* and the diatom *Cyclotella nana* supported little or no growth, while bacterial food did not support growth of *Conopeum* cultures.

5. Combinations of algal food produced good colony growth. A three-species mixture was better than a two-species mixture, suggesting differential yet additive nutritional contributions by each algal species.

6. Increased food concentrations supported increased growth only up to a certain concentration. This growth response varied with salinity.

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