THE EARLY LIFE HISTORY OF THE NEW ENGLAND CLAM DRILLS, POLINICES DUPLICATUS (SAY), POLINICES HEROS (SAY), AND POLINICES TRISERIATA (SAY) (NATICIDAE: GASTROPODA)

JAMES ELDEN HANKS

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University of New Hampshire, Ph.D., 1960
Zoology

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May 27, 1960
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"The relation between an animal community and a biocoenosis, however, cannot be solved, until we have acquired a detailed knowledge of the biology of each single species of invertebrate living in a 'community,' and until we have cleared up their requirements as regards food, salinity, temperature, substratum, surrounding animals, and - above all - their breeding habits and mode of larval development."

Dr. Gunnar Thorsen, 1946.

SECTION I.

INTRODUCTION

It would be difficult to estimate the impetus given marine ecology by the publication in 1946 of Dr. Gunnar Thorson's comprehensive volume on the early life histories of Danish marine bottom invertebrates. This work and his subsequent papers on marine bottom communities consider the methods and provide the basic data for comparative studies elsewhere. In addition, Thorson has indicated what complementary research is necessary if we are to reach a clear understanding of the ecology of the level sea bottom. He has placed prime emphasis on the study of life histories of species that belong to taxonomic groups which have a wide geographic distribution. In particular, a knowledge of the type of larval development, the length of larval life, and the larval food requirements is essential to any consideration of the problem of how bottom communities are formed and how they maintain themselves.

Through his stimulus, studies have been undertaken along a similar plan by biologists at various laboratories elsewhere. As a result, we are beginning to acquire quantitative data on the faunal populations existing on the shallow areas of the continental shelves along the major land masses of the world (Thorson 1958).

In contrast, the equally important and correlative research concerning the biology of the component species has not been undertaken to the same extent. Thus we still lack a knowledge of the life history and autecology of even the majority of dominant organisms present in the bottom communities which have been mapped for the coastal waters of the
United States. This may be attributed, at least in part, to the notable lack of marine laboratories in north temperate regions that maintain year-round sea water systems (Hiatt 1951).

Rearing of larvae of marine invertebrates in mass cultures is a relatively new development in the field of marine biology and no standard procedures exist that will assure success. However, such studies must be undertaken on a larger scale than at present if we are to have the means of solving many of the problems facing marine biology. This was emphasized by a number of contributors to the Symposium on Perspectives in Marine Biology held at Scripps Institution of Oceanography on March 24 - April 2, 1956. In particular, the opening paper at this meeting by Dr. K. M. Rae (1958), Parameters of the Marine Environment, poses a strong challenge to marine biologists. He said, "...until we become more adept at bringing marine animals under our control ... we are not well prepared to take advantage of many of the excellent techniques of the experimentalist, be he a biochemist, a biophysicist, a geneticist, or a microbiologist."

The author's employment as a Graduate Fellow at the Woods Hole Oceanographic Institution (referred to hereafter as W.H.O.I.) in the summer of 1957 provided an excellent opportunity to enter into a research project of this type. The problem undertaken was a study of the reproduction and early life history of three species of gastropod mollusks (Polinices; Naticidae).

This and other genera of the family Naticidae are frequently a part of level bottom communities from the high-arctic to the tropics wherever pelecypod mollusks constitute the dominant fauna (Thorson 1958).
The majority of the species described are known to be carnivorous, feeding primarily on the pelecypod mollusks available. In carrying on this predatory existence, they remain in the substratum a large portion of the time, either feeding or in search of prey. Thus they are essentially a part of the infaunal population. In this respect they occupy a rather unique habitat amongst gastropod bottom dwellers, which are generally associated with the epifaunal population.

Their role as secondary consumers places these gastropods near the top of the trophic level system for bottom communities. This predatory aspect relates directly to such ecological considerations as standing crop, material removed, and production (Clarke 1946). In addition, such predation has a significant effect on the commercial shellfish catch in New England for such species as the quahog, Mercenaria mercenaria (Linné), the soft-shell clam, Mya arenaria Linné, and the skimmer clam, Spisula solidissima (Dillwyn).

A major effort in studies with species that have pelagic stages is the rearing of the larvae to successful metamorphosis. In order to increase the chances that all phases of the problem could be completed, three species of clam drills common in New England waters, Polinices duplicatus (Say), P. heros (Say), and P. triseriata (Say) were used as research animals. It was felt that this would also provide valuable comparative data for closely related species.

From previous studies of the effects of temperature and salinity on the rate of predation by P. heros and P. duplicatus (Hanks 1952, 1953) and with other predaceous gastropods (Hanks 1957a), the author had acquired considerable background information regarding the biology of the adult drills. Much additional data was available from shellfish
investigations carried out by biologists at other laboratories in New England and southern Canada.

The problem was formulated with an ecological viewpoint. The aims of the research program were the investigation of reproduction, larval development, metamorphosis, and the habits of recently metamorphosed drills. This work was carried out in the laboratory where animals could be readily observed and where their environment could be easily controlled. The information gained from such observations and experiments would then be applied to a discussion of three ecological factors: larval mortality, food requirements of larvae, and temperature limitations to the distribution of *Polinices*.

It was considered that this approach might aid in solving some of the problems encountered by fishery biologists. It would also provide information useful to the design of field experiments with invertebrate bottom animals.

The primary research aims included:

a. To find methods for maintaining drills in mature sexual condition throughout the year and obtaining egg collars at all times of year.

b. To observe the normal development of the eggs and larvae of the three species of drills.

c. To determine the period of free-swimming, pelagic larval existence.

d. To study the food requirements of the pelagic larvae.

e. To observe the feeding habits and determine the food of recently metamorphosed drills.

f. To determine the size and/or age at which drills become sexually mature.
Secondary research aims, dependent on the success of the primary aims, included studies of:

a. The effect of different water temperatures on the development of eggs and larvae.

b. The effect of the substratum and/or available food as an inducement for metamorphosis of larvae.

c. The food value, as measured by larval growth, of various species of algae.
SECTION II.

REVIEW OF THE LITERATURE

The Family Naticidae is represented in marine communities from the arctic waters of Greenland (Thorson 1936) to the tropical waters of the Indian Ocean (Natarajan 1957). Thus, various species of naticids are frequently included in regional faunal lists. Despite this, the biology of these gastropods has not been treated extensively in the literature except from somewhat specialized points of view. Published studies on naticids center about three aspects of research. These are: their method of feeding, their impact as predators of commercial mollusks, and their role in bottom communities.

Most of the papers which discuss the method of feeding deal primarily with the problem of how the drills penetrate the shell of their pelecypod prey. An early work is that of Schiemenz (1891) who proposed a method of chemical "boring." Ankel (1937, 1938) extended this research and also concluded that the boring was a chemical process. Since no acid was demonstrated to be present in the animals by either of these investigators, Ankel suggested that an enzyme, "Calcase," was involved. Ankel considered this shell dissolving agent to be secreted from a "boring gland" (Bohrdruse) located at the base of the proboscis of naticid drills.

Carriker (1943) continued research on this problem using the muricid drill, Urosalpinx cinerea Say. He provides a detailed description of the functional anatomy of the proboscis of this species.
His observations of drilling by *U. cinerea* led him to conclude that penetration of the prismatic layer of a pelecypod shell is aided by a secretion from a glandular structure located in the anterior mid-ventral region of the foot. This gland was discovered by Fretter (1941) in two British drills, *Nucella (= Thais) lapillus* (Linne) and *Ocenebra erinacea* (Linne), and is called the accessory proboscis. At that time, Fretter considered that the accessory proboscis did play some role in the boring process. However, after further research (Fretter 1946) she concluded that the function of this gland is the secretion of an adhesive material which allows the drill to maintain a firm position on the shell during the drilling process. Although this gland is not found in naticid drills, it has certain histological similarity to the "boring gland" present on the proboscis of naticids (Fretter 1941, 1946; Fischer, 1922).

Jensen (1951a, 1951b) offers evidence for exclusive mechanical boring by naticids through the walls of egg cases of certain elasmobranch fish (Raiidae). He also cites the boring of prosobranch egg capsules (*Sipho curitus*) by *Natica* sp. as reported by Thorson (1935). Since neither of these egg cases showed any effect when exposed to 1 or 2 per cent solutions of sulphuric acid, Jensen concluded that the holes present were bored by the action of the radula alone.

Since then, Jensen's views have received further support. Gunter (1952) considers the efficiency of the radula apparatus sufficient to effect boring. Turner (1953) performed experiments which showed that *P. duplicatus* can penetrate the shell of a soft-shell clam, *M. arenaria*, that has been coated with paraffin or Plaster of Paris (hydrated calcium sulphate). Since both of these
substances are insoluble in dilute acid and would not be acted on by a specific calcium softening enzyme, it appeared that mechanical boring alone was responsible in both cases.

Carriker (1955) further describes the boring process by U. cinerea as mechanical rasping alternating with a chemical softening. In his most recent studies with the muricid drills, U. cinerea and Eupleura caudata Say, Carriker (1959) has shown that drills which had the accessory proboscis removed by surgery could not bore through the valves of small oysters until such time as this gland had regenerated. Experiments in which the complete proboscis was removed similarly indicated that boring could not be resumed until the proboscis apparatus had been regenerated.

This would appear to establish that the accessory proboscis of muricid drills plays a role in the boring process. Although this has not been shown for the naticid "boring gland," its histological similarity would at least suggest a like function. This problem cannot be satisfactorily answered until a substance responsible for softening the shell is identified and its action demonstrated.

Fisheries biologists have conducted a number of studies on the predator-prey relationships between the naticids and various species of mollusks. Along the North Atlantic coast, the three species of Polinices considered herein are known to be serious predators of commercial shellfish populations, particularly of the soft-shell clam, M. arenaria, and the quahog, M. mercenaria. It is toward this aspect of their biology that most of the research on naticids has been directed.
Gould (1870) comments on the predatory habits of New England naticids indicating that early American conchologists were aware that these gastropods fed on pelecypod mollusks.

Reports resulting from investigations of New England shell-fisheries at the turn of the present century mention *Lunatia* (= *Polinices*) as a predator of clams and quahogs. In Rhode Island, Mead and Barnes (1903) showed from field studies that a single *Polinices* could consume up to 8 clams in a 12-day feeding experiment. Belding (1930) also mentions *Polinices* as a serious pest to the soft-shell clam fishery in Massachusetts. Based on field experiments, he estimated that a 2-inch *Polinices* could destroy 26 clams per month at summer water temperatures.

Investigations on *Polinices* were initiated at approximately the same time by biologists at the Atlantic Biological Station, St. Andrews, New Brunswick. Although most of this work has not been published, it is available on loan from the Librarian of the Atlantic Biological Station. Melville (1930) reported on some aspects of the natural history of *P. heros* including observations on boring and feeding, rate of locomotion, and mating.

The general decline of commercial shellfish resources over the last 25 years has directed attention as to what extent predation by drills is involved in the lowering of shellfish yields. The two main questions are: what portion of natural mortality results from predation, and what effect does predation have on the success of clam-farming operations. Work in this field has been conducted primarily with the aim of determining: how destructive clam drills are, and if effective control measures can be developed.
Canadian research is reported by Stinson (1946), Wheatley (1947), Larocque (1948), Thurber (1949), and Giglioli (1949, 1952).

Information is contained in these papers on population density, mating habits, egg collar (= midus of some authors) production, and the results of intensive experimental studies on the control of egg collars and adults for both P. heros and P. triseriata.

Similar research has been carried out on P. duplicatus in the New England area. Turner, Ayers, and Wheeler (1948) consider that clam drills may often be responsible for destruction of small clams to such an extent that successful sets may be wiped out in the ensuing summer and so go unnoticed. Experiments by Turner (1949) indicated that clam drills were responsible for nearly 31.7 per cent of the clam mortality in planted beds. Sawyer (1950) carried out laboratory feeding studies with P. duplicatus at summer (21° C.) and fall (10° C.) water temperatures. He concluded that feeding was correlated with temperature because feeding rates were highest during the summer months. Turner (1951) considered the secondary food supply of small non-commercial pelecypod species available to P. duplicatus populations. He concluded that small drills, 12 mm. opercular length or less (Figure 1), could exist on the small prey species in areas barren of larger commercial mollusks. While surviving at near starvation levels, these drills could be immediately destructive to any plantings of seed clams.

Chin (1952) studied the method of prey selected by P. heros. His experiments indicated that four factors were interrelated in selecting prey. These were: shell thickness, size preference and/or limitation, species preference, and accessibility. He did not consider his experiments conclusive as to how the drills located their prey. He did,
however, show that drills would usually choose "stream-paths" which contained living pelecypods at the source in preference to similar "stream-paths" without prey species present.

Hanks (1952, 1953), working with P. duplicatus and P. heros, extended the laboratory studies of feeding rates through a range of water temperature from 2° C. to 21° C. and a range of salinity from 6 o/oo to 32 o/oo. This research established feeding rates for both species over most of the annual temperature cycle and for upper estuarine to open oceanic conditions.

Research on the biology of those naticid species which inhabit the continental shelf has been carried out primarily by Danish biologists within the scope of their level sea-bottom studies.

Thorson has reported on many biological aspects of the naticid gastropods recovered from a number of areas, in particular Denmark, Greenland, and the Gulf of Iran. His papers include information on reproduction and larval development (1946), comparative shell morphology supporting the "Apex Theory" (1950), and north-south distribution of naticids as influenced by water temperature (1958).

Thorson considers that the presence of these macro-predators constitutes a good indication of the uniformity of ecological conditions over large areas of level sea-bottom. He cites as evidence the occurrence of a single species (Natica groenlandica (Moller) as a dominant organism along with two pelecypod mollusks (Area glacialis Gray and Pecten groenlandicus G. B. Sowerby) at depths of 1000 to 2000 meters from the arctic through warm temperate and even to subtropical areas (1936). He contends that with sufficient knowledge of the biology of naticids, these animals will be extremely useful in
interpreting bottom community ecology for the deep ocean areas where quantitative sampling is, as yet, not possible (1958).

Giglioli's paper (1955) on the egg masses of a number of Atlantic species of naticids contributes significantly to our ecological knowledge. He has devised a key for identifying egg collars with the species that produced them. This should have wide application in bottom study work, since drills are frequently not recovered from the areas where their egg collars are dredged.

Clarke (1956) observed feeding on eggs of *P. heros* by a common intertidal snail, *Nassarius trivittatus* (Say). This is of considerable interest, since few enemies of adult drills are known. Various sea birds and bottom-feeding fish are the most notable, and they do not appear to account for any significant amount of predation on drills.

Although drills usually attack their pelecypod prey by boring a hole through the shell prior to feeding, Turner (1955) has observed instances of successful predation on the razor clam, *Ensis directus* Conrad, by *P. duplicatus* without boring. Where observed, it appeared that a slime envelope, secreted around the clam by the drill, acted in some way to anesthetize the clam. In this manner the drill could feed by inserting its proboscis between the valves of the clam.

Turner (1958) also reports the effect of nutrition on the color of the callus portion of the shell of *P. duplicatus*. His experiments demonstrate that well fed drills have a pearly-white callus while starved drills have a dark brown callus. This is significant to the ecologist, in that it provides a means of assessing the food available to any specific population of *P. duplicatus*. 

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Although the rearing of larvae of marine invertebrates is by no means a new endeavor, most investigators have used larvae collected from the plankton at various stages of development. This method was used successfully by Thorson (1946), but he had the advantage of a team of workers.

Other investigators have used artificially fertilized ova to study the cleavage stages and larval development. Such "finger-bowl" techniques appear satisfactory for strict embryological and morphological studies as evidenced by Costello's recent handbook (1957). They will not, however, serve to answer questions concerning the ecology of invertebrate larvae.

In recent years, it has been found essential to rear invertebrate larvae under culture conditions in order to determine the effects of such environmental factors as temperature, salinity, and food. The development of culture methods for the larvae of pelecypod mollusks has received attention largely because of their commercial aspect. Such methods have been reviewed by Korringa (1952) and Loosanoff (1954). Much less attention has been given to methods for rearing other species of invertebrates. Studies such as those of Lebour (1937, 1944) on British prosobranch gastropods and decapod crustaceans and of Mortensen (1938) on echinoderms are notable exceptions.

The significance of culture methods is well shown by the experiments of Day and Wilson (1934) and Wilson (1932, 1937). This work indicated the importance of a suitable substratum as an inducement to metamorphosis of larvae of a number of invertebrate animals. Conclusive results such as these can be obtained only when populations of larvae of known age are reared under controlled environmental conditions.
SECTION III.

GENERAL METHODS AND MATERIALS

A. Collection of Animals and Egg Collars.

1. Polinices duplicatus

All specimens of P. duplicatus were obtained from the tidal flats at Barnstable Harbor, Massachusetts. Initial collections of approximately 100 adult drills (25 mm. opercular length or larger), were made in the summer and early fall of 1957. These animals were kept in the laboratory tanks as a spawning stock. Several collections of juvenile P. duplicatus (4 to 10 mm. opercular length) were also made in this same area for use in growth rate and sexual maturity studies.

Most of the studies of the development of eggs and larvae within the egg collars were conducted with collars produced by drills maintained in the laboratory. However, some of the studies were conducted with collars of P. duplicatus obtained from the tidal flats at Quissett Harbor, Massachusetts. Although P. duplicatus was the only species of drill observed at this station, only those collars with the scalloped-shaped lower edge (Figure 2), clearly identifying them with P. duplicatus, were collected for study.

2. Polinices triseriata

Through the courtesy of Dr. William Burbank (Department of Zoology, Emery University, Georgia) 3 adult P. triseriata were recovered from a dredge haul in 30 feet of water outside Menemsha Harbor, Massachusetts.

1/ The taxonomy of the genus Polinices has not been adequately studied to date. It should therefore be noted that various synonyms for the three species studied herein exist. These are: Lunatia, Natica, Neverita, Ampullaria and Euspira.
These animals were kept in tanks with running sea water where they produced several egg collars during the summer months.

Three collections of *P. triseriata* egg collars were made. One collection was from Starboard Island, Maine. Although the collars were packed in damp seaweed for transport to the recirculating sea water system at the University of New Hampshire, the eggs and larvae did not survive, perhaps because of excessively warm air temperatures during the period of transport. These collars were preserved for examination.

Two subsequent collections of collars were made by Dr. George H. Moore (University of New Hampshire) in the vicinity of St. Andrews, New Brunswick. Again, one collection did not survive transporting and the collars were examined and discarded. The other collection, which was kept in an ice-cooled plastic container, survived the trip to the laboratory at W.H.O.I. These collars were kept in running sea water where the eggs developed to metamorphosed drills.

Extensive dredging and bottom sampling was undertaken in Vineyard Sound, Massachusetts in the summer of 1958 by biologists of the U.S. Bureau of Commercial Fisheries (anonymous 1958). The author had the opportunity of going on two of these cruises and of examining the dredged material as it was unloaded on deck. However, no recoveries were made of adult *P. triseriata* or their egg collars.

**3. *Polinices heros***

About 100 adult *P. heros* (35 mm. opercular length or larger) were collected through the dredging operations in Vineyard Sound. These animals were taken from 30 to 40 feet of water in various areas of the Sound where quahog, *M. mercenaria*, populations were present. This group
of drills was kept in laboratory tanks and constituted the spawning stock for this species.

Two collections of juvenile P. heros (5 to 8 mm. opercular length) were made at York Beach, Maine. These were kept in running sea water in the laboratory for growth rate studies.

All collars of this species used for observation of development of eggs and larvae were produced by the laboratory population. Collars of P. heros were collected from the tidal flats at St. Andrews, and also from the dredging in Vineyard Sound. However, none of these collars survived transporting to the laboratory and were discarded after examination for stages in larval development.

B. Methods for Maintaining Drill Populations in the Laboratory.

Investigators working with eggs and larvae of marine invertebrates have usually depended on two methods in order to obtain material. One is by collecting these stages from the plankton. The other method is by rearing eggs and larvae in the laboratory, starting with artificially or naturally spawned gametes produced during the natural reproductive period of the animal concerned. Both methods have distinct disadvantages. Among those for plankton collections are: the difficulty of correct identification, the need to isolate sufficient numbers of individuals, and the problem of reconstructing larval development by tracing stages through a series of plankton samples. For the reared material the disadvantages include: population fluctuations from year to year which may curtail research programs because of insufficient spawning stock, and, frequently, the impossibility of obtaining "ripe" animals at the time needed, since the maturation period for the gametes is dependent on a number of ecological factors.
In an effort to overcome these difficulties in carrying out larval studies throughout the year, temperature conditioning methods were developed for some species of pelecypods (Loosanoff 1945; Loosanoff and Davis 1950) that will induce "out-of-season" gametogenesis and spawning. In this way, eggs and larvae can be made available at any season of the year.

Such methods have not been extended to other invertebrates. Thus the initial phase of research with Polinices was the development of a method which would induce these gastropods to produce egg collars at all seasons of the year.

This was accomplished by keeping populations of drills at summer water temperatures throughout the year and providing them with an adequate food supply. For this purpose, drills were kept in a wooden tank (36" x 18" x 8") with ½ inches of sifted beach sand covering the bottom. A sea water heating unit (Loosanoff 1949; Hanks 1953) was connected to the laboratory sea water system. This unit provided a flow of warmed water at an approximate rate of 1 to 2 liters per minute. An immersion thermostat (Appendix II) located in the heating unit was used to adjust the water temperature to between 18° and 20° C. In this manner, a relatively constant summer temperature was maintained without difficulty even when the normal winter water temperature was as low as 2° C.

Under these conditions a group of 25 to 30 drills was maintained with little effort. Various species of pelecypods were used as a food source depending on availability. The edible mussel, Mytilus edulis Linné, constituted the bulk of the food. These mussels were obtained from the dock pilings at W.H.O.I. and were readily consumed by all three
species of drills. Under such conditions, populations of *P. duplicatus* and *P. heros* fed actively, mated, and produced egg collars throughout the year.

C. Rearing Techniques for Developing Eggs and Larvae.

The general procedure for culturing the larval stages was as follows: Egg collars were collected from the spawning tank as soon as they were observed on the surface of the sand. The date and time of collection was recorded and designated as the "time produced" by a female. These collars were then individually isolated in 1-gallon polyethylene containers.

In the summer months, when the laboratory sea water temperature was between 18° and 23° C, the containers were kept on a wooden rack and supplied with a constant flow of sea water during the first week or so of egg and larval development. The flow of sea water was then turned off and the sea water in the container was changed daily. Standing water was necessary to prevent the escape of larvae when they were released from the collar. During the remainder of the year, when laboratory sea water temperatures were lower than 18° C., the containers were placed in a heated fresh water bath maintained at a temperature suitable for the development of eggs and larvae. Again, sea water in the containers was changed each day.

The water bath consisted of a wooden tank (5½" x 28" x 10"). A number of ½" x 1½" wooden slats were attached to the bottom of the tank to allow circulation under the containers when they were sitting in the bath. A 50-foot flexible lead heating cable (Appendix II) was wound between the slats. The tank was filled with fresh water to minimize corrosion of the cable. By placing a thermostat in the bath, it was
possible to maintain a desired water temperature up to 25° C. within 
± 1° C. In this manner, eggs and larvae could be kept at suitable 
temperatures for development regardless of seasonal temperature changes.

After release from the collar, the swimming larvae were kept in 
sea water in polyethylene containers or glass jars of approximately 10-
liter capacity. Under these conditions, the sea water had to be changed 
every 2h hours. This minimized the increase of competitive organisms 
such as protozoan ciliates. It also prevented any excess accumulation 
of metabolites, either from the larvae or from the phytoplankton added 
as food, which might be toxic to the larvae. A few cultures, in which 
the sea water was changed every 48 hours, had excessive larval mortality. 
Such cultures survived only a few days.

In order to change the sea water, it was necessary to carefully 
remove the larvae from the culture. The shells of the larvae are 
extremely thin, and if damaged the larvae do not survive. Screens of 
number 7 or 8 bolting silk, such as that used for plankton nets, were 
constructed for removing the larvae. However, they clogged quickly and 
were difficult to clean. Wire screens of stainless steel were found to 
work best (see Appendix II for sizes and dimensions of screens useful in 
larval culture work). Screens of other metals and alloys should not be 
used since they release ions that are generally toxic to invertebrate 
eggs and larvae. A number 100 screen was adequate to retain the larvae 
of Polinices while most undesirable material, such as clumps of dead 
algal cells, was passed through. To avoid injury to the larvae, the sea 
water was siphoned to the screen through rubber tubing. Larvae collected 
on the screen were then washed into a fingerbowl using a plastic wash-
bottle. They survived such conditions during the short time necessary
for cleaning the containers and refilling them with filtered sea water. A sample of larvae for observation was usually taken at each change of water.

Once larvae are released from the collar, they must be fed some phytoplankton organisms. Since the cultures were changed daily, a quantity of algal food was added at each change. In order to add an approximately equal amount of this algal food at each feeding, cell counts of the algal cultures were made routinely. Throughout most of the study, this was done using a standard glass hemocytometer cell. Such counts were made to determine the number of cells per milliliter of algal culture. From this it was possible to calculate the volume needed to provide the desired cell count in the larval culture. Algae were usually added to provide a cell count of 200,000/ml. in the larval culture although other concentrations were used at times. When the algal cultures approached maximum cell density, the amount added each day for food was in the range of 50 to 100 ml.

It was satisfactory to add phytoplankton food in this manner when working with a single food species. However, because of differences in the size of different species of algae, it was not satisfactory when using two or more species of algae in comparative feeding studies. Equal numbers of cells do not necessarily give equivalent amounts of food. Thus the equal cell volume method of Davis and Guillard (1958) was used in such studies as it gives a more accurate basis of comparison. This involves centrifuging a 10 ml. sample of algal culture in a Hopkins tube and measuring the wet-packed cell volume. By doing this for each species of alga used, the volume of algal culture needed to provide an equivalent quantity of packed cells per milliliter of larval culture can
then be calculated (see Appendix I for a discussion of algal culture methods).

As mentioned above, the sea water was changed daily during the colder months when larval containers were kept in the heated water bath. This bath was maintained at a temperature of about 23° C. In order that no thermal shock would occur to the larvae when the cultures were changed, it was necessary to have a large volume of warmed, filtered sea water available each day. This was supplied by a heating unit of similar design but of larger size than the unit described for the spawning tank. This unit consisted of a 50-gallon drum with a 75-foot section of \( \frac{3}{4} \)-inch lead pipe coiled into it. Four heating rods (Appendix II), each rated at 1000 watts, were used to warm the fresh water that surrounded the coil. The water temperature was controlled by a thermostat which could be adjusted to provide a flow of sea water heated at a desired temperature within \( \pm 1 \)° C. By filling a 20-gallon polyethylene container each morning with sea water at 25° C, filtered through cotton and glass wool, a supply of warm sea water was maintained for changing cultures.

A number of other special techniques were used during various portions of the study. These are included in the sections that follow where they are pertinent to the results.
SECTION IV.

OBSERVATIONS AND EXPERIMENTS

A. Reproduction

As is characteristic of most marine prosobranch gastropods, members of the genus Polinices are dioecious. The sex of mature drills can be distinguished by visual examination of the external genitalia. Adult males have a penis located on the right side of the body posterior to the right tentacle and just dorsal to the separation of the visceral mass and the foot. The penis of P. duplicatus is moderately large in proportion to the size of the animal, averaging in length about one-fourth of the shell height (Table 1). A similar size ratio is described by Wheatley (1947) for adult male P. heros and P. triseriata. In all three species the penis is dorso-ventrally flattered in cross section and curves somewhat to the right, the tip being directed away from the body. When the foot of a male drill is normally extended as it crawls about, the penis rests within the mantle cavity and is not visible. If the operculum is grasped firmly with forceps before the body is retracted, the body can be withdrawn from the shell far enough to observe the penis without injury to the animal.

Table 1. P. duplicatus: Penis Length and Shell Height of Ten Adult Males

<table>
<thead>
<tr>
<th>Shell Height (mm.)</th>
<th>Penis Length (mm.)</th>
<th>Shell Height (mm.)</th>
<th>Penis Length (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>10</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>37</td>
<td>9</td>
<td>23</td>
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<td>34</td>
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<td>21</td>
<td>5</td>
</tr>
<tr>
<td>32</td>
<td>8</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>29</td>
<td>7</td>
<td>17</td>
<td>4</td>
</tr>
</tbody>
</table>

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Adult female drills bear the vaginal opening at the terminus of a small papilla located on the right side in a similar position as the penis of the male. However, the papilla is not as readily observed as is the penis. Because of this, determination of the sex of mature specimens of all three species of drill studied was accomplished by considering the absence of a penis as evidence of femaleness.

When the shell is removed from a _P. duplicatus_, exposing the visceral mass, the gonad is easily located in mature specimens. From June to September, the normal reproductive season, the color of the gonad is usually distinctively different in male and female drills. The color is characteristically cream to yellow in females and chestnut-brown in males. However, some variation in the color of the gonads has been observed making this characteristic of doubtful validity for determining the sex of individual animals. As the gonads contain mature gametes during this period, it is preferable to make a smear preparation of the gonad tissue. This can be done by making a small incision in the gonad and removing some of the tissue with a pipette. Examination of this tissue with a compound microscope will show either ova or spermatozoa. Ova obtained in this manner appear to be in different stages of maturation since they are present in various sizes. The largest ova measure about 100 micra. These have a distinct germinal vesicle and are apparently ready for fertilization. They are, however, somewhat oval in shape compared with the spherical shape of fertilized eggs observed in recently produced egg collars. Ova removed from the ovary in this manner are extremely fragile. Often, the cell wall ruptures while they are being observed under the microscope. The spermatozoa are flagellated, and though inactive when first removed,
become quite active within a few minutes after being placed in sea water on a depression slide.

In both sexes the gonad is located dorsal to the digestive gland which constitutes the bulk of the spiral portion of the visceral mass. The gonad is embedded in the digestive gland and extends back nearly to the tip of the visceral mass.

The vas deferens begins ventro-lateral to the testis. It is also embedded in the digestive gland and is much convoluted as it passes along the gland to enter the mantle cavity. This duct then passes along the wall of the mantle cavity until it penetrates the penis.

The oviduct is similarly situated but is less convoluted than the vas deferens. As it passes along the digestive gland, the oviduct becomes wider and the wall becomes thick and muscular. After entering the mantle cavity, it continues to a small papilla that bears the vaginal opening.

In order to determine the individual sexes present in large collections of drills, it is usual to sacrifice the animals. The shell is cracked and removed. This allows direct observation of the presence or absence of a penis. *P. duplicatus* of 10 to 12 mm. shell height or smaller cannot be accurately sexed by this method, since males of this size have not developed a penis. In *P. triseriata*, a smaller species, individuals can be sexed at a size of about 6 mm. shell height. Specimens smaller than this cannot be sexed. Again this results from the absence of external genitalia (Giglioli 1949). When drills of these small sizes appear in collections, they are usually grouped as immatures without reference to sex.

Small *P. heros*, 10 to 12 mm. shell height or smaller, collected at
York Beach, Maine, did not have external genitalia present. Specimens of this species between this size and approximately 25 mm. shell height were not obtained during this study. Giglioli (1949) also notes a lack of small *P. heros* in Canadian waters. Thus, it has not been determined at what size *P. heros* develops external genitalia.

During 1949 and 1950, Turner made extensive collections of *P. duplicatus* at Barnstable Harbor, Massachusetts. The measurement and sex of individual drills was recorded for a number of these collections. This unpublished data was made available to the author and indicated that nearly equal numbers of males and females were present on the flats during the summers of those years.

Sex determinations of two collections of *P. heros* were made by the author. One group of 150 specimens from Vineyard Sound, Massachusetts contained 81 females and 69 males. Data on a collection of this species from St. Andrews, New Brunswick was available from 90 specimens kindly sent to the author in 1952 by Dr. Carl J. Medcof, Atlantic Biological Station. This group contained 49 females and 41 males.

In studies of *P. triseriata* in southern Canada, Wheatley (1947) found a predominance of females over males of about 4 to 3. No comparative data for this species was obtained in conjunction with this study since no populations were located in New England waters.

The unpublished data of Turner also show that the average size of male drills is smaller than the average size of females in any of the individual collections made at Barnstable Harbor, Massachusetts. In addition, observations indicate that the male member of a mating pair of *P. duplicatus* is smaller than the female. The difference in size is on the order of 10 mm. opercular length (Table 2).
Table 2. *P. duplicatus*: Opercular Length of Male and Female Members of Mating Pairs

<table>
<thead>
<tr>
<th>Pair</th>
<th>Opercular Length mm.</th>
<th>Size Differential mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>46</td>
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<tr>
<td>4</td>
<td>24</td>
<td>37</td>
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<tr>
<td>5</td>
<td>18</td>
<td>29</td>
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<td>6</td>
<td>23</td>
<td>31</td>
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<tr>
<td>7</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>42</td>
</tr>
</tbody>
</table>

Six of the pairs listed above were recovered from the laboratory spawning tank. The drills in the tank had been randomly selected from one of the collections made at the Barnstable Harbor station. Two of the pairs were collected on the tidal flats at the same station.

A similar difference in size between the sexes is reported for mating pairs of *P. heros* and *P. triseriata* recovered from field populations (Stinson 1946; Giglioli 1949).

Among other phyla of invertebrate animals, the sexes are sometimes separated during the colder months of the year. This has been noted for the green crab, *Carcinoides maenas* (Linne). Data collected by the U. S. Bureau of Commercial Fisheries in southern Maine suggest that female crabs of this species overwinter in burrows in the banks of marsh creek systems. In contrast, the male crabs appear to migrate in the fall from the creeks into deeper, subtidal water and do not return until the following spring (Hanks, Robert W., personal communication).
All three species of Polinices herein considered burrow into the flats in the late fall, where they usually remain below the surface throughout the winter and early spring months. Occasionally, a few drills can be found crawling about as late as December or as early as April when low tides and bright sunlight combine to warm the surface on the flats.

Insofar as the author is aware, the drills recovered by Turner (unpublished) on November 7, 1950 at Duxbury, Massachusetts constitute the last collection made for that time of year. Although not abundant, 315 specimens of P. duplicatus were obtained by covering a large area of flat during the period of low tide. Of these, 147 were males and 168 were females. The estimated sea water temperature at the time of this collection is 10° C. This estimate is based on a water temperature of 11.5° C. recorded by Turner at Barnstable Harbor, Massachusetts on November 17, 1950.

Since the number of drills on the surface of the flat at Duxbury was considerably less than it had been in the same area in the previous summer (Turner, personal communication), it appears that the bulk of the population had already burrowed into the flat for the remainder of the winter.

Thus, from the evidence available, it does not appear that any separation of the sexes takes place by migration in populations of Polinices that live in the intertidal zone. Therefore, males and females should be present in about equal numbers at the beginning of the reproductive season in the spring. Under such conditions, it would be possible for all mature females to mate.

This would result in a high reproductive potential in that all adult female drills should be capable of producing egg collars containing fertilized eggs.
Observation of *P. duplicatus* at Barnstable and Quissett Harbors indicated that mating takes place throughout the summer. Although mating is primarily a nocturnal activity, a few mating pairs were seen on the surface of the flats at both stations from late May through the middle of August whenever collections of drills were obtained. Most of such pairs were not in copulation but were moving over the surface of the flat in a "pre-conjugal march" similar to that described by Giglioli (1949) for *P. triseriata*. Giglioli considers that this activity precedes the actual copulation, which he believes always takes place below the surface of the flat. Although paired *P. duplicatus* go through this same "marching" prior to copulation, they do not always burrow into the flat to copulate. A number of mating pairs of *P. duplicatus* were observed in the spawning tank. In a typical mating, the propodium (anterior portion of the foot) of the male is supported by a portion of the foot and shell of the female. Only the posterior half of the foot of the male is in contact with the substratum. In some cases, when the male is quite small relative to the female, the male is entirely supported by the female throughout the period of mating. The drills may remain in this paired position for as long as two hours. The female moves over the surface of the flat continually and the male is more or less passively carried along with her. This is the typical position and movement which Giglioli calls the "pre-conjugal march." After a varying period of time, the female may burrow; copulation then takes place below the surface. In the relatively few cases where copulation has been observed to take place on the surface of, rather than in, the substratum, it was clearly observed that the penis, which is capable of considerable vascular extension, passes dorsal to the
foot of the male and enters the mantle cavity of the female to penetrate the vagina. The members of the pair then separate and no resumption of mating activity was observed once this separation had occurred. Pairs disturbed by physical means while in copulation did not attempt to remate. However, pairs disturbed while crawling along the surface would often resume this activity and complete the reproductive act.

Since the female drill normally produces the egg collar below the surface of the substratum, it is not possible to observe the actual formation process. Some direct observations were made on the formation of collars by *P. duplicatus* just before they were completed when a portion of the collar was visible above the surface. These observations were supplemented by disturbing females while they were forming collars and recovering the portion of the collar produced to that time. Observations from both sources indicate that the body of the collar is formed from a jelly-like material secreted by the foot. This material apparently hardens somewhat after contacting the sea water. By the action of the foot, reflected upwards against the shell, the jelly is formed into a thin ribbon of about 1 mm. in thickness. The eggs are then deposited into this jelly from the vaginal opening. Since the collar is formed below the surface, sand grains adhere both to the outside of the jelly and are pressed into it while the collar is being shaped against the shell. Portions of collars recovered by digging the female from the sand while the collar was being formed show that the complete width of the collar is produced and the eggs deposited in a nearly simultaneous operation. The characteristic shape of the collar is imparted as the collar is being formed.
When egg collars, both whole and in section, were examined under the microscope, the eggs appeared to be deposited somewhat randomly and each in a single gelatinous capsule. However, a number of collars of *P. duplicatus* were recovered from the spawning tank in which portions of the collar had no sand grains embedded in the jelly. These portions were quite transparent and the eggs were clearly visible. Examination of these areas showed the eggs were actually deposited in continuous gelatinous cords which were wound back and forth in the jelly (Figure 3). This is a typical method of egg deposition among nudibranch and prosobranch gastropods which have gelatinous egg cases. It appears that when the sand grains are pressed into the collar this pattern of deposition of eggs is obscured. Although this was observed only for collars of *P. duplicatus*, it is probable that the same method of deposition of eggs occurs for the other two species studied.

Giglioli (1955) has shown that the egg collars of different species of naticids are characteristic and identifiable as such. His descriptions are adequate for the identification of the egg collars of the three species dealt with in this study. It was also found possible to identify portions representing as little as 1/6th of the whole collar of these species. In addition to differences in the shape of collars of different species, there are also differences in the size of the eggs and the number of eggs per capsule. These are also described by Giglioli (1955) for many of the species of naticids. The external features of the collars of *P. duplicatus*, *P. heros* and *P. triseriata* are shown in Figures 2, 4, and 5.

Although it is generally correct to state that the characteristics of a collar are specific for each species, it should be noted that certain exceptions occur. Over a period of two years of maintaining
P. duplicatus in the laboratory for spawning purposes, a number of collars were produced that did not have the characteristic external shape for this species. The usual abnormality was a lack of scalloping along the basal edge of the collar. Since no other species was present in the spawning tank, there could be no doubt but what these collars were produced by P. duplicatus. These aberrant collars were not produced by a single female since they were recovered from more than one spawning tank and from different groups of drills.

In order to determine if such aberrant collars contained normal eggs that would develop to normal larvae, two of these collars were isolated in separate plastic containers with sea water for observation of development. The eggs cleaved normally and developed to early veliger larvae. These larvae were released from the collar after 12 days at 18° to 20° C., which is the same as for collars of normal shape. Released larvae swam actively and fed on the added phytoplankton. Thus the abnormal shape of these collars did not appear to affect the eggs or larvae which they contained.

The aberrant collars are estimated to have been produced on the order of about 1 in 100 under laboratory conditions. No such collars have been observed by the author in collections from field populations of P. duplicatus. Stinson (1946) and Giglioli (1949) report that some imperfect collars of both P. heros and P. triseriata were obtained during their studies of these species. However, the imperfections they noted were mainly incomplete sanding of the collar, leaving transparent areas, rather than any abnormal shape of the collar itself.

One explanation for the production of such aberrant collars concerns the crowded condition of the drills in the small space of the spawning
tank. It seems possible that overcrowding might lead to a female drill being disturbed by the movement of other drills during the formation of a collar. Such a disturbance might affect the rate of formation such that the normal shaping of the collar would not occur. Since it takes a female drill 4 to 8 hours to produce a complete collar, an interruption in the process might result in an abnormal shape.

Two experiments were conducted with P. duplicatus to test the hypothesis that physical disturbance was the cause of the formation of abnormally shaped collars. In the first experiment, a female drill in the process of forming a collar was disturbed a number of times with a glass rod pushed below the surface of the sand. When this collar was recovered after completion, it had the normal characteristic shape for this species. For the second experiment, two additional drills were pushed below the surface of the sand beside a female in the process of forming a collar. Yet, after they had moved away, the female continued to form the collar. When this collar was recovered after completion, there was no evidence of any interruption along the length of the collar and the shape of the collar was normal. Thus, it could not be shown that physical disturbance resulted in the formation of aberrant collars.

The significance of these aberrant collars formed by P. duplicatus lies in the fact that collars as yet unidentified to species have been dredged from deep water off the Canadian Atlantic coast (Giglioli 1952, 1955). The number of such unidentified collars that were recovered led Giglioli to believe that they were produced by an unknown species of naticid. It seems possible, however, that these collars may have been abnormally shaped specimens of known species of Polinices or Natica. Providing adequate collections of such unidentified collars could be

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obtained, it should be possible to maintain them in the laboratory until
the larvae were released and then to rear the larvae to metamorphosis.
If the growth rate of naticids in general is at all comparable to that
for P. heros and P. duplicatus, it would be possible to determine whether
these individuals were a described species by about one month after
metamorphosis. In that period of time, the drills should have grown to
a size of about 5 to 10 mm. shell height. At that size, identification
can be made from characteristics of the shell and radula.

The normal breeding season and period of collar formation for the
three Polinices studied is during the warmer months of the year. This
generally includes a period from the middle of June through the end of
August. Some variation occurs from year to year in the beginning and
ending dates of collar formation. Field observations on the three
species were made on populations at a number of locations from southern
Massachusetts to southern Canada.

The earliest observation of egg collars of P. duplicatus was on
April 11, 1958. This was reported to the author by Mr. Charles L.
Wheeler (Biologist, Division of Marine Fisheries, Commonwealth of
Massachusetts) for collars present in a shallow section of Tisbury
Great Pond, Marthas Vineyard, Massachusetts. No measurement of the sea
water temperature was taken with this observation, but the water tempera-
ture recorded at the W.H.O.I. laboratory at this time was 9° C.
Collars were not present that year at the Barnstable or Quissett Harbor
stations until the second week of June. Thus it seems probable that the
water temperature in Tisbury Great Pond was somewhat higher than at the
station where P. duplicatus populations were under routine observation.
This could account for the much earlier date of spawning at Tisbury
Great Pond.

Egg collars of *P. duplicatus* were common throughout the summer of both 1957 and 1958 at the Barnstable and Quissett Harbor stations when the sea water temperature was above 17° C. In 1957, collars were present when these stations were visited on June 12th. In 1958, collars were first observed at Quissett Harbor on June 16th and at Barnstable Harbor on June 19th. Both stations had been visited one week prior to these dates and no collars had been present. Collars were also found on the flats until August 26, 1957 and August 29, 1958 at Quissett Harbor and on August 27, 1957 and August 30, 1958 at Barnstable Harbor. No collars were observed at either station in either year when the first September visit was made.

Although subtidal populations of *P. heros* are present in Vineyard Sound, no intertidal populations were located in the vicinity of Cape Cod, Massachusetts. A few collars were collected at St. Andrews, New Brunswick the later part of August, 1957. These were not obtained by the author and the sea water temperature was not taken at the time of collection. The monthly average surface sea water temperature at St. Andrews for the month of August that year was 13.5° C. (Lauzier 1958). A number of collars were dredged from Vineyard Sound in July, 1958 in 30 to 40 feet of water. Bottom water temperatures at the stations dredged ranged from 16° to 19° C.

Although 3 adult specimens of *P. triseriata* were taken by dredging near Menemsha Harbor, Massachusetts, no populations of this species were found in the intertidal areas of Cape Cod, Massachusetts. Only three collections of *P. triseriata* egg collars were obtained during this study. One was from Starboard Island, Maine where a number
of collars were collected from the tidal flats on April 26, 1957. The surface sea water temperature at the time of collection was 6.2° C. Two other collections were made in the vicinity of St. Andrews, New Brunswick; one in early June, 1957 and one in late August, 1957. The average monthly surface sea water temperature for those months was respectively 10.3° C. and 13.5° C. (Laузier 1958).

Two egg collars were produced by the 3 P. triseriata kept in the laboratory in running sea water at natural temperatures. The water temperature at the time the collars were produced on April 20th and 22nd was 9.5° C.

These observations indicate that these three species have their normal period of reproduction during the summer months over the whole of the area covered in this study.

In order to obtain experimental data for comparison with the field observations on water temperature limitations to the deposition of egg collars, 150 specimens of P. duplicatus were placed in a wooden tank (18" x 36" x 16") in the laboratory. Four inches of sifted beach sand was placed in the bottom of the tank and a constant flow of sea water provided. Mussels were added to the tank as often as necessary to maintain an excess supply of food for the drills. The temperature of the sea water running to the tank followed the annual temperature cycle from a winter low of about 2° C. to a summer high of about 21° C. Since the drills fed, mated and produced egg collars under such conditions, it was assumed that the environment was similar, even though not identical, to their natural habitat. There were two obvious factors which were quite different from those existing on the tidal flats. One was the large number of drills confined in the rather small
space of the tank. The other was a lack of any tidal cycle. The water level was maintained at about a depth of 1 foot by placing an overflow hole near the top of the tank.

These drills were observed regularly for a full year from the time the tank was set up in August, 1957. Records of the water temperature were kept, and the number of collars produced were noted at 3 to 5-day intervals. Collars were removed from the tank after each observation in order that they would not be recorded twice. Table 3 shows the number of collars produced each month as well as the water temperature range for the same period. A few mortalities occurred during the period of observation. Dead drills were replaced by drills from another tank which also had been kept at normal water temperatures.

Table 3. Number of Egg Collars Produced Each Month by 150 P. duplicatus During One Annual Cycle of Sea Water Temperature

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of collars produced</th>
<th>Monthly Temperature Range (° C.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>1957</td>
<td></td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>82</td>
<td>21</td>
</tr>
<tr>
<td>October</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>November</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>December</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>1958</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>February</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>March</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>April</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>May</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>June</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>July</td>
<td>87</td>
<td>22</td>
</tr>
<tr>
<td>August</td>
<td>97</td>
<td>22</td>
</tr>
</tbody>
</table>

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The first collars were deposited within a week after the start of
this experiment. The water temperature at that time was 21° C.
Although the number decreased, collars continued to be produced through-
out the early fall as the water temperature lowered from 21° C. to 13° C.
This was considerably later than at the field stations where collars were
not produced after the end of August. The water temperature at the field
stations had not dropped below 18° C. at that time. When the temperature
in the laboratory tank dropped below 13° C., collars were produced only
intermittently. Often two observations would pass without any collars
being found and then one or two would be deposited. Collars were
produced at all temperatures down to 7.6° C. when a collar was recovered
on December 11, 1957. From that date onward, no collars were produced
until the following spring, a period of about four months. On April 11,
1958, two collars were produced when the water temperature was 8.4° C.
This indicates that egg collars can be produced at water temperatures
considerably below those at which they are produced in the field.

Mating pairs of drills were not observed in the laboratory tank
during the early spring. Unless sperm from a mating which had occurred
during the previous summer was retained by the females over the winter
for fertilization of ova, mating must have taken place unobserved below
the surface of the sand. Evidence is available that a female P. duplicatus
need mate only once in any one breeding season in order to
produce fertilized eggs in a number of collars during the same season
(Hanks 1953). No evidence is available for Polinices to confirm the
possibility of sperm being retained in a female over the winter for
fertilization of ova during the next breeding season. It is known for
the oyster drill, Urosalpinx cinerea, that individual females, isolated
in perforated plastic containers, can produce egg capsules in two successive breeding seasons (Hanks, unpublished). Eggs in the capsules produced during the second breeding season, when no mating could have occurred, developed normally to metamorphosed animals. Thus it seems probable that sperm received in the first season were retained in the female and served to fertilize the ova deposited in capsules during the second breeding season. It is therefore possible that a similar over-winter retention of viable sperm by females occurred in the laboratory population of *P. duplicatus*.

It has been established for the oyster, *Crassostrea virginica* (Gmelin), that there is a period after the summer spawning season in which unspawned ova or sperm are resorbed. The gonad then enters an indifferent stage (Loosanoff 1942). During this stage of indifferent sex cells, spawning cannot be induced even if the oyster is warmed to normal summer water temperatures. This latent period for oysters in southern New England is from the end of the spawning season in September until December or January. By that time, oysters can be taken into the laboratory and, after conditioning to normal summer water temperatures, will develop "ripe" gonads. They can then be induced to spawn. Under natural conditions, new gonad tissue starts to form at the end of the indifferent period in October. This development is of short duration, however, as it ceases with the onset of cold winter weather. The gonad development commences again in the early spring when the water temperature begins to approach 10° C. Development continues and the oysters attain "ripe" gonads, in condition for spawning, sometime in June.

In order to determine the condition of the testes and ovaries of
P. duplicatus during the period after the summer spawning and throughout the colder months of the year, drills were sacrificed at four different water temperatures (15°, 10°, 5°, and 2° C.) below those of normal spawning. Smear preparations of the gonad tissue were made. Microscopic examination of these smears showed that sperm and ova were present at all months of the year. Thus, in terms of the presence of gametes, it did not appear that an indifferent period of gonad development occurred. P. duplicatus, therefore, appeared to be in "ripe" reproductive condition at all times of the year even though no mating occurred at low water temperatures under field conditions.

A few P. duplicatus were removed from the holding tanks from time to time after the water temperature was lower than 15° C. These drills were placed in a spawning tank in which the water temperature was raised to between 18° and 20° C. over a 72-hour period. Drills treated in this manner became active and fed on mussels within 12 to 24 hours after exposure to temperatures above 15° C. After 10 to 12 days at a water temperature of 18° to 20° C., these drills began to produce egg collars. This was true for drills moved from tanks where the water temperature was as low as 2° C. From this evidence, it appears that temperature is at least the primary stimulus controlling breeding and production of egg collars, since the gonads of both males and females are in condition to produce mature gametes throughout the late fall, winter, and early spring months.
B. Development of Eggs and Larvae Within the Egg Collar and Release of Larvae from the Collar

_P. duplicatus_ is the more abundant of the three species studied in the region of Cape Cod, Massachusetts. It does not occur in Canadian waters and therefore has not been discussed to any extent in reports originating from that area. Giglioli (1955) has given a brief description of the egg collar of _P. duplicatus_ on the basis of preserved specimens. Some details of the development of eggs and larvae of _P. heros_ and _P. triseriata_ have also been discussed by Giglioli (1949, 1952, 1955). Because of this, more emphasis was placed on the study of the developmental stages of _P. duplicatus_ than on the other two species. However, all three species were studied under similar environmental conditions, and comparative data were obtained.

Collars recovered from the spawning tank were placed in plastic containers. These were filled with running sea water or filtered sea water, changed daily, depending on whether or not the water had to be heated to maintain a temperature sufficiently high for eggs and larvae to develop. By cutting away a \( \frac{1}{2} \)-inch portion of the width of a single collar at frequent intervals, it was possible to follow the development of eggs to larvae and of larvae to the time of their release from the collar.

A majority of the collars were collected from the spawning tank between 8:00 and 8:30 A.M. A final observation of the spawning tank was made each evening during this portion of the study. Thus the bulk of the collars were produced sometime during the night and could have been formed no more than 12 hours prior to their recovery.
1. *Polinices duplicatus*

A number of collars were obtained at the time of completion by the female drill as they were pushed to the surface of the sand. All such collars contained only uncleaved eggs.

At that stage of development, each egg is enclosed in a capsule of clear, gelatinous material. This capsule measures between 200 and 250 micra in diameter and is covered by a thin pellicle. The eggs are opaque, cream colored, and generally spherical in shape. They measure between 90 and 105 micra in diameter. The capsules are irregularly spaced within the collar and are arranged in a staggered pattern. This results in a two-layered appearance when the collar is viewed in cross section. Capsules are present right up to the apical and basal edges of the collar. In all capsules examined, none was found that contained more than 1 egg. Table I gives the calculated number of eggs for 10 separate collars of *P. duplicatus*.

Observations of the development of eggs and larvae were made by two techniques. First, small portions of a collar, maintained at 18° to 20° C., were removed and examined to determine the stage of development. Second, a number of eggs were removed from this collar and maintained in a fingerbowl of sea water kept at 18° to 20° C. By frequent observation of these eggs, the time of development for various stages was determined.

The first technique proved to be the most satisfactory, since veliger larvae could be obtained only when development was allowed to take place within the collar. Eggs removed from the collar developed for the next 12 to 24 hours. They did not survive longer than that even when fresh changes of sea water were made at frequent intervals. The rate of development of the various stages was slightly faster for the
eggs removed from the collar. It seems probable that this was due to the somewhat higher temperatures obtained when a microscope lamp was directed on the fingerbowl in order to observe development of the eggs.

Table 4. Number of Eggs Contained in Egg Collars of P. duplicatus

<table>
<thead>
<tr>
<th>Collar</th>
<th>Capsules per sq. cm.</th>
<th>Eggs per Capsule</th>
<th>Eggs per Collar *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>1</td>
<td>5700</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>1</td>
<td>5700</td>
</tr>
<tr>
<td>3</td>
<td>112</td>
<td>1</td>
<td>4500</td>
</tr>
<tr>
<td>4</td>
<td>92</td>
<td>1</td>
<td>6000</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>1</td>
<td>5500</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
<td>1</td>
<td>5200</td>
</tr>
<tr>
<td>7</td>
<td>89</td>
<td>1</td>
<td>5500</td>
</tr>
<tr>
<td>8</td>
<td>96</td>
<td>1</td>
<td>5700</td>
</tr>
<tr>
<td>9</td>
<td>109</td>
<td>1</td>
<td>6100</td>
</tr>
<tr>
<td>10</td>
<td>103</td>
<td>1</td>
<td>5500</td>
</tr>
<tr>
<td>Average</td>
<td>101</td>
<td>1</td>
<td>5600</td>
</tr>
</tbody>
</table>

* Number of eggs per collar calculated on basis of total length and width of collar and rounded off to the nearest 100.

Table 5 gives the average time required for the development of the various stages as determined by a number of observations. The times listed are based on eggs and larvae allowed to develop in the collar with the exception of the formation and withdrawal of the polar lobes which had to be observed on eggs kept under continuous observation. Because the time of fertilization could not be determined, the time of development is taken from the formation of the first polar lobe.

Polar bodies were observed on some eggs prior to and just after the first cleavage. However, they were not seen routinely nor on a sufficient number of eggs to make it possible to determine the time of their appearance. All developing eggs and larvae were at about the same stage of development in the sections of collars that were removed. This.
suggested a relatively synchronous rate of development for eggs present in any one collar.

Table 5. Time of Development of Egg and Larval Stages of *P. duplicatus* in Egg Collars Maintained at a Water Temperature of 18° to 20° C.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time of Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of 1st polar lobe</td>
<td>Zero time</td>
</tr>
<tr>
<td>Spherical egg; 1st polar lobe withdrawn</td>
<td>1 hr. 15 min. *</td>
</tr>
<tr>
<td>First cleavage - 2 cells</td>
<td>3 hrs. 15 min.</td>
</tr>
<tr>
<td>Second polar lobe formed</td>
<td>3 hrs. 10 min.</td>
</tr>
<tr>
<td>Second polar lobe withdrawn</td>
<td>½ hrs. 5 min.</td>
</tr>
<tr>
<td>Second cleavage - 4 cells</td>
<td>6 hrs. 45 min.</td>
</tr>
<tr>
<td>Third cleavage - 8 cells</td>
<td>9 hrs. 20 min.</td>
</tr>
<tr>
<td>Fourth cleavage - 16 cells</td>
<td>12 hrs. 35 min.</td>
</tr>
<tr>
<td>Ciliated, motile trochophore</td>
<td>26 hrs.</td>
</tr>
<tr>
<td>Shelled veliger</td>
<td>2 to 3 days</td>
</tr>
<tr>
<td>Veliger released from collar</td>
<td>10 to 12 days</td>
</tr>
</tbody>
</table>

*Minutes are rounded off to the nearest 5-minute period.*

From about 2½ hours to 2 days, the larvae were ciliated trochophores, turning slowly within the capsule. This was followed by the veliger larvae with a transparent shell. The veligers remained 7 to 10 days in the collar, showing only slight measureable growth (a total increase of about 15 micra in shell length).

2. *Polinices heros*

Observations of the development of this species were made on eggs and larvae from a number of collars which were produced in the laboratory. Since most of the data on development were similar to that for *P. duplicatus*, only those points of distinct difference will be mentioned.

The eggs of *P. heros* measure between 95 and 110 micra in diameter and are generally spherical in shape. They are opaque and light-yellow to cream in color. The eggs are contained within a capsule of gelatinous
material enclosed in a thin pellicle. The capsule is spherical in shape and measures between 725 and 800 micra in diameter. In sharp contrast with _P. duplicatus_ and _P. triseriata_, each capsule contains a large number of eggs. Counts of the number of eggs per capsule were made on portions of 20 collars of _P. heros_. Table 6 shows the number of eggs per capsule, which may vary from 21 to 48, as well as the calculated number of eggs contained in collars of this species.

In view of the large number of eggs that occur in each capsule, it seemed pertinent to determine whether all of these eggs developed to veliger larvae or if some of the eggs present were nurse eggs. The presence and function of nurse eggs have been described for the egg cases of other prosobranchs such as _Buccinum undatum_ Linne (Portmann 1925, 1926). For species in the family Naticidae, however, nurse eggs are reported to occur only in the capsules of egg collars of _Natica catena_ (da Costa), an eastern Atlantic species found from the North Sea to the Mediterranean (Giglioli 1955). Ankel (1930) has described the nurse-egg feeding for this species. Only a few of the eggs develop to veliger larvae. The remainder of the eggs cease development after undergoing atypical cleavage and are fed upon by the few larvae that do develop to veligers.

For those invertebrates known to have nurse eggs, the number of eggs per capsule varies somewhat with different environments (Thorson 1946). Since, in all cases known, the nurse eggs are consumed prior to the hatching of a pelagic larval stage, Thorson further considers the presence of nurse eggs to be of considerable ecological significance. He suggests that the number of eggs present will be directly related to the length of time that larvae will remain within the egg capsules.
Table 6. The Number of Eggs Contained in Egg Collars of P. heros

<table>
<thead>
<tr>
<th>Collar</th>
<th>Capsules per sq. cm</th>
<th>Capsules per Capsule (average of 5 capsules)</th>
<th>Capsules per Collar</th>
<th>Eggs per Collar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91</td>
<td>4.7</td>
<td>3100</td>
<td>159,800</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>4.3</td>
<td>4900</td>
<td>161,700</td>
</tr>
<tr>
<td>3</td>
<td>89</td>
<td>21</td>
<td>6900</td>
<td>164,900</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>22</td>
<td>8700</td>
<td>191,500</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>4.2</td>
<td>7400</td>
<td>217,800</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>36</td>
<td>6700</td>
<td>212,800</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>28</td>
<td>7600</td>
<td>113,100</td>
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<tr>
<td>8</td>
<td>92</td>
<td>39</td>
<td>2900</td>
<td>111,100</td>
</tr>
<tr>
<td>9</td>
<td>87</td>
<td>37</td>
<td>6700</td>
<td>217,900</td>
</tr>
<tr>
<td>10</td>
<td>91</td>
<td>33</td>
<td>4800</td>
<td>158,100</td>
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<tr>
<td>11</td>
<td>103</td>
<td>27</td>
<td>3500</td>
<td>94,500</td>
</tr>
<tr>
<td>12</td>
<td>97</td>
<td>29</td>
<td>8200</td>
<td>237,800</td>
</tr>
<tr>
<td>13</td>
<td>87</td>
<td>4.3</td>
<td>5600</td>
<td>210,800</td>
</tr>
<tr>
<td>14</td>
<td>85</td>
<td>30</td>
<td>7900</td>
<td>237,000</td>
</tr>
<tr>
<td>15</td>
<td>96</td>
<td>1.8</td>
<td>8100</td>
<td>388,800</td>
</tr>
<tr>
<td>16</td>
<td>83</td>
<td>2.4</td>
<td>5700</td>
<td>136,800</td>
</tr>
<tr>
<td>17</td>
<td>97</td>
<td>21</td>
<td>7200</td>
<td>151,200</td>
</tr>
<tr>
<td>18</td>
<td>92</td>
<td>1.5</td>
<td>5800</td>
<td>261,000</td>
</tr>
<tr>
<td>19</td>
<td>102</td>
<td>25</td>
<td>8000</td>
<td>220,000</td>
</tr>
<tr>
<td>20</td>
<td>93</td>
<td>4.1</td>
<td>3900</td>
<td>159,900</td>
</tr>
</tbody>
</table>

Average 94 34 6200 200,600

* Number of capsules and eggs per collar calculated from total length and width of collar and rounded off to nearest 100.

In order to determine whether or not nurse eggs were present in egg capsules of P. heros, a small portion of each of five recently produced collars was isolated in a plastic container with filtered sea water. The containers were placed in a water bath to maintain the temperature at 18° to 20° C. and the sea water was changed each day. Each piece of collar contained between 30 and 50 capsules. Capsules observed at the start of the experiment contained only uncleaved eggs.
A few capsules were removed daily from each of the five pieces of collar and examined with a compound microscope. Development was followed until swimming veliger larvae were present. In no instance was there any apparent difference in the stage of development among the several eggs within a single capsule. While cleavage stages were present, all embryos were in a close range of stages. As development progressed to the trochophore stage, the earlier cleavage stages were no longer observed in the same capsules. When the larvae had developed to shelled veligers, the remaining capsules were examined and no earlier stages of development could be found. It was therefore concluded that nurse eggs do not occur within the egg capsules of *P. heros*, as evidenced by the lack of any cleaving eggs in capsules which contained the ciliated trochophore stage. Since this is a prefeeding stage, nurse eggs would have had to be present in such capsules if they were to be available as food for the veligers.

The development of eggs of this species to the shelled veliger stage was similar to that of *P. duplicatus* both as to stages and length of time. The initial shell of the veliger is colorless and transparent. Later, the shell takes on a yellowish-brown color, but retains its transparency.

Although the time of development of the various stages was about the same as that for *P. duplicatus*, the total average time of development to release of larvae from the collar was somewhat less for *P. heros* than for *P. duplicatus*. At a water temperature of 18° to 20° C., the time of development to release for *P. heros* was 8 to 10 days as compared with 10 to 12 days for *P. duplicatus*. Since these data were not obtained from simultaneous experiments, it seems probable that this is
not a significant difference between these two species.

3. *Polinices triseriata*

Three collars were produced by the drills maintained in laboratory tanks. Each collar was isolated in a plastic container with running sea water at a temperature of 18° to 20° C. Small portions of each collar were removed from time to time and broken apart to release the egg capsules in order to determine the stage of development of eggs or larvae.

Two of the collars collected in the vicinity of St. Andrews, New Brunswick were returned successfully to the laboratory at W.H.O.I. These were also kept in warmed, running sea water for observation of the development of larvae. This made it possible to obtain comparative data for field and laboratory produced collars. When first observed, the development of larvae in the St. Andrews collars had reached the veliger stage.

Collars recovered from the spawning tank within 12 hours after being formed all contained uncleaved eggs. They were yellow in color and measured between 325 and 350 micra in diameter. Each egg is surrounded by a firm, gelatinous material which becomes more fluid as development progresses. This is covered by a thin pellicle. The capsules measure between 850 to 1080 micra, considerably larger than the capsules of the other two species. Because of their large size, each capsule is distinctly visible in the collar and bulges somewhat from the surface of the collar, which presents a bumpy appearance. Capsules are not present to the very edge of the apical and basal rim of the collar as they are in collars of the other two species.
Giglioli (1949) has observed 2 eggs in a single capsule of *P. triseriata* on a few occasions and Stinson (1946) reports rare instances of 3 eggs being present in a single capsule. However, in all of the capsules examined by the author only one egg was present in each capsule. Table 7 shows the number of eggs contained in collars of *P. triseriata*.

Table 7. The Number of Eggs Contained in Egg Collars of *Polinices triseriata*

<table>
<thead>
<tr>
<th>Collar</th>
<th>Capsules per sq. cm.</th>
<th>Eggs per capsule</th>
<th>Eggs per collar *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>1</td>
<td>720</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>1</td>
<td>404</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>1</td>
<td>738</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>1</td>
<td>429</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>1</td>
<td>561</td>
</tr>
<tr>
<td>6 **</td>
<td>36</td>
<td>1</td>
<td>396</td>
</tr>
<tr>
<td>7 **</td>
<td>32</td>
<td>1</td>
<td>387</td>
</tr>
<tr>
<td>8 **</td>
<td>14</td>
<td>1</td>
<td>928</td>
</tr>
</tbody>
</table>

Average 38 1 570

* Number of eggs per collar calculated on the basis of total length and width of collar.

** Formalin preserved specimens collected near St. Andrews, New Brunswick.

The small number of collars available for study made it necessary to retain most of the capsules for the purpose of obtaining metamorphosed drills. Consequently, it was not desirable to sacrifice many of the developing eggs in order to determine the time of development of the cleavage stages. The observations that were made indicated that cleavage to the 4-cell and 8-cell stage was completed within 2 hours after the collars were collected. This gives something of the order of 36 hours from the time of formation of the
collar. A ciliated trochophore was present by 72 hours. This is a motile stage, turning slowly within the capsule. Development from this point is relatively slow. Shelled veliger larvae were not observed in any of the collars before 11 to 15 days. These larvae measured between 450 and 500 micra in shell height. They were motile and rotated within the capsule by action of the ciliated velum.

Veliger larvae removed from the capsule at this time were able to swim actively in the sea water. They survived only 12 to 24 hours, even though they were provided with moderate amounts of algae for food.

The veligers remained within the capsule for a period of 4 to 5 weeks, increasing in size to between 700 and 800 micra in shell height. The larval shell, at first colorless and transparent, slowly developed a rose color which later changed to a dark reddish-brown. As the larvae grew, the typical dextral asymmetry of the adult shell was assumed. This could first be observed at a size of 600 to 700 micra, when 1/2 to 2/3rds of a whorl was present.

Metamorphosis was not observed. It occurred after 7 to 8 weeks and at a size of 800 to 900 micra. Metamorphosed drills remained within the collar for another 1 to 2 weeks until they were released by the collar disintegrating. Such drills measured 0.9 to 1.0 mm. in shell height and possessed a well developed foot. When placed on a sand substratum, they burrowed beneath the surface and crawled actively about the container.

Table 8 shows the time of development for the various stages of

P. triseriata observed in the 5 collars studied.
Table 8. Time of Development (Days) of Egg and Larval Stages of *P. triseriata* at 18° to 20° C.

<table>
<thead>
<tr>
<th>Source</th>
<th>Cleavage Stages</th>
<th>Ciliated Embryo</th>
<th>Colorless Veliger</th>
<th>Pigmented Veliger</th>
<th>Metamorphosed Drill</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Andrews</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>12</td>
<td>61</td>
</tr>
<tr>
<td>St. Andrews</td>
<td>--</td>
<td>--</td>
<td>17</td>
<td>40</td>
<td>67</td>
</tr>
<tr>
<td>W.H.O.I.</td>
<td>1 to 2</td>
<td>6</td>
<td>14</td>
<td>31</td>
<td>57</td>
</tr>
<tr>
<td>W.H.O.I.</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>W.H.O.I.</td>
<td>1</td>
<td>5</td>
<td>15</td>
<td>36</td>
<td>59</td>
</tr>
</tbody>
</table>

4. Release of Veliger Larvae from Egg Collars

The release of veliger larvae from egg collars of *P. duplicatus* and *P. heros* was observed. Although no differences in the process were noted for these two species, the data on larval release reported here is for *P. duplicatus*.

The veligers remained within the collar until they were released by the collar disintegrating. Collars maintained at 18° to 20° C. started to disintegrate after a period of 10 to 12 days by a softening of the gelatinous matrix which binds the sand grains. The collar loses its characteristic shape as the basal rim begins to settle on the substratum, and finally the whole collar flattens out on the substratum. The sand grains in the matrix are thus loosened and the larvae swim out of the collar. When the release of larvae is complete, only the apical rim of the collar remains. Under laboratory conditions it took from 1 to 8 hours for a collar to disintegrate completely. In the field, where tidal currents and wind induced waves are present, collars were observed to disintegrate in as short a period as 2 hours. However, when wind conditions are moderate, collars on the tidal flats did not completely disintegrate until they had been exposed to two periods of flood tide.

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This was determined by marking collars with small stakes just as they were starting to flatten along the basal rim and observing them at intervals over two tidal cycles. It seems probable that collars produced subtidally would require some intermediate length of time between 2 and 4 hours to completely disintegrate and release their larvae.

A number of experiments were conducted to determine whether the presence of living larvae within a collar influenced the length of time for the collar to disintegrate. Eggs and larvae were killed in the collar by dipping it for 30 seconds in a fresh-water bath heated to 125° F. The collars were then returned to sea water at 18° to 20° C., but they still required the usual 10 to 12 days to reach the point of disintegrating. Observations on eggs and larvae so treated showed that they had all been killed and had started to decompose. There was no difference in the total period of time for the collar to disintegrate regardless of whether the eggs and larvae were killed as soon as the collar was recovered or at some period during the normal development prior to the disintegration. Thus it was concluded that the presence of living larvae had no bearing on the disintegration of the collar. Since it was not possible to remove eggs or larvae from collars, it cannot be said whether their presence in the collar, even though dead, had an effect on the disintegration process. The decomposition products may play some role in influencing disintegration, but this would not be significant to the natural process.

These observations and experiments on the disintegration of egg collars indicate that it is a process independent of larval development. This is in disagreement with the opinion of Giglioli (1949, 1955) that
the larvae are responsible for the disintegration of the collar. While his observations indicated that this possibility existed, he offered no experimental data to support such a conclusion.

5. Effect of Low Temperature on Development

Since egg collars of *P. duplicatus* were produced in the laboratory at water temperatures down to 6° C., it was of interest to determine the fate of eggs produced and maintained at temperatures lower than those of the summer breeding period.

Experiments were first conducted to determine if eggs produced at temperatures lower than 18° C. were viable and would develop normally. Collars produced by drills living at water temperatures of 6° C., 7.5° C., 9.9° C., 11.7° C., and 14.8° C. were isolated in containers of sea water at the same temperature. The containers were then placed on racks on the water table at room temperature. Thus the temperature of the sea water in the containers increased slowly over a period of 8 hours after which the container was placed in a water bath set at 20° C.

Eggs in collars from all initial temperature levels developed normally and completed development to the release of swimming veliger larvae. No abnormal development was observed at any stage and there was no apparent mortality of eggs or larvae within the collars.

It therefore appeared that egg collars produced at all temperatures down to and including 6° C. contained eggs which would develop normally if maintained at a summer water temperature.

With this established, other experiments were conducted in which egg collars produced at low water temperatures were maintained at the same temperatures in order to determine if the eggs would develop.

Eggs in two collars produced and maintained at 15.2° C. developed
somewhat slower than eggs kept at 18° to 20° C. The shelled veliger stage was not observed before 6 days, which was twice the time of development at 18° to 20° C. Eight days later these larvae were released from the collar. This gave a total of 14 days for completion of development within the collar as compared with the 10 to 12 days established for 18° to 20° C. A single collar produced and maintained at 11.7° C. released veliger larvae after 17 days of development. This was the lowest water temperature at which larvae were released from the collar.

One collar produced at a water temperature of 9.9° C. and maintained at this temperature and below showed eggs in late cleavage stages after 14 days. In order to determine if eggs developing at this slow rate would complete their development to larvae, the collar was cut in half. One half was kept at the normal winter water temperature below 10° C. The other half was warmed slowly to 20° C. and kept at this temperature. The eggs in the warmed portion of the collar developed normally and released veliger larvae after 10 days. The eggs in the half of the collar kept below 10° C. continued to develop slowly and had reached the early veliger stage after 25 days. Continued observation for the next 30 days showed that development to the shelled veliger stage took place and that these larvae were active within the capsule. During this period of 55 days, the sea water temperature decreased from 9.9° C. to 2° C. The collar was firm and showed no sign of disintegration.

Since the larvae showed no change during the next 8 days, the sea water was shut off and the container placed on a rack at room temperature. The collar released larvae during the night and disintegrated.
completely during the next morning. The water temperature at that
time had reached 15° C. Thus, it was apparent that the low water
temperature was preventing the release of the veliger larvae.
Whether the larvae could have survived the next 2 to 3 months until
normal spring water temperatures reach this level was not determined,
but it seems probable that such larvae could not exist for this
length of time without algal food.

From this data, it appears that collars produced between 10° and
18° C. and maintained at temperatures in this range will have normal
development of eggs and larvae. It further appears that collars will
release larvae at water temperatures between 10° and 18° C. even
though the rate of development is considerably slowed.

At temperatures between 10° and 2° C., however, development of
eggs to larvae was very slow and no veliger larvae were released at
these temperatures over a period of 1 month after this stage had been
reached. This suggests that, even though development of larvae to the
veliger stage could take place in egg collars produced in the late
fall, it is unlikely that such larvae could exist within the collar
throughout the winter to be released when spring water temperatures
had risen above 10° C.
C. Development of Pelagic Larvae and Metamorphosis

As soon as swimming veliger larvae began to emerge from the egg collars in the hatching containers, a small amount of algal culture was added to the sea water. This was usually in the amount of 50 to 100 cc., depending somewhat on the density of cells in the algal culture. As a rule, sufficient algal culture was added to give the 1 gallon of sea water a slight tinge of the color of the algae. This was slightly in excess of the needs of the larvae released from a single collar as evidenced by the presence of algal cells in the water when sampled 24 hours later. The bulk of the larvae were released within a 15 to 20-hour period at 18° to 20° C. After all of the larvae were released, they were collected by pouring the culture through a number 100 stainless steel screen. Larvae were then washed from the screen into a container of freshly filtered sea water which had been warmed to the same temperature as the water in the hatching container. This was done in order to prevent any thermal shock. With each change of water, fresh algal culture was added. This procedure was repeated at 24-hour intervals throughout the rearing period. Attempts to extend the water change to a 48-hour interval were not successful. Some cultures survived for a week on this basis, but ultimately died out. Since aeration of cultures did not alter this mortality, it appeared that toxic metabolites might be responsible rather than oxygen deficiency. Whether such metabolites were produced by the algae, the larvae, the bacteria present or from other sources was not determined.

On release from the collar, larvae were observed to have no food visible in the digestive tract as ascertained by viewing the animal
through its transparent shell. Within 30 minutes after adding algae to the cultures, the digestive tracts of the larvae had taken on the pigmentation of the algal species being used. Microscopic examination of such larvae showed the digestive tracts contained large numbers of algal cells.

If no food was added, all larvae died off within 24 to 48 hours. This suggests that an abundance of food must be available if larvae are to survive the first few days after release, and that the water passing through the laboratory sea water system did not contain sufficient algal food to meet the needs of the larvae. This is supported by the fact that recently metamorphosed quahogs, M. mercenaria, kept in running sea water at W.J.O.I. do not show any growth over long periods of time (Turner, personal communication).

Within 48 hours, growth of the larvae could be observed as new shell deposited along the lip of the aperture. Shell growth was not continuous but with a number of distinct increments in a manner similar to the growth lines on pelecypod mollusks. Individual increments were too small to measure. However, measurements made on larvae from a number of different cultures indicated an increase of approximately 3 to 5 micra of shell over a 4-day growth period.

Newly emerged larvae lack the spire on the shell that is typical of the adults. The spire could be distinguished only after 7 to 10 days of growth had occurred, at which time the larval shell assumed the dextral pattern of the adult.

During the summer and fall of 1957, the algal species Phaeodactylum tricornutum was used as the food for the larvae of P. duplicatus and P. This alga was formerly considered to be the diatom, Nitzschia closterium forma minutissima, but has recently been removed from this group and given an undetermined systematic position within the Chrysophyta (Hendey 1954; Lewin, J.C. 1958).
This alga had been used previously to rear larvae through to metamorphosis for the quahog, *M. mercenaria*, (Turner and George 1955) and the mud snail, *Nassarius obsoleta* Say (Scheltema 1956).

Algal food was added to give a cell count of 200,000/ml. of larval culture. Veligers of *P. duplicatus* and *P. heros* did feed on this alga, as evidenced by the presence of cells within their digestive tracts.

No difficulty was experienced in rearing larvae through the first 15 days. However, only a small amount of growth took place and no larvae metamorphosed. Since mortalities to the larvae were not sudden, it appears that *P. tricornutum* was not toxic. Two possibilities that could account for this gradual mortality are that this alga might be deficient in some essential growth substance or it might be indigestible and that it might be too large for easy ingestion by the veligers.

In order to circumvent this problem, three other smaller species of algae were reared in pure culture for use as larval foods. They are listed in Table 9, along with their taxonomic position and cell dimensions.

| Table 9. Species, Class and Size of the Algae Used in the Various Feeding Experiments |
|-----------------------------------------------|------------------|-----------------|
| Species                                      | Class            | Size            |
| *Phaeodactylum tricornutum*                  | Bacillariophyceae| 3 x 30 micra    |
| *Dunaliella euchlora*                        | Chlorophyceae    | 6 x 12 micra    |
| *Nannochloris atomus*                        | Chlorophyceae    | 3 x 5 micra     |
| *Isochrysis galbana*                         | Chrysophyceae    | 3 x 5 micra     |

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Because of the problems involved in culturing the last three species of algae in Table 9, they were used in successive experiments in rearing larvae rather than in simultaneous experiments. *N. atomus* and *D. eucn-
lorum* gave somewhat better results as larval food than *P. tricornutum*.
When added to the larval cultures at cell concentrations of 200,000/ml.,
the larvae added measurable shell growth in 2 days. Larval mortalities
were also less than for cultures fed on *P. tricornutum* during the first
7 to 10 days of rearing. After a 15 to 20-day period, however,
mortalities increased and the majority of the larvae were dead by 22 days.
A few larvae developed to the "swimming-crawling" or pediveliger stage
that just precedes metamorphosis. Again, no larvae metamorphosed.

Attempts were then made to rear larvae using *I. galbana* as the food
algae. Of the four species used, this was the most difficult to culture
in any quantity. However, by frequent subculturing, it was possible to
maintain sufficient amounts for feeding a number of 3-liter larval
cultures. The same concentration of cells was used for this alga as for
the other species.

*I. galbana* was chosen since it had been shown to be a "good" food
species for the larvae of the oyster, *C. virginica*, and quahog, *M.
mercenaria* (Davis and Guillard 1958). With larvae of both these
pelecypods, it produced relatively better growth than a number of other
algae tested.

*I. galbana* was the only alga fed in pure culture to veligers that
resulted in successful metamorphosis of *P. heros* larvae. It also gave
a faster rate of growth per unit of time and higher survival rates for
larvae of *P. duplicatus*. Even so, no larvae of this species meta-
morphosed.
Two larval cultures of *P. heros* which were fed *I. galbana* over
the first 15 days, followed by equal amounts of *I. galbana* and *P.
tricornutum*, showed the best results in terms of the number of larvae
that metamorphosed.

The problem of an adequate algal food for *Polinices* veligers was
not solved by these experiments. It was shown, however, that one
species, *I. galbana*, was superior to the three other species tested,
*P. tricornutum*, *N. atomus*, and *D. euchlora*, as a food for these larvae.
It also appeared that feeding of mixed algal species might offer the
best means of providing adequate food over a long pelagic larval period.
It is possible that *N. atomus* or *D. euchlora* might also have given
satisfactory results if they had been used with another species as a
mixed food.

Successful rearing to metamorphosis occurred only in the summer ....
and fall of 1958, during which time the cultures received treatment with
antibiotics (see below). It seems probable that this was a significant
factor in rearing larvae of *P. heros* through the approximate 30-day
pelagic period to metamorphosis.

In many cases where high mortalities occurred in larval cultures,
an abundance of bacteria was observed within the shells of dead larvae.
On two occasions, colonies of a pinkish-colored bacteria were found
growing on the sides of the culture container. Many swimming veliger
larvae in these cultures also had the same pinkish bacterial growth on
their shells and velum. Both cultures died out completely within 24
hours even though the larvae were removed by screening and placed in
another container of freshly filtered sea water. Attempts to grow this
bacterium on nutrient agar plates, using material removed from the sides
of the container as well as infected larvae, were unsuccessful.

Thus, while no evidence was obtained that bacteria were directly responsible for larval mortalities, it was at least suspect. This opinion is supported by the fact that bacteria have been isolated from cultures of dying quahog larvae, *M. mercenaria*, and demonstrated to be lethal to healthy larvae of this species when cultures were inoculated (Guillard 1959).

In an effort to depress bacterial growth, some cultures of larvae were reared with the addition of antibiotics. In these experiments, Penicillin G (Crystalline-Potassium) and Dihydrostreptomycin sulphate were added to the cultures at each 24-hour change of sea water. Guillard (personal communication) found high concentrations of either of these antibiotics toxic to oyster, *C. virginica*, and quahog, *M. mercenaria*, larvae. Based on this and experiments by Oppenheimer (1955) as to the effect of antibiotics on the development of pelagic eggs of marine teleosts, it appeared that a dose of 100,000 units (75 mg. per liter for penicillin 1/ and 100 mg. of streptomycin) would be adequate to prevent bacterial growth without any adverse effect on the larvae.

Initial experiments were conducted by dividing larvae obtained from a single egg collar into two containers, one for antibiotic treatment and the other as a control. Each culture was treated similarly in regard to changing of the water and the amount of algal food added. In all cases, the larvae in the cultures treated with antibiotics had a longer survival period than for their controls.

1/ Based on 1650 units per mg. for Penicillin G No. 526, Eli Lilly Company.
Larvae from treated cultures lived as much as 7 to 10 days longer than their untreated siblings. The treated larvae also did not have as much material fouling the surface of the shell as did the untreated larvae.

Since neither treated nor untreated cultures produced metamorphosed larvae, these experiments were conclusive that bacterial growth alone was not responsible for mortalities to late veliger stages. The improved survival of antibiotic treated cultures, however, was such that antibiotics were added to all larval cultures routinely from this point onward.

The use of antibiotics in larval culture work certainly deserves more attention but was considered beyond the scope of this study in view of the variety of antibiotics available. It is encouraging to note that the use of antibiotics for this purpose is being investigated at the Haskins Laboratory, New York City (Provasoli, et al., 1959).

A problem often encountered in larval cultures was the presence of contaminant organisms. Although glass wool and cotton filters were found to keep out small crustaceans, such as copepods and decapod larvae, quite satisfactorily, developing eggs of some of these animals occasionally passed the filter. Initially, the filtered sea water was stored in a 20-gallon polyethylene container until used. On a few occasions, copepod nauplii and decapod zoeae developed in this filtered water and were therewith passed into the cultures of veligers. Since these organisms were retained with the veligers when the cultures were screened for water changes, they remained in the cultures throughout the rearing period.
On two occasions, copepod populations became so dense that the veliger larvae were discarded due to doubt as to what portion of the algal food they could obtain. In the experiments in which different algae were being compared for their food value to veliger larvae, it was necessary to assume that the copepods were using a sufficient portion of the available algal food to negate the experiment.

Crab zoeae are a lesser problem since they cannot reproduce in the culture as do the copepods. However, zoeae feed on the larvae and their presence in large numbers reduces the veliger population considerably over a 20 to 30-day period.

Ciliate protozoans were probably the most frequent contaminant of the larval cultures since they were not retained by the sea water filtering device. The high density of algal cells added to feed the larvae offered ideal food conditions for many forms, both motile and attached. Motile ciliates are small enough to pass through the screens when the cultures are changed, and thus only a few are transferred with each water change. However, under these apparent optimal food and temperature conditions, their reproductive rate is very high and the population rebuilds itself rapidly.

Stalked ciliates are perhaps a more serious problem. Although they are also competitors for algal food along with the motile forms, their attachment makes it difficult to reduce their numbers. It was often necessary to wash culture containers with a strong "Chlorox" solution to kill colonies of these animals. Even this was not totally successful in removing them from the culture since many were attached directly to the shells of the veliger larvae. In some instances, where larvae were reared in culture over 25 days, as many as 20 stalked
protozoa were found attached to the shell of a single veliger. Observations of such larvae, when removed and placed in Petri dishes, indicated they were so weighted with attached ciliates that they could not swim up from the bottom of the container. Some larvae had protozoa attached near the aperture of the shell in such numbers that their vela were prevented from expanding fully. Such larvae could not swim but merely rotated slowly while resting on the bottom of the dish.

Under such conditions, it is doubtful that larvae could feed adequately and many mortalities may have resulted from enforced starvation.

It is probable that heavy infestations of this type of protozoa are confined to culture conditions. Some success at controlling copepod contaminants in algal cultures have been effected through treatment with low dilutions of organic compounds (Loosanoff, Hanks and Gamaros 1957). Continued research along these lines should contribute methods for control of other groups of animals.

The various protozoan forms observed in the cultures appeared to be competitors of the veliger larvae for available food. Even when such protozoan populations were very dense, there was no evidence in the digestive tracts of the larvae that they were consumed as food organisms.

Certain heterotrich protozoa are known, however, to be predators on larvae of the quahog, *M. mercenaria*, and the American oyster, *C. virginica*, under similar culture conditions (Loosanoff 1959).

Five of the 36 larval cultures of *P. heros* produced metamorphosed drills. No direct observation of a metamorphosing individual was made although cultures of 20 days and older were
observed at frequent intervals. Large larvae were selected from such cultures, isolated in depression slides and observed every half hour. It would appear that the period of metamorphosis is quite short, for in two cases within 6 hours the veliger larvae had metamorphosed.

Recently metamorphosed *P. heros* measured 0.5 to 0.7 mm in shell height and resembled the adult drill in general shape. The shell is a relatively transparent brownish-yellow in color with fine striations running at right angles to the plane of growth.

Although larvae of *P. duplicatus* were reared successfully to the pedoveliger stage, no metamorphosed drills were obtained. Wilson (1937) and Scheltema (1956) have shown that metamorphosis of annelid and gastropod larvae can be induced or retarded depending on the suitability of the substratum presented. In view of such evidence, experiments were conducted along similar lines using pedoveligers of *P. duplicatus* from 28-day cultures. Sand was obtained from the Quissett Harbor station which supports a population of this species. It was felt that this substratum should have present any desirable properties which might induce metamorphosis of the larvae. Cultures without substratum served as controls. Over a 6-day period, during which sea water was siphoned off and replaced each day and 50 ml. each of *I. galbana* and *P. tricornutum* were added for food, none of the 25 larvae placed in each container metamorphosed and all were dead after 7 days. These experiments were repeated using first a fine-screened beach sand and second a mud-sand mixture. No metamorphosis of larvae occurred in either case.

As *P. heros* had been shown to be carnivorous on small bivalves immediately after metamorphosis, it seemed possible that the presence
of such prey might contribute toward inducing metamorphosis. In order to test this hypothesis, 3 culture containers were prepared: a control with no animals or substratum, a quantity of Gemma but no substratum, and Gemma plus Quissett Harbor sand. In each of the containers, pedoveliger stage larvae were added, sea water was changed daily, and algae were added. During an 8-day experimental period no metamorphosis occurred, and all of the larvae died.

From these experiments, it appears that no metamorphosis-inducing substance occurred in the three substrata offered and that the presence of prey animals did not induce metamorphosis. However, since 100 per cent larval mortality occurred in both experimental and control groups after about one week, these results are not conclusive.
D. Food and Feeding of Recently Metamorphosed Drills

Of considerable importance in this study was the question of how soon after metamorphosis young drills started to feed and what type of food they consumed. A knowledge of whether or not the change from the herbivorous diet of the larvae to the carnivorous diet of the adult occurred abruptly at metamorphosis would be significant to our understanding of the predation pressure which these gastropods exert on the pelecypod members of the community. The survival of recently metamorphosed drills and bivalves would appear to depend to a large extent on the time factor involved for the change-over in food habits by the drills.

The feeding habits of recently metamorphosed drills are also of significance to the commercial shellfisheries. Any predation on bivalves of a 1 to 2-mm. size range would be likely to take place unnoticed. Yet such predation could account for what otherwise might be considered a lack of a successful "set" of bivalves. Likewise, a dependence on and availability of small sized prey could result in the success or failure of metamorphosing drills to establish themselves in a particular locality.

In order to determine if drills were carnivorous as soon as they had metamorphosed, it was necessary to have available a dependable supply of small bivalves and other organisms which might serve as food. One method of supplying such food organisms would be to rear larval quahogs, M. mercenaria, to the setting stage. However, this is quite time consuming since these cultures would need the same care as is required for rearing the larvae of the drills. It would also be necessary to maintain successive cultures in order to be assured of
having available recently metamorphosed quahogs small enough for newly metamorphosed drills to feed on successfully.

Another approach would involve collecting recently "set" bivalves from their natural environment. Newly "set" soft-shell clams, M. arenaria, are available during the early summer months, but dense sets are sporadic in occurrence. If located, this species can be collected in the intertidal zone in large numbers attached to such algae as Enteromorpha and Ulva. Similarly, recently set mussels, M. edulis, can be obtained from wharf pilings or on artificial collectors placed in the water during the spring months. Since both of these species are available only during the spring and early summer, they are not satisfactory as a continuous food supply. In addition, they soon grow too large to be suitable as food organisms for the small drills.

Aside from bivalve mollusks, newly set barnacles appeared to be sufficiently small to be a potential source of food. These animals are quite easily collected by placing chicken-wire bags of empty bivalve shells in an intertidal area where barnacles are known to attach to rocks and pilings. Good "sets" of barnacles can usually be obtained in this manner if the shell bags are put out in early March and observed frequently between then and the latter part of April. After a "set" of young barnacles has been obtained, the shell bags are placed near the upper tide level where they will not be submerged in sea water for more than 1 to 2 hours on each tidal cycle. Since the barnacles cannot feed when exposed to the air, they will have a slow rate of growth under such conditions and will remain for a considerable time at a size small enough to be used as food by drills.
While considering various sources of small bivalves, it was recalled that the gem clam, *Gemma gemma* (Totten), one of the smallest of our New England pelecypods, is an ovoviviparous species. Eggs are retained within the female throughout development to metamorphosed animals. Since these newly metamorphosed individuals measure 0.5 to 1.0 mm. in length at the time they are discharged by the adult, it appeared that they might be a source of food for small drills. A single female *Gemma* may contain as many as 15 to 20 juveniles. These are easily obtained by placing the adult clam in a Petri dish with sea water and crushing the shell. If the body tissue is then teased apart with fine needles, the juvenile clams can be recovered without any damage. Most of the developing individuals in any one adult female are at approximately the same stage of development. Since *Gemma* breeds throughout the summer, it is often necessary to crush a dozen or so clams in order to find one or two that contain the newly metamorphosed individuals.

To observe feeding activities of newly metamorphosed drills, they were removed from the culture containers and placed in Petri dishes containing filtered sea water. Different types of food materials were then presented and the actions of the drills observed.

When newly metamorphosed *P. heros* were presented with plant material, such as concentrations of algal cells, small portions of macro-algae or rock surfaces bearing algal growth, they did not feed. Although the drills moved over the surface of such material, they did not remain in place as when feeding or show any of the usual responses of the adults to a food stimulus, such as extending the proboscis. Observation of the surface of the macro-alga showed no area where drills had rasped. It
thus appeared that this species was an obligate carnivore as soon as
metamorphosed.

Various animal foods were then presented to the drills including
newly "set" barnacles, excised tissue from mussels and clams, small
living mussels and juvenile Gemma.

Newly metamorphosed _P. heros_ did not feed or show any attempt to
feed on barnacles. This was somewhat surprising since newly meta-
morphosed oyster drills, _U. cinerea_, have been observed by the author
to feed voraciously on small barnacles.

Although mussels small enough for these feeding experiments were
not obtainable at all periods when metamorphosed drills were available,
it was possible to conduct some feeding trials using these bivalves.
Drills reacted to the presence of the mussels as soon as they made
direct contact with them. The small drills moved around the mussels
and appeared to examine them by touching the edges of the shell with
their foot. One drill attempted to wrap its foot around a mussel but
the foot was not large enough to enclose the mussel. After a period
of 11 minutes, this drill released the mussel and moved away. Although
other mussels were approached in a similar manner, none was bored into
or consumed. Since the mussels were about three times the size of the
drills, it seems probable that they were too large for the drills to
attack successfully.

Although adult _P. heros_ are reported to feed on the remains of
dead fish and crabs (Chin 1952; Turner, personal communication), as well
as living mollusks, newly metamorphosed drills did not feed on excised
clam tissue when it was presented. Drills placed directly on small
portions of clam tissue moved away without attempting to feed. When
pieces of tissue were placed at distances of a centimeter or so from the drills, they did not move directly to the tissue. They did not, however, avoid the tissue and passed over it frequently without making any attempt to feed.

When juvenile Gemma were used as the food organism, successful feeding followed. In a number of feeding experiments, a dozen or so of these small clams were pipetted into a dish containing 6 drills. The drills did not appear to be directly attracted to the small clams. Their movements were quite random until they reached a point a few millimeters away from an individual clam. From this distance they moved directly to the clam, first moving around the clam and touching it with the foot along the edges of the shell, then crawling over the clam. Clams became attached to the metapodium (posterior portion of the foot) and were then dragged along behind the drill. It appeared to adhere in this position due to a mucous material present on the foot. With a clam in this position, a drill would move about the dish for a period of 15 to 20 minutes. This procedure is similar to that of adult drills attacking bivalves which are on the surface of the sand. The noticeable difference is that adult drills enclose the clam in the metapodium by folding the ventral side of the foot completely around the clam (Figure 6). In a few instances, a young drill would attach 2 or 3 clams to its foot and carry them around the dish.

Each time a drill was observed moving about the dish with a clam attached to its foot, the other drills and clams were removed. After carrying the clam for a varied period of time, the drill then wrapped its foot around the clam. This is the typical action of adult drills when boring is started (Figure 7). After the drill and clam had
separated, the shell of the clam was examined with a microscope. In all cases, a small hole was present in one of the valves and the clam tissue had been completely rasped from the inside of the shell.

Since development to metamorphosis takes place entirely within the collar of *P. triseriata*, large numbers of recently metamorphosed drills of this species were available for feeding experiments. Various food substances were presented in a similar manner as for *P. heros*. Again, no plant material was fed upon or appeared to stimulate the drills in any way. This species also did not attempt to feed on small barnacles.

When presented small mussels, the small *P. triseriata* attached them to the metapodium and carried them about the dish. After a period of about 15 minutes, the drill enclosed the mussel completely in its foot while boring and feeding on the mussel tissues. Drills also attacked and consumed juvenile *Gemma* in a similar manner. Since *P. triseriata*, at the time of release from the collar, are about 1/3rd larger than newly metamorphosed *P. heros* and did successfully attack and feed on mussels, while the smaller *P. heros* were not able to, the indications are that the size factor was what prevented *P. heros* from feeding on this species of bivalve.

While conducting feeding experiments with *P. triseriata* and *Gemma*, a few recently metamorphosed mud snails, *N. obsoleta* were introduced into the Petri dish by accident. This gastropod is a common estuarine species and occurs in the same general habitat as *Gemma*, *M. arenaria* and the three *Polinices* species. Although the small *Nassarius* are capable of much faster locomotion than small *P. triseriata*, individuals of each species would occasionally meet in their movements about the
dish. Whenever this occurred, the drill would quickly attach itself to the shell of the Nassarius. The Nassarius would then move actively about the dish with the drill attached. This action often lasted as long as 2½ hours but no movement by the Nassarius dislodged the drill. Examination of the Nassarius shells indicated that during this period of being carried about, the drill was able to bore a hole through the shell and feed on the tissue.

Since larvae of P. duplicatus were not successfully reared to metamorphosis, it was not possible to determine their feeding habits at metamorphosis. However, a few small individuals of this species, 2 to 4 mm. in shell height, were collected from the flats at Barnstable Harbor. Using data on size at metamorphosis and growth for P. heros and P. triseriata, it appears reasonable to estimate the age of these drills as about 4 to 6 weeks after metamorphosis. Drills of this size fed on both adult Gemma and small mussels but did not feed on macro-algae. From these results, it seems probable that this species is also an obligate carnivore as soon as it has metamorphosed.

Data on feeding rates are available for adult oyster drills, U. cinerea (Carriker 1955; Hanks 1957) and the adults of the three species of Polinices studied herein (Giglioli 1949; Sawyer 1950; Hanks 1952, 1953). In addition, Carriker (1957) established feeding rates in laboratory experiments using newly released U. cinerea preying on newly "set"quahogs, M. mercenaria. The feeding rate, in terms of the number of clams consumed per drill per day, established by Carriker for small U. cinerea (8.9 for the average of 14 feeding experiments) was much higher than for the adults of this species (0.2 for the average of 6
feeding experiments) at similar water temperatures (Hanks 1957). If this difference in voracity is general for small individuals of carnivorous gastropod species, we must especially look to this level of predation for any maximum limiting effect upon bivalve species within a community.

In order to obtain data on the rate of feeding of recently metamorphosed P. heros and P. triseriata, a number of experiments were conducted to determine the length of time necessary to complete the boring and feeding process. Drills were removed from rearing containers and placed in fingerbowls with filtered sea water and a number of small Gemma, 0.5 to 1.0 mm. in length, were added. As soon as an individual drill attacked a clam, all other drills were removed. Feeding activity was observed with a binocular microscope. The feeding period was considered as the time between the drill wrapping its foot about the clam and the release of the empty valves. Water temperature for these experiments was in the range of 18° to 20° C. Since Gemma, in nature, live near or on the surface of the flats, most experiments were conducted without a substratum in order to provide clear observations. Results of these experiments for both species are given in Table 10.

Table 10. Feeding Time for Recently Metamorphosed P. heros and P. triseriata on Juvenile Gemma gemma

<table>
<thead>
<tr>
<th>Species</th>
<th>Shell Height (mm)</th>
<th>Time Elapsed During Feeding (to nearest 5 min.)</th>
<th>Feeding Rate (Clams per drill per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. heros</td>
<td>0.6</td>
<td>2 hrs. 45 min.</td>
<td>8.7</td>
</tr>
<tr>
<td>P. heros</td>
<td>0.6</td>
<td>3 hrs. 25 min.</td>
<td>7.2</td>
</tr>
<tr>
<td>P. heros</td>
<td>0.7</td>
<td>2 hrs. 20 min.</td>
<td>10.2</td>
</tr>
<tr>
<td>P. heros</td>
<td>0.7</td>
<td>2 hrs. 10 min.</td>
<td>11.1</td>
</tr>
<tr>
<td>P. heros</td>
<td>0.8</td>
<td>2 hrs. 40 min.</td>
<td>9.0</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>0.9</td>
<td>3 hrs. 50 min.</td>
<td>6.3</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>1.0</td>
<td>2 hrs. 35 min.</td>
<td>9.3</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>1.2</td>
<td>3 hrs. 20 min.</td>
<td>7.2</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>0.8</td>
<td>3 hrs. 45 min.</td>
<td>6.4</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>0.9</td>
<td>2 hrs. 15 min.</td>
<td>10.7</td>
</tr>
<tr>
<td>P. triseriata</td>
<td>1.0</td>
<td>2 hrs. 55 min.</td>
<td>8.2</td>
</tr>
</tbody>
</table>
These results show an average feeding rate (extrapolated from the time of consumption of 1 clam by 1 drill) for *P. heros* of 9.2 clams per drill per day and for *P. triiseriata* of 8.0 clams per drill per day.

In order to compare these observed feeding times with those of a more nearly natural situation, 3 experiments were conducted using a number of *P. triiseriata* and *Gemma* maintained in a container for a few days without being disturbed. Feeding rates established in these experiments are given in Table 11. The water temperature ranged between 20° and 22° C., and the water was changed daily during the experiment by siphoning to avoid disturbing feeding drills.

**Table 11. Feeding Rates of Newly Metamorphosed *P. triiseriata* on *Gemma gemma***

<table>
<thead>
<tr>
<th>Number of Clams</th>
<th>Number of Drills</th>
<th>Feeding Period in Days</th>
<th>Number of Clams Consumed</th>
<th>Feeding Rate (Clams per Drill per Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>5</td>
<td>2</td>
<td>21</td>
<td>2.1</td>
</tr>
<tr>
<td>35</td>
<td>5</td>
<td>4</td>
<td>32</td>
<td>1.6</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
<td>3</td>
<td>28</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The rate of feeding shown in Table 11 is probably more realistic than those extrapolated from a single feeding period in Table 10. Although the feeding rate for newly metamorphosed *P. triiseriata* is higher than for adults of this species as reported by Giglioli (1949), it does not approach the highest feeding rate for newly released oyster drills, *U. cinerea*, (19.0 clams per drill per day) reported by Carriker (1957). In his experiments he had present a much larger quantity of food species (200 to 300) than were used in the *Polinices* experiments reported here. This may account for some of the difference in feeding rate since the clam drills would have spent more time...
searching for the prey between feedings than would the oyster drills in Carriker's experiments.

Present evidence indicates that both recently metamorphosed P. heros and P. triseriata have considerably higher feeding rates than adults of the same species.
E. Growth and Sexual Maturity of Young Drills

In order to determine the rate of growth of young P. heros, an experimental group was placed in a wooden tank with a sand substratum. A constant flow of sea water was provided and small mussels were placed in the tank for food.

Six individuals, measuring 6 to 8 mm. in opercular length, were selected from a collection of small drills obtained at York Beach, Maine. These animals were kept in sea water at natural temperatures for a period of about 18 months. Since seasonal differences in the rate of feeding are reported for P. heros (Hanks 1952, 1953), it was assumed that the growth rate of this experimental group would be comparable to the rate of growth of P. heros in natural populations with an abundant food supply. In order to assure that the drills had sufficient food to allow for maximal growth, mussels were continually added to the tank to replace those consumed.

As this experiment was started in August, 1957 and terminated in February, 1959, it included only one summer period. P. heros is known to have a higher rate of feeding at warm water temperatures than at cold and to feed throughout the water temperature range of 2° to 23° C. (Hanks 1953). Thus only one high level feeding period occurred during the experiment. It is therefore possible that a greater overall growth might have been attained over a similar 18 months if the period chosen included two summers and only one winter. Regardless of this possibility, it should not alter the growth during any particular warm or cold period.

In order to determine the difference between growth during the summer and growth during the remainder of the year, three sets of measurements
of the drills were made after their initial size was taken at the start of the experiment. Table 12 shows the growth of the 6 drills during this experiment.

Table 12. Growth of Young P. heros Kept at Natural Sea Water Temperatures and with a Maximum Supply of Food *

<table>
<thead>
<tr>
<th>Initial Size</th>
<th>Size After 9 Months</th>
<th>Size After 13 Months</th>
<th>Size After 18 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 29, 1957</td>
<td>22.5° C.</td>
<td>June 2, 1958</td>
<td>15.4° C.</td>
</tr>
<tr>
<td>7.0</td>
<td>10.0</td>
<td>20.0</td>
<td>21.0</td>
</tr>
<tr>
<td>7.0</td>
<td>9.0</td>
<td>19.0</td>
<td>25.0</td>
</tr>
<tr>
<td>8.0</td>
<td>9.0</td>
<td>21.0</td>
<td>22.0</td>
</tr>
<tr>
<td>6.0</td>
<td>11.0</td>
<td>18.0</td>
<td>26.0</td>
</tr>
<tr>
<td>7.0</td>
<td>12.0</td>
<td>22.0</td>
<td>25.0</td>
</tr>
<tr>
<td>8.0</td>
<td>10.0</td>
<td>18.0</td>
<td>19.0 ***</td>
</tr>
<tr>
<td>Av. 7.1</td>
<td>10.2</td>
<td>19.7</td>
<td>23.0</td>
</tr>
</tbody>
</table>

* Measurements listed are length of the operculum to the nearest mm.

** Animal dead when recovered from the tank for measurement.

The data in Table 12 indicate that small P. heros do grow during the colder periods of the year. The average size of the 6 drills increased over the 9-month period from August, 1957 to June, 1958 from 7.1 mm. to 10.2 mm.; an increase of 3.1 mm. For the shorter cool period of five months from September, 1958 to February, 1959, the increase in average size was 3.3 mm. Yet for the 4-month period of warm temperature from June, 1958 to September, 1958, the increase in average size was 9.5 mm. This represents a threefold increase in average size over the increase for either of the two colder periods and for a shorter actual period of growth.
These data also indicate a rate of growth for young *P. heros*, under optimal food conditions, of approximately 2.4 mm. per month during the summer period and of 0.5 mm. per month during the remainder of the year.

*P. heros*, of average size 7.1 mm., had nearly the same increase in average size (3.1 mm.) for a 9-month period of growth as the same individuals at average size 19.7 mm. had (3.3 mm.) for a 5-month period of growth. It is probable that the slightly faster growth during the 5-month period (0.6 mm. per month) was due to the fact that the size of the mussels added for food was increased as the drills grew larger. This would tend to provide a somewhat greater amount of food from each mussel consumed.

An experiment was conducted to determine at what size *P. duplicatus* reach sexual maturity in terms of mating and producing egg collars. A collection of young drills of this species was obtained from the flats at the Barnstable Harbor station. Twenty individuals, 6 to 8 mm. in opercular length, were selected and placed in a wooden tank containing 1 inch of sand substratum. Since the normal period of reproduction is in the summer, a flow of sea water was provided at a temperature range of 18° to 20° C. This served to keep the rate of feeding at a high level in order that these drills could approach a maximal growth rate. Small mussels were placed in the tank for a food supply. The experiment was terminated after 86 days when the first egg collar was produced. At that time the drills were removed and measured. Table 13 shows the initial size of the drills and their size at the end of the experiment.

The egg collar recovered measured 50 mm. in basal diameter and 16 mm. in height. The average size of the 16 drills present at the termination
of the experiment was 11.7 mm. and the maximum size of an individual was 14 mm.

Table 13. Size of Small P. duplicatus Before and After 86 Days of Feeding at 18° to 20° C. *

<table>
<thead>
<tr>
<th>Initial Size</th>
<th>Number Present</th>
<th>Size After 86 Days</th>
<th>Number Present **</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 14, 1957</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0 mm.</td>
<td>6</td>
<td>14.0 mm.</td>
<td>2</td>
</tr>
<tr>
<td>7.0 mm.</td>
<td>11</td>
<td>13.0 mm.</td>
<td>2</td>
</tr>
<tr>
<td>6.0 mm.</td>
<td>3</td>
<td>12.0 mm.</td>
<td>6</td>
</tr>
<tr>
<td>11.0 mm.</td>
<td>3</td>
<td>10.0 mm.</td>
<td>1</td>
</tr>
<tr>
<td>9.0 mm.</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The measurements listed are the opercular length to the nearest mm.

** Four dead animals removed during the experiment.

As this collar was produced during the night, the actual deposition was not observed nor the specific female responsible ascertained. It seems probable, however, that the collar was produced by one of the larger individuals measuring 13 to 14 mm. No mating pairs were observed on the surface during the course of this experiment.

Two other small collars were produced after these drills were returned to the tank, after which this group of drills was sacrificed in order that the individuals could be sexed. Of the 16 drills present, 9 were females, 4 were males, and 3 could not be sexed due to the absence of external genitalia. All of the 9 females measured 12 mm. or larger. Of the 4 males, 2 measured 12 mm. and 2 measured 11 mm. The 3 unsexed individuals measured 9 mm., 9 mm., and 10 mm.

From these data it appears that a female P. duplicatus can mate and produce egg collars at a minimum size of 12 mm. while a male is capable
of successful mating at a minimum size of 11 mm.

Two of the small egg collars produced were allowed to develop in warm sea water. Both collars released swimming larvae after 12 days. These larvae were examined and found to be of the same size and appearance as the larvae from larger collars. Since the larvae fed and appeared normal, no attempt was made to culture them to metamorphosis.

The author has never observed such small egg collars at any of the areas where collections of P. duplicatus have been taken. Although collar measurements are seldom given, no mention of small sized collars is contained in the reports of Canadian workers.

It seems possible that the conditions of the experiment were such that maturing drills were in much closer proximity than they would be under field conditions. Also, large differences in size between the sexes may make it impossible for successful mating to take place. Thus while drills of 11 to 14 mm. are capable of reproducing, it is doubtful if this occurs to any extent in the field due to the infrequency of chance meeting of small drills of the opposite sex.

This experiment also provided data showing the rate of growth of P. duplicatus at warm water temperatures. The average size of the 20 individuals at the start of the experiment was 7.2 mm. At the end of the experiment, the average size of the 16 survivors was 11.7 mm., giving an increase in average size of 4.5 mm. and a rate of growth of approximately 1.5 mm. per month. This is somewhat less than the rate of growth per month for P. heros (2.4 mm.) at a similar water temperature range. It is probable that this difference in rate of growth is a reflection of the difference in adult size between these two species.

Within the New England area, the author has never collected P. duplicatus
larger than about 1.5 mm. opercular length while specimens of *P. heros* collected on Cape Cod often measure as much as 65 mm. opercular length. This is in general agreement with such handbooks as Miner (1950) and Abbott (1954) which list the maximum size for *P. duplicatus* as 2 1/2 inches (shell height) and for *P. heros* as 4 3/2 inches in shell height.

As large numbers of newly hatched *P. triseriata* were available on occasion, growth studies of this sized drill were conducted. A finger-bowl of 8 inches in diameter was filled to a depth of 1 1/2 inch with fine sand recovered from the surface of the flat at the Barnstable station. Filtered sea water was added to a depth of about 2 inches. An estimated 300 to 400 Gemma were then placed in the bowl. After they had burrowed into the sand, 100 *P. triseriata* were pipetted into the bowl. These were drills which had been released from a laboratory-produced egg collar 3 days prior to the experiment and had been kept in a one-gallon plastic container with aerated, filtered sea water without food organisms. The majority of the drills used measured 0.9 to 1.0 mm. in shell height, a few being 0.7 and 0.8 mm.

Most of the tiny drills righted themselves and burrowed into the sand within 5 minutes after being placed in the bowl. A few remained retracted in the shell for some time before extending the foot and burrowing. Within an hour, however, all of the drills were below the surface. The fingerbowl was then placed on a water table such that a flow of cool sea water passed around it, providing a water temperature of 18° to 20° C. in the bowl. The sea water was siphoned off daily and fresh, filtered water added. A small amount of algae, *Nannochloris atomus*, was added at each water change to provide a food supply for the Gemma. Daily observations were made with a binocular microscope.
After 24 hours, 5 gaping Gemma were recovered from the surface of the sand, each with a hole through one valve and all the soft parts missing.

After a week, during which time numerous Gemma shells were removed, the surface of the sand was turned over with the aid of a small spatula. By this means, 18 drills were uncovered, each of which had a Gemma wrapped in its foot.

A few drills crawled up the side of the bowl and out of the water. By marking the outside of the bowl with red crayon, it was determined that these drills did not return to the water, but, instead, withdrew their body into the shell and attached firmly to the wall of the bowl. Six drills, allowed to remain in this position for 48 hours, did not recover when removed and placed in running sea water. It appeared that they had died from dessication. Therefore, during the first week of the experiment, drills were brushed from the sides whenever they were observed out of the water. This procedure was unsatisfactory both in terms of drill mortality and the intermittent feeding activity. The water was vigorously aerated from this point onward using an air stone suspended just below the surface of the water. Under these conditions only an occasional drill was found out of the water.

At the end of 2 weeks, a sample of 25 drills was obtained by turning over the surface of the sand. All of these animals were recovered at a depth of 2 to 3 mm. below the surface of the sand. Although a few were located by probing below a small bump of sand visible on the surface, most did not leave such a characteristic mark.

These drills were examined with a binocular microscope to ascertain if new shell had been deposited. This could be easily determined since
new shell, laid down at the lip of the aperture, was cream to buff in color as compared to the rose to reddish-brown color of the shell of newly hatched drills. The amount of growth of shell was about 0.1 to 0.2 mm. for each of the 25 drills. These animals were returned to the bowl and the experiment continued.

After 43 days, all the drills present in the bowl were recovered by sifting the sand through a fine mesh screen. Only 91 animals were recovered; the other 9 were assumed lost either when the water was changed or by escape from the bowl. Measurements of the group recovered are given in Table 11.

| Table 11. Growth of Newly Hatched *P. triseriata* After 43 and 73 Days of Feeding at 18° to 20° C. |
| Days | Number of Drills | Size |
| Start | 100 | 0.6 to 1.1 mm. (majority 0.8 to 1.0 mm.) |
| 43 (91 recovered) | | |
| 14 | 2.0 to 2.5 mm. |
| 16 | 1.0 to 1.9 mm. |
| 15 | less than 1.0 mm. |
| 16 (dead) * | less than 1.0 mm. |
| 73 (86 recovered) | | |
| 17 | 3.0 to 3.5 mm. |
| 32 | 2.0 to 2.9 mm. |
| 12 | 1.0 to 1.9 mm. |
| 9 (dead) ** | 1.0 to 1.5 mm. |
| 13 (dead) ** | less than 1.0 mm. |

* 11 of the 16 cannibalized
** All 22 cannibalized

These data indicate that 15 of the living drills did not grow during the 43 days of the experiment while 60 had some measurable growth of new shell. Of the latter, however, only 11 showed any considerable amount of
growth of shell, i.e., on the order of 1.0 to 1.5 mm. over the original size.

Since no drills had been observed on the surface where the bored Gemma were found, it was surprising to find that 16 of the drills were dead. As noted in Table 11, 11 of these had been bored and consumed by other drills. No cause could be determined for the death of the other 5 drills.

The remaining 75 drills were returned to the bowl, a new supply of Gemma added, and the experiment continued for an additional 30-day period. Again, all drills present were recovered by sifting the sand. Measurements of this group of 86 animals are also given in Table 11. Twenty-two of these had been bored and consumed.

New shell growth was on the order of 1.0 to 1.5 mm. for the 17 drills in the largest size group. After the 73-day experimental period, most of the drills showed some growth of new shell. Only 3 animals did not indicate any growth had taken place. The maximal growth that could have been attained by an individual drill was approximately 3.0 mm.

It appears that growth up to 3.0 or 4.0 mm. is about the maximum that could be expected with an optimal food supply during the summer months. This is about the size of small P. duplicatus that were collected at the Barnstable Harbor station during August and early September. Thus, it seems a safe assumption that this species also has a similar rate of growth during the first few months after metamorphosis and that the animals in this size range represent the "set" of the same summer.

No information can be given for the growth of recently metamorphosed P. heros as a sufficient number were not reared for experiments and small individuals were not located at any of the field stations.
Giglioli (1949) also notes the lack of tiny P. heros in field collections in Canada and their whereabouts at this size is at present undetermined.

Although cannibalism by both P. heros and P. duplicatus is known to occur in nature as well as in laboratory tanks (Giglioli 1949; Hanks 1953), the mortality of small drills by this means was higher than might be expected with the abundance of pelecypod food present. Cannibalism accounted for approximately 12 per cent of the drill mortality during the first 43 days and 25 per cent over the next 30 days. Under laboratory conditions in tanks, P. duplicatus did not cannibalize unless all other food species had been absent for some period of weeks. The small number of bored shells of Polinices observed in the field at the various stations visited, suggest that cannibalism by adult drills occurs only in the scarcity of other mollusk species. Yet the food supply of Gemma present with the small drills appeared to be far in excess of their needs. When the drills were sifted out at 43 and 73 days, over half of the Gemma recovered were alive.

These data suggest that the food pressure exerted on small drills is greater than for adults. This appears to force them to attack whatever mollusk prey they contact rather than act in the selective pattern of the adults as reported for P. heros (Chin 1952) and for P. duplicatus (Hanks 1953). This possibility is further supported by the successful attacks by small P. triseriata on small mud snails, N. obsoleta, even though Gemma were present (Hanks 1957b and see SECTION IV, D.).
SECTION V.

DISCUSSION

The results of this study of the early life history of three species of Polinices (P. duplicatus, P. heros, and P. triseriata) are pertinent to some of the current problems encountered by marine ecologists.

A. The Loss of Larvae During the Free-swimming Pelagic Period

Marine invertebrates which have a pelagic larval stage in their life history usually produce a large number of eggs. Conversely, those lacking a pelagic stage of development produce a distinctly lesser number of eggs. Within a community, populations of species with either type of development frequently do not show significant fluctuations in numbers from year to year. Even for invertebrates which often do show large annual fluctuations in the size of the population, as for example clams, mussels and oysters, the highest density of individuals never approaches the potential indicated by the quantity of eggs produced. Davis and Chanley (1956) have shown that a single female oyster can produce as many as 48.8 million eggs in one spawning and that females will spawn a number of times during one reproductive season. In the waters of Long Island Sound, where a regular annual spawning by adult oysters is known to occur, heavy sets of young oysters are infrequent and the most successful sets never reach the possible maximum based on egg production (Loosanoff and Nomejko 1956). While it is probable that some mortality occurs at metamorphosis or immediately following, there appears little doubt that it is during
the pelagic existence that the greatest loss of larvae takes place. Such mortality is usually called larval "waste," although this is somewhat anthropomorphic.

No accurate method has yet been devised to measure planktonic larval mortality as it occurs. Thorson (1946) has suggested an indirect method for estimating this mortality which is applicable to data obtained in this study. His reasoning is as follows: Some closely related species of marine invertebrates may show contrasting modes of larval development; i.e., one species with pelagic development, another species with non-pelagic development. When these species live under similar environmental conditions, it seems a fairly safe assumption that after metamorphosis both species will have an equal opportunity for survival to adult life. If it is known that the adult populations of both species show only moderate fluctuations and the number of eggs produced by a single female of each species during one breeding season can be determined, it should be possible to estimate the loss of individuals in the plankton for the species with pelagic larvae. This loss would be the difference in the number of eggs produced by the two species in question and would include the mortality from all sources (disease, starvation, consumption by other animals, etc.).

Of the three species studied herein, P. triseriata has a non-pelagic larval development, the larvae metamorphosing within the egg collar. The other two species, P. duplicatus and P. heros, have a period of pelagic larval existence.

Studies by Turner (unpublished) indicate that populations of P. duplicatus at Barnstable Harbor, Massachusetts do not fluctuate
appreciably from year to year. Stirson (1946) and Giglioli (1949) have shown that this is also the case for populations of *P. heros* and *P. triseriata* in southern Canada. Since the latter two species coexist at one of the Canadian study areas (Belliveau Cove, Nova Scotia), they are exposed to the same environmental conditions. Although co-existence is not found to the same extent for *P. heros* and *P. duplicatus*, there are areas, notably from Boston Harbor south to Duxbury and Plymouth, Massachusetts, where these two species do occur together in moderate abundance. No mixed populations of *P. triseriata* and *P. duplicatus* are known to the author. Thus it would appear that these three species satisfy Thorson's criteria.

As indicated in Tables 4, 6 and 7, the average number of eggs present in the egg collar of these species is as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Average Number of Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polinices heros</em></td>
<td>203,600</td>
</tr>
<tr>
<td><em>Polinices duplicatus</em></td>
<td>5,600</td>
</tr>
<tr>
<td><em>Polinices triseriata</em></td>
<td>570</td>
</tr>
</tbody>
</table>

Although the exact number of egg collars that are produced by individual females of these species over one summer’s spawning period is not known, the information that is available indicates that individual females of each species produce approximately the same number of egg collars within a season. Data for *P. duplicatus* (Hanks 1953) and Table 3 above, suggest that something on the order of 2 to 4 egg collars are produced by one female during the summer reproductive period. Although similar data were not obtained for *P. heros* and *P. triseriata*, collar recovery from spawning tanks indicated the same order of magnitude of egg collar production by these species. In addition, the reports of Wheatley (1947), Thurber (1949) and Giglioli (1949) show that collections
of adult *P. heros* and *P. triseriata* were nearly equal for both species at Belliveau Cove, Nova Scotia. Similar collections of egg collars of *P. heros* and *P. triseriata*, obtained at this same station by Larocque (1948), further indicate that egg collars of both species occur in about equal numbers. This supports the contention that no disproportion exists in the number of egg collars produced by females of either species.

The various data mentioned above strongly indicate that the total number of egg collars produced by females of the three species studied is approximately the same and that it is possible to consider the differences in biotic potential for these species to be represented by the difference in the number of eggs deposited in the egg collars of each species. Thus the average number of eggs present in an egg collar of *P. heros* (200,600) in contrast to the average number of eggs present in collars of *P. triseriata* (570) would indicate that a loss of approximately 200,000 larvae of *P. heros* occurs during the period of pelagic existence. A similar comparison between *P. duplicatus* (5,600 eggs per collar) and *P. triseriata* (570 eggs per collar) would indicate a loss of approximately 5,000 larvae of *P. duplicatus* during their pelagic life. This represents a loss of larvae of approximately 99 per cent for *P. heros* and 91 per cent for *P. duplicatus*.

All three *Polinices* species will presumably face similar factors of natural mortality from metamorphosis through adult life. However, *P. triseriata*, lacking a pelagic stage, should maintain a relatively constant population level since small drills are released from the egg collar near the same site at which they were produced. Populations of *P. heros* and *P. duplicatus* certainly encounter a much more precarious
existence. A small increase in natural mortality to the pelagic larvae, 1 per cent for *P. heros* and 9 per cent for *P. duplicatus*, would result in the loss of a complete year-class of individuals. In view of this possibility, it is somewhat surprising that population densities of these two species are fairly constant. This may be indicative of relatively uniform ecological conditions to which the pelagic larvae are exposed from year to year or a balance of factors; i.e., a decrease in predation by zooplankton is associated with a decrease in phytoplankton food available, producing the same end result.

B. The Food Problem of Pelagic Larvae

A critical requirement for the survival of herbivorous, pelagic larvae is an adequate food supply of phytoplankton. Such a food supply is usually considered from a quantitative viewpoint since studies of the basic productivity of a particular marine environment include all species of phytoplankton present. Thus, the presence of large numbers of single-cell algae in a relatively restricted area of water (commonly known as "a bloom") is frequently taken as evidence of optimal food conditions for herbivorous larvae.

However, Thorson (1960) has pointed out the importance of the size of the various species of algae in regard to their being usable as food by planktonic larvae. Considering prosobranch larvae, he has concluded that the maximum diameter of an alga that can actually be ingested is within the range of 5 to 45 micra. This is based on measurements of the larval esophagus and the anterior portion of the digestive tract of a large number of species. Ciliary currents alone seem to be responsible for the movement of ingested algae through these portions
of the larval digestive system. Thorson suggests that the action of the cilia is impeded or stopped if particles of a larger diameter than these are consumed in any quantity.

Recent feeding studies with clam and oyster larvae (Davis and Guillard 1958), using a number of pure algal cultures as foods, showed a considerable difference in the rate of larval growth depending on which species of alga was fed to the larvae. Their experiments also suggest that much of this difference in food value may be attributed to differences in the toxicity of metabolites produced by the algae. Although their experiments did not conclusively demonstrate it, these authors consider that such toxic metabolites may be retained in the algae, rather than being released into the water, and thus affect the larvae only after the algae are ingested.

The toxic effect to fish of high concentrations of metabolites produced by the dinoflagellate, Gymnodinium breve, is known from field observations (Gunter, et al., 1945) and has been demonstrated in the laboratory (Ray and Wilson 1957). There is no direct evidence for similar mortalities to populations of zooplankton in the sea, but Wilson (1958) and Loosanoff (1958) have reported studies which certainly are suggestive of such a mechanism.

The feeding studies with larvae of P. heros and P. duplicatus did show a difference in food value for the four species of alga that were used. Larvae fed on Phaeodactylum tricornutum exclusively did not survive to metamorphosis and showed only slight growth. Since P. tricornutum has been used successfully at W.H.O.I. to rear other prosobranch larvae (Scheltema 1956) as well as pelecypod larvae...
(Turner and George 1955), it did not appear that a toxic metabolite was involved. The difference in size of *P. tricornutum*, 3 x 30 micra, as compared with *Isochrysis galbana*, 3 x 5 micra, (the only single algal species which resulted in the successful rearing of *P. heros* larvae to metamorphosis) favored the probability that *P. heros* larvae could not ingest sufficient numbers of *P. tricornutum* to provide an adequate amount of food. The fact that *Nannochloris atomus*, also measuring 3 x 5 micra, proved to be the second best alga in food value (larvae of *P. duplicatus* developed to the late pedo veliger stage when fed on this species exclusively) further supports the probability that the size of the alga used was of prime importance.

Whether due to size, harmful metabolites or other factors, an algal species which shows a high value as a food for some planktonic larvae is by no means a universally "good" larval food as shown by the results of the larval feeding experiments. This is supported by successful rearing of other invertebrate larvae with *P. tricornutum*, as mentioned above. In addition, both *N. atomus* and *Dunaliella euglena* gave better results with *Polinices* larvae than when they were used as food for clam and oyster larvae (Davis and Guillard 1958).

The food problem for rearing planktonic larvae of marine invertebrates is of great importance to the advancement of marine ecology. We are in need of more laboratory studies to evaluate pure and bacteria-free algal cultures as food for larvae of other invertebrate groups. We also need complemental field studies to determine what species of algae are actually present in the larval environment and their abundance and seasonal fluctuations. This information is essential before we can expect to discover the role that algal food plays in the successful
existence of planktonic larval stages.

C. The Limiting Effect of Water Temperature on the Distribution of Polinices

Hutchins (1947) has given a lucid explanation of how water temperature affects the north-south distribution of marine intertidal invertebrates. He lists four critical temperature conditions which can act as distributional boundaries for such organisms. These are:

1. Minimum temperature for survival - Winter poleward boundary.
4. Maximum temperature for survival - Summer equatorward boundary.

The distribution limits for a single species, poleward or equatorward, will obviously depend on whether the critical survival temperature or repopulation temperature would be exceeded by any movement of the species out of its normal range.

Since data were obtained during this study on the effect of low sea water temperatures on reproduction as well as egg and larval development, it is of interest to apply this toward a possible explanation of the poleward distribution of Polinices. Only P. duplicatus, which has its northern boundary at Boston Harbor, Massachusetts, will be considered since most of the experimental work was done on this species.

Previous research (Hanks 1952, 1953) has shown that sea water temperatures down to and including 2° C. were not lethal to adult P. duplicatus over a period of exposure of about 1 month. However, drills of this species, maintained in experimental tanks, did not feed at sea water temperatures of 5° and 2° C. over a period of about 6 weeks.
Since \textit{P. heros} did feed at a water temperature of 2° C., it appeared that while this temperature was not lethal to \textit{P. duplicatus}, it would set some limitation in terms of feeding to the northern boundary of the species. One would expect this to occur at a point where the low temperature limitation on feeding was of sufficient duration to cause starvation mortalities. However, since \textit{P. duplicatus} did survive without food for a period about equal to the natural winter period of water temperature less than 5° C. in the area of southern New England, it does not appear that this factor alone would set the northern boundary for the species at Boston Harbor.

The next consideration is the effect of low temperatures on reproduction. Field observations indicated that egg collars were not produced by \textit{P. duplicatus} when sea water temperatures dropped below 17° C. This restricts the reproductive period in waters along the coast of southern Massachusetts to about 3 months. However, the coastal waters north of Boston have progressively shorter periods when temperatures are above 17° C. Thermograph records for 1958, kept at the U. S. Bureau of Commercial Fisheries Laboratory at Boothbay Harbor, Maine (Hanks, R. W., personal communication), show that the sea water temperature at their dock reaches a daily maxima of 17° to 18° C. and daily minima between 12° and 14° C. during July and August. On no occasion did a daily average water temperature exceed 16.2° C. in the summer of that year. Temperature records obtained on an intertidal flat, with the thermograph bulb placed a few inches into the substratum, also showed a few daily maxima of 18° to 22° C. (recorded at low tide when the flat was exposed to direct sunlight) and daily minima between 10° to 13° C. Thus at a point somewhat
north of Boston Harbor one would expect the period of 17° C. water
temperature to be insufficient to initiate the reproductive process
and for egg collars to be produced. This would be the northern
boundary for reproduction.

Finally, there is the consideration of the effect of low water
temperature on the development of eggs and larvae of _P. duplicatus_,
as well as the tolerance of the pelagic, veliger larvae to such
temperature levels.

In experimental studies, development of eggs through cleavage
stages to veliger larvae occurred at five different temperatures from
15° to 2° C., even though the rate of development was considerably
slowed at temperatures below 12° C. Veliger larvae were not released,
however, from egg collars maintained at water temperatures below 10° C.

Although larvae of _P. duplicatus_ did not metamorphose at any sea
water temperature to which they were exposed, no evidence was obtained
that low temperatures were lethal to the veliger stages. Veligers
that were released from egg collars maintained at temperatures
between 15° and 11.7° C. swam actively and fed on the added phyto-
plankton. However, these larvae had a slower rate of development at
these temperature levels as shown by the small amount of new shell
added in comparison with larvae reared at 18° to 20° C.

From the data available, it appears that the period of minimum
summer temperature necessary for reproduction exerts the most
influence on this species in setting the northern boundary limit.
Adult _P. duplicatus_ can live throughout the annual range of sea water
temperature occurring in the New England area. Eggs will develop to
veliger larvae and these larvae can live and feed at water temperatures
considerably less than those at which they naturally occur in the field.

Under natural field conditions, the fact that *P. duplicatus* does not produce egg collars at water temperatures below 17° C. would appear to limit any permanent northern extension of this species to areas where this temperature does not prevail over a number of weeks during the summer. However, this temperature limitation does not explain the rather sharp northern boundary at Boston Harbor. Other contributing environmental factors need to be determined before such geographical boundaries are fully understood.

As Dr. Alfred C. Redfield, W.H.O.I. and Harvard University, has reminded the author on numerous occasions, "All of the answers are available. It remains for the marine biologist to ask nature the right questions."
SECTION VI.

SUMMARY OF PRINCIPAL RESULTS

1. The normal breeding season of Polinices duplicatus (Say) is from June through August when the sea water temperature is above 17 ° C., although egg collars have been observed as early as April 11th in certain shallow, protected waters.

2. Although mating by P. duplicatus is generally a nocturnal and subsurface activity, diurnal and surface matings were observed in the laboratory.

3. Egg collars of P. duplicatus are formed in their complete width and given their characteristic shape as they are produced; the eggs are deposited in the collar in a continuous, gelatinous cord. Although egg collars of P. duplicatus are generally distinctive for this species, collars of aberrant shape do occur thereby invalidating identification from external characteristics alone.

4. Under laboratory conditions, P. duplicatus can produce egg collars from the middle of April to the middle of December over the range of sea water temperature from 22° C. to 6° C.

5. Gonads of mature P. duplicatus contain ova or spermatozoa throughout the annual sea water temperature cycle, even though breeding and egg collar production in the field is restricted to the warmer months of the year.
6. Populations of 25 to 30 *P. duplicatus*, maintained at a sea water temperature of 18° to 20° C. and provided with an adequate supply of food, will breed and produce egg collars throughout the year. Similar groups of this species, taken from sea water temperatures as low as 2° C. and brought slowly to 18° to 20° C. water, will commence feeding after 1 day and produce egg collars after 10 to 12 days.

7. Egg collars of *P. duplicatus* have gelatinous capsules measuring 200 to 250 micra in diameter, each of which contains 1 egg; the average number of eggs per collar is 5600. Collars of *Polinices heros* (Say) have capsules measuring 725 to 800 micra in diameter and can contain from 21 to 48 eggs per capsule; the average number of eggs per collar is 200,600. Collars of *Polinices triseriata* (Say) have capsules measuring 850 to 1080 micra in diameter which contain 1 egg per capsule (rarely 2 or 3); the average number of eggs per collar is 570.

8. As measured from the formation of the first polar lobe, the time of development of eggs and larvae of *P. duplicatus* at a water temperature of 18° to 20° C. is about 12½ hours for cleavage through the 16-cell stage, about 26 hours for the appearance of a ciliated trophophore, 2 to 3 days for the shelled veliger stage, and 10 to 12 days for the release of these larvae from the collar.

9. Nurse eggs are not present in capsules of the egg collar of *P. heros*.

10. Both *P. duplicatus* and *P. heros* have a pelagic, herbivorous larval stage following release from the egg collar. Development of eggs and larvae to metamorphosis is completed within the egg collar of *P.*.
triseriata; total time from production of the collar to release of metamorphosed drills from the collar is about 56 days at a sea water temperature of 18° to 20° C.

11. Experimental data indicate that egg collars of P. duplicatus disintegrate as a result of exposure to sea water for certain periods of time depending on the water temperature. No evidence was obtained that developing larvae influenced the breakdown of the collar.

12. Egg collars produced in the laboratory at sea water temperatures of 14.8°, 11.7°, 9.9°, 7.5°, and 6.0° C. all showed normal development of eggs and larvae when brought slowly to a water temperature of 18° to 20° C.

13. The lowest sea water temperature at which an egg collar was maintained throughout the developmental period until veliger larvae were released was 11.7° C.

14. Although egg collars of P. duplicatus maintained at sea water temperatures below 10° C. showed development of eggs to veliger larvae, release of these larvae did not take place within a period of one month. However, these larvae were released from the collar within 12 hours when the collar was brought slowly to a water temperature of 15° C.

15. Pelagic larvae of both P. duplicatus and P. heros feed on phytoplankton as soon as they are released from the egg collar; in the absence of sufficient algal food, the larvae die within 24 to 48 hours.

16. Larvae of both P. duplicatus and P. heros showed observable deposition of new shell within two days after release from the egg collar;
shell was added at a rate of 3 to 5 micra for a 4-day period of growth at 18° to 20° C.

17. Of the four species of algae used in larval feeding studies, Isochrysis galbana proved to be the best food in terms of larval growth. A mixed algal food, consisting of equal amounts of Phaeodactylum tricornutum and I. galbana, produced the greatest number of metamorphosed larvae of P. heros when fed to veligers which had previously been fed for 15 days on only I. galbana.

18. The addition of antibiotics, Penicillin G. (Crystalline Potassium) and Dihydrostreptomycin sulphate, to larval cultures resulted in a longer survival of veliger larvae of P. duplicatus and P. heros as compared with untreated control cultures.

19. The length of pelagic larval existence of P. heros, from the time of emergence from the egg collar to metamorphosis, is about 30 days, when maintained in laboratory cultures at a sea water temperature of 18° to 20° C. The recently metamorphosed P. heros measure 0.5 to 0.7 mm. in shell length.

20. The pelagic larval period of P. duplicatus was not determined since no larvae metamorphosed in spite of experimental efforts to induce metamorphosis with different substrata and the presence of a food species, Gemma. Pedoveligers, which were similar to those of P. heros just prior to metamorphosis, were present after about 30 days at a sea water temperature of 18° to 20° C.
21. Both recently metamorphosed *P. heros* and *P. triseriata* are obligate carnivores and appear to use small pelecypods and gastropods as their main source of food. It was found that a young drill could consume a single clam, *Gemma gemma*, in from 2 to 3 hours at a sea water temperature of 18° to 20° C.

22. The average feeding rate for recently metamorphosed *P. triseriata* is 1.9 clams per drill per day, as determined experimentally at a sea water temperature of 18° to 20° C.

23. Experimental growth studies with juvenile *P. heros* (6.0 to 8.0 mm. opercular length) indicate a rate of growth of 2.4 mm. of new shell deposited per month during the summer when sea water temperatures range from about 15° C. to 22° C. and a rate of 0.5 mm. per month during the remainder of the year when water temperatures range from less than 15° C. down to 2° C., all under optimal food conditions.

24. Experimental growth studies with juvenile *P. duplicatus* (6.0 to 8.0 mm. opercular length) indicate a rate of growth of 1.5 mm. of new shell deposited per month under optimal food conditions and at a sea water temperature of 18° to 20° C.

25. Experimental studies indicate that female *P. duplicatus* reach sexual maturity at 12 mm. opercular length and that males of this species reach sexual maturity at 11 mm. opercular length. However, field observations suggest that reproduction is not common among drills at this size level.
26. Experimental growth studies with newly released *P. triseriata* indicate a rate of growth of approximately 3.0 mm. of new shell deposited over about a 10-week period after release from the egg collar under optimal food conditions and at a sea water temperature of 18° to 20° C.
SECTION VII.

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APPENDIX I.

CULTURE METHODS FOR MARINE ALGAE

Although growing algal cultures, per se, was not a specific aim of the research here reported, it did assume a major role in obtaining the information desired. An adequate source of algae is the first problem to be met by any biologist interested in rearing large numbers of planktonic larvae of marine invertebrates. Thus it seems of particular value to consider the basic equipment and techniques used in growing large quantities of algae in the laboratory.

The following information is drawn from many sources. It does, however, make use of personal experience in maintaining cultures of a number of algal species over a two-year period. It is intended primarily as an aid to other workers in growing cultures and in avoiding some of the difficulties.

The size of the culture to be grown is governed primarily by the quantity of algae that will be needed for the daily feeding of larvae. It is possible to draw off about one-half of a culture each day, add an equal amount of fresh nutrient medium and have the culture return to its initial cell density within 24 hours (see Ketchum and Redfield 1938). If more than one-half of the culture is used daily or a 24-hour growth period between use is not feasible, it is necessary to maintain two or more cultures. Alternate use of these allows the necessary growth period for maintaining a high cell density.
Ehrlemeyer flasks of from 5 to 8 liter capacity or 5 gallon, wide-mouth, pyrex carboys are satisfactory culture containers both for size and convenience. To allow for maximum photosynthetic and reproductive activity, algal cultures need some continuous source of light. A fluorescent light is adequate for flask culture, particularly when supplemented by aluminum foil reflectors to light all sides of the culture. However, such external lighting is not adequate for obtaining a high cell density of algae when carboys are used. An efficient light source for carboy cultures is a glass U-tube of red, neon gas so constructed that it can be placed inside the carboy. Compared with most types of light sources, neon tubes radiate very little heat. The unit is enclosed in a pyrex test tube which is inserted into the culture through the mouth of the carboy. Although it is necessary to have a current transformer to operate such a unit, lighting the culture from the center in this manner gives much better results.

Since most algal species so far used for mass cultures grow best at temperatures between 15° and 18° C., it is usually necessary to provide some method for cooling the cultures. This can be accomplished by having the culture container sitting in a running fresh-water bath when tap water temperatures are sufficiently low, by maintaining cultures in a room where air conditioning is available, or by keeping cultures in some type of refrigerated water bath. Even though internal neon light units produce a minimum of heat, it is advisable to direct a flow of fresh water around the tube to assist in keeping the temperature of the culture within the desired range.
In order to supply a continuous source of carbon for algal growth, compressed air, containing the normal complement of carbon dioxide, is bubbled into the bottom of the container. This also serves to keep the culture stirred and prevents any settling of the algal cells. An open-end glass tube is adequate for this purpose. Aeration stones should be avoided since they tend to collect a film of plant life and become clogged. The compressed air should be passed through a cotton filter to prevent oil droplets and other foreign materials from contaminating the culture. It is possible to obtain a growth approaching the maximum cell density by bubbling carbon dioxide into the culture in place of compressed air (Burlew 1953). However, this procedure is relatively expensive and would probably be of advantage only when dealing with an algal species that does not respond well to the usual methods.

Figures 8 and 9 show the standard culture arrangement used for this study and a breakdown of the equipment involved. Scott (1943) figures such a culture arrangement and gives some discussion of methods for mass culturing algae.

There are two methods in general use for growing cultures of marine algae. One method uses natural sea water enriched with added nutrient salts, etc. The other method uses artificial sea water also enriched with nutrients. These methods have developed from the studies of Allen and Nelson (1910) as a modification of methods for fresh-water algal culture set down by Miguel (1890-93). They have been improved on considerably in recent years by microbiologists studying the physiology of marine algae (Provasoli, McLaughlin and...
Droop 1957). Some success has been attained using commercial fertilizers as a source of nutrients (Loosanoff and Engle 1942), but the nutrient requirements for many algal species are very specific and can best be met by the addition of exact amounts of nutrients which have been determined experimentally as necessary for successful cultures.

A. Natural Sea Water Method

Although many modifications exist for the amounts of the nutrients to be added, the formulae suggested below will give good results for most estuarine species of algae. It is used at W.H.O.I. as a general culture medium.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water 20 to 28 °/00</td>
<td>1 liter</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>150 mg.</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>20 mg.</td>
</tr>
<tr>
<td>Ferric Sequestrene</td>
<td>10 mg.</td>
</tr>
<tr>
<td>Trace elements</td>
<td></td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.0196 mg. (0.005 mg. Cu)</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>0.0114 mg. (0.003 mg. Zn)</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>0.022 mg. (0.005 mg. Co)</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>0.360 mg. (0.1 mg. Mn)</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.0126 mg. (0.005 mg. Mo)</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Thiamin HCl</td>
<td>0.2 mg.</td>
</tr>
<tr>
<td>Biotin</td>
<td>1.0 ug.</td>
</tr>
<tr>
<td>B-12</td>
<td>1.0 ug.</td>
</tr>
<tr>
<td>Silicon (necessary only for diatom cultures)</td>
<td>Na₂SiO₃ 30 to 60 mg. (*)</td>
</tr>
<tr>
<td>Ammonia (required only for certain species)</td>
<td>NH₄Cl 50 mg.</td>
</tr>
</tbody>
</table>

(*) This requirement varies with the species, culture density, and natural seasonal fluctuations of silicon in sea water.

For convenience, it is possible to prepare the above nutrient additions in three stock solutions as follows:
1. Salts (*

\[
\begin{align*}
\text{NaNO}_3 & \quad 150 \text{ mg.} \\
\text{NaH}_2\text{PO}_4 & \quad 20 \text{ mg.}
\end{align*}
\]

To one liter of distilled water.

2. Iron and Trace elements (*

\[
\begin{align*}
\text{FeSequestrene} & \quad 10 \text{ mg.} \\
\text{Trace (As given above)} & \quad \text{To one liter of distilled water.}
\end{align*}
\]

3. Vitamins (**)

(As given above) To one liter of distilled water.

(* Autoclave after preparation to sterilize.

(** Since vitamins are not stable in solution at room temperatures, this stock solution should be kept frozen. By placing the stock solution in 5 to 10 cc. screw-cap test tubes, it can be quickly thawed for use.

To prepare the culture medium, add one milliliter each of stock solutions 1., 2., and 3. to each liter of sea water. Only glass-distilled water should be used to prepare these solutions. Metal ions carried over from regular distilling apparatus are somewhat toxic to algae and usually lethal to invertebrate larvae in very low concentrations.

In order to assure that other species of algae that occur naturally in sea water will not contaminate a culture, it is necessary to boil the water for 5 to 10 minutes and then cool it prior to the addition of the desired algal inoculum. The same procedure is followed before adding fresh medium to a culture. Bacterial contamination is thereby kept at a minimum since sterile stock solutions of nutrients are employed with the exception of the vitamins which are destroyed by heat.
The best results are usually obtained if sterile techniques are used throughout giving bacteria-free cultures. It is difficult, however, to maintain sterile cultures under conditions of routine removal of culture and addition of fresh medium. Thus all cultures used in this study for feeding larvae were considered to have bacteria present.

B. Artificial Sea Water Method

This method was developed at W.H.O.I. by Turner (unpublished) as a means of avoiding the boiling of large quantities of sea water for the preparation of media. While it is much the simpler method to employ, it will not give good results with all species of marine algae. The method was first used to supply large volume cultures of *Phaeodactylum tricornutum*. By reducing the salinity of the sea water from 30 o/oo to approximately 27 o/oo, the author was able to extend the method to grow cultures of *Nannochloris atomus* as well. These two species were the only ones that could be grown successfully by this method. It seems probable, however, that by further modifying the method to make use of added nutrients for enrichment rather than the use of commercial fertilizer for this purpose, it could be used to grow other algal species.

Tap water 1 liter
"Neptune Salts" (Appendix II) 30 gm.

This will make an approximate 30 o/oo

Nutrients are supplied by the addition of 2 to 3 grams of commercial fertilizer, "Vigoro," to the initial 10 liters of medium and a similar amount with every alternate addition of 5 liters of fresh medium.

Since the chemical treatment of public water supplies varies considerably in different areas, the use of tap water may not always result
in successful algal cultures. If it is suspect that chlorination, copper additives or other treatments are excessively high, it will be necessary to use distilled water to prepare medium by this method. Again, only glass-distilled water should be used.

Pure cultures of various marine algae can be maintained in test tubes on solidified agar medium using techniques similar to those for the culture of bacteria. The tubes should be stored at approximately 18° C. in a well lighted room. A general practice is to use screw-cap tubes with the caps loosely seated. This allows access to the air while minimizing bacterial contamination. Cotton plugs serve equally well for this purpose.

The isolation and identification of marine algae requires considerable experience (Droop 1954). In addition, the study of the nutrients, trace elements, and vitamin requirements of algae is a specialized field of botanical research. Therefore, it seems worthwhile to record for the New England area those laboratories where a variety of stock cultures are routinely maintained. These are:

1. Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

2. Haskins Laboratories, 305 E. 13rd Street, New York 19, N. Y.

3. Biological Laboratory, U. S. Bureau of Commercial Fisheries, Milford, Connecticut

Although it is not a direct function of these laboratories, the microbiologist on the staff is usually willing to supply other research workers with an amount of algae sufficient to start large cultures. Since some algal species have very specific nutrient requirements, it is well to
inquire about this prior to attempting to grow cultures of algae other than those listed in this study.

LITERATURE CITED


(*) These publications contain extensive bibliographies covering all phases of algal culture research.
APPENDIX II.

SPECIAL EQUIPMENT AND MATERIAL

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immersion heating rods</td>
<td>Central Scientific Supply, 79 Amherst Street, Cambridge, Massachusetts</td>
</tr>
<tr>
<td>Immersion thermostat</td>
<td>Fenwal Inc., Ashland, Massachusetts</td>
</tr>
<tr>
<td>Lead heating coil, flexible, 40 foot - 350 watts.</td>
<td>Sears, Roebuck and Co., Boston, Massachusetts</td>
</tr>
<tr>
<td>Neon light tubes</td>
<td>Made on order in desired size by most companies manufacturing neon signs.</td>
</tr>
<tr>
<td>&quot;Neptune Salts&quot;</td>
<td>Westchester Aquarium Supply Company, 1511 Mamaronect Avenue, White Plains, New York</td>
</tr>
<tr>
<td>Wire screens, stainless steel</td>
<td>Newark Wire Cloth Co., Newark, New Jersey</td>
</tr>
<tr>
<td>No. 100 149 micron mesh</td>
<td></td>
</tr>
<tr>
<td>No. 250 61 micron mesh</td>
<td></td>
</tr>
<tr>
<td>No. 325 44 micron mesh</td>
<td></td>
</tr>
</tbody>
</table>
### APPENDIX III.

**STATIONS AT WHICH POLINICES WERE COLLECTED**

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Species Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnstable Harbor,</td>
<td>41° 43' N</td>
<td><em>P. duplicatus</em></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>70° 20' W</td>
<td></td>
</tr>
<tr>
<td>Menemsha Harbor,</td>
<td>41° 20' N</td>
<td><em>P. triseriata</em></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>69° 46' W</td>
<td></td>
</tr>
<tr>
<td>Quissett Harbor,</td>
<td>41° 32' N</td>
<td><em>P. duplicatus</em></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>70° 40' W</td>
<td></td>
</tr>
<tr>
<td>St. Andrews,</td>
<td>45° 05' N</td>
<td>Egg collars of <em>P. triseriata</em> and <em>P. heros</em></td>
</tr>
<tr>
<td>New Brunswick</td>
<td>67° 04' W</td>
<td></td>
</tr>
<tr>
<td>Starboard Island,</td>
<td>44° 36' N</td>
<td>Egg collars of <em>P. triseriata</em></td>
</tr>
<tr>
<td>Machias, Maine</td>
<td>67° 23' W</td>
<td></td>
</tr>
<tr>
<td>Vineyard Sound,</td>
<td>41° 30' N</td>
<td>Egg collars and specimens of <em>P. heros</em></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>70° 35' W</td>
<td></td>
</tr>
<tr>
<td>York Beach,</td>
<td>43° 10' N</td>
<td><em>P. heros</em></td>
</tr>
<tr>
<td>Maine</td>
<td>70° 37' W</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX IV.

FIGURES AND PLATES

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--- | ---
1. Shell of Polinices heros | 1
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Figure 1. Shell of Polinices heros. Line drawing showing various shell measurements and morphological terms mentioned in the text.

Figure 2. Egg collar of P. duplicatus. The scalloping along basal rim is characteristic of the collar of this species (X 1/2).
Figure 3. Egg capsules of *P. duplicatus*. Photomicrograph of an unsanded portion of the egg collar (X 140).

Figure 4. Egg collar of *P. heros* (X 1/2).
Figure 5. Egg collar of *P. triseriata* (natural size).

Figure 6. *P. heros* carrying a soft-shell clam, *Mya arenaria*, (natural size).
Figure 7. *P. herbos* feeding on a small quahog, *Mercenaria mercenaria,* (natural size).

Figure 8. Phytoplankton culture arrangement. Five-gallon carboy containers showing air and water-cooling tubes attached.
Figure 9. Components of neon light units used in carboys. Left to right: air tube, neon tube, and test-tube water jacket.
Figure 10. Shell of *P. heros* in apical view (X 2).

Figure 11. Shell of *P. heros* in aperture view (X 2).
Figure 12. Shell of *P. duplicatus* in apical view (X 2).

Figure 13. Shell of *P. duplicatus* in aperture view (X 2).
PLATE 8

Figure 14. Shell of *P. triseriata* in apical view (X 3).

Figure 15. Shell of *P. triseriata* in aperture view (X 3).