F. M. Truesdale

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CHESAPEAKE BAY INSTITUTE
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ECOLOGICAL STUDIES OF THE LARVAL DEVELOPMENT OF RHITHROPANOPEUS HARRISII (XANTHIDAE, BRACHYURA)

N. A. Chamberlain

TECHNICAL REPORT XXVIII

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D. W. Pritchard
Director

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ABSTRACT

The larvae of Rhithropanopeus harrisii were successfully reared in isolation in the laboratory using copepod nauplii as the sole food source. Laboratory cultures fed this and other diets were used to examine environmental effects on the development and mortality of the larvae. Every individual successfully completing larval development went through four zoeal stages and one megalopa before metamorphosis to the first crab stage. Larvae exposed to both algae and nauplii developed much more slowly then those exposed to nauplii alone. Larvae exposed to algae alone died before their first moult but lived longer than those which were fed nothing. The mechanism of this apparent inhibition of larval development by algae is unknown.

Cultures maintained at 1‰ and 15‰ salinity were not successful, those at 3‰ salinity had high mortality, and those at 6‰ and 10‰ salinity were most successful with mortalities of 0 to 20%. Development at 30°C was found to be about twice as rapid as at 15°C at salinities where mortality was low. The results of these experiments were used in the design of a study of a natural population.

A recently described tracer technique using the dye rhodamine B and highly sensitive fluorometers was employed in the estimation of exchange of water between a small embayment containing a natural population of Rhithropanopeus harrisii and outside areas in Chesapeake Bay. By taking daily plankton samples during the first few weeks of the spawning season, the development of larvae spawned at the same time was followed. The observed daily loss of these larvae during their period of planktonic life was corrected for loss of larvae from the

embayment through exchange of water, to give an estimate of natural mortality of 42%. The addition of larvae from outside areas by exchange was quite small due to low exchange and low concentrations of larvae in the waters outside the embayment. The rate of larval development in the embayment was approximately the same as for laboratory cultures under similar conditions.

INTRODUCTION

During the early years of microscopy a number of authors described previously unknown planktonic animals which later proved to be brachyuran larvae. The first of these was probably Leeuwenhoek in 1686 (Gurney, 1942). Bosc in 1802 erected the genus Zoea for one of these larvae and placed several species in it (Gurney, 1942). Zoea is the name now used for the larval stages of the Brachyura. The systematic position of these organisms was naturally unclear, and evidence for their taxonomic placement was not available until Thompson (1828) described the hatching of brachyuran eggs into zoeae and the metamorphosis of zoeae into crabs. His evidence was strongly contested by most of the authorities of the day since at that time metamorphosis was not thought to occur in Crustacea. It was not until the 1840's that decapod metamorphosis was accepted.

Thompson's interest in the plankton led him to describe the use of plankton nets and pumps. Since many texts (e.g. Sverdrup, et al., (1942) continue to credit Muller with the first use of the plankton net in 1846, it may be of interest to quote here from one of Thompson's papers (1828).

> "Few of these marine animals [plankton], except some of the larger and more conspicuous, have as yet been observed, so that the investigation of them holds out the promise of a rich harvest to the Naturalist and a vast field of exploration replete with novelty and interest; to accomplish this object however, he must use the greatest diligence, seizing every opportunity when the way of the ship does not exceed three or four miles per hour, to throw out astern a small towing net of gause, bunting, or other tolerably close material, occasionally

> drawing it up, and turning it inside out into a glass vessel of sea water, to ascertain what captures have been made; when a ship goes at a greater rate, and in stormy weather, a net of this kind might be appended to the spout of one of the sea-water pumps, and examined three or four times a day, or oftener, according to circumstances.
>
> The luminosity or speakling of the sea by night, is a phenomenon which never fails to attract the attention of voyagers the most incurious, and having been found in the greater number of instances, to be produced by marine animals, first led the author into the use of the towing net...
>
> ... the author, who in towing for luminous animals, during a voyage from the Mauritius in 1816, discovered the species figured in Plate I..... [a zoea]."

Most studies of brachyuran larvae have been reconstructions of larval stages from plankton collections. In most areas this method is inherently poor since other undescribed brachyuran larvae are also present. Due to the unique distribution of a crab found with no other brachyuran species in the very low-salinity waters of the upper Miramichi estuary in New Brunswick, Connolly in 1925 was able to describe its larvae from the plankton. This species, Rhithropanopeus harrisii (Gould, 1841) Rathbun, 1898, is typically found in low-salinity or fresh water (Rathbun, 1930).

This same situation was found to exist in areas of the upper Chesapeake Bay. Though methods are now available for the laboratory culturing of brachyuran larvae (Chamberlain, 1957), such a field situation is ideal for studying problems of natural populations. Areas containing populations of adult Rhithropanopeus harrisii were then examined to find the most desirable

location for a field study of the larvae of this species. One of the biggest problems encountered in the study of estuarine plankton populations is that of differentiating between real changes in population and apparent changes due to water movements. Often, in studies of this sort, this problem is minimized by making the area of study very large, sometimes including an entire estuary (Bousfield, 1955). Such a solution is not possible when one of the requisites of the study area is that few, if any, similar species be present. This study attempts to surmount the problem of water movement by selecting an embayment which has a low exchange of water with other areas, and employing a newly described tracer technique for the estimation of exchange.

The purpose of this study is the examination in the field of problems raised by laboratory work on larval development.

This approach is made possible by the prior development of successful laboratory culturing techniques (Chamberlain, 1960).

The work presented here is divided into three parts:

1. the description of the larvae, based on laboratory culturing to supplement Connolly's general description and to ensure accurate identification of plankton collections,
2. the culturing of the larvae in the laboratory under varying environmental conditions to provide basic information concerning the ecological requirements of the larvae,
3. the field studies, including the description of the habitat, estimates of water movement and plankton collections.

DESCRIPTION OF THE LARVAE

Connolly (1925) has pictured and given a general description of the four zoeal stages and the one megalopa of Rhithropanopeus harrisii (Fig. 1). The following description, based on larvae reared from the egg in the laboratory, gives further details of the development of the appendages. The shape and general location of the setae on the appendages were found to be identical with those of Neopanope texana sayi as given in the description by Chamberlain (1961). For that reason the appendages are described but not pictured here.

Some indication of variation in setation is given in the description. This variation is based on examination of several individuals of each stage from sponges of ten adults collected in the study area.

First Zoea

The eyes are not movable at this stage.

Cephalothorax - The zoeal stages have long rostral and dorsal spines and very short lateral ones. The relative lengths of these spines do not change appreciably during zoeal development.

First Antenna - A simple appendage bearing 4 or 5 setae terminally.

Second Antenna - Smooth to tip with the exception of a small dorsal seta near the base.

Mandible - Simple with no palp present.

First Maxilla - Coxopodite with 5 or 6 setae. Basipodite with 5 setae. Endopodite with two segments, the proximal bearing a single seta, the distal bearing 6 setae.

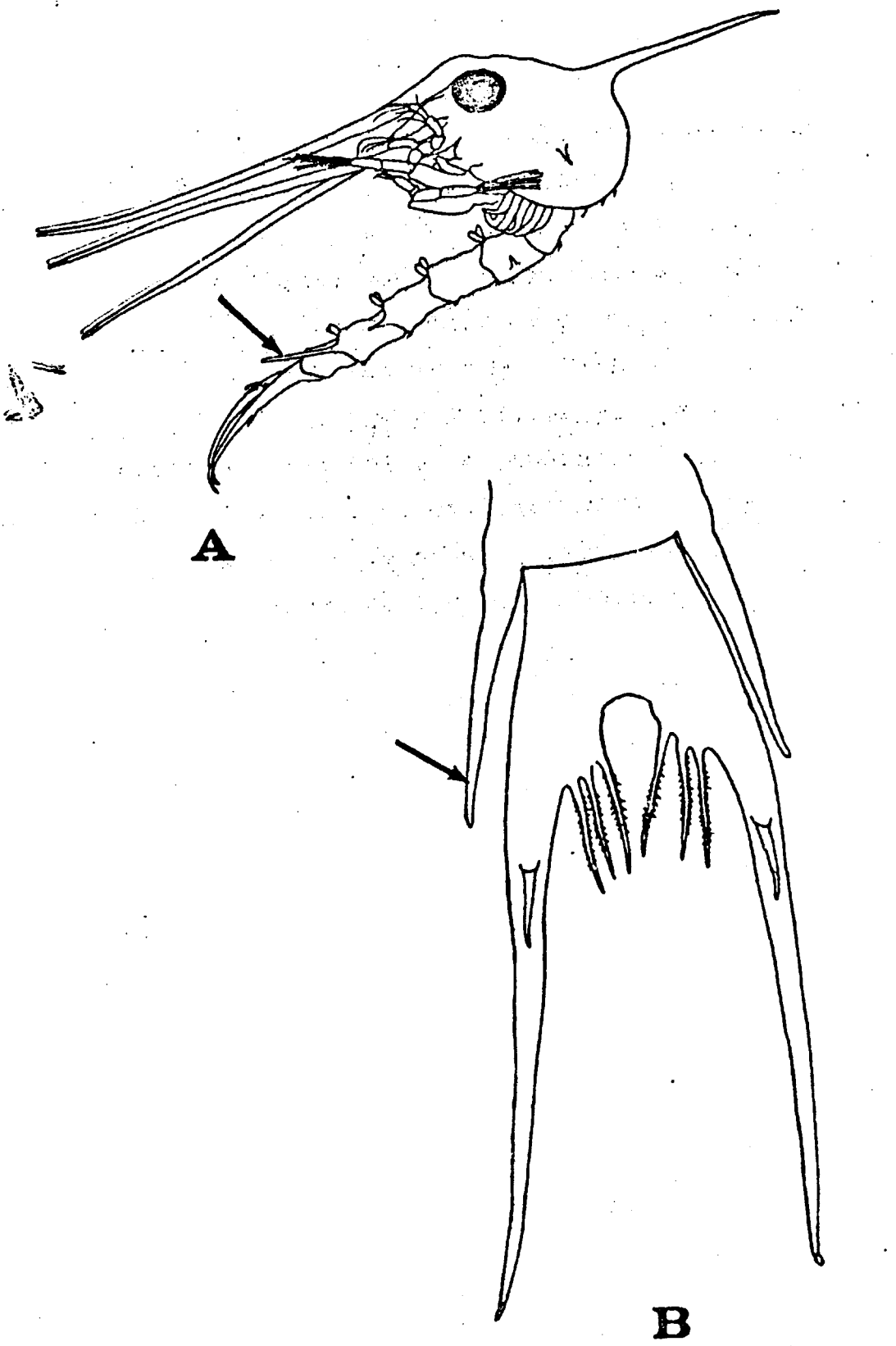

Figure 1. Rhithropanopeus harrisii. A: Third zoea. B: Telson of first zoea. Arrows indicate diagnostic character. From Connolly (1925).

Second Maxilla - Coxopodite with 6 to 8 setae. Basipodite with 7 or 8 setae. Endopodite with 7 setae. Scaphognathite with 5 setae.

First Maxilliped - Precoxa with a medial lobe bearing a single seta. Protopodite bearing 9 or 10 setae. Endopodite with five segments bearing setae (from proximal to distal segments) 2 or 3, 2, 1, 2, 4. Exopodite with two segments, the first bearing no setae, the terminal one bearing 4 swimming setae.

Second Maxilliped - Precoxa bearing no setae. Protopodite bearing 4 setae. Endopodite with three segments bearing setae (proximal to distal) 1, 1, 5. Exopodite with two segments, the first bearing no setae, the second with 4 swimming setae.

Third Maxilliped - Rudimentary.

Pereiopods - All five pairs are rudimentary. None are chelate.

Abdomen - Six segments, the sixth being fused with the telson. The pleopods are not evident at this stage. The first five segments have two setules on their dorsal posterior margins. The second segment bears a short (50μ) mid-lateral projection. The third, fourth, and fifth segments bear ventro-lateral projections (40μ, 60μ, and 225μ respectively) on their posterior margins. The long ventro-lateral projection on the fifth segment extends beyond the junction of the cornua of the telson in this and in all later zoeal stages. This character is the most striking difference between this species and other similar xanthid species (Fig. 1B). The telson is bicornuate with each cornu bearing three setae on its medial margin and a short spine on its mid-dorsal surface.

Second Zoea

The eyes are now movable.

First Antenna - A simple appendage bearing 5 setae terminally.

Second Antenna - No change except in size.

Mandible - No change except in size.

First Maxilla - Coxopodite with 6 setae. Basipodite with 7 setae. Endopodite with two segments, the proximal bearing a single seta, the distal bearing 6 setae. A single epipodal seta is now present.

Second Maxilla - Coxopodite with 7 or 8 setae. Basipodite with 6 or 7 setae. Endopodite with 6 or 7 setae. Scaphognathite with 9 or 10 setae.

First Maxilliped - Precoxa with a medial lobe bearing a single seta. Protopodite bearing 10 setae. Endopodite with 5 segments bearing setae (proximal to distal) 3, 2, 1, 2, 5. Exopodite with two segments, the first bearing no setae, the second bearing 6 swimming setae terminally.

Second Maxilliped - Precoxa with no setae. Protopodite with 3 or 4 setae. Endopodite with three segments bearing setae (proximal to distal) 1, 1, 4. Exopodite with two segments, the first bearing no setae, the second with 7 swimming setae.

Third Maxilliped - No change except in size.

Pereiopods - No change except in size.

Abdomen - The pleopods now appear as slight ventro-lateral swellings near the posterior margins of the second through the fifth segments. A pair of medial setules is now present at the junction of the cornua of the telson.

Third Zoea

First Antenna - No change except in size.

Second Antenna - A small bud is now present at the location of the basal seta.

Mandible - No change except in size.

First Maxilla - Coxopodite with 7 setae. Basipodite with 8 setae. Endopodite with two segments, the proximal bearing a single seta, the distal bearing 6 setae.

Second Maxilla - Coxopodite with 7 setae. Basipodite with 8 or 9 setae. Endopodite with 8 or 9 setae. Scaphognathite with 15 to 17 setae.

First Maxilliped - Precoxa with a medial and a lateral lobe, the medial one bearing a single seta, the lateral bearing no setae. Protopodite bearing 10 setae. Endopodite with five segments bearing setae (proximal to distal) 2 or 3, 2, 1, 2, 5. Exopodite with two segments, the first bearing no setae, the second with 8 swimming setae.

Second Maxilliped - Precoxa with a lateral bud bearing no setae. Protopodite with 4 setae. Endopodite with three segments bearing setae (proximal to distal) 1, 1, 4. Exopodite with two segments, the first bearing no setae, the second bearing 9 swimming setae.

Third Maxilliped - No change except in size.

Pereiopods - The first pair are now chelate.

Abdomen - The sixth segment is now separate from the telson. The pleopod buds are now quite distinct (20μ to 50μ) on the second through the fifth segments.

Fourth Zoea

First Antenna - A basal and a sub-terminal bud are now evident. Eight setae are borne terminally.

Second Antenna - The endopodite bud is now about 250μ in length.

Mandible - An anterior dorsal bud is now present.

First Maxilla - Coxopodite with 8 or 9 setae. Basipodite with 9 or 10 setae. Endopodite with two segments, the proximal with 1 seta, the distal with 6 setae.

Second Maxilla - Coxopodite with 8 setae. Basipodite with 9 or 10 setae. Endopodite with 8 or 9 setae. Scaphognathite with 20 or 21 setae.

First Maxilliped - Precoxa with a medial and a lateral lobe, the medial one bearing 2 setae, the lateral bearing no setae. Protopodite with 9 or 10 setae. Endopodite with five segments bearing setae (proximal to distal) 3, 2, 1, 2, 5 or 6. Exopodite with two segments, the first bearing no setae, the second with 9 swimming setae.

Second Maxilliped - Precoxa with a lateral bud bearing no setae. Protopodite with 4 or 5 setae. Endopodite with three segments bearing setae (proximal to distal) 1, 1, 4. Exopodite with two segments, the proximal bearing no setae, the distal bearing 11 swimming setae.

Third Maxilliped - No change except in size.

Pereiopods - No change except in size.

Abdomen - The pleopods on segments two through five are now much longer and are biramous. The sixth segment has a small pair of pleopod buds.

Megalopa

Cephalothorax - The dorsal and lateral spines are no longer present on the now depressed carapace. The rostrum is short and notched.

First Antenna - Now well segmented at the base and incompletely segmented distally. The first segment bears 4 or 5 setae, the second none, the third 5, the fourth 5, the fifth and sixth together 8 or 9, and the seventh 3 or 4 terminal setae. A single short segment with 4 terminal setae is borne laterally on the third segment.

Second Antenna - Nine or ten segments with segmentation not complete between the fifth and eighth. The first segment bears 4 or 5 setae, the third 1, the fifth 2, the eighth 2, the ninth and tenth 4 each.

Mandible - The palp now bears 3 or 4 setae.

First Maxilla - Coxopodite with 9 or 10 setae. Basipodite with 12 setae. Endopodite with two segments, the proximal bearing a single seta, the distal with 4 setae. A single epipodal hair is present.

Second Maxilla - Coxopodite with 5 or 6 setae. Basipodite with 11 setae. Endopodite with a single seta. Scaphognathite with 25 to 28 setae.

First Maxilliped - An epipodite is now present and bears 3 to 4 setae. Exopodite with two segments, the proximal bearing a single seta, the distal bearing 3 setae. Endopodite with 5 setae. The protopodite is two-lobed, the more distal lobe bearing 18 to 19 setae, the more proximal bearing 12 setae.

Second Maxilliped - Epipodite with 5 setae. Exopodite with two segments, the proximal bearing 2 setae, the distal bearing 4 or 5. Endopodite with four segments bearing setae (proximal to distal) 3, 1, 4, 7.

Third Maxilliped - Protopodite with 12 or 13 setae. Epipodite with 18 to 20 setae. Endopodite with five segments bearing setae (proximal to distal) 15 to 17, 11 to 13, 5 or 6,

5 to 7, 5 to 7. Exopodite with two segments, the proximal with 4 setae, the distal with 6 setae.

Pereiopods - These are in the form of the adult with the exception of the chelae which are both the same size in this stage.

Abdomen - The abdominal segments now have short posteriolateral projections and no others. The pleopods are biramous on segments two through five and simple on segment six. The exopodites of all pleopods bear setae; 9 or 10 on the first four pairs (abdominal segments two through five), and 5 or 6 on the last pair (abdominal segment six).

Discussion

In the above description the ranges of setation include the few values for setation (on the antennae and on the exopodites of the maxillipeds) described by Connolly. There is one discrepancy in the time of appearance of an appendage, however. The bud of the endopodite on the second antenna is described here as first appearing in the third zoea. Connolly describes "some specimens" in the second zoeal stage as having this bud. The measurements given by Connolly are compatible with those of this study.

The characteristics most easily used on both living and preserved material for distinguishing among the zoeal stages are: the number of setae on the exopodites of the maxillipeds (the swimming setae), the state of development of the pleopods, and size. For the four zoeal stages the length from tip of rostrum to tip of dorsal spine is: first, 2.2 mm; second, 2.5 mm; third, 3.4 mm; fourth, 3.8 mm.

It is interesting to note the striking similarity between the larvae of Rhithropanopeus harrisii and those of Neopanope

texana sayi. Even in details of setation there are wide areas of agreement (cf. Chamberlain, 1961). The parataxa erected by Aikawa (1929, 1937) included the classification of Rhithropanopeus harrisii as taken from Connolly and, therefore, did not show the "hair formulae". From the present description these formulae are: second maxilla, 2-5(7) or 3-5(8); second maxilliped, 5-1-1 or 4-1-1. These formulae as well as the other characteristics used by Aikawa overlap or are identical with those of Neopanope texana sayi. The hair formulae are used by Aikawa as 'supporting characters', therefore some variation in the formulae does not alter the placement of these two species in his parataxon Xanthozoea. Although the ranges of these two species are the same, the habitats in which they are found are quite different (Rathbun, 1930). Neopanope is found in high-salinity water and Rhithropanopeus in very low-salinity or even fresh water. The effect of studies of this sort on decapod systematics will undoubtedly become important when more of them have been completed

LABORATORY CULTURING

General Methods

The techniques developed earlier for rearing xanthid larvae (Chamberlain, 1957, 1961) are generally those used in this study. Adult female crabs carrying eggs are brought into the laboratory and maintained in isolation until the eggs hatch. Upon hatching, the larvae are removed and placed in isolation in small containers with filtered sea water and food. Food and water are changed each day.

In this study the culture water was filtered through 0.45μ (HA) Millipore filters before use. Glass-distilled water was used for dilution to the required chlorinity. Chlorinities were determined by titration with silver nitrate.

For development through the zoeal stages the larvae were placed in 25 ml polystyrene tissue culture flasks (Falcon Plastics Company) containing 10 ml of culture water. At the megalopa stage the larvae were transferred to fingerbowls.

Cultures were maintained at 15°C and 30°C in constant-temperature boxes, and at 24°C in an air-conditioned laboratory. Maximum variation of these temperatures was ± 2°C.

Larvae representing each stage of development were preserved in 3% formalin in the culture water in which they had been grown. Appendages of the preserved specimens were removed by dissection with fine tungsten needles.

Feeding

Five sets of isolation cultures with 10 cultures in each set were prepared, with each set having a different diet. The diets were: (1) copepod nauplii, (2) *Artemia* nauplii, (3) copepod

nauplii and clumps from old cultures of the unicellular algae Dunaliella tertiolecta (Chlorophyta) and Isochrysis galbana (Chrysophyta), (4) the above algae alone, and (5) no food. These cultures were maintained at 24°C in water with a salinity of 6‰. Table I shows the duration of each zoeal stage under these conditions. It can be seen that there is essentially no difference between cultures fed the two diets of nauplii. The copepod nauplii were obtained from the plankton and maintained for short periods in the laboratory in mass culture. Most adult copepods in the plankton samples were of the genus Acartia. The nauplii are probably also of this genus. Since Artemia nauplii are much easier to obtain, they were used in later experiments. The rate of development of zoeae fed both nauplii and algae was only half that of zoeae fed nauplii alone. This slow development was general throughout the stages. Larvae fed algae alone did not moult and died after 6 to 10 days. Larvae which were not fed died within 3 days without moulting.

Temperature and Salinity Effects

Fifteen sets of isolation cultures with 10 cultures in each set were prepared to examine the effects of temperature and salinity. One set was maintained in every combination of the following temperatures and salinities: 15°C, 24°C, and 30°C; 1‰, 3‰, 6‰, 10‰, and 15‰ salinity. The mortality during larval development is given in Table II. Some individuals in each set survived the first moult. No individual completed larval development at the salinities 1‰ or 15‰. Mortality was greater than 50% at salinity 3‰. At salinities 6‰ and 10‰ mortality was minimal.

Table I

Duration of the zoeal stages when cultured with different diets.

Diet	Duration of each stage (days)				Total duration (days)	Sample size*
	1	2	3	4		
Copepods	4-5	2	2-3	4-6	13 - 16	9 (10)
Artemia	4-5	2-3	2	4-6	13 - 15	9 (10)
Artemia and algae	8-10	5	4-5	9-11	27-31	8 (10)
Algae	6-10**					
No food	1-3 **					

* First figure is total number completing zoeal development. Figure in parentheses is original number in sample.

** No survival after this time.

Table II

Temperature and salinity effects on percent larval mortality.

	Temperature (°C)		
Salinity (‰)	15	24	30
1	100	100	100
3	60	40	80
6	10	10	20
10	0	10	20
15	100	100	100

Tables III, IV, and V show the duration of each stage at the three temperatures at salinities of 3‰, 6‰, and 10‰ respectively. The sample size indicates the number of larvae successfully completing larval development. There seem to be no essential differences in developmental times at these three salinities. However, developmental time can be seen to increase regularly with decreasing temperature.

Table III

Duration of the zoeal stages versus temperature at 3‰ salinity.

Temperature (°C)	Duration of each stage (days)				Total duration (days)	Sample size
	1	2	3	4		
15	6-7	5-6	3-4	5-7	20 - 24	4
24	4-5	2-3	2-3	4-6	14 - 17	6
30	3-4	2-3	2-3	3	11 - 12	2

Table IV

Duration of the zoeal stages *versus* temperature at 6‰ salinity.

Temperature (°C)	Duration of each stage (days)				Total duration (days)	Sample size
	1	2	3	4		
15	6-7	5	3-4	5-8	20 - 24	9
24	4-5	2-3	2	4-6	13 - 15	9
30	3-4	2	2-3	3-4	11 - 13	8

Table V

Duration of the zoeal stages versus temperature at 10‰ salinity

Temperature (°C)	Duration of each stage (days)				Total duration (days)	Sample si
	1	2	3	4		
15	6-7	4-5	3-5	5-7	19 - 24	10
24	4	2-3	2-3	4-5	13 - 15	9
30	3-4	2	2-3	3-4	11 - 13	8

FIELD STUDIES

Description of the Area

The criteria for a suitable embayment for field work were the presence of an adult crab population, low exchange of water with outside areas, and location close enough to dock and storage facilities of the Chesapeake Bay Institute Field Laboratory to allow daily sampling. After a number of embayments had been investigated, Lake Ogleton located 2 miles southeast of Annapolis, Maryland, was selected (Fig. 2).

The embayment is about 2 km long and 0.3 km wide with a surface area of 0.55 km^2 and a drainage area of about 1.8 km^2. The volume at low water is about 1,300,000 m^3 and the intertidal volume, 134,000 m^3. The maximum depth is 3.5 m and the mean depth 2.2 m. The mouth is only 75 m wide at low and 150 m at high tide. The depth at the mouth is 2 m but this is not the effective sill depth since there is a higher sill (1.0 m) outside the present mouth at the location of bars marking the location of an older mouth (Fig. 3). The only source of fresh water for Lake Ogleton is runoff. The total recorded temperature range throughout the embayment during the first period of larval development (June 5 to June 19, 1961) was 21°C to 25°C. The total recorded chlorinity range during this period was 3.2‰ to 3.4‰ (5.8‰ to 6.2‰ salinity).

Extensive mussel beds (Modiolus demissus) were found at the mouth of the embayment extending well inside particularly on the northeast side. These beds contain most of the population of Rhithropanopeus harrisii in and around the embayment. The dominant attached plant in the area is Potamogeton perfoliatus var. bupleuroides (Fern.) Farwell. This plant encircles Lake

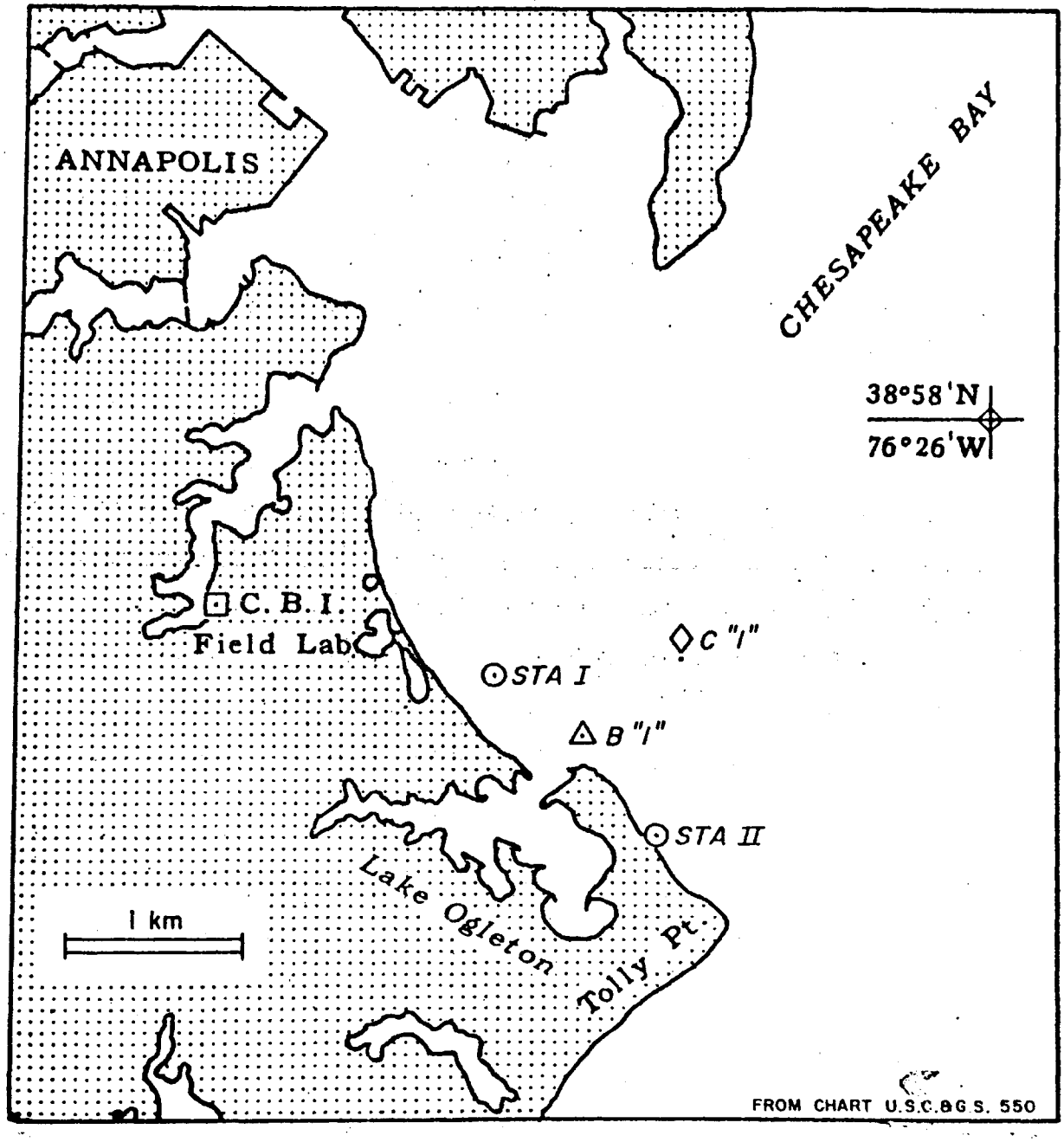

Figure 2. Lake Ogleton and surrounding area.

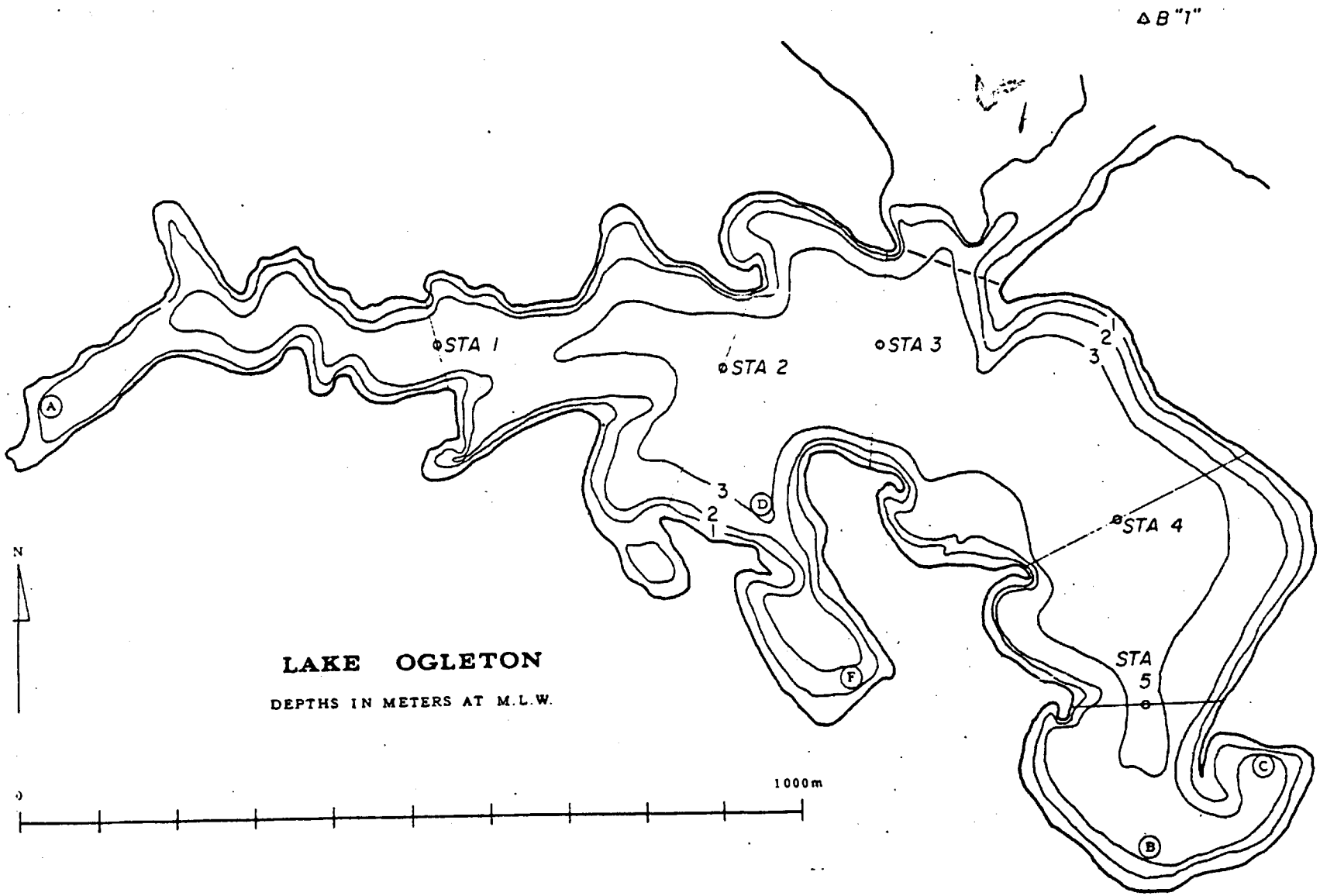

Figure 3. Lake Ogleton.

Ogleton at 1- to 2-meter depths during the summer. Anacharis canadensis Michx. was found occasionally as was free-floating Myriophyllum spicatum L. after June, 1960.

General Survey

During the spring of 1960, plankton sampling showed the first spawning of Rhithropanopeus harrisii to occur on May 27. Daily sampling for the next three weeks showed that larvae of Rhithropanopeus were extremely abundant, that there were no other brachyuran larvae present, and that it was possible to follow, at least qualitatively, the development of the larval population. The first occurrence of larvae in the water appeared as a burst of spawning and subsequent spawning was very light during the next week. The concentration of larvae outside the embayment was found to be very much less than inside. It was thus possible to see that the first occurrence of second-stage larvae was probably due to the development of the first stage larvae which had been spawned on May 27. This reasonably clear-cut situation continued throughout the larval development of the first-spawned group.

The larvae were successfully reared in the laboratory and the time for the development of the various stages was found to be about the same in the laboratory for chlorinities and temperatures similar to the field situation.

In 1961 plankton collections by net and pumped samples showed that the maximum concentration of zoeae was located at depths between 0.5 m and 2.0 m during the day and from 2.0 m to the surface at night. Maximum concentrations of megalopae occurred at night from the surface to 2 meters. During the day the megalopae were found in smaller concentrations at 1 to 2 meters

Very few zoeae or magalopae were found in the bottom meter of water even though oxygen was present there. Water samples taken occasionally during collecting and at three-hour intervals at all depths during one night of plankton sampling never showed an oxygen concentration below 3 $m\ell/\ell$. Megalopae were occasionally found in empty mussel shells on the bottom and perhaps spend much of the day on submerged shell and vegetation (as Aufwuchs).

Estimates of primary production were obtained on two days during the early part of the spawning season in June, 1961. The radiocarbon method (Steeman Nielsen, 1952; Ryther, 1956) and the method requiring chlorophyll concentrations and light data (Ryther and Yentsch, 1957) were used. In the first method $Na_2C^{14}O_3$ was added to a bottled water sample which was then incubated for 5 to 10 hours at the depth from which it was taken, filtered, and the filter pad assayed for C^{14} in a flow counter. Radiocarbon uptake in the dark was much higher than is usually reported, perhaps due to large quantities of suspended solids (about 60 mg/ℓ) in the water. The Secchi disc values were 1.3 meters.

Both methods gave results of about 0.3 to 0.5 mg C assimilated per m^3 per day for the upper two meters. These values are low by about an order of magnitude for estuarine waters during this season. Although the two methods are not thought to measure exactly the same process (Ryther and Yentsch, 1957), their agreement suggests that the actual phytoplankton production in the embayment was low.

Collections of Rhithropanopeus showed that the spawning season lasts from late May to early September. Young crabs hatched in June grew by September to the size of the smallest spawning individuals in May, but their ovaries were not well

developed by September nor were any of them found to be gravid. Most of the adult population in summer appears to be one year old with a small percentage of two-year-olds and occasional older individuals.

Exchange Estimation

An attempt was made to estimate the rate of exchange of water, and therefore larvae, between the embayment and outside waters by an examination of the changing chlorinity distribution. In this method (Pritchard and Bunce, 1959) the change in mean chlorinity of an embayment over some period of time is considered to be due to exchange of water with outside areas with some constant chlorinity (determined by averaging the observed chlorinities for the period). Since the volume of the outside area is very large with respect to the volume of the embayment, dilution by water from the embayment is neglected Mixing is assumed to be complete within the embayment.

This method gave reasonable results in early spring (5.6% exchange per tidal cycle between April 19 and April 27), but by late May the method could no longer be used due to the homogenei of the chlorinity distribution. As is common in embayments in the Chesapeake Bay, Lake Ogleton has a higher chlorinity in the spring than does the adjacent Bay. Greatly increased discharge in spring from the Susquehanna River reduces the surface chlorin of the Bay more rapidly than adjacent embayments can adjust to by exchange. With sufficient time, the chlorinity of the embayments is reduced to that of the surface Bay water. Lake Ogletor reached homogeneity with the surface water of the adjacent Bay during the time of first spawning. The calculation of exchange by the chlorinity method described above would therefore have been based on chlorinity differences so small as to make the

results meaningless. Another method for the estimation of exchange was thus necessary.

The method employed was a modification of a tracer technique described by Pritchard and Carpenter (1960) using the dye rhodamine B (available commercially in acetic acid solution from du Pont de Nemours) and a Turner Model 111 fluorometer modified to excite a sample to fluorescence with the green (546 mμ) line of mercury. The background fluorescenceof natural waters is about 1000 times smaller at this wavelength than at ultraviolet wavelengths. A continuous flow cuvette allows underway sampling.

The plan was to introduce enough dye, as homogeneously as possible, into the embayment to give a final concentration about one hundred times the sensitivity of the fluorometer. This was to be done a few days before the onset of spawning. The embayment was then to be sampled to obtain a daily estimate of mean dye concentration. Rate of loss of dye could then be computed and thus an estimate of exchange obtained. In 1960 the first spawning occurred during the last week in May. The dye was, therefore, released at the beginning of the last week in May, 1961. On May 22, the background fluorescence within and immediately outside the embayment was found to range from 10 to 20 parts per trillion (10^{12}) rhodamine B equivalent with a mean of 15 parts per trillion. This value was subtracted from all subsequent values used for computation.

The dye was introduced on May 24. Sufficient methanol was mixed with 13.6 kg of rhodamine B in acetic acid solution to reduce the density to about that of the water and to reduce the viscosity for ease in pumping. Through an error in calculation, this weight of dye was about 10 times the weight which

was planned. This error proved to be extremely fortunate since the initial loss of dye was quite high. The rhodamine solution was then introduced with a metering pump into the discharge sic of a high-volume deck pump, led over the side, and continuously discharged at a depth of about one meter. The boat, 20 feet long with two 45 horsepower outboard engines, was run at consta speed over the entire embayment for 6 hours, the duration of th morning flood tide. Due to the failure of the method of introduction to mix the dye as deeply into the water as was expected and the error leading to the release of ten times "too much" d the entire surface of Lake Ogleton glowed brilliant pink.

The sampling procedure was designed to give a fairly comp picture of dye distribution to allow a good estimation of mean concentration. A submersible pump housed in a fairing which could be lowered while under way to a depth of 1.5 m was clamp in a set of gimbals at the stern to allow lateral swing of the housing. Water was pumped continuously through the cuvette in the fluorometer. Fluorescence was indicated on a servo dia on the fluorometer and recorded continuously on a Rustrac Mode recorder. Each day that a complete sampling program was carri out, a continuous record was made of dye concentration at a dep of 0.3 m from station A through stations 1 - 5 and B to statio C; from station 2 through D to F; along each of the 5 transver sections containing stations 1 through 5; and from station 3 o of the mouth to the end of detectable dye concentration (Fig. A continuous record was also made of dye concentration at a de of 1 m from station 1 through stations 2, 3, and 4 to station and dye concentrations were determined from surface to bottom using another submersible pump lowered on hydrographic wire at each station, 1 - 5. Due to difficulties with the equipment,

complete data were not obtained before May 30, but were obtained for 11 days during the following three weeks. The data were corrected for background fluorescence and temperature and plotted on separate charts for the depth intervals 0-0.5 m, 0.5-1.5 m, 1.5-2.5 m, and 2.5-3.5 m. Contour lines were then drawn through the plotted data so as to divide each depth interval into horizontal segments containing homogeneous or nearly homogeneous water. The area of each segment was then found by planimeter and this area, multiplied by its dye concentration and depth interval, gave the mass of dye in each segment. The masses were then summed to give the total mass of rhodamine in the embayment for that day.

The following are some of the sources of error in the determination of exchange by the above technique.

During the entire experiment the background fluorescence was assumed to be 15 parts per trillion rhodamine B equivalent on the basis of measurements on May 22. During the course of the experiment, the background of the waters outside the embayment dropped to 10 parts per trillion and remained there. Since this is the water than entered the embayment during exchange, the actual exchange was slightly lower than that calculated. This difference of 5 parts per trillion is less than 0.5% of the mean concentration on June 5 and less than 1.0% of the mean concentration on June 19. Its effect on the mean exchange rate was therefore neglected.

Since tide records were not available for Lake Ogleton, the actual effect of the height of the tide on the dye concentration is not known. On the basis of tide records for Baltimore and Solomons Island (Fig. 4) and tide predictions for Lake Ogleton, this error is assumed to amount to no more than a few percent of the calculated values.

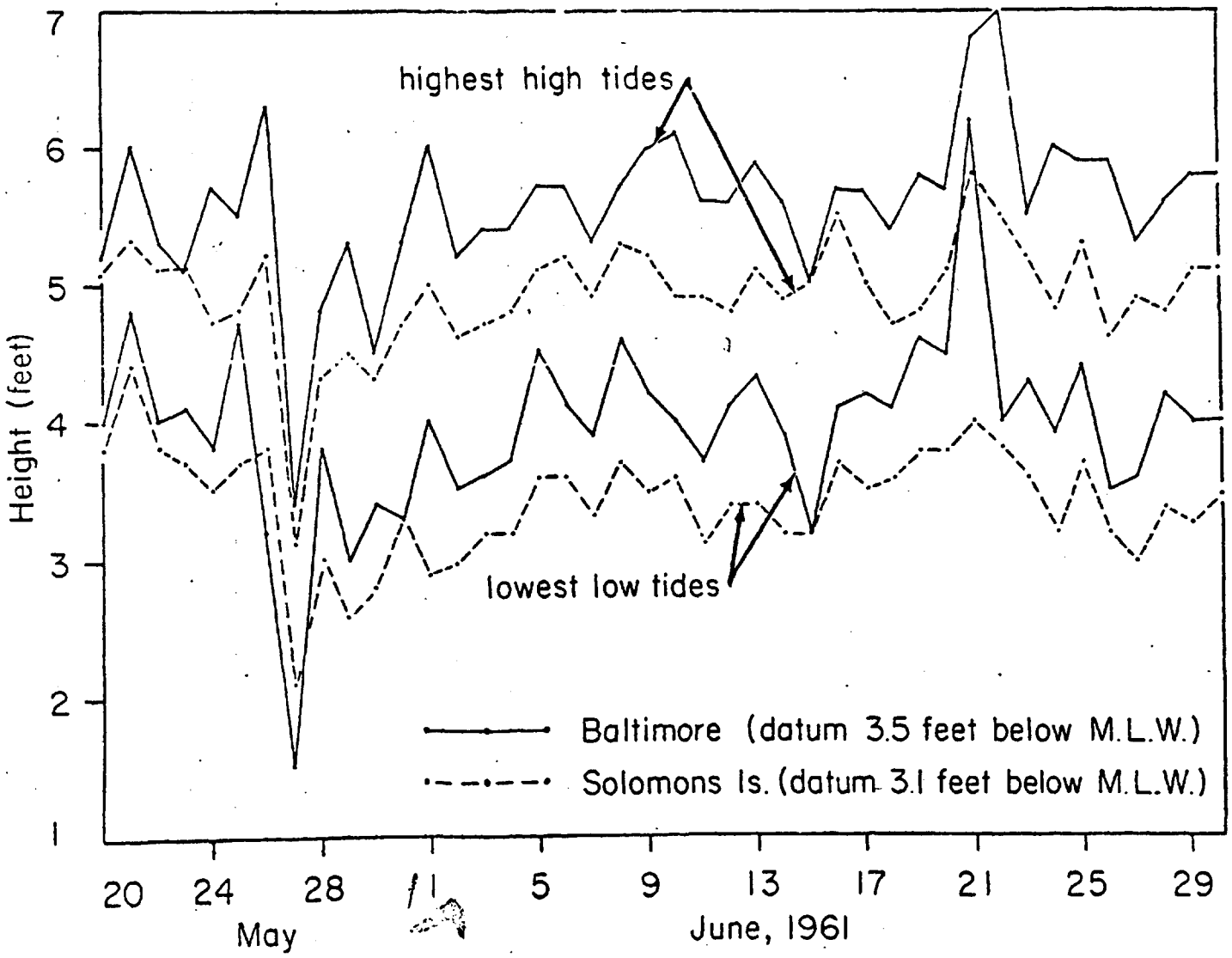

Figure 4. Daily highest high and lowest low tides at Baltimore and Solomon's Island, Maryland. From U.S.C. & G.S. tide records.

The effect of absorption and release of rhodamine B by bottom sediments and suspended solids is not known. If dye was absorbed during the first period of larval development, June 5 through June 19, the calculated exchange is higher than the real value. If, on the other hand, dye which was absorbed before June 5 was released to the water during the next two weeks, the calculated exchange is too low.

The fluorometer used was calibrated before and after the field work and found to be stable in use.

The errors introduced in plotting and contouring the raw data were estimated by deliberately biasing the contours to give both a maximum and a minimum possible concentration value for the data on June 5, and a similar pair of extreme values for June 19. The difference in percent of dye lost during this period as calculated by using the mean concentrations resulting from high bias on June 5 and low bias on June 19 and vice versa was 2.5%. This was considered small, since the percentage of dye lost during this period as calculated from the unbiased contours was 48.8%.

Some data on the degradation of rhodamine B by light are given by Carpenter (1960). From these data it is evident that a correction for loss by light degradation should be applied to long-term experiments in nature. A preliminary field experiment in which a solution of raw Bay water and dye was bottled and suspended at various depths showed that the loss of dye was much larger than had been expected on the basis of laboratory experiments with distilled water. The following experiment was therefore made to obtain an estimate of dye loss through light degradation. Three sets of Pyrex bottles were suspended at 0.3, 1.0, and 1.6 m for 21 days. One set of bottles contained raw Bay

water and rhodamine B, another contained Bay water which had been autoclaved at 15 psi for 20 minutes and rhodamine B then added aseptically, and the third set contained distilled water and the dye. The Secchi disc values for the water in which the bottles were suspended ranged from 1.2 to 1.5 m during the experiment. The dye remaining in the bottles after 21 days is shown in Table VI. The lower losses in the sterile bottles are thought to be due to absence of bacterial action. More data are obviously needed to determine more accurately the effect of light on rhodamine B degradation in natural waters.

The mean degradation of the dye by light during this experiment, extrapolated to 2.2 meters (the mean depth of Lake Ogleton), is taken to be 15%. In order to use this correction most effectively, some estimate of the deviation about this mean should be considered. The standard deviation is, therefore, considered to be 5% since it is thought to be highly unlikely that the true value of the mean does not lie between 0% and 30% (Table VI).

The calculated amounts of dye remaining in Lake Ogleton for the month following the release on May 24 are shown in Table VII and Figure 5. Tide records from Baltimore (20 miles north) and Solomons Island (40 miles south) show that the maximum tidal ranges for the period occurred on May 26 and the several days following, and on June 21 (Fig. 4). Wind velocities were highest during these times. The curve showing loss of dye can thus be explained as follows. The greater loss of dye during the first few days following its introduction is due to the fact that in the initial dye distribution most of the dye was in the upper two meters. Each ebb therefore removed a greater fraction of dye at this time than later. During the last few days of May, large tidal ranges and high winds both removed large quantities of dye and mixed the remaining dye more homogeneously in the

Table VI

Degradation of rhodamine B *versus* depth.

Dye solution	Depth (m)	Loss in 21 days (%)
Distilled water	0.3	27.8
	0.3	28.0
	1.0	19.0
	1.6	15.9
Raw Bay water	0.3	41.7
	1.0	33.0
	1.6	28.6
	1.6	27.4
Sterile Bay water	0.3	20.1
	0.3	18.8
	1.0	8.8
	1.6	5.2
	1.6	4.5

Table VII

Dye remaining in Lake Ogleton
(not corrected for light degradation).

Date	Total dye (grams)
May 24	13600 *
30	2430
June 1	1159
2	746
5	384
7	346
8	336
12	262
14	259
18	212
19	198
22	118
26	98

* This figure is the amount of dye introduced. All others are calculated from sampling.

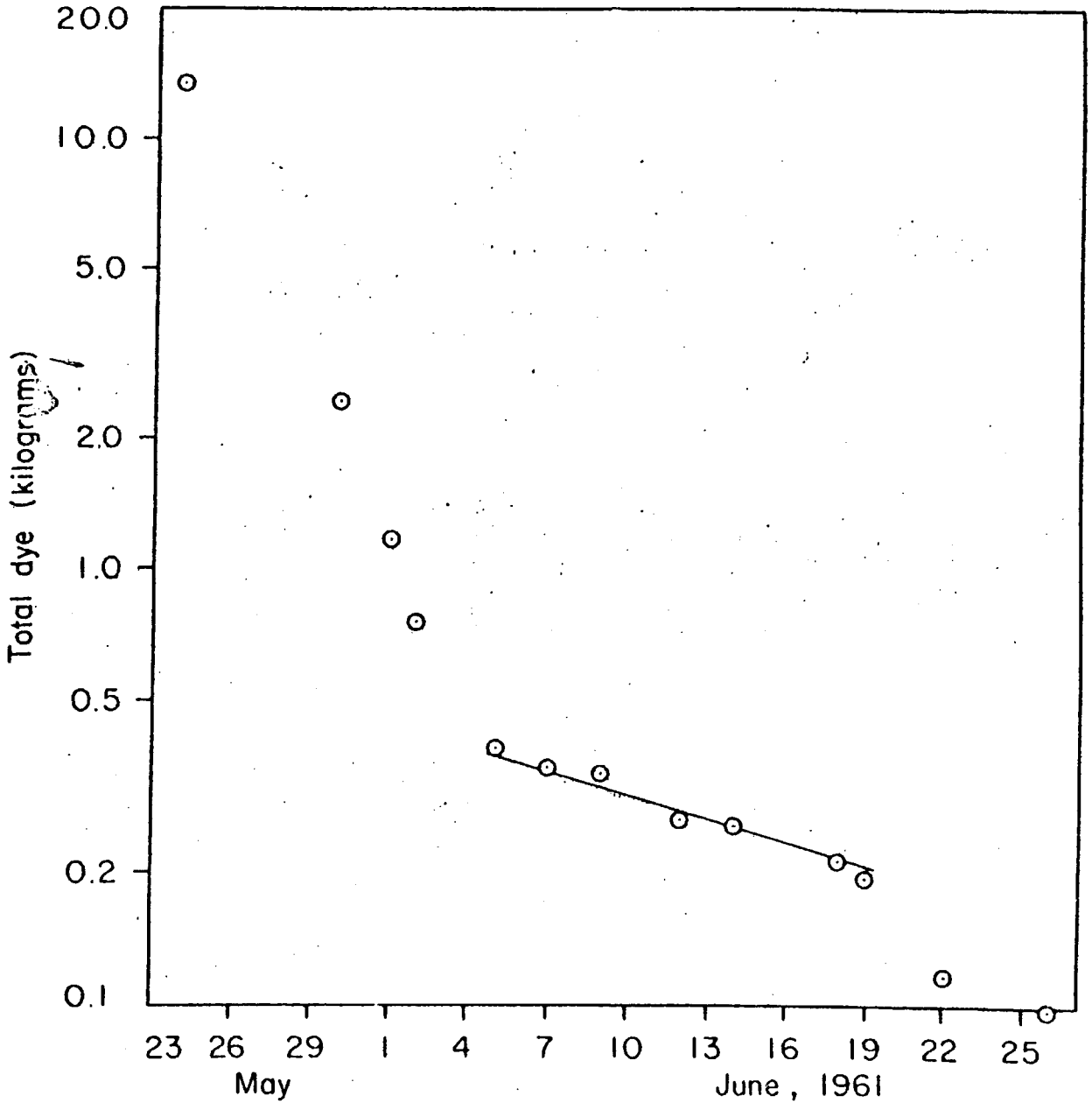

Figure 5. Dye remaining in Lake Ogleton. Value shown for May 24 is weight of dye introduced.

embayment. During the next two weeks both winds and tidal ranges were minimal and the rate of loss of dye was therefore also a minimum. On June 21 winds and tides were again high resulting in a sharp increase in dye loss. The mean rate of dye loss between June 5 and June 19, the period of first larval development, was calculated by regression analysis to be 2.29% per tidal cycle with a standard error of estimate of 0.64% per tidal cycle. Figure 5 shows this regression line.

Quantitative Plankton Collections

Zooplankton was collected daily by towing a nylon net 35 cm in diameter at the mouth and with square mesh openings of 250 μ on a side. The net was equipped with a 500 ml bottle which was also used for storage of the sample after preserving in 3% formalin neutralized with basic magnesium carbonate.

The determination of the area to be sampled outside the embayment so as to obtain an estimate of the larval concentration in the water flowing into Lake Ogleton was made with free-floating current drogues (Pritchard and Burt, 1951). The drogues consist of two perpendicular 1.6 m by 0.6 m planes fixed in the form of an X and hanging vertically 0.6 m below a small float. At wind velocities below 5 knots, the movements of these drogues are considered to indicate current velocity and direction. On both ebb and flood the direction of the current paralleled the shore for some 800 m off the mouth (Fig. 6). The maximum velocities immediately outside the mouth were 0.4 knot (0.2 m/sec) at mid-tide. During the ebb, water from Lake Ogleton ran closely along the shore in very shallow water until it reached Tolly Point (Fig. 6). After passing Tolly Point, it joined the high-velocity ebb of the main part of the Bay and was almost certainly permanently lost to Lake Ogleton. About half the water leaving the embayment

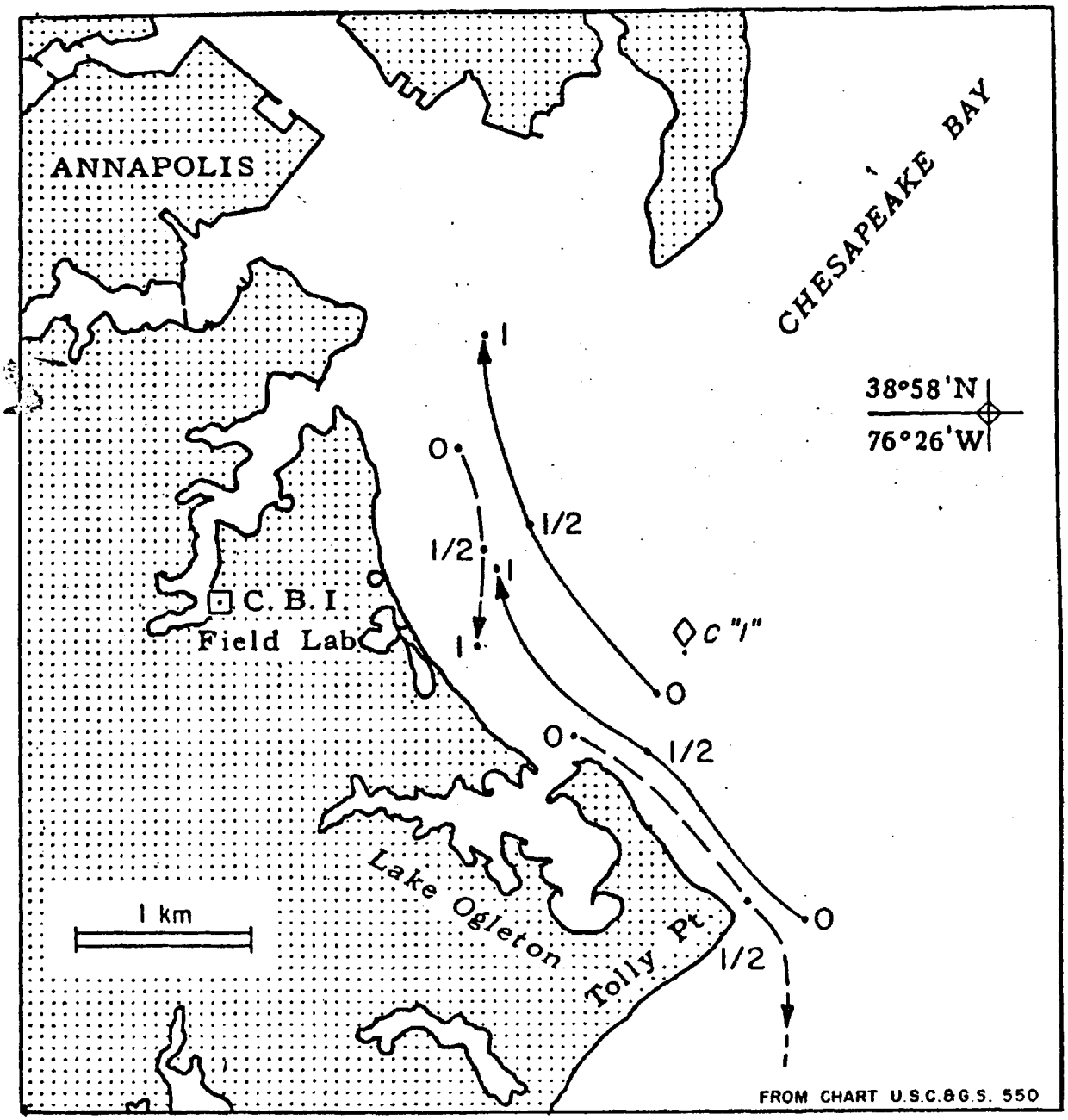

Figure 6. Lake Ogleton and surrounding area showing streamlines of tidal current as indicated by drogues. Figures indicate fractions of ebb and flood tides.

on each ebb was calculated to pass Tolly Point. The path of the water on the flood tide is from Tolly Point close along the shore toward the mouth of the embayment. It should be pointed out that these results do not necessarily describe the "normal" or mean tidal movement of the water but rather the movement during periods when wind conditions allowed measurements by free-floatir drogues. The actual movement of water during any ebb or flood may differ, perhaps drastically, from the above description depending on wind conditions.

Based on the above information, plankton collections were planned from station I to beacon 1, beacon 1 to station II, and beacon 1 to can 1 (Fig. 2).

The routine tows for determining larval concentration within the embayment were always made from station 1 to station 4 and, immediately following, from station 4 to station 1. During these tows, the mouth of the net was kept at a depth of about one meter Each entire sample was examined under a dissecting microscope and the larvae of each stage counted. The results of these counts for Lake Ogleton are shown in Table VIII and Figure 7. The concentration of larvae is shown as larvae per cubic meter. This concentration was calculated by dividing the number of larvae in the sample by the volume of water represented by a cylinder with the diameter of the mouth of the net and the length of the tow. Thus the true concentration of larvae was almost certainly higher than these values. Since the net was kept clean throughout the investigation and did not shrink, the difference between the volume represented by the above cylinder and the volume of water actually passing through the net is assumed to have remained constant. The two tows in opposite directions were taken to minimize the effects of water movement and are treated as one sample. The differences between these two tows (about 20%) were

Table VIII

Daily concentrations of larvae in Lake Ogleton (larvae per meter^3). Underscored numbers refer to larvae considered to have been hatched June 5.

Date	Zoeal Stages				Megalopae
	1	2	3	4	
June 2	0	0	0	0	0
3	0.1	0	0	0	0
4	3.5	0	0	0	0
5	<u>29.7</u>	0	0	0	0
6	<u>25.0</u>	0.1	0	0	0
7	<u>22.3</u>	0.1	0	0	0
8	<u>23.3</u>	0.1	0	0	0
9	7.6	<u>20.7</u>	0.1	0	0
10	2.0	<u>19.0</u>	0.1	0	0
11	4.4	<u>18.2</u>	3.7	0	0
12	12.5	2.2	<u>14.0</u>	0.1	0
13	32.1	0.1	<u>14.6</u>	0.2	0
14	24.2	1.0	5.1	<u>13.2</u>	0
15	24.7	0.3	0.4	<u>13.1</u>	0
16	17.0	7.1	3.2	<u>11.6</u>	0
17	10.8	18.2	1.0	<u>11.0</u>	0
18	16.4	23.6	5.7	<u>10.8</u>	0.1
19	18.9	20.9	2.3	4.1	0.6
20	27.5	26.8	13.2	0.2	0.1

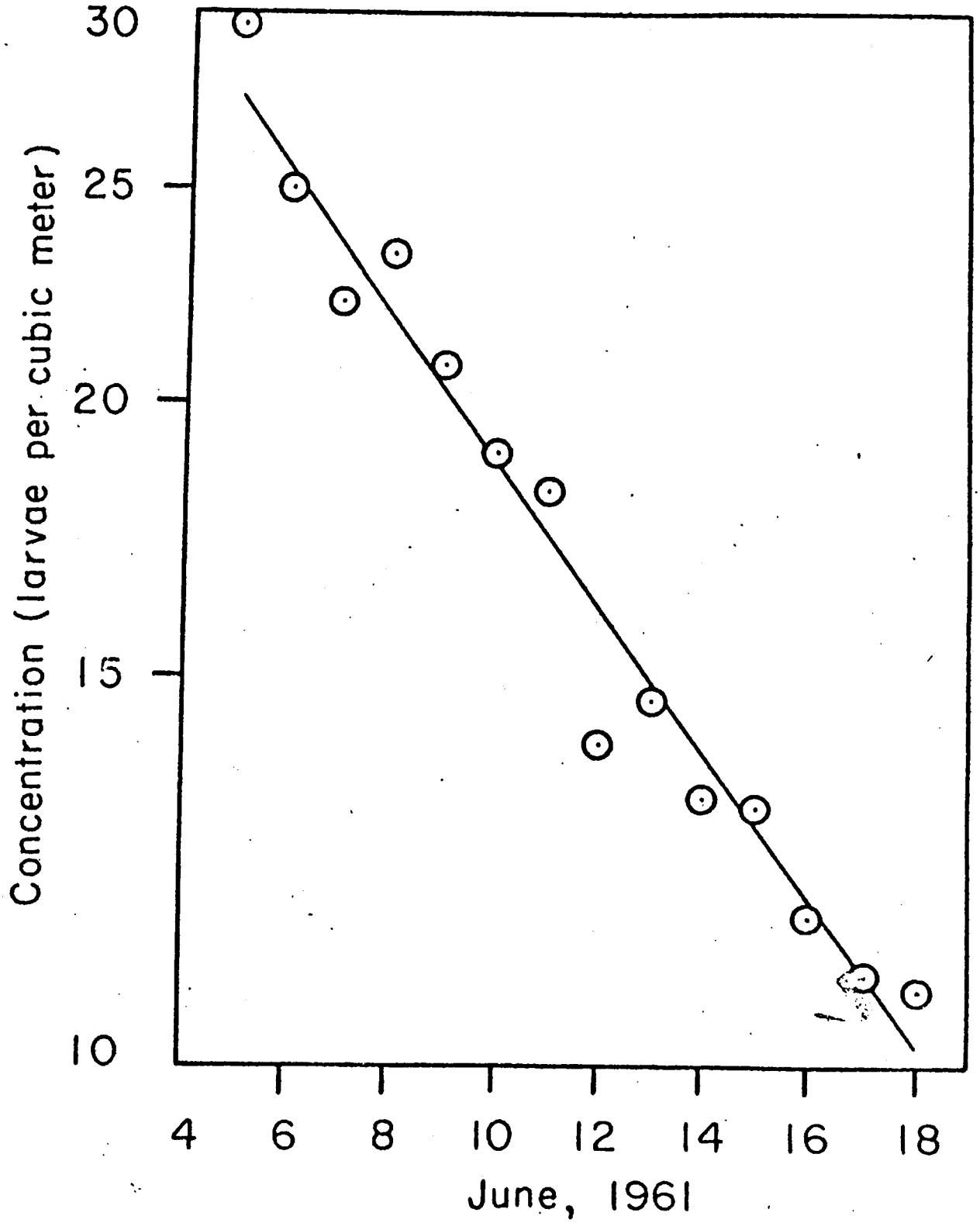

Figure 7. Observed concentration of larvae in Lake Ogleton.

found to be no greater than has been reported for simultaneous collections (Barnes, 1949).

The underscored numbers in Table VIII show the concentration of larvae considered to have been hatched on June 5. Figure 7 is taken from these underscored numbers.

The mean rate of disappearance of larvae was calculated from these data by regression analysis to be 3.84% per tidal cycle with a standard error of estimate of 0.88% per tidal cycle. Figure 7 shows this regression line. The standard error of estimate here, as in the calculation of dye loss, reflects deviations from the mean caused by sampling errors as well as real deviations from the mean rate. There is no reason to expect mortality to be constant or to be normally distributed about a mean value.

The decrease in concentration of the dye (due to light degradation and to exchange) and the larvae is assumed to be governed by the following equation:

$$\frac{dC}{dt} = -kC$$

where C = concentration

t = time (in tidal cycles)

k = rate constant

Integrating this equation, one obtains the equation used in the computation of the three rates:

$$\ln \frac{C}{C_o} = - kt$$

The rate constants are assumed to be additive.

The mortality of the larvae is defined here as the disappearance of larvae not accounted for by loss due to exchange of water. It is, therefore, the difference between the observed rate of loss of larvae based on plankton collections and the

calculated rate of loss of larvae through exchange. Since the concentration of larvae in the water outside the embayment never exceeded 10% of the concentration inside, this correction was neglected. The three rates and their standard error of estimate are:

observed loss of larvae =
3.84% per tidal cycle $\pm$ 0.88% per tidal cycle;
observed loss of dye =
2.29% per tidal cycle $\pm$ 0.64% per tidal cycle;
light degradation of dye =
0.39% per tidal cycle $\pm$ 0.10% per tidal cycle.

Thus the mean mortality of the larvae is 3.84% - 2.29% + 0.39% = 1.94% per tidal cycle. The standard errors of estimate for the three rates are summed to give a range of 0.32% to 3.56% per tidal cycle which has a probability of 0.31 (if the distribution are normal, $p = (\sigma)^3 = (0.68)^3 = 0.31$.) The total mortality is, therefore, 42% with a range of 8.6% to 63.1% ($p = 0.31$). Since each standard error of estimate is larger than it would be if based solely on experimental error (the actual rates in nature are not constant), the true value of the mortality almost certai lies within the above range and very likely lies even closer to the mean.

DISCUSSION

The use of copepod nauplii in culturing provides a much more natural food source than the usual Artemia nauplii. While it is gratifying to see that larval development requires about the same time with these two food sources, copepod nauplii are preferable for use in further experimentation. This is particularly true for feeding experiments which clearly need to be done. The apparent inhibition of larval development by algae has been noted previously in other xanthids (Chamberlain, 1961) and in the shrimp Palaemonetes (Broad, 1957), but the mechanism is entirely unknown. The experimental results can be explained by a restriction of intake of valuable nutrients by ingestion of nutritionally inert material. This is the explanation postulated by Broad. Algae are probably not toxic to the larvae since they live longer when fed algae than when not fed at all. It is possible that algae lack an essential nutritional factor or that such a factor if present is not obtainable by the larvae.

Quantitative feeding experiments, which have never been carried out with decapod larvae, should help to clarify this inhibition. The efficiency with which the larvae obtain energy from algae and nauplii could be examined and crude analyses of the diets could be obtained. Eventually synthetic diets might be attempted in order to define essential nutrients.

Developmental times of the larvae in nature were found to be in good agreement with results of laboratory culturing at comparable salinity and temperature. Comparison of salinity ranges in nature with those which permit successful culturing in the laboratory awaits studies of the relation between salinity and larval distribution patterns in nature. The effects of various

salinities on the adults and on hatching are not known. The present work indicates that the presence of adults in fresh water is probably due to migration after the crab stages have been reached.

The importance of larval culturing in systematics has been discussed previously.

The success of the field work was dependent in part on the low and relatively constant exchange rate during the period of observed larval development. While this situation existed partly through chance (e.g. low wind velocities in mid-June, 1961) it should be noted that the simultaneous appearance of the first large population of larvae and the first low exchange rate in June is not necessarily a coincidence of independent events. If the first actual spawning occurred during the time of high exchange the larvae may well have been dispersed into areas outside the embayment and so not observed at all.

The calculated mortality of the planktonic larvae is much lower than was expected. If the mortality of these larvae is generally this low, very high mortalities obviously must occur in the postlarval megalopa or early crab stages since each adult breeding pair of crabs produces several thousand larvae in a season. Previous work (Chamberlain, 1961) with laboratory culture of Neopanope texana sayi demonstrated that of 160 isolation cultures over half of the 16.9% total mortality occurred in the moult from the fourth zoea to the megalopa. Since plankton collections do not adequately sample megalopae, the data presented here do not help to clarify this problem. Sampling methods adequate for quantitative collecting of the megalopa and early crab stages need to be developed.

The combination of methods used in this study should be useful for the study of many estuarine planktonic populations.

SUMMARY

1. The larvae of Rhithropanopeus harrisii were successfully reared in isolation from eggs to the crab stages.
2. A morphological description of larval appendages of Rhithropanopeus harrisii is given, confirming and complementing an earlier general description (Connolly, 1925).
3. The larvae hatched as first-stage zoeae and developed through three more zoeal stages and one megalopa before metamorphosis to the first crab stage.
4. The effect of temperature and salinity on both the developmental time and the mortality of the larvae is described. Laboratory cultures showed that optimum salinities for larval development were 6‰ to 10‰.
5. The duration of the larval stages was twice as long when the zoeae were fed copepod nauplii and algae as when fed nauplii alone. Larvae did not survive when fed algae alone but lived longer than those not fed at all. The mechanism of the apparent inhibition of development by algae is unknown.
6. The exchange of water in an embayment containing a natural population of larvae with adjacent Chesapeake Bay waters was estimated by the use of an introduced tracer. This exchange, along with daily plankton data, was used in the estimation of developmental times and mortality of the larvae in nature. Natural developmental times were found to be similar to those found in the laboratory. Natural mortality was found to be much lower than was expected and the implications of this are discussed.

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