INSTAR BEHAVIOR OF
CHAOBORUS PUNCTIPENNIS SAY

by

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INTRODUCTION

Larval instars of Chaoborus exhibit a behavioral pattern unique in Class Insecta, that of diurnal vertical migration. The first two instars are entirely planktonic while the third and fourth are benthic during the day and planktonic at night. This is a study to determine any behavioral differences between the larval instars during the vertical migration.

Generally, chaoborid adults first appear in early May, and after two or three consecutive days of warm quiet weather, swarms are likely to occur. Emergence begins in early evening and continues throughout the night with adults dispersing over a wide area. During daylight hours they may be found quiescent in bushes and shrubs, but as the sun sets and the wind dies down, mating begins. After mating, females fly in swarms over the lake where oviposition occurs (Lindquist *et al*. 1943). In the warmer temperatures of shallow water (20 to 24°C.) the eggs will hatch within 24 hours while those eggs in the deeper cooler waters will not develop as rapidly (Juday 1921).

The young larvae are transported by currents out into the lake where they are entirely planktonic, occupying the hypolimnion during the day and migrating into the upper water at night (Lindquist *et al*. 1943). The first and second larval instars exhibit this behavior until they are approximately one-third grown (8 to 15 days). They then enter the mud and spend about one-half their time as a member of the benthic community and about one-half their time as a member of the planktonic community. The larvae take part in the diurnal migration with a maximum of 50 per cent of the benthic larval population leaving the mud shortly after sunset, migrating to the surface waters to feed, and returning to the mud shortly after sunrise. Duration of the third and fourth instar may be from 2 to 36 weeks depending upon the time of year the eggs are oviposited. Juday (1921) found that the larvae of Chaoborus were predominant in the deeper portions of Lake Mendota Wisconsin, with as many as three times the number within the 20 meter contour as there were within the 8 to 20 meter contour. There is a negligible number in water where the depth does not exceed 5 meters. Most of the pupation, however, occurred in shallower water.

Scott and Opdyke (1941) and Lindquist and Deonier (1942) reported inshore migrations of larvae and pupae before the time of emergence. They suggest that the larvae and pupae migrate to shallower water because the higher temperature of the water favors transformation. The pupal period lasts from two to three days under optimal conditions, with emergence beginning in the early evening in the shallower waters (Deonier 1943). A life cycle of two months may be a general feature of the biology of Chaoborus living under warm summer conditions in temperate climates or under tropical conditions (MacDonald 1956).

Larvae of Chaoborus are more numerous from November to April than at any other time of the year. During this time there are no losses in the overwintering larvae due to pupation, and because the losses due to other causes are not great, the number remains uniformly high. As the water temperature increases in

April the rate of pupation increases and results in a marked decrease in the benthic population during June. This decline in numbers continues until the minimum for the year is reached in August. With the decrease in temperatures during September there is a corresponding decrease in pupation resulting in an over-all increase in the number of larvae (Juday 1921, Lindeman 1942).

Also involved in the seasonal distribution are the ablittoral and adlittoral migrations. During the fall and early winter the abundance of bottom living larvae decreases in the shallow areas and greatly increases in the deeper areas. A shoreward movement of the overwintering larvae occurs during the spring and, for a short time, the largest populations of mature larvae are taken in shallow areas (Wood 1956).

DESCRIPTION OF STUDY AREAS

Saratoga Lake is a eutrophic lake in the Hudson River drainage located in southeastern Saratoga County, New York. It has an area of 10,722 hectares and is one of the largest lakes in New York occurring at lower elevations (62 meters above sea level). The lake is 14 kilometers long and has a maximum width of 2.5 kilometers. Maximum depth is 29 meters with 840 hectares over 12 meters while 3459 hectares are less than 9 meters. It has regular, and for the most part, sandy shores which are free of vegetation except in a few protected bays. The nature of the bottom variously consists of gravel, muck, rock, and sand. The water source is primarily surface water with Fish Creek being the main tributary to the lake (Fig. 1). The history of the lake shows

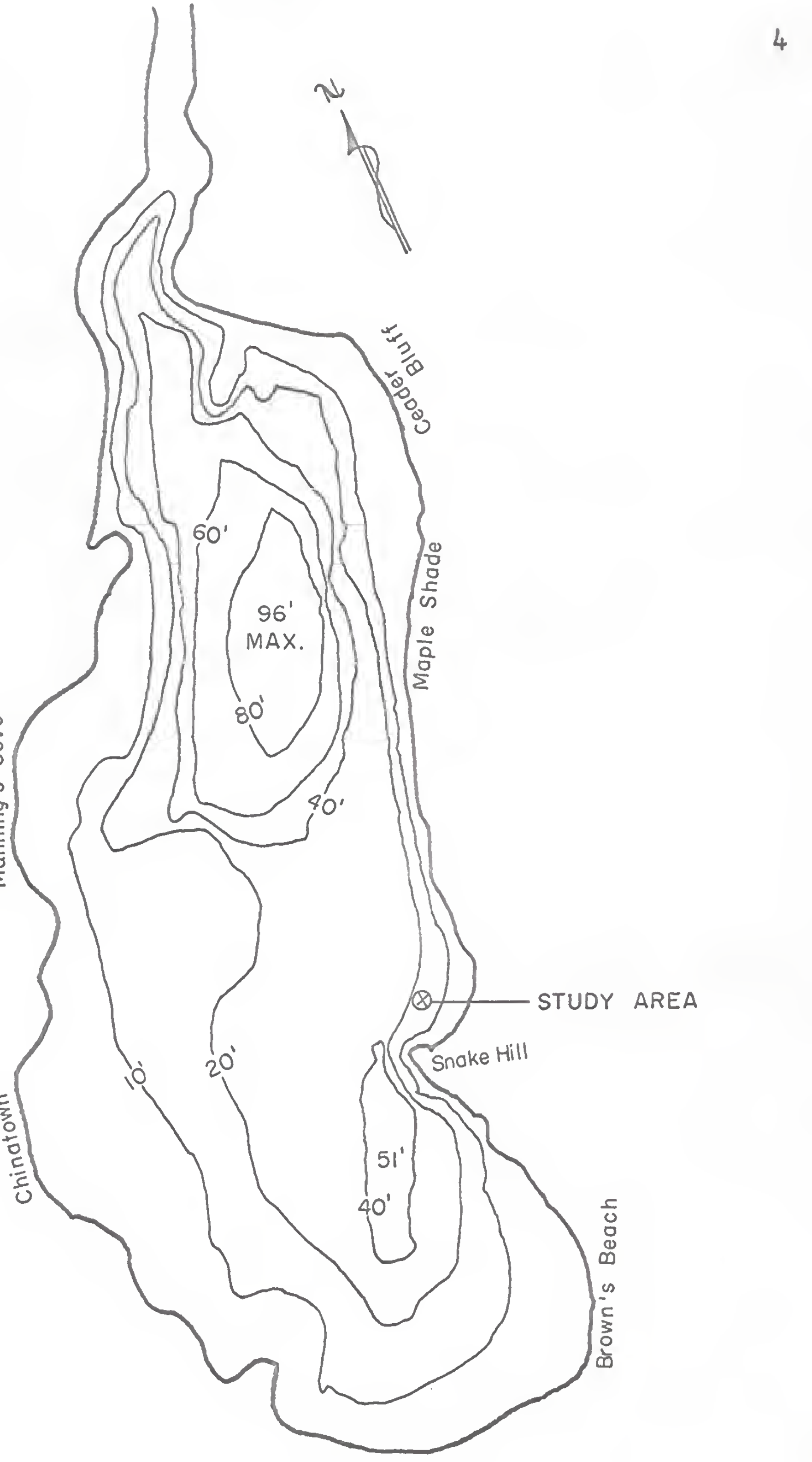

FIGURE I. SARATOGA LAKE, NEW YORK

a high rate of production. The lake is slightly alkaline with a surface pH of 8.2 to 8.5 with no free carbon dioxide, while the bottom pH is 7.1 with free carbon dioxide present. Summer stratification occurred with all but 17.5 per cent of the oxygen removed during the summer months (Adams 1932).

Blue-green algae were most abundant in warm waters of Saratoga Lake during the latter part of the summer. Heavy and persistent alga blooms were apparent through August and early September. There were heavy growths of Lyngbya, Aphanizomenon, and Coelosphaerium. Numerous species of insects were present, with an abundance of Chaoborus punctipennis Say. During the months of June, July, and August the latter are in such great numbers that they become a nuisance to residents and summer campers. Larvae of this species comprise a major portion of the zooplankton collected during the hours of darkness. Also found in great numbers in the zooplankton population were copepods (Diaptomus and Cyclops), cladocerans (Bosmina and Daphnia), and rotifers (Polyarthra and Synchaeta).

Tuttle Creek Reservoir is located in northeast Kansas, on the Big Blue River, in Riley, Pottawatomie, and Marshall counties. Construction of the dam was completed in 1962, and the reservoir reached the top of the conservation pool April 29, 1963. The conservation pool is at an elevation of 1075 feet (328 meters) above sea level, and the reservoir at this level covers an area of 21,480 hectares with a maximum depth of 21 meters. The reservoir is 38 kilometers long and has a maximum width of 2.5 kilometers (Fig. 2).

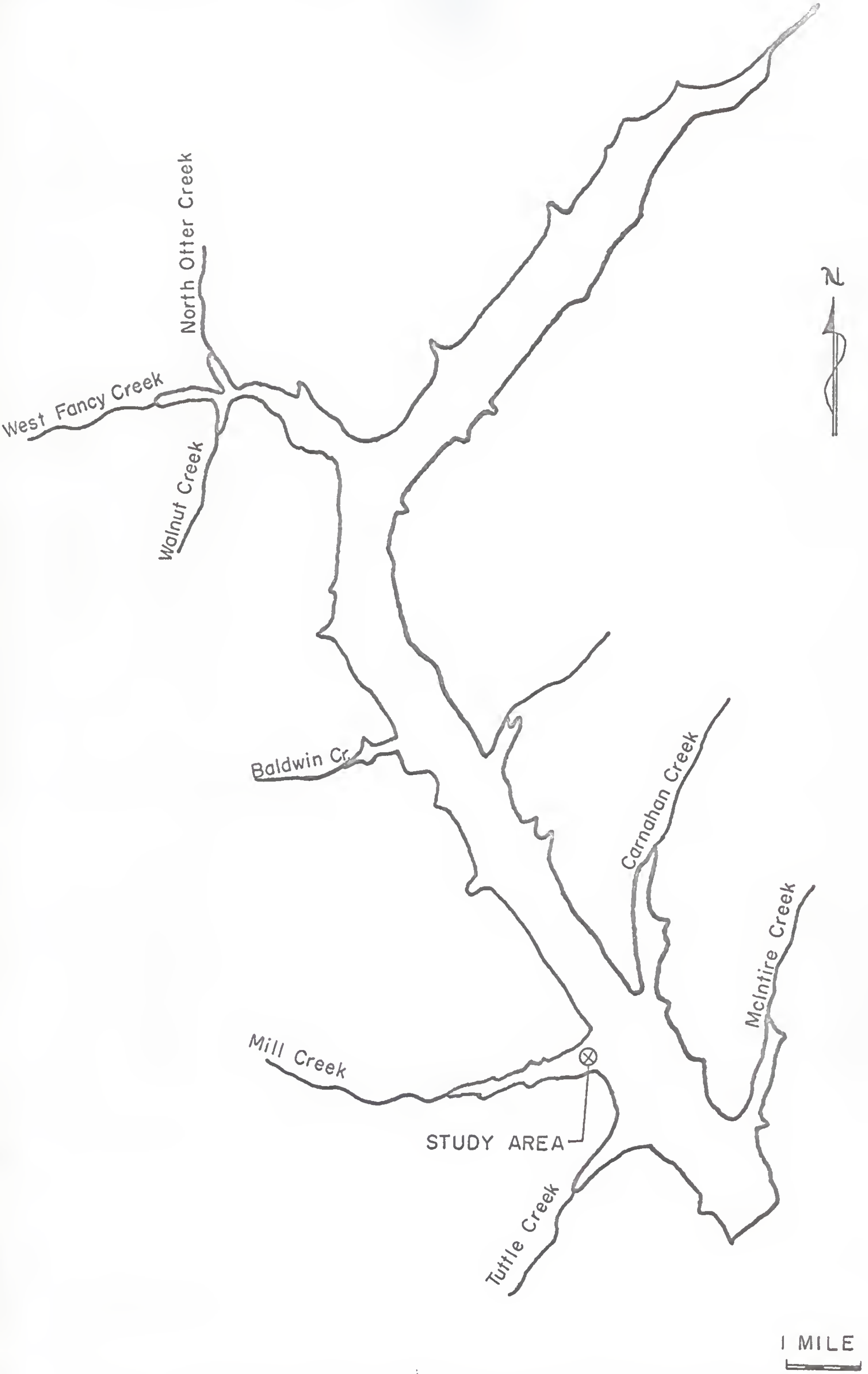

FIGURE 2. TUTTLE CREEK RESERVOIR, MANHATTAN, KANSAS.

As is true with many reservoirs there was a high initial rate of production due to the large amounts of organic matter and nutrients in the impounded basin. Stratification and oxygen depletion in the hypolimnion may occur for short periods of time during the summer, but strong persistent winds cause frequent overturns. Chaoborus punctipennis Say and cyclopoid copepods are common in plankton samples with the latter being more numerous.

MATERIALS

All samples were taken with a standard plankton tow net with a diameter of 30 centimeters, the bag being made of #10 nylon bolting cloth. A screw cap from a pint mason jar was permanently attached to the cod end of the bag with a radiator hose clamp. This facilitated sampling in that the collecting bottles (#503 pint-size mason jars) could be easily screwed onto the end of the net. A maximum and minimum registering thermometer was used for determination of air temperature and water temperature at various depths. A 15-meter line was calibrated by wrapping friction tape around the line at every meter, with a wider band of tape at 5-meter intervals. A weight was attached to one end. This line was employed in depth determination as well as for the temperature series. A dissecting microscope was used in separating larvae from the sample. Measurements were made with a compound microscope with a calibrated ocular.

METHODS

The first sampling at Saratoga Lake was to standardize procedures and to find a suitable sampling area. The area selected was at the mouth of a protected bay northeast of Snake Hill, where the water was 8 meters deep (Fig. 1). All samples resulted from five minute tows taken by rowing at a constant rate across the mouth of the bay. Points of reference were selected to ensure that all samples would be taken from the same area. To standardize sampling depths a known amount of line was let out, and the boat was rowed at a constant low rate. The exact amount of line necessary to sample the bottom waters was determined empirically by shortening the line by one-third meter on each successive sample until no evidence of bottom materials could be found in the net. One-half this amount of line was released and the boat was rowed at the same rate to sample the waters at approximately 4 meters. To sample the surface waters enough line was released so that the net was just beneath the surface when the boat was rowed at the same rate.

The sampling area selected at Tuttle Creek Reservoir was at the mouth of Mill Creek Cove where the water was 8 meters deep (Fig. 2). Sampling methods were identical to those employed at Saratoga Lake, with the use of a motor boat being the only exception. The Tuttle Creek samples also resulted from five minute tows.

A total of 164 plankton samples were taken from Saratoga Lake during the summer of 1964, and that same autumn 35 samples were taken from Tuttle Creek Reservoir. On each sampling date complete weather data was obtained from the United States Weather Bureau, and a water temperature series was taken at the study area. Sampling was initiated at Saratoga Lake on June 26, 1964. The first samples were taken in water 2.5 meters deep. Samples were taken at the surface every half-hour beginning at 2100 hours. Because of the low yield (25 larvae/sample), the next samples (June 29) were taken in water 4 meters deep. A second series of samples with a low yield (25 larvae/sample) prompted a move into deeper waters, and on July 14, samples were taken in water 8 meters deep. The yield from the latter samples was sufficient in numbers for analysis, and all subsequent samples were taken at this depth. On July 21, the first of two 24-hour sampling periods was initiated at Saratoga Lake. Samples were taken every three hours from 0700 to 1900, then every hour until 0600. Five minute tows were made at three depths: 0, 4, and 8 meters. On August 12-13, a second 24-hour series of samples was obtained in the same manner as were the July 21-22 samples. On October 24, 1964, samples were taken at Tuttle Creek Reservoir every half hour from 1700 to 1900, and then every three hours until sunrise. The same three depths sampled at Saratoga Lake were also sampled at Tuttle Creek Reservoir.

All samples were fixed with formalin in the field and stored for laboratory analysis. In the laboratory larvae were removed from samples with the aid of a dissecting microscope, and were placed in vials for further study. Samples taken at Saratoga Lake were diluted with tap water to facilitate separation of larvae. It was necessary to dilute the August samples 12:1 with tap water due to the amount of algae present. A total of 33,349 larvae were collected during sampling, and each of these was categorized according to instar.

Determination of instar was made with the aid of a compound microscope with a calibrated ocular. Total body and head-capsule lengths were measured for the first 1000 specimens. Total body length measurements were made from the juncture of the prehensile antennae with the head-capsule, to the posterior end of the last abdominal segment. Head-capsule measurements were made from the juncture of the prehensile antennae with the head-capsule, to the suture separating the latter from the thoracic segments. The number of setae on the anal fan was also determined for the first 1000 specimens. The measurement which varied least, and was thereby the most accurate, was that of the head-capsule (Appendix). For the next 2000 individuals, instars were determined by measuring only the head-capsule length. After measuring approximately 3000 larvae, it became an easy task to separate the larvae under the compound microscope merely by a visual survey of the field under low power (100X). Periodically the visual counts were checked by making accurate measurements of the head-capsules, and the accuracy never dropped below 95 per cent with the majority of the checks showing 100 per cent accuracy.

Data obtained from the three 24-hour sampling periods were analyzed by members of the Kansas State University Statistics Laboratory. An I.B.M. 1410 computer was utilized in making Chi-square tests comparing temporal distributions of the immature with the mature larvae, as well as comparing distributions of the third with the fourth instars. The null hypothesis for the analysis was that there was no significant difference in the temporal distribution of the larvae. A 95 per cent C.I. was selected, and all values greater than the Chi-square value for (k-1) degrees of freedom would lead to the rejection of the null hypothesis. The alternative hypothesis was that a significant difference did exist in the temporal distribution of the larvae which would indicate a difference in migratory behavior.

RESULTS

The immature larvae and pupae comprised a greater percentage of the population in shallower water than in deeper water. The greatest numbers of larvae were found in the deeper water, even though transformation and emergence was found to occur in the shallower water (Table 1).

A comparison of temporal distributions indicates a similarity in the behavior of larval instars in shallow waters (4 meters) and deeper waters (8 meters), the only major difference being the total numbers found at each depth.

Table 1. Percentage of pupae, and immature larvae at various depths.

Date	Depth (meters)	Percentage of immature larvae	Percentage of pupae	Larvae per sample
6/26	2.5	13	2	25
6/29	4.0	4	3	25
7/14	8.0	1	.1	1071

On July 21, no larvae were taken at the surface from 0700 to

1600. Immature larvae first appeared at the surface at 1600, increased at 2200, and then underwent minor fluctuations until the maximum numbers were obtained at 0500 (53 larvae). The third instars first appeared in large numbers at 2100, and increased rapidly to their maximum at 2200 (1101 larvae). They decreased until 2400, and then displayed a slight recovery at 0100. They were absent at the surface by 0600. The fourth instars also appeared at 2100, and reached their maximum at 2200 (903 larvae). They decreased in numbers until 2400 when the fourth instar became dominant. Pupae were first observed at 2300 (Fig. 3).

At 4 meters, no larvae were taken until 1500 when the immature larvae first appeared. They gradually increased and reached their maximum at 0400, then decreased with a small peak observed at 0600. The third instars first appeared at 2100 and reached their maximum at 2200. The fourth instars also appeared at 2100 and reached their maximum at 2200. They displayed major peaks at 0200 and again at 0500. The third and fourth instars alternated dominance with the third being dominant from 2200 to 0200, and again briefly from 0300 to 0400. The fourth instars were dominant at all other times (Fig. 4).

Small numbers of immature larvae were found at 8 meters throughout the 24-hour sampling period. They increased at 1600 reaching their maximum at 0400. The third and fourth instars first appeared at 2000 and reached their maximum together at 2100, one hour earlier than at the surface and 4 meters. The third and fourth instars decreased rapidly until 2300. The third instar recovered and reached a second peak at 0300, and then decreased

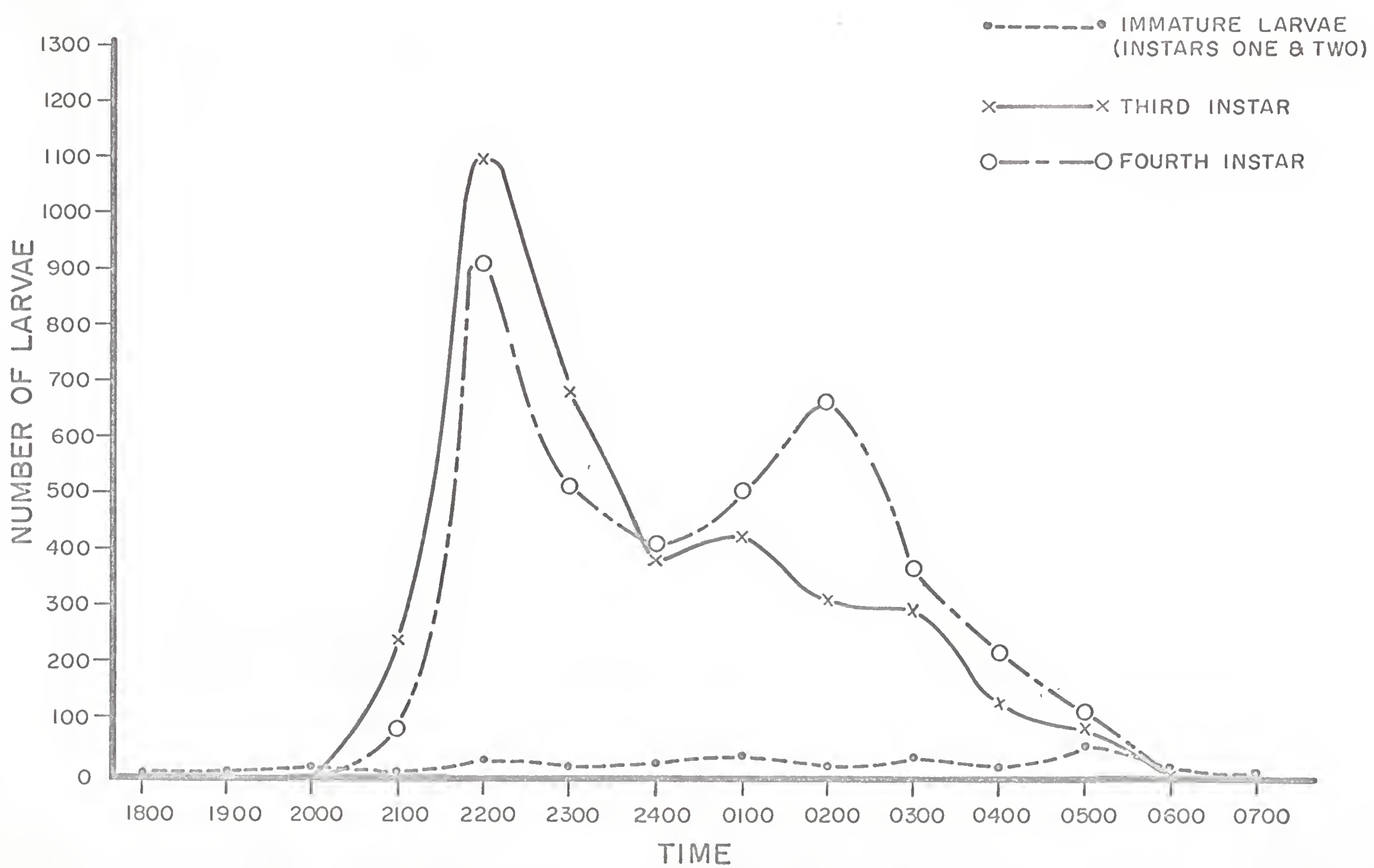

FIGURE 3. TOTAL NUMBER OF LARVAE COLLECTED AT THE SURFACE ON JULY 21-22. (SARATOGA LAKE)

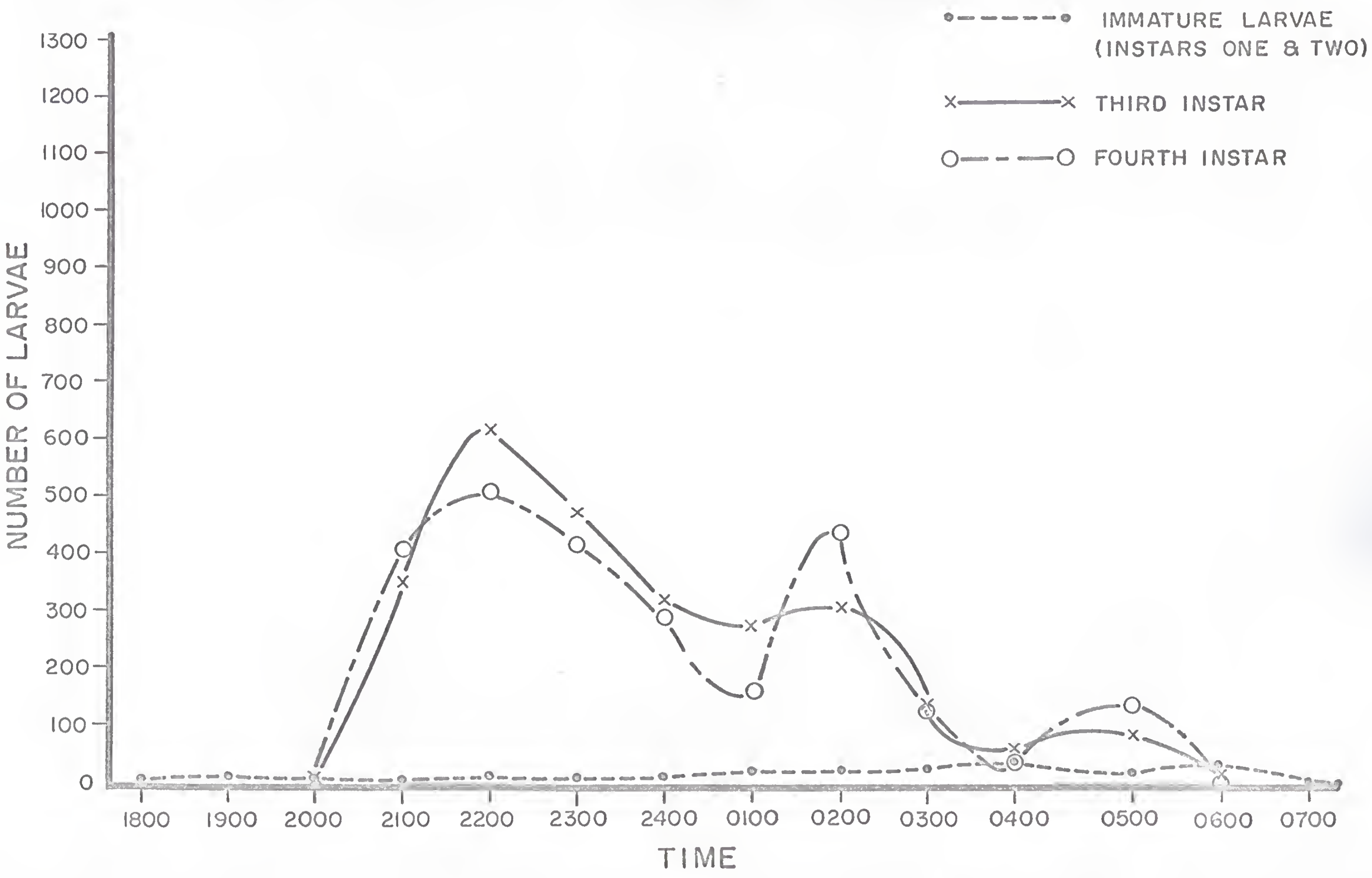

FIGURE 4. TOTAL NUMBER OF LARVAE COLLECTED AT FOUR METERS ON JULY 21-22. (SARATOGA LAKE)

rapidly. The fourth instar also recovered after its 2300 minimum and reached a second high at 0100. It displayed another recovery at 0500, and was absent in the hypolimnion by 0600. The third instar was dominant until 2400 when the fourth instar became the dominant form (Fig. 5).

Chi-square analysis comparing immature larval distribution with that of mature larvae collected on July 21-22 showed a significant difference in temporal distribution at each depth, indicating a difference in migratory behavior (Table 2).

Table 2. Chi-square values for July 21-22 samples (immatures vs. matures).

Depth in meters	Chi-square	DF	P
0	71.04705	16	$<.05$
4	1283.51350	16	$<.05$
8	1550.03170	16	$<.05$

Values greater than 26.30 indicate a significant difference in temporal distribution.

Chi-square analysis comparing the temporal distribution of the third and fourth instars on July 21-22 also indicates a significant behavioral difference (Table 3).

Table 3. Chi-square values for July 21-22 samples (third vs. fourth instars).

Depth in meters	Chi-square	DF	P
0	247.38257	8	$<.05$
4	533.57953	8	$<.05$
8	88.95975	8	$<.05$

Values greater than 15.51 indicate a significant difference in temporal distribution.

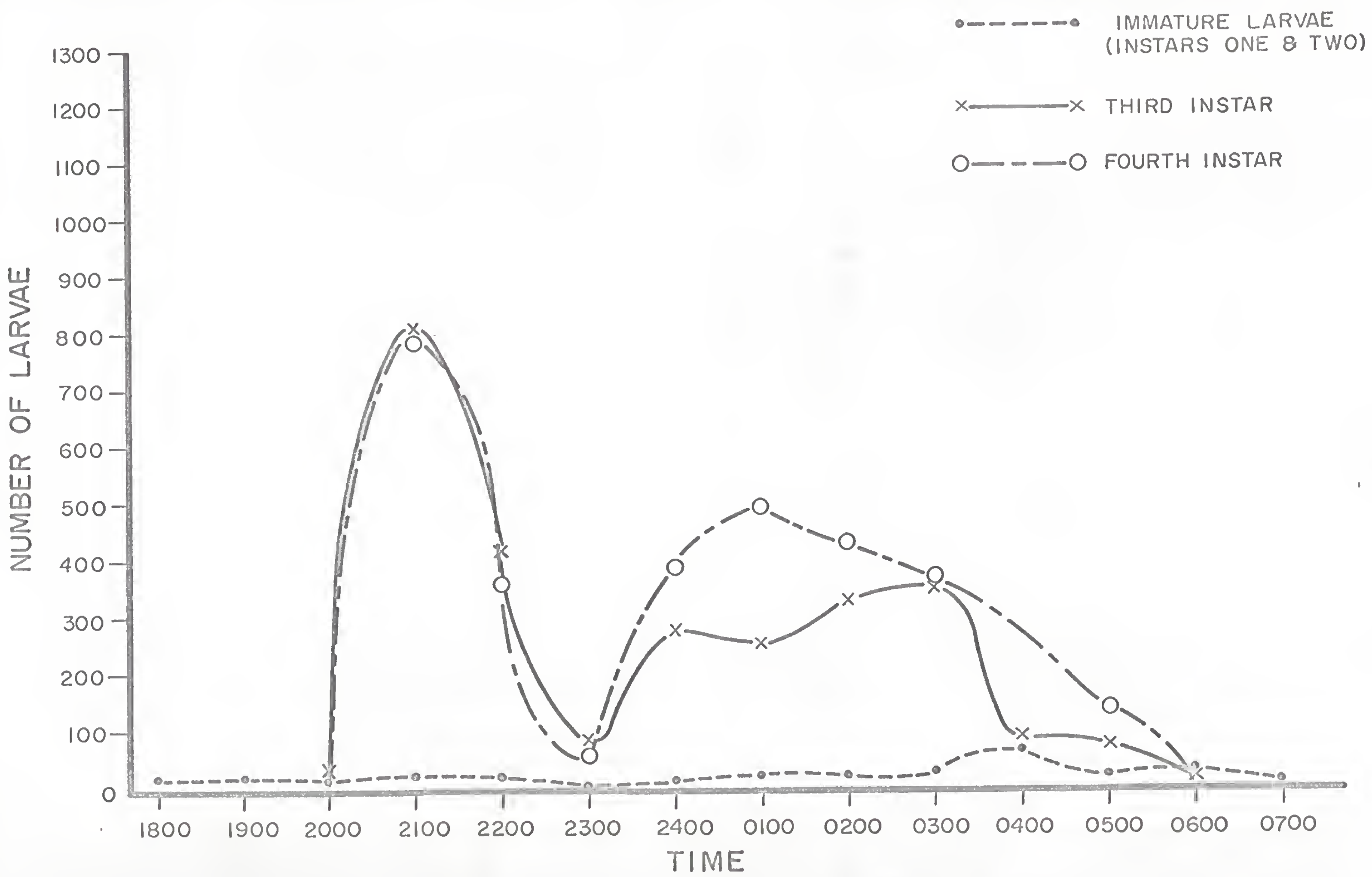

FIGURE 5. TOTAL NUMBER OF LARVAE COLLECTED AT EIGHT METERS ON JULY 21-22. (SARATOGA LAKE)

The results obtained from the August 12-13 samples were similar to the July results, with a few minor exceptions. The numbers of larvae collected were slightly greater in August and the fourth larval instars were dominant in all samples. The surface samples were similar to those taken in July (Fig. 6). The samples taken at 4 meters differed in that the immature larvae remained low in numbers throughout the sampling period, and showed no fluctuations as were observed in July. The third and fourth instars displayed a behavior in August similar to that of July, with the major exception being that the fourth instar was dominant throughout the sampling period (Fig. 7). No larvae were collected at 8 meters until 1200. The immature larvae showed a similar behavior on both sampling dates. A second recovery of the third instars occurred at 0100 rather than at 0300 as was observed in July. The fourth instar again showed the same behavior on both sampling dates. The pupae did not appear in the August sample until 2400, two hours later than observed in July (Fig. 8).

Chi-square analysis comparing the temporal distribution of immature larvae with that of mature larvae on August 12-13 incicated a significant behavioral difference (Table 4).

Table 4. Chi-square values for August 12-13 samples (immatures vs. matures).

Depth in meters	Chi-square	DF	P
0	91.02336	16	$<.05$
4	1245.57810	16	$<.05$
8	1478.17470	16	$<.05$

Values greater than 26.30 indicate a significant difference in temporal distribution.

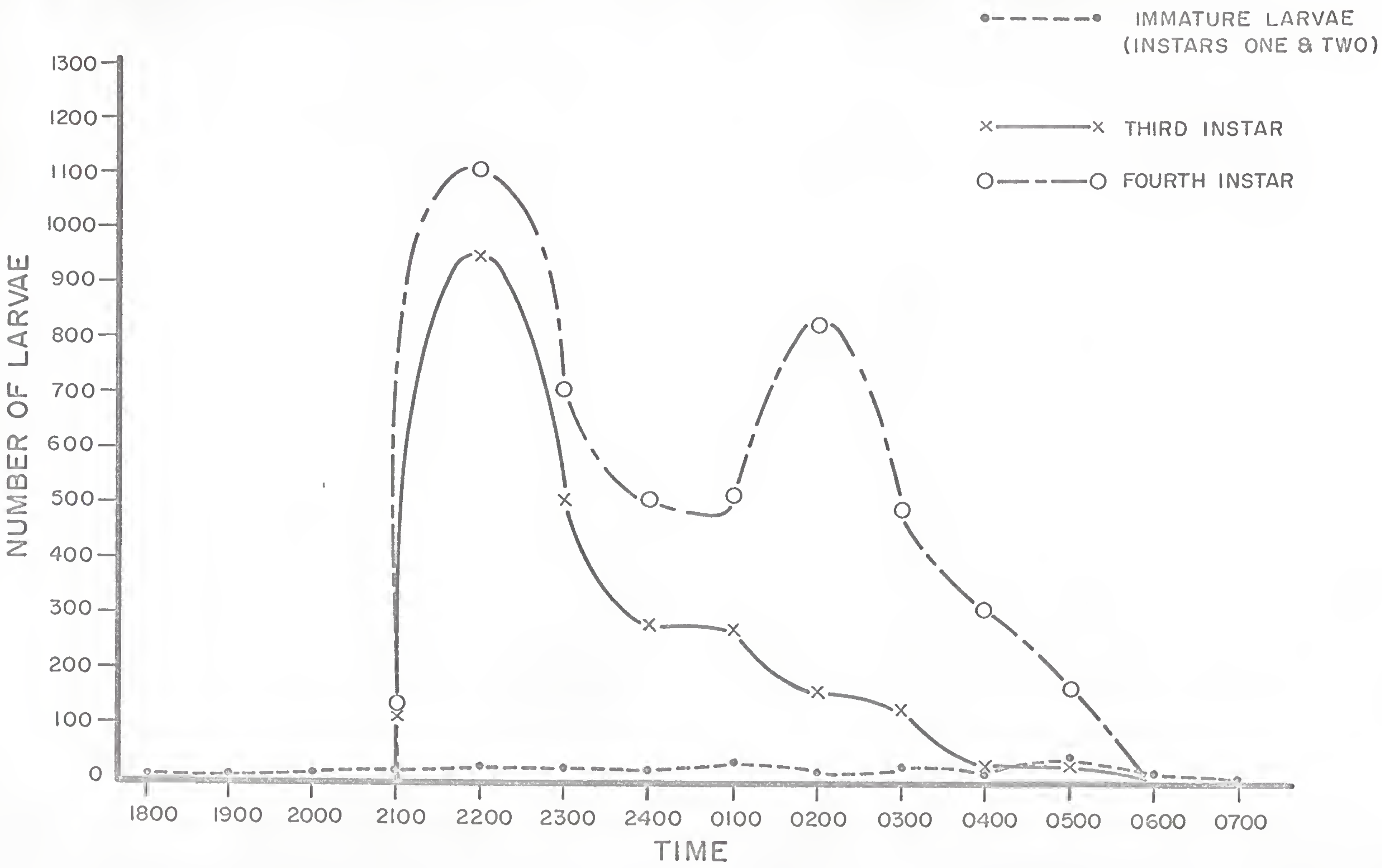

FIGURE 6. TOTAL NUMBER OF LARVAE COLLECTED AT THE SURFACE ON AUGUST 12-13. (SARATOGA LAKE)

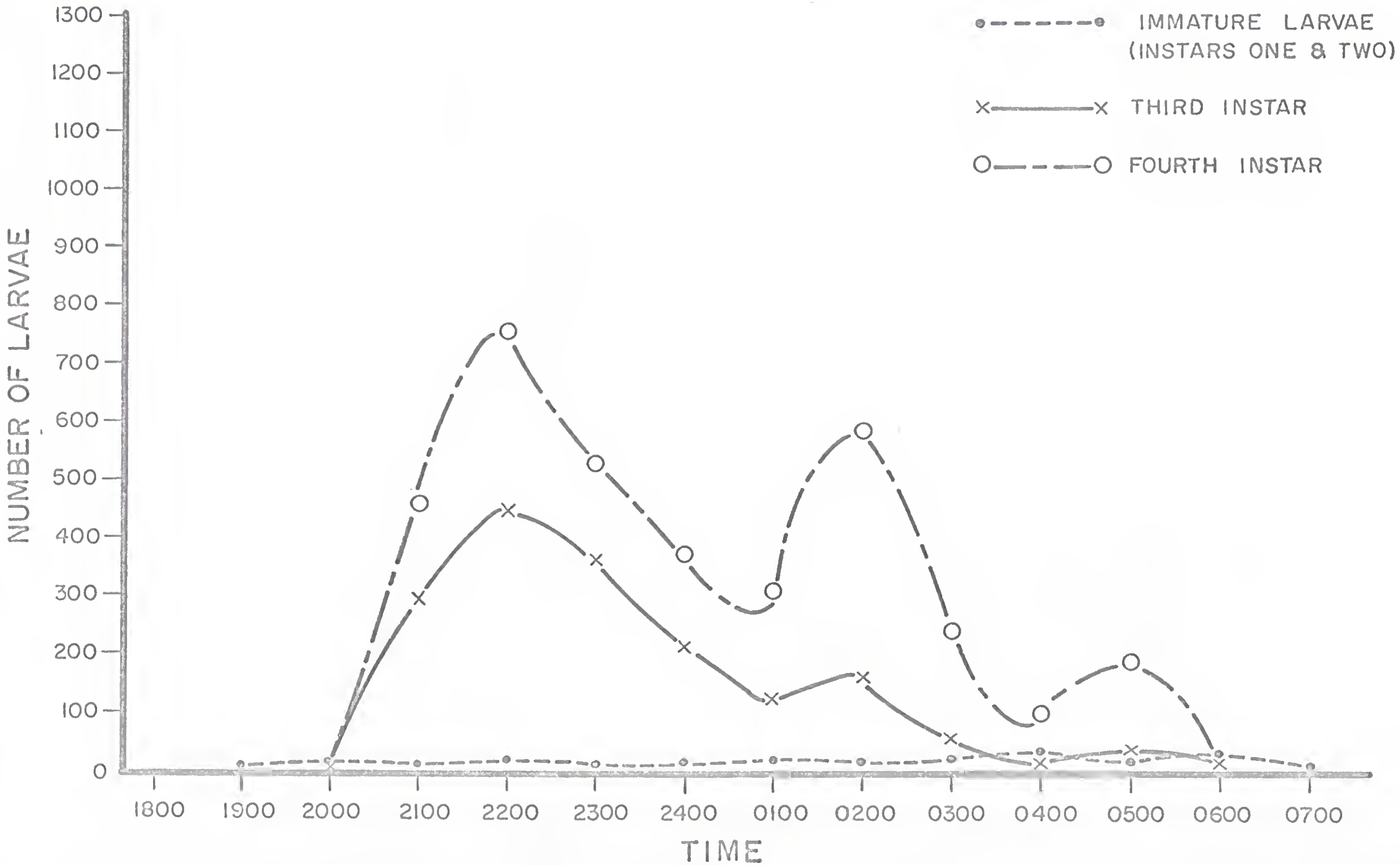

FIGURE 7. TOTAL NUMBER OF LARVAE COLLECTED AT FOUR METERS ON AUGUST 12-13. (SARATOGA LAKE)

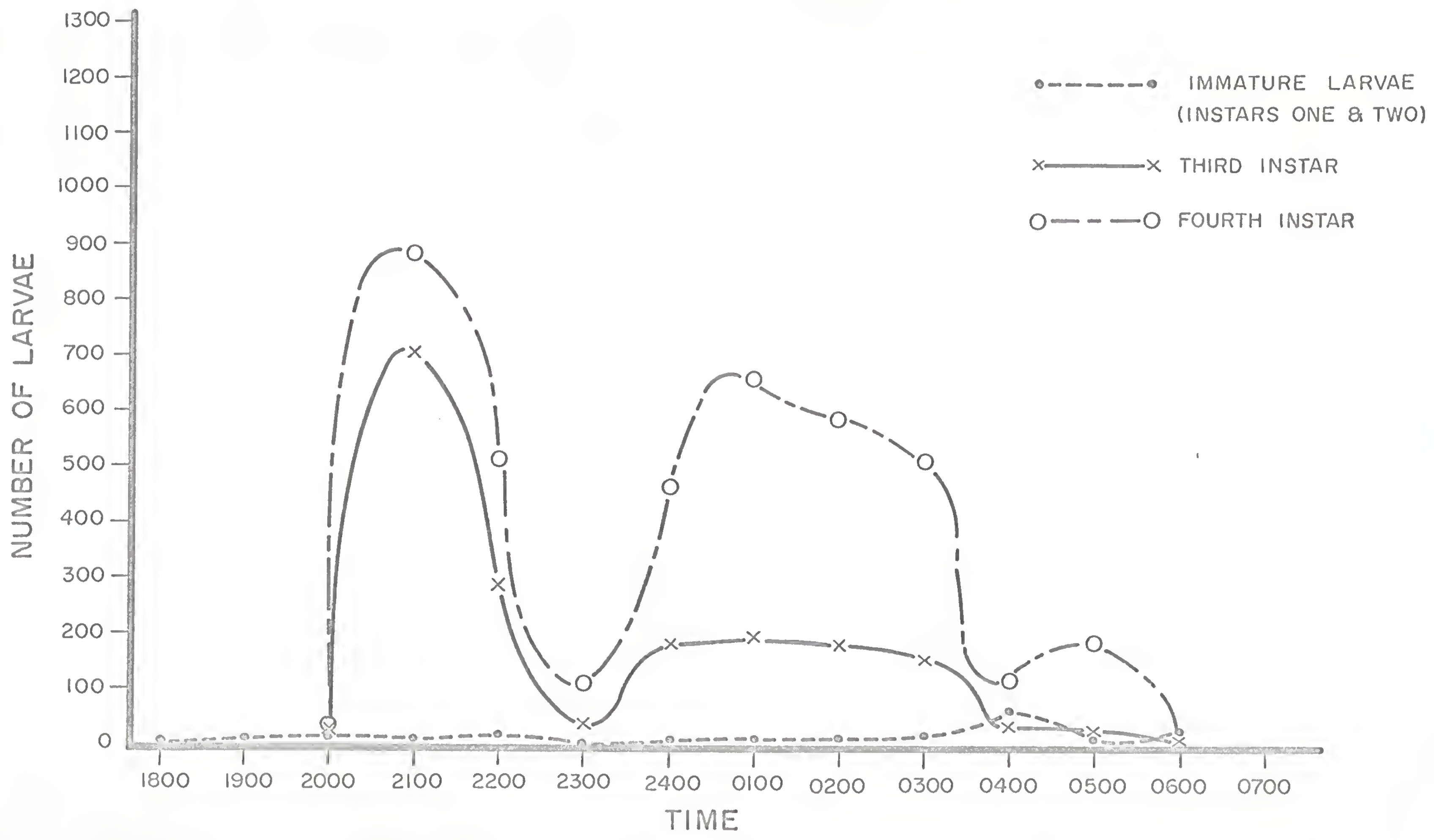

FIGURE 8. TOTAL NUMBER OF LARVAE COLLECTED AT EIGHT METERS ON AUGUST 12-13. (SARATOGA LAKE)

The values obtained from the Chi-square test comparing the temporal distribution of third instar with that of the fourth instar on August 12-13 indicated a significant behavioral difference (Table 5).

Table 5. Chi-square values for August 12-13 samples (third vs. fourth instars).

Depth in meters	Chi-square	DF	P
0	183.71373	8	$<.05$
4	123.24536	8	$<.05$
8	256.04250	8	$<.05$

All values greater than 15.51 indicate a significant difference in temporal distribution.

In the October 24-25 samples from Tuttle Creek Reservoir immature larvae were present in small numbers at the surface at 1724, and increased to their maximum at 1800. There was a subsequent decrease until 1900 when the numbers leveled off and remained constant throughout the remainder of the night. The third instars also appeared in the samples at 1724 and reached their maximum at 1800. A second high was observed at 0612, and was followed by a sharp decrease in numbers. The fourth instars did not appear until 1800 and increased to their maximum at 2400. They decreased during the remainder of the night and were absent in the 0648 sample. Fourth instars were dominant throughout the night except for two brief periods when the third instars were dominant—1724 to 1836 and 0512 to 0648 (Fig. 9).

In the 4-meter samples immature larvae were present in small

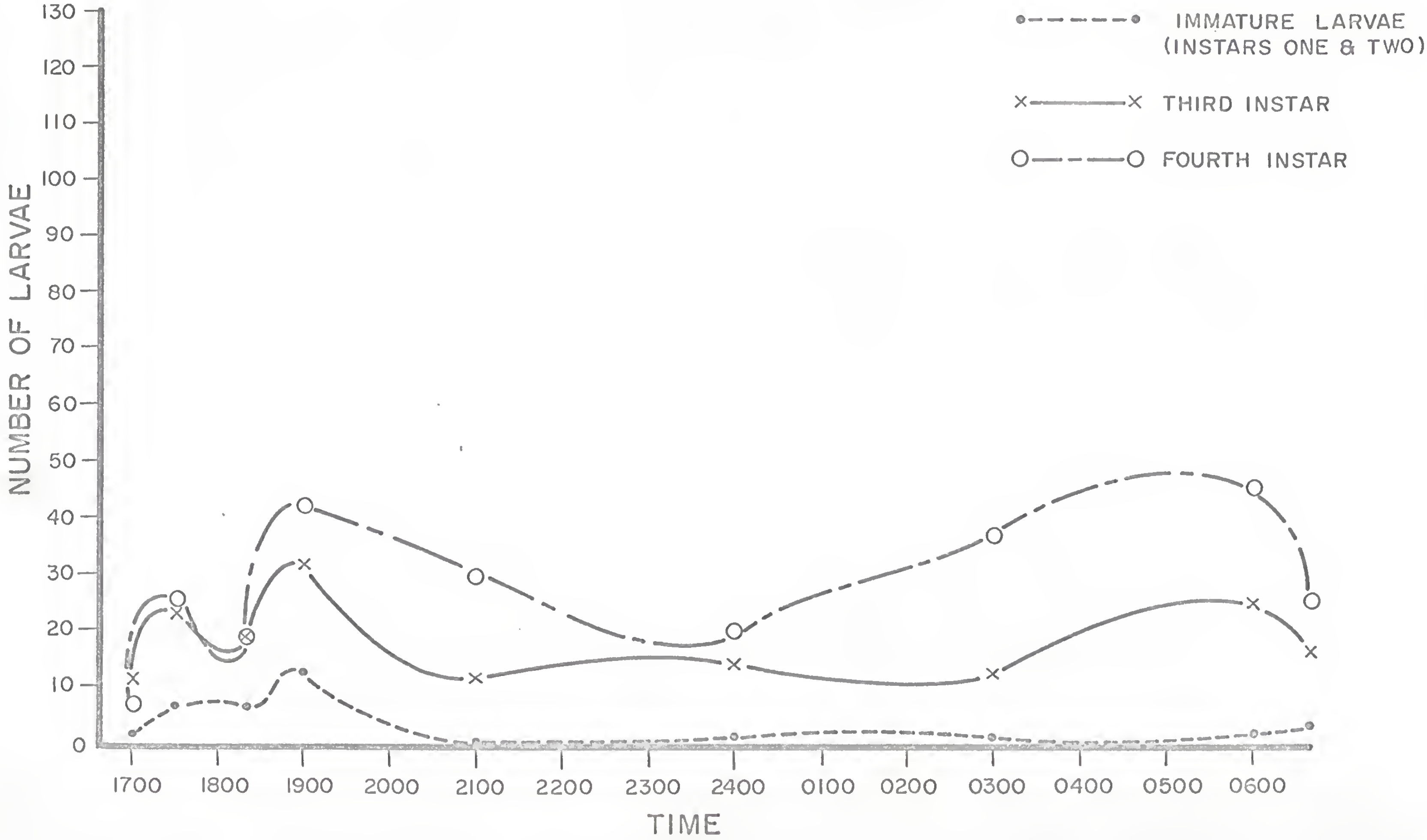

FIGURE 9. TOTAL NUMBER OF LARVAE COLLECTED AT EIGHT METERS ON OCTOBER 24-25. (TUTTLE CREEK)

numbers at 1712. They increased and reached their maximum at 1748 and were absent in the 0636 sample. The third instars were present in large numbers at 1712 and increased rapidly until they reached their maximum at 1748. There was a subsequent decrease until 2400 when the nightly minimum was reached. They again increased and were still present in numbers in the 0636 sample. The fourth instars did not appear until the 1748 sample when they were at their maximum. They decreased rapidly to a low at 1924. A secondary peak was observed at 2112, followed by a decrease until 0636 when the numbers were again increasing. The fourth instar was dominant throughout the entire sampling period except for the initial 1712 sample (Fig. 10).

At 8 meters immature larvae were present in low numbers at 1700 and reached their maximum at 2100. The third instars were present at 1700 and increased to their maximum at 1900. A second high was observed at 0548, and there was a decrease in numbers at 0700. The fourth instars were present at 1700 and reached their maximum at 1900. A low was observed at 2400, and a complete recovery was made at 0548. A subsequent decrease was observed; however, they were still present in numbers at 0700. The fourth instars were the dominant form from 1748 to 0700. No pupae were collected at Tuttle Creek Reservoir (Fig. 11).

Chi-square analysis comparing the distribution of immature larvae with that of mature larvae at Tuttle Creek Reservoir showed that there was no significant difference in the temporal distribution of larvae at the surface and 4 meters. A significant difference was observed at 8 meters (Table 6).

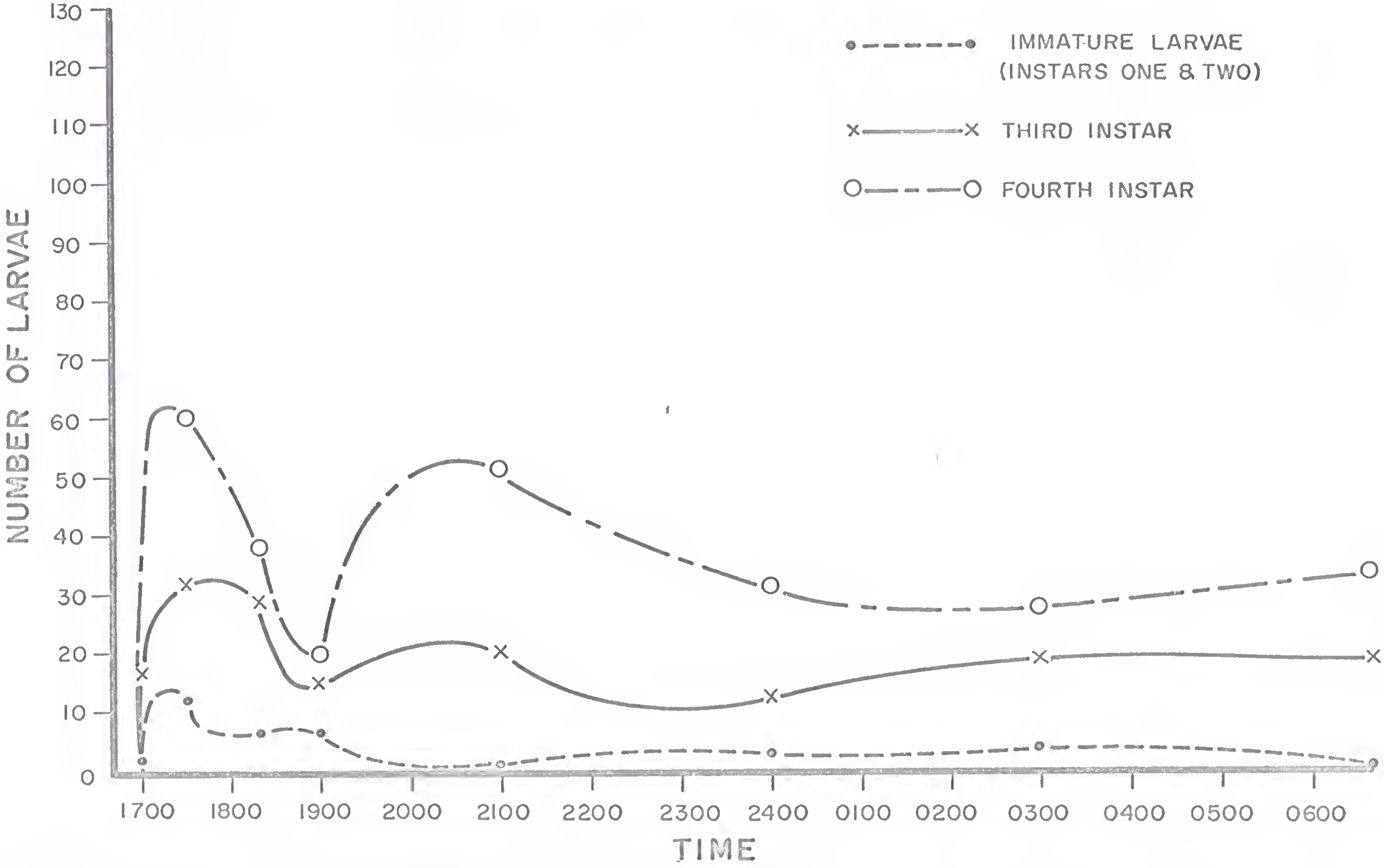

FIGURE 10. TOTAL NUMBER OF LARVAE COLLECTED AT FOUR METERS ON OCTOBER 24-25. (TUTTLE CREEK)

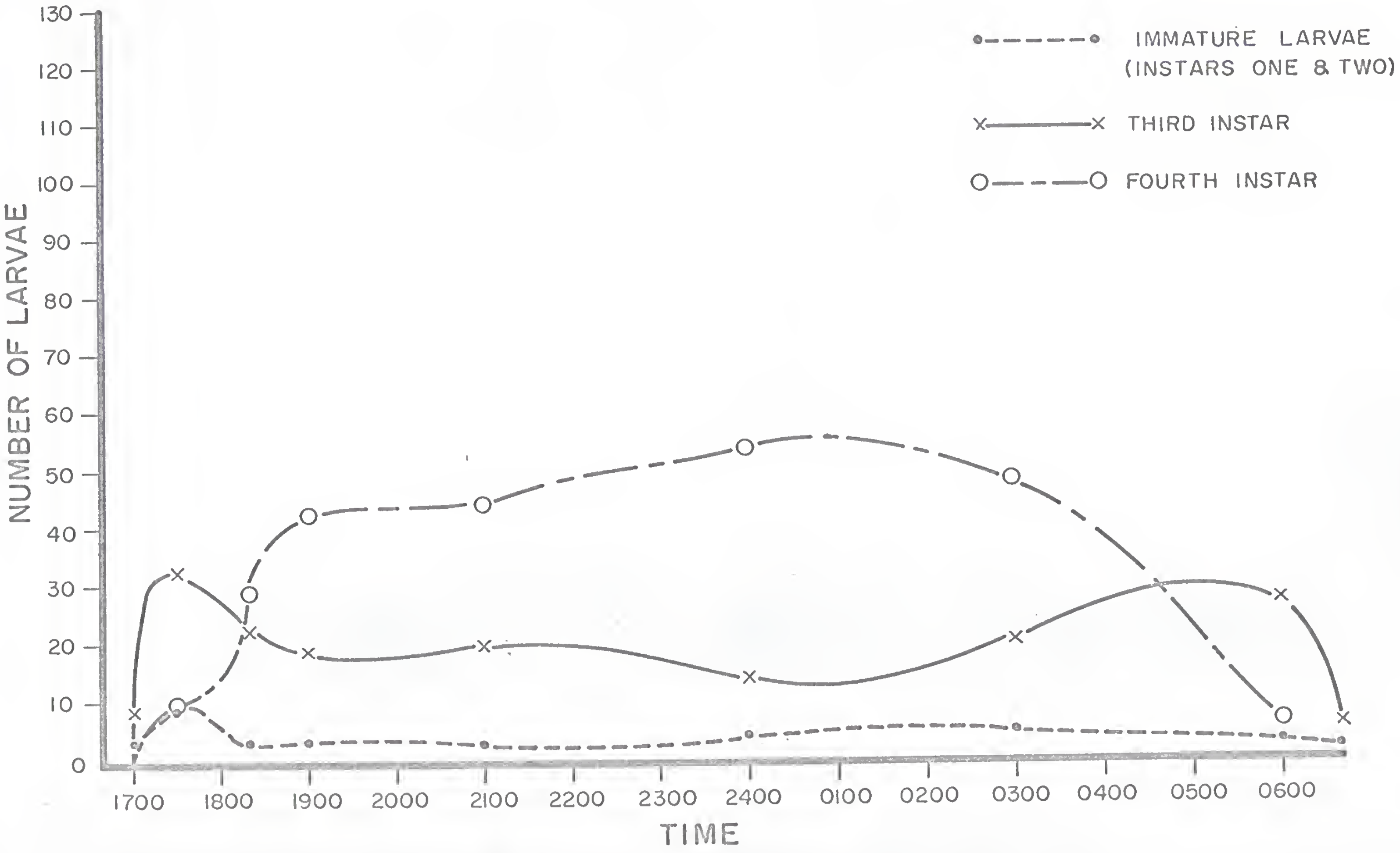

FIGURE 11. TOTAL NUMBER OF LARVAE COLLECTED AT SURFACE ON OCTOBER 24-25. (TUTTLE CREEK)

Table 6. Chi-square values for the October 12-13 samples (immature vs. mature larvae).

Depth in meters	Chi-square	DF	P
0	16.08001	10	$<.05$
4	15.23880	10	$<.05$
8	73.28531	10	$>.05$

Values greater than 18.31 indicate a significant difference in temporal distribution.

Chi-square analysis comparing the distribution of third with that of the fourth larval instar at Tuttle Creek Reservoir showed that there was no significant difference in the temporal distribution of larvae at 4 and 8 meters. A significant difference was observed at the surface (Table 7).

Table 7. Chi-square values for October 12-13 samples (third vs. fourth instars).

Depth in meters	Chi-square	DF	P
0	41.67574	5	$>.05$
4	5.61837	5	$<.05$
8	9.24443	5	$<.05$

Values greater than 11.07 indicate a significant difference in temporal distribution.

DISCUSSION

Vertical Migration

Birge (1897) found that Chaoborus larvae were present in considerable numbers at the surface of Lake Mendota after sunset but were rarely found at the surface during the day. He suggested that the larvae moved toward the surface at night together with the crustacea on which they feed. Juday (1904) observed the diurnal migration of Chaoborus and stated that the larvae came to the surface at Lake Mendota to feed on the crustacean population which also exhibited this migration. Juday (1921) found that the larval population in Lake Mendota reached the surface one and one-quarter hours after sunset with the greatest numbers of larvae taken two and one-half hours after sunset. They declined in numbers three and one-half hours after sunset and remained substantially the same until one-half hour before sunrise. In the present study it was found that larvae at Saratoga Lake reached the surface 30 minutes after sunset with the greatest numbers observed one and one-half hours after sunset. They declined in numbers three and one-half hours after sunset; however, the population recovered two hours later, then declined in numbers and were absent at the surface one-half hour before sunrise. Juday (1921) also suggested that the migratory behavior was not a simple light phenomenon. The bottom strata for sometime before sunset were in total darkness, yet observations showed that emergence from the mud was closely related in time with the setting of the sun. It thus appears from the evidence of other authors that this migration may be an adaptation for feeding and that it is not dependent on light alone.

Behavioral Differences Between Immature and Mature Larvae

The first reference to any behavioral difference between the immature larvae and mature larvae was made by Muttkowski in 1918. He stated that the immature larvae were planktonic at all times while the mature larvae were found to be benthic during the day and planktonic at night. This behavior has been reported by most investigators since 1918 (Juday 1921, Eggleton 1931, Miller 1941, Lindquist and Deonier 1943, Wood 1956, MacDonald 1956). This same behavior was observed in the present study at Saratoga Lake. Mature larvae were rarely found in samples collected during the daylight hours, whereas immature larvae were commonly found in the hypolimnion during the day. Both the immature and mature larvae were found to take part in the migration; however, data showed a significant difference in temporal distribution during this migration. Since the environmental stimulae affecting the chaoborid larvae are the same for each instar, the difference in temporal distribution must then be due to instar specific differences in response to these stimulae.

If the migration of Chaoborus is a feeding response as postulated by Birge (1897) and Juday (1904, 1908, 1921), one can explain the apparent behavioral difference of immature and mature larvae during the migration by their feeding habits. It has been reported by Deonier (1943) that the first and second instars feed on planktonic rotifers whereas the third and fourth instars feed on copepods. Any distributional difference between copepods and rotifers would be reflected in the behavior of the immature and mature larvae during their migration. Both the copepods and planktonic rotifers undergo vertical migration; however, they reach their surface maxima at different times. Juday (1904) reported that the maximum number of copepods occurred at the surface one-half to two and one-half hours after sunset. Pennak (1953) reported that the planktonic genera of rotifers exhibit a 24-hour cycle of vertical movements in accordance with variations in subsurface illumination. Beginning in the late afternoon a slow drift upwards begins and maximum abundance occurs near the surface in the early morning (3 to 5 A.M.). At dawn a downward migration begins, until maximum concentrations are found in the deep waters about noon. The maximum concentration of third and fourth instar Chaoborus at the surface of Saratoga Lake would correspond with a maximum surface population of copepods as reported by Juday (1904). The maximum numbers of first and second instars would correspond with the maximum surface population of the rotifers as reported by Pennak (1953). It thus appears from the data obtained at Saratoga Lake that the migration of both the immature larvae and mature larvae closely follows the migrations of those organisms upon which they prey.

A diurnal migration of Chaoborus larvae was also evident at Tuttle Creek Reservoir; however, behavioral differences between the immature and mature larvae were evident only from data collected at 8 meters.

Behavioral Differences Between Third and Fourth Instars

Although the feeding habits of the third and fourth instars are the same (Deonier 1943), a Chi-square analysis comparing the temporal distribution of third and fourth instars showed a significant distributional difference for those samples from Saratoga Lake. This distributional difference can be attributed to behavioral differences in the migratory pattern. The behavior of the third and fourth instars was similar until 2400 when the fourth instars showed a secondary peak in numbers while the third instars failed to recover and gradually decreased in numbers until sunrise. This decrease can be attributed to the fact that the third instars leave the surface earlier and presumably reenter the mud in numbers sooner than the fourth instars. This behavioral difference is evidence that there may be a difference in the food requirements. The fourth instar being the larger, and the last feeding stage in the life cycle of Chaoborus could require more food than the third instar. Carthy (1958) states that reactions which lead certain invertebrate organisms to food may be reversed when the animal is satiated. If the food requirements are less for the third instar, they would be satiated before the fourth instars. It thus appears probable that once the third instars have fed sufficiently a reversal of the reaction which initially led them to food would cause them to return to the sediments sooner than the fourth instars. This reversal in the sign of response after satiation has been demonstrated in other invertebrates. Lees (1948) found that a hungry sheep tick

Ixodes ricinus crawls to the tip of a grass blade and quests when its host comes near. When it has had its blood meal it descends into the grass. Herter (1928) reported that the leech *Hemiclepsis marginata* no longer moves towards shadows above it when it is fully fed. The data obtained at Saratoga Lake seems to indicate that a similar reversal in the sign of reaction may be involved in the behavioral differences exhibited by the third and fourth instars.

Analysis of the Tuttle Creek Reservoir samples showed a significant difference in temporal distribution between the third and fourth instar distribution at the surface. However, differences at 4 and 8 meters were not significant.

The two major factors that could possibly account for the behavioral differences implied by the distributions observed at Saratoga Lake and Tuttle Creek Reservoir are (1) the dissimilarity of the two environments, and (2) the time of the year that samples were collected. Juday (1904) observed that the diurnal migration of one species of *Chaoborus* could vary from one lake to another due to both biotic and abiotic differences in the environment. Worthington and Ricardo (1936) found that in Lake Edward and Bunyoni (East Africa) all larval instars of *Chaoborus* were planktonic; however, the third and fourth instars of the same species were benthic during the day in other lakes sampled. Hunt (1958) reported that *Chaoborus* larvae were entirely planktonic in Deep Lake, Florida. It appears that larval behavior can vary with environment.

Many seasonal factors may also be involved in the behavioral difference observed. Saratoga Lake samples were collected during the summer stratification, whereas Tuttle Creek Reservoir samples were collected during homothermal conditions (Table 8). Marzolf (In press) found that the benthic amphipod Pontoporeia affinis shows a definite response to the thermal structure of the water column through which it is migrating. A discontinuous thermal structure was found to inhibit the vertical migration of the organism. It is possible that the discontinuity layer present in Saratoga Lake could have modified the migratory behavior of the chaoborid larvae. Since samples were collected at Tuttle Creek Reservoir during homothermal conditions this thermal factor would influence the migratory response in a different manner than under stratified conditions.

Table 8. Comparison of the temperature series taken at Saratoga Lake (August 12-13, 1964) with that taken at Tuttle Creek Reservoir (October 24-25, 1964).

Depth (meters)	Temperature at Saratoga (Degrees Celsius)	Temperature at Tuttle Creek (Degrees Celsius)
0	23.3	14.5
1	22.2	14.5
2	21.1	14.5
3	20.5	14.5
4	20.5	14.5
5	20.0	14.5
6	14.2	14.5
7	12.9	14.3
8	12.7	14.4

Due to the seasonal variation in sampling there was a difference in population composition. Juday (1921) reported that pupation at Lake Mendota ceased when the water temperature reached 17.7° C. The water temperature during the October sampling at Tuttle Creek Reservoir was only 14.5° C. Hence the assumption has been made that pupation had ceased and the overwintering population was being sampled. The Saratoga Lake samples contained both newly hatched larvae and pupae. It is difficult, therefore, to compare data obtained from the two study areas; however, the samples collected at Saratoga Lake did provide data which showed that instar behavior varied during the migration. The behavioral differences between the larvae at Tuttle Creek Reservoir and at Saratoga Lake seem to indicate that behavior can vary from one environment to another or from one season to another, and this provides an interesting area for further investigations.

Horizontal Distribution

The June 26, June 29, and July 14 samples at Saratoga Lake provided evidence that the *Chaoborus* larval population varied both qualitatively and quantitatively with depth. Larger numbers of larvae were sampled in areas where the water was deeper. Samples taken in water 2.5 and 4 meters deep contained an average of 25 larvae, whereas samples taken in water 8 meters deep contained an average of 1071 larvae. Lindquist and Deonier (1943) also observed a definite positive correlation between depth and number of *Chaoborus* larvae collected. Samples taken in water 4 to 5 meters deep contained an average of 32 larvae, whereas samples taken in water 5 to 6 and 8 to 10 meters deep showed an average of 103 and 185, respectively. Hermes (1937) reported that the greatest number of larvae was collected in water greater than 6 meters deep, and that very few larvae were taken in shallower water. Juday (1921) observed that the greatest number of larvae occurred where the water reaches a depth of 20 meters. The number within the 20-meter contour was three times the number within the 8 to 20-meter contours.

Qualitative differences in the larval population taken from water of various depths were observed on the sampling dates listed above. The immature larvae comprised a greater percentage of the population in the shallow depths, and decreased in percentage with an increase in depth. Samples taken in water 2.5 meters deep contained 13 per cent immature larvae; 4 and 1 per cent of the population were immature larvae in water 4 and 8 meters deep, respectively. Lindquist and Deonier (1943) observed that at times during the summer the shallow water near shore showed the heaviest concentrations of immature larvae. These larvae were observed to move to greater depths as they increased in size. Berg (1937) found a high density of immature larvae of Chaoborus near shore, and these larvae moved gradually into the deeper water still leading their planktonic life. Later they entered the mud at these greater depths. The fact that a majority of eggs hatch in the warm shallow water may explain the high percentage of immature larvae in shallow waters (Juday 1921, and Lindquist and Deonier 1942). Juday (1921) postulated three factors that could possibly explain the fact that most eggs hatch in shallow water, and most larvae occur in the deep waters. (1) There may be an active migration of the immature larvae into the deeper waters. Such active horizontal migrations have been found to occur during the life cycle of Chaoborus. Adlittoral pupal migrations (Lindquist and Deonier 1942) and seasonal adlittoral and ablittoral migrations (Wood 1956) are examples of this horizontal migratory behavior. It is probable that such an ablittoral migration of the immature larvae could also occur. (2) Currents could act to carry the immature larvae into deeper waters. Currents may be the agents which cause many eggs to concentrate in shallower waters, so it is plausible that currents could also act as the distributive agent for the immature larvae. (3) There may be a greater loss of mature larvae in shallow water due to predation. The immature larvae are much smaller and less available as prey, while the mature and pupae may have their populations diminished by predation. This could eventually cause the greater proportions of immature larvae in shallow waters. The latter factor may be involved but there is little evidence to evaluate its significance.

Saratoga Lake pupae were found to comprise a greater percentage of the planktonic population in the shallower water (2.5 and 4 meters deep) even though the greatest numbers of larvae were found in deeper waters. Two per cent of the population were pupae in water 2.5 meters deep; 3 per cent and .1 of a per cent of the population were pupae in 4 and 8 meters of water, respectively. Lindquist and Deonier (1943) found that bottom samples did not indicate that the pupal population was greater near shore; however, emergence was invariably greater at that point. They suggested that as the pupae leave the mud during the night they exhibit a migration seeking quieter or warmer waters for transformation. Scott and Opdyke (1941) also recognized that such a migration of pupae must occur. Wood (1956) found that most of the pupation occurred in shallower water. He found that 78 per cent of the pupae collected were in water 3 to 6 meters deep, 10 per cent and 12 per cent were collected in water 6 to 8 and 8 to 10 meters deep, respectively. Borutsky (1939) suggested that the pupae migrated to shallower depths where the higher temperature of the water favored transformation.

Behavior of the Pupae

In the June 21-22 samples at Saratoga Lake the pupae were found to appear at the surface two hours after the larvae (2300). Samples at 8 meters show that they began leaving the mud at 2100. The August 12-13 samples at Saratoga differed slightly from the July samples in that the pupae did not appear at the surface until 0100 (three hours after the larvae). They did not leave the mud until 2400. The data from Saratoga Lake is similar to the data collected by Lindquist and Deonier (1942) who found that the larvae ascended from the bottom earlier than the pupae, and that the pupae were seldom recovered near the surface before 2400. Juday (1921) found that the pupae reached the surface 15 minutes after the larvae and were found in maximum numbers at 2200.

A total of 176 pupae were collected at Saratoga during the two 24-hour sampling periods. Of these only 19 were collected at the surface, 52 at 4 meters, and 105 at 8 meters. These data suggest that only 10 per cent of the pupae that left the mud ever reached the surface at a depth of 8 meters. Three possible explanations for this data immediately come to mind. (1) The pupae may not take part in the migration, and only those ready for transformation migrate to the surface. Those not ready to pupate leave the mud, remain in the hypolimnion during the night and return to the mud before sunrise. (2) There could be a high mortality rate during pupal migration due to predation. Pupae are not transparent as are the larvae, and are not as efficient in swimming, therefore are more likely victims of fish predation. Miller (1941) has reported a high mortality rate for pupae. Even though the pupae make up a small percentage of the population, he found that the stomachs of Lake Trout contained a high percentage of *Chaoborus* pupae. (3) The adlittoral migrations as described by Lindquist and Deonier (1943), Scott and Opdyke (1941), and Wood (1956) could only occur in the hypolimnion. If this were the case the pupae would leave the mud each night and instead of migrating to the surface would undergo an active shoreward migration in the hypolimnion. The only pupae that would be found at the surface at 8 meters would be those ready to emerge. Any one of the three hypotheses or a combination of these could explain the data; however, further investigation would be necessary before one could draw definite conclusions.

No pupae were found in the October 24 samples at Tuttle Creek Reservoir. The temperature of the water was sufficiently low to prevent pupation. The larvae collected were assumed to be from the overwintering population and, therefore, would not be expected to pupate until the following spring. Juday (1921) found that pupae first appeared as the water temperature increased in the middle of June and then appeared regularly until late August when pupation and subsequent emergence ceased as the water temperature decreased below 17.7° C. The water temperature at Tuttle Creek Reservoir during the October samples was 14.5° C.

Emergence

Emergence at Saratoga Lake was observed to be greatest in areas where the depth ranged from 2.5 to 5 meters, and began in the early evening and continued throughout the night. Juday (1921), Lindquist and Deonier (1942) also found that emergence was greatest at these shallower depths. The observations made at Saratoga Lake agree with those of Juday (1921) who also reported that emergence of *Chaoborus punctipennis* Say began in the early evening and continued throughout the night. Lindquist and Deonier (1942) used cages on the water's surface to trap emerging adults and thereby determine the time of emergence of *Chaoborus astictopus*. Examination of the cages up to 11 P.M. did not disclose any emerged adults, whereas examinations from 6 to 8 A.M. always showed adults in the cages. They, therefore, concluded that emergence occurs between 11 P.M. and 6 A.M. It thus appears that the time of emergence can vary with species and environment.

SUMMARY

Chaoborus larvae have long been the object of scientific investigations because of their migratory behavior. This paper analyzes the behavioral differences of the larval instars during the diurnal vertical migration of the chaoborid larvae. The data obtained during this study indicate that the feeding habit of an organism is an important factor influencing its behavioral patterns. It also demonstrates the phenomenon of a species modifying the behavior of another species within a particular food chain.

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APPENDIX

Table 9. Measurements for twenty-five first instars, randomly selected from samples taken at Saratoga Lake.

Number of setae on anal fan	Head-capsule length (millimeters)	Total body length (millimeters)
14	.16	2.18
12	.16	1.71
12	.16	1.63
12	.15	2.11
14	.16	2.04
14	.16	2.30
13	.16	2.11
14	.15	2.11
14	.16	2.31
12	.14	2.25
12	.15	1.94
12	.15	2.23
12	.16	2.09
13	.16	2.15
14	.16	2.18
12	.15	1.93
12	.15	2.19
12	.16	2.06
14	.16	2.35
14	.17	2.31
13	.16	2.19
12	.15	2.09
14	.16	2.11
12	.13	2.19
12	.16	1.86

Number of setae:	Total Numbers:
12	13
13	3
14	9

Range of head-capsule length: .13mm. to .17mm.

$\bar{X}$ of head-capsule length: .156mm.

Range of total body length: 1.63mm. to 2.35 mm.

$\bar{X}$ of total body length: 2.094mm.

Table 10. Measurements for twenty-five second instars, randomly selected from samples taken at Saratoga Lake.

Number of setae on anal fan	Head-capsule length (millimeters)	Total body length (millimeters)
14	.29	3.68
14	.32	4.03
15	.31	3.57
14	.33	3.43
14	.29	3.86
14	.33	3.54
15	.34	3.56
14	.33	4.50
14	.32	3.76
14	.33	3.50
13	.36	3.17
14	.32	3.86
14	.32	3.64
14	.33	3.85
14	.34	4.20
14	.35	3.74
14	.29	3.44
14	.29	3.24
14	.32	3.62
14	.32	3.56
14	.33	3.65
14	.35	2.50
14	.33	3.93
15	.34	3.04
14	.33	4.01

Number of setae:	Total numbers:
13	1
14	21
15	3

Range of head-capsule length: .29mm. to .36mm.

$\bar{X}$ of head-capsule length: .324mm.

Range of total body length: 2.50mm. to 4.50mm.

$\bar{X}$ of total body length: 3.668mm.

Table 11. Measurements for twenty-five third instars, randomly selected from samples taken at Saratoga Lake.

Number of setae on anal fin	Head-capsule length (millimeters)	Total body length (millimeters)
16	.61	6.19
16	.62	5.19
16	.61	5.56
16	.62	5.03
16	.62	5.11
17	.65	5.43
16	.58	5.08
16	.64	5.11
15	.63	5.20
16	.61	5.56
17	.65	5.19
16	.58	5.72
16	.61	4.81
16	.62	5.24
16	.58	5.02
17	.62	5.62
15	.63	5.24
17	.60	4.48
16	.58	4.40
15	.66	6.16
16	.61	4.44
15	.64	4.44
17	.59	5.25
16	.61	5.51
16	.63	4.57

Number of setae:	Total numbers:
15	4
16	16
17	15

Range of head-capsule length: .58mm. to .66mm.

$\bar{X}$ of head-capsule length: .616mm.

Range of total body length: 4.40mm. to 6.19mm.

$\bar{X}$ of total body length: 5.182mm.

Table 12. Measurements for twenty-five fourth instars, randomly selected from samples taken at Saratoga Lake.

Number of setae on anal fan	Head-capsule length (millimeters)	Total body length (millimeters)
18	.93	6.94
18	1.04	9.40
18	1.03	9.18
18	1.06	6.17
18	1.01	7.04
17	1.06	8.16
18	1.04	6.59
18	1.07	8.37
18	.96	8.65
18	1.04	7.74
18	1.07	7.69
18	1.06	7.83
18	1.08	7.98
17	1.08	6.94
18	1.02	7.28
18	1.04	5.94
18	1.06	7.04
18	1.02	9.71
19	1.08	7.56
17	1.03	6.56
18	1.08	10.04
20	1.06	9.96
19	1.02	6.38
18	1.03	8.54
18	1.04	9.10

Number of setae:	Total numbers:
17	3
18	19
19	2
20	1

Range of head-capsule length: .93mm. to 1.08mm.

$\bar{X}$ of head-capsule length: 1.04mm.

Range of total body length: 5.94mm. to 10.04mm.

$\bar{X}$ of total body length: 7.686mm.

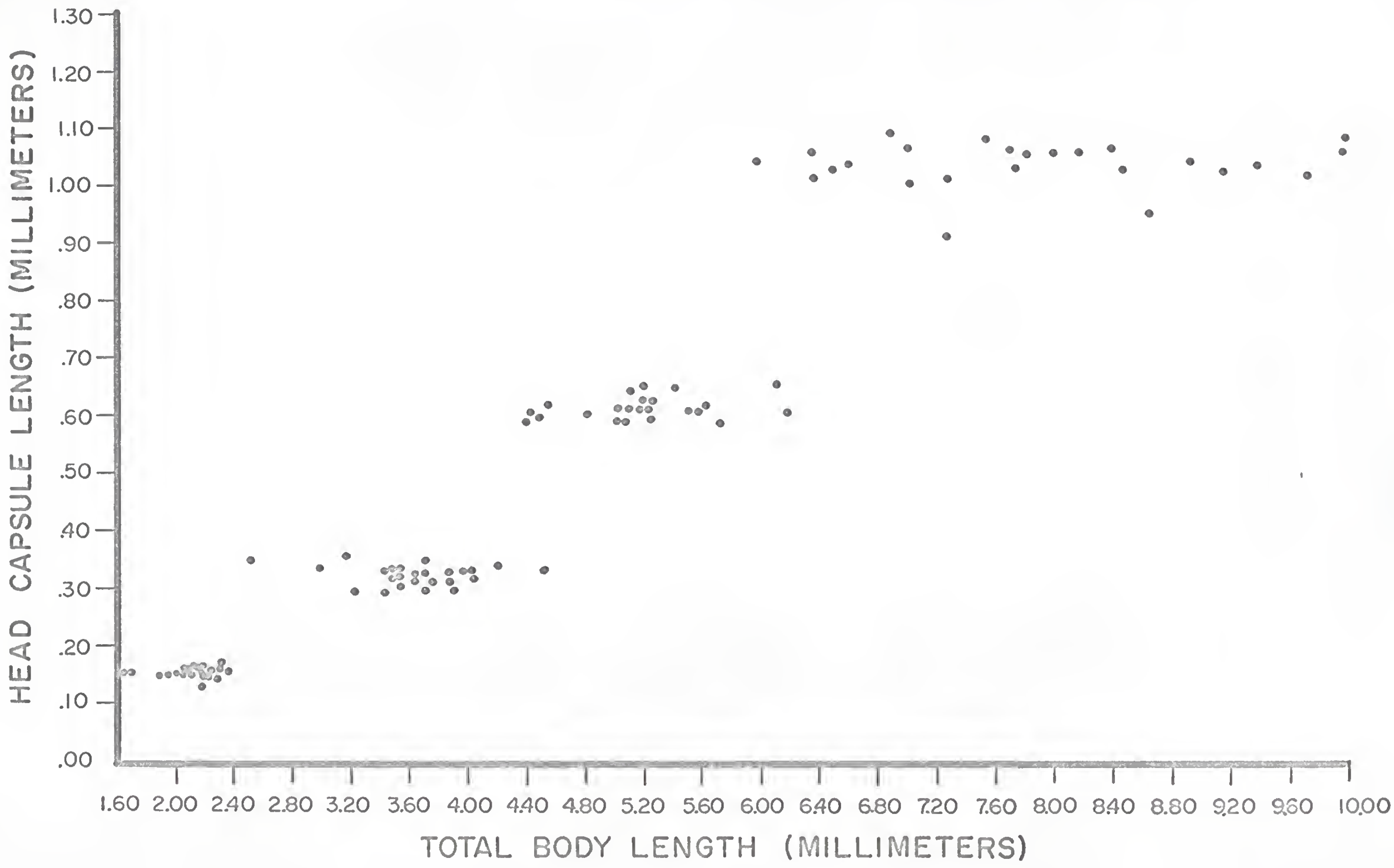

FIGURE 1. HEAD CAPSULE MEASUREMENTS VS. TOTAL BODY LENGTH MEASUREMENTS.

INSTAR BEHAVIOR OF *CHAOBORUS PUNCTIPENNIS* SAY

by

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B. S., St. Bernardine of Siena College, 1960

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This is a study to determine any behavioral differences between the four larval instars during the diurnal vertical migration of *Chaoborus punctipennis* Say. A total of 164 plankton samples were collected at Saratoga Lake, New York, during the summer of 1964, and 35 samples were collected at Tuttle Creek Reservoir, Kansas, in the autumn of 1964. A total of 33,349 larvae were collected, and each of these was categorized according to instar. Chi-square tests were made comparing distributions of the immature larvae (instars one and two) with the mature larvae (instars three and four), as well as comparing the distributions of the third and fourth instars.

A definite vertical migration was observed at both Saratoga Lake and Tuttle Creek Reservoir. The immature larvae were found to be planktonic at all times while the mature larvae were found to be benthic during the day and planktonic at night. Data obtained at Saratoga Lake showed a significant difference in temporal distribution of the immature and mature larvae during this migration indicating a behavioral difference. Data obtained at Tuttle Creek Reservoir showed an apparent behavioral difference between the immature and mature larvae at 8 meters; however, the difference in temporal distribution at 4 meters and the surface was not significant.

A Chi-square analysis comparing the distributions of the third and fourth instars showed a significant distributional difference for those samples collected at Saratoga Lake. This distributional difference can be attributed to behavioral differences in the migratory pattern. Analysis of the Tuttle Creek Reservoir

samples showed a significant difference between the third and fourth instar distributions at the surface; however, the differences in distribution at 4 and 8 meters were not significant.

Data collected from Saratoga Lake showed that the larval population varied both quantitatively and qualitatively with depth. Pupae were rarely collected at the surface until 2400 hours, and of the 176 pupae collected only 19 were collected at the surface, 52 at 4 meters, and 105 at 8 meters. No pupae were found in the samples taken at Tuttle Creek Reservoir.