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A Comparison of Four Stocks of Lake Trout from the Upper Great Lakes with Respect to Early Development

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A COMPARISON OF FOUR STOCKS OF LAKE TROUT FROM THE UPPER GREAT LAKES WITH RESPECT TO EARLY DEVELOPMENT

Final Report To

The Great Lakes Fishery Commission

For Research Entitled

Movements of Lake Trout Fry:
Environmental and Genetic Factors

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I. INTRODUCTION

The purpose of my study was to evaluate the hypothesis that stocks of lake trout (Salvelinus namaycush) inhabiting the upper Great Lakes region differ significantly with respect to rates of morphological and behavioral development of sac-fry. My interest was motivated primarily by the continuing failure of stocked lake trout in Lake Michigan to develop into self-sustaining populations. The hypothesis that this failure reflects genetic maladaptations has been advanced (Loftus 1976, Brown 1981). I will argue that lake trout populations differ with respect to the timing of hatching, emergence, skeletal development, and intestinal development and that those differences reflect adaptations to ancestral spawning environments. I will suggest, in keeping with arguments recently advanced by Balon (1981), that adaptations are not expressed by synchronously altered rates of development but by altered timing of phenomena relative to one another. I will first present descriptive data on lake trout early life history and development.

A few notes on terminology are in order. First, I will use the term sac-fry for any lake trout that has hatched but which retains some remnant of the yolk-sac.

This is a useful, simple, descriptive, and widely-used term. Second, in keeping with Balon's (1980) terminology, I will use where necessary the term eleutheroembryo for the period between hatching and the start of emergence and the term alevin for the period from start of emergence to completion of yolk-sac absorption.

EARLY BEHAVIOR AND DEVELOPMENT OF LAKE TROUT, A REVIEW

My review summarizes information on movements and morphological development of lake trout sac-fry from hatching through yolk-sac absorption. Hatching and emergence are two conspicuous events of importance in that period.

The time of hatching is partially dependent on the time of spawning. Spawning and early incubation take place during fall, a season of declining temperature. Time of spawning is apparently controlled by many environmental conditions including light (Royce 1951, McCrimmon 1958), temperature (Martin 1957), and wind (Martin 1957). Martin and Olver (1980) review these papers. Time of spawning may be under some genetic control; lake trout strains reared under identical conditions in the Marquette State Fish Hatchery show different stock-specific spawning times (John

Driver, personal communication). Of particular interest here is the difference between eastern Lake Superior and Green Lake strains; the latter spawn later.

Movements immediately after hatching are poorly known. Stauffer (1978) reported benthic dispersal by sac-fry in aquaria beginning immediately after hatching. Field data confirming pre-emergence dispersal are lacking. In connection with my experiments reported in Appendix 1, emergence traps were placed over eggs in Trout Lake, Wisconsin. While most captures occurred between May 13 and June 2, several fry were captured before March 17. These captures several months prior to general emergence (probably very near the time of hatching; see 1981 data below) indicate a vertical component of either pre-emergence benthic dispersal or predator avoidance.

The phenomenon of emergence itself is not well defined. It is widely believed that migration to the surface is essential for swimbladder inflation (Tait 1960). Balon (1980, see summary below) describes a period of alternating benthic and free-swimming activities.

Balon (1980) describes sac-fry behavior in its context of morphological development. In this and the following four paragraphs I summarize his results where directly relevant to my thesis. He identifies two phases in the

time from hatching to the completion of yolk-sac absorption; the eleutheroembryo phase and the alevin phase. These are each further subdivided into two steps. Balon's fish were incubated at 4.4 C or at 9.5 C. The timing of events differed between the two groups.

In Balon's terminology, hatching marks the start of step 1 of the eleutheroembryo phase. Median hatching times were 404 and 440 degree-days in two sub-groups of fish reared at 4.4 C. For two sub-groups of fish reared at 9.5 C the times were 463 and 473 degree-days. Average total lengths at hatching were 15 mm at 4.4 C and 17 mm at 9.5 C. No bones were calcified at hatching but cartilage was present in many bones and cartilaginous rods of neural and hemal arches had developed along the entire notochord. Some neural arches were formed, that is the rods had fused, by 462 degree-days at 4.4 C. Balon describes the behavior of fish in this step as follows: "Freshly hatched eleutheroembryos rolled or jumped as a result of rapid tail-trunk beats. All reacted to contact by rapid movements but there was no reaction to changes in light intensity or to rapid flashes of light. Active movements in the first half of this step or more correctly, a short time after hatching, were probably an adaptation for dispersal away from empty egg coats and hatching enzymes.

Since lake charr eggs are not covered by a layer of gravel in the redds, but are usually incubated in crevices of rocky outcrops, a premature onset of photophobia could interfere with the dispersal. Except for the convulsive movements inside the egg coats before hatching, and for a brief period of time after hatching, this step can be considered as an interval of inactive eleutheroembryos."

Balon distinguishes a second step in the eleutheroembryo phase, the period preceding emergence. He characterizes this step as starting with the onset of strong photophobia, "any light source now induced violent movements away from it." He mentions that eleutheroembryos remained aggregated during this step. For fish reared at 4.4 C, this step began after 524 degree-days and the fish were 22.4 mm in total length. There was a full set of 62 neural arches. Calcification began during this step with one fish reared at 4.4 C showing the beginning of calcification after 590 degree-days. "The edges of the teeth on the upper and lower jaws and the segmented joints of the caudal rays were calcified." Other structures that were calcified during the last week of this step included the premaxillae and the anterior edges of the cleithra. "The intestine of most fish was filled with carotenoids until the end of this step. By then the internal mucous

layer of the intestine had developed dense regular folds along the entire length." Parr marks began to be apparent in fish reared at 4.4 C but not in fish reared in warmer water.

The first step of the alevin phase begins when the photophobic reaction weakens and the fish emerge from their hiding places. For fish reared at 4.4 C, this step began after 651 degree-days, for fish reared at 9.5 C it began after 779 degree-days. "They occasionally assume a free-swimming position above the bottom, with the head directed against the current, but tend more often to hide under objects, or rest on the bottom, to escape from direct light...Immediately after emergence the fish began to snap at particles recognizing food items only when taken into the mouth...In fish incubated neither in cold nor warm water the swimbladder filled immediately after emergence, although the fish alternated between free swimming, maintaining a position above the substrate, and resting on the bottom." Total lengths of fish at the start of this step were 26.9 mm at 4.4 C and 26.0 mm at 9.5 C. In fish reared at 4.4 C, parr marks were apparent at the start of this step. Calcification developed in 19 rays of four segments each in the caudal fin, in the edges and central parts of six hypurals and stegurals, and in bones.

The start of the second step identified by Balon in the alevin phase is characterized by inflation of the swimbladder. This step began after 743 degree-days for fish at 4.4 C and after 1140 degree-days for fish at 9.5 C. The total lengths of fish in the two groups were 26.6 to 28.3 mm at 4.4 C and 28.3 to 28.9 mm at 9.5 C. The fish had no external yolk sac at the start of this step. Calcification of vertebral rings began.

The role of dissolved oxygen concentration in influencing time of hatching and movement is not well understood. Balon (1980) asserts that chronic low-oxygen level retards development and thus delays hatching but that acute low level induces premature hatching.

Temperature is directly correlated with rate of development but, other than that, its role is poorly known. I use degree-days, the sum of daily temperatures, as a reference for development. Unfortunately, degree-days preceding any developmental event varies depending on the particular thermal regime. Thus in Balon's results (1980) hatching occurred after fewer degree-days in fish incubated at 4.4 C than for those at 9.5 C.

I know of no literature on the role of temperature changes in influencing fry movement, but it seems logical that rapid temperature increase during spring turnover

might cue emergence or dispersal.

RATIONALE FOR THE STUDY

The stock concept, the idea that individual fish species are assemblages of locally adapted interbreeding populations, is widely accepted and was the subject of a recent symposium (Can. J. Fish. Aquat. Sci., 38(12), 1981). The hypothesis to be evaluated here is an extension of that concept. The hypothesis is plausible; lake trout stocks of the upper Great Lakes have been sufficiently isolated from one another and utilize sufficiently diverse habitats for the development of eggs and sac-fry that differences in early behavior and development should have evolved. If the hypothesis is true it carries important implications for reestablishing self-sustaining stocks of lake trout in Lake Michigan.

APPROACH

The approach to evaluating this hypothesis was, first, to develop a description of sac-fry behavior and development under simulated natural conditions. Earlier studies do not include direct observations under controlled

conditions matching those in nature. Furthermore no accounts are available in the literature in which behavioral events and physical processes are correlated in a systematic, well-defined manner. Chapters II, III, and IV present my data and some conclusions related to this first goal.

My second goal was to experimentally compare sac-fry of four stocks of lake trout with regard to behavioral events and physical processes that seemed measurable and relevant. The earlier descriptive data helped define and select variables for comparison and provided a basis for evaluating the significance of any observed differences. Experimental data and my conclusions are presented in Chapters V nd VI.

II. PRELIMINARY EXPERIMENTS

In the winter and spring of 1979 sac-fry in Trout Lake were found in emergence traps as early as March 17 and as late as June 9, with 4% (5 of 116) emerging before March 17 and 86% emerging between May 13 and June 2. I doubt that the fish captured in March were precocious or developed to the extent of the fish captured in late May. Their occurrence in the emergence traps (approximately 50 cm above the substrate) was more likely a consequence of predator avoidance, i.e. they moved vertically when disturbed by crayfish, mudpuppies, sculpins or other benthic organisms. Stauffer (1978) reported movement of newly-hatched sac-fry, but no evidence exists for unprovoked vertical forays of the magnitude indicated here.

During the winter and spring of 1980 experiments were conducted to assess the extent of vertical and horizontal movement by sac-fry. The hypothesis that vertical movement can be induced by crayfish was tested experimentally.

On November 2, 1979, gametes were obtained from ripe males and females in Trout Lake. The eggs were fertilized immediately and placed in enclosures on the bottom of Trout Lake at a depth of 7 m and temperature, at the time of placement, of 7.6 C. On February 11, 1980, 800 eggs were brought from the lake, then at 1.5 C, to the lab where they

were placed in a holding tank with flow-through Trout Lake water at 4.0 C.

Two experiments were conducted. In the first, fry in the presence and absence of crayfish were observed under red light. Horizontal and vertical movements were measured and response to crayfish activity was described. In the second, the influence of crayfish activity on vertical movement was assessed in a controlled experiment. All crayfish used in the experiments described in this chapter were adult female Orconectes propinquus with carapace lengths between 30 and 35 mm.

EXPERIMENT 1

Methods

Fry were observed in a Plexiglas aquarium 68 cm long, 28 cm wide and 19 cm deep. The aquarium was divided with screens into three sections, two large observation areas 31 cm x 28 cm in bottom area and a small central area where lake water flowed into the tank. Twenty-four small stones, each about 7 cc, were distributed over the bottom of each of the two observation areas; most of the aquarium floor was clear. One of the two areas contained a crayfish during observation sessions. Each of the two areas was stocked with 20 eggs on February 24. Eggs and fry were not handled after that date.

The aquarium was kept in darkness except when observations were being made. Observations were made under light filtered with a Kodak Safelight Filter #1 to exclude wavelengths under 610 nm. The light source, a portable darkroom lamp with a 15 watt bulb, was generally placed over the aquarium, although it was sometimes moved. The observer usually watched from below the aquarium, occasionally viewing from the side to assess vertical movement.

The floor of the aquarium was marked into labelled 1 cm squares. The observer selected individual fry haphazardly for observation and watched each for ten minutes. The fry's position was recorded verbally on a tape recorder each time it moved from one square to another as were any vertical movements or responses to other fish or crayfish.

Observations were made on March 15, March 18, and April 19, 1980. Those dates correspond approximately to 426, 438, and 590 degree-days after fertilization, respectively. On the earlier dates, some eggs had not yet hatched; by April 19 all had hatched.

Results

March 15

Twenty-five fry were observed with no crayfish present (there were fewer than 20 hatched fry present so several were observed more than once). The water temperature was 4 C. All fry were observed to move. The average horizontal distance travelled was 41 cm per 10 minutes. Vertical movement was less common but seven fry left the aquarium floor farther than two cm at least once. The average height reached was 5.6 cm and one fry achieved a height of 14 cm before falling to the aquarium floor. All fry were negatively buoyant.

The fry at this stage had a marked tendency to aggregate. I have no basis for concluding that the fry were drawn together a) by mutual attraction, b) by shared affinity for certain micro-habitats, or c) by physical restriction of forward movement; I simply observe that they were found in aggregations. During most of the observation period, one or two aggregations of two to ten fry were present. The aggregated fry lay with their heads toward the aquarium corner, undulating vigorously.

Much of the horizontal movement was seemingly in response to physical contact by other fry. Individuals or entire aggregations repeatedly moved away when disturbed by the arrival of another fry.

March 18

Twenty-two ten minute observations were made with one crayfish present in the aquarium. The general pattern was as above. On 19 occasions a fry was observed to move. The average horizontal distance travelled (including data for immobile fry) was 19 cm per 10 minutes.

When the crayfish was placed in the aquarium at the beginning of my observations, two live eggs and 18 hatched fry were present. By the completion of the twenty-two observations, the two eggs as well as three fry had been eaten. Fry move in response to crayfish but the response to physical contact was not always strong. Excluding the three captures, only 3 of 5 fry touched by the crayfish moved. Only one of these swam vertically to escape. Fry showed no sign of response to the crayfish unless physically touched and, when touched, showed no evidence of knowing where the crayfish was. Movement then was seemingly random, not necessarily directed away from danger.

April 18

Twenty-one fry were observed when aquarium water temperature had reached 5 C. By this time 590 degree-days had elapsed, bringing the fry near the time of emergence.

Several distinct behavior patterns were observed. Two fry spent their entire ten minute observation (and probably the entire two-hour observation session) swimming at the surface. They were near enough to the surface to, on one occasion, bump a bubble, but far enough from it so that no disturbance of the surface was visible. These two showed no sign of negative buoyancy. Several fry were seen swimming in mid-water, although none could be followed for long so we don't know if swimming at that depth was sustained. Among fry still at the bottom, there was variety. Several, resting upright on the bottom, moved gradually while undulating their bodies. Forward movement seemed incidental to the undulations. Occasionally fish cruised briefly within a centimeter of the bottom; one for nearly three minutes.

No aggregations were seen. Fish were extremely sensitive to contact from others and, when touched by another, darted so rapidly that they could not be followed. Sometimes fish swam several widths (about 30 cm) of the aquarium within seconds after physical contact.

Response to contact by crayfish was similarly violent

although, as earlier, the fish showed no awareness of the crayfish's location. Vertical movement was common after contact by crayfish, sometimes followed by a return to the bottom at the same spot, still within danger. The fry showed no ability to sense the crayfish's presence unless touched. I had the impression that the fish were too quick and responsive to be vulnerable to crayfish predation, but after the crayfish was left with the fish overnight three could not be found.

The fish generally did not appear to respond to the observer's movement in red light. However, on at least one occasion a fry seemed to move abruptly when the observer raised a hand.

EXPERIMENT 2

Methods

Eighteen plastic boxes (Fig. 2-1), each with an emergence trap, were placed on the floor of a 364 liter aquarium. The boxes were cubes, 15 cm on a side. Wide-mouthed plastic bottles, 15 cm tall and 7 cm in diameter were fitted, upside down, into the tops of the boxes. A solid translucent plastic funnel was placed in the mouth of each bottle with the wide opening down. Walls of the boxes and the bottles were partially removed and the openings covered with screen. Stones, 300 to 500 cc, were

placed in a single layer at the bottom of each box. Initially, the small opening of each funnel was plugged with a cork.

The aquarium was supplied continuously with water from Trout Lake and excess water drained out via a standpipe. Several airstones kept the water outside the boxes circulating. The aquarium was kept in darkness except during the tri-weekly checks described below. At those times illumination was through a Kodak Safelight Filter #1.

On February 24, 10 eggs were placed in each box, funnel plugs were removed from four boxes and an adult female crayfish was placed in two of the boxes with plugs removed. Three weeks later, on March 16, the four boxes without funnel plugs were removed from the aquarium and four more boxes were similarly handled. The process was repeated four times, with six boxes (three with crayfish) handled in the last set.

When a box was removed, number of eggs in the box and fry (both alive and dead) and number of fry in the bottle were counted. These data would provide the bases for inferences about the roles of crayfish as predators and as stimulators of vertical movement by fry during each of four periods in development.

Results

The influence of the crayfish as egg predators is seen throughout the period (Table 2-1, Fig. 2-2). At each date fry or eggs were missing in enclosures with crayfish but all were accounted for whenever crayfish had not been present.

The influence of crayfish on emergence, defined here as appearance in the bottle, is not conclusively demonstrated by these limited data. The relevant statistic is percent of live fry that had emerged. No consistent difference is seen between boxes with and without crayfish.

Fry moved vertically at a date (April 5, 520 degree-days) well before emergence as defined in the following chapter (573 degree-days). Fish emerging at the earlier dates had a large yolk-sac and no cryptic coloration such as the parr marks or silvering apparent in fish found in the emergence traps on May 18. See Appendix 2 for photographs of fry on April 5, April 27, and May 18.

III. A DESCRIPTIVE STUDY OF SAC-FRY BEHAVIOR AND DEVELOPMENT

Balon (1980) has provided the only detailed description of physical and behavioral development in lake trout sac-fry. He postulates several developmental correlates of behavioral events. His study has three shortcomings: First, he provides no precise operational definitions for the variables he measured; for example, we do not know how he measured the time of swimbladder inflation, nor do we know how he measured response to light or what he means by "emergence". Second, his data seem not to have been collected in a systematic fashion; we do not know what his experimental protocol was. Third, his fish were not reared under a thermal regime matched to any naturally occurring conditions. In my studies described in this chapter I attempted to collect data that would permit description of the changes in sac-fry behavior and the concomitant changes in skeletal development, coloration, digestive tract development, and swimbladder inflation.

METHODS

Egg Handling and Incubation

Gametes were obtained from ripe wild adults from Trout Lake. The parents are identified as T6 and Td in Table

3-1. Both parents are believed to be derived from the ancestral Trout Lake population; the female was unclipped and thus was conceived naturally in the lake, although possibly by stocked parents, and the male had an adipose clip, indicating that his parents were captured in Trout Lake and that he was incubated in the local hatchery (McKnight, 1977). Gametes were taken and eggs fertilized on October 28, 1981 and held in a holding tank at 8 C to 9 C until November 1 when they were transferred to shelters on a natural spawning area in Trout Lake at a depth of 7 m. Lake temperature was 6 C on the day of transfer.

The eggs were removed from the lake on March 15, 1981 (411 degree-days after fertilization) and transferred to two holding tanks in the laboratory. The holding tanks were fed continuously with lake water from Trout Lake. One tank was maintained at lake temperature from that time on (Fig. 3-1); the other was always slightly warmer. The two groups of fish were kept separated throughout the study and will, in most cases, be referred to separately.

Lighting was always by overhead incandescent bulbs. The photoperiod was 12 dark:12 light at the start of the laboratory observations and was changed twice, remaining at 10 dark:14 light for most of the study; these photoperiods only approximate the coinciding natural photoperiods (see Fig. 3-2). The light was controlled to intensify gradually

in the morning and to diminish slowly at the end of the light period each day.

Observation Aquaria

Observations were made in aquaria such as illustrated in Fig. 3-3. Each aquarium was divided into a small chamber (10 cm long, 2.5 cm wide, 30.5 cm deep) where water entered from above and a larger chamber (10 cm long, 7.5 cm wide, and 30.5 cm deep) from which water exited. The two were connected by a screened window located in the lower half of the dividing panel; water flowed into the front chamber, through the screen panel, and out through a hole in the rear chamber. Except for the screen panels, aquaria were constructed entirely of transparent plastic.

The lower third of the front chamber was filled with transparent glass marbles, 2.2 cm in diameter (not shown). A hole (not shown) in the outer face of the smaller chamber and 2 cm from the bottom was plugged with a cork and water could be drawn out through the cork using a hypodermic syringe; this allowed measurement of dissolved oxygen concentration using the Winkler method (APHA, 1976). Water temperature in each aquarium was matched to that in one of the two holding tanks so that fish transferred from holding tanks to the aquaria for observation experienced no change in water temperature. Water entered the aquaria at a rate

of about ten milliliters per three seconds. The observation aquaria were subject to the same light regime as described above for the holding tanks.

Horizontal runways were constructed in the colder holding tank to assess light intensity preferences of five fish. Five runways were used, each 9.5 cm long, 2.5 cm wide and 1.5 cm deep; each was completely enclosed, with openings for light only in the front and rear (away from light source). One fish was placed in each runway for the duration of the experiment (one escaped and was replaced). None of the fish had access to air. A movable opaque panel was used to cover either the left or right half of all runways and thus to produce a light gradient in each.

Observation Protocols

Fry to be observed were placed in one or another observation aquarium at least twelve hours prior to observation; they were not physically disturbed from that time until after completion of data collection. Selection of the fry from the holding tanks was not strictly random; fry were chosen haphazardly from those that had hatched.

Fry were observed for fifteen minute sessions. The observer sat without moving for three minutes before each fifteen minute session. When fish were observed singly (with no other fish present) position was noted every 30

seconds. This was recorded as "bottom" (touching the aquarium floor), "substrate" (touching a marble but not touching the floor), "water" (above the substrate but farther than 2.5 cm from the water surface) or "surface" (within 2.5 cm of the surface). Also recorded, beginning April 7, was total time spent moving, which included both swimming above the substrate (including periods when the fish was still in the sense of there being no whole-body displacement) and moving within the substrate. Following observation sessions where fish were observed singly, a second fifteen minute period was devoted to continued observation and written recording of descriptive information about fry movement. Throughout this thesis I will reserve the term "movement" to refer only to displacement of the whole fish or to swimming above the substrate in which the fish holds one position; a fish was not "moving" only if it was resting on the substrate. The term "beat" will refer to finning. Descriptive data are summarized in Appendix 3.

For each of ten weeks beginning March 25, four fry were observed from each of the two holding tanks. The fry were observed singly as described above, both before sunrise and at midday.

Observations before sunrise were made using light filtered through a Kodak Safelight Filter #1, light

consisting of wavelengths greater than 610 nm. The light source was either a 15 watt bulb or, after May 8, a 25 watt bulb. The lamp was placed behind the aquarium so that the light passed through the aquarium toward the observer. After May 12, the light was directed downward from directly over the aquarium. In either case the light was not moved after the start of the three-minute pre-observation period.

Other fry were observed in pairs or quartets. The same handling and observation procedures were followed. The only data presented here from those observation sessions pertain to direct interactions; numbers of contacts between fish and numbers of responses were recorded. Where two fish were found touching each other at the start of an observation period, the fact of continuous contact was recorded, and nothing else. When one fish moved and touched another, "2" was recorded as the number of contacts and the number of fish (0, 1 or 2) that seemed to alter their behavior as a result of the contact was recorded as number of responses.

In some instances fry were observed after a crayfish was placed in the aquarium. Number of contacts between crayfish and fish (this time, of course "1" was recorded when a crayfish touched a fry) and number of responses by the fry (0 or 1) were recorded.

Each day the opaque panel was left covering one half

of the horizontal runways in the colder holding tank. The position of each fry (light side or dark side) was noted after the lights came on the following morning. The panel was moved to cover the other half. After at least one hour, during which there was no human activity around the aquarium, the position of each fry was again noted. Fish that were in the darker half both times were considered to prefer dark; the percentage preferring dark was recorded.

Whenever fry were transferred from the colder holding tank to an observation aquarium, the numbers of "benthic" and "emerged" fry were determined. Those terms are used here to mean resting on the bottom and swimming, respectively. An effort was made to select representatives of each group during the period when both were represented.

Number of unhatched eggs and number of dead fry or eggs were recorded daily for each holding tank until all eggs had hatched.

Photography, Preservation, and Processing

Each week eight fish that had been observed singly were photographed live (one series of photographs is in Appendix 2). Four of those were from the colder holding tank and four from the warmer one. Two from each group of four were preserved in 10% buffered formalin and two were preserved in Davidson's solution A and B (Shaw and Battle,

1957). Four additional fry from each holding tank were also preserved, half in each solution, so a total of 16 fry were preserved each week. One fry from each weekly set of fish preserved in Davidson's solution was photographed after preservation (see Appendix 2).

All individually observed fish were cleared and stained for bone and cartilage following the method described by Dingerkus and Uhler (1977). One stained skeleton from each weekly set was photographed (see Appendix 2).

The following data were obtained from stained specimens that had been preserved in formalin:

1) "Skeleton length" - the distance from the anterior-most stained part to the posterior-most stained part,
2) "Amount eaten" - 0 if no food is visible in the lower gut (below the pylorus), 1 if one discrete bolus is visible, and 2 if more than that is visible,
3) "Number of neural arches" - the number of neural arches where blue stain (or clear outline of the structure) forms a complete arch. This was as determined under 12X magnification,
4) "Number of ossified teeth" - the number of teeth on the right dentary (lower jaw) with any amount of red stain,
5) "Endogenous gut contents" - 0 if no granular stained material is visible in the lower gut, 1 if some such

material is visible with difficulty (25X magnification), and 2 if the material is clearly visible, and

6) "Development of internal gut folds" - 0 if no folds are visible (25X magnification) in the lower gut, 1 if folds are visible along the gut margin as the fish is viewed from the side, and 2 if the folds are sufficiently pronounced to be visible as lines crossing the gut.

In descriptions below, bones will be termed "ossified" when any amount of red stain is visible and "chondrified" when any amount of blue stain is visible.

Stained specimens that had been preserved in Davidson's solution had somewhat different staining characteristics. They were used only for assessment of swimbladder inflation since in them, unlike those preserved in formalin, the swimbladder was often visible after staining and clearing.

RESULTS

Behavior

Until about 600 degree-days had elapsed fish were benthic and inactive, between approximately 600 and 700 degree-days individual fish became mobile, and after 700 degree days all fish were active (Figs. 3-4 and 3-5).

Almost all of the movement indicated in these figures is swimming, not movement within the substrate so the activity indicated in these figures can be termed emergence. Both groups of fish, cold- and warm-water reared, emerged over about the same range of degree-days.

The picture of total immobility prior to emergence contradicts the findings of the preceding chapter and study of the descriptive notes in Appendix 3 shows it to be misleading; movement was noted on most dates of observation. One fry swam nearly to the surface, a distance of 30 cm, after only 436 degree-days, a point preceding 50% hatch of his cohort. Bouts of tail beating were seen regularly throughout the benthic period with movement often apparently restricted either because the fish was swimming into the wall or because it could not fit into a crack or between a marble and the lower edge of the aquarium. In the aquaria observed the preceding year, fry moved, on the average, more than 20 centimeters per ten minute observation period (see Chapter III). That far exceeds the rates indicated in Appendix 3 for fish of equal age. The difference can be attributed to either the effects of other fry present in the former study or to the greater substrate complexity in the later one.

The timing of emergence is about the same when measured in different ways. In Fig. 3-4 an emergence curve

is superimposed for fish in the colder holding tank; it indicates the percent of fish in the holding tank that were above the bottom at the times that the individually observed fish were transferred to the observation aquaria. Its correspondence with the other data shown suggests that both measure the same emergence phenomenon. In Fig. 3-5 a superimposed curve shows data from the experiment described in the following chapter; the percentage of aquaria that have reached average emergence (defined below) is plotted against degree-days since fertilization. Again the superimposed curve and the observational data correspond.

Individual fish can differ widely in timing of emergence; one fish (Fig. 3-4) was actively swimming by day after 573 degree-days but another was still immobile by day after 673 degree-days. This apparent variation in emergence times might be partially an artifact of a transition period for each individual fish, when the fish is benthic part of the time and pelagic part of the time. The time from 573 to 673 degree-days will be defined here for convenience as the emergence period. It is a period of variability between fish and within fish; individual fry differ from each other during this time and the behavior of each is variable.

Throughout the period of emergence differences in activity between day and night are seen, most commonly

showing greater activity by day (Fig. 3-6). The notes in Appendix 3, representing contiguous but different observation periods, confirm this pattern.

Appendix 3 also suggests a diurnal periodicity in movement during the period from hatching to emergence. In those early weeks, however, the apparent pattern is toward greater activity by night. In 24 cases where notes are available for individual fish observed before 573 degree-days both under red light and white light, activity was greater under red light in 12 cases, indistinguishable (based on the notes) in ten cases, and greater under white light in only two cases.

Cumulative thermal experience of lake trout fry is of greater importance than daily temperature in controlling emergence. Data for fish reared in cold water (Fig. 3-4) and fish reared in warmer water (Fig. 3-5) are displayed in separate graphs in order to allow comment on this point. As noted above, the interval of emergence was similar for the two groups when measured in degree-days; cold-water-reared fish emerged between 573 and 673 degree-days while warm-water-reared fish emerged between 623 and 686 degree-days. These ranges corresponded to daily temperature ranges of 6.6 to 8.6 C and 8.5 to 10.1 C, respectively.

Except for some results near or during the time of

emergence, fry did not respond strongly to contact with other fry (Fig. 3-7). The change suggested by these data, from seeming indifference to contact to some responsiveness, parallels but does not exactly match the impressions left by the observations of the preceding chapter; there, fry observed at about 430 degree-days were apparently responsive to contact but nevertheless inclined toward aggregating while fry observed at about 590 degree-days were unambiguously sensitive to any contact and did not aggregate. The limited data shown regarding responses to crayfish support the idea that crayfish can be important influences in sac-fry movements.

The preference for dark decreased from about 80% in dark at 500 degree-days to 20% in dark at 700 degree-days (Fig. 3-8). The change corresponds approximately to the beginning of the emergence period.

Development

Appendix 2 illustrates changes in the shapes, colors and skeletons of fry as they grow through the period from hatching to completion of yolk-sac absorption.

Yolk-sac absorption is not complete until after the emergence period (as defined above). The yolk is easily seen after 579 degree-days and a small bulge is visible in a side view after 681 degree-days. Fry were not fully

"buttoned-up" until after that time. Parr marks are discernible as early as 550 degree-days but, in the series of preserved fish shown in Appendix 2, are not conspicuous until after 698 degree-days.

The first signs of skeleton ossification occur around the time of emergence. Figure 3-9 displays number of ossified teeth (defined above) as a function of degree-days. The fish that showed ossification earliest were preserved after 579 degree-days (one of those, #61, was the earliest to show extensive activity; see Fig. 3-4). By the end of the emergence period (673 degree-days) the average number of ossified teeth was about eight. The youngest fish to have a cleithrum ossified to any detectable degree was also #61, at 579 degree-days. That fish and one other preserved on the same day were the first to have detectably ossified maxillae or premaxillae. No dentaries were ossified until 681 degree-days. No joints in caudal lepidotrichia were ossified until after 758 degree-days. The youngest fish with any detectable ossification of vertebral rings was 778 degree-days old. Fish reared in cold water did not seem to differ from those reared in warmer water with respect to the timing or rate of ossification of teeth (see Fig. 3-9). That was true of other measures of development (see Figs. 3-10, 3-11, and 3-12). The two groups are therefore not distinguished in

this discussion.

The youngest fish with any neural arches was preserved after 539 degree-days. As Fig. 3-10 indicates, the number of fused neural arches is a highly variable index which, nevertheless, seems to increase linearly as a function of degree-days through the emergence period. The rate of increase decreases after that point paralleling an apparent decline in the rate of increase in skeleton length (see Fig. 3-11). Of course, that apparent decline is based on very little data and no claim is made here as to its statistical significance.

Variables related to gut development and feeding were "amount eaten", "endogenous gut contents", and "development of internal gut folds". The values of those variables are displayed in Fig. 3-12. Two fish had eaten prior to any sign of emergence. Endogenous material in the gut was present at the end of the emergence period in some fish. With only one exception, internal gut topography was well developed by the start of the emergence period.

In fish preserved in Davidson's solution and examined for swimbladder status after clearing and staining, the earliest clear evidence for inflation was in two fish preserved after 579 degree-days. In those fish the lower gut was pushed to the lower right side of the abdomen.

IV. BEHAVIOR AND DEVELOPMENT OF SAC-FRY, A SUMMARY AND DISCUSSION

Outline:

I. Summary of results from preceding two chapters. This will include integration of known information.

   A. Sac-fry are active between the times of hatching and emergence. This is clear from both data sets and confirms the results of Stauffer.

      1. Movement is sometimes spontaneous, that is, not induced by conspecifics or predators.

      2. Movement is sometimes caused by predators and probably by conspecifics.

      3. Dispersal can occur between the time of hatching and emergence but its magnitude is probably insignificant in deep, complex Great Lakes spawning reefs.

      4. Movement during this period is quite possibly limited by light (i.e. by dark preference).

      5. My data do not confirm Balon's distinction between the early and later parts of this period.

   B. Emergence occurs after about 573 degree-days (April 27 in 1981) under natural conditions in Trout Lake.

      1. There is a period of mixed activity for each fish, sometimes it swims, sometimes it rests.

      2. Swimbladder inflation coincides roughly to the start of

emergence, as do initiation of feeding and reduced photophobia.

3. Dispersal is probably great after emergence starts since the fish become subject, periodically, to surface water movement and are, for periods, actively swimming.
4. Yolk-sacs are not fully absorbed until many days after emergence.
5. Emergence is probably not cued by water temperature changes.

C. The relative timing of emergence and various development indices is somewhat different for my fish than reported by Balon. This comparison will utilize a tabular display of my results and Balon's.

II. Discussion of implications for stock comparison experiment.

A. The only conspicuous and, therefore, easily measurable change in behavior is emergence. If changes in light sensitivity or activity occurred during the eleuthero-embryo phase, they are too inconspicuous to serve well in comparing stocks.

B. Ossification of teeth, formation of neural arches, skeletal growth, and development of internal gut folds are all processes that show measurable changes during the emergence period, and therefore can serve in comparing development of stocks if specimens from the stocks are obtained during the emergence period.

V. EXPERIMENTAL COMPARISON OF FOUR STOCKS

If the failure to reestablish self-sustaining populations of lake trout in Lake Michigan is a consequence of genetic maladaptation of stocked parent fish, we might hypothesize that the adaptations of importance are to spawning environment. In this chapter I will examine the related hypothesis that among modern lake trout populations we can demonstrate differences in behavior and development of sac-fry.

The preceding chapters provide a solid basis for comparing lake trout stocks; events and processes that can be readily observed and measured and that occur during the sac-fry period have been identified and defined. Those events and processes will be the dependent variables in the experiment described below.

METHODS

Gametes were obtained from four sources: a wild lake trout population in western Lake Superior, a hatchery brood stock at the Marquette (Michigan) State Fish Hatchery, a hatchery brood stock at Crystal Springs (Wisconsin) Hatchery, and a wild population in Trout Lake, Wisconsin. Information on the parent fish is summarized in Table 3-1.

The parent fish from western Lake Superior were

captured by the Wisconsin Department of Natural Resources at Gull Island Shoal, a shallow reef near Bayfield, Wisconsin in the Wisconsin waters of Lake Superior. They bore no fin-clips, indicating that they were not hatchery-reared fish; they had been spawned naturally. Gull Island Shoal was used as a spawning ground by indigenous lake trout (Coberly and Horrall 1980) and it is likely that unclipped fish captured there are descendents of the native population (Bruce Swanson, personal communication).

The parent fish from Marquette were all second generation broodstock derived from native inshore (i.e. nets set at 0 to 35 m) populations in the Michigan waters of Lake Superior between Copper Harbor and Munising (John Driver, personal communication).

The parent fish from the Crystal Springs represent the last remaining isolated population derived from native lake trout of Lake Michigan. They are derived from a stocked population of lake trout in Green Lake, Wisconsin, a population believed to be derived principally from the deep spawning native lake trout of southern Lake Michigan referred to in Chapter I but also partially derived from shallow-spawning Lake Michigan natives. A fraction, possibly 30% (John Driver, personal communication) of one generation of males in the lineage leading to the current

Crystal Springs Hatchery population was of eastern Lake Superior descent. Thus the present Crystal Springs population (and its descendents used in my experiments) should be regarded as carrying a gene pool principally but not exclusively derived from Lake Michigan and probably derived in large part from the deep-spawning natives of southern Lake Michigan.

The fin-clips of parent fish captured in Trout Lake (see Table 3-1) indicate whether or not they were planted and, if so, what the source of the parents was (McKnight 1977). Trout Lake has been stocked from all the sources described here. Unclipped fish were spawned naturally but the origin of their parents cannot be determined. The clipped fish from which gametes were taken for this experiment were all derived from parents captured in Trout Lake, but, again, the original gene pool from which they were derived cannot be determined.

Table 5-1 summarizes relevant information concerning handling of gametes. Gametes from each source were held in dry containers (i.e. no water was added, the gametes were moistened only by natural gonadal fluid) at 1 C from the time the parent fish were stripped (date of gametes in Table 5-1) until shortly before fertilization. After being brought to the temperature of the water to be used for fertilization, the gametes were mixed in that water on the

dates and at the times indicated in Table 5-1.

Within each stock as many crosses as possible were attempted. Figure 5-1 indicates the crosses that ultimately yielded animals used in the experiment described here; other crosses also yielded fertile eggs but high egg mortality during the winter eliminated them. A small sub-sample of water-hardened eggs from each cross was held in the lab for determination of fertilization rates and for measurement of egg diameters.

Eggs were transferred from a laboratory holding tank to shelters on a spawning area 7 m deep in Trout Lake. Dates of transfer are indicated in Table 5-1. Holding-tank and lake temperatures were monitored throughout the study. Shelters were of two types: large boxes and small bottles.

On February 22, 1981 eggs were retrieved from the lake and held at 1 C for two days until transfer to experimental aquaria. The aquaria were fed by Trout Lake water and temperatures (recorded twice daily) approximated those of the warmer holding-tank described in Chapter III (see Fig. 3-1). The experimental aquaria were identical to those described in Chapter III; eighty were used. The aquaria were fed by siphon from a common head-tank. The siphons from the head-tank entered larger plastic tubes which carried the water to individual aquaria. The flow to each aquarium (and therefore the temperature of each) could be

controlled by changing the distance below head-tank water level of the end of the siphon leading to the larger tube. The aquaria were arranged in two tiers of 40 aquaria each. Water warmed somewhat in transit to the aquaria so in order to keep water temperatures in the lower tier equal to those in the upper tier it was necessary to have slightly faster flow to the lower aquaria.

As in the earlier chapter, the front compartment of each aquarium was partially filled with transparent marbles to simulate rocky substrate. Water was withdrawn regularly from four reference aquaria for monitoring of dissolved oxygen concentration. Lighting was as described for the aquaria used for observations reported in Chapter III except that all 80 aquaria were held in darkness from the time the eggs were transferred until March 11.

When eggs were transferred to the aquaria, assignment of individual crosses to particular aquaria was done randomly. Some crosses were represented in more than one aquarium. Five eggs (and fry, see below) were placed in the front chamber of each aquarium unless fewer than five were available from the particular cross. Three (occasionally more) eggs and fry were placed in the rear chamber when enough were available. A few eggs hatched between the time of removal from the lake and placement in the aquaria. When five animals were selected from a larger

number for placement in the two chambers of an aquarium the selection of sac-fry and the chamber they were assigned to was determined by a random process so that whether or not an egg was hatched was not a factor in where it went.

Data

The numbers of live eggs, dead eggs, live fry and dead fry were determined once every two days until April 12 when detritus accumulation on the marbles made continued determination difficult, nine live eggs were not yet hatched at that time. These determinations were made "blind"; the observer did not know which aquaria were used for each stock. For each egg known to hatch, the time, in days and in degree-days (the number of degree-days to an event was always the sum of the daily temperatures for the days preceding an event), preceding hatching was determined and the average for each aquarium computed. That average is referred to below as "time preceding hatching". Where eggs had not hatched by April 12 (2, 1, 2, and 4 from Bayfield, Marquette, Crystal Springs, and Trout Lake, respectively) a hatching date of April 13 was assigned. Where eggs were hatched already when placed in the aquaria (14 from Trout Lake and one from Crystal Springs) a hatching date of February 23 was assigned.

The numbers of fry in each aquarium that were in each

of five places were determined six days each week; the five positions were bottom, substrate, substrate surface, water, and surface. These data were also gathered "blind". After detritus accumulation made determination of the first three difficult, only the latter two were recorded. An individual fry was defined as "emerged" on a given day if it was above the substrate. Emergence dates for individual fry were defined in the following manner:

1) When one or more fry were emerged on two consecutive days, the first of the two was defined as the day of emergence of one fry.
2) If more than one fry were emerged on that day, it was defined as the day of emergence for all that emerged then.
3) On subsequent days, each day on which the number of emerged fry exceeded the earlier total was defined as the day of emergence of another fry.
4) Average time preceding emergence was computed for each aquarium, both in days and in degree-days. That average is referred to below as "time preceding emergence".

On May 1, all fry were removed from the rear chamber of each aquarium. At this time only approximately five percent of the aquaria with Trout Lake fish had reached average time preceding emergence; 602 degree-days had

elapsed for those fish while 618, 594, and 594 degree-days had elapsed for fish from Bayfield, Marquette, and Crystal Springs, respectively. They were preserved in 10% buffered formalin for later clearing and staining for bone and cartilage using the methods described in Chapter III. The following data were taken from the stained fish: "skeleton length", "amount eaten", "number of neural arches", "number of ossified teeth", "endogenous gut contents", and "development of internal gut folds". See Chapter III for definitions of these terms. Average values for individual aquaria were computed for each dependent variable.

Analysis

In the analyses summarized here it was assumed that variation among crosses is very small compared with variation within crosses. That assumption is supported by analyses of within stock variability made using data from Trout Lake fish, the stock with the largest amount of data. In these analyses, time to hatching (in degree-days) was the dependent variable. In a two-way ANOVA, with fixed maternal and paternal effects assumed and with the assumption of no interaction, the parental effects were not statistically significant, although the p-value associated with paternal effect approached .10. When a random-effects model (with a term for interaction) was applied to the

Trout Lake data and maximum likelihood estimates of the variance components were derived, the variance among fathers was estimated to be only 7.6% of the error-variance and the maternal and interaction variance components were estimated to be zero.

Unless specified otherwise all statistical tests were one-way analyses of variance with origin of fish (i.e. stock) as the factor of interest. Where that test indicated statistically significant differences among stocks, the S-method (Scheffe, 1959) was employed to identify which differences explained the result. Analysis of covariance was used to eliminate several factors as explaining between-stock differences.

RESULTS

Stocks differed significantly with respect to "time preceding hatching" (Fig. 5-2); the p-value in a one-way ANOVA was less than .005. In pair-wise comparisons using the S-method, Bayfield fish differed from each of the non-Lake Superior groups at a very high level of statistical significance; p-values for those comparisons were less than .005. The difference between Bayfield fish and Marquette fish, the other Lake Superior group, was nearly statistically significant at the .05 level. All

other pair-wise comparisons fell short of the .10 level of significance. The date on which the Bayfield fish passed mean hatch (477 degree-days) was March 31; they had experienced the mean time preceding hatching for Trout Lake fish (441 degree-days) twelve days earlier.

Differences among stocks were also very highly significant with respect to "time preceding emergence" (Fig. 5-3); the p-value in a one-way ANOVA was less than .005. The pair-wise differences that are statistically significant at the .05 level are those between Bayfield and Trout Lake and between Marquette and Trout Lake.

Differences between stocks with respect to time preceding hatching cannot be explained by differences in sizes of the eggs. Figure 5-4 displays the data as linear function of egg diameter. A regression line is shown, the best fitting straight line when effect of stock is ignored. Inclusion of stock as an independent variable improves the fit to a very high degree of statistical significance; the p-value is less than .005.

Similarly, differences between stocks with respect to time preceding hatching cannot be explained by differences in lengths of the mothers. Figure 5-5 illustrates this point. Again, inclusion of stock as a dependent variable (after fitting mother's length linearly) reduces unexplained variability significantly; the p-value is less

than .005.

Similar results are found when time preceding emergence is considered; the differences between stocks are not a result of differences in size of mother or of differences in size of eggs.

Type of incubation chamber does not explain the observed differences between stocks. As mentioned above, two types of shelters were used to incubate eggs in Trout Lake; these were 1) small plastic bottles and 2) large boxes with mesh troughs. Figure 5-6 shows values of time preceding hatching for the four stocks with data from aquaria stocked with eggs incubated in bottles distinguished from data from aquaria stocked with eggs incubated in boxes. Considering the "bottle-data" only, Bayfield and Trout Lake values differ at the .005 level of statistical significance. Considering "box-data" only, Marquette, Crystal Springs and Trout Lake differ at the .005 level of statistical significance.

In considering the data on variables describing physical development, it should be kept in mind that all groups had not lived the same number of degree-days since fertilization at the time the fish were preserved on May 1. This is because fertilization took place on different dates for the four groups (see Table 5-1). The numbers of degree-days experienced prior to May 1 were 618, 594, 594,

and 602 for fish from Bayfield, Marquette, Crystal Springs, and Trout Lake, respectively.

The four groups differed significantly with respect to "number of neural arches" (Fig. 5-7) and with respect to "skeleton length" (Fig. 5-8), two variables that are highly correlated between groups. Also, the groups with the largest and smallest values, Trout Lake and Crystal Springs, respectively, had the largest and smallest eggs, respectively (see Fig. 5-4). Within the Trout Lake specimens the correlation between skeleton length and number of neural arches was strong and positive. With reference to neural arch development the significant pair-wise differences were between Trout Lake and Marquette and between Trout Lake and Crystal Springs.

The groups differ significantly with respect to gut-fold development (Fig. 5-9) variable also; the p-value is less than .005. The pair-wise difference that is significant in a S-test is that between Trout Lake and Marquette.

The four groups did not differ with respect to "amount eaten" (Fig. 5-10), "number of ossified teeth" (Fig. 5-11), or "endogenous gut contents" (data not shown).

VI. CONCLUSIONS AND DISCUSSION

I. Differences between stocks

A. There are clear differences.

1. The stocks differ with respect to time of hatching.

2. The stocks differ with respect to time of emergence.

3. The stocks differ with respect to certain morphological variables but these differences are probably directly related to differences in egg size.

B. The differences among stocks are not parallel for all dependent variables. Most conspicuous here is that the Crystal Springs (lower Lake Michigan) fish hatch and emerge early but show retarded morphological development (number of neural arches, especially) relative to Trout Lake or western Lake Superior fish.

II. Implications for re-establishing self-sustaining stocks in Lake Michigan. This section will be a projection, based on the data of this study and known thermal regimes at the four ancestral spawning areas, of hypothetical hatching and emerging times of each stock at each location. Also included will be speculation about the importance to each site of different rates of morphological development.

III. Unanswered questions.

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Table 2-1. Crayfish predation and fry emergence data.

Each date indicated at the left is the end of a three week period during which ten fry in each of the four or six boxes were free to move into the emergence trap. The numbers of days and degree-days to that date (after fertilization) are indicated as is water temperature on that date. Each row contains data for one box and emergence trap. Presence or absence of a crayfish is indicated. The number of eggs and fry, in the box and in the bottle, alive and dead, are given. "Percent emerged" is the percentage of live fry that were in the bottle.

Date	Days	Degree-Days	Temp (C)	Cray-fish	Box Eggs Alive	Box Eggs Dead	Box Fry Alive	Box Fry Dead	Bottle Fry Alive	Bottle Fry Dead	Missing	Percent Emerged
March 17	134	430	4.1	Yes	0	0	6	0	0	0	4	0
March 17	134	430	4.1	Yes	1	2	3	0	0	0	4	0
March 17	134	430	4.1	No	6	0	4	0	0	0	0	0
March 17	134	430	4.1	No	2	1	6	1	0	0	0	0
April 5	154	520	5.0	Yes	0	0	1	0	3	0	6	75
April 5	154	520	5.0	Yes	0	2	0	0	0	0	8	0
April 5	154	520	5.0	No	0	0	6	0	4	0	0	40
April 5	154	520	5.0	No	0	1	4	0	5	0	0	55
April 17	176	652	7.0	Yes	0	1	1	0	7	0	1	88
April 17	176	652	7.0	Yes	0	0	4	0	3	0	3	43
April 17	176	652	7.0	No	0	0	7	1	2	0	0	22
April 17	176	652	7.0	No	0	8	1	0	1	0	0	50
May 18	197	857	11.5	Yes	0	0	1	0	6	0	3	86
May 18	197	857	11.5	Yes	0	0	2	0	6	0	2	75
May 18	197	857	11.5	Yes	0	0	1	1	5	0	3	83
May 18	197	857	11.5	No	0	0	1	2	6	1	0	86
May 18	197	857	11.5	No	0	0	1	1	8	0	0	89
May 18	197	857	11.5	No	0	7	0	0	3	0	0	100

Fig. 2-1. Fry enclosure and emergence trap.

Translucent plastic boxes with screened windows on four sides (only one is shown here) were used as fry enclosures. Bottles, also with screened windows, were fitted into the tops of the boxes. Inverted plastic funnels in the mouths of the bottles allowed emerging fry to enter the bottles but restricted return to the box.

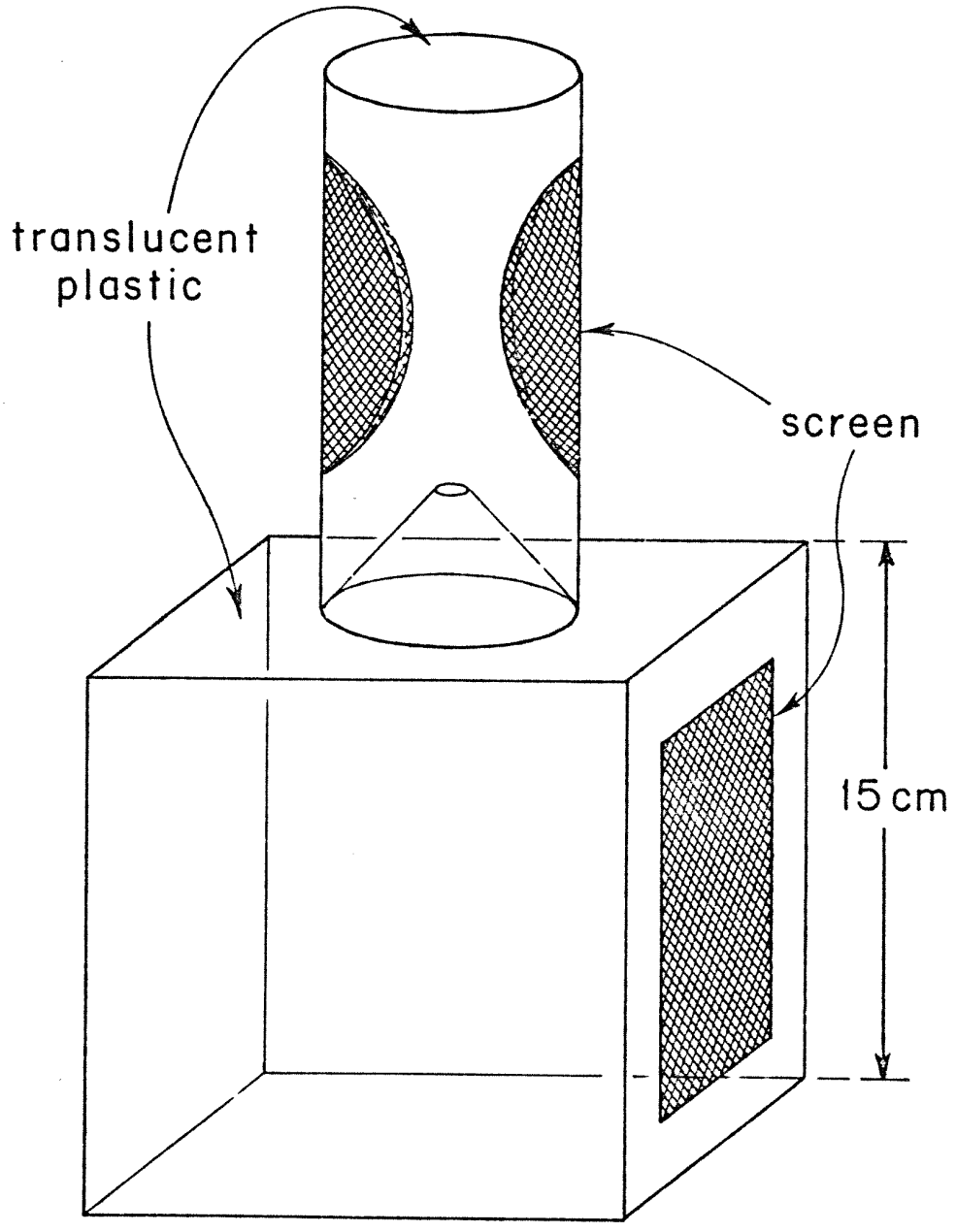

translucent
plastic
screen
15 cm

Fig. 2-2. Crayfish predation and fry emergence data.

"Percent emerged" is the percentage of live fry that were in the emergence trap at the end of the three week period of access. "Percent presumed eaten" is the percentage of the ten originally stocked eggs that were unaccounted for. Numerals beside data points denote numbers of coinciding values.

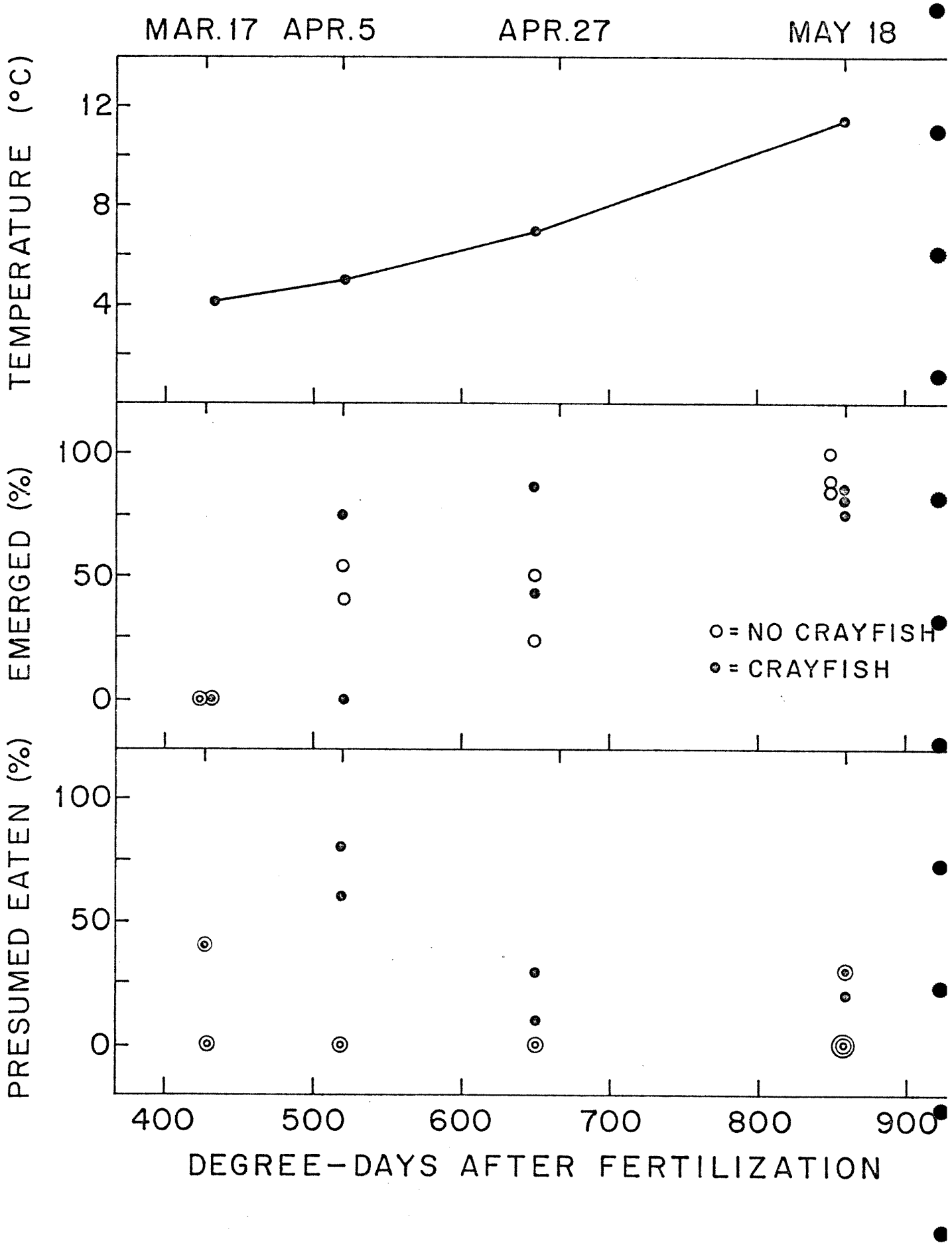

MAR. 17
APR. 5
APR. 27
MAY 18
TEMPERATURE (°C)
12
8
4
EMERGED (%)
100
50
0
○ = NO CRAYFISH
● = CRAYFISH
PRESUMED EATEN (%)
100
50
0
400
500
600
700
800
900
DEGREE-DAYS AFTER FERTILIZATION

Table 3-1. Parents of fish used in 1980-81 experiments.

Information on the parents of all fish studied in 1980-81 is displayed. The identification codes are used in the text to indicate which parents were the source of gametes used in the individual experiments. (a) Lengths of individual Bayfield males were recorded but not associated with identification numbers; the numbers ranged from 64.5 to 75.7 cm. (b) Total lengths of Marquette fish were estimated from fork-lengths.

Source	Sex	ID	Total Length (cm)	Egg Diam. (mm)	Fin Clip
Bayfield	m	B1	(a)	--	none
Bayfield	m	B2	(a)	--	none
Bayfield	m	B3	(a)	--	none
Bayfield	m	B4	(a)	--	none
Bayfield	m	B5	(a)	--	none
Bayfield	f	Ba	77.5	5.65	none
Bayfield	f	Bb	83.3	5.79	none
Bayfield	f	Bc	81.3	5.84	none
Bayfield	f	Bd	77.5	6.00	none
Marquette	m	M1	66.8(b)	--	none
Marquette	m	M2	75.6	--	none
Marquette	m	M3	71.9	--	none
Marquette	m	M4	70.7	--	none
Marquette	m	M5	74.6	--	none
Marquette	f	Ma	69.9	5.70	none
Marquette	f	Mb	65.0	5.90	none
Marquette	f	Mc	66.7	5.99	none
Marquette	f	Md	70.9	5.77	none
Marquette	f	Me	60.9	6.00	none
Crystal Spr.	m	C1	44.7	--	none
Crystal Spr.	m	C2	45.7	--	none
Crystal Spr.	m	C3	48.2	--	none
Crystal Spr.	f	Ca	42.3	5.00	none
Crystal Spr.	f	Cb	47.2	5.26	none

Fig. 3-1. Rearing temperatures.

The fish described in Chapter III were reared in two holding tanks. The daily water temperatures in those tanks are plotted here along with periodically measured water temperature at 7.5 m in Trout Lake (large dots).

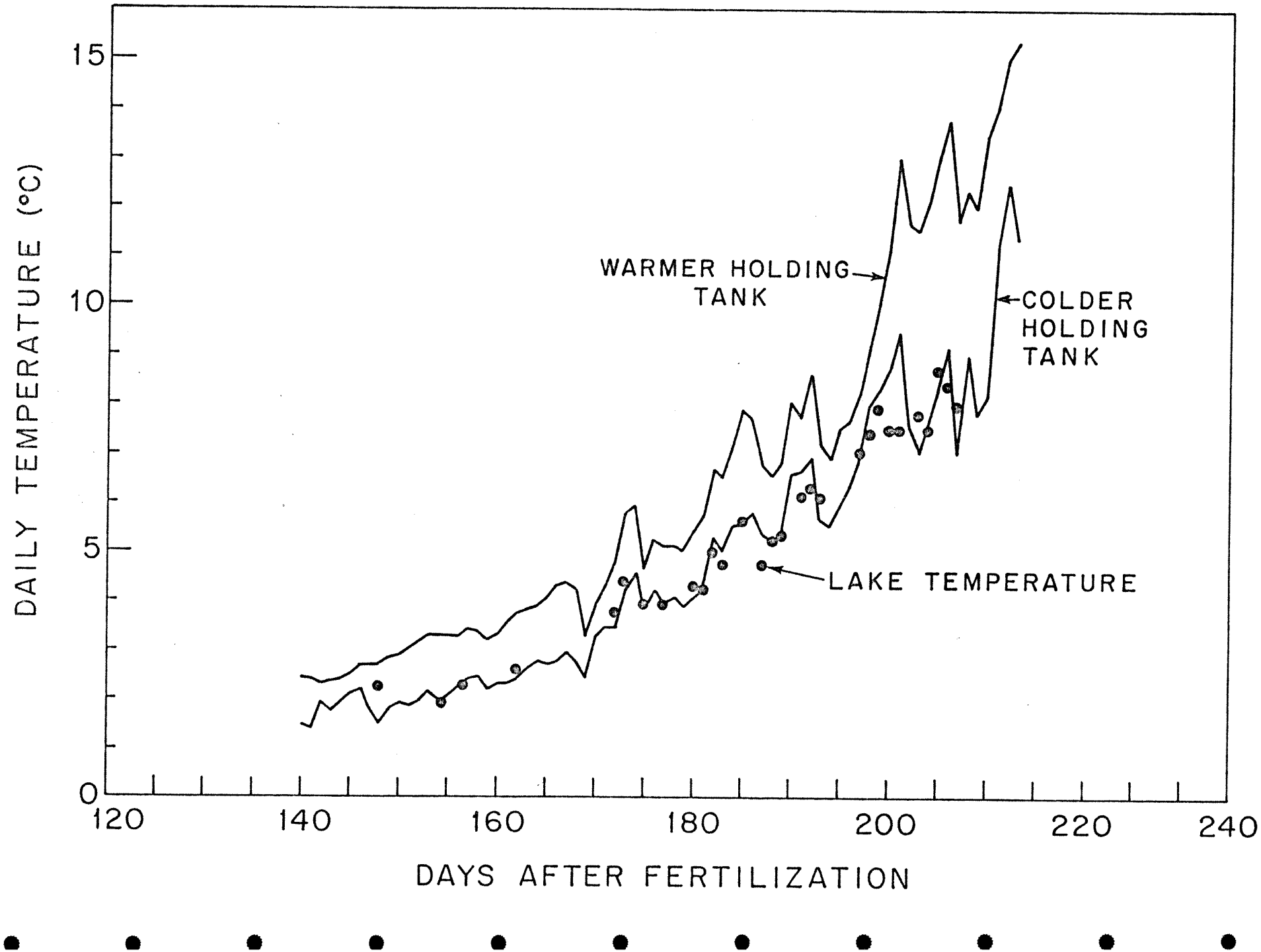

15
10
5
0
DAILY TEMPERATURE (°C)
120
140
160
180
200
220
240
DAYS AFTER FERTILIZATION
WARMER HOLDING TANK
COLDER HOLDING TANK
LAKE TEMPERATURE

Fig. 3-2. Natural and laboratory photoperiods.

The number of minutes of daylight at Trout Lake is plotted for the first day of each month over the number of degree-days experienced by fish reared in the holding tank matched, in temperature, to Trout Lake. Those monthly values are connected by broken lines for clarity only; intervening values were not determined. The number of hours of illumination in the laboratory is also shown. At the top, the total wattage of the overhead incandescent bulbs is displayed.

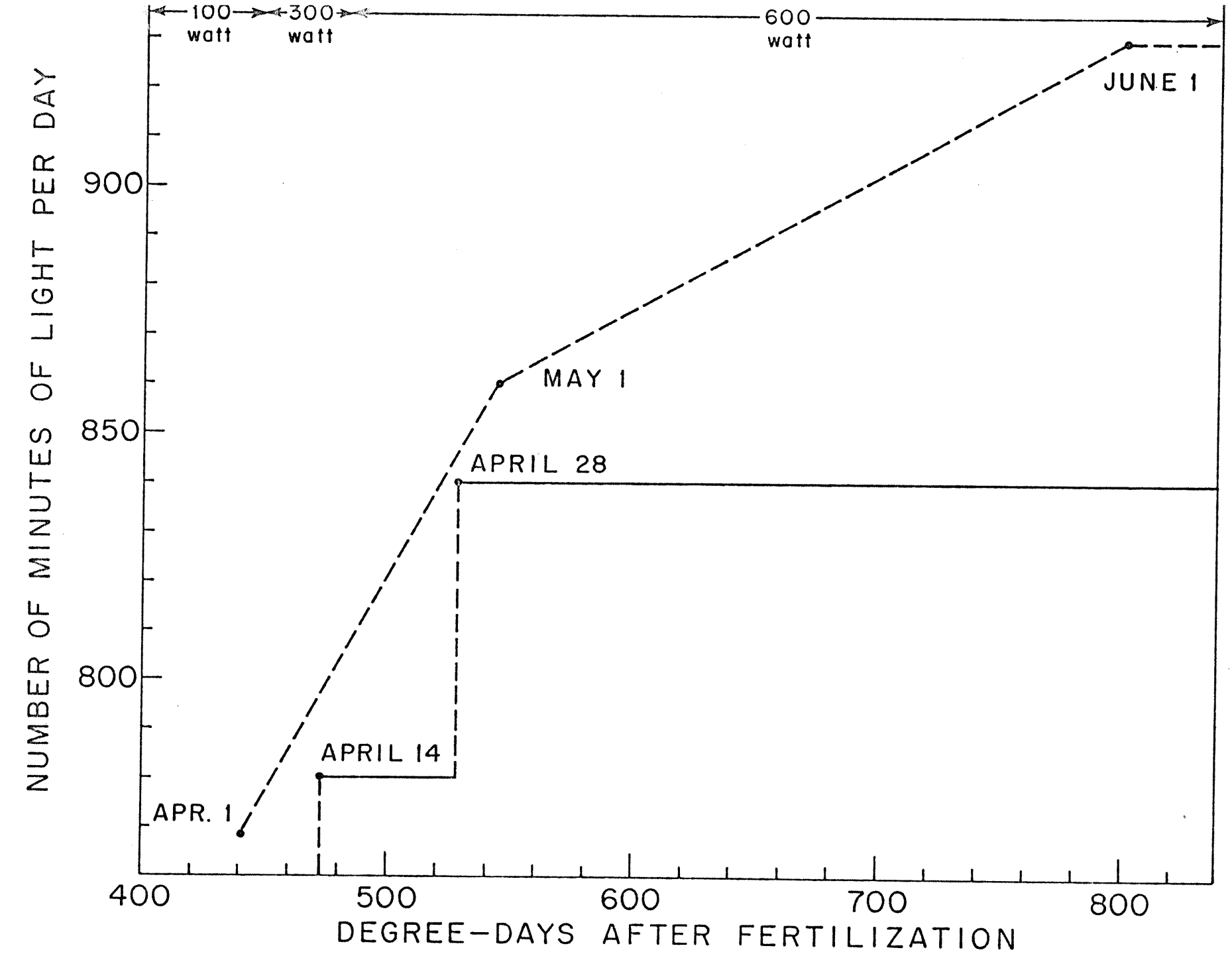

100 watt
300 watt
600 watt
JUNE 1
MAY 1
APRIL 28
APRIL 14
APR. 1
900
850
800
400
500
600
700
800
NUMBER OF MINUTES OF LIGHT PER DAY
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-3. Observation aquarium.

Fish were observed in aquaria such as this. The aquaria were constructed of clear plastic except for the screen in the dividing panel. Water entered the smaller front chamber, passed through the screen and exited from the rear chamber. The front chamber was partially filled with transparent glass marbles, 2.2 cm in diameter. The cork allowed withdrawal of water through a hypodermic needle for measurement of dissolved oxygen.

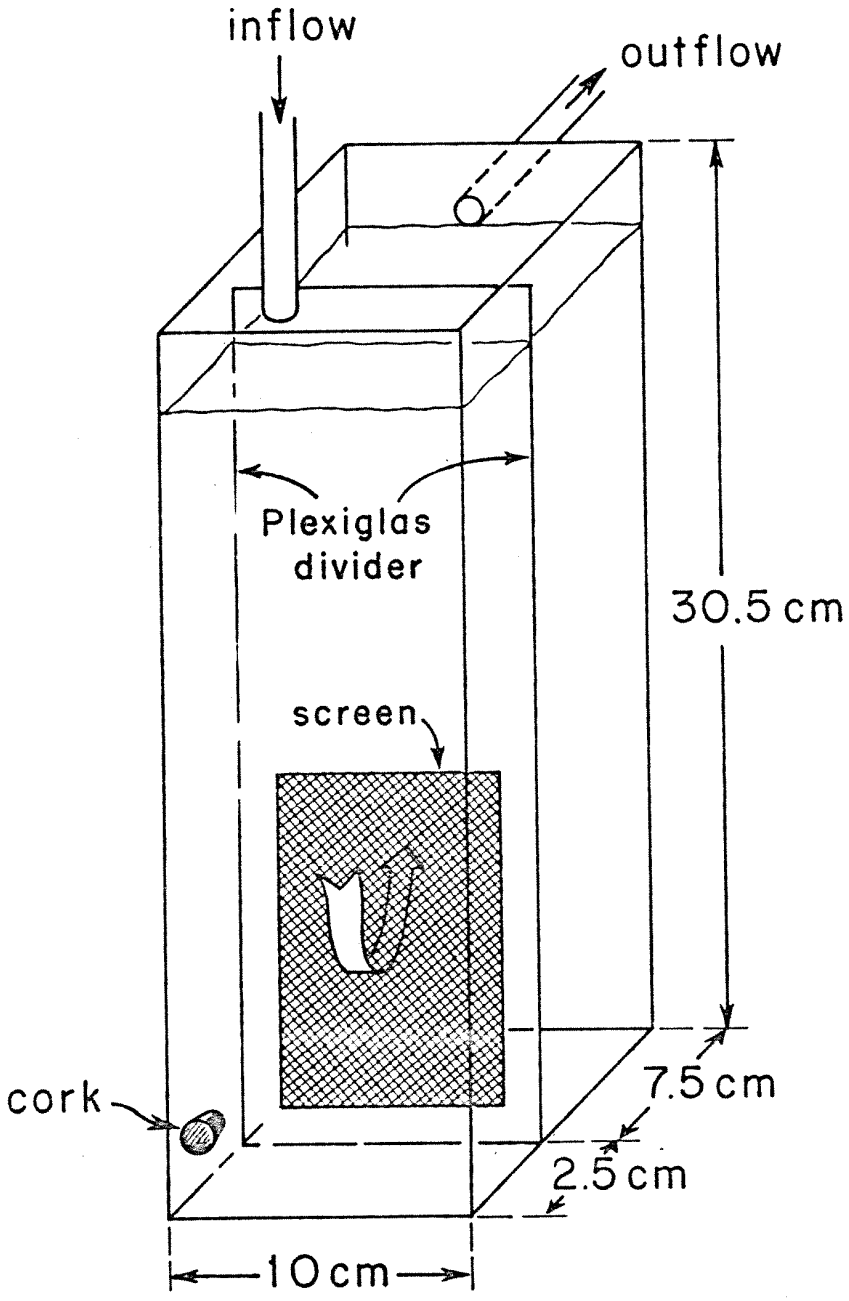

inflow
outflow
Plexiglas
divider
30.5 cm
screen
cork
7.5 cm
2.5 cm
10 cm

Fig. 3-4. Movement of sac-fry in water held at lake temperature.

Movement of individual sac-fry in white light (solid dots) is shown as a function of time after fertilization. Movement as defined here includes immobility in the water column. Individual fry were observed for 15 minutes. These data are for fish held at lake temperature. The superimposed dotted line is the percent of the cohort that were actively swimming when the observed fish was transferred to the observation aquarium. The solid line indicates daily temperature. The number of degree-days at which 50% of the cohort was hatched is shown. Small numerals denote numbers of points coinciding.

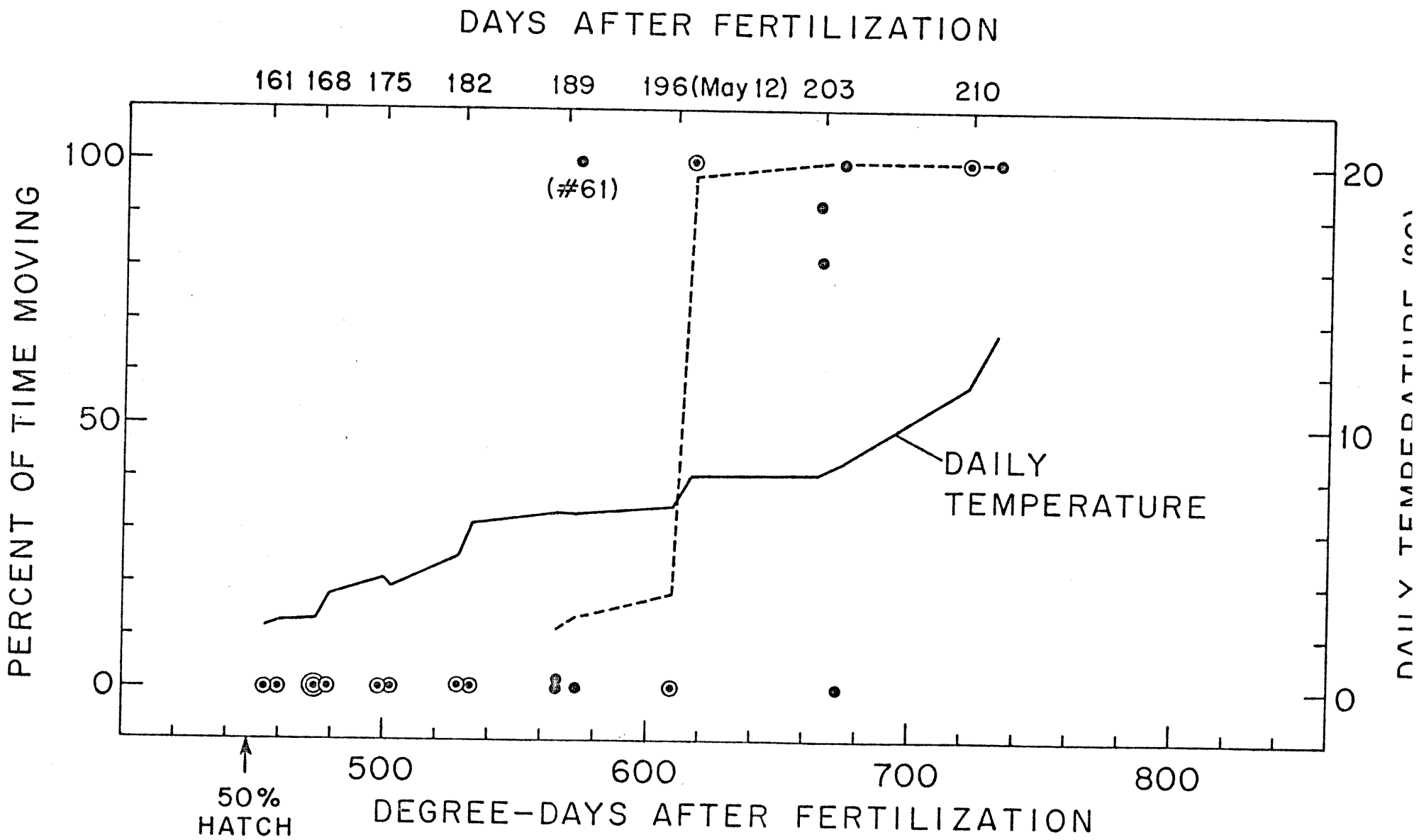

DAYS AFTER FERTILIZATION
161 168 175 182 189 196(May 12) 203 210
100
50
0
PERCENT OF TIME MOVING
(#61)
DAILY
TEMPERATURE
20
10
0
500
600
700
800
50%
HATCH
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-5. Movement of sac-fry in water kept warmer than lake temperature.

As in Fig. 3-4, movement is plotted here for observations made under white light. These data are for fish reared in the warmer holding tank. Daily water temperature is shown, as in Fig. 3-4. The superimposed unlabelled curve indicates the percent of aquaria (among those containing fish of Trout Lake parentage) having reached mean emergence in the experiment reported in Chapter V. Small numerals denote numbers of points coinciding. left out ?

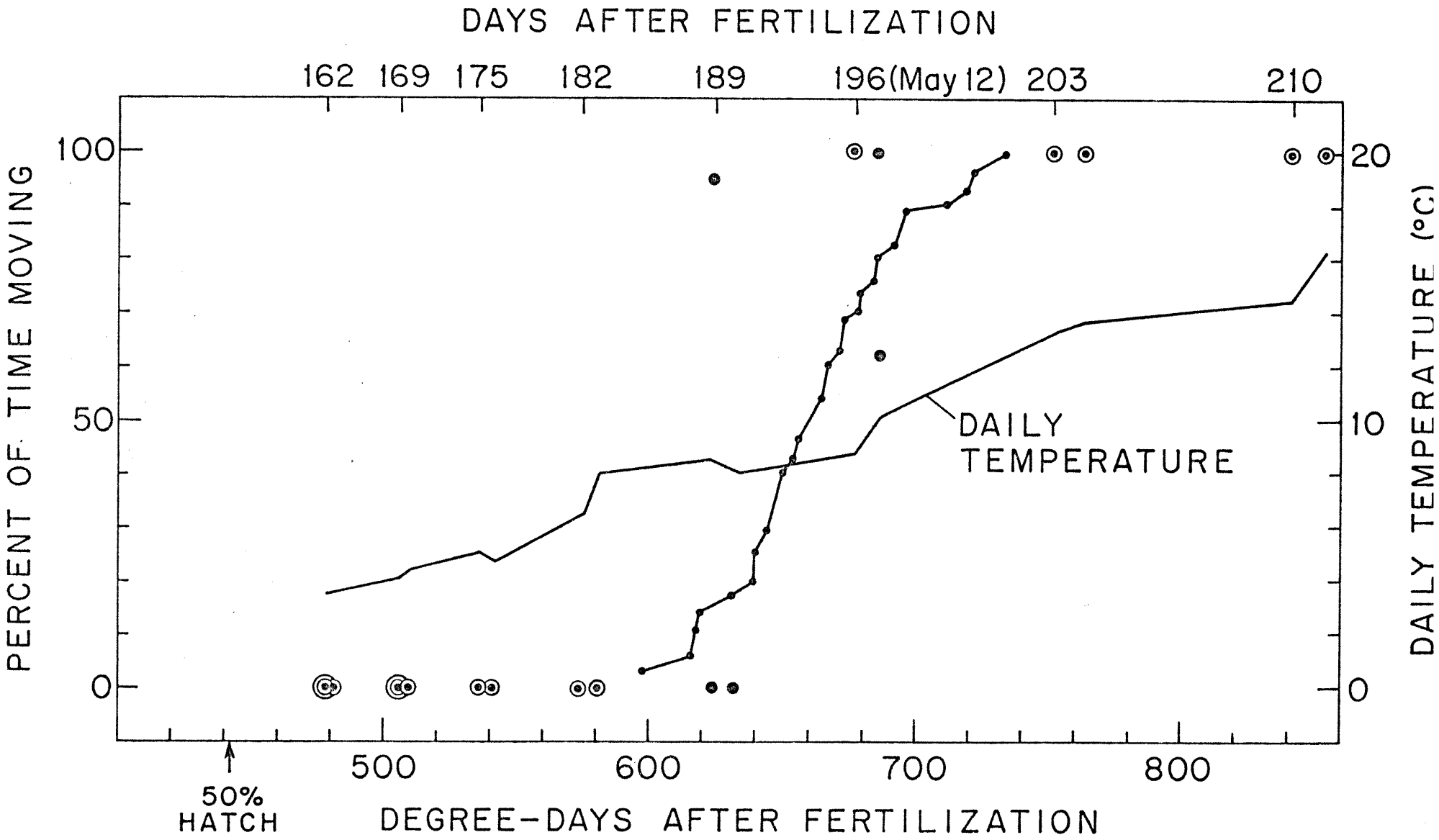

DAYS AFTER FERTILIZATION
162 169 175 182 189 196(May 12) 203 210
PERCENT OF TIME MOVING
100
50
0
DAILY TEMPERATURE (°C)
20
10
0
DAILY TEMPERATURE
500 600 700 800
50% HATCH
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-6. Diurnal activity pattern, warmer water.

Individual fish were observed for fifteen minutes by day (overhead incandescent bulb) and by night (portable red-light source) for fifteen minutes in each session. The differences between percent of time moving by day (Figs. 3-4 and 3-5) and percent of time moving by night are plotted here over degree-days elapsed since fertilization. Fish reared in cold water (lake temperature) and fish reared in warmer water are distinguished. Small numerals denote numbers of points coinciding.

left out

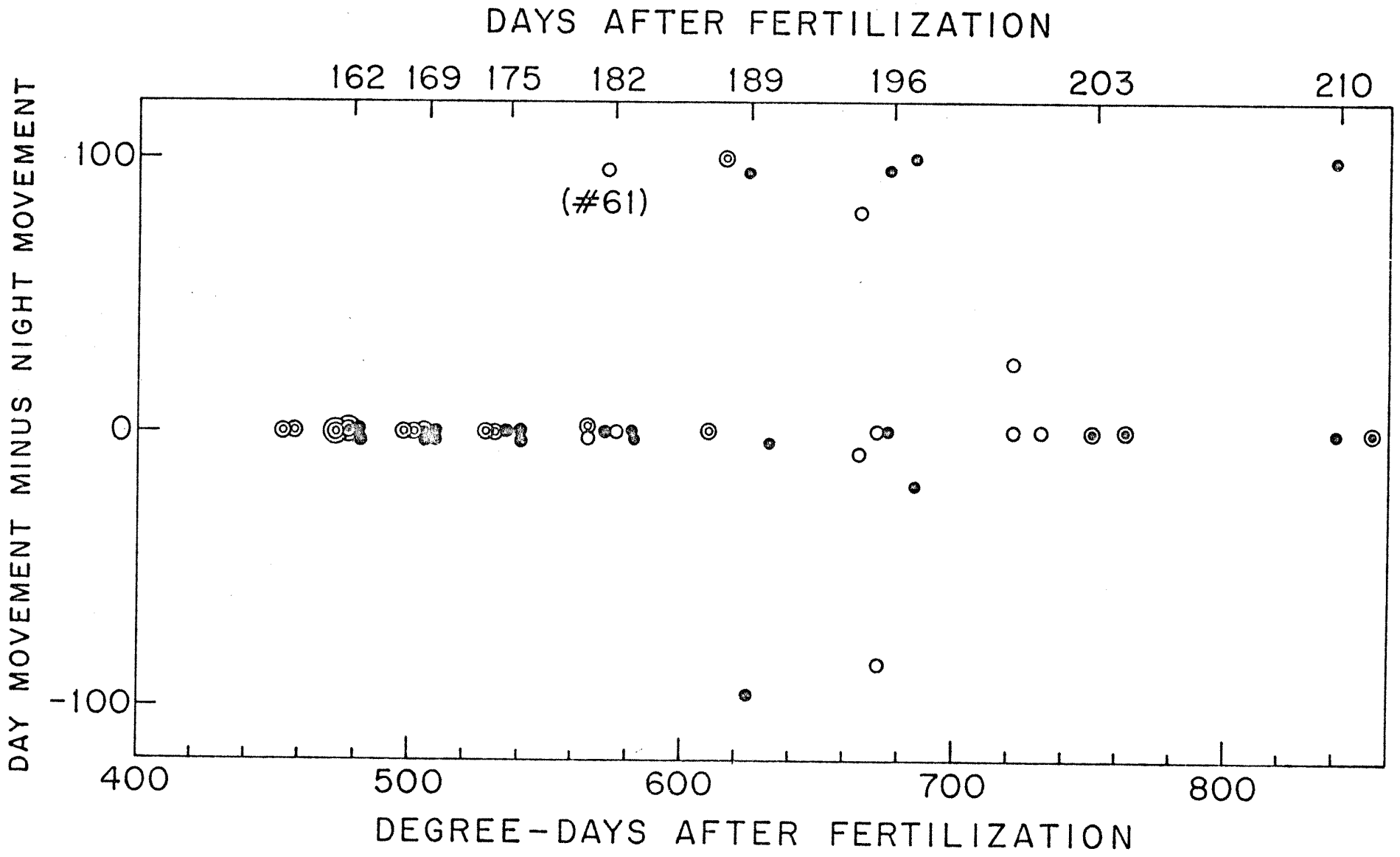

DAYS AFTER FERTILIZATION
162 169 175 182 189 196 203 210
100
0
-100
(#61)
DAY MOVEMENT MINUS NIGHT MOVEMENT
400 500 600 700 800
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-7. Response to contact.

Percent of contacts by fish (dots) or crayfish (X's) that were followed by conspicuously altered behavior is shown for individual fish observed by white light. Numerals denote numbers of coinciding points.

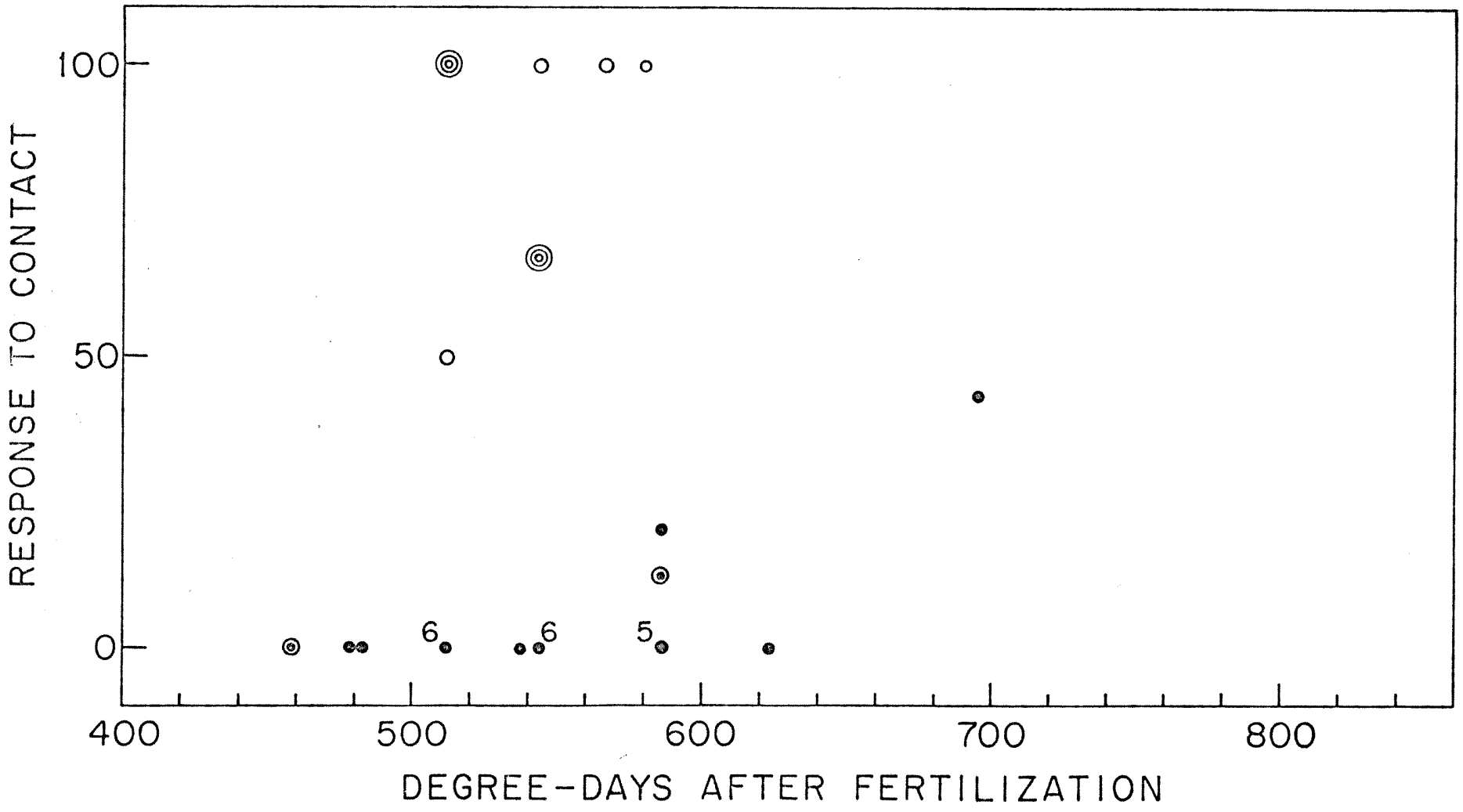

RESPONSE TO CONTACT
100
50
0
6
6
5
400
500
600
700
800
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-8. Change in dark preference with time.

Each point represents the percentage of four or five fish choosing the darkened end of a horizontal runway. Points are connected for clarity. Data were taken daily but time is expressed in degree-days to facilitate comparison with other figures.

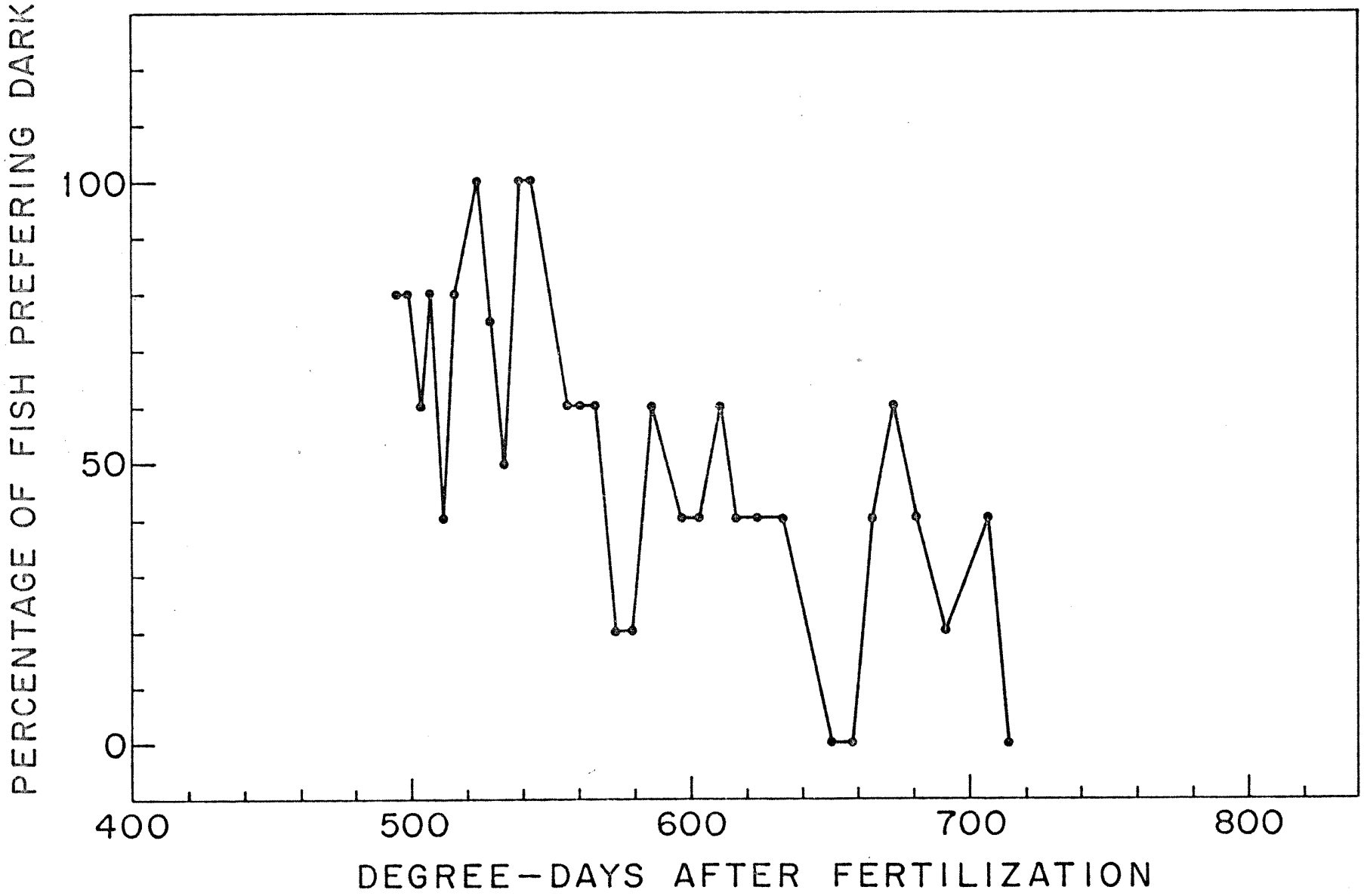

PERCENTAGE OF FISH PREFERING DARK
100
50
0
400
500
600
700
800
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-9. Ossification of teeth.

Number of ossified teeth (see text for definition) is plotted over degree-days since fertilization for individual fish. Fish reared in colder water (lake temperature) and fish reared in warmer water are distinguished. Numerals indicate numbers of coinciding points.

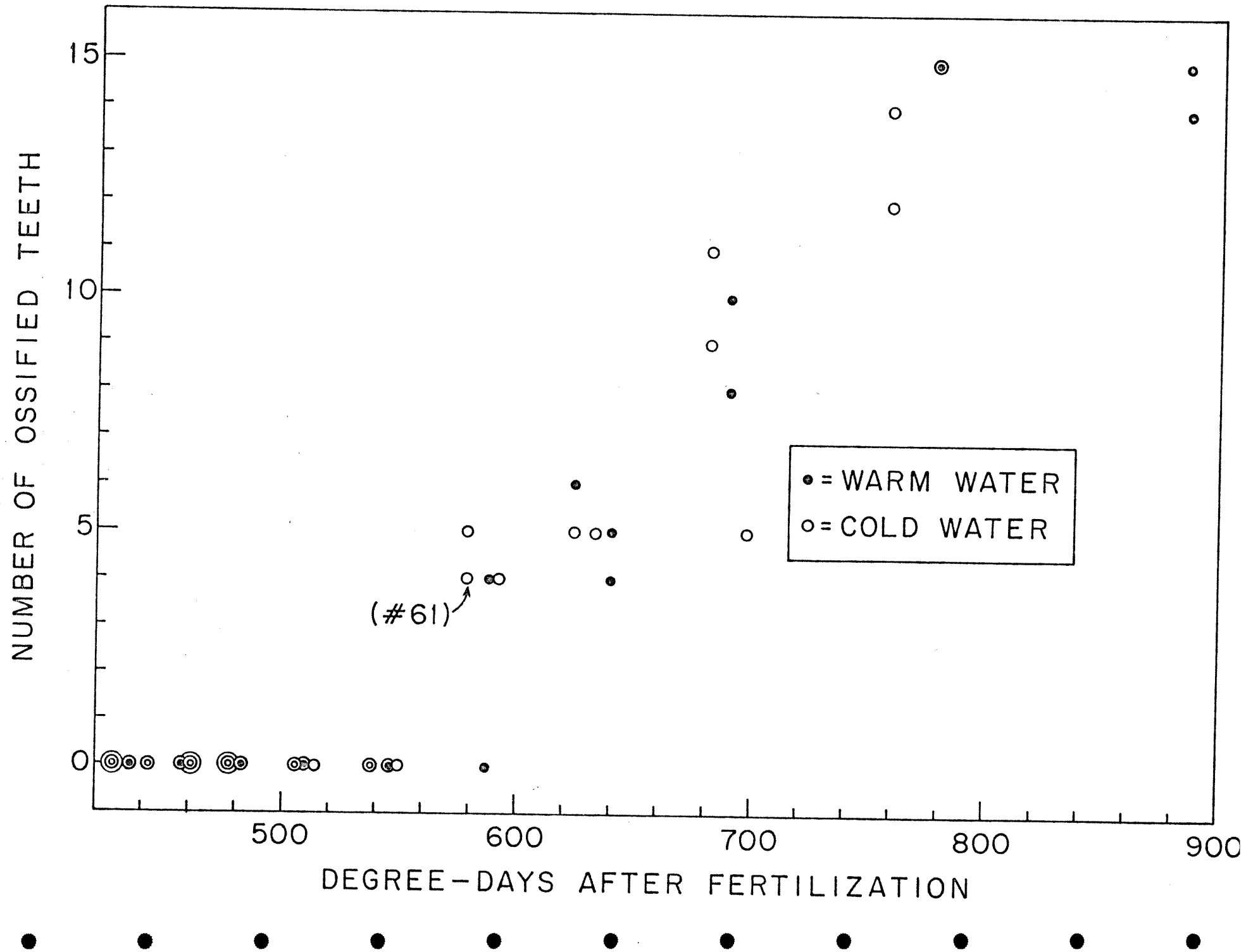

15
10
5
0
NUMBER OF OSSIFIED TEETH
500
600
700
800
900
DEGREE-DAYS AFTER FERTILIZATION
● = WARM WATER
○ = COLD WATER
(#61)

Fig. 3-10. Number of neural arches.

Numbers of neural arches (see text for definition) are plotted over degree-days since fertilization. Fish reared in colder water (lake temperature) and fish reared in warmer water are distinguished. Numerals denote numbers of coinciding points.

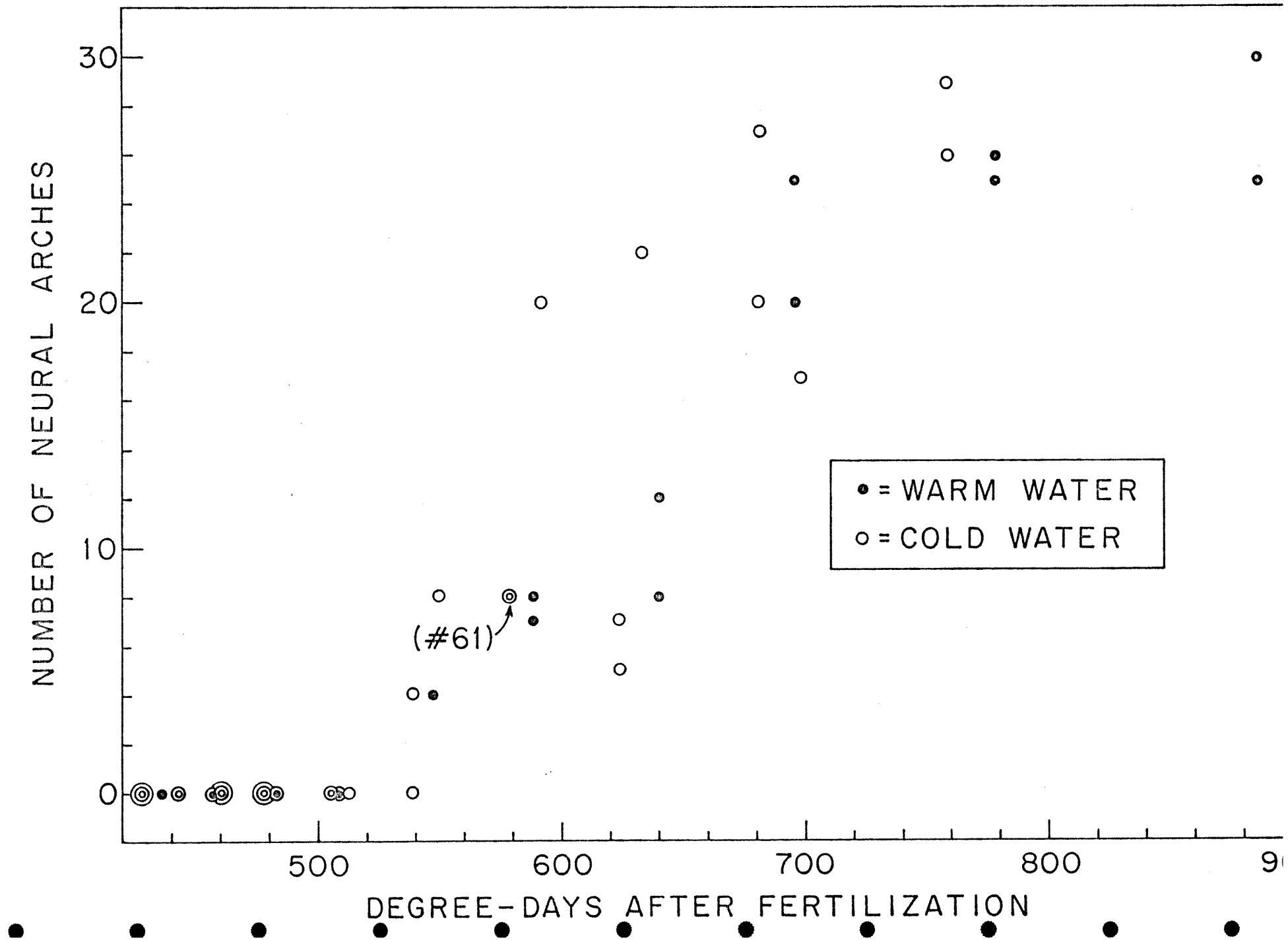

NUMBER OF NEURAL ARCHES
30
20
10
0
500
600
700
800
DEGREE-DAYS AFTER FERTILIZATION
(#61)
● = WARM WATER
○ = COLD WATER

Fig. 3-11. Skeleton length.

Skeleton length (see text for definition) is plotted over degree-days since fertilizaton. Fish reared in colder water (lake temperature) and fish reared in warmer water are distinguished. Numerals indicate numbers of coinciding points.

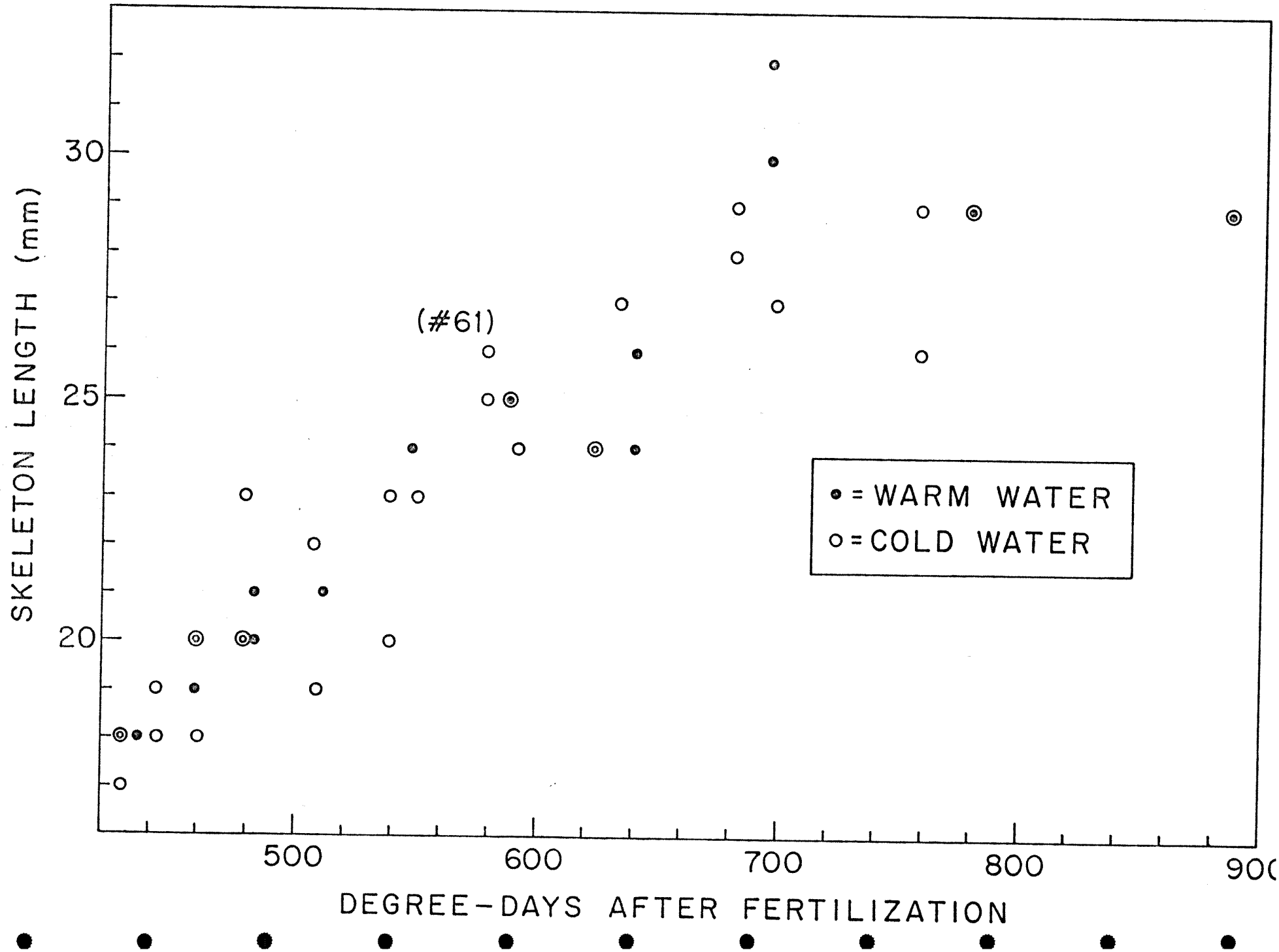

SKELETON LENGTH (mm)
30
25
20
(#61)
● = WARM WATER
○ = COLD WATER
500
600
700
800
DEGREE-DAYS AFTER FERTILIZATION

Fig. 3-12. Gut development and feeding.

Three variables related to feeding (see text for definitions) are plotted over degree-days since fertilization. Fish reared in colder water (lake temperature) and fish reared in warmer water are distinguished. Numerals denote numbers of coinciding points.

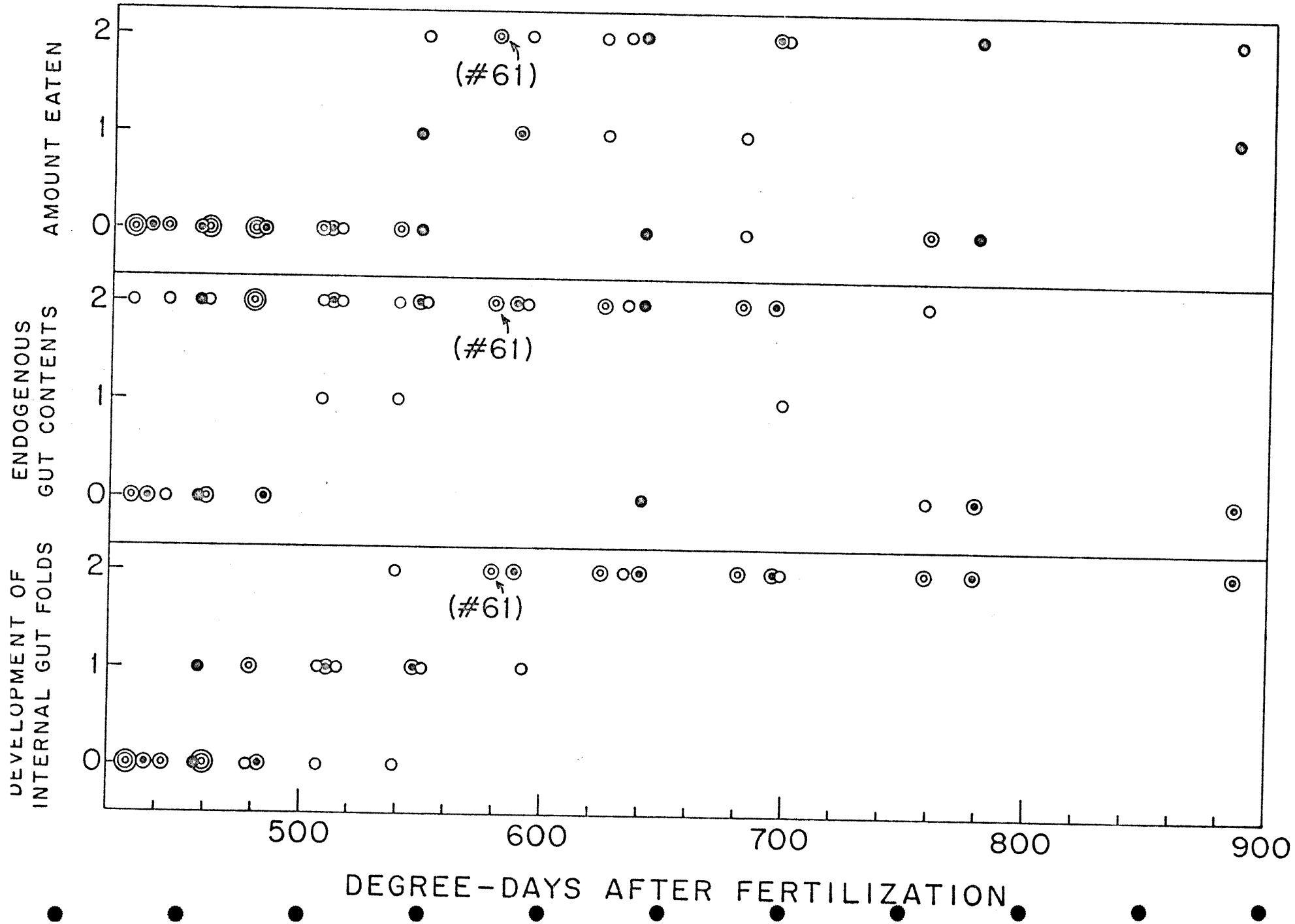

AMOUNT EATEN
ENDOGENOUS GUT CONTENTS
DEVELOPMENT OF INTERNAL GUT FOLDS
(#61)
(#61)
(#61)
0
1
2
500
600
700
800
900
DEGREE-DAYS AFTER FERTILIZATION

Table 5-1. Important dates and times in handling of gametes and fertilized eggs.

Source of Eggs	Date Gametes Were Taken	Date of Fertilization	Time of Fertilization	Date of Placement in Trout Lake
Marquette	27-X	29-X	1500	31-X
Crystal Springs	29-X	29-X	1400	31-X
Trout Lake	28-X	28-X	1800	31-X
Bayfield	27-X	27-X	1800	3-XI

Fig. 5-1. Crosses used in comparison of stocks.

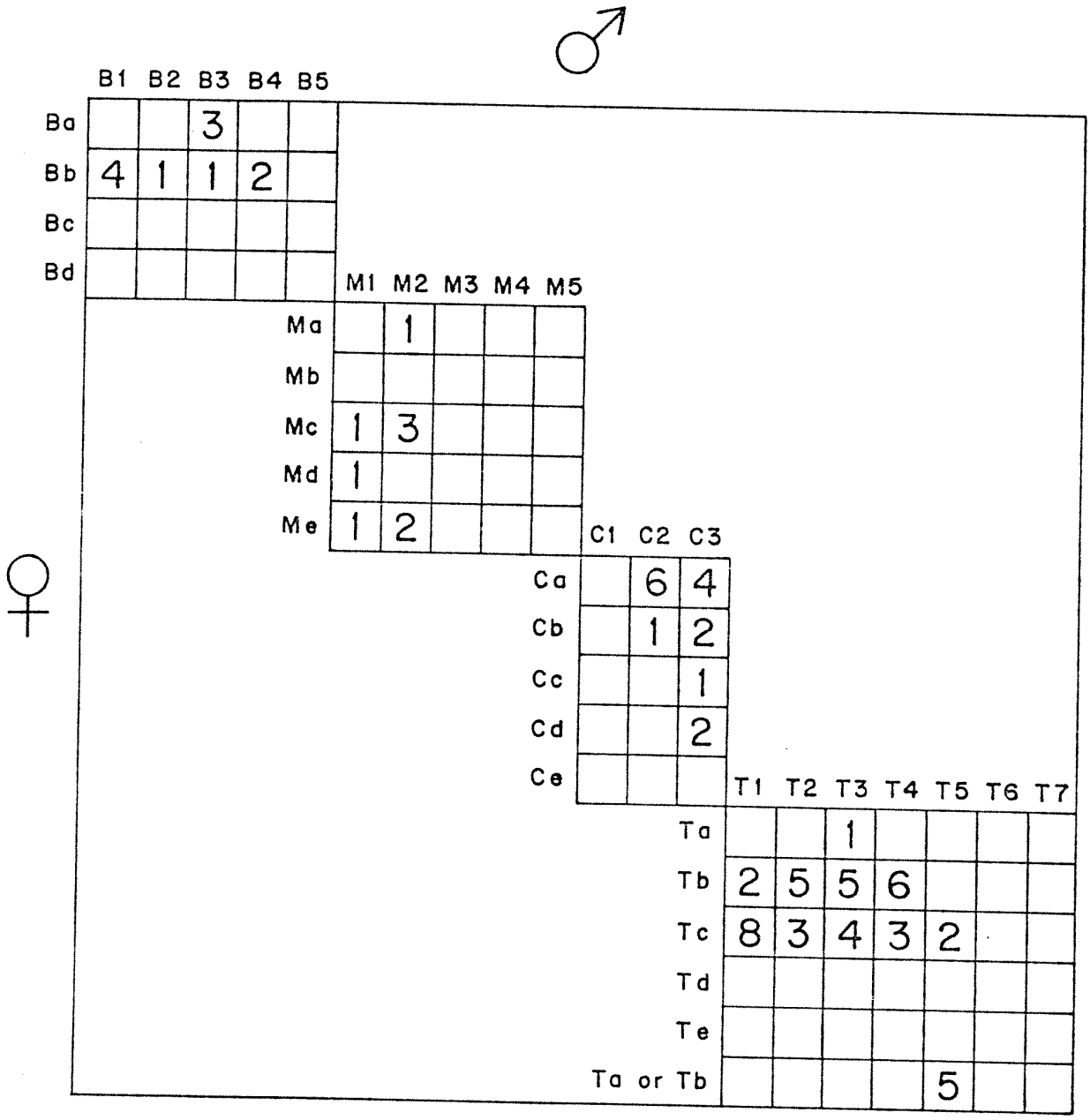

♂
B1 B2 B3 B4 B5
Ba
Bb
Bc
Bd
4 1 1 2
3
M1 M2 M3 M4 M5
Ma
Mb
Mc
Md
Me
1 3
1
1 2
1
C1 C2 C3
Ca
Cb
Cc
Cd
Ce
6 4
1 2
1
2
T1 T2 T3 T4 T5 T6 T7
Ta
Tb
Tc
Td
Te
Ta or Tb
1
2 5 5 6
8 3 4 3 2
5
♀

Fig. 5-2. Times preceding hatching for four stocks.

Data for individual aquaria are shown. Within each stock, data for distinct crosses are separated laterally; vertical lines connect within-cross replicates. Open circles represent means for the four stocks. For each stock vertical bars denote a total range of four standard errors of the mean. Standard error estimates assume a) equal error variance for each stock and b) insignificant parental effect within stocks. Numerals denote numbers of coinciding points.

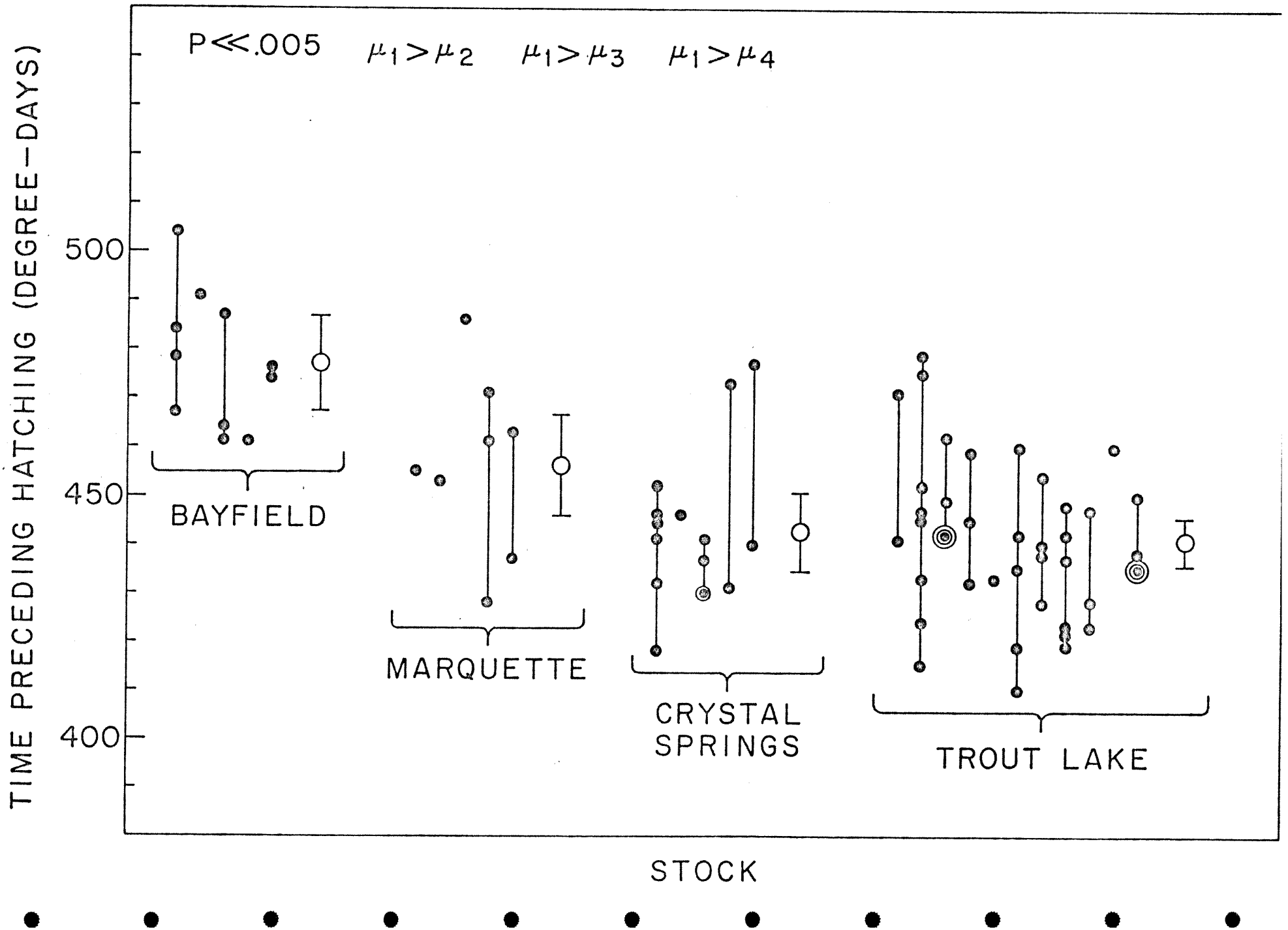

P≪.005
$\mu_1 > \mu_2$
$\mu_1 > \mu_3$
$\mu_1 > \mu_4$
TIME PRECEDING HATCHING (DEGREE–DAYS)
500
450
400
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-3. Times preceding emergence for four stocks.

Data for individual aquaria are shown as in Fig. 5-2. See Fig. 5-2 caption for clarification.

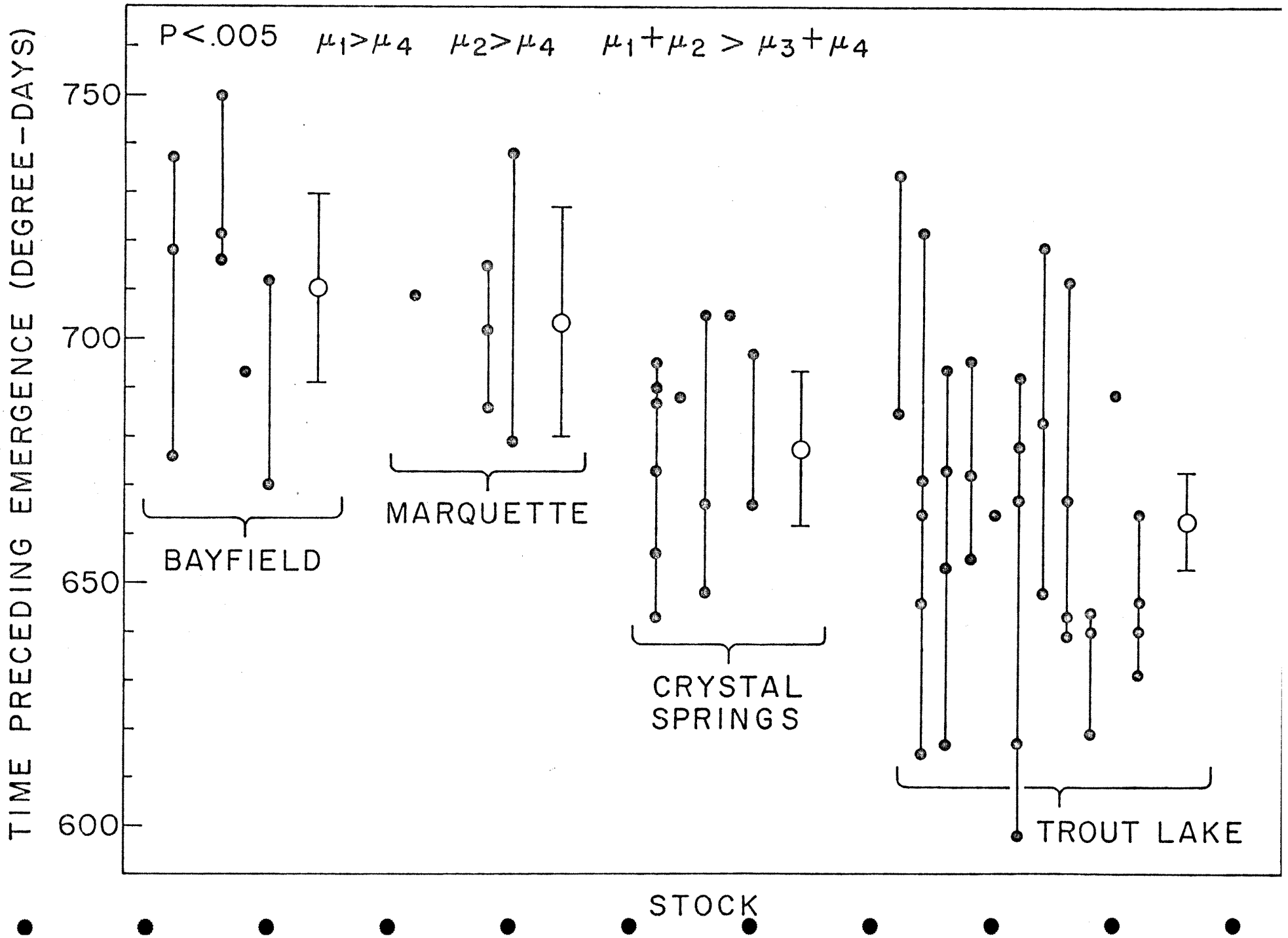

P<.005 $\mu_1>\mu_4$ $\mu_2>\mu_4$ $\mu_1+\mu_2>\mu_3+\mu_4$
TIME PRECEDING EMERGENCE (DEGREE-DAYS)
750
700
650
600
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-4. Time preceding hatching as a function of egg diameters.

Data for individual aquaria are shown. This figure is analogous to Fig. 5-5, only the independent variable is different. See Fig. 5-5 for clarification.

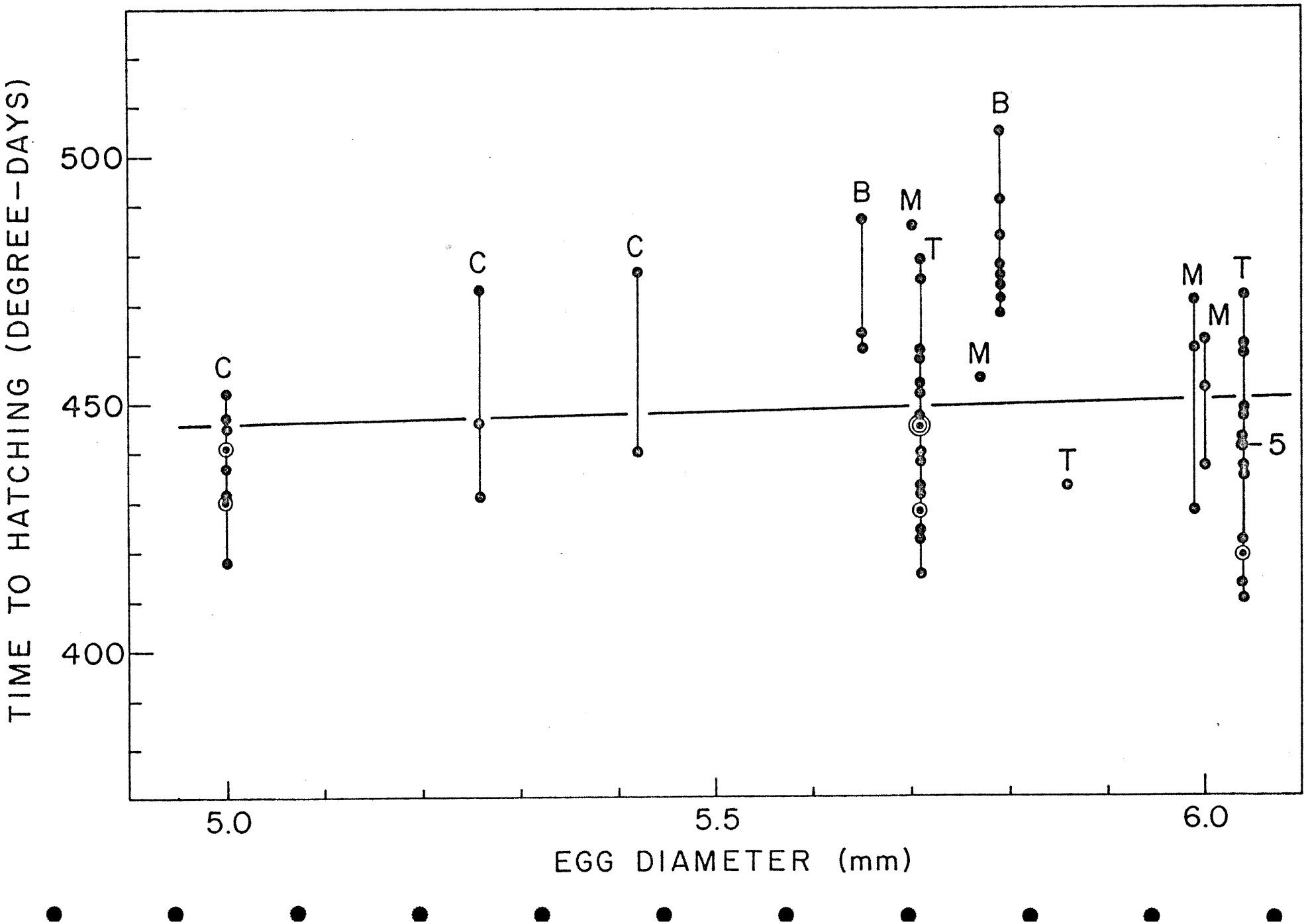

TIME TO HATCHING (DEGREE-DAYS)
500
450
400
5.0
5.5
6.0
EGG DIAMETER (mm)
C
C
C
B
M
T
B
M
T
M
M
T
5

Fig. 5-5. Time preceding hatching as a function of mother's length.

Data for individual aquaria are shown. The best fitting (least squares) straight line is shown. Vertical lines connect data for individual mothers. Capital letters indicate which stock the data are from: B, Bayfield; M, Marquette; C, Crystal Springs; T, Trout Lake. Numerals denote numbers of coinciding points.

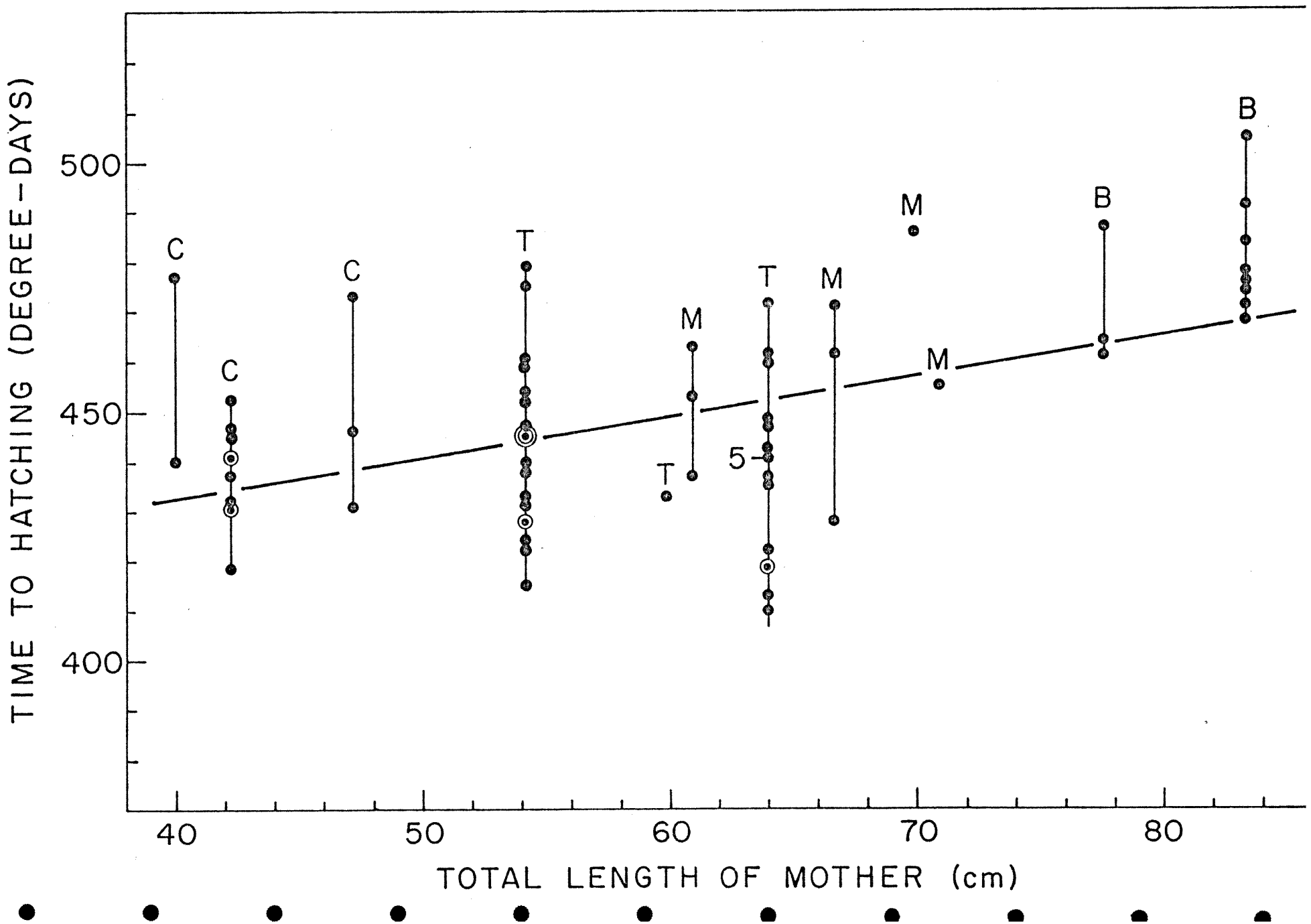

TIME TO HATCHING (DEGREE-DAYS)
500
450
400
40
50
60
70
80
TOTAL LENGTH OF MOTHER (cm)
C
C
C
T
M
T
T
5
M
M
M
B
B

Fig. 5-6. Effect of type of egg shelter on time preceding hatching.

Data for individual aquaria are shown. Solid circles denote aquaria that were stocked with eggs incubated in small bottles (see text); open circles denote aquaria stocked with eggs incubated in large boxes. Numerals denote numbers of coinciding points.

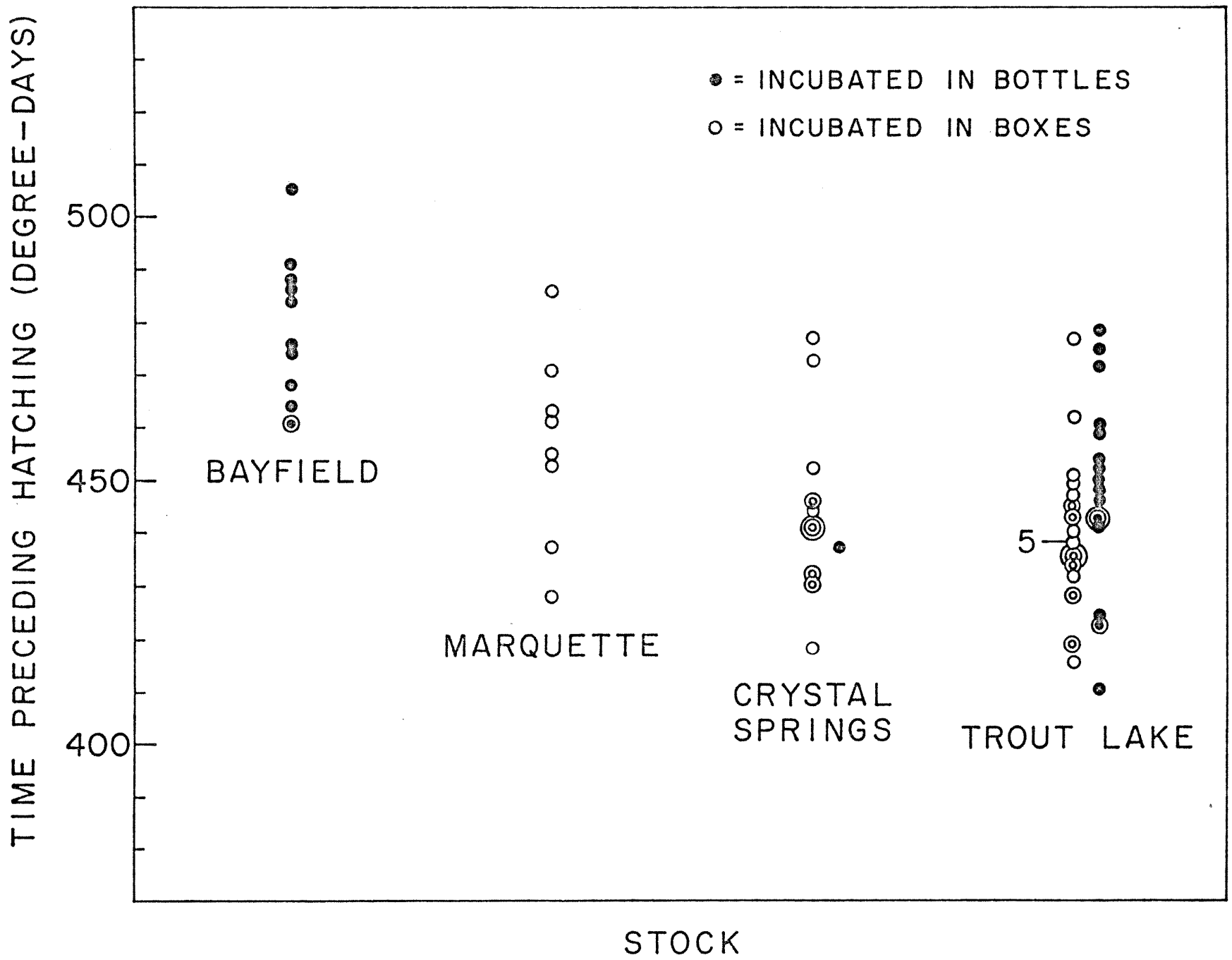

TIME PRECEDING HATCHING (DEGREE-DAYS)
500
450
400
● = INCUBATED IN BOTTLES
○ = INCUBATED IN BOXES
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
5
STOCK

Fig. 5-7. Number of neural arches on May 1 for four stocks.

Data for individual aquaria are shown as in Fig. 5-2. Open circles with vertical bars represent group means and four-standard-error ranges as in Fig. 5-3. Numerals denote numbers of coinciding points.

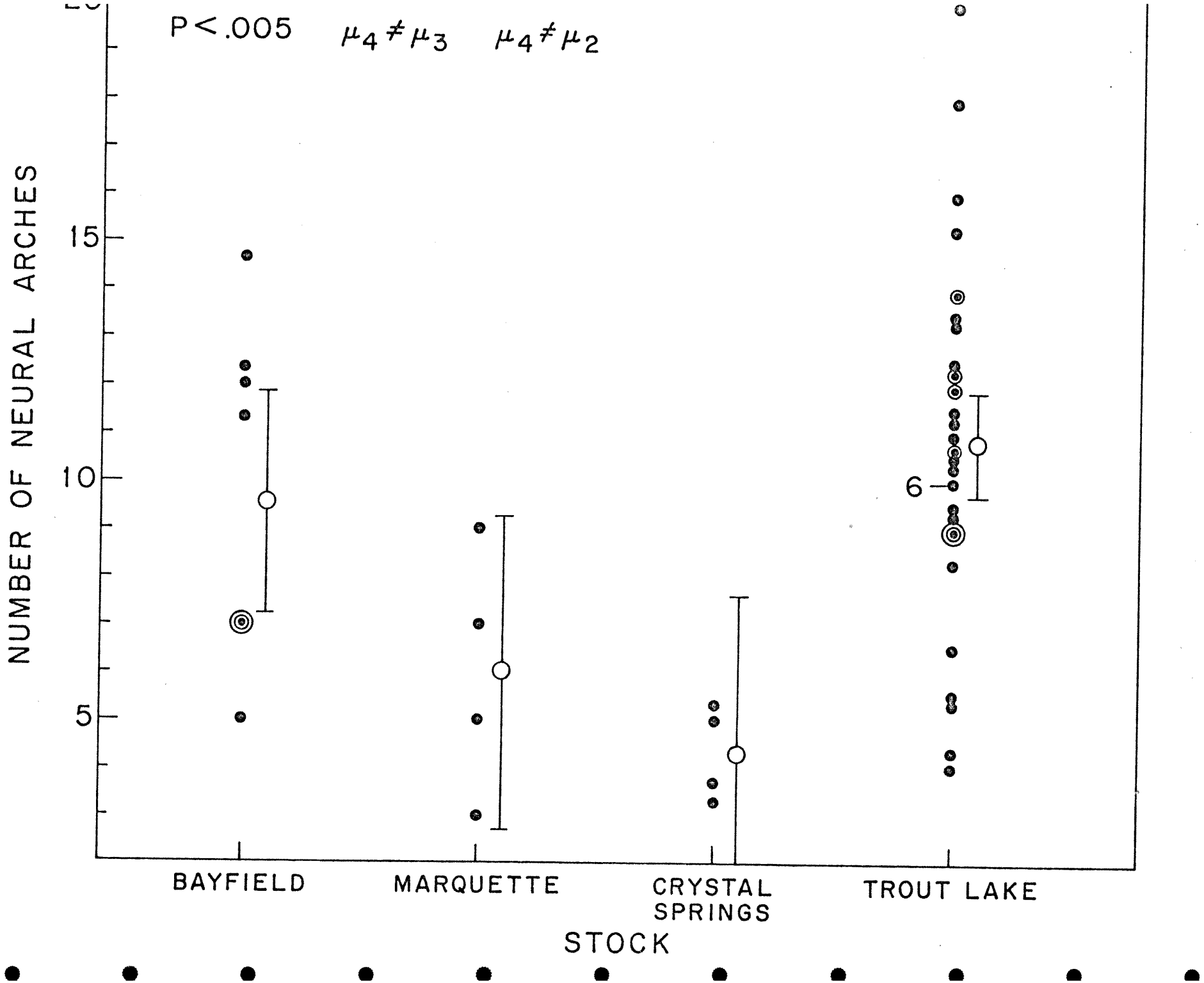

P<.005 $\mu_4 \neq \mu_3$ $\mu_4 \neq \mu_2$
NUMBER OF NEURAL ARCHES
15
10
5
6
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-8. Skeleton length on May 1 for four stocks.

Data for individual aquaria as displayed here as in Fig. 5-2.

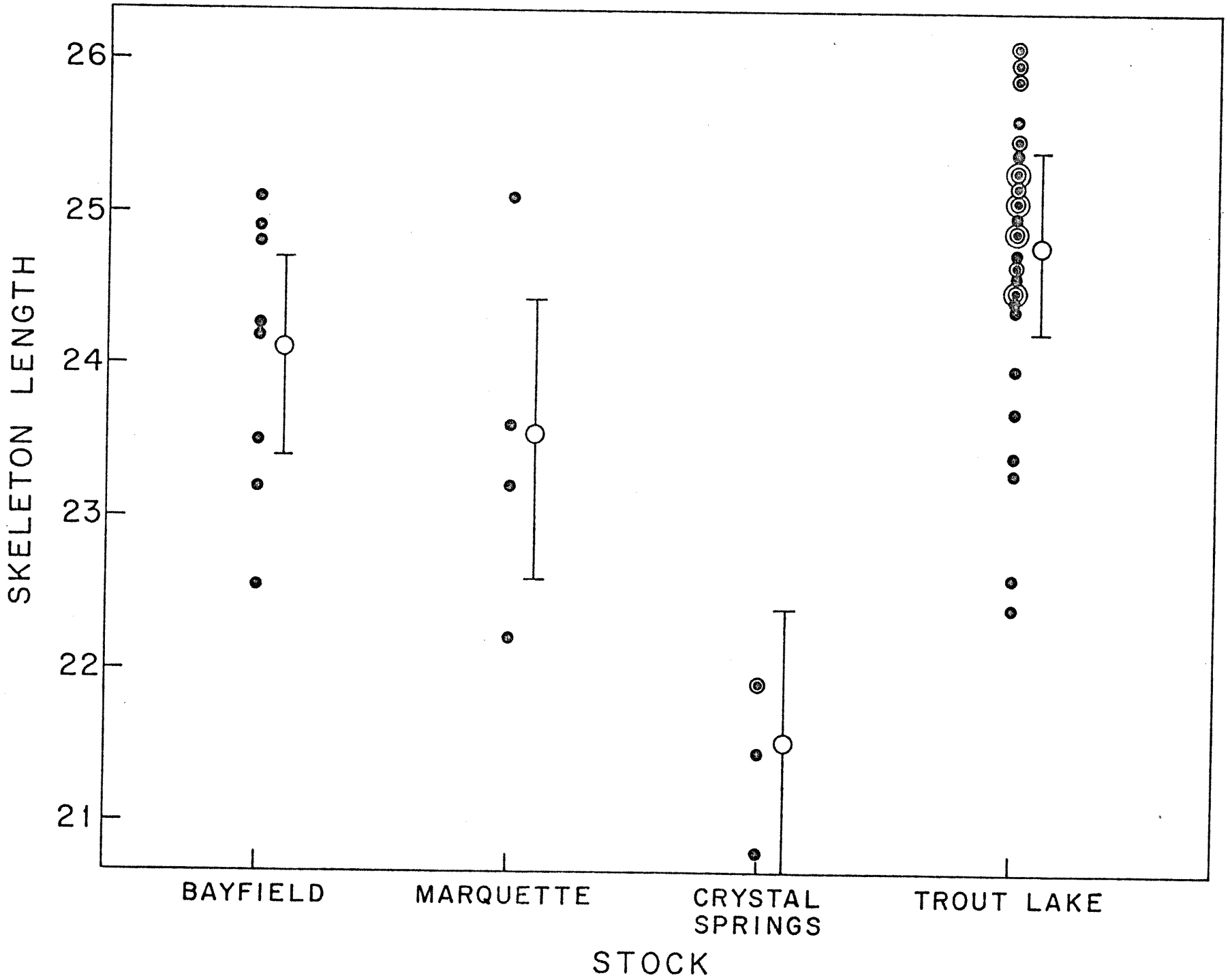

SKELETON LENGTH
26
25
24
23
22
21
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-9. Gut fold development on May 1 for four stocks.

Data for individual aquaria are displayed as in Fig. 5-2.

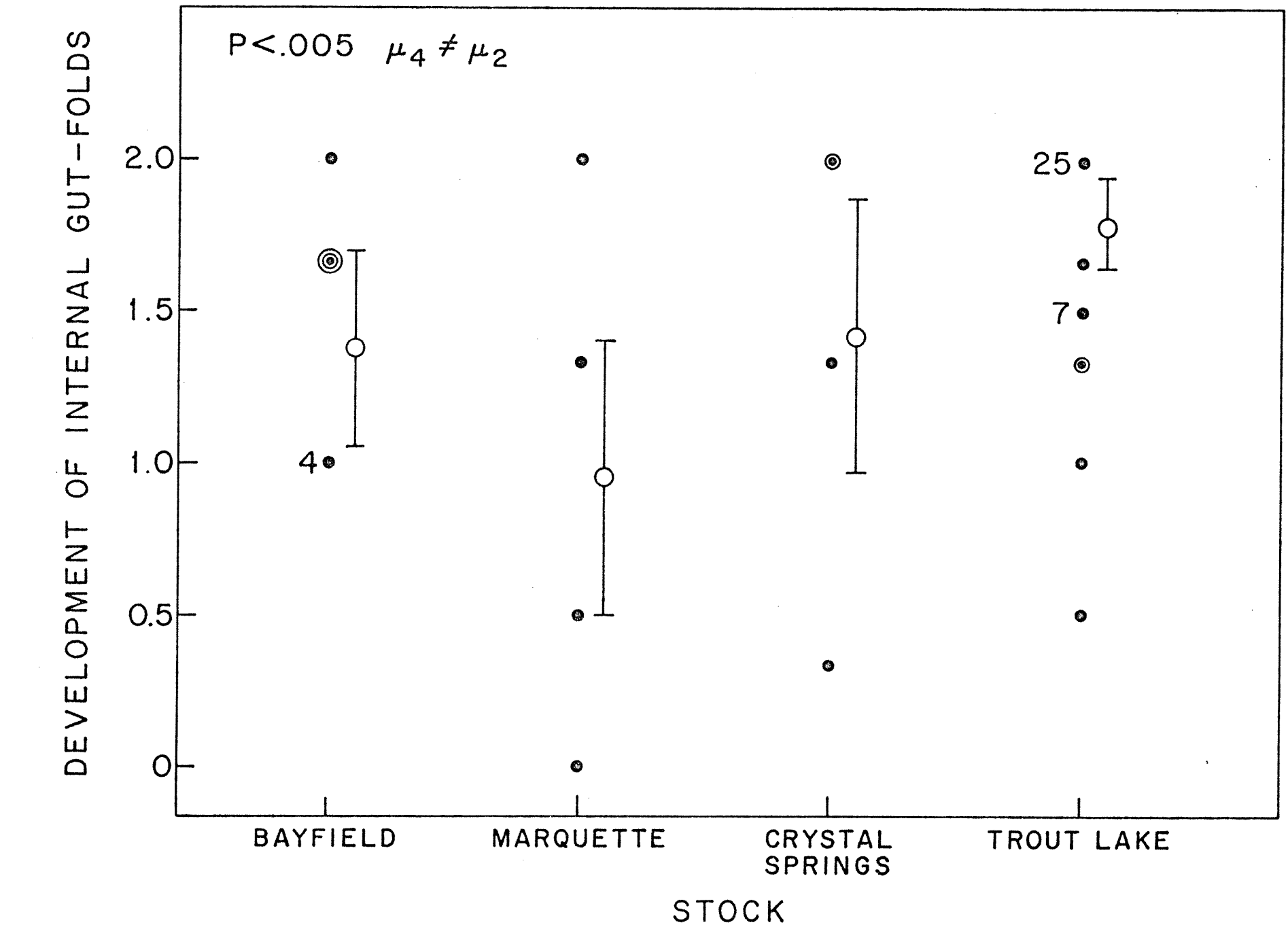

P<.005 $\mu_4 \neq \mu_2$
DEVELOPMENT OF INTERNAL GUT-FOLDS
2.0
1.5
1.0
0.5
0
4
25
7
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-10. Amount eaten on May 1 for four stocks.

Data for individual aquaria are displayed as in Fig. 5-2 except that error bars are absent.

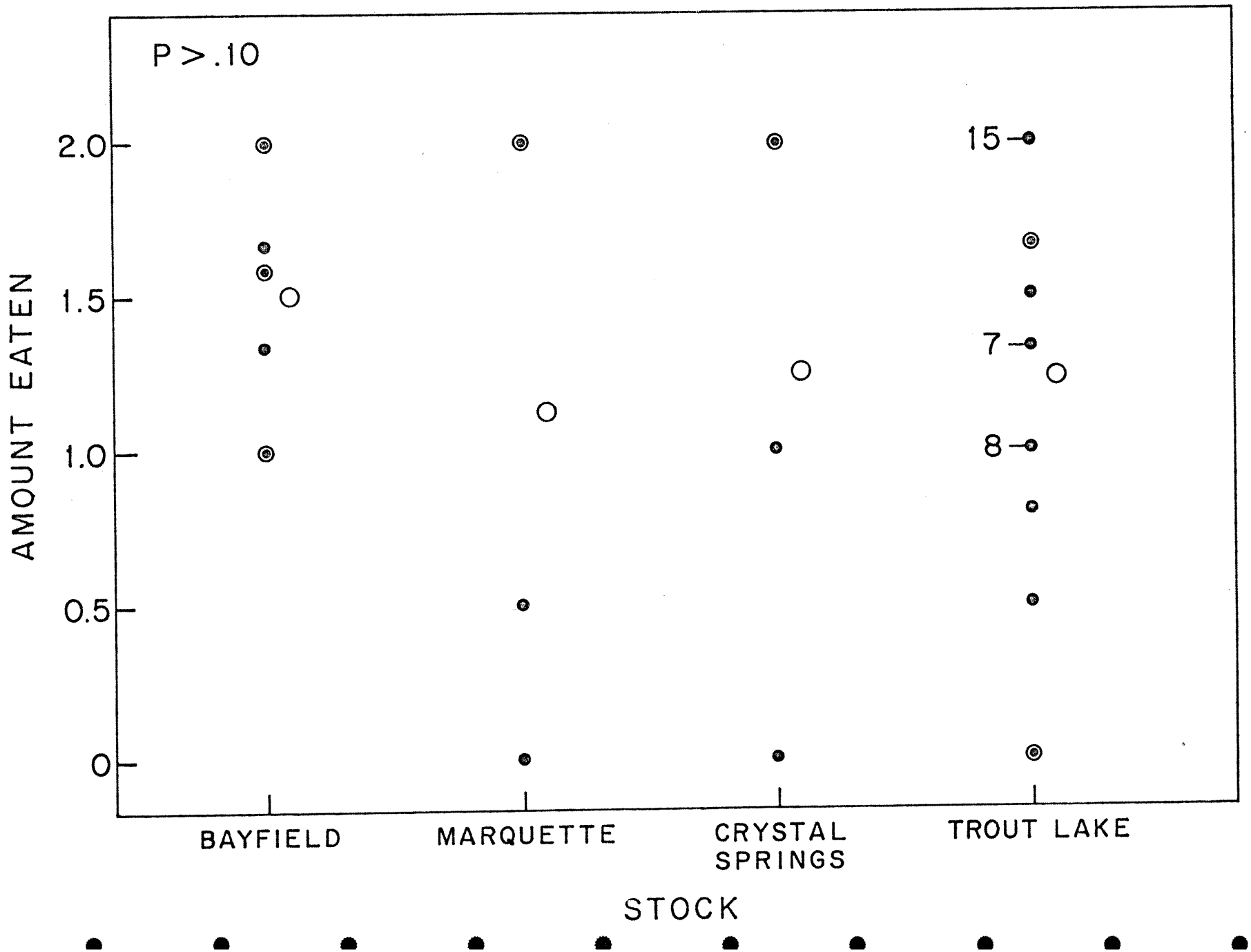
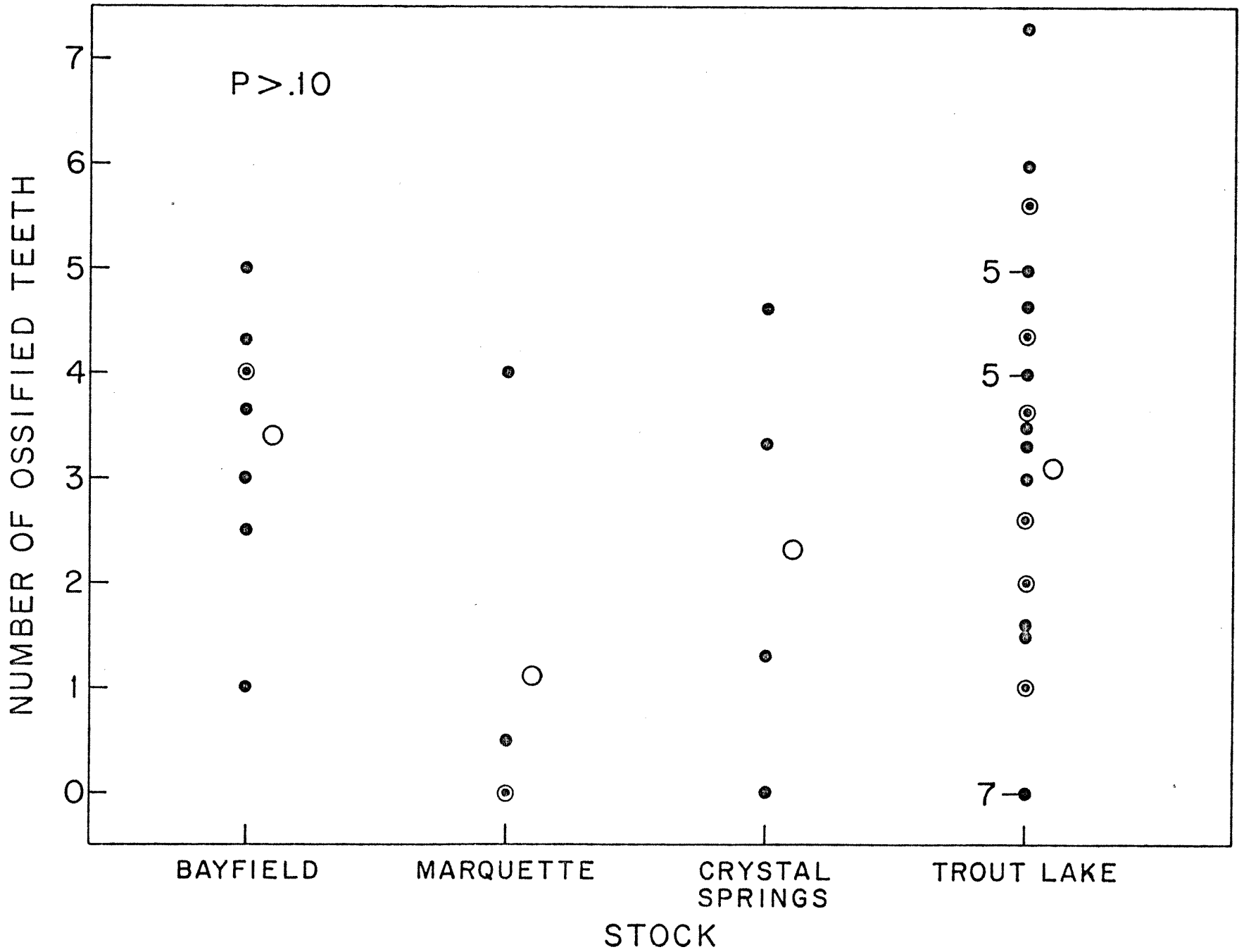

P > .10
AMOUNT EATEN
2.0
1.5
1.0
0.5
0
15
7
8
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

Fig. 5-11. Number of ossified teeth on May 1 for four stocks.

Data for individual aquaria are displayed as in Fig. 5-2 except that error bars are absent.


P>.10
NUMBER OF OSSIFIED TEETH
0
1
2
3
4
5
6
7
5
5
7
BAYFIELD
MARQUETTE
CRYSTAL SPRINGS
TROUT LAKE
STOCK

APPENDIX 1

CRAYFISH PREDATION ON LAKE TROUT EGGS IN TROUT LAKE, WISCONSIN

SYNOPSIS

Substrate composition is an important factor determining lake trout egg survival. Crayfish as well as other large predators consume significant numbers of eggs even when the eggs are deposited over rocky substrate. Artificial predator exclosures reduce mortality even where rocky shelter is available. Crayfish species Orconectes virilis and O. propinquus differ in tendency to eat accessible lake trout eggs.

INTRODUCTION

Lake trout (Salvelinus namaycush) deposit their eggs in late fall and provide no parental care (Royce 1951). The substrate must provide protection from predators including many fish species (Machniak 1975); the mudpuppy, Necturus maculosus (Hacker 1957); and crayfish. Predation is considered one of the principal mortality factors guiding adaptations in spawning behavior and spawning site selection (Balon 1975).

In 1932 Orconectes virilis was believed to be the predominant crayfish species throughout the lakes of northern Wisconsin (Creaser 1932) including Trout Lake. Since then O. propinquus has displaced O. virilis as the predominant species in Trout Lake. Some evidence suggests that natural reproduction by lake trout in Trout Lake has declined in recent years (McKnight 1977), possibly coincident with the increase in abundance of O. propinquus.

Efforts to establish natural reproduction by planted lake trout in Lake Michigan have, for the most part, failed (some natural reproduction by stocked lake trout has been demonstrated in Grand Traverse Bay, Stauffer 1978). The role of predators in that failure is not known although in Lake Superior survival of eggs spawned by planted fish is reasonably high (8.9%) when the eggs are sheltered from

large predators (Peck 1978). Stauffer and Wagner (1976) found that more eggs were recovered (dead or alive, hatched or unhatched) when planted in covered containers than when planted in uncovered ones, although low sample sizes precluded firm inferences. Predation on lake trout eggs by several fish species known in Lake Michigan is well documented (Stauffer 1978, Wagner 1978). Crayfish (usually not identified to species but known to include O. virilis, James Lorman, pers. comm.) have been found on several spawning areas in Lake Michigan (Keillor et al. 1977, Stauffer and Wagner 1976).

The purposes of our experiments were to (1) evaluate predation on lake trout eggs by O. propinquus and other predators on a natural spawning reef, (2) determine the importance of rocks as cover in reducing egg predation in nature, and (3) compare predation rates among Orconectes species.

METHODS

Field Experiments

The study site, a lake trout spawning reef in Trout Lake, Wisconsin, was a rocky area about 100 m from shore near the inflow of Allequash Creek. The depth was six to ten meters.

Four boxes, each .23 m deep and .38 m^2 in internal

area, were placed at each of five locations on the study site. One box at each location was designed to allow access by fishes and mudpuppies but exclude crayfish. Another box at each location was designed to exclude fishes and mudpuppies but enclose crayfish. Five adult male O. propinquus were stocked in each of those. Those crayfish densities (13 m^{-2}) were comparable to total natural crayfish density in the area but far above the natural density of comparably sized animals. A third box at each location was designed to exclude all large predators. Also at each location were two controls from which no large predators were excluded. One control was the fourth box; it had open sides. The other was simply a designated area of the substrate.

In November fertilized lake trout eggs obtained from fish netted in Trout Lake were placed in basins (25 cm in diameter and 10 cm deep) inside each of the 25 boxes or control areas. In each of two experiments the surviving eggs were counted twice during the week following placement, first after eggs were placed among rocks and again after the rocks had been removed. The percent mortality between inspections was computed. Rocks used to shelter eggs in the experimental basins were gathered from the surrounding substrate. They varied greatly in size but averaged approximately 750 cc and were piled two layers

deep in each basin.

In late November developing eggs were again placed among rocks on the bottom of Trout Lake but survival was assessed at the time of swim-up in spring using emergence traps patterned after those of Collins (1975). The boxes were emptied after the fall experiments. Rocks were piled in the center of each and, on November 22, 1978, 100 developing eggs were placed among the rocks within each. Four or five adult male O. propinquus were placed in each box designed to exclude fishes and mudpuppies but enclose crayfish. The various boxes and controls were left undisturbed for three months. On February 17, 1979, emergence traps were placed over each. The traps fit inside the boxes and covered the rocks among which the planted eggs were incubating. Large predators were, from then on, excluded from all developing eggs except controls unless they had already entered a box. In the controls crayfish or other demersal predators could still reach the eggs by crawling under the edge of the traps.

Laboratory Experiments

Two-way factorial experiments with substrate at three levels (large rocks - approximately 1000 cc, small rocks - approximately 50 cc, and bare sand) and species at two levels (O. virilis and O. propinquus) were conducted at

different temperatures as the lake water cooled from 10 C to 2 C. Experiments were conducted in flow-through aquaria supplied with water from Trout Lake at the Trout Lake Biological Station of the University of Wisconsin-Madison. Crayfish were studied in sets matched by carapace length (smallest 28 mm, largest 44 mm). Each contained four (later experiments) or five (early experiments) adult male O. propinquus or O. virilis. The rocks were closely packed in two layers over the sand. In each aquarium five eggs per crayfish were scattered over the bottom at the start of each experiment, but after addition of the crayfish. After 32 hours (including 13.5 hours of dark and 18.5 hours of light from 15 watt incandescent bulbs suspended individually over each aquarium) the surviving eggs were counted.

RESULTS

Field Experiments

Results of fall experiments (Fig. A-1) were analyzed in a series of sign tests in which paired differences between box types within locations were used. Data from the first two experiments were combined in these comparisons giving a sample size of ten for each test. No effort is made here to adjust p-values to correct for multiple comparisons.

Enclosed crayfish (O. propinquus) ate eggs whether the eggs were among rocks ($p<.004$) or not ($p<.002$), but ate more when the eggs were not among rocks ($p<.002$). Natural predators including crayfish (O. propinquus) ate eggs whether among rocks ($p<.05$) or not ($p<.002$), but ate more when the eggs were not among rocks ($p<.002$). Natural predators including crayfish ($p<.02$) and natural predators not including crayfish ($p<.02$) ate more eggs than enclosed crayfish when eggs were not among rocks. Boxes designed to keep crayfish out but let fish in were occasionally invaded by crayfish which were removed when discovered. Those boxes might have excluded mudpuppies as well. Enclosed crayfish ate an average of 18% (SE = 5) of eggs placed among rocks and 60% (SE = 12) of eggs not placed among rocks in 2 to 4 days.

No consistent statistically significant differences exist among controls, crayfish exclosures, and crayfish enclosures for eggs that were placed among rocks. This is because of extreme variability in predation on sheltered eggs.

In the winter experiments no emerging fry were captured from eggs deposited among rocks in controls that did not exclude any large predators. Where fish and/or crayfish were excluded, eggs survived to be captured as fry in 13 of 15 boxes.

Survival of eggs not eaten by large predators was good. Eggs used in the first of the fall field experiments were fertilized seven days before planting and, among those not eaten, 92% survived six days with one-half of the mortalities occurring in a single basin. The eggs used in the second experiment were planted 13 days after fertilization and 99.7% of those not eaten survived five days. In one exclosure at least 18% of eggs planted 22 days after fertilization, survived 193 days until capture. Since the capture efficiency of the emergence traps is unknown, the actual survival might have been higher. All eggs used in the experiments were held at lake temperature until planting.

Laboratory Experiments

Statistical analyses of laboratory experiments indicated that (Fig. A-2):

a) The size of rocks in the substrate influences egg predation by crayfish.

b) O. virilis and O. propinquus have different rates of egg predation.

c) Differences between crayfishes are important when eggs are exposed.

d) Egg consumption rate is positively correlated with

water temperature.

A two-way factorial analysis with five observations per cell was employed. Crayfish species and substrate were the two factors. Data for all experiments were combined and temperature was introduced as a linear covariate. The interaction between substrate and species was statistically significant ($p<.025$). Thus, both substrate and species were significant in their own right at one or more levels of the other factor.

DISCUSSION

The composition of the substrate is a critical factor in lake trout egg survival. Eggs deposited where they are exposed to predators will not survive. Natural predators, including or excluding O. propinquus, eat essentially all eggs not placed among rocks. Predators other than crayfish eat such accessible eggs more rapidly than O. propinquus alone. While deposition among rocks improves short-term survival, long-term survival is still low. We were unable to detect any long-term survival unless some predators were artificially excluded. When all large predators are excluded, the probability of survival to swim-up is increased. Our survival rate of 8% (median of five values) was close to the 8.9% found under similar conditions by

Peck (1978) in Lake Superior.

Predators other than O. propinquus accounted for most of the predation on eggs not placed among rocks. However, since exposed eggs are doomed, it is predation on eggs deposited among rocks that concerns us in a) assessing the roles of crayfish and other large predators and b) comparing crayfish species as trout egg predators. Each predator group (fishes and mudpuppies; crayfish) apparently can be important in long-term survival when eggs are placed among rocks, since artificially excluding either group alone, increases the proportion of eggs surviving to swim-up. In comparing O. propinquus and O. virilis as egg predators, laboratory data for predation on sheltered eggs is relevant. When large rocks sheltered eggs, inter-species differences were less pronounced than when eggs were fully exposed. No inter-species differences existed when eggs were deposited among small rocks. Thus, for crayfish of the same size and abundance the significance, for lake trout, of a species shift from O. virilis to O. propinquus might be slight. Since O. propinquus is a smaller species than O. virilis, the relation between crayfish size and egg predation should be studied, particularly where eggs are placed among rocks typical of natural spawning areas.

Planting lake trout eggs in containers providing

shelter from large predators is a technically feasible alternative to rearing them in hatcheries and then planting as fingerlings or yearling fish. We have shown that reasonably high survival to the swim-up stage can be expected. Artificially excluding predators significantly decreases mortality even where natural shelter is available.

ACKNOWLEDGEMENTS

Support was from the National Science Foundation (DEB 77-22223) and the National Oceanic and Atmospheric Administrations' Office of Sea Grant, Department of Commerce, through an institutional grant to the University of Wisconsin. We thank Thomas Kern, Walter Haag, Douglas Stamm, Ross Horrall, and the personnel of the Wisconsin Department of Natural Resources at Bayfield and Woodruff.

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Fig. A-1. Egg predation under field conditions.

Mortality of eggs when accessible to different predators for short periods in fall or all winter. Eggs were placed among rocks or were exposed in open plastic wash basins. Values are medians of five replicates.

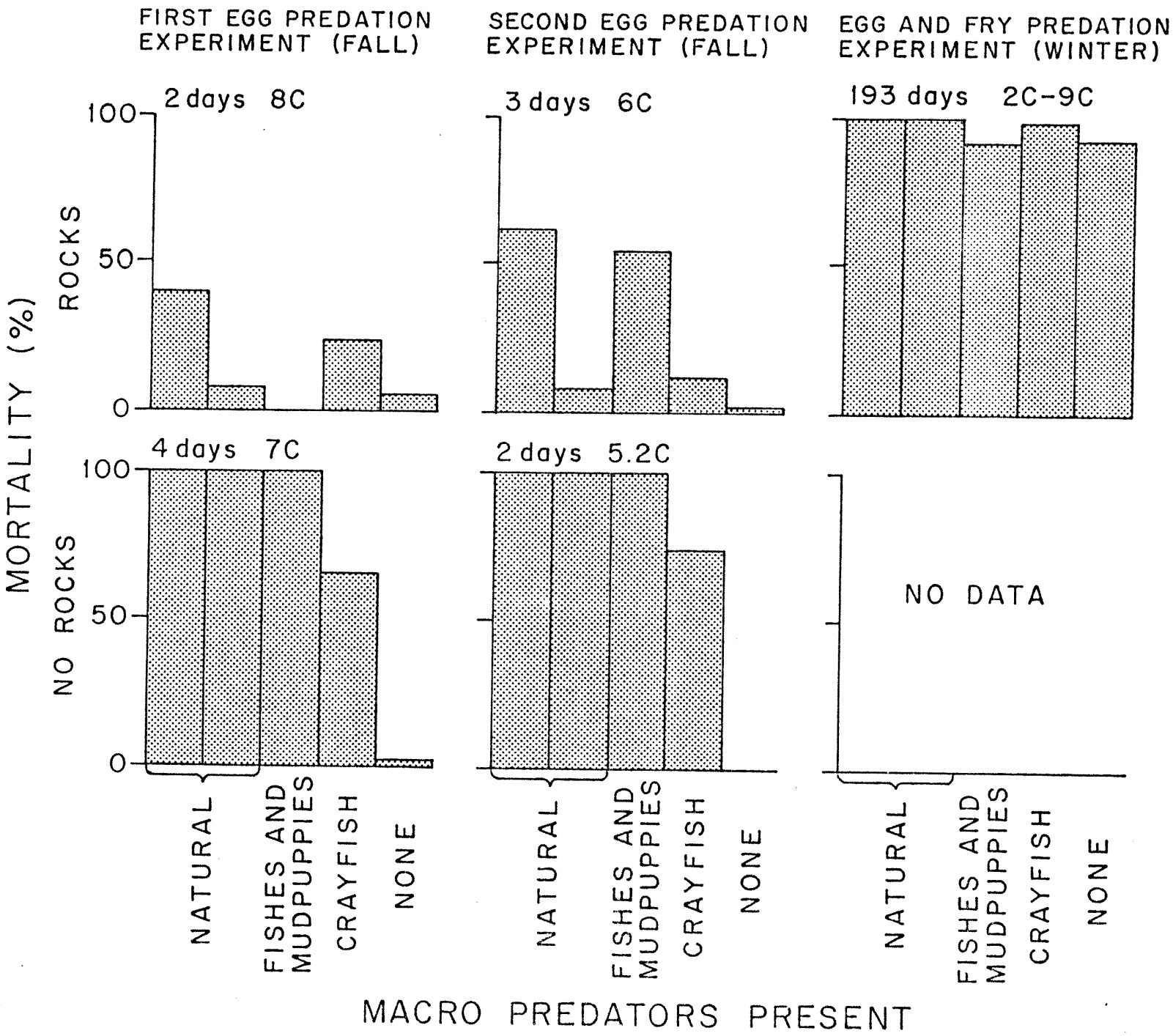
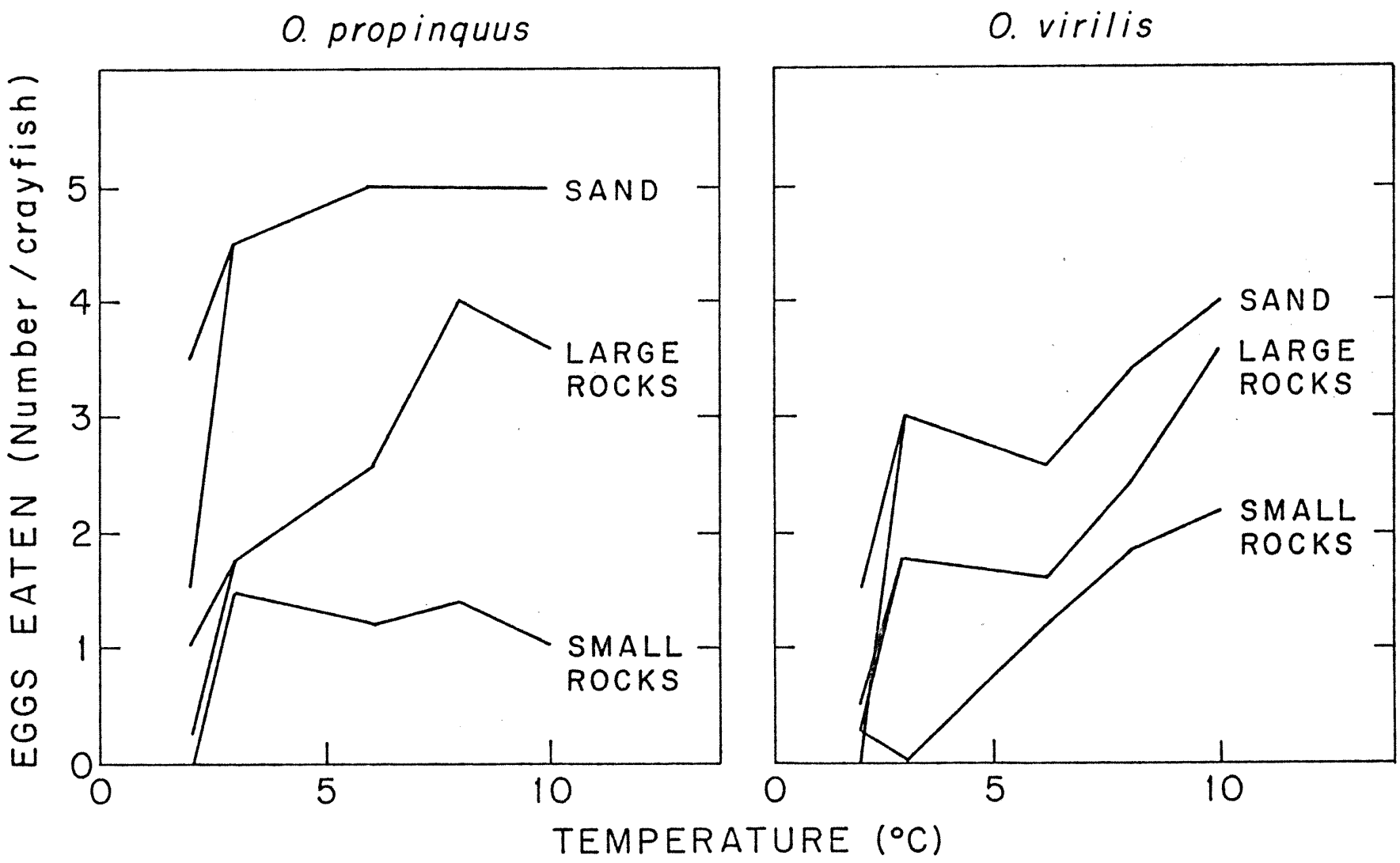

FIRST EGG PREDATION EXPERIMENT (FALL)
SECOND EGG PREDATION EXPERIMENT (FALL)
EGG AND FRY PREDATION EXPERIMENT (WINTER)
2 days 8C
3 days 6C
193 days 2C-9C
4 days 7C
2 days 5.2C
NO DATA
MORTALITY (%)
ROCKS
NO ROCKS
100
50
0
NATURAL
FISHES AND MUDPUPPIES
CRAYFISH
NONE
MACRO PREDATORS PRESENT

Fig. A-2. Egg predation under laboratory conditions.

Numbers of eggs eaten by O. propinquus or O. virilis when they were allowed to forage for eggs on three substrate types in aquaria at 2 - 10 C temperatures. Two observations at 2 C. Maximum possible value is five eggs per crayfish.

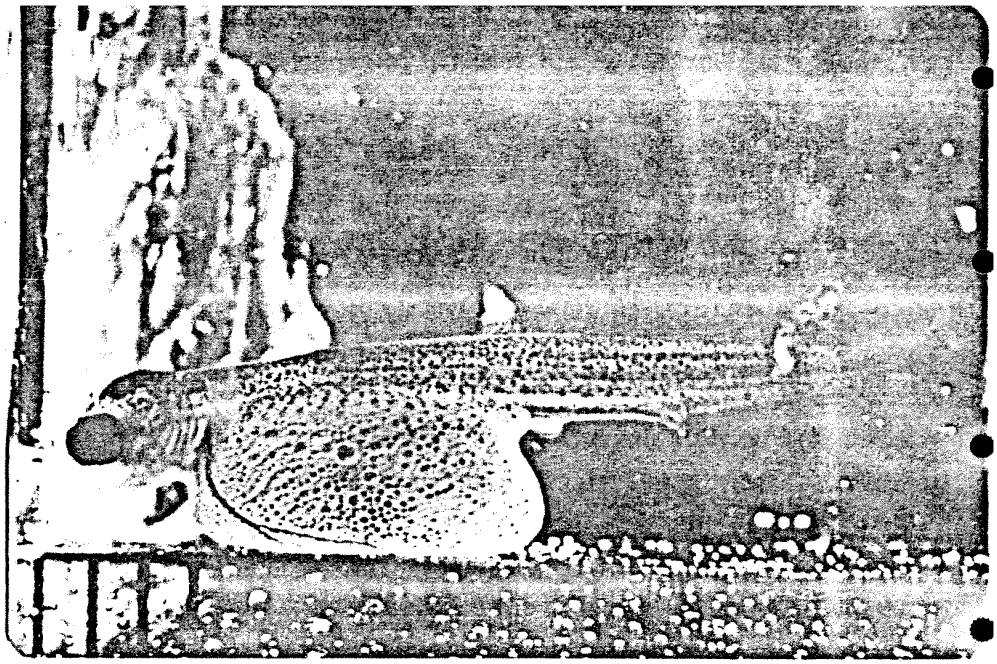

O. propinquus
O. virilis
EGGS EATEN (Number / crayfish)
SAND
LARGE ROCKS
SMALL ROCKS
SAND
LARGE ROCKS
SMALL ROCKS
0
1
2
3
4
5
0
5
10
0
5
10
TEMPERATURE (°C)

APPENDIX 2

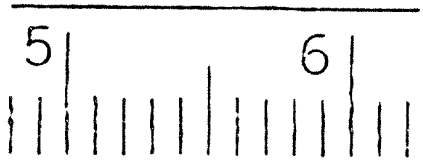
PHOTOGRAPHS OF SAC-FRY

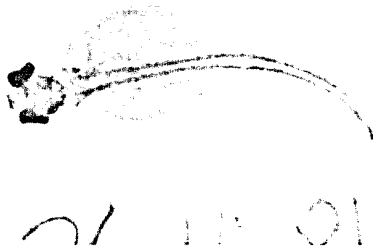
Photographs are included here from a sequence of dates in the sac-fry period. There are three photographs for each date; a live fish, a fish preserved in formalin, and a fish cleared and stained using the methods described in the text. A millimeter scale was photographed with each fish to allow measurement. Each page is labelled with date, number of days since hatching and number of degree-days since hatching. The three pictures from each date represent three different fish. The date indicated with each set of three photographs is the date on which the live fish was photographed. The preserved specimens were sometimes preserved as much as two days later.

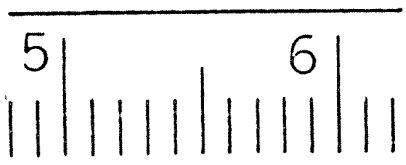
The final page of photographs shows live fish photographed on 5-IV-80, 27-IV-80, and 18-V-80. These fish were used in the experiments described in Chapter II. The dates correspond to approximately 520, 662, and 857 degree-days respectively.

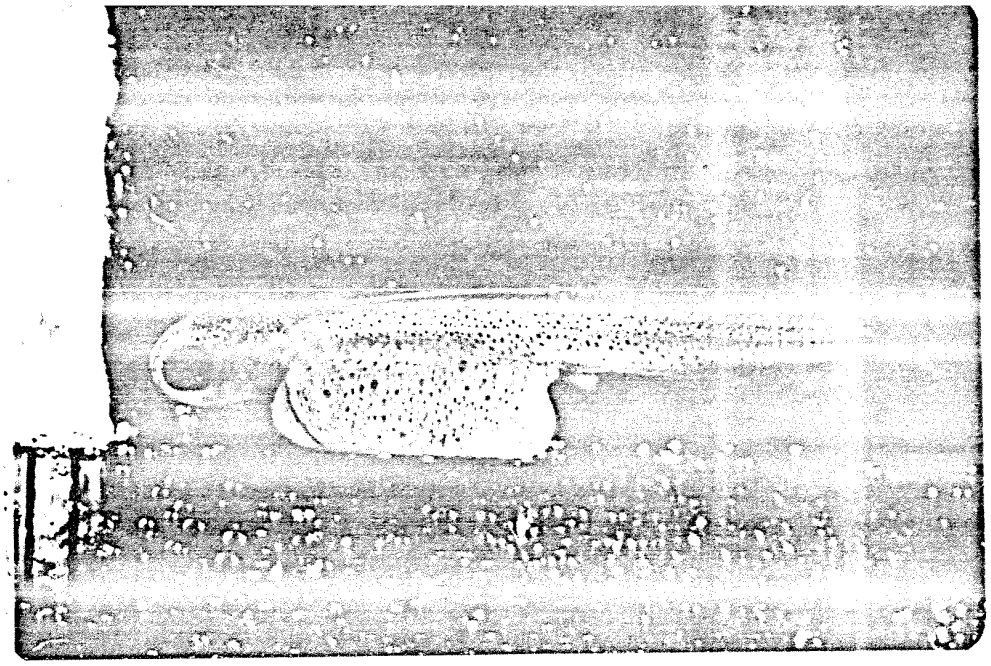


Date: 26-III-81
Days: 149
Degree-days: 429

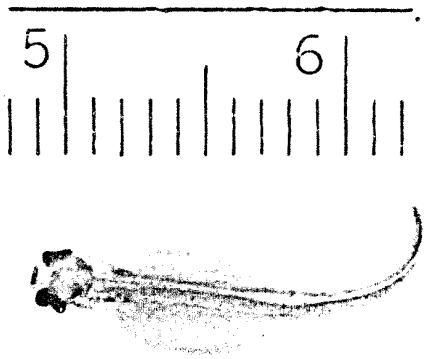


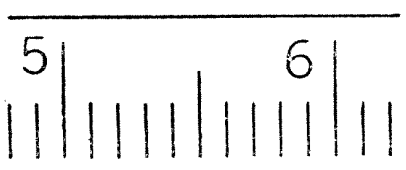


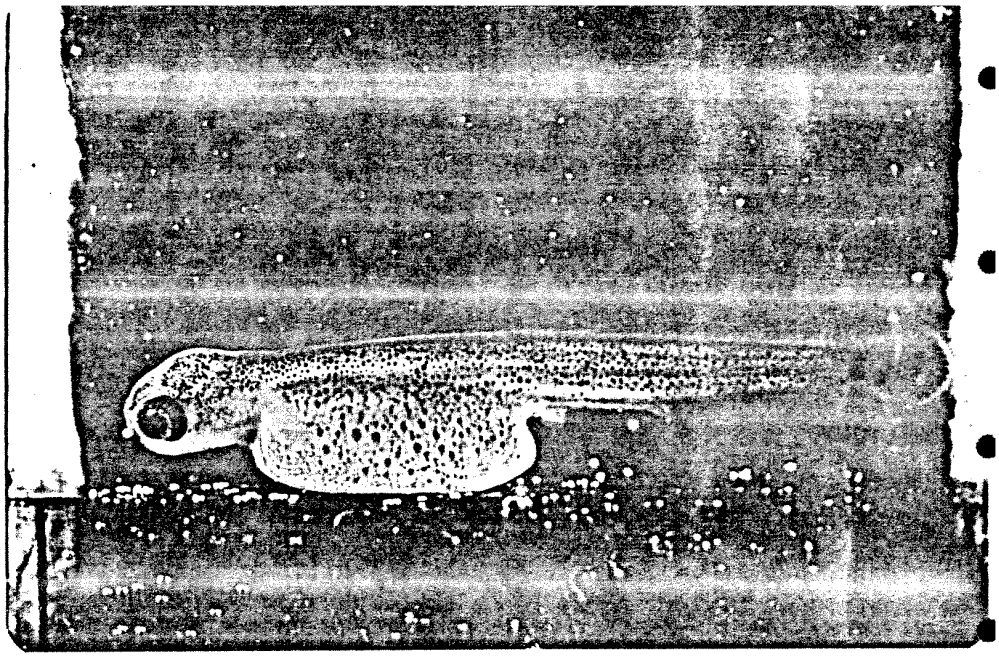




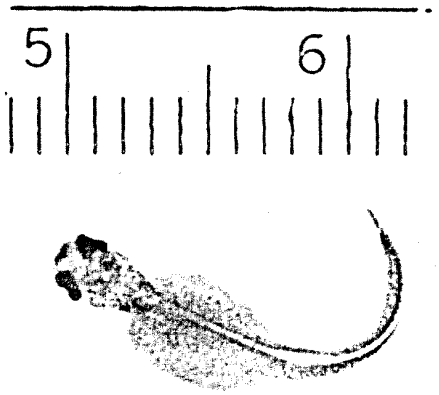
Date: 2-IV-81
Days: 156
Degree-days: 443

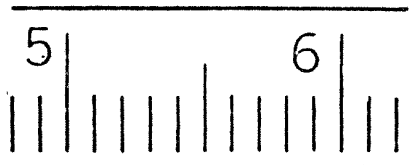




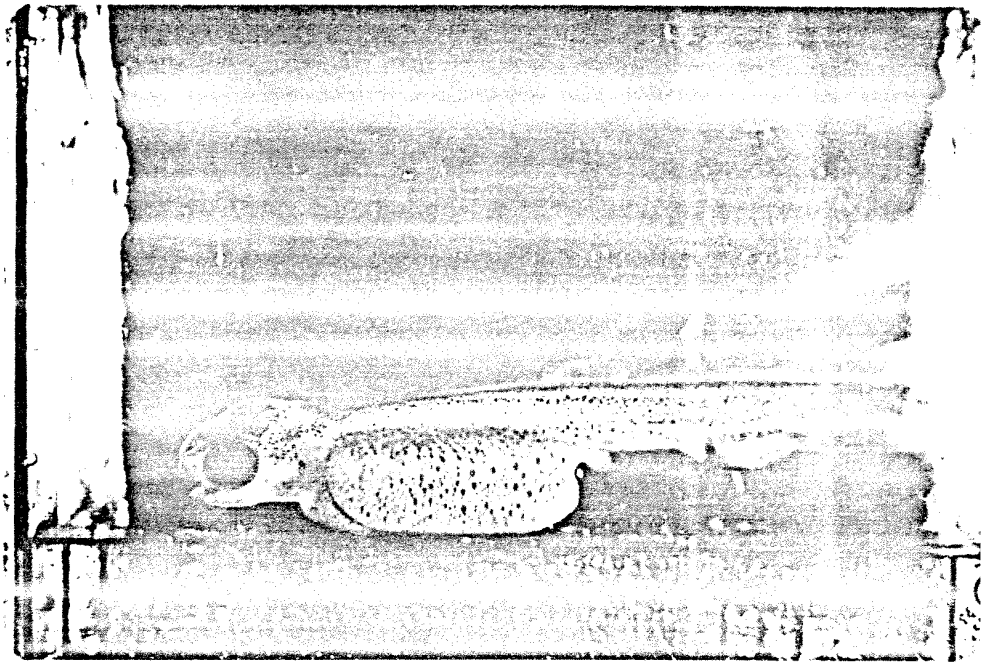


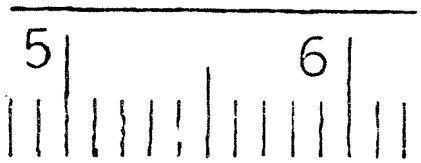
Date: 9-IV-81
Days: 163
Degree-days: 460



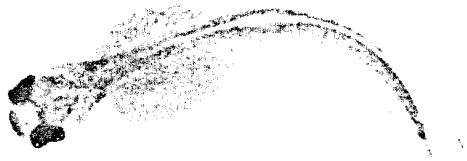


A2-5

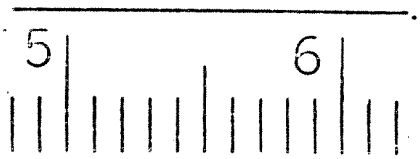


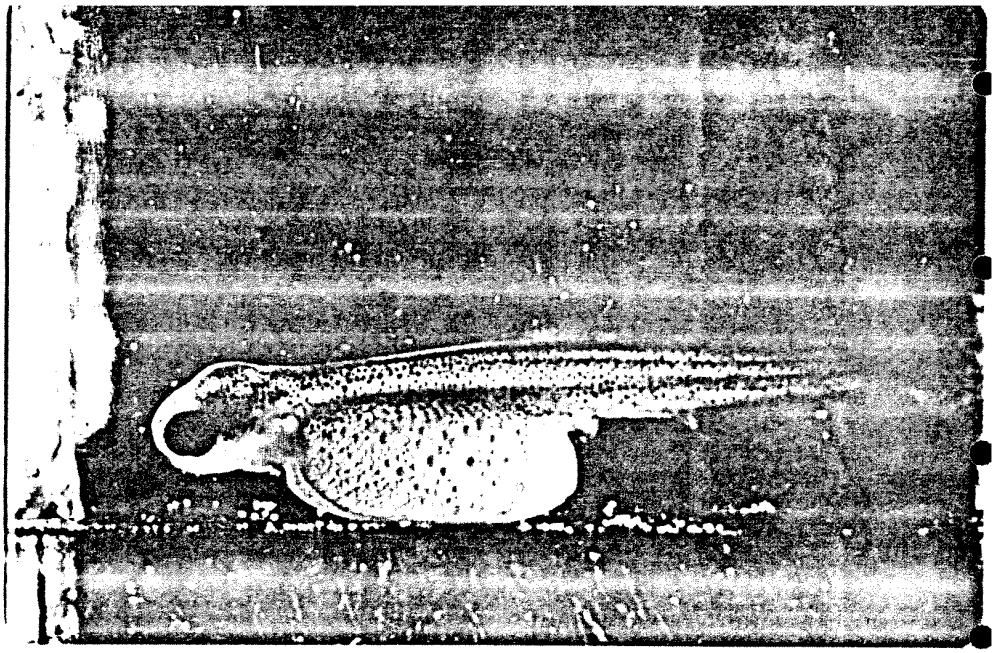


Date: 16-IV-81
Days: 170
Degree-days: 479



16-IV-81





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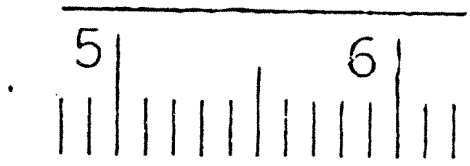
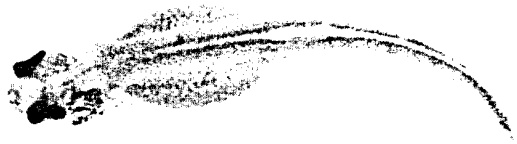
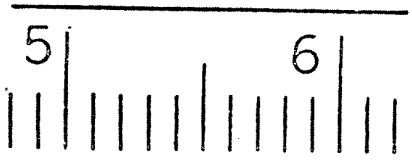
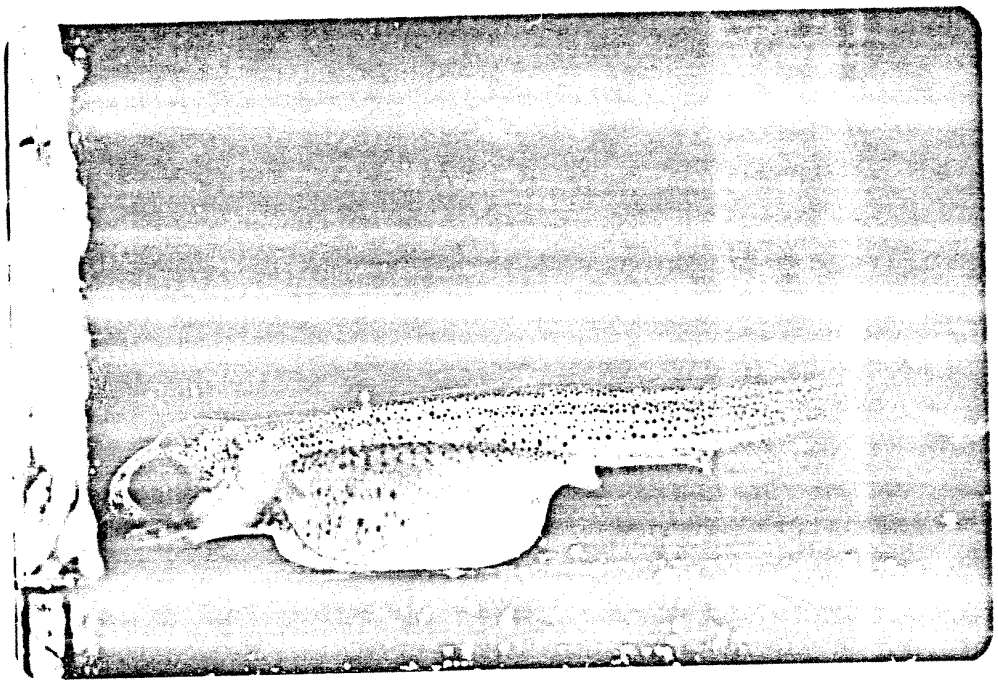
Days: 177
Degree-days: 507

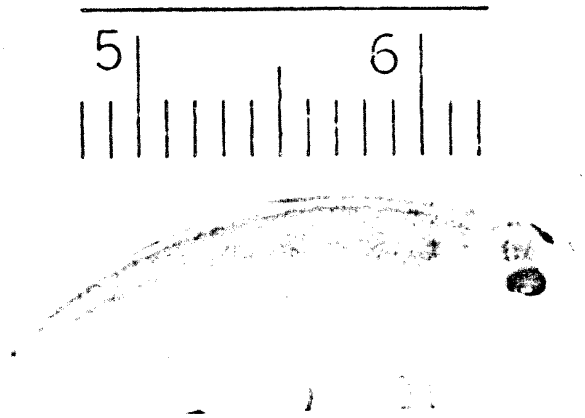


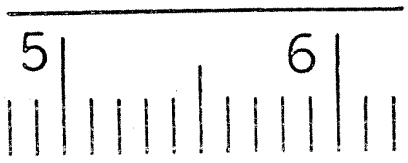


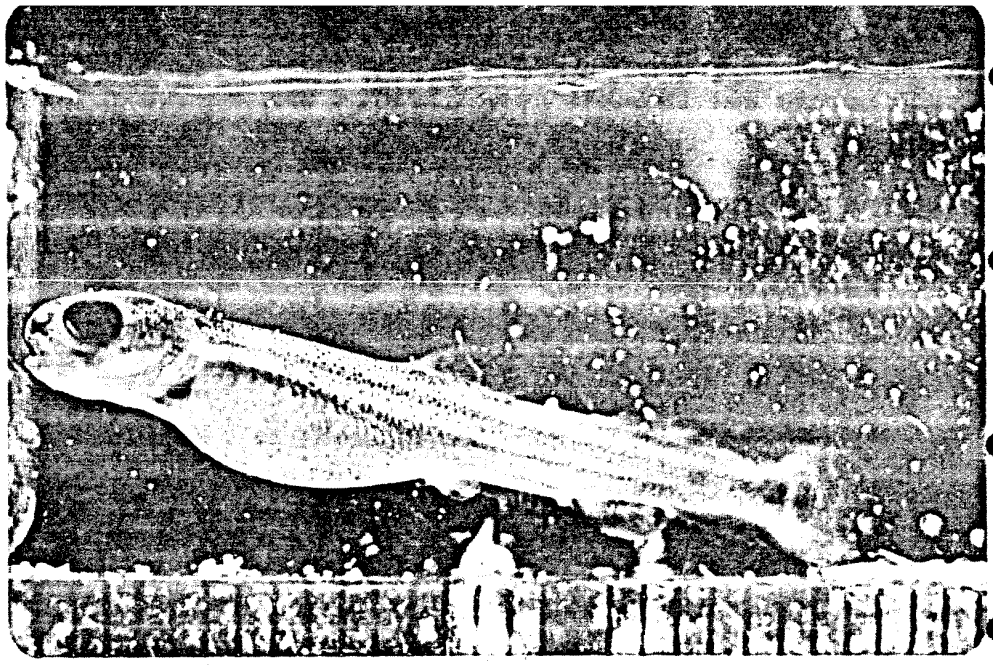


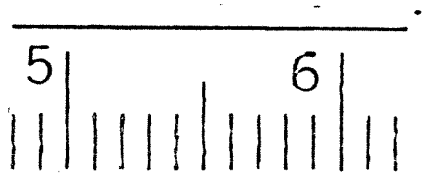


Date: 30-IV-81
Days: 184
Degree-days: 539

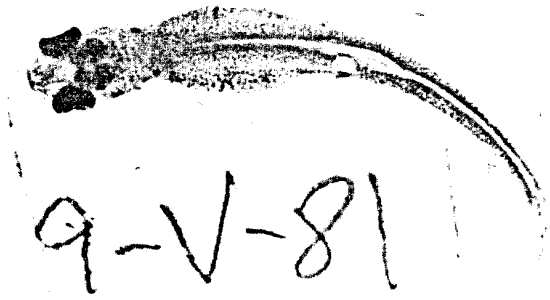


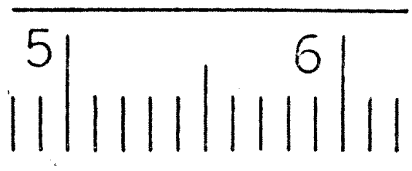


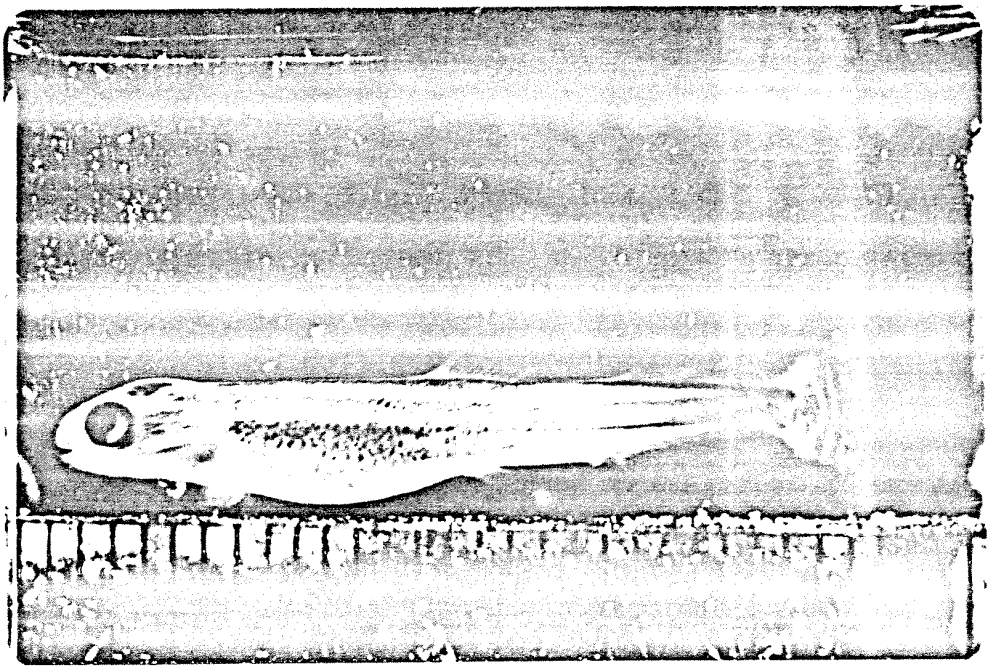


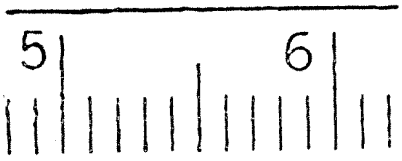


Date: 7-V-81
Days: 191
Degree-days: 579









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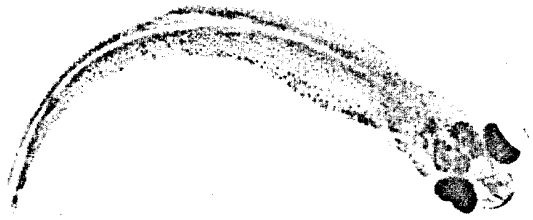
Degree-days: 624



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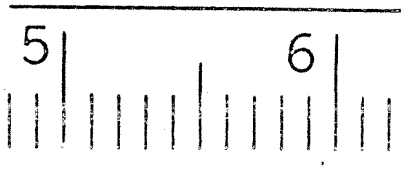

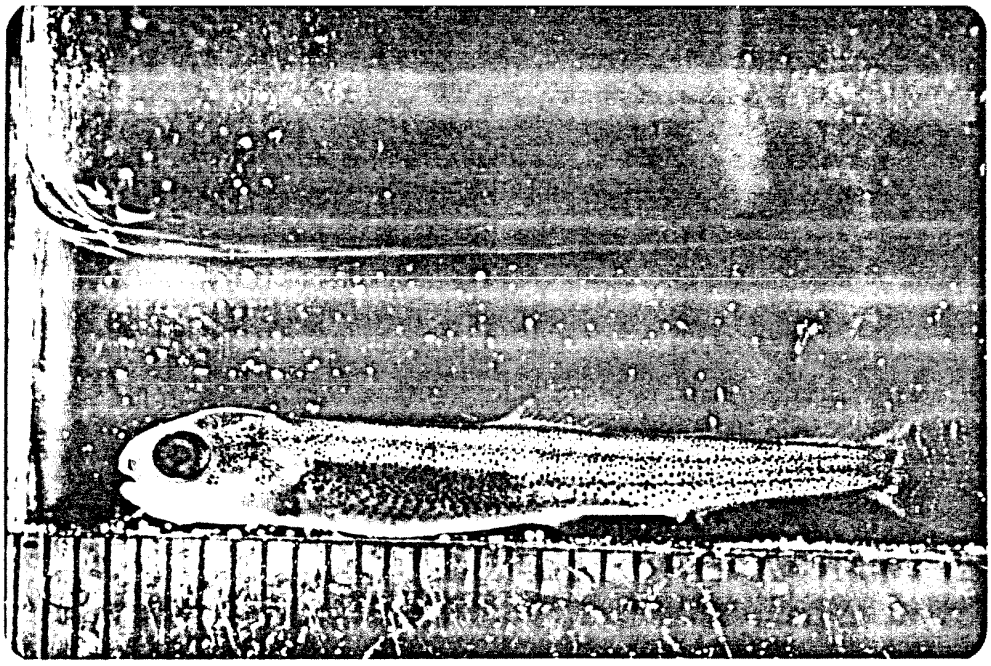

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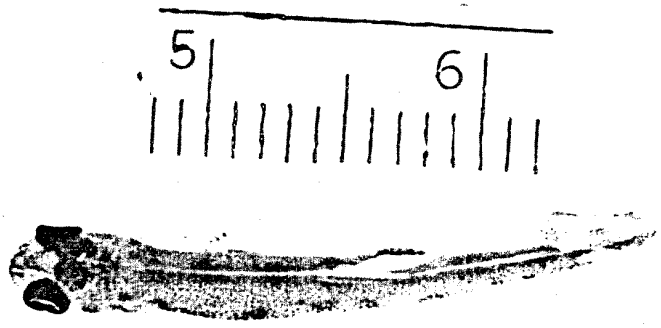
Days: 205
Degree-days:681



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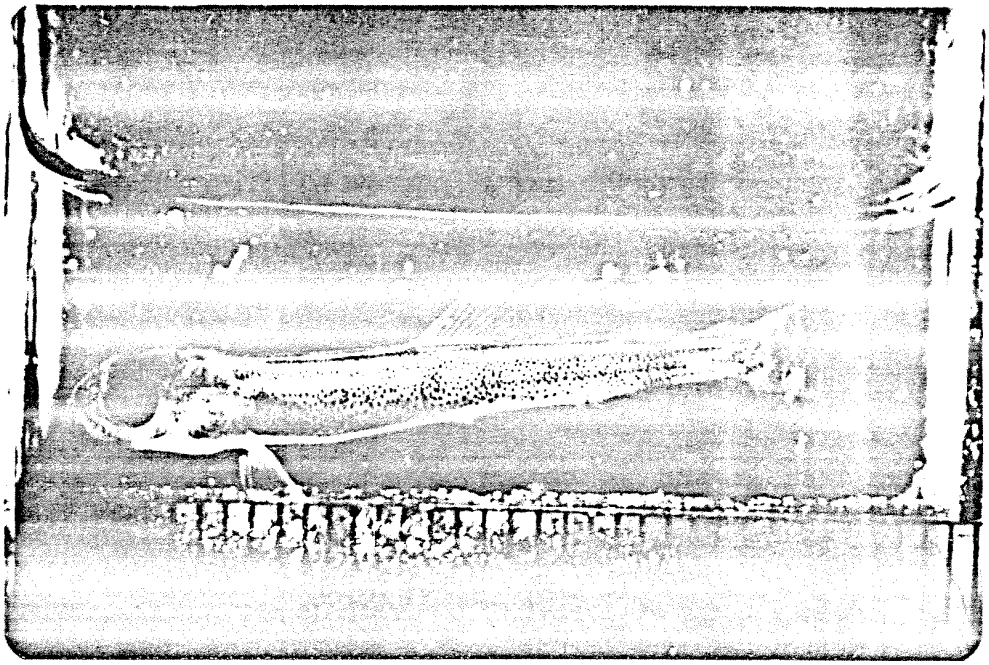
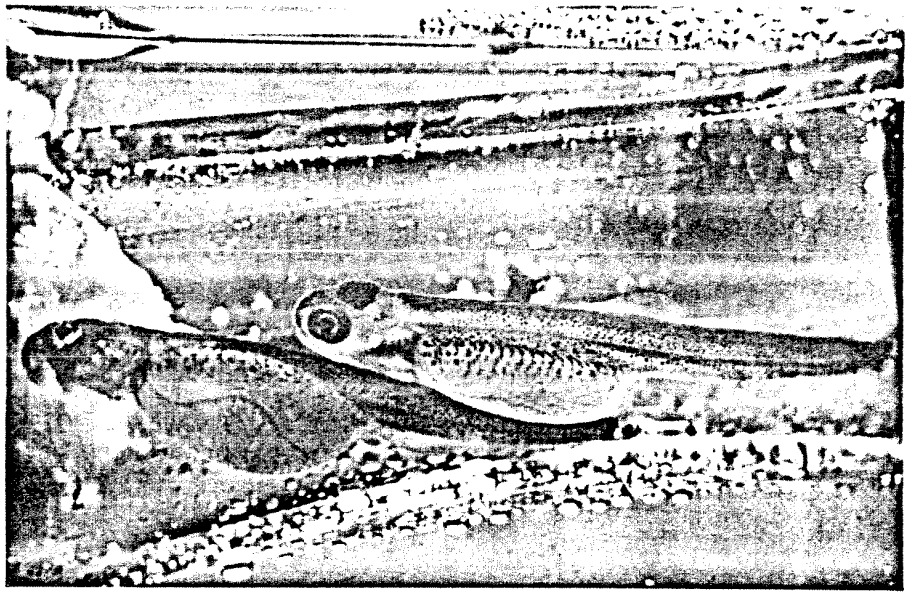
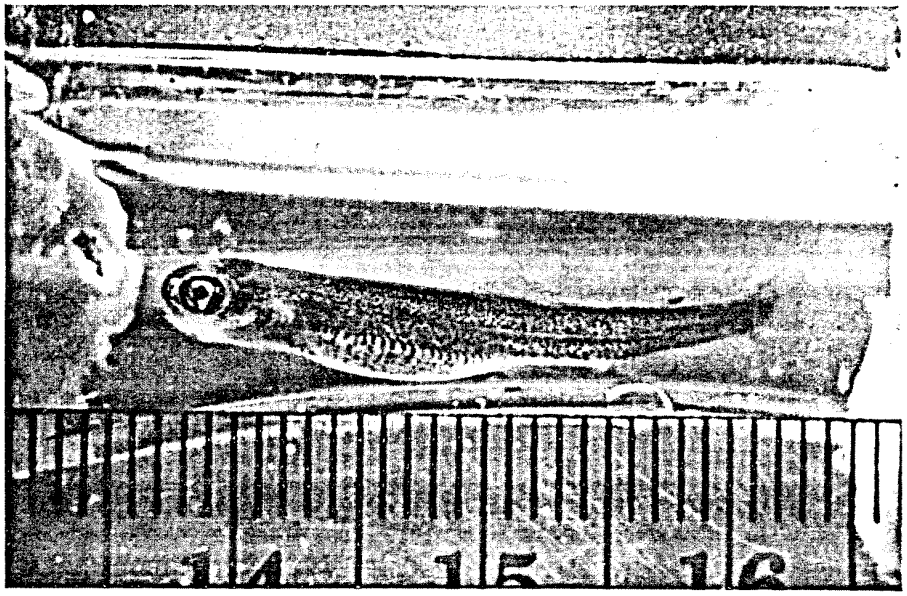
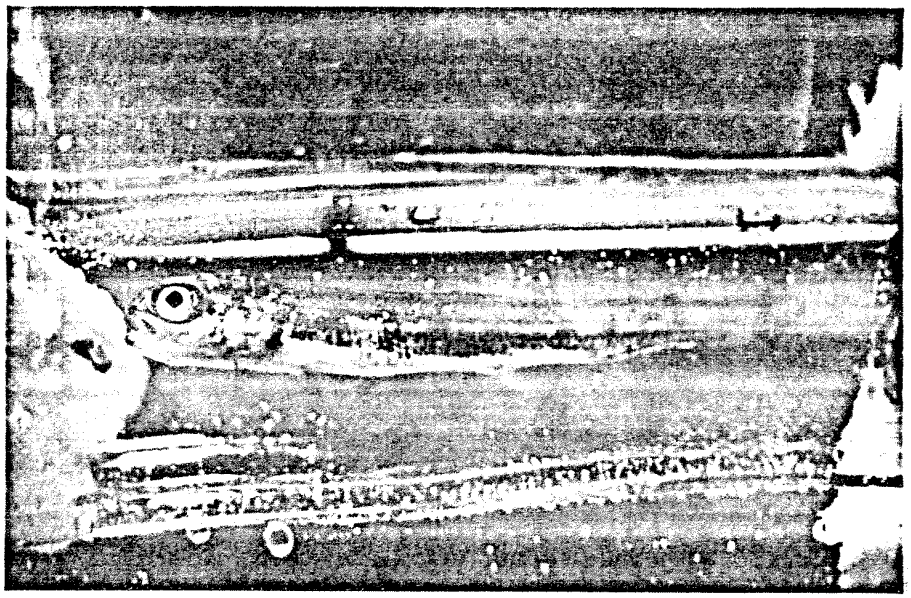
Degree-days: 761






14
15
16



APPENDIX 3

NOTES ON DIRECT OBSERVATION OF FISH

This is a compendium of descriptive data collected as described in Chapter III. It has been edited in the transcription to permit consistency in terminology. The term "movement" here means not immobile on the bottom or on a marble, it includes movement within the substrate as well as all swimming, with or without whole-body displacement. A fish is termed "still" if it is above the substrate and no displacement is occurring; it may be finning. Fish that are above the substrate and not still are "mobile." A fish is "at the surface" if it is within 2.5 cm of it. If it is described as "below the surface," it is also, by implication, above the substrate, otherwise its presence in the substrate would be specified. The term "nipping" refers to repeated touching of the surface (breaking the surface) with the snout.

In observations made on or before May 12 and under red light, the light was positioned behind the aquarium and shone toward the observer, unless otherwise stated. After that date the light was positioned to shine down into the aquarium, unless otherwise stated. In observations after May 12, the inflow of water was redirected to the rear chamber during the observation sessions.

After May 12, the descriptive record specified behavior in terms of activity, position and posture. As summarized here, possible activities are "resting" (in

substrate, either bottom or marbles), "still" (but above the substrate), or "mobile" (either within the substrate or above it). The term "moving" is used here and elsewhere to mean anything except immobility in the substrate; it includes what I describe as "still." Possible positions are "surface", "below surface", or "substrate" (sometimes specifically "bottom" or "marbles"). Possible postures (applying only to fish above the substrate) are horizontal, tilted head-up, and tilted head-down.

Occasionally fish raced vertically the entire depth of the aquarium two times or more; these excursions seemed sometimes to be caused by an inadvertent movement by the observer and sometimes by interactions with other fish. They are recorded here as "rapid vertical excursions." Fish occasionally "jumped", broke the surface and extended, sometimes, nearly a full body length into the air. These exertions were typically a response to observer movement and rarely occurred during formal observation periods.

In the original record and in this summary, activity, position and posture are recorded as they occurred together. The data are arranged here chronologically. Thus, they are not in proper sequence by degree-days. "Time after fertilization" is the time (in days and in degree-days that had elapsed prior to the observations but after fertilization. "Fish ID" is an identification number

assigned to each distinguishable fish. It allows comparison of daytime and nighttime activity of individual fish. "Holding tank" indicates whether the fish was reared in cold water (matched to Trout Lake, see Fig. D) or somewhat warmer water. "Water temperature" is the temperature of the observation aquarium. "Light or dark" indicates whether the observations were made before sunrise by red light (dark) or midday by white light (light).

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
25-III-81	148	434	3	warm	2.4	D	No movement for first 15 minutes. Vigorous tail beating, no movement in second 15.
25-III-81	148	434	2	warm	2.6	D	Pelvic fin beats irregularly. Tail beating is sporadic, then vigorous. About 4 inches of movement in 15 minutes. Moved 1 cm vertically.
25-III-81	148	434	2	warm	2.6	L	Lying motionless on its side at first. Weak tail beats, in bouts of up to 15 sec.
25-III-81	148	434	3	warm	2.6	L	Lying motionless on its side.
26-III-81	149	429	4	cold	2.4	D	No movement for first 15 minutes. Lying on its side. Some horizontal movement (a few inches) and one vertical foray of 1 cm. Rests upright between bouts of activity. Vigorous tail beats with head against the wall.
26-III-81	149	429	5	cold	2.3	D	In front left corner, facing observer, on side. Vigorous but episodic tail beating, no movement, probably blocked by a marble.
26-III-81	149	436	6	warm	2.6	D	This fish attained an elevation of about 1 cm, then "swam back and forth and then up to nearly the surface (30 cm)." Later moved around on the bottom.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
26-III-81	149	436	7	warm	2.6	D	Moved back and forth a few times and up as high as about 3 cm (the second layer of marbles) a few times.
31-III-81	154	439	8	cold	2.2	D	Moved about 15 cm along the bottom. Made four ineffectual vertical movements, no more than 2 cm each.
31-III-81	154	439	9	cold	1.8	D	Made repeated vigorous bouts of tail beating with head in a crack under the aquarium divider; seemed to be trying to get through. Rested on its side on bottom.
31-III-81	154	439	8	cold	2.3	L	No movement.
31-III-81	154	439	9	cold	2.3	L	No movement.
1-IV-81	155	455	2	warm	3.0	D	Initially at rest, upright. Bouts of tail wagging sometimes result in 2 cm of movement on bottom. One bout, with head in corner, appears to be directed to getting into a crack there.
1-IV-81	155	455	3	warm	2.9	D	Initially at rest on its side. No movement.
1-IV-81	155	455	2	warm	3.0	L	At rest, upright. No movement.
1-IV-81	155	455	3	warm	3.0	L	At rest on side. No movement.

Date	Time after Fertilization		Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
	Days	Degree-Days					
8-IV-81	162	479	2	warm	3.4	D	At rest on side. Bouts of tail beats resulted once in fish becoming upright and once in a 1 cm elevation gain. Rests upright sometimes and on its side sometimes.
8-IV-81	162	479	3	warm	3.3	D	At rest upright initially. Bouts of tail beating and short movement along the bottom. Always rests upright.
8-IV-81	162	479	1	warm	3.4	D	At rest upright. Bouts of tail beats result in no movement; fish is apparently blocked.
8-IV-81	162	479	2	warm	3.5	L	At rest upright. Bouts of tail beats with no movement during first 7-1/2 minutes. No activity during second 7-1/2 minutes.
8-IV-81	162	479	3	warm	3.5	L	At rest upright. No tail beating.
8-IV-81	162	479	1	warm	3.5	L	At rest upright. No tail beating.
14-IV-81	168	474	9	cold	1.9	D	No movement. Bouts of tail beating.
14-IV-81	168	474	8	cold	1.8	D	No movement. No tail beating.
14-IV-81	168	474	14	cold	1.9	D	No movement. No tail beating during second 15 minutes but there had been some earlier.
14-IV-81	168	474	9	cold	2.6	L	No movement. No tail beating.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
14-IV-81	168	474	8	cold	2.6	L	No movement. No tail beating.
14-IV-81	168	474	14	cold	2.6	L	No movement. No tail beating.
15-IV-81	169	507	3	warm	3.5	D	No movement. No tail beating. Head toward the light.
15-IV-81	169	507	19	warm	3.5	D	The fish started facing the light. It moved about 8 cm in a series of short bouts of tail beating.
15-IV-81	169	507		warm	3.5	D	Two fish were present through a mix-up. One was #2. One did not move, the other (not specifically watched) was more active and even did some swimming in the substrate.
15-IV-81	169	507	3	warm	4.2	L	No movement. No tail beats.
15-IV-81	169	507	19	warm	4.1	L	No movement. No tail beats.
15-IV-81	169	507		warm	4.1	L	No movement. No tail beats.
21-IV-81	175	499	25	cold	4.3	D	Six cm of horizontal movement on bottom. Two, 2 cm vertical forays.
21-IV-81	175	499	26	cold	4.2	D	No movement.
21-IV-81	175	536	27	warm	5.0	D	No observations.

Date	Time after Fertilization		Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
	Days	Degree-Days					
21-IV-81	175	536	28	warm	5.0	D	No observations.
21-IV-81	175	499	25	cold	4.2	L	No movement.
21-IV-81	175	499	26	cold	4.1	L	No movement.
21-IV-81	175	536	27	warm	5.0	L	No movement. Bouts of tail beats with resting on side.
21-IV-81	175	536	28	warm	5.0	L	No movement. Bouts of tail beats with resting on side.
22-IV-81	176	503	29	cold	4.0	D	Horizontal movement on the bottom of 5 cm, in bouts.
22-IV-81	176	503	30	cold	4.0	D	One horizontal movement of 1 cm.
22-IV-81	176	542	31	warm	4.8	D	No movement.
22-IV-81	176	542	32	warm	4.9	D	No movement.
22-IV-81	176	503	29	cold	3.9	L	Horizontal movement on the bottom of 8 cm, in bouts.
22-IV-81	176	503	30	cold	3.9	L	No movement.
22-IV-81	176	542	31	warm	4.7	L	No movement.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
22-IV-81	176	542	32	warm	4.7	L	No movement
23-IV-81	177	507		cold	4.0	D	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	507		cold	4.0	D	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	547		warm	2.9	D	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	547		warm	4.9	D	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	507		cold	4.0	L	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	507		cold	3.9	L	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	547		warm	4.8	L	Four fish present. One, chosen for observation, didn't move.
23-IV-81	177	547		warm	4.8	L	Four fish present. One, chosen for observation, didn't move.
28-IV-81	182	528	41	cold	4.9	D	Horizontal movement on the bottom of 8 cm, in bouts.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
28-IV-81	182	528	42	cold	4.9	D	No movement.
28-IV-81	182	575	43	warm	6.2	D	No movement.
28-IV-81	182	575	44	warm	6.3	D	No movement.
28-IV-81	182	528	41	cold	5.1	L	No movement.
28-IV-81	182	528	42	cold	5.0	L	No movement.
28-IV-81	182	575	43	warm	6.5	L	Horizontal movement on the bottom of 5 cm, in bouts.
28-IV-81	182	575	44	warm	6.5	L	No movement.
29-IV-81	183	533	45	cold	4.8	D	Moved horizontally 2.5 cm and vertically 5 cm.
29-IV-81	183	533	46	cold	4.7	D	No movement, bouts of tail beating throughout.
29-IV-81	183	581	47	warm	6.1	D	No movement.
29-IV-81	183	581	48	warm	6.1	D	Horizontal movement of 13 cm, in bouts.
29-IV-81	183	533	45	cold	6.2	L	No movement, some tail beats.
29-IV-81	183	533	46	cold	6.1	L	No movement. Very regular bouts of tail beating; one sequence segment of inter-bout times was (in seconds): 18, 17, 17, 17, 19, 15, 14, 13, 14, 16, 17, 15, 19, 21, 21, 17, 14.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
29-IV-81	183	581	47	warm	8.0	L	No movement.
29-IV-81	183	581	48	warm	8.0	L	No movement. Regular bouts of tail beating with inter-bout times (in seconds) of 20, 12, 20, 15, 18, 11 in one sequence segment.
30-IV-81	184	539		cold	5.1	D	Total horizontal movement on bottom of 10 cm, in bouts. Two vertical forays of 2.5 and 1 cm. There were four fish present; only one was watched carefully.
30-IV-81	184	539		cold	5.1	D	Four fish present, one watched. One horizontal movement of 1 cm.
30-IV-81	184	588		warm	7.5	D	Four fish present, one watched. One horizontal movement of 4 cm.
30-IV-81	184	588		warm	7.5	D	Four fish present, one watched. Three horizontal movements of 1, 3 and 1 cm. (One fish, not watched, was at or near the surface the whole time.)
30-IV-81	184	539		cold	5.9	L	Four fish present, one watched. Three horizontal movements of 3 cm each.
30-IV-81	184	539		cold	5.9	L	No movement. Four fish present.
30-IV-81	184	588		warm	7.1	L	No movement. Four fish present.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
30-IV-81	184	588		warm	7.1	L	Four fish present. Two horizontal movements of 1 cm each.
5-V-81	189	566	57	cold	5.9	D	No movement.
5-V-81	189	566	58	cold	5.9	D	No movement.
5-V-81	189	624	59	warm	7.3	D	Continuous nipping at surface. No vertical movement.
5-V-81	189	624	60	warm	7.3	D	No movement.
5-V-81	189	566	57	cold	6.7	L	Horizontal movement of 7 cm, in 6 bouts.
5-V-81	189	566	58	cold	6.7	L	No movement.
5-V-81	189	624	59	warm	8.5	L	No movement.
5-V-81	189	624	60	warm	8.5	L	Continuous nipping.
6-V-81	190	573	61	cold	5.8	D	No observations, fish still benthic in early observations.
6-V-81	190	573	62	cold	5.8	D	No observations, fish still benthic in early observations.
6-V-81	190	632	63	warm	6.9	D	No movement. The red light was directed from above.

Date	Time after Fertilization		Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
	Days	Degree-Days					
6-V-81	190	632	64	warm	6.9	D	Fish lost.
6-V-81	190	573	61	cold	6.6	L	The fish rarely nipped the surface; it spent most of the time hovering horizontally about 2 cm below the surface.
6-V-81	190	573	62	cold	6.6	L	No observations, fish still benthic in early observations.
6-V-81	190	632	63	warm	8.1	L	No observations, fish still benthic in early observations.
6-V-81	190	632	64	warm	8.2	L	Fish lost.
12-V-81	196	610	69	cold	6.0	D	No observations, fish still benthic in early observations.
12-V-81	196	610	70	cold	6.0	D	No observations, fish still benthic in early observations.
12-V-81	196	678	71	warm	7.7	D	No movement. Resting on glass marbles. The red light was directed from above.
12-V-81	196	678	72	warm	7.7	D	The fish was nipping continuously. Red light from above.
12-V-81	196	610	69	cold	6.9	L	No observations.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
12-V-81	196	610	70	cold	6.9	L	No observations.
12-V-81	196	678	71	warm	8.8	L	No observations.
12-V-81	196	678	72	warm	8.8	L	No observations.
13-V-81	197	616	73	cold	6.6	D	Resting on bottom.
13-V-81	197	616	74	cold	6.6	D	Resting on bottom.
13-V-81	197	686	75	warm	8.4	D	Resting on marbles.
13-V-81	197	686	76	warm	8.4	D	At surface, tilted head-up, still. At surface, tilted head-up, mobile.
13-V-81	197	616	73	cold	8.1	L	At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, tilted head-up, still.
13-V-81	197	616	74	cold	8.1	L	Rapid vertical excursions. At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, mobile.
13-V-81	197	886	75	warm	10.1	L	At surface, horizontal, still. At surface, horizontal, mobile. Rapid vertical excursions.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
13-V-81	197	886	76	warm	10.1	L	Resting on marbles. Rapid vertical excursions. At surface, horizontal, mobile. At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, horizontal, mobile. Below surface, horizontal, still.
14-V-81	198	624		cold	7.5	D	Four fish present, all resting on bottom or marbles.
14-V-81	198	695		warm	9.4	D	Four fish present, one chosen for observation. Resting on bottom. At surface, tilted head-up, still. At surface, tilted head-up, mobile.
14-V-81	198	624		cold	8.5	L	Four fish present, one observed. At surface, tilted head-up, mobile. At surface, tilted head-up, still. Below surface, tilted head-up, slowly ascending. Rapid vertical ascent. Resting in substrate.
14-V-81	198	695		warm	10.5	L	Four fish present, one observed. Resting in substrate. At surface, tilted head-up, still. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) At surface, tilted head-up, mobile. Rapid vertical movement. At surface, horizontal, still. At surface, horizontal, mobile. Below surface, tilted head-up, slowly ascending. Below surface, tilted head-down, slowly descending. "Jumping" above the surface.
19-V-81	203	665	77	cold	7.2	D	Resting on bottom. Moving along the bottom.
19-V-81	203	665	78	cold	7.2	D	At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, horizontal, mobile. Below surface, tilted head-down, mobile.
19-V-81	203	753	79	warm	11.0	D	Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. Below surface, horizontal, still. Sudden descent.

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
19-V-81	203	753	80	warm	11.0	D	At surface, tilted head-up, still. At surface, horizontal, still. Below surface, tilted head-up, still. Below surface, tilted head-up, slowly ascending.
19-V-81	203	665	77	cold	8.1	L	At surface, tilted head-up, still. At surface, tilted head-up, mobile. "Jumping" above the surface. Below surface, horizontal, mobile. Below surface, tilted head-up, mobile. Below surface, tilted head-down, mobile. Resting in substrate. Rapid vertical excursions.
19-V-81	203	665	78	cold	8.1	L	Resting in substrate. At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-up, mobile. Below surface, tilted head-down, mobile. Rapid vertical excursion.
19-V-81	203	753	79	warm	13.3	L	At surface, horizontal, still. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-up, slowly ascending.
19-V-81	203	753	80	warm	13.3	L	At surface, tilted head-up, still. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-up, ascending slowly. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. Below surface, horizontal, still. Below surface, horizontal, mobile.
20-V-81	204	673	81	cold	8.7	D	Rapid vertical excursions. At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-down, mobile.
20-V-81	204	673	82	cold	8.7	D	Resting in substrate. Moving in substrate. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-up, slowly ascending. "Jumping" above surface.
20-V-81	204	765	83	warm	13.0	D	At surface, tilted head-up, mobile. At surface, horizontal, still. At surface, horizontal, mobile. Below surface, tilted head-up, mobile. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, horizontal, slowly ascending.
20-V-81	204	765	84	warm	13.0	D	At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, still. At surface, horizontal, still. Below surface, horizontal, moving.
20-V-81	204	673	81	cold	8.6	L	At surface, tilted head-up, still. At surface, tilted head-up, mobile. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) At surface, horizontal, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-down, mobile. Rapid vertical excursions.
20-V-81	204	673	82	cold	8.6	L	Resting in substrate. Moving in substrate.
20-V-81	204	765	83	warm	13.7	L	Rapid vertical excursions. At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, still. At surface, horizontal, mobile. Below surface, tilted head-up, mobile. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile.
20-V-81	204	765	84	warm	13.7	L	Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile.
21-V-81	205	681		cold	4.4	D	Four fish present, one observed. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) Resting in substrate. At surface, tilted head-up, still. At surface, tilted head-up, moving. At surface, horizontal, still. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-up, slowly ascending. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. Below surface, tilted head-down, slowly ascending. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, horizontal, slowly ascending. "Jumping" above surface.
21-V-81	205	778		warm	13.3	D	Four fish present, one observed. At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, still. Resting in substrate. Rapid vertical excursions. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.) Below surface, horizontal, mobile.
21-V-81	205	681		cold	9.1	L	Resting in substrate. Moving in substrate. "Jumping" above surface. Rapid vertical excursions. At surface, tilted head-up, still. At surface, tilted head-up, mobile. At surface, horizontal, still. At surface, horizontal, mobile. Below surface, tilted head-up, still. Below surface, tilted head-up, mobile. Below surface, tilted head-up, slowly ascending. Below surface, horizontal, still. Below surface, horizontal, mobile. Below surface, tilted head-down, still. Below surface, tilted head-down, mobile.
21-V-81	205	778		warm	14.5	L	Rapid vertical excursions. Resting on bottom. At surface, horizontal, still. At surface, horizontal, mobile. At surface, tilted head-up, still. At surface, tilted head-up, mobile. Below surface, tilted head-up, still. (contd.)

Date	Time after Fertilization: Days	Time after Fertilization: Degree-Days	Fish ID	Holding Tank	Water Temp. (C)	Light or Dark	Description
							(contd.)
							Below surface, tilted head-up, mobile.
							Below surface, tilted head-down, still.
							Below surface, tilted head-down, mobile.
							Below surface, horizontal, still.
							Below surface, horizontal, mobile.

Source	Sex	ID	Total Length (cm)	Egg Diam. (mm)	Fin Clip
Crystal Spr.	f	Cc	48.9	4.82	none
Crystal Spr.	f	Cd	40.0	5.42	none
Crystal Spr.	f	Ce	45.9	5.01	none
Trout Lake	m	T1	55.6	--	LP
Trout Lake	m	T2	50.0	--	LP
Trout Lake	m	T3	56.9	--	Ad
Trout Lake	m	T4	64.5	--	Ad
Trout Lake	m	T5	56.4	--	LP
Trout Lake	m	T6	52.1	--	Ad
Trout Lake	m	T7	53.3	--	none
Trout Lake	f	Ta	59.9	5.86	Ad
Trout Lake	f	Tb	64.0	6.04	Ad
Trout Lake	f	Tc	54.1	5.71	none
Trout Lake	f	Td	78.7	--	none
Trout Lake	f	Te	57.4	--	LP

(a) Lengths of individual Bayfield males were not recorded; they ranged from 64.5 to 75.7.

(b) Total-lengths of Marquette fish were estimated from fork lengths.