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THE LIFE HISTORY AND FOOD RELATIONS OF *EPISCHURA LACUSTRIS* FORBES (COPEPODA:CALANOIDA)

by
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of the requirements for the degree of
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INTRODUCTION

It has been suggested by Hardy (1956) that planktonic copepods are more numerous than all other metazoan animals combined. Whether or not this is an exaggeration, the importance of calanoid copepods in natural waters has long been recognized by virtue of their sheer numbers. Experimental studies on individual species under controlled laboratory conditions have been a profitable and even indispensable approach to our understanding of the role of these animals in nature. Yet, such studies have been almost entirely limited to but a few marine species. The present investigation was undertaken in order to gain firsthand information about certain salient features of the biology of Epischura lacustris Forbes, a common fresh-water calanoid. It was selected for study because, in spite of its frequent appearance in the plankton of our larger lakes, almost nothing was hitherto known of its life history or ecology. Further, the animal was readily available and relatively easy to recognize macroscopically in the adult stage, since it was the largest copepod present in the collections. Larval development, feeding habits, and reproductive activities were investigated by observing living animals. In addition, periodic collections from a southeastern Michigan lake provided the basis for determining the seasonal distribution of the species in nature.

The best known copepod is the marine calanoid Calanus

finmarchicus (Gunnerus) (summary by Marshall and Orr, 1955). No fresh-water zooplankter has been studied, in scope or intensity, to a comparable degree. Seasonal studies of natural populations of Calanus, such as that of Marshall, Nicholls, and Orr (1934), owe much of their thoroughness to a detailed knowledge of the morphology, development, and reproductive behavior of the species, accumulated by several workers over many years. Of equal importance, and forming an indispensable background for making interpretations of changes observed in nature, have been extensive experimental studies with living animals. Research with Calanus has developed principally in two directions: that relating to the acquisition and supply of food and that involving respiratory rates, the interest being focussed on "balancing the budget" between the copepod's food requirements and the amount of food normally available to it in the sea.

Epischura lacustris, a relatively large planktonic species, has been described as a typical summer form inhabiting deep cold lakes of temperate North America (Marsh, 1933; Pennak, 1953). Its distribution extends from the northeastern coastal states and provinces west to Minnesota and the Northwest Territories according to Wilson (1959), who further states that in northern Canada it may be found associated with E. nevadensis Lilljeborg. The species, the type for the genus, was first described by Forbes in 1882, who found it a conspicuous member of the October plankton in Grand Traverse Bay, Lake Michigan. It has been recorded on numerous lists of planktonic organisms (e.g., Birge, 1897; Chandler, 1940; Davis, 1954, 1959a; Forbes,

1882, 1891; Herrick and Turner, 1895; Juday, 1903; Marsh, 1893, 1895, 1897, 1903; Sars, 1915; Wells, 1960; Wilson, 1929) but little can be learned from these lists as to the animal's seasonal distribution. In most cases the data presumably were based on adult individuals taken during the summer and fall months; at the same time it seems unlikely that distinctions were consistently made between adult and late copepodid stages.

The most complete study of the limnetic distribution of E. lacustris was made in Lake Nipissing, Ontario, by Langford in 1938. Collections were made with a plankton trap from May to September for two successive years and all copepodid stages were considered together. Nauplii were not distinguished from those of the other copepods present. Langford believed the population's horizontal distribution to be nearly random, but numbers taken in any one sample were so low than an adequate treatment of changes in population size was not possible. Adults of the species are known to be active swimmers and regularly make extensive vertical migrations (Juday, 1903; Langford, 1938; Marsh, 1897; Welch, 1952; Wells, 1960). Marsh (1897) has suggested an association between these daily vertical movements and a richer food supply near the surface. Nothing definite has been reported about the kinds and amounts of food utilized by the animal as is the case with most other fresh-water calanoids. Despite the scarcity of winter collections, available records point to the animal's absence from the plankton during this presumably unfavorable season. The

method of survival from year to year has yet to be demonstrated.

Marsh (1900) published a preliminary description of certain changes in male development with the intention of doing a more thorough treatment at a later time. Unfortunately this work never appeared. His figures are not detailed nor were the larval stages identified. In any case, the larval stages of E. lacustris have not hitherto been described.

The natural food of copepods has been investigated in a variety of ways. In summarizing the status of our knowledge of the trophic relations of the three dominant groups of limnetic zooplankton (Cladocera, rotifers, and copepods, especially calanoids), Edmondson (1957) has emphasized the need for precise knowledge about food demands of individual species. Other authors have expressed similar views (Fryer, 1957; Gurney, 1931; Welch, 1952). Even the more refined and direct methods of assessing the suitability and/or availability of food seem to be accompanied by difficult technical problems. The relative importance of the major components of the seston (including nonliving tripton) as sources of food is in fact largely unknown for most zooplankton species. In contrast there is detailed information for Calanus on ingestion rates, preferences, size, and assimilation of food materials (summary by Marshall and Orr, 1955). Techniques which have proven successful in studying food utilization in Calanus have not been used in investigations with fresh-water calanoids.

All calanoids in which food acquisition has been investigated are filter feeders. Nearly identical food-filtering mech-

anisms have been described for Calanus and a relatively unrelated fresh-water calanoid, Diaptomus gracilis Sars (Cannon, 1928; Lowndes, 1935; Storch and Pfisterer, 1925). The feeding movements, being intimately associated with those used in swimming, suggested an automatic and indiscriminate removal of suspended particles. Subsequent gut examinations have served to confirm this idea. However, particle size and shape often appear to be of critical importance for the degree of effective retention by the maxillary filters in a variety of calanoids (reviews by Jørgensen, 1955; Marshall and Orr, 1955, 1955a). Selection of one kind of food in favor of another has been demonstrated experimentally in Calanus (Harvey, 1937). Selective feeding by other species in nature has been reported by Fryer (1954) and Conover (1956).

Although diatoms doubtless serve as its principal source of food, Calanus is capable of capturing larger organisms. Indeed, Marshall and Orr (1958) believe that the copepod may rely largely on predation for survival when, as during the winter months, algae are scarce and food requirements cannot be met solely by phytoplankton filtration. Digby (1954) has made the suggestion that the large decline in the overwintering stock might be due to cannibalism. The fresh-water calanoid Heterocope appears to be a consistent predator of the plankton (Bossone and Tonolli, 1954; Elster, 1936, 1954; Naumann, 1923); in fact, Burckhardt (1944) has described a situation in several Swiss lakes in which the predatory activities of H. saliens has resulted in wholesale depletion of zooplankton populations.

The significance of specific differences in food selection has received little more than theoretical consideration. Hutchinson (1951) has presented evidence to indicate that coexisting, closely related species of limnetic copepods consistently vary in body size. He suggested that permanent coexistence could be explained by the species' selecting food of different particle size such that interspecific competition for food is negligible. The mechanisms involved in selective feeding are unknown. According to Fryer (1957), shape and consistency as well as size may play a role in determining what organisms are eaten by Acanthocyclops viridis in nature. Little consideration has been given to this problem in calanoids. Some are known not to feed for rather long periods of time, and there is evidence that Calanus is not obliged to ingest continuously while actively swimming (Gauld, 1951; Marshall and Orr, 1955a). Moreover, Lowndes (1935) has reported that Calanus may bring certain mouthparts into play to divert the feeding current away from collecting surfaces. Esterly (1916) has observed Calanus to scatter food by a flick of the maxillipeds after it has accumulated it on the filtering setae.

Distinguishing copepod larval stages requires careful detailed observation. For the limnologist confronted with the task of segregating individuals of two or more coexisting species, the problem poses special difficulties. While the copepodid stages resemble a miniature adult, the nauplii of nearly all planktonic species are so similar that they can be distinguished only with difficulty. The general morpholo-

of individual instars in calanoids has been described by Oberg (1906), Grandori (1912), Dietrich (1915), and Ewers (1930), and thoroughly summarized by Gurney (1931). For generic and specific distinctions, descriptions by Elster (1932), Ravera (1953), Wuthrich (1948), and especially Humes (1955) are of value.

In the literature on fresh-water calanoids, population studies of a particular species over an entire year are scarce. A few examples of the most detailed investigations are those of Comita (1956), Comita and Anderson (1959), Bossone and Tonolli (1954), Elster (1936, 1954), Humes (1955), Klein (1938), and Ravera (1954, 1955). In the more elaborate of these, all twelve life-history stages were segregated and special pains were taken to obtain quantitative information about a variety of structural changes in the populations. For the most part, however, causal interpretations have been highly speculative or neglected, doubtless due to a lack of precise knowledge about the ecological requirements of the species concerned. It is becoming increasingly clear that some kinds of information necessary to interpret changes in natural populations can be obtained only under controlled conditions.

The papers by Humes (1954, 1955) on the systematics and life history of Epischura massachusettsensis Pearse and the studies of Elster (1932, 1936, 1954) on Heterocope borealis Fischer deserve special mention. Their morphological descriptions are among the most thorough to be found in the literature dealing with free-living copepods. Humes (1954) included a

comprehensive review of the adult morphology of the seven recognized species of Epischura. Humes (1955) described larval development and seasonal distribution. Qualitative plankton collections from a small, shallow pond were taken over a 13-month period. The appearance of the species was associated with pronounced changes in the physical conditions of the pond. E. massachusettsensis is the only species of the genus for which the nauplius stages have been described or in which their seasonal occurrence has been investigated. Certainly one of the most comprehensive studies of any fresh-water calanoid is the work of Elster (1932, 1936, 1954), in which the population of H. borealis in the Bodensee was examined in relation to the entire plankton community. Laboratory observations on certain aspects of feeding and reproductive behavior, egg production and hatching, and rate of development provided a wealth of information necessary in the analysis.

The species composition of limnetic zooplankton communities in terms of relative abundance has received little attention. Pennak (1957) has reviewed this subject in connection with the "niche" concept, attempting to demonstrate the relative simplicity of associations of species at any one time. E. lacustris seems consistently to be associated with a number of other calanoids, most commonly including Diaptomus oregonensis Lill., D. minutus Lill., and Limnocalanus macrurus Sars (Birge, 1897; Juday, 1903; Langford, 1938; Marsh, 1893). In the Great Lakes additional coexisting species are D. ashlandi Marsh, D. sicilis Forbes, D. siciloides Lill., and Senecella

calanoides Juday (Chandler, 1940; Davis, 1954, 1959a; Marsh, 1929; Wells, 1960). Also noteworthy are the repeated observations that populations of E. lacustris are often composed of relatively few individuals. For example, Juday (1903) in a comparative study of the plankton of eight Wisconsin lakes stated that sparse numbers prevented him from making quantitative analyses. As previously mentioned, Langford (1938) reported an extremely small population in Lake Nipissing; Birge (1897), Davis (1959a), and Wells (1960) have noted similar situations in Lakes Mendota, Erie, and Michigan, respectively.

The present work advances our knowledge of the larval development, ecological life history, and feeding behavior of a widely distributed fresh-water calanoid copepod by augmenting a field study with laboratory experiments and observations. The descriptive part concerns the larval identification of E. lacustris and the seasonal occurrence of all twelve stages during a 15-month period in Whitmore Lake, Michigan. Egg viability in relation to temperature was investigated with particular reference to the appearance of the copepod in nature. Matters pertaining to food were pursued by examining the contents of the digestive tract, measuring the filtration rate, and by assessing the suitability of a variety of organisms by offering them to laboratory cultures of animals.

GENERAL METHODS

Life History

Field collections

Plankton samples were taken from a small boat in the northeast basin of Whitmore Lake. Throughout the period of study, Epischura was found to be scarce or absent from the shallow areas surrounding this basin, circumscribed by its steeply sloping sides, as shown in Fig. 1. Large qualitative tows were taken with a #10 standard silk plankton net on 59 dates from March 2, 1956, to June 12, 1957. On the average the plankton was sampled once every six days during ice-free conditions. The aperture of the net's mesh (0.158 mm square) was just fine enough to retain the first nauplius stage. In an effort to obtain representative samples, the net was towed from near the bottom (17 m or more) to the surface several hundred feet across the deepest part of the lake, except during the winter when a series of vertical hauls was made through the ice at a single station.

Surface and air temperatures were recorded on each visit to the lake. Every few weeks the vertical distribution of temperature was determined by lowering to the bottom of the deepest depression a Whitney resistance thermometer on a calibrated line.

Unlike the majority of fresh-water copepods which carry their eggs in one or a pair of ovisacs, E. lacustris sheds

them directly into the water. Since eggs oviposited even at the lake's surface might be expected to sink to the bottom before they could hatch, bottom samples were dredged and examined for the presence of eggs. As a result of finding them there in abundance, routine bottom sampling was undertaken in an attempt to relate the occurrence of viable eggs to the copepod's seasonal appearance in the plankton. Monthly collections were made with an Ekman dredge (six inch size) during the first half of 1957. The samples, taken along a transect extending from near shore to the profundal region, reflected a variety of sediment types.

Morphological treatment

Distinguishing nauplius stages was accomplished only after eggs, procured from freshly captured females, were allowed to hatch in the laboratory and the development of the resulting larvae followed through one or two molts. Immature copepodids were relatively easy to recognize because of their similarity to the adult. Individual nauplius stages were compared with those of E. massachusettsensis (Humes, 1955), and all instars were identified by noting differences in body size and in the state of development of the appendages. Body length measurements of more than 200 nauplii and 150 copepodids were made. The adult second maxilla, the appendage which in calanoids serves as a filter, as well as the sexually dimorphic fifth legs of the last three copepodid stages were described and drawn.

Through experience in examining a large number of cope-

pods, living individuals of the species could be distinguished simply by scanning a fresh plankton sample with a dissecting microscope. The distinctive position of the swimming appendages in the living nauplius and the animal's characteristic swimming behavior made this possible, thus obviating time-consuming microscopic examination under high magnification, usually required to recognize nauplius stages in preserved plankton collections.

Living instars were photographed to illustrate some of their characteristic features. Photographs of the egg and six nauplius stages were taken with a 35 mm camera mounted on a Zeiss Plankton Microscope designed by Utermohl. Copepodids were photographed with a Bausch and Lomb binocular dissecting microscope fitted with a Micro-Ibso attachment and a Leica camera box. Specimens were immobilized by rapid cooling to near freezing or by immersion in 10^{-4} concentration of MS 222 (tricaine methylsulphonate).

Figures were drawn with the aid of a camera lucida at 275 and 430 magnifications. To determine detail a 95X oil immersion lens was used. Examinations of whole specimens and dissected appendages were made using animals previously stored for a few days in a mixture of 20% glycerine in 70% alcohol. This made them easier to manipulate and to dissect and prevented the material from drying out.

Instar analysis of Whitmore Lake population

The seasonal distribution of instars was determined by recording their presence in each of the preserved plankton

samples. The method was essentially the same as that employed by Humes (1955), no attempt being made to obtain quantitative data.

On occasion it was necessary to examine the entire contents of a given sample. Those in which a relatively large number of Epischura were encountered were subsampled and only a fraction of the original sample studied. Twelve instars were segregated. The numbers of adult males and females (Copepodid stage VI) were recorded and adult females further divided according to the proportion carrying ripening eggs in their oviducal diverticula. The transparency of the body and the dark opacity of the eggs made these individuals easy to pick out. Usually 100 adults, taken at random from each sample, were used to estimate the sex ratio. For a measure of the occurrence of egg production, in most cases 50 adult females were examined. On a few dates, a fewer number of adults were examined due to the small number of individuals present in the collections.

Egg viability

It was noted that the length of time necessary for eggs to hatch in the laboratory varied considerably, often by as much as several weeks. Yet all eggs examined appeared structurally similar. The discovery that large numbers of viable eggs rested in the bottom mud some months after the major period of egg production in the fall of the year established that there were overwintering or resting eggs. Although the term "resting" egg is usually reserved for special thick-

walled eggs known to be produced by some species of calanoids (e.g., Limnocalanus macrurus), it is commonly supposed that ordinary eggs may occasionally overwinter in an extended incubation period (Gurney, 1931; Pennak, 1953). It seemed reasonable to expect to explain differences in incubation time on the basis of temperature. In the lake, eggs would be exposed to different temperatures depending on the time of year. Eggs laid during the warm months of the year would experience low temperatures only in the lower water layers. Eggs produced in the late fall, on the other hand, would encounter continually decreasing temperatures and ultimately almost uniformly cold conditions regardless of depth.

Two methods were used to investigate egg viability. One employed eggs laid in the laboratory by freshly captured animals so that the time of laying and usually the number of eggs per female were known. The other involved recording the frequency of hatching of eggs occurring in mud samples by noting the presence of Nauplius I individuals. Culture vessels containing either the laboratory-laid eggs or small quantities of mud in filtered lake water were exposed to one of two constant temperatures: 20.0° C (18.7 to 21.5° C) and 5.0° C (4.7 to 5.2° C). The vessels were examined at intervals varying from one to seldom more than ten days, and as nauplii appeared, they were removed by decanting and counted. It was possible to retrieve virtually all nauplii due to their tendency to swim up from the bottom of the culture vessels. Unhatched eggs and sediment remained undisturbed.

Water removed was replaced by filtered lake water kept at the experimental temperature.

It was recognized that while enroute from the lake to the laboratory and especially during the time required to isolate the gravid females, plankton and mud samples were subjected to fluctuating and generally increased temperatures. For experimental material destined to be kept at 20° C the effect may not have been significant in that 20° was for the most part higher than any pre-experimental temperature likely to have been encountered. In any case the time elapsing between collecting and maintaining the material at the desired temperature was kept to a minimum.

In isolating females whose eggs were to be exposed to the lower temperatures, special precautions were taken to avoid elevated temperatures. Living plankton was stored in a refrigerator immediately upon return from the lake. Subsequently, small subsamples were examined and ovigerous individuals isolated and promptly returned to the refrigerator where oviposition was allowed to take place at a temperature of about 5° C. The number of eggs laid was not counted directly but was estimated from the number of nauplii subsequently recovered.

The procedure with eggs taken in mud samples was much the same as that used for laboratory-laid eggs. Bottom sampling was restricted to a period (January to June, 1957) during which no sexually mature Epischura were present in the lake. Consequently, all eggs taken in mud samples must have been produced by the population of the preceding season, or even

earlier. Freshly collected mud was allowed to settle, then decanted, and stored in sealed jars at each of the two experimental temperatures. Decanting effectively removed the few microcrustaceans present. Eggs were retained in the settled portion. No reliable method was found to distinguish the eggs of Epischura from those of the other crustaceans in the bottom debris. Therefore, the eggs were not removed nor was it possible to estimate the numbers actually present. A small amount (about 10 grams) of moist mud was placed in each of several culture dishes, flooded with filtered lake water, and thereafter maintained at one of the experimental temperatures. Stored moist mud samples which had been shown to contain a large number of viable eggs sometimes were subsampled again and treated similarly.

Food Relations

Feeding studies were initiated in an effort to prolong the laboratory survival of mature female Epischura taken from live plankton hauls in connection with egg viability and larval identification work. The problem of determining food preferences was approached first by exposing laboratory populations to known species of phytoplankton suspended in filtered lake water. Differences in growth and survival of the animals, maintained in small aquaria, were recorded according to the algal organism offered.

Gut dissections of both experimental and freshly captured Epischura provided direct evidence of the kinds of food con-

sumed, and variations in fecal pellet production by laboratory animals were noted. As could be inferred by carefully examining the contents of the fecal pellets, the degree of digestion was also taken into account in assessing food suitability.

In order to substantiate and, if possible, amplify the results of these preliminary observations, measurements of the rates at which the copepod ingested different algae were made. The phytoplankton species used were largely those which seemed to be consistently associated with significant differences in growth and survival of the copepods in the earlier work. The method involved determining the change in phytoplankton concentration occurring during the period in which Epischura were allowed to graze upon it. In order to keep the algae in constant suspension, a grazing apparatus, designed to accommodate 12 experimental bottles simultaneously, was constructed. A brief description of the equipment appears later.

Finally, the possibility was investigated that crustacean plankton itself might be an important item in the animal's natural diet. A variety of crustaceans were offered to stage III, IV, V, and adult copepods and the rate was noted at which the different ones were consumed. These consisted primarily of diaptomid, cyclopoid, and brine shrimp nauplii as well as those of Epischura itself. Daphnids, other cladocerans, and late-stage copepods also were tested. The suitability of crustaceans as the sole source of food was examined by rearing individual Epischura which were periodically supplied with freshly-hatched Artemia larvae.

Laboratory rearing

The prospects of eventually establishing a self-perpetuating laboratory population of Epischura at no time were considered very likely. Still, the most direct approach in obtaining detailed information about the animal's food habits seemed to bring the copepods in from nature from time to time and to study them under a variety of controlled conditions. In maintaining animals for purposes of trying to raise them on different kinds of foods, three procedures ultimately were used. Conditions common to all three, as they seemed to be best for survival, were a medium consisting of microfiltered lake water (Reeve Angel paper #202), a controlled temperature of 20.0° C (±1.5), and indirect fluorescent illumination (not measured). It was found that at about 10.0° C, adults had difficulty in feeding.

Cultures initiated with adults and other copepodid stages were kept in 8-10 liter aquaria allowing as much as a liter of medium per animal. The copepodid's comparatively large size permitted easy observation for censusing. For rearing nauplii, small Petri dishes were used in order to change the medium at more frequent intervals. Up to a dozen young nauplii were maintained in each of several cultures containing about 10 ml of lake water and offered fresh dilute concentrations of algae every few days. Epischura raised on Artemia larvae were isolated in jars of about 100 ml capacity. The cultures were initiated with 30 stage V copepodids of both sexes. For each individual daily records were kept on

mortality, molting, egg production, and number or prey consumed. Bacterial contamination resulting from uningested food was minimized by daily replacement of lake water.

In determining what algae to use, convenience of culturing, size, and similarity to forms represented in the lake's plankton were considered. The significance of size and shape of filterable food particles was mentioned previously. Accordingly, a wide range in size among foods to be tested was chosen. Average sizes varied from about a 4 μ diameter for Chlorella vulgaris to that of Pediastrum biradiatum, which measured approximately 68 μ in diameter and 10 μ in thickness. Except for Chlamydomonas, all genera were represented in the plankton of Whitmore Lake and occurred throughout much of the year.

Blue-green algae and diatoms were not used in rearing. Their ommission may not be as serious as would first appear. The former, although dominant in the lake's summer flora, are not generally recognized as important in crustacean nutrition. Species of Anabaena, Oscillatoria, Fragilaria, and Synedra were isolated from living plankton samples but failed to multiply in any of the culture media used. Two species of diatoms were subsequently obtained from the Culture Collection of Algae, Department of Botany, Indiana University, Bloomington, Indiana, and were cultured successfully. Their suitability was evaluated by measuring their rates of ingestion by Epischura as is described in a later section.

The phytoplankton offered to laboratory populations were:

Species	Indiana University culture number
Ankistrodesmus sp.	
Chlamydomonas reinhardi	
Chlorella vulgaris	#397
Oocystis sp.	
Pediastrum biradiatum	# 37
Scenedesmus quadricauda	# 77

The three species indicated by culture numbers, corresponding to those published by Starr (1956), were procured from the Indiana University collection. Ankistrodesmus and Oocystis were obtained by isolating individual cells or coenobia from settled volumes of Whitmore Lake surface water.

The liquid nutrient medium which satisfied the growth requirements of the six algal species sufficiently to permit mass culturing was found to be a slight modification of culture medium #10 reported by Chu (1942). Its composition was as follows:

Constituent	Concentration in grams per liter
$Ca(NO_3)_2$	0.08
K_2HPO_4	0.02
$MgSO_4.7H_2O$	0.025
Na_2CO_3	0.02
$NaSiO_3$	0.025
Fe citrate citric acid	0.0004 Fe^{+++}
50 ml of soil extract per liter	

Unialgal mass culturing was carried out in 100 ml of medium contained in 350 and 500 ml Erlenmeyer flasks using a constant illumination provided by two 40-Watt, daylight type, fluorescent tubes at a temperature of 20.0° C (±1.5). One ml inoculations to fresh sterile medium were made periodically so as to have algal populations in active cell growth when needed.

In supplying each of the laboratory populations of Epischura with one of the algal species, care was taken to use cultures in a relatively active state of growth. Feeding consisted of washing, centrifuging, and resuspending a sample of the culture in filtered lake water before adding it to the appropriate aquarium and Petri dish containing the copepods. Nauplius populations were given a change of medium and fresh algae every two or three days. Water in the larger aquaria was replaced only rarely, and algal food, eventually allowed to accumulate on the bottom of each tank, was offered less frequently. A record was kept of mortality. This record was less complete for the few copepodid populations which had managed to reproduce, in that no attempt was made to census nauplii, which in at least one aquarium appeared in abundance. However, the times of the occurrence of copulation, egg formation, and newly-hatched larvae were noted. Occasionally, one or more animals were sacrificed in order to examine gut and fecel pellet contents.

Dried eggs of Artemia salina were hatched in brine and the nauplii rinsed in fresh water prior to introducing them to vessels containing isolated copepods. Generally the brine

shrimp remained actively swimming for twelve or more hours. None metamorphosed beyond the second nauplius stage. It was possible to obtain a fairly accurate count of the numbers consumed as fresh media and food were supplied daily and the nauplii for the most part were taken in whole. Almost invariably one or more brine shrimp were found untouched at the end of each 24-hour period, thus food supply doubtless was in excess of the copepod's needs.

Gut analysis

Gut analyses were made by placing live animals individually in a clean drop of water on a glass slide and compressing them with a cover slip. The technique was similar to that described by Fryer (1957) in that by smearing the extruded contents across the slide it was possible to identify individual components. Remains arising from the rectum and foregut frequently could be distinguished.

The use of gut analyses in determining the natural food of copepods is open to question. Gurney (1931) believed that it is essentially impossible to recognize the food once it has entered the digestive tract. On the other hand, Fryer has relied entirely on gut examinations to demonstrate specific food habits of a variety of fresh-water cyclopoids. Undoubtedly, certain food organisms, because of delicate structure and digestibility, become unrecognizable immediately or soon after being ingested, whereas others resist digestion and apparently are not seriously affected by passing through the alimentary tract. Moreover, the fact that partly digested organisms

usually cannot be distinguished from ingested organic detritus sheds little light on the question of whether the latter is a major source of copepod nutrition, as maintained by some investigators (Naumann, 1923; Järnefelt, 1940; Pennak, 1955). The consensus appears to be that remains which are recognizable do not necessarily portray food preferences of natural populations (Edmondson, 1957; Marshall and Orr, 1955).

Accordingly, the guts of freshly-captured limnetic Epischura were examined only after algal food preferences of experimental animals had been investigated. The analyses were expected to give merely an indication of the kinds of materials eaten in nature. Ninety-six adult and stage V copepods, taken in August and September, were studied individually as soon as possible after collection, and recognizable remains in their guts were recorded.

Measurement of filtering rate

The method of employing concentrations of suspended food particles before and after exposure to filter-feeding copepods as a means of determining ingestion rates is a familiar one (Fuller and Clarke, 1936; Fuller, 1937; Gauld, 1951; Marshall and Orr, 1955a; Conover, 1956; Edmondson, 1957). A major difficulty seems to arise from an inability to determine to what extent the confinement of the animals interferes with normal feeding activity. Similarly, measures taken to insure a constant suspension of food, such as agitating experimental vessels so as to make different foods equally available to the copepods, may inhibit filtering behavior. The method must

therefore be used with caution by the investigator concerned with absolute filtration rates. On the other hand, insofar as they may be considered to affect filtering rates uniformly, standard treatments are not likely to alter seriously genuine food preferences.

At least as serious are other factors which undoubtedly influence measurements of filtering rates, and a remarkable fact in all studies to date is the great variation in the results, even under supposedly similar conditions. Differences attributable to such things as cessation of feeding, crowding of experimental animals, inhibitory substances produced by senescent algal cultures, variations in food concentration, and inevitable errors in counting are difficult to take into account. Typically, the procedure has been to average the results from a large number of experiments, although some investigators have taken the maximum values as the most reliable. In the present work, measurements of the rates at which different algae were ingested were taken as a measure of the food's relative suitability, although for reasons to be explained later, the highest rates were thought to actually approximate those normally occurring in nature.

One hundred and four experiments with the following nine species of algae were run in 13 series. Eleven experiments were discarded without counting, due to abnormally high mortality and accidental spilling. The organisms are ranked according to their approximate mean size, determined by direct measurement of cells or colonies.

Species	Indiana University culture number	mean size in μ
Chlorella vulgaris	# 397	4 dia
Navicula pelliculosa	# 645	10 X 3
Nitzschia closterium f. minutissima	# 642	19 X 3.5
Chlamydomonas reinhardi		15 X 11
Oocystis sp.		14 X 9
Scenedesmus quadricauda	# 77	20 X 14
Ankistrodesmus sp.		62 X 10
Pediastrum biradiatum	# 37	10 x 65 dia
Closterium littorale	# 736LB	20 X 150

Three of these, Navicula, Nitzschia, and Closterium, are additions to ones previously used in rearing the copepods. The diatoms were included because they are presumably important in the diet of calanoids. Closterium was chosen on account of its large size and the fact that it was easily cultured. It was found that the best growth was obtained in the three species by using heavier concentrations of soil extract.

The grazing apparatus consisted of a simplified modification of one described and used by Conover (1956). Two drums, each equipped with six holes into which experimental bottles were inserted and held securely in place, were mounted on a shaft which was turned by a 1/2 hp electric kymograph motor. A rheostat on the motor made it possible to rotate the drums at a speed as low as two rpm (slower than in Conover's equipment). Slow rotation rates were thought to have had the least

inhibitory effect on the copepod's feeding behavior, yet were more than adequate to keep the phytoplankton in constant suspension. Experiments were run at a temperature of 20.0° C (±1.5). For any one series, temperature variations were minimized by submerging the drums in a large water bath equipped with a heating element and by maintaining the entire apparatus in a room with a constant temperature of 10.0° C (±1.5).

Freshly-captured copepods were segregated according to stage and then conditioned in filtered lake water prior to their introduction into experimental bottles. All but ten experiments involved adult Epischura, usually of one sex. Animals were conditioned by allowing them to remain 24 hours or more at the experimental temperature in microfiltered lake water. Only active, healthy appearing individuals then were selected for use in the experiments. Each species of phytoplankton was centrifuged, washed, and resuspended in filtered water, the suspension further diluted to give a desired algal concentration. Approximate concentrations were estimated with the aid of a Sedgwick-Rafter counting cell according to methods described by Welch (1948). Between five and 21, but more often ten animals were introduced into each of a series of glass-stoppered bottles of known volume, filled with suspended food. Bottle volumes averaged about 125 ml. One bottle, containing no animals, was used as a control; all were then mounted on the grazing apparatus and the experiment run in the dark. The experimental period varied from between ten and 24 hours; most often it was 16 hours.

The filtration rate was determined from differences in phytoplankton concentrations between experimental and control bottles, as measured by Sedgwick-Rafter counts. It was based on the equation:

$$F = v(\ln C_o - \ln C_t),$$

after Gauld (1951) as modified by Conover (1956), where F is the volume of water swept free of food in 24 hours, v the volume of water per animal, C_t the experimental algal concentration after 24 hours, and C_o the control algal concentration after 24 hours.

Immediately following an experiment, after removal and brief examination of animals, 100 ml of the thoroughly mixed contents of each bottle were preserved with Lugol's iodine solution (American Public Health Association, 1959). Enumeration procedures with the Sedgwick-Rafter cell followed essentially those prescribed by Serfling (1949). Depending on the size and concentration of organisms, one of two magnifications was employed and the volumes represented by their corresponding microscope fields calculated. A minimum of 100 organisms and 30 fields, distributed symmetrically over the chamber, were counted. The mean of individual field counts was set at five or more; rarely this requirement necessitated concentrating the original sample. To evaluate the reliability of the counts, the coefficient of variation (relative standard deviation), based upon the range of ten observations, was estimated for each sample mean (Snedecor, 1946). It was used to calculate the standard error, a function of sample size, in percentage

of the mean (relative error). Counting additional fields beyond the minimum number, arbitrarily set at 30, served to control the latter; in a few instances as many as 100 fields per sample were counted.

RESULTS

Life History

Postembryonic development

The egg. - The entire series of developmental stages from egg to adult, in which the living instars appear in ventral view, is shown in Plates 1 and 2. Summer and overwintering eggs produced at different times of year are represented in Figs. a and b of Plate 1 respectively. As mentioned previously, they could not be distinguished morphologically. The summer eggs were retrieved from a specimen collected August 3, 1958. They hatched within 24 hours. The overwintering or resting eggs were oviposited in the laboratory by animals taken in the plankton on October 2, 1956. No instance of hatching among the more than 2000 eggs subsequently retrieved was observed as late as early March, 1957 when the photograph was taken. Yet all managed to hatch by April of that year. As is evident in the photographs, clutches laid in the fall sometimes were composed of slightly larger eggs than those produced in summer, but there seemed to be no consistent trend in this relation or in the change in clutch size. Fig. c of Plate 1 illustrates part of a clutch of eggs produced by laboratory-reared Epischura which had been isolated at Copepodid stage V and fed brine shrimp larvae. They were not fertilized and eventually disintegrated.

Hatching requires but a very few minutes. Rupturing of

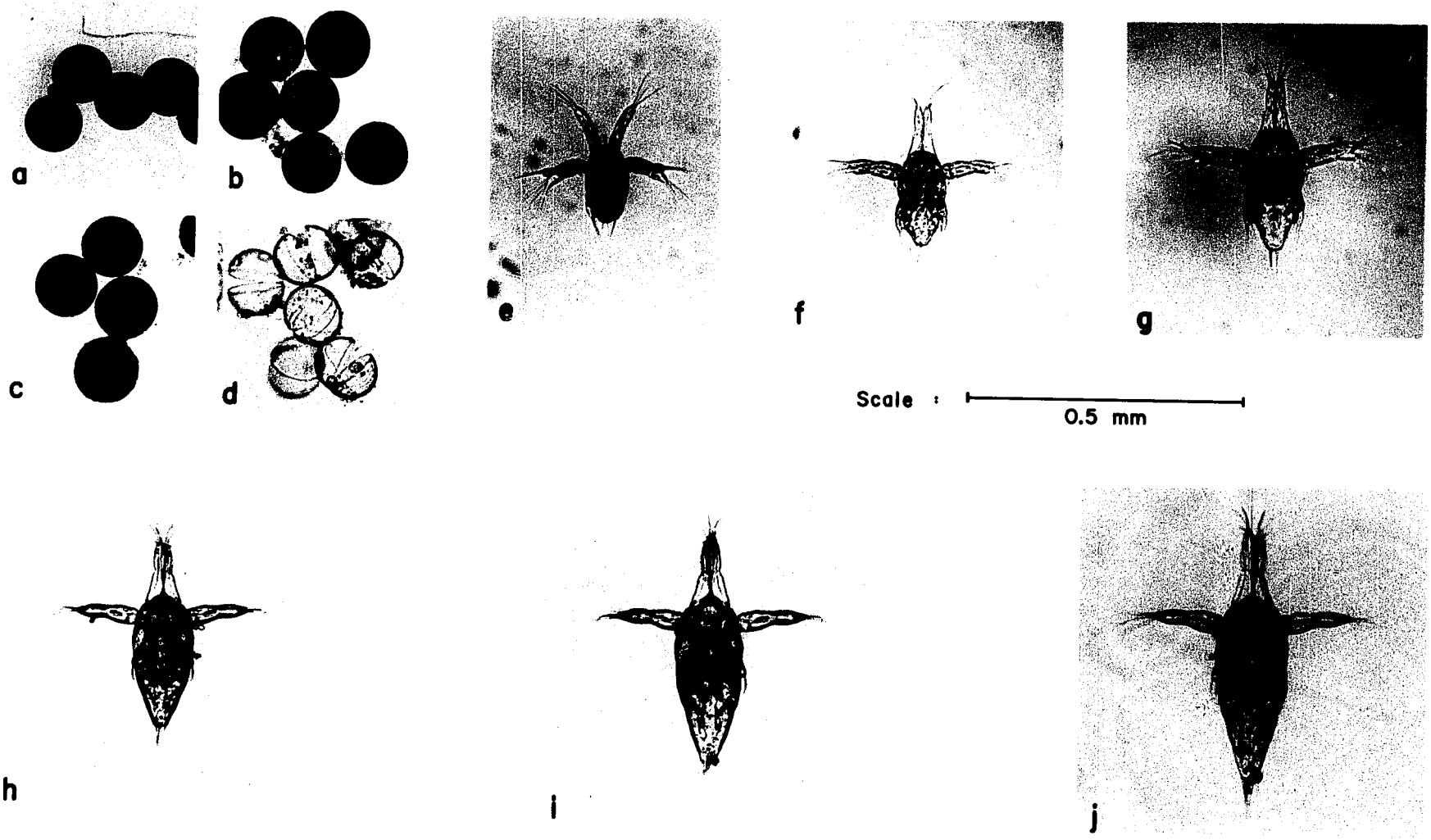


PLATE I. Egg and Nauplius stages of *Epischura lacustris* Forbes. (a - c) Egg, (d) Egg shell, (e - j) Nauplius I to Nauplius VI. Photographs of living animals, all to the same scale (about X85).

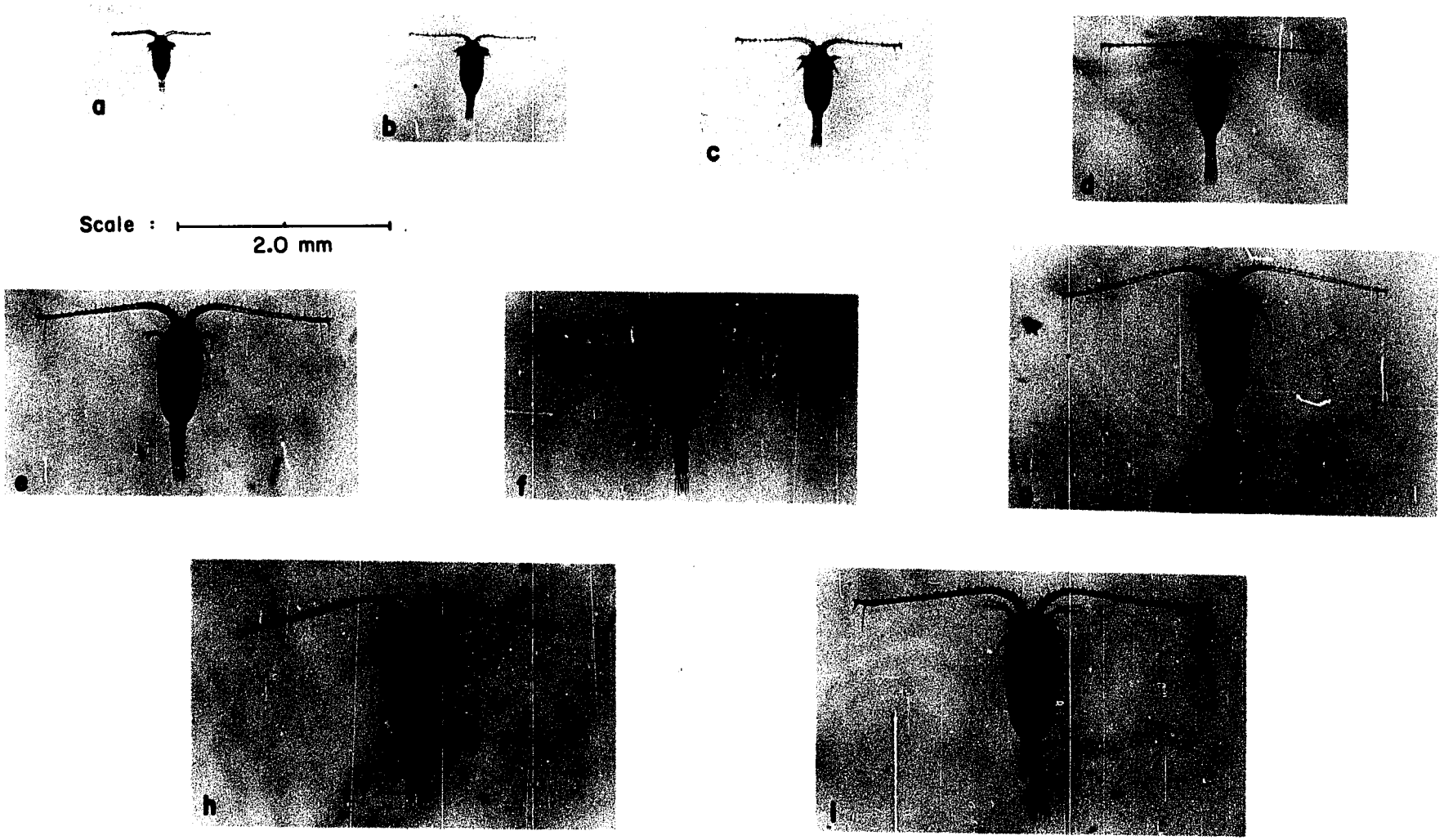


PLATE 2. Copepodid stages of *Epischura lacustris* Forbes. (a - d) Copepodid I to IV, (e) female and (f) male stage V, (g) virgin female and (h) male stage VI, (i) fertilized female stage VI. Photographs of living animals, all to the same scale (about X16).

the shell appears to follow a predetermined line of dehiscence resulting in a nearly complete pair of identically shaped hemispheres. The hemispheres do not completely separate but remain attached along a short hinge-like segment. After escape of the nauplius, the two discarded transparent halves remain gaping open at an angle of about 30°. Some of these features are clearly seen in Fig. d of Plate 1. The presence of an inner membrane, common to the eggs of all eucopepods in which the hatching process has been described (summary by Davis, 1959b; Elster, 1936), was not positively verified. In Calanus it is of such a delicate and hyaline nature that it may be easily overlooked (Marshall and Orr, 1955). Apparently it is most obvious, as a spherical bubble containing the nauplius, during its period of swelling and subsequent emergence from the ruptured outer shell or membrane. Although this sequence of events in the hatching process of E. lacustris was not observed, it is entirely possible that the finely tattered debris frequently found associated with discarded shells (barely discernible in Fig. d) is in fact the remains of inner membranes.

Hatching in Heterocope borealis and Diaptomus gracilis has been thoroughly described by Elster (1936), but this work apparently was overlooked by Davis (1959b). Eggs of the former are quite large (0.23 mm in diameter); in both a delicate hyaline "bubble", homologous to an inner shell swells osmotically. The outer shell ruptures by a "meridional crack", with the two halves gaping apart to allow escape of the bubble containing the nauplius. The entire process lasts from three to

eight minutes. The observations of hatching in Epischura, while less complete, appear to conform precisely with those made by Elster.

Nauplius stages. - Nauplius stages, with the exception of stage I, were most readily distinguished from those of other copepods by a peculiar apposition of the first pair of antennae. These appendages do not function in locomotion but remain in a fixed position. As illustrated in Plate 1 (Figs. f - j), they appear at first glance as a single structure projecting anteriorly. This feature alone makes possible the identification of larvae of E. lacustris in living plankton samples even though dominated by the nauplii of other copepods. Whether the character is unique to the species is not known. It is strongly suggested in the figures of entire nauplii of Heterocope borealis in Elster's detailed monograph, but not in those of H. saliens by Ravera (1953). It should be noted, however, that the latter are somewhat diagrammatic and that both investigators presumably based their descriptions on preserved rather than living material. In Epischura, upon preservation or death the first antennae are drawn more or less apart so that the feature is almost always lost.

Referring again to Plate 1, the first nauplius stage specimen was newly hatched in the laboratory; the other specimens shown were taken directly from Whitmore Lake plankton. Consequently, from the figures alone, a reasonably accurate estimate of natural size increase among the first six instars is possible. Epizoic colored microorganisms were found attached to several individuals. A number are noticeable on the body

and second pair of antennae of Nauplius stage IV in Fig. h. They were not identified. Their occurrence seemed to be related to the time of molting since newly metamorphosed specimens generally were devoid of such growths.

For positive species identification, preserved nauplii and living specimens of Nauplius stage I require high power microscopic examination. On the other hand, more or less gross characters were useful in identifying nauplii to suborder or genus. Cyclopoids were most easily distinguished from calanoids on the basis of body shape and development of caudal setae. The former have a body which is pear-shaped, never laterally compressed, but sometimes flattened; in the latter, the body is compressed and usually elongate. Similarly, caudal or furcal setae are invariably symmetrical in cyclopoids, but after stage I always asymmetrical in calanoids. These features, shown semi-diagrammatically in Plate 3 (Figs. a - d), require no painstaking manipulation or dissection to be observed clearly. In contrast are other diagnostic features which, while at least as applicable, are much more difficult to see. For example, Ewers (1930) and Gurney (1931) refer to masticatory structures on the second antenna and mandible, relative flatness of distal segments and length of the first antenna, and the stage in which certain appendages first appear as rudimentary lobes.

Nauplii of Diaptomus may be distinguished from those of Epischura by the setation of the first and second segments of the first antenna. As these two genera regularly occur together, their larval stages frequently may be confused. Body

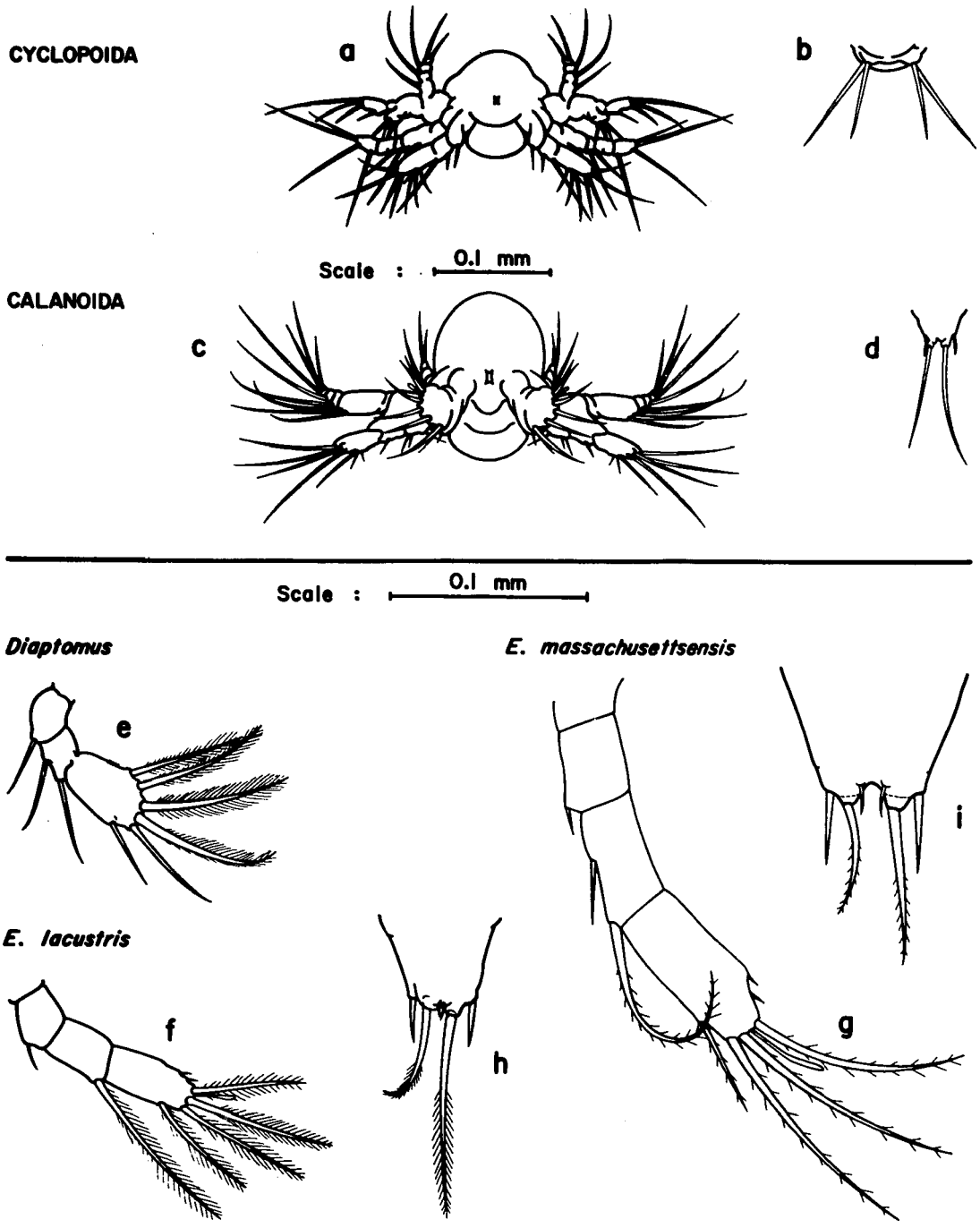


PLATE 3. Morphological characters useful in distinguishing nauplii of different species. (a) anterior view and (b) caudal setae of *Mesocyclops edax*; (c) anterior view and (d) caudal setae of *Epischura lacustris*; (e), (f), and (g) first antennae of *Diaptomus minutus*, *E. lacustris*, and *E. massachusettsensis* respectively; (h) and (i) caudal setae of *E. lacustris* and *E. massachusettsensis*. (a) through (g) Nauplius stage III; (h) and (i) Nauplius stage V. (g) and (i) after Humes, 1955.

dimensions have only a limited value in species identification. Humes' generic distinction may now be emended to include E. lacustris. In this species, the most proximal seta is indeed very short, as in E. massachusettsensis, but the middle seta is absent altogether. These characters are illustrated by Figs. e, f, and g of Plate 3 which are lateral views of the first antennae of Nauplius stage III of Diaptomus minutus, E. lacustris, and E. massachusettsensis, respectively. Nauplii of D. minutus were reared from the egg after isolating live ovigerous females from the plankton.

Other distinctions between the two species of Epischura were found. Setation is much more developed in E. lacustris; stage for stage, setae are not only proportionately longer but more heavily feathered. This difference is most noticeable on distal segments and caudal areas (Plate 3, Figs. f, g, h, i; Plate 4). For E. lacustris, an inhabitant of the open water of deep lakes, an increase in surface area no doubt serves as an aid to flotation. E. massachusettsensis, on the other hand, is known only from small and extremely shallow ponds. Similarly, a higher degree of asymmetry is apparent in the caudal setae. Humes (1955) noted that the middle seta of the right caudal ramus in E. massachusettsensis is slightly shorter than that on the left and bent somewhat dorsally. In E. lacustris, there is not much difference in length, but the curvature of the large, right caudal seta is more pronounced in all nauplius stages (Plate 4, Figs. g through l). In Nauplius stage VI (Plate 4, Figs. l and m), this seta is usually directed dorsally with the tip pointing forward.

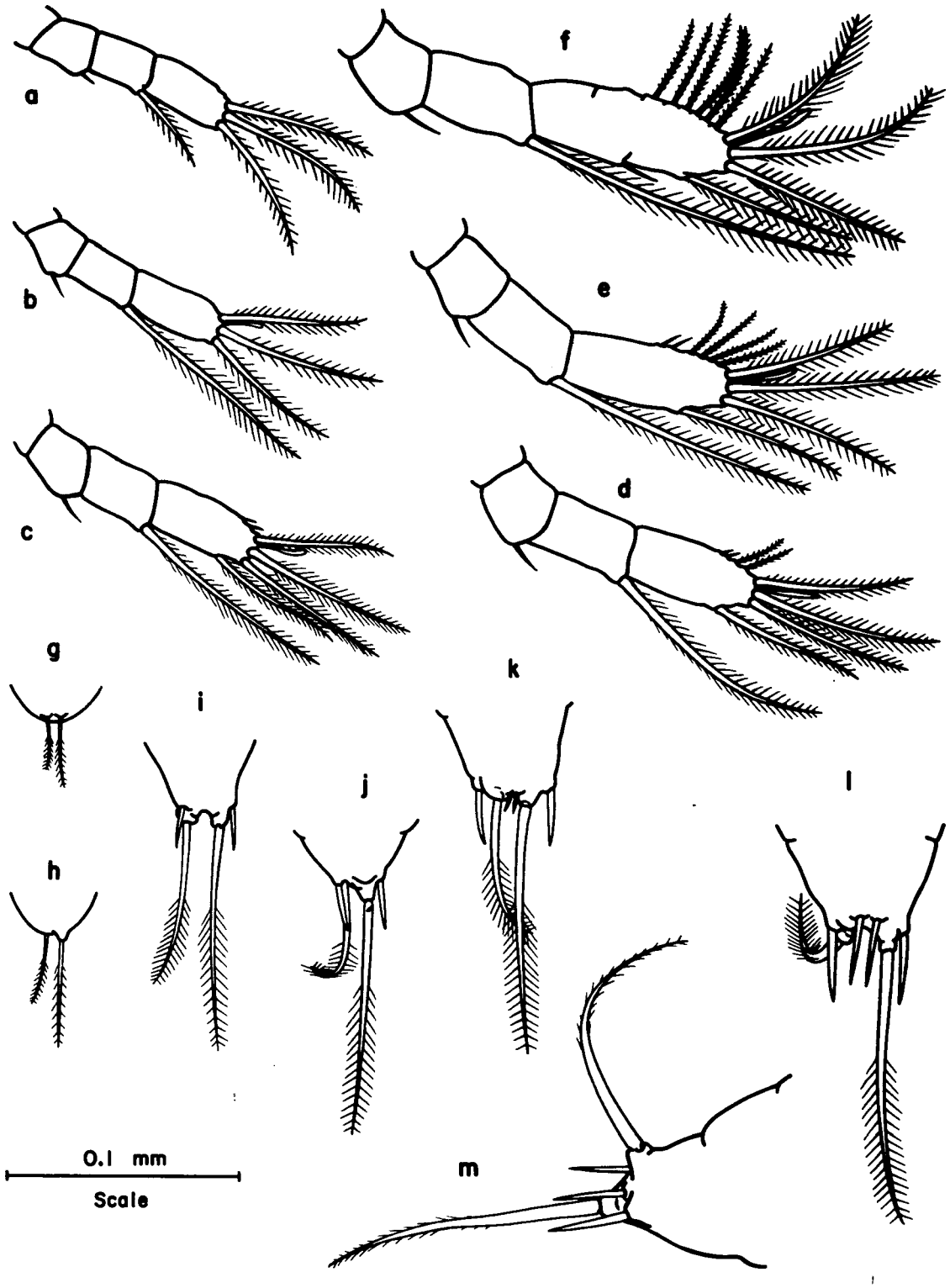


PLATE 4. First antenna and caudal setae of six Nauplius stages of *Epischura lacustris*. (a - f) first antenna, (g - l) caudal setae in ventral aspect, of stages I through VI respectively. (m) caudal setae of stage VI as observed from the right side.

In other respects, the morphology of the nauplius stages of the two species is in close agreement (cf. first pair of antennae, Plate 4, Figs. a - f).

Copepodid stages. - The copepodid stages of the genus Epischura are more or less adequately known, and in two species, E. baikalensis and E. massachusettsensis, they have been described in detail (Garber, 1941; Humes, 1955). Therefore a description of the fifth pair of legs of both sexes and certain general features of the morphology of these stages in E. lacustris was included in the present study.

All copepodid stages of Epischura and Heterocope have one character in common: each furcal ramus is equipped with three well-developed plumose terminal setae, a slender, dorsal, inner seta, and usually a reduced or spiniform outer seta (Wilson, 1959; Humes, 1955, 1954; Borutskii, 1947; Marsh, 1933, 1900; Elster, 1932; Juday and Muttkowski, 1915; Sars, 1900; Schacht, 1898; Lilljeborg, 1889; Forbes, 1882). The character is used by Wilson (1959) to distinguish genera of North American calanoids. These three large setae are very noticeable in E. lacustris and are not difficult to make out in some of the photographs shown in Plate 2.

A common and frequently necessary practice in identifying copepod larval stages is to associate them with the species that are known to occur in a particular body of water. In the Calanoida, copepodid stages follow a similar pattern of development with respect to segmentation and appearance of appendages, so that by following a standard formula individual instars may be told apart. As was expected, development in

E. lacustris follows the basic formula described by Wilson (1959), and corresponds strikingly with that of E. massachusettsensis.

As in other calanoids, species of Epischura may be clearly distinguished on the basis of the fifth legs alone. As far as is known, this applies not only to the adult but to developmental stages of either sex. These appendages in E. lacustris have been described for the adult, although not in precise detail (Forbes, 1882; Marsh, 1900, 1933). For the larva (Copepodids IV and V), the one previous description was neither thorough nor accurate.

Plate 5 illustrates the fifth pair of legs as it appears in specimens taken from Whitmore Lake. In general the development agrees with that described for E. massachusettsensis. The agreement is very close in the female, especially in stage IV, although even here the distal segment is narrower and its spines slightly more attenuated in E. lacustris. This distinction was noted in the adult stage by Humes (1954), who further remarked on the variable number of spines which has been reported for the female armature of the distal segment in E. lacustris. Forbes (1882) described six spines, four terminal and one on each side. Schacht (1898) reexamined the type specimens plus material taken in the same locality as the type (Normal, Illinois), and other specimens collected in Illinois, Indiana, and Michigan. He reported a constant number of seven spines, three on the outer margin, three terminal, and one on the inner margin. Humes (1954) found seven similarly arranged spines in specimens from Lake Erie.

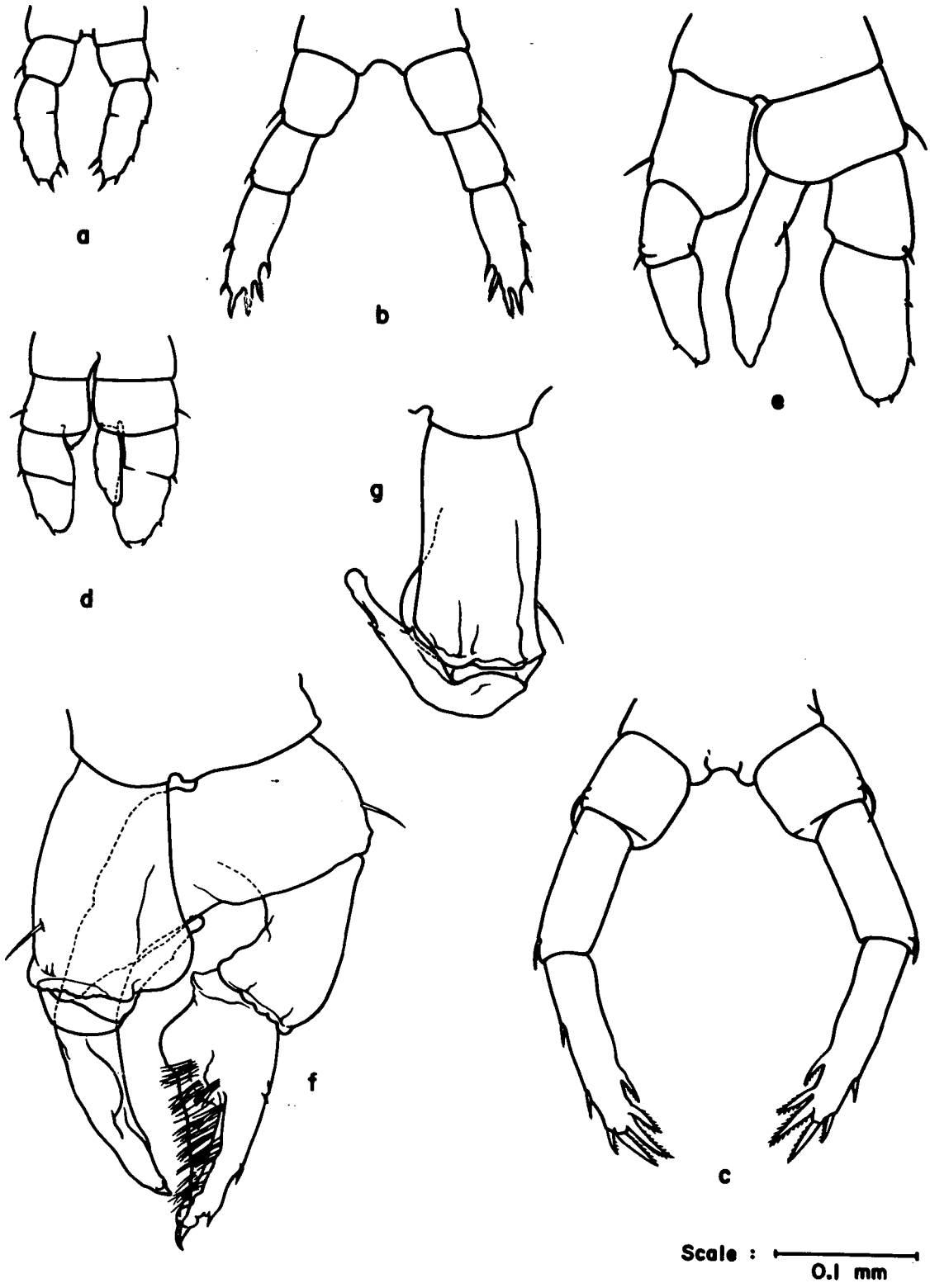


PLATE 5. Fifth pair of legs of *Epischura lacustris*. (a-c) female and (d-f) male Copepodid stages IV, V, and VI respectively. (g) male right fifth leg in posterior view.

Females from the Whitmore Lake population usually possess six spines arranged as shown in the original figures of Forbes. However, a few individuals were found to have six on one leg and seven on the other. Examples appear in Figs. b and c of Plate 5. It may be noted that fine spinules first appear in stage V. On the distal segment they are restricted to spines located terminally on the inner margin (in E. massachusettsensis they occur only in the adult stage but on all the spines of the distal segment). Only one specimen was encountered which had seven spines on both legs, so it was probably of rare occurrence in the population.

The female fifth legs of adult specimens from Lake Michigan (off Grand Haven) and Lake Winnebago, Wisconsin, also were examined by the writer. The Lake Michigan material possessed seven spines, but in Lake Winnebago the number varied from six to eight, sometimes in the same individual.

Intraspecific variation in the number and arrangement of these spines has been reported for E. nevadensis (Forbes, 1893; Marsh, 1933) and E. nordenskioldi (Schacht, 1898). In the former species, the position of the spines was found to be different in the two legs of a single individual, but there has been no previous report for any species of a variable number within a single specimen.

Other cases of this kind in calanoids are well known, especially in diaptomids (Wilson, 1959). In E. lacustris, for example, the armature of the second exopod segment of the male left fifth leg is similarly variable (Schacht, 1898).

Often it is armed with an outer distal spine. In specimens from Whitmore Lake this structure is usually lacking in the adult (Fig. f Plate 5), although present in the preceding instars, and was neither described nor figured by Forbes.

It seems fair to conclude that Forbes' original description was correct for his material, and that the number and distribution of the spines on the third exopod segment of the female fifth legs in Epischura should be considered at present as characters which vary intraspecifically.

The spermatophore is a persistent and characteristic structure in the adult female. It occurs singly, firmly attached to the genital somite (Plate 2 Fig. i), and only very rarely is a fully metamorphosed animal found lacking one. Unlike the situation in Calanus (Marshall and Orr, 1955) and many diaptomids, only one case was observed in which an Epischura possessed an extra one, and no instances were recorded of stage V females (Plate 2 Fig. e) or males bearing spermatophores. Therefore, copulation no doubt is confined to a limited period immediately following the female's final molt.

Growth factor. - Body length measurements, summarized in Table 1, were of practical value in preliminary identification of instars. E. lacustris is considerably smaller than E. massachusettsensis and it was of interest to compare their growth patterns. Growth factors, or the ratios of lengths between two succeeding instars, were calculated for the two species. They are recorded as mean values (i.e., average for all instars) in Table 2, along with those determined for a number of other related calanoids. Despite the size difference,

Table 1. - Mean sizes and extreme ranges of eggs, nauplii, copepodids, and adults of Epischura lacustris.

Stage	Number of measurements	Body length from tip of rostrum to end of caudal rami, (mm)		Growth Factor
		Range	mean	
egg*	7	0.105 - 0.110	0.108	
NI	47	0.117 - 0.131	0.120	
NII	52	0.145 - 0.161	0.152	1.267
NIII	31	0.174 - 0.207	0.188	1.237
NIV	45	0.221 - 0.268	0.237	1.261
NV	12	0.277 - 0.316	0.288	1.215
NVI	16	0.325 - 0.360	0.348	1.208
CI	11	0.490 - 0.541	0.518	1.488
CII	15	0.592 - 0.709	0.650	1.255
CIII	25	0.782 - 0.876	0.823	1.266
CIV ♂	17	0.998 - 1.064	1.039	1.262
CIV ♀	16	1.011 - 1.186	1.074	1.305
CV ♂	14	1.300 - 1.511	1.394	1.341
CV ♀	20	1.372 - 1.524	1.469	1.368
CVI ♂	17	1.608 - 1.748	1.681	1.206
CVI ♀	16	1.722 - 1.867	1.795	1.222

* diameters of living eggs

Table 2. - Mean growth factors for a variety of calanoids of the Family Temoridae. Values calculated from length data given in indicated references.

Species	Adult size (mm)	Mean Growth Factor	Reference
Epischura lacustris	♂ 1.68 ♀ 1.80	1.273 1.281	
Epischura massachusetts-ensis	♂ 3.13 ♀ 3.40	1.278 1.287	Humes (1955)
Eurytemora hirundoides	♂ 1.53 ♀ 1.60	1.283 1.287	Davis (1943)
Eurytemora velox	♂ 1.47 ♀ 1.77	1.269 1.284	Gurney (1931)
Heterocope borealis	2.35	1.211	Elster (1936)

the average relative increase in length for each sex is in remarkably close agreement in the two species.

It has been theorized that among crustaceans in general there is a fixed relative increase in size at each successive molt, which is characteristic for each species and sex. The idea, known as "Brooks's law" and attributed to Fowler (1909), has received only partial substantiation. It has been discussed by Skogsberg (1920) in his studies on marine ostracods and examined in marine and fresh-water copepods by Sewell (1912) and Gurney (1929, 1931). Sewell's account attempted to explain dimorphic adult forms by postulating entirely different male and female growth factors leading to alternative possibilities of development. A growth factor of 1.26 generally has been taken as normal. However, Gurney did not find a sufficiently uniform or specific growth rate in fresh-water diaptomids to support Sewell's theory. He considered an empirically determined growth factor not much exceeding 1.3, except for the molt from Nauplius stage VI to Copepodid I, to be typical. The change in shape from nauplius to copepodid was offered to explain a higher ratio of increase at this point of development.

In successive instars of Limnocalanus johanseni, Comita (1956) reported a fairly uniform growth factor which averaged less than 1.21, but curiously the length ratio between the last nauplius and first copepodid was one of the very smallest in the series (1.08).

In North American fresh-water calanoids, sexual differentiation appears first at Copepodid stage IV. In Epischura,

structural differences in the right first antenna and fifth pair of legs are quite noticeable. A sexual difference in body length appears in this stage in E. lacustris (Table 1) and becomes progressively stronger in the two subsequent stages; a pronounced sexual size difference does not occur in E. massachusettsensis until the adult stage. Humes measured over 1100 individuals yet found no size overlap among individual instars. This seems to be true for E. lacustris as well, although a much smaller number of specimens was measured. In diaptomids for which data are available, successive instars are often of the same size (Wuthrich, 1948). Based on mean lengths, growth factors in instars of E. lacustris ranged from 1.206 to 1.488 (Table 1), the latter value falling between nauplius and copepodid stages. This degree of variation is not unusual.

Mean growth factors for a number of fresh and brackish-water temorids (Table 2) demonstrate that usually there is only a slight variation between species or sexes. Considering the difficulty in obtaining accurate measurements and the fact that size is definitely known to vary with food supply in at least some species, such differences are probably not significant. No doubt, among copepods, a more or less fixed relative increase in size at each molt does exist; certainly it has a practical application in the study of larval development.

Seasonal occurrence in Whitmore Lake

Description of the lake. - Whitmore Lake, located 12 miles north of Ann Arbor in Washtenaw and Livingston Counties (T. 1 S., R. 6 E., Sec. 5, and T. 1 N., R. 6 E., Sec. 32), has

an area of 677 acres, a mean depth of 14.2 feet, and neither inlet nor outlet. It has a well developed littoral zone of sand and peat dominated by rooted aquatic vegetation and a major depression in the northeast end which extends to a maximum depth of 69 feet (Fig. 1). Certain aspects of its physical and chemical characteristics, some of which indicate pollution from domestic drainage, have been reported by Hooper (1956).

The net plankton was variable and frequently abundant. Occurring at least most of the year in prevalent numbers were the crustaceans Diaptomus oregonensis Lilljeborg, D. minutus Lilljeborg, Cyclops bicuspidatus thomasi S. A. Forbes, Mesocyclops edax (S. A. Forbes), Daphnia retrocurva Forbes, D. galeata Sars mendotae Birge, Diaphanosoma brachyurum (Lieven), Bosmina coregoni Baird, and rotifers of the genera Keratella, Kellicottia, and Synchaeta. Besides Epischura, less conspicuous or sporadically appearing forms were Diaptomus sicilis S. A. Forbes, Daphnia pulex Leydig, Chydorus sphaericus (O. F. Muller), Leptodora kindtii (Focke), Latona sp., Polyarthra sp., and Asplanchna sp.

Phytoplankton organisms were identified from periodic surface tows. Usually a #20 mesh net was used. Forms commonly present during the period of growth and development of the Epischura population were:

Cyanophyta

Anabaena	Chroococcus	Oscillatoria
Aphanocapsa	Gloeotrichia	Polycystis
Coelosphaerium	Lyngbya	

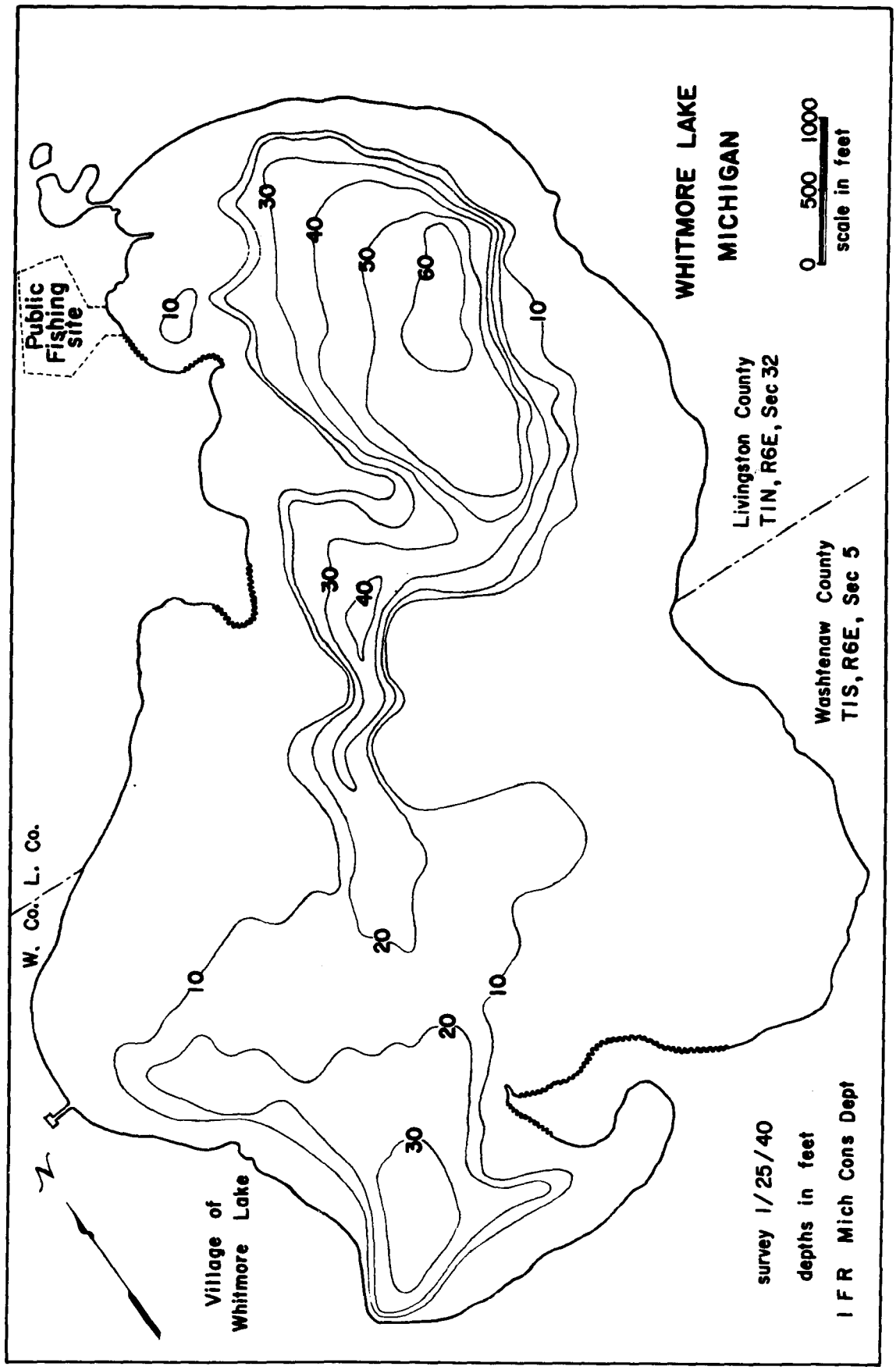


Fig. 1. Contour map of Whitmore Lake, Michigan.

Chrysophyta

Asterionella	Cyclotella	Navicula
Botryococcus	Dinobryon	Synedra

Chlorophyta

Ankistrodesmus	Mougeotia	Sphaerocystis
Closterium	Oocystis	Spirogyra
Crucigenia	Pediastrum	Volvox
Dictyosphaerium	Scenedesmus	Volvulina

Pyrrophyta

Ceratium

Additional forms at other times included Micrasterias and Staurastrum in early spring, Gomphosphaeria, Gyrosigma, Melosira, Gloeocystis, Glenodinium and abundant numbers of Fragillaria in the fall.

Temperature distribution. - Surface and air temperatures for each date of collection are recorded in Table 3; subsurface temperatures in the northeast basin in Table 4. These data were the basis for constructing the depth-time diagram shown in Fig. 2. Surface temperatures ranged from a maximum of 26.5° C on July 16, 1956 to a low of 0.22° C recorded on February 18, 1957; bottom temperatures (18.5 m) from 9.81° C to 2.80° C on October 22, 1956 and February 18, 1957, respectively. An ice cover existed for a short time at the outset of the study (March 2 to April 3, 1956), and again for a period of nearly four months the following winter (November 22, 1956 to March 17, 1957). It reached a thickness of over 16 inches on February 18, 1957.

Table 3. - Sampling dates and surface temperatures, Whitmore Lake, 1956-1957.

Date	Temperature (°C) Surface	air	Date	Temperature (°C) Surface	air
1956					
2 Mar	01.5	-03.0	20 Sep	17.5	07.5
21 Mar	03.23	05.0	23 Sep	16.75	16.0
3 Apr	(lake ice-free)		25 Sep	16.5	15.0
24 Apr	07.87	06.0	2 Oct	17.0	19.5
5 May	14.5	18.5	8 Oct	14.5	21.0
12 May	17.42	24.0	15 Oct	17.0	25.5
22 May	19.0	28.0	22 Oct	16.03	22.0
28 May	19.5	23.0	27 Oct	13.5	18.0
2 Jun	20.5	14.5	8 Nov	13.0	06.5
6 Jun	19.5	24.0	19 Nov	07.48	10.5
11 Jun	21.50	21.0	22 Nov	(ice cover)	
15 Jun	25.0	28.0	1957		
20 Jun	24.0	24.0			
25 Jun	24.5	26.5	19 Jan	02.40	03.5
29 Jun	23.5	21.0	18 Feb	00.22	02.0
3 Jul	26.21	26.0	5 Mar	02.5	13.0
7 Jul	23.5	26.0	17 Mar	(lake ice-free)	
12 Jul	25.0	22.5	22 Mar	03.5	05.0
16 Jul	26.5	25.0	28 Mar	03.75	04.5
20 Jul	23.5	23.0	11 Apr	05.0	06.0
25 Jul	25.0	26.0	18 Apr	06.48	10.5
30 Jul	25.0	23.0	25 Apr	16.06	22.0
3 Aug	24.0	24.5	30 Apr	17.0	18.5
7 Aug	24.5	25.0	4 May	14.0	08.5
15 Aug	25.05	21.0	9 May	16.78	21.0
20 Aug	25.0	19.0	14 May	17.0	19.0
24 Aug	22.0	17.5	18 May	15.5	10.0
28 Aug	23.5	26.0	23 May	16.0	19.5
1 Sep	25.0	21.0	28 May	17.24	17.0
6 Sep	24.0	19.0	1 Jun	20.5	19.0
11 Sep	20.0	18.5	4 Jun	21.0	22.0
15 Sep	20.5	13.0	12 Jun	21.5	21.0

Table 4. - Vertical distribution of temperature, Whitmore Lake, 1956-1957 (°C).

Date	Depth in meters										
1956	0	1	2	3	4	5	6	7	8	9	10
21 Mar	3.23	3.19	3.19	3.16	3.14	3.14	3.14	3.13	3.12	3.12	3.14
24 Apr	7.87	7.79	7.74	7.55	7.50	7.07	7.00	6.93	6.85	6.60	6.52
12 May	17.42	16.90	16.38	16.04	15.33	13.75	12.40	11.50	10.66	9.75	8.77
11 Jun	21.50	21.48	21.30	21.22	21.01	19.32	17.80	16.75	16.05	15.66	13.65
3 Jul	26.21	26.21	26.15	26.12	26.08	25.93	23.40	21.45	19.00	15.62	13.26
15 Aug	25.05	24.70	24.55	-	24.08	-	23.52	22.66	20.26	16.87	14.35
23 Sep	16.75	16.55	16.51	16.50	16.50	16.50	-	16.31	-	16.22	15.88
22 Oct	16.03	15.96	15.75	15.67	15.55	15.45	15.34	15.20	-	15.06	14.45
19 Nov	7.48	-	-	7.29	-	-	7.21	-	-	7.11	7.10
1957											
19 Jan	2.40	2.42	2.44	2.44	2.50	2.54	2.65	2.70	2.72	2.86	2.96
18 Feb	0.22	1.50	2.04	2.09	2.16	2.19	2.22	2.25	2.29	2.30	2.38
28 Mar	3.75	3.73	3.60	3.60	3.57	3.56	3.56	3.55	3.49	3.51	3.52
18 Apr	6.48	6.24	6.17	5.93	5.70	5.62	5.60	5.58	5.51	5.30	5.22
25 Apr	16.06	15.90	15.90	15.88	14.25	11.82	10.75	9.84	9.33	8.43	8.21
9 May	16.78	16.78	16.75	16.75	16.70	16.68	16.62	16.56	16.13	14.49	9.45
28 May	17.24	16.48	16.43	16.41	16.17	16.08	15.92	15.79	15.63	15.55	14.65

Table 4. - Continued

Date	Depth in meters										
1956	12	14	16	17	17.5	18	18.5	19	19.5	20	20.5
21 Mar	3.17	3.17	3.22	3.22	-	3.29	-	3.32	-	3.40	3.42
24 Apr	-	5.89	5.30	5.28	-	5.25	-	5.22	-	5.21	
12 May	7.67	6.28	5.78	5.58	-	5.44	-	5.40	5.40		
11 Jun	10.24	7.00	6.03	5.96	-	5.91	-	5.91	5.90	5.90	
3 Jul	10.39	9.11	8.60	8.53	-	8.21	-	8.18			
15 Aug	10.59	9.41	-	8.68	-	8.57	-	8.48	-	8.33	
23 Sep	13.88	10.00	9.48	9.33	-	9.18	-	9.10	9.03		
22 Oct	13.22	11.55	9.98	9.87	-	9.81	9.81				
19 Nov	7.00	6.98	6.96	6.95	-	6.92	-	6.90	6.90		
1957											
19 Jan	2.98	3.00	3.05	3.05	-	3.36	-	3.52			
18 Feb	2.44	2.52	2.62	2.72	-	2.76	2.80				
28 Mar	3.53	3.57	3.60	3.60	3.75						
18 Apr	5.20	5.12	5.10	5.00	5.17						
25 Apr	6.45	5.25	5.23	5.23	-	5.18	5.10	5.10			
9 May	7.20	6.42	5.61	5.55	-	5.43	-	5.42	-	5.40	
28 May	9.22	6.33	5.90	5.80	-	5.78	-	5.75	5.78		

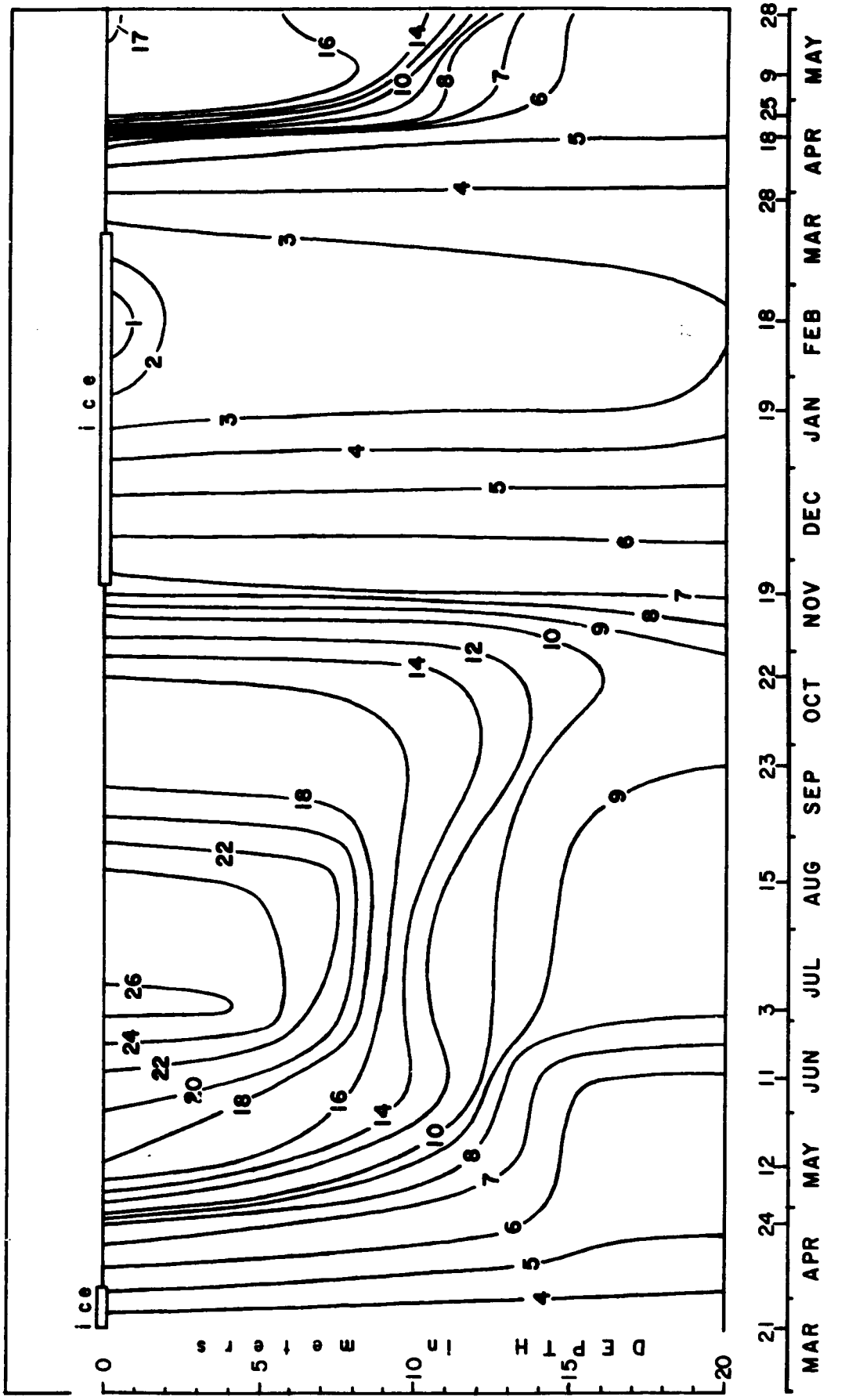


Fig.2. Whitmore Lake isotherms in degrees centigrade, 1956-1957.

Permanent thermal stratification was established in both years early in May, preceded by nearly homothermal conditions for much of March and April. Otherwise the rates of heating were quite different. In 1956, a relatively late thaw was followed by gradual uniform heating. In 1957, there was at first a longer period of instability, then extensive, rapid heating, and finally in May only a slight temperature change, so that by the first of June, temperatures in the upper water layers were still considerably higher than in the previous year. The fall overturn in 1956 was followed rather quickly by surface freezing.

Instar distribution. - Adults of E. lacustris were present in Whitmore Lake from May or early June to mid-November, with an abrupt decrease in numbers after October. The seasonal occurrence of individual instars from March 2, 1956 to June 12, 1957, is shown in Fig. 3. During the winter, a period marked by a permanent ice cover, only eggs persisted in the bottom mud, and neither adults nor immature stages were to be found. Nauplii first appeared at the time of the spring thaw. In 1956, Nauplius stages I through V were present in the earliest collection, April 24, three weeks following the melting of the ice. The next year, a sample taken on March 22, just five days after the lake became ice-free, contained only stage I and stage II nauplii; so it is likely that hatching had already commenced some few days before the ice had melted, perhaps about March 13. Nauplius stage III appeared six days later, but after that a precise picture of an orderly succession of

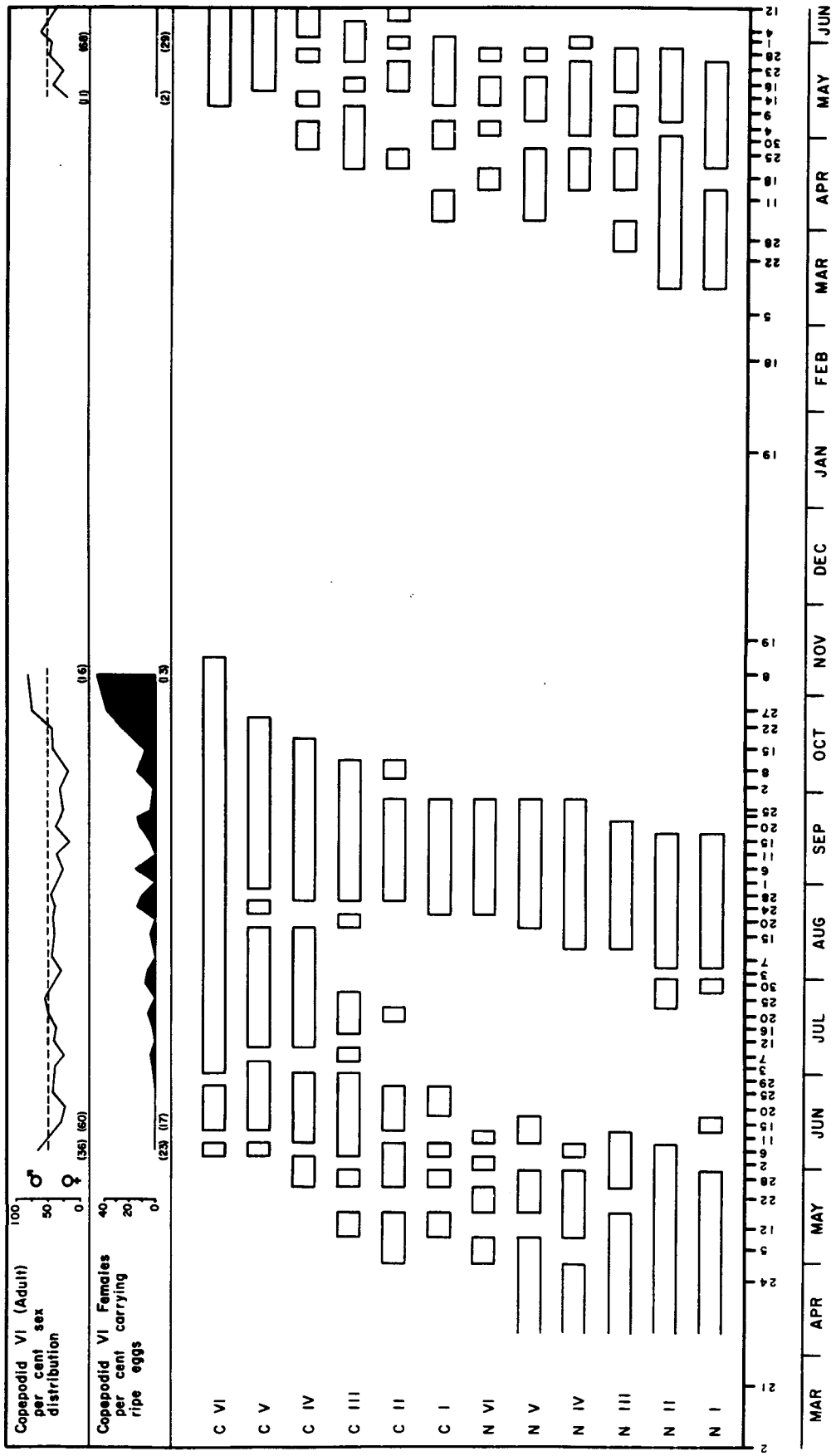


Fig. 3. Seasonal occurrence of instars of *Epischura lacustris* in Whitmore Lake, Michigan. N = nauplius, C = copepodid. Data on adult sex distribution and presence of gravid females were based on 100 and 50 mature specimens respectively for each date, except at times indicated by numbers in parentheses.

instars is obscure. Breaks in the data to do with the sequence and continuity of individual stages in the spring of both years are a result of the sparseness of the population. In Whitmore Lake, Epischura was conspicuous on account of its size, not its numbers. Probably, at no time did it represent more than about five per cent of the total standing crop of calanoids present (four species). Usually it was much less.

Mortality among the young nauplii must have occurred on a large scale in April and May. Typically, the first two stages were relatively common in spring collections, but Nauplius III and succeeding stages became increasingly scarce. While it is likely that a complete examination of all the specimens in each sample would have eliminated some of the gaps in the record, it was considered not worth the time which would have been required.

Despite this discontinuity, it is possible from Fig. 3 to estimate the growth rate from the time of hatching to the appearance of the adult stage. Development in the spring required about 64 days in 1956 and about 55 days in 1957, or an average of five to six days per stage. The difference may have been partly the result of different rates of heating in the two years. In 1956, the first gravid females appeared on July 7, which gives a period of egg maturation of approximately 27 days. About two weeks later, a large influx of young larvae made its appearance. This summer generation, presumably resulting from eggs produced by the mature members of the spring population, grew rapidly and reached adulthood in perhaps 38 to 40 days (July 22 to August 30). The duration of each stage of the summer brood up to the last two or three instars is fairly well

defined in Fig. 3; this indicates a higher population density as a consequence of conditions more favorable to hatching and growth.

Egg production continued at a variable but generally increasing rate throughout the summer and fall, reaching a peak on the last day (November 8) that any Epischura were found in the collections. Only 13 females were present in that sample, so the population had already largely disappeared, but six of them were gravid.

Spring and summer broods were not clearly distinguishable in adult and late copepodid stages. As a consequence gravid females from both generations may have existed together during the last of the season. However, it seems reasonable to suppose that eggs destined to overwinter were laid by animals produced during the summer, and that the sharp cessation of hatching shortly after mid-September corresponds to the end of the spring brood's egg production. This would give a date of about September 15 for the first appearance of gravid summer-generation females, which thereafter laid nothing but resting eggs. Temperature conditions in the summer and fall are of interest. The lake was strongly stratified during the period of summer growth in August and early September, with surface temperatures close to 20° C or above, and the bottom slightly below 9° C. After mid-September there was a gradual drop in the temperature of the epilimnion from about 18° C to near 13° C by the first of November. At the same time, the hypolimnion reached a maximum bottom temperature of close to 10° C. Such conditions may have been unfavorable for hatching, larval growth, or both. The

influence of temperature on hatching is taken up in the following section.

An estimate of the adult sex ratio is illustrated in Fig. 3. On most dates males outnumbered females; the reverse was true for a short period prior to the population's collapse in the fall. Females may take longer to reach maturity, probably live longer, and undoubtedly are hardier than males, which may partly account for the relative increase in their numbers toward the end of the season. In the laboratory, male mortality was very high, especially following copulation.

The act of mating was observed several times, although the initial seizure by the male invariably was missed. Handling under bright light may have interfered with normal behavior as spermatophore transfer was not witnessed. It may be noted that Calanus has never been known to copulate in the laboratory; therefore the possibility of rearing it through one complete generation can automatically be ruled out. Elster (1936) reported that copulating pairs of Heterocope borealis separated upon handling but that the entire process lasted only a few minutes. In Epischura it may continue for at least a few hours. The strong, pincer-like structure on the abdomen and the swimming legs of the male are used to hold the female. An eventual shifting brings the two ventral sides together with the animals heading in opposite directions. The male's abdomen is wrapped closely around that of the female. His fifth legs are then in a position close to the female's genital segment; meanwhile he is being dragged upside-down by the female's steady forward progress. Elster observed the attachment of the spermatophore

by the fifth legs of a male Heterocope on one occasion. He further noted that following mating, the male's right first antenna, which functions in grasping the female's abdomen or furcal ramus, remained in a flexed position. Apparently this appendage serves a similar purpose in Epischura as the geniculate antenna frequently was found to be strongly flexed in males which had mated in laboratory cultures.

Egg viability

Laboratory-laid eggs. - Table 5 summarizes the fate of eggs retrieved from freshly-caught Epischura and kept at constant temperatures of 5.0° C and 20.0° C in the laboratory. Censuses were made at frequent but irregular intervals as indicated in the left-hand column. Usually they were continued for a period of several weeks depending upon the occurrence and frequency of hatching. In one case, all eggs hatched within one day, and in another, periodic inspections were continued for a period of more than nine months. Animals counted and removed at each census were usually stage I nauplii, although sometimes they had reached stage II.

Hatching at 20.0° C in Series A required less than 24 hours. It was typical of summer eggs which presumably were produced by spring-generation Epischura and which undoubtedly encountered comparable or warmer temperatures in waters of the epilimnion during their maturation. In contrast were the extended incubation periods of eggs in the other 20.0° cultures (Series B, C, E, G) collected in September and October. Most hatching was delayed for up to several days but in one case it was postponed for over two months. While 20.0° is consider-

Table 5. - Viability and hatching record under controlled temperatures, of eggs of Epischura lacustris oviposited in the laboratory. Numbers refer to newly-hatched nauplii, removed and counted at each census.

Series	A	B	C	D	E	F	G	H
Date of collection (1956)	28 Aug	25 Sep	2 Oct	2 Oct	8 Oct	8 Oct	22 Oct	22 Oct
Date eggs were laid	29 Aug	25 Sep	2-7 Oct	2-7 Oct	8-12 Oct	8-12 Oct	22-29 Oct	22-29 Oct
Temperature (°C)	20	20	20	5	20	5	20	5
Inspection dates 1956								
Aug 30	45							
31								
Sep 1-25								
26		0						
27		0						
28		0						
29		0						
30		14						
Oct 1- 7		0	0	0				
8-12		0	3	0	0	0		
13-15		-	2	0	0	0		
16-18		-	7	0	20	0		
19-21		0	14	0	0	0		
22-24		-	0	-	0	-	0	0
25-27		-	7	-	4	-	0	0
28-31		0	0	-	0	-	0	0
Nov 1- 3		-	0	0	0	0	7	0

Table 5. - Continued

Nov	4- 7		0	0	-	0	-	14	-
	8-12		-	2	0	2	0	1	0
	13-15		-	0	-	0	-	0	-
	16-18		0	0	0	0	0	1	0
	19-21			0	-	0	-	0	-
	22-24			0	-	0	-	3	-
	25-27			3	0	0	0	0	0
	28-30			0	-	0	-	1	-
Dec	1-10			0	0	0	0	0	0
	11-20			1	0	0	0	0	0
	21-31			0	0	0	0	0	0
1957									
Jan	1-31	(weekly inspections)		0	0		0	0	0
Feb	1-28				0		0		0
Mar	1- 8				0		0		0
	9-14				1035		53		0
	15-16				369		11		0
	17-18				390		13		0
	19-20				255		9		0
	21-22				68		1		0
	23-24				21		2		0
	25-31				0		0		0
Apr	1- 6				47		1		0
	7-10				0		2		0
	11-20				0		0		0
	21-30				0		0		0
May	1-10				0		0		0
	11-20								0
	21-31								0
Jun	1-10								0
	11-20								0
	21-30								0

Table 5. - Continued

Jul 1-10								-
11-20								0
21-29								0
30								0
31								167
Aug 1- 4								88
5-10								46
11-16								21
17-20								0
21								0
Number of clutches	3	3	11	200	5	10	7	20
Total number of eggs	45	37	128	2185	44	92	108	322
Mean number of eggs per clutch	15.0	12.3	11.6	10.9	8.8	9.2	15.4	16.1
Number of eggs rotting	0	23	26	0	4	0	21	0
Number of eggs left unhatched	0	0	53	0	14	0	60	0
Total per cent hatching	100	38	30	100	59	100	25	100

ably above the temperatures to which the eggs normally would have been exposed for most of the period, it is possible that some hatching did take place in nature. A large number of eggs failed to hatch within the experimental period and many of them decomposed (Table 5). The infrequency of hatching for several weeks prior to the last census suggests that they would not have survived the winter. If they were destined to overwinter on the floor of the lake under relatively stable and reduced temperature conditions, the experimental exposure to 20.0° C may well have inhibited normal development. Nonetheless the data imply that in September a change in the egg's response to temperature took place. A genuine physiological difference would lend support to the hypothesis that in nature eggs laid after mid-September were produced by summer-generation Epischura.

The results of the 5° C experiments (Series D, F, H in Table 5) are of immediate interest in that not a single instance of hatching among the estimated 2599 eggs involved was noted for at least five months. In two of the series, hatching started almost simultaneously during the second week of March, 1957, and was explosive. Two weeks later, 97% of the eggs in each flask had hatched; within a month, all had. The results of the final experimental series (H), involving eggs laid toward the end of October, show an even greater arrest in development. A postponement of more than nine months is remarkable, but such discrepancy seemed contradictory and was puzzling at first. The explanation no doubt lies in the treatment received by the three culture flasks. Upon being placed in the refrigerator they were stoppered so as to reduce water loss by evaporation. The

discovery that two of the stoppers were perforated whereas that used in the Series H flask was not, was made toward the end of July. This unintentional procedure evidently resulted in near anaerobic conditions in the latter. When unstoppered a strong H_2S odor was noted, and upon examination the contents were seen to be incompletely decomposed. The bodies of several microcrustaceans were nearly intact and even recognizable. The eggs looked normal. It was decided on July 29 to attempt to induce hatching by aerating and washing the material and by raising the temperature. Egg hatching commenced shortly thereafter, a striking demonstration of the resistance of resting eggs to oxygen depletion and the inherent toxicity of hydrogen sulfide. It lasted less than three weeks. The numbers of discarded shells and retrieved nauplii were in close agreement, which suggests a 100 per cent survival. Very likely the eggs might have been able to withstand these conditions considerably longer. In nature, such adaptation may be of real significance in certain situations.

An experimental temperature of 5° C is similar to that found over much of the bottom of the lake during the winter. In Series D and F (Table 5) the outburst of hatching during March conforms so precisely with the time that larvae first appeared in the plankton in 1957 (previously judged to have been about March 13) that the coincidence must be more than accidental. A rise in temperature preceding the spring thaw, however slight, would seem to be the logical choice as the major factor responsible for stimulating the hatching process. Yet this is not supported by the experimental results. As far

as is known, the temperature in the refrigerator was controlled within narrow limits during the entire period of observation. That no prolonged rise in temperature took place is certain. The data hint at the possibility of an internal regulatory mechanism which could be interpreted as a provision for larval production at a time favorable for survival. In such a case, temperature might be expected to play a significant but perhaps secondary role. A different experimental design than the one used would be necessary to investigate the matter.

Davis (1959b) was unsuccessful in attempting to demonstrate a hatching enzyme among several fresh-water species of copepods. Investigations to date indicate that the inner membrane expands by osmotic inbibition of water, and that the pressure exerted ruptures the outer membrane. Changes prior to normal hatching include an increase in the inner membrane's permeability as well as a steadily increasing osmotic pressure of the fluid within it. Davis suggested that the former was brought about by "chemicals produced by the larva" and that the latter was caused by the excretion or secretion of external metabolites. He further reported that hatching can be arrested by subjecting the eggs to solutions of concentrated sugar.

Seasonal changes in clutch size in E. lacustris were not investigated as such. But the numbers given in Table 5 were typical for the species throughout the period of egg production in Whitmore Lake. The smallest number observed was six, the maximum 28, but a wider range may have existed. The two-month trend indicated in Table 5 may have some general significance (e.g., in relation to food supply). It was observed that large

clutches occurring in frequent bursts were typical of individual animals during October and November.

Eggs laid in nature. - The benthic distribution of resting eggs occurring in bottom samples and a record of their hatching are given in Table 6. Presumably these eggs had overwintered but one season, but the dredge may have exhumed eggs, perhaps from the deeper sediments, which actually had been lying there much longer. The inability to know how many eggs were present in the samples as well as the effects of inevitably rapid temperature changes immediately following collection hinders interpretation. Yet, one aspect of the distribution appears unmistakably clear: viable eggs occurred only in the soft "muck" associated with deep and consequently cold water; from sediments of coarse sand and peat mixed with sand, at depths of nine meters or less, they were absent. Evidently, eggs which were deposited the previous fall in relatively shallow areas had failed to survive. In the absence of reduced fall temperatures many if not most may have hatched before the onset of winter. If so, the larvae must have been subjected to unfavorable conditions, as they clearly did not occur in numbers large enough to make their appearance in the plankton collections.

Resting eggs were present on each sampling date during the six-month period from January to June, 1957. While it is decidedly risky to make use of quantitative comparisons, certain trends in the hatching record of benthic eggs seem to be related to the seasonal occurrence of immature stages in nature. The great majority of eggs kept at 5° hatched in March and April. Relatively few nauplii were retrieved from the June 1

Table 6. - Benthic distribution and hatching record of eggs of Epischura lacustris. Numbers refer to nauplii removed and counted at each inspection of Whitmore Lake mud samples maintained in the laboratory under controlled temperature conditions. Bottom types indicated by M:black mud; SP:mostly sand and peat; CS:coarse sand.

Series	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
Sampling date (1957)	19 Jan			18 Feb		5 Mar							11 Apr				30 Apr		9 May				1 Jun	
Depth in meters	17		9	16.5		2	8	17					16				7.5	16	15				19.5	
Bottom type	M		SP	M		CS	SP	M					M				CS	M	M				M	
Temperature (°C)	5	20		5	20	20		5	20				5	20			20		20				20	
Inspection dates 1957																								
Jan 11-20	0	3	0																					
21-31	5	48	0																					
Feb 1-10	0	29	0																					
11-20	1	76	0	1	0																			
21-28	2	9	0	3	12																			
Mar 1-10	18	1	0	14	11	0	0	27	41															
11-20	22	5	0	42	14	0	0	7	45															
21-31	0	0	0	0	4	0	0	0	33															
Apr 1-10	3	3	0	0	9	0	0	0	76															
11-20	0	0	0	9	3	0	0	0	9				5	26										
21-30	32			53				38	0				30	18										
May 1-10	0			0				6	3	0			1	20			0	10	0					
11-20	0			0				0	0	5			0	20	0		0	20	10					
21-31	-			-				-	2	30			-	7	0		0	7	9					
Jun 1-10	0			0				0	3	9			0	1	3		0	0	11	10			1	
11-20	-			-				-	4	0			-	3	2		0	1	0	21			9	
21-30	0			0				0	9	2			0	7	1		0	1	2	8			2	
Jul 1-10									1	6				5	4			0	6	2			3	
11-20									3	0				0	2			5	3	1			7	

Table 6. - Continued

Series	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
Jul 21-31									0	6				2	0			2	0	0			2	
Aug 1-28																								
29-31											0					0					0			
Sep 1-10											37					0					0			
11-20											0					0					0			
21-30											0					0					0			
Oct																								
Nov																								
Dec																								
1958																								
Jan																								
Feb 1-26												0										0		0
27-28												0										0		0
Mar 1-10												0										1		0
11-20												0										0		0
21-31																								
Totals	83	174	0	122	53	0	0	78	229	58	37	0	36	109	12	0	0	46	41	42	0	1	24	0

sample although it was taken from the very deepest part of the lake. The liberation of nauplii in May, June, and even July (20° series) seems to indicate that an overwintering stock is still potentially available as late as early summer. However, it is possible that these eggs were simply induced to hatch by exposing them to a temperature much higher than they would have encountered in the lake. So it might be supposed that many would have normally failed to hatch. Clearly, a more immediate response was evoked in all 20° experiments.

Subsequent treatment of the original samples, which had been stored as semisolid mud in sealed containers, is of interest in this connection (Series J, K, L, O, P, T, U, V, X, Table 6). By flooding small subsamples with lake water it was usually possible to recover nauplii months after the collection was made. The most striking example of arrested development and a presumably adaptive resistance is demonstrated by Series V, in which an egg taken from the lake floor on May 9, 1957, did not hatch until mid-March, 1958. This must be taken at the very least as a total delay of 16 months from the time of laying.

In the lake, a large number of resting eggs probably do not hatch at all. It seems reasonable from Fig. 3 and the data in Table 6 to set the date for the cessation of spring hatching in 1957 as about June 1 or a little earlier. The decline in hatching of experimental eggs during this period is in general agreement with the absence of young stages from the plankton.

Resistance to adverse conditions does not include those of freezing or drying. No instances of hatching were noted among eggs which were either air-dried or brought to the freezing

point, even briefly. Eggs sink to the bottom soon after being laid, and they may be found in the bottom mud at any time of year. All may be potential resting eggs which are adapted to withstand prolonged periods of reduced temperatures or anaerobic conditions. Those laid in the fall serve to tide the species over the winter.

The seasonal reproductive cycle of Epischura lacustris in Whitmore Lake is very similar to that of Heterocope borealis in the Bodensee (Elster, 1936). All the eggs produced by Heterocope are resting eggs. In the years 1930-1932, egg-laying occurred from August to December but hatching lasted only about half as long, from March to mid-May. Elster estimated that the eggs sank at a rate of six cm per minute, so that they would have had time to reach the bottom (170-250 m) in no more than two days. It was calculated that the mean density during the latent incubation period amounted to over one million eggs per square meter of mud surface, but that 95 per cent subsequently died without hatching. Beginning in May, widespread mortality among the entire larval population was so severe that only about 10 per cent of the individuals lived to maturity.

Long after the natural period of hatching, eggs lying on the bottom were still viable. It was possible to induce all of the living ones to hatch in the laboratory by exposing them first to an elevated temperature (13.5° to 16° C) and subsequently returning them to a temperature close to that (4° C) of the lake's floor. Elevated temperatures alone inhibited hatching. In other experiments, a definite rhythm in the frequency of hatching under controlled temperature conditions was observed.

It coincided with the time of hatching in nature. Elster was unable to relate the period of hatching to a particular environmental stimulus ("external factor"), but thought that the influence of temperature at the bottom of the Bodensee could be ruled out on account of its uniformity.

Heterocope was found to be strongly monocyclic, yet the mean life span was something less than one year. Its superabundant production of eggs, however biologically wasteful, undoubtedly insures survival. Elster considered such overproduction among pelagic zooplankters an extreme case of competition against parthenogenetic reproduction in cladocerans. A similar situation appears to be characteristic of E. lacustris.

Food Relations

Utilization of Algae

Laboratory populations

Ingestion and survival. - Rearing Epischura on algal organisms was only partly successful. While it was not possible to establish a single population which perpetuated itself much beyond one generation, favorable growth and survival were obtained in several cases.

Active, healthy animals regularly ingested all six of the species of phytoplankton to which they were exposed. Despite the artificiality of providing starving copepods with excessive amounts of but one kind of food, the ingestion of such diverse organisms points to automatic filtration as the common means of food acquisition. Under these circumstances, essentially any suspended particulate matter of suitable size might be expected to be eaten. In the laboratory, Epischura readily ingested Euglena, yeast cells, and carmine particles, as well as crustaceans.

Successful growth and survival occurred only in populations supplied with Pediastrum biradiatum, but even in these, mortality was disappointingly high, especially among nauplii and freshly-captured adults. Data derived from censusing algal-fed cultures are reported in Table 7. The cultures were kept in 8-10 liter aquaria and initiated with copepodids of various stages. Only the most reliable estimates of the duration of individual instars are included. They are limited

Table 7. - Survival, growth, and reproduction of individual *Epischura lacustris* in laboratory populations supplied with different species of algae. N = naupllius; C = copepodid. *denotes animal died in stage.

Algal food provided	Estimated number of days in stage: NI-CI	CI	CII	CIII	CIV	CV	CVI	sex	Reproduction
Starved (filtered lake water)			5	1*		2	3*	♂	
						3	4*	♂	
							3*	♂	
						12	7*	♀	
							6*	♀	1 clutch
Ankistrodesmus sp.					2-3	8*			
						20	3*	♀	
Chlamydomonas reinhardi		5	11*						
				2-4	6	3*			
						5	6*	♀	
Chlorella vulgaris			2*						
				3*					
				3-5*					
					2*				
						2	2*	♀	
							4*	♀	
						3	1*	♂	
							5*	♀	
Oocystis sp.				1-2	5-7	7*		♀	
					6	9	2*	♀	
						3	8*	♂	
							9*	♀	1 clutch
Pediastrum biradiatum				4	5	7	58*	♀	4 clutches (a)
	16	3	4	2-3*					
	17-19	3	4	4	4-6	5	23*	♀	2 clutches (b)
				2	3	4-5	14*	♂	(c)
					1-2	3-4	21*	♂	
Scenedesmus quadricauda				3	4	7*			
					2	12	19*	♀	(d)

(a) eggs produced on following days after final adult molt: 14, 28, 42, 50
(b) eggs produced 17 and 21 days following final adult molt
(c) known to have mated twice
(d) gravid at time of death

to only a few animals for each species of alga, but are representative of the overall results obtained in rearing experiments.

The estimated number of days in each stage recorded in Table 7 requires some explanation. Individual copepods were identified as to stage before being placed in aquaria. Periodic observation revealed approximately how many days later the animal either died or molted. This is the first number to appear opposite a particular food for each specimen reported in the table. Nauplii were too small and sometimes too numerous to be adequately censused. Therefore only the total length of time spent in these stages was estimated by noting the day nauplii were first seen, and later, the day Copepodid stage I appeared.

Starved immature animals usually molted once before dying and lived about a week. In contrast were copepodids provided with Pediastrum. For example, one female matured rapidly from stage III and produced a quantity of eggs during the two months of her adult life. Chlorella, on the other hand, must be considered not only the least suitable of the algae tested, but the evidence suggests that it was worse than no food at all. None of the animals exposed to it survived for more than a few days and molting was exceptional. Such a response might be attributed to an antibiotic substance. One produced by this species of Chlorella was found by Ryther (1954) to inhibit feeding and longevity in Daphnia. Epischura cultured on the other algal foods fared somewhat better than those starved.

Several individuals molted more than once and some survived three weeks or more. However, no eggs were produced by copepods which had managed to reach the adult stage in the aquaria. Further, there was a tendency for animals to remain in a given stage longer than ones reared on Pediastrum. In Table 7, this is especially noticeable for Copepodid stages II and V reared on Chlamydomonas, Ankistrodesmus, and Scenedesmus. Under certain conditions, then, these algae were sufficient to sustain the animals for a while but did not allow for more than minimal growth. It should be noted that a few females, while not gravid at the time of collection, did produce eggs after a few days in the aquaria. To attribute this to utilization of the algae available to them is, of course, unjustified.

In Pediastrum-fed populations survival was best in cultures initiated with young copepodids. This was the case with most Epischura which lived for very long after reaching adulthood. The female specimen which lived longest (74 days) is of particular interest. Fourteen days after her final molt she produced the first of a series of at least four clutches of eggs; the second one two weeks later is known to have contained 26 eggs. There was some evidence of yet a fifth clutch being laid during the fifth week. This particular individual apparently mated within the first few days of adulthood, but the exact date isn't known. Only eight eggs were produced in the last clutch, about a week before the animal died. One of her clutches gave rise to nauplii which were allowed to remain in the same aquarium. A few of these subsequently developed into

copepodids and one even managed to reach stage III, the same stage from which its mother was reared. It died in the process of molting. Difficulty in molting was of common occurrence among late nauplii and young copepodids. Sometimes the process lasted several hours and resulted in abnormal development of posterior swimming appendages.

In one other case, a female reared on Pediastrum succeeded in completing the life cycle from the egg through sexual maturity. Although mortality among the nauplii from which she developed was very high, with most larvae dying as nauplii, she lived about 62 days under laboratory conditions. After attaining full growth she was able to produce two clutches of eggs. Such success must be attributed to an efficient utilization of Pediastrum as a food source.

The data in Table 7 further indicate a sexual difference in survival and perhaps growth rate. Males generally did not live as long but may have developed faster. Adult males introduced into the aquaria almost always were short-lived.

Feeding in the young stages. - More frequent censusing of nauplii was possible by maintaining them in small Petri dishes which could be examined efficiently. As a result, more accurate estimates of survival of particular instars were obtained.

All but a very few animals failed to develop beyond stage II. Among those that did only in the case of cultures supplied with Pediastrum was there successful growth. This seemed surprising at first since nauplii might be expected to be unable to ingest such a large form. Yet, as in the case of copepo-

dids, gut examinations revealed that all six algal species offered as food were regularly eaten by nauplii. Further, it was found that Nauplius I does not feed as the anus is not yet developed. Feeding begins in Nauplius II. This explains the high mortality encountered in the second instar. In some specimens small colonies of Pediastrum, measuring about 25 μ in diameter, were observed in the digestive tracts. It should be noted that in Calanus the first feeding stage is Nauplius III (Marshall and Orr, 1956a), but that in Heterocope, as in Epischura, the anus is first apparent in the second nauplius stage (Elster, 1932).

The results served to confirm those obtained in rearing experiments involving mixed larval stages and adults kept in large aquaria. Development from the egg to Copepodid I required a minimum of about 18 days. The duration of each stage is included in a summary in Table 8 and is discussed in a later section. A few individuals managed to grow into young copepodids when fed Pediastrum, but none matured. They often died in the process of molting and appeared smaller than comparable instars taken from the lake. For instance, one Copepodid I measured 0.483 mm in length (mean size of this stage in the natural population was 0.518 mm, Table 1). Chlorella-fed nauplii died sooner than starved ones, sometimes in stage I but usually in stage II. When supplied with one of the other four algal species, growth was variable, but no animals developed beyond Nauplius stage V.

Digestion. - Fecal pellets were retrieved from copepods

which had been actively feeding. Those produced by nauplii were very small, transient, and extremely difficult to recognize. Some tiny, pale ones were collected from late-stage nauplii but were too few in number to obtain evidence for digestion. They contained nothing identifiable.

The number and appearance of fecal pellets produced by adults and other copepodids varied according to the kind of food but not always with the amount of material in the gut. Many animals with apparently full guts failed to yield any at all, which may mean that they had stopped feeding. Consistently large, dark pellets were found associated with copepods which had been feeding on Pediastrum. Usually the hindguts of such animals appeared likewise dark and full. How long food normally was retained in the gut is not known. Not nearly as many pellets were produced with the other algae and they were smaller and paler. In some cases, small, transparent objects resembling "ghost" pellets (cf. Marshall and Orr, 1955), containing nothing identifiable, were observed. Occasionally, a pellet, produced at least several days after the animal had been taken from the lake, contained tiny diatom frustules of a naviculoid type. Evidently, some materials may remain in the digestive tract for a week or more.

Cells and coenobia were usually taken in whole. Those of Chlamydomonas, Ankistrodesmus, and particularly Pediastrum showed definite signs of more or less complete digestion. The cell contents appeared dark brown in some cases, colorless or void in others. But digestion may be only partial, as pellets

were seen to contain apparently unharmed, intact, green cells or coenobia on several occasions. This was most common among active animals with full guts, and was thought to be directly related to the concentration of phytoplankton in the aquaria. Conclusive evidence of digestion of Scenedesmus and Oocystis was not available, in spite of the copepod's apparent utilization. In the case of Chlorella, none of the fecel pellets contained recognizable remains. Only a few were recovered but evidently the copepods had stopped feeding, and presumably the alga was left undigested in the gut.

The suitability of Closterium littorale was not tested by rearing. However, it was discovered in filtering experiments that large and well defined pellets were produced by the copepod after only a brief period of feeding on this desmid. A group are shown in Fig. 4. They were compacted and larger (about 180 X 40 μ) than those produced in response to any of the other algae. They contained a number of almost completely digested cells in addition to some which appeared unharmed. For the most part, the cells were not broken up.

Fryer (personal communication) is of the opinion that cells totally enclosed in cellulose (e.g., Pediastrum) must be virtually undigestible unless ruptured. In fresh-water cyclopoids, Fryer (1957) has reported that "almost any green or blue-green alga such as Scenedesmus or Cosmarium which is swallowed without having its walls ruptured, is almost certain to emerge from the anus undamaged." This is not the case in Epischura and the explanation must be other than pos-



Fig. 4. Fecal pellets produced by an adult female *Eischura* after feeding on *Closterium littorale.*

tulating the presence of a cellulase. It is very possible that the problem involves some basic aspects of nutrition in natural populations and should receive more attention in the future.

Experimental feeding of Calanus has shown that the shape, size, and color of the fecal pellets vary with the kind of food (Marshall and Orr, 1955; Raymont and Gross, 1942). The number produced in nature has been taken as an index of grazing rate by Harvey et al. (1935). Generally, the number was found to increase with an increasing concentration of food cells. In some interesting experiments on the effect of food on egg production, Marshall and Orr (1952) found that Chlorella was taken into the gut in sizeable numbers but apparently passed through intact. Judging from the number of eggs laid, they concluded that the alga was no better than starvation.

On the basis of fecal pellet production and content, then, Pediastrum again must be considered as a more or less satisfactory food for E. lacustris. This is not to say that it is an ideal food in the sense that one should expect to find it being selected by the copepod in nature. The high mortality among animals provided with this species is difficult to interpret in terms of overall suitability. But clearly, a few individuals actually thrived on it; under experimental conditions it was greatly preferred to Ankistrodesmus, Chlamydomonas, Oocystis, Scenedesmus, and above all, Chlorella. The last-named alga appeared to be decidedly harmful, at least in high concentrations.

Growth; duration of instars. - Individual lengths of life of successfully reared Epischura may be compared with estimates derived from sampling the natural population. Differences in temperature as well as in kinds and amounts of food would be expected to influence the growth rate. Yet, if it is assumed that Pediastrum was adequate or better for the most successful of experimental animals, then by adding together the minimum time for each stage certain limits may be placed on the time necessary for one complete generation of the species in nature. In the same way the total life span may be approximated. Table 8 summarizes the duration of individual instars of experimental Epischura, including the time required for egg maturation and the total length of life. Since there was poor survival of males in the laboratory, the observed life span of 59 days may be short of what actually occurs in the lake. On the other hand, judging from the seasonal occurrence of instars, 98 days may represent a good estimate of the length of life of the females.

In Whitmore Lake, the period of larval development of the spring generations in 1956 and 1957, from the time of hatching of overwintering eggs to the adult stage, was estimated as 64 and 55 days, respectively. That of the 1956 summer brood is of more direct interest because of more comparable temperature conditions. This estimate, 38 to 40 days, is in striking agreement with the duration of larval development of experimental Epischura (Table 8). It would appear that conditions in both situations were nearly optimal, and allowed for maximal

growth. If so, it might be supposed that the estimate represents a theoretical limit for the growth rate of the species. In the summer plankton immature stages were present in sufficient numbers to permit the sequence of instars to be followed rather closely. This was at a time of relatively uniform, high temperatures in the epilimnion (Fig. 2), which correspond with experimental temperatures within a few degrees. Perhaps a considerable part of larval development occurred in the epilimnion. The value for the period of development in nature is thought to be reliable; considering that minimal times were used to esti-

Table 8. - Duration of postembryonic developmental stages of Epischura lacustris, estimated by censusing laboratory populations reared on Pediastrum biradiatum, (20° C).

Stage		Duration in days
Egg : laying to hatching		<1
NI		1
NII		3-4
NIII		<3
NIV		3+
NV		3
NVI		4
CI		3
CII		4
CIII		4
CIV		5
CV	♂	4
	♀	6
Total larval development		38-40
CVI	♂ as adult	21*
	♀ to egg maturation	14
	♀ as adult	58*
One complete generation		54
Entire life span	♂	59
	♀	98

* maximum time these stages lived in laboratory

mate the duration of experimental development, perhaps the close agreement should be expected.

In a previous section (p. 55 and Fig. 3), females in the natural population were judged to require about 27 days for egg ripening in the spring and early summer (June 6 to July 3, 1956). But later in the year, it was thought that summer-generation females first reached adulthood by the end of August and began to lay eggs about mid-September. The latter date was arrived at indirectly by suggesting that the sharp cessation of hatching in late summer corresponded to the end of the spring generation's egg production. Further, it was assumed that summer-generation copepods produced only resting eggs. This tentative estimate of about two weeks for egg maturation is supported by the fact that laboratory Epischura produced eggs within 14 days in one case, 17 in another (Tables 7 and 8), following their final molt. The minimal time, then, for the completion of one generation was calculated to be 54 days (Table 8). In the lake, depending on the time of year, it is usually much longer.

Gut content of Epischura in nature

Gut analyses were made before it was discovered that the copepod was able to capture and devour relatively large zooplankters. The presence of plant materials in the digestive tracts was noticeable from merely a cursory look at whole, living animals. The foreguts especially were often distinctly green. Consequently, it was with the intention of identifying the kinds and relative amounts of the algae present that de-

tailed examinations were undertaken.

A large percentage of the guts (42 of 96) contained nothing but unidentifiable mush (Fig. 5). In several other cases, unfortunately, only the most general of identifications were possible. As a result, the categories were established necessarily on an arbitrary basis, which makes it difficult to draw conclusions about algal food preferences in nature, much less to relate the data to results of experimental feeding. For example, a considerable fraction of the vegetable remains were recognizable only as "indeterminate" or "colonial" algae. Perhaps the most important single finding was the unexpected presence of large numbers of copepods. It appeared certain that widespread predation had taken place. This discovery led to further direct observations on the suitability of various zooplankters as food.

Diaptomids greatly predominated. Of the 50 Epischura whose guts contained recognizable material, copepodids were present in 17, and nauplii in 12 (Fig. 5). Nauplii of cyclopoids were found in six guts. Skeletal remains showed that except for really large individuals the prey was usually ingested whole. It was surprising to find as many as four intact bodies of sub-adult Diaptomus crammed into one digestive tract along with skeletal fragments of perhaps several others. Most of the material listed as miscellaneous Crustacea consisted of the mandibles, other mouthparts, furcal rami, and similar chitinized parts of what was taken to be largely diaptomid prey. Thin exuviae of nauplii were very delicate and

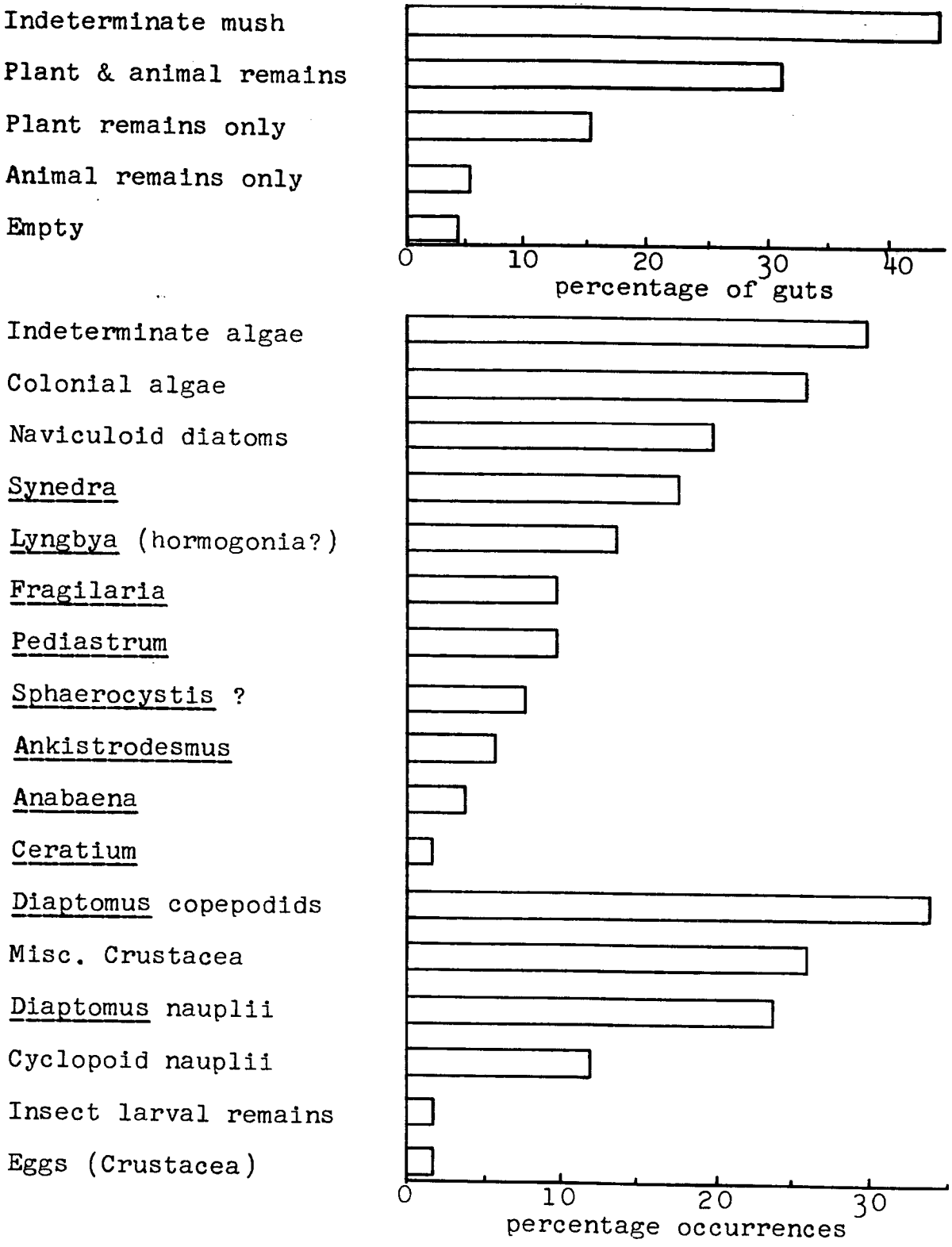


Fig. 5. - Gut composition of adult and Copepodid stage V Epischura lacustris shortly after collection from Whitmore Lake; above, general; below, itemized.

difficult to find, so undoubtedly some were missed. Numerically, then, it is probable that nauplii greatly prevailed over later stages, since they would not be expected to persist in the gut as recognizable remains as long. Evidently, large copepods were mutilated by the heavy mandibles prior to ingestion.

The absence of cladocerans, rotifers, and cyclopoid copepodids from the diet of Epischura is conspicuous. While it is possible that a few small daphnids and loricate rotifers could have been overlooked, the explanation must lie elsewhere. It should be noted, however, that in August and September only Keratella and Kellicottia were at all common, but both were greatly outnumbered by the crustacean plankton. Large cyclopoids and cladocerans apparently either were capable of avoiding capture or were unacceptable.

Unidentifiable contents recorded as indeterminate mush were mostly from the foreguts and varied in color from green to brown. Animal remains typically were associated with the latter color. The bulk of the unidentifiable phytoplankton was thought to have consisted mostly of colonial forms. Most of the cells, even when imbedded in a gelatinous matrix, were highly compacted, often filling much of the gut. Some colonies were tentatively identified as Sphaerocystis; others resembled Dictyosphaerium, Coelosphaerium, and Pandorina.

The itemized list of algal remains shows that naviculoid diatoms and Synedra occurred in 20 and 18 per cent of the guts, respectively. The former were mostly very small forms, but neither was abundant numerically. It is just possible that

they had been derived from the digestive tracts of prey. Fryer (1957) has shown that the guts of carnivorous cyclopoids after feeding on herbivorous prey often give the impression that the copepod itself had eaten the algae. Most other algal components in the gut of Epischura were negligible. Several were recorded on the basis of finding a single specimen. For example, Pediastrum occurred in five guts but less than twice that number of colonies were seen. While this species was at no time very common in the plankton, there is insufficient evidence to suggest that it was a preferable item of food in nature. Some infrequently-occurring algae (Anabaena, Ceratium) may have been eaten accidentally. They may even represent unsuitable foods if it is assumed that the ability to recognize them at all is indicative of resistance to digestion.

Smears of crushed fecal pellets revealed some interesting items. The pellets were retrieved from vessels in which Epischura had been kept for a day or two following capture. Most noticeable were chitinized remains. Even a few transparent fragments of nauplius exuviae were found. Algal components that were readily identifiable included small naviculoid diatoms, intact cells of Chlorella, and two trichomes of Lyngbya. The diatom frustules were unbroken, but judging from their translucent to pale brown appearance, their contents had been completely digested. On the other hand, the short Lyngbya filaments, perhaps hormogonia, appeared virtually untouched. Scattered cells of Chlorella also apparently passed through

the gut unharmed.

Gut analyses established that Epischura lacustris is an omnivorous although not an indiscriminate feeder. Diaptomid copepods were preferred on the one hand and probably colonial algae on the other; only larger organisms were broken up before being swallowed. It appears that copepods form a considerable and perhaps essential part of the diet of adult and sub-adult animals. Pediastrum was eaten but may not be a preferable food item. Some inspections of the digestive tracts of preserved Epischura from Lake Winnebago, Wisconsin, in which Pediastrum was very abundant in the plankton on the date of collection (May 21, 1958), gave evidence that the alga was only occasionally ingested. Of 15 specimens examined, the alga was found in three, and altogether but seven colonies were seen.

Considering the significance of the itemized composition, it appears that Gurney's (1931) pessimistic view about the value of gut examination is not without foundation.

Filtering rate

The results of 93 individual filtering rate experiments are reported in Table 9. Nine experiments involved stage V copepodids supplied with five of the species of phytoplankton, but only one determination was made for stage IV (feeding on Closterium). Of the 1,073 copepods used in this work, 62, or just under six per cent, died during the experiments, in spite of precautions taken to insure that only healthy animals were selected. In only one case was there any evidence that death was caused by injury due to predation so feeding was

Table 9. - Results of filtering rate experiments on Epischura lacustris, listed according to the phytoplankton species supplied. Adult animals unless otherwise noted. Numbers in parentheses refer to immature animals which molted during an experiment.

NUMBER Experimental series and temperature		PHYTOPLANKTON species age (days)	PHYTOPLANKTON concentration (cells or colonies per ml)	DURATION of test (hrs)	ANIMALS number, sex and stage	ANIMALS mortality	VOLUME filtered (ml per copepod per day)
I	21.0°C	Ankistrodesmus sp.					
	4	9	2,800	12	5 ♂	0	3.36
	5	9	2,800	12	5 ♀	0	1.23
	6	9	2,800	12	5 ♂	0	0.82
	10	7	70,000	24	21 ♂	1	0.34
	11	7	70,000	24	20 ♀	0	0.32
II	20.2°C						
	3	14	135,000	24	21 ♀	3	0.13
	7	6	140,000	24	20 ♀	2	1.27
	9	6	140,000	24	16 ♀	0	1.08
II	20.2°C	Chlamydomonas reinhardi					
	4	9	3,500	24	20 ♀	4	1.65
	8	9	3,500	24	18 ♂	6	1.60
III	19.5°C						
	10	7	1,850	24	17 ♂	5	0.72
	11	7	1,850	24	12 ♀	2	2.42

Table 9. - Continued

IV	18.7°C	*Chlamydomonas reinhardi*					
	2	9	4,000	20	9 ♂	1	6.04
	9	9	4,000	12	10 ♀	0	-0.04
VIII	20.0°C						
	6	17	15,000	20	10 ♀	1	4.34
XII	21.1°C						
	8	8	60,000	16	10 ♀	0	4.99
	9	8	60,000	16	10 ♂	0	1.79
	10	8	60,000	16	12 ♀	0	5.51
II	20.2°C	*Chlorella vulgaris*					
	2	9	200,000	24	20 ♀	4	0.87
	6	9	200,000	24	19 ♂	4	0.78
III	19.5°C						
	7	7	16,000	24	20 ♂	3	0.32
	8	7	16,000	24	10 ♀	3	0.62
IX	20.5°C						
	1	13	350,000	14	10 ♀	0	-1.05
	6	13	85,000	14	10 ♀	0	-2.96
	9	13	175,000	14	10 ♂	1	-0.39
	10	13	175,000	14	10 ♀	1	3.78
	11	13	175,000	14	10 ♀	1	0.30

Table 9. - Continued

XII	21.1°C	Chlorella vulgaris					
	2	8	85,000	16	10 ♀	2	-0.84
	3	8	85,000	16	17 ♀ CV(2)	0	1.62
	4	8	85,000	16	9 ♀	1	0.35
VII	20.7°C	Closterium littorale					
	1	8	1,700	16	10 ♀	1*	7.19
	2	8	1,700	16	10 ♂	0	6.86
	4	8	850	16	9 ♀	0	7.09
	5	8	850	16	10 ♂	0	7.98
VIII	20.0°C						
	1	10	900	16	10 ♀	1	4.21
	2	10	900	16	18 ♀ CV(6)	0	4.41
	3	13	950	16	10 ♀	0	11.44
	4	13	950	16	10 ♂	0	6.90
	5	13	950	16	11 ♀♂ CIV(4)	1	1.70
XI	21.0°C						
	2	7	1,200	16	9 ♀	1	5.42
	3	7	1,200	16	17 ♀ CV(4)	0	7.08
	4	7	1,200	16	10 ♀	0	6.51
	6	17	1,800	16	9 ♀	0	6.38
	7	17	1,800	16	11 ♂	0	5.34
	8	17	1,800	16	10 ♀	0	8.97
	10	17	12,000	16	10 ♀	0	7.90
X	21.0°C	Navicula pelliculosa					
	1	6	9,000	16	10 ♀	0	4.37

* accidentally crushed

Table 9. - Continued

	2	6	9,000	16	10 ♂ CV(6)	0	3.67
	3	6	9,000	16	17 ♀ CV(2)	0	3.16
	5	9	18,000	16	10 ♀	0	2.71
	6	9	18,000	16	11 ♀	1	2.26
	7	9	18,000	16	10 ♂	0	3.39
	8	9	18,000	16	18 ♀ CV	0	3.42
	9	9	18,000	16	10 ♀	1*	3.68
	11	9	163,000	16	10 ♀	1	1.71
IV	18.7°C	Nitzschia closterium f. minutissima					
	1	9	2,600	20	18 ♀	2	0.07
	4	9	2,600	20	5 ♂	1	0.34
	6	9	2,800	12	19 ♀	0	-0.15
	7	9	2,800	12	10 ♂	2	0.29
VIII	20.0°C						
	11	5	11,000	20	10 ♀	1	2.78
XII	21.1°C						
	5	12	98,000	16	10 ♀	0	2.05
I	21.0°C	Oocystis sp.					
	1	7	45,000	24	18 ♀ gravid	0	0.45
III	19.5°C						
	4	7	7,000	24	20 ♂	0	0.78
	5	7	7,000	24	20 ♂♀	2	0.67

* accidentally crushed

Table 9. - Continued

V	18.6°C	*Oocystis sp.*					
	1	2	1,200	20	8 ♀ CV	0	0.10
	5	2	1,200	20	8 ♀♂	0	0.82
	6	2	1,200	20	8 ♂	0	-0.25
	7	2	1,200	12	8 ♀	0	2.35
	9	2	1,200	12	8 ♂	0	4.96
VI	19.6°C						
	1	4	10,000	10	10 ♀	0	1.88
	2	4	10,000	10	10 ♂	0	3.95
	4	9	3,500	10	8 ♀	0	2.58
	5	9	3,500	10	10 ♂	1	1.56
	7	11	1,500	10	10 ♀	1	1.51
	8	11	1,500	10	10 ♂	0	2.20
VII	20.7°C	*Pediastrum biradiatum*					
	7	8	2,000	16	10 ♀	0	7.07
	8	8	2,000	16	10 ♂	0	5.80
	10	8	980	16	10 ♀	1*	7.64
	11	8	980	16	10 ♂	0	9.15
XIII	20.0°C						
	2	13	600	16	10 ♀	0	6.32
	3	13	600	16	10 ♂	0	4.76
	5	16	8,500	16	10 ♀	0	3.34
	6	16	8,500	16	10 ♂	0	2.89
	8	23	54,000	16	10 ♀	0	6.23
	9	23	54,000	16	10 ♂	2	1.83

* accidentally crushed

Table 9. - Continued

III	19.5°C	Scenedesmus quadricauda					
	1	8	8,500	12	10 ♂	0	0.93
	2	8	8,500	12	10 ♀	0	1.86
V	18.6°C						
	3	8	700	20	8 ♀ CV	1*	0.45
	4	8	700	12	6 ♀	0	1.02
	8	8	700	12	7 ♂	0	0.10
VI	19.6°C						
	10	19	33,000	10	10 ♂	0	4.11
	11	19	33,000	10	10 ♀	0	5.94
IX	20.5°C						
	7	12	3,000	20	8 ♀ CV	0	2.08

* injured

probably not completely normal. Nonetheless, consistently significant filtering rates were obtained with some of the algae tested.

One of the most obvious features of the calculated rates, expressed as volumes of water swept clear of particulate food by a copepod in 24 hours, was the great amount of variation between different experiments under presumable identical conditions. As mentioned previously, large individual variations are usual in studies of filtering rate in calanoid copepods. For example, even in radioactive tracer experiments on single specimens of Calanus, unaccountable deviations sometimes amounted to upwards of two orders of magnitude (Marshall and Orr, 1955a, 1956). Some of the conditions of individual experiments are indicated in Table 9, but an analysis of their effects on feeding was outside of the scope of this study. Among the factors undoubtedly influencing the measurements, errors introduced by sampling and counting may be largely compensated for by averaging the results. On the other hand, since the effects of such things as the health of the animals, their confinement, substances produced by senescent algae, and so on, must be considered of an inhibitory nature, it could be argued that maximal values would be the most realistic.

It will be noted than in seven instances (Table 9), computations resulted in negative values. As meaningless as they may appear to be, they serve to emphasize a further source of error which is inherent in the method. The filtering equation

is based on the assumption that differences between control and test-bottle concentrations are solely attributable to feeding. Yet, within the duration of the experimental periods used, significant differences inevitably would be expected as a result of differential growth of the algae, at least in some rapidly reproducing species, even in the absence of feeding. Consequently, assuming for the moment that feeding is negligible, there is every reason to suppose that growth in an experimental bottle would occasionally exceed that in its control bottle, and a negative rate would result. Whether the presence of copepod waste products further complicates the problem by affecting algal growth isn't known. Conover (1956) found that excretory wastes of Acartia had no marked or regular effect on the growth rate of the diatom Skeletonema costatum, which was used as food. The problem hasn't been investigated by other workers. But if the findings of Conover may be accepted in the present study, it seems fair to conclude that discrepant F values caused by differential algal growth are compensatory, so that in a given group of experiments, low or negative rates are offset by correspondingly high ones.

Accordingly, it appeared that the most reliable estimates of food suitability as determined by filtering rates would be obtained by averaging the results of individual experiments for a given food. Ranges and means for adult Epischura together with the number of experiments for each food species are given in Table 10. On the average, the highest rates were obtained with Closterium and Pediastrum, the lowest with

Table 10. - Summary of results of filtering rate experiments on adult Epischura lacustris: means and extreme ranges for each phytoplankton species tested.

Species	Number of experiments	Calculated F (ml filtered per copepod in 24 hours)	
		Range	Mean
Ankistrodesmus sp.	8	0.13 to 3.36	1.07
Chlamydomonas reinhardi	10	-0.04 to 6.04	2.90
Chlorella vulgaris	11	-2.96 to 3.78	0.16
Closterium littorale	13	4.21 to 11.44	7.09
Navicula pelliculosa	6	1.71 to 4.37	3.02
Nitzschia closterium f. minutissima	6	-0.15 to 2.78	0.90
Oocystis sp.	13	-0.25 to 4.96	1.96
Pediastrum biradiatum	10	1.83 to 9.15	5.50
Scenedesmus quadricauda	6	0.10 to 5.94	2.33

Chlorella and Nitzschia. Consumption of Navicula, Chlamydomonas, and Scenedesmus indicated that they were distinctly inferior to Closterium but somewhat more suitable than either Oocystis or Ankistrodesmus.

The validity of correlating food preferences with mortality is questionable, but it is of interest to note that among the three species of algae most readily eaten total death rate amounted to little more than one-half of one per cent. But in experiments with Chlorella, Chlamydomonas, and Nitzschia, nine per cent of the animals died. Some Epischura may have actually starved to death, but the possibility of noxious substances being produced by Chlorella is supported by a rather high mortality of 13 per cent.

Guts of animals feeding on Navicula were usually well packed and dark, with fecal pellets in evidence. Not only did few copepods die but they almost always appeared active and healthy at the end of the experiments. Feeding rates with this species were the least variable of any of the algae tested. The two lowest rates occurred in experiments in which a copepod died. Yet the relative success with Navicula was rather surprising considering its small size and the low rate at which Nitzschia was eaten (a diatom somewhat larger than Navicula). One plausible explanation for the discrepancy was disclosed in the process of making cell counts. In the case of Navicula, but not of Nitzschia, many cells were found to be agglutinated to such a degree that in order to get statistically reliable counts, it was necessary to shake the contents of each bottle

with a small amount of detergent. It is entirely possible that clumps and strings of such cells formed an object of filterable size which could have been readily ingested by Epischura, but that single cells ordinarily passed through the copepod's maxillary filters. Presumably, then, Nitzschia was in most cases virtually unavailable to the animal. This explanation is supported by the fact that in lower concentrations feeding was negligible (Series IV, Table 9), but in rich concentrations moderately high rates were encountered.

Marshall and Orr (1955a) reported a comparable situation in feeding experiments in Calanus. When offered a mixture of the small flagellate Dicrateria and a larger diatom, the copepod was found to ingest both in large numbers, but was unable to feed on the flagellate alone. The explanation was given that Dicrateria was too small to be efficiently removed by itself, but in the mixture became entangled in the mucus exuded by the diatom.

The range of food concentrations used in the entire series extended well above those normally occurring in nature. It has been fairly well established that the volumes filtered by calanoids are usually depressed in extremely rich concentrations (Marshall and Orr, 1955a; Conover, 1956). Both mechanical inhibition and satiation have been offered as possible reasons. However, in Calanus supplied with μ-flagellates (size below 10 μ) which are too small to be effectively retained, the volume filtered has often been greatest in the highest concentrations rather than in the lowest. The

suggestion was made by Marshall and Orr that a gradual clogging of the filtering setae would show up in just such a way, and they concluded that even in their highest natural concentrations, smaller nannoplankton species could not serve as an important source of food. In the present study, it is reasonable to suppose that some algae, other than Nitzschia, which in lower concentrations would have been unfilterable, were frequently eaten by Epischura.

The results of most experiments indicate that crowding and long confinement may have inhibited active feeding. Usually, the highest rates were obtained when each animal was allowed a volume of at least ten ml and the experiments were terminated in 16 hours or less. Experimental Calanus have been found by Marshall and Orr (1955a) to filter more effectively when the volume was increased from 20 ml per copepod to twice that amount. Gauld (1951) kept individual Calanus in 100 ml beakers, which may be one reason why his estimates of the volume filtered are uncommonly high. Conover (1956), however, provided for less than two ml per animal in experimentally-fed Acartia, yet had no reason to believe that feeding had been suppressed. It should be noted that Epischura lacustris is appreciably smaller than Calanus (which attains a length of 3.4 mm or more) but larger than the species of Acartia with which Conover worked (1.2 mm). Apparently, the question of crowding has been a matter of trial and error.

The data show no obvious relation between filtering rate and either food concentration (except as noted for Nitzschia)

or the age of the algal cultures. Three of the species (Chlorella vulgaris, Navicula pelliculosa, and Scenedesmus quadricauda), are those with which Ryther (1954) demonstrated inhibitory effects of senescent cultures on the grazing rate of Daphnia magna. In view of the several variables and sources of error in the present work, the absence of a trend among the relatively few observations for any one species is not surprising.

The maximum rates (with Closterium and Pediastrum) may actually represent a reliable index of the filtering potential of Epischura in nature. Cultures of these two species certainly were less subject to experimental error arising from differential growth because their reproductive rates were ever so much lower than those of the other algae. It was found, for example, that changes in population density of resuspensions of both Closterium and Pediastrum over a 16-hour experimental period usually were hardly distinguishable from regular variations in counting. A volume of about 10 to 12 ml per copepod per day, then, may be taken as the upper limit of the amount of water from which adult Epischura is able to remove suspended matter in its natural habitat. For comparative purposes, filtration values of different instars of Calanus are given below (Table 11). The data are taken from Marshall and Orr (1956a) and arranged according to mean size of the animals. Despite differences in methodology as well as in the temperatures used, the agreement for instars of comparable size is good, especially for maximum values. Unfortunately, the value for stage IV Epischura is based on only one measurement, and

since an animal died in that experiment, probably it should be considered an underestimate. Other data for immature Calanus are available in the literature, but considering the various techniques employed by different workers, the values given in the table are probably among the most reliable. For a given size, then, Epischura is able to filter about the same amount

Table 11. - Comparative filtering rates: Calanus finmarchicus feeding on Syracosphaera elongata at 14.5° C, (from Marshall and Orr, 1956a), and Epischura lacustris feeding on Closterium littorale at 20° C.

Stage		Size (length in mm)	Volumes filtered (ml per copepod per day) Maximum	Mean
Calanus	CIII	1.65	9.21	4.45
Epischura	CVI	1.80	11.44	7.09
Calanus	CII	1.25	6.45	2.95
Epischura	CV	1.47	7.08	5.74
Calanus	CI	0.94	2.81	2.12
Epischura	CIV	1.06	1.70	1.70*

* one experiment only

of water as Calanus. No information on respiration for these stages in either species is available so that feeding rate data cannot be compared with food requirements. However, as in adult Calanus, it appears likely that a gap exists between the amount of phytoplankton food normally present in nature and the metabolic needs of the animals. Acartia is much more efficient in filtration. Conover (1956) estimated the rate at 20° C to be

about 20 ml per copepod per day for the adult. Yet it is of a size about comparable to Calanus stage II (1.2 mm).

In summary, data on filtering rate substantiate the results of laboratory rearing. Large algal organisms are preferred, and perhaps only in concentrations in which cells become agglutinated can organisms smaller than about 15 μ be effectively retained. Pediastrum and Closterium were readily digested without the rupturing of their cell walls, and the basis of their suitability must be partly a matter of size. Nitzschia as well as Chlorella appear to be too small. Some forms undoubtedly resisted digestion to some extent, and the production of substances which inhibit feeding was again indicated. It is suggested that the relative unsuitability of Ankistrodesmus and Scenedesmus may be partly due to an undesirable shape.

Predatory Activities

Preferences

Some microcrustaceans were readily devoured by Epischura whereas others were definitely unacceptable (Table 12). Direct observations were made by confining groups in depressions of 2 ml capacity into which single Epischura, usually stage V and VI, were introduced. Calanoid nauplii, including its own, Artemia larvae, and to some extent younger stages of cyclopoid copepods were the most preferred prey. Ingestion of larger copepods was less frequent and appeared to be related to size and the ability to escape capture. Cyclopoids were usually

Table 12. - Predation by *Epischura lacustris* as determined by direct observation. Eaten in large numbers (+++), occasionally (++), rarely (+); uneaten but sometimes mutilated (-); untouched (--).

Species	Approximate Size (length in mm)	Individual *Epischura*			
		Adult	CV	CIV	CIII
Artemia salina nauplius I, II	0.4 - 0.6	+++	+++	++	+
Diaptomus minutus - adults	1.0	+	-	--	
Diaptomus sp. copepodids	0.5 - 0.8	++	++	+	--
nauplii	0.1 - 0.2	+++	+++	+++	+++
Epischura lacustris CV - CVI	1.4 - 1.8	--	--		
CIII	0.8	-	--		
CI - CII	0.5	++	+	--	
nauplii	0.1 - 0.3	+++	+++	+++	+++
Mesocyclops edax adults	1.2 - 1.5	--	--		
Cyclopoida copepodids	0.6 - 1.0	+	--	--	
nauplii	0.1 - 0.3	++	++	++	
Daphnia sp. adults	1.0	--	--		
young	0.2 - 0.5	--	--		
Diaphanosoma brachyurum	1.0	--	--		
Bosmina coregoni	0.5	--	--		

successful in avoiding contact by their sudden and even violent jumping movements.

In contrast, there was no evidence that cladocerans, regardless of size, were ever harmed, much less eaten. Apparently they were unsuitable and were simply rejected. The possibility that they were difficult to seize or were otherwise protected by the rigidity and shape of the carapace also may have been of importance.

Most prey were taken in whole. A few instances were noted in which a copepod's body was cut nearly in two through the thorax, and sometimes immature Epischura, after seizing and mangling their victims, did not eat them. But usually even dismembered animals were consumed as readily as whole living ones. Their being ingested whole was really remarkable considering that many of the preferred prey were a third or more the size of Epischura. No less surprising were the numbers eaten. On one occasion, an adult female consumed 12 diaptomid nauplii, five Epischura nauplii, and three small cyclopoid copepodids in about four hours. In another case, during a three-hour period, eight Artemia were consumed by a single individual. The average daily number of brine shrimp consumed during a one-month period ranged from 3.2 to 5.6 for individually-reared copepods. Feeding was typically restricted to a few hours immediately following the daily introduction of fresh food and media. Consequently the animals must have been satiated most of the time. Apparently the guts were first crammed full and further feeding awaited digestion and clearance. Fecal pellets produced

from brine shrimp digestion were large, soft, and easily broken. But there was no indication that digestion in well-fed animals was less complete than in starved ones.

The characteristic manner of capturing prey was carefully noted. No chase appeared to have been involved. Small nauplii generally were swept into the mandibular region by the filtering currents, but otherwise contact was fortuitous and depended on strictly arbitrary movements. Moreover, response by Epischura was only momentary, limited to the brief period of physical contact of a prey with its appendages, unless capture was successful. Victims were firmly held by the mouthparts and swimming legs, and sometimes were carried about for several minutes. After seizure there were irregular but rapid motions of maxillae and mandibles. Despite the close confinement, however, perhaps only about ten per cent of the collisions actually ended in capture. In nature, the incidence must be much less.

The fundamental similarity in feeding habits of Epischura lacustris and European species of Heterocope, provides another example of the striking parallelism of these forms. Elster (1936) investigated food preferences of H. borealis by direct observation and detailed gut analyses. In several small alpine lakes, Bossone and Tonolli (1954) and Burckhardt (1944) demonstrated the dependence of populations of H. saliens on those of other members of the zooplankton community, especially diaptomids and rotifers. These studies have confirmed some earlier observations by Naumann (1923).

Elster reported widespread predation and cannibalism, although phytoplankton was regularly eaten. Nauplii were believed to have fed largely on small algae, but certainly a part of their diet included Asterionella, Fragilaria, Synedra, and Cyclotella, as well as diaptomids (presumably young larvae)! Cyclotella was thought to have been especially suitable as there was indirect evidence that it was selected. In adult guts, recognizable remains ranged from pennate diatoms to nearly intact bodies of adult copepods. But in all stages examined, the ingested food was often broken up and sometimes even "pulverized". Later stages became progressively more predaceous, feeding extensively on nauplii and other copepodids, but frequently taking adult Diaptomus and Cyclops, in addition to rotifers (species not indicated). Cladoceran remains were noticeably scarce and consisted solely of filtering combs and skeletal fragments. While unquestionably omnivorous, late instars were thought to have relied mainly on predation for survival.

Heterocope can devour larger organisms relative to its own size than can Epischura. In a few instances, Elster watched the capture of adult individuals by other adults as well as by Stage V animals. In the laboratory, reciprocal responses were limited to distances of less than one mm, both escape and capture being attributable to "chance and arbitrary reaction".

Elster compared the feeding apparatus of Heterocope with that of Diaptomus gracilis. In the former, while the basic calanoid filtering function is retained, a slower and intermittent rhythm of the mouthparts was noted. On the basis of

observations using quince jelly, soot, and carmine, Elster believed that much less water passed through the maxillary filters. Filtration was not measured. In D. gracilis filtration by the second maxilla, according to Elster, accounts for all the food ingested, but in Heterocope, the appendage apparently serves two purposes. It seems to be modified to allow for both greater mobility and the passage of prey toward the mouth. Its "powerful, dagger-like, distal setae" were considered to function in "actively seizing and holding prey".

Artemia as food

The majority of the Epischura reared on Artemia thrived on them. Despite a rather heavy and customary initial mortality within the first week, 12 of the original 30 animals were still active after 32 days, at which time culturing was discontinued. All but three metamorphosed to stage VI in an average time of less than four days. Eggs were produced by 13 of the 19 females, all virgins, and were distributed among an estimated 32 separate clutches. The number of eggs was not counted as many of them were eaten before they could be retrieved. Some are shown in Plate 1, Fig. c. At least three Epischura produced as many as four clutches apiece.

It seems certain that most deaths must have resulted from something other than inadequate food. More likely, it was a general failure to adjust to conditions imposed by confinement following an initial shock of capture. Very few copepods failed to eat at all, but some died in the process of molting. Occasionally several cultures became heavily contaminated with

bacteria, despite daily changes of water, and this may have been detrimental. Further, many Epischura became gradually encrusted with debris arising from the fragmented bodies of their prey. Swimming was impeded but whether the animals were seriously affected in other ways isn't known.

The results demonstrate at the very least that the food requirements of Epischura may be completely satisfied by a diet of crustaceans. Prey very much larger than Artemia are probably only rarely consumed in nature, but a dependence on immature copepods is strongly indicated.

The Filtering Appendage

The second maxilla was examined in detail in an attempt to associate structure with observed feeding behavior. In this appendage (in early terminology, the first maxilliped), the distances between adjacent setules might be expected to determine the lower size limit of filterable particles. On the assumption that feeding in calanoids is essentially automatic, they have been taken as an estimate of the size of the foods which are most effectively retained (review by Jørgensen, 1955). However, algal organisms which have been shown experimentally to be important as food cover a rather wide range in size, and a close relationship between particle size and food preferences has yet to be established. Moreover, following Elster, it might be supposed that the second maxilla in some calanoids is to a large extent adapted for seizing prey.

According to Gurney (1931) and Marshall and Orr (1956a),

the appendage is formed of a coxa bearing two endites, a basipod also with a pair of endites, and a five-segmented, distal endopod, in which the first two segments project as endites. The endites in turn bear a series of long plumose setae. In Calanus, there are a total of 29 setae of which 20 form a broad sheet in one plane. In Heterocope and Epischura (Fig. 6) altogether there are only 20 or 21 setae, respectively. But six (in Heterocope) or seven are so reduced that they must be of little or no importance in filtration. The remaining 14 setae form the major filtering surface, and in E. lacustris usually bear either two or three rows of setules, as shown. In the two most proximal setae, the setules are irregularly spaced and project in all directions. The entire appendage is much simpler than in Calanus, as well as in at least several species of Diaptomus, in regard to number, arrangement, and relative length of the setae and setules.

Over most of the filtering area, it can be seen that the distance between setules lies between 3 and 12 μ, not counting the fine feathering on the tips of many of the setae. In the great majority of cases it is more uniform, ranging from about 6 to 10 μ. The sizes of the algae used in filtering experiments are also shown in Fig. 6. That the filtering surface should be expected to adequately retain all but the three smallest species seems clear. Average-sized Oocystis and Chlamydomonas theoretically could pass only rarely. On the other hand, both diatoms are of a size that only if oriented with their long axes in the direction of water flow, would

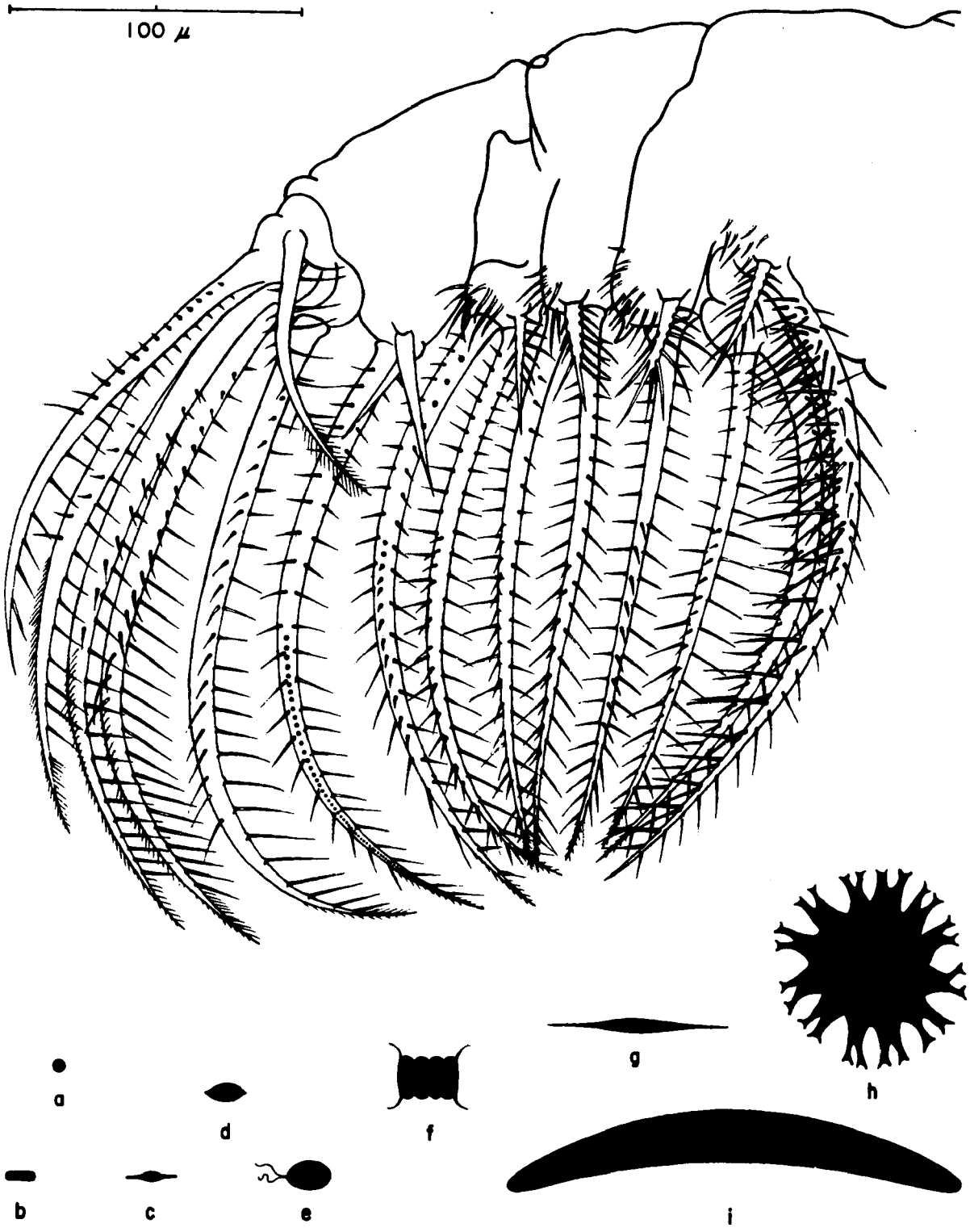


Fig. 6. Left second maxilla of *Epischura lacustris* adult female viewed from the right. Approximate mean sizes of algal organisms used in feeding experiments represented by (a), *Chlorella vulgaris*; (b), *Navicula pelliculosa*; (c), *Nitzschia closterium f. minutissima*; (d), *Oöcystis sp.*; (e) *Chlamydomonas reinhardi*; (f), *Scenedesmus quadricauda*; (g), *Ankistrodesmus sp.*; (h), *Pediastrum biradiatum*; (i), *Closterium littorale*.

they be expected to escape the filters altogether. Jørgensen believed that in Harvey's (1937) experiments, the failure of Calanus to feed on Nitzschia closterium f. minutissima (subsequently reidentified as Phaeodactylum tricornutum) could be attributed to the same thing. It might be supposed that an occasional cell of Ankistrodesmus similarly could be transported lengthwise through the filtering apertures, but this must have been unusual. It seems equally clear, then, that particle size cannot adequately account for differences in filtering rate, and that the sizes of the algae tested with Epischura correspond only roughly with food preferences.

Marshall and Orr (1956a) measured the minimum distance between setules in all stages of Calanus, and found that it varied from 2 to 11 μ, but showed little variation with stage. Yet the size of the organisms which were effectively filtered was nearly always greater than 10 μ, except in very rich concentrations when smaller cells gradually clogged the filtering setae. Therefore it was not possible to demonstrate a distinct correlation between aperture size and the size of the food. They further concluded that nauplii feed on much the same food as the adult except for really large cells, which is at variance with the more popular concept that immature stages require smaller food organisms (Jørgensen, 1955). It substantiates the observations of Storch (1928), however, and seems to be supported by observations of Heterocope as well.

It may be unrealistic to expect that the distances between setules should be closely related to the size of the

food. In nature, an accumulation of suspended detritus together with only a few large algal cells would probably form a fine meshwork retaining much smaller particles than could otherwise be filtered. The importance of the relation between palatability and the shape and consistency of foods as well as the significance of adsorption of very small particles remain to be investigated.

The function of the seven reduced setae shown in Fig. 6 is not known. Those borne on the endites of the coxa and basipod possess a double row of rather heavy setules and the endites themselves bear many projecting bristles of about the same thickness. In Epischura, the proximal endite of the endopod has a pair of small naked setae which appear as sharply pointed spines. In Heterocope, they are represented by a single feathered seta. Lastly, a reflected seta, about twice as long as the others and sparsely feathered, occurs at the tip of the appendage.

Of more immediate interest perhaps are the six long curved setae of the endopod, which were thought to be used by Heterocope to grasp prey (Elster, 1936). In E. lacustris they are certainly among the longest and stoutest of the setae, and may even be strong enough to seize active zooplankters. These distal setae in E. massachusettsensis are somewhat more robust relative to the others (Humes, 1954, Fig. 9). In Heterocope borealis, their development is even more pronounced (Elster, 1932, Fig. 77). Some have a diameter nearly twice that of the more proximal ones, and they do appear not unlike a series

of curved, powerful daggers.

The morphological similarity of the second maxilla in the three species attests to a close generic affinity (cf. Marsh, 1933), since the structure of this appendage is quite different in other members of the Family Temoridae (e.g., Eurytemora velox, Gurney, 1931, Fig. 279). But the specific variation in setation further suggests a direct association with the degree of predatory feeding.

DISCUSSION

Fresh-water calanoids spend a large part of their life span in an immature condition. It follows that the ability to distinguish the larvae of various species occurring together in the plankton must be considered indispensable to the value and accuracy of any serious study of their ecology. The relatively few published accounts of copepod larval stages indicate that further study will provide information about systematic relationships which are obscured in the adult (see Gurney, 1931, pp. 22 ff.).

It is well recognized that familiarity with the appearance and behavior of live material can greatly simplify the task of identifying many fresh-water organisms (Edmondson, 1959). In the case of Epischura lacustris, living nauplii were readily distinguishable on the basis of the position of the first antennae, a feature which, incidentally, may have additional taxonomic interest to the systematist. Yet upon death or preservation, it ceases to be of diagnostic value.

In Whitmore Lake the free-swimming stages of E. lacustris are found only from March to November, or roughly that part of the year during which the lake is ice-free. The descriptions presented in the preceding section make it possible to differentiate its immature stages from those of most other species with which it is known to occur. Like Heterocope, the ability of E. lacustris to produce large numbers of resting eggs adapts

it to withstand winter conditions. The length of the incubation period varies seasonally. Experimental evidence indicates that normally eggs laid by the spring generation hatch within a day or two, whereas the fall generation produces only resting eggs which lie dormant on the floor of the lake until the following spring. An indirect influence of temperature both before and after egg-laying appears largely responsible for the distinction. For an extended incubation period in nature, it may even be necessary that the eggs be deposited in cold bottom waters of deep, thermally stratified lakes, as such conditions typify the species' habitat throughout its range. Due to the shorter period for summer growth in its northernmost limits, E. lacustris might then be expected to produce only resting eggs which result in but one generation per year. If so, its life cycle in northern lakes would closely conform with those of E. massachusettsensis and Heterocope borealis.

E. lacustris may spend up to 50 per cent of its life as an immature animal. Judging from estimates reported for other calanoids this is not unusual. Calanus regularly occurs as stage V throughout the winter months (Marshall, Nicholls, and Orr, 1934); in Lago Maggiore, Diaptomus vulgaris requires about 75 days to reach sexual maturity (Ravera, 1954). Among certain monocyclic forms, the period of larval development varies from about a month or more (Limnocalanus johanseni, Comita, 1956; Epischura massachusettsensis, Humes, 1955) to over 100 days (Heterocope borealis, Elster, 1936, 1954); in some instances it may last an entire year (Diaptomus ashlandi, Comita and Anderson, 1959; D. laciniatus, Ravera, 1954).

Natural mortality would be expected to take a heavy toll among the immature stages; previous mention was made of Elster's estimate that only about 0.5 per cent of the eggs produced by Heterocope in the Bodensee develop into sexually mature adults. It follows that the numerical superiority and persistence of immature individuals in a natural population attach special significance to their activities. Yet there are relatively few published records which deal with the larvae of individual species of copepods. An unreliable picture of species associations appears to be one of the inevitable results. A recent example is the study by Wells (1960). Although he frankly admits that sampling methods "unquestionably allowed the loss of valuable data", the lack of assurance that distinctions between adult and sub-adult animals were consistently made is difficult to resolve. Moreover, it seems clear that the assertion that limnetic communities are dominated momentarily by one species of copepod, one cladoceran, and one rotifer (Pennak, 1957) is based largely on incomplete data. The statement that "adequate sampling in 'typical' lakes shows that, on the average, 80 per cent of all limnetic copepods belong to one species, ..." is, at the very least, premature, since Pennak's literature references include not a single study in which immature stages were treated quantitatively. Considering only the adult limnetic copepods of Lake Michigan, Wells (1960) has shown that seven species may be taken regularly in the same sample; similarly, the adults of as many as four species of calanoids coexist in Lake Erie (Davis, 1959a). While these situations may be somewhat unusual, they are probably not exceptional. Even here a

quantitative evaluation of the species composition of the community is unwarranted without knowledge of the numbers of larvae present. A reasonably complete enumeration of all the organisms sampled is easily as important as "adequate sampling".

It is not surprising that the problem of routine identification of immature copepods has been neglected. More often than not, available keys require detailed examination and difficult dissection. Yet the fact remains that frequently in any sample of net plankton the number of larvae typically exceeds that of the adults. That a more complete realization of copepod taxonomy may be derived from a knowledge of the detailed morphology of their larvae was clearly evident in the present study after examining a key to the developmental stages of Heterocope constructed by Elster (1932, pp. 231-233). All of the distinctions used in it apply not only to Heterocope borealis and H. saliens (as pointed out by Elster), but equally well to Epischura lacustris and E. massachusettsensis. Verification is still needed for other species of these genera. It is just this sort of information which may lead to a more satisfactory systematic arrangement of the Copepoda, and eventually to a universal key of broad application. To the writer's knowledge, the only serious attempt at such an ambitious project is the work of Borutskii (1960).

The tendency in quantitative plankton studies to look for numerical correlations between standing crops of phytoplankton and zooplankton is a familiar one. Reductions in marine diatom populations as a result of zooplankton grazing have received much attention (Clarke, 1939; Fleming, 1939; Gauld, 1950;

Riley, 1946, 1947; Riley and Bumpus, 1946). Similar observations on fresh-water organisms have been published (Berg and Nygaard, 1929; Davis, 1954; Anderson, Comita, and Engstrom-Heg, 1955; Comita and Anderson, 1959). While obviously not all declines in phytoplankton should be attributed to grazing, any assessment of the demands of zooplankton populations for food requires a thorough knowledge of the feeding behavior and food preferences of the species concerned. Such information is rarely available for fresh-water zooplankters. The present work, for example, is the first experimental effort to determine feeding rates or algal food preferences of a fresh-water calanoid.

Although many animals are known to prey on zooplankton, neither the effect of predation on the plankton community as a whole nor the significance of predation within the community has received much attention. The lack of an obvious relationship at any point in time between zooplankton populations and their ultimate food supply in the form of phytoplankton might be partly a result of predation. Edmondson (1957) has stressed the point that it is unrealistic to expect the relation to be a simple one. The role of Leptodora and Bythotrephes in zooplankton consumption has been shown by Mordukhai-Boltovskaia (1958) to be of at least the same order as that of fish. Chaoborus larvae apparently devour large numbers of planktonic crustaceans during their pelagic existence (Deonier, 1943; Main, 1953). Certain cyclopoid copepods are highly carnivorous; in fact, some are known to attack and destroy small fish (Davis, 1959). Yet, in lieu of direct observation, it is decidedly unsound to make generalizations about the feeding habits of even

closely related species (Fryer, 1957). Curiously, the fact that some species of calanoids regularly eat other zooplankters appears to have largely escaped notice. It is difficult, for example, to find outside of the foreign literature any reference to studies dealing with the feeding behavior of Heterocope.

Pennak (1946, 1949, 1955) has strongly criticized attempts to demonstrate a direct dependency of zooplankton on phytoplankton in fresh waters. On the grounds that "the usual plankton diatom cells and colonies (Asterionella, Synedra, Melosira, Fragilaria, and so on) are often too large to be ingested intact by cladocerans and the small calanoid and cyclopoid copepods", Pennak (1955) has contended that limnetic crustaceans rely heavily on fine particulate detritus and bacteria for food. Obviously this author was unaware of the studies of Elster, Fryer, and Burckhardt; moreover, anyone who has examined living net plankton should be familiar with the habit of copepods, particularly adult cyclopoids, of devouring both plant and animal organisms sometimes only slightly smaller than themselves. If, as Pennak believes, many biologists have an erroneous idea of the complexity of limnetic zooplankton communities, it may be due to an oversimplification of even the meager information on species composition and feeding behavior presently available. Even if Pennak is essentially correct about the nutritional dependency of many small crustaceans on detritus and bacteria, the evidence he offers is not convincing. Furthermore, his reasons for believing that limnetic grazers do not rely on intact algal cells and that therefore phytoplankton does not constitute a serious limiting factor are inadequate.

Epischura lacustris, like Heterocope, is an omnivorous feeder. The materials it ingests cover a wide size range and a considerable part of its natural diet consists of other copepods but not of cladocerans. Experimentally, the animals can subsist if not actually flourish on certain algae (e.g., Pediastrum and Closterium), whereas others are definitely unsuitable. There is increasing evidence that substantially different kinds of food materials are utilized by various limnetic calanoids. While reliable information is incomplete for any single species, it is unrealistic to assume that either fresh-water zooplankton as a whole or even its major taxonomic groups represent but a single trophic level.

The significance of cannibalism in natural populations is difficult to evaluate. Elster (1954) thought that it was of secondary importance in controlling either the size or the success of the Heterocope population in the Bodensee. It is possible that since hatching in Heterocope, as in E. lacustris, occurs on the lake floor, the larger predaceous individuals normally encounter young stages only sporadically in the epilimnion, where presumably the former are most active in feeding. Elster had evidence that nauplii were more abundant in the bottom water layers, and perhaps not until they have attained considerable size do they migrate in large numbers into the warmer surface waters.

A final comment about food relations seems pertinent. While the basic feeding mechanism of calanoids appears to be one of filtration, the structure of the second maxilla may provide an index as to the capacity of a species to consume

its fellow zooplankters. Certain features as described in the previous section, namely the stoutness of the proximal setae, loss of feathering, and reduced filtering surface in Heterocope and in at least some species of Epischura, are proposed as just such structural modifications. This concept is not entirely new. Lowndes (1935) was convinced that in a number of calanoids the maxillae and maxillipeds "are in general raptorial, and not filtratory", and that they "cannot possibly function as strainers of microscopic organisms." According to him, females of Euchaeta norvegica feed almost exclusively on Calanus finmarchicus, and Limnocalanus macrurus is one of several predatory fresh-water species. It must be more than coincidental that Epischura, Heterocope, and Limnocalanus are among the largest fresh-water calanoids, and that both Elster and Marshall and Orr, independently, have provided evidence that young stages feed on much the same kind of food as the adults; furthermore, the fact that populations of E. lacustris are usually small and are characteristically if not invariably found associated with diaptomids in no way disputes the thesis that they depend on these smaller and almost ubiquitous animals for food. In future studies bearing on the trophic interactions of the zooplankton, it should prove profitable to explore the relation between body size and the predatory habits of calanoid copepods.

SUMMARY

1. As part of a study of the seasonal life history of Epischura lacustris in Whitmore Lake, Michigan, from March 2, 1956, to June 12, 1957, the eleven pre-adult instars were identified by a detailed examination of specimens both reared from the egg and taken in the plankton.

2. The nauplii may be distinguished from those of Diaptomus and Epischura massachusettsensis by the setation of the first and second segments of the first antenna. In living collections, they could be separated from those of the other copepods present by the position of the first pair of antennae. Developmental changes at each molt followed in most respects those of the larger E. massachusettsensis, and agreed with key distinctions outlined by Elster (1932) for Heterocope borealis.

3. Body length measurements of successive instars indicated no overlap in size but showed a consistently uniform percentage increase at each stage. Sexual differentiation was determined on the basis of the structure of the fifth swimming legs. The armature of the distal segment of the female fifth leg was found to vary, sometimes within the same individual.

4. In Whitmore Lake the population was found to be dicyclic. The animals occurred in the plankton of the northeast basin only during ice-free conditions. Nauplii first appeared at the time of the spring thaw and reached sexual maturity by July. A second generation appeared in August, apparently a

direct result of the earlier brood's reproduction. After mid-November neither adults nor immature stages were to be found.

5. There is no ovisac. Eggs are laid free in the water and sink to the lake floor where they may be found in soft muck throughout the year. They can withstand anaerobic conditions and high concentrations of H_2S, but neither freezing nor drying. The hatching process agrees with that described for Heterocope.

6. Egg production increased in intensity after September. Eggs laid by the first generation required only a day or two to hatch. Yet those produced in the fall lay dormant in an extended incubation period until the following spring. An indirect effect of temperature was thought to account for the distinction between resting and non-resting eggs; yet preliminary experiments suggested that resting eggs hatched at a time favorable for survival due to an internal regulatory mechanism. Under certain conditions development was arrested for as long as 16 months in the laboratory.

7. Feeding is mainly by filtration, but is neither indiscriminate nor completely automatic. Laboratory experiments demonstrated a distinct preference for two kinds of phytoplankton and certain microcrustaceans. Algae were grown individually in mass culture. Their relative suitability was tested in laboratory populations on the basis of copepod survival, growth, fecal pellet production, and reproduction, and further assessed by determining filtering rates, using differential cell counts from ten to twenty-four hour experiments. Predatory behavior was examined by offering individual Epischura various cladoc-

erans, copepods, and brine shrimp larvae.

8. Rearing on phytoplankton was only partly successful. Only in populations fed Pediastrum biradiatum was there satisfactory growth and reproduction. The life cycle from egg to sexual maturity was completed on two occasions. One female lived 74 days in culture and produced four clutches of eggs. Animals appeared to do somewhat better with Scenedesmus quadricauda and Ankistrodesmus than with either Chlamydomonas reinhardi or Oocystis. Chlorella vulgaris was worse than no food at all.

9. Nauplius I does not feed as the anus is not yet developed. Carnivorous habits and filtering rates of young stages were not investigated. Larvae utilized the same algae as did the adults, except for really large cells. Feeding in late-stage Epischura appeared to have been inhibited at a temperature of 10°C.

10. Filtering rate measurements demonstrated distinct algal food preferences despite considerable variation between individual experiments. Among experiments which resulted in the highest rates were those utilizing algal species which were found to have been the most successful in rearing laboratory populations of Epischura. The maximum rates for adults occurred in vessels containing the largest algae, Closterium littorale and Pediastrum biradiatum. They averaged, respectively, 7.09 and 5.50 ml of water swept free of algae per copepod per day. The maximum rate (11.44 ml) was encountered in exposing adult females to Closterium.

11. The mean filtering rate of adults supplied with Chlorella was found to be negligible, further evidence of the unsuitability of this alga as food. Those for the other algae ranged from about 1.0 ml per copepod per day for Nitzschia closterium f. minutissima to about 3.0 ml for Navicula pelliculosa. Among factors considered responsible for the observed differences were size of the food and in a few cases its tendency to clump. Escape of small cells through the maxillary filters probably occurred but did not adequately account for rate differences. Chlorella may have produced substances which inhibited feeding.

12. The numbers and appearance of fecal pellets were directly related to filtering rates. Usually algae were ingested whole. Preliminary experiments with sub-adult animals showed an increase in rate with an increase in body size. Comparing instars of similar length, Epischura seems capable of filtering about the same amount of water as Calanus finmarchicus.

13. Independent estimates of the time required for larval development were made by censusing laboratory populations and by noting the succession of instars in the natural habitat. The two were in striking agreement: 38 to 40 days. Similarly, the time for one complete generation was calculated to be about 54 days; estimates of the entire life span were 59 days for males and 98 for females. The long duration of the larval period in many calanoids should not be overlooked in describing the species composition of limnetic communities.

14. Gut analyses of freshly-captured adult and stage V Epischura demonstrated that the copepod is omnivorous. While

much of the material was essentially unrecognizable, the most abundant items were diaptomid copepods and colonial algae. Remains of cladocerans, adult cyclopoids, and rotifers were noticeably absent. That Epischura lacustris may rely heavily on diaptomid copepods for food in nature is further suggested by its species associations throughout its range.

15. Experimental Epischura devoured large numbers of calanoids, cyclopoid larvae, and Artemia nauplii, but refused (or failed to seize) Diaphanosoma, Bosmina, and small to large Daphnia. The intensity of carnivorous feeding increased during development. Rarely prey were mangled. Epischura flourished on a diet of Artemia, devouring them whole. Small crustaceans are swept into the oral region by filtering currents, but capture of larger animals is fortuitous.

16. The structure of the second maxilla, basically a filtering appendage, may reflect carnivorous feeding in calanoids. The similarity of E. lacustris, E. massachusettsensis, and Heterocope in their larval development, reproductive behavior, and seasonal life history, appears to apply to their feeding habits as well. Predation in calanoid copepods as it bears on limnetic phytoplankton-zooplankton relationships was briefly discussed.

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