BY CHARLES A. KOFOID.

The limestone region of Kentucky covers an area of about eight thousand square miles. A portion of this region, especially in the neighborhood of Mammoth Cave, is devoid of the usual system of streams which afford surface drainage. The St. Louis limestone which underlies the Chester sandstone is honeycombed by caverns hollowed out by the underground water-courses, and the roof of sandstone has fallen in in many places forming sink-holes some of which are several thousands of acres in extent. It is reported, say Hovey and Call ('97), that there are four thousand of these sink-holes in Edmonson County alone. At the bottom of the sink-hole a more or less open passage-way leads to the cavern below. These myriad sink-holes drain off the surface water into the underground water-courses which eventually make their way to Green River, the only open stream of the vicinity. The total length of this underground system is estimated by Professor Shaler, says Packard ('89), to be one thousand miles. Echo River is a part of this subterranean system found in Mammoth Cave, in a tubular cavern accessible for the length of half a mile. Its width, according to Hovey and Call ('97), varies from twenty to two hundred feet. At times of high water the entire cavern is filled by the stream, but at low water, when the stream is most visited, the archway overhead varies in height from five to thirty feet and the depth of the water has about the same range. Barometric observations indicate that the level of Echo River is about twenty feet above that of the local surface stream known as Green River. Experiments with floating chaff have demonstrated that the subterranean water system of Mammoth Cave opens in certain large springs along Green River though I have found no data upon this point pertaining

directly to Echo River. It is known, however, that Echo River has some connection with other parts of the water system of the cave.

As above stated the only known source for the water in these cave streams is the surface waters of the neighborhood which make their way through the sink-holes, or by seepage, into the caverns below. The abundance of the sink-holes has prevented any extensive development of surface streams and I have found no reference to "lost" streams in the literature at hand. Hovey and Call ('97) state that many of the outlets of the sinkholes have been closed up artificially to prevent accidents to domestic animals and that this has resulted in the formation of deep pools. It is evident from these facts that the planktonts can have but limited opportunity to breed in the surface waters before they reach the underground system of the cave.

The temperature of the water in Echo River was found by Mr. Hovey (see Packard, '89, p. 9) to be 55° F. on Aug. 13-15, 1881, thus having, at this time at least, about the temperature of the air of the cave and the rock walls $(53^{\circ}-54^{\circ})$. I have found no records of water temperatures at times of flood, or in December, the month in which the collections here reported upon were made.

In December, 1898, Professor C. H. Eigenmann of the University of Indiana made a towing-net collection in the waters of Echo River which he preserved in formalin and kindly sent to me for examination. The collections were made with a net of No. 12 silk bolting cloth, a silk whose apertures will easily allow many of the smaller organisms usually found in plankton to escape. When, however, the pores of the net become clogged with organisms or debris the filtration is more efficient though less rapid. Collections made with nets of this cloth may thus contain representatives of all the organisms present in the plankton but not in their relative numbers.

The total volume of the catch received from Professor Eigenmann after standing twenty-four hours was 1.75 cubic centimeters. It consisted almost entirely of a semi-flocculent

sediment, colored reddish brown by the salts of iron from the limestone leachings, together with a few fragments of vegetable debris and traces of finely powdered quartz. Superficial examination revealed a few Entomostraca upon the surface of the sediment while inspection with the microscope increased the evidence for the presence of plankton organisms in the cave waters. All of the material was carefully examined under a low power (35 diameters) and the organisms listed and removed for identification when it was possible. About one-third of the catch was examined under a higher power (175 diameters). The following is a list of the organisms observed together with such biological data as could be gathered from the material:

LIST OF SPECIES IN THE PLANKTON OF ECHO RIVER.

ALGAE.

Phycochromaceae.

1. Oscillaria sp.

Two filaments were found of twenty and fifty-five cells respectively.

2. Ulothrix sp.

Three fragments of filaments of fifteen to forty cells.

Bacillariaceae.

3. Nitzschia linearis Smith.

A single dead frustule.

PROTOZOA.

Rhizopoda.

4. Amoeba limax Duj.

A single well preserved individual was found which has been referred to this species. It lacks all indications of pseudopodia such as are usually to be seen upon A. proteus when taken in plankton and killed in formalin. The body measured $15 \times 45 \mu$, was somewhat wider posteriorly than anteriorly, and was abruptly truncate at both ends. A single well defined nucleus and a number of food vacuoles were visible but no trace of a contractile vacuole could be found. It may be that this is but a form of *A. proteus*, but for the reasons above given it has seemed best to retain Dujardin's name for the form. *Amoeba* has been found by me occasionally in river planktons in company with testaceous rhizopods.

5. Difflugia globulosa Duj.

Two specimens of this common rhizopod were found, one of which was but an empty shell while the other was a normal individual. Next perhaps to D. lobostoma this species is the commonest of rhizopods in the plankton of rivers. In Lake Michigan also (Kofoid, '95) it is an abundant planktont.

6. Centropyxis aculeata var. ecornis Leidy.

Two examples only were found, both of which were empty shells. *Centropyxis* is a littoral species abundant among water plants and shore debris. I have rarely found it in living condition in river planktons.

Choanoflagellata.

7. Salpingoeca amphoridium J. Clark.

A cluster of six loricae was found attached to the scale of a moth, and two smaller clusters to fragments of vegetable debris. In the individuals examined the neck of the lorica was somewhat shorter than it is in the typical *S. amphoridium*. No trace of collar or flagellum could be detected. *Salpingoeca brunnea* Stokes has been abundant in the plankton of the Illinois River, being found sessile upon *Melosira* and other plankton diatoms and algae. The specimens found lack the truncate base and the brown color characteristic of the typical *S. brunnea*. It has seemed best to refer the form to the variable and cosmopolitan *S. amphoridium*.

Flagellata.

8. Colacium vesiculosum Ehrbg.

Rather common upon Cyclops, attached to the carapace and

appendages. This is an abundant parasite of *Cyclops* in epigean streams.

Suctoria.

9. Podophrya cyclopum Clap. & Lach.

Common upon *Cyclops*, especially upon the bases of the antennae and upon the carapace. This form, like the one preceding, is frequent upon *Cyclops* in other waters.

PORIFERA.

10. Spongilla fragilis Leidy.

A small number of spicules resembling those of the skeleton of this common species were found. Exact identification is impossible without the gemmulae, of which none were to be discovered. Sponge spicules are frequently to be found in river waters.

VERMES.

Nematoda.

11.

A single small nematode worm, evidently young, about 0.35 millimeter in length was found. A single large nematode egg occurred in the collections.

Oligochaeta.

12. —

A fragment one millimeter in length from the posterior end of a small enchytraeid worm occurred in the collection.

Rotifera.

13. Rotifer sp.

But a single rotifer was found in the whole collection. This was a much contracted specimen belonging to the Philodinadae of which, owing to its condition, specific identification was impossible and even generic questionable.

ARTHROPODA.

Crustacea.

Ostracoda.

14. Limnocythere sp.

Two specimens, one of which was young, belonging to this or a related genus were found. The fully grown specimen had been feeding recently. Ostracoda are frequently found in the plankton of streams though they belong properly to the bottom or to the littoral fauna.

Copepoda.

15. Diaptomus sp.

The mutilated and empty carapace of a single specimen was found. Identification of the species was impossible.

16. Cyclops viridis var. americanus Marsh.

Represented by a single adult female. This is a common summer form in streams and lakes.

17. Cyclops bicuspidatus Claus.

This was the most abundant species of the genus and was represented by about seventy individuals of which about two-The two sexes were about equally reprethirds were adult. sented. The immature forms were principally females and were almost fully grown. Several of the adult females had egg-sacs attached and a number of free eggs were found in the collection. Some of the adult females were unusually large, the length, excluding caudal setae, being 1.75 millimeters. The usual length is a little more than one millimeter. The antennae were somewhat shorter than usual, reaching only to the posterior border of the first segment. The caudal furcae are also somewhat shorter than they often are found, the proportions of length to width being but four or five to one. In river and lake specimens, especially the latter, the furcae may be as much as nine times as long as they are wide. This species is abundant in the Great Lakes and appears in the plankton of the Illinois River during the cooler parts of the year.

18. Cyclops albidus Jurine.

This species was represented in the collection by about thirty specimens of which but three were males. A single egg-bearing female was found, though but a few of the females were immature. This species also is frequently found in river planktons.

19. Cyclops serrulatus Fischer.

But one specimen was found, a small egg-bearing female. The furcae were noticeably curved and divergent and were armed externally with heavy hooked spines. This variable species is widely distributed especially in creek waters.

The facts that the Cyclopidae were present in considerable numbers in Echo River and that some of them were carrying eggs seem to indicate that they might be regarded as normal members of the stygian fauna. A few observations, however, render this inference of questionable validity.

1st. All the individuals examined had eyes which did not appear to differ from normal eyes in any marked degree. Whatever pigment was present in the living animal had been removed by the action of the formalin, as is usual in collections preserved in either alcohol or formalin.

2nd. The proportion of dead individuals represented by more or less disintegrated bodies was large, larger in fact than usual in collections from epigean waters. Several specimens were fungoused and three instances of infection by an opaque whitish spore-like growth, which by some writers has been referred to *Sporotrichum*, were noted.

3rd. The entire absence of nauplii and of the larval stages would suggest that the activity in breeding had been recently checked. The number of eggs present in the ovisacs was frequently below the normal. It hardly seems possible that all the nauplii—if any were present—should have escaped through the net.

4th. Less than ten per cent. of the individuals showed any trace of food contents in the intestinal tract, indicating a suspension of feeding. 5th. The species are all common epigean forms and are probably continually carried into the cave by the waters tributary to the cave streams, and are as continually removed by the outflowing currents. Under these conditions these species of *Cyclops* should be regarded as adventitious and temporary members of the cave fauna and not as permanent residents since the conditions of access and the continual changing of the waters do not permit the establishment of a permanent colony.

Hexapoda.

20. _____

A single small dipterous larva, 0.8 millimeter in length, too young for further identification. Dipterous larvae, especially of *Corethea* and of *Chironomus*, are frequently found in the plankton of lakes and streams.

The collection also contained a few insect eggs of three different sizes.

Of the twenty organisms above listed but three are plants, and these not usually found in plankton. They were also present in but insignificant numbers. Of the seventeen animal planktonts, three are Rhizopoda, one a flagellate, and one a suctorian, in all five Protozoa of which but one may be called a planktont. The sponges, nematodes, oligochaetes, and rotifers are cach represented by a single species no one of which is a typical plankton organism. Aside from the unidentified ostracod all of the five Entomostaca found occur in the plankton of epigean streams. The single dipterous larva is at the best but an unimportant representative of the plankton. Of the total number of organisms the following only may be cited as typical planktonts:

> Difflugia globulosa, Diaptomus sp., Cyclops viridis americanus, Cyclops bicuspidatus, Cyclops serrulatus, Cyclops albidus.

The following parasitic or attached organisms are to be classed as passive planktonts:

> Salpingoeca amphoridium, Colacium vesiculosum, Podophrya cyclopum.

The representatives of the littoral fauna and flora which were found include the following:

> Oscillaria sp., Ulothrix sp., Nitzschia linearis, Amoeba limax, Centropyxis aculeata var. ecornis, Spongilla fragilis, Nematode worm, Enchytraeid worm, Rotifer sp., Limnocythere sp., Dipterous larva.

The chief characteristics and noticeable features of this collection are the absence of plant life, especially of diatoms, the absence of rotifers, the predominance of Copepoda, and the presence of a considerable range of littoral species, chiefly attached or bottom-living forms.

In addition to the species above listed the collection contained other evidences of life, the scales of Lepedoptera for example being very numerous. These were of a number of different types and were much more abundant than they are in ordinary stream waters. The stellate hairs of the elm (Ulmus*americana*), the spores of *Alternaria*, and comminuted fragments of vegetation were also present much as in surface waters. The collection also contained a noticeable quantity of hyphae and knotted mats of mycelium for which a subterranean origin seems most probable. There were also present a number of the calcareous shells of fossil Foraminifera, derived presumably from the oolitic limestone of the cave.

The plankton of the cave streams doubtless plays an imporant part in the oecology of aquatic cave life since it may be the primal source of the food supply of the larger Crustacea, the blind-fish, and the cave salamanders. The animal plankton which is swept into the cave or which develops in its waters can find little plant life to support it. Plants—other.than the fungi—do not seem to thrive in the total darkness, so that any permanently established colony of animal planktonts in cave waters must either depend upon the uncertain accessions of food from the surface waters or adapt itself to the supply furnished by the fungi. The fact that most of the Copepoda in the collection had not been feeding recently would seem to indicate a scanty food supply.

Previous observations upon the microscopic fauna and flora of the waters of Mammoth Cave seem not to have been made upon towing-net collections, and they report other forms than those here listed. With the possible exception of the dipterous larva all the species found in this plankton collection were never before reported from the waters of the cave. Dr. Tellkampf ('45) examined the water of the cave, making some hasty sketches which he later submitted to Professor Ehrenberg of Berlin. This examination resulted in the following list. Under the circumstances the identification is uncertain and no attempt will be made here to enter into the question of nomenclature and synonomy:

From Serena's Bower, 9 miles from entrance of the cave-

Monas colpoda, Monas socialis, Bodo sp. From River Styx— Chilomonas emarginata, Kolpoda or Chilodon cucullus.

On May 3, 1874, Professor Packard ('89) examined the water in Wandering Willie's Spring, a pool not far from the mouth of the cave, and reports the following as present:

> Vibrio, Colpoda (?), Nassula or Prorodon, Paramoecium (?).

In addition Professor Packard ('89) describes a *Canthocamptus* for which he proposed the name *stygius* from this same spring. No other Copepoda, so far as I can ascertain, have been reported from the cave prior to this paper.

Ehrenberg ('54) in his "Microgeologie" (fide Packard, '89, p. 26), adds to the list of Tellkampf the following:

Biddulphia (?), Gallionella (?), Synedra ulna.

The micro-fauna of European caves has been studied by Claus, Joseph, and Schmeil and the fragmentary data of this paper accord well with their more extended results. Thus Joseph ('82) records two "new" species of Cyclops from the Carnolian Caves which Schmeil ('94) believes to be C. albidus and C. serrulatus, two of the species here reported from Echo River. His list also includes Branchipus pellucidus, Estheria coeca, Leptodora pellucida, and Cypris stygia. In previous papers he deals with other groups of stygian invertebrates. Of the Rhizopoda he ('79d) finds only an amoeba, which he describes as A. cellarum, though in all essential particulars it resembles A. proteus. The only other Protozoa found by Joseph ('79b) were some unidentified attached ecto-parasites of the cave Crustacea and other cave animals, and a new member of the Peridinidae, Peridinium stygium, which occurred in a pool near the mouth of a cave near Adelsberg. He further states that in a score of years' collecting in caves he has found examples "of more than half of the groups of Infusoria" principally in parts of the caves where bat excrement is found. No specific identifications are given. Of the nematodes he ('79c) records fourteen species from the rubbish at the mouth of the cave, and several others belonging to the genus Plectus from the deeper parts of the cave in bat excrement. In Recca Cave another species of Plectus occurs. Of the Rotifera nine species were reported from the Karst cave region by Joseph ('79a). These are said to belong, one each to Trochosphaera (sic) and Lepadella, two to Hydatina, and two to a new genus Apodoides,

while the other three remain unidentified. Specific identifications are not given and it seems probable that the generic identifications are subject to revision. The caves from which these species were secured are said by the author to receive large amounts of flood water in March from surface streams. A single oligochaete *Enchytraeus cavicola* is described by Joseph ('80) from the cave at Potiskavez; this worm was frequently taken from the stomach of the cave salamander.

Schmeil ('94) in a critical paper describes the Copepoda which he secured from the cave region in which Joseph worked. As above stated he concludes that Joseph's species were only well known epigean forms. He finds no particular stygian forms, reporting from Magdalen Cave five species, Cyclops bisetosus Rehberg, C. viridis Jurine, C. dybowskii Lande, C. serrulatus Fischer, and C. prasinus Fischer. In the living condition some of the individuals showed a marked reduction in the amount of pigment in the eye. Claus ('93) had previously reported from Recca Cave five species of Cyclops, viz: C. bisetosus Rehberg, C. bicuspidatus Claus, C. vernalis Fischer, C. strenuus Fischer, and C. serrulatus Fisher. Thus three of the four species here reported from Mammoth Cave have been found in the waters of European caves. In addition to the Copepoda, Schmeil ('94) reports from Magdalen Cave two Ostracoda, both apparently new, belonging to the genera Cypris and Typhlocypris.

There is little doubt that more extended collections made in various localities in waterways of Mammoth Cave and at different seasons of flood and low water would considerably increase the list of species which make their way into the cave with the surface waters, and may possibly lead to the discovery of some peculiar stygian planktonts, or more likely of some sessile or bottom-living forms of micro-organisms.

The character of this collection renders inevitable the conclusion that the plankton of Echo River—in this instance at least—has been recently derived from epigean waters. It presents few if any typical stygian forms. The recurring access

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of surface waters provides for the renewal and maintenance of this subterranean plankton and effectually prevents the development of any peculiar cave species. It is also possible that currents of air and visitors might carry into the cave the germs of many minute forms which could there develop when they found proper conditions of moisture within the cave. The additions to the cave fauna from this source must, however, be insignificant in comparison with the ever recurring contributions of the surface drainage.

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