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The Behavior of a Parasitic Copepod, *Lernaeopoda Edwardsii* Olsson

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THE BEHAVIOR OF A PARASITIC COPEPOD, *LERNAEOPODA EDWARDSII* OLSSON

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CONTENTS

I. GENERAL REMARKS

The data presented in this paper were obtained during July and August of 1912, while the author was in the service of the Wisconsin Fish Commission, studying an outbreak of parasitic copepods in the trout hatchery at Wild Rose, Wis. The brook trout, *Salvelinus fontinalis*, were found to be attacked by the copepod *Lernaeopoda edwardsii*. Most generally this parasite attaches itself to the filaments of the gills, but sometimes it is found on the gill operculum, the roof of the mouth, and on the pectoral and pelvic fins. For its identification, I am indebted to Professor C. B. Wilson. To the Commissioners of Fisheries of the State of Wisconsin, and their employes, especially Mr. Zalsman, foreman of the Wild Rose hatchery, my thanks are due for many courtesies shown me. To Professors E. A. Birge, George Wagner, and A. S. Pearse I wish to extend my best thanks for their many helpful suggestions.

II. DESCRIPTION OF THE FREE-SWIMMING COPOPOD

Lernaeopoda edwardsii, like all known species of the *Lernaeopodidae*, is parasitic during almost its whole life. The duration of its free-swimming existence is very short, perhaps not more than two days. Its nauplius and metanauplius stages are passed within the egg sac of the mother, and the animal hatches

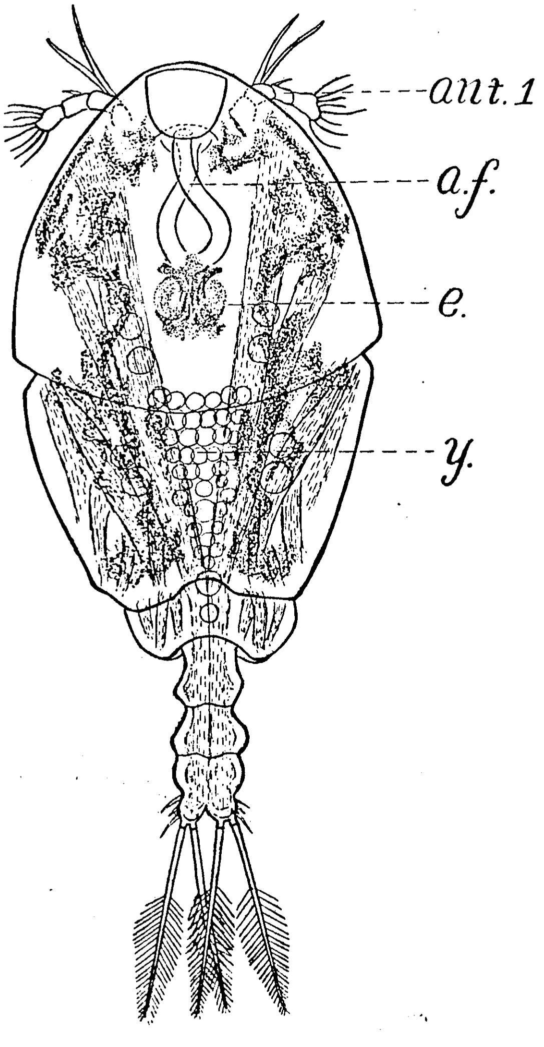

FIGURE 1. Lernaeopoda edwardsii. Dorsal view, free swimming stage, x 173.5
a. f. =attachment filament.
ant. 1=first antennae.
e =x-shaped eye.
y =yolk.

into a fully developed larval form, which immediately begins an active hunt for its host. During this stage of its life history the copepod does not feed, its nourishment being derived from the yolk which it carries over from its embryonic development (figure 1, y).

The larva is minute in size, about 0.726 m. m. in length. Running along each side of its dorsal surface are two well defined brownish streaks of pigment. A characteristic x-shaped copepod eye occupies the middle of the head (figure 1, e).

The head is broad, elliptical, and bears the mouth parts and a peculiar attachment filament. The mouth parts are situated on the ventral surface and consist of the first antennae, the second antennae, the mouth tube, stationed between the second antennae, the mandibles, the two pairs of maxillae (the first and the second), and the maxillipeds. The attachment filament is located beneath the head, and is made up of two parts:—(1) a broad circular, mushroom-like body whose position is between the first antennae, and (2) a tube-like structure, which makes its way backward from the posterior region of the mushroom body as far as the eye, and then turns upward in one circular loop, passing underneath the first part of the tube, and continues to ascend until it reaches the level of the posterior margin of the mushroom body, where it is attached to the head (figure 1, a, f).

The thorax has two segments and these bear the two biramous swimming feet (Fig. 2 s. f. 1 and s. f. 2), which end in broad laminated bases—the respective exopods and endopods. Each exopod terminates in four long feathery setae, whereas the endopods contain seven of these plumose structures. The feet are operated by a system of strongly developed dermal muscles situated along the dorsal side of the body.

The abdomen is rather slender and contains three segments. The last of these is supplied with four feather-like setae, as well as three pairs of smaller seta-like appendages, that are vestigial in character.

The head and the first thoracic segments comprise the main bulk of the copepod's body. Figures 1 and 2 illustrate, respectively, a dorsal and a ventral view of the free-swimming copepod.

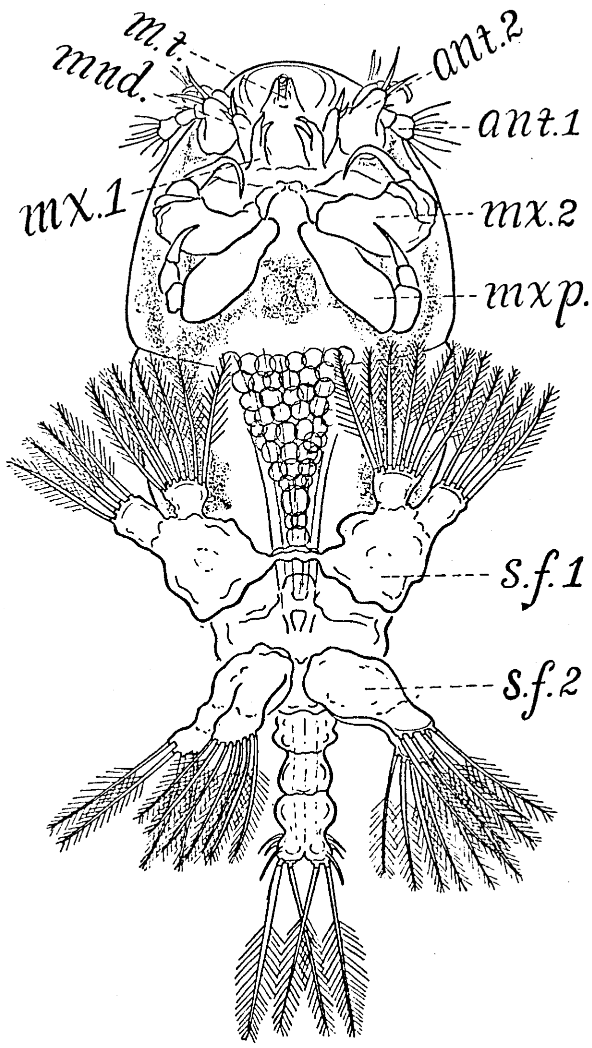

FIGURE 2. Lernaeopoda edwardsii. Ventral view, free swimming stage, x 173.5

ant. 1=first antennae.
ant. 2=second antennae.
mnd. =mandibles.
m. t. =mouth tube.
mx. 1=first maxillae.
mx. 2=second maxillae.
mxp. =maxillipeds.
s. f. 1=first pair of swimming feet.
s. f. 2=second pair of swimming feet.

III. HATCHING THE ORGANISM

In order to obtain enough material for experimentation, it became necessary to hatch the copepods artificially. The first efforts were confined to attempts to hatch the parasites in aquaria within the hatchery, but this proved a failure. Conditions here were far from normal, and the attempt was soon abandoned.

Outdoor experiments were next tried. Two large tanks were constructed, which were eight feet long by three and one-half feet wide and two and one-fourth feet high. The frame of these tanks was made of wood, while the sides consisted of coarse wire netting. The interior faces were lined with fine linen gauze so as to prevent the escape of any copepods. Figure 3 is a photograph of one of the tanks.

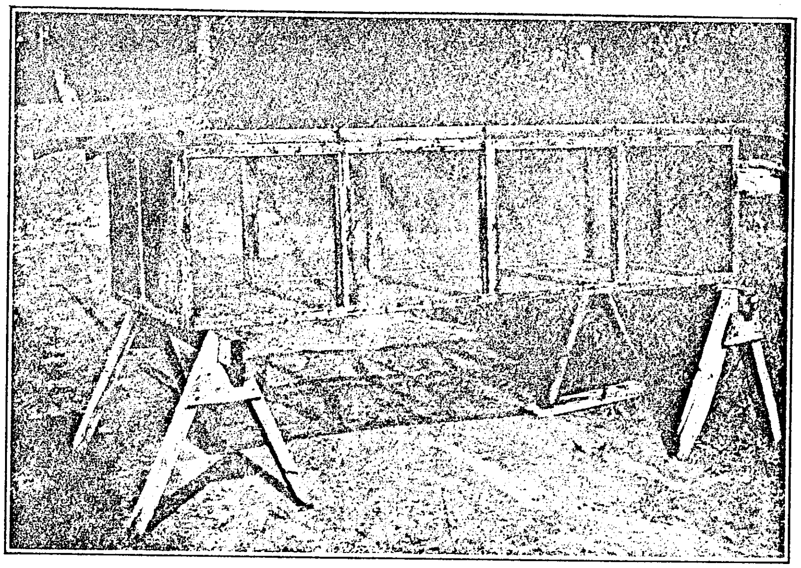

FIGURE 3. Hatching tank

Simply stationing these tanks in one of the open hatchery ponds proved insufficient, for, through lack of circulation, trout placed into them very quickly succumbed. So the tanks were placed in such a way that the water from some feed pipes ran directly into them. This arrangement proved entirely satisfactory. Figure 4 shows the manner in which the tanks were placed within the pond. The water can also be seen entering them.

Before putting any of the infected fish into the tanks, the water in the feed pipes was thoroughly examined in order to determine whether *Lernacopoda edwardsii* existed in it. Fine linen bags were tied over the outflows, thereby catching the organisms brought in by the water. The contents of these bags were looked over under the microscope three times daily. Fifteen examinations of these catches did not reveal a single parasitic copepod, and this convinced me that the water was free from the infection. One hundred parasitized trout were next introduced into each tank, and the covers placed over them. Two days later, the first batch of free swimming larvae was obtained—about two dozen of them. This was on July 17th, and almost every day after that until September 5th, when the investigator left the hatchery, from a dozen to three dozen free swimming parasites were secured.

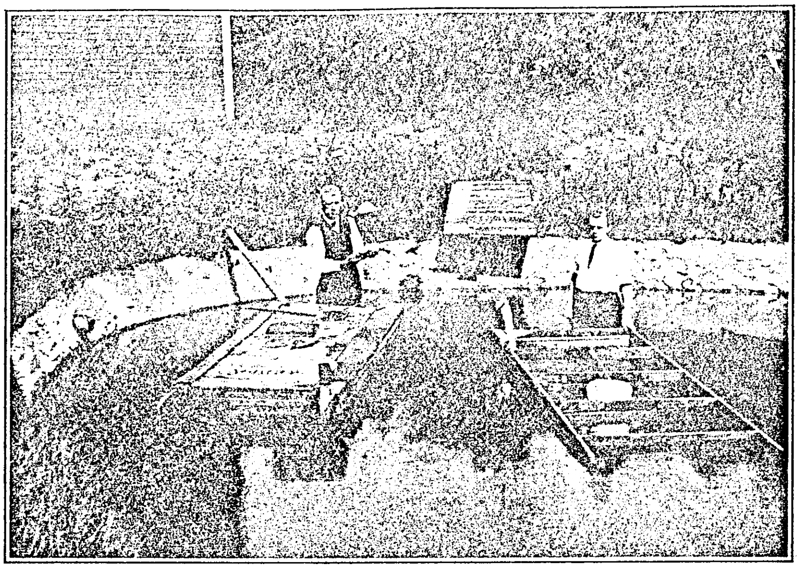

FIGURE 4. Tanks in position

The animals were scooped up with an open tow net, which was dragged along the surface of the water. The net had an aluminium cup attached to its tapering end. In the bottom of this cup there was an opening covered by a tightly fitting screw-cap. By unscrewing this cap, each haul could easily be transferred from the cup to another receptacle without losing any of the gathered organisms. Generally, each catch was strained through a fine linen plankton sieve, in order to condense the haul for examination. By means of a pipette the copepods were segregated from the other organisms, and introduced into a clean dish of water, where they were allowed to remain until needed.

IV. MOVEMENTS OF THE LARVAE UNDER NORMAL CONDITIONS

In the free living stage, the copepod swims about actively, with a snappy, dart-like spiral motion. Its mouth parts are, at the same time, moving incessantly, always ready to grasp their host and thereby insure the further development of the organism. Movement through the water is accomplished by the two biramous swimming feet on the thorax. In their normal resting attitude, the feet are held in an oblique position, with their setae pointed toward the head of the organism. The abdominal portion of the copepod is somewhat bent, at an angle of about 20° with the long axis of the body.

Motion is produced by the contraction of the powerful dermal muscles, which cause the swimming feet to dart backwards, thus shooting the organism ahead. The copepod, in its motion, passes through the same angle as that which the swimming feet traversed in the act of propelling the body. The actual distance covered by the copepod through one stroke of its feet is about an inch. Further motion is accomplished by a repetition of the same process; the animal thus travels in a snappy, spiral path (figure 5). When motion ceases, Lernaeopoda usually takes an upright position, with its long axis almost perpendicular to the surface of the water. Soon it turns upside down, and slowly begins to sink to the bottom, where it may assume a ventral or a dorsal position, depending entirely on which side of the body strikes the bottom first.

Oftentimes the copepod is found adhering to the side of the glass dish nearest the window, or it may sometimes suspend itself on the surface film of the water. In such cases, the larva maintains an upright position, while the first antennae are stretched out horizontally, making an angle of almost 90° with the long axis of the body. By means of these antennae the copepod is enabled to cling to the sides of the dish or to suspend itself from the surface film. In the latter case, surface tension undoubtedly plays the important rôle in maintaining the animal. Parker (1901), has observed similar behavior in the marine copepod *Labidocera aestiva.* He concluded that the antennae serve as means of attaching the copepod to fixed objects or to the water film. My observations are in accord with his.

The peculiar spiral movement of the copepod is of great significance. Many of the Protozoans and Rotifers have for a long time been known to move in this way. Jennings, more than any other investigator, has made a careful study of the problem, and has shown that a spiral path is of great adaptive value to the lower organisms. He has found (1901, '04, '06) that most of the Infusorians, especially the "hunter ciliates," move in a spiral course. In his work on Paramecium we get a thorough analysis of this type of locomotion. Discussing the adaptiveness of this movement, Jennings says:

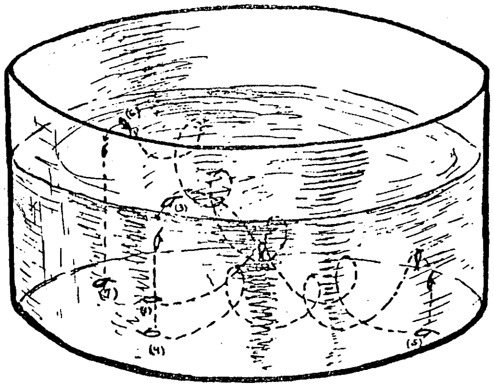

FIGURE 5. Normal movements of larva.

"The problem solved by the spiral path is as follows: How is an unsymmetrical organism, without eyes or other sense organs that may guide it by the position of objects at a distance, to maintain a definite course through the trackless water, where it may vary from the path to the right or to the left, or up or down, or in any intermediate direction? It is well known that man does not succeed in maintaining a course under similar but simpler conditions. On the trackless, snow-covered prairie, the traveler wanders in circles, try hard as he may to maintain a straight course, though it is possible to err only to the right or left, not up or down as in the water. Paramecium meets this difficulty by revolution on the axis of progression, so that the wandering from the course in any given direction is exactly compensated by an equal wandering in the opposite direction. Rotation on the long axis is a device which we find very generally among the smaller water organisms for enabling an unsymmetrical animal to follow a straight course. The device is marvellously effective, since it compensates with absolute precision for any tendency or combination of tendencies to deviate from a straight course in any direction whatsoever."

"The normal movements of Paramecium are adaptive in another respect. The same movements of the cilia, which carry the animal through the water, also bring it its food. Thus Paramecium is continually receiving samples of water in front of it. Since in its spiral course the organism is successively pointed in many different directions, the samples of water it receives likewise come successively from many directions. The animal is given opportunity to try the various different conditions supplied by the neighboring environment."

In the case of Lernaeopoda, we meet with a similar condition. The organism, like Paramecium and the other Infusorians, circles through the water in its characteristic way, "trying" the water, so to speak, in every direction. Of course, the animal being a parasite throughout most of its life, must come in contact with the proper host in order to carry on its further development. In other words, the copepod, like Paramecium, must meet its food in order to exist.

It is questionable whether the larva perceives objects through its ocellus. Even if it did, however, the visual range would, in all probability, be so short that the organism could not see its host in the water. It thus becomes a question of the copepod searching out its host by random movements, or perishing. Chance plays the greatest part in its ever meeting the host. The movements of the animal, therefore, are apparently adaptive. The copepod darts up and down, circles in this direction and in that. Its movements bring it into as many different localities as possible. If one path happens to bring it in contact with the necessary host, attachment immediately takes place, and the life of the individual and its progeny is secure. If, however, the organism does not meet the host, it tries other regions. The copepod perishes if its movements do not meet success within a given time.

V. REACTIONS TO CONTACT

Lernaeopoda is often found adhering to small bits of alga and other substances that float about loosely in the water. A glass rod or a needle may be slowly brought in contact with the body of the copepod, without producing the avoiding reaction. The organism may even adhere to the object for some period. When an object rapidly approaches the animal, it immediately shifts its position, thus avoiding the obstacle. Tapping or jarring a dish containing the copepod, even blowing on the surface of the water, or allowing a drop of water to fall directly above a resting copepod, calls forth the avoiding response; the animal quickly leaves its initial position and moves to other regions. When a copepod is picked up in a pipette and transferred to another dish of water, it almost always sinks to the bottom and remains motionless for a few seconds. Then it begins to dart about actively. In general, Lernaeopoda reacts positively to contact stimuli which are weak in character, whereas to strong stimuli it is negative.

VI. REACTION TO GRAVITY

Normally, Lernaeopoda responds positively to gravity. Its specific gravity is heavier than that of water, and it therefore tends to sink to the bottom. This was determined by the following experiment. Six copepods were placed in a test-tube filled with water and this was then tightly corked. When the tube was held in a vertical position, the organisms were observed to sink to the bottom, with their bodies almost vertical and their heads downward. As soon as they reached the bottom of the tube, the animals assumed a horizontal position, either ventral or dorsal, and began to move about actively. When the tube was reversed the larvae again sank to the bottom in their characteristic way. When the side of the test-tube was lightly tapped, while the copepods were passively sinking downward, they were observed to instantly dart upward again. However, as soon as the disturbing agent ceased acting, the animals again began to sink, until they reached the bottom, and here they moved about in their peculiar way. It becomes evident that this is not exactly a case of geotactic response but merely a result due to the high specific gravity of the copepod.

VII. REACTIONS TO LIGHT

1. *Behavior in daylight.*—Seven copepods were placed into a round dish of water, and allowed to remain near a window for some time. Soon all the organisms gathered near the window. Rarely did one leave the area. If a copepod, through its motion, happened to be brought into the opposite side of the dish, it soon began to adjust itself accordingly, swimming about actively until it was brought into the region of greatest illumination.

When a hand was passed between the window and the glass dish, the shadow caused the copepods to momentarily dart about. They did not, however, leave the most highly illuminated side of the dish, but actively moved about in it. The response in this case is similar to what Whitman (1898) has observed in the leech Clepsine. If a hand is passed over a dish containing a number of Clepsine, the shadow causes the animals to stretch in every direction, as if trying to reach something. Bateson (1887), has found that a passing shadow also causes a similar response in shrimps and prawns.

Obviously, this mode of behavior is of great value in procuring these animals their food. As Mast (1911) says, "The important point is that the shadow in itself is of no particular importance, but what follows may be."

In direct sunlight, the copepods were found to behave similarly. The reaction was observed many times and the positive phototropism was very striking.

2. *Behavior in artificial light.*—All experiments with artificial light were carried on during the evenings. The hatchery was situated a good distance from any of the neighboring dwelling houses, and it was also surrounded by tall trees, so that the laboratory was perfectly dark on the nights when experiments were conducted.

Five copepods were placed into a circular dish, eight and one-half inches in diameter, and this was allowed to remain in darkness for about an hour. The current was then switched on to a 60 c. p. Mazda bulb, which had previously been arranged in position about a foot from one side of the dish. When first illuminated, all the copepods were found to be at the bottom; but soon they began to dart about in every direction, ultimately making their way into the lighted area, where they remained. When the light was shifted to the opposite side of the dish, the organisms also changed their position. They moved about actively and in a few moments all had made their way across the dish, to the light side. An object, such as the hand, passed between the light and the dish, produced the same reaction as that brought forth by the shadow in ordinary daylight.

When an aqueous solution of alum was intercepted between the dish containing the free swimming copepods and the light, in order to absorb the heat from the rays, the organisms were found to behave similarly. Of course, the water in the dish served the same purpose to some extent. It is seen then that it is the light which causes the copepods to orient and not the heat produced by the electric bulb.

In another series of experiments twelve of the animals were used, and the same results were obtained. Here the electric bulb was slowly shifted in a circle around the dish, and the copepods slavishly followed the light. It is remarkable to note with what precision the organisms shift, when the position of the light is gradually changed. Time and time again, the animals made the complete round of the dish.

The definiteness with which the copepods orient to light is even more easily discerned when an oblong dish is used. Eight copepods were placed into a glass dish whose dimensions were eight inches long, one and one-fourth inches high, one and one-fourth inches wide, and were allowed to remain in the utter dark for about an hour. Then the current was switched on to a 60 c. p. Mazda globe, which was previously arranged in place at one end of the dish. The sudden flash caused the organisms to leave the bottom and to swim actively about in every direction. Shortly, however, they all made their way into the most highly illuminated region and remained there. Now a similar electric lamp, stationed at the opposite end of the dish, was turned on, while at the same time the first globe was extinguished. The copepods soon began to shift their position. One after another, they circled through the water, actively making their way from the dark side of the dish to the lighted region. In two instances, the organisms were timed. In the first case it took the copepods five minutes to traverse the entire length of the dish, while in the second instance the time was six minutes.

3. *Behavior in light of low intensity.*—In light of low intensity, the copepod does not react quite as definitely as in strong light. This was brought out by the following experiment. An oblong glass dish was used, whose dimensions were the same as those of the oblong dish used in the previous experiment. Eight copepods were placed into it, and with a 60 c. p. Mazda lamp these were attracted to one end. Then the current was turned on to a 1 c. p. bulb stationed at the opposite end, while at the same time the other globe was extinguished. But the organisms paid little attention to the faint glimmer. Two or three of them were observed to travel a very short distance to the light, but no definite orientation occurred. When a 4 c. p. bulb was substituted for the 1 c. p. lamp, the same behavior resulted, no specific orientation taking place. If, however, an 11 c. p. globe was switched on, the animals oriented definitely, but slowly They all moved to the illuminated side of the dish. A 16 c. p. bulb brought about this reaction more readily, and globes of higher intensity increased the positive behavior accordingly.

From these observations it becomes evident that the definiteness of orientation of the copepod varies with the intensity of illumination. Any increase in the illumination brings about a corresponding increase in precision of the specific reaction of the animal. Yerkes (1900), has noticed a similar behavior in Daphnia and Cypris.

The above experiments tend to bear out what was observed concerning the behavior of the copepods in their normal surroundings. While conducting hatching experiments it was noticed that whenever the covers of the hatching tanks were removed, the copepods would suddenly bob up to the surface of the water from underneath. This was observed many times and the regularity of its occurrence was very striking. The only explanation that could be found for this behavior was that the increase of illumination within the tanks attracted the animals to the surface. With this in mind, the foregoing series of light experiments was undertaken. The results have convinced me of the fact that in ordinary daylight, especially in sunlight, the free swimming copepods move about close to the surface waters. In weak light, and in total darkness they sink passively to deeper regions.

The migrations of water dwelling organisms is a problem that has been studied by a number of investigators. Giesbrecht (1892), in his splendid report on the pelagic copepods of Naples, states that Weismann (1877), was the first one to attribute the upward and downward movement of pelagic organisms to the responses of these animals to light of different intensities. However, the first investigators to determine this question experimentally were Groom and Loeb (1891), who worked on the nauplii of the barnacle *Balanus perforatus.* In the laboratory, these investigators found that when the young were subjected to light of strong intensity, they reacted negatively, whereas in faint light, they were positive. These reactions were similar to the behavior of the nauplii in the open sea, where the larvae are found clinging to the surface of the water at night only, while during the day they swim about in deeper regions. Based on these observations, Groom and Loeb came to the following conclusion: "Das starke Licht bei Tage treibt die Tiere in die Tiefe, das schwache Licht, das auch in der Nacht vom Himmel ausgesandt wird, zwingt sie wieder in die Oberfläche emporzusteigen."

Loeb (1893) has brought forth additional evidence among marine copepods as well as other animals, such as Limulus and the Annelid Spirographis, which tends to strengthen the theory that light causes the periodic migrations of pelagic organisms. In this paper, however, Loeb calls attention to other factors besides light, such as gravity, which may also play an important part in determining these periodic movements.

Parker (1901), in his studies on the daily migrations of the marine copepod *Labidocera aestiva,* found that light was the most important factor in the vertical movements of these organisms. "*Labidocera aestiva* frequents the surface of the sea from sunset to sunrise. From sunrise to sunset, it is presumably in deeper waters. Its migrations are explained as follows: Females rise to the surface with the setting sun, because they are positively phototropic to faint light and negatively geotropic; they descend into deep water with the rising of the sun, because they are negatively phototropic to strong light (their negative geotropism being overcome by their negative phototropism); the males follow the females in migration, because they are probably positively chemotropic toward the females."

Juday (1904), has studied the diurnal migrations of fresh water Plankton Crustacea, and found that most of these organisms come to the surface of the water at night, especially during the early part of the evening. Through most of the day, however, the animals occupied deeper water. He calls attention to the fact that diurnal movements are rather complex phenomena, and that they cannot be attributed to a single factor alone. Each lake presents different conditions, and these modify the behavior accordingly. The downward movement of the Plankton Crustacea he attributes to light, but he maintains that other factors, such as temperature, food, and the substances dissolved in the water may control the upward migrations of the animals.

Esterly (1907), working with Cyclops also emphasizes light as the directive agent in the migrations of this form.

It is thus seen that amongst marine as well as fresh water organisms we find a periodic migration. Light seems to play the most important factor in these movements. We must not, however, leave out of consideration such important factors as food, temperature and gravity, for these may also influence migrations, as was shown by Loeb (1893), Parker (1901) and Juday (1904).

In the case of Lernaeopoda we meet with an organism that offers an exception to the general rule of vertical migrations. Here we find the larva attracted by strong light, and it swims about close to the surface of the water during the day time, even overcoming its natural tendency to sink downward. At night, on the other hand, the animal does not react to weak light, and responds to the pull of gravity, thereby sinking to deeper regions. Clearly this behavior is of great value to the stability of the species. The migrations of the copepod are identical with the movements of its host. During the day trout generally feed near the upper surface of the water, whereas, at night they frequent lower regions.

VIII. REACTIONS TO HEAT

Loeb (1893), while experimenting with the copepod *Temora longicornis*, found that increasing the temperature of the water caused positively phototropic individuals to become negative, and vice versa; decreasing the temperature of the water brought about a change in normally negative individuals and caused them to become positive. Holmes (1901), found that increasing the temperature hastens, or may even induce, positive reactions among certain amphipods. This same author (1905), also found that an increase in temperature caused Ranatra to accentuate its positive phototaxis, while a decrease tended to produce the negative reaction. Yerkes (1900), could not induce any changes in the behavior of Daphnia and Cypris to light by varying the temperature. Parker (1901), also met with no success when he tried to induce changes in the light reactions of Labidocera by similar methods.

In the present experiments with heat, it was found that increasing the temperature of the water caused no change in the light reaction of Lernaeopoda. Six copepods were placed in an oblong dish of water, whose temperature was 58° F. These were then attracted to one end of the receptacle by a 60 c. p. Mazda lamp. At the opposite end, water that had been heated to near the boiling point was slowly poured at intervals a few minutes apart. As soon as the warmer water reached the animals they became very active, darting about rapidly; but none of them left the illumined side of the dish. As more hot water was supplied, the movements became slower, until at the temperature of 81° F. all of them were dead in the region of positive phototaxis. It was thus seen that increasing the temperature does not alter the behavior of the copepods to light.

IX. REACTIONS TO CHEMICALS

The effect of chemicals was next tried, with two purposes in view: first, to determine whether chemicals could reverse the behavior of the copepods to light, and second, to find out the solution of the chemical necessary to kill the parasitic organism while in this free-swimming stage of its existence. Only the first of these problems will be discussed here. The other will be dealt with in another paper on the economic aspects of *Lernaeopoda edwardsii*.

The chemical experiments were all performed at night, when the only source of illumination was a 60 c. p. Mazda globe stationed about a foot from the receptacle containing the copepods. Most of them were also confirmed in diffuse day-light. The observations were made in oblong glass dishes whose dimensions were eight inches long, one and one-fourth inch wide, and one and one-fourth inch high. The number of copepods used in each case was four or more.

Before applying a reagent, the copepods were first attracted to one end of the dish and then the chemical was slowly poured, drop by drop into the opposite end of the vessel. The following table gives a condensed statement of the results obtained. The sign + is used whenever the copepods reacted positively to light, whereas the sign — is used when the reaction to light was negative.

Chemical used	Lowest percentage of chemical causing death	Time required to kill copepod	Behavior to light until death
Sodium chloride	1.2	15 min.	+
Potassium chlorate	0.2	4 "	+
Calcium chloride	0.85	4 "	+
Hydrochloric acid	0.08	2 "	+
Sulphuric acid	0.015	3 "	+
Tartaric acid	0.45	2 "	+
Oxalic acid	0.3	2 "	+
Copper sulphate	0.2	5 "	+
Acetic acid	0.1	3 "	+ (indication of reversal)
Nitric acid	0.03	16 "	+ (indication of reversal)
Magnesium sulphate	1.6	45 "	2+, 2—
Hydrogen peroxide	9.0	18 "	—

Copper sulphate was found to affect the copepods differently during the day than at night. In the dark room a solution of 0.2 per cent copper sulphate caused all the copepods to die in about five minutes. In daylight, however, a two per cent solution of the chemical affected the animals very little. After remaining in this medium for twenty minutes they appeared to be as active at the end of this time as at the beginning. When the solution was increased to three per cent the organisms died in about four minutes. Evidently these differences in the behavior of the free swimming copepods depend upon differences in their physiological states, which may perhaps have been due to minute differences in the environment of the animals at the times when they were secured; perhaps the individuals used during the day were younger, and therefore possessed greater vitality.

In acetic acid, nitric acid, magnesium sulphate and hydrogen peroxide the copepods gave indications of reversal in their behavior to light.

Acetic acid.—Acetic acid was slowly added until the water became a 0.1 per cent solution of the chemical. As the acid reached the copepods they became very active. One of them circled clear across the dish from the side nearest the light to the opposite end. The next moment, however, it moved into the light again, where it remained till death. In three minutes all of the copepods died in the illuminated region.

Nitric acid.—This chemical was slowly added to a dish of water containing four copepods. When the medium became a 0.03 per cent solution, the animals became very active, darting rapidly about in the light for nearly ten minutes. Then two of them died, while the other two moved a little towards the opposite side. The latter reaction was but momentary, however, and the copepods again returned to the illuminated region. One now began to move away from the lighted side, but when it traveled about one-third the distance it returned into it again and remained there till death. No actual reversal took place although there were very strong indications of the organisms trying to avoid the chemical.

Magnesium sulphate.—These observations lasted from 8.22 P. M. to 9.10 P. M. When the magnesium sulphate was added to the water gradually, the copepods remained positive until the concentration reached one per cent. Here the addition of the chemical was suspended, and after a wait of a few moments, one copepod was seen making its way from the lighted region, while the others kept moving about within it. When a card was suddenly flashed between the dish and the light, the passing shadow caused the negative copepod immediately to become positive again. When more magnesium sulphate was added until the solution was 1.6 per cent, two copepods died, while the other two became negative. Passing a shadow between the light and the dish brought them back again into the positive region, but as soon as this ceased, they again became negative.

One persisted in remaining here, while the other copepod darted back into the lighted side of the dish. About three minutes later the organisms died.

The following evening this same experiment was repeated. When the concentration reached 1.7 per cent, two copepods became negative. One of these became positive again. The concentration of the solution was now increased until it reached a strength of four per cent, but the copepods behaved as before, one remaining negative, while the others were positive. Every now and then one of the positive three would dart away from the light, but the next moment it would again become positive. The copepods were not killed in this solution in thirty minutes. We thus meet a parallel with that noticed in the discussion of the reaction to copper sulphate,—a difference in the physiological state of the copepods.

Hydrogen peroxide.—This chemical caused a distinct reversal in the behavior of the animals to light. When the water became a nine per cent solution of the chemical, all of the copepods, which up to this time had been darting about in the lighted region, suddenly reversed their direction and began to move away from the light. One died almost instantly, while the other three traveled about three-fourths the length of the dish. The motion of these copepods now became very slow; their dorsal muscles were affected to such a degree that the swimming feet could no longer beat with enough force to propel them through the water. The mouth appendages also stopped moving, and fifteen minutes later the copepods died.

From these results it becomes evident that the chemicals used have little effect on the behavior of the copepods to light. In a few cases a reversal in the orientation was observable. This was produced by the addition of hydrogen peroxide, magnesium sulphate, nitric and acetic acids. In the last three cases, however, the reversal may have only been incidental, for but one or two of the copepods became negative while the others remained positive. Generally those that did become negative remained in this region but a short while, and soon returned to the illuminated territory. In all the tests, when the chemical reached the animals they became very active, as if trying to get away from something that was affecting them severely. But as the concentration of the medium was gradually increased, the motion of the copepods became slower, until death finally overtook them in the lighted side of the dish. The organisms are so strongly positive in their reaction to light, that they persist in going toward the source of greatest illumination, even though they are brought into regions that are dangerous to their existence.

X. INFECTION EXPERIMENTS

In the hatchery ponds, as well as in the natural streams *Lernaeopoda edwardsii* was found to attack the brook trout only. A great many rainbow trout as well as German-brown trout were examined and not a single case of infection by the copepod was observed. Furthermore, suckers that were kept in the hatchery ponds, as well as those thriving in neighboring trout streams were examined, but were found to be clean and healthy. Now the question arose: What explanation could be offered for this choice on the part of the copepod for the brook trout only? In attempting to determine this a series of infection experiments were undertaken.

Three groups of three copepods were placed into three separate dishes of water. Into each dish was then placed, respectively, the gills of freshly killed brook, rainbow and German-brown trout. The fresh blood of the gills soon began to diffuse in the water and the behavior of the copepods was noticed. The copepods in the first dish into which the gill of the brook trout was thrown, became very active, darting about in every direction, as if they were in search of something. In the other two dishes, which contained the gills of the rainbow and German-brown trout, no such reactions of the copepods were noticeable. Two of the animals came in contact with the brook trout gill and began to attach themselves. In the other two dishes no attachment took place, in spite of the fact that the organisms came in contact with the gills a number of times.

The manner in which the copepod attaches itself to the gills of the brook trout is very interesting. Wilson (1911), in his paper on the development of *Achtheres amblophitis* Kellicott, a copepod of the family Lernaeopodidae, parasitic on the gills of the rock bass, describes the attachment of the organism as follows: "Of course, it is practically impossible to actually witness the fastening of the larva. But what has been observed in the chalimus larva of the Caligidae, we can infer what occurs here. The outer end of the attachment filament is enlarged into the mushroom form already described, and is filled with adhesive fluid. It lies just inside the frontal margin, covered only by a very thin outer cuticle. Doubtless, the larva rubs its frontal margin against the skin of the gill arch of its host and in this way burrows through the slime and outer integument to the solid tissue underneath, holding on meanwhile with its powerful maxillipeds. At the same time the thin covering of the frontal margin of the parasite is broken through and the end of the filament is brought in contact with the gill arch, to which it adheres firmly. By moving away from the point of attachment the coiled filament is drawn out of the body of the larva. As it comes forth the larva grasps it between the claws at the tip of the second maxillae."

This process of attachment as surmised by Wilson, however, was not found to be the method by which *Lernaeopoda edwardsii* attaches itself to the gills of the brook trout. By the aid of the microscope I observed the process of attachment four times. As soon as the copepod comes in contact with the filament of the gill, its mouth parts are inserted into the flesh, and by means of the powerful claw-like second maxillae it begins to rasp the filament until it forms a cavity within it. As soon as this occurs, the anterior portion of the copepod's head, the frontal margin, is brought in contact with the cavity and the enclosed attachment filament is injected into the hole. The spherical mushroom body adheres to the flesh and the regenerating tissue of the gill soon encloses it tightly, thereby fastening the organism firmly. The mouth parts are then withdrawn from the flesh of the gill filament. In this condition the parasite remains attached for a short time. Then the second maxillae detach the posterior region of the attachment filament from the head margin and they themselves become permanently attached to this end of the filament. Degeneration soon sets in and the organism changes considerably. The female copepod remains thus attached throughout life, while the male remains attached in this way until shortly before it is mature for copulation.

Wilson believes that the transference of the filament from the frontal margin to the tips of the second maxillae takes place at the time of fixation to the host, and that the larvae are never

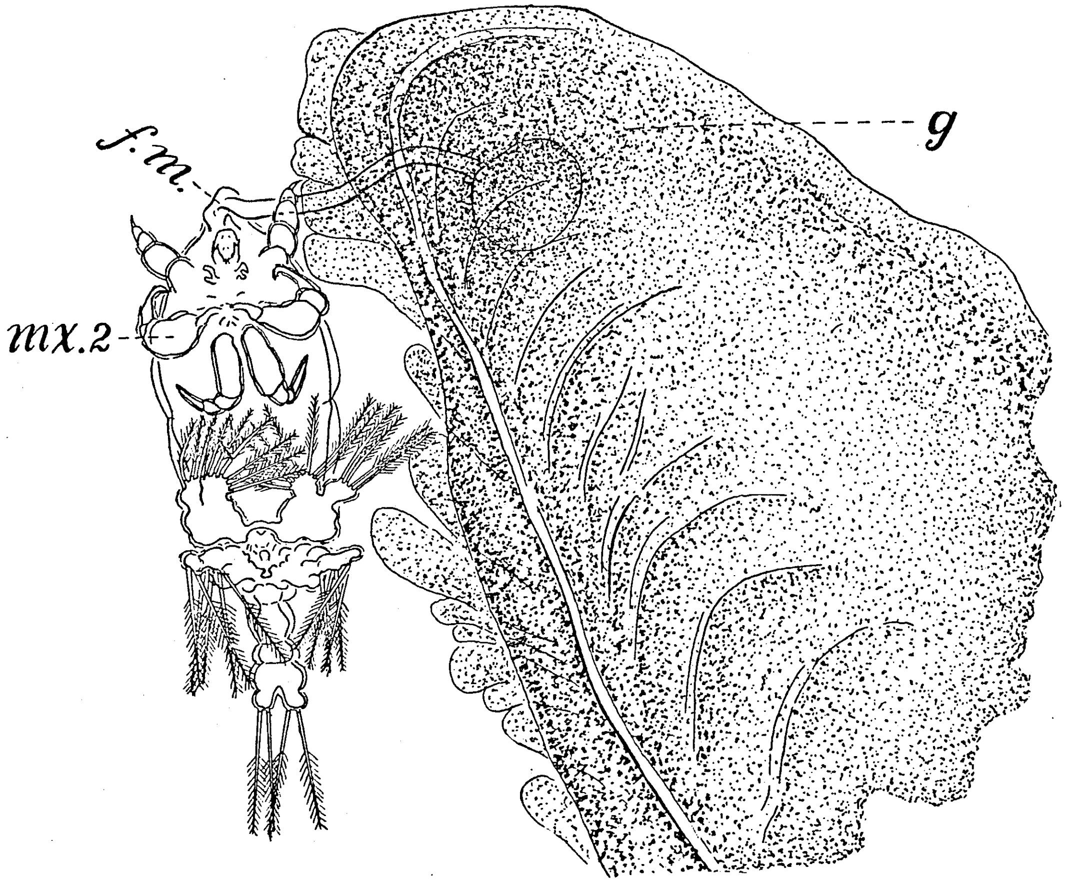

FIGURE 6. Lernaeopoda edwardsii. Larva attached by frontal margin, x 110
f. m. =frontal margin.
g. =gill.
mx. 2=second maxilla.

found fastened by their frontal margins. Figure 6 is a camera lucida drawing of a larva of *Lernaeopoda edwardsii* thus attached.

Let us now come back to the infection of the fish. In order to study the problem further, a floating cage was sunk in one of the hatching tanks, and into it were placed healthy specimens of brook trout, rainbow trout and perch. The fish were examined two days after the sinking of the cage and the brook trout were found to be the only species infected; the others were all clean. Five days later the perch died, but their gills revealed no infection by Lernaeopoda, their death was apparently due to a fungus which had developed on them. The rainbow trout were also found to be free from the copepod.

Lefevre and Curtis (1912), in their recent paper on the artificial propagation of fresh water mussels, note a similar preference on the part of the hookless glochidia of the genus Lampsilis for certain fishes. However, they have found that the glochidia may attach themselves to a number of different kinds of fish, although their choice for one is generally predominant. The glochidia are therefore not quite as exclusive in their choice as are the free swimming Lernaeopoda. The reactions observed in the glochidia and in the copepod are, undoubtedly, chemical in nature. In the case of the copepod there must be something in the blood or other secretions of the brook trout which attracts the parasite to this species of fish. This chemical substance, or substances, must be wanting in the other fish and they are, therefore, not parasitized by *Lernaeopoda edwardsii*.

XI. CONCLUSIONS

From the foregoing discussion it becomes evident that *Lernaepoda edwardsii* is well adapted to its parasitic mode of life. It moves about with a darting spiral motion, thereby covering a maximum of territory with the amount of energy it expends. In many respects this method of locomotion is similar to that of the hunter ciliates. The organism is so strongly attracted by intense light that during the day it frequents the upper regions of the water, though it is normally positively geotropic. In this position the copepod is very favorably situated, for the brook trout feed near the surface waters throughout most of the day, and hence the parasite is readily accessible to them. At night, on the other hand, the copepods are no longer attracted by light, and since they possess a greater specific gravity than the water, they passively sink to deeper regions. Here, also, their position is advantageous because after sunset the trout frequent the lower strata of water, and they are thus stationed close to the parasites where infection may occur and the life of the copepod thus insured.

XII. SUMMARY

1. *Lernaeopoda edwardsii* is a parasitic copepod which attaches itself to the gills of the brook trout. It attacks this species of trout only, the rainbow as well as the German-brown trout being immune from it. Undoubtedly, its reaction to the brook trout is a chemotactic one.

2. The free-swimming existence of the organism is very short, about two days at the most, and during this stage of its life history it swims about with a darting spiral motion.

3. When the copepod stops moving through the water, it sinks to the bottom in an upright position, with its head down. When it strikes bottom it may rest either on its ventral or dorsal side, depending entirely on which side of the animal strikes the bottom first. Undoubtedly the organism sinks because its specific gravity is greater than that of water.

4. Lernaeopoda is strongly positive in its reactions to intense light. In light of low intensity, however, it is indifferent. Because of its affinity for strong light, the copepod moves about near the surface of the water during most of the day. At night, the animal remains indifferent to weak light, and through its specific gravity it sinks to deeper waters.

5. Increasing the temperature of the water does not change the behavior of the organism to light.

6. Some chemicals have little effect on the behavior of *Lernaepodao edwardsii* to light. No reversal in the positive behavior of the larva could be induced through the use of sodium chloride, potassium chlorate, copper sulphate, calcium chloride, hydrochloric, sulphuric, tartaric and oxalic acids. In hydrogen peroxide, magnesium sulphate, nitric and acetic acids indications of reversal were noticeable.

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