Argulus foliaceus. A Contribution to the Life History.

By Frederick Noad Clark. *Read October 9th*, 1902.

Classification, Literature, and Introduction.

The subject of my paper this evening is *Argulus foliaceus*, of the class Crustacea, sub-class Entomostraca, family Argulidæ, genus Argulus. Biologically, it is more nearly related to the Copepoda than to any other family of the Entomostraca. So far as my information goes, *foliaceus* is the only species known in Great Britain, although others such as *A. coregoni* occur on the Continent, and *A. catostomi* in America. Furthermore one genus only is known. Little has been written in England concerning this interesting creature since Baird's account in his "Natural History of the British Entomostraca," published by the Ray Society in 1850. Short notes are given by Murray in his "Economic Entomology," and in Cuvier's "Animal Kingdom," vol. iii. Amongst other authorities may be mentioned Dana and Herrick, 1837; Gerstäcker, Jurine, 1806; Leydig, and Thorell. By far the best description is that of Professor C. Claus (Vienna), published in the "Zeitschrift für wissenschaftliche Zoologie," vol. xxv, 1875, in which he gives an exhaustive account of the development and organisation of *A. foliaceus*.

Commonly called the "fish louse," *Argulus* is found parasitic on various fresh-water fish, *e.g.* carp, roach, tench, stickleback, etc. It is from the *Gasterosteus aculeatus*, or three-spined stickleback, I have obtained my specimens. It is recorded to have been found on frog and toad larvæ, and on the minnow, but seldom on pike, perch, or salmon-trout. My observations commenced in 1896, since when I have had good opportunities of studying its life history, having repeatedly bred them from the egg to the adult stage, and so on again. By reason of its exceptional structural character, the elaborate nature of its apparatus for attachment to its host, the interesting details of its metamorphosis, and the curious organs and mode of reproduction, I venture to say that a study of the Argulidæ will well repay the scientific observer.

I will refer later on to a more detailed description of the anatomy of this creature, and for the present will describe it briefly as a transparent, jelly-like animal of a greenish hue, the bulk of whose body appears as a slightly convex carapace, which entirely covers the various organs with the exception of the eyes, swimming-feet, and tail (so called). The adult female measures 8 mm. long by 6 mm. broad. The male is slightly smaller, and may be distinguished from

EXPLANATION OF PLATE I.

ARGULUS FOLIACEUS.

FIG. 1.—Stickleback, with two Arguli (adult female) attached. Two thirds size.

FIG. 2.—Ova of *Argulus foliaceus*, photographed under water. Magnified 15 diameters.

FIG. 3.—Larva, just emerged. × 36 diameters.

FIG. 4.—Female larva, third stage. × 15 diameters.

FIG. 5.—Adult male. × 15 diameters.

FIG. 6.—Adult female. × 15 diameters.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

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the female by the absence of the ovary and the two black spots on each lobe of the tail, which are so evident in the female even at an early stage.

Development and Metamorphosis.

The egg (Fig. 2) is of a spherical ovoid shape, measuring ·35 mm., laid in rows three to six in breadth, of a pale yellow colour, and glued to its neighbour with an albuminous material. When freshly laid the egg is quite soft, and whitish opaque in appearance. The albuminous matter rapidly becomes coagulated, and is seen to spread over and extend outside the rows of eggs in short, ribbon-like processes, obviously for the purpose of adhesion to the object on which the eggs are laid. The egg is composed of three layers—the outer, tough, granular, and slightly brown in colour; the middle coat, which is much thicker, is more transparent, though somewhat granular in texture; the inner coat being transparent and membranous. The egg mass has the appearance of closely agglutinated starch granules almost crystalline and transparent. Previously to hatching, the eyes of the larva may be plainly seen, about the fifteenth day after oviposition. The contained larva makes its exit from the upper surface of the egg by cutting a longitudinal slit, the tail end first emerging.

The newly emerged larva (Fig. 3) measures ·8 mm. in length, and immediately sets forth in quest of a host, to which it attaches itself and commences feeding. Even at this early stage it is well provided with organs for this purpose, although in a much modified degree. The eyes are relatively large. The swimming-feet are undeveloped, their functions being undertaken by two pairs of limbs bearing setæ, and situated at the base of the eyes. These give to the larva a darting motion similar to the Daphnia. A powerful pair of triple-barbed hooks or foot-jaws (which are replaced at a later stage by suckers) answer the purpose for attachment to the host. There are two pairs of antennæ—the anterior, or first pair, consists of a simple hook bearing on one side of its base an organ resembling a palpus, its function being probably the same; the second pair has three joints terminated by a claw, and united at its base to the first pair of swimming setæ. I have no doubt that these two pairs of clawed antennæ are the means by which the larva makes its exit from the egg. Of these setæ, the anterior pair at the base of the second antennæ are situated on a stalk, and are four in number of equal length, with one smaller. Each of these is finely covered with minute cilia, and, being articulated at the base, may be moved in any direction by the owner. The second pair of setæ are at the base of the first pair of foot-jaws, and adjoin the mouth organs; they bear three setæ each as above. In this larval state the modification of the antennæ into swimming organs is a character of the larvæ of the higher orders of Crustacea. The second pair of foot-jaws in the

adult is represented by a very similar structure with five joints, having two terminal hooks and a taste-pad.

On the third day an important change takes place, when the argulus makes its first moult. It then measures 1 mm. The first and second pairs of setæ are shed, the second pair of clawed antennæ still remaining. The four pairs of swimming-feet proper are now developed, the basal joints carrying two others, each of which has hairs, one singly, the other divided into two branches, which in their turn are covered with minute secondary hairs, which require the higher powers of the microscope to resolve. The first pair of swimming-feet at this stage are more developed than the others, and up to the eighth day are the only pair in active motion. At the apices of the lobes of the tail-plate are two tubercles, each terminated by three hairs; at later stages these gradually recede between the lobes. Their use I am unable to determine. The first pair of foot-jaws have now four barbs to each claw, as well as an adjacent plain one. Surrounding the mouth, and attached to the base of the foot-jaws, are several chitinous teeth. These are probably used for making the incision in the body of the host prior to feeding.

The second moult corresponds to the third stage in the life of the larva, and occurs from the eighth to the tenth day after hatching (Fig. 4). The increase in size is but slight, being only 1·5 mm. The sex may now be determined by the presence of the black spots on the tail-plate in the case of the female. The male organs are not yet developed. The most notable change is at the base of the first pair of foot-jaws, which are now becoming enlarged preparatory to a remarkable change which occurs after the next moult. On the under surface of the front of the carapace, a few spines appear, whilst the swimming-feet are gradually developing hairs and performing their proper functions. The second pair of antennal hooks are still retained, and the tubercles at the extremities of the tail-plate have approached nearer the fork of the two lobes.

The third moult, or fourth stage, commences about the sixteenth day, and a considerable increase in size is noticed (2·5 mm.). The male organs are also evident. The second pair of antennal hooks are cast, as are also the first pair of foot-jaws. These have become modified into sucking-discs, which have been gradually developing for the past three or four days at the base of the foot-jaws. Portions of the hooks still remain attached to the margin of the discs.

About the twenty-fourth day the fifth stage begins. The most important change is the shedding of the hooks above mentioned, the merest rudiments remaining. The first and second pairs of swimming-feet have each developed a small, recurved branch, which arises from the base of the fork of the two main branches on the dorsal side. The growth is now very rapid.

A few days later a fifth moult takes place. The suckers have now thrown off the rudiments of the hooks, and the *Argulus* has reached the perfect state. Its measurement at this stage is from 4 to 5 mm.

EXPLANATION OF PLATE II.

FIG. 7.—Eye (left). × 72 diameters.

FIG. 8.—Antenna (left). × 36 diameters.

FIG. 9.—Sucker (front view). × 36 diameters.

FIG. 10.—Swimming-feet of adult female, showing the recurved branches. Left half section of thorax. × 12 diameters.

FIG. 11.—Genital organs of male (left pair), situated on the third and fourth swimming-feet. × 45 diameters.

FIG. 12.—Tail-plate of adult female. The two black spots are the receptaculi seminis. The papillæ referred to in the text lie between the receptaculi and appear as two spines with their apices pointing towards the middle line. × 24 diameters.

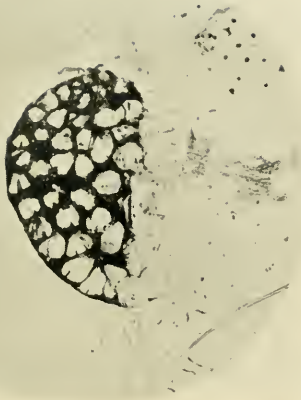

FIG. 7.

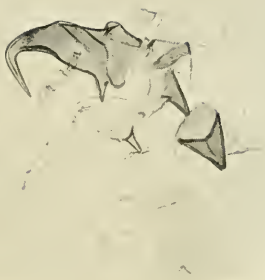

FIG. 8.

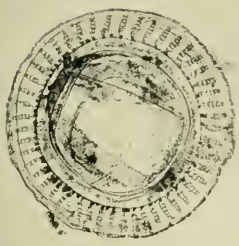

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

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Exuviation now continues at intervals of a few days up to and during adult life. The cast skin has a most perfect resemblance to the animal it has left, the minutest structures being retained in the cast. The suckers alone appear to be shed separately from the body structures. I have observed ten moults in the same individual, but the length of time elapsing between these is longer as the season advances and the temperature is lower. The early development also of all organs in the young is largely influenced by the chances of feeding and temperature. Opinions vary as to the age at which the larva enters the second stage; Claus mentions five days, and Baird six days after hatching, but my observations go to show that the first moult takes place on or before the third day.

Structural Description and Organisation.

The carapace or shield is of a transparent greenish hue, cordate in shape, the anterior margin being rounded off, the posterior having a deep Λ-shaped indentation, the angle of which is occupied by the thorax.

The eyes are of a deep violet colour, enclosed in a membranous sac, and are areolar, like those of the Branchiopodæ (Fig. 7). They are situated on the dorsal surface of the carapace immediately below the antennæ. This pair of eyes (for there exists another eye) consists of about forty facets or kernel-shaped crystalline bodies in each eye; the dark pigment patches radiate from the base in five rows, the interstices being of a greenish-yellow colour. There is a channel surrounding each, through which may be seen blood bodies in motion. The eye itself is capable of a slight twitching, rotary movement. The single eye above referred to has three convex corneal lobes encircling a triangular mass of dark pigment, and is placed in the middle line of the carapace on the dorsal surface, just above the mouth, at the apex of a triangle having for its base the paired eyes. Baird apparently described this organ as the brain, but its lenticular structure shows it to be without doubt an auxiliary eye.

The antennæ are on the ventral surface of the carapace immediately above the paired eyes, but nearer to the middle line (Fig. 8). They consist of two pairs, the upper pair having three joints each, the lower four joints. Attached to the upper pair are a pair of acutely curved hooks having sharp points. Under the microscope they are seen to be traversed by a channel. At their bases are four hard, chitinous teeth. The structural difference between male and female antennæ is slight; the latter are the larger of the two.

The mouth or siphon is a curious structure, and occupies a central position below the single eye. It has the appearance of a prominent pouch, and bears two pairs of mandibles and maxillæ. These are minute, transparent, serrated plates, which are placed at

the entrance to the œsophagus. There are also an upper and an under lip. The whole structure is controlled by strong chitinous tendons connected with the muscles of the carapace. At the upper end of the siphon we find a sheath containing a long telescopic proboscis, called a poison-sting by Claus. It is a beautifully slender and exquisitely pointed weapon, capable of movement in any direction ; it may be extended or retracted very rapidly. Its function is difficult to determine. Claus thinks that by reason of its irritating effect on the skin of the fish that it induces inflammation and a flow of blood to the punctured part. As will presently be shown, the blood of the fish forms the food of the parasite.

The thorax has four slightly defined segments, some say five, best seen in the larval state. These correspond to the four pairs of swimming-feet which are attached. It commences at the base of the mouth, and contains the large and small stomachs, and at its lower extremity the dorsal heart, where it is terminated by the tail-plate. In the gravid female the ovarium occupies the whole of its ventral surface.

Describing the feet in their proper order, one must commence with the pair of foot-jaws (so called). These arise from a little below the mouth on both sides, and lie in the middle of the carapace. Each consists of five cylindrical joints terminated by two claws and a fleshy, claw-like pad. Numerous spines cover the surface of each joint. To each basal joint are attached three strong chitinous teeth, whilst four similar teeth unattached to the foot-jaws lie between their bases. The foot-jaws have an independent movement, and in this respect differ from the four pairs of swimming-feet. Their use is apparently to assist the *Argulus* in attaching itself to the fish, and in locomotion. They undergo little change in structure from the earliest stage to the adult.

The swimming-feet are most important organs (Fig. 10). There are four pairs placed on either side of the thorax. From the basal joint of each foot proceed two branches of about equal length, bearing pectinate hairs. These latter have a secondary structure of beautiful plumose cilia, which may be stained with nitrate of silver to render them more visible. The first and second pairs in both male and female bear short branches, which arise from the basal joint near the fork of the two main branches on the dorsal side, and curve backwards. Some authorities state that these recurved branches occur also on the third pair, but the most careful observation on my part has failed to reveal such. In the case of the male, as will be afterwards noticed, the organs of generation are situated on the third and fourth pairs of swimming-feet. The corresponding feet in the female have no special organs.

The suckers are prominent organs, lying a little below the paired eyes, but farther apart. They are the structures referred to in my notes on the fourth larval stage, where they replaced the first pair of foot-jaws. They are fleshy, cylindrical appendages, cartila-

ginous, with membranous margins which are fringed with many jointed rays (Fig. 9). The extreme margin is ciliated and perfectly circular, its diameter measuring 0·72 mm. Four muscles are attached to each for the purpose of producing a vacuum in the sucker and thus enabling the parasite to hold on securely to its host whilst feeding, whilst ovipositing, or as claspers during copulation.

The tail-plate at the extremity of the thorax is a flat, oval, bilobed organ. Its use is primarily as a rudder. Some consider it to be an organ of respiration. In the male it contains a portion of the reproductory organs, which are called the testicles by some authorities. In the female it has two well-defined oval black spots (one at the base of each lobe of the tail-plate) (Fig. 12). They are called the receptaculi by Claus, and their presence readily enables one to distinguish the female. Numerous spines and glands appear on its surface; some of the latter are very curiously provided with a tail-like duct. Inside the margin of the lobes is a blood-channel.

The *Argulus* has an armament of hooks and spines of varying size and structure, the under surface of the carapace being studded with them. All point in one direction—that is, to the tail portion. There are some very curious spines on the under surface of the joints of the foot-jaws, which are probably some special sense organs; some are bifid and trifid, and not unlike the scales of the lepidoptera. Numerous gland-cells exist on the surface of the integument in addition to those on the tail-plate.

The genital organs of *Argulus* are probably the most interesting of all, and their position, structure, and method of copulation are remarkable.

The male genital organs are situated on the third and fourth pairs of swimming-feet on both sides of the thorax, and are thus in duplicate (Fig. 11). On the anterior margin of the first joint of the fourth pair is a brown-coloured, conical tubercle of a horny nature. Corresponding to this we find on the posterior margin of the first joint of the third pair of feet a vesicle called the semen-capsule, filled with a transparent fluid, apparently for fecundation. Previous to copulation this fluid is opaque and darker than the surrounding tissues. Five minutes after copulation this opaque matter becomes absorbed, leaving a transparent sac. In the middle line of the thorax and near to the base of the tail-plate is a dark purple-coloured organ, the contents being granular. This is called the semen bladder, and from it proceed two branches. The tail-plate, as before mentioned, contains two brown-coloured patches or so-called testicles in either lobe, but in what manner these and the last-mentioned structures communicate with the male fecundating organs is not very clear.

The female organs comprise the ovary, the oviduct, the two receptaculi, and the papillæ. The ovary of the gravid female occupies the whole of the ventral surface of the thorax. At puberty the outer coat is covered with numerous pigment-cells of typical structure.

The oviduct has its orifice between the fourth pair of feet. The receptaculi seminis are two dark-coloured, oval-shaped bodies situated at the base of each lobe of the tail-plate, and, as their name implies, are the receptacles for the male fertilising element (Fig. 12). Claus says emphatically that there is no connection between them and the ovary; that the female fertilises the ova as they pass from the oviduct, by inserting in them the papilla conveying the male element. This is more effectually accomplished by the upward motion of the tail-plate during oviposition, and thus pressing the point of the papilla into the egg. The papillæ referred to are situated on the dorsal side of the tail-plate immediately above each receptaculum, but a little nearer to the orifice of the oviduct. They are minute pointed hooks, which lie concealed within a pouch-like sac. They communicate with the receptaculi by means of delicate tubes.

Physiological Functions.

The circulatory system is in a rudimentary stage, so to speak. The heart or dorsal vessel is situate in the last segment of the thorax, immediately above the base of the tail-plate. Its pulsations take place once every second, when the blood, containing small round diaphanous globules, may readily be seen in circulation. The best situations for observing this are in the outer margin of the tail-plate and in the channels surrounding the paired eyes; beyond these there do not appear to be any definite vessels wherein the blood circulates.

The brain and nervous system are connected with all the vital organs of the body, the former being situated immediately above the single eye. Thence proceed, by means of central ganglia, branches to the paired eyes, antennæ, suckers, foot-jaws, feet, and reproductory organs.

The muscular system is well developed, particularly in the region of the suckers and feet, where the striped muscle bundles may be seen. There are several chitinous bands connected with the muscles, which actuate the movement of the carapace and mouth organs. There are also four oval-shaped chitin rings, two on either side of the carapace.

Concerning the respiratory system little is known. According to Claus there is no satisfactory ground for supposing that there exists a definite respiratory area; that the constant motion of the swimming-feet is probably connected with respiration, their structure being similar to the branchiopoda and their use the same. Some observers consider the tail-plate to be an organ of respiration.

The alimentary system is somewhat complex. The main canal, or stomach intestine, as it is called, commences at the base of the mouth organs and occupies about one half of the thorax. It is then joined by a smaller channel or intestine, which terminates at the anal opening. The large intestine has two main branches on either

side, which ramify throughout the larger portion of the carapace. It has been suggested that these ventral branches serve as a store for food, so that the *Argulus* is enabled to live for several days, or even weeks, away from its host.

The reproductory system will now be considered. The generative organs having been described, I will give a short summary of my observations on the copulation of the *Argulus*. This frequently takes place when attached to the fish, and at this period the female is seen to be full of ova. The male takes up a position on the back of the female and places the tail-plate underneath that of the female. The third and fourth feet of the male are seen to be clasped together at their bases, the branches of which are bent under. This position is taken up alternately with either lobe of the female tail-plate, and contact appears to take place just over the receptaculum. The corresponding feet on the opposite side of the male are held motionless during this time, whilst the first two pairs of feet continue their usual movements. Under the microscope the semen-capsule on the third foot of the male is seen from the dorsal side to be placed under the tubercle on the fourth leg. It appears to me that the semen is transferred to the receptaculum from the male semen-capsule by means of this tubercle, possibly by pressure thereon, but exact observation is very difficult, if not impossible. The change in the appearance of the semen-capsule from opacity to transparence, before and after copulation, has already been noticed. Copulation may be performed with either side of the organs of both male and female. Claus says that the female requires copulation on both receptaculi. Leydig is of opinion that the capsule of the male is placed over the papilla of the female. Copulation may last during several hours.

Oviposition.

Under natural conditions ovipositing commences about the end of April. There are at least two broods during the year. In confinement I have records of ova laid on November 29th and hatching on May 6th the following year; also others laid as late as December 27th, when the weather was very cold and frosty. On the other hand, some batches laid on August 30th and September 2nd remained over the winter and hatched on April 10th. I have also observed two batches of eggs hatched on February 27th, when the weather was mild, and continue hatching throughout March. *These became adult on June 27th, and laid ova which hatched on July 20th* (23 days).

Statistics which I have taken from twenty cases of oviposition show that the average time for the laying of the egg until hatching occupies *twenty-five days*, and hatching continues over three or four days. The opinions of competent authorities differ widely on this point. Temperature no doubt influences the length of period between oviposition and hatching, for I find that the later broods before hatching

occupy a longer time. Oviposition is also arrested if the Arguli are deprived of food. The number of eggs laid vary from 300 to 500, and I have a record of one *Argulus* of 633 eggs laid at intervals. Eggs are laid in rows of from three to six broad.

Whilst ovipositing the three first pairs of feet are in motion, the fourth pair being held rigid, with their bases (which are somewhat prominent) pressed together over the entrance to the oviduct, as in the act of straining. After depositing an egg the *Argulus* raises itself higher up on the object on which the eggs are laid, and, moving a little from side to side, causes the eggs to be placed in proper order in the several rows. The average time occupied during the depositing of each egg is eight seconds. I have already described under the heading of female organs the mode of self-fecundation of the egg by means of the papillæ, and I have only to add that the protuberances at the base of the fourth pair of feet materially assist the *Argulus* during this operation. The *Argulus* generally dies soon after completing ovipositing. I have noticed that ova laid in confinement are invariably fertile. My experiments were carried on by means of test-tubes blocked with cotton wool and placed in the shade at a moderate temperature.

Concluding Remarks on the Habits and Life History.

To the casual observer the most noticeable feature of the *Argulus* is the incessant motion of the swimming-feet, in which the four pairs participate simultaneously. The pair of foot-jaws have an independent movement. The *Argulus* frequently makes a series of somersaults, and when about to attach itself to its host the current of water caused by the movement of the fish's fins gently carries it on to the fish. When once attached by means of the powerful suckers, all efforts of the fish to shake it off are in vain. The movement of the swimming-feet just described is never arrested during life, even when the *Argulus* is at rest on its host, or when ovipositing. Occasionally one sees the *Argulus* maintain for an hour or more a whirling motion.

The food of the *Argulus* is blood-plasma, procured by means of the proboscis and mouth organs. The incision made on the fish is very minute, but a good deal of inflammation is evident. The favourite position of the parasite appears to be the gills, but it is also found on the fins and tail, underneath the gills, and even on the eyes. They remain attached without once leaving their host for as much as three or four days at a stretch; in fact, the newly-hatched Arguli immediately attach themselves to the fish, and only leave it to undergo their metamorphosis. On the other hand, the newly-hatched larvæ have been known to live five days without the host, and I have a record of an adult *Argulus* living fourteen days without food. Soon after the death of the host the parasites forsake it. If

hungry, or pressed for food, the stickleback will sometimes swallow the Arguli, but generally speaking they are avoided, and if swallowed are ejected from the mouth. Their abundance is probably due to their being distasteful to their enemies. Some I had in confinement were readily attacked by a *Notonecta*, and as speedily killed.

As to their capture, I have taken as many as a dozen on one fish, and about the middle of June, when fish were plentiful, nearly every-one captured had parasites attached. In captivity the Arguli, if numerous, will attach themselves to the number of thirty or forty on one fish, and needless to say the host lives but a short time after. I once took a young roach with two parasites attached, which remained so in captivity twenty-five days. The *Argulus* is frequently captured unattached, Mr. S. W. Kemp having supplied me with several specimens so taken. My observations as to their existence in the adult state during the winter have been corroborated by a capture made by Mr. Kemp in a pond at Alperton, under natural conditions, as early as March 15th. Winter eggs also exist, as I have previously mentioned. I am of opinion that delay in hatching and development is mainly a question of temperature, as, for instance—a batch of ova hatched on October 30th did not reach the third stage until November 28th, and the fourth stage on December 12th.

The average length of life of an *Argulus* is probably over six months, and I have kept several such. They are frequently victims of a filamentous fungoid disease precisely similar in its nature to that of its host. Vorticellæ also infest the *Argulus* from its earliest stages, some small captured specimens having been found almost entirely covered with colonies of Vorticellæ.

A remarkable capture of three *A. foliaceus* is recorded by Isaac C. Thompson, F.L.S., collected by Dr. G. H. Fowler from the Faeroe Channel, 1896-1897, in the "Zoological Society's Proceedings," 1898, showing that the *Argulus* will live in salt water. These presumably had been conveyed to sea by the medium of some fresh-water fish.

I have tried various methods of staining these transparent animals, with a view to a better differentiation of their structure, such as the aniline dyes, picro-carmine, osmic acid, hæmatoxylin, etc. Nitrate of silver, $\frac{1}{10}$ per cent., is the only reagent which has given me any satisfaction, and this only in staining the various ciliated organs. For their preservation as permanent specimens formalin is useless, as it renders the tissues opaque. Glycerine and water, equal parts, is the best means of killing them by immersion alive. For mounting as microscopic objects I can recommend Farrant's medium.

My specimens were taken from the Grand Junction Canal, Paddington.