

THE ATTACHED YOUNG OF THE CRAYFISH *CAMBARUS CLARKII* AND *CAMBARUS DIOGENES*

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A REMARKABLE fact in the life history of the crayfish is that the young associate with the mother for many days after leaving the egg, being at first firmly fastened to her and later going back to her for protection until finally quite independent.

As pointed out in *The American Naturalist*, March, 1904, *Cambarus affinis* molts twice while fast to the mother and leaves her only in the third stage. Some facts as to the character of this incipient family life in an American *Astacus* from Oregon will be given in another communication. The object of the present note is to describe the association of parent and offspring in two more species of *Cambarus* and to compare this with what is found in *C. affinis* and in *Astacus*. The illustrations are all of *C. clarkii*.

The young of *C. clarkii* were obtained from eggs laid in confinement by adults shipped from New Orleans, November 18, 1904; some 18 out of 61 surviving the journey. Two of these active, prawn-like and brilliant red crayfish, one male and one female lived in a shallow sink of warmish water during the winter and by March 25, 1905, the single female had the abdominal basket full of many very small and very dark-colored eggs. These eggs were already in the stage H of Reichenbach but differed from that in having the abdomen larger. Each egg was about $1\frac{1}{2}$ mm. in diameter and partook of the exceptionally vivid coloring of the adult, the large oil-like yolk drops being wine colored instead of yellow as in *Cambarus affinis*.

When received in November, three of the females examined had only minute yellow eggs in the ovaries and no sperm in the annuli, while the males had small testes but yet mature sperm in the vasa deferentia. It would thus appear that the season of conjugation may be late autumn or winter, and that of laying early spring, but this can be determined only by observations in the field.

By April 17 the eggs had become coated over with a dark deposit, but the embryo within was far advanced and easily escaped when pressure caused the egg case to spring open. With Zeiss 2. A. it was evident that the embryo was clothed in a loose cuticle, or cast off shell, which loosely invested the tips of the first and second antennæ, the chelæ, the walking-legs, the abdomen and thorax as well as the ends of the gills when torn out of the gill chamber.

These embryos were now essentially the same as when they hatched three days later. The eyes were almost sessile and with the pigment restricted to a narrow crescent and this pigment reflected yellow light but appeared black by transmitted light. The yolk was still a large dark mass of saddle-bag shape. The tips of the fourth and fifth legs were strangely bent back like hooks while the tips of the claws of the chelæ did not as yet seem to be recurved. All over the body the extremely dark crimson pigment cells again emphasized the agreement of embryo and parent in intensity of coloration.

But the detail of anatomy of the telson was the most important character for understanding the subsequent attachment of the young to the parent. The abdomen ended in a simple, flat, rounded telson that bore a row of simple spines along its posterior edge as seen with 2. D. in figure 3. The spines were fourteen or fifteen on each side symmetrically placed right and left, and a group of seven or eight of them on each side, near the median plane, seemed to push off the loose cuticle, which on the middle plane, was close to the body. The spines, or better, papillæ, were highly refractive and clear except that some showed granules and some vacuoles in their homogeneous contents. Some of them had small protrusions at the tips as if paste-like material had extruded from within.

The spines in the special group, right and left, converged, arched over, met and seemed grown together. On the animal's left the spines 7 and 11 were grown together at their tips while 8 and 10 seemed fused together at the tips into one continuous arch and the same was true of 9 and 12. With higher power, 4 mm. 4.45 comp. oc., tufts of fine threads, or fibrils were seen diverging from the tips of many spines to pass, posteriorly, often beyond the tips of other spines. Some of these threads passed out to the

loosened cuticle and seemed fastened to it. On the right of the specimen fine wavy lines suggested secreted films rather than fibrils.

As will be seen later these specialized spines are glandular structures that make the telson adhere firmly to the cast-off cuticle and thus make possible the "telson thread" of the hatching larva.

The small number of spines so grouped, together with the fact that the telson of earlier embryonic stages is incised on the middle of the posterior edge suggests that these 7 or 8 spines may be comparable to the 7 or 8 spines seen on each side of the incised telson of the lobster embryo (Fig. 72; Herrick; *The American Lobster*) before it molts at the time of hatching and is in a stage which Herrick compared to a protozoa, or other early larva. On this basis a very remote ancestral state has been retained to the extent that its spines have been applied to the new use of attaching the larva to its cast cuticle.

Before speaking of the hatching larvæ it must be recalled that all crayfish eggs are fastened to the pleopods of the mother by a hardening mass whose origin is somewhat in dispute. In *C. clarkii* the pleopods of the mother were so translucent that the transverse striation of the muscles was seen through the exoskeleton and with 2. D. the gland cells that are supposed to take part in fixing the eggs to the pleopods were seen as polygonal areas of secretion droplets separated by clear lines.

All over the bases of the pleopods these areas were massed together but the terminal part had them arranged in transverse bands that crossed the anterior face and extended into the sides but left the posterior face without glands. On the bands anteriorly were small tubercles each with a number of tubes passing from the glands to the surface and near these were some short, sharp setæ which occurred again at the tips of the pleopods.

While it is possible that these sharp setæ act in pricking the eggs and liberating an adhesive material as claimed by Williamson for crab's eggs, and that the glands of the pleopods have nothing to do with fastening the eggs, yet this seems very improbable, as the eggs are fastened to a large mass of material similar to the egg case and stalk and which binds all the long plumose setæ together and is most probably the product of the pleopod glands.

The small first pleopods also had glands and setæ like the others but were more colored with large, arborescent, red cells.

When fastened to the mother each egg was in a remarkably elastic case which had a rough, dirty outside layer and a clear inner layer containing the same microscopic droplets seen in *C. affinis* and similar to the droplets coming from the pleopod glands. Each case was continued on one side as a long stalk that in turn was continuous with the hardened secretion binding together the plumose setæ along the edges of the pleopods. The stalk was hollow though flat and wide and was a continuation of the dirty outer layer of the egg case, separating from the inner layer on one side to form a large hollow bell or tent.

Between the egg and the egg case was a variable amount of coagulum showing fibrils in it.

By April 17th some of the larvæ had hatched while others were not yet out of the egg cases. The young, figure 1, had the usual embryonic look of crayfish at hatching; a huge swelling of the head region owing to the presence of much yolk there; a weak development of the locomotor part of the head-thorax so that the five pairs of weak legs all arose posterior to the middle of the head-thorax; a weak, down-bent abdomen of little use in locomotion; eyes almost sessile and of little size or perfection. These larvæ were transparent and showed the heart beating rapidly and the scaphognathites rapidly baling the water out of the gill chambers. The dark area in figure 7 represents the dark red yolk mass; and the scattered dots, the aborescent pigment cells that were thickly scattered over the head-thorax and abdomen with but few upon the third maxilliped, base of antenna, three basal segments of the antennule and some few segments of the periopods.

Normally the larvæ remained upon the mother and did not move about, and when pulled off and put on the bottom of the dish they could not stand up but could progress by lying upon the side and flapping the abdomen.

At hatching, figure 1, the young were so weak they would have dropped to the bottom but for the "telson-thread" which is the cast off cuticle pulled out into a thread or band and fastened at one end to the telson of the larva by the special telson spines described above and at the other end to the inside of the egg case. As the egg case still remains fast by its stalk to the mother the larva is hung suspended from the mother till able to use its claws

and obtain a hold by them to the egg stalk or to parts of the material covering the plumes of the pleopods.

In hatching the larva escapes not only from the egg case but from its loose cuticle and this cuticle, where it covers the abdomen, is pulled inside out, but leaves the telson spines fast as before to the inside of the cuticle over the tip of the telson. The cuticle is so strong that larvæ may be picked up by the telson thread and their weight does not break it even when hanging in the air.

The attachment of the cast cuticle to the inside of the egg case seems to be an indirect one; apparently the larval cast cuticle is in some way fast to the egg membrane and that in turn adherent to the inner of the two layers that makes the egg case, but this was not definitely seen. In many eggs the embryo when young lies upon the side of the egg near the stalk and we suspect some relation between the region of fertilization and of stalk formation. Later, when the embryo hatches, it goes out back foremost through a crack in the case opposite to the stalk. In the old embryos the tip of the telson is carried forward to near the eyes and not far from the stalk of the egg case and in that same region of the egg is found the connection of egg case to embryonic cuticle. Possibly there may be some common factor, as gravitation, that determines at fertilization the position of the embryo, the place for formation of the egg stalk and the connection of egg case and larval cuticle.

The part played by the special telson spines in holding the larva fast to the telson thread is shown in figures 2 and 4, which show how the wrinkled telson thread is connected to fibrillar material, fastened to and interlocked with the long, curved and arched spines. In passing from the condition shown in figure 3 to that in figure 4 the cuticle over the abdomen has been pulled off and turned inside out and is now free from the larva except where held by the material furnished by the glandular spines.

When the young get hold of the mother pleopods with their claws they soon break the telson thread, but a short end of it long remains fast to the telson and the main mass still recognizable is fastened to the egg case.

In this first larval stage these young crayfish were about $4\frac{1}{2}$ mm. long from tip of telson to a point between the eyes where the rostrum turned down close against the head and was concealed between the eyes. The antennæ were $1\frac{1}{2}$ mm. long.

The accompanying camera sketches from specimens hardened in Worcester's liquid show the generally imperfect state of the appendages of the first larva, which lived for a few days an inert embryo-like existence fastened to the mother and not eating but only rapidly aerating and circulating its blood as the yolk was being transformed. The first antenna, figure 8, has only four segments in its exopodite and in its endopodite and agrees with most of the other appendages in being devoid of setæ. This bareness of the appendages of the first larval stage was first pointed out in the English *Astacus* by Huxley and seems common to all crayfish larvæ in their first stage. In place of setæ there are but a few spinules at the tips of the first antenna and on the basal segment there is a small ear-pit; but as yet the entire appendage would seem of no use as a sense organ.

The second antenna, figure 9, has only 24 segments in the slender part of its filament, beyond the three large broad segments, and the exopodite scale bears a blunt process and a row of few, sharp spines. The tubercle upon which the nephridial canal opens is, as in all young crayfish, proportionally very large.

The mandible, figure 10, has a smooth edge with no teeth and is probably not used. The first maxilla also, figure 11, is very simple and probably of no use.

The second maxilla, on the other hand, figure 12, bears the large scaphognathite which is very active in removing water from the gill chamber. The setæ along the edges of the scaphognathite, though represented in the figure as smooth, were in reality, under 2. D., set with five side branches so that in this only actively moving appendage the setæ are present as plumes that would seem to be of use in striking against the water and in making the appendage fit more closely into the passage leading out of the gill chamber.

The three maxillipeds, figures 13, 14, 15, are strangely lacking in setæ except upon exopodite of the first where there is a row of sparsely plumose setæ. The gills begin as a large podobranch and a slender anterior arthrobranch on the second, figure 14, and on the third, figure 15, there are two arthrobranches. The projecting lobe at the base of the epipodite, or lamina bearing the filaments of the podobranch, is conspicuously large in all larval crayfish and here bears a few, acicular setæ. Probably these

lobes and setæ aid respiration in making the inlet water more free from dirt.

The chelæ, figure 16, are long and strong but as yet not specialized as cutting organs. The tips of the claws are recurved as Huxley first found them to be in the English *Astacus* so that once shut upon a penetrable mass they could scarcely be loosened by the larva, figure 17. By means of these locking tips the young become fastened to the egg stalks and to the hardened secretion on the mother's pleopod setæ so that they probably remain fixed in one spot all the time they live in the first stage. The simple, acicular setæ seen along the edge of the claw, figure 17, may possibly aid in tactual reflexes to enable the larva to shut its claw on suitable substances.

The next two pairs of legs are very like the chelæ, but more slender, short and weak.

The fourth leg, figure 18, with no claw, has its arthrobranchs much reduced, the anterior having but slight protuberances to represent lateral filaments and the posterior being quite smooth.

The fifth leg has no gills at all associated with it; the pleurobranch of *Astacus* being absent not only in the adult *Cambarus* but in the earliest larva here as well as in *C. affinis* and, as long ago determined by Faxon, in *C. rusticus*. The branchial formula is thus the same in the larvæ as in the adults.

On the abdomen the appendages have the incompleteness of all crayfish larvæ. The first pair are not begun and the sixth pair are forming under the exoskeleton within the base of the telson. The other four pairs are very small and apparently quite useless structures each projecting towards its fellow crosswise under the abdomen and with the endopodite more anterior and the exopodite more posterior. As seen in figure 19, the endopodite is somewhat the larger and both endopodite and exopodite are very simple and show but slight suggestions of spines at their tips.

The telson, figure 2, is a simple, elongated, flat plate showing within its clear substance radiating lines ending at the marginal spines and also the outlines of the long exopodites of the sixth pleopods lying along on each side of the rectum and anus. On the ventral side, figure 1, the base of the telson is quite protuberant over the part of the enclosed pleopod that will be the exopodite.

In larvæ that have been in the first stage a few days and are about ready to molt it is obvious that the radiating lines in the posterior part of the telson are the glands secreting the setæ which will replace the marginal spines at the next molt. In a prepared section of the posterior part of the telson of such a larva, figure 5, the old cuticle is separated from the epidermis by a space across which the tips of the forming setæ pass toward the hollow bases of the old spines. Each old spine has a new seta beneath it but as there are also other setæ the second larvæ will have more setæ than the first had spines; the long plumes, however, figure 7, are slightly fewer in the second larvæ than the spines in the first.

Each developing plumose seta seems a flat plate ending in a fine central thread and with its edges frayed out in short fine threads. The base of each is deep within the epidermal ingrowth that forms the secreting gland. Each gland seems a row or rod of cells, indicated by large nuclei in a common protoplasm in which no cell walls were seen. The longitudinally striated base of the plumose seta forms the axis of the rod of cells. The space between the radiating glands was in part occupied by blood, staining, like the setæ, yellow, while the nuclei were red in borax carmine and orange G.

Similar, but less developed rods of cells were also seen in sections of the internal buds of the sixth pleopods where they were forming plumose setæ that projected into a bag surrounding the pleopod.

By April 24th, when some of the larvæ had begun to molt, it was evident that something was abnormal, as some larvæ in both the first and the second stages fell away from the mother and died. The mother also died, April 28th. The hatching was prolonged more than is probably normal so that many first and second stage larvæ were found side by side for a few days. Some of the young in the second stage remained with the mother for a few days but made excursions away from her and again returned as was the habit for the second stage in *Astacus leniusculus*, but it is not certain that this was normal in *C. clarkii*. While upon the mother these young held firmly with their chelæ, but they let go when the dead female was lifted out of the water. When upon the bottom of a dish they were able to stand up and walk feebly, and after a day, they swam backwards on their sides by flapping

the abdomen. They tended to climb over one another and one held so fast to a dead fellow that it could be shaken loose only with difficulty. They also climbed up on to the dead female and on to a piece of Canton flannel where they held fast by their chelæ for a time and then got down and swam actively if disturbed by a pipette.

Though the larva in the second stage may thus go away from the mother it doubtless returns even into the third stage as Faxon records finding upon the abdomen of museum specimens larvæ with characters evidently of the third stage.

The second stage young, figure 6, were still so translucent that in the abdomen the digestive tract and the ventral ganglia could be plainly seen.

In *C. affinis* it was noticed that the young, in passing from the second to the third stage, was suspended from its cast cuticle by an anal thread which bound its anus to that of the cast off cuticle and as the claws of that cuticle still held fast to the mother the young was prevented from dropping away from the mother till able to take hold again with its new claws. In *C. clarkii* the same arrangement may prevail though it was noticed only in two larvæ that died just after molting. In these there was a long thread that issued from the anus and, passing down through the hollow cast off cuticle of the abdomen, was fastened at the bottom of it to the flat telson. By the strain of the anal thread the cast off abdominal cuticle had been telescoped; the old telson being dragged up against the collapsing rings of the cast cuticle. As in *C. affinis* this anal thread was only the cuticular lining of the intestine not cast off entirely at the same time with the external cuticle and thus serviceable in binding the larva to its old shell. If this tardiness in casting the lining of the intestine is normal in *C. clarkii* it would seem a useful means of holding the young to the mother when soft and helpless at molting time, provided the young molts while upon the mother, which is probably the case even if it has some freedom of motion in the second stage.

In contrasting the second stage, figure 6, with the first, figure 1, we find an increase in size, the body being now $5\frac{1}{2}$ mm. long with antennæ 3 mm. long, and some advance in the proportions of the body and in the perfection of the limbs. While the head-thorax

still contains much yolk it is less swollen and more elongated while the abdomen is relatively larger and it is more useful as its telson bears a fringe of setæ.

The limbs are changed but little, yet they now bear some setæ though these are too small to show under low magnifications, figure 6. The rostrum is still triangular, but sharp, and though it is still bent down between the eyes it can be seen from a dorsal view and also from a side view, figure 6, where its base is visible near the eyes which are now decidedly stalked.

The first antenna had six segments in its exopodite and in its endopodite and the former bore five sense setæ, three on the terminal and two on the fifth segment. The ear was a wide open cavity with three or four finely barbed setæ along its external border.

The second antenna now had a long spine and a row of 19 or 20 plumose setæ on its scale and its filament contained 34 segments some of which seemed to be dividing.

The mandible edge was now no longer smooth but had six or seven teeth on its free edge and three above the palpus.

The spines at the tips of the chelæ, figure 20, stood at about right angles and were but slightly recurved. Along each edge of the claws was a row of peculiar spines having a thick edge and a narrow blade, figure 20, which tended to be cracked or striated across its length. These cutting or rasping spines are a specialization not found in the first stage when the claws are used only as hold-fasts.

On the abdomen no new appendages were found but the four pleopods present were now well provided with plumose setæ. The telson of the second stage, figure 7, compared with the first stage, figure 2, shows a great increase in size and the addition of a row of barbed setæ in place of the simple marginal spines. The sides of the telson protrude so much where the enclosed sixth pleopods have enlarged that its margin is somewhat three lobed; all the setæ are upon the middle or posterior lobe, and are symmetrically placed right and left. In the middle line there are no setæ; and right and left they begin short and suddenly reach the greatest length and then, as seen in figure 7, are long enough to make an efficient increase in the length and area of the telson as an organ for striking against the water in swimming.

In the figure the dotted lines represent the enclosed pleopods and in them the radiating setæ glands in which are forming the plumes to be expanded at the next molt. Studied in life with Zeiss 2. D. these glands were long tubes from each of which projected a plume, the tip of which turned to one side in the space between the edge of the pleopod and the enveloping sac. In the same way the posterior end of the telson showed long tubular glands forming a set of plumose setæ to replace those already present. The tip of each new plume projected slightly from its gland into the hollow base of the existing plume, which would be cast off with the cuticle of which it is a part, at the next molting. All these setæ seen in formation in long tubes are richly barbed plumes that later come into use when suddenly exposed to the water at the next molt.

Only some five or six of these specimens of *C. clarkii* survived to change into a third stage, April 29 to May 1st, but these agreed with all known crayfish of the third stage in having a complete tail-fan, with both telson and widely expanded sixth pleopods together forming a very large area for resistance to the water and set all along the combined edges with the above long plumose setæ.

Though these few individuals seemed weak they both walked and swam easily. The color had now become a darker flesh-color from the crowding of red pigment cells, but the area about the stomach was lighter and on each side of the stomach there was a small, narrow, dark band representing the yolk.

As above stated it is probable that in nature the larvæ in the third stage remain with the mother for a time, and then gradually become entirely independent.

While the conditions seem to have been so unfavorable for *C. clarkii* that the young were weak and probably somewhat abnormal in their actions this was not the case with the young of another crayfish, *C. diogenes* Girard as kindly determined by Walter Faxon. April 8, 1906, ten females with eggs in late stages, three females without eggs and twenty-two males were obtained from Talbot Co., Maryland, by a collector who stated that they usually breed in May and are caught walking about in ditches.

The eggs were nearly black, or upon a few females dark brown, and of great size, being $2\frac{1}{2}$ to 3 mm. in diameter, while in *C. affinis* they are $1\frac{3}{4}$ to 2 mm. and in *C. clarkii* only $1\frac{1}{2}$ mm.

The young were hatching upon six of these females May 22nd and just before this an examination of the embryos showed a delicate loose cuticle over each tip of the chela, over the abdomen, and over the body, and an egg opened in strong sugar solution, and then put into water showed a cuticle swelling up all over the antennæ, the chelæ and the abdomen. But when carefully dissected it seemed that this cuticle was not a case over each appendage but rather that it was a large bag over the thorax, a side pouch over the abdomen, a large side pouch over all the pereopods and a side pouch over all the gills. Probably, however, there are two thin membranes, an outer vitelline membrane of irregular form when stretched over the protuberant regions of the animal and an inner, real cast-off cuticle, that goes over each appendage; for some dissections showed the embryo inside a delicate spherical bag fastened to the inside of the egg case, and observations upon the hatching larvæ seemed to show them drawing out the limbs from separate envelopes.

At the end of the telson there were groups of spines fastened to the cuticle by refractive fibrillar coagulum. On each side a group of six spines arched over and connected very much as in *C. clarkii*, figure 3, and here the cuticle was thrust off further, while on the middle line it was close to the telson.

In one individual the actual hatching lasted forty-five minutes; the egg case cracked open opposite to the stalk and the embryo slowly "oozed" out back forward. During this process some movements of the legs were seen as well as a rhythmic pulsation of the lateral lobe of the liver lying close to the yolk mass on each side of the body, and swaying movements of the yolk mass. This tube was filled with yellow liquid for ten or twelve seconds and then grew narrow and white for about two seconds and again filled. It seemed as if the tube were contractile itself, but the yellow liquid may have been forced into it and so have caused it to distend. In either case the rhythmic filling would seem useful in aiding in digestion of the yolk, which was the only available food so far. Should it prove that the adult liver also rhythmically fills and empties it would be an interesting addition to the anatomical and physiological evidence advanced by H. Jordan (Pflüger's Archiv, 1904,) to show that the 'liver' is the chief organ for absorption as well as secretion.

As soon as out of the egg case the larva began to kick its legs and in a few minutes the scaphognathite slowly moved, stopped and began again, finally establishing a rapid rhythm. On adding carmine, the currents made by the scaphognathite were visible and its movement seemed comparable to a scooping motion of a hollowed hand, the fingers downward, thus forcing the water through the dorsal part of the respiratory passage as the fingers closed the lower part and then rising up to close the upper part and prevent a back set of water into the passage way.

Once out of the egg case the larva was still fastened to it by a telson thread consisting of a short string from the telson spines to a large crumpled mass that seemed a cast off cuticle and lay just within the gaping egg case and was fastened to it, inside, by the intervention of an expanded membrane which may possibly have been the old vitelline membrane. This membrane was bound to the inside of the egg case by a few short fibrils over a round area smaller than the base of the egg stalk and often near it. Thus suspended the larva moved its legs weakly and now and then shut its claws and violently flapped its abdomen without breaking loose from the telson thread. Soon the larvæ became fast by their claws to the egg stalks or to the material on the plumose setæ of the mother's pleopods.

In this first stage the larvæ remained cowered down close to the pleopods and were so firmly fastened to the mother by their claws that they did not break loose when the pleopod was thrown into Worcester's liquid, though they jerked their legs and powerfully and violently flapped their abdomens. Those left locked to the pleopods of the mother lived three to four days and then molted into a second stage, May 26.

They were very large, 5 to 6 mm. long when stretched out and $4\frac{1}{2}$ mm. as they lay with the weak abdomen carried forward under the thorax and were very attractive objects because of the swaying of the dark red and golden yolk mass, the contractions and change in color of the lobes of the liver spread like the fingers of a hand deep in over the back, and of the fiery, ruby-red, neuron-like, branching pigment cells spangled over a body so translucent as to show the white blood corpuscles hurried along the vessels over the red yolk, along the sinus at the edge of the carapace and out and in through the legs and antennæ like shuttles.

Camera lucida sketches of the first larva of *C. diogenes* showed it larger than even the second stage of *C. clarkii* but in simplicity and proportions essentially like the first stage. As usual in hatching crayfish the appendages were almost all bare of setæ; the eyes were nearly sessile; the rostrum a small triangle close to the body and between the eyes. The yolk far forward in the head-thorax distended that region and left the region for the gills and pereopods of less extent.

In the first antennæ there were four segments in the larger, club-like exopodite and also in the slender, smaller endopodite and there were no sensory setæ.

The second antennæ were carried curved backward and downward but not close against the thorax as in *C. affinis* and each had only spines upon its scale and 35 segments on the slender part of the filament.

The mandible had no teeth but its edge was very slightly waved where the epidermal cells seemed about to secrete slight thickenings.

The scaphognathite used as a baling organ also was exceptional amongst the appendages in bearing plumose setæ which formed a row along the edge and were longer and more easily seen than in *C. clarkii*.

The gills were larger and with more side filaments than in *C. clarkii* but were suddenly reduced upon the fourth pereopod so that the anterior arthrobranch had but a few filaments and the posterior none. On the last thoracic somite there were no gills, as is the case in all the young of *Cambarus* thus far studied.

The four pairs of pleopods had the endopodites slightly longer than the exopodites and the entire appendage was very much longer than in *C. clarkii* and with evident spines on both tips.

The telson with its enclosed pleopods was very much like that of *C. clarkii* and bore on its posterior edge the same kind of spines, about 14 on each side, six of which were specialized gland ducts arched over and joined together and bound to the telson string very much as in *C. clarkii*, figure 4. Inside the telson there were again the radiating glands making the plumose setæ of the second stage and a day before molting, the tips of the plumose setæ extended from their glands so far along, posteriorly, between the

epidermis and the loosened cuticle as to pass by several spines. Each new seta had its lateral barbs closely appressed against its axis. Moreover the new cuticle extended inward to line part of the seta gland so that in macerated specimens these cuticularized tubes ending abruptly, strongly recalled the like tubes that go in along the setæ of the earth worm. Probably at molting these sleeves become everted and so allow the sudden extension of the setæ to a length equal to the length of those tubes added to the length of setæ already lying between the old and new cuticles.

Molted into a second stage the young *C. diogenes* were $8\frac{1}{2}$ mm. long, $1\frac{1}{2}$ wide and 2 deep and had antennæ 5 mm. long. Until the next molt—some five days, May 26th to 31st,—they remained upon the mother's pleopods, but were not so firmly fixed as before as they fell off when put into Worcester's liquid.

While in most respects the larva was essentially like the second larva of *C. clarkii* a number of differences were noted.

The rostrum was less bent down than in *C. clarkii* and was long and pointed and visible from the side as its tip extended out beyond the eyes; its sides moreover were not straight as in *C. affinis* but arched so that something of the adult character was already expressed.

The first antenna was yet concave on the upper side of its base to fit against the eye and had in it a large open ear pit bordered externally by a few small spines and one very imperfect plumose seta. Beneath these spines the plumose setæ of the third stage were seen in formation. The exopodite bore seven sense setæ, three on the sixth and two on the fifth and on the fourth segments. The exopodites and endopodites were each divided into six segments.

The second antenna had some 13 to 15 plumose setæ on its scale and 38–42 segments on its filament.

The tips of the chelæ were still slightly recurved but as above noted the larvæ did not seem very firmly fastened by them.

Though the telson was larger than in *C. clarkii* its fringe of sparsely plumose setæ were noticeably shorter. There were about twenty on each side. In the base of the telson the large sixth pleopods showed a long exopodite, with a transverse joint, suggesting an index finger lying along the side of the telson while the endopodite was bent crosswise like a thumb.

A dissection of one of these second larvæ revealed a mass of membranous material and both simple and plumose setæ in the intestine suggesting that these larvæ may eat the egg cases and setæ from the mother's pleopods.

The creature was still translucent enough to show the ventral ganglia through the exoskeleton of the abdomen and was dotted over with pigment cells of stellate form, which when expanded were light red and when contracted very dark, while deeper in were diffuse and indefinite blue cells. In the antennæ and legs as well as in the antennal artery the corpuscles were going outward rapidly and returning somewhat more slowly in wider vessels.

Two days before molting into the third stage the new inner cuticle was already formed and the new setæ projected into the bases of the old. The yolk had become reduced to a small dark remnant on either side and even to the naked eye the gastroliths were conspicuous as two pink-white opaque areas, one on each side of the stomach enveloped in a clear glassy coat.

The third stage began by June 1st and had the adult character of a tail-fan made of the telson and the fully expanded sixth pleopods all fringed with perfect plumose setæ. These larvæ were $10\frac{1}{2}$ to 11 mm. long, 3 wide and $2\frac{1}{2}$ deep and expanded the tail-fan about 4 mm. while the antennæ were 6 mm. long.

These third stage larvæ when recently molted were still somewhat translucent and of a faint pink color with red-tipped claws and though the stomach was plainly visible the gastroliths were lacking on the outside. But within the actively moving stomach was a brownish liquid containing white particles or in some cases whole gastroliths moved about actively. In some cases the intestine contained colored material in its anterior part.

The specific gravity of the larvæ had so changed that they now floated in Worcester's liquid though the first and second stages sank; they were also less resistant to this fluid and died more quickly than when younger.

When the larvæ had changed into the third stage it was noted that the six mothers no longer had egg cases and cast cuticles upon their pleopods and as their fæces contained parts of plumose setæ of adult size it may be that they aided in cleaning off their pleopods though there is some evidence that the second larvæ may eat off

that material and Soubeiran stated that the young of an *Astacus* ate the egg cases and larval skins.

The third stage larvæ stayed near the mother some ten days or more, often, when disturbed, climbing on one another and crowding under and upon the mother, but after that they were quite independent and seemed to have no association with the mother though kept in the same small aquarium.

Walking and swimming the young sought food over the bottom of the aquarium and in a day cleaned off all the brown deposit from a spray of *Myriophyllum* and when another piece was given them ravenously set about tearing off and eating the bacterial slime and algal growths. When given *Chara* they seized an internode with their mouth parts and pushed it with their feet somewhat as a dog gnaws a bone, but when pieces of internode were cut off for them they seized them by one end and walked about sucking the contents out. Such a larva holding its head high and supporting a stick longer than its body, held by its mouth parts straight out in front of it, ludicrously suggested the enjoyment of a huge stick of candy. Animal food in the shape of a dead comrade was eagerly seized and pulled to pieces and a small earthworm was eaten up in a few hours.

Living thus, at a temperature of 85° F., the young were very active and darted away from the shadow of an object outside the water. After two weeks some molted without much change of size but by July 3rd some were 13 to 15 mm. long and the only survivor, July 15th, 18 or 19 mm. long.

From the above account it appears that the young of *Cambarus clarkii* and *Cambarus diogenes* associate with the parent in the first and second stages and in part of the third and this sort of family life is aided both by special recurved tips on the chelæ and by a peculiar telson thread; and as this is true also in *C. affinis* as well as in an *Astacus* of France and one in Oregon it is probably a general fact for all species of these two genera. Moreover all these crayfish show in the young structural characters and habits that make them unfit for free life like that of their marine relatives, the lobsters, and better fit them for a life of protected association with the mother with whom they live as in a kind of elementary family.

In this departure from ancestral conditions *C. affinis* has gone farther than *Astacus* in the following respects. In the first stage and in the second stage the telson is more reduced and both pairs of antennæ are more simple and to some extent this is also true in the other species of *Cambarus* here described. Thus in the first stage, *Astacus* has 50 to 66 spines along both the posterior and lateral edges of the telson while the three species of *Cambarus* have spines only upon the posterior edge and they are less than 30. *Astacus* also has in the first stage five segments in endopodite and exopodite of the first antenna and 50 in the filament of the second antenna while the three species of *Cambarus* have but four in the first case and 25 to 35 in the last.

In the first larval stage the three species of *Cambarus* thus agree amongst themselves and depart from *Astacus* in the direction of simplicity which is presumed to be a secondary reduction in connection with protected life upon the mother.

In the second larval stage *C. affinis* alone has spines merely and no plumose setæ upon its telson and is thus most remote from fitness for the active life of its ancestors. In the second stage *Astacus* is most like a free form in having its telson fringed with much more perfect and numerous plumose setæ than are found in *C. clarkii* or *C. diogenes*. In *Astacus* also the first antenna has its ear-pit well overarched by a row of plumose setæ but in *C. clarkii* there are only 3 or 4 plumes, in *C. diogenes* but one plume and in *C. affinis* only minute spines and no plumes. In *Astacus* the second antenna has 54 segments, in *C. diogenes* about 40, in *C. clarkii* 34 and in *C. affinis* 39. In *C. affinis* alone is there a retention of simple spines such as occur in the first larval stage, so that the scale of the second antenna here still bears no plumes.

Thus in the second stage *C. affinis* is most removed from *Astacus* but *C. diogenes* and *C. clarkii* depart less from the ancestral *Astacus*-like form.

Likewise in habit the three species of *Cambarus* agree in remaining attached to the parent during the second stage but in *Astacus*, however, as far as known, the larvæ become free in the second stage.

Apparently also *Cambarus* is more fitted to family life than is *Astacus* by having the anal thread at the time of passing from the first into the second stage.

From consideration of the larval life we come to the same general conclusion as that generally drawn from study of adult anatomy and geographical distribution, namely that *Cambarus* is a more highly evolved form than *Astacus* and that *C. affinis* is one of the higher, more specialized forms of the genus.

As to the relative position of *C. clarkii* and *C. diogenes* there is, however, doubt and discrepancy. The adult characters seem to leave no doubt that *C. clarkii* is much the more primitive, less specialized and more like *Astacus* of the two. But in the adjustment of the larva to family life *C. diogenes* would seem to have progressed less far than *C. clarkii*, at least in the first stage *C. diogenes* has more segments in its second antenna and in the second stage more sense setæ in the first antenna as well as more segments in the second antenna. On the other hand *C. clarkii* would be more primitive in having more setæ over the ear-pit and if in nature the young actually get loose from the mother in the second stage they would be more like *Astacus*.

Yet in future study of crayfish it would seem that regard should be paid to the first three larval stages as aids in determining the relative positions of the species and their probable derivation from ancestral forms.

From a thorough study of larvæ of many species and from experiments in cross breeding some idea might be got as to the nature of the causes that seem to be leading some of the more evolved crayfishes to develop further that association of parent and offspring which forms in the crayfish a simple stage in family life.

December 20th, 1906

EXPLANATION OF PLATES.

All the figures were drawn with camera lucida and the Zeiss lenses indicated and were reduced to one third in diameter. They represent the first and second stages of *Cambarus clarkii*. Figures 1, 3, 6, 17 are from living and the rest from preserved specimens, fixed in Worcester's liquid.

- FIG. 1. Larva just hatched from egg case with which it is connected by the telson-thread: 2.90 mm. a a.
- FIG. 2. Dorsal face of telson of first larva with attachment of telson thread and internal pleopods, etc. 2. A.
- FIG. 3. Dorsal face of end of telson teased out from egg about to hatch, showing investing cuticle over the glandular marginal spines. 2. D.
- FIG. 4. Enlargement of part of such a view as Fig. 2, showing attachment of telson-thread to certain marginal spines. 2. D.
- FIG. 5. Composite of surface views and sections of part of margin of telson of first stage. 6.4 mm.
- FIG. 6. Right side of living larva in second stage. 2.90 mm. a a.
- FIG. 7. Dorsal face of telson of second stage. 2. A.
- FIG. 8. Dorsal face of left antennule of first stage. 2. A.
- FIG. 9. Ventral face of left antenna of first stage. 2. A.
- FIG. 10. Outer face of left mandible of first stage. 2. A.
- FIG. 11. Outer face of left first maxilla of first stage. 2. A.
- FIG. 12. Outer face of left second maxilla of first stage. 2. A.
- FIG. 13. Outer face of left first maxilliped of first stage. 2. A.
- FIG. 14. Outer face of left second maxilliped of first stage. 2. A.
- FIG. 15. Outer face of left third maxilliped of first stage. 2. A.
- FIG. 16. Posterior face of left chela of first stage. 2. A.
- FIG. 17. Dorsal view of tip of chela of first stage alive. 2. D.
- FIG. 18. Posterior face of left fourth pereopod of first stage. 2. A.
- FIG. 19. Anterior face of a left pleopod of first stage. 2. A.
- FIG. 20. Tips of chela of second stage. 2. D.



