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THE YOUNG OF THE CRAYFISHES
ASTACUS AND CAMBARUS

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BY

E. A. ANDREWS

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Commission to whom this memoir has been referred :

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ADVERTISEMENT.

The present memoir by Professor E. A. Andrews, of the Johns Hopkins University, on "The Young of the Crayfishes *Astacus* and *Cambarus*," forms part of Volume XXXV of the Smithsonian Contributions to Knowledge.

The memoir describes and illustrates the young of two kinds of crayfishes, one from Oregon and one from Maryland, which represent the two most diverse forms found in North America. Of these, one genus, found only in North America, is widely distributed all over the United States, except the Pacific Slope; while the other is restricted almost exclusively to the Pacific Slope in North America, and at the same time it is almost the only genus in Europe and Asia. This memoir fills a gap in the knowledge of these common animals that still remained notwithstanding the extensive researches of Huxley and many others.

It determines the form and habits of the first, second, and third larval stages; gives the first detailed description and illustrations of the appendages of the first and second stages; describes the hitherto unknown nature of successive mechanical attachments of the offspring to the parent; and opens up the problem of the nature and causes of the incipient family life in the crayfish.

The new facts and comparisons add to the data for solution of the important problems of the geographical distribution and the origin of the species of crayfish, and they furnish a basis for practical application to the problems of artificial culture and introduction of new kinds of crayfish.

In accordance with the rule adopted by the Smithsonian Institution, the work has been submitted for examination to a commission consisting of Dr. Walter Faxon, of the Museum of Comparative Zoölogy, at Cambridge, Massachusetts; Prof. W. K. Brooks, of the Johns Hopkins University, and Prof. W. P. Hay, of Howard University, who recommended its publication in the present series.

CHAS. D. WALCOTT,
Secretary.

SMITHSONIAN INSTITUTION,
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The Young of the Crayfishes *Astacus* and *Cambarus*.

By E. A. ANDREWS.

INTRODUCTION.

The object of the present paper is to figure and describe the external forms and the appendages of the early larval stages of the crayfishes *Astacus* and *Cambarus* and to illustrate the details of the connections that exist between these larvæ and the mother.

It is well known that the genus *Cambarus* is found only in North America and here only to the east of the Rocky Mountains, while the genus *Astacus* is the only one found in Europe and Asia and in America is found almost exclusively west of the Rocky Mountains. It is thus natural that the history of scientific knowledge of crayfishes has been first the study of *Astacus* in Europe and later the study of *Cambarus* in the eastern United States. The *Astacus* of the Pacific States remains less well known.

Despite all the work that has been done upon these common animals, several parts of their life histories have received scant attention. The geographical distribution and systematic description have been studied in detail by Hagen, Faxon, Huxley, Ortmann, and others; the embryology minutely observed by Rathke and by Reichenbach; and the general knowledge of crayfish natural history added to again and again since the days of Roesel von Rosenhof. Yet little attention has been given to the study of the young crayfish after it leaves the egg; a comparative neglect that naturally arose from the centering of scientific interest upon the larval changes of marine crustacea in which remarkable metamorphoses occur.

When these metamorphoses were established by Vaughn Thompson and others it was already known from the work of Rathke that the crayfish hatches from the egg in essentially the adult shape and thus passes through no series of metamorphoses. Interest in the crayfish young was then restricted to the fact that it was exceptional in having no metamorphosis. In the preoccupation of students of crustacean life histories in study of metamorphoses the young of the crayfish were left without any illustrations excepting only those given by Rathke to show the condition of the embryo when nearly ready to hatch and the two wood cuts given by Huxley. These latter illustrations were evidently

made from specimens preserved in alcohol, and, however excellent for their purpose, fail to give the just proportions of the larvæ, since all alcoholic specimens of young crayfish are much swollen and distorted.

While two more figures of the young crayfish have recently been given, (Andrews, '04) details of external structure are still lacking.

Having obtained the young of both *Astacus* and *Cambarus* by hatching the eggs in the laboratory, it seemed well to fill in some of the gap in our knowledge of the early larval life of crayfish, and especially so as the details of a curious mode of connection with the parent were here first made evident.

HISTORICAL.

A brief statement of the history of our knowledge of the young of crayfish will show that as yet the character of the appendages in the early stages, the exact number of stages present in the life of the young while associated with the mother, and the nature of the means by which the young are held attached to the mother have waited discovery and illustration.

To Roesel von Rosenhof belongs the credit of an enthusiastic appreciation of the care of the mother crayfish for the young; the observation that the young are transparent and like the parent; the description of their crowding upon the abdomen of the parent and of their finally forsaking her after a few days, during which, however, they would return to her at times as if recalled by a signal.

Rathke ('29) was chiefly concerned with the embryology of the crayfish, but also described something of the growth in proportions and in internal anatomy of the young after hatching and gave figures of the embryo and of some of its appendages shortly before hatching.

Reichenbach ('86) also added to his classic study of the embryology only a figure of the abdomen of a recently hatched larva.

Soubeiran ('65) measured young crayfish grown at the farm of Clairfontaine and recorded their moultings and rates of growth. More facts of this same kind were gathered by Chantran ('70, '71) from prolonged study of larvæ reared in the laboratory of M. Coste. Chantran also discovered a peculiar filament that held the young to the egg-shell after hatching and he finally convinced himself that the young ate their egg shells and their cast-off skins. Some other observations upon the size and times of moulting of young crayfish in Sweden were also made by Steffenberg ('72).

Huxley ('79) illustrated the recently hatched larvæ by two wood cuts, one showing the early larva fastened to the maternal pleopods by its peculiarly re-curved claws, which he first described and figured, and the other a dorsal view of such a larva. He rectified the previous statement that the young at hatching are exactly like the adult and pointed out their differences in lack of setæ,

in lack of abdominal appendages on the first and sixth somites, and in various proportions.

In America Hagen ('70) in his fundamental study of our crayfish observed the young of *Cambarus* attached to the mother in alcoholic specimens and recorded the dimensions of young in which the sexes were externally recognizable. From like specimens Faxon ('85) in his revision of American crayfish was able to make a number of comparisons with specimens of young *Astacus pallipes* from France, and also to demonstrate the important facts that the young *Cambarus* agreed, in general, with *Astacus* as described by Huxley, but that in the early larva as in the adult, *Cambarus* showed no vestige of the gill found on the last thoracic somite in *Astacus*. These facts were verified by Steele ('02), who for the first time described living larvæ of *Cambarus* hatched from eggs in the laboratory and also recorded facts as to the habits and sizes of young larvæ. From like living material Andrews ('04) added details of hatching and behavior of these larvæ with figures of side views of living larvæ in the first and in the second stages and noted the occurrence of two successive attachments of the larvæ by means of special structures.

To complete that last preliminary paper by adding details and many new facts observed in *Cambarus* and to describe similar stages in our American *Astacus* is the purpose of the present paper.

III. *ASTACUS LENIUSCULUS*.

As far as known, no observations have been made upon the life histories and habits of any American crayfish of the genus *Astacus* and as the following facts were gained from animals kept in Baltimore far from their native habitat they will need the corroboration of future studies made in the Pacific States, but for the present they supply all our knowledge of the young of American *Astacus*.

The material for study of the young *Astacus* was obtained as follows: Sixty-four specimens were received February 23, 1904, by express from Portland, Oregon, and, though packed only in wet excelsior, ten females and eighteen males survived the journey and lived in running water in the laboratory for some time. Shipped without selection of sex, the sixty-four were found to be made up of thirty-one females and thirty-three males, which indicates an equal distribution of the sexes. The males, however, seem to have endured the journey better than did the females.

During March, April, May, and June these crayfish died slowly one by one, leaving one survivor in October. As they were fed from time to time with small oligochætæ, with fragments of crayfish and with pieces of frogs, the cause of the very slow and lingering deaths was not evident, but probably the food was not sufficient for such large and active crayfish.

It was decided that this *Astacus* was probably *Astacus leniusculus* Dana as distinguished from *A. Trowbridgii* by Faxon ('85) and the following observations upon their habits in captivity seem to be all that is known of this species, beyond its specific characters.

The largest specimen was a female measuring 155 mm. from tip of rostrum to end of telson, 205 mm. from tip of chela to tip of telson, 40 mm. wide across the thorax and 35 mm. deep; the abdomen was 45 mm. wide and its tail fan expanded to a width of 70 mm., exclusive of the setæ. With only about one-half of its eggs left upon its pleopods this female weighed 106 grams.

The smooth clean shells and large well colored claws gave these crayfish an attractive and decidedly lobster-like appearance, which was enhanced by their very active pugnacious nature. In shallow water they quickly responded to approaching objects and readily threw themselves into a defensive attitude, leaning back with the whole anterior region raised high from the bottom of the tank, thrusting their brilliantly colored claws above them high into the air and either holding them wide apart and open or clashing them together towards an approaching object, towards which they lunged, or even seemed to spring.

Their quickness to react to distant objects, their quick reflexes and irritability led me to suppose they might well be carnivorous and their clear colors suggested a life in clear water, but the collector reported that the Willamette River, whence they came, was a rather muddy stream, though formerly used as water supply for Portland. In captivity they were nocturnal, lying quiet and away from the lighter parts of the tank in the daytime and crawling about in the night. They sometimes injured one another and also ate parts of their dead fellow-crayfish and, as is so common with many kinds of crayfish, there were cases of regeneration, which may have followed from injuries caused by their pugnacious and carnivorous habits. One male 95 mm. long, when received, had two regenerating limbs. The left chela was represented by its original basal two segments and by a delicate new limb only 9 mm. long protruding from the truncated end of the second large segment. This little, bluish protuberance was made of six movable segments and it was movable upon the old limb whence it sprang, so that to make the seven normal movable segments of a complete limb a reduction in the number of movable joints must take place, probably by the base of the new growth ceasing to move upon the old second segment. The other regenerating limb was the third left walking leg and here again the old second segment bore a soft protuberance, which, however, was as yet not segmented. Thus in this *Astacus*, as is common in other crayfish, regenerations had started from the preformed breaking plane, and in handling this same specimen, though dead, the right third walking leg fell off at the breaking plane.

In captivity shedding, or ecdysis, took place in early summer, in some; thus on June 11, when the water had risen from 9° C. in March to 22° C., one large male cast its shell as one perfect piece containing also the "teeth" and other part of the lining of the "stomach." The creature was then quite soft and easily indented by touch upon its carapace; it lay inert but could be made to crawl in a somewhat palsied way and even to flap its abdomen. On each side of the heart region the carapace bore a white, indented, scar, as if made by the claws of some other crayfish, so that the advantage of concealment at this season as practiced by other crustacea doubtless applies here as well. The entire shell was covered by a slimy soft mucus and its colors were brighter than before, the under side of the legs showing also more blue. As seen from below, the flesh of the abdomen had a peculiar coagulated appearance.

The new carapace had a length of 57 mm. and the old of 43 mm., and had enlarged in diameter from 25 to 28 mm. The cast shell had one broken antenna, 53 mm. long, which was replaced in the new shell by a perfect antenna 68 mm. long. A week later this crayfish was active and keenly seized and ate a large lumbriculus.

Two more males cast their shells June 15 and one of them was attacked by others when only the carapace had been shed and the abdomen was as yet in its old shell; one-half of the thorax and part of the abdomen were devoured. The other large crayfish was able to flap its abdomen vigorously when lifted out of the water though its body and chelæ were soft and flaccid. In both cast shell and new one the rostrum was broken off and had evidently not been regenerated. Still another large male, received in October, 1904, cast its shell May 19, 1905, and could move about though still soft.

The breeding season of these crayfish was far advanced when they were received in February. In many cases the males had no sperm left in the deferent ducts and the females had laid their eggs, which in four dead and six live specimens formed large dark masses attached to the abdominal limbs, or pleopods, and to the sternal hairs of the abdomen. Contrary to expectations, these eggs were still alive and it was found possible to rear them and to get the subsequent stages in the life history as described below. In the hope of getting light upon the early part of the breeding season, another lot of thirty-seven crayfish of the same species were got from the same place October 29, 1904; but here again the beginning of the breeding season had passed. The only two survivors on arrival were both males, and three others recently dead were also males, so that here again the vitality of the males seems to exceed that of the females. In all there were twenty-two males and fifteen females. Five of the females had eggs upon the pleopods and these were in early cleavage, showing some twenty nuclei migrating to the surface. It would thus seem that egg-laying takes place in the autumn, probably in October, and subsequent observations

showed that the eggs are carried by the female all the winter and hatch in the spring. The eggs on each female were about 500 in number and formed a dark brown or more usually nearly black mass all over the under side of the abdomen. Each egg was very large, about 2.5 mm. in diameter, and as in other kinds of crayfish, enclosed in a complete capsule of hardened secretions that extended as a slender stalk to fix each to the setæ on the pleopods, or, in some cases, to the setæ on the sternal ridges of the abdomen. All the eggs seemed in good condition except a cluster of four or five among the brown eggs of one female and these few were overgrown with a fungus.

The darker eggs plainly showed embryos in the stage represented by Reichenbach ('86) as J, fig. 12, standing out as a whitish area on one side of each egg. Thus the embryo had already advanced to a condition in which the embryonic area occupied a considerable part of one-half of the spherical egg. There was still a wide margin between the appendages and the well elevated wall that surrounded all the posterior part of the embryo. The eyes, two pair of antennæ, mandibles, maxillæ, maxillipeds, and five periopods were well marked. The posterior four periopods and the abdomen projected forward over the thorax so that the abdomen reached to the first maxillæ. The eggs of one female showed in addition to the above embryo many nuclei scattered over the nonembryonic areas of the egg and plainly seen against the brown background of yolk.

In one female the eggs were covered by a dark deposit that had to be scraped off before the glossy egg capsule and the contained embryo could be seen.

A few eggs were greenish and covered by a deposit that could be scraped off; when these were opened, or when boiled, the contained embryo was found to be in the stage II of Reichenbach.

In one black egg the heart of the embryo was seen to beat very faintly, and after the eggs had been kept in water twenty-four hours many eggs showed the heart beating and were kept in the hope that they would develop. In water about 9° C. the eggs that were still attached to the abdomen of living females did develop, though very slowly, as will be seen from the following results.

After nine days, March 2, the embryos were perceptibly enlarged with longer antennæ and abdomen, the second antennæ reaching back nearly half way to the end of the limb-bearing region. The heart, now lying in a plane at right angles to the ventral surface and above the base of the abdomen, was beating so strongly as to give a decided jerk to the thoracic limbs. In one embryo it beat at the rate of 66 to the minute and in another at 82. Inside the outer egg capsule there was evidently a delicate inner membrane investing the embryo.

For six days more the only change noted was a slight increase in size and the extension of the second antennæ beyond the middle of the limb-bearing region.

Four days later a marked widening of the anterior part of the embryonic area had taken place and the slow growth of the abdomen had brought its tip up to the posterior edge of the mouth. In another week the still larger embryos had antennæ reaching nearly to the end of the depressed, limb-bearing, region and the abdomen reached forward over the mouth as far as the bases of the second antennæ.

Embryos of this age, some twenty-five days after they were received, were very attractive objects seen through the transparent egg capsules. The transparent limbs stood out strongly contrasted against the dark red yolk mass that still took up the major part of the spherical egg. On each side of the egg the boundary of the yolk mass was slightly incised where the "liver" was forming. When such embryos were plunged a moment into boiling water and then put into cold water a mere scratch of a needle sufficed to cause the tough outer capsule to open with explosive force and the embryo was readily removed, leaving even its thin membrane sticking to the inside of the capsule.

During another week there was but little increase in the length of the appendages, but through the transparent walls of the limbs and body a few blood corpuscles were seen passing along in the large median thoracic artery and in the antennæ and periopods.

Ten days later, April 5, when the water had risen from 9° to 11°, the size had markedly increased and the embryo instead of being restricted to a flat region upon one side of the sphere could no longer be seen from a single point of view, since it now extended in a curved surface over one-half of the egg. The long antennæ now met one another and their tips overlapped at the deep notch where the abdomen joined the thorax. The legs and chelæ had grown long enough to overlie the abdomen and to conceal its tip. Such embryos were nearly in the stage K of Reichenbach and were prettily colored. The carapace had bright red pigment along its ventral border; the dark red-brown yolk took up less than half the bulk of the egg and was divided by a deep fissure into anterior and posterior lobes which were encroached upon by the "liver" which was conspicuously colored. In one its contents were red and in fifteen greenish and translucent or else white-yellow and opaque. The large eyes had also some pigment formed in them.

Eleven days later the elongated walking legs and chelæ reached forward over the posterior edges of the eyes. Pigment cells were as yet not seen in the above limbs, but were conspicuous in the first and second antennæ and in the abdomen as well as the thorax. The delicate inner membrane was seen loosely investing the tips of the chelæ like a cast-off exoskeleton.

Some six days later the embryo had passed beyond the stage K and was nearly ready to hatch. The limbs were even longer, so that the chelæ reached over part of the eyes and the antennæ overlapped one another the whole width

of the abdomen in such a way that the left passing posterior to the right had its tip on the right side and the right passing anterior to the left had its tip on the left side. While all the eggs were still alive and had been kept well aërated by the swaying movement of the pleopods that the mother makes, seemingly for this purpose, yet some of them showed the effects of such a very long existence in the water by being covered over upon the outside of the egg capsule with deposits which in some cases were mixed with growths of minute fungi and in some with vorticellæ. The eggs seemed under tension and burst open when thrown into hot Worcester's liquid; the touch of a needle to the live egg also caused its capsule to pop open.

As seen under a pocket lens, these eggs nearly ready to hatch present a most attractive appearance. The carapace is spangled with branching, vermilion, pigment cells that are especially numerous along its ventral edges and near the base of the abdomen, which is broad and also well pigmented with the same kind of cell. The antennæ and antennules have both red and blue pigment cells on their basal part, but these cells are not numerous and are entirely absent from the filaments. The chelæ and walking legs have some few scattered pigment cells. The still large dark red-brown yolk mass has the forked, light-colored, "liver" projecting into it. The eyes are darkly pigmented in all their central parts, while the surface is still clear and transparent for some distance inward.

The actual hatching of the eggs took place on one female April 25-27, and on another May 1-6, and was prolonged over several days, that is, not all the eggs on one female hatched at the same time, but whether this is normal or induced by the artificial conditions remains to be found out from study of these crayfish in their native waters. These eggs had thus required 62 to 64 days in one case and 67 to 71 days in the other to develop from the well advanced embryo of stages H and J of Reichenbach to the hatching larva. The temperature of the water had slowly risen from 9° C. to 14° C.

When a female died before the eggs hatched, it was found possible to hatch the eggs by cutting off the pleopods of the mother and fastening them to pieces of floating cork so that the eggs would be suspended in well aërated water.

In hatching, the egg capsule burst open over the back of the embryo, and usually opposite to the egg stalk, and then the embryo slowly glided out backward, much in the same way as has been described for *Cambarus* (Andrews, '04).

In all crayfish, and in many other crustacea, the eggs remain firmly fastened to the mother during the whole period of development and when the embryo escapes from the egg-shell the old shell remains still fastened by a strong stalk that is stuck to the maternal setæ. In this *Astacus* as the em-

bryo slowly emerged from the egg capsule it was evidently in a very inactive, helpless state, soft and unable to use its limbs, so that one might expect it to drop away from the egg-shell, fall to the ground, and continue its life, if at all, away from the mother. However, it is well known that young crayfish remain for some time upon the abdomen of the mother, which they do not leave till they are well able to swim and to walk. This period of interrelation between mother and offspring deserves special study and we will describe more in detail than has hitherto been done for any crayfish the remarkable structures used in ensuring the connection of mother and young from the moment of hatching up to the time of real independence and free life.

Instead of dropping away entirely from the egg-case, each soft, helpless, larva hung attached to the inside of the egg-case by a delicate thread which was firmly fastened at one end to the inside of the egg-case and at the other end to the telson of the larva. The soft, pink-colored larvæ thus at first hung out from the egg capsules like the pulps from burst grape-skins and were then prevented from entirely falling away from their capsules by these threads, so that they suggested the seeds of the "cucumber tree" dangling out of their pods. As the young remained limp and helpless for some time these "telson threads," as we may call them, appeared to be of great use, since without them the larvæ would have fallen to the bottom of the water and having lost connection with the parent have had small chance of survival, lacking the protection or aëration furnished by the mother.

Such telson threads are doubtless found in the European *Astacus*, for in a footnote added by M. Robin to Chantran's paper ('70), we read: "J'ai pu constater, à l'aide du microscope, comme l'a montré M. Chantran à l'Académie, que les petits restent pendus sous l'abdomen de la mère, par l'intermédiaire d'un filament hyalin, *chitineux*, qui s'étend d'un point de la face interne de la coque de l'œuf jusqu'aux quatre filaments les plus internes de chacun des lobes de la lame membraneuse médiane de l'appendice caudal. Ce filament existe déjà lorsque les embryons n'ont encore atteint que les trois quarts environ de leur développement avant l'éclosion." And the same general fact is mentioned in the report of the committee awarding the Montyon prize to Chantran (C. R., 75, 1872, p. 1341). Of the above passage Huxley ('80, p. 352) says: "Is this a larval coat? Rathke does not mention it and I have seen nothing of it in those recently hatched young which I have had the opportunity of examining."

The exact mode of attachment of this filament, or telson thread, and its probable nature will be described below in connection with the telson of the larva of this American *Astacus* and later on in considering the like structure in *Cambarus*.

The size of the larva in its first stage is indicated by the following rough

measurements of preserved specimens: Length of head-thorax, 4 mm.; width, 3 mm.; depth, 3 mm. Length of abdomen, 5 mm. Length of antenna, 6 mm. Length of chela, 5 mm.

Returning to the account of the hatching young, it is to be noted that the activities of the young were but slowly acquired; here and there amongst the mass of eggs and young upon the female some larvæ showed feeble movements of the scaphognathites and, later, rhythmic respiratory movements; the long antennæ projecting into the water moved somewhat, the legs and chelæ sometimes moved and the claws opened and shut. Upon escaping from the spherical egg-case the larva became but little straightened out and remained essentially a spheroidal head-thorax with a weak abdomen bent in under it and with soft, pendent limbs. However, in from one to six hours the limbs reaching about, the claws opening and shutting and the abdomen sometimes flapping up and down, it was seen that the chelæ managed to get hold of the stalk of the egg-case. Henceforth the larvæ held fast by the chelæ though for a time still fastened by the telson thread also.

The pleopods of the parent were now covered over with a mass of flesh-colored young, showing slight movements and conspicuously marked by the two-lobed, red yolk mass, by the dark eyes and by the yellowish "liver" areas. The dark yolk masses showing through the pale bodies gave somewhat the general appearance represented in figure 2, which was made from a photograph of a living female shortly after the eggs (excepting one) had hatched.

When forcibly torn loose from the mother the recently hatched larvæ, too spheroidal to rest on their ventral side and unable to stand on their legs, lay for two days on their sides, kicking their legs but unable to walk; when, however, much disturbed, they managed to swim forward along the bottom of the dish by flapping their abdomens, though they still remained on their sides. When offered a piece of rough string, such young seized it and remained suspended in the water, holding fast by their chelæ. In this way some larvæ were kept suspended in running water and successfully carried into later larval stages away from association with the mother.

This tending to seize hold with the chelæ is accompanied by a tendency to push far in amongst the general mass of young attached to the pleopods, so that in a few days all the young are densely crowded together in a compact mass and their long chelæ are seen to reach far in and to be fastened either to the stalks of egg-cases or to the coagulum that binds the setæ together on the pleopods. Generally both chelæ grasped the same egg stalk but not always and one larva was seen holding by one chelæ to an egg stalk on one pleopod and by the other chelæ to an egg stalk upon the next pleopod. As the rhythmic movements of the pleopods continued after the young were hatched, this larva was in danger of having its chelæ stretched apart.

The fixation of the chelæ was a gradual process; at first the claws of the chelæ were not opened even when those of the walking legs were opened and shut, but soon the chelæ claws opened and shut and soon seized hold of any solid object accessible. Sometimes the object seized was again let loose but before long the chelæ had reached in among the mass of young and eggs far enough to find and fix upon one of the egg stalks, which are of a material and size seemingly well fitted for the attachment of the claws. Henceforth the chelæ seemed to remain always fast and their structure as described below indicates that once imbedded in the material of the egg stalk the tips of the claws could scarcely be liberated by the crayfish. This fixation was first made out in the English crayfish by Huxley ('80), who inferred that when once fast it would be difficult, or impossible, for the claws to open again.

This use of the chelæ to obtain attachment to the mother was exercised with what appeared to be very earnest effort and once successful it was found that the telson thread was soon ruptured. And then if the larvæ were disturbed they flapped their abdomens up and down and it was seen that the telson thread had broken so that a piece of it was still attached to the telson and was waved about by the telson like a bit of rag fastened to it. Henceforth the young held fast by the chelæ only.

The general appearance of this mass of young on the mother was peculiar since the rounded head-thorax was the chief part visible in each and this was of light color with a striking bilobed or horseshoe-shaped, dark red, yolk mass across it. The legs and abdomen were concealed and the dark eyes were generally out of view. With bent heads and outstretched limbs their attitude ludicrously suggested one of supplication.

Thus they remained for some days. When disturbed the young made tramping movements with their legs but did not move from the place to which they were fixed by their long chelæ. The abdomen, carried down under the thorax somewhat as in the embryo, was not readily moved but with sufficient stimulus from a needle point was flapped rapidly back and forth. When a larva was forcibly pulled off from the mass its chelæ, still attached, were stretched out to their fullest extent and when the larva was released the chelæ contracted and made the larva spring back into place where its limbs and abdomen were again drawn in under the thorax and the creature became again one of the herd of "bison" presenting only their humped backs to the observer. If by stronger pulling the larvæ were torn loose from the mother the chelæ parted from the egg stalks without breaking and reaching about seized hold of adjacent objects such as the antennæ of other larvæ. Left to themselves on the mass, these separated larvæ soon got back again amongst the crowd; but if put upon the bottom of the dish they did not yet stand up but only gyrated about by flapping their abdomens.

The long antennæ remained for the most part low down amongst the general mass and did not yet project up above the level of the rounded backs; they were, however, carried out in front and not, as in *Cambarus affinis*, tucked in between the legs.

Fastened thus to the mother, the larvæ remained some days and then casting off their shells passed into a second larval stage which also lived upon the mother. In one female kept in running water at 17° C., the duration of the first larval stage was only four days, but some young of another female which were kept suspended from strings hanging in water in a warm room remained five to seven days before changing to the second stage and their fellows kept in water at 14° C. remained eleven to thirteen days in the first stage. During this long existence in the first larval stage the only change noted was a slight darkening of the color which, owing to the scattering of bright red, branching, pigment cells on a white background appeared to the naked eye flesh-color.

Before considering the transition from the first to the second stages we will describe the details of the external form and appendages of the first larva.

A side view of the living larva (fig. 3), suggests embryonic incompleteness in that the antennæ, abdomen, and limbs are carried downward in a way not adapted to locomotion, while the globose cephalothorax and large eyes with short stalks are features of an embryo rather than of an active larva. The dark mass seen in the figure was the still conspicuous red yolk mass which from the dorsal view (fig. 4), was balanced right and left in the anterior half of the cephalothorax. In life the larva was translucent yet brilliantly colored by the scattered pigment cells indicated in black in the figures and which were absent only from the terminal filaments of the antennæ and from most of the segments of the legs. In the side view the first and second antennæ are conspicuous, the three maxillipeds are seen in part, the chelæ are very long and heavy and the four walking legs are long and weak. The abdomen bears only four pairs of pleopods and these are small, weak, and bifid. The first and sixth pleopods are not seen and the abdomen ends in a simple telson in place of the locomotor fan of the later larvæ and adults. The larva is evidently very defective in locomotor apparatus, has its sensory organs not perfected, and is specialized in its strong clinging organs, the chelæ, and in its large digestive apparatus for utilization of the stored-up yolk. It is still essentially embryo-like in structure and in mode of dependent life, but is exposed free to the water.

The same general features are shown in the dorsal view (fig. 4), which shows the split-open egg capsule and its stalk, connected by a slender thread to the telson of the larva, a "telson-thread" that is fast at one end to the peculiar fan-like telson of the larva and at the other end to the inside of the ruptured egg capsule. It will be noted that the head-thorax though globoidal is considerably elongated and does not have the swollen sides shown in Huxley's

figures of the English *Astacus*, which, however, were doubtless drawn from alcoholic specimens, and in *A. leniusculus* the action of alcohol is to cause great distortion of the branchiostegites. As compared with the adult, however, the proportions of the head-thorax are embryonic and there must needs be much greater elongation as well as lateral and vertical changes to bring about adult proportions.

While both from side and top views (figs. 3 and 4), the characteristic rostral spine of such crustacea seems absent, full front and diagonal side views (fig. 5) show the rostrum to be well developed and armed with lateral spines, but so bent down between the eyes as to be of no such use as a defense as it later will be in active stages of the larva. The habit of the first stage which clings to the parent is thus correlated with imperfections of defensive armament as well as with presence of food yolk and imperfections of locomotor organs. Among the latter characters may be reckoned the smooth surfaces of the limbs and absence of setæ that later will increase the areas of resistance for striking against the water as well as furnish means of sense perception. The lack of setæ represented in figures 3 and 4 is still more striking in enlarged views of the limbs and is in strong contrast to the hirsute character of all parts of the active larvæ and of the adults, and this absence of setæ gives the larvæ a decidedly embryonic appearance.

The pronounced incompleteness of locomotor organs is also associated with the shortness of the thoracic region bearing legs; thus the chelæ arise farther from the anterior than from the posterior ends of the head-thorax and leave little space for the walking legs, while the anterior region containing the yolk is greatly developed in size.

Next taking up in sequence the nineteen pairs of appendages of the adult we find them represented in the first larva by seventeen pairs that have in the gross, as made out by Rathke for embryos about to hatch, the essential morphology of the adult appendages but lack the setæ and differ in proportion as will be seen from the following account and illustrations.

The whole exterior of the larva in its first stage is covered by a chitinous exoskeleton of such resistance that when the young were thrown into Worcester's liquid they did not die for several minutes; the appendages were cut off separately from such hardened embryos and gave the views represented in the following illustrations.

The first antenna stands out horizontally in front of the head (figs. 3, 4) and is straight; as seen in figure 6, it has three basal segments, five in exopodite and in endopodite. The segmentation of the endopodite is very obscure. The terminal segment in both endopodite and exopodite bears three obscurely pointed spines, one of which, in the exopodite, is long and apparently of the same character as the sensory setæ found there in later stages. On the long

basal and next segment of the protopodite there are a few blunt spines as indicated in figure 6. This also shows the auditory organ as an open pit on the upper surface of the basal segment, in the part that is swollen out laterally.

The second antenna (fig. 7), though it has a long filament, is still embryonic in proportions. The base consists of two short segments, the first of which bears the very large prominence that faces inward and has the opening of the green gland, or kidney, within its depressed top. The second segment bears a few blunt distal spines of large size. The exopodite is a very large flat scale ending in a blunt point and bearing some sixteen blunt spines along its serrated inner and anterior edges. The endopodite consists of three large basal segments and of a long round filament of many segments, forty-five to fifty, the first of which is long and slender, while the following ones are each about one-third as long as the first. The terminal segments are again more long and slender. As indicated in figure 7, there are a few spines at the distal edges of some of the terminal segments and of some of the others near the tip. In the adult there may be 125 segments.

The mandibles (fig. 8) have a well developed cutting edge which, however, is smooth and not toothed as it is in all later, functional, stages and there was no evidence found that these organs were used. The palpus has three segments and is smooth except for the distal face of the third segment which is sparsely set with rather acute spines, many of which are shown in figure 8, and more of which are present upon the inner aspect and not seen from this point of view. The palp is thus a blunt club with terminal spines.

The first maxilla (fig. 9) is very small and made of two flat plates and a somewhat rounded and blunt endopodite of curved finger-shape. Here for the first time we meet with a few small, plumose setæ along the outer edge of the distal segment. The two flat plates that represent the protopodite are spinulose at the ends, much as in the palp of the mandible. The distal piece is also armed with a row of a few spines along its proximal edge as seen in figure 9. The ends of both plates are set with spines on the face toward the mouth; the proximal plate is rounded, the distal one truncated to form a jaw-like organ.

The second maxilla (fig. 10) bears the long scaphognathite which has a dense row of plumose setæ all along its extensive free edges. There are also a few plumose setæ at the base of the endopodite, as in the first maxilla (fig. 9). As above noted one of the first muscular activities acquired after hatching is the slowly developed rhythmic beat of the scaphognathite, and with this use of this appendage there is present an armament of plumose setæ lacking elsewhere in the locomotor organs of the larva. While these plumose setæ are not used in locomotion their function as flexible areas of resistance to the water which the scaphognathite bales out of the branchial chamber is akin to that of locomotor setæ. The rest of the second maxilla is nearly bare of setæ, but there

are a few, long hair-like setæ standing out from the external face of the protopodite. The protopodite has the same two plates seen in the first maxilla (fig. 9), but each is deeply cleft, so that four free tips project toward the mouth. The four tips each bear blunt spines on the outer and distal faces as shown in figure 10, while upon the inner faces toward the mouth they are all set with longer, sharp, curved spines that are claw-like. The endopodite though longer and more slender than in the first maxilla is still very simple and not segmented.

The first maxilliped (fig. 11) shows the protopodite again reduced to two flat plates much as in the first maxilla (fig. 9), and there are simple spinules on their cutting edges. The endopodite is small and simple, intermediate between that of the second and first maxillæ. In place of the scaphognathite there are two movable parts; a long flat plate, the epipodite, which is without setæ though sparsely spinulose on its posterior edge; and a very long and prominent exopodite. The exopodite has a very long swollen basal part with very long plumose setæ on its outer edge as shown in figure 11 and is elsewhere naked. The terminal part is long, slender and with a very few spines at its tip. As the base of the exopodite lies over the distal end of the epipodite it is not readily seen that the epipodite has a short truncated extension suggesting the anterior end of the scaphognathite as well as the evident posterior blade that reached back into the gill chamber and is comparable to the like portion of the scaphognathite.

The second maxilla (fig. 12) is more complex; the two segments of the protopodite are subordinate in mass and extent to the greatly developed endopodite and gill structures, but they bear a few plumose setæ upon their inner edges. The endopodite resembles that of the first maxilla in position, general form and curvature, but is not only larger but subdivided into five segments and bears spines. In addition to the spines shown in figure 12, there are also long curved ones on the inner face of the terminal segment. Compared with that of the first maxilliped, the exopodite of the second has a very narrow basal part which is without plumose setæ but bears a few long spines on its external edge. The epipodite is present as a long, curved lamina, bilobed at the tip, and along its inner face are borne the numerous blunt filaments of the gill, podobranch. This podobranch is free at its tip, but elsewhere adnate to the lamina and bearing two rows of blunt side papillæ or gill filaments which are directed toward the apex, and increase in size in each row from base to apex. The epipodite lamina has a few plumose setæ on the rounded ridge at its base and along its edges are scattered curved, short hooks, while its emarginate tip bears a few blunt, fringe-like papillæ. In addition to the above gill there is one arthrobranch that is shown in figure 12 to have a long slender stem ending bluntly and bearing two rows of blunt, curved, finger-like lateral filaments

which may each end with a small, blunt spine. The third maxilliped (fig. 13) exhibits the typical morphology of this organ in the adult; a somewhat two jointed basal protopodite bears a large five-jointed endopodite of great size, a long slender exopodite, a large epipodite and podobranch; and two arthrobranchiæ arise from the region connecting the appendage to the body.

The endopodite bears spines upon all its segments and the protopodite has a couple of small spines upon its distal segment and a plumose setæ upon its proximal segment. The exopodite, in contrast to that of the second maxilliped, has a shorter and more slender basal segment devoid of spines, while the second segment has several spines at and near its tip. The lamina of the epipodite has the same characters as in the second maxilliped, but the plumose setæ along its basal ridge are twice as many. The podobranch is like that of the second maxilliped. Of the two arthrobranchiæ the anterior one is much like its homologue on the second maxilliped, while the posterior one, nearer the observer in figure 13, is smaller and more simple with fewer lateral filaments.

Coming next to the ambulatory appendages, we find the usual large chelæ, the two pairs of slender chelate and two pairs of non-chelate legs (figs. 14, 15, 16, 17, 18). In these appendages of the first larva there are, as in the adults, no exopodites, and even the remarkable exopodite setæ of later larvæ and of adults are absent in this first stage, thus adding to the simplicity of the limbs, which is also expressed in the entire absence of plumose setæ and the presence of but few sharp spines.

The chela (fig. 14) has the recurved terminal hooks first made out by Huxley in the English *Astacus*, and which lead to the firm locking of the chelæ to the egg stalk, as above narrated; and the opposing edges of the claw are serrated from the presence of sharp spines pointing toward its tip. The chela bears very large sharp protuberances along the inner edge of the meropodite segment, of no apparent use, while the great length and thickness of the whole limb is apparently necessary in that firm holding of the larva to the mother which resists the force of the maternal pleopods that swing the larvæ back and forth.

The epipodite and gills of the chelæ are like those of the third maxilliped.

The following two legs (figs. 15, 16) are like one another in every way except in proportion, the first being shorter than the second. Each has a sharp claw with spines pointing toward the tip, but the tips are not recurved as is the case in the big chelæ. The gills on these two appendages are like those of the chela, but there is in addition a slender simple gill upon the body wall near the arthrobranchiæ. This pleurobranchia is a single filament with no lateral outgrowths and may be regarded as rudimentary at this stage, as it is also in the adult.

The remaining legs (figs. 17, 18) have terminal segments almost like those

of the preceding legs, but there being no opposing outgrowth of the propodite, there is no claw. The legs increase in length and in slenderness from before back in the series behind the chelæ. The penultimate leg (fig. 17) has a longer pleurobranch associated with it but otherwise its gills are as in the preceding limb. The last leg, however (fig. 18), has its gills suddenly reduced; in place of the epipodite and podobranch there are but a few plumose hairs such as stand upon the basal ridge of the epipodite of the preceding somites. The arthrobranchs are entirely absent and there is but one gill which is a pleurobranch, which, however, in place of being a simple filament or rudiment, resembles a reduced or simple arthrobranch in that it has about seven short lateral processes in two imperfect rows.

The branchial formula of the first larval stage of *Astacus leniusculus* is then as is given in the table below. This was found to be just the same in the adult of this species and it is said to be the same in the English *Astacus palipes*, except that the latter lacks the rudimentary pleurobranch on the somite of the first leg.

	Podo-branchiæ.	Arthrobranchiæ.		Pleuro-branchiæ.	Total.
		Anterior.	Posterior.		
Somite of 2d maxilliped	1	1	0	0	2
Somite of 3d maxilliped	1	1	1	0	3
Somite of chela	1	1	1	0	3
Somite of 1st leg	1	1	1	1 R	3 + 1 R
Somite of 2d leg	1	1	1	1 R	3 + 1 R
Somite of 3d leg	1	1	1	1 R	3 + 1 R
Somite of 4th leg	0	0	0	1	1
	6	6	5	1 + 3 R	18 + 3 R

The above illustrations of the separate appendages of the head-thorax are the first ones as yet given of any larval *Astacus*, since the previous illustrations of European forms are only the small maxilla and maxilliped of an embryo not yet hatched as depicted by Rathke ('29, fig. 29), the tip of the chela shown by Huxley ('80, fig. 8), and the under side of the abdomen with its pleopods figured by Reichenbach ('86).

Upon comparing the adult appendages of *Astacus leniusculus* with those of first larva as above described, the fundamental agreement in morphology was obvious, but there were the following differences which all suggest a lingering on of embryonic characters into the life outside the egg-shell. Throughout all the appendages there was a marked lack of setæ correlated with evident lack of locomotion and probable weakness of sensory activity. Excepting the chelæ the cephalothoracic appendages had no obvious use. The first antenna, having

but five segments in its exopodite and in its endopodite, is but embryonic compared with the adult that has at least thirty-five in its exopodite and almost as many in its endopodite, a difference which is of great moment when we consider the repetition of special sense organs that are found on many successive segments of the exopodite of the adult. The auditory organ also is apparently of no functional value in the early larva. The number of segments in the filament of the second antenna must also increase greatly to form the one hundred and twenty-five of the adult; an increase apparently brought about by interpolating new segments at various places by division of the old ones into two.

While all the adult appendages of the head-thorax are represented in the larva at hatching, this is not the case for the abdomen, for the sixth pair of abdominal appendages are not externally present and the first pair which in the adult male are so essential are absent in the first larva as they also are in the adult female. The other abdominal appendages are four pairs of simple pleopods which hang down beyond the pleural plates of the abdomen so that they are seen from a side view (fig. 3). Each pleopod (fig. 19) is as in the adult composed of a short and a long segment that make up the protopodite and of two simple terminal plates, the endopodite and the exopodite. These are slightly curved and armed at the ends and to some extent on the edges with small weak spines and they entirely lack the plumose setæ that makes them useful in the adult for fanning the water. The illustration is of the anterior face of the left pleopod of the second abdominal segment and shows that the exopodite is longer and wider than the endopodite, while in the adult the exopodite is much the shorter and smaller in the female pleopods that bear the eggs and in the male pleopods that transfer sperm. The appendages of the sixth somite though not externally free are yet present and of large size though imperfect in development and lie within the substance of the telson, as can be seen in transparent living larvæ. It is their presence which swells out this region ventrally and gives rise to the protuberance seen from the side view (fig. 3). Looking at the telson from above (fig. 20), the very imperfect future sixth pleopods are seen as two somewhat less translucent areas, right and left in the anterior part of the telson and each having an outline suggesting that of a mitten. Only later will these concealed buds of the sixth appendages burst out after a moulting and expand as the very large lateral parts of the caudal fan, so essential in quick locomotion. This retention of these appendages within the telson in *Astacus* was known to Huxley ('80), and figured by Reichenbach ('86).

The telson requires special consideration in connection with the "telson-thread," as mentioned above. The telson itself is a very large plate with nearly circular outline and is thin posterior to the above region occupied by

the buds of the pleopods on either side of the intestine and anus. In structure it is essentially a translucent, vascular, connective tissue mass over which are scattered brilliant vermilion pigment cells, indicated in black in figure 20, and which is covered by a thin epidermis and thin chitinous cuticle. Along most of the free margin of the telson is a row of blunt, stiff papillæ, or spines. In all there are about sixty-six of these spines arranged symmetrically, half on each side of the median plane. In the figure of *Astacus* given by Reichenbach ('86) there are but twenty-five spines or dentations on each side of the median line and this may well be a character of systematic value. In addition to the thirty-three lateral spines of *Astacus leniusculus* there were six or seven smaller, blunt spines on each side which stand in between some of the larger ones, one small one between two larger ones, and generally not so near the edge but more up on the dorsal surface of the telson.

The interior of the telson has a radiated appearance like that figured by Remak and by Reichenbach and which was referred by Huxley to the disposition of vascular canals; but in our present larvæ this radiation is due to long delicate lines passing centrally inward, one from each lateral spine and from some of the smaller spines, thus making the divergent system radiating from near the anal region as shown in figure 20. Subsequent events show that these lines are the forming plumose setæ for the perfect locomotor telson of later larval stages. Each when enlarged (figs. 21, 22) is a bundle of fibrillæ that are very small in comparison with the nuclei of the epidermis as each bundle of very many fibrils is but once or twice the diameter of a nucleus. These radiating lines are in fact to be likened to compressed bottle brushes which later will expand as the perfect locomotor plumes on the telson of the second larval stage (fig. 23). The plumes are being made within epidermal tubes or glands and between the successive radiating glands the vascular spaces, also radiating, form the justification for Huxley's interpretation.

During the first hours of larval life the telson is connected to the inside of the egg case by the long telson thread represented in figure 4. This is a translucent, chitin-like membrane, or flat ribbon, showing a striated appearance due to fine wrinkles in it (fig. 20), but otherwise apparently homogeneous. Though seemingly but superficially attached to the telson the contact is a very firm one so that when the larva succeeds in getting hold of the egg stalk and finally flaps its abdomen strongly enough the telson thread is broken before it is torn loose from the telson. When enlarged (fig. 21) the mode of attachment of the very thin but tough membrane is seen to be, that ten of the marginal spines of the telson bear special hook-like projections that are fast to the membrane. These few spines are different from the others though some of the adjacent ones have somewhat of the same structure at their tips. While in figure 21 the spines and their processes are represented in black, they are in nature trans-

parent, colorless and very inconspicuous, especially the processes which are hyalin, myelin-like protrusions, suggesting the products of glandular activity. While many of these protrusions end bluntly others seem to be continued as fine threads that are fast to the membrane or telson thread. Moreover, many of the protrusions bridge over the space from one spine to the next and are continuous with adjacent protrusions as if they had flowed out when viscid and then coagulated. Such bridges make adjacent spines into hooks that hold fast to the membrane, but the ultimate and essential fastening of the membrane to the telson is by fine threads of some coagulated material that seems to be a continuation of the grosser protrusions figured in black in figure 21.

It would appear from the statement of M. Robin cited above (page 15) that in the *Astacus* studied by Chantran there are but eight spines used for attachment to the telson thread.

The origin of these peculiar glandular spines, for such they seem to be, is to be sought in the embryo. At the time of hatching the egg capsule breaks and for a brief period the embryo may still be enveloped in a very delicate membrane which passes over the abdomen and all about the telson. At that period the spines of the terminal part of the telson abut against the investing membrane as shown in figure 22, which is from a specimen just hatching and killed in Pereny's liquor. Here are shown the epidermal nuclei, the striations, or forming plumes of the future telson, continuing up through the body of the spines, only three of which are drawn; and at the tips of the spines fountain-like masses of blunt protrusions, swollen at the tip and in many cases pressed against the membrane. It would seem that a viscid mass had been poured out from the tips of the spines and that this oozing out in threads had become firmly soldered to the membrane.

As far as made out the origin of the supporting telson thread is thus as follows. The thread is really a membrane and when the embryo is hatching this membrane is spread like a loose skin all over the embryo inside of the egg case. When the embryo hatches it also sheds this membrane, coming finally to pull its abdomen out of the part of the membrane that surrounds the abdomen like a long bag. The bottom of the bag is, however, fastened to the tip of the telson as indicated in figure 22, so that the creature cannot get entirely free from the bag but pulling out its abdomen pulls up the bottom of the bag and turns the bag inside out. The struggles of the larva drag the membrane into a long thread, and one end of this remains attached to the telson spines as seen in figure 21. Once this is accomplished the fact that the clear thread is really a cast-off membrane would not be suspected, since it seems a homogeneous, finely wrinkled thread that might well be a secretion.

The similar structure in the European *Astacus* was mentioned merely as a filament as cited above, page 15, but Huxley intuitively queried if it might be a larval skin.

The other end of the telson thread remained fast inside the egg capsule (fig. 4), and this attachment is as important as the above described attachment to the telson in making the thread of use to secure the larva from being lost. How the connection of the inner membrane to the egg capsule was brought about was not determined but it was existent long before the embryo hatched. Embryos three days before hatching killed in Worcester's liquid and soaked a week in five per cent potash showed an outer egg capsule, an inner shell and a membrane that was loose and visible over the chelæ and over the deeply bifid telson which already bore terminal spines. And embryos nine days before hatching showed the same double shell and membrane, but no telson spines.

The telson thread is thus a thin membrane formed about the embryo and early fastened by radiating fibrils (fig. 4) to the inside of the inner of the two layers of the egg capsule. Later in the life of the embryo this membrane becomes also fastened to the telson by secretory activity of the terminal spines. When the embryo hatches the membrane is ruptured and in part turned inside out and drawn into a thread-like form, fastened at both ends. Other facts regarding the telson thread will be given below in the description of *Cambarus*, in which it also exists.

The passage from the first to the second larval stages was seen in some larvæ lying in the bottom of a dish, and in others that had fixed themselves to strings. In these the old larval skin burst open and the second larva, as it were, "oozed" out backward for several minutes and its chelæ and abdomen remained longer inside the old skin but were then suddenly withdrawn. For a few minutes the larva in its new stage lay stretched out straight, as if dead but then flopped its abdomen, moved its legs, got upright and walked and even swam backward and finally crawled up into the piles of other young in the same second stage.

While the larva in the first stage was inactive and remained always fastened to the mother, the second larva was active and finally abandoned the mother though for a time still associated with her. Upon casting off their first larval skins the larvæ in the second stage leave those skins fastened by their chelæ to the egg stalks on the mother's pleopods, and are free to crawl about over the pleopods of the mother amongst their numerous fellow-larvæ. Soon these larvæ descend the pleopods and make short excursions under the abdomen of the resting mother and over various parts of the mother's body, finally wandering off over the bottom of the aquarium for short distances to return frequently to the mother again.

The mother thus had fastened to her pleopods a large mass of old egg stalks and capsules to which were fastened the cast-off skins of the larvæ, and over this mass crawled the active larvæ till after a few days the egg cases and cast skins as well as egg stalks were found to have disappeared leaving the ple-

opods clean but still occupied by the larvæ. In the *Astacus* in France Chautran ('71) finally convinced himself that the larvæ ate their cast skins and the egg capsules; and the same probably occurs in *Astacus leniusculus*.

The habits of the second larvæ showed much greater diversity than was possible in the attached larvæ in the first stage but through the early part of the second stage a tendency to climb seemed a dominant feature of their lives. When a number were put into a dish by themselves they tended to climb up onto one another to form a mass but if put back with the mother they soon climbed up onto her pleopods where they held on so firmly that when a pleopod was cut off and thrown into 70 per cent alcohol, some of the larvæ still retained their hold though most of them did not. When the larvæ alone were in a dish with a spray of myriophyllum they climbed up it and crawled together in a mass between the plant and the glass; but they did not climb up onto a piece of cotton cloth hanging down in the water from a floating cork. Even when the mother was dead, the young twenty hours after passing into the second stage continued to hold firmly to the maternal pleopods. But after three more days the young had ceased to huddle together so much, and crawling about over the bottom of the aquarium, and sometimes swimming, they were at times carried away with the current of water. Though some of the larvæ concealed themselves under ooze and dead leaves at this time, others continued to hold on to the abdomen of the dead mother for four or five days, when the abdomen was cut off and fastened at the surface of running water, but about May 18 these larvæ also dropped to the bottom and lived there. This climbing instinct can then be satisfied in various ways, and when thirteen larvæ were removed and put into a dish with another female bearing young, but few minutes sufficed for five of the thirteen to find and to climb up onto the pleopods of the strange female. The possibility of resolving these habits of the young second stage crayfish into so-called tactic phenomena, into chiefly geotactic and stereotropic responses, will be considered in connection with some observations upon the young of *Cambarus*.

The general form of the second stage as represented in figures 23 and 24 is obviously more like the adult than like the first stage as is true also of the habit. Comparing these figures with 3 and 4 there is a noteworthy change in size; after casting off the first skin the crayfish measured 11 mm. from tip of rostrum to end of telson, exclusive of the long fringe of plumose setæ which made the length 12 mm. if the setæ were included, and so greatly enlarged the area of telson available for locomotion. The thorax was 2 mm. wide and about 2.5 mm. deep. The cephalothorax was 6 mm. and the abdomen 5 mm. long; the telson was 2 mm. wide without the setæ, and 4 mm. with the setæ. The antennæ were 10 mm. and the chelæ 8 mm. long. All these measurements were taken from preserved material and show that the animal was now a large larva.

Its brilliant color added to its size made it an attractive larva resembling a young lobster after the swimming stages. As indicated in black in the above figures the pigment cells were scattered over the head thorax and abdomen and more sparingly over the chelæ, walking legs and basal parts of the antennæ. To the naked eye the larva walking on the bottom of a dish seems light flesh-color, translucent and inconspicuous, but the chelæ look red, the eyes are dark and the yolk is still a very evident dark, red-black mass of bilobed form across the middle of the head thorax. The liver lobes anterior and posterior to the yolk were noticeably yellowish and greenish. The abdomen was flesh colored for the most part, but the telson was nearly colorless and with a fringe of white, clear setæ so long as to suggest a peacock's tail. On the first abdominal somite the densely crowded pigment formed a conspicuous cross-band (fig. 23). Another such aggregation of pigment was found posterior to the eye and external to the base of the rostrum. In addition to the color due to the much branched red pigment cells, indicated in black in figures 23 and 24, there soon came to be a variable amount of blue color not so readily seen and due to large blue pigment cells. In strong light the red pigment often stood forward on a background of blue. The blue was evident on the basal part of the antenna and antennule, on the mandible and its palp, but not on the maxillipeds. On the dorsal side of both thorax and abdomen there were some blue, faint, scattered areas internal to the red.

As shown in figures 23 and 24, the cephalothorax in passing from the first to the second stage had become long, narrow, and angular with a long gothic rostrum standing straight out in front between the eyes on a level with the back. The rostrum also had large lateral spines at its base and half way out its length.

In walking about these larvæ carried the antennæ and the red chelæ forward and the abdomen straight out behind as in figure 23, and not bent in under the thorax as in the first stage. However, when not walking the abdomen was bent as in figure 24. As in a young lobster the slow walking was quickly replaced, at alarm, by rapid backward swimming caused by flapping the abdomen with its extensive telson fan. As the larvæ went about more and more away from the parent, they became more individual and more complex in their movements; they were seen to scrape the backs of their heads with their legs, to raise their chelæ as if in defense when a shadow passed over them, and in other ways to act like an adult crayfish much more than did the sluggish and simple first larvæ.

In watching one of these second larvæ slowly walking, the movements of the five long limbs seemed to be as follows. The fifth, fourth, and third limbs standing out at the sides of the body (fig. 23), were so bent as to hold the body high up above the bottom of the dish and swing back and forth as the

chief locomotor organs. The chelæ were held in readiness in front of the animal and the second limbs (fig. 23) were bent inward under the body with the tip forward so that in moving forward the body rode over these appendages as upon levers with very little movement of the base of these appendages. The third appendages always pointing forward swung from an angle of about 45° with the side of the body to one of about 70° . The fourth limbs had a very long swing from a forward position about 45° with the side of the body back past 90° with the side of the body. The fifth limbs had a very short, hobbled, movement like the third but always directed backward, from about 100° to 120° with the side of the body.

In taking up the appendages of the second larva, in sequence (figs. 25 to 39) it will be noticed that the relative nakedness of the first larva has given place to a hirsute condition, indicated in figures 23 and 24; setæ occur upon the antennæ, chelæ, legs, pleopods as well as the sides of the abdomen and, as the separate sketches show, upon all the other appendages. The active second larva has thus come into possession of sensory and locomotor setæ lacking in the imperfect first stage, and similar to those of the adult stages.

The first antenna (fig. 25) still has the same general form as in the first stage (fig. 6), it has five segments in exopodite and in endopodite but it is noticeably more finished in being well armed with setæ. The narrow part of the proximal segment bears a sharp spine upon its inner side. The auditory pit on the basal segment is now well guarded by a row of plumose setæ passing from the outer edge inward and spread across the orifice of the pit. There is also a row of sparsely branched plumes along this upper face of this segment and parallel to its inner edge and in addition there are a few other setæ arranged as in figure 25. The second and the third segments bear long plumose setæ on their inner sides and long, stiff spine-like setæ on the external sides of their distal ends. The endopodite and the exopodite each bear a few long stiff spine-like setæ at the distal ends of their five segments and in addition the characteristic blunt sense clubs of this appendage are now evident. These organs are placed in groups upon the inner and lower faces of the third, fourth, and fifth segments of the exopodite. The third segment has a cluster of three upon its distal edge, the fourth has a group of two at its distal edge while the fifth has a group of three at its middle part, where its diameter suddenly diminishes. As all these sensory clubs face downward they are foreshortened in the above figure and in reality are much longer than shown.

The second antenna (fig. 26) has increased greatly in length over its former state (fig. 7). Its basal parts are more angular and the excretory cone on the basal segment is relatively very much smaller while the spines of the second segment are borne upon a large, scale-like protuberance. The exopodite scale bears a row of long, plumose setæ all along its outer edge while in the first

stage there were only simple spines to anticipate some of these plumes. The three large segments of the exopodite are more angular than before and now bear a few setæ while the filament has some fifty-four segments of the form and proportions shown in figure 26. As these bear needle-like setæ on their distal edges the filament seems under a low magnification somewhat like a brush.

The mandible is not only greatly enlarged but more complex in having an effective cutting edge no longer smooth but serrated by seven unequal angular projections (fig. 27). The palp is more complex in having more numerous long, acicular setæ both on the exterior and interior faces of its terminal segment and a very few sparsely-branched plumes on the distal part of its second segment. When folded down the palp fits into a deep depression on the inner face of the mandible (fig. 28), and the proximal border of this depression is irregularly dentated with rounded protuberances. The exoskeleton over these dentations and over the sharp teeth of the cutting edge is now very thick and horny, being solid as far back as the second line in figure 28. While the acicular setæ over the terminal segment of the palp appear smooth under Zeiss 2 A, they are really set with short, fine, side branches along their distal halves as seen with 2 D, and they have rather blunt points so that they would seem to aid in a brush-like use of the palp.

The first maxilla (fig. 29) has progressed beyond its former stage (fig. 9), chiefly in the outgrowth of long setæ in place of blunt spines and also in the addition of setæ where there were no outgrowths at all. The setæ are of two kinds, a few plumose and many acicular; the latter are found chiefly on the ends of the two plates of the protopodite where they replace simple spines, while the plumes are chiefly lateral. On the basal, or first segment, however, there is somewhat of a transition, since its proximal border is set with setæ that extend out to the tip as very long and sparsely branched plumes that thus extend close up to the acicular setæ. These latter under 4 D are seen to be set with very few and fine side branches so that they are really somewhat plumose. The similarly placed acicular setæ upon the second segment, however, show no side branches but are really smooth. The small tuft of setæ already present in the first stage at the base of the endopodite remains but little changed in the second stage.

The second maxilla has undergone like changes (fig. 30). The terminal spines of the first stage (fig. 10) are replaced by plumose setæ and a few more long plumes are added. Here again the plumose setæ of the proximal edge of the first segment extend out as far as the acicular setæ of the apex. The endopodite has a few long plumose setæ on its distal part in addition to the cluster at its base. The scaphognathite is but little changed, the setæ along its outer edge being so bent down that they do not show their full length in the view represented in figure 30. However, at the posterior tip of this respiratory

organ there is a peculiar growth of one or two setæ. These are very long and though appearing quite smooth and hair-like, with 2 D, they are seen to be really set with short, fine side bristles, and they are sharp pointed. On the inner faces of the four lobes of the protopodite there are now many long, sharp, curved, setæ.

In the first maxilliped (fig. 31) the process of substituting plumes and acicular setæ for spines has been carried on in the same general way. The cutting edges of the protopodite now bristle with setæ most of which are simple, some straight, some with curved tips; but the tendency to run the plumose setæ up to the tip of the basal segment is carried so far that these plumes take entire possession of this basal segment and only the second segment has acicular setæ. Even on the second segment a few plumes come up nearly to the tip, on the distal side. The endopodite bears but a few plumose setæ while the long exopodite has in addition to the former series of plumes along the outer edge of the basal part (fig. 11) several remarkably long and conspicuous plumes near its tip. The terminal part of the exopodite is cylindrical and tends to become segmented and it is from its last and its penultimate segments that the long plumes project and form a terminal brush. The flat epipodite scale has still a few minute, blunt papillæ along its posterior edge (figs. 31, 11), and on its outer and inner edges a few of the remarkable exoskeletal hooks characteristic of these organs.

The second maxilliped (fig. 32) has changed chiefly in adding plumes and setæ. The gills remain as before (fig. 12) but are longer. The protopodite has added longer and more numerous plumose setæ and a very few acicular ones. The exopodite has substituted for its few terminal spines a brush of several remarkably long, strong plumes (fig. 32), and for the sparse spines along the outer edge of its basal segment a few setæ which proximally are sparsely plumed and distally smooth without barbs. The endopodite looks much changed owing to the development of many long and stout acicular setæ over its terminal parts and inner edges. The setæ of the endopodite are smooth as seen with 2 A, but with 2 D some five or six on the inner face of the second segment and again on the distal edge of the fourth segment are finely barbed. In the latter position two at the corner toward the exopodite have their fine lateral branches flattened like saw teeth, so that they resemble the cleansing setæ upon the penultimate segment of the fifth leg of the adult, elsewhere described (Andrews, '04). The fifth, or terminal segment of the endopodite, is armed with long, smooth, stout, spine-like setæ, with blunt points.

The third maxilliped has added but few plumes but many very long acicular setæ (figs. 33, 13). The plumes are a noticeable bunch at the tip of the exopodite and a few on its basal part, as well as very few on the protopodite and an increased number at the base of the epipodite. The gill region is now

for the first time provided with the phenomenally long hair-like coxopoditic setæ found in the adult and in all but the first larval stages on this and all the following thoracic somites. As seen in figure 33, these setæ arise external to the exopodite and add a conspicuous element to the appendages as they are longer than the entire exopodite and coil about like stiff wires. The endopodite bristles with exceedingly long, sharp needle-like setæ and has no real plumes whatever, though with 2 D it was evident that some of the longest needles on the terminal segment were very finely barbed. On the anterior, and inner, face of that segment there were also numerous stout setæ with flat saw-teeth like those above described, on the corner of the penultimate segment of the endopodite of the second maxilliped.

The chela (fig. 34) has greatly increased in size, as shown in comparing with fig. 14, and now has simple acicular setæ scattered over it but no plumose setæ. The terminal claw is no longer used as a holdfast and has no longer recurved tips. Henceforth of use as a cutting shears it now bears a large tooth on each blade as well as the rows of spines formerly present. At the base of the appendage the gills have increased in size and there is a long tuft of coxopodite setæ, which, however, are short in proportion to the enormous endopodite.

The four walking legs (figs. 35, 36, 37, 38) though much larger than in the first stage (figs. 15, 16, 17, 18) have not increased as much as have the chelæ and they retain their relative proportions and sizes. Like the chelæ they now bristle with acicular setæ and show no plumose setæ. However, at the distal edge of the penultimate segment of the fourth and of the fifth legs (figs. 37, 38) there are a few of the saw-like "cleansing setæ" previously referred to as occurring in the adult. The gills have changed only in size; the coxopodite setæ are very long threads, but only few in number and on the last leg reduced to one.

The branchial formula in the second stage was thus just like that above given for the first stage.

On the abdomen of the second stage there were still but four pairs of pleopods since the first were not yet formed and the sixth still remained inside of the telson. But each pleopod was now so well provided with plumose setæ that the appendage simulated a locomotor organ. The larvæ also now had the adult habit of swinging the pleopods back and forth and so producing currents in the water which may well be of aid in respiration as they would change the water supplied to the inhalent openings of the gill chambers. Each pleopod (fig. 39) had grown greatly in length as compared with its first appearance (fig. 19); the long plumose setæ arose from the distal parts of the exopodite and endopodite and resembled the plumes upon the exopodites of the maxillipeds (figs. 31, 32, 33). The exopodite was still the longer and the endopodite the

shorter of the two flat lobes that bear setæ so that the adult relationship in size of these parts was not yet arrived at.

While the telson of the first larva was nearly circular, in the second it was swollen laterally (fig. 23), and became thus transversely elongated since the sixth pleopods had now grown within it as very large lateral masses. The posterior edge of the telson was somewhat incised on the median plane and thus recalled the early bilobed condition found in the embryo some days before hatching.

The long plumose setæ of the telson (fig. 23) that aid the larva in swimming are the expanded products of the radiating glands seen within the telson of the first stage (fig. 20).

Thus provided with effective swimming setæ and more numerous sensory setæ the second stage larva gradually depends less and less upon its mother and finally leaves her altogether. After some eight to ten days these active larvæ cast off their shells and passed into a third stage.

The third larva was in the main very like the second but it had advanced a very important step in freeing its sixth abdominal appendages which henceforth are not inside the telson, but lying by the side of it to make the effective tail-fan that is used in rapid locomotion. Some hours before shedding the second larva plainly showed the sixth pleopods as dark red, partly opaque masses within the base of the telson and after shedding these appendages were expanded as is shown in figures 40 and 42. As both the end of the telson and the edges of these great flat sixth pleopods are set with plumose setæ the combined fringe of setæ augments the surface used by the larva in escaping backward by vigorous blows of the telson and sixth pleopods against the water.

The details of this effective and very large sixth pleopod which has been forming slowly on each side within the base of the telson ever since the larva came out of the egg, that is from some two to three weeks, are shown in figure 42, which shows the dorsal face of the left appendage of the sixth abdominal somite. This appendage joins onto the sixth somite and lies by the side of the telson as indicated in figure 40. The protopodite bears a prominent spine over the base of the endopodite; the endopodite is armed with two spines near its edge and the exopodite with five spines, along the edge of the two segments into which it is divided, as in the adult, by a movable hinge. Scattered over the surface are a few relatively short acicular setæ. It will be noted that the plumes along the edges of both endopodite and exopodite are arranged to make a most effective fan since those of the endopodite overlap some of those of the exopodite, when, as in figure 42, the exopodite is not extended as far as possible away from the median plane. At times the exopodite may be shut in under the endopodite like a part of a fan.

The real length of the terminal plumes is somewhat greater than shown in

the figure since they are foreshortened there; they do not stand out in straight continuation of the plane of the telson and sixth appendage but are curved downward so that their tips tend to point forward under the animal. The entire fan is thus somewhat concave on the ventral side and in life the larva carries its abdomen with its tip close to the substratum upon which it walks or stands (fig. 41), and seen from above the fan is foreshortened. Both the habit of carrying the fan inclined downward and the curved growth of the plumose setæ combine to make the fan a hollow scoop, which form would seem to be a more efficient one for striking the water forwards and thus propelling the animal backward. The forward bending of the setæ by making it more difficult for them to be forced back beyond the plane of the stiff parts of the fan would seem to make them a more efficient addition to the striking surface.

The period of life in which the effective tail fan is formed by the liberation of the sixth pleopods has hitherto remained unknown for the genus *Astacus*. Huxley ('80) says: "I imagine * * * that the appendages of the sixth abdominal somite are at that time (during the first ecdysis) expanded, but nothing is definitely known at present of these changes." Faxon ('85) records that specimen of *Astacus pallipes* 11 mm. long and ten days old still had the sixth pleopods enclosed within the telson and supposed that they would be set free after the second or third moult. Other observations seem to be lacking.

The telson seen foreshortened in the natural position figure 40, is seen in its true proportions in figure 43. From the circular form of the first stage (fig. 20) it has passed through the transversely elongated form of the second stage (fig. 23) to its present complex and angular form. By an imperfect transverse hinge it is now divided into an angular anterior part and a rounded posterior part. The fact that this transverse division of the telson does not show till the third larval stage is of interest in connection with the fact that this seems one of the recent acquisitions of the highest crayfish. In the lobsters and other marine forms, as well as in all the crayfish of the Southern Hemisphere, the telson is not at all divided and amongst the Potamobine or higher crayfish of the Northern Hemisphere the division of the telson is much more perfect in the highest forms, such as *Cambarus affinis*, less pronounced in some lower forms as *Cambarus Clarkii* and in *Astacus leniusculus*, which is doubtless less specialized than *Cambarus*, the division of the telson in the adult is by no means a perfect one.

In figure 43 are shown groups of acicular setæ symmetrically placed right and left; the rounded, terminal lobe is the only part bearing plumose setæ. These plumes stand in a single row and on the right and the left begin anteriorly as short setæ followed by others that very soon are much longer and of about constant length along the posterior border till near the median line when there is a sudden falling off in length and one very short setæ ends the series.

Just dorsal to this row of long plumes there is a row of much fewer and relatively short acicular setæ, shown in black in figure 43. In the reentrant angle between the two lobes of the telson, each side, there is a rounded spine similar to the one formed on the sixth pleopod (fig. 42) where the basal lobe of the exopodite joins the distal lobe.

Beyond this perfection of the locomotor apparatus of the abdomen the third larva differs but slightly from the second though there are differences in size, proportion, color and habits. Comparing figure 40 with figure 23 it is seen that the abdomen is wider, the cephalothorax more cylindrical, the limbs stouter, the entire aspect more heavy and crayfish-like.

The third larva is still sufficiently transparent to allow the heart to be seen through the shell, under the microscope, beating at about three times a second; the stomach and intestines also show dimly as dark areas.

The animal is not conspicuous upon sand or mud; its color is, as before, light with fine, dark-red specks. The eyes are black and the chelæ not red but pink. The liver region is greenish but the dark yolk so long conspicuous has disappeared with the perfection of sensory and locomotor organs. Where the head-thorax ends posteriorly there is a dark rim caused by concentration of pigment cells there and the abdomen still has a dark band across the dorsal side of the first somite. The region above the heart is quadrangular and very pale in color. Back of each eye there is a dark longitudinal band. The groove between the head and thorax dorsally is light and the pigment anterior to it is more dense. Under the microscope the arborescent red pigment cells often have a blue background and in some regions there are arborescent yellow cells amongst the red ones.

The considerable increase in size in passing from the second to the third stage may be seen by comparing the measurement given above, page 28, with the following. In third stage a larva measured 14 mm. from rostrum tip to edge of telson and 15 mm. to end of plumose setæ. The antenna was 12 mm. and the chela 10 mm. long. The width of the thorax was 3.5 mm. and its depth 4 mm. The telson was 2 mm. wide and the fan formed by it and the sixth pair of pleopods 5 mm. without the plumose setæ, and 6 mm. with them. The length of the head-thorax was 7 mm. and of the abdomen 7 mm.

In the third stage the larvæ were active and voracious, walking and swimming with ease and speed so that they were hard to catch. When kept together they soon lost chelæ in fight with one another and greedily devoured their dead fellows. When a piece of frog's muscle was put into a dish with these larvæ they seized as soon as they came into contact with it and holding the main mass with their chelæ and other claws dragged it backward while tearing off fragments with their mouth appendages. Thus twenty-three to twenty-five days after hatching larvæ in the third stage which had had no flesh food, unless

it were the chelæ of other larvæ, possessed well developed responses in the presence of flesh food. When meat was put on green ooze on the bottom of small aquaria containing such larvæ they showed no sign of being aware of it till in contact with it when they seized it ravenously with their mouth parts and then holding the mass with their chelæ jerked back like a dog tearing meat from a bone, so strongly as to pull off mouthfuls. When frightened away from the meat, which was then placed one-third of an inch to one side of its former position in the ooze, the larva returned to the place where the meat had been and seemed to masticate the ooze there and very slowly found the piece of meat again as if through touch, taste, or smell and not at all by sight.

The pugnacity of the third larvæ resulted in loss of limbs and specimens with legs pulled off at the "breaking joints" and also rapidly regenerating there were seen. Chantran stated that larvæ of *Astacus* would regenerate lost limbs in seventy days while the adult males required one and a half to two years and the females three or four years.

Excepting the newly expanded last abdominal appendages all of the appendages agreed with those of the second larva in most all details, but they were larger. The first antenna, however, in place of the eight sense clubs of the second stage, had eleven. These were placed as follows on the exopodite: four in a group on the distal segment; three at the distal end and one upon the middle of the under side of the penultimate segment; two at the distal end of the antopenultimate segment and one at the distal end of the next segment. Just as in the second stage the ear cavity was protected by a row of plumose setæ arching over it from its external border.

The long filament of the antenna was often broken near the tip but contained from 60 to 65 segments and some few of the terminal ones were constricted about the middle as if they might divide at the next moult.

The acicular setæ of the filament were about one-half as long as the segment, from the distal ends of which they arose in whorls of five or six. Toward the tip of the filament these setæ were much longer than in the second larva.

The mandibles, maxillæ, maxillipeds, chelæ, and walking legs with their gills and setæ were the same as in the second stage except for increase of size.

Upon the abdomen the pleopods also were as in the second stage, except in the case of the expanded sixth pair above described. As yet no appendages were seen upon the first abdominal somite but as in this *Astacus* no appendages were found there in the adult female it may be that only female larvæ were examined. The four pleopods of the somite anterior to the sixth still had the exopodite longer than the endopodite, as was also the case in the new sixth pleopods (fig. 42). In the adult this relative size of exopodite and endopodite is reversed in the abdominal organs that serve as secondary reproductive organs.

Thus in the male the first and the second somites bear the remarkable male sperm conductors in which the endopodites greatly exceed the exopodites or else form the only part developed while in the four following pleopods the larval relation is not much changed in the adult, as the exopodite is as large as the endopodite or, in the sixth pair, larger, and in all these four the function is not sexual but locomotor, or perhaps respiratory. In the female the same state is found in the sixth pair, the first pair are lacking, while the four other pairs have the endopodite much longer than the exopodite and both are used as reproductive organs, as supports for the eggs and larvæ.

Some of the larvæ of the third stage were kept from May 18 to October 2, and some of the moultings and increase in size noted, but no details of the gradual completion of adult structure were studied.

The fourth larval stage was reached by a moult after the larva had lived in the third stage twelve to fourteen days and was a little over one month old. The length then increased from 15 mm. to 17 mm. The color was no longer bright but dull and inconspicuous, pale grey densely spotted with almost black pigment and scarcely any flesh color though the tips of the chelæ were pink. There was a marked transverse band of bluish across the posterior edge of the carapace and the abdomen was much lighter than the thorax.

The fourth stage had advanced beyond the third in one important particular since now for the first time the appendages of the first abdominal somite were seen in some specimens, which were probably males. These pleopods, however, were as yet but very simple rounded knobs which projected from the sternal ridge of the first somite downward and decidedly inward, toward one another, and were about one-tenth of a millimeter long.

At the end of June when the larvæ had been in the fourth stage about a month they passed into a fifth stage which was nineteen millimeters long and in color red-brown or in some cases decidedly bluish. Kept in running water with water plants and tubifex for food and at a temperature as high as 21.5° C., they climbed about actively upon the plants or else remained buried in the ooze.

From some of the larvæ left in a closed aquarium with algal ooze from June 16 to October 2, there remained one survivor five months and a week old that measured 30 mm. in length, 6 mm. in width of thorax, 12 mm. in width of telson-fan, and 25 mm. along the antenna. This crayfish having areas for the ends of the oviducts upon the antepenultimate legs was a female, and it had no appendages upon the first abdominal segment. The color was bright, finely speckled over with brown; the legs lighter; the antennæ dark; the chelæ purplish with red and blue spots and there was still a blue transverse band across the posterior edge of the thorax. The eyes were brown. The shell and flesh were still translucent so that the intestine showed through the dorsal side of the abdomen.

This larva buried itself in the ooze but was so active as to be caught with difficulty. By October 20, it had shed and grown to a length of 34 mm., with antenna 32 mm. long, so that with the food that had been given it it had gained 4 mm. in length in eighteen days. This larva lived till February, 1905, and died when nine months old.

Another lot of larvæ kept in running water from July 16 to October 2, also yielded but one survivor which had increased from less than 26 mm. to 55 mm. and had a telson-fan 25 mm. wide, thorax 13 mm. wide, and antenna 36 mm. long. This fine young crayfish five months and a week old looked like the adult and was a male with well formed male stylets upon its first abdominal somite. The body was opaque and dark; greenish-blue and brown finely speckled; the legs lighter; the chelæ with much blue and with the conspicuous flesh-colored area that the adult has at the angle of the claw. The posterior edge of the carapace was dark blue and the antennæ were bluish at the base fading to brownish at the tip. The under side of the body was pale flesh-color, or colorless with some clear blue.

A third lot of larvæ, 13 mm. long on May 27, and in the third stage, were kept in more favorable conditions, that is, running water in a large tank, with mud, plants, and sunlight, and on October 2 there were six survivors. The measurements of these larvæ, five months and a week old are given in the table below:

Larva	A	B	C	D	E	F
Length	56	60	63	58	58?	52
Width	13	15	15	15	15	14
Antenna length . . .	51	52	52	50	55	45
Sex	♂	♀	♀	♀	♀	♂

The males A and F had well formed male organs upon the first abdominal somite while the four females had no appendages there, but the largest females B and C had two blue spots upon that somite that suggested some connection with a possible appendage. The colors of these large young were those of adults, and though all reared in the same tank they showed individual differences in: general color, some being more dark olive, others brown; in size and color of the spot at the angle of the claw, which in one was reduced to a mere light nodule and in others was a very large area; in color of under side of claw which was dark or else bright flaming-red.

The eight young reared to an age of five months and one week thus measured 30, 55, 56, 60, 63, 58, 58, 52 or an average of 54 mm.

Tabulating the facts above recorded as to the size and length of duration of the larval stages as far as they were followed we find the following:

Stage.	Duration.	Length of body.	Habit.
1	4-13 days	9 mm.	Fast to parent.
2	8-10 "	11 mm.	Free and gradually becoming independent.
3	12-14 "	14-15 mm.	Independent.
4	30 "	17 mm.	Independent.
5	?	19-20 mm.	Independent.

The number of moults necessary to increase the larva from the fifth stage when about 20 mm. long to the autumnal larvæ ranging from 30 to 63 and averaging 54 is not known. The amount of increase at each moult above tabulated was about 3 mm. In one case above cited a larva which had grown only to 30 mm. in the autumn, when well fed quickly gained 4 mm. probably in one moult. We might expect then an increment of 3 or 4 mm. at each moult, and growth from the 20 mm. length of the fifth stage to the average autumnal length of 54 mm. may have taken from 8 to 11 moults thus making an estimated total of 12 to 15 moults the first growing season. The size reached was, however, evidently determined in part by food. On such basis it may have taken even more moults to produce the large autumn young 60 and 63 mm. long. And at all events the above larva 30 mm. long having been kept in a closed aquarium should be disregarded in reckoning the average. Rejecting this the average for the seven others reared in running water would be 57. To attain this average size it may well have required at least nine moults after the fifth stage.

It seems then not improbable that this *Astacus*, under these conditions moulted at least a dozen times while growing to a length of over two inches in the first five months of its life. Four stages were observed in the first two months when the length had not extended to four-fifths of an inch, and probably twice as many stages were necessary in the following three months to bring the length up to over two inches.

The only previous records of the rate of growth of young *Astacus* seem to be those of Soubeiran, Chantran, and Steffenberg. Soubeiran ('65) from measurements of a crayfish in a French crayfish farm concluded that they did not moult more than once in the first year, and were 50 mm. long when one year old. Chantran ('70) thought the young crayfish moulted five times in 85 to 100 days of July, August, and September and no more till the end of the next April. The first moult was at ten days after hatching and the other four at intervals of twenty to twenty-five days each. These results were modified by his further studies, also in the laboratory, and later ('71) he stated the number of moults in the first summer was eight and that the temperature influenced the

number of moults so that there were six in the second summer, if hot. Finally Steffenberg ('72) gave the lengths of the *Astacus* larvæ in Sweden as 8.5 mm. at hatching; 11 mm. in the second stage; 13 mm. in the third stage; 15 mm. in the fourth stage.

Astacus leniusculus thus agrees closely with the *Astacus* of Sweden in the length of the larva in the first, second, third, and fourth stages. As far as the facts go it seems evident that *Astacus leniusculus* probably has more larval stages than have been described for the first year young of the French *Astacus*, but the differences are probably due more to difference in food and in temperature than to any innate differences in the species.

With this close agreement in larval life between the American and European *Astacus* and the demonstrated success of rearing in the laboratory the large young from the eggs brought overland, the culture of the American *Astacus* should be as successful when undertaken as has been crayfish culture in Europe. As elsewhere remarked (Andrews, :06³; :06⁴) the introduction of the western *Astacus leniusculus* into Eastern waters might not only prove of economic value but also help to throw light upon the interesting problem of the nature of the causes that have brought about the present remarkable geographical distribution of crayfish. And the geographical distribution of crayfish is intimately connected with the origin of species in this group.

CAMBARUS AFFINIS.

As elsewhere described (Andrews, :04) this common crayfish of Maryland and adjacent States lays its eggs in the spring, and the development of the young can be followed in the laboratory. Preparatory to laying, the females carefully cleanse the parts of their bodies to which the eggs are to be attached and the eggs flow out of the oviducts into a mucous mass which covers the pleopods upon which, after some special "turning" movements of the female, the eggs are found attached each by its own stalk (Andrews, :06).

The hatching young thus find themselves upon the abdomen of the parent and here, as in *Astacus*, there are special contrivances which prolong the connection of parent and offspring for some time after hatching so that the attainment of a free and independent existence is a slow and gradual process.

The special arrangements used in the attachment of the egg and of the larvæ in the first and second stages form a series of interesting adjustments between the adult and the next generation. These successive means of association of mother and offspring will be described in what follows.

We will first consider the egg and the maternal appendages.

The eggs, while numerous, three to six hundred according to the size of the female, are small, scarcely 1.5 mm. as compared with 2.5 mm. in *Astacus leniusculus*. Some few become fastened to setæ upon the abdominal sterna but

most are fastened to plumose hairs upon the pleopods of the second to fifth somites inclusive, and some account of these pleopods may be given here in connection with questions as to how the eggs and larvæ are attached to the mother.

The posterior face of the fourth left pleopod of a female about to lay is represented in figure 44. It will be noted that the endopodite is longer than the exopodite, and both bear a fringe of long plumose setæ on their right and left edges. The setæ are shorter toward the base of the exopodite and of the endopodite while on the protopodite there are but a few plumose setæ in two tufts upon the inner edge. The long setæ make of the pleopod a wide fan or flat brush since the setæ lie close together and like the wing feathers of a bird form a rather flat firm expanse. The protopodite has a basal part containing several calcified plates in a soft membrane, and a long segment that is well calcified except for a triangular soft area toward its base on the posterior face. The endopodite is made of two segments and the exopodite of one. The endopodite and the exopodite are also somewhat annulated in appearance owing to the grouping of the cement glands. The groups are opaque white and from each side tend to run together across the posterior face. Distally they do not meet but proximally they meet and make cross bands. Still farther toward the base the glands cover the entire surface more and more completely. The non-glandular areas are clear and not opaque, and in the figure are represented dark.

The anterior face differs from the posterior (fig. 44) in a greater development of glands which formed transverse bands more nearly all the way to the tip. As so many of the glands are to the right and left near the setæ, they are well placed to smear their secretion over the setæ.

Toward the tips of the pleopods the exoskeleton is so translucent that with Zeiss 2 D, the striation of the muscle fibers, the branched connective tissue cells, granular blood corpuscles, and the polygonal gland cells may be seen. The gland cells are about the diameter of a muscle fiber and larger than a blood corpuscle.

In most cases the plumose setæ spring from over the glands, and the base of a seta is as thick as two gland cells. The setæ have a large central cavity and a thick wall which is highly refractive and clear and is of unequal thickness so that it projects into the cavity in lumps or waves, and gives the distal part of the axis of the seta a somewhat segmented aspect. At the base each seta is articulated to the exoskeleton, and its central cavity is constricted by a clear refractive thickening of the wall that leaves very little communication between the cavity of the setæ and the cavity of the body.

The side branches of the seta spring out not only along its sides but also, scatteringly, along its posterior face so that the plumose seta is more like a bottle brush than like a flat feather. While the side branches generally make

a wide angle with the main axis, toward the tip they become more nearly parallel with it and thus a fine terminal brush is formed by the main axis coming gradually to an acute point in the midst of the surrounding side branches.

The eggs are fastened to these plumose hairs by a secretion that probably comes out of the glands of the pleopods (Andrews, :06²). They then look as in figure 45, which represents the anterior face of a pleopod cut off in the afternoon of April 18, 1905, from a female that had laid the night before. Most of the pleopod is concealed by the eggs which are opaque yellow balls and very elastic. The plumose setæ are all bound together by a common cement or mass of glaire so that the individual setæ are not seen. From this mass a clear, flat, glassy band of material goes out to each egg. Upon separating the eggs these bands are seen as clear, flat stalks, continuous at one end with the mass that binds the setæ together and at the other end with the envelope about the egg. While the shorter bands are flat and wide the longer bands are more string-like and some few are twisted. Though these stalks may cross one another and be more or less intertwined they are not fastened to one another. As the setæ spring chiefly from the side of the pleopod and the eggs are tied to the setæ, the eggs may be combed out, as it were, into groups on each side of the endopodite and of the exopodite. Where the stalk joins the egg it is enlarged as a bell, or tent, full of liquid and its edges are continuous with the outer layer of the egg capsule.

Each egg had then its own separate stalk, though a very few exceptions showed two stalks connected with an egg at different points and with a common extension running over the surface of the egg from one stalk to the other.

Thus very firmly attached to the mother, the eggs are waved back and forth by the mother who regulates the movements of the pleopods in accordance with the oxygen supply in the water, and thus they slowly develop till they hatch. The old egg cases and stalks still remain fast to the pleopods, and are of use to the larva as a means of prolonging its life of dependence upon the mother.

We will next describe the way in which the first larva is connected with the mother.

In hatching, the larva comes slowly out of the egg capsule through a rent along its back, in such a way as to draw out the legs and abdomen last of all as represented in figure 8 in a previous paper (Andrews, :04). In fact the tip of the abdomen remains inside the egg case long after the soft, helpless larva is extruded and left dangling down into the water. And all these newly hatched larvæ would fall to the bottom were it not for a firm attachment of the tip of the abdomen inside the egg case. As it is some time before the respiratory movements become perfect, as the limbs only gradually acquire ability to move, and as the body is globose and the creature cannot stand on its legs, the larva would

probably perish but for this temporary fastening of the abdomen that tides it over the weak period till it can reach up and take hold of the egg stalk as indicated in figure 9 of the above paper.

This attachment of the abdomen is the same phenomenon found in *Astacus* and is brought about by a telson thread; but as the eggs and larvæ of *Cambarus* are so much smaller and as the tip of the abdomen remains inside the egg capsule the facts are not so readily made out and in a previous notice (Andrews, :04) the telson thread was spoken of as proceeding from the anal region, though further study shows it to be fastened to glandular spines of the telson edge as in *Astacus*.

In *Cambarus affinis* the tip of the abdomen is fastened by a short thread to a crumpled membrane which lies inside of the spheroidal egg case and is firmly fixed to the egg case on the side near the stalk. The short thread and the membrane together are comparable to the long membranous thread that in *Astacus* allows the larva to hang far down from the egg case. For convenience we will describe the two parts of the telson thread separately. The short part fastened to the abdomen remains on larvæ 48 hours old though then broken off from the more membranous part inside the egg case. The short part may be whipped up and down in the water like a lash when the larva flaps its abdomen (fig. 50).

At hatching, the telson (fig. 46) is a simple rounded, translucent lobe, with minute spines on each side of the median plane, which formerly fastened it to the telson thread. In this ventral view of a recently hatched larva the pleopods of the fourth and fifth somites are seen free while the sixth somite has its pleopods as lobes inside the telson on each side of the anus. The terminal part of the telson is traversed by radiating lines which point to spines along the edge of the telson. These lines are in reality rows of cells that are to make the plumose setæ of later larval stages and the rest of the translucent flat telson is filled by a parenchymatous mass traversed by blood spaces in which float blood corpuscles.

The telson thread arising from the edge of the telson is a flat band that is readily twisted and shows a striation due to fine wrinkles of the stiff chitin-like material composing this very strong but thin and translucent membrane.

Twenty hours after hatching, the telson had changed form, become more quadrangular and its terminal part was somewhat three lobed (fig. 47). And as the spines to which the telson thread was attached were all on the middle lobe it seemed as if the pull of the telson thread might have aided in making the middle region protrude as a lobe. At this time the cuticle of the larva was separating from the body in preparation for the moult from the first to the second stage.

While the form of the telson is thus so different from that of *Astacus* (fig. 20), the mode of attachment of the telson thread is fundamentally the same. Figure 48 shows the tip of the telson of a larva torn out of its shell just before hatching; the thread is fastened to five or six spines on each side of the median plane in the same way as in *Astacus* (fig. 21). Thus nearly the same number of spines are specialized in both crayfish for attachment of the thread though in *Cambarus affinis* the entire number of spines is much less and they are found only on the posterior part of the edge of the telson.

All the spines are glass clear, ice-like in refraction; the lateral ones are bent toward the tip of the telson and the five or six specialized glandular spines converge toward a center as shown in figure 48. The lateral spines toward the posterior end tend to show blunt brushes and secreted lobes at their tips, and thus form somewhat of a transition to the effective specialized spines.

These specialized central spines are much longer and thicker and bent, often at right angles. Some are fused together by their blunt ends and all seem to have flowed out at the tips as a mass which is now fibrous and which binds all of them to each other and to the telson thread. They seem comparable to paste tubes which should squeeze out a myelin-like substance that could coagulate as strong fibrils.

The appearances suggested that the rows of gland cells that later make the plumose setæ of the later larva, had previously, in late embryonic life, secreted a substance which oozed out of the hollow spines and set into a firm cement; but these cells no doubt were also active in making the cuticular walls of the spines themselves, and no sharp line seemed drawn between the substance of the cuticular spines and the material that issued out of their tips. Both are presumably the same exoskeleton and made from ectoderm cells that later make other exoskeletal secretions in the form of plumose setæ. In *Astacus* (fig. 21) the distinction between spine and secretion was more evident, but in this *Cambarus* (fig. 48) the spines are so minute that details are not as readily seen.

While the mode of attachment of the telson thread to the telson is thus the same in *Astacus* and in *Cambarus*, the thread itself differs in appearance in the two crayfish, in the former being pulled out into a long thread, in the latter being for the most part a wrinkled mass of membrane within the egg case. While in *Astacus* the thread is apparently a cast off embryonic skin, this is by no means obvious in *Cambarus* and an interpretation of its meaning was had only from the following facts. When an egg ready to hatch was scratched with a needle the outer egg case came off and the larva popped out alive but still enclosed in a thin spheroidal membrane. This membrane was firmly fastened to the outer egg case by one small area towards which the legs converged and which lay opposite the claws. When the egg case was pulled it remained so fast to the membrane that both were drawn out of shape rather than

separate. The part of the egg case attached to the membrane was often near to the stalk of the egg case, which led one to infer that the attachment of egg case and membrane and the making of the egg stalk might have some common cause very early in the life of the egg, but no observations were made to decide this. Left to itself a few minutes, such an embryo removed from the shell burst the membrane without the aid of the egg case. Its telson was fastened by its special glandular spines to the inside of the membrane, and soon it reached about with its claws and seized hold of the membrane. This bag-like membrane was thus the same thing as the telson thread of normal hatching. When dissected from an embryo this membrane was seen to be a bag fast to the telson spines, but with no observed special envelopes for the limbs as would be the case were it a cast skin. Before hatching, the membrane was stretched tightly all over the abdomen except where the two special groups of glandular telson spines were, and there the membrane was raised up as two swellings, one right and one left, much higher than the spines themselves. The space between the membrane and the spines was occupied by a mass of seemingly liquid lumps which in some cases with 4 D showed a finely fibrous material amongst the clear spines which, in places, extended out to the membrane like a fibrous coagulum binding the spines to the membrane. On the median line the membrane was close down against the telson so that the secreted masses right and left seemed to have locally pushed the membrane away from its original connection with the telson.

This membrane was well developed in embryos of stages J to K of Reichenbach but upon dissecting, these embryos dropped out of the membrane, as the telson was then not yet fast to the membrane, though the membrane was firmly fastened to the egg case near the stalk. In the early stage F there was also a membrane over the body and this was loose over the slightly projecting, small abdomen.

It seems probable that the telson thread of *Astacus*, which is a cuticle formed over the embryo when its limbs are well advanced and thus has tubular outgrowths to cover the limbs, is represented in *Cambarus* by a telson thread having the form of a sac-like membrane formed so early that the small limbs receive no special envelopes. In both cases there is a special outgrowth to cover the abdomen, but while in *Astacus* this is a long bag, in *Cambarus* it is scarcely recognizable as a separate region.

In both, by some unknown process that is imagined to be associated with fertilization phenomena, the membrane is made fast to the outer egg case, and in both the membrane becomes fastened to the embryo by the activity of certain telson glands. A diagram of *Astacus* would represent it as escaping from a cuticle when hatching, a cuticle fastened to the egg case at one point, and near that point fastened, inside, to the telson of the larva. A diagram of *Cambarus*

would represent it as casting off a membrane at hatching which is fastened as in *Astacus* but lacks the tubular coverings for the limbs. Here again the small size of *Cambarus* may have led to misinterpretation of what is plain in *Astacus*.

Having described this temporary, mechanical association of parent and young we will next consider the more active association that lasts during the rest of the first larval period.

The larvæ, supported for a time by the telson thread, soon established a second connection with the mother by seizing hold of the egg case, the egg stalk, or in many cases the matted pleopod setæ, with their chelæ, and thus, for a time, were fastened both by the telson thread and by the chelæ. While the eggs hung loosely from the pleopod, figure 45, the young crayfish had the habit of reaching in their chelæ as far as possible amongst the egg stalks and pulling themselves close to the pleopods so that they became densely crowded together in a solid mass (fig. 52). In life this mass of young covering the pleopod is a curious sight suggesting mammalian young crowding for maternal milk. Each held itself close to the pleopod, and when disturbed drew itself all the closer as if eager to remain. The part exposed to view is chiefly the rounded head-thorax which is flesh-colored but is conspicuously marked by the enclosed saddle-shaped dark yolk. Here and there the legs and abdomen are seen in profile (fig. 52), but generally the abdomen and legs are under the body, the abdomen being bent somewhat as in a brachyuran (fig. 50) and a full dorsal view shows chiefly the simple head-thorax (fig. 49). The larvæ thus seem egg-like, inert and inactive.

The long-stalked egg cases stand out above the backs of the larvæ as do also a few belated eggs which may hatch some forty-eight hours after the rest (fig. 52). By this time many of the larvæ have broken the telson thread and when disturbed make slight movements with their legs and flap the abdomen to which is fastened, like a small handkerchief, the narrow telson end of the thread, while the membranous sac remains within the egg case.

So closely do the larvæ crowd together that only few of the pleopod setæ can be seen. Figure 52 represents a case in which some of the larvæ had been removed.

While the color of the first larva is light flesh-color to the naked eye, under the microscope the creature is clear, and colorless, except for the scattered, arborescent, vermilion pigment cells over the carapace, abdomen, basal parts of antennæ and some few segments of the legs, and except for the reddish pigment in the eyes and for the large red-brown yolk mass.

The shape of the larva when alive is retained very well in specimens killed in Worcester's liquid, though in many other liquids the head-thorax swells and is abnormally glubose, while the branchiostegites roll back and expose the gills.

In neither the dorsal nor the side view (figs. 49, 50) can the rostrum be well seen, but in a front view (fig. 51) it is seen to be pointed, though so bent downward between the eyes as to be of no use as a protection.

The body is thus rounded and embryonic in proportion and lacks all the angularities of adults or of active larvæ.

With the clinging helpless state of the larvæ is associated a lack of ordinary use of the antennæ. The second antennæ, which in larval and adult life of crustacea are carried out in front of the animal, are here folded down under the thorax (fig. 50), between the right and left series of legs. This peculiar position of the antennæ is not found in *Astacus* and here is brought about at the time of hatching, for in the embryo these antennæ grow backward along the edge of the carapace external to the bases of the limbs. Soon after hatching the legs get astride the antennæ and this apparently useless position of the antennæ is maintained during the rest of the life of the larva in its first stage.

This peculiar position of the antennæ as well as the down bent rostrum were observed in another species of Cambarus, *C. rusticus* by Faxon ('85); and later Steele (:02) described the rostrum as bent down in *C. gracilis* and in other species and the legs, antennæ and abdomen of *C. virilis* as lying under the thorax; thus it seems possible that the first stage of *Cambarus* in general differs from *Astacus* in these characteristics of the antenna.

The appendages of *C. affinis* in this first stage resemble those of *Astacus leniusculus* in being without the setæ of later stages, but they differ not only in being very much smaller but in being, in some cases, more simple. Thus the first antenna (fig. 53) has only four segments in the endopodite and in the exopodite in place of five, and the tip of the endopodite bears no sensory club.

The antenna (fig. 54) is remarkably short, as is seen on comparing that figure with figure 7, and figure 50 with figure 3. The filament is bent back and has but twenty-five segments, or about half as many as *Astacus*. In the adult there may be 150 segments. Carried as it is under the thorax it reaches only to the beginning of the abdomen while in *Astacus* the antenna if in such a position would reach about to the end of the abdomen when stretched out.

The mandible (fig. 55) has the same simple form as in *Astacus* (fig. 8).

The first maxilla (fig. 56) is like that of *Astacus* (fig. 9), but more simple in lacking the few filose setæ and in having fewer spines.

The second maxilla (fig. 57) as in *Astacus* (fig. 10), has a row of plumose setæ along the entire edge of the scaphognathite and is elsewhere as simple.

The maxillipeds (figs. 58, 59, 60) represent in miniature the structure seen in *Astacus* (figs. 11, 12, 13) with slight increase in smoothness due to the presence of fewer setæ and spines. There are important simplifications, however, in the gills which in some cases have but half as many side filaments as in *Astacus*.

The chelæ (figs. 61, 62) have the same recurved tips as in *Astacus*. Soon

after hatching the larva opens its claws widely (fig. 50), and after some failures fastens them to the egg stalk, or to the setæ of the mother's pleopods. Once firmly locked in the hardened secretion that makes the egg stalks and binds the setæ together, these larval chelæ remain fast during the first stage. And even after the larva has escaped from its cuticle and passed into the second stage the old cuticles are left firmly hung to the mother by the cast-off chelæ.

As in *Astacus* the chela has at first a cutting edge set with a row of few and simple spines, but when the first larva is ready to moult, the loosening of the cuticle reveals the fact that each spine will be replaced by one that is serrated (fig. 62), owing to the presence of flat plates along the posterior face of the new spines. In addition the second stage will have on its claw some spines not represented in the first stage and also near the tips of the claw some long, simple setæ in place of the blunt spines there in the first stage. This figure shows that the recurved tips will be abandoned in the second stage since there are already formed tips that are but slightly curved hooks, to take the place of former recurved tips, one of which was broken off in this specimen.

The chela besides being so small and weak, is inferior to that of *Astacus* in having its gills less developed, the anterior arthrobranch being very short and simple and with but few side filaments.

In the four walking legs (figs. 63, 64, 65, 66), we find the same proportions as in *Astacus* but the pleurobranchiæ are absent and the arthrobranchiæ are more simple, especially the anterior ones.

The branchial formula for the first stage is thus the same as in the adult and is as follows:

	Podo- branchiæ.	Arthrobranchiæ.		Pleuro- branchiæ.	Total.
		Anterior.	Posterior.		
Somite of 2d maxilliped	1	1	0	0	2
Somite of 3d maxilliped	1	1	1	0	3
Somite of chela	1	1	1	0	3
Somite of 1st walking leg	1	1	1	0	3
Somite of 2d walking leg	1	1	1	0	3
Somite of 3d walking leg	1	1	1	0	3
Somite of 4th walking leg	0	0	0	0	0
	6	6	5	0	17

That the pleurobranch of the last thoracic somite of *Astacus* was absent from *Cambarus rusticus* larvæ 4 mm. long and evidently just out of the egg was observed by Faxon ('85) and it is probable that no members of the genus *Cambarus* have remnants left in the early larvæ of that pleurobranch still found in *Astacus*.

As in *Astacus* the abdomen in the first stage of *C. affinis*, just as in some other species of *Cambarus* studied by Faxon ('85), has only four pairs of evident pleopods. The sixth pair are but partly formed and enclosed within the telson while the first pair exist here as minute buds readily overlooked and not found in *Astacus* nor as yet described in other species of *Cambarus*. The four evident pleopods (fig. 67) have equal exopodites and endopodites in contrast to the proportion found in *Astacus* (fig. 19). The beginnings of the first pair of pleopods are but minute rounded elevations of the sternal ridge of the first somite (as indicated in fig. 3; Andrews, :06), and contain a small mass of nucleated epidermal cells. The appendages of the sixth somite are large internal buds that cause the side part of the telson to be very thick as they fill all its interior on each side of the anus. As this region is very translucent each pleopod can be seen to have a long outer and a short inner lobe (figs. 46, 47).

The first larva is in several ways more imperfect than the same stage in *Astacus*, and as it lives inactive and fastened to the maternal pleopods it seems but an extension of the embryonic period in preparation for the second stage, and not at all a self supporting organism. The second stage is made way for by the gradual loosening of the cuticle of the first stage, as above noted in case of the claws of the chelæ, and by the addition within the old cuticle of the new exoskeletal structure of the second stage. This was evident in the telson of a larva twenty hours after hatching, where the old cuticle was raised up the length of the old telson spines and new spines had grown out across this space to enter the hollows of the old spines. Later, just before the moult, the space between the old and new cuticle was twice the length of the old spines, which were still fastened to the remnant of the telson thread, and the new spines still projected across the space into the old hollow spines.

The moulting into the second stage took place after the first stage had lived this dependent and preparatory life for about forty-eight hours and the larva resulting had the form shown in figures 68 and 69. These second larvæ unlike the second stage of *Astacus* did not gradually become separated from the mother and live isolated, but remained again adhering to the pleopods till a second moult brought them into the third stage which was the first free larval form. Thus the pleopods were still covered with the crowded larvæ. Figure 70 represents a pleopod with only part of its load of second stage larvæ as many had been removed.

In the following description it will be shown that in the second larva also there is a peculiar temporary mechanical connection of young and parent.

Each pleopod in life was covered as with a mass of animated jewels of pink and garnet colors. The flesh-colored larvæ were still strongly marked by the yolk that remained in each as a garnet red mass of saddle-bag shape. No longer firmly fixed by the chelæ, the larvæ soon reached about for firm objects to

which to hold, or kicking their legs about, swinging their pleopods and flapping their abdomens formed an animated restless mass quite unlike the inert one seen in the previous period. Thus active, though holding fast, the larvæ were no longer densely crowded in close to the pleopods as in the first stage (fig. 52), but loosely aggregated (fig. 70).

The old larval cases shrivelled and not readily recognizable as more than crumpled membranes, still remained fast by their chelæ and the old egg cases were yet fixed by their stalks to the maternal pleopods. Side by side on old egg stalks were the pairs of living claws and the empty cuticles of old cast off claws. Thus the second larvæ lived amidst a mass of former envelopes,—the ghost-like phantoms of their former selves and the rigid cradles of those selves.

While many larvæ holding fast to the pleopod hairs and to egg stalks tend to pull themselves in close to the pleopod so that they become buried amongst the egg stalks and empty cases, a few larvæ at the periphery of the mass may dangle free in the water, as in the lower right corner of figure 70. These are larvæ which have recently cast off the first cuticle and are still in a soft, helpless condition. Before these larvæ succeed in reaching up and seizing hold of some part of the general mass they are in danger of falling to the bottom where they might be lost, though when they are removed it is found that they can stand and walk, with difficulty. However they seem strongly controlled by an instinct to climb, crawling over one another in heaps when removed from the mother and swimming only when thrown into Perenyi's liquid and possibly many would eventually climb up onto the mother. At all events they are prevented from falling away from the mother by a fine thread which, as indicated in figure 70, passes from the posterior end of the larva to the crumpled, cast-off cuticle which still is firmly fixed to the mother. This thread may be called the anal thread since it actually comes from the anus and not from the rim of the telson as did the supporting thread of the first larva.

But before discussing this anal thread some general features of the second larva may be considered. The abdomen is still carried bent down as seen in figures 69 and 68, but the cephalothorax is more elongated and narrow than in the first stage and ends in a sharp pointed toothed rostrum that projects out between the eyes and is only slightly bent down. The legs are longer and may now be used for walking and the antennæ stand out in front of the animal as if useful feelers, in strong contrast to their apparently useless position in the first larva (fig. 50). This increase in length and change of position of the antennæ gives the mass of young in the second stage (fig. 70) a much more living aspect than was possible in the first stage (fig. 52). The abdomen when straightened out is seen to end still as a simple rounded telson (fig. 71) with the large sixth pleopod still within it on each side the anus and with a terminal part that has its middle lobe set off but slightly from the side lobes. The edge of the telson

bears only simple spines as in the first stage (fig. 46), and these spines are not divided into groups (fig. 72), but all seem equally useless since none act as glands and there is no telson thread formed in the second stage. The middle lobe bears spines all along its edge, some sixteen on each side replacing the fewer spines of the first stage (fig. 48). Passing in from each spine there is still a radiating line of cells which is active in making the plumose setae which we miss in this second stage but which will be expanded in the third stage.

It is to the larva of the above structure that the anal thread is fastened and this may now be considered in connection with this telson that has progressed so little beyond its first form. As seen in figure 71, the anal thread was in life a clear narrow ribbon running from the anus a distance greater than the length of the telson to be fast to a crumpled mass which proved to be the cast-off cuticle. This thread was fast at one end inside the intestine of the larva and at the other was fast to the cast cuticle by being continuous with it at the edge of the cast off anal opening. In fact the anal thread is nothing more nor less than the old cuticular lining of the intestine of the first larva still continuous with the cast-off cuticle of the abdomen and not yet entirely loosened from the intestine of the second larva. An examination of the recently shed larva showed that the cuticle of the larva had become loose all over the body and some distance into the intestine, but that farther in it still adhered to the epithelial lining of the intestine.

When the larva has moulted, the old cuticle of the abdomen is found to be telescoped and its old telson joined by a short cord to the new telson as shown in figure 73; A. If the old cuticle is then pulled with forceps it does not break loose but the thread comes out of the anus of the larva and is thus made longer, as shown in figure 73 B, by a shortening and crumpling of the intestine. There is thus a posterior part of the intestine (*y*) in which the old cuticle is loose and an anterior part (*x*) where it is still fast, the region (*a*) being the first place met with where the cuticle lining of the intestine does not pull away from the intestinal wall. Hence the region (*y*) is thrown into folds and the region (*x*) stretched and pulled bodily toward the anus when tension of the anal string pulls the region (*a*) toward the anus. The anal thread is the loose lining of the intestine as far as it is pulled out of the anus and while in origin a tubular cuticle it is stretched out as a flat ribbon which seems made of clear wire-like fibres, but in reality is only thrown into longitudinal folds due in part to the longitudinal ridges which sections show are present in the intestine. In figure 74 is seen more enlarged the region of the intestine where the loosened cuticle running up from the anus arrives at the region (*a*) where it is still fast to the epithelial walls, as made out in preparations cleared in Bela Haller's liquid.

Though actual ecdysis was not seen, it is evident that what happens in the moulting of the first larva to the second stage, is that the lining of the intestine

for a certain distance up from the anus, delays in casting off its cuticle. The animal bursting out of its head-thorax cuticle and freeing its limbs from their cases would next pull its abdomen out of its old cuticle, but the old cuticular lining of the intestine remains firm where continuous with the outside cuticle at the edges of the anus, and acts like a string tied to the old telson and to the inside of the abdomen far up in the intestine. If the larva then drops out of its cuticle and is too weak to seize hold of the old cuticle, or neighboring firm objects, it will dangle suspended by the anal thread and the tension on this thread will pull it out of the anus as far as possible. By puckering the posterior part of the intestine and by dragging the anterior part of the intestine backward there is thus drawn out of the anus an anal string which, however, is not as long as the abdomen, by any means. The old telson being a stiff plate and the old walls of the abdomen being elsewhere like the bellows of a camera, the pull of the weight of the larva along the telson thread drags the old telson against the cast-off abdominal rings and telescopes the abdominal cuticle as if the front board of a camera were pulled back against the bellows by a string inside. The tendency of the telson string would be to turn the abdominal cuticle of the cast-off case inside out as a hand holding fast to a sleeve would turn the sleeve inside out when the arm was withdrawn, but the presence of a firm, board-like telson at the bottom of the sleeve allows only a telescoping of the cuticle. By this means the old abdominal walls are so shut up that the free anal string from the old anus to new anus is no longer inside the old abdomen, but largely free in the water, as indicated in the diagram on page 54.

Soon after hatching, presumably, the cuticular lining of the intestine becomes loosened along the regions (*a*) and (*x*) and then the anal thread is pulled out from the intestine as is usual in moulting. In the meantime the larva becomes strong enough to reach about and take hold of the old larval skin or of other objects fastened to the pleopods and does not need the anal string as a means of support.

By this delay in the casting off of part of the cuticular lining of the intestine an advantage to the larva seems to result. Both in the first stage and in the second stage the larva has a mechanical means of fixation to its mother during the brief period when it is unable to use its claws for this purpose. Reviewing the life of the crayfish up to the third stage with the aid of a rude diagram (page 54), with reference to the association of mother and offspring, we have seen: the egg fastened to the pleopod setæ by a material that hardens soon after the egg is laid; the larva hung for awhile by its telson thread; then holding by its claws as well as telson thread till the latter breaks when claws alone hold it locked to the mother; then dangled loose as a helpless second stage supported by an anal thread till able to take hold with its claws and cast off the thread, that is, to finish the moulting; finally moulting again and as an active

third larva living no longer mechanically bound to the mother, with whom it gradually ceases to associate. The mechanical supports, the telson thread and the anal thread, are left hanging with the egg stalks, egg cases and the other part of the cast off membrane and cuticle; all fast to the pleopod setæ which are bound together in a secreted mass. All this material soon disappears, apparently being eaten up by the third larva before it leaves the mother to hunt other food in wider fields.

EXPLANATION OF DIAGRAM.

Pl. = pleopod of mother; Pl. H. = setæ of pleopod; 1 = cast cuticle of first larva; St. = egg stalk; Sh. = egg shell; M. = membrane inside shell; Tf. = telson thread, which is broken; Af. = anal thread; = second stage on the mother's pleopods held by anal thread till the claws have taken hold.

Of the two threads which we here describe as mechanical means for preserving the association of the mother and offspring and prolonging the maternal protection beyond the egg stage over larval periods that are especially helpless and not self supporting, the first, the telson thread, is a secretion of glands active before the larva hatches combined with an adherence of the larval envelope to the egg shell that was determined very early in embryonic life. The second thread, the anal thread, is a much more simple and temporary attachment brought about by a delay in casting off the cuticular lining of the intestine and thus utilizing what is cast off as a means of keeping the larva fastened to its old cuticle till the claws can lay hold of some firm object.

When the second larva finally becomes fastened by its claws it remains some six days in a condition of little activity. It is dependent upon the mother for physical support though in a state intermediate between the invariably fixed first larva and the wandering third larva. The shape of the claws (fig. 84) shows that they would not become as firmly locked as did the claws of the first larva (figs. 61, 62), though they might stick firmly into soft material.

When larvæ in the second stage were put into Kleienberg's picrosulphuric they flapped their abdomens for some minutes but did not loosen their claws from the pleopod of the mother; when put into alcohol, however, they dropped off, and when into Worcester's liquid they all broke loose and fell to the bottom where they lay kicking their legs for some time as they were not so readily killed as were the third larvæ.

The second larva lives much as did the first larva and in structure is very like it but it differs not only in the above mentioned characteristics of the telson and mode of fixation to the parent but also in the size, proportions, and amount of armament with setæ of some of the appendages as will be seen when the nineteen pairs of appendages are taken up in sequence.

The first antenna (fig. 76) has grown much longer than it was in the first stage (fig. 53), and the basal segment of the exopodite has divided into two so that there are now five in place of four segments. And as the figure shows there are now a few setæ upon endopodite, exopodite, and protopodite and of these there are five blunt sensory clubs on the inner face of the exopodite. Two of these are close together on the distal end of the fourth segment, two are close together on the swollen basal half of the fifth and the remaining one stands alone on the narrow terminal half of same segment. The ear is still a simple deep pit without as yet any setæ along its edges but with only seven or eight spines or teeth seen with 2 D along its external edge where the second stage of *Astacus* has plumose setæ.

The second antenna (fig. 77) has greatly grown in length and in perfection of form and as above noted (fig. 69) is no longer carried in the remarkable position it occupied in the first stage (fig. 50). Yet while in a position to be of use as a sense organ the antenna is still markedly lacking in setæ: the exopodite scale bears only a row of few spines and the long endopodite bears but a few acicular setæ toward its tip. With its increase in length there has been also an increase in number of segments in the filament which has now 36 beyond the three large basal segments in place of the 22 of the first larva.

The mandible (fig. 78) has added a row of small acicular setæ along the medial face of the end segment of the palpus, and developed sharp teeth along the heavy cutting edge of its base. When the palpus is folded down into the hollowed face of the mandible two teeth are dorsal and five ventral to the tip of the palpus.

The first maxilla (fig. 79) has changed but little, but its spines are longer and sharper and it has developed a few, minute, acicular setæ.

This stationary state is still more pronounced in the case of the second maxilla (fig. 80), which has only grown sharp setæ in place of the blunt terminal spines. The changes in the first maxilliped are also the development of setæ in place of spines and the addition of a few setæ (fig. 81).

In this second maxilliped (fig. 82) this substitution and addition of setæ is more pronounced but there is a noticeable retrogression in the gill which is more simple, no longer having any lateral filaments.

The third maxilliped (fig. 83) again, as compared with the first stage (fig. 60), has quite long acicular setæ in place of blunt spines, and a few sparsely plumose setæ, noticeably a group at the tip of the exopodite. While the posterior gill is longer the anterior is here also somewhat reduced.

The chela now used as before for clinging to the mother has its tips somewhat recurved (fig. 84), but they are much straighter than in the more firmly fixed first stage (fig. 61). There are added a few acicular setæ and the claw is more like the adult in the development of rasp-like spines along its edges in place of the simple spines of the first stage which, as seen in figure 62, were being replaced during the first stage by the toothed spines of the second stage.

The four walking legs (figs. 85, 86, 87, 88) have changed chiefly in the addition of a few terminal setæ and of a few exopodite, thread-like setæ.

The branchial formula is thus the same in the second as in the first stage.

On the abdomen the four functional pairs of pleopods (fig. 89) are now long and slender but very simple and not yet fringed with setæ, though the spinules on the edges of the exopodite and endopodite are more numerous than in the first stage.

The appendages of the first abdominal somite exist as yet only in the form of minute round knobs (fig. 4; Andrews, :06), but slightly larger than in the first stage. The appendages of the sixth somite are still within the telson (fig. 71). When the larva is nearly ready to moult into a third stage these pleopods make the telson protrude laterally more than in the above figure through the substance of the telson, allowing one to see the form both of the pleopods and their setæ. When they are expanded, at moulting into the third stage, these pleopods have the appearance shown in figure 90.

The second larva remained fastened to the mother for six days, apparently eating nothing, and as the yolk-mass gradually diminished it seems probable that the larva still subsisted upon the original supply of energy taken from the ovary as yolk. Before the moult into the third stage the gastroliths became quite conspicuous as blue areas showing through the body on each side of the stomach.

The actual moult occupied but a few minutes and as usual the head-thorax came out of the old shell first, then the legs were withdrawn from their cases and finally a few flaps of the abdomen freed the larva completely. There being no telson or anal thread this time, the larva at once left its old cuticle and climbed upon the pleopod of the mother. The increased strength and size of the larva with the perfection of its limbs and caudal-fan would make the danger of being lost on falling away from the egg much less than it was in the preceding stages

as the larva was now well able to follow its strong instinct to crawl upward and so recover its position on the mother, or, failing that, to live independently, as it soon did.

In the following description of the third larva we will emphasize its manner of association with the parent.

In general form the young crayfish in this third stage (figs. 90, 91) is now for the first time like the adult, yet the great size of the eyes and of the exopodite scales of the antennæ together with the very wide expanse of the tail fan and its long swimming plumes give the larva the aspect of a pelagic larva or of the adult of some lower form of marine decapod. In fact the ability to swim freely in great leaps which the third larva soon exercises and the translucency of the body together with the big eyes and long antennæ and very slender long legs and chelæ make the larva seem much like a small shrimp.

The rostrum is now directed straight forward and is armed with large lateral spines that exaggerate the gothic style of the adult. All the appendages are now provided with long setæ which make them sparsely hirsute as seen under the microscope, but only on the exopodite scale of the antenna and upon the telson and sixth pleopods are the setæ set in rows that suggest a locomotor function. The use of the row of plumes on the scale of the antenna is not obvious but the long plumes all along the edge of the telson fan seem to add to its area effective in locomotion.

The animal was still translucent and within it the liver showed as a narrow green band external to the large mandibular muscle, extending forward a short distance and backward nearly to meet its fellow in the region of the heart.

The sixth pleopods were now no longer inside the telson and the telson had become specialized as in the adult into an anterior and posterior part separated by a distinct movable hinge. Through the clear dorsal exoskeleton was seen the anus and a large mass of muscle on each side in the anterior region and in the posterior part of the telson the old radiating glands, which had eventually perfected the long setæ that a high power showed to be plumose.

The pigment cells represented by dark dots in figures 90 and 91, had become more numerous than before but were still chiefly arborescent red cells, though in many regions there were large blue cells not represented in the above figures though they now altered the ground tone of the animals. Posterior to the eyes the crowded pigment cells formed a streak along the base of the lateral spines and more to the rear a rounded area over the attachment of the great mandibular muscle. This round area on each side was especially conspicuous from the dense crowding there of blue pigment mixed with red. The dark aggregation of pigment cells across the anterior edge of the abdomen still remained. The yolk area and its color were quite gone and on each side of the stomach a blue area indicated the gastrolith. Owing to the fineness of the

network of pigment cells on the tips of the chelæ these areas appear conspicuously dark red.

While the young in the third stage still remained on the mother for a while, they were free to move about and soon became more and more independent. A few hours after moulting some of the young were found walking about on the bottom of the aquarium. When disturbed they leaped backward and upward several inches by strong strokes of the abdomen and looked not unlike shrimp, while, when they walked, their habit of holding the anterior part of the body high up in the water as well as the attitude of the slender chelæ gave them the alert, nervous look of shrimp-like creatures and made them look very unlike the adult.

The young climbed about on the pleopods of the mother, over her side and back, upon her mouth parts and eyes and, though at first densely crowded under the abdomen of the mother, more and more frequently they walked off to greater distances, always returning to the abdomen if possible. The same larvæ that at times left the mother in the water, clung fast to her when she was lifted out of the water. But by violently shaking the mother in the water all the young could be shaken off. When two days after moulting into the third stage some sixty larvæ were thus shaken off and left in the same dish with her all but twelve had returned to the mother's pleopods in twenty minutes. As many as a hundred young were seen walking about the aquarium and climbing up onto water plants for a time and later all but a few had returned to the mother.

At first, however, the young did not wander far from the mother and when in a darkened aquarium the mother stayed in one spot for two hours the small faecal casts of the young over the bottom of the aquarium were almost all very near to the mother.

In about a week this association of mother and offspring was gradually given up and more and more of the young failed to return to the mother.

In nature it is possible that the mother and young separate quite soon, especially if the mother wanders about, but as yet nothing is known of the natural life of this crayfish when carrying the young. It may be that the female then lives in holes or cavities and then the young might long remain with her. In captivity the females with eggs will dig holes in the mud and live in them as long as the water is well aerated, but come out when the water is not running. When kept in a small dish the female had no chance to escape from the young and in some cases some of the young remained with the mother and crawled over her three weeks and three days after leaving the egg, but when such females were put into a large tank they walked away and hid themselves, leaving the young scattered about.

Possibly the restricted artificial conditions both prolonged the duration of association of mother and young and lessened the variety of external stimuli so that there was a certain amount of domestication. By close confinement and by supply of abundant food to be given the mother and young without effort on their part, it would appear possible to make of the crayfish a domesticated animal with a more prolonged association of mother and offspring. Such experiments would build upon the ground that the crayfish is preëminently a creature in which embryonic life has been continued on as a series of stages, ancestrally free, but now dependent upon the mother and with special organs to ensure that dependence.

The young that came down from the pleopods took food and eagerly devoured fragments let loose from the mouth parts of the mother when she was feeding. At such times the young climbing on the mouth parts of the mother seemed in danger from those rapidly vibrating appendages but always seemed to be shoved aside and not devoured. When a couple of tubifex were put into the water and seized by a female, the young also took hold and either carried off pieces or continued to hold on while the worm was being dragged into the mouth of the female. Thus the young were drawn up with the food to the mouth of the female, but when between the maxillipeds the young leaped away and none seemed to be injured.

The accompanying illustrations (figs. 92 and 93), being from photographs of living crayfish in water but poorly represent the pinkish mass of active larvæ crowding under the abdomen of the mother or the separate young climbing over the back of the mother or walking about in the dish. They serve, however, to show the crouching attitude of the mother, the size of the young in this third stage and the general character of this active dependence of young upon mother which was long since admired by Roesel von Rosenhof (1775), and might be likened to the clustering of hen and chicks or of sow and pigs. Roesel von Rosenhof's statements regarding the association of the young and parent *Astacus* are as follows:

“Wenn die Mutter dieser kleinen Krebse, nachdem selbige sich zu bewegen angefangen, zuweilen bei ihrem Futter stille, oder sonst ruhig sitzt, so begeben sich solche von ihr etwas weg und Kriechen um sie herum; merken sie aber nur im geringsten etwas feindliches, oder sonst eine ungewöhnliche Bewegung im Wasser, so scheint es, als ob sie die Mutter, sich zurück zu begeben, durch ein Zeichen erinnerte; indem sie allezusammen geschwind unter den Schwanz zurück fahren, und sich wieder auf einen Klumpen zusammensetzen, worauf sich die Mutter sammt selbigen mit möglichster Eilfortigkeit, in Sicherheit begiebt, welche sie aber etliche Tage darauf, nach und nach verlassen.”

The suggestion of a possible signal to recall the young raises the question as to the nature of the means by which the young associate with the parent.

The interrelation of mother and young in the crayfish would seem a profitable field for study of comparative family life and the following few facts suggest lines of inquiry.

It is not evident that the mother gets any advantage from the clinging of the young to her body, though if in the third stage they eat off the old egg stalks and cases, the cleansing of the pleopods so caused might satisfy in the mother something akin to whatever it may be that causes her to laboriously cleanse that region just before the eggs are laid; but judging from the long endurance of the load of eggs and larvæ and from the frequency of dirt and parasites upon the pleopods it seems hardly probable that the female would feel an instinct gratified when the pleopods are clean again. That the female is at all conscious of the presence of the larvæ remains to be found out by evidence not yet at hand; however, when a lot of young were put into a dish with a male and they climbed over him he cleaned them off from his abdomen and seemed to be annoyed by their climbing over his head, but when the same young were put with a large female that had reared her own young long enough and naturally separated from them some weeks before, they climbed up onto her without her showing any evident sign of being affected by their presence upon her chelæ, thorax and abdomen. When a female was feeding and the young climbed upon her chelæ she did not seem aware of them but reacted eagerly to a tubifex near her chelæ. Whether, then, the young are simply tolerated, [as are the small parasitic leeches over the body of crayfish,] as a continuation of the eggs that are part of the body, or whether the females have special responses to stimuli given by the young is not known, but the former seems not improbable.

The young, on the other hand, act as if they were strongly affected by the mother but this may be due to a few simple reflexes and without any complex visual or other conception of the mother's existence. At all events there is no evidence that the young distinguish one female from another and when two mothers were in the same dish some young of one climbed up onto the other. Even when the young shaken off a female when they were in the third stage were put in a dish with a female carrying her young in the second stage they climbed up amongst the younger larvæ of the strange female and seemed content. When the young were shaken off two females in separate dishes and the females exchanged, the young climbed up onto the strange females; in a few minutes all but three were upon one female and all but seven or eight upon the other though she was moving about. Some young *C. affinis* in the second stage were taken off from the mother and put with some young of *C. Diogenes* of the second stage also removed from the mother and all were then put into a dish with another female *C. Diogenes* bearing young in the second stage. All the young got upon the foster mother and continued there, though the *C. affinis* were

scarcely more than one-half the length of the other larvæ. The young that are shaken off dart, swimming, about in the water but soon settle to the bottom and climb up upon one another in heaps or try to climb up at the corners of the dish and upon water plants.

The tendency to climb onto the mother is very strong so that when a female walks along swinging her pleopods laden with young she often gathers up free young that are walking about, as these when touched by a pleopod may not spring away but turn and climb onto the mass. In fact early in the third stage a larva that fell to the bottom, apparently for the first time, stood with head end elevated and quickly responded to the presence of a passing pleopod. Even if lying upon their backs they quickly seize and mount the pleopod of a passing female. A piece of white cheese-cloth, however, did not appear to stimulate them to climb upon it and when a lump of rough cement, a model of a toad, was left in the aquarium very few got on it, most of them preferring the mother.

Possibly the young receive chemical stimulus from the mother that aids them in returning to her. That they responded to some chemical stimuli is most probable and the following facts may be interpreted on this basis. When the mother feeds, the young gather under her mouth and even after the food is gone they remain as if excited by chemical substances coming from the mouth parts of the female. It was also found that while the young climb onto a dead female and even over the exposed surface of the freshly broken abdomen, after some days they remain on the rest of the surface but not upon the broken surface where chemical substances due to decay probably existed.

During the time the larvæ in the third stage associate with the parent the large collections of old egg shells and skins on the pleopods disappear and are probably eaten up by the larvæ since their intestines contain minute setæ that might come from their old cuticle and since in *Astacus*, Chantran ('71) found that the larvæ eat their shells and cast off cuticles. It is therefore possible that one reason the young remain with the mother is that this food supply on the abdomen acts as a stimulus to them.

However, along with whatever chemotactic movements there may be, there are other factors concerned in the association of the young with the female as indicated by the few following experiments, which tend to show that the young are controlled in part by responses to light and to gravitation and contact. Thus when a small paste-board table was put under the water the young collected on the under side of the horizontal paste-board and remained standing upon it, in an inverted position. When the paste-board was then turned upside down the young scattered in a few minutes so that half were off the paste-board and some were under it in its new position. In crawling under such a table the young reared up and seemed to be trying to reach it above their heads as if

influenced by it before they touched it. When a small glass table was used the young also collected under it and reached up as if trying to climb up to it. When dark paste-board was laid on part of the glass, above, twenty-two larvæ had collected under that part of the table shaded by the paste-board, in two hours, and none under the clear part.

It may be imagined from these indications that the larvæ in the third stage have a tendency to climb upward due to negative geotactic responses, that they prefer the shade as being photophobic and that they come to rest standing upon a solid object as being thigmotactic. Assuming some such responses together with some chemotactic phenomena, possibly all the habits of the young in reference to the mother may be explained; possibly not.

The association of the young and the mother, however brought about, endures in *Cambarus affinis* during two larval stages and a part of a third. In *Astacus leniusculus*, however, it is the second and not the third stage which gives up the association with the mother. Whether this is a generic difference cannot be determined without extensive observations, as almost no data are yet at hand. That *Cambarus* in general have three dependent stages seems probable from the following few facts that have been recorded.

Faxon ('85) saw specimens of *C. Clarkii* 7 mm. long upon the abdomen of the mother (as previously recorded by Hagen, '70), in which the tail-fan was perfect so that with the ability to lead a free existence there was still the habit of associating with the mother. In *C. affinis* larvæ in the first stage are 4 mm., in the second 5 mm., and in the third 7-8 mm. long, and it seems probable that the above recorded *C. Clarkii* were also in a third stage. The same author saw upon the mother *C. gracilis* 9 mm. and *C. Bartonii* 10 mm. long, with perfect tail fan. These two species may well have three stages of association as in *C. affinis*. Also he records *C. rusticus* just hatched and 4 mm. long as being so embryonic that we may assume they would remain with the mother as long as *C. affinis* does. On the other hand Steele (:02), who first observed the living larvæ of *Cambarus* associated with the mother, records *C. gracilis* 7 to 8 mm. long with a bent rostrum as if in the first stage though so large. And in a more detailed study of *C. virilis* she describes the first stage and then a second stage nine days old with the tail fan complete and other features of the third stage of *C. affinis*, but as she says the larva six days old could swim it seems probable that in both these species as in *C. affinis* there are three stages in the period of association, the second of which is easily overlooked.

There is then no obstacle to assuming that we may expect to find in *Cambarus* that the larvæ remain with the mother through a first, a second and part of a third larval stage and that in *Astacus* they remain through the first and part of the second only.

The active third larvæ becoming independent lived some eighteen days in all before moulting again to pass into a fourth stage and these in turn were followed through more stages till eventually adults were reared in the laboratory. Without describing the gradual changes of the early larvæ to the details of adult form we may now give a more complete record of the rates of growth of young crayfish than has hitherto been possible as a number of new observations have been added to those previously published, Andrews (:04), and certain points as to the period of sexual maturity determined. We will give certain facts as to the number of moults, as to the rate of growth, as to the development of the external male organs, as to the ratio of the sexes and as to the arrival of sexual maturity and the ability to breed in captivity.

Observations made upon the young of a single female which laid March 28, 1903, and whose eggs hatched May 18, 1903, gave the times of moulting and rates of growth of the larvæ represented in the following table:

Stages.	Dates.	Duration.	Size.	Increase.	Habit.
1st stage.	May 18-May 20	2 days	4 mm.	. . .	Attached to mother.
2d stage.	May 20-May 26	6 days	5 mm.	1 mm.	Attached to mother.
3d stage.	May 26-June 13	18 days	8 mm.	3 mm.	{ Associated with mother for about one week and then free.
4th stage.	June 13-July 1	17 days	12 mm.	4 mm.	
5th stage.	July 1-July 6	5 days	15-18 mm.	3-6 mm.	Free.
6th stage.	July 6-July 17	11 days	21 mm.	3-6 mm.	Free.
7th stage.	July 17- ?	?	29 mm.	8 mm.	Free.

These same young were kept till October 6, 1903, without observation and the eight survivors then measured as follows: 62, 55, 53, 49, 50, 45, 43, 41 mm. in length. The average is nearly 50 and as far as can be judged from the above table such a large larva as that measuring 29 mm. in the seventh stage would probably have passed through at least four or five moults to become the large one 62 mm. long and smaller larvæ would have required as many or more moults if the increment was as large as in passing from the sixth to the seventh stage; but if the increase was the average for the above seven stages, scarcely 4 mm., then at least six more moults may have taken place. Other data show that the rate of growth is very different in individuals and in some cases seemed to depend directly upon food supply so that the number of moults during the first summer is probably not by any means constant but different in individuals. However, we are sure that at least seven stages may be passed through and probably eleven to thirteen larval stages may occur in the first summer of the creature's life outside the egg. Yet data given below show that the larvæ may remain but 20 mm. long in the fall, owing, probably, to insufficient food arresting them when in the sixth stage.

After October there were no moults till the following warm season, the lengths remaining constant during the winter. Just as the growth in the first summer was restricted to the five months of May, June, July, August, and September, so in the second summer there was an increase of 25 mm. during that time and though the moults were not observed there may well have been four or five during this second summer. In a few exceptional cases a moult took place in the early spring before May and a gain of 6 mm. was observed then. After a second winter of no moults there followed a third summer of growth in which a single observation showed that a specimen 70 mm. long December, 1902, was 76 mm. long July 1, 1903, an increase of 6 mm. probably due to one moult. As this same individual increased to 90 mm. by October, 1903, it probably moulted two or three times or in all three or four times during this third summer.

We thus have evidence for seven and probably as many as twelve moults the first summer, four or five in the second summer, three or four in the third summer, and in the fourth summer perhaps only one or two moults to judge from a single case of 8 mm. increase.

Without reference to the number of moults, observations upon the amount of growth of young crayfish hatched from eggs laid in the laboratory and kept under various artificial conditions in the laboratory, were made and are recorded below as a substitute for data never yet obtained as to the rate of growth of American crayfish in the open.

The following list gives the length in millimeters from tip of rostrum to end of telson of 101 young hatched in May, kept in six different tanks in different years and under somewhat different conditions and measured in October when four months old.

I. 62, 53, 53, 49, 50, 45, 43, 41.

II. 60, 59, 52, 52, 45, 39, 40, 33, 30, 22.

III. 41, 38, 24, 56, 32, 44, 43, 39, 39, 33, 35, 36, 32, 34, 30, 23, 30, 29, 27, 32.

IV. 47, 39, 33, 27, 20, 23, 31, 38, 30, 31, 28, 30, 35, 35, 35, 34, 36, 42, 46, 52, 46, 45, 46, 41, 39, 40, 36.

V. 43, 51, 41, 49, 43, 37, 38.

VI. 55, 43, 55, 48, 38, 50, 40, 40, 53, 46, 40, 42, 40, 43, 50, 45, 44, 48, 50, 49, 37, 57, 44, 45, 43, 46, 46, 36, 38, 38.

It is obvious that there was great individual difference in size attained in the first four months of life both under different conditions and when kept in apparently the same conditions, but of course subject to different chances of food supply even in one tank.

The first group of eight survivors ranged from 41 to 62, with an average of nearly 50.

The second group of 10 survivors ranged from 22 to 62 with an average of 43.

The third group of 20 ranged from 23 to 56 with an average of 35.

The fourth group of 26 ranged from 20 to 47 with an average of 36.

The fifth group of only 7 ranged from 37 to 51 with an average of 43.

The sixth group of 30 ranged from 36 to 57 with an average of 45.

The large size of the crayfish in the first group was undoubtedly due to greater care taken to feed them and the differences in other groups were probably due to differences in food supply and also in temperature.

Judging from the above 101 measurements the young four months old may vary in length from 20 to 62 mm. but while the average of all was 41 mm. the average of different experiments ranged from 35 to 50.

We may then expect this crayfish to be nearly two inches long in the autumn of its first year of life.

Measurements made in the autumn and again in the next spring showed that there was no growth from October to May, except in rare cases in which a moult occurred in early spring. The crayfish thus started in their second summer of life with a length of nearly two inches and were the same size when twelve months old as when four months old.

The rate of growth in this second summer may be inferred from some few measurements made in October upon crayfish reared from eggs laid in the laboratory and measured then, when sixteen months old. Of five larvæ measuring May 26, 1904, 62, 55, 53, 50, 62 mm. the three survivors in October, 1904, measured 75, 80, and 72 mm., an average of 76 mm. From the average, 58, of the above five to the average 76 of the three survivors there was an increase of 18 mm., or a gain of 30 per cent in length in this second summer. And records showed that one of these larvæ had grown from 62 to either 75 or to 82, that is added 13 or else 20 mm. to its length, or increased 21 per cent or else 32 per cent.

Another group of young left three survivors of 56, 70, 75 mm., or on the average 67 mm. when 16 months old to represent the seven which were 40-45 mm. long when twelve months old; they had thus probably added 25 mm. or gained more than 50 per cent in the second summer.

A third group of young, twenty in number, ranging from 23 to 56 mm. when twelve months old, left only two survivors at sixteen months, which measured 70 and 79 mm., or an average of 74: there was thus an increase from the average 35, of 39 mm. But records of the individuals showed that the few survivors were probably 43 and 56 mm. long in the spring and had grown only 23 and 27 mm. in each case which would be an increase of 53 per cent and of 63 per cent.

The above few data indicate a growth in the second summer of sometimes 50 per cent of the length; that is a crayfish two inches long the end of the first summer may be expected to be three inches long the end of the second summer,

when sixteen months old. But here again the individual differences are very great.

In the second winter there was probably no growth; two crayfish 75 and 80 mm. long in October, 1904, were the same length in May, 1905, and, in general, moulting during the early spring was rarely observed.

The only observation upon the growth of crayfish reared from the egg and kept during the third summer was the following: two individuals 70 and 79 mm. long when twenty-four months old left one survivor 90 mm. long in October, 1903, when 28 months old. It had thus increased either 11 or else 20 mm. in the third summer, that is, it added only 14 per cent or 20 per cent to its length.

Probably then a crayfish may grow in the third summer from a length of three inches to be somewhat short of four inches.

The one young crayfish kept during its third winter did not change but measured 90 mm. in October, 1903, and the same again in May, 1904.

In its fourth summer this crayfish grew to a length of 98 mm. by October but died then when three years and four months old, having grown but 8 mm. or barely 9 per cent in this summer. This may indicate a great diminution in rapidity of growth as the maximum size is approached so that the crayfish one hundred and twenty millimeters long that are taken in the Potomac may well be six or seven years old; but from one specimen we cannot say that the rate is naturally lessened in the fourth year and a possible yearly increment of one inch would make the large crayfish of five inches only four years old.

The age of *Cambarus affinis* would seem to be roughly determinable from the formula $A = L - V$, where A = the age in years ending in May, L = the length in inches and V = one, in conditions of maximum favorableness, but in unfavorable conditions V may become zero and in very old large crayfish V may be a negative quantity.

Soubeiran ('65) gave the length of *Astacus* in France as 50 mm., 70 mm., 90 mm., 110 mm., 125 mm., in the first to fifth years inclusive, that is with an annual increment of 20 mm., which, however, became less in the fifth year, so that he could not tell the age of crayfish 160 mm. long nor of any very old ones 190 mm. long.

Compared with this *Astacus*, *C. affinis* starting as a much smaller egg caught up in size in the first summer and for two years showed just about that same increment of 20 mm. annually, but then probably began to grow with more diminished speed than did the French *Astacus*.

The sexes of these young crayfish are early determined and in the third larval stage the female shows the beginning of the annulus and the male the external openings of the deferent ducts, as elsewhere described (Andrews, :06). But the difference between the appendages of the first abdominal somite in

male and in female are first well seen in the fourth stage when the female still bears minute tubercles and the male long papillæ to represent the appendages of this somite. From that time on to the autumn the distinctive characters of the sexes become perfected so that the annulus of the female is much like that of the old crayfish, and the first abdominal appendages, or stylets, of the male are also much like those of the full grown crayfish. It is thus very easy to recognize the sexes by external characters when they are four months old. The actual length in millimeters of the male stylets in crayfish of four months is given in the following table:

Length of body . . .	55	53	49	45	43	60	52	52	45	39	40	33	30
Length of 1st stylet .	9	9	9	8	7	10	9	9	7	6	6	5	4
Length of 2d stylet .	10	10	9	7	11	10	10	8	7	7	5	4	

The second stylets are thus longer than the first and as much as 18 per cent to 20 per cent of the length of the body so that the stylets are in about the proportions they will be in larger crayfish since specimens 115 mm. long have stylets 18 and 22 mm. long.

Some examinations of various catches of adult crayfish indicated that there is no great disparity in number of males and females and though it is not known whether one sex or the other is subject to greater mortality in early larval life, the following observations tend to show that the sexes are about equal when four months old so that it may be that the eggs are about equally male and female. Observed at four months: of 26, there were 15 male and 11 female; of thirty, 11 were male and 19 female; of 7, 4 were male and 3 female; of 19, 10 male and 9 female; of 10, 8 male and 2 female; of 8, 5 male and 3 female. Of the entire 100, 53 were male and 47 female. With no marked disparity in numbers and with well formed external organs the two sexes when four months old and about two inches long often have well developed sexual instincts. Thus in October a male 55 mm. long, 15 mm. wide, with tail fan 22 mm. wide, antennæ 48 and 51 mm. long and entire expanse from tip of chelæ to end of telson only 75 mm., was seen to try to conjugate with a sister 62 mm. long when four months eighteen days old. Another female had a mass of sperm transferred to its annulus by a male of like age when four months old and 57 mm. long. Many cases were seen in which the four months young had conjugated, thus of 19 females four months old, in one aquarium, five bore sperm given by males of like age. Though the sperm transferred by these young males was apparently perfect it seemed doubtful if these unions would lead to fertile eggs but when a number of four months females thus provided with sperm by males of like age were kept isolated from all males during the winter they laid eggs in the spring which developed and thus demonstrated not only that the females could lay eggs when but a year

old but also that these eggs would develop after union with males of the same age, union which moreover took place in the previous autumn so that these experiments also showed that the sperm got in the autumn and kept all winter would fertilize the eggs, parthenogenesis being, supposedly, out of the question. In *Astacus*, Chantran stated that the eggs were laid only ten to forty-five days after union but in *Cambarus affinis* as many as 230 days may elapse. The data on which the conclusions rest are as follows. A crayfish 62 mm. long laid eggs when forty-seven weeks old, and these eggs hatched eight days after the mother's birthday; this female received sperm the preceding autumn from a male of like age and after that was kept isolated. Three other females were supplied with sperm in the autumn and laid eggs in the spring when one year old and kept isolated from males. Another female 57 mm. long laid eggs May 26, 1904, when one year old, having hatched May 18, 1903, and after being kept isolated after conjugation with a male of like age in the autumn of 1903. Another female only 50 mm. long laid eggs when about eleven months old, April 29, 1905.

It thus seems demonstrated that a crayfish growing the first summer to the length of about two inches and receiving sperm from a male of like age in the autumn may lay fertile eggs the next spring when scarcely a year old.

When older, 16 months old and about three inches long, that is in the second autumn of their lives, crayfish were seen to conjugate and to lay fertile eggs the following spring when two years old. In one case it was shown that a female which laid eggs when 62 mm. long and not quite one year old again laid eggs the next spring when 80 mm. long and but a few days over two years of age.

Though it is possible that artificial conditions may have made these crayfish that reproduce when a year old precocious, it is probable that this *Cambarus* comes to sexual maturity much sooner than does the *Astacus* of Europe of which both Soubeiran ('65) and Chantran ('70) state that it reproduces first in its fourth year, though the latter says the males were ready for conjugation at the beginning of their third year.

The fact that one female *Cambarus affinis* laid in two successive years is in contrast to the statement credited to Steffenberg ('72) that the female *Astacus* in Sweden hiding away during the winter, if fecundated, breeds only every other year.

It is finally to be noted that not only did the young of *C. affinis* reared in captivity lay fertile eggs but the young of the next generation also, so that there would seem to be no obstacle to the establishment of a permanent race of domesticated crayfish bred in captivity.

COMPARISONS AND CONCLUSIONS.

The chief differences between the young of *Astacus leniusculus* and those of *Cambarus affinis* are to be found in the first and second stages and are differences in size, in habit, and in structure.

The differences above described may be enumerated as follows. In *Cambarus affinis* the larva coming from a small egg about 1.75 mm. in diameter is about 4 mm. long and lives only two days before moulting. In *Astacus leniusculus* the larva coming from a very large egg, 2.5 mm. in diameter, is about 9 mm. long and lives four or more days before moulting. The first larval stage in *Cambarus* has a simplified telson not well armed but having only 26 spines which are upon its posterior edge only, while the *Astacus* has 66 spines that are set along the lateral as well as the posterior edges. The abdominal appendages differ in that the first pair are absent in *Astacus* while barely recognizable in *Cambarus* and the four following have equal exopodites and endopodites in *Cambarus* while in *Astacus* the exopodite is longer. The telson thread is short in *Cambarus* and long in *Astacus*, and in *Cambarus* there seems no part of it a recent cuticle cast off by the embryo with well developed limbs as is the case in *Astacus*. The first antenna in *Cambarus* is more simple in having only four in place of five segments in its exopodite and in its endopodite and in lacking the sense-hair of *Astacus*. The second antenna in *Cambarus* has 25 segments while *Astacus* has 50; moreover, *Cambarus* carries this antenna bent backward close to the body between the legs while *Astacus* carries it forward, but depressed. In *Cambarus* the first maxilla lacks the few plumose setæ of *Astacus* and has fewer spines; and the maxillipeds have fewer setæ and spines, as well as gills of more simple structure. The gill formula of *Cambarus* is already that of the adult and is therefore more simple than that of *Astacus*, but besides this generic difference the gills of *Cambarus* are more simple in structure, in this first larval stage, in many cases, especially the anterior arthrobranchs of the pereopods.

In the second larval stage the habits of the two are different in that the young *Cambarus* still remain inactively fixed upon the mother and are aided in doing so by an anal thread, while the young of *Astacus* soon wander away from the mother. The former are about 5 mm. long and live about six days before moulting while the latter are 11 mm. long and live 8 to 10 days before moulting. In *Cambarus* the rostrum is more bent and not so efficient as a protection and the setæ over the entire animal are much less developed. The abdomen of *Cambarus* has only weak spines upon its telson and these are along its posterior edge only, while in the active *Astacus* there is a complete fringe of long plumose setæ on all the edges of the telson. In *Cambarus* the first antenna is less perfected; in having only 5 segments in its exopodite and 4 in its endo-

podite while *Astacus* has 6 in each; in having but 5 sensory hairs in place of 8 in *Astacus* which has advanced further as is shown by the arrangement of these hairs, and in having the ear pit still bare while in *Astacus* it is guarded by a fringe of plumose setæ along its edge. In *Cambarus* the second antenna has 39 segments and its scale has a row of a few spines only while in *Astacus* there are 54 segments and the scale is fringed with plumose setæ. In *Cambarus* the maxillæ and maxillipeds have but few setæ and no plumose ones except on the tip of the last maxilliped and the edge of the scaphognathite, while in *Astacus* the plumes are conspicuous and show a tendency to extend out to the cutting edges of the protopodites and to thus replace the simple setæ by highly specialized forms of setæ with lateral barbs. The scaphognathite in *Cambarus* is more simple in lacking the peculiar long plume-like setæ at its posterior end. The chelæ are used by *Cambarus* to hold fast to the mother and their tips are somewhat less straight than in *Astacus*. In *Cambarus* also the remarkably long exopoditic setæ of *Astacus* are not developed.

The first two stages in which the larvæ of these crayfish differ most are also the stages in which crayfish are peculiar in having an intimate association of larvæ with parent. From the generally accepted standpoint that crayfish have been evolved from marine ancestors which had pelagic, or at least, active larvæ that left the mother as soon as they were hatched, the association in the crayfish is a new acquisition. The differences between the larvæ are also presumably of recent origin since they make the early stages more unlike than later stages, while if there were a common ancestor the greater divergence would be in the most remote descendants and stages, until some newer change modified the early larvæ and thus "falsified" the record.

Some of the new modifications in structure that go along with the new habits of association of larvæ and mother may be recognized as distinct from the small size and other characters that might belong to active larvæ. The characters in the first and second stages that seem inimical to free larval life and to be adapted to the parasite-like life upon the mother are; partly those negative traits which an embryo might have in protected sedentary life and which here may be in fact but the lingering on outside the egg of conditions formerly found only within the egg; and partly directly adapted additional characters that are peculiar to this life of larval dependence upon the pleopods of the mother, and of no use elsewhere.

The embryo-like characters of the first larval stage are as follows. The spheroidal head-thorax and accompanying position of limbs and of abdomen that make walking and true swimming impossible and force the creature to lie upon its side if removed from its natural attachment to the mother. The weak, imperfect state of the locomotor organs; slender legs, simple telson, absence of utilizable sixth pleopods. The lack of useful defensive organs, that is the

bending down of the rostrum between the eyes and the small number and insignificance of the telson spines. The imperfect state of the sense organs; namely the short eye stalks and incomplete eyes, the open unguarded ear-pit, the complete or nearly complete absence of sense setæ on the first antenna and the naked character of the other appendages and whole body over which only a few simple spines take the place of the innumerable sensory and locomotor setæ of active larval stages. The lack of development and of use of the first and sixth pairs of abdominal appendages; the imperfect development and shortness of the first and second antennæ; the lack of teeth on the edge of the mandible and the apparent lack of use of any of the numerous mouth parts and appendages concerned in later stages in getting and eating food. The passive habit as a fixed dependant with physiology like that in the embryo, except for the addition of active respiratory movements; the pose of the body with the limbs and abdomen beneath the thorax and antennæ depressed.

The special characters of the first larva that are neither embryonic nor such as a free larva would have are the two means of attachment to the mother. The first is the existence of recurved tips on the long strong chelæ that, combined with the instinct of the larva, enable it to seize hold of and become locked to the material on the mother's pleopods. The second is the telson-thread that holds the larva to the mother till it can get locked with its claws. This contrivance is formed in advance of its use both by early adhesions of membranes and by later special secretions of telson glands so that its usefulness is dependent upon a series of modifications of the ancestral history.

In brief the first larval stage is embryonic in proportions and activities and in lack of the perfection of sensory, feeding, locomotor, and defensive organs to be expected in a free larva. It is actively specialized for its peculiar association with the parent by the locking claws and telson thread.

The second larval stage is transitional in structure from an embryonic to a free larval state and in habit it remains still a fixed dependant in *Cambarus* though in *Astacus* gradually becoming but loosely associated with the parent. In both crayfish the second larva is still imperfect in lacking the sixth pleopods and is embryonic in still containing yolk. It also has imperfections in sensory and locomotor setæ that may be interpreted as retrogressions from a more perfect ancestral state toward a yet more simple state like that of the first larva; presumably the association with the mother was first acquired in the first stage and has later extended, as yet, less thoroughly through the second stage.

Supposing the ancestors of crayfish to have hatched as active larva the first and second stages at present are new, in so far as they are specialized for attachment to the mother and in that they are inactive and imperfect, while the third stage may be compared more directly with ancestral larvæ and is older in its general structure.

While the absence of the locomotor sixth pleopods in the first and second stages of crayfish young might be regarded as indicating that these stages had never been represented by free ancestral forms but were merely embryos prematurely removed from the egg to live a pseudo-larval life upon the mother's pleopods, yet the near marine relative of the crayfish, the lobster, likewise has no available sixth pleopods in its first and second stages though free and even pelagic, and we are free to assume that the first stages of the crayfish are modified representatives of free ancestral larvæ.

Such ancestors may have been much like the lobster and at present the third larval stage of the lobster is like the third in the crayfish in that the sixth pleopods are then first expanded; but it is not till the fourth larval stage in the lobster that the length of antennæ and perfection of tail-fan is developed enough to be comparable to the third stage in the crayfish. While the first and second stages of the crayfish are well adjusted to life upon the mother's pleopods, the first, second, and third stages of the lobster are well fitted for pelagic life in retaining the locomotor exopodite of the pereopods and short antennæ. The fourth stage of the lobster first acquires long antennæ, perfect tail fan and reduced exopodites, and is in the main very like the third stage of the crayfish; in fact the adult state is rather suddenly imitated in the third stage of the crayfish and in the fourth stage of the lobster. If these two stages are supposed to be homologous we may suppose that the process of reduction of metamorphoses that has already gone so far in the lobster (Herrick, '95), has advanced a little more in the crayfish so that only two larval stages exist where there used to be three.

The greater length of the antennæ in the earlier stages of the crayfish and the earlier presence of the 2nd-5th pleopods may be imagined as secondary changes from the lobster-like state, possibly connected with development of crawling habits and abandonment of pelagic life, for the long antennæ would be of more use to a creeping animal and the pleopods perhaps of more use in connection with respiration and sensory examination of water to an animal living on the bottom, or possibly in holes, and needing a supply of water to the gill region and information of the nature of the material beneath the abdomen.

In the departure of crayfish from marine lobster-like ancestors the changes have been in the abolition of most of the free swimming contrivances, the acquisition of some arrangements better fitted for a crawling life and finally the retention of embryonic states along with special new adjustments in connection with a life of quasi-parasitism upon the mother.

We imagine the ancestral crayfish to have given up pelagic larvæ, to have developed crawling larvæ, and with these to have lately begun an association of larva and parent that forms an early phase in family life. If continued in the same lines there might ultimately result a crowding back of hatching nearer

to the time of egg laying and a retention of embryo-like larvæ upon the mother along with reduction in number of larval stages, that would allow for a long family life and protection of young nearly to the adult state.

That *Cambarus* has departed from the ancestral state more than has *Astacus* is shown by the following considerations. In *C. affinis* the telson is more reduced, that is its shape is less like that of an ancestral swimming organ and its protecting spines are only upon the posterior edge and not along the side as well, and, moreover, there are but 32 as compared with 66 in *A. leniusculus* or, probably, 50 in one *Astacus* of Europe. *Cambarus affinis* holds its second antennæ bent back under the thorax out of the way of jostling neighbors upon the pleopods but in a position of no use as a feeling organ. This antenna is also very short and has but 25 segments as against 50 in *Astacus*. In *Cambarus* the appendages of the first abdominal segment, which will ultimately be sexual organs in the male, are started in the first stage though lacking till much later in *Astacus*, as far as known. And the young develop to sexual maturity within four months, thus shortening the period of non-sexual life remarkably. Rathke ('29) found no signs of stylets in male *Astacus* 1 inch 3-4 lines long while we find them 0.1 mm. long in *C. affinis* 17 mm. in length. The telson thread of *Cambarus* seems made less evidently from a recent larval skin and is apparently of earlier origin than in *Astacus*, as it seems to arise further back in the embryonic life; or we should say the larval history of *Astacus* is the more primitive in having a more complete representation of a lost larval stage still evident in a complete cast cuticle within the egg. But while there is a cuticle cast off near the time of hatching in both *Astacus* and the lobster we may assume from comparison of these larvæ that the first stage of the lobster is represented within the egg of the crayfish and that therefore the cuticles cast at hatching are not homologous.

In the second stage of *Cambarus* there are also signs of further recession from ancestral states. Thus the rostrum is less developed and not as efficient as a defense as it is in the more active *Astacus*. The second larva of *Cambarus* still lacks most of the plumose setæ that are present in the second *Astacus* and is thereby less fit for free life and is also more like an embryo. The second stage of *Cambarus* lives upon the mother while in *Astacus* it becomes free: in *Cambarus* it is fast by its chelæ and when it moults a delay in casting off the cuticular lining of the intestine makes an anal thread that mechanically continues the attachment of the young to the mother beyond what is found in *Astacus*. The telson of *Cambarus affinis*, even in the second stage, has only spines in place of the locomotor plumes of *Astacus* and is thus evidently unfit for free life. *Cambarus* also has not yet developed sense setæ upon the second segment of the exopodite of the second antenna and is thus not as advanced as *Astacus* is; and the ear pit is less perfected in not having the setæ along its edge. The

second antenna is less perfect in having only 39 in place of 54 segments and in still having only spines in place of plumes upon its scale. In *Cambarus* also the second stage is notably lacking in almost all plumose setæ and is thus further removed from fitness for free active life.

The third stage of *Cambarus* though rivaling *Astacus* in perfection of locomotor and sensory apparatus still associates longer with the mother and thus suggests a future period when the larval dependence may be extended so that more than two larval stages may remain upon the mother.

Though the young of *Cambarus* are better fitted to stay with the mother, and do so for a longer period of their life as reckoned in stages, the actual number of days in which the association continues is not essentially different in *Cambarus* and in *Astacus*. In the former the larvæ is fixed on the mother for a week and then remains in association for another week or more. In the latter the larva is fixed to the mother four to fourteen days and then is associated for only a few days more, as far as was made out in laboratory culture of *A. leniusculus*, while Chantran ('70) states the young of a European species are fixed for ten days and then go and come for ten days more.

The conclusion that *Cambarus* has advanced further than *Astacus* in the adaptation of its young to a life of association with the mother is in harmony with the relative positions of these two genera as determined by anatomy and geographical distribution. The absence of the pleurobranchiæ in *Cambarus* and the presence of a specialized sperm receptacle, the so-called annulus ventralis, as well as the corresponding specialization of the male stylets, are some of the important characters that show the adult *Cambarus* to be more highly evolved than *Astacus*, and Ortmann (:02) has shown how the evolution of all the species of *Cambarus* may have taken place since divergence from *Astacus* like ancestors in the region of Mexico.

We may therefore suppose that as *Cambarus* has migrated over the middle and eastern United States it has become split up into the sixty odd species now found and in some as in *C. affinis* has made more perfect the association of young and parent already present in the *Astacus* ancestor.

As all the crayfish are primarily fresh water or land dwellers it is natural to seek to connect their possession of an elementary family state with their departure from their ancestral marine life and it is easy to imagine that the species might be benefited by the young being protected by the mother when either living in holes or moving from place to place till of larger size and more perfect structure. But it is not obvious that the advantage would be greater in fresh water than in salt water life, and as we know the lobster has already greatly shortened its ancestral series of metamorphoses and that the crayfish family-life is possible only after such shortening of metamorphoses, we seem, in the lack of evidence as to when the association found in the crayfish really began,

to have no grounds for connecting fresh-water life with these peculiarities of crayfish. The marine ancestors of crayfish may have already acquired some connection of young and parent before leaving the sea and in fact the life in bays and estuaries would be one in which the dangers of loss of young if set free early and swept out to sea, would make an attachment to the parent especially valuable! And, later, the main advance of crayfish ancestors having been presumably up rivers and across country from river to river, the advantage of having the young not set free early when they might more easily drift down stream or be devoured, but when they were large enough and able to crawl and to hold to the bottom, would perhaps lead to a continuance and perfection of family life.

But while it is easy to speculate on the origin of the characters of crayfish on the assumption of utility and the working of natural selection, the conclusions are of doubtful value in our present relative ignorance of their actual life. Moreover, the nature and the amount of differences in the hard parts and in the larval history that distinguish one kind of crayfish from another are such as to raise the question whether utility and natural selection have played any part in their formation or in their perfection. All the specific and generic characters of crayfish may be as useless as color differences, and they may have suddenly arisen perfected as we see them, or they may have progressed in certain lines for long periods of time independent of external agencies. We need more evidence from observation and from breeding experiments before concluding that such characters as the shape of stylets and annulus or of rostrum and of spines, absence or presence of one gill filament more or less, or the behavior of telson glands and the presence of feathered setæ instead of simple spines, have ever in any manner been connected with utility to the species or with the survival or the extinction of individuals. Until the contrary is proven we may regard these as the unmeaning by-products of unknown activities in the living protoplasm.

LITERATURE.

ANDREWS, E. A.:

- :04. Breeding Habits of Crayfish. Amer. Nat., vol. 38, pp. 165-206, 10 text figs.
- :04¹. Crayfish Spermatozoa. Anatomische Anzeiger. xxv, pp. 456-463, 7 figs.
- :06. Ontogeny of the *Annulus ventralis*. Biol. Bull., vol. 10, pp. 122-137, 25 text figs.
- :06². Egg-laying of Crayfish. Amer. Nat., xI, No. 473, pp. 343-356.
- :06³. Geographical Distribution of Crayfish. J. H. Univ. Circular, May, 1906, pp. 100-103.
- :06⁴. The Future of the Crayfish Industry. Science, N. S., vol. xxIII, No. 60, pp. 983-986.

CHANTRAN:

- '70. Observations sur l'Histoire naturelle des Écrevisses. Compt. Rend. Acad. Sci. Paris, vol. 71, pp. 42-45.
- '71. Nouvelle observations sur le development des Écrevisses. Compt. Rend. Acad. Sci. Paris, vol. 73, pp. 220-221.

FAXON, W.:

- '85. A revision of the Astacidae. Mem. Mus. Comp. Zoöl., vol. 10, no. 4, vi + 186 pp., 10 pl.

HAGEN, H. A.:

- '70. Illustrated Catalogue of the Museum of Comparative Zoölogy at Harvard College. No. 3. Monograph of the North American Astacidae. Mem. Mus. Comp. Zoöl., vol. 2, no. 3, viii + 109 pp., 11 pls.

HERRICK, F. H.:

- '95. The American Lobster. Bull. U. S. F. C., Washington, 252 pp., 54 pls.

HUXLEY, T. H.:

- The Crayfish: An introduction to the study of zoölogy. New York, 8vo, xiv + 371 pp., illus.

ORTMANN, A. E.:

- :02. The geographical distribution of fresh-water decapods and its bearing upon ancient geography. Proc. Amer. Phil. Soc., vol. 41, pp. 267-400.

RATHKE, H.:

- '29. Ueber die Bildung und Entwicklung des Flusskrebsses. Leipzig, 97 pp., 5 pls.

REICHENBACH, H.:

- '86. Studien zur Entwicklungsgeschichte des Flusskrebsses. Frankfurt am Main. 137. pp., 14 pls.

ROESSEL VON ROSENHOF:

- 1755. Der Monatlich-herausgegebenen Insekten Belustigung. III, pp. 305-350, pls. LIV-LIX. Nurnberg.

SOUBEIRAN, L.:

- '65. Sur l'Histoire naturelle et l'Education des Écrevisses. Compt. Rend. Acad. Sci. Paris, vol. 60, pp. 1249-1250.

STEELE, MARY:

- :02. The Crayfish of Missouri. Publ. Univ. Cincinnati, Bull. 10, pp. 1-50, 10 pls.

STEFFENBERG:

- '72. Bijdrag Til Kanne domen om flodkraftens natural historia. Abstract in Zoöl. Record. IX.

EXPLANATION OF PLATES.

All figures drawn with camera lucida with Zeiss lenses specified, with few exceptions noted.

They are printed one-third the diameter of the drawings, except Figs. 92 and 93, which are nearly life size.

ASTACUS LENIUSCULUS.

PLATE I.

- Figure 1. Larva issuing from egg-shell. 290 mm., aa.
2. Pantograph enlargement of photograph of living female to show young, several hours after hatching, crowded upon the under side of abdomen of mother.
3. Right side of living larva soon after hatching. 290 mm., aa.
4. Dorsal view of living larva just hatched, showing split egg-case and long filament connecting it to telson of larva. 290 mm., aa.
5. Front and oblique side views of eyes and rostrum to show its curvature. 2. aa.
6. Dorsal face of left antennule of first stage. 2. A.
7. Under face of left antenna of first stage. 2. A.
8. Outer face of left mandible of first stage. 2. A.
9. Outer face of first maxilla of first stage. 2. A.
10. Outer face of second maxilla of first stage. 2. A.
11. Outer face of first maxilliped of first stage. 2. A.
12¹. Outer face of second maxilliped of first stage. 2. A.

PLATE II.

- Figure 13. Outer face of third maxilliped of first stage. 2. A.
14. Posterior face of left chela of first stage. 2. A.
15. Posterior face of left second walking leg, first stage. 2. A.
16. Posterior face of left third walking leg, first stage. 2. A.
17. Posterior face of left fourth walking leg, first stage. 2. A.
18. Posterior face of left fifth walking leg, first stage. 2. A.
19. Anterior face of one of left pleopods of first stage. 2. A.

PLATE III.

- Figure 20. Dorsal face of telson of first stage, showing internal radiations, marginal spines, and attachment of telson thread. 2. A.
21. Enlarged views of attachment of telson-thread to certain glandular spines on the posterior edge of the telson of first stage. 2. D.
22. Detail of three such glandular telson spines still covered by cuticle of embryo. From a larva just hatched, but not yet shed. 4. D.
23. Dorsal view of living larva in second stage. 290 mm., aa.
24. Left side of living larva in second stage. 290 mm., aa.
25. Upper face of left antennule of second stage. 2. A.
26. Under face of left antenna of second stage, with flagellum represented in two isolated parts. 2. A.

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¹In this and the subsequent five figures the anterior arthrobranch has been dotted for distinctness. --

PLATE IV.

- Figure 27. Outer face of left mandible of second stage. 2. A.
 28. Inner face of left mandible of second stage. 2. A.
 29. Outer face of left first maxilla of second stage. 2. A.
 30. Outer face of second maxilla of second stage. 2. A.
 31. Outer face of first maxilliped of second stage. 2. A.
 32¹. Outer face of left second maxilliped of second stage. 2. A.
 33. Outer face of left third maxilliped of second stage. 2. A.
 34. Posterior face of left chela of second stage. 2. A.

PLATE V.

- Figure 35. Posterior face of left second walking leg, second stage. 2. A.
 36. Posterior face of left third walking leg, second stage. 2. A.
 37. Posterior face of left fourth walking leg, second stage. 2. A.
 38. Posterior face of left fifth walking leg and pleuro branch. 2. A.
 39. Posterior face of a left pleopod of second stage. 2. A.

PLATE VI.

- Figure 40. Dorsal aspect of living larva in the third stage in normal attitude which makes the telson fore-shortened. 290 mm., aa.
 41. Side view of living third-stage larva kept quiet by alcohol. 290 mm., aa.
 42. Dorsal aspect of the left newly expanded pleopod of the sixth segment of the third-stage larva. 2. A.
 43. Dorsal aspect of the telson of third-stage larva. 2. A.

CAMBARUS AFFINIS.

PLATE VII.

- Figure 44. Posterior face of fourth left pleopod of adult female. 290 mm., aa.
 45. Eggs attached to pleopod of adult female about twenty hours after being laid. 290 mm., aa.
 46. Ventral aspect of posterior part of abdomen of first larva, showing attachment of telson thread, the concealed sixth and free fifth and fourth pleopods. From a recently hatched larva. 2. A.
 47. Dorsal aspect of telson and sixth abdominal somite of first larva twenty hours after hatching. 2. A.
 48. Ventral aspect of terminal middle part of telson of first larva dissected out of egg-shell, showing attachment of telson thread to certain spines. 2. D.
 49. Dorsal aspect of first larva as seen in Worcester's liquid. 290 mm., aa.
 50. Right side of first larva, seen in Worcester's liquid. 290 mm., aa.
 51. Front view of eyes and rostrum of first larva, seen in Worcester's liquid. 2. aa.
 52. Pleopod covered by larvæ in the first stage and 48 hours old; alive; some few removed. Camera lucida on dissecting stand, Steinheil Aplanatic, No. 9.
 53. Dorsal aspect of left antennule of first stage. 2. A.
 54. Lower face of left antenna of first stage. 2. A.
 55. Outer face of left mandible of first stage. 2. A.
 56. Outer face of left first maxilla of first stage. 2. A.
 57. Outer face of left second maxilla of first stage. 2. A.

¹ In Figs. 32 to 37 the anterior arthrobranch is dotted for distinctness.

- Figure 58. Outer face of left first maxilliped of first stage. 2. A.
59. Outer face of left second maxilliped of first stage. 2. A.
60. Outer face of left third maxilliped of first stage. 2. A.

PLATE VIII.

- Figure 61. Posterior face of left chela of first stage. 2. A.
62. End of chela of first stage, showing new cuticle and spines within old. 2. D.
63. Posterior face of left second walking leg of first stage. 2. A.
64. Posterior face of left third walking leg of first stage. 2. A.
65. Posterior face of left fourth walking leg of first stage. 2. A.
66. Posterior face of left fifth walking leg of first stage. 2. A.
67. Posterior face of left pleopod of first stage. 2. A.
68. Dorsal aspect of second larva, in Worcester's liquid. 290 mm., A.
69. Left side of second larva, living, copied from Andrews, :04.
70. Part of pleopod with some young in second stage left upon it. Drawn living, with dissecting stand and Steinheil Aplanatic, No. 9.
71. Ventral aspect of telson of second stage with included sixth pleopods and anal thread issuing from anus; living. 2. A.
72. Ventral view of right half of telson of second stage to show marginal papillæ. 2. D.
73. Telson and part of intestine removed, with attached anal thread and portion of cast shell, ventral views. In A normal relation is shown; in B the puckering of the intestine when the cast telson shell is pulled away from the telson. 290 mm., A.
74. Portion of intestines and chitinous lining, made transparent to show the limit of the cast-off lining (*y*) and the region (*x*) where still attached. 2. D.
76. Dorsal aspect of left antennule of second stage. 2. A.
77. Under face of left antenna of second larva. 2. A.

PLATE IX.

- Figure 78. Outer face of left mandible of second larva. 2. A.
79. Outer face of left first maxilla of second larva. 2. A.
80. Outer face of left second maxilla of second larva. 2. A.
81. Outer face of left first maxilliped of second larva. 2. A.
82. Outer face of left second maxilliped of second larva. 2. A.
83. Outer face of left third maxilliped of second stage. 2. A.
84. Posterior face of left chela of second stage. 2. A.
85. Posterior face of left second walking leg, second stage. 2. A.
86. Posterior face of left third walking leg, second stage. 2. A.
87. Posterior face of left fourth walking leg. 2. A.
88. Posterior face of left fifth walking leg, second stage. 2. A.
89. Posterior face of left pleopod of second stage. 2. A.
90. Dorsal aspect of living larva in third stage. 290 mm., aa.
91. Left side of living larva in third stage, reduced $\frac{1}{2}$ from 2 aa.

PLATE X.

- Figure 92. Photograph of side view of adult female and its young in third stage climbing under and about the body.
93. Photograph of dorsal side of adult female, with its young in third stage climbing over its back.

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FIGURE 92

FIGURE 93

CAMBARUS AFFINIS: THIRD STAGE

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