Natural History of Cambarus immunis. Progress Report. K. E. Goellner Second Semester 1939 - 40.

First attempts at writing this report resulted in detailed discussions which seem now to be unwarranted in view of the work completed in 1939 by Peter Tack at Cornell University. His thesis has been examined and it does not look as if there were enough difference between his work and mine to justify trying to build a thesis out of my data. However I propose to cont indue parts, at least, of the study, so as to obtain certain corroborative or contradictory data bearing on the natural history of this and other species of crawfish.

Marking experiments: It was necessary before successful laboratory tests could be completed to give up trying to "balance" the aquarian and to install aerators. Most of the crawfish died when plants alone were used to supply oxygen; eating and tearing up of the plants by the crawfish made balancing doubly difficult. Before aeration the dissolved oxygen in the tank waters averaged about 3 ppm ; after aeration, it has averaged about 8 ppm .

Tagging (pairs). First marking attempts were made with pairs of celluloid discs, with a center hole in each, fastened one on each side ${ }^{\text {of }}$ a crawfish's abdomen by means of a nickel pin thrust through one abdominal somite. This method is used by the U.S.B.F. in the shrimp investigations. Pins and discs were secured through Dr. Lindner of the Bureau. Five crawfish were tagged and two left untagged in Tank A. Nos 1 and 2 were pinned through abdominal somite 2; no. 3 through somite 3 ; no. 4 through somite 4 , no. 5 between somite 3 and 4. All tagged on march 15.

Results. Nos 1, 2, and 3 died the first time they tried to
molt. Notes on their struggles to shed were taken but are irrelevant here. ivo. 5 died unexplainably 24 days after tagíing. No. 4, tagged at somite 4, did succeed in one ecdysis, with a little help from me, beginning on April 6 and finishing on April 12. However he still bears scars from the struggle.

Another crawfish was marked by means of a trout jaw tag fastened through the exopodite of one uropod, near the base. He tore this away within five days and molted successfully. the next night. In another case a nickel pin was thrust upward through the sternite, muscles and tergite of the abdomen of a small specimen, then bent over so it could not silp out. This specimen died trying to molt the next day.

The method has been given up without further tri al because of the obvious difficulties imposed upon the crawfish in ecdysis. Only at ecdysis did the pins and tags cause them apparent trouble; no infection or corrosion of rins was observed. It is a method which could be used in the field, as far as ease of manipulation goes.

Tattoo. It was next hoped to inject spots of dyes of some sort beneath the ventral abdominal hypodermis as a mark for future recognition. A suspension of Chinese ink (black) was given up because of difficulty in detecting artificial black spots in crawfish already naturally discolored and spotted in the field.

A water suspension of carmine was tried. First difficulties were in adjustment of proper depth of needle in a live struggling specimen; also in injection of the right amount of dye. Too little could not be seen; too much discolored the whole abdomen. After preliminary trials; three specimens were "spotted" with carmine on April $\leq$.

One specimen received enough carmine between the 3 rd and 4 th pleopods on' the right side to discolor the whole abdomen ventrally. between 3 anad A distinct spot/persisted through a molt which occurred 3 days later, and until death 22 days later. Cause of death was not apparent.

Another was given a "neat" spot of pink on the right side of the first intersegmentad membrane. This spot persisted, (tho it took on the aspects of a scar, which would be hard to recognize in the fiedd), until ecdysis occurred after 50 days. After ecdysis the stain was still visible, but faint and diffused, not a spot. That is the condition at present, with the additional note that the whole ventral abdomen has recen tly become pink.

The third specimen received carmine spots beneath the right 3rd and 5th, and left 4 th intersegmental membranes. These spots, though distinct at first, became too diffuse for recognition as individual spots after 32 days. After ancecaysis at about 50 days, there are no spots at all but only a faint pink stain diffused over the ventral abdomen.

This method gives some promise but would, I believe, be extremely difficult to use in the field because of the delicacy in technioue reduired. Cold wet fingers in a high wind, etc., could never use it propenly.

Pleura clipping. At Dr. Hubb's'suggestion ettempts were made at clipping the projecting pleura of the crawfish abdomen. This techniaue is easily performed with fine scissors even in the field. A growing set of field observations indicates that injuries and scars to the pleura a very infrequent in nature. Extent and
speed of regeneration are the obvious question marks, and as yet I can not say how long inflicted injuries will persist and be recognizable.

In the laboratory 12 crawfish, 6 in each tank, have been marked by various combinationsof clipped pleura, on one side or both sides, and up to all five pleura on one side. Most specimens seem not to suffer from the clipping, although three have died in Tank A, one in Tank B. Individual records for each specimen have been kept on separate cards, observation being directed mainly at changes after ecdysis.

Summing up results in the tanks, to date, of the 6 clipped in $A$, two were killed and eaten before undergoing a molt. A third died three days after an ecdysis. Only very slight regeneration, rather a healing only, had occurred in this case. Two have not nolted yet (after 50 days). The sixth has molted twice in 43 days and tre "clips" are still easily recognizable, though about one-half regenerated.

In tank $B$, one of the six was killed by his fellows 43 days after clipping, the marks having persisted recognizably, though about two-thirds regenerated, through three ecdyses. The others have molted from one to three times, with attendant regeneration from one-third to three-fourths complete. These have been observed from 43 to 50 days and are sill healthy. The youngest and smallest, a o of 13.8 mm . cephalothorax length at the start has molted three times and shows the most regeneration. However the marks are still recognizable, and may be even after regeneration is complete, though I would not predict this. There is some reason to believe that if the pleura are clipped deeply enough, that is, close enough to the abdominal muscles, regeneration may be checked considerably.

At any rate because of the length of time reauired laboratory tests, and the convenience of the method, it was decided to try pleura clipping in the field in connection with the periodic censuses at the Fenton Ponds and at Geddes Creek ( 7 miles E. of Ann Arbor). No predicityon was made as to the results. It was simply felt that what data was secured would be of interest. Accordingly the following marking was done by clipping of pleura in the field, each specimen being sexed, measured (cephalothorax length), described as to shell condition, condition of first pleopods ("andropods") in males, presence of eggs, etc., before being clipped with a specific code number, as R12L4, or R4L35 ( $R$ and $L$ indicating right and left). General notes as to place of capture and release were also recorded. During measuring and marking the crawfish are kept in water in pails and are returned to the point of capture within an hour.


Data for each recovered crawfish is sumarized on individual cards giving all information recorded in field data.

As yet, the data from recoveries too scanty for detailed analysis, but it ean be said that such marking promises to give valuable data on actual growth, breeding condition, etc., of individual specimens in the field; also possibly on population numbers. Regeneration in recovered specimens seems hardly to have begun and the method seems suttable as a temporary mark, at least. Large numbers of marked crawfish must be released to insure frequent recoveries. Therefore the method of mariking must be suited to field conditions.

Ventral tagging. The most recent attempt at marking is one proposed by Van Bonde (1928)\% for the Cape Crawfish. A nickel pin is put through a celluloid tag, then the pin point is bent back to form a barb, thus: . This barb is inserted from the ventral side into the abdomen of the crawfish, to one side of the midine, and turned so as to catch over the large abdominal muscles. This pin, then, passes through only the thinnest part of the exoskeleton (beneath the abdomen) and of 5 specimens recently tagged, with some variations, one hes successfully molted past the pin, suffering no ill effects. This mefhod may be the best yet.

* Union S. Africa Fish and liarine Biol. Surv. Rep't 6(3):1-4.

Periodic censuses; Collections have been made at the Fenton Fonds and the Geddes Creek at intervals of two weeks, except when weather has been bad or something else has interfered. At these times a sample of the crawfish are seined out of the pond or creek and measured (cephalothorax length) and sexed; condition of male sex organs (Form I or II) is recorded, also presence of eggs or young, hardness of shell, and any dther pertinent notes. The marking work ties in nicely with these censuses and is done simultaneously. All crawfish are returned to point of capture, except a few which are preserved. The data give information on growth rate of the groups, changes in breeding condition, frequency of molting, changes in sex ratio, span of life, duration and frequency of egg-bearing, etc.

Preliminary graphs showing the results of the censuses are given on the following three sheets. Cephalothorax lengths (abscissas) in one millimeter intervals are plotted against numbers of individuals. Females are plotted separately from males, while Form II and juvenile males are plotted below a line with Form I males above. Some samples are obviously rather small, sometimes because of an apparent scarcity of crawfish, sometimes through unfortunate lack of time to work longer. Censusing was begun at Geddes Creek on March 20, at Fenton Pond III on April 15, at Fenton Pond I on May 10.

Without attempting enalysis of the graphs, it can be seen that they show a considerable average size difference between the Bond I population and specimens from Fond III and Gedaes Creek, the latter being especially small. Changes in the male "andropod" condition can be seen, especially in Geddes Creek between liay 11 and May 26. The sample from Fond I on kay 24 was altogether too small; more crawfish simply could not be found. Eut the graph is


Geddes Creek, Mor. 20,1940


Geddes Creek, Aori 17, 1940



Finton Pond I



Fenton Pand I; May 10,1940


included to show that some Form I males of May 10 had molted to Form II by May 24. Graphs for the entire season's changes should be interesting. I believe the data will be useful for statistical treatment, in genereal, especially because in Geddes Creek the census includes, I believe, most of the crawfish present; and in the Fenton Ponds I shall be able to get a mass sample in the fall at draining time.

Miscellaneous notes. Egg-bearing females of various sizes have been preserved for data on reproductive capacity. Counts of these eggs were to be checked against unextruded eggs in ovaries of females. Counting has not progressed far enough to say what the results would be. I believe Taak should have counted more than 37 egg somples.

Examinations of stomachs of preserved specimens collected on different dates is still in preliminary stages and indicates the presence of larval insect fragments and plant fragments. I have also learned that most stomachs are empty unless the specimen is killed in strong formalin, and has holes punched in the carapace to aid penetration. A list of foods acceptable to laboratory crawfish has been kept.

As to the occurrence of blue immunis, I am still unable to say I have seen a true blue specimen. There is a confusing variation in color in this species, from brown to blue and lavender, and it is difficult to describe the differences in field notes.

Among the predators mentioned by Tack, the mink is not listed. I am sure a mink was competing with me for the few crawfish specimens available at Fenton in February and March. His tracks were conspicuous, but though I trailed him, I could find no faeces, nor crawfish fregments.

I am accumulating a few notes on occurrence of Branchiobdellids on immunis and preserving specimens for identification by Dr . Goodnight. These worms lose no time in transferring themselves from a fresh-cast exuvia to the soft skin of the crawfish.

Trapping crawfish at Fenton was not successful during late winter and ought to be recommenced. Am not sure why my traps caught so few specimens. The cage in which I had captive crawfish, in Fenton Pond I, became exposed to severe drought conditions in early May with the result that the specimens all died. Am not sure the data from this cage have any value.

Conclusion. I fear that Dr. Tack's thesis precludes my going on with this work for my thesisk but I do believe some parts, that is, the marking and censuses, ought to be continued as of interest in themselves and worthy of publication when sufficiently completed.

