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Effect of Degree of Injury upon rate
of Regeneration and rate of Molting
in the Crayfish, *Cambarus Blandingi*

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EFFECT OF DEGREE OF INJURY
UPON
RATE OF REGENERATION AND RATE OF MOLTING
IN
THE CRAYFISH, CAMBARUS BLANDINGI

BY

ARTHUR THOMPSON EVANS

THESIS

FOR THE

DEGREE OF
BACHELOR OF ARTS

IN

GENERAL SCIENCE

COLLEGE OF SCIENCE

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Arthur Thompson Evans

ENTITLED *Effect of Degree of Injury upon Rate of
Regeneration and Rate of Molting in the crayfish, Cambarus
Blandingi.*

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF *Bachelor of Arts in General Science*

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The problem of effect or degree of injury upon the rate of regeneration has been studied in several forms by different investigators but with lack of uniformity in their conclusions. This set of experiments was undertaken for the purpose of clearing up, if possible, some of the apparent discrepancies in former data. Recent investigators, working upon various forms, have brought out the effect of different factors upon regeneration which had not been controlled in former experiments. A knowledge of these factors has aided materially in eliminating elements which otherwise would have tended to obscure the results of the experiment.

This experiment records data not only upon the effect of degree of injury upon the rate of regeneration but also its effect upon the rate of molting.

The experiment was conducted in the Zoological Laboratory at the University of Illinois, under the direction of Doctor Charles Zeleny, to whom the writer is indebted for his many kind and valuable suggestions. The author is also indebted to Miss Margaret Taggart for her aid in identifying the animals used.

METHOD

The experiments were conducted upon the crayfish *Cambarus blandingi*. Throughout the experiment care was taken to keep conditions alike for all individuals and to eliminate all factors which seemed likely to invalidate the results. The one factor which was different in the two compared sets of crayfish was that of degree of injury. Some of the factors which would be likely to influence the rate of regeneration and the methods of eliminating them follow:

AGE: In selecting animals for the experiment, care was taken that all were as near the same size as it was possible to obtain them, it being assumed that animals of the same size are of relatively the same age. It is known from previous experiments that younger animals regenerate lost parts more rapidly than do older ones.(1) In this experiment only very young material was used. A probable source of error lies in the fact that animals of the given size may not necessarily be of the same age.

Laboratory History: An animal removed suddenly from its natural environment and placed in an entirely new one is undoubtedly affected by such a change. It is quite essential for the best results that all animals have the same laboratory histories. In this experiment all animals have had the same laboratory histories.

Temperature: Rate of regeneration is known to vary with the temperature.(2) This factor was eliminated by making pairs of animals which molted at the same time. Where this was not possible animals molting at nearly the same time were paired. The mean temperature of the laboratory was about 22 degrees C. This varied at times from 18 to 26 degrees C.

Sex: The rate of regeneration may be different in the two sexes.(3) The possibility of such a difference affecting the results of the present study is very slight, as the animals used were so young that it was difficult to distinguish the difference between the sexes.

1. Zeleny, 1905

3. Zeleny, 1905

2. Morgan, "Regeneration"

Food: Different investigators studying the effect of food upon the rate of regeneration have reached no general conclusions.(4) Since food may affect the rate of regeneration, an effort was made to keep the amount and kind of food the same for all individuals.

Time of Operations with Respect to the Molt: It is quite probable that operations performed at various times after molting might affect the rate of regeneration as well as that of molting. An attempt was made to eliminate any error which might occur because of this factor by performing all operations at the same lapse of time after the molting process.

Individual Variation: Individual variation cannot be entirely eliminated. By the use of large numbers the probability of error is reduced to a minimum.

The advantages of such a form as the crayfish for experimental purposes are (1) the molting habit sets a definite period from which to work, (2) the appendages have a definite breaking joint which insures all operations being at the same level, (3) the hard carapace reduces the liability to injury in handling, (4) it is easily obtained, (5) it is easily kept. In this set of experiments two separate lots of crayfish were used. The two lots were collected at different times, but all individuals in the same lot were collected at the same time. Series I contained 33 individuals, Series II 54 individuals. During the course of the experiment several individuals died; some also did not molt until very late in the experiment, probably because they had molted just before being captured. The experiment does not contain data on these, as it was terminated as soon as ten pairs of animals had molted, in

4. Morgan, Durbin

each series.

After the animals had been brought into the laboratory each was immediately isolated in a four ounce bottle containing from one to one and one-half ounces of water. The bottles were then arranged on a table in the following manner: small strips of wood one inch thick were nailed about six inches apart on the top of the table. The bottles, the mouths of which had been previously covered with cheese-cloth held in place by a rubber band to prevent the animals from escaping, were then inclined upon the table with the necks resting upon the strips of board. In this position the lower end of the bottle contained enough water easily to cover the animal, while in the higher end the water was quite shallow.

All of the animals were fed on the same days. In general the feeding was done twice each week. The food consisted of chopped beef, tubifex worms, and elouea. For fear of contamination meat was never allowed to remain in the bottles longer than from two to four hours. The water was always changed and the bottles thoroughly washed after each feeding of meat. When Tubifex and Elouea were used as food they were allowed to remain with the animal. As they were live foods the danger of contamination was eliminated.

Operation: In each series there were two degrees of injury; (1) the lesser, which consisted in the removal of the right chela alone; and (2) the greater, which consisted in the removal of both chelae. In all cases the operations were performed on the second day after the first recorded molt. This was observed so that the

animals might recover from the shock of molting and and also to allow the tissues to expand fully and harden before being removed.

In all cases regeneration measurements represent the length of the propodite of the large chela.

The operations were performed by pinching the chela below the propodite with a pair of pointed forceps. This causes the animal to cast the chela at the definite breaking joint. All measurements of the regenerated parts were made with a micrometer under the low power of a compound microscope. In this way measurements could be made up to 1/100 of a millimeter. The thoracic measurements were made with a pair of calipers.

All regenerating appendages were removed on the second day after the third molt. In the data the number of days recorded represents the number of days in two molting periods. It was necessary to use two molting periods instead of one, because some of the chelae had not regenerated after the second recorded molt.

DATA

In the tables the two Series have been arranged so as to show the relation of the regeneration in the cases of both the greater and the lesser degree of injury when compared with each other. In Series I, No. 27, with the lesser degree of injury, and in Series II, No's 23 and 33, with the greater degree and No's 2, 27, and 29, with the lesser degree of injury, were not included in the results. The regeneration in the case of each of these seems not to have been normal, so it was concluded that some factor other than those eliminated had entered in, in the case of each of these individuals. It will be noticed, however, that the results are

not altered, even if these individuals are included in the data, but the difference between the greater and the lesser degree of injury is made even greater.

In Series I the regeneration in the case of the animals with the greater degree of injury exceeds the regeneration in the lesser by .30 of a millimeter or an increase of 8%. In Series II, after three of the individuals which regenerated very slowly were left out, the greater degree of injury showed an increase in regeneration over the lesser of .17 of a millimeter or an increase of 5%. In the case of both Series I and II the cephalo-thoracic lengths were almost equal. The average length of the animals in Series I, in the case of both the greater and the lesser degree of injury was 13.9 millimeters; the length of those in Series II with the greater degree of injury was 11.9 millimeters; while the average length of those with the lesser was 11.7 millimeters.

The tables also show a decided increase in the rate of molting of the animals with the greater degree of injury, as compared with the animals with the lesser degree of injury. In Series I the increase was five and one-half days while in Series II the increase was four and one-third days.

In selecting the animals for an experiment, even though great care is taken to obtain specimens of exactly the same size, one is never able to do so. In studying the rate of regeneration some method must be adopted that will afford a direct method of comparing different-sized animals. For this purpose no better way is afforded than by the use of Specific Amount and Specific Rate of Regeneration.

Specific Amount of Regeneration: By the specific amount of regeneration is meant the amount of regeneration which takes place in one chela, or the average amount which takes place in the case of two chelae, divided by the thoracic length of the animal. The specific amount of regeneration in both Series I and II was greater in the case of the animals with the greater degree of injury. In Series I the greater exceeded the lesser by .20 of a millimeter, or an increase of 7%. In Series II the greater exceeded the lesser by .13 of a millimeter, or an increase of nearly 5%.

Specific Rate of Regeneration: The specific rate of regeneration is obtained by dividing the specific amount of regeneration by the number of days during which regeneration is going on. This gives a value which represents the rate of regeneration per unit of cephalo-thoracic length per day. The specific rate of regeneration showed an increase in the case of both Series I and II. The amount the greater degree of injury exceeded the lesser in Series I was .0009 of a millimeter, or an increase of 16%; while in the case of Series II the greater exceeded the lesser by .0011 of a millimeter or an increase of 22%.

DISCUSSION OF RESULTS

Results of the Present Experiment: All data collected seems to point conclusively to the fact that the rate of regeneration in the crayfish is increased by additional injury. Also the data show that increased injury causes an increase in the rate of molting. As the results of Series I and II verify each other it would seem very probable that the different degrees of injury are direct causes.

Just why the results are such is hard to explain. It may be that in the case of an animal with both chelae removed the animal is incapacitated and that the need of a means of defense as well as a means of capturing its food may tend to cause the chelae to grow more rapidly. The theory has already been advanced that exercise may cause the regenerating parts to develop more rapidly. It seems quite probable that this may be true. Another assumption is the fact that the chela on the animal with the lesser degree of injury may not develop so rapidly due to the fact that the one remaining chela increases in efficiency.

The increase in the molting may be directly associated with the increase in the rate of regeneration. It is assumed that molting is caused by the growing animal becoming too large for its old covering. It may be that the increase in molting is due to the abundance of nourishment due to the absence of the two large chelae. As these chelae are large, muscular, much-used appendages they undoubtedly require a large amount of nourishment. This supply not being required by them might be utilized and cause the rapid development of other parts. This would cause the carapace to become too small much sooner than it otherwise would, and so increase the rate of molting. If this is the case, then a crayfish with one removed chela should show an increase in molting over the rate of molting in a normally controlled lot of crayfish.

FORMER WORK: In 1905, Zeleny, working on the crayfish, advanced the theory that the rate of regeneration was increased, up to a certain limit, by additional injury to the specimen. This limit he called the optimum limit. Above this limit further injury would

undoubtedly cause a decrease in the rate of regeneration.

In 1906, Emmel, working on the young lobster, observed that the rate was not increased with additional injury but was decreased. This fact was observed by Emmel while working upon the effect of injury upon the rate of molting. It is possible that some factor affecting the rate of regeneration was not controlled in the experiment, as he was working primarily for the effect of the degree of injury upon molting. Emmel also found that increased injury decreased the rate of molting. In his work on the lobster he draws his conclusions from two Series, D and E. In the D Series he removed one chela while in the E Series he removed the two chelae as well as the third and fourth pair of walking legs. This fact may account for the decrease in the rate of molting. It is quite possible that in the case of the greater degree of injury he has exceeded the optimum limit to which Zeleny refers. If this is the case then the results agree with Zeleny's as well as this paper. As the optimum is not as yet determined Emmel's conclusions are open to question.

It is also a well known fact that the young lobster is very hard to rear. It is a question of doubt whether so young an animal as Emmel used would be able to withstand the shock of having three pair of legs removed and still be able to regenerate normally. A smaller degree of injury would have undoubtedly given the problem a fairer test.

Another question which comes up is whether an animal with so great a degree of injury would be able to feed itself sufficiently. Investigators working on the effect of food upon the rate of re-

generation have come to no general conclusions, however, until it is proven otherwise we will have to assume that food does affect the rate of regeneration.(5)

In 1907, Scott, working on *Fundulus heteroclitis* brought forth data to prove that there was neither an increase nor a decrease in the rate of regeneration with regard to the degree of injury. Whether cuts can be made on the tail of *Fundulus heteroclitis* at exactly the same levels is a question of doubt. Failure in this respect may account for Scott's results.

In 1907, Stockard carried on a series of experiments upon the rate of regeneration in the arms of *Cassiopea Xamachana*. In these experiments Stockard found that the disk diameter of the animal decreased with the greater injury. In figuring the specific rates of regeneration he used the original disk diameters. In this case he found the rate of regeneration to decrease with the greater degree of injury. If, however, the final disk diameter is used it is found that the rate of regeneration increases with the greater degree of injury. The question which presents itself is whether the original or final disk diameter should be used. It is quite possible that the wrong interpretation may have been placed upon the data.

CONCLUSION

Thus far it seems quite certain that the rate of regeneration is increased with an increase in the amount of injury, in the crayfish, up to a certain limit. As the crayfish is one of the few forms that has been worked on with the same results, it seems very

likely that they are true. Nevertheless, additional work on this form as well as the forms in question will no doubt be watched with interest.

SUMMARY

I. In the crayfish *Cambarus blandingi* it was found that the rate of regeneration was increased in the case of each of two removed chelae, over the rate of regeneration in the case of only one removed chela.

II. In the crayfish *Cambarus blandingi* it was found that the rate of molting was increased with additional injury. Specimens with two removed chelae were found to molt more rapidly than those with only one removed chela.

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SERIES I

Specific Amount of Regeneration in the Crayfish in the Cases of One
and Two Removed Chelae

Table for Two Removed Chelae

Crayfish No.	Days bet. Molts	Cephalo- Thoracic length in mm.	Length of Propodite in mm.			Sp. Amt Regen. mm	Sp. Rate Regen. mm
			right	left	average		
1	51	12.5	4.51	4.68	4.59	.367	.0071
4	54	12.5	4.47	4.54	4.51	.360	.0066
7	51	15.0	4.26	4.26	4.26	.284	.0055
8	45	11.5	3.97	3.50	3.73	.324	.0072
11	45	15.0	4.33	4.33	4.33	.288	.0064
17	32	12.5	3.55	3.57	3.56	.284	.0088
5	42	12.5	4.26	4.33	4.29	.343	.0081
31	40	14.5	3.82	3.90	3.86	.266	.0066
28	54	12.5	3.40	3.40	3.40	.272	.0050
21	46	16.0	4.75	4.77	4.76	.297	.0064
Average					4.13	.308	.00671

Series I Continued

Table for One Removed

Chela

Crayfish No.	Days bet. Molts	Cephalo- Thoracic length in mm.	Length of right	Propodite in mm. left average	Sp. Amt Regen. mm	Sp. Rate Regen. mm.
2	54	11.5	3.97		.346	.0064
3	54	13.0	4.40		.338	.0062
9	46	12.5	4.26		.340	.0073
12	66	13.0	3.12		.240	.0036
14	58	14.0	3.90		.278	.0048
16	41	14.5	3.92		.270	.0065
19	37	14.5	4.26		.294	.0079
20	61	13.0	2.98		.230	.0037
30	43	14.0	3.69		.263	.0061
27*	45*	15.5*	.71*		.046*	.0010*
Average	51-1/9	13.4	3.83		.288	.0058

* 27 was not counted in the results. The amount of regeneration was quite small and was evidently affected by some factor which was not controlled.

SERIES II

Specific Rate of Regeneration in the Crayfish in the Case of Both
One and Two Removed Chelae

Table for Two Removed Chelae:-

Crayfish No.	Days bet. Molts	Cephalo- Thoracic length in mm.	Length of Propodite in mm.			Sp.Amt Regen. mm	Sp. Rate Regen. mm
			right	left	average		
6	52	13.0	3.50	3.62	3.56	.273	.0052
9	39	12.5	3.74	3.74	3.74	.300	.0076
21	70	12.0	3.32	3.40	3.36	.280	.0040
23*	77	11.0	1.77	2.34	2.05*	.186*	.0027*
30	46	11.0	3.12	3.06	3.09	.280	.0060
33*	22	11.0	1.72	1.47	1.59*	.144*	.0065*
45	49	12.0	3.47	3.40	3.44	.286	.0058
48	39	13.0	3.90	3.90	3.90	.300	.0076
50	48	12.0	3.47	3.57	3.52	.293	.0061
Average	49.1	11.9			3.51	.287	.0060

* NO!s 23 and 33 were not counted as they were much smaller than
the average.

Series II Continued

Table for One Removed

Chela

Crayfish No.	Days bet. Molts	Cephalo- Thoracic length in mm.	Length of Propodite in mm.			Sp. Amt Regen. mm	Sp. Rate Regen. mm
			right	left	average		
2*	45	12	1.02*			.085*	.0018*
3	52	13	3.62			.279	.0053
4	60	12	3.26			.271	.0045
11	54	12	3.30			.275	.0050
22	48	11	2.92			.265	.0055
27*	38	10	1.27*			.327*	.0033*
29*	48	10	.56*			.056*	.0011*
37	58	12	3.55			.295	.0050
38	76	13	3.40			.261	.0034
Average	53.4	11.7			3.34	.274	.0049

* Nos 2, 27 and 29 were not used in figuring up the mean as some factor not controlled probably entered in.