- Beamish, F. W. H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of several species. Can. J. Zool. 42:177-188.
- Beamish, F. W. H. and P. S. Mookherjii. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, Carassius auratus L. Can. J. Zool. 42:161-175.
- Hickman, C. P., Jr. 1959. The osmoregulatory role of the thyroid gland in the starry flounder, Platichthys stellatus. Can. J. Zool. 37:1006-1023.
- Keys, A. B. 1930. The measurement of the respiratory exchange of aquatic animals. Biol. Bull. (Woods Hole) 59:187-198.
- Mann, K. H. 1965. Energy transformations of fish in the River Thames. J. Anim. Ecol. 34:253-275.
- Winberg, G. G. 1956. Rates of metabolism and food requirements of fish. (Transl. from Russian.) Fish. Res. Bd. Can., Transl. Ser. 194. 239 p.

## SOME EFFECTS OF SALINITY ON TWO POPULATIONS OF RED SWAMP CRAWFISH.

### **Procambarus clarki (Girard)**<sup>1</sup>

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#### ABSTRACT

Salinity tolerance was compared between an inland population of red swamp crawfish, Procambarus clarki, from Baton Rouge, Louisiana, and a coastal marsh population from Grand Chenier, Louisiana.

Newly hatched crawfish from each population were killed in less than one week in salinities of 15, 20 and 30 ppt. Crawfish, 30 mm in total length, withstood salinities up to 20 ppt, but died in 30 ppt in two to three days. Crawfish, 40 to 120 mm in total length, showed no significant mortality after one week in salinities up to 30 ppt.

Thirty-millimeter crawfish exposed to salinities of 0, 10, 20 and 30 ppt for four weeks grew very little when fed fresh fish flesh, tropical fish food pellets, and Oedogonium sp. All 30-mm crawfish in 30 ppt died. Growth varied inversely with salinity.

Forty- to fifty-mm crawfish held in 0, 10, and 20 ppt salinity for four weeks had average increases in weight of 4.4, 13.5 and 4.9%, respectively. They were fed mixed green algae, which they ate continually. Growth in 10 ppt was significantly greater (<.05) than that in 0 or 20 ppt.

#### INTRODUCTION

The red swamp crawfish, Procambarus clarki (Girard), is becoming an important food in Louisiana and in other parts of the country. Because of improved processing techniques, crawfish can be obtained

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nearly any time of year. More harvestable crawfish are needed to meet this increased demand and greater utilization.

The natural habitat of red swamp crawfish produces only a part of the crop needed. Although the population of crawfish may be sufficient, difficulties in harvesting in swamps limit the yield. To supplement the natural crop, crawfish are being cultivated in ponds or in rice fields. On these farms impoundments are made by constructing levees around several acres of land and pumping in water from a local source. The water level is lowered in summer and raised in fall to produce maximum yields of crawfish (LaCaze, 1966). Fresh water is used on virtually all crawfish farms.

Louisiana has over 4,000,000 acres of marsh (O'Neil, 1949) unsuitable for agriculture. Water in this marsh is subject to change in salinity from 1 ppt to over 30 ppt. This fluctuation is due primarily to seasonal precipitation and evaporation; but extremely high tides, such as those associated with hurricanes, can greatly increase salinity.

Crawfish ponds in this area would sometimes necessarily be filled with brackish water. Survival, growth and reproduction of crawfish may be affected. Viosca (1961) stated that a small amount of salt in water may be beneficial to crawfish. There is a natural population of red swamp crawfish in coastal marsh around Pecan Island, Vermilion Parish, Louisiana (LaCaze, 1966). In spring of 1965 this area yielded an unusually large crop of crawfish (Louisiana Department of Agriculture and Immigration, 1966). A very high tide associated with the fall equinox flooded this marsh in October, 1964. Although the crop size was probably increased simply by favorable water depth, presumably the high tide brought in a quantity of sea water, which did not measurably harm the crawfish. During the same season crawfish production was poor in other parts of Louisiana because of low water.

This study was conducted to determine acute and chronic effects of salinity on red swamp crawfish, and to compare the salinity tolerance of an inland population to a coastal marsh population.

#### MATERIALS AND METHODS

#### Collection of Crawfish and Water

Experiments were conducted with red swamp crawfish collected from ponds on Ben Hur Farm, East Baton Rouge Parish, Louisiana, and at Grand Chenier, Cameron Parish, Louisiana. Crawfish were identified according to Penn (1959). Except in one growth experiment effects of salinities were compared on crawfish of approximately equal total lengths from both sites.

Newly hatched crawfish, 8 to 9 mm in total length, were obtained from females captured from burrows in early December. Each female with young was placed into a gallon jar in the field, and held in the laboratory until the young were needed for experiments. Just enough pond water was provided to allow females to lift young into the air if oxygen were depleted in the water.

Intermediate size crawfish, 30 to 90 mm in total length, and adult crawfish, 90 to 120 mm in total length, were collected with dip nets and seines at both sites. These crawfish were acclimated 24 hours before salinity tolerance tests.

Water for control media (0 ppt salinity) was collected from the natural environment with the crawfish, and water for higher salinities was taken from the Gulf of Mexico. Gulf water was diluted with distilled water to produce salinities of 10, 15, and 20 ppt. Compensation was made for evaporation by adding distilled water to test solutions each day. Salinities were determined by titrating with mercuric nitrate (American Public Health Association, 1960).

#### Acute Effects

Newly Hatched Young. Four experiments were conducted with 8to 9-mm crawfish. Young were shaken from females into a white porcelain pan. Appropriate numbers of crawfish were randomly drawn with the edge of a stiff feather, then placed in test media for one week. Test 1. Fifteen crawfish from each population per treatment were exposed to 0 and 10 ppt salinity.

Test 2. Fifteen crawfish from each population per treatment were exposed to 0 and 30 ppt salinity.

Test 3. Fifteen crawfish from each population per treatment were exposed to 0, 10, 20, and 30 ppt salinity.

Test 4. Fifteen crawfish from each population per treatment were exposed to 0 and 15 ppt salinity.

Newly hatched crawfish were observed and mortality recorded at 3-, 6-, 12-, or 24-hour intervals. A wooden applicator was used to lightly touch each crawfish. If the crawfish responded to this stimulus in any way, it was considered alive.

Intermediate and Adult Crawfish. Acute effects of salinity were tested once each with 30-mm and 60-mm crawfish and twice with 90to 120-mm crawfish. Experimental animals were selected at random and placed in test solutions for one week.

Test 1. Nine 30-mm crawfish from each population per treatment were exposed to salinities of 0, 10, 20, and 30 ppt.

Test 2. Three 60-mm crawfish from each population per treatment were exposed to salinities of 0, 10, 20, and 30 ppt.

Test 3. Five 90- to 120-mm crawfish from each population per treatment were exposed to salinities of 0 and 30 ppt.

Test 4. Three 90- to 120-mm crawfish from each population per treatment were exposed to salinities of 0, 10, 20, and 30 ppt.

Observations were made daily and deaths were recorded. Crawfish which did not move when touched were recorded as dead.

#### Chronic Effects

Two experiments were conducted to test effects of salinity on growth of red swamp crawfish.

Test 1. Five 30-mm crawfish from each population per treatment were exposed to salinities of 0, 10, 20, and 30 ppt for four weeks. They were fed every other day, and all uneaten food was removed on alternate days. One-half cubic centimeter of fish flesh was given at each feeding to each crawfish for two weeks. Tropical fish food was fed for the third week, and *Oedogonium* sp. was fed for the fourth week. Weights and lengths were recorded weekly, water was changed weekly, and deaths were recorded daily.

Test 2. Ten 40- to 50-mm crawfish from Ben Hur Farm per treatment were exposed to salinities of 0, 10, and 20 ppt for four weeks. They were given a continuous diet of mixed green algae and alligator weed (Alternanthera philoxeroides). Weights and lengths were recorded biweekly, water was changed weekly, and deaths were recorded daily.

Numbers of days crawfish lived and per cent gains in weight were used as measurements for statistical analysis. Results of experiments were tested with analysis of variance at the five per cent significance level.

#### RESULTS AND DISCUSSION

#### Acute Effects

Newly Hatched Young. Young crawfish from Grand Chenier had a higher salinity tolerance than those from Ben Hur Farm. Solutions of 10 ppt were sublethal for one week, but 15, 20, and 30 ppt salinity killed all young crawfish in less than one week.

Test 1. From a total of 60 crawfish none died from either population in 0 or 10 ppt salinity in one week.

Test 2. Only two of 15 Ben Hur crawfish and none from Grand Chenier died in 0 ppt in one week, but all died in less than 24 hours in 30 ppt salinity.

Test 3. Six of 15 Ben Hur crawfish died in 0 ppt in one week, but no Grand Chenier crawfish died in this concentration. Six from Ben Hur Farm and three from Grand Chenier died in 10 ppt and all 60 crawfish from the two populations died in 20 and 30 ppt (Figures 1 and 2). The differences in number of days young lived in the four treatments were significant. Effects of 0 and 10 ppt were not significantly different, but 20 and 30 ppt salinity each significantly increased mortality.

Test 4. Eight of the 15 Ben Hur crawfish and none of the Grand Chenier crawfish died in 0 ppt in one week, but all 30 died in 15 ppt (Figure 3). Treatment effects were significantly different.





Average numbers of dead crawfish

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If all newly hatched crawfish die in water between 10 and 15 ppt salinity, a hurricane in September or October, when most hatching occurs (Penn, 1943), could virtually destroy the crop for the next spring. More work should be done to determine the critical limits of salinity tolerance of red swamp crawfish eggs, embryos and newly hatched young.

Intermediate and Adult Crawfish. Crawfish, 30 mm in total length, showed little mortality in 0, 10, and 20 ppt, but died rapidly when exposed to 30 ppt. Kendall and Schwartz (1964) compared salinity tolerance of Orconectes virilis and Cambarus b. bartoni. They used salinities of 0, 6, 14, and 33 ppt to test the two species. One-half the crawfish exposed to 33 ppt died within 96 hours, but the other concentrations did not significantly affect survival of either species. Test 1. Two, zero, two, and four of nine 30-mm Ben Hur crawfish in each solution died in one week in 0, 10, 20 and 30 ppt, respectively (Figures 4 and 5). None of the Grand Chenier crawfish died in 0 or 10 ppt, but four and six died in 20 and 30 ppt, respectively. Crawfish of this size from the two populations were not significantly different in their salinity tolerance. Statistical removal of effects of 30 ppt salinity on the remaining data indicated comparable mortality in 0, 10 and 20 ppt.

Test 2. One of three 60-mm Ben Hur crawfish each died in 20 and 30 ppt salinity in one week, but none from Grand Chenier died. Neither origin of crawfish or treatments had significant effects on mortality.



Average numbers of dead crawfish



Average numbers of dead crawfish

Test 3. One of five 90- to 120-mm crawfish died after five days in 0 ppt and one died after one day in 30 ppt, but no Grand Chenier crawfish died in one week in 0 ppt and two died after four days in 30 ppt.

Test 4. None of the three Ben Hur crawfish in each treatment died in one week, but one Grand Chenier crawfish died after two days in 20 ppt and two died after two days in 30 ppt.

Effects of origin of crawfish and treatments were nonsignificant for adult crawfish. Apparently adult crawfish can tolerate salinities approaching that of sea water (35 ppt) for a short time. Bogucki (1934) found that Astacus fluviatilis tolerated 66% sea water for one month. This would enable crawfish to withstand sea water brought into coastal marsh by hurricanes and other causes of unusually high tides.

#### Chronic Effects

Test 1. Survival was poor in 30-mm crawfish from Ben Hur Farm (Figure 6). Deaths were as follows: Two of five in 0 ppt, three in



Average numbers of dead crawfish 10 ppt, two in 20 ppt, and five in 30 ppt. Only two of five Grand Chenier crawfish died in 20 ppt. One died after 15 days and one died after 22 days. All five in 30 ppt died in two days.

Crawfish rejected fish flesh, but they ingested tropical fish food pellets and *Oedogonium* sp. Hughes (1966) and Hubschman (1967) recommended beef liver and macerated fish flesh, respectively, but these rations fouled water unless it was filtered or changed daily.

The mean percent gain in weight after four weeks for crawfish in salinities of 0, 10 and 20, ppt was: Ben Hur, 27.0, 23.0, and 1.0; Grand Chenier, 16.2, 12.6, and 4.7 (Figures 7 and 8). Effects of origin of crawfish and salinity levels on growth were nonsignificant.

Because of the loss of observations on Ben Hur crawfish, and the resultant large standard error of the treatment means (4.12), the second experiment was conducted with Ben Hur crawfish only.

Test 2. Two of 10 crawfish each died in 10 and 20 ppt, but missing observations were calculated with an iterative scheme (Snedecor, 1956). Crawfish fed on mixed green algae continually, occasionally nibbling on alligator weed. The highest mean growth was 13.5% in 10 ppt, and the lowest was 4.4% in 0 ppt. Mean growth in 20 ppt was 4.9% (Figure 9). Standard error of the mean was 2.0. The difference in rate of weight gain was significant between 10 ppt and the other two salinities.



Salinity in ppt

Figure 7. Average per cent gain in weight of red swamp crawfish, 30 mm in total length from Ben Hur Farm, East Baton Rouge Parish, Louisiana, after four weeks in 0,10,20 and 30 ppt salinity.



Salinity in ppt.

- Figure 8.
- Average per cent gain in weight of red swamp crawfish, 30 mm in total length, from Grand Chenier, Cameron Parish, Louisiana, after four weeks in 0,10,20 and 30 ppt salinity.



Figure 9. Average per cent gain in weight of red swamp crawfish, 40 to 50 mm in total length from Ben Hur Farm, East Baton Rouge Parish, Louisiana, after four weeks in 0, 10, 20, ppt salinity. Data are too limited to make any conclusions regarding growth, but there are some trends apparent. Crawfish, 30-mm in length, grew faster in lower salinities in the first growth experiment, but the Ben Hur crawfish, 40 to 50 mm in length, demonstrated a different trend in the second experiment. The latter crawfish in 10 ppt may have grown faster because of increased essential minerals in the slightly more saline water, but those in 20 ppt may have been unable to cope with the increased osmotic stress, so they could not utilize the increased supply of minerals. These larger animals were nearly a month older and 10 to 20 mm longer than those in the first growth experiment. They may have had physiological changes occurring with sexual maturation. Crawfish exoskeletons thicken before spawning occurs. The greater need for minerals to produce this thickening may be supplied by 10 ppt salinity; however, this may be the maximum concentration crawfish can tolerate osmotically and still function normally. Much more work is needed to determine if red swamp crawfish can be successfully cultivated in brackish water.

#### CONCLUSIONS

1. Tolerance to salinity was directly proportional to size of the crawfish. Newly hatched young were killed by 15 ppt salinity and above; intermediates were killed by 30 ppt, and adults tolerated 30 ppt for one week with no significant mortality.

2. Up to 10 ppt salinity increased growth, but 20 ppt and higher retarded growth.

3. The two populations showed no significant difference (<.05) in tolerance to salinity, except that newly hatched young from coastal marsh were more resistant than those from the inland population.

4. Based on this study, no conclusions can be made regarding the feasibility of raising crawfish in coastal marsh. Research in ponds in the marsh would certainly yield more conclusive results.

#### ACKNOWLEDGMENT

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#### LITERATURE CITED

- American Publication Health Association. 1960. Standard methods for the examination of water and waste water, including bottom sediments and sludges. 11th ed. Amer. Public Health Assoc., Inc., New York. 626 p.
- Bogucki, M. 1934. Recherches sur la regulation de la composition minérale du sang chez l'écrivisse (Astacus fluviatilis, L.). Archives International du Physiology 38:172-179.
- Hubschman, J. 1967. Effects of copper on the crayfish, Orconectes rusticus (Girard). I. Acute toxicity. Crustaceana 12(1):33-42.
- Hughes, J. 1966. Use of the red crawfish, *Procambarus clarki* (Girard) for herbicidal assays. Proc. 20th Annu. Conf. Southeastern Assoc. Fish and Game Comm. (In press).
- Kendall, A. and F. Schwartz. 1964. Salinity tolerance of two Maryland crayfishes. Ohio J. Sci. 64(6):403-409.
- LaCaze, C. 1966. More about crawfish. Louisiana Conservationist 18(5 and 6):2-7.
- Louisiana Department of Agriculture and Immigration. 1966. Crawfish farming in Southwest Louisiana. 2 p., mimeo release, unpublished.
- O'Neil, T. 1949. The muskrat in the Louisiana coastal marshes. Louisiana Dep. Wild Life and Fisheries, New Orleans, Louisiana. 152 p.
- Penn, G., Jr. 1943. A study of the life history of the Louisiana red crawfish, *Cambarus clarki* Girard. Ecology 24(1):1-18.

with a summary of their distribution within the state. Tulane Studies in Zoology 7(1):3-20.

Snedecor, G. 1956. Statistical methods. Iowa State College Press, Ames, Iowa. 534 p.

Viosca, P., Jr. 1961. Crawfish culture, mudbug farming. Louisiana Conservationist 13(3):5-7.

# EFFECTS OF SEWAGE EFFLUENTS ON FISHES IN UPPER PATUXENT RIVER, MARYLAND

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#### ABSTRACT

A survey on fish population was made in the upper Patuxent River, situated between the metropolitan centers of Washington, D. C. and Baltimore, Maryland, in the summer of 1966. This stream has received effluents of eight secondary domestic sewage treatment plants. Data obtained by two series of fish collections with a 10-foot-and-one-fourthinch mesh seine during June 17-July 14 and August 5-24 were compared with water quality data (dissolved oxygen, conductivity, and pH). The effects of sewage effluent on the fish species diversity and fish abundance in this stream were evaluated.

Results of this study indicate that chlorinated sewage effluent reduced species diversity and fish abundance immediately below the seven effluent outfalls where dissolved oxygen remained at 4.2-10.6 ppm and pH value was 6.9-8.9 (above the limit of fish tolerance). Species diversity and fish abundance gradually increased downstream below the Laurel sewage outfall (the largest) from one species and one fish to 13 species and 194 fish in a 3.6-mile section of the stream while the dissolved oxygen gradually decreased from 6.5 ppm to 3.5 ppm and the pH value only changed from 7.4 to 7.2. This demonstrates that dissolved oxygen, regarded as the most damaging factor for fish in sewage pollution, was not a decisive factor for fish distribution below a chlorinated sewage outfall. Sewage toxicity (perhaps mainly chlorination) appears to be the causative factor.

In the various polluted areas located downstream (13 stations), where dissolved oxygen ranged from 7.5 ppm to 3.0 ppm and sewage toxicity decreased to such a degree that it was no longer harmful to fish life, a change in species composition with a fairly constant number of species was found at each station. Species diversity was similar to that found at three unpolluted upstream stations where dissolved oxygen was above 8.5 ppm. This suggests that a species shift rather than a reduction in species diversity is the principal effect of sewage pollution due to organic deoxygenation of the water and the additional nourishment. The species shift suggests two phenomena: (1) The number of ecological niches for fish species in a stream may not be affected by the reduction in dissolved oxygen (not applicable below 3 ppm) and by the increased nutrients as a result of sewage pollution. Niche structure, however, will be changed. (2) There is a continuous spectrum in the minimum range of oxygen requirements for each species in a fish community. The open habitat left by the pollution-sensitive species can be taken over by a pollution-tolerant species. This also suggests that there is no actual border line such as 5 ppm or 6 ppm, the current dissolved oxygen criteria for fish, that can guarantee safe propagation of all species.