

STARVATION, ENERGY RESERVES, AND
AGGRESSION IN THE CRAYFISH *ORCONECTES VIRILIS* (HAGEN, 1870)
(DECAPODA, CAMBARIDAE)

BY

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INTRODUCTION

The deprivation of basic biological requirements often are associated with increases in agonistic behaviors. The deprivation of one such requirement, food, has been related to increases in aggression in a number of vertebrates (Hinde, 1970; Hogan, 1971; Scott, 1958; Ulrich & Symannek, 1969). Such a relationship has also been demonstrated in several Crustacea (Hazlett, 1966; Hazlett & Estabrook, 1974).

Field observations on *Orconectes virilis* in southeast Michigan (Hazlett, Rittschof, & Rubenstein, in progress) have indicated seasonal variations in aggressiveness which could be related to changes in food availability and/or energy reserves. The present study was carried out in the laboratory to measure the relationship between starvation, energy reserves, aggression, and locomotion in the crayfish *O. virilis*.

MATERIALS AND METHODS

The crayfish used were male *Orconectes virilis* between 36 and 44 mm carapace length collected in a stream in Pinckney, Michigan. The observations were carried out between July 29 and August 29, 1972. Because most of the crayfish in the collection stream were marked as part of another study, few unmarked individuals of the desired size range and sex were available. Thus some variance in moult stage and cheliped "completeness" were present in the test animals. These factors will be considered below.

After collection, all animals were fed for 3-6 days. Then pairs were placed in "starved" or "fed" tanks. Individuals were either marked with fingernail polish on the back of their carapace or identified by individual peculiarities in coloration. Each ten gallon aquarium was equipped with an autoclaved sand bottom, undergravel filter and aeration system, and two small clay pots facing in opposite directions (for "burrows"). The tanks were shielded from direct sunlight to decrease algal growth and no observable growth developed in the "starved" tanks.

The animals in the starved tanks were given no food for one to two weeks.

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The fed animals were given Tetramin fish flakes, clumps of algae, and bits of shrimp to excess every day. All behavioral observations were carried out between 2000 and 2200 hours, a period of peak activity as indicated by pilot observations on diel activity. The crayfish were observed on Days 0 (first day of separation into starved and fed tanks), 7, and 14.

Two animals to be tested were taken from their tanks, placed in a ten gallon observation aquarium with a wooden partition between them and allowed to adjust for 10 minutes. The aeration in the observation tanks was turned off during observations and a red light was used for all observations. The sand in the observation tanks had been autoclaved prior to placement in the tanks. Temperature of the room in which the tanks were held was approximately 24° C during the period of the tests.

Three behavioral measures were taken: 1) locomotory rate, 2) aggressive level, and 3) win probability during encounters between starved and fed crayfish. Locomotory rate was measured by recording the number of grid lines crossed, ends of lines marked on outside of aquaria, by animals during a five minute period just prior to their interaction with each other. This period was started five minutes after placement in the observation aquaria.

To measure aggressive level, animals from the same treatment group (but from different aquaria) were paired against each other (i.e. fed vs. fed, starved vs. starved). The number of fights or tension contacts (Bovbjerg, 1956) and the number of seconds spent fighting during a 15 minute period were recorded.

The second measure of aggressiveness was to record the number of fights won (other animals retreated) by each crayfish in interactions between a starved and a fed animal. Only the first interaction was used to determine winners since past experience has such a marked effect on aggressive behavior in *O. virilis* (Rubenstein & Hazlett, 1974).

At the end of behavioral observations, the animals, identified by code numbers, were given to a non-observer for physiological measurements. A total of 18 starved and 18 fed animals were measured. The animals were placed in individual plastic bags and quick-frozen by immersion in a dry ice and methonal mixture. Subsequently the hepatopancreas was removed and the posterior four segments of the abdomen cut off while the specimens were still frozen. The hepatopancreases and tail sections were weighed on a Roller-Smith torsion balance. Subsequently samples of both body parts were assayed for total carbohydrate content by the method of Ashwell (1955) and for total protein content by the method of Lowry et al. (1951). Carbohydrate levels rather than lipid levels were measured, in part because of the apparent differing results with regard to lipid utilization in starving crayfish (Speck & Urich, 1969; Grasynski, 1970).

RESULTS

The locomotory rates of crayfish, grouped by time and treatment are shown in table I. Clearly the patterns of locomotory rates were different for the two

groups, the starved animals being more active than the fed animals on Day 7 and less active on Day 14. The difference in levels of locomotion among starved animals on Days 7 and 14 was significant ($t = 3.5$, $p < 0.005$), but the difference between starved and fed animals was not significant ($t = 1.70$, $p > 0.05$, for Day

TABLE I

Average locomotory rate (number of lines crossed) for two treatment groups on the three days measured

	Day 0	Day 7	Day 14
Starved (n=12)	19.0	20.2	11.6
Fed (n=12)	20.6	15.4	16.1

7 and $t = 1.69$, $p > 0.05$ for Day 14). Also, on day 14, the starved animals were significantly less variable in their locomotion than the fed animals ($F = 3.06$, $p < 0.05$).

The number of fights and seconds spent fighting between starved pairs and fed pairs is shown in table II. The difference between starved and fed crayfish in

TABLE II

Aggression between pairs of crayfish of similar treatment groups, measured in terms of number of fights and total seconds spent fighting

	Day 7	Day 14
Starved: fights	122	65
seconds	1443	1101
Fed: fights	80	81
seconds	1000	1124

total number of fights observed on Day 7 is significant (chi-square = 8.6, $p < 0.01$). However, on Day 14 there was no difference in the number of fights (chi-square = 1.8, $p > 0.05$). The total number of seconds spent fighting followed a similar pattern (chi-square = 80.0, $p < 0.01$ for Day 7, chi-square = 0.2, $p > 0.05$ for Day 14). For both locomotion and fighting, the fed animals were rather consistent, while the starved animals varied.

The numbers of fights won by individuals in mixed treatment pairs are shown in table III. While the starved animals won more fights after one week of treatment, although not significantly so (chi-square = 3.6, $p > 0.05$), there was

TABLE III

Number of fights won (initiated) by animals in various categories

	Day 7		Day 14	
	Starved wins	Fed wins	Starved wins initiated	Fed wins initiated
Starved larger	6 (8)	2 (0)	4 (5)	3 (2)
Fed larger	6 (4)	5 (7)	1 (3)	4 (2)
Equal sized	4 (4)	0 (0)	2 (2)	2 (2)
Totals	16 (16)	7 (7)	7 (10)	9 (6)

definitely no difference after two weeks (chi-square = 0.2, $p > 0.10$). When these data are examined with reference to the size relationships of the two animals, the effects of starvation on aggressiveness are more marked. On Day 7, in 11 out of 19 pairs where there was a size difference, the fed crayfish was larger (yet fed crayfish lost 12 of those 19 fights). On Day 14, the fed crayfish was larger in just 5 out of 12 pairs, yet won 7 of those 12 fights. The number of fights in various categories differ on Days 7 and 14 because of 1) three deaths that occurred, 2) deliberate non-pairing if an animal was about to moult, and 3) some animals were only observed for one week (and then used for physiological measurements).

The results of the physiological measurements are shown in table IV. There were no significant differences in the absolute organ weight means of the two

TABLE IV

Mean values (\pm standard error) of carbohydrate and protein levels in animals starved or fed for two weeks. Eleven specimens for each measure

Measure	Starved	Fed
Abdomen: carbohydrate in $\mu\text{g}/\text{mg}$ tissue	1.53 \pm 0.06	4.53 \pm 0.60
protein in $\mu\text{g}/\text{mg}$ tissue	53.0 \pm 0.30	69.0 \pm 0.30
Hepatopancreas: carbohydrate in $\mu\text{g}/\text{mg}$ of tissue	11.30 \pm 5.50	17.99 \pm 20.30
protein in $\mu\text{g}/\text{mg}$ of tissue	40.0 \pm 0.10	70.0 \pm 0.10

groups. However, the differences between starved and fed animals in relative carbohydrate and protein levels in the tail were very significantly different ($p < 0.01$ by t tests on means) at the end of two weeks treatment. Both protein and carbohydrate levels were lower in the starved animals. The levels in the hepatopancreases were just significantly different ($p \leq 0.05$) in the starved and fed animals, in part due to higher variability in the values for that organ. Interestingly, the variance in carbohydrate values was significantly lower in starved animals ($p < 0.01$ by F statistic) both for the tail and hepatopancreas. In the three animals starved for just one week, and then analyzed, the physiological measurements were almost identical to the values for animals starved for two weeks. This was true for the relative levels of both protein and carbohydrate.

DISCUSSION

The results clearly indicate that starvation for one week results in increases in activity in the crayfish *Orconectes virilis*. The increases in locomotion could explain, in part, the increased rate of within-group aggressive activity since more movement in the same area (bottom of ten-gallon aquarium) means more frequent social contact. A constant aggressive level plus more frequent social contact could result in a higher rate of fighting (Andrews, 1957). However, the results of the mixed pairings plus subjective evaluations of the interactions indicate that aggressive levels were higher after one week of starvation, i.e. the increased number of fights among starved animals was not just a result of alterations in locomotory

rate. The animals starved for one week more frequently walked about in aggressive postures (Bovbjerg, 1956; Heckenlively, 1970; Rubenstein & Hazlett, 1974), and reacted to the presence of another crayfish at a greater distance (larger individual distance). The number of fights which were initiated by the starved animals (table III) also can be taken to indicate a higher aggressive level. Many of the fights observed on Day 7 were very intense, involving many rapid movements, marked physical contact, and long periods of interlocked chelae.

Very similar results in terms of all three behavioral measures were obtained for the hermit crab *Calcinus tibicen* after seven days starvation (Hazlett, 1966). Starved individuals of the spider crab *Microphrys bicornutus* tended to win fights over fed individuals (Hazlett & Estabrook, 1974), although the effects were marginal in comparison to other factors affecting aggression.

The relative decrease in locomotion and aggression in the animals starved for two weeks was a bit surprising, but consistent with the kinds of changes seen in other species after long periods with lowered food input (Hall, 1964; Keys, et al., 1950; Miller, 1957; Southwick, 1967). Since these crayfish had no known food input (not even the usual algae growing in aquaria), the rather quick reversal of activity levels may not be too unexpected. The fact that starved animals initiated ten of the sixteen mixed pair fights observed after two weeks starvation, but won only seven, suggests they lacked the ability to "carry through."

A complicating factor in interpreting these data is the fact that some animals used were in the process of ecdysis. This process alters almost all aspects of a crayfish's biology (Gorell, 1971; McWhinnie & Mohrherr, 1970; Speck & Urich, 1971). It appeared that the two fed animals which moulted between observation days were normal in their behavior, while the four starved animals which moulted were particularly lethargic. The physiological strain of ecdysis would be particularly marked in starved animals. Several starved animals which moulted were among the very lowest in protein and carbohydrate levels. A second complication was that several animals were in the process of regenerating one cheliped, thus one was smaller. In the mixed pairings at the end of one week of treatment, three pairs involved a starved animal with a smaller cheliped vs. a normal fed animal while there was just one case of the opposite condition. However, in the first week's mixed pairs there were six cases where a starved crayfish won despite smaller carapace length and/or cheliped, while just two cases of fed animals winning with such handicaps occurred.

The physiological measures taken indicated very clear differences between the two treatment groups at the end of two weeks. In some of the measures there was almost no overlap in values for individuals from the two groups. Yet in their behavior in terms of aggressive activity and win probability, there was no observable difference in the animals treated (starved or fed) for two weeks. Apparently more exact or different behavioral measures would be needed to measure behavioral expression(s) of the definitive physiological differences.

ZUSAMMENFASSUNG

Der Einfluss von Nahrungsentzug auf das aggressive Verhalten des Krebses *Orconectes virilis* wurde im Labor untersucht. Nach einer Woche Nahrungsentzug kämpften die Versuchstiere öfter und länger und gewannen öfter als Kontrolltiere. Nach zwei Wochen Hungern waren diese Unterschiede nicht mehr zu beobachten.

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