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Effect of starvation and drought on the activity of the freshwater crayfish, *Procambarus clarkii*

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Abstract
This study was designed to investigate the effect of starvation and drought on the adult *Procumbarus clarkii*. Samples were captured from the River Nile, Dakahleyia province, Egypt. Measurements were the animal weight and length and survival rate. The experiment was divided into four groups, control with food and water, starved with water only, drought with food only and starved – drought without any food or water groups. Crayfishes showed great tolerance for 14 weeks ($P < 0.05$). The body weight of the crayfish was more affected than the body and carapace lengths due to the loss of its chelae during starvation. In the starved groups, survival rate of the crayfish declined because its aggressive behavior such as killing each others (66.6%). Crayfishes could not tolerate drought for a long time (5 days). The body weight, length, and the carapace length were minimally influenced during drought, while the survival rate was dramatically affected within few days. The combined effect of both stressors affected the animal survival more drastically within four days (100, 50 and 0%). It can be concluded that starvation and drought caused serious changes in *P. clarkii* behavior, biometry and survival rate, which determine the ability of crayfish to adapt to one or more of the environmental changes.

Keyword: *Crayfish, Drought, Starvation, Survival rate*

1 Introduction

P. clarkii had been introduced into many countries all over the world due to its highly tolerance to the unfavorable conditions such as poor water quality, temperature fluctuations, low oxygen concentrations and desiccation beside its extraordinary production rate in farming (Huner and Lindqvist, 1995). *P. clarkii* range in size from 8 cm to 13 cm in length, hard outer skeleton or carapace, which protected the body and made it rigid, the abdomen had wedge-shaped stripe. Elendt (1989) found that, *Daphnia magna* could survive under starvation condition dependent on the amount of energy reserved and their subsequent allocation to maintenance, growth, and/or reproduction. Dickson and Giesy (1982) studied the effect of starvation on *P. clarkii*. They indicated that after 30 days of starvation, the dorsal muscle of the tail of starved group contain more ATP and adenylate than the control group. They explained that increasing the condition of starvation associated with increased motor activity that may cause greater phosphoadenylate concentration. In addition, during starvation period, crayfish metabolize lipids, proteins, and carbohydrates from various organs in the body to meet their energy demands. The bathypelagic mysid *Gnathophausia ingens* can survive 19 weeks of starvation (Hiller-Adams and Childress, 1983).

Dall (1974) reported that the rock lobsters, *Panulirus longipes* survived more than 3 months of starvation in the laboratory. During dry season, crayfish hide in burrows, become inactive and its feeding and growth were greatly reduced (Rhoads, 1976). Hydrological conditions caused crayfish avoid disperse long distances to avoid long period of drought by hiding in burrow in local substratum during the dry downs (Kushlan and Kushlan, 1979). Extreme drought can cause mortality effects on animals like fishes due to decrease oxygen concentrations, high temperatures and other factors (Brochet, 1977 and Brooker et al., 1977). Therefore, the purpose of the present work was to study the effect of starvation, drought alone or in combination on the biometry and activity of the freshwater crayfish, *P. clarkii*

2. Materials and Methods

2.1. Animals collection

Samples of *P. clarkii* was used in this study were captured from the River Nile at EL-Mashaia region, EL Mansoura, Dakahleyia province, Egypt. Samples were collected from the middle of the River Nile by nets in an area of about 4 km in length and ranged from 150 to 200 m in width. Adult males and females of nearly the same size, complete organs and highly active were chosen. Animals were transported to the laboratory and were put in suitable glass aquaria with 50 cm height, 40 com width and 60 cm length and covered with wire cover.

Animals were fed regularly on different kinds of agricultural plants as fresh leaves of lettuce, potato tubers, corn and beans. The physiochemical parameters of the water were measured. Temperature ranged from 25-30 °C; dissolved oxygen from 4-7 mg/l; consumed oxygen from 2.4-5.5 mg/l; pH from 7.5-7.8 and ammonia from 0.06-0.3 mg/l. 100 % water change was done every other day for the corresponding aquaria with semi static method.

2.2. Experimental design

The total number of the animals was 72, with nearly the same size and weight. The experiment divided into four groups, three replicates and 18 animals/group. Each replicate contains six animals (3 males and 3 females). The first group, were considered as control group and supplied with mud (10-20 cm depth), water (10-20 cm depth) and food. The second group, were considered as starved animals supplied with mud (10-20 cm depth) and water (10-20 cm depth) only but without food. The third group, were considered as drought animals and supplied with mud (10-20 cm depth) and food but without water. The fourth group, were considered as starved - drought animals and supplied with dried mud (10-20 cm depth) only and without food or water.

Body weight, length, carapace length and survival rate in the control and experimental groups were measured at the end of every week until 14 weeks.

2.3. Statistical analysis

Data were presented as Mean ± Standard Deviation and analyzed using Minitab software package (version 16 for Windows) for simple regression analysis where Y is the variable, X is the time and R^2 is the coefficient of determination. Probability values when $P \leq 0.05$ were defined as significant.

3. Results

3.1. Effect of starvation on biometry and survival rate of adult *P. clarkii*

a- Effect of starvation on biometry

From the experimental observations, the animals ate the mud, their excreta, attacked each other, and ate the weak animals between them during the period of starvation. The mean body weight of the control group increased from 38.17 ± 0.52 g on the 1st week to the 42.6 ± 0.33 g on 14th week. While mean of the body weight of starved group decreased from 41.1 ± 0.71 g on1st week to 41.2 ± 0.96 g on 14th week (Table 1). There was a significant relationship ($P < 0.05$) between the body weight of *P. clarkii* and time in the control where **Y** = 1764.37 + 0.0446 X and $\mathbf{R^2}$= 95.43%, and of the starved group where **Y** = -3966.96 + 0.099 X and $\mathbf{R^2}$ = 82.60%.

The body length of control group increased from 10.43 ± 0.08 cm on the 1st week and the maximum to 10.78 ± 0.03 cm on 14th week. While the body length of starved group decreased from 10.68 ± 0.13 cm on 1st week to 10.66 ± 0.08 cm on 14th week (Table 1). There was a significant relationship ($P < 0.05$) between the body length of *P. clarkii* and time of the control group where **Y** = 117.833 + 0.00317 X and $\mathbf{R^2}$= 91.44%, and of the starved group where **Y** = -279.855 + 0.00718 X and $\mathbf{R^2}$ = 80.48%.

The carapace length of the control group increased from 5.37 ± 0.27 cm on 1st week to 5.58 ± 0.02 cm on 14th week. While the carapace length of starved group decreased from 5.42 ± 0.28 cm on 1st week to 5.36 ± 0.03 cm on 14th week (Table 1). There was a significant relationship ($P < 0.05$) between carapace length and time of *P. clarkii* and time of the control group where **Y** = 81.1833 + 0.00214X and $\mathbf{R^2}$= 82.99%, and of the starved group where **Y** = -123.54 + 0.00318937 X and $\mathbf{R^2}$ = 67.30%.

b- Effect of starvation on survival rate

In the control group, the survival percentage of *P clarkii* was 100% at 1st week and reached the minimum percentage 66.6% at 14th week. In the starved group, the survival percentage was 100% at 1st week and minimum percentage was 0% at 14th week (Table 1). The relationship of survival rate and time was statistically significant ($P < 0.001$) for the control and starved group where **Y** = 131.899 - 0.00323 X and $\mathbf{R^2}$ = 80.3%., and **Y** = 396.473 - 0.0097 X and $\mathbf{R^2}$ = 97.5% for control and starved groups, respectively.

3.2. Effect of drought on biometry and survival rate of adult *P. clarkii*

a- Effect of drought on biometry

From the experimental observations, the animals stopped feeding and burrowing in the mud during the period of drought. The body weight of the control group increased from 38.14 ± 0.7 g on the 1st day to the 38.3 ± 0.4 g on 5th day. While the body weight of drought group decreased from 32.27 ± 0.29 g on 1st day to 17.7 ± 0.17 g on 5th day (Table 2). There was no statistically significant relationship ($P > 0.05$) between the body weight of *P. clarkii* and time of the control group, where **Y** = -322.257 + 0.00891 X and $\mathbf{R^2}$= 63.51%, and the drought group where **Y** = 82620.3 - 2.04378 X and $\mathbf{R^2}$ = 90.50%.

The body length of the control group increased from 10.43 ± 0.46 cm on 1st day to 10.47 ± 0.09 cm on 5th day. While the body length of the drought group decreased from 10.02 ± 0.13 cm on 1st day to 9.8 ± 0.03 cm on 5th day (Table 2). There was no statistically significant relationship ($P > 0.05$) between the body length of *P. clarkii* and time of the control group where **Y** = -173.597 + 0.00455 X and $\mathbf{R^2}$= 35.14%, and of the drought group where **Y** = 1140.45 - 0.027 X and $\mathbf{R^2}$ = 0.00%.

The carapace length of the control animals ranged from 5.37 ± 0.05 cm on 1st day to 5.5 ± 0.02 cm on 5th day when compared to the drought group (Table 2). The carapace length of the drought animals did not affect so much where it ranged from 4.95 ± 0.12 cm on 1st day to 4.60 ± 0.11 cm on 5th day. The relationship between the carapace length of *P. clarkii* and the time (days) was not statistically significant ($P > 0.05$) where **Y** = -11.0035 + 0.00040X and 1916.24 - 0.0472 X and $\mathbf{R^2}$= 0% and 35.14% for the control and drought groups, respectively.

Table 1. Body length and weight, carapace length and survival rate of control and starved groups

Investigation period	body length (cm) of control group	body length (cm) of starved group	carapace length (cm) control group	carapace length (cm) starved group	body weight (g) control group	body weight (g) starved group	Survival rate of control group (%)	Survival rate of starved group (%)
1st week	10.43 ± 0.08	10.68 ± 0.13	5.37 ± 0.27	5.42 ± 0.28	38.17 ± 0.52	41.1 ± 0.71	100	100
2nd week	10.47 ± 0.18	10.68 ± 0.48	5.37 ± 0.1	5.38 ± 0.03	38.28 ± 0.25	39.98 ± 0.92	100	100
3rd week	10.5 ± 0.33	10.70 ± 0.06	5.34 ± 0.23	5.38 ± 0.03	38.55 ± 0.34	40.02 ± 0.76	100	83.3
4th week	10.55 ± 0.43	10.70 ± 0.23	5.37 ± 0.45	5.38 ± 0.03	39.07 ± 0.56	41.04 ± 0.93	83.3	83.3
5th week	10.68 ± 0.63	10.71 ± 0.53	5.48 ± 0.84	5.39 ± 0.03	39.64 ± 0.12	40.86 ± 0.36	83.3	83.3
6th week	10.64 ± 0.45	10.69 ± 0.09	5.48 ± 0.28	5.38 ± 0.03	39.77 ± 0.33	40.48 ± 0.35	83.3	47.9
7th week	10.64 ± 0.02	10.68 ± 0.86	5.48 ± 0.29	5.37 ± 0.03	39.86 ± 0.76	41.95 ± 0.94	83.3	66.6
8th week	10.66 ± 0.04	10.68 ± 0.38	5.48 ± 0.39	5.38 ± 0.03	40.12 ± 0.66	41.95 ± 0.35	83.3	50
9th week	10.68 ± 0.12	10.63 ± 0.88	5.5 ± 0.75	5.37 ± 0.03	41.2 ± 0.34	41.0 ± 0.37	83.3	50
10 week	10.68 ± 0.03	10.63 ± 0.09	5.5 ± 0.02	5.37 ± 0.03	41.2 ± 0.35	41.0 ± 0.45	83.3	50
11th week	10.68 ± 0.37	10.63 ± 0.08	5.5 ± 0.19	5.37 ± 0.03	41.26 ± 0.56	40.99 ± 0.96	83.3	33.3
12th week	10.68 ± 0.09	10.64 ± 0.11	5.5 ± 0.08	5.36 ± 0.03	41.06 ± 0.46	41.25 ± 0.66	66.6	16.6
13th week	10.78 ± 0.07	10.66 ± 0.09	5.58 ± 0.04	5.36 ± 0.03	42.6 ± 0.35	41.3 ± 0.95	66.6	16.6
14th week	10.78 ± 0.03	10.66 ± 0.08	5.58 ± 0.02	5.36 ± 0.03	42.6 ± 0.33	41.2 ± 0.96	66.6	0

Note, *n* = 6

Table 2. Body length, weight, carapace length and survival rate of control and drought groups

Investigation period	Body length (cm) of control group	Body length (cm) of drought group	Carapace length (cm) of control group	Carapace length (cm) of drought group	Body weight (g) of control group	Body weight (g) of drought group	Survival rate of control group (%)	Survival rate of Drought group (%)
1st day	10.43 ± 0.46	10.02 ± 0.13	5.37 ± 0.05	4.95 ± 0.12	38.14 ± 0.7	32.27 ± 0.29	100	100
3rd day	10.43 ± 0.14	10.01 ± 0.12	5.38 ± 0.09	4.90 ± 0.15	38.2 ± 0.2	26.73 ± 0.28	100	16.6
5th day	10.47 ± 0.09	9.8 ± 0.03	5.5 ± 0.02	4.60 ± 0.11	38.3 ± 0.4	17.7 ± 0.17	100	0

Note, *n* = 6

b-Effect of drought on survival rate

Drought did not change the survival percentage in the control group, while the survival rate of drought animals decreased from 100% on 1^{st} day to 0% on 5^{th} day (Table 2). The relationship between survival rate of *P. clarkii* and experimental time was not significant ($P > 0.05$) **Y** = 1.54578 - 1.3513 X and 5917.02 - 0.1464X and $\mathbf{R^2}$ = 0% and 84.10% for the control and drought groups, respectively.

3.3. Effect of drought - starvation on biometry and survival rate of adult *P. clarkii*

a- Effect of drought - starvation on biometry

The body weight of the control group recorded 38.14 ± 0.5 g on 1^{st} day and 38.2 ± 0.2 g on 4^{th} day. On the other hand, drought and starved group recorded 40.05 ± 0.09 g on 1^{st} day and 30.13 ± 0.23 g on 4^{th} day (Table 3). The relationship of the body weight and time was not statistically significant ($P > 0.05$). Where, **Y** = -322.257 + 0.00891X and 58539.6 - 1.4477 X and $\mathbf{R^2}$ = 63.51% and 87.70% for the control and drought- starved animals, respectively.

The body length of the control animals did not change and the mean was 10.43 ± 0.12 cm on 1^{st} day and 10.47 ± 0.11 cm on 4^{th} day of the experiment. However, in the drought - starved animals, the body length decreased slightly from 10.63 ± 0.15 cm on 1^{st} day to 10.20 ± 0.08 cm on 4^{th} day (Table 3). The relationship was statistically insignificant ($P > 0.05$) between the body length and experimental time of the control and drought -starved groups. The control group was **Y** = -173.597 + 0.00455 X and $\mathbf{R^2}$ = 35.14%, and the drought - starved group was **Y** = -2059 + 0.0512 X and $\mathbf{R^2}$ =73.78%.

The carapace length did not affect in the control and drought - starved group with values 5.37 ± 0.06 and 5.36 ± 0.05 cm on 4^{th} day, respectively (Table 3). The relationship between the carapace length of *P. clarkii* and the experimental time was statistically insignificant ($P > 0.05$). where **Y** = -11.0035 + 0.000405 X and -868.264 + 0.0216 X and $\mathbf{R^2}$ = 0% and 35.14% for the control and drought - starved groups, respectively.

b- Effect of drought - starvation on survival rate

The drought and starvation affected and changed the survival rate. The maximum survival rate was 100% animals at 1^{st} day and the minimum percentage of survival rate at 4^{th} day was 0%. Survival rate of the control group did not change during the investigation period and the percentage of survival rate was 100% (Table 3). The relationship between the survival rate of *P. clarkii* and the experimental time was statistically insignificant was ($P > 0.05$) for the control group (**Y** = 1.54578 - 1.351 X and $\mathbf{R^2}$= 0%). However, for the drought - starved group, the relation was statistically significant ($P \leq 0.05$, **Y** = 5734.73 - 0.1418 X and $\mathbf{R^2}$ = 98.65%).

Table 3. Body length, weight, carapace length and survival rate of control and drought - starved groups

Investigation period	Body length (cm) of control group	Body length (cm) of drought – starved group	Carapace length (cm) of control group	Carapace length (cm) of drought – starved group	Body weight (g) of control group	Body weight (g) of drought – starved group	Survival rate of control group (%)	Survival rate of drought – starved group (%)
1^{st} day	10.43 ± 0.12	10.63 ± 0.15	5.37 ± 0.07	5.47 ± 0.08	38.14 ± 0.5	40.05 ± 0.09	100	100
3^{rd} day	10.43 ± 0.09	10.72 ± 0.11	5.37 ± 0.05	5.47 ± 0.1	38.2 ± 0.3	32.12 ± 0.13	100	50
4^{th} day	10.47 ± 0.11	10.20 ± 0.08	5.37 ± 0.06	5.36 ± 0.05	38.2 ± 0.2	30.13 ± 0.23	100	0

Note, $n = 6$

4. Discussion

In the present work, the 14 weeks of starvation periods decreased the body weight, total body length and carapace length of *P. clarkii* by decreasing it. *P. clarkii* tolerated for a long time without food and the relationship between survival rate and time of this study was statistically significant ($P < 0.05$). When environmental conditions were unfavorable for normal activity, the ability to regulate the mobilization of energy reserves could potentially maximize the survival time of an individual (Storey and Storey, 1990). The effects of starvation on crustacean metabolism were well documented and involve the major physiological systems of the body. *P .clarkii* could remain in the burrows for 4–5 months until water levels rise either through natural rains or artificial flooding in ponds. If there is little or no food available in the burrow, it was hypothesized that the crayfish feed minimally during this time (Jaspers and Avault, 1969). Morsy (2001) proved that, starvation affected *P. clarkii* by decreasing in total body weight to 0.5 ± 0.28 g. In addition, decrease in total body length reached to 3 cm and a slightly decrease in carapace and abdominal length and survival rate to 9% after more than seven weeks of starvation. Dickson and Giesy (1982) proved that Physiologic responses to starvation while in the burrow might be a key factor in determining individual species success. During starvation, crayfish had been reported to metabolize lipids, proteins, and carbohydrates from various organs in the body to meet these energy demands and that can cause depletion of these important biological molecules (Hazlett et al., 1975 and Speck and Urich, 1969). The main nutrient storage sites in crustaceans are hepatopancreas and abdominal muscle that utilized during starvation (Schirf et al., 1987 and Jones and Obst, 2000). Ansell (1973) and Hazlett et al. (1975) indicated that starvation at early stages caused changes in motor activity patterns. Starvation can cause decrease in the whole animal and individual tissue metabolic rates for most species (Marsden et al., 1973 and Newell, 1973). Powell (2000) indicated that all seven of the enzymes measured in both the tail muscle and hepatopancreas of *P. clarkii* had decreased in total activity by 50% or more compared to fed controls and the loss in enzyme activity was directly related to loss of tissue mass. In the current study, the change in the weight of fed and starved *P. clarkii* could be attributed to the metabolism of stored energy yielding constituents.

The presented study indicated that, drought had side effects on *P. clarkii* life and caused high or complete mortality. Drought affected the river water quality by increasing total dissolved solids and their constituent ions, elevated ammonia and biochemical oxygen demand, and decreased dissolved oxygen and suspended sediments (Anderson and McCall, 1968; Anderson et al. 1972; Stefan and Combs, 1978; Muchmore and Dziegielewski, 1983). Responses to drought were different from species to another. In fish drought had lethal effect due to low oxygen concentrations, high temperatures and other factors Brochet, 1977; Brooker et al., 1977). On the other hand, the response for invertebrates might be diverse, which cause declines and stability of populations of various species (Moth Iversen et al., 1978; Taylor, 1983; Canton, 1984; Cowx et al., 1984; Kownacki, 1985). *P. clarkii* was able to tolerate dry periods of up to four months (Henttonen and Huner, 1999). During dry season, crayfishes were inactive in burrows by reducing feeding and growth rates (Rhoads, 1976) and affected its ability to reach the hyporheic zone (Dyer et al., 2014).

In conclusion, drought influenced on *P. clarkii* behavior, biometry and survival rate more than starvation. Both stressors alone or in combination may affect population diversity of this animal in a given ecosystem.

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