

Annual and Proecdysial Variations in Urine Production in Crayfish¹

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THE CRUSTACEAN X organ-sinus gland complex has been extensively studied and shown to regulate such diverse activities as molting, integumentary and retinal pigment movements, blood glucose levels, ovarian development, and many others. The elucidation of the controlling mechanisms of these activities is complicated by the interdependent relationships among them. Further complications arise from the fact that in crustaceans, as in most organisms, seasonal and life cycles may be superimposed upon daily cycles and changes.

The neuroendocrine control of water metabolism has been studied previously in relation to proecdysial uptake of water. Scudamore (1947) observed that increased water content in the tissues was responsible for the abnormal weight increases in crayfish induced to molt by eyestalk extirpation. Sinus gland implants partially prevented this increased water content, suggesting that factors from the X organ-sinus gland complex are responsible for maintaining proecdysial water metabolism in animals with intact eyestalks.

Rapid responses to osmotic stress are necessary for homeostatic salt and water balance. Riegel (1960) reported that the handling of crayfish resulted in a spontaneous hyperglycemia, prolonged cessation or marked decrease in urine production, and sometimes glycosuria. Such an effect on urine production may suggest neural and/or hormonal control. Further evidence for a fast-acting principle for salt and water balance in the crayfish was suggested by Kamemoto et al. (1966).

Our previous studies on the crayfish *Procambarus clarkii* (Kamemoto et al., 1966; Kamemoto and Ono, 1967, 1968b) have suggested that neuroendocrine factors from the brain and

eyestalks are involved in the regulation of salt and water metabolism. In these studies it was demonstrated that the removal of the eyestalks resulted in increased flow of urine in animals held in tap water. In the course of studies on urine flow rates, it was observed that there was a seasonal variation in the production of urine. Based on these observations, studies were undertaken to determine the annual variations in urine flow and the relationship of urine production and the proecdysial stages in these crayfish. The results of these studies are reported here.

MATERIALS AND METHODS

Procambarus clarkii were collected from fresh waters in leeward Oahu, Hawaii, and were held in running tap water. Animals were collected throughout the year for the determination of variations in urine flow. Specimens used ranged from 13.1 to 43.3 grams in weight. Because of the nature of the apparatus used in the collection of urine, the chelae were removed at least one day before urine was collected. The crayfish weighed approximately 30 percent less after the removal of the large chelae, the wet weight being estimated after blotting the animal with cheesecloth. All experiments were conducted at room temperatures of 21° to 22° C.

Urine flow was determined as described previously (Kamemoto and Ono, 1968a). The apparatus was placed in a container of constantly aerated water and urine was collected over a 24-hour period. The volume of urine produced was expressed as percent of body weight (without chelae) per 24 hours.

Eyestalk removal was effected by bilaterally ligating the eyestalks at the bases. The animals were then returned to tap water, and urine flow was determined one day later for a 24-hour period. These collections were made throughout the year and compared with the urine flow of normal intact animals.

To determine the effects of proecdysial stages

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on urine flow, eyestalks were removed from animals to induce molting. Urine was collected over 24-hour periods on succeeding days. These animals were fed on liver on alternate days. Molting stages were determined by the method of McWhinnie (1962) after sacrificing the animal and removing the gastroliths. Gastrolith diameter at the longest axis was measured to the nearest millimeter. The carapace length was measured from the tip of the rostrum to the posterior end of the carapace. The gastrolith (G) to carapace (C) ratio was taken to indicate the proecdysial stage of the animal.

In our studies on urine collection in normal animals, only two animals with any degree of

gastrolith formation was obtained. This was due to the difficulty in determining the early proecdysial stages without sacrificing the animal. The use of brittle, obviously late proecdysial animals and soft, postecdysial animals was avoided because of the nature of the apparatus, requiring the firm clamping of the animal around the anterior cephalothorax.

RESULTS

Urine production in normal and eyestalk ligated animals throughout the year is presented in Figure 1. Individual and mean values over three-month periods are given. As can be seen,

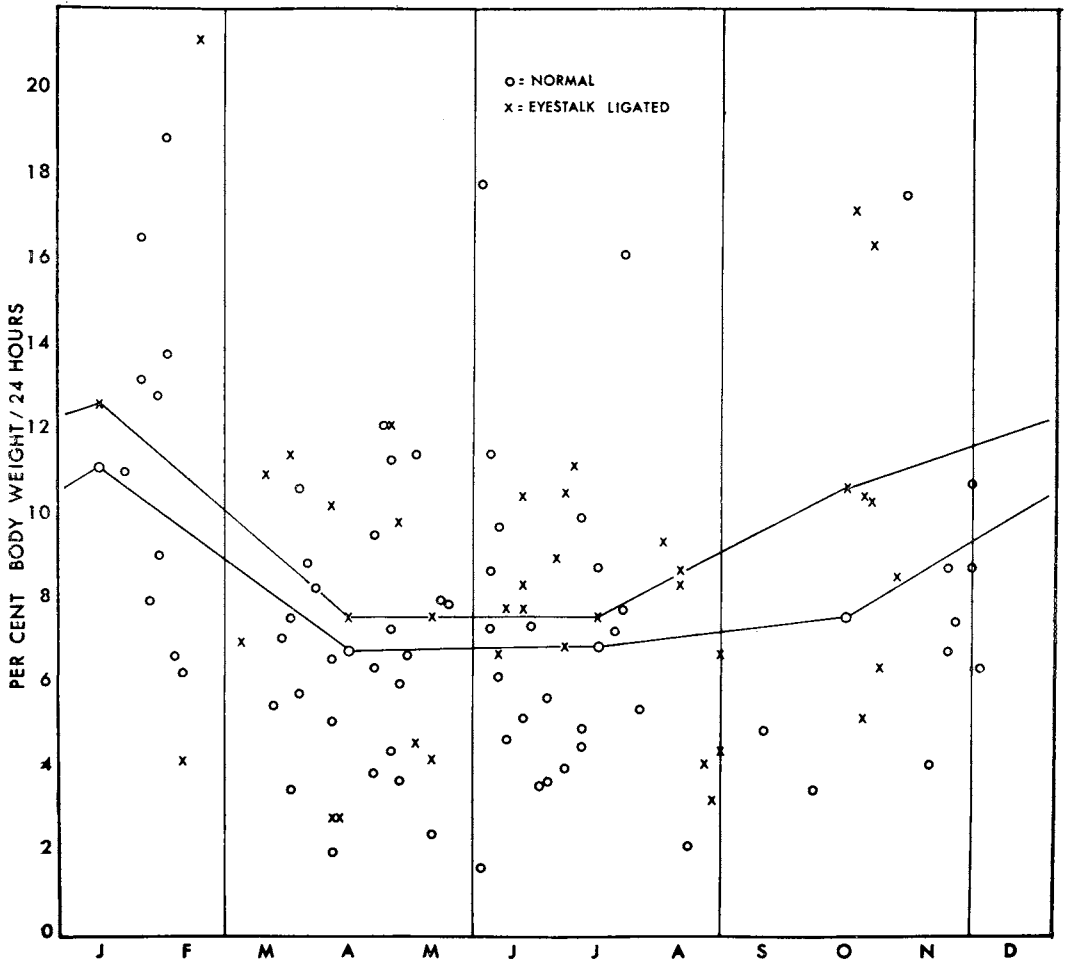


FIG. 1. Urine production in normal and eyestalk-ligated *Procambarus clarkii* throughout the annual cycle. Individual values and means values over 3-month periods are presented.

TABLE 1
URINE PRODUCTION AND G/C RATIOS IN *Procambarus clarkii* ON SUBSEQUENT DAYS AFTER
EYESTALK LIGATION (MARCH, 1968)

DAYS AFTER LIGATION	NO. OF ANIMALS	URINE VOLUME (% body wt/24 hr)			RANGE OF G/C
		Mean	S.D.	Range	
1	6	7.6	4.0	2.8-11.5	0-0.09
2	4	6.4	3.6	2.4-10.6	0-0.08
3	3	8.0	5.6	2.1-13.2	0-0.10
4	2	7.0	3.4	4.6-9.4	0.07-0.09
5	2	5.8	1.6	4.4-7.1	0
6	2	12.0	1.6	10.9-13.2	0.09-0.10
8	4	6.2	5.7	0.1-12.5	0-0.16
9	1	8.0	—	—	0.16
10	2	6.7	1.7	5.5-7.9	0-0.16
11	2	5.2	3.3	2.9-7.6	0.11-0.16
12	1	8.3	—	—	0

there was considerable variation in urine production of both normal and eyestalk ligated animals during any time of the year. However, the lowest urine volumes were obtained during the late spring and summer months and the highest values were obtained during the winter months. Throughout the year, eyestalk ligated animals produced more urine than did normal, intact animals.

In an effort to determine the relationship of

TABLE 2

URINE PRODUCTION IN EYESTALKLESS *Procambarus clarkii* (MOLTING STAGES DENOTED BY G/C RATIOS)

G/C RATIO	URINE VOLUME
	(% body weight/day)
0.08	10.6
	8.4
	8.7
0.09	13.2
	9.4
	10.9
0.10	8.8
	2.2
0.11	2.9
	1.8
	16.3
0.13	2.4
0.14	3.8
0.16	0.1
	7.6

urine volume to molting stage, a series of experiments was initiated in March. Crayfish were induced to enter proecdysial stages by eyestalk ligation, and urine was collected in succeeding days after ligation. The results are presented in Table 1. The range of G/C ratios are given also for the various groups of animals. There is no apparent correlation between urine flow and the number of days elapsed after eyestalk ligation. Except for the high values obtained for crayfish six days after eyestalk ligation, urine production, despite much variation, did not vary significantly with the number of days after ligation.

As expected, gastroliths were found in greater frequency in groups of eyestalkless animals studied on progressively later days after eyestalk ligation. However, the average G/C ratios did not increase steadily on successive days after eyestalk ligation. One animal had been without eyestalks for 12 days before urine was collected and no gastroliths could be discerned.

Table 2 relates urine volume with the G/C ratios. There was a marked decrease in urine production (except for the 16.3 percent obtained from one animal with a G/C ratio of 0.11) when the G/C ratio approached and exceeded 0.10.

DISCUSSION

The annual molting cycle of crayfish in temperate climates consists of a spring molt, a short summer intermolt, a summer molt, and a long

winter intermolt (Scudamore, 1947; Prosser and Brown, 1961). Information on molt cycles of crayfish in Hawaii is not available. Casual observations, however, suggest that although molting may occur throughout the year, a major molt season occurs in April and May. The urine volumes obtained during this molting season were relatively low whereas the greatest urine volumes were obtained during the late winter (intermolt) period. It must be pointed out, however, that urine was collected from only two animals with gastroliths during the course of this study. Therefore, it can not be stated with certainty whether the annual variation in urine flow is or is not related to the molt cycles. The annual cycle pattern occurs without manifestations of gastrolith formation and may be distinct from the molt cycle.

There is little doubt that diuresis occurs in these animals during the winter. Riegel (1968) has stated that winter and spring crayfish have swollen antennal glands, that the labyrinths are vivid green as contrasted with yellow labyrinths during the summer, and that these winter animals are diuretic. He has also suggested qualitative and quantitative differences in the urine collected from various parts of the antennal gland during the winter and summer months.

The statistically significant differences between urine production obtained from normal and eyestalkless crayfish as reported earlier (Kamemoto and Ono, 1967, 1968*b*) occurred during the summer months. Such changes in urine volumes in normal and eyestalkless decapod crustaceans have been reported also for *Eriocheir sinensis* by de Leersnyder (1967) and for *Metopograpsus messor* by Kato and Kamemoto (1968). Mean urine volumes of eyestalkless crayfish, although not statistically significant, appear to be greater than those of normal animals throughout the annual cycle. During these periods, the annual cycle of urine production may buffer the effect of eyestalk removal.

Urine flow in eyestalkless animals drops drastically as the G/C ratio increases to and exceeds 0.10. Urine flow is lowest as the animal approaches ecdysis, while high urine flow was obtained from animals in early and mid premolt stages. McWhinnie (1962) reported that in the crayfish *Orconectes virilis*, G/C ratios of 0.18 to

0.22 were obtained at ecdysis, and G/C ratios of less than 0.05 correspond to early premolt or stages D₀-D₁. Stevenson et al. (1968) designate the G/C ratios in *Orconectes sanborni* as D₁: 0.10 to 0.13; D₂: 0.13 to 0.16; D₃: 0.17 to 0.19. Based on these ratios, it appears that the reduction in urine flow occurs first in the mid premolt stages.

Scudamore (1947) showed that the increase in weight in *Cambarus immunis* and *C. virilis* after eyestalk ablation, averaging 3.84 percent after seven days, was due to increases in tissue water content. Such increases in water content with the approach of ecdysis were also demonstrated in other crustaceans (Baumberger and Olmstead, 1928; Guyselmann, 1953) and could be promoted by the increased water intake (Scudamore, 1947; Kamemoto et al., 1966; Mantel, 1967; Thompson, 1967; Kato and Kamemoto, 1967, 1968; Kamemoto and Ono, 1968*b*) and the decrease in urine outflow in mid and late proecdysis as demonstrated in the present study on *Procambarus clarkii*.

Kamemoto et al. (1966) and Kamemoto and Ono (1968*b*) suggested that eyestalk removal led to increased permeability of the body surfaces which resulted in the greater influx of water in crayfish. Increased permeability to tritiated water was also shown by Thompson (1967) in eyestalkless freshwater crab *Pseudohelphusa jouyi*. Similar increases in permeability occur in crustaceans approaching ecdysis. Mantel (1967) showed that the permeability of the foregut of *Gecarcinus lateralis* increases as ecdysis is approached. Whether there are seasonal changes in permeability or water movement independent of the molting cycle has not been investigated.

Recent studies on permeability using tritiated water suggest that the water uptake may be mainly due to drinking rather than through the external body surfaces. In permeability studies on crayfish, Rudy (1967) found that the expected volume of urine was only 2.6 percent body weight/day. The difference between this figure and direct measurements of urine flow may be attributed to drinking. Scudamore (1947) observed that crayfish ate voraciously after eyestalk extirpation and our observations confirm this. These findings suggest that the

seasonal, and perhaps the molting stage, variations in urine production may be due to differences in feeding and drinking habits.

The annual variations in urine production may be the result of increased or decreased permeability of the body surfaces or gut and may be influenced by brain-eyestalk neuroendocrine system factors as suggested previously (Kamemoto and Ono, 1968*b*). The decreased urine flow during the latter part of proecdysial stages may be due to the inability of the antennal glands to eliminate the excessive amounts of water entering the animal. However, Riegel's experiments (1961) on water loading of *Orconectes virilis* demonstrate that the crayfish kidney is able to eliminate large quantities of injected water. Also, the urine flow in late proecdysial animals is reduced to about 25 percent of that by early proecdysial animals. It seems plausible that the decreased urine flow before molt is under direct regulation of some control mechanism. However, no evidence of hormonal control of the antennal gland function is currently available.

Our studies reported here clearly demonstrate the influence of the annual and molt cycles on the production of urine in the crayfish, and further emphasize the importance of the recognition of annual, daily, and other physiological cycles in experimental animal biology.

SUMMARY

1. Annual and molt cycle variations in the production of urine by the crayfish *Procambarus clarkii* was studied.

2. There is considerable variation in urine production in normal and eyestalk ligated animals during the year. Urine production is greatest during the winter and lowest during late spring and summer.

3. There appears to be no correlation between the number of days following eyestalk ligation and urine production. However, there is a decrease in urine production by eyestalkless animals in the mid and late proecdysial stages.

4. It is suggested that the increase in weight and water content of the crayfish as it approaches ecdysis may be due to both increased uptake of water and decrease in urine formation, which

may be regulated through the brain-eyestalk neuroendocrine system.

LITERATURE CITED

- BAUMBERGER, J. P., and J. M. D. OLMSTEAD. 1928. Changes in the osmotic pressure and water content of crabs during the molt cycle. *Physiological Zoology*, vol. 1, pp. 531-544.
- DE LEERSNYDER, M. 1967. Le milieu intérieur d'*Eriocheir sinensis* H. Milne-Edwards et ses variations. II. Étude expérimentale. *Cahiers de Biologie Marine*, vol. 8, pp. 295-321.
- GUYSELMAN, F. B. 1953. An analysis of the molting process in the fiddler crab, *Uca pugnator*. *Biological Bulletin*, vol. 104, pp. 115-137.
- KAMEMOTO, F. I., K. N. KATO, and L. E. TUCKER. 1966. Neurosecretion and salt and water balance in the Annelida and Crustacea. *American Zoologist*, vol. 6, pp. 213-219.
- KAMEMOTO, F. I., and J. K. ONO. 1967. The effects of eyestalk ligation on salt and water balance in the crayfish, *Procambarus clarkii*. *American Zoologist*, vol. 7, p. 723.
- . 1968*a*. Urine flow determinations by continuous collection in the crayfish *Procambarus clarkii*. *Comparative Biochemistry and Physiology*, vol. 27, no. 3, pp. 851-857.
- . 1968*b*. Neuroendocrine regulation of salt and water balance in the crayfish *Procambarus clarkii*. *Comparative Biochemistry and Physiology*, vol. 29, no. 1, pp. 393-402.
- KATO, K. N., and F. I. KAMEMOTO. 1967. Implications of neuroendocrine regulation of the osmotic concentration in the grapsid crab *Metopograpsus messor*. *American Zoologist*, vol. 7, pp. 722-723.
- KATO, K. N., and F. I. KAMEMOTO. 1968. Neuroendocrine involvement in osmoregulation in the grapsid crab *Metopograpsus messor*. *Comparative Biochemistry and Physiology*, vol. 28, no. 2, pp. 665-674.
- MANTELL, L. H. 1967. The foregut of *Gecarcinus lateralis* as an organ of water balance. *American Zoologist*, vol. 7, p. 765.
- MCWHINNIE, M. A. 1962. Gastrolith growth and calcium shifts in the freshwater crayfish, *Orconectes virilis*. *Comparative Biochemistry and Physiology*, vol. 7, pp. 1-14.

- PROSSER, C. L., and F. A. BROWN, JR. 1961. Comparative animal physiology. 2nd ed. Philadelphia, W. B. Saunders Co. 688 pp.
- RIEGEL, J. A. 1960. Blood glucose in crayfishes in relation to moult and handling. *Nature*, vol. 186, p. 727.
- 1961. The influence of water-loading and low temperature on certain functional aspects of the crayfish antennal gland. *Journal of Experimental Biology*, vol. 38, pp. 291–299.
- 1968. Analysis of the distribution of sodium, potassium and osmotic pressure in the urine of crayfishes. *Journal of Experimental Biology*, vol. 48, pp. 587–596.
- RUDY, P. P. JR. 1967. Water permeability in selected decapod Crustacea. *Comparative Biochemistry and Physiology*, vol. 22, pp. 581–589.
- SCUDAMORE, H. H. 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiological Zoology*, vol. 20, pp. 187–208.
- STEVENSON, J. R., R. H. GUCKERT, and J. D. COHEN. 1968. Lack of correlation of some proecdysial growth and developmental processes in the crayfish. *Biological Bulletin*, vol. 134, pp. 160–175.
- THOMPSON, L. C. 1967. Osmotic regulation in the Mexican freshwater crab, *Pseudothelphusa jouyi* Rathbun. *American Zoologist*, vol. 7, p. 736.