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Elimination of Diurnal Rhythm of Respiration by Methyl Parathion in the Crab, *Oziotelphusa senex senex* Fabricius

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Rhythmicity is a fundamental property of organisms. Diurnal rhythms in physiological activities like color change (Brown et al. 1953), oxygen consumption (Ramamurthi and Sainath Janak 1973), blood sugar level (Reddy et al. 1986), feeding and locomotor activity (Surendra Reddy 1978) were reported in several crustaceans. Circadian rhythms in Oziotelphusa senex senex have been studied earlier with reference to blood sugar level (Reddy et al. 1986) oxidative metabolism (Reddy 1991) and acetyl cholinesterase activity (Reddy et al. 1985 a). Measurement of oxygen consumption not only indicates the metabolic rate in animals, but also provides an index of response to stress conditions (Reddy and Ramamurthi, 1981). An attempt is made here to study the diurnal variations in oxygen consumption of the fresh water crab, Oziotelphusa senex senex and also to determine the effect of methylparathion, an organophosphorous compound, on the variations in oxygen consumption.

MATERIALS AND METHODS

Adult, healthy, 30-35 g crabs (Oziotelphusa senex senex) were collected from paddy fields around Tirupati (India). They were kept singly in a 1-L glass aquaria and acclimated to laboratory conditions for 10 d. Ambient temperature was maintained at 25 ± 1°C and relative humidity at 80 ± 5% under a 12 : 12 light: dark regimen. The crabs were fed twice weekly with frog muscle ad libitum. The medium was changed daily. Only uninjured, healthy, intermolt (Stage C_4 according to Reddy, 1990) male crabs in the weight range of 30-32 g were selected to obviate variations in oxygen uptake due to sex, size and molt cycle (Ramamurthi and Sainath Janak 1973; Reddy et al. 1985b; Reddy 1990).

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Technical grade methylparathion (O-O dimethyl-O-(4-nitrophenyl)-mono-thiophosphate of 95% purity was obtained gratis from Bayer (India) Ltd. Lethal limits were calculated by the probit method (Finney 1964) and the LC_{50} value was found to be 1 ppm for 48 hr. Hence 1/5th of the 48-hr LC_{50} concentration (0.2 mg/L) was selected for sublethal treatment. At this concentration crabs survived and apparently normal even after prolonged exposure (60 d).

Crabs were divided into two batches of 360 each. One batch of crabs were exposed to 0.2 mg/L of methylparathion solution. Other batch of crabs served as controls and were maintained under the same conditions with the exception that no pesticide was introduced. Whole animal oxygen uptake was measured according to Saroja (1959) at 0800, 1200, 1600, 2000, 0000 and 0400 hr. The crabs were dissected out just after measuring the whole oxygen uptake. The hepatopancreas and chelate leg muscles were isolated and used immediately for oxygen uptake (Umbriet et al. 1959). Ten specimens were used for each determination. The experiments were repeated on five consecutive days to assess the consistency of rhythm. Differences between crests and troughs were analyzed using Student's t-test (Pillai and Simha 1968).

RESULTS AND DISCUSSION

A diurnal rhythm of oxygen uptake in the whole animal and in the isolated organs of Oziotelphusa has been observed (Fig.1). The maximal oxygen consumption was registered at 0000 hr of the day and then it showed a gradual decline as day approached, being minimum at 1200 hr. The tissue oxygen consumption also exhibited a similar rhythm. Here also the maximum oxygen consumption was registered at 0000 h alternating with a nadir at 1200 hr. The 24-hr was arbitrarily divided into two spans: 0800, 1200 and 1600 hr, occurring during light hours (light span) and 2000, 0000 and 0400 hr occurring during dark hours (dark span). The average oxygen uptake during the two spans were presented in Table 1. Oxygen uptake was high during the dark span in both the tissues and in whole bodies. The differences between light span and dark span values of oxygen uptake were statistically highly significant at $P < 0.001$. Methylparathion exposure decreased the respiratory rate of whole animal and of both tissues. Methylparathion exposure also disrupted the rhythm of respiration.

The present study demonstrates the occurrence of a diurnal rhythm of oxygen consumption in crab. Rhythmic variations in oxygen consumption were reported earlier

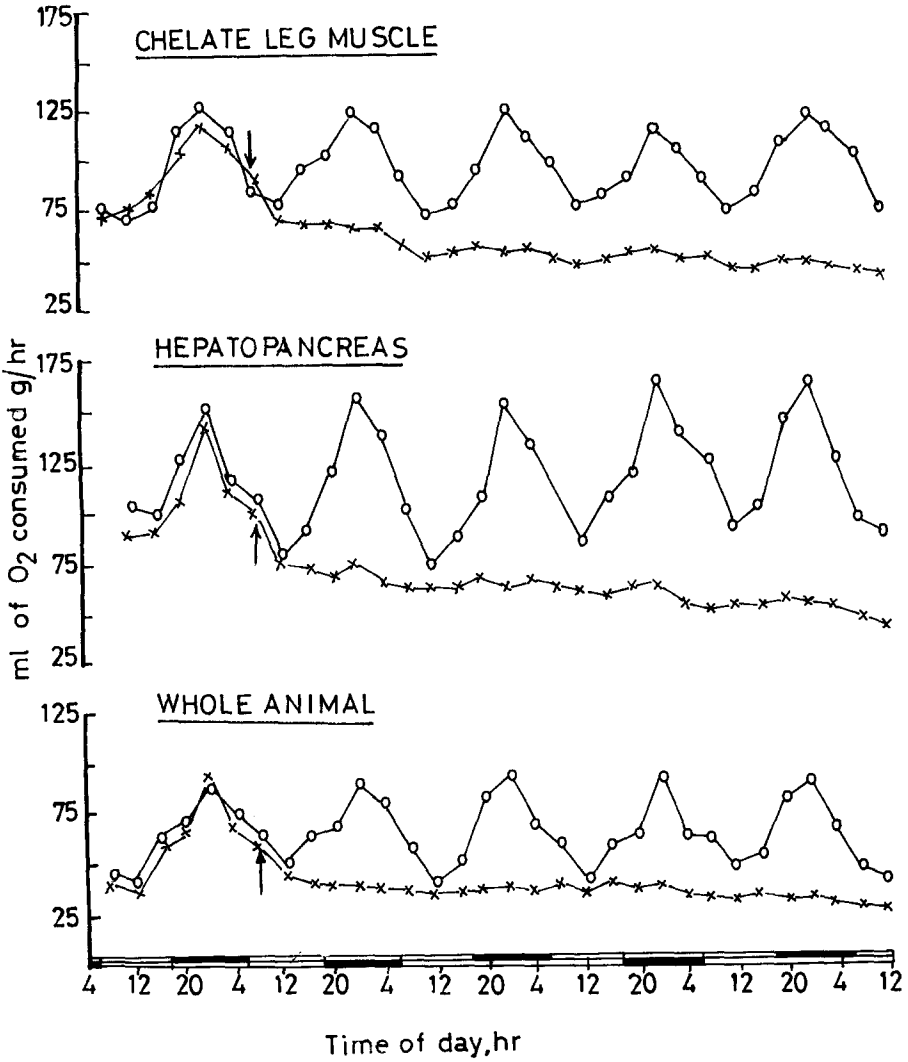

Figure 1. Changes in whole animal and tissue (hepatopancreas and chelate leg muscle) oxygen uptake of control (O——O) and methylparathion (0.2 mg/L) exposed (X——X) crabs over a period of 5 d. Oxygen uptake was measured at 4-hr time intervals. Each point is a mean of ten individuals. The arrow indicates time of exposure of crabs to methylparathion.

in crabs Carcinus maenas (Arudpragasam and Naylor 1964), Uca pugnax (Barnwell 1966) and in Paratelphusa hydrodromous (Ramamurthi and Sainath Janak 1973). Rhythmic variations in oxygen consumption in Oziotelphusa may be related to rhythmic variations in locomotor activity (Surendra Reddy 1978). Surendra Reddy (1978) observed overt locomotor activity in the crab, Oziotelphusa, around midnight (0000 hr). The

Table 1. Average oxygen consumption (mL of O_2 consumed/ g/hr) in whole animal, hepatopancreas and chelate leg muscle of Oziotelphusa senex senex during light (0800 to 1600 hr) and dark (2000 to 0400 hr) spans of day.

Source	Light span	Dark span
Whole animal	54.61±6.71	72.36±8.44*
% change		32.50
Hepatopancreas	100.42±8.12	132.14±8.79*
% change		31.58
Chelate leg muscle	92.71±5.49	112.25±7.31*
% change		21.08

Values are mean ± S.D. of 60 individuals. For computation of % change and evaluation of 'p' for dark span crabs, light span crabs served as controls.
* $p < 0.001$.

metabolic rhythm is found to be synchronous with the period of active locomotion. This is coupled with cyclical fluctuation in physiological variables such as blood sugar levels (Reddy et al. 1986) heart beat (Surendra Reddy 1978) and acetylcholinesterase levels (Reddy et al. 1985a) which all crest during the active phase.

Methylparathion exposure decreased respiratory rate both at whole animal level and at tissue level. Clogging of respiratory lamellae and destruction of gill epithelium observed in the gill of crab exposed to methyl parathion (Kishore and Katadore 1982) could be responsible for the observed depression in oxygen uptake. In addition to the gill damage, decreased blood pigment content observed during organophosphorous intoxication (Koundinya and Ramamurthi 1979) may also interfere with respiratory process with resultant respiratory suppression.

Methylparation exposure also disrupted the respiratory rhythm. Similar disruption of circadian rhythm has been reported in fish exposed to metasystox, an organophosphorous compound (Natarajan 1985). Disruption of circadian rhythm of respiration has been reported in endocrine-manipulated crabs (Reddy 1991). Respiration in the crab, Oziotelphusa, is under the control of respiration inhibiting hormone of eyestalks (Reddy and Ramamurthi 1981). Bilateral eyestalk ablation eliminated the diurnal rhythm of aerobic metabolism (Reddy 1991). Earlier, Reddy et al. (1986) demonstra-

ted repetitive discharges from crustacean neurons and release of hormones from eyestalks in pesticide-exposed crabs. It is conceivable in the present case that methylparathion might cause disruption of discharge of neurosecretory material including respiration inhibiting hormone from the neurosecretory cells that synthesize it in a similar way, resulting in disruption of respiration rhythm. This disruption of rhythm of respiration may have a significant effect on the survival ability of the animal in its physical environment.

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