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Sub-lethal impact of carbaryl on food utilization in the freshwater prawn *Macrobrachium malcolmsonii*

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Abstract

This study determines the toxic effect of carbaryl (Sevin® 50% W.P) on the food utilization parameters in intermoult juveniles of the prawn, *Macrobrachium malcolmsonii*. The prawns (4.5-5.0 cm in length and 1.0-1.25 g wet wt.) were exposed to three sub-lethal concentrations of carbaryl (5.15, 7.73 and 15.47 $\mu g\ l^{-1}$) for duration of 40 days. The toxic medium was renewed daily. The prawns were fed ad libitum with known energy quantity of boiled goat liver on daily basis. The overall wet weight gain was calculated. The energy lost through unconsumed food (15-60%), faeces (15-109%), ammonia excretion (9-27%) and moults (13-26%) of the prawns were calculated. The feeding rate, the rate and efficiency of absorption, the metabolic and food conversion rates and the gross and net food conversions efficiencies were found to be significantly declined ($p<0.05$) in test prawns when compared to that of the control. The energy lost through faeces, ammonia excretion and exuvia was found to be significantly elevated ($p<0.05$) in test prawns than that of the control. The effect of carbaryl on the bioenergetics parameters was severe in the highest sub-lethal concentration, less in the intermediate concentration and least in the lowest sub-lethal concentration. The results indicated that decrease in feeding, absorption, metabolism and food conversion are interdependent and toxicity of carbaryl diverting energy from production to maintenance pathways, which ultimately resulting in declined growth of *M. malcolmsonii.*

Key words

Bioenergetics, Prawn, *Macrobrachium malcolmsonii*, Carbaryl

Introduction

Pesticide pollution affects many non-target organisms including human being (Svensson *et al*., 1994; Azmi *et al*., 2009). The carbamate pesticides are most widely used for agricultural and residential applications. Enormous amount of these components, such as aldicarb, carbofuran, carbaryl etc., are used worldwide. Unlike organochlorine pesticides, the carbamates do not persist long in the environment. However, these are also toxic to non-target organisms (Bhavan *et al.,* 1997a, Bhavan and Geraldine, 2002, 2009). Carbamates are most rapidly degrading pesticides. In mammals these are rapidly metabolized, and excreted in urine (David and Kumaraswmi, 1988; Hassal, 1990). The physiological action is more reversible than that of organophosphorous. Some of the degradation products of carbamates are more persistent than the parent component (Lagadic *et al*., 2000). Carbaryl has a depressive effect on mitochondrial respiration without affecting the phosphorylation complex (Moreno *et al*., 2007). The toxic effect of pesticides are known to alter the behavioural pattern, growth, reproductive potential and susceptible to diseases of crustacean by affecting a variety of biochemical and physiological mechanisms (Mortimer and Connel, 1995; Geraldine *et al*., 1999; Bhavan and Geraldine, 1997, 2000a,b, 2001, 2002, 2009; Collins and Cappello, 2006; Ali *et al.,* 2007). Of these, growth serves as composite measure of the whole organisms function and ecological fitness. The growth rate is one of the most powerful factors determining economic performance in shrimp farming (Wyban *et al*., 1987). Thus, a bioenergetic description of growth can provide a fruitful approach to the study of sub lethal effects of pollutants on organisms.

Food intake is one of the most important factors regulating the level of metabolism. Detection of dearangrments in metabolism is most sensitive parameters of stress, since it integrates many processes, including enzyme activity and modulation, substrate pools and physiological response. Disruption of energy balance in various aquatic animals has been studied to determine the metabolic distress caused by pollution (Wong *et al.,* 1993; Singh *et al.,* 1996; Bhavan and Geraldine, 2000b; Wang *et al*., 2005; Soucek, 2007; Tong *et al*., 2010).

The economically important freshwater prawn, *Macrobrachium malcolmsonii* is found in abundance and constitutes a major fishery in the Cauvery River, an important perennial river of Tamilnadu, South India. Carbaryl appears toxic to *M. Malcolmsonii* (Bhavan *et al*., 1997a). Carbaryl was found to have been accumulated in tissues of *M. malcolmsonii* even after allowed for depurate the insecticide (Bhavan *et al.,* 1997b). Carbaryl toxicity causes reduction in concentrations of protein, carbohydrate, glycogen, free sugar and lipid, it also cause decline in activity of glutathione S-transferase and acetylcholine esterase, caused derangements in the gills and hepatopancreas, and altered the intensity of polypeptide bands in *M. malcolmsonii* (Bhavan and Geraldine, 2002, 2009). However, there is a paucity of literature relating to the toxic effects of carbaryl on energy allocation in aquatic animals in general and *Macrobrachium* in particular. Therefore, the present investigation was conducted to study the changes that occur in food utilization parameters, such as feeding, absorption, metabolism and conversion in the juveniles of *M. malcolmsonii* following exposure to three sub-lethal concentrations of carbaryl.

Materials and Methods

Healthy juveniles of the freshwater prawn, *M. malcolmsonii*, were collected from the lower Anicut of the river Cauvery, Tamil Nadu, South India. The juveniles were acclimatized to laboratory conditions with ground water for two weeks in 1000 lit. capacity cement aquarium. The prawns were fed with boiled and chopped goat liver *ad libitum*. During acclimation the water was adequately renewed daily.

Sevin® 50% W.P., (containing 50% active ingredient of carbaryl, 1-Naphthyl methylcarbamate) purchased from a local agrochemical service centre was directly dissolved in double distilled water to prepare solutions of the required concentration. Based on 96 hr LC_{50}, 77.370 µg l^{-1} (Bhavan *et al*., 1997a), three sub-lethal concentrations (1/15th, 1/10th and 1/5th of the 96 hr LC_{50}) were chosen as 5.15, 7.73 and 15.47µg l^{-1} respectively.

In the present study, 120 intermoult juvenile prawns used were equally divided into four groups with 30 individuals each (average length: 4.5-5.0 cm and body mass: 1.0 - 1.25 g). One group served as control; the other groups were exposed to three sub lethal concentrations of carbaryl. Each group comprised of three aquaria (15 lit. capacity), with 10 juveniles in each aquarium. The experiment was carried out for 40 days. Water medium was gently siphoned out daily and replaced by medium containing freshly prepared carbaryl solution. The experiment was conducted in triplicates.

Estimation of food utilization parameters: The energy balance was determined in the present study was based on the IBP formula (Petrusewicz and Mac Fadyen, 1970), this is C= (P+E) +R+F+U, where C = food consumed; P = growth; E = exuvia; R = material lost as heat; F = faeces and U = nitrogenous excretory products.

Food consumed (C): Each animal was uniformly allowed to feed *ad libitum* on boiled and chopped goat liver (1.0 g wet wt.) for 12 hr d^{-1} (6.00_{pm} - 6.00_{am}). Every day, the unconsumed food was separately collected into a filter by the siphoning method and dried in a hot air oven 90°C for 48 hr and weighed. To estimate the dry weight of food consumed, a sample of food was dried everyday and the dry weight of the unconsumed food was subtracted from the dry weight of the food offered.

Faeces (F): The faeces were collected daily by siphoning into a blotting silk-filter. This was then dried in a hot air oven at 90°C for 24 hr, weighed and stored.

Nitrogenous excretory product (U): The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of Solorzano (1969). The energy loss occurring by ammonia excretion was calculated using the ammonia calorific quotient 1 mg NH_3: 5.9 cal. (Elliot, 1976).

Exuvia (E): During daily renewal of test medium the moults, if any were collected. After blotting the adhering water, the moults were weighed and dried overnight in a hot air oven (90°C) and the dry weight was then recorded. The number of moults per individual was also observed. Since exuvia (E) constitutes part of the converted energy in crustaceans, in the present study, the energy loss through exuvia was considered to be part of conversion. The production of new tissues (= conversion P) was calculated by adding the exuvial weight to the gain in total weight of the prawn and the actual growth was calculated subtracting the exuvial weight from the gain in total weight.

Growth (P): The term conversion has been used to refer to growth, *i.e*., P of the IBP terminology. As already mentioned above, prior to the commencement of the experiment, the test prawns were starved for 24 hr inorder to evacuate the undigested food consumed the previous day. Subsequently, the wet (live) weight of the each individual was determined at the commencement of the experiment. To estimate the initial dry weight of the test individuals the 'sacrifice method' was adopted (Maynard and Loosli, 1962). A group of five juvenile prawns of similar live weight and experimental state served as control to determine the initial weight and energy content. These prawns were sacrificed and dried in a hot air oven at 90°C till they attained constant weight. The dry weight and energy content of these prawns were considered to represent those of the test individual at the commencement of the experiments.

During exposure period, the mortality in control and experimental groups was found to be negligible. On the 40th days, the morphometric data, such as the final length and weight of all prawns in triplicate was measured; the prawns were then sacrificed and dried in a hot air oven as defined above to estimate the energy content. The energy content of dried food, prawn, faecal matter and exuvia was determined by the oxygen Bomb calorimeter (Parr 1042), and the energy value was expressed in calories.

The food energy consumed was measured as the difference between the energy content of food offered and that of the uneaten food. The quantity of absorbed food energy was calculated by subtracting F and U from C. Conversion of growth is the sum of energy channelled to somatic growth (P) and exuvia (E). The efficiency of absorption was calculated by relating the food absorbed to the food consumed. Gross conversion efficiency (K_1) and net conversion efficiency (K_2) were calculated as percentage of conversion to consumption and absorption respectively. Feeding rate (FR), absorption rate (AR), conversion rate (CR) and metabolic rate (MR) were all calculated by dividing the respective amounts of energy by initial live weight of the prawn per unit time in days. Following the estimations of C, F, U and P the metabolism (R=Respiration, material lost as heat) was calculated.

The rate of energy utilization in various parameters was expressed in terms of g.cal g^{-1} live wt. of the prawn d^{-1}. The data were analysed statistically by adopting student 't'-test (Zar, 1984) manually.

Results and Discussion

Table 1 depicts the food utilization in *M. malcolmsonii* exposed to sub lethal concentrations of carbaryl. In test prawns, the food utilization parameters, such as feeding, absorption, metabolism, conversion and growth were found to decline when compared with control. The decline was most pronounced in the highest sub lethal concentration of carbaryl (26-73%) in comparison with intermediate (21-47%) and the lowest sub lethal concentrations (13-24%). In test prawn, the energy lost through exuvia (13-26%), faecal output (13-109%), and ammonia excretion (9-27%) were found to elevate over control. In these parameters, the energy lost over control was maximum in the highest sub lethal concentration (26, 109 and 27% respectively) in comparison with energy lost in intermediate (20, 59 and 15%) and the lowest (13, 13 and 9%) sub lethal concentrations.

The growth rate of test prawns exposed to sub lethal concentrations of carbaryl was found to lower (22-69%) than that in control (Table 1). Similar reductions in growth rate have been reported in *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000b), in green lipped mussels, *Perna viridis* due to pesticide pollution (Wang *et al*., 2005), in *Oreochromis mossambicus* exposed to Arsenic (Tsai and Liao, 2006), in *Centropomus parallelus* due to sodium dodecyl sulphate (Rocha *et al*., 2007), in the grass shrimp, *Palaemonetes pugio* due to copper and cadmium toxicity (Manyin and Rowe, 2009) and in the amphipod, *Melita longidactyla* exposed to 1,2-dichlorobenzene (Tong *et al*., 2010). The observed reduction in the growth rate of test prawns is due to reduction in food consumption, absorption, metabolism and conversion as reported in the snail, *Indoplanorbis exustus* exposed to dimecron (Singh *et al*., 1996) and in *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000b).

In this study, the lower food absorption recorded (absorption rate = 21-67%; absorption efficiency = 13-27%) in test prawns exposed to sub lethal concentrations of carbaryl may be due to retention of food for a longer period in the alimentary canal because of malfunctioning of digestive system. This might have led to reduction in food consumption (15-60%). This hypothesis is highly valid in this study as the energy lost through faecal out-put was higher in test prawns. A similar phenomenon has been reported in *I. exustus* exposed to dimecron (Singh *et al*., 1996) and in *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000b). The recorded higher energy loss through faecal output suggests that a considerable quantum of food offered was neither digested nor absorbed. This indicates the fact that nutrients offered got wasted under carbaryl stress as suggested in *Labeo rohita* under the stress of monocrotophos (Ramaneswari and Rao, 2006).

Table - 1: Food utilization in *M. malcolmsonii* exposed to sub lethal concentrations of carbaryl (values exposed as g.cal g^{-1} d^{-1}) for 40 days.

Parameters	Control	Sub-lethal exposure		
		5.15 µg l^{-1}	7.73 µg l^{-1}	15.47 µg l^{-1}
Feeding rate (FR)	33.51 ± 1.47	28.47 ± 1.44 (15)	19.30 ± 1.59 (42)	13.32 ± 1.04 (60)
Absorption rate (AR)	32.45 ± 1.69	25.49 ± 1.41 (21)	17.25 ± 1.39 (46)	10.65 ± 1.30 (67)
Absorption efficiency (AE) %	97.52 ± 1.53	84.49 ± 1.45 (13)	76.52 ± 1.58 (21)	70.65 ± 1.38 (27)
Conversion rate(CR) P	7.50 ± 0.73	5.70 ± 0.67 (24)	4.14 ± 0.65 (44)	2.01 ± 0.38 (73)
Exuvia (E)	0.30 ± 0.05	0.34 ± 0.03 (13)	0.36 ± 0.06 (20)	0.38 ± 0.06 (26)
Growth rate (GR)= P+E	7.81 ± 0.78	6.04 ± 0.70 (22)	4.51 ± 0.72 (42)	2.40 ± 0.45 (69)
Faecal output (F)	0.66 ± 0.06	0.75 ± 0.06 (13)	1.05 ± 0.06 (59)	1.38 ± 0.30 (109)
Ammonia excretion (U)	3.85 ± 0.30	4.20 ± 0.25 (9)	4.44 ± 0.42 (15)	4.91 ± 0.36 (27)
Excretory rate (ER) =F+U	4.51 ± 0.36	4.96 ± 0.31 (9)	5.49 ± 0.48 (21)	6.29 ± 0.65 (39)
Metabolic rate (MR)	29.25 ± 1.47	23.22 ±1.49 (20)	15.44 ± 1.46 (47)	9.94 ± 0.89 (66)
Gross conversion efficiency (GCE) %	17.62 ± 1.43	14.26 ±1.44 (19)	10.65 ± 1.11 (39)	7.37 ± 0.91 (58)
Net conversion efficiency (NCE) %	18.82 ± 1.43	15.75 ±1.41 (16)	11.75 ± 1.14 (37)	8.80 ± 1.05 (53)
Overall weight gain (wet wt. in g)	0.85 ± 0.10	0.71 ± 0.10 (7)	0.65 ± 0.10 (24)	0.50 ± 0.09 (41)

Each value is mean ± SD of three observations, Values observed in sub-lethal exposure groups are statistically significant at $p<0.05$ (5.0 % level) over control group, Values given in parenthesis represent percentage (%) difference over control

The soluble nitrogen excreted in the form of ammonia represents the catabolic by-product of protein and purine breakdown. In test prawns, the higher rate of ammonia excretion (9-27%) recorded suggests a considerable energy lose. Similar observation has also been reported in *Oreochromis mossambicus* exposed to deltamethrin (Khanee *et al*., 1992) and in *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000b).

The exoskeleton of crustaceans is mainly consists of protein, chitin and minerals (Sarac *et al*., 1994). Lose of considerable quantities of these materials should have occurred as a result of moulting, and therefore, the test prawn must metabolize a large quantity of nutrients to develop a new exoskeleton. In fact, the energy lost through moult was higher (13-26%) in test prawns. It has been reported that in fiddler crab, *Uca pugilator* exposed to DDT, a high energy lose through exuvia was due to accelerated moulting because of induction of moulting hormone and ecdysone (Weis and Mantel, 1976). A decrease in inter moult period due to acceleration of moulting has been reported in *Palaemonetes argentinus* exposed to chlorpyrifos and endosulfan (Montagna and Collins, 2007). It has been reported that the pesticide, methoprene act as an endocrine disruptor in the lobster, *Homarus americanus*, altered the synthesis and incorporation of chitoproteins and affects the normal pathway of cuticle synthesis and the quality of the post-molt shell (Walker *et al*., 2005).

In the present study, the reduction in food consumption and the need for allocation of a greater proportion of absorbed/ assimilated food energy for maintaining various metabolic processes associated with stress possibly contributed to the reduction in the food conversion (GCE: 19-58%; NCE: 16-53%). The observed reduction in net conversion efficiency (16-53%) suggests that the test prawns were under stressful environment and that a greater proportion of the energy absorbed from the food was being used for maintenance rather than growth. This is evident from the higher energy lose recorded through metabolism (20-66%) of test prawns. Further, it is suggested that the reduction in absorption/ assimilation should have effectively lowered the catabolic processes, which in turn ultimately affect the growth of test prawns.

The results are in agreement with our previous bioenergetics work in *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 2000b). The mode of action of endosulfan and carbaryl is different as these pesticides belong to different categories, organochlorine and carbamate respectively. However, the sub lethal impact of carbaryl showed similar effect to that of endosulfan (Bhavan and Geraldine, 2000b) as evidenced by different food and energy utilization parameters.

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