CHAPTER II

EFFECT OF PENTROTHION ON ECDYSIS IN THE FRESH WATER

PRAWN MACROBRACHIUM LAMARII
1.0 INTRODUCTION

Though there has been a great deal of research on the process of moulting which is a dominant aspect of crustacean physiology, associated with growth, there have been very few studies to evaluate, the toxicity of pesticides to them, at different stages of moulting cycle. Conklin and Ranga Rao (1977) evaluated the toxicity of sodium penta-chlorophenate to the grass shrimp, *Palaemonetes pugio*, at known stages of the moulting cycle using standard 96 hr bioassays, long-term exposures and exposures for varying periods during the moulting cycle.

Fingerman and Fingerman (1977) first reported that, the persistent, industrial pollutant Aroclor 1242, a polychlorinated biphenyl (PCB) has an inhibitory effect on moulting in the fiddler crabs, *Uca pugilator*. In fact, Aroclor 1242 completely inhibited ecdysis among the eyestalkless crabs. However, the octachlorodibenzofuran produced only a relatively slight inhibition of the rate of moulting (Fingerman and Fingerman, 1977).

Investigations of acute toxicity of copper to the crayfish, *Orconectes rusticus*, indicated that many of the test organisms died in the act
of ecdysis (Hubschman, 1967). Duke et al. (1970), Nimmo et al. (1971) suggested increase in the sensitivity of the pink shrimp, *Penaeus duorarum* to a polychlorinated biphenyl Aroclor 1254 during or soon after ecdysis. Armstrong et al. (1976) observed the same sensitivity of the Dungeness crab, *Cancer magister* to methoxychlor. Recent investigations on the toxicity of chromium to *Daphnia pulex* (Lee and Buikema, 1979), of copper and zinc to *Crangon crangon* (Price and Uglow, 1979), and of chlorine to *Penaeus kerathurus* (Saroglia and Scarano, 1979) have also revealed an increased susceptibility of crustaceans to aquatic pollutants at the time of, or shortly after, moulting. Most studies have concentrated on marine crustaceans but little information exists on the effect of pesticides regarding freshwater prawns in relation to moulting.

The phase from one moult to the next comprises the following stages (Drach, 1939). (1) Premoult or precydys - a period of active preparation for moult (stage D₀ to D₄), (2) Moult or ecdysis - the splitting and shedding of the old, partially reabsorbed cuticle (stage E), (3) Post moult or metecdysis - a period of rapid deposition of new chitin, inorganic
salts and a period of tissue growth (stage A and B)

(4) Intermoult - a period of time during which the processes normally associated with moulting are absent (stage C).

In evaluating the toxicity of chemicals to adult crustaceans, the physiological status of the animal in relation to the moult cycle should be considered. The relative toxicity at different stages of the moult cycle may vary depending on the type of chemical tested, the species examined, the relative thickness of the cuticle and frequency of ecdysis (Conklin and Rao, 1977). During the period immediately following ecdysis, the new thin cuticle is relatively more permeable and less protective than the thicker and calcified exoskeleton present during the intermoult period.

Ultrastructural changes induced by sodium pentachlorophenate in the grass shrimp, Palaemonetes pugio, in relation to the moult cycle were observed by Daniel and Ranga Rao (1978). There is not much literature available to evaluate the toxicity of pesticides to crustaceans at different stages of the moult cycle.
For the present toxicity studies in relation to moult cycle, Fenitrothion was used as a toxicant, which is extensively used to control the agricultural pests in Marathwada region. Considerable amount of work has been done on the effects of Fenitrothion on various metabolic parameters of fish (Koundinya et al. 1978; Koundinya and Ramamurthi, 1978a, b; 1979a, b, c; 1980), fresh water snail, (Ramana Rao et al., 1978, and 1980). Bhagyalakshmi (1981) has evaluated the impact of insecticide Fenitrothion on aspects of behaviour and metabolism of rice field crab, Oziotelphusa senex senex. The present work was undertaken to observe the toxicity, moult accelerating effects, of Fenitrothion on edible freshwater prawn, Macrobrachium lamergii at different moult cycle.

2.0 MATERIAL AND METHODS

The fresh water prawns Macrobrachium lamergii were collected from Paithan near Aurangabad. They were acclimatized at least for three days in shallow water tanks. The average length (rostrum to telson) of the prawn used for the present study was 38 mm. The prawns were kept individually in well aerated, one litre capacity jars containing 500 ml of tap
water at a temperature of 27°C ± 2°C under
12 hour light and 12 hour dark conditions. Care
was taken to dechlorinate the tap water (pH 6.5 -
7.0) before supplying it to the experimental prawns.

The prawns were not fed during short term
(96 - hour) static bioassays. During long term
exposures the test animals were fed with few
pellets of wheat flour on alternate days. The
water was changed and a fresh emulsion of the
pesticide was added daily.

One of the methods used in identifying the
stages in the moult cycle, involves an examination
of the progress in formation of new setae during
the proecdysial period (Drach and Tchernigovtzeff
1967; Rao et al., 1973). Each prawn was placed on
a glass microscope slide, with a drop of freshwater
under the telson. The telson and uropods were
gently spread with the help of a probe and the edges
of the uropods were examined microscopically (100 x
magnification) for signs of epidermal retraction
(apolysis) and progress in the formation of new setae
(neosetogenesis). Thus the prawns of different
stages of the moult cycle were sorted out and used.
Observations were carried out through three moult.
Acute toxicity of Penitrothion to *M. lamerrii* at different stages of the moult cycle was observed. Intermoult (stage C) prawns, and prawns in early proecdysial (stage D₀), late proecdysial (stage D₃-D₄), and ecdysis (stage E) were exposed to different concentration of Penitrothion. The observations were made, on the mortality rate and median lethal concentration (LC₅₀) was found out for each group using toxicological statistics method (James Busvine 1971).

A sub-acute chronic experiment was conducted for a period of 60 days to determine the effect of various concentration of Penitrothion on successive intermoult periods. Forty intermoult prawns were divided into four equal groups. One group served as control when others were exposed to 0.0004; 0.0008; 0.001 ppm of Penitrothion. The prawns were kept individually in glass jars. Each prawn was examined microscopically on alternate days to assess the progress in the moult cycle. The jars were examined daily for cast of exoskeleton or mortality.
Observations were carried out and critical period of the moult cycle was noted on prawns exposed to 0.001 ppm. Twenty prawns were exposed to Fenitrothion throughout the proecdysial period and allowed to complete ecdysis in the medium. Other groups were transferred to tap water at different stages of moult cycle $D_1$, $D_2$, $D_3$, $D_4$ and $E$. A group of prawns was kept in tap water throughout the proecdysial period and allowed to complete ecdysis in that medium. Then they were placed in 0.001 ppm Fenitrothion within a few hours after ecdysis.

Experiments were conducted using a total number of 30 prawns *M. lamerrii*, which were at the same stage of the moult cycle 'stage C' and in the same size 35 mm, average rostrum-telson length. Ten prawns were maintained as the control, and the other twenty prawns were kept in 0.0003, 0.0006 ppm Fenitrothion. The control water and experimental media was changed daily. The prawns were fed with wheat-flour on alternate days. Cast exuvia were collected, and washed with glass distilled water. Care was taken not to include exuvia which had been partially eaten by the moulted prawn. The exuvia were dried at 110°C for 48 hr and dry weight was taken.
3.0 OBSERVATIONS AND RESULTS

The process of moulting was observed in *Macrobrachium lamertii*. The newly moulted prawn was unable to swim. Death from cannibalistic-like behaviour was observed in groups.

3.1 Identification of the stages in the moult cycle

During the post ecdysial period, 'stage A' and 'B' (Figure 1a and 1b) additional layers of cuticle are secreted, calcified and these processes are completed in intermoult stage 'stage C' of the moult cycle. Intermoult prawn shows no evidence of epidermal retraction or neosetogenesis (Fig. 2). During early proecdysis 'stage D₀' epidermal retraction is seen (Fig. 3) in which neosetogenesis is started. In stages D₃ - D₄ extremely well developed new setae were observed.

3.2 Acute toxicity in relation to moult cycle

Short-term (96 hr) toxicity test was carried out to determine the acute toxicity of organophosphate, Fenitrothion to *Macrobrachium lamertii* at different stages of moult cycle. The median lethal concentrations (LC₅₀); computed, using toxicological
Fig. 1 a: The apical setae of a uropod of *M. lamertii* in a moult stage A. Note the lack of internal cone X 150.

Fig. 1 b: The apical setae of a uropod in a moult stage B. Note the developing internal cone X 150.
Fig. 2: The outer edge of a uropod of an intermoult 'stage C' M. lamerrii. The epidermis shows no evidence of retraction from the old cuticle X 150

Fig. 3: The apical setae of a uropod of M. lamerrii in moult stage D. Note the epidermal retraction and neosetogenesis
Table - 1

Short term toxicity test results (LC$_{50}$ in ppm) of Fenitrothion to *M. lamerrii* at different stages of the moult cycle.

<table>
<thead>
<tr>
<th>Stage</th>
<th>24 hour</th>
<th>48 hour</th>
<th>72 hour</th>
<th>96 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermoult</td>
<td>0.00741</td>
<td>0.00575</td>
<td>0.00346</td>
<td>0.00287</td>
</tr>
<tr>
<td>Stage D$_0$</td>
<td>0.0039</td>
<td>0.00346</td>
<td>0.00251</td>
<td>0.00181</td>
</tr>
<tr>
<td>Stage D$_3$-D$_4$</td>
<td>0.00141</td>
<td>0.00121</td>
<td>0.000912</td>
<td>0.00063</td>
</tr>
</tbody>
</table>
Short-term (96 hr) toxicity of Fenitrothion to *M. lamellii* at different stages of the moult cycle.
statistics, James Busvine (1971) are indicated in Table 1. The LC₅₀ values for intermoult prawns are 0.0074 ppm; 0.0057 ppm; 0.0034 ppm and 0.0028 ppm for 24, 48, 72 and 96 hours respectively, while the stage D₀ and D₃ - D₄ prawns have 0.0039 ppm, 0.0034 ppm, 0.0025 ppm, 0.0018 ppm, and 0.0014 ppm, 0.0012 ppm, 0.00091 ppm; 0.00063 ppm. Relative toxicity at different moulting stage is shown graphically in figure 4.

3.3 The effect of various concentrations of Fenitrothion on moulting frequency

The average intermoult period for the control prawn was 18 days. The intermoult period in the control increased fairly constantly (Fig.5). Exposure to Fenitrothion in 0.0005 ppm did not differ much in intermoult period from control prawns. In 0.0006, 0.0009, 0.001 ppm of Fenitrothion the moulting frequency is increased. In 0.001 ppm the duration decreased from 15 days at the beginning to 11 days at the third moult, although only half of the prawns survived the third moult. Prawns exposed to 0.001 ppm Fenitrothion were put into fresh tap water immediately after finishing the third moult; after one further moult the successive intermoult increased sharply. In higher
FIG. 5.

SUCCESSIVE INTERMOULT PERIODS OF MACROBRACHIUM LAMERRIT

EXPOSED TO DIFFERENT CONCENTRATIONS OF FENITROTHION
concentration (0.004 ppm) average intermoult period could not be calculated since the animals died soon after ecdysis. In an alternative method of presentation (Fig. 5) each successive intermoult period was plotted.

3.4 Critical period of the moult cycle

90% mortality was observed in prawns which were exposed to 0.003 ppm Fenitrothion throughout the proecdysial period and allowed to moult in the same concentration. On the other hand the prawns exposed to the same concentration till the stage D₁, D₂, D₃ or D₄ are transferred to tap water before ecdysis showed 10% and 15% mortality. In control prawns no postecdysial mortalities occurred. It was interesting to note that when prawns were exposed to 0.002 ppm Fenitrothion, immediately after ecdysis, 65% of them died (Table 2).

3.5 Effect of Fenitrothion on Exuvial weight

Exuvia from prawns exposed to 0.0006 ppm Fenitrothion were heavier in dry weight than controls. Dry weight of exuvia from prawns exposed to 0.0003 ppm Fenitrothion did not differ from that of control prawns. Dry weight of the control prawns' exuvia was 20.87 mg
### Table - 2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Exposure time in 0.003 ppm after one moultig</th>
<th>The stage at which prawns were transferred to freshwater</th>
<th>Percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>11 days</td>
<td>$D_1$</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>13 days</td>
<td>$D_2$</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>15 days</td>
<td>$D_3$</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>16 days</td>
<td>$D_4$</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>17 days</td>
<td>$E$</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>19 days</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>8</td>
<td>Exposure in 0.002 ppm after moultig</td>
<td>-</td>
<td>65</td>
</tr>
</tbody>
</table>
The dry weight of exuvia from *M. lamerrii*, exposed to different concentrations of Fenitrothion.
+1.25 whereas exuviae of prawns exposed to 0.0003 ppm and 0.0006 ppm of Fenitrothion were 21.45 mg ± 1.442 and 29.22 mg ± 2.672 respectively (Fig. 6).

4.0 DISCUSSION

The results in the present study indicate that the relative toxicity at different stages of the moult cycle may vary depending on the concentration of the pesticide used, and the physiological status of the prawns. As the integument of the animal was very soft immediately after moultling, the newly moulted prawns might be killed by other animals. In *Macrobrachium lamerrii* and other Crustacea, cannibalism has been correlated with ecdysis. (Bovbjerg, 1953; 1956; Momot, 1967; Forster and Beard, 1974 and Dugan et al., 1975).

Generally there is no avenue of escape, when the attacker is in moult stage C and the animal under attack is in moult stage A. A similar type of behaviour was noticed on *M. lamerrii* in groups and cannibalistic-like behaviour was completely prevented in the present investigation by keeping the experimental prawns individually in separate jars.
During each period of the moult cycle, various metabolic processes occur. Moulting in Crustacea has been reviewed by Carlisle and Knowles (1959). In proecdysis stage $D_0$, $D_1$ and $D_2$ the cuticle thins as materials are reabsorbed to be used in the formation of the new cuticle. The thin cuticle was noticed morphologically by simple touch and the microscopical observations were in perfect agreement with the epidermal retraction and neosertogenesis.

During ecdysis, the old, partially reabsorbed cuticle is split and shed. The animals immediately increased in size because of uptake of water from the environment. This is the most critical period for the prawns when they are exposed to pesticides. The present investigation results are discussed in the following paragraphs. When the cuticle is hard enough to permit normal body movements, the animal begins feeding, and the water taken in to swell the body during the moult, is replaced by tissue growth.

The acute toxicity study of the freshwater prawn, $M.\ lamerrii$ in relation to moult cycle, exposed to different concentrations of Fenitrothion indicated that,
all the stages of the moult cycle did not exhibit the same effect. Having lower LC$_{50}$ value than intermoult and early proecdysial stages, the late proecdysial stage (D$_3$ - D$_4$) exhibited increased sensitivity to Fenitrothion. Conklin and RangaRao (1978) reported that in 96-hour bioassays, the shrimp *Palaemonetes pugio*, in later stages of the proecdysial period exhibited a greater sensitivity to Na-PCP than that exhibited by shrimp in the intermoult and early proecdysial stages of the moult cycle. Our results are in agreement with their reports (Fig. 4). Inspection of all the LC$_{50}$ values obtained including immature and mature *M. lamerrii* reveals that the 96 hours LC$_{50}$ value (0.00063 ppm) obtained for late proecdysial *M. lamerrii* was the lowest of all the LC$_{50}$ values reported here. The 96 hour LC$_{50}$ value obtained for proecdysial shrimps is the lowest of all the LC$_{50}$ values reported previously for adult crustaceans and is comparable to those for fish and larval crustaceans (Conklin and RangaRao, 1978).

VanDija *et al.* (1977) noticed increased sensitivity in relation to moultng in the larvae of *Palaemonetes varians* whereas it was not so in the adult *Palaemonetes pugio* as observed by Conklin
and RangaRao (1978). This may be due to the fact that the larval crustaceans moult often and possess much thinner cuticles compared to adults. Hence, the magnitude of changes in the permeability of cuticle of larval forms in relation to the moult cycle may not be as great as that in adult.

Fingerman and Fingerman (1977) reported that Arclor 1242 drastically inhibits the rate of moultng of fiddler crab lacking either both eyestalks or four walking legs, and the octachlorodibenzojuran produced only a relatively slight inhibition of the rate of mouling. RangaRao et al. (1978) reported that, in Palaemonetes pugio, depending on the concentration under, Na-PCP, caused either a complete inhibition or regeneration, or a delay in initiation of limb bud growth, without altering the intermoult duration. In the present study, the interval between the first and second ecdysis in the control prawns was not significantly different from that of prawns exposed to 0.0005 ppm. In higher concentration, average intermoult period could not be found since the prawns died immediately after ecdysis.
The results obtained in 0.0006, 0.0009, 0.001 ppm Fenitrothion showed the increased moulting frequency. Hon-Cheng Chen (1980) found out that, zinc and copper significantly shortened the intermoult period of prawn Palaemon elegans, and prawns in higher concentration of metals other than mercury either did not grow or decreased in size following the moult. It might be considered that the shorter intermoult period found in pre-mature prawns is suggestive of a positive response to get rid of absorbed metals on the exoskeleton. Following a transfer of prawns to fresh sea water no further metals should be adsorbed on the exoskeleton, but the prawns still underwent the next few molts with shorter intermoult period. Further more, larval development was retarded by heavy metals. It was therefore, Hon-cheng Chen (1980) concluded that the presence of adsorbed metals on the exoskeleton does not play an important role in the moultng process. As we observed, the successive intermoult periods became longer in the control and shorter in Fenitrothion solutions e.g., three molts in the control took 57 days but only 45 days in 0.001 ppm Fenitrothion. The increased moulting frequency may be due to the metabolic stress which exerts to remove the rigid exoskeleton.
Bengtsson (1975) on minnows noticed that even a small stimulus with zinc was sufficient to induce pronounced reaction with an initial hyperactivity and Organophosphates are known to have effects on the neuromuscular junctions. The behavioural responses have, often been used as the sensitive measures of stress syndrome in organisms experiencing it (Olla, 1974, Eisler, 1979 and Miller 1980). It is a known fact that the physiological and behavioural adaptations are the parameters which have been used to study the relative stress conditions due to pollutants. Further work is needed to assess the physiological effect of organophosphates in moulting frequency.

Conklin and Rao (1977) reported that the grass shrimp, Palaemonetes pugio, is very sensitive to Na-PCP during the period immediately following ecdysis. The prawns M. lamerrii exposed to 0.003 ppm Fenitrothion throughout the proecdysial periods and allowed to moult in the same medium showed 90% mortality. From the same concentration when the prawns were transferred to tap water before ecdysis only 10 or 15% mortality occurred. The duration of exposure to Na-PCP during the period of proecdysis was not the major factor, affecting the postecdysial
survival of grass shrimp (Conklin and Rao, 1977). When *M. lamerrii* were exposed to 0.001 ppm Fenitrothion concentration immediately after ecdysis, majority of the prawns died. This increased sensitivity of the postecdysial shrimp appeared to be due to nearly 30-fold increase in the uptake of the biocide following ecdysis (Daniel et al., 1977).

Observing ultrastructural changes induced by Na-PCP in the grass shrimp *Palaemonetes pugio* in relation to the moult cycle Daniel et al. (1977) confirmed that due to the increased permeability of the new untanned cuticle immediately after ecdysis, it seems likely that the peripheral epithelium became inundated with Na-PCP. The subsequent entry of Na-PCP into the haemolymph and its transport into other major organs may account for the concurrent degeneration of the gut and hepatopancreatic tissue. Now one can conclude that, among all the stages of the moult cycle of freshwater prawn *M. lamerrii*, Stage 'E' especially the period immediately after molting is the most critical period for the toxic effect of Fenitrothion.

The weight of exuvia depends on factors such as the thickness of the cuticle, the degree of calcification and the extent of resorption occurring
proceeding ecdysis. Anita and Conklin (1977) reported that the calcium concentration in the exuvia from control shrimp did not differ from that of shrimp exposed to Na-PCP indicates that this compound may not specifically affect the deposition or resorption of calcium. With the aid of secretion of the moulting fluid, a partial resorption of the exoskeletal contents may be accomplished during the proecdysial stages of the moult cycle (Passano, 1960). A reduction in the extent of resorption may lead to the shedding of a heavier exoskeleton. Our results show that M. lamertii exposed to higher concentration of Fenitrothion has higher moulting frequency and the exuviae are heavier in dry weight. From this one can assume that, since the moulting frequency is induced by Fenitrothion the exuvia is cast off even before the complete resorption of the exoskeletal contents.
5.0 SUMMARY

The onset and progress of proecdysial preparation is identified by the epidermal retraction and progress in neosetogenesis in the uropod setae of *M. lamerrii*. The intermoult prawn (stage C) has no epidermal retraction and it requires 10 to 14 days for completing premoult preparation. When neosetogenesis is completed (Stage D), the prawn moult within two or three days.

On the acute toxicity of Fenitrothion to freshwater prawns *M. lamerrii* at known stages of moult cycle, Fenitrothion is more toxic to moulting than to non-moult, intermoult prawns.

Moultng is the most critical period in the moult cycle, when prawns are exposed to pollutant. But if Fenitrothion exposed prawns are transferred to freshwater before moulting, the mortality rate, has been reduced.

Chronic exposure of *M. lamerrii* to Fenitrothion, alters the duration of intermoult period. Fenitrothion causes dose-dependent moult accelerating effect in the prawns *M. lamerrii*.

The exuvia is heavier in dryweight, which may be due to the fact that, the exuvia is cast off even before the complete resorption of the exoskeletal contents.


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