Crustacean Molting: Regulation and Effects of Environmental Toxicants

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Abstract

In crustaceans the growth of the animal occurs by shedding of old exoskeleton and formation of new exoskeleton. Immediately after ecdysis the newly synthesized cuticle up takes water to expand new exoskeleton thereby size. Molt cycle in crustaceans is under the control of several regulatory hormones, internal and external factors. The predominant hormones molt inhibiting hormone (MIH) and ecdysteroids act in a controversial manner to one another in regulation of molt. It is also identified that the methyl farnesoate (MF) induces molting by inducing the synthesis and release of ecdysteroids from Y-organs. Besides several other hormones and internal molecules like opioids and neurotransmitters along with toxicants (xenobiotics, chemicals and metals) are also involved in the regulation of crustacean molting. Toxicity of aquatic pollutants leads to retardation of growth and delays molting, besides influence mortality and causes huge loss to crustacean farming. This review presents the advances in the field of crustacean molting and its regulation.

Keyword

Crustacean Hormones; Ecdysteroids; Methyl Farnesoate; Halloween Gene

Contents

• Introduction
• Endocrine regulation of Molting
• Inhibition of MIH on phantom (phm) gene expression in Y-organ
• Toxicity of chemicals that mediated endocrine disruption on growth

Introduction

Crustaceans especially crabs are rich in protein and also known as poor man's protein. Worldwide population was increasing year by year and feeding billion people through aquaculture. So crustacean rearing has become significant and developed culture methods. On the other hand crustacean industry has its own complications like availability of quality yield etc., in addition to lack of ways to enhance the growth of animal. In crabs the vegetative growth (somatic growth instead of reproductive) of the animal is regulated by special process called molting that is shedding of old exoskeleton and synthesizing of new exoskeleton. The molt cycle in crustaceans is under the control of several regulatory hormones, internal and external factors. The predominant hormones molt inhibiting hormone (MIH) and ecdysteroids act in a controversial manner to one another in regulation of molt. It is also identified that the methyl farnesoate (MF) induces molting by inducing the synthesis and release of ecdysteroids from Y-organs. Besides several other hormones and internal molecules like opioids and neurotransmitters along with toxicants (xenobiotics, chemicals and metals) are also involved in the regulation of crustacean molting. Toxicity of aquatic pollutants leads to retardation of growth and delays molting, besides influence mortality and causes huge loss to crustacean farming. This review presents the advances in the field of crustacean molting and its regulation.

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Introduction

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An inhibitory hormone that regulates molt belongs to CHH family neuropeptides are synthesized from neurosecretory cells, located in medulla terminalis X-organ (XO) Sinus gland of eyestalk. Among the inhibitory hormones, Molt inhibiting hormones (MIH), a type II peptide secreted from XO sinus gland suppresses ecdysteroid synthesis by YO[2].

In addition to above limitations of molting, one of the most prevailing problem nowadays is contamination of harmful chemicals lead (Pb) copper (Cu), Zinc (Zn) etc. due to urbanization increased the use of these metals in various ways. Several publication evidences clearly state the chemicals released into water bodies. As it sediments in fatty tissues because of high lipophilicity of organ chlorine. Some of compound as polychlorinated biphenyls, DDT, HCB are observed to get incorporated in fatty tissues as observed in shore crab C. maenas. Heptachlor epoxide, dieldrin, endosulfan, chlorine, DDT and metabolites HCHs were found to accumulate in C. granulate [3].

Endocrine Regulation of Molting

The neurosecretory system of the ES consists of a group of peptidergic neurons clustered in the medulla terminalis X-Organ (MTXO) and their bulbous axonic terminals that constitute the SG, which is a neurohemal organ that releases a number of peptide hormones into the hemolymph [4]. Molting in decapod crustaceans is controlled by the eyestalk X-organ/sinus gland complex, which secretes molt-inhibiting hormone (MIH), a neuropeptide that inhibits ecdysteroid production by a pair of Y-organs (YOs) located in the cephalothorax [5] and serves as the linkage between neurological signaling and steroidal control of processes such as molting and embryo development [6], CHH (crustacean hyperglycemic hormone) were identified from American lobster (H. americanus) that contributed to regulating carbohydrate metabolism [7]. However, one hormone also contributed to the regulation of molting [7]; while, the other stimulated oocyte maturation [8]. MOIH (mandibular organ inhibiting hormone) negatively regulates the secretion of methyl farnesoate from the mandibular organ and its associated regulatory activities and GH (Gonadal inhibiting hormone) also called vitellogenin-inhibiting hormone (VIIH) negatively regulates aspects of gonadal maturation [9]. In addition to the above neuropeptides the XO
The sinus gland and the neurotransmitters like Xanthurenic acid, 3-hydroxy L-kyurenin and serotonin. Thus the process of molting is under the control of eyestalk peptide hormones secreted by XO sinus gland [1]. The involvement of eyestalk peptides and other molecules secreted by eyestalk on regulation of molting is described below as shown in Figure 1.

Figure 1: General Scheme to summarize the results concerning neuropeptide hormones from eyestalk and their mode of action on ecdysteroid synthesis by Y-Organ from cholesterol. CHH - Crustacean hyperglycemic hormone, MIH-molt inhibiting hormone, VHI-vitellogenin/gonad inhibiting hormone, MOIH-mandibular organ inhibiting hormone, Extra eyestalk factor-RPCH- red pigment concentrating hormone (RPCH) and PDH. XA-xanthurenic acid. Cholesterol synthesis pathway involves STAR-steroidogenic acute regulatory protein, cytP450-cytochrome P450.

Role of molt inhibiting hormone (MIH) on molting

The surgical extirpation of the eyestalk ablation results in a shortened molt cycle interval, while the implantation of the eyestalk ablation contents restores this interval. A factor has been implied that normally inhibits the molting process and it has been named the MIH. It belongs to class II peptide and separated into two subgroups A and B. Subgroup A acts physiologically as MIH, B regulates gonadal maturation in addition to molting in Callinectes sapidus [10]. Main function of MIH is to regulate ecdysteroid pathway by inhibiting conversion of ketodiol and 25 deoxyecdysone by binding to receptors on epidermis of YO [5]. MIH induces an increase in cAMP and cGMP by binding to receptors on eyestalk peptides and other molecules secreted by eyestalk on regulation of molting is described below as shown in Figure 1.

Responsiveness of YO to MIH differs through molt stage. Whereas, Ca++ increase during premolt and enhance the activation of two intra cellular enzymes, protein kinase C and phosphodiesterase (PDE). Both PKC and PDE stimulate the increase of ecdysteroid levels on premolt stage. But this is reversed on incubation of YO with inhibitor of PKC and PDE IBMX which authentically supress ecdysteroid secretion and drops their levels at postmolt stage and intermolt stage [17]. But this is reversed on addition of Calcium ionosphere (A23187).

Role of Ecdysteroids on molting

Crustaceans appear to have the same enzymes for ecdysteroid biosynthesis as insects. An inhibitor of steroid 5-reductase, L-645390, blocks the conversion of cholesterol to 7DC in the YO of M. mercenaria [18]. The 5-reductase that converts 4-diketol to 5-diketol is a cytosolic enzyme in YO cells that requires NADPH for activity [19]. Orthologs of nvd, nmg/sro, spo, phm, dib, sad, and shd have been identified in the Daphnia pulex genome [20-22], and a cDNA encoding Phm has been cloned from Kuruma prawn, Marsupenaeus japonicus [23]. Nvd and spo are located adjacent to each other in the D. pulex genome [20]. The M. japonicus Phm and D. pulex Phm have five conserved motifs present in insect Phm (WxxxR, GxExDTT/S,
The CHHs are the most abundant neuropeptides in the SG. Their central role on the regulation of carbohydrate metabolism has been reviewed [36]. CHH family peptides are the pleotropic hormones with multifunctional and involves in biological activities like blood glucose regulation, molting and inhibition of methyl farnesoate synthesis, lipid metabolism regulation, vitellogenin and ovariian maturation. CHH amino acid sequence was first determined in species like shore crab *C. maenas*. As it is separated into two subgroups A and B. Both the isoforms A and B have a hyperglycemic effect, in additional to that A subgroup possess molt inhibiting activity and B subgroup stimulate oocyte growth [37]. Both their values vary according to molt and reproductive cycle. In the species like *C. maenas* CHH shows sequence homology with MIH. This confirms that CHH has inhibitory effect on ecdysteroidogenesis even though 20 times slower than MIH. Binding of CHH to the specific receptor guanylyl cyclase II (GC II) on the membrane of YO elevated the levels of cGMP and inhibits regulation of ecdysteroidogenesis [38].

Role of Vitellogenesis/ Gonad inhibiting hormone (VIH) on molting

In crustacean females, the late phase of gonadal maturation to form mature ova is called vitellogenesis. Usually the inhibitory role of GIH on vitellogenesis is observed in females, at the same time the occurrence of GIH in males is also recorded and this provides the additional role of GIH in males, assumed to be regulating molt [39]. Structural similarities among GIH and MIH in accordance to number of cysteine residues, their location provide the existence of homogeneity or similarity [40]. Thus similarity between GIH and MIH support the hypothesis phenomena of involvement of VIH in molting. GIH might have a molt-inhibiting function because female lobsters molt only after hatching of their larvae when the CHH and GIH hemolymph levels are low in *H. americanus* [41].

Molting and reproduction are complex hormone mediated process, and are also regulated by several other internal and external factors [42]. The similarity of VIH, GIH with that of MIH confers the involvement of induction of molting at previtellogenic stage in *C. quadricarinatus* [43].

Role of opioids on molting

Mancillas et al. [44] first declared the presence of opioid as small peptides in various species like spiny lobster *Panulirus interruptus*, red shrimp, and in crayfish *P. clarkia* by using various techniques like immunohistochemistry, RIA, HPLC. Fingerman et al. [45] reported the presence of two types of pesticides methionine-enzkephalin and leucine-enzkephalin from neuroendocrine complex of eyestalk of fiddler crab *U. pugilator*. Later, based on experimental evidences proved the role of opioids like methionine-enzkephalin as a neurotransmitter that regulates the secretion of hyperglycemic hormone in fiddler crab *U. pugilator*, movement of red pigment molecules that regulates chromatophores and on ovariian maturation in *U. pugilator*. Recently confirmed that opioids, leucine-enzkephalin have another crucial role on regulation of molting in the fresh water crab *O. senex senex* [46]. Complete descriptions of opioids were represented in review [47].

Influence of other eyestalk factors

Role of Xanthurenic acid, 3-OH-K on molting: Biogenic amines and peptide neuroregulators are known to modulate the release of some neuropeptide hormones from the SG [48]. These XA and 3-OH-K are...
also secreted by XO sinus gland and fully identified by MS and NMR and their structures. Among them the 3-OH-K is the circulating form and which converts into an active XA form by the action of an enzyme amino transferase [49]. Aminotransferases present in hemolymph, eyestalk and YO. The inhibitory action of both XA and 3-OH-K is studied to mediate through binding their respective receptors present on the YO [49]. There by suppress the secretion of Ecdysteroids from YO. By interfering with the synthetic co enzymes like Cytochrome C (Cyt C) and Cytochrome P450 (CytP450) at the site of iron porphyrin [50]. But the suppressive action of XA, 3-OH-K is studied to differ from species to species and also in stage specific [51].

Role of serotonin on molting: Serotonin (5-hydroxytryptamine or 5HT) is a neurotransmitter secreted by XO sinus gland of Eyestalk. The released serotonin is studied to stimulate the release of molt inhibiting hormone from eyestalk, thus have inhibitory role on molting. The inhibitory role of serotonin is by stimulating the release of variable hormones of molting such as PDH, CHH, GSH, MIH and Neurone depresssing hormone. On the other hand it is having suppressive role on MF secretion at the same time the progression of ovarian maturation by serotonin was examined in P. clarkia, white pacific shrimp L. vannamei [53] and tiger shrimp P. semisulcatus [54]. Further the suppressive role of serotonin on growth was evidenced by where the shrimp fed with Mannon oligosacharide 3 g kg-1 showed progressed molting compared with that of shrimp fed with serotonin. This supports the inhibitory role of serotonin on molting. But more recently Sainath and Reddy [55] stated that serotonin has no effect on molting in O. senex senex. Thus in depth and clarification studies are required in this direction.

MIH Inhibition of Phantom (Phm) Gene Expression on YO

Though it is well known that the process of molting is regulated by MIH, unfortunately the exact mechanism of action at gene expression level of MIH is unknown. Limited results persist regarding the involvement of Halloween genes and their regulation in molting [23]. YO ecdysteroid synthetic pathway suggest that the P450 mono-oxygenases, encoded by the Halloween genes Phantom (phm), Disembodied (dib), Shadow (sad), and Shade (shd) can bind multiple substrates. Phm apparently can hydroxylate 5β-diketol or 5β-ketodiol at C25; Dib apparently can hydroxylate 5β-diketol, 3D2, 22dE, 5β-ketodiol, or 5β-ketotriol at C22; Sad apparently can hydroxylate 3D2dE, 2.25dE, or 2dE at C2; and Shd apparently can hydroxylate 5βdE, 3D2E, or ecdysone at C20. However, the specificities of the Phm, Dib, Sad, and Shd enzymes are such that the C25→C22→C2→C20 order of hydroxylations is maintained [56]. These Halloween genes are studied to express in various parts of body like prothoracic gland, fat body, midgut etc. [57]. Unlike in crustaceans, in insects the prothoracic gland hormone known as prothoraccotropic hormone (PTTH) is studied for its positive regulation of molting [58]. Further the identification of Halloween gene orthologues in Daphnia ecdysteroidogenetic pathway [21] represents the involvement of Halloween genes in molting of crustaceans. Based on the above studies the involvement of phm genes (Member of Halloween gene family) in molting and their expression levels at different molt stages were studied and were observed to regulate in stage specific manner [23]. The expression of phm was confined to YO at all stages of molting though minor expression was observed in ovaries at mature stages which was the basis for selecting the phm gene expression as the limitation in the above studies. The results clearly proposed that the levels of phm expression were high during pre-molt stages and expression was suppressed during the intermolt stages, which was due to the inhibitory action of MIH mediated by binding to the receptors on YO [23]. On the other hand the expression of phm in insects was observed in ovary also suggesting that on degradation of prothoracic gland diameter metamorphosis the ovary adopts the function of ecdysteroidogenesis [59]. In addition to the difference in the expression levels of phm gene the levels of Cyp4c15 (type of cytochrome P450) were also regulated though the role of Cyp4c15 in molting was not known. This provides an idea that the MIH suppresses the molting by affecting the CYP gene expression in Y-organs. The role of Halloween genes in insect molting is mediated by initiating the transcription factor FTZ-F1 observed in Drosophila [60] Similarly though the Orthologues of EcR, USP, FTZ-F1 were found in crustaceans but their possible contribution in molting is not clear and which needs further research in this direction [61].

Toxicity of Chemicals that Mediated Endocrine Disruption on Growth

Ecdysteroids regulate aspects of embryo development, growth (molting), and reproduction (perhaps vitellogenin synthesis). Accordingly, chemicals that interfere with ecdysteroid signaling have the potential to elicit profound adverse effects on crustacean populations. Chemicals with anti-ecdysteroidal activity in crustaceans have been identified that function as either ecdysteroid synthesis inhibitors or ecdysteroid receptor antagonists. Chemicals with anti-ecdysteroidal activity include many of the classic estrogen receptor agonists of vertebrate. However, studies with ecdysteroid-responsive insect cells have demonstrated that non-steroidal EcR agonists are rare [62]. The binding of an environmental chemical to the EcR will more likely result in inhibition of ecdysteroid signaling. Testosterone exposure causes abnormal embryo development of daphnids similar to that observed with fenarimol [63]. Administration of exogenous 20-hydroxyecdysone protected embryos against this toxicity of testosterone indicating that testosterone interfered with normal ecdysone signaling. Additional studies indicated that testosterone elicited anti-ecdysteroidal activity by inhibiting the EcR [64]. The binding of an environmental chemical to the EcR will more likely result in inhibition of ecdysteroid signaling. Among chemicals shown to elicit 20-hydroxyecdysone-like activity in crustaceans are ponasterone A and RH 5849. Ponasterone A is a steroid that was first isolated from plants that has high-ecdysteroidal activity in insects [65]. Exposure of D. magna to ponasterone A stimulated premature ecdisys [66]. RH 5849 accelerated molting; and, in the barnacle B. amphitrite, RH 5849 enhanced attachment and metamorphosis of the larvae [67]. Recent development of a crustacean EcR reporter gene construct may stimulate screening efforts aimed at identifying chemicals that harbor this activity [68]. Several studies have reported effects of environmental chemicals that are consistent with interference with ecdysteroid signaling, though a precise mechanism of action was not established. The chemicals 4-nonylphenol [69], propiconazole [70], and bisphenol A [71], have elicited effects in crustaceans consistent with anti-ecdysteroidal activity. The fungicides propiconazole [72] and fenarimol [73] inhibit cytochromeP450 (CYP) enzymes that are critical to ecdysteroid synthesis. Both of these chemicals may inhibit ecdysteroid synthesis through this enzyme inhibition. The 4-Nonylphenol is an antagonist of the insect ecdysteroidreceptor in vitro [62]. Bisphenol A was proposed to elicit anti-ecdysteroidal activity through a receptor cross-talk.
mechanism [71,74]. Thus, it is mechanistically plausible that all of these compounds elicit toxicity to crustaceans via perturbations in ecdysteroid signaling. Relatively few studies have been performed that evaluated perturbations in ecdysteroid signaling in crustaceans by xenobiotics.

Axenobiotics is a foreign chemical substance found within an organism that is not normally, naturally produced by or expected to be present within that organism and which affects the organism. In this regard the crustacean industry nowadays facing many problems due to increasing urbanization day by day i.e. contamination of water bodies by harmful pesticides, metals, plastic and sewage, from agriculture and industrial activity which are getting assimilated and affecting the normal physiological process of aquatic organism as shown from (Figure 2). Various estrogenic compounds such as Arochlor 1242, PCB29, DES, endosulphan, diethyl phthalate and are detected in the fatty tissues and are studied to affect molting [75]. It is found to effect or delay molting either by disrupting the synthetic pathway of chitin of exoskeleton by degrading the enzyme chitobiase (N-acetyl-β-glucosaminidase) or by disturbing the ecdysteroid receptor axis (EcR) which heterodimerize with crustacean retinoid X receptor (RXR) [76] an central event in molting.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Types</th>
<th>Effect on ecdysis</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Metals</td>
<td></td>
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<tr>
<td>Lead</td>
<td></td>
<td>Delays molt in U. pugilator</td>
<td>Weis 1978</td>
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<td>Zinc</td>
<td></td>
<td>Less inhibitory in U. pugilator</td>
<td>Weis 1980</td>
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<tr>
<td>Mercury</td>
<td></td>
<td>Retards limb regeneration. Delay molting in U. pugilator</td>
<td>Weis 1980</td>
</tr>
<tr>
<td>Selenium</td>
<td></td>
<td>Delays molting in Daphnia magna. Alters chitin molt.</td>
<td>Schudde et al. 1990</td>
</tr>
<tr>
<td>TBT</td>
<td></td>
<td>Inhibits calcium resorption. Retards molting in follicle emb C. rajadhari</td>
<td>Nalgudi et al. 1990, Weis et al. 1987</td>
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<tr>
<td>Pesticides</td>
<td>Xenobiotics</td>
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<tr>
<td>DDT, Organochlorine compounds, Diabenzofurans.</td>
<td></td>
<td>No-effect of molting.</td>
<td>Weis et al. 1997b</td>
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<td>PCB, Aroclor 1242, Diethylphthalate</td>
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<td>Chitin inhibits molting in U. pugilator</td>
<td>Fingerman &amp; Fingerman 1977</td>
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<tr>
<td>Aromatic hydrocarbons benzene, Diaclathrinophthalates</td>
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<td>Delays molting in Daphnia magna</td>
<td>Cumwith et al. 1981, 1982</td>
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<tr>
<td>Cyclodiene, heptachlor</td>
<td></td>
<td>Delays molting in Bosurus americanus</td>
<td>Snyder and Mulder 2001</td>
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</table>

**Figure 2**: Effects of toxicants on endocrine disruption.

Adverse effects of some of Pesticides such as Organochlorine compound DDT, PCB, HCHs on molting were studied and found to accumulate in tissues of burrowing crabs C. granulata [77]. On the other hand no effect on molting on exposure of carbamate, Malathion and parathion and progressive molting on exposure to DDT was studies in U. pugilator.

Diflubenzuron (Dimilin) a chitin inhibitor was found to increase mortality near ecdysis on exposure to higher concentrations [78]. Baer and owens [79] investigated that Aroclor 1242, 2,4,5-trichlorobiphenyl (PCB29), diethylphthalate and Methoxycyl decreases chitobiase activity at epidermis and results in inhibition of molting in D. magna (Snyder and Mulder, 2001). Delay in molting by various pesticides Heptachlor in H. americanus [80] and dioxins, dibenzoferans, benzene and dimethylnaphthalene in C. sapidus are also reported. Similarly Feeding with 2,3,7,8-TCDD dioxin found to retard regeneration and molting in C. sapidus. Some endocrine modulators, estrogenic agents like DES and endosulfan are observed to delay the molting in Cladoceran. Diet containing sodium pentachlorophenate, 2,4,5-trichlorophenol or 2,4,6-trichlorophenol was found to retards limb regeneration, in Palaemonetes pugio but does not alters molt cycle [81].

Though metals such as sodium, potassium, calcium and magnesium are required for normal physiological functions of organism some heavy metals like cadmium, zinc, mercury, manganese, chromium, cobalt, nickel and selenium are very toxic to flora and fauna. Heavy metals are observed to interfere with the biochemical events involved in physiological process. These heavy metals interfere with hormones and manipulate their release and thus affect the physiological events like molting, limb regeneration, blood glucose levels and reproduction. In crayfish Astacus leptodactylus exposure to cadmium caused impairment of nuclear pynosis, mitochondrial dis organization, abnormal development and collapse of Golgi vesicles and fragmentation of endoplasmic reticulum by accumulating in central nervous system, thus affecting the normal physiological metabolism [82]. On exposure of cadmium results delayed molt in eyestalk ablation C. granulata [83] and P. clarkii [84]. On feeding 10 ppm cadmium for 10 days caused damage to neurosecretory cells in brain eyestalk ganglia and also observed that males developed resistance when compared to females [85]. Cadmium when fed in combination with lead and mercury got accumulated in the brain and inhibited central nervous system, sensory ganglia, and sulphhydryl group containing enzymes in crayfish P. clarkii. Zinc is also observed to have profuse effect on limb generation when combined with methyl mercury than alone and in combination with cadmium.

In addition to the effects of cadmium and zinc other heavy metals like Selenium delays molting in Daphnia magna, arsenic (in the form of CCA: (chromated copper arsenate) retarded regeneration in U. pugilator in a dose dependant manner, chromium affects the neurosecretory cells in brain and thoracic ganglia of the shrimp, Peneaus monodon and Lead, retards limb regeneration and molting of U. pugilator are also reported.

Organometallic compound Tributyltin (TBT) extensively used in antifouling paints also retards molting and produces abnormalities in regenerates. It gets interfered in the calcium reabsorption, an essential event in the molting and inhibits the exoskeleton formation as observed in C. rajadhari [86]. Reddy et al. [87] found the impact of TBT at initial and final stages of limb regeneration in freshwater prawn C. rajadhari. Further Reddy et al. [88] proposed that low dosage of TBT will not show any effect on first two molts, it shows significant changes after third molt.

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Page 6 of 8


