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Research Article

Behavioural Toxicity of Arsenic Trioxide: Alteration in Auto-Grooming Behaviour of a Freshwater Prawn, *Macrobrachium lamarrei*

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Abstract

Auto-grooming is the act of cleaning body parts which is a robust behaviour in *Macrobrachium lamarrei* (Arthropoda: Crustacea: Decapoda), a highly abundant native freshwater prawn species in India. The first and fifth pairs of thoracic appendages are the major grooming appendages, which show prominent signs of adaptive modifications. Grooming activity in *M. lamarrei* is a complex pattern, which is streamlined into two major groups, Anterior Grooming (AG) and Posterior Grooming (PG), depending on the body area. Anterior grooming is broadly divided into Carapace Grooming (CG) and Ventral Cephalothorax Grooming (VCG) and Posterior Grooming is further divided into Ventral Abdomen Grooming (VAG) and Dorsal Abdomen Grooming (DAG).

Prawns were exposed to 1.72 ppm of arsenic trioxide for 15 days and the dose was found to be non lethal. Therefore, we selected this non-lethal concentration for a 24 h exposure schedule to study different grooming patterns. We report for the first time that 1.72 ppm of arsenic trioxide induced notable auto-grooming alteration in this species and the prawns were found to spend considerably more time to groom each body part compared to the control. It is concluded that grooming patterns are reliable indices of stress or sensitivity towards heavy metals in aquatic invertebrates.

Keywords: Behavioural Toxicology; *Marobrachium lamarrei*; Auto-grooming

Introduction

Innate behaviours are genetically programmed in animals and are performed in a specific sequence, ethologically termed as fixed action pattern. It is a fact that behaviour is an instant expression of neural activities. Recent researchers are emphasizing behavioural evaluation to assess the neural activity in an animal body. Therefore, recent trends in neurobiological research include the assessment of behavioural markers. Auto-grooming or grooming is the process of taking care of body surface by removing unwanted microscopic organisms and particulate matters from the body surface. However, research on rodent grooming activity unravelled a wide variety of its purposes; auto-grooming make the animals more detectable and less dangerous to nearby conspecifics and it is a potential action to attract mates in voles [1]. Grooming has been extensively studied in rodents, mainly mice. The fixed action pattern of grooming in mice was described as a cephalocaudal event, which implies that grooming occurs in a specific sequence, which starts from the head and then proceeds to the lower body and finally terminates at the tail [2,3]. Grooming activity is a robust innate behaviour in animals and is considered as a marker owing to its sensitivity to numerous stressors, several neuronal drugs (both anxiolytic and anxiogenic drugs) and hormones (steroids) [4]. In mammals abnormal grooming is established as a manifestation of neuropsychiatric disorder [5].

Among invertebrates, the decapod crustaceans are widely known for their auto-grooming behaviour. The thoracic appendages are essential in executing routine tasks like feeding, defence, mate guarding, locomotion and auto-grooming. These appendages are adapted to perform auto-grooming and microscopic structures on the appendages make them effective for minute grooming activities [6]. In Hawaiian river prawn, *Macrobrachium grandimanus*, the first and fifth pairs of pereiopods, third maxillipeds and the major chelipeds are mostly used for grooming [7]. *Macrobrachium grandimanus* adopt interesting mechanisms for auto-grooming such as scraping of the whole body, brushing of different parts and also selecting a specific part of the body, which needs to be groomed. In *M. rosenbergii*, auto-grooming is classified as a secondary behaviour, which was defined as less important to other behaviours like feeding, mating and aggression [8]. Grooming activity in invertebrates was studied in decapod crustaceans (prawns), which mainly concentrated on the adaptive modifications in the appendages for grooming different parts of the body. There are a few reports on considering auto-grooming as a marker in case of stress in invertebrates; Corophium volutator, an amphipod (crustacean) demonstrated change in grooming frequency due to pollutant exposure [9].

Macrobrachium lamarrei is a major freshwater prawn species in India and adjacent countries. It is found in all types of freshwater bodies; thus we have selected this species to consider as a model organism to study behavioural toxicology.We aimed to analyse and compare the behavioural pattern of *M. lamarrei* in arsenic free and arsenic contaminated water; precisely to note the alteration in

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Table 1: Several environmental contaminants induced behavioural effects on *M. lamarrei.*

Contaminants	Treatment concentration (mg/L)	Treatment hours	Behavioural effects
Mercuric chloride	0.002	60	Loss of balance [11].
Copper sulphate	0.075	30	Scaphognathite oscillation and heart beat: increase and later decrease [12]; scraping of body parts by chelipeds; huge mucous secretion by the gill region [13].
Nickel chloride	65.77	96	Increase in locomotion, scraping of body surface by chelipeds and aggression. Whitening of the abdominal tissue along with heavy mucous secretion in the gill region, followed by decrease in locomotion and aggression and black marks on the margins of uropod. Loss of balance during walking [14].
Cadmium chloride	0.195	96	Loss of balance [11].
Aldrin	0.008,0.017,0.021	96	Hyperactivity [15].
Phosphamidon	0.66, 1.33,1.66	96	Unnatural swimming and aggression [16].

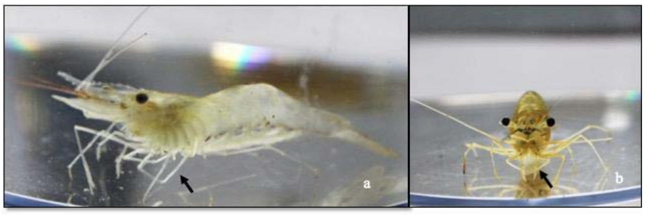

Figure 1: Cephalothorax grooming activity in *M. lamarrei* photographed from (a) side view and (b) frontal view. In photograph a, it is clear that the anterior tip first pair of chelipeds is inside the branchial chamber (through the ventral side of the cephalothorax). Black arrows in part a and b indicate the chelipeds.

Figure 2: Abdomen grooming in *M. lamarrei* photographed from (a) side view and (b) frontal view. White bottom arrow in the part a indicate the first pair of chelipeds cleaning the abdomen with eggs; while the top arrow shows the curvature taken by the prawn for the cleaning process. The bottom black arrow in the part b indicate the first pair chelipeds cleaning the ventral side of abdomen; while the top black arrow indicates the fifth pair of pereiopods cleaning the hard exoskeleton of the abdomen towards the telson region. The prawn in part b is not bearing any eggs.

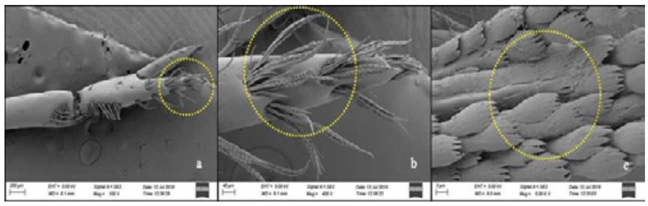

Figure 3: Electron micrographs of (a) whole anterior portion of the first cheliped; (b) higher magnification shows tufts of filaments in the tip of the cheliped or dactyl; (c) higher magnification shows microscopic structures on the filaments.

grooming activity due to toxic metalloid exposure. As grooming is already indicated as an index of stress in mammals [10] we have focused to address grooming as an index of heavy metal induced stress, sensitivity and alteration of neural activity in aquatic invertebrates (Table 1). Enlists the behavioural effects of different contaminants on *M. lamarrei.*

Methodology

Prior to an organized experiment, to evaluate the alterations of auto-grooming behaviour in *M. lamarrei*, preliminary laboratory observations revealed a specific auto-grooming behaviour pattern in this species. Prawns were sampled from ponds (natural habitat) and were maintained in the laboratory in clear tap water. Twenty prawns were acclimatised for seven days in arsenic free tap water; the toxicity range was predetermined; 1.72 ppm arsenic trioxide was found to be non toxic in an exposure regimen of 15 days. Thereafter prawns were transferred to thisnon-lethal dose for 24 hours for videography. Assessment of the behaviour pattern was undertaken by recording the behaviour of prawns executed in both clear tap water (control) and arsenic dosed water.The entire study and experiments were done in glass aquaria. Temperature was maintained between 25-270 C. Food (flour globules) was given ad libitum, once every day at 4pm considering their nocturnal habit. The recording was done in a Canon EOS DSLR camera (High Definition mode), using 18-55 mm lens, manual focusing, without a tripod. The video-graphic evaluation of auto-grooming in both arsenic contaminated and arsenic free water was conducted followed by manual quantification from the recorded videos. The quantification was done in terms of time spent for grooming.

Scanning electron microscopy

While analysing the behaviour pattern, the thoracic appendages were identified to be used effectively for the purpose of grooming. A prawn was sacrificed, and the appendages were dissected out and fixed in 3% glutaraldehyde overnight, to see the adaptive modifications by scanning electron microscopy (SEM). Samples for SEM were then dehydrated using 30% - 50% - 70% - 90% - 100% ethanol (30 minutes at each step). Critical Point Drying (CPD) was done to eliminate the alcohol and the super dried samples were then gold coated to observe under a SEM which was done at the University of Burdwan, West Bengal, India.

Results and Discussion

Analysis of auto-grooming patterns in *Macrobrachium lamarrei*

The body of *Macrobrachium lamarrei* is roughly divided into two parts; the outer hard exoskeleton (non-living part) and soft organs under the exoskeleton (like, gills mouth, eyes, abdomen, eggs). Grooming or cleaning of these parts is specifically executed by efficient

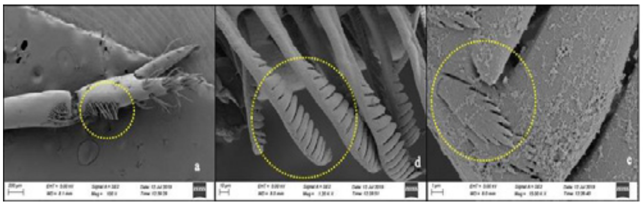

Figure 4: Electron micrographs of (a) whole anterior portion of the first cheliped; (d) higher magnification shows comb like structure on the propodus; (e) higher magnification shows microscopic projections on the combs.

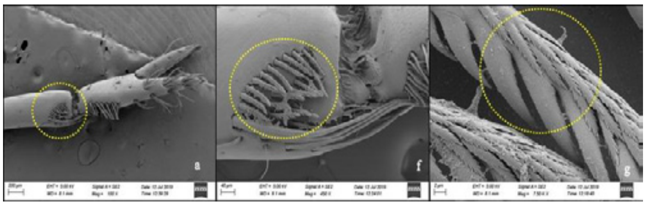

Figure 5: Electron micrographs of (a) whole anterior portion of the first chelipeds; (f) higher magnification shows dense setation on the propodus; (g) higher magnification shows microscopic leafy structures on the setae.

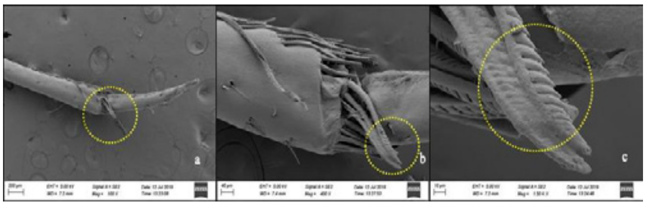

Figure 6: Electron micrographs of (a) whole anterior portion of the fifth pereiopod; (b) higher magnification shows dense setation (c) higher magnification shows microscopic structures on the setae.

use of the first pair of chelipeds and the fifth pair of pereiopods.

Depending on the intricate observation study of the grooming pattern, we have categorised the phenomenon as anterior grooming and posterior grooming, depending on the body regions. The anterior grooming broadly includes carapace grooming (exoskeleton) and cephalothorax (living part) grooming. Folding of the jointed first pairs of chelipeds enable them to approach inside the carapace to groom and clean the branchial chamber (Figure1a,b) and also to groom and clean the top and sides of the carapace.

Posterior grooming includes abdomen cleaning. Abdomen of a prawn is a segmented part with discrete joints, with relatively small postural degree of freedom. Apparently, a prawn's abdomen-grooming posture would depend on the relative angles of the abdomen. These angles make a favourable posture for the animal to clean the abdomen, both dorsal and ventral regions. The abdomen is covered by a hard dorsal exoskeleton. *M. lamarrei* cleans the ventral part of the abdomen with the first pair of chelipeds (Figure 2a) .Very rarely they use their second chelipeds to clean the abdomen. The fifth pair of pereiopods mainly cleans the dorsal part of the abdomen (Figure 2b).

Adaptive modifications in the grooming appendages of *Macrobrachium lamarrei*

Adaptive modifications of the grooming appendages have been

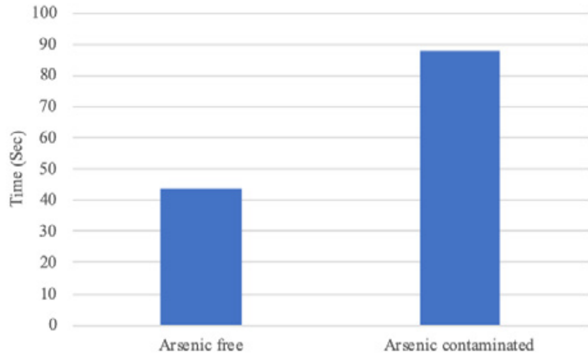

Figure 7: Comparative auto-grooming rate of *M. lamarrei* in both arsenic free and arsenic contaminated water.

described in different species [6,17]. Accordingly SEM analyses demonstrated in the present investigation intricate modifications in the key grooming appendages.

The above figures demonstrate the intricate, finer adaptations (microscopic structures) on the grooming appendages. The first pair of chelipeds is the major grooming appendage. Electron micrographs of this appendage show different types of setation on its anterior portion (Figure 3a). Higher magnifications show long filamentous grooming structures which originate in tufts or bunch from a socket like structure (Figure 3b). The filaments have human palm like structures with pointed finger like microstructure (Figure 3c). The propodus have two separate varieties of setae (Figures 4a & 5a). The first type on the propodus is comb like microstructures with blunt blades (Figure 4d). The comb blades have human palm like microstructures with pointed finger like projections (Figure 4e). The last type of setae on the propodus has bilayers of leafy structures with pointed ends revealed at the higher SEM magnification (Figures 5f,g).

The fifth pairs of pereiopods are secondary appendages used for grooming but not as frequently as the first pairs of chelipeds. It is used only for the grooming of the exoskeleton of the abdomen. The anterior region of the fifth pereiopods has dense setation bearing leafy or comb like microstructures with pointed blades (Figures 6a-c).

Microscopic study of the appendages clearly indicates that they are appropriately modified to increase the efficacy of the auto-grooming act. The first chelipeds are found to be responsible for cleaning most of the body; for this reason, they have diverse setation on them, which probably helps to efficiently clean the interior corners of the branchial chamber and the genital region. The fifth pereiopods are not used for interior cleaning as evidenced by a simple pattern of setation.

Macrobrachium lamarrei is a sensitive species and have a prominent grooming performance. Auto-grooming pattern is described in several other prawn species, however, unlike other prawns, *M. lamarrei* does not show prominent antennal grooming mechanisms. However, antennal grooming is very essential as the antennule and the antennal flagellum are sensory organs for chemoreception and olfaction.

Comparative profile of auto-grooming in arsenic free and arsenic contaminated water

Quantification of grooming activity in terms of time spent

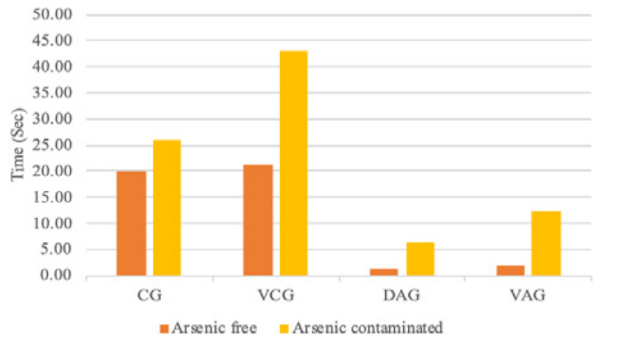

Figure 8: Comparative assessment of the subtypes of auto-grooming rate of *M. lamarrei* in both arsenic free and arsenic contaminated water.

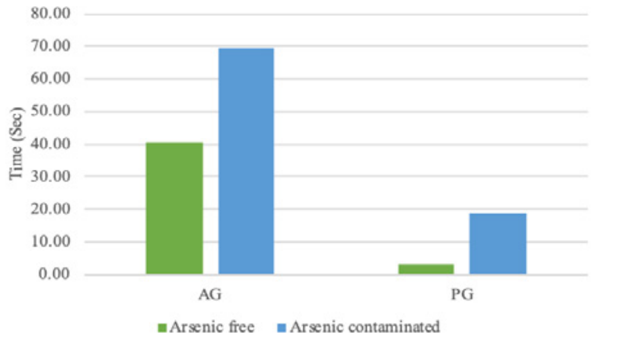

Figure 9: Comparative anterior grooming and posterior grooming rate of *M. lamarrei* in both arsenic free and arsenic contaminated water.

is a convenient way to distinguish between patterns. (Figure 7) demonstrates that in water contaminated with 1.72 ppm of arsenic trioxide noticeably increase in grooming activity in this species. This concentration of arsenic trioxide is not acute for this species. Depending on the auto-grooming pattern (described earlier) the process can be divided into four major groups CG = Carapace Grooming, where the carapace is groomed; VCG = Ventral Cephalothorax Grooming, where the inner part of cephalothorax is groomed through the ventral region; DAG = Dorsal Abdomen Grooming, where the dorsal part of the abdomen is groomed and VAG = Ventral Abdomen Grooming, where the ventral abdomen is groomed. The division is made considering the distribution of vital organs and body position. CG and VCG belong to anterior grooming (AG) and DAG and VAG represent posterior grooming (PG).

Subtypes of auto-grooming in *M. lamarrei* also demonstrate the same scenario (Figure 8). All the subtypes have enhanced grooming activity in the contaminated environment as compared to the arsenic free water. The mean rate of VCG, DAG, VAG in arsenic contaminated ambience is more than double the rate of VCG, DAG, VAG in arsenic free water (Figure 8).

Analysis of the subtypes of AG suggests that the mean rate of CG and VCG in arsenic free water are approximately same. However, in arsenic contaminated water, the mean rate of VCG is more or less double the mean rate of CG. Similarly, in PG, mean rates of DAG and VAG in arsenic free water do not differ much. However, in arsenic contaminated water, the mean rate of VAG is approximately twice the mean rate of DAG. The ventral region of the body, which is not covered by the exoskeleton, is groomed more in the arsenic contaminated water and arsenic is found to induce a higher rate of grooming activity in the soft visceral part of the body (Figure 8).

In normal arsenic free condition the average rate of AG is considerably higher than the rate of PG; probably, the anterior region of the body have relatively vital organs, morphologically more complex and susceptible to accumulation of more unwanted matters than the posterior region. In arsenic contaminated water, the mean rate of AG and PG increased significantly against the rate of grooming in arsenic free condition (Figure 9).

Conclusion

Arsenic is a known neurotoxicant, which can modulate neuronal activities in animals. Our study establishes a fundamental approach to consider grooming behaviour as an index or marker of sensitivity and stress caused by toxicants in aquatic invertebrates using *M. lamarrei* as a model. Studying robust behaviours can give us indications of the status of neuronal activities in an organism. This study further reveals a significant change in the pattern of grooming activity in *Macrobrachium lamarrei* due to a metalloid toxicity. We conclude that a non-lethal concentration of arsenic trioxide can trigger neuronal activity in such a way that it can induce changes in the grooming pattern of the study organism, within only 24 hours of exposure.

References

1. Ferkin MH, Leonard ST. Self-grooming as a form of olfactory communication in meadow voles and prairie voles (Microtus spp.). Kalueff AV. LaPorte JL. Bergner CL, editors. In: Neurobiology of grooming. Cambridge University Press. 2010; 19-45.
2. Berridge KC, Aldridge JW, Houchard KR, Zhuang X. Sequential super-stereotypy of aninstinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. BMC Biol. 2005; 3: 1–16.
3. Fentress JC. Expressive contexts, fine structure, and central mediation of rodent grooming.Ann N Y Acad Sci. 1988; 525:18–26.
4. Kalueff AV, LaPorte JL, Bergner CL. Neurobiology of grooming behaviour. New York: Cambridge University Press. 2010.
5. Kalueff AV, Stewart AM, Song C, Berridge KC, Graybiel AM, Fentress JC. Neurobiology of rodent self-grooming and its value for translational neuroscience. Nat Rev Neurosci. 2016; 17: 45-49.
6. Bauer RT. Adaptive modification of appendages for grooming (cleaning, antifouling) and reproduction in the crustacea. Watling L. Thiel M, editors. In: Functional morphology and diversity. University Press Oxford. 2013; 327-364.
7. VanMaurik LN, Wortham JL. The grooming behaviour of the Hawaiian river prawn Macrobrachium grandimanus. J Crustac Biol. 2011; 31: 617-622.
8. VanMaurik LN, Wortham JL. Grooming as a secondary behavior in the prawn Macrobrachium rosenbergii (Crustacea, Decapoda, Caridea). Zookeys. 2014; 457: 55-77.
9. Kirkpatrick AJ, Gerhardt A, Dick JT, McKenna M, Berges JA. Use of the multispecies freshwater biomonitor to assess behavioral changes of Corophium volutator (Pallas, 1766) (Crustacea, Amphipoda) in response to toxicant exposure in sediment. Ecotox Environ Safe. 2006; 64: 298-303.
10. Kalueff AV, Aldridge JW, Laporte JL, Murphy DL, Tuohima P. Analyzing grooming microstructure in neurobehavioral experiments. Nature Protoc. 2007; 2: 2538-2544.
11. Murti R, Shukla GS. Acute toxicity of mercuric chloride and cadmium chloride to freshwater prawn, Macrobrachium lamarrei (H. Milne Edwards). Acta

Hydrochim Hydrobiol. 1984; 12: 689-692.

12. Singh P. Effect of sub-acute exposure of copper sulphate on oxygen consumption and scaphognathite oscillations of fresh water prawn Macrobrachium lamarrei (Crustacea- Decapoda). Int J Adv Res. 2014; 2: 88-93.

13. Lodhi HS, Khan MA, Verma RS, Sharma UD. Acute toxicity of copper sulphate to fresh water prawns. J Environ Biol. 2006; 27: 585-588.

14. Verma RS. Acute toxicity of nickel to fresh water prawns. Turk J Zool. 2012; 36: 534-542.

15. Omkar, Murti R, Shukla GS. Effect of aldrin on the carbohydrate metabolism of a freshwater prawn Macrobrachium lamarrei (H.M. Edwards) Crustacea, Decapoda. Acta Hydrochim Hydrobiol. 1984; 12: 549-552.

16. Omkar, Upadhyay VB, Shukla GS. Impact of phosphamidon on the carbohydrate metabolism of a freshwater prawn, Macrobrachium lamarrei. Environ Res. 1986; 41: 591-597.

17. Wortham JL, VanMaurik LN, Price WW. Setal morphology of the grooming appendages of Macrobrachium rosenbergii (Crustacea: Decapoda: Caridea: Palaemonidae) and review of decapod setal classification. J Morph. 2014; 275: 634-649.