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Effect of inorganic arsenic (As III) on growth and feeding of the Indian River prawn *M. malcolmsonii*

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Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT:

Experiments were conducted to study the effect of sodium arsenite (As III) on survival, growth, and feeding of Indian River prawn *Macrobrachium malcolmsonii*. Significant differences ($p<0.05$) in prawn survival were observed between 0.10 and 0.50mg/l of arsenic levels. Arsenic had a significant ($p<0.05$) effect on prawn growth as the prawns grew faster at control compared to 0.5 and 0.75mg/l of arsenic. Arsenic level in water had a significant effect on the feed intake of prawns. Feed intake increased in the 0.25mg/l of arsenic levels compared with 0.50 and 0.75mg/l of arsenic levels. Feed intake ranged from 3.06±0.24% body weight at control (without arsenic) to 2.48±0.22% at the 0.75mg/l of arsenic level. In the muscle tissue of the prawn, a pronounced effect of arsenic was noted on calcium ATPase. Activities of this enzyme and also magnesium ATPase decreased in the muscle tissue of the prawn with an increase of arsenic concentrations.

Key Words:

Arsenic, survival, growth, feed intake, ATPase, *Macrobrachium malcolmsonii*

INTRODUCTION:

Arsenic (As) is ubiquitous and toxic trace element. Both inorganic and organic forms of arsenic are present in the environment and the inorganic form seems to be more toxic and slightly more accumulated in some aquatic species than the organic form. Trivalent As may show an adverse effect on aquatic biota and is considered more toxic than the inorganic pentavalent form (Hall and Buston 1982). Arsenic contamination is mainly caused by the use of arsenic pesticides, industrial activities including its profuse uses in textiles, clothes, dyes, depilatory agent, glass, enamels and mining operations. Arsenic contamination of ground-

water is a natural occurring high concentration of As in deeper levels of groundwater, which became a high-profile problem in recent years due to the use of deep tubewells for water supply in the Ganges Delta, causing serious arsenic poisoning to large numbers of people. A 2007 study found that over 137 million people in more than 70 countries are probably affected by As poisoning of drinking water. Arsenic contamination of ground water is found in many countries throughout the world including the USA (Twarakavi and Kaluarachchi 2006).

Arsenic is a carcinogen which causes many cancers including skin, lung, and bladder as well as cardiovascular disease. The occurrence of Arsenic in ground water was first reported in 1980 in West Bengal in India. In West Bengal, 79 blocks in 8 districts have Arsenic beyond the permissible limit of 0.05 mg/l. The most affected areas are on the eastern side of Bhagirathi river in the districts of Malda, Murshidabad, Nadia, North 24 Parganas and South 24 Parganas and western side of the districts of Howrah, Hugli and Bardhman. The occurrence of Arsenic in ground water is mainly in the aquifers upto 100 m depth. The deeper aquifers are free from arsenic contamination. Apart from West Bengal, arsenic contamination in ground water has been found in the states of Bihar, Uttar Pradesh, Assam & Chhattisgarh. Arsenic in ground water has been reported in parts of 15 districts in Bihar, 9 districts in Uttar Pradesh and one district each in Chhattisgarh and Assam (Central Groundwater Board, 2010). The presence of arsenic in ground water could be the source of this element in surface water depending on the suitable environmental conditions. There are few reports about the physiological and biochemical responses of fish to arsenic (Leah *et al.* 1992), whereas considerable attention has been given to the study of the effect of arsenic on human being and selected mammals (Biswas *et al.* 1998). Humtsoe *et al.* (2007) found that when *Labeo rohita* was exposed to arsenic, the activity of enzymes likes acid phosphates, alkaline phosphatases, glutamate pyruvate tranaminase and glutamate-oxaloacetate transaminase in muscle and liver was significantly reduced. The Indian river prawn *Macrobrachium malcolmsonii* is the second largest natantian of the world, after *M. rosenbergii*. This species is strictly restricted to the riverine region of India including Pakistan, Burma, Bangladesh and Srilanka. *M. malcolmsonii* is the only economically important river prawn, which migrates up to the point of origin of river systems and their small tributaries. Moreover, this species has also got tremendous potential for aquaculture practices. Nowadays, this valuable resource is in alarming stage due to the various human activities like overexploitation of broods and juveniles, discharge of industrial wastes, construction of dams etc. along the river.

In general, water that is free of toxic pollutants can be used for the aquaculture. However, some naturally occurring minerals can make some water more suitable than others. Generally in freshwater prawn hatchery and grow-out system, well water is frequently used as a major source of freshwater in all the stages of freshwater prawn culture practices in some states of India. However, this well water may contain arsenic, and some other metals, which may have some negative impact on growth and survival of aquatic animals either fish or crustaceans and could minimize the production as a result of reduction in growth rate and survival. Therefore, the present investigation was undertaken to study the impact of inorganic arsenic (As III) on the survival, growth, some biochemical parameters and feeding of the Indian river prawn *Macrobrachium malcolmsonii*.

MATERIALS AND METHODS:

Collection of prawn

Two hundred juveniles of giant river prawn *M. malcolmsonii* were collected from Kalinga hatchery, Guriapukari, 5km from the institute. The juveniles were 90 days old having an average weight of 6.07± 1.2g and average length of 7.3± 1.5cm of the same stock. Maximum care was taken while transporting the juveniles from the hatchery site to the laboratory and juveniles were brought in buckets of well-oxygenated water and stocked in ten plastic buckets, thirty liter of well-oxygenated water. The juveniles were acclimatized for seven days under laboratory condition (temperature 26.0±1.0°C, conductivity 313±0.005, dissolved oxygen 6.2±0.5mg/l, pH 7.3±0.5, alkalinity130±3mg/l as $CaCO_3$, and water hardness 107±2mg/l as $CaCO_3$). During acclimatization, water exchange was done on

alternate days and the prawns were fed with pelleted feed @2% body weight twice daily at 0900h and 1800h. The experiment was conducted in 15 circular plastic tough of 50 l capacity. Tap water was used for the experiment. Each tank was provided with an aeration system for oxygenating the tap water continuously.

Arsenite treatment

Stock solution of 1000 mg/l arsenic was prepared by dissolving required quantity of sodium arsenite ($NaAsO_2$) in deionized water. Four concentrations of arsenic (0.10, 0.25, 0.50, 0.75 mg/l) were used for this experiment in four different toughs along with controls in separate toughs to assess their effect on the growth and feed utilization of *M. malcolmsonii*. The doses of arsenic were chosen arbitrarily. Three replicates were maintained for each concentration of arsenic and also for the controls. No addition of any chemicals was made to the controls. The medium was thoroughly mixed after the addition of chemicals in the tanks and the water is replaced by respective concentration by water exchange of arsenic in every alternate days. Six juveniles were kept for study in each tank for 45days exposure (this duration is sufficient to study the effect of arsenic on prawn) for all the concentrations including controls. All the tanks were aerated for 24h/day. Mussel shells and plastic pipes were used at the bottom of each tank to allow prawns to distribute themselves better throughout the water in order to reduce cannibalism associated with crowding.

Feed intake

Prawns were fed with pelleted feed @ 2% body weight twice daily at 0900h and1800h. The proximate composition of feed was analyzed well before using in the experiment (protein-40-43%, fat-4-8%, nitrogen free extract-30-33%, moisture-5-7% and ash-7-8%). Observations were made every morning at 0900h. Dead prawns and molted exoskeletons were removed from the tank whenever they appeared. Fecal matter was also siphoned off on alternate days and filtered then place in a petridish and dried in room temperature. The percentage utilization of feed was expressed as (A-B) X100/A. Where, A is the amount of feed supplied (gm) and B is the amount of feed remaining after 2 h of feeding.

Chemical analysis

The physico-chemical characteristics of water such as temperature, pH by electronic pH meter, dissolved oxygen by Winkler's method, total alkalinity by methyl red indicator and sulphuric acid titration method, total hardness by EDTA and eriochrome black-T indicator method and conductivity were measured by electronic conductivity meter. The physico-chemical characteristics of water were analyzed at the beginning of the experiment and at an interval of five days alternatively only to assess the physico-chemical characteristics of the water.

At the end of the experiment, weigh of each prawn in wet condition for all the treatments including controls was measured individually. Mortality was calculated for all the experimental treatments including controls. Average daily growth (g/d) was calculated at the end of the experiment. Total arsenic estimation in the whole body prawn was done by using the HG-AAS technique (Perkin-Elmer atomic absorption spectrophotometer equipped with a hydride generation system). Hydride generation was performed using a 3% (w/v) $NaBH_4$ in 1% NaOH solution. The radiation source was a hollow cathode lamp of arsenic used at a wavelength of 193.7 nm and a spectral slit width of 0.7 nm. Prawn samples were mineralized by wet way (Nitric acid 1:1 with hydrogen peroxide), then burned in a muffle furnace and reduced to As (III) by addition of KI in the ascorbic acid (Celechovska *et al.* 2005).

Enzyme analysis

For enzyme analysis, prawns *M. malcolmsonii* collected from the laboratory experimental tank at the end of the experiment and killed by decapitation, and muscle tissue were dissected out separately and placed in petridishes. Measured quantities of tissues at wet condition were taken in eppendorf tube for the analysis of calcium and magnesium ATPases (Roy and Chainy, 1996). For each treatment, five muscle tissues were taken as replicates. Enzyme activity was expressed as

µmol inorganic phosphate liberated from ATP per minute per mg protein. Protein concentration was determined by the procedure of Lowry et al. (1951).

Statistical analysis

The data were analyzed using PC-SAS programme for Windows, Release v6.12 (SAS Institute Inc., Cary, USA) to assess the level of significance at 5% level. Analysis of variance was performed with the parametric procedure of General Linear Model (GLM). Duncan's Multiple Range Test was used for comparison of data of each of the variables among the treatments.

RESULTS AND DISCUSSION

Some important water quality parameters from *M. malcolmsonii* juveniles exposed to waters of different arsenic levels had the following characteristics: temperature 26±2.6˚ C, pH7.31±0.2, conductivity 0.31±0.04 dS/m, dissolved oxygen 5.92±0.5 mg/l, total alkalinity 132±8 mg/l as $CaCO_3$, and total hardness 106±6 mg/l as $CaCO_3$. The water quality parameters were optimum for the growth of the prawn.

Survival rate, weight gain and average feed intake of *M. malcolmsonii* exposed to different concentrations of arsenic are shown in table 1. Survival rate of the prawn was maximum in control (without treatment) and minimum in the highest arsenic (0.75mg/l) level. Significant differences ($p<0.05$) in prawn survival were observed between 0.10 and0.50mg/l of arsenic levels. However, no significant differences in survivals were recorded among 0.25, 0.50 and0.75mg/l of arsenic levels. Survival rates of *M. malcolmsonii* juveniles following 45 days exposure to control (without arsenic), 0.10, 0.25, 0.50 and 0.75 mg/l of arsenic were 100, 93.3, 88.3, 83.3 and 83.3%, respectively. Arsenic had a significant ($p<0.05$) effect on prawn growth as the prawns grew faster at control compared to 0.5 and 0.75mg/l of arsenic. Average daily growth were 0.037±0.007, 0.033±0.004, 0.030±0.002,0.014±0.004 and 0.010±0.006 g/day at control, 0.10,0.25,0.50 and 0.75mg/l of arsenic levels, respectively. No significant differences ($p>0.05$) in prawn growth could be observed between 0.5 and 0.75mg/l of arsenic levels. Also, no significant differences ($p>0.05$) in prawn growth were noted among control, 0.10 and0.25mg/l of arsenic levels. Arsenic level in water had a significant effect on the feed intake of prawns. Feed intake increased in the 0.25mg/l of arsenic levels compared with 0.50 and 0.75mg/l of arsenic levels. Feed intake ranged from 3.06±0.24% body weight at control (without arsenic) to 2.48±0.22% at the 0.75mg/l of arsenic level. Significant differences ($p<0.05$) in feed intake were observed due to higher level of arsenic exposures. The highest As accumulation 0.07±0.01 mg/kg fresh weight) occurred at 0.75 mg As/l, whereas the lowest accumulation (0.008±0.002 mg/kg fresh weight) occurred at 0.10 mg As/l treatment. The As content in the whole body fish in control (without As treatment) was in trace amount. The present study demonstrates that the level of arsenic influences the survival, growth and feed intake of *M. malcolmsonii*. Mortality of juveniles increased with increasing arsenic concentrations. Further, *M. malcolmsonii* juveniles exposed to higher levels (0.50 and0.75mg/l) of arsenic showed a reduction in growth and feed intake compared with the control over 45 days of exposure. De Foe (1982) reported that survival and growth of fathead minnow for 30 days juveniles exposed to As (V) in flow-through condition were no different from control levels at the two lowest concentrations tested (100 and 530 µg/l) while low survival and decreased growth occurred at the highest concentrations tested (1500-14000 µg/l). Rankin and Dixon (1994) reported that feed behavior, survival and growth of rainbow trout exposed to arsenite for 181 days in flow through aquaria were significantly different from the control (<20 µg/l) at the highest exposure concentration (9640 µg/l) but were not significantly affected at the two lowest concentrations (760 and 2480 µg/l). Reduced growth in the post larval stages of Indian prawn, *Penaeus indicus* at 40, 80 and 160 µg/l was reported by Rajkumar (2013). However, in the present study the adverse effect was noticed in the juvenile stages of *M. malcolmsonii* at comparatively higher doses of 500 and 750 µg/l. The sensitivity of a species to growth effects caused by arsenic exposure may be influenced by the relative growth rate of the species. Moreover different life stages could also be one of the reasons for the different tolerance levels of a particular chemical.

Table 1. Effect of arsenic on mean (.SD) growth, survival and feed intake of juvenile *Macrobrachium malcolmsonii*, raised for 45 days. Values followed by the same superscripts in a column are not significantly different at the 0.05 levels.

Treatments (mg/L)	Initial wt. (g)	Final wt. (g)	Weight gain (g)	Average growth (g/d)	Survival (%)	Feed intake (% body wt)	Accumulation (mg/kg fresh weight)
Control	4.60±0.32	6.27± 0.35	1.67± 0.16a	0.037± 0.008a	100a	3.06± 0.24a	trace
0.10	6.39± 0.41	7.90± 0.18	1.51± 0.09a	0.033± 0.004a	93.3± 3.4a	3.04± 0.21a	0.008± 0.002a
0.25	7.53± 0.46	8.88± 0.12	1.35±0.08a	0.030±0.006a	88.3± 4.6a,b	3.00± 0.16a	0.018±0.006a
0.50	6.28± 0.24	6.93±0.24	0.65± 0.07b	0.014±0.006 b	83.3± 4.3b	2.74± 0.20b	0.047±0.008c
0.75	5.58± 0.32	6.04±0.16	0.46±0.08b	0.010± 0.008b	83.3± 4.6b	2.48± 0.22b	0.070± 0.010d

The mean and standard deviation of enzyme activities after exposure to arsenic are presented in table 2. In the muscle tissue of the prawn, a pronounced effect of arsenic was noted on calcium ATPase. Activities of this enzyme decreased significantly ($p<0.05$) in the muscle tissue of the prawn with an increase of arsenic concentrations. Though there was a decrease in magnesium ATPase activity in the muscle of the prawn exposed to the higher concentration of arsenic (0.75mg/l), the decrease was not significant. Humtsoe *et.al*. (2007) studied the effect of arsenic at 96 and 144 µg/l concentrations for 30 days on the activity levels of phosphatase, alkaline phosphatase, glutamate pyruvate transaminase and glutamate- oxalo acetate transaminase in muscle and liver tissues of *Labeo rohita*. They reported that all the enzyme activities were reduced and a significant variation in the activities of these enzymes was also noted after exposure to arsenic. It has been reported that the variation in enzymes activities in heavy metal treated fish is due to increased permeability of the cell as well as the direct effect of the heavy metal on the tissues (Roy 2002). Therefore, significant depletion of enzymes in the prawn exposed to arsenic observed in the present study can be attributed to increased arsenic levels in the muscle tissue. Further more, accumulation of arsenic in muscles could be the possible reason for variation of enzyme activities. Arsenic in the form of arsenate can also resemble phosphate, which is used by cells for energy and signaling. By displacing phosphate in enzymes or signaling proteins, arsenic can block energy production and normal cell signaling (Dartmouth toxic Metal Research 2005). Many of the toxicological effects of arsenic, especially the trivalent form as in the case of the present study are believed to be associated with its reaction with cellular sulfhydryl (-SH) groups (Peters 1949; Peters 1963; National Academy of Sciences 1977). Thus tissues rich in oxidative systems often affected particularly the gastrointestinal tract, kidney, liver, lung and epidermis. The overall effect produced by the consequent inhibition of enzyme systems essential to cellular metabolism is the depression of fat and carbohydrate metabolism and cellular respiration and thus, the reduced growth was observed in the present study at higher doses of arsenic. In this study, arsenic accumulation in the whole body of the fish increased with increasing concentrations of this element. Average levels of Arsenic in various fish tissues (muscle, gill and liver) of 10 fish species collected from the Salek lakes (Slovenia) ranged from 0.02 to 0.44 mg/kg ww, respectively (Petkovsek *et al.* 2011). Total arsenic value ranged from 0.72 to 2.23 mg/kg in rainbow trout (*Oncorhynchus mykiss*) muscle (Harkabusova 2009).In this study, no biota-like filamentous algae, net plankton, macro invertibrates, etc. were present except prawn, thus whatever small amount of As was detected in the prawn would have been reduced drastically within the normal ecological conditions of a pond.

Table 2. Enzyme activities in the muscles of *Macrobrachium malcolmsonii* exposed to arsenic for 45 days. Activity is expressed as .mol phosphate formed per mg protein (mg-1 protein) per minute. Values followed by the same superscripts in a column are not significantly different at the 0.05 levels. Values are mean of five determination± S.D.

Treatments (mg/L)	Ca^{2+} ATPase	Mg^{2+} ATPase
Control	0.134 ± 0.009^{a}	0.545 ± 0.004^{a}
0.10	0.076 ± 0.006^{b}	0.537 ± 0.003^{a}
0.25	0.068 ± 0.006^{b}	0.499 ± 0.004^{a}
0.50	0.046 ± 0.005^{b}	0.496 ± 0.003^{a}
0.75	0.019 ± 0.002^{c}	0.475 ± 0.003^{a}

CONCLUSION

The present study demonstrates that the level of arsenic influences the survival, growth, and feed intake of *M. malcolmsonii*. Mortality of juveniles increased with increasing arsenic concentrations. Further, *M. malcolmsonii* juveniles exposed to higher levels (0.50 and 0.75mg/l) of arsenic showed a reduction in growth, calcium and magnesium ATPase concentrations of the muscle and feed intake of the prawn compared with the control over 45 days of exposure.

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