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Research Article

PHYSIOLOGICAL RESPONSES OF HEART OF TAILLESS FRESH WATER FLEA *SIMOCEPHALUS VETULUS* (CRUSTACEA-CLADOCERA) UNDER COPPER SULPHATE STRESS

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ABSTRACT
The cladoceran "tailless fresh water flea" *Simocephalus vetulus* (Crustacea-Cladocera) is well established as a model for eco-toxicology and being the sensitive crustacean can serve as better bio-indicator of metallic pollution. The myogenic heart of the *S. vetulus* is regulated by inhibitory cholinergic neurons that modulate cardiac function, including heart rate and susceptibility to cardio-active drugs known to affect the human heart. Present study deals with the effect of copper sulphate (96hr LC_{50}, 25%, and, 10% of 96hr LC_{50} values, 0.037, 0.092, and 0.037mg/l respectively) on physiology of heart beat rate of *S. vetulus* for 96hr, 7, 14 and 21 day exposure. The mean heart beat rate of acute (96hr LC_{50}) was 297.8 and for control 418.6 beats/min., sub-acute (25% 96hr LC_{50}) 429.6, 392.6 and 345.4 beats/min. for 7, 14 and 21days respectively and for control 460.4, 450.0, and 465.2 beats/min. for 7, 14 and 21days respectively, and chronic (10% of 96hr LC_{50}) was 415, 398.8 and 385.6 beats/min. for 7, 14 and 21days respectively, comparing with control being 460.4, 450.0 and 465.2 beats/min. for 7, 14 and 21days respectively. The mechanisms of copper toxicity in reference to heart beat rate of *S. vetulus* have been discussed which can serve as better biomarker in reference to metal toxicity.

Keywords: *Copper Sulphate, Heartbeat, Simocephalus vetulus, Tailless Fresh Water Flea*

INTRODUCTION
Rapid industrialization, intensive chemical use in agriculture and anthropogenic activities had inverse effect on aquatic ecosystem there by adversely affecting flora and fauna (Tiwari *et al.,* 2008). Heavy metals like copper are common contaminants from agricultural and industries of minerals, petrochemicals, paints, where copper is often used as an algaecides, it may eventually reach water bodies as run offs (Nandini *et al.,* 2007; Blake *et al.,* 2014). Cu an essential element assists with metabolic activities and acts as a catalyst for cellular respiration. Excess copper can result in oxidative stress during displacement of zinc in proteins and disruption of internal ion balance in gills of aquatic organisms (Gheorghiu *et al.,* 2010).
In general, invertebrates are more sensitive than fish to copper toxicity (USEPA, 2007). Cu is one of the heavy metals, that is required at low concentrations but at higher levels it becomes toxic to zooplankton (Gama-Flores *et al.,* 2007).
Cladocerans are important aquatic organisms because they transfer energy from primary producers to consumers of higher trophic level, such as fish. At the same time, they are commonly used in measuring pollution level, drinking water quality and in testing of human body fluids, due to their macroscopic size, easy culture methods, short life span and parthenogenetic mode of reproduction (Hanazato, 2001; Smirnov, 2014).
The heart, in cladocera is reduced as a bulbous chamber in first true segment (Wagler, 1927), in Branchiura, heart is triangular located in last thoracic segment (Martin, 1932), in Decapoda, heart is triangular sac, situated in pericardial sinus, above the hepatopancreas (Lodhi *et al.,* 2009). Cladocerans possess an open circulatory system and a myogenic heart (Maynard, 1960; Yamagishi *et al.,* 2000; McMahon, 2001; Smirnov, 2014). Heart beats and the flow of hemolymph can be easily observed, as cladocera are translucent (Maynard, 1960; Smirnov, 2014). Toxic effect of heavy metals on cardiac

physiology has been studied in fishes (Al-Yousuf and El-Shahawi, 1999; Ashraj, 2005) and crustaceans (Tiwari *et al.,* 2008; More, 2011; Rajaretnam and Stanley, 2015). Toxicity of copper on physiology of heart beat has been investigated in crustaceans (Ketpadung and Tangkrock-olan, 2006; More, 2011; Singh, 2014; Rajaretnam and Stanley, 2015) and other aquatic animals (Scott and Major, 1972; Brown *et al.,* 2004). But less in reference physiology of heart beat to metal toxicity in cladocerans (Fernandez-Gonzalez *et al.,* 2011; Rajaretnam and Stanley, 2015).

S. vetulus are small crustaceans that are inexpensive, easy to care and translucent colors. The latter property allows to easily observing the beating heart without dissecting and sacrificing the animals. Keeping this in view, the present study was under taken to evaluate the effect of copper sulphate on the physiology of heart beat rate of the "tailless freshwater flea" *S. vetulus* due to the heavy metal toxicity.

MATERIALS AND METHODS

Culture and Maintenance

S. vetulus (Crustacea-Cladocera) were collected from the freshwater pond located near Ikkar railway station at Haridwar (Uttarakhand), India. The samples were brought to laboratory and their culture was prepared by method of Davis and Ford (1992).

Preparation of Toxicant: Stock solution of Copper sulphate ($CuSO_4.5H_2O$: AR: Merck Specialities Private Limited, Mumbai, India) was prepared by dissolving weighed amount of salt in double distilled water. Two to three drops of glacial acetic acid was added to stock solution so as to prevent the precipitation. The experimental medium was analyzed for its physico-chemical parameters (Temperature, pH, Dissolved oxygen and Total hardness) as per standard methods (APHA *et al.,* 2012).

Experimental Set up and Recording of Heart Beat: Acute, sub-acute and chronic effect of copper sulphate on physiology of heart beat rate was carried out on adults and neonates (age < 24 hr) of *S. vetulus* obtained by isolating from the stock culture.

The heart beat recording procedure was based on that of Baylor (1942). A small amount of cotton was placed into a 1.5 ml glass well. A *S. vetulus* was added with enough aged medium water to immerse the animal. The cotton prevented the *S. vetulus* from swimming but still allowed some movement. Heart beat rate was recorded by a Panasonic DMC-LZ8 camera. Heart beat rate was determined by playing a 06-second portion of the recording in slow motion with the help of movie maker software and counting the beats/min.

Statistical Analysis: All the experiments were replicated thrice and results were analyzed with Student's t-test and one-way ANOVA using Minitab software. All error bars represent the standard errors of the means.

RESULTS AND DISCUSSION

Results

S. vetulus possessed a myogenic heart. Like some arthropods, its heart was located dorsally, posterior to the brain, and was slightly flattened, elongated as translucent body and heart beat was easily observed without dissecting the animals (Plate: 1).

In the present study, exposure of *S. vetulus* to copper sulphate showed marked alterations in heart beat rate during acute, sub-acute and chronic test. The experimental medium was analyzed for its physico-chemical parameters (Temperature- 23±2°C, pH- 7.3±0.2, Dissolved oxygen- 6.5±0.2 mgL^{-1} and Total hardness- 290± 2.4 mgL^{-1}) as per standard methods of (APHA *et al.,* 2012). Acute effects of copper sulphate on physiology of heart beat of *S. vetulus* are summarized in Table 1, Figure 3, sub-acute Table 2, Figure 4 and chronic exposure are summarized in Table 2, Figure 5.

A significant decrease in heart beat rate was observed in response to 96hr LC_{50} (0.37mg/L) of copper sulphate, the decrease in heart beat rate being 297.8±4.17bpm in comparison to control being 418.6±5.30bpm.

The differences in heart beat rate of exposed and control of *S. vetulus* were moderately significant ($t= 4.00$, $p<0.01$) at 24h; highly significant ($t= 10.92$, $p<0.001$; $t= 13.00$, $p<0.001$ and $t= 17.89$, $p<0.001$) at

48, 72 and 96 hr. The overall fluctuations in heart beat rate from 24-96h were insignificant (F=1.22, p>0.05) in control and moderately significant (F=32.71, p<0.01) in exposed animals.

In sub-acute (25% of 96h LC_{50}) exposure the heart beat rate was also lower in experimental animals than controls throughout the experiments (Table 2 and Figure 4). The differences in heart beat rate in control and experimental animals were moderately significant at 7 day (t=4.25, p<0.01) and highly significant 14 day (t=9.05, p<0.001) and 21 day exposure (t=27.93, p<0.001). The overall fluctuations were found to be significant in control (F=2.99, p<0.05) and moderately significant in exposed (F=105.85, p<0.01) from 7 to 21 days of exposure.

In chronic (10% of 96h) exposure the heart beat rate was also lower in experimental animals than controls throughout the experiments (Table 2 and Figure 5). The differences in heart beat rate in control and experimental animals were highly significant at 7 day (t= 5.49, p<0.001), 14 day (t= 9.69, p<0.001) and 21 day exposure (t= 19.42, p<0.001). The overall fluctuations were found to be significant in control (F=2.99, p<0.05) and moderately significant in exposed (F=12.37, p<0.01) from 7 to 21 days of exposure.

S. vetulus exhibited a significant decrease in heart rate immediately following the 25% and 10% of the 96hr LC_{50} values the heart rate.

PLATE: 1

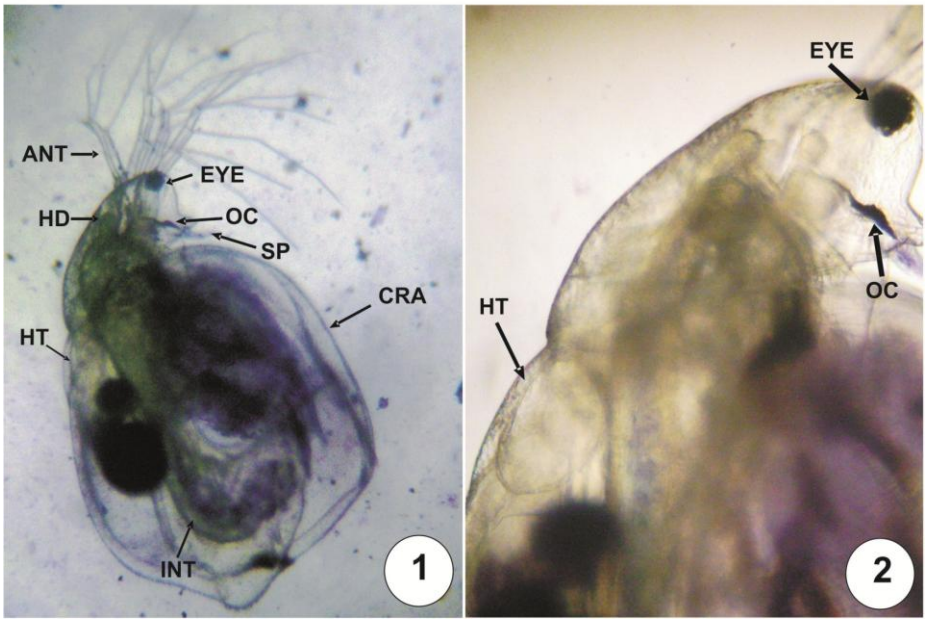

Figure 1: Photograph of Tailless Water Flea, *Simocephalus Vetulus*
Figure 2: Photomicrograph of Anterior Region of *S. Vetulus*
ANT=Antennae, CRA=Carapace, EYE=Eye, HD=Head, HT=Heart, INT=Intestine, OC=Ocellus, SP=Sensory Papillae

Table 1: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Acute Exposure

Duration of Exposure (in Hours)	Heart beat/min. (Mean ±SE)	
	Control	Experimental
24	409.8±5.88	369.8±8.08**
48	405.2±4.63	344.8±3.02***
72	412.6±4.30	334.8±4.15***
96	418.6±5.30	297.8±4.17***

Note- ** denote differences in mean to be moderately significant at p<0.01
*** denote differences in mean to be highly significant at p<0.001

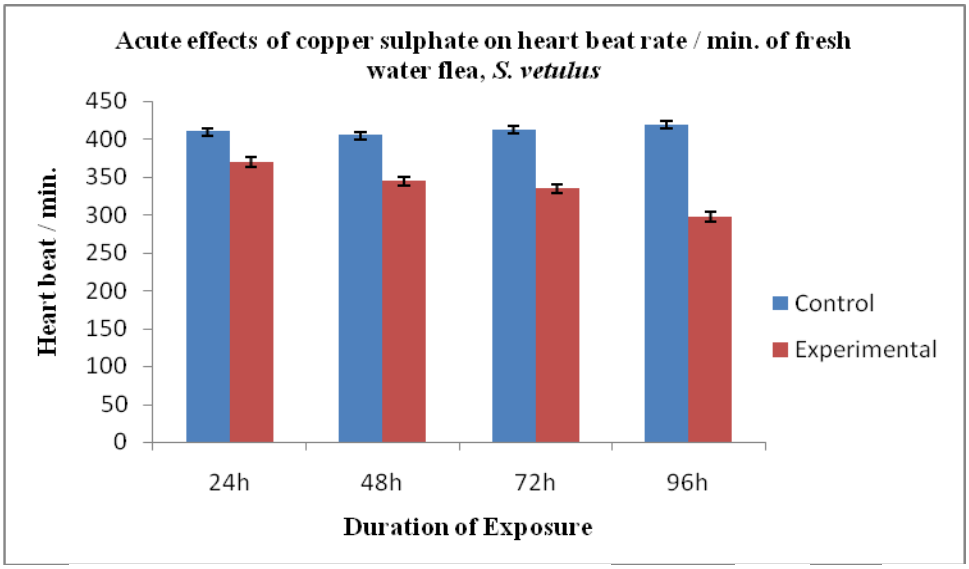

Figure 3: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Acute Exposure

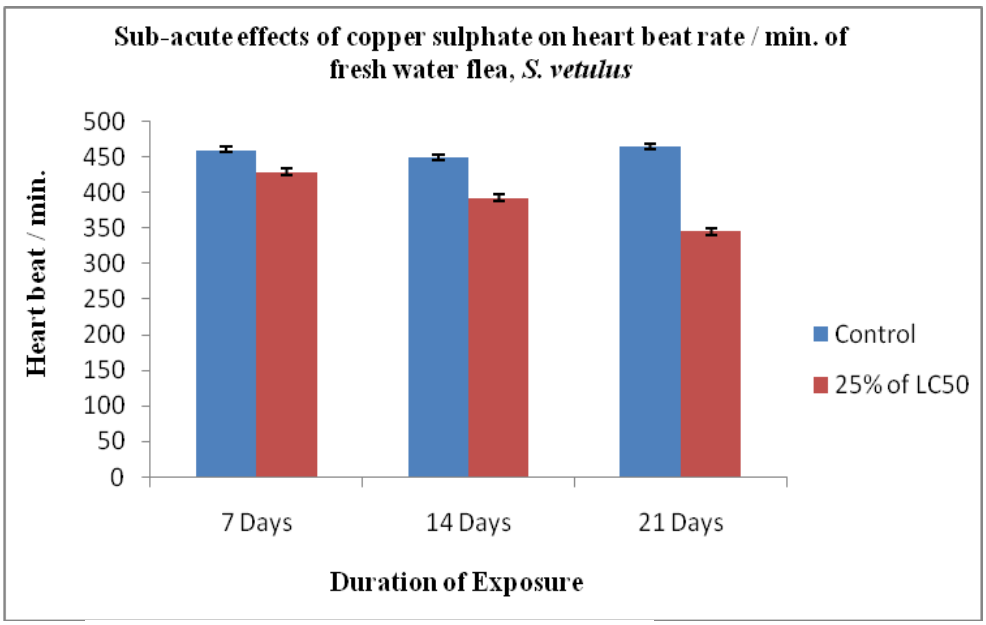

Figure 4: Effects of Copper Sulphate on Heart Beat/Min. of *S. vetulus* after Sub-Acute Exposure (25% of 96hr LC_{50})

Table 2: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Sub-Acute and Chronic Exposure

Duration of Exposure (Days)	Heart Beat/min. (Mean ±SE)		
	Control	Experimental	
		25% of 96h LC_{50}	10% of 96h LC_{50}
7	460.4±6.59	429.6±2.99**	415.0±5.00***
14	450.0±2.77	392.6±5.70***	398.8±4.49***
21	465.2±3.07	345.4±2.99***	385.6±2.71***

Note- ** denote differences in mean to be moderately significant at $p<0.01$
*** denote differences in mean to be highly significant at $p<0.001$

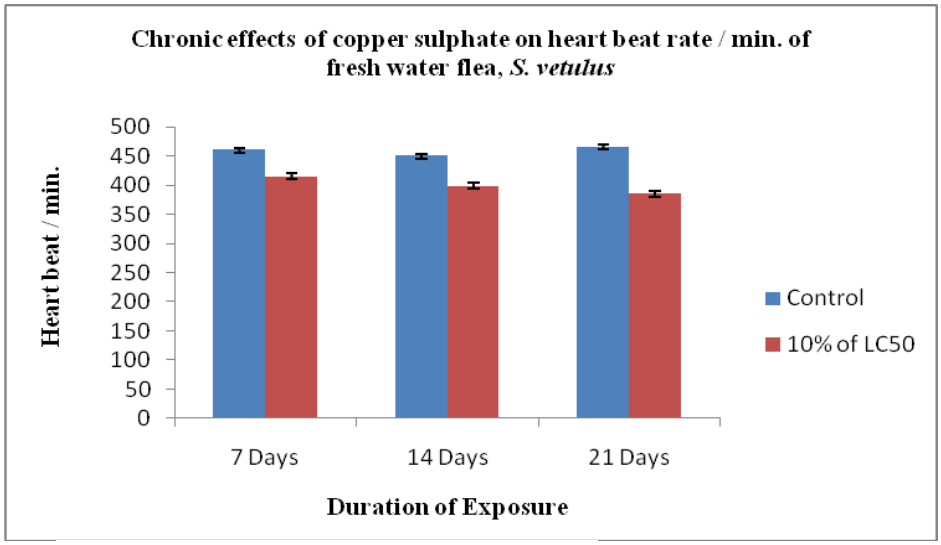

Figure 5: Effects of Copper Sulphate on Heart Beat/Min. of *S. Vetulus* after Chronic Exposure (10% of 96hr LC_{50})

Discussion

The results revealed that copper induced marked decrease in heart beat of *S. vetulus* during acute, sub acute and chronic exposures. Heart beat activity in cladocerans and crustaceans have been studied and mentioned to the wide range of stimuli *i.e.* tactile, chemical, pesticides, external electric field, temperature and hypoxic rate of breathing structures (Mac Mohan and Wilkens, 1975 (*Homarus americanus*); Usanov *et al.,* 1999 (*D. magna Straus*); Usanov *et al.,* 2001b (*D. magna*); Ketpadung and Tangkrock-olan, 2006 (*Portunus pelagicus*); Kaas *et al.,* 2009 (*D. magna*); Chung *et al.,* 2012 (*Procambarus clarkii* and *Macrobrachium rosenbergii*). The rate of heart beat is faster in smaller animals of a species and slower in larger species *Barytelphusa guerini* (More, 2011).

The heart beat rate is also affected and influenced by various environmental conditions and toxicant present in aquatic environment and can be used as sensitive bio-monitoring techniques for aquatic bodies (Ketpadung and Tangkrock-olan, 2006 ; Kaas *et al.,* 2009; Medesani *et al.,* 2011).

Various heavy metals also have direct effect on heart beat rate, which is very well known (Tiwari *et al.,* 2008; Kozak *et al.,* 2010). Copper sulphate toxicity showed significantly decreased heart rate throughout the experimental period, during acute (96hr LC_{50}), sub-acute (25% 96hr LC_{50}) and chronic (10% 96hr LC_{50}) exposure periods.

High concentration of copper sulphate, the thermoresistance of the cardiac muscle of *D. magna* is significantly decreased (Pashkova *et al.,* 1998).

This possibly result in decreased impulses in the cardiac nerves reduction the hemolymph flow so that the heart beat rate decrease under acute, sub-acute and chronic conditions to the toxicant. Decrease in heart beat rate observed in the present study is similar to observations of the other crustaceans *Barytelphusa guerini* (More, 2011). Heart beat frequency has been studied in many crustacean species in response to environmental stressors and chemicals (Tiwari *et al.,* 2008; Singh, 2014). Decrease in heart beat in later stage of experiment may be due to altered carbohydrate metabolism (Radhakrishnaiah and Bussapa, 1986). Crustacean's heart beat rate differs under different environmental conditions and rhythm varies in temperature, pH, size, sex, presence and absence of embryo (Seidl *et al.,* 2002; More, 2011). Changes in heart rate of other aquatic animals are well known indicators of some maladjustment in the environment (Williams *et al.,* 1991; Brown *et al.,* 2004; Kozak *et al.,* 2010).

Conclusion

It is evident from present investigation that heavy metal copper, despite being an micronutrient. Significantly lower the heart beat of Tailless water flea, *S. vetulus* in acute ($t=17.89$, $p<0.001$), sub-acute

(t=27.93, p<0.001) and chronic exposure (t=19.42, p<0.001). Due to their translucent colors heart beat can be counted without dissecting and sacrificing the animals. *S. vetulus* can serve as better bioindicator and heart beat rate can serve as biomarkers in reference to metal toxicity.

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REFERENCES

Al-Yousuf MH and El-Shahawi MS (1999). Trace Metals in *Lethrinus lentjan* Fish from the Arabian Gulf (Ras Al-Khaimah, United Arab Emirates): Metal Accumulation in Kidney and Heart Tissues. *Bulletin of Environmental Contamination and Toxicology* **62**(3) 293-300.

APHA, AWWA, WPCF (2012). *Standard Methods for the Examination of Water and Waste Waters*, 22nd edition, (APHA, Washington, USA).

Ashraj W (2005). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. *Environmental Monitoring and Assessment* **101**(1-3) 311-316.

Baylor ER (1942). Cardiac pharmacology of the cladoceran, *Daphnia*. *Biological Bulletin* **83** 165–172.

Blake D, Nar M, D'Souza NA, Glenn JB, Klaine SJ and Roberts AP (2014). Treatment with Coated Layer Double Hydroxide Clays Decreases the Toxicity of Copper-Contaminated Water. *Archives of Environmental Contamination and Toxicology* **66** 549–556.

Brown RJ, Galloway TS, Lowe D, Browne MA, Dissanayake A, Jones MB and Depledge MH (2004). Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. *Aquatic Toxicology* **66** 267-278.

Chung YS, Cooper RM, Graff J and Cooper RL (2012). The acute and chronic effect of low temperature on survival, heart rate and neural function in crayfish (*Procambarus clarkii*) and prawn (*Macrobrachium rosenbergii*) species. *Journal of Molecular and Integrative Physiology* **2** 75-86.

Davis L and Ford P (1992). *Toxicity Reduction: Evaluation and Control*, (Technomic Publishing Company Inc., Lancaster, Pennsylvania, USA).

Fernandez-Gonzalez MA, Gonzalez-Barrientos J, Carter MJ and Ramos-Jiliberto R (2011). Parent-to-offspring transfer of sublethal effects of copper exposure: Metabolic rate and life-history traits of *Daphnia*. *Revista Chilena de Historia Natural* **84** 195-201.

Gama-Flores JL, Castellanos-Paez ME, Sarma SSS and Nandini S (2007). Life table demography of *Ceriodaphnia dubia* (Cladocera) exposed to copper at different levels and periods. *Journal of Environmental Biology* **28**(3) 691-696.

Gheorghiu C, Smith DS, Al-Reasi HA, McGeer JC and Wilki MP (2010). Influence of natural organic matter (NOM) quality on Cu-gill binding in the rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* **97** 343-352.

Hanazato T (2001). Pesticide effects on freshwater zooplankton: an ecological perspective. *Environmental Pollution* **112** 1-10.

Kaas B, Krishnarao K, Marion E, Stuckey L and Kohn R (2009). Effects of melatonin and ethanol on the heart rate of *Daphnia magna*. *Impulse: The Premier Journal for Undergraduate Publications in Neuroscience* 1-8.

Ketpadung R and Tangkrock-olan N (2006). Changes in oxygen consumption and heart rate of the blue swimming crab, Portunus pelagicus (Linnaeus, 1766) following exposure to sublethal concentrations of copper. *Journal of Environmental Biology* **27**(1) 7-12.

Kozak P, Policar T, Fedotov VP, Kuznetsova TV, Buri M and Kholodkevich SV (2010). Effect of chloride content in water on heart rate in narrow-clawed crayfish (Astacus leptodactylus). *Know and Mana of Aquatic Ecology* **08** 394-395.

Lodhi HS, Shukla S and Sharma UD (2009). Studies on the structure of heart of two freshwater prawns under light microscope. *Uttar Pradesh Journal of Zoology* **29**(1) 01-10.

Mac Mohan BR and Wilkens JS (1975). Respiratory and circulatory responses to hypoxia in lobsters, *Homarus amercanus*. *Canadian Journal of Experimental Zoology* **50** 165-170.

Mano H, Ogamino Y, Sakamoto M and Tanaka Y (2011). Acute toxic impacts of three heavy metals (copper, zinc, and cadmium) on *Diaphanosoma brachyurum* (Cladocera: Sididae). *Limnology* **12** 193-196.

Martin MF (1932). On the morphology and classification of *Argulus*. *Proceedings of The Zoological Society of London* 755-806.

Maynard DM (1960). Circulation and heart function. In: Waterman, T.H. (edition), *The Physiology of Crustacea*. (Academic Press, New York, USA) **1** 161-226.

McMahon BR (2001). Control of cardiovascular function and its evolution in Crustacea. *Journal Experimental Biology* **204** 923-32.

Medesani DA, Cervino CO, Ansaldo M and Rodríguez EM (2011). Effects of parathion on cardiac rate, ventilatory frequency and hemolymphatic gas levels in the estuarine crab *Neohelice granulata* (Decapoda, Brachyura). *Journal of Toxicology and Environmental Health Sciences* **3**(3) 74-79.

More A (2011). Sub-lethal effects of copper sulphate, zinc sulphate and cadmium sulphate on rate of heart beat of fresh water crab *Bary Telphusa Guerini*. *Scholars Research Library* **2**(6) 437-440.

Nandini S, Picazo-Paez EA and Sarma SSS (2007). The combined effects of heavy metals (copper and zinc), temperature and food (*Chlorella vulgaris*) level on the demographic characters of *Moina macrocopa* (Crustacea:Cladocera). *Journal of Environmental Science and Health Part A* **42** 1433-1442.

Pashkova IM, Andronikov VB and Korotneva NV (1998). Changes in *Daphnia magna* cardiac muscle thermo resistance in copper sulfate contaminated medium. *Tsitologiya* **40**(4) 275-281.

Radhakrishnaiah K and Bussapa B (1986). Effect of Cd in carbohydrate metabolism of the fresh water field crab. *Oziotelphusa sensex*. *Journal of Environmental Biology* **7**(1) 17-21.

Rajaretnam AS and Stanley SA (2015). Studies on the toxicological effects of bimetals on the cladoceran, *Daphnia magna* and examination of histopathological effects through Transmission Electron Microscopy (TEM). *Journal of Chemical and Pharmaceutical Research* **7**(4) 506-511.

Scott DM and Major CW (1972). The Effect of Copper (II) on Survival, Respiration, and Heart Rate in the Common Blue Mussel, *Mytilus edulis*. *Biological Bulletin* **143**(3) 679-688.

Seidl MD, Pirow R and Paul RJ (2002). Water fleas (*Daphnia magna*) provide a separate ventilatory mechanism for their brood. *Zoology* **105** 15-23.

Singh P (2014). Effect of sub-acute exposure of copper sulphate on oxygen consumption and scaphognathite oscillations of fresh water prawn *Macrobrachium lamarrei* (Crustacea-Decapoda). *International Journal of Advanced Research* **9**(2) 88-93.

Smirnov NN (2014). *Physiology of the Cladocera*, (Academic Press, Elsevier) 196.

Tiwari KJ, Lodhi HS, Tripathi R, Shukla S and Sharma UD (2008). Effects on Lead on Heart Beat Rate of freshwater Prawn, *Macrobrachium dayanum* (Crustacea-Decapoda). *Environment and Ecology* **26**(2) 207-210.

Usanov DA, Shishkin GG, Usanov AD and Skripal AV (2001b). Effect of external alternating magnetic fields on heart rate of a freshwater crustacean *Daphnia*. *Biom Tekhnol Iradio* **8** 57-61.

Usanov DA, Skripal AV and Skripal AV (1999). Synchronization of the heartbeat rate of Daphnia by an external electric field. *Technical Physics Letters* **25** 157-158.

USEPA (2007). *Aquatic Life Ambient Freshwater Quality Criteria-Copper*, (Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington D.C, USA) EPA/822-R-07-001.

Wagler E (1927). Branchipoda, Phyltopoda. In: *Handbuch der Zoologic* **3**(1) (W. Kiskeenthal, W. & Krumbach, T. edition), (Der Goug, Berlin, Germany) 309-398.

Williams TM, Kooyman GL and Croll DA (1991). The effect of submergence on heart rate and oxygen consumption of swimming seals and sea lions. *Journal of Comparative Physiology B* 160 637-644.

Yamagishi H, Ando Y and Matsuzaki O (2000). Myocardial depolarizing response to glutamate in the myogenic heart of the branchiopod crustacean *Triops longicaudatus*. *Zoo Science* **17** 27-32.