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# Induced thermal stress on serotonin levels in the blue swimmer crab, *Portunus pelagicus*

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## ABSTRACT

The temperature of habitat water has a drastic influence on the behavioral, physiological and biochemical mechanisms of crustaceans. Hyperglycemia is a typical response of many aquatic animals to harmful physical and chemical environmental changes. In crustaceans increased circulating crustacean hyperglycemic hormone (CHH) and hyperglycemia are reported to occur following exposure to several environmental stress. The biogenic amine, serotonin has been found to modulate the CHH levels and oxidation of serotonin into its metabolites is catalysed by monoamine oxidase. The blue swimmer crab, *Portunus pelagicus* is a dominant intertidal species utilized throughout the indo-pacific region and is a particularly important species of Palk bay. It has high nutritional value and delicious taste and hence their requirements of capture and cultivation of this species are constantly increasing. This species experiences varying and increasing temperature levels as it resides in an higher intertidal zone of Thondi coast. The present study examines the effect of thermal stress on the levels of serotonin and crustacean hyperglycemic hormone in the hemolymph of *P. pelagicus* and analyzes the effect of the monoamine oxidase inhibitor, pargyline on serotonin and CHH level after thermal stress. The results showed increased levels of glucose, CHH and serotonin on exposure to 26 °C in control animals. Pargyline injected crabs showed highly significant increase in the levels of CHH and serotonin on every 2 °C increase or decrease in temperature. A greater CHH level of 268.86 ± 2.87 fmol/ml and a greater serotonin level of 177.69 ± 10.10 ng/ml was observed at 24 °C. This could be due to the effect of in maintaining the level of serotonin in the hemolymph and preventing its oxidation, which in turn induces hyperglycemia by releasing CHH into hemolymph. Thus, the study demonstrates the effect of thermal stress on the hemolymph metabolites studied and the role of pargyline in elevating the levels of serotonin and CHH on thermal stress in the blue swimmer crab, *P. pelagicus*.

## 1. Introduction

The temperature of habitat water has a drastic influence on the behavioral, physiological and biochemical mechanisms of crustaceans. Crustaceans have unique kind stress responsive mechanisms and they are well known to use that mechanisms controlled by neural and endocrine centers. Hyperglycemia is a typical reaction of crustaceans to harmful physical and chemical environmental changes. Hyperglycemia generally occurs following exposure to differing stressors, such as emersion [1], cold shock [2], anoxia and carbon dioxide [3], nitrite [4], pollutants [5] and parasitic infection [6] in crustaceans.

The release of glucose into the hemolymph is mediated by Crustacean Hyperglycaemic Hormone (CHH) through the mobilization of intracellular glycogen stores [7]. Researchers have noted that production of CHH after stressful periods during premolt is completely from the sinus gland (SG); apparently CHH from the gut is certainly not involved in the stress response [8]. Still other authors have suggested that non-SG-CHH could mediate a localized regulation of cellular glucose metabolism. Under periods of stress, localized release of CHH in the nervous system could be important in meeting the metabolic requirement of neural cells or play neuromodulatory roles in addition to endocrine functions [9]. Hence, although the contribution of this form of CHH may be insignificant to glucose regulation at the organism level, it could contribute to the regulation of the secreting organ local glucose metabolism [10].

Biogenic amines have been found to modulate their release of various neurohormones from crustacean neuroendocrine organs. Biogenic amines present in crustacean nervous systems [11,12] have been reported by Luschen et al. [13] to produce hyperglycemia in intact and eyestalk ablated shore crab, *Carcinus maenas*. The role of 5-hydroxytryptamine (5-HT/Serotonin) as an important neuromodulator of hormonal secretion has been well documented

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for several *crustacean spp.* [12]. Inhibition of the release of MIH by 5-HT has also been described by Mattson and Spaziani [14] in the crab, *Cancer antennarius. In vitro* and *in vivo* experiments carried out in the crayfish, *Procambarus clarkii*, and the crab, *Uca pugilator*, have shown that 5-HT stimulates the release of the stimulatory factor, Gonad stimulating hormone (GSH) [15]. Van Herp and Kallen [16] and Fingerman [17] have reviewed the stimulatory effect of 5-HT on CHH. Serotonin has been reported to stimulate CHH release from sinus glands of the crayfishes, *Orconectes limosus* and *Astacus leptodactylus* [18,19]. Serotonin increases the level of hemolymph glucose by inducing the release of crustacean hyperglycemic hormone in the crayfish *O. limosus* [18], in *Fenneropenaeus indicus* [20].

Direct demonstration of CHH release as a response to stressful conditions had to await methods for determination of circulating CHH in the hemolymph. Radioimmunoassays (RIAs) for CHHs of *C. maenas, O. limosus,* and *Cancer pagurus* have been developed and proved sensitive enough to measure CHH in relatively small hemolymph samples [21,22]. These assays have been used to measure CHH levels hemolymph under different experimental conditions in *O. limosus* [23], *Homarus americanus* [24] and in *C. pagurus* made hypoxic by emersion [22]. In addition, an ELISA, which proved to be of higher sensitivity than the RIA, has been developed for crab CHHs [25].

Serotonin is an amine neurotransmitter synthesized by enzymes that act on tryptophan and/or 5-hydroxytryptophan. Serotonin is stored in presynaptic vesicles and released to transmit electrochemical signals across the synapse. Serotonin is synthesized through 2-step process, involving a hydroxylation reaction (catalyzed by tryptophan-5-monooxygenase) and then a decarboxylation process catalyzed by aromatic L-amino acid decarboxylase. Serotonin is stored in presynaptic vesicles and released to transmit electrochemical signals across the synapse. Serotonin is then oxidized into 5-hydroxyindole acetaldehyde in the presence of monoamine oxidase (MAO) and its further catabolism into 5-hydroxylindole acetic acid is mediated by aldehyde dehydrogenase.

Pargyline is a monoamine oxidase inhibitor with antidepressant activity. Pargyline selectively inhibits MAO type B, an enzyme catalysing the oxidative deamination and inactivation of certain biogenic amines within the presynaptic nerve terminal. By inhibiting the metabolism of those biogenic amines in the brain, pargyline increases their concentration and binding to postsynaptic receptors. Increased receptor stimulation may cause down regulation of central receptors which my attribute to pargyline's anti-depressant effect.

Injection of pargyline has been found to inhibit the monoamine oxidase activity and elevate the presence of 5-HT content in the eyestalk and brain of *U. pugilator* [26]. Higher concentration of pargyline has been observed to result in a greater reduction of 5-HT degradation into 5-HTPH in the eyestalk of crayfish, *P. clarkii* [27]. The blue swimmer crab, *Portunus pelagicus* is a dominant intertidal species utilized throughout the indo-pacific region and is a particularly important species of Palk bay experiencing varying and increasing temperature levels. Thus a better understanding of the relationship between tolerance mechanisms against temperature in this species in laboratory condition may give rise to the possibility of more efficient control of their mortality and its higher degree of adaptability to thermal regimes. The present study examines (a) the effect of thermal shock on the levels of stress indicators i.e., hemolymph serotonin, CHH and glucose and (b) the activity of pargyline, the monoamine oxidase inhibitor, on the level of serotonin after thermal shock in the blue swimmer crab, *P. pelagicus.*

## 2. Material and methods

### 2.1. Animal collection and maintenance

Adult intermolt female crabs were procured from local fishermen of Thondi coast with the carapace length of 10 ± 1 cm and 80 ± 5 g wet weight. All the crabs were immediately transferred to the laboratory and introduced into the tank containing pre aerated filtered sea water. Crabs were acclimatized for one week in tanks containing 10–15 cm of sand at the bottom, at about 34 ± 2 ppt salinity and at a rearing temperature of 28 ± 0.5 °C and were fed with clam meat. Feeding was stopped 24 h prior to the experiment.

### 2.2. Heat shock treatments

The crabs were divided into four experimental groups consisting of 30 crabs each for stress treatments.

Experimental group 1: This experimental group was exposed to 24 °C.

Experimental group 2: The crabs were exposed to 26 °C.

Experimental group 3: The crabs were exposed to 30 °C.

Experimental group 4: This experimental group was exposed to 32 °C.

After 3 h of thermal stress, the experimental crabs were injected with either phosphate buffered saline or pargyline prepared in phosphate buffered saline. Groups of ten crabs each were injected with pargyline (100 mg/kg) or PBS was injected through the arthroidal membrane at the base of a swimming leg. The crabs that did not receive any injection served as control.

The crabs were then reintroduced into their ambient temperature (28 ± 0.5 °C). After a recovery period of 3 h, hemolymph was sampled from both control and experimental crabs and stored at −20 °C to access the levels of glucose, CHH and 5-HT.

### 2.3. Determination of hemolymph glucose levels

Determination of glucose was achieved by using the glucose oxidase method [28] in the Multiwell format. Hemolymph samples were thawed and centrifuged at 1000 rpm for 10 min at room temperature to drive Cell Free Hemolymph (CFH) from the sample. The glucose mono reagent (Span Diagnostics Ltd., India) of 1 ml was added to 10 μl CFH and was incubated for 10 min at room temperature. The sample (200 μl) was then added to well plates and read with the ELISA reader (Cyberlab Inc., USA) at 492 nm.

### 2.4. Quantification of hemolymph CHH and serotonin levels

HPLC-purified CHH standard from the crayfish *Orconectes limosus* (0.3–20 fmol; Fig. 1) and the anti-*Carcinus maenas*-CHH raised in rabbit were used for CHH quantification. Quantification of serotonin was carried out with serotonin creatinine sulfate standard (0.02–2500 ng/ml; Fig. 2) and the serotonin primary antibody raised in rabbit. Hemolymph CHH and 5-HT levels in the hemolymph were determined using indirect ELISA [29,30].

The cell free hemolymph samples (CFH) was mixed 1:1 v/v with coating buffer (0.2 M Sodium carbonate-bi carbonate buffer, pH-9.4) [5,22] and 100 μl was loaded in each well. The plate was incubated at 4 °C overnight. After washing with washing buffer (10 mM Phosphate Buffered Saline, pH-7.4 and 0.1% Tween 20) the plate was blocked with 100 μl of blocking buffer (10 mM PBS, 0.1% Tween 20, 2% BSA) for two hours at room temperature. After washing, the wells were incubated with anti-*Carcinus maenas*-CHH or anti-serotonin antibody (Sigma, S5545) (dilution 1:10000 in blocking buffer) for 2 h at room temperature. Wells were then washed and incubated with the secondary antibody, Anti-Rabbit

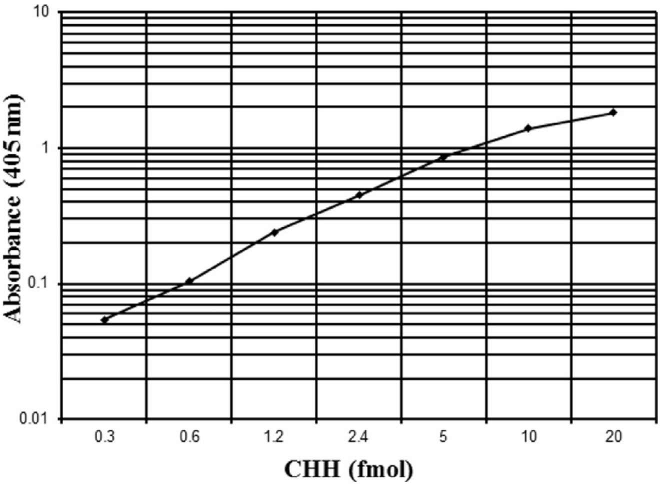

**Fig. 1.** Standard graph of Indirect ELISA using serially diluted CHH as standard.

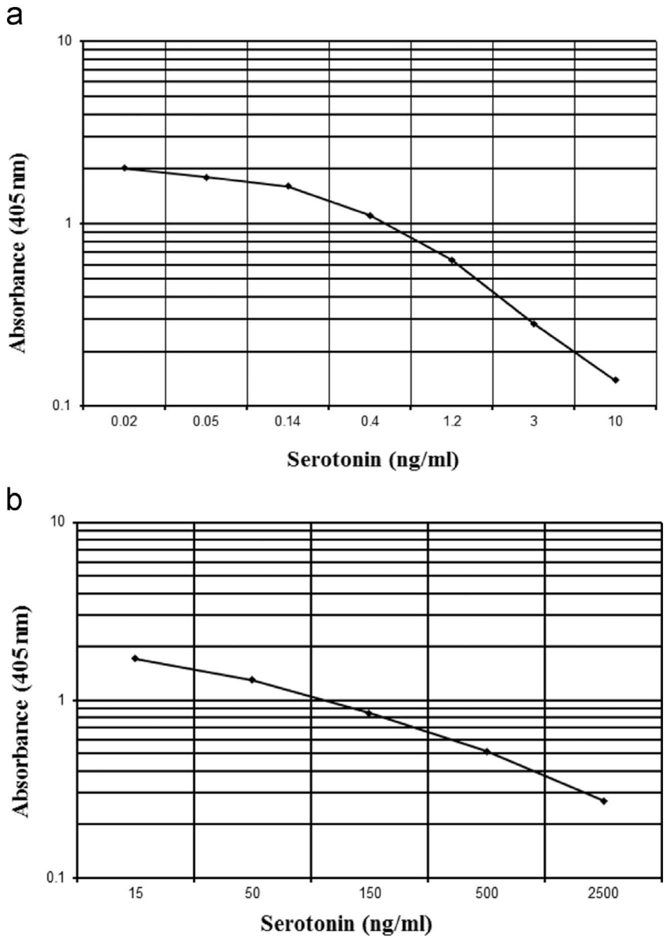

**Fig. 2.** (a) Standard graph of Indirect ELISA using serially diluted Serotonin as standard, (b) standard graph of Indirect ELISA using serially diluted Serotonin as standard.

IgG-Peroxidase (Sigma, A4914), (dilution 1:500 in blocking buffer) for 2 h at room temperature. Again, the wells were washed and 100 μl of TMB substrate (1 mg Tetramethyl benzoate, 1 ml DMSO, 9 ml 0.5 M Phosphate Citrate buffer, 2 μl 30% Hydrogen peroxide) was added into each well to initiate the enzymatic reaction. The plate was incubated in dark for 10–30 min at 37 °C. The reaction was stopped by adding 2 M $H_2SO_4$. Finally, multiwell plates were read at 450 nm on ELISA-reader (Cyberlab Inc., USA).

### 2.5. Statistical analysis

The results obtained in the present study were subjected to Student's *t*-test and Analysis of Variance (ANOVA) to determine whether the variations in the hemolymph constituents are statistically significant between the groups studied.

## 3. Results

### 3.1. Effect of thermal stress on hemolymph glucose

Crabs of the control group showed a significant increase in glucose level (215.7 ± 2.79 μg/ml) when there was 2 °C drop in the temperature (Fig. 3). Though there was an increase in the glucose level to 106.86 ± 2.86 μg/ml at 30 °C, the levels dropped back to that of the ambient temperature (75 ± 3.67 μg/ml) when the temperature was increased to 32 °C (75.34 ± 3.06 μg/ml) ($t < 0.01$).

Crabs that received PBS showed significantly lower levels of glucose both on heat and cold stress when compared to that of the ambient temperature (28 °C) (118.86 ± 2.59 μg/ml) ($t < 0.1$). Significant increase in the levels of glucose was observed in pargyline injected crabs on heat and cold shock, with the maximum being 406.92 ± 2.66 μg/ml at 4 °C increase in temperature (32 °C) ($t < 0.01$). Heat shock was able to increase the glucose levels on every 2 °C rise in temperature whereas decrease in the glucose level was observed on 2 °C fall in temperature in all the experimental groups. Elevated levels of hemolymph glucose was recorded on thermal stress in pargyline injected crabs when compared to control and PBS injected crabs ($P < 0.001$).

### 3.2. Effect of thermal stress on hemolymph CHH

Significant increase in hemolymph CHH level (182.96 ± 2.23 fmol/ml) was observed on 2 °C decrease in temperature (26 °C) in control crabs when compared to that of ambient temperature (145.98 ± 2.26 fmol/ml). Highly significant decrease in CHH level to 42.18 ± 1.91 fmol/ml was observed when the temperature was increased by 2 °C ($t < 0.001$) (Fig. 4).

Insignificant variations were observed in PBS injected crabs on 2 °C rise (78.82 ± 1.44 fmol/ml) or fall (56.74 ± 2.41 fmol/ml) in temperature than the crabs at 28 °C (73.05 ± 1.54 fmol/ml) ($t > 0.01$). Hemolymph CHH tend to increase gradually on heat and cold shock in pargyline injected crabs. Though the variations

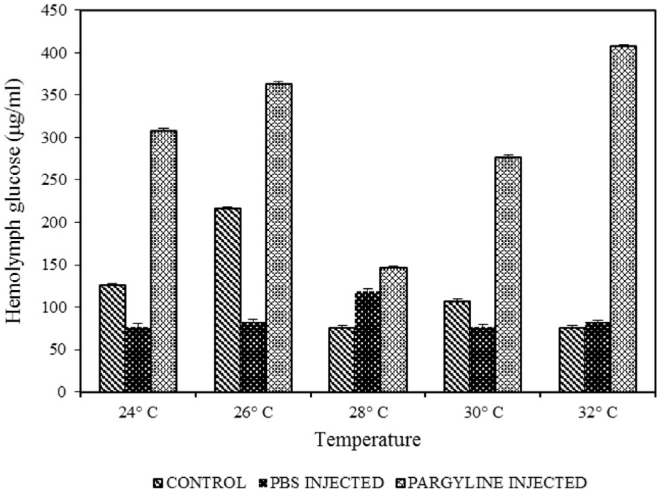

**Fig. 3.** Variations of hemolymph glucose level on thermal stress in *P. pelagicus*.

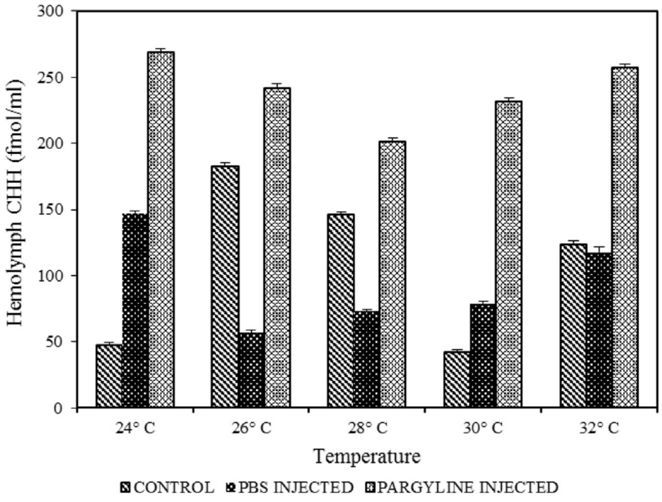

**Fig. 4.** Variations of hemolymph CHH level on thermal stress in *P. pelagicus*.

observed were less significant, increase in the CHH level was observed on every 2 °C rise or fall in temperature. A maximum CHH level of $268.66 \pm 2.87$ fmol/ml was observed at 24 °C ($t < 0.05$). Gradual increase in level of hemolymph CHH was observed on cold shock and heat shock in PBS treated and pargyline treated crabs with higher levels recorded in pargyline treated crabs ($P < 0.01$).

### 3.3. Effect of thermal stress on hemolymph serotonin

Increase in hemolymph serotonin level was observed on 4 °C fall ($94.87 \pm 2.60$ ng/ml) ($t < 0.01$) and rise ($77.16 \pm 3.12$ ng/ml) ($t < 0.05$) in temperature when compared to the ambient temperature ($71.66 \pm 1.92$ ng/ml) in control animals.

A short fall in the level of serotonin to $43.94 \pm 4.05$ ng/ml at 26 °C followed by a sudden increase to $93.53 \pm 3.77$ ng/ml at 24 °C was observed in PBS treated animals ($t < 0.01$). Pargyline injected crabs displayed a gradual increase in serotonin level with every 2 °C fall or rise in temperature. Maximum level of serotonin was observed at 24 °C ($177.69 \pm 10.10$ ng/ml) and at 32 °C ($166.62 \pm 11.29$ ng/ml) ($t < 0.001$). The hemolymph serotonin concentration of pargyline injected group crabs was significantly higher when compared to control and PBS injected group crabs ($P < 0.001$) (Fig. 5).

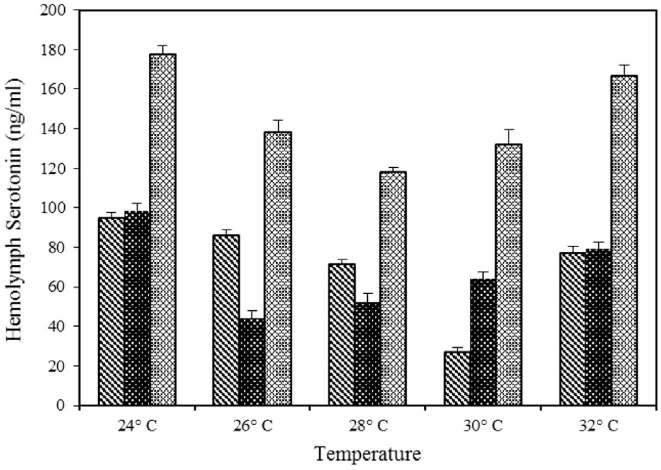

**Fig. 5.** Variations of hemolymph serotonin level on thermal stress in *P. pelagicus*.

## 4. Discussion

It is known from several crustacean studies that stressors of various natures (extreme temperature, hypoxia, organic and inorganic pollutants, bacterial infection, etc.) induce hyperglycemia and/or CHH release [22,29–33]. Hemolymph glucose concentration can change significantly with altered physiological and environmental conditions. Our study demonstrates that induced thermal stress has significant effect on the levels of hemolymph levels of CHH and glucose at 26 °C resulting in hyperglycemia, while even higher temperatures or lower temperatures can furthermore alleviate hyperglycemia. Similarly, an increase in water temperature was found to increase blood CHH in *C. pagurus* [34] and *P. clarkii* [35]. The increase of CHH in response to thermal stress may be related either to the hypoxic conditions existing in seawater and resulting from elevated temperatures or to increased general metabolism at higher temperatures. Our results are consistent with observations made on thermal stress on crabs by Chung and Webster [8].

Serotonin has long been known to have a potent hyperglycemic effect by controlling the production of CHH from its endocrine centers [36]. Studies of Lee et al. [37,38] suggest that serotonin induced hyperglycemia in *P. clarkii* is mediated by types 1 and 2 serotonin receptor. The inactivation of 5-HT by monoamine oxidase (MAO) was tested by using pargyline, an inhibitor of MAO activity. Significant increase in the hemolymph glucose, CHH and serotonin levels on pargyline injection was observed which may be due to the stop of oxidation of 5-HT by inhibiting monoamine oxidase, thereby retaining the maximum level of serotonin released during the stress. The results of the present study are in correlation with those demonstrated earlier in *U. pugilator* [26,27].

Fingerman et al. [26] have observed pargyline to prevent oxidation of 5-HT by monoamine oxidase. Consequently, an excess of 5-HT was suggested to be present in the nervous system and lead to abnormal release of red pigment-dispersing hormone in the crabs on a white background, resulting in red pigment dispersion. Rodriguez-Sosa et al. [27] reported that within the range 0.8–10 mmol $l^{-1}$, pargyline induces a dose-dependent increase of 5-HT content and a simultaneous decrease in the content of 5-HTPH. Control groups of eyestalks incubated in van Harreveld's solution did not show any significant changes in either 5-HT or 5-HTPH within the 2 h period. These researchers have suggested the two of the common mechanisms of 5-HT inactivation to be present in the crayfish eyestalk: uptake of 5-HT, which is blocked by fluoxetine [39,40], and 5-HT oxidation by MAO, which is inhibited by pargyline, as demonstrated earlier in *U. pugilator* [26,41] but not detected by Barker et al. [42] and Kennedy [43] in the lobster *H. americanus*.

We observed that thermal stress, which results in hyperglycemia, is a potent stimulator for the elevation of hemolymph CHH. Our ELISA is sensitive enough to monitor levels of CHH by repeated sampling of small volumes of hemolymph from the same animal. Handling and sampling did not stimulate CHH release into the hemolymph to a significant degree. Therefore, the influence of some environmental stresses could be studied without interference from this handling stress. Another advantage of the ELISA is that hemolymph samples can be assayed directly, without extraction or hormone enrichment procedures. Our results demonstrated that measurement of hemolymph CHH is a promising tool to monitor stress responses in crabs, and, more generally, to study the role of CHH in the metabolic regulation of crustaceans

The observations that pargyline evoked hyperglycemia during stress in the present study it is evident that MAO activity is present in the eyestalk of *P. pelagicus* and is consistent with the hypothesis that 5-HT functions normally as a neurotransmitter for release of CHH. The 5-HT depletors would cause a loss of 5-HT which would then be unavailable to elicit release of additional CHH. On the other hand, pargyline would prevent oxidation of 5-HT by monoamine oxidase. Consequently, an excess of 5-HT would be present in the nervous system and lead to hyperglycemia to meet the effect of stress on carbohydrate metabolites. Moreover, pargyline helps in the better quantification of glucose, CHH and 5-HT levels on thermal stress. And our research findings would pave way to study the effect a wide array of neurotransmitters on various neurohormones controlling different metabolic activities with the help of drugs such as pargyline and fluoxetine in crustaceans.

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## Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2015.11.005.

## References

[1] F. Durand, N. Devillers, F.H. Lallier, M. Regnault, Nitrogen excretion and change in blood components during emersion of the subtidal spider crab *Maia squinado* (L.), Comp. Biochem. Physiol. 127A (2000) 259–271.

[2] C.M. Kuo, Y.H. Yang, Hyperglycemic responses to cold shock in the freshwater giant prawn, *Macrobrachium rosenbergii* J. Comp. Physiol. 169 (1999) 49–54.

[3] M.R. Hall, E.H. Van Ham, The effect of different types of stress on blood glucose in the giant freshwater prawn, *Penaeus monodon* J. World. Aquacult. Soc. 29 (1998) 290–299.

[4] A.C.K. Benli, H.Y. Yildiz, Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*, Aquacult. Res. 35 (2004) 1388–1390.

[5] S. Lorenzon, M. Francese, E.A. Ferrero, Heavy metal toxicity and differential effects on the hyperglycemic stress response in the shrimp *Palaemon elegans*, Arch. Environ. Contam. Toxicol. 39 (2) (2000) 167–176.

[6] A. Powell, A.F. Rowley, Haemolymph changes in the shore crab, *Carcinus maenas,* infected by the parasite barnacle *Sacculina carcini*, Dis. Aquat. Organ. 80 (2008) 75–79.

[7] G.D. Stentiford, E.S. Chang, S.A. Chang, D.M. Neil, Carbohydrate dynamics and CHH effects of parasitic infection in Norway lobster *Nephrops norvegicus*, Gen. Comp. Endocrinol. 121 (2001) 13–22.

[8] G.M. Chung, S.G. Webster, Dynamics of *in vivo* release of molt inhibiting hormone and crustacean hyperglycaemic hormone in the shore crab, *Carcinus maenas.* Endocrinology 146 (2) (2005) 5545–5551.

[9] E.S. Chang, S.A. Chang, B.A. Beltz, E.A. Kravitz, Crustacean hyperglycemic hormone in the lobster nervous system: localization and release from cells in the sub-oesophageal ganglion and thoracic second roots, J. Comp. Neurol. 414 (1999) 50–56.

[10] E.S. Chang, S.A. Chang, E.P. Mulder, Hormones in the lives of crustaceans: an overview, Am. Zool. 41 (2001) 1090–1097.

[11] G.A. Kerkut, C.B. Sedden, R.J. Walker, The effect of DOPA, α-methyl DOPA and reserpine on the dopamine content of the brain of the snail, *Heliz aspersa*, Comp. Biochem. Physiol. 18 (1966) 921–930.

[12] M. Fingerman, R. Nagabhushanam, R. Sarojini, P. Sreenivasula Reddy, Biogenic amines in crustaceans: identification, localization and roles, J. Crust. Biol. 14 (1994) 413–437.

[13] W. Luschen, A. Willig, P.P. Jaros, The role of biogenic amines in the control of blood glucose level in the decapod crustacean, *Carcinus maenas*, Comp. Biochem. Physiol. **105C** (1993) 291–296.

[14] M.P. Mattson, E. Spaziani, 5-hydroxytryptamine mediates release of molt inhibiting hormone activity from isolated crab eyestalk ganglia, Biol. Bull. 36 (1985) 347–358.

[15] G.K. Kulkarni, R. Nagabhushanam, G. Amaldoss, R.G. Jaiswal, M. Fingerman, *In vivo* stimulation of ovarian development in the red swamp crayfish *Procambarus clarkii* by 5-hydroxytryptamine, Invert. Reprod. Dev. 21 (1992) 231–240.

[16] F. Van Herp., J.L. Kallen, Neuropeptides and neurotransmittes in the X-organ sinus gland complex, an important neuroendocrine regulation center in the eyestalk of Crustacea, in: G.B. Stefano, E. Florey (Eds.), Comparative aspects of Neuropeptides, Manchester University Press, Manchester, 1991, pp. 211–221.

[17] M. Fingerman, Endocrine mechanisms in crayfish with emphasis on reproduction and neurotransmitter regulation of hormone release, Am. Zool. 35 (1995) 68–78.

[18] R. Keller, J. Beyer, Zur hyperglykämischen Wirkung von Serotonin und Augenstielextrakt beim Flusskrebs *Orconectes limosus*, Z. Vergl. Physiol. 59 (1968) 78–85.

[19] G.E.C.M. Strolenberg, F. Van Herp, Mise en evidence du phenomene d'exocytose dans la glande du sinus d' *Astacus leptodactylus* sous l'influence d'injection de serotonin, C. R. Acad. Sci. 284D (1977) 57–60.

[20] S. Santhoshi, V. Sugumar, N. Munuswamy, Serotonin modulation of hemolymph glucose and crustacean hyperglycemic hormone titers in *Fenneropenaeus indicus*, Aquaculture 281 (1–4) (2008) 106–112.

[21] R. Keller, Radioimmunoassays and ELISAs: Peptides, in: L.I. Gilbert, T.A. Miller (Eds.), Immunological Techniques in Insect Biology, Springer, Heidelberg, 1988, pp. 253–272.

[22] S.G. Webster, Measurement of crustacean hyperglycaemic hormone levels in the edible crab *Cancer pagurus* during emersion stress, J. Exp. Biol. 199 (1996) 1579–1585.

[23] R. Keller, H. Orth, Hyperglycemic neuropeptides in crustaceans, in: A. Epple, C. Scanes, M. Stetson (Eds.), Progress in Comparative Endocrinology, Wiley-Liss, New York, 1990, pp. 265–271.

[24] E.S. Chang, S.A. Chang, R. Keller, P. Sreenivasula Reddy, M.J. Snyder, L.S. Jeffrey, Quantification of stress in lobsters: crustacean hyperglycemic hormone, Stress Proteins Gene Exp. Am. Zool. 39 (1999) 487–495.

[25] R. Keller, B. Haylett, I. Cooke, Neurosecretion of crustacean hyperglycemic hormone evoked by axonal stimulation or elevation of saline $K^+$ concentration quantified by a sensitive immunoassay method, J. Exp. Biol. 188 (1994) 293–316.

[26] M. Fingerman, W.E. Julian, M.A. Spirtes, R.M. Kostrsewa, The presence of 5 -hydroxytryptamine in the eyestalks and brain of the fiddler crab *Uca pugilator*, its quantitative modification by pharmacological agents and possible role as a neurotransmitter in controlling the release of red pigment dispersing hormone, Comp. Gen. Pharmacol. 5 (1974) 299–303.

[27] L. Rodriguez Sosa, A. Picones, G. Calderon Rosete, S. Islas, H. Arechiga, Localization and Release of 5-Hydroxytryptamine in the crayfish eyestalk, J. Exp. Biol. 200 (1997) 3067–3077.

[28] N.W. Tietz, Clinical guide to laboratory tests, S.W. Saunders Co, Philadelpia (1976), p. 238.

[29] J. Levenson, J.H. Byrne, A. Eskin, Levels of serotonin in the hemolymph of *Aplysia* are modulated by light/dark cycles and sensitization training, J. Neurosci. 19 (18) (1999) 8094–8103.

[30] M. Fingerman, M.M. Hanumante, U.D. Deshpande, R. Nagabhushanam, Increase in the total reducing substances in the haemolymph of the freshwater crab, *Barytelphusa guerini*, produced by a pesticide (DDT) and an indolealkylamine (serotonin), Experientia 37 (1981) 178–179.

[31] P.S. Reddy, M. Devi, R. Sarojini, R. Nagabhushanam, M. Fingerman, Cadmium chloride induced hyperglycemia in the red swamp crayfish, *Procambarus clarkii*: possible role of crustacean hyperglycemic hormone, Comp. Biochem. Physiol. 107C (1994) 57–61.

[32] S. Lorenzon, P.G. Giulianini, E.A. Ferrero, Lipopolysaccharide- induced hyperglycemia is mediated by CHH release in crustaceans, Gen. Comp. Endocrinol. 108 (1997) 395–405.

[33] E.S. Chang, R. Keller, S.A. Chang, Quantification of CHH by ELISA in hemolymph of the lobster, *Homarus americanus* following various stress, Gen. Comp. Endocrinol. 111 (1998) 359–366.

[34] D.C. Wilcockson, J.S. Chung, S.G. Webster, Is crustacean hyperglycemic hormone precursor-related peptide a circulating neurohormone in crabs? Cell Tissue Res. 307 (2002) 129–138.

[35] H. Zou, C. Juan, S. Chen, H. Wang, C. Lee, Dopaminergic regulation of crustacean hyperglycemic hormone and glucose levels in the hemolymph of the crayfish *Procambarus clarkii*, J. Exp. Zool. 298 (2003) 44–52.

[36] S. Lorenzon, P. Edomi, P.G. Guilianin, R. Mettulio, E.A. Ferrero, Role of biogenic amines and CHH in the crustacean hyperglycemic stress response, J. Exp. Biol. 208 (2005) 3341–3347.

[37] C.Y. Lee, P.F. Yang, H.S. Zou, Serotonergic regulation of crustacean hyperglycemic hormone secretion in the crayfish, *Procambarus clarkii*, Phys. Biochem. Zool. 74 (3) (2001) 376–382.

[38] C.Y. Lee, S.M. Yan, C.S. Liau, W.J. Huang, Serotonergic regulation of blood glucose levels in the crayfish, *Procambarus clarkii*: site of action and receptor characterization, J. Exp. Zool. 286 (2000) 596–605.

[39] R.W. Fuller, D.T. Wong, D.W. Robertson, Fluoxetine, a selective inhibitor of serotonin uptake, Med. Res. Rev. 11 (1991) 17–34.

[40] N.H. Chen, M.E.A. Reith, Effects of locally applied cocaine, lidocaine and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships, J. Neurochem. 63 (1994) 1701–1713.

[41] M. Fingerman, R.E. Schultz, B.P. Bordlee, D.P. Dalton, Twenty-four hour variation of 5-hydroxytryptophandecarboxylase and monoamine oxidase activities in the eyestalks of the fiddler crab, *Uca pugilator*, Comp. Biochem. Physiol. 61C (1978) 171–175.

[42] D.L. Barker, P.B. Molinoff, E.A. Kravitz, Octopamine in the lobster nervous system, Nat. New Biol. 263 (1972) 61–62.

[43] M.B. Kennedy, Products of biogenic amine metabolism in the lobster: sulfate conjugates, J. Neurochem. 30 (1978) 315–320.