Effect of water temperature on hemocytes and histopathological findings in freshwater Crayfish (*Astacus leptodactylus*)

A Samnejhad [1] M Afsharnasab [2] and S Kakoolaki [2]

[1]Specialized veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2]Department of Aquatic Animal Health & Diseases, Agricultural Research, Education and Extention Org., Iranian Fisheries Science Research Institute, Tehran, Iran

Recived: July 2015 Accepted: November 2015

Abstract

In this research, the effect of different water temperature on THC (Total hemocyte count), variety of hemocyte cells and histopathological differences in Crayfish *Astacus leptodactylus* were studied. This study was designed in three groups in triplicate with 20 Crayfish in each glass aquarium. The rate of temperature in three groups were 10,15, 20, and 25 °C, respectively. The results indicated that the differences of THC value between Group1 (69± 19.00) and Group 3 (18± 12.49) were significant after 48 h . After 168 h, also the differences of THC value between Group 3 (9± 6.24) with Groups 1 & 2 (35.33± 7.02 &32.33± 8.73) were significant. The highest and lowest density of hemocyte cells were belong to semigranular cells (SGC), granular cells (GC) and hyaline cells (HC), with estimated values of 59-64%, 28-36% and 3-8% respectively. The result of histopathology in heaptopancreas, gill and heart in Group 3 affecting of high temperature showed the distribution of hemocyte aggregation and pyknosis of nucleus within vaculation of hepatopancreas. In Groups 1 and 2 the differences of hepatopancreas and gill lamella were lower and no pathological changes of heart were observed. No significant changes of digestive tract were observed in all treatments.

Keywords: *Astacus leptodactylus*, total hemocyte count, histopathology.

Correspondence A Samnejhad, Specialized veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran (e-mail: Drsam.arash@gmail.com)

Introduction

Crayfish are known to play a significant role in some freshwater systems. Some species may be used as bioindicators for ecosystem function, although a general applicability is still debated (Furdeer & Reynolds 2003). Crayfish have also been viewed as ecological engineers, as they are important actors in altering habitat conditions by digging and transferring substrate (Statzner, Fievet, Champagne, Morel & Herouin 2000). The narrow-clawed crayfish Astacus.leptodactylus is one of the five species of the Astacidae family that are endemic to the European continent (Perdikaris, Koutrakis, Saraglidou & Margaris 2007). In Iran, Aras dam reservoir as a natural habitat of A. leptodactylus has an important role in export of this species to European countries (Matinfar 2007). Crayfish has an open circulating system which is filled with hemolymph containing hemocytes. These hemocytes originate from the hematopoietic tissue and mature hemocytes can be divided into the following three cell types based on their structural and functional features: hyaline cells (HCs), semigranular cells (SGCs), and granular cells (GCs) (Soderhall & Smith 1983). The hyaline cells are small, spherical and have no or few granules. These cells constitute ≤ 5% of all mature hemocytes and they have important role in phagocytic removal of microbial pathogens and dead cells. The SGCs contain small granules and participate in encapsulation as well as phagocytosis. Both GCS and SGCs store the components of prophenoloxidase activating system and participate in cytotoxic reaction in addition to generate and store AMPs and other immune effectors (Soderhal, Wingren, Jhansson & Bertheussen 1985). All three hemocytes contain

variable amount of antioxidant enzymes, which act in host defense mechanisms (Bell & Smith 1995). Hemocytes take part in prophenoloxidase activation system (Soderhal, Johansson & Smith 1988) and contain phenoloxidase (Po), bactericidins and lectins (Takahashi, Itami & Kondo 1995). Crustacean hemocytes are normally produced in the hematopoietic tissues. These are located in dorsal anterior part of thorax and on top of the cardiac stomach in lobsters (Hose, Martin, Tiu & Mckrell 1992). Crayfish hemocytes play important roles in the initiation of several immune responses and production of antimicrobial peptides (Jiravanichpaisal, Lee, Kim, Andren & Soderhall 2007). Hemocytes also play a role in surveillance of healthy and damaged basement membranes and also to encapsulate and destroy aberrant tissue (Liu 2008). On the other hand, crayfish hemocyte have importance role in cellular defense mechanisms as well as in release of humoraldefence molecule. Thereby the hemocyte will initiate several immune response such as activation of the prophenoloxidase activating system, initiation of the coagulation system and production of antibacterial peptides (AMPs) (Soderhall & Cerenius 1998). The research demonstrated that hemocytes play a major role in crustacean immune system. First, they eliminate foreign particles in the hemocoel though phagocytosis, encapsulation and nodular aggregation. Secondly, hemocytes participate in wound healing by cellular clumping and begin coagulation processes though the release of factors required for plasma gelatination (Kakoolaki, Sharifpour, Soltani, Mousavi, Mirzargar & Rostami 2010). Several studies have showed that the cellular immune response mainly phagocytosis and encapsulation by hemocytes. Hemocytes can internalize various particles including bacteria, yeast, sephadex, and dsrna (Liu 2008). The number of circulating hemocytes in crayfish have shown to react to different stressors and diseases and could be used as indicators either Crayfish condition or environmental stress. Hemocyte counts may provide an indication of subacute physiological effect in crustaceans (Smith 1991), and changes in hemocyte count have been shown to be a suitable indicators of stress in some species (Lorenzon, Francese, Smith & Ferrero 2001). Accordingly, this research determined the effect of temperature on total hemocyte count and hemocyte cells as well as histopoathology changes in freshwater A. leptodactylus.

Materials and Methods

Management and Collection of samples

One – hundred and eighty freshwater crayfish, A. leptodactylus (weight range of 25-40g) were collected from Aras dam reservoir in West Azerbaijan province and transported to National Artemia Research Center (Urmia) in October 2010. The samples were acclimated in two tanks containing chlorinated and well-aerated freshwater at 10-15 °C in the laboratory for two days. During the acclimation and experiment time, they were fed once per day with commercial diet (trout, Biomar and Blood worm). Water of aquariums were exchanged once per day and the uneaten food was cleaned up regularly. Three water temperature groups were selected as 15±1°C (Group 1), 20±1 °C (Group 2) and 25±1°C (Group 3). Also the measured values of pH and dissolved oxygen were 7-8 and 5.0- 5.5 ppm, respectively. A total of nine glass aquariums (three groups in triplicate) were prepared and each contain 20 specimens of crayfish.

Before experiment, the crayfishes were disinfected by oxytetracyclin antibiotic at concentration of 100ppm for 24 h and then washed with chlorinated and disinfected water. Then all transferred to glass aquariums.

Haemolymph Sampling

Haemolymph was obtained from the haemocoel in the second abdominal segment. For preventing coagulation, haemolymph sample were mixed 1:1 with an anticoagulant solution (Smith & Soderhall 1983) (26 mm Citric Acid, 30 mm Trisodium Citrate, 10 mm EDTA (Ethylene Diamine Tetra – Acetic acid) (pH = 5.4). Haemolymph samples were transported in differential gamma plastic tubes for further THC measurement and maintained in icebox in period of analysis. The haemolymph samples (one sample from each aquarium) were

withdrawn from crayfish for measuring of THC and variety of hemocyte cells within interval hours of 2, 6, 12, 24, 48, 96, 192 and 240 h, after affecting of temperature. In this study, implementation of moribund and diseased crayfish was mostly sampled and the number of samples were 12 at each time.

Total Hemocyte Count and variety of cell hemocyte

Hemocytometer was used for counting of hemocytes. A drop of the anticoagulant - haemolymph mixture were transferred on a hemocytometer and the rate of THC (total hemocyte count) measured by the light microscope (Jiang, Yu & Zhou 2004). Also the different types of hemocytes and their relative ratios were calculated .

Histopathology

Tissue samples (3 specimens of crayfish from each aquarium) including heart, gill and hepatopancreas were also collected at 2, 6, 12, 24, 48, 96, 168, and 240 h under affecting of selected temperature. Then their tissues were fixed in Davidsons fixative for 48 h and at the end transferred to 70% ethylic alcohol. In following, the tissue samples were processed for histhopathological study according to Bell & Lighthner (1988) method, and sections were prepared for H&E staining and viewed under light microscopy.

Statistical analysis

A one way ANOVA test was used to compare the differences of THC values in different temperature at 95% confidence interval ($P<0.05$). Multiple comparisons along with the Tukey test was conducted to find any significant difference among Groups. All statistical tests were evaluated using the SPSS 18.0 software.

Results

THC and Hemocytes cell changes

The results obtained revealed (Table 1) that the value of THC in Group 1(T= 10-15°C) at 48 h effecting by temperature in comparison with Group 3 (T= 25°C) significantly increased ($P<0.05$). Also there was significant difference in THC between Group 3 (T=25°C) and Group 1(T= 10-15°C) and the value of THC in Group 3 significantly decreased in comparison with Group1 ($P< 0.05$). Also the value of THC in Group 3 comparing to Group 1 significantly decreased at 168 h ($P< 0.05$). In other durations , no significant difference in THC was observed between different groups ($P>0.05$). Also the highest and the lowest value of hemocyte cells were semigranolocyte cells (SGC), granulocyte cells (GC) and hyaline cells (HC), respectively. There was no significant differences between measured same treatment replicates too.SGC cells were round, ovoid or fusiform with high percent of granules in the cytoplasm. Granular cells were round or ovoid with many granules in the cytoplasm and hyaline cells were spherical or ovoid cells without or with few granules. The rough estimated values for semigranular , granular and hyaline cells were 58-65, 29-37 and 3-8%, respectively.

Histhopatology findings

In histopathology observations, distributed and aggregated hemocytes were observed in haepatopancreas, excluding the Group 3. (Fig 5). Also distributed orlow aggregated hemocyte were observed in Group 2 (T=20°C) (Fig7), with lower amount of aggregated hemocytes comparing to Group 3. But no differences were observed in Group 1 (Fig 8). As well as vaculation and decreasing of hemocyte cells in haepatopancreas were observed by passing time in crayfish specimens under effect of higher temperature (Fig 6), but these appearance was not observed in haepatopancreas tissue in Groups 1 and 2 (Fig 8).

According to histopathological observations of gills, the rate of hemocyte cells show a descending trend in time passes. In crayfishes affecting by high temperature (T=25°C) aggregated hemocyte along with the low normal cells of gill lamella were seen (Fig 9). Also distribution of low hemocyte cells in Groups 1 & 2 were observed, but with lower changes in comparison with Group 3.

Histopathological finding of heart showed that in crayfish affecting to high temperature (T=25°C),

Table 1 The mean±SD of THC at difference hours after affecting by temperature

THC (Hemocyte× 10^4)								
Sampling times (h)								
	2	6	12	24	48	96	168	240
Groups	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Group1 T=10- 15°C	17.34 36.00±	86.67± 45.54	117.33± 71.28	42.67± 22.59	69.00± 19.00*	34.33± 14.01	35.33± 7.02	75.33± 20.42
Group 2T=20°C	10.01 31.76±	29.54 77.00±	42.85 85.67±	34.33± 11.71	45.67± 14.57	24.33± 8.50	32.33± 8.73	71.33± 8.32
Group 3T=25°C	18± 5.29	82.33± 8.62	11.59 86.33±	19.33± 10.40	*18.00± 12.49	22.00± 11.35	9.00± 6.24*	61.67± 4.16

(*) show significantly differences of THC in similar times.

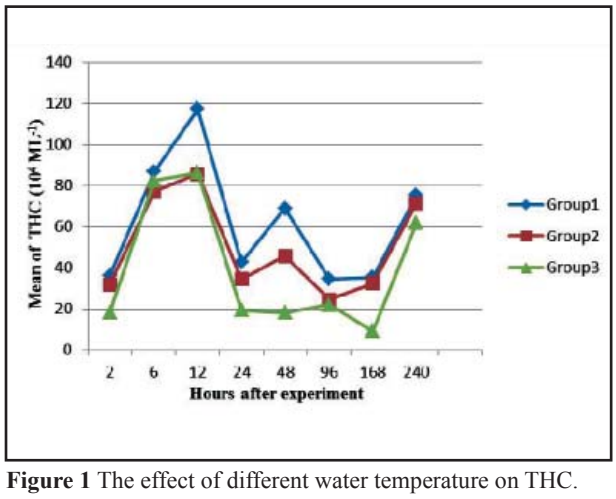


Figure 1 The effect of different water temperature on THC.

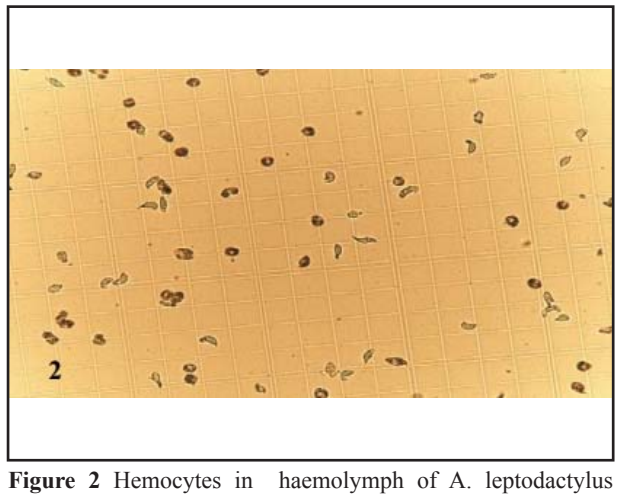
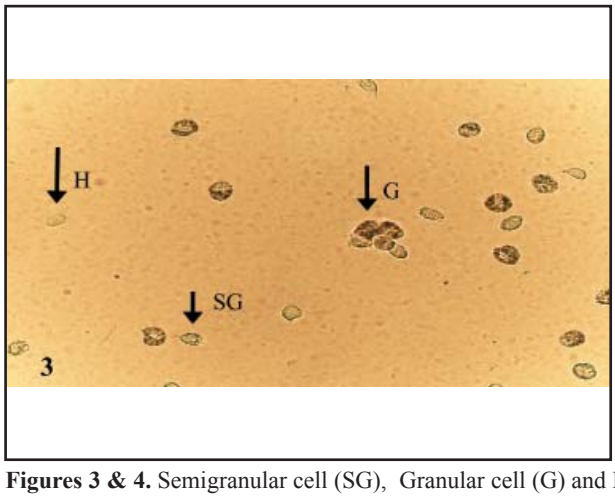


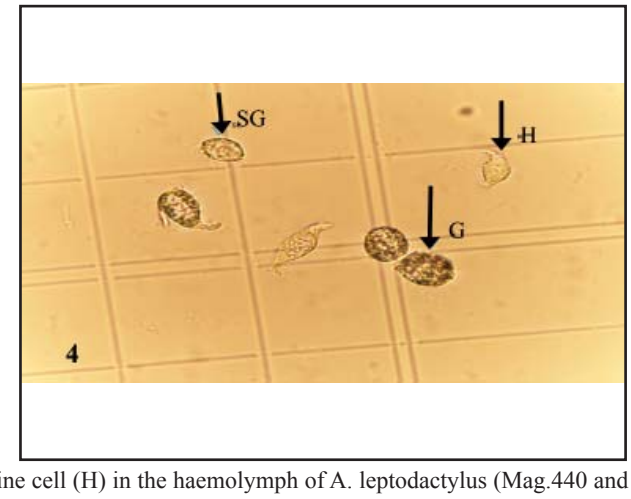
Figure 2 Hemocytes in haemolymph of A. leptodactylus (Mag.220).

aggregated and distributed hemocytes in myocardium were observed (Fig 11), but no differences observed in hearts of Groups 1 and 2 (Fig 12). Also no differences observed in digestive system of all 3 Groups of crayfishes, and digestive system was completely healthy (Fig 13).

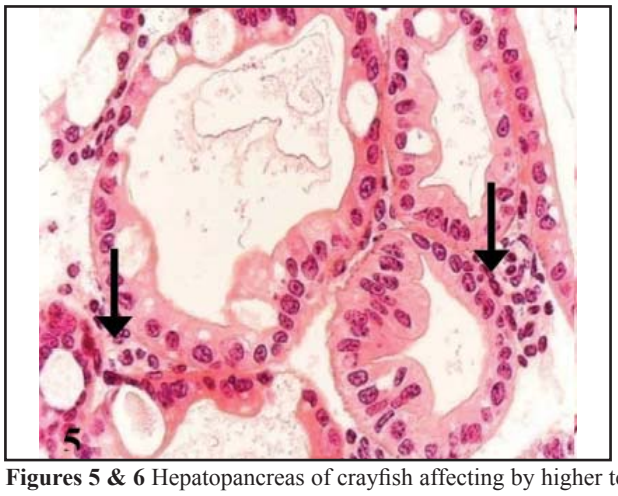
Discussion

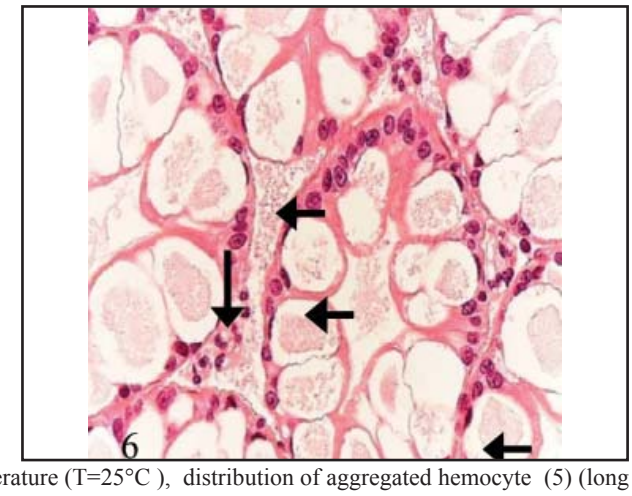
Hemocytes in crayfish, similar to the other crustaceans, play an important role in the host immune response (Jiravanichpaisal, Lee & Soderhall 2006a), such as early non-self recognition, phagocytosis, encapsulation, melanization and cytotoxicity. In crustaceans, the number of freely circulating hemocytes varies in response to environmental stress, endocrine activity during the moulting cycle, and infection. Exposure to non-self molecules can cause a dramatic in total hemocyte count and the animals may die of an infection that they otherwise usually resist (Johansson, Keyser, Sritunyalucksana & Soderhall 2000; Jiravanichpaisal, Lee & Soderhall 2006a). In our study, there was no significant differences between three Groups of crayfishes affecting by temperature in early time (2,6,12,24 h) after experiment. It can be concluded that the crayfish immune system to resist with stressor agents caused by temperature and the mean of THC could not be changed before 24 h after affecting of crayfishes to temperature. But the mean of THC showed significant differences between crayfish affecting to high temperature (Group 3, T=25°C) with Group 1(T=10-15°C) at 48 h after experiment time. This finding showed that the high temperature as a stressor factor due to decrease of hemocyte cells and destruction of immunological system function and finally will lead to death of crayfish. Also there was significant differences between Group 3 with Groups 1 and 2 at 168 h after affecting of temperature. This implies





Figures 3 & 4. Semigranular cell (SG), Granular cell (G) and Hyaline cell (H) in the haemolymph of A. leptodactylus (Mag.440 and 880, respectively).





Figures 5 & 6 Hepatopancreas of crayfish affecting by higher temperature (T=25°C), distribution of aggregated hemocyte (5) (long arrow) and vaculation and low hemocyte cells (long arrow) with many of pyknotic nuclei by passing time were observed (6) (short arrow)(H&E/Mag.440).

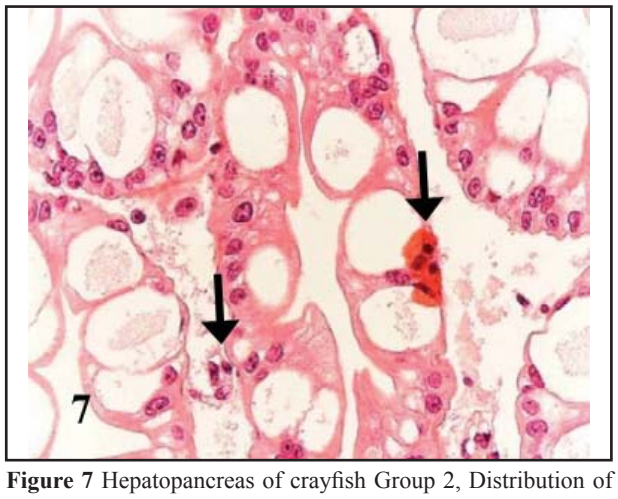


Figure 7 Hepatopancreas of crayfish Group 2, Distribution of low hemocyte cells are observed (short arrow) (H&E/Mag.440).

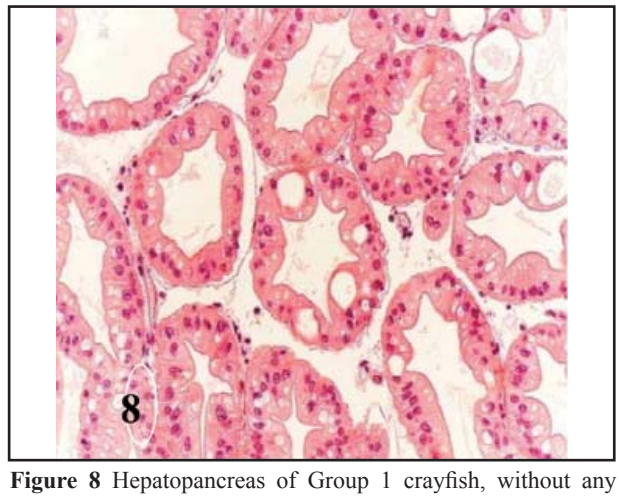


Figure 8 Hepatopancreas of Group 1 crayfish, without any significant changes and all hepatopancreas cells are healthy (H&E/Mag.220).

that the high temperature in Group 3 comparing to Groups 2 & 1 due to decrease of circulating hemocyte cells and weakness of immune system by passing time. The number of circulating hemocytes in crayfish have shown to react to different stressors and diseases (Soderhall, Johansson & Smith 1988)

Figure 9 Aggregated hemocytes (short arrows) with decreasing of normal cells of gill lamella in Crayfish affected to high temperature (T=25ċ) (H&E/Mag.440).

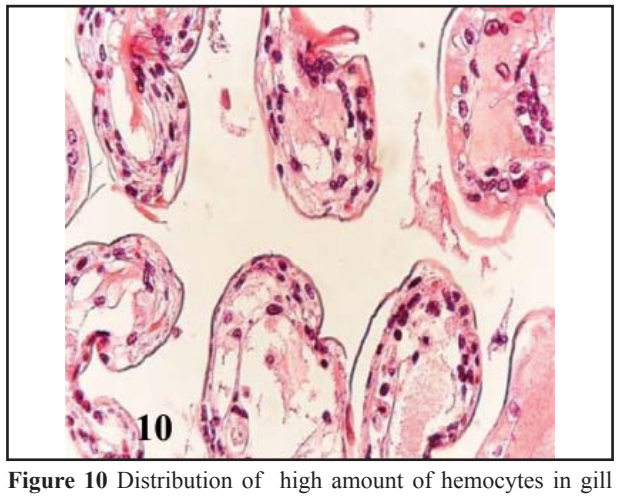


Figure 10 Distribution of high amount of hemocytes in gill lamellas (short arrows) in Groups 1 & 2 of crayfish (H&E/Mag.220).

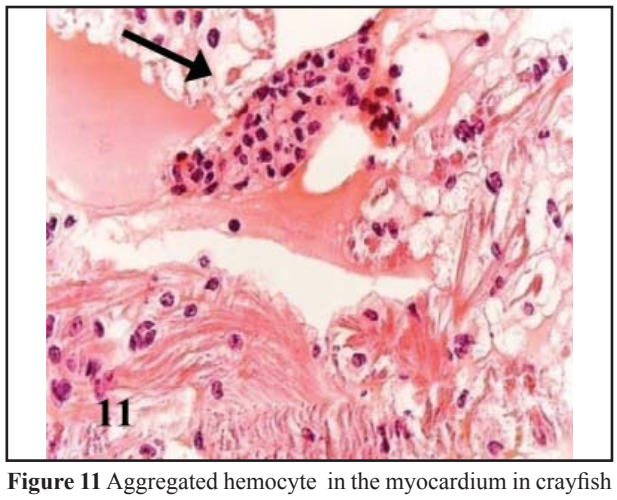


Figure 11 Aggregated hemocyte in the myocardium in crayfish affecting to high temperature (T=25°C) (H&E/Mag.220).

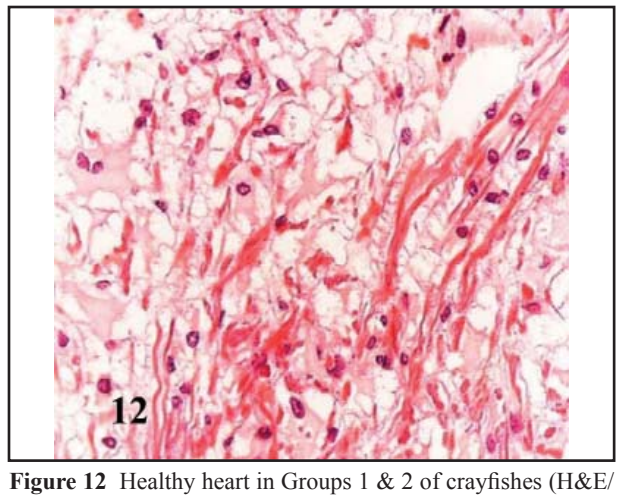


Figure 12 Healthy heart in Groups 1 & 2 of crayfishes (H&E/Mag.220).

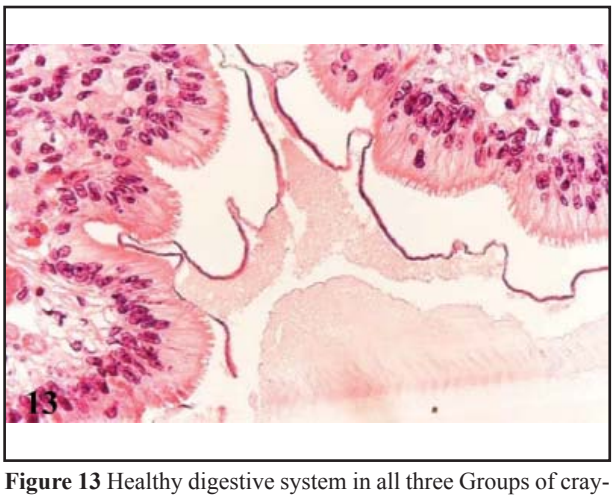


Figure 13 Healthy digestive system in all three Groups of crayfishes under affecting to temperature (H&E/Mag.220).

and could be used as indicators of either crayfish condition or environmental stress. Several studies indicated that the decline in THC under the normal THC range could be taken as indication of worsening condition in crayfish (Jussila 1997). Some studies indicated that various environmental factors, such as fluctuations in temperature and dissolved oxygen, influence the crustacean immune system on a daily basis (LeMoullac, Soyez, Saulnier, Ansquer, Avarre & Levy 1998; Lemollac & Haffner 2000), whereas captive crustaceans are typically subjected to constant ambient conditions. The present study indicated that there were no significant differences observed in THC between Groups 1 and 2 during of experiment . This finding showed that the rate of temperature couldn't affect on the immune system of crayfish in treatment groups, and differences of THC were not significant in Groups 1 and 2 . These facts suggest that stress inducing the high temperature by passing time of experiment leading

to destruction of immune system and differentiation and decreasing of THC in A. leptodactylus, respectively. The Crayfishes finally will die due to high temperature and decreasing of hemocyte cells. The variations in number of hemocytes are mainly regulated by release from the hematopoietic tissue, perhaps complemented by storage and release of hemocytes at other sites. Several workers have attempted to find proliferation of circulating hemocytes. Some reserachers have reported evidence for this, whereas others have not detected it (Johansson, Keyser, Sritunyalucksana & Soderhall 2000). Some studies indicated that three groups of hemocytes including hyaline cells (HC), small granular cells (SGC) and large granular cells (LGC) were identified in shrimp Fenneropenaeus indicus that the highest and the lowest value of hemocyte cells were detected as SGC and HC, respectively. In our study three types of hemocyte cell including SGC, GC and HC were determined in agreement with the previous crustacean hemocyte (Bauchau 1981; Hose, Martin & Gerard 1990; Johansson, Keyser, Sritunyalucksana, & Soderhall 2000; Taylor & landman 2009), that the highest and the lowest value of hemocyte cells were semigranular SGC, GC and HC, respectively. Our finding showed that the hyaline cells were comprised the lowest value of hemocyte cells in A. leptodactylus that it probably showed that these cells have low participation on immune system of crayfish in normal condition of temperature (T=10-15°C). But the value of this cells had no significant differences with other hyaline cells in normal condition of temperature in A. leptodactylus. But our research demonstrated that the SGC had the highest value of hemocyte cells. These cells probably were the main defense cells in crayfish . It is suggested that the rate of high temperature caused stressing condition and hemocyte aggregation in these organs. But the rate of hemocytes were lower and vaculation of hepatopancreas was seen by passing time that it probably can cause the decreasing of immune system of crayfish due to high temperature. Also low aggregation of hemocytes in hepatopancreas in Group 2 and in gill lamella in Groups 1 and 2 were seen but it wasn't significant comparing to Group 3 affecting to high temperature of which the low temperature can be the reason of this occurrence. n this research the rate of mortality due to high temperature in Group 3 was higher in comparison with Groups 2 and 1, that it can be considered as stress of high temperature in this group.

References

Bauchau A.G. (1981) Crustaceans. In: Ratcliffe, N.A. and Rowley, A.F. (eds): Invertebrate blood cells. Academic Press, London, USA. Vol. **2,** 336-420.

Bell T.A. & Lightner D.V. (1988) A hand book of normal penaeid shrimp histology, world aquaculture society, Baton Rouge, Louisiana, 114p.

Bell K.L. & Smith V.J. (1995) Occurrence and distribution of antioxidant enzymes in the haemolymph of the shoe crab Carcinus maenus. *Marine Biology* **123(4),** 829-835.

Fureder L. & Reynolds J. (2003) 1s Austropotamobius pallipes a good bioindicator? Bulletin Français de la Pêche et de la Pisciculture **370-371,** 157-163.

Hose J.L., Martin G.G. & Gerard A.S. (1990) A decapods hemocyte classification scheme integratiny morphology, cytochemistry and function. *Biological Bulletin* **178,** 33-45.

Hose J.E., Martin G.G., Tiu S. & Mckrell N. (1992) Patterns of hemocyte production and release throughout the moult cycle in the penaeid shrimp Sycionia ingentis. *Biological Bulletin* **183,** 185-189.

Jiang G., Yu R. & zhou M. (2004) Modulatory effects of ammonia–N On the immune system of Penaeus japonicus to Virulence of white spot syndrome Virus. *Aquaculture* **241,** 61-75.

Jiravanichpaisal P., Lee B.L. & Soderhall K. (2006a) cell–mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization. *Immunobiology* **211,** 213-236.

Jiravanichpaisal P., Lee S.Y., Kim Y.A., Andren T. & So-

derhall I. (2007) Antibacterial peptides in hemocytes and hematopoietic tissue from freshwater Crayfish Pacifastacus leniusculus: Characterization and Expression pattern. *Developmental and Comparative Immunology* **31,** 441-455.

Johansson M.W., Keyser P., Sritunyalucksana K. & Soderhall, K. (2000). Crustacean haemocytes and haematopoiesis. *Aquaculture* **191,** 45-52.

Jussila J. (1997) Physiological responses of Astacid and Parastacid Crayfish (Crustacea: Decapoda) to condition of intensive culture. Kuopio university publications C. *Natural and Environmental Sciences* **67,** 136p.

Kakoolaki S., Sharifpour I., Soltani M., Mousavi H.A.E., Mirzargar S. & Rostami M. (2010) Selected morpho-chemical features of hemocytes in farmed shrimp. Fenneropenaeus indicus in iran. *Iranian Journal of Fisheries Sciences* **9(2),** 219-232.

LeMoullac G. & Haffner P. (2000) Environmental factors affecting immune response in crustacean. *Aquaculture* **191,** 121-131.

LeMoullac G., Soyez C., Saulnier D., Ansquer D., Avarre J.L. & Levy P. (1998) Effects of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp Penaeus stylirostris. *Fish and Shellfish. Immunology* **8,** 621-629.

Liu H. (2008) Functional studies of some immune relevant genes in a crustacean. Acta universitatis upsaliensis. Digital comprehensive summaries of Uppsala Dissertations from the faculty of science and technology 435, 61pp.

Lorenzon S., Francese M., Smith V.J. & Ferrero E.A. (2001) Heavymetals affect the circulating haemocyte number in the shrimp Palaemon elegans. *Fish and Shellfish Immunology* **11,** 459-472.

Matinfar A. (2007) Prancipal program of Freshwater Crayfish *Astacus leptodactylus*. Jahad agriculture organization. *Iranian Fisheries research Organization* **10,** 118p.
Perdikaris C., Koutrakis E., Saraglidou V. & Margaris N. (2007) Occurrence of the narrow-clawed crayfish *Astacus leptodactylus* Eschscholtz, 1823 in the River Evros in Greece. *Bulletin Français de la Pêche et de la Pisciculture* **385,** 45-52.

Smith V.J. (1991) Invertebrate immunology phylogenetic, ecotoxicological and biomedical implications. *Comparative Haematology International* **1,** 61-76.

Smith V.J. & Soderhall K. (1983) B-1,3-glucan activation of crustacean hemocytes in vitro and inviro. *Biological Bulletin* **164,** 299-314.

Soderhall K. & Smith V.J. (1983) Separation of the haemocyte populations of Carcinusmeanas and other marine decapods , and prophenoloxidase distribution. *Development and Comparative Immunology* **7,** 229-239.

Soderhall K., Wingren A., Jhansson MW. & Bertheussen K. (1985) The cytot reaction of hemocytes from the freshwater Astacus astacus. *Cellular Immunology* **94(2),** 326-332.

Soderhall K., Johansson M.W. & Smith V.J. (1988) Internal defence mechanism. In: Freshwater Crayfish: biology, management and exploitation. Eds. Holdich, D.M., Lowery, R.S. Croom Helm, London, 213-235.
Soderhall K. & Cerenius K. (1998) Role of the prophenoloxidase activating system in invertebrate immunity. *Current Opinion in Immunology* **10,** 23-30.

Statzner B., Fievet E., Champagne J.Y., Morel R. & Herouin H. (2000) Crayfish as geomorphic agents and ecosystem engineers Biological behavior affects sand and gravel erosion in experimental streams. *Journal of Limnology and Oceanography* **45(5),** 1030-1040.

Takahashi T., Itami T. & Kondo M. (1995) Immunodefence system in crustacean. *Fish Pathology* **30(2),** 141-150.

Taylor S. & Landman J.M. (2009) Flowcytometric characterization of freshwater Crayfish hemocytes for the examination of physiological status in wild and captive animals. *Journal of Aquatic Animal Health* **21,** 195-203.

تأثیرات درجه حرارت آب بر روی هموسیت‌ها و یافته‌های هیستوپاتولوژی در شاه میگوی آب شیرین (*Astacus leptodactylus*)

آرش سام نژاد[1*]، محمد افشارنسب[2]، شاپور کاکولکی[2]

۱ فارغ التحصیل بهداشت و بیماریهای آبزیان، دانشکده تخصصی دامپزشکی دانشگاه آزاد اسلامی واحد علوم و تحقیقات تهران، تهران
۲ موئسسه تحقیقات علوم شیلاتی کشور، صندوق پستی ٦١١٦-١٤١٥٥ تهران

چکیده

در تحقیق حاضر تأثیرات مختلف درجه‌حرارت آب بر تعداد، تنوع سلول‌های هموسیت و تغییرات هیستوپاتولوژی در شاه‌میگوی آب‌شیرین مورد بررسی قرار گرفت. این مطالعه در ۳ گروه با ۳ تکرار و ۲۰ عدد شاه‌میگو در هر آکواریوم مورد طراحی قرار گرفت. میزان درجه‌حرارت در ۳ گروه به ترتیب ۱۵-۱۰، ۲۰و ۲۵°C بود. نتایج نشان می‌دهد که تغییرات هموسیت‌های کل بین گروه ۱ (۱۹± ۶۹) و گروه ۳ (۴۹/۱۲± ۱۸)، بعد از ۴۸ ساعت از آزمایش معنی‌دار بود. همچنین بعد از ۱۶۸ ساعت از آزمایش، تغییرات هموسیت‌های کل بین گروه ۳ (۶/۲۴ ± ۹) با گروه ۱ (۲/۷± ۳۳/۳۵) و ۲ (۸/۷۳ ±۳۳/۳۲) معنی‌دار بود. بالاترین و پایین‌ترین میزان سلول‌های هموسیت به ترتیب سلول‌های نیمه‌دانه‌دار، سلول‌های دانه‌دار و سلول‌های هیالین بودند. سلول‌های نیمه‌دانه‌دار ۶۴%-۵۹%، سلول‌های دانه‌دار ۳۶%-۲۸% و سلول‌های هیالین ۸%-۳% از کل سلول‌های هموسیت را تشکیل دادند. نتایج هیستوپاتولوژی در گروه ۳ مواجه شده با درجه‌حرارت بالا در ارگانهای هپاتوپانکراس، آبشش و قلب، تجمعات هموسیت‌ها و پیکنوزه‌شدن هسته‌ها با واکوئله‌شدن هپاتوپانکراس را نشان داد. در گروه‌های ۱و۲ تغییرات هپاتوپانکراس و تیغه‌های آبشش پایین‌تر بوده و هیچ تغییر آسیب‌شناسی در قلب مشاهده نشد. هیچ تغییر آسیب‌شناسی در دستگاه گوارش درهر ۳ گروه در طول زمان‌های مختلف مشاهده نشد.

واژه‌های کلیدی: شاه میگوی آب شیرین، هموسیت کل، سلول‌های نیمه دانه‌دار، سلول‌های دانه‌دار، سلول‌های هیالین، هیستوپاتولوژی.

*نویسنده مسئول: Drsam.arash@gmail.com