Acta Aquatica Turcica

E-ISSN: 2651-5474

Home Page: https://dergipark.org.tr/actaquatr 18(4): 505-512, 2022 DOI: 10.22392/actaquatr.1150615

Research Article **Araştırma Makalesi**

The Effects of Environmental Variables and Morphometry on Hemocyte Structure in the Hemolymph of Portunid Crab, *Carcinus aestuarii* Nardo, 1847 in Çardak Lagoon (Çanakkale Strait)

Çardak Lagünü'nde (Çanakkale Boğazı) Bulunan Portunid Yengeç, *Carcinus aestuarii* Nardo, 1847'nin Hemolenfinde Çevresel Değişkenlerin ve Morfometrinin Hemosit Yapısına Etkileri

Seçil Acar[1,*], A. Suat Ateş[1]

[1]Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, 17100, Çanakkale

*Corresponding author: secilkolsal@gmail.com

Received: 29.07.2022 **Accepted:** 18.10.2022 **Published:** 01.12.2022

How to Cite: Acar, S., & Ateş, A. S. (2022). The effects of environmental variables and morphometry on hemocyte structure in the hemolymph of portunid crab, *Carcinus aestuarii* Nardo, 1847 in Çardak Lagoon (Çanakkale Strait). *Acta Aquatica Turcica, 18*(4), 505-512. https://doi.org/10.22392/actaquatr.1150615

Abstract: This study aims to determine the relationship between hemocyte count and hemocyte types with environmental variables and morphometric measurements in *Carcinus aestuarii*. A total of 240 crabs were seasonally collected in Çardak Lagoon June 2018, October 2018, February 2019, and May 2019. The average carapace length (CL) was 44.66±0.44 mm, the mean carapace width (CW) was 37.71±0.38 mm and the mean weight was 28.49±0.88 g. Crab specimens were anesthetized on ice for 10 min, and 500 µL of hemolymph was collected from the legs of each crab using a 1-mL plastic syringe. After sampling, hemolymph samples were mixed with the anticoagulant, which was applied to a Thoma slide and examined under a microscope at X40 magnification. The total hemocyte count (THC) was calculated as $13.91\pm8.08x10^6$ (cells/mm^3). Differential hemocyte count (DHC) 53.25±0.6% for hyalinocyte, 26.15±0.23% for semi-granulocyte, and 20.6±0.06% for granulocyte and 3 different cell types were observed. The most dominant cell type is hyalinocyte cells.

Keywords
- Total hemocyte counts
- Hyaline
- Granular
- Physicochemical variables
- Mediterranean Green Crab

Özet: Bu çalışma, *Carcinus aestuarii*'de hemosit sayısı ile hemosit tipleri arasındaki ilişkiyi çevresel değişkenler ve morfometrik ölçümlerle belirlemeyi amaçlamaktadır. Çardak Lagünü'nde Haziran 2018, Ekim 2018, Şubat 2019 ve Mayıs 2019'da mevsimsel olarak toplam 240 yengeç toplanmıştır. Ortalama kabuk uzunluğu (CL) 44.66±0.44 mm, ortalama kabuk genişliği (CW) 37.71±0.38 mm ve ortalama ağırlık 28.49±0.88 g' idi. Yengeç örnekleri buz üzerinde 10 dakika süreyle anesteziye tabi tutuldu ve 1 mL'lik plastik bir şırınga kullanılarak her bir yengecin bacaklarından 500 uL hemolenf toplandı. Örneklemeden sonra hemolenf örnekleri bir Thoma lamına uygulanan antikoagülan ile karıştırıldı ve X40 büyütmede mikroskop altında incelendi. Toplam hemosit sayısı (THC) $13.91\pm8.08x10^6$ (hücre/mm^3) olarak hesaplandı. Diferansiyel hemosit sayısı (DHC) hiyalinosit için % 53.25±0.6, yarı granülosit için % 26.15±0.23 ve granülosit için % 20.6±0.06 ve 3 farklı hücre tipi gözlendi. En baskın hücre tipi hyalinosit hücreleridir.

Anahtar kelimeler
- Toplam hemosit sayısı
- Hyaline
- Granuler
- Fizikokimyasal değişkenler
- Akdeniz yeşil yengeci

1. INTRODUCTION

Crustaceans have an open vascular system with large numbers of hemocytes circulating freely in the hemolymph. (Matazzo & Marin, 2010). Circulating hemocytes of crustaceans and other invertebrates play an important role in immunity and can be used as an indicator of physiological

states. (Battison et al., 2003). Total cell count and differential cell count are useful in determining the physiological state of an organism (Battison et al., 2003). They perform functions such as phagocytosis, encapsulation, and fragmentation of foreign cells and provide information on local populations' health status. (Petri et al., 2006; Adeogun et al., 2015). Furthermore, hemocytes play critical roles in wound healing and defense mechanisms against parasites, viruses, and bacteria. (Matazzo & Marin, 2011). Hose et al. (1990) classified crustacean hemocytes based on morphology, cytochemistry, and function and distinguished three cell types; hyaline cells, granulocytes, and small and large granule cells. While hyaline cells initiate coagulation, small and large granule hemocytes participate in phagocytosis and encapsulation, respectively. Hemolymph numbers differ between species, as does the variation in the crustacean hemocyte population between species. (Adeogun et al., 2015; Sawyer, 1970; Clare & Lumb, 1994). While hemolymph number varies by species it is also affected by environmental factors. Age, sex, season, capture method, sexual fatigue, length, weight, water temperature, pH, diet, and other factors affect the hemolymph structure living (Başusta & Şen, 2004).

Ecological indicators are widely used in monitoring, evaluating, and managing the ecosystem because they can help simplify environmental and ecosystem complexity and provide valuable information for further risk assessment. THC and hemocyte types, which are physiological variables, are used as ecological indicators in determining the stressors and health status of living. Considering that hemocytes are immune responding cells, the balance of total hemocyte density in aquatic invertebrates over a physiological range is an important variable of the cell-mediated immune response (Mello et al., 2010; Matozzo et al., 2016; Burgos-Aceves & Faggio, 2017, Qyli et al., 2020). It is known that the increase in total hemocyte count is the most common response to environmental stressors (Coles et al., 1994).

Carcinus aestuarii which is an important representative of the Mediterranean Lagoons can tolerate physicochemical variables. Although there are many studies conducted on *C. aestuarii* in Turkey, there is any study on the hemolymph structure of the species. This study aims to determine the total number of hemocytes and the percentages of hemocytes of *C. aestuarii* in Çardak Lagoon and their relationship with environmental variables.

2. MATERIAL AND METHODS

2.1. Crab Sampling

Carcinus aestuarii samples were collected (Çanakkale Strait) seasonally in Çardak Lagoon in June 2018, October 2018, February 2019 and May 2019 using a static traditional eel trap. Crabs were sampled as 60 individuals per season. Environmental variables such as salinity, temperature, pH, and dissolved oxygen in the lagoon water were measured in real-time using a YSI 556 model MPS in *situ*. A digital caliper was used to measure the carapace length (CL) and carapace width (CW) of each crab (in mm). A digital scale was used to determine total wet body weight (W) (in 0.001 g).

2.2. Total Hemocyte Count (THC) and Differential Hemocyte Count (DHC)

Crab specimens were anesthetized on ice for 10 min before 500 L of blood was collected from the walking legs of each crab using a 1-mL plastic syringe. After dilution with a 1:2 citrate buffer/ethylenediaminetetraacetate (EDTA) solution (NaCl 0.45 mol L-1, glucose 0.1 M, sodium citrate 30 mM, citric acid 26 mM, EDTA 10 mM, pH 4.6, stored at 4 ^{o}C) in Eppendorf tubes, a manual count was performed on a Thoma slide to calculate the total number of hemocytes (THC) (Soderhall & Smith 1983). After sampling, hemolymph samples were mixed with the anticoagulant, which was applied to a Thoma slide and examined under a microscope at X40 magnification. To identify hemocyte morphology, 0.1 ml of hemolymph was mixed on a glass slide with 0.1 ml of a 1.2% trypan blue solution in seawater. Hemocyte morphotypes were identified, and 100 cells from each slide were counted. For wet staining, one drop of hemolymph was placed on a slide and thin smears were

immediately viewed with a 100x light microscope, while for permanent staining, three drops of hemolymph were placed on individual slides, smeared, and fixed with absolute methanol for three minutes with three types of dyes: Giemsa, which was applied for 10 minutes to determine the best stain for crab hemolymph morphology. The stains were rinsed with distilled water, and the slides were examined under the microscope for the different cell morphotypes. Giemsa staining provided the best results for the hemocyte morphology of *C. aestuarii.*

2.3. Statistical Analysis

The relationship between the sexes and the seasons and THC counts was tested using the t-test. Statistical relationships between environmental variables measured at seasons and hemocyte parameters were determined using Pearson correlation. Statistical analysis was performed using SPSS 25.

3. RESULTS

3.1. Environmental Variables

Seasonal differences in salinity, temperature, and dissolved oxygen were observed during the study (Table 1). The average temperature values according to the seasons were between 10.80 and 25.67. Average salinity values according to seasons were 20.35±0.12-23.19±0.2‰. Dissolved oxygen values were between 7.5±0.78 mg L^{-1} and 9.14±1.64 mg L^{-1} and pH values were 8.67±0.06 - 8.85±0.02.

Table 1. Temperature, salinity, dissolved oxygen, and pH values measured in sampling seasons

	Temperature (°C)	Salinity (‰)	Dissolved Oxygen (mg L^{-1})	pH
Summer	25.67 ±2.15	20.35±0.12	7.5±0.78	8.85±0.02
Autumn	18.92±3.73	21.17±0.56	7.8±1.5	8.8±0.02
Winter	10.80±1.13	23.19±0.25	9.14±1.64	8.67±0.06
Spring	16.13±4.05	21.94±0.43	8.93±1.21	8.81±0.03

A total of 240 individuals (120 females and 120 males), were examined. Several morphometric measurements of the crabs are shown in Table 2. The average carapace length (CL) was 44.66±0.44 mm, the mean carapace width (CW) was 37.71±0.38 mm and the mean weight was 28.49±0.88 g. The mean hemocyte count in hemolymph was calculated as 13.91±8.08x10^6. The range of total hemocyte count was from 5.54±1.22x10^6 to 24.5±6.48x10^6 (cells/mm^3). While the average hemocyte count in females is 13.84±8.97x10^6 (cells/mm^3) and 13.88±6.48x10^6 (cells/mm^3) in males.

Table 2. Average CL, CW, W, and THC values by sex.

Sex	N	CL (mm)	CW (mm)	W (g)	THC (cells/mm^3)
Female	120	40.51±0.38	34.57±0.36	19.91±0.75	13.84±8.97x10^6
Male	120	48.82±0.6	40.85±0.53	37.08±1.16	13.88±6.48 x10^6
Total	240	44.66±0.44	37.71±0.38	28.49±0.88	13.91±8.08 x10^6

N: The number of specimens, CL: Carapace length (mm), CW: Carapace width (mm), W: Weight (g), THC: Total Hemocyte Counts (cells/mm^3)

The t-test was applied to find the relationship between several morphometric variables (CL, CW, and THC) in female and male individuals. CL, CW, and THC levels in females and males are statistically significant ($p=0.00$; $p<0.05$).

The relationships between the environmental variables and THC and morphometric characteristics are shown in Table 3. A statistically significant and strong correlation was found between THC and carapace length CL ($r_s=.638$; $p<0.05$). THC is positively correlated to temperature and pH and negatively correlated to salinity and oxygen levels.

Table 3. Correlations between environmental variables and morphometric measurements

	T(°C)	S(‰)	DO (mg L^{-1})	pH	THC (cells/mm^3)	CL (mm)	CW(mm)	W(g)
T	1	-.937**	-0.540	.667*	0.153	0.406	0.098	0.051
S	-.937**	1	.624*	-.721**	-0.253	-0.388	-0.096	-0.082
DO	-0.540	.624*	1	-0.046	-0.105	-0.170	0.014	0.056
pH	.667*	-.721**	-0.046	1	0.473	0.296	0.010	0.013
THC	0.153	-0.253	-0.105	0.473	1	.638*	0.343	0.527
CL	0.406	-0.388	-0.170	0.296	.638*	1	.823**	.747**
CW	0.098	-0.096	0.014	0.010	0.343	.823**	1	.592*
W	0.051	-0.082	0.056	0.013	0.527	.747**	.592*	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

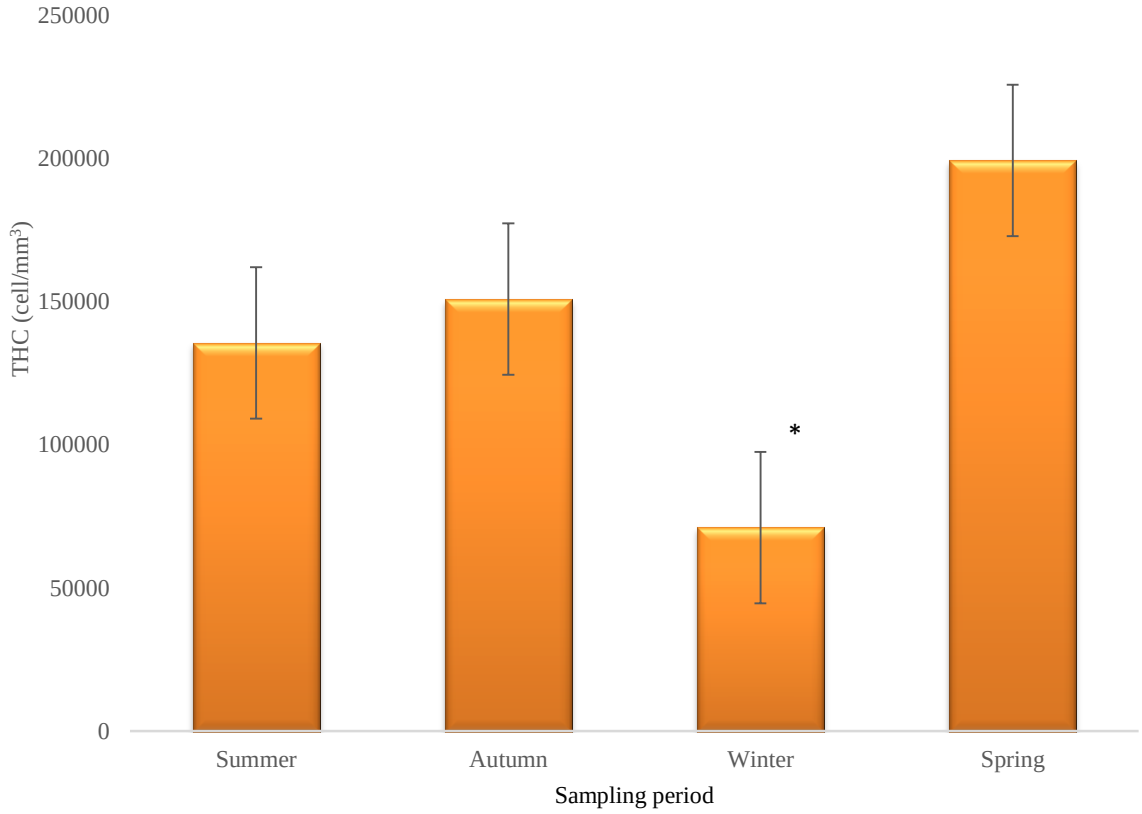

Figure 1. Total THC in sampling seasons

The number of THC in each season is shown in Figure 1. The highest THC count was recorded in the spring and the lowest in the winter season. The statistical differences between seasons were not significant, but the association between the winter season and THC count was statistically significant ($p=0.00$; $p < 0.05$).

Hemocytes in the hemolymph of *C. aestuarii* individuals were divided into hyalinocytes, semigranulocytes, and granulocytes. Hyalinocytes were free of granules or sometimes contained very few small intracellular inclusions. Semigranular cells contained fewer small and generally less refractive granules. Granular cells were filled with numerous large, highly refractory granules. The most abundant cell type was counted as hyalinocyte. Three different types were determined for hemocytes: Hyalinocytes, Semigranulocytes, and Granulocytes (Table 4). While the number of

hyalinocytes (55.32±0.55%) and semigranulocytes (28.13±0.5%) was higher in females, the number of granulocytes (26±0.07%) was calculated to be higher in males.

Table 4. The number of differential hemocyte counts (DHC) in *C. aestuarii*

Sex	Hyalinocyte (%)	Semi-granulocyte (%)	Granulocyte (%)
Female	55.32±0.55	28.13±0.5	16.55±0.05
Male	48.46±0.45	25.54±0.58	26±0.07
Total	53.25±0.6	26.15±0.23	20.6±0.06

4. DISCUSSION

The number of hemocytes in *Carcinus aestuarii* and their relationship to hemocyte species were investigated in several studies that evaluated the effects of pollutants and various environmental stressors on hemolymph quality (Matozzo and Marin, 2010; Aliko et al., 2015; Mancuso et al., 2019; Gürkan, 2019; Qyli et al., 2020). Here we tried to define the relationships between crab morphometry, environmental variables, and hemocyte structure.

In this study, the highest THC count was found in the spring, and it was observed that the THC count decreased in the winter period. Matazzo et al. (2013) found a significant relationship between season and THC and counted the highest THC content in *C. aestuarii* for summer. In both sexes of *C. aestuarii*, the lowest THC level was measured in the winter months, when the lowest seawater temperature was recorded. This result shows a possible correlation between water temperature and THC. The t-test showed a statistically significant association between sex and THC. In addition, THC levels showed a significant positive correlation with the carapace length of the crabs. The correlations between sex and carapace length, and THC support the findings of other studies. The number of hemocytes was found to increase with growth (Matozzo, 2013). In their study, Türeli et al. (1999) found higher, total hemocyte counts, hyaline, granular, and semigranular cells in the blue crab *Callinectes sapidus*, although the females were morphometrically small. Dutta et al. (2021) found more hemocytes in females than in males in their hemocyte study of another portunid crab, *Scylla serrata*. In this study, although the values of weight carapace length, carapace width of female individuals are lower than those of male ones, hyaline, semigranular, values are found to be higher. Also in this study, the THC count was found to be very similar between female and male individuals and the number of granular cells was higher in males, in contrast to other studies. Although the exact cause of the sex-specific variation is not known, it is generally thought that the reason for the high hemocyte counts in females is reproductive (Türeli, 1999; Pugazhvendan & Soundarjan, 2012; Dutta et al., 2021).

According to the relevant literature, the density of each hemocyte type varied between crustacean species. Matazzo & Marin (2010) stated that the hyaline cell type was determined to be 44%, semigranular 27%, and granular 28%. In this study, although hyaline cell type was predominant, it was calculated at 58%, semigranular at 26%, and granular at 13.6%. Qyli et al. (2020) found in their study that under stress conditions, *C. aestuarii* mainly increases the number of granular cells and decreases the number of hyaline cells. Bauchau and Plaket (1973) found in their study that granular cells are about eight times more abundant than hyalinocytes. Although the significance of this apparent variability in the relative proportions of each hemocyte type among crustacean species remains unclear, it is thought to be caused by the effects of molting, feeding, reproduction, disease, and environmental pollutants. Granular cells are known to play an important role in phagocytosis. The functional differences between these two cell types have not been fully elucidated herein and in other studies. However, the role of hyalunocytes is not fully understood. Hyalunocytes are smaller and less granular than granulocytes and have a large central nucleus. However, both cell types are thought to be involved in the cytotoxic immune response (Wang et al., 2012). The total number of hemocytes and

the differential number of hemocytes thus provide an informative way of assessing the general physiological condition of an animal (Battison et al., 2003; Qyli et al., 2020). Because crab hemocytes are involved in the immune response, their defense against pathogens and environmental contaminants, as well as their survival success, is highly dependent on the number, proportion, and cellular integrity of circulating immunocytes.

5. CONCLUSION

In this study, which was conducted in the Çardak lagoon, the relationship between the hemolymph structure of *C. aestuarii*, one of the most important representatives of the lagoon areas, and the environmental and morphometric variables was discussed. Total hemocyte count is positively correlated to temperature and pH and negatively correlated to salinity and oxygen levels. Consequently, this study showed that the temperature, carapace length (CL), and sex significantly the number and types of hemolymphs.

ACKNOWLEDGEMENTS

The authors would like to thank commercial fisherman Hasan Şenses Tülümen for sampling in this study.

FUNDING

No financial support was received in the conduct of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

The authors equally contributed to the present study.

ETHICAL STATEMENT

The material used in this article is invertebrate species therefore, ethics committee approval is not required for this study.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the corresponding author upon reasonable request.

REFERENCES

Adeogun, A. O., Salami, O. A., Chukwuka, A. V., & Alaka, O. O. (2015). Hematological and serum biochemical profile of the blue crab, *Callinectes amnicola* from two tropical lagoon ecosystems. *African Journal of Biomedical Research*, *18*(3), 233– 247.

Aliko, V., Hajdaraj, G., Caci, A., & Faggio, C. (2015). Copper induced lysosomal membrane destabilization in haemolymph cells of Mediterranean green crab (*Carcinus aestuarii*, Nardo, 1847) from the Narta Lagoon (Albania). *Brazilian Archives of Biology and Technology*, *58*(5), 750-756. https://doi.org/10.1590/S1516-89132015050244

Bachau, A. G. (1981). Crustaeceans. *Invertebrate blood cells*, *2*, 385-420.

Bauchau A. G, & Plaquet J. C. (1973). Variation du nombre des hémocytes chez les Crustacés Brachyoures. *Crustaceana*, *24*(2), 215-23. https://doi.org/10.1163/156854073X00380

Başusta A, & Şen D. (2004). Keban Baraj Gölü'nde yaşayan *Acanthobrama marmid* (Heckel,1843) de kan parametrelerinin incelenmesi. *Turkish Journal of Veterinary and Animal Sciences*, *28*(1), 1-6.

Battison, A., Cawthorn, R., & Horney, B. (2003). Classification of Homarus americanus hemocytes and the use of differential hemocyte counts in lobsters infected *with Aerococcus viridans* var. *homari* (Gaffkemia). *Journal of Invertebrate Pathology*, *84*(3), 177-197. https://doi.org/10.1016/j.jip.2003.11.005

Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Burgos-Aceves, M. A., & Faggio, C. (2017). An approach to the study of the immunity functions of bivalve hemocytes: physiology and molecular aspects. *Fish and Shellfish Immunology*, *67*, 513–517. https://doi.org/10.1016/j.fsi.2017.06.042

Clare, A. S., & Lumb, G. (1994). Identification of hemocytes and their role in clotting in the blue crab *Callinectes sapidus*. *Marine Biology*, *118*(4), 601-610. https://doi.org/10.1007/BF00347507

Coles, J. A., Farley, S. R. & Pipe, R. K. (1994). Effects of fluoranthene on the immunocompetence of the common marine mussel, *Mytilus edulis*. *Aquatic Toxicology*, *30*(4), 367–379. https://doi.org/10.1016/0166-445X(94)00051-4

Dutta, S., Biswas, B., & Guha, B. (2021). Sex specific quantitative estimation of total haemocytes in mud crab, *Scylla serrata* (Forskâl, 1775): An evidenced based report. *Journal of Entomology and Zoology Studies*, *9*(4), 439-441. https://doi.org/10.22271/j.ento.2021.v9.i4f.8814

Gürkan, M. (2018). Effects of three different nanoparticles on bioaccumulation, oxidative stress, osmoregulatory, and immune responses of *Carcinus aestuarii*. *Toxicological & Environmental Chemistry*, *100*(8-10), 693-716. https://doi.org/10.1080/02772248.2019.1579818

Hose, J. E., Martin, G. G., & Gerard, A. S. (1990). A decapod hemocyte classification scheme integrating morphology, cytochemistry and function. *Biology Bulletin*, *178*(1), 33-45.

Mancuso, M., Zaccone, R., Carella, F., Maiolino, P., & Vico, G. D. (2013). First Episode of Shell Disease Syndrome in *Carcinus aestuarii* (Crustacea: Decapoda: Portunidae) in the Volturno River. *Journal of Aquaculture Research & Development*, *4*(5), 191. https://doi.org/10.4172/2155-9546.1000191

Matozzo, V., & Marin, M. G. (2010). A first cytochemical study of hemocytes from the crab *Carcinus aestuarii* (Crustacea, Decapoda). *European Journal of Histochemistry, European Journal of Histochemistry*, *54*(1). https://doi.org/10.4081/ejh.2010.e9

Matozzo, V., Gallo, C., & Marin, M. G. (2011). Effects of temperature on cellular and biochemical parameters in the crab *Carcinus aestuarii* (Crustacea, Decapoda). *Marine Environmental Research*, *71*(5), 351–356. https://doi.org/10.1016/j.marenvres.2011.04.001

Matozzo, V., Boscolo, A., & Marin, M. G. (2013). Seasonal and gender-related differences in morphometric features and cellular and biochemical parameters of *Carcinus aestuarii* from the Lagoon of Venice. *Marine Environmental Research*, *89*, 21-28. https://doi.org/10.1016/j.marenvres.2013.04.007

Matozzo, V., Pagano, M., Spinelli, A., Caicci, F., & Faggio, C. (2016). *Pinna nobilis*: a big bivalve with big hemocytes? *Fish and Shellfish Immunology*, *55*, 529–534. https://doi.org/10.1016/j.fsi.2016.06.039

Mello, D.F., Proenca, L.A., & Barracco, M.A. (2010). Comparative study of various immune parameters in three bivalve species during a natural bloom of *Dinophysis acuminata* in Santa Catarina Island, Brazil. *Toxins*, *2*(5), 1166–1178. https://doi.org/10.3390/toxins2051166

Petri D., Glover C. N., Ylving S., Kolas K., Fremmersvik G., Waagbo R. & Berntssen M. H. G. (2006). Sensitivity of Atlantic salmon (*Salmo salar*) to dietary endosulfan as assessed by

hematology, blood biochemistry, and growth parameters. *Aquatic Toxicology*, *80*(3), 207-216. https://doi.org/10.1016/j.aquatox.2006.07.019

Pugazhvendan, S. R., & Soundararajan, M. (2012). Quantitative changes of total haemocytes count during metamorphosis and reproduction in the insect *Chrysocoris purpureus* (Hemiptera: Pentatomidae). *African Journal of Basic & Applied Sciences*, *4*(5), 143-145. https://doi.org/10.5829/idosi.ajbas.2012.4.5.6576

Qyli, M., Aliko, V., & Faggio, C. (2020). Physiological and biochemical responses of Mediterranean green crab, *Carcinus aestuarii*, to different environmental stressors: Evaluation of hemocyte toxicity and its possible effects on immune response. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *231*, 108739. https://doi.org/10.1016/j.cbpc.2020.108739

Sawyer, T. K., Cox, R., & Higginbottom, M. (1970). Hemocyte values in healthy blue crabs, *Callinectes sapidus*, and crabs infected with the amoeba, *Paramoeba perniciosa*. *Journal of Invertebrate Pathology*, *15*(3), 440-446. https://doi.org/10.1016/0022-2011(70)90188-6

Söderhäll, K. & Smith, V.J. (1983). Separation of the haemocyte populations of *Carcinus maenus* and other marine decapods, and prophenoloxidase distribution. *Developmental & Comparative Immunology*, 7(2), 229-239. https://doi.org/10.1016/0145-305X(83)90004-6

Türeli, C. (1999). *İskenderun Körfezi'ndeki Mavi Yengeç (Callinectes sapidus RATHBUN, 1896)'in Biyolojik Özellikleri* [Doctoral Thesis, Çukurova University].

Wang, Y., Hu, M., Chiang, H. W. L., Shin, P. K. S., & Cheung, S. G. (2012). Characterization of subpopulation and immune-revelated parameters of hemocytes in the green-lipped mussel *Perna viridis*. *Fish and Shellfish Immunology*, *31*, 381-390. https://doi.org/10.1016/j.fsi.2011.08.024