



Lysosomal Membrane Stability Dynamics and Hemocyte Composition in Hemolymph-Affected *Carcinus aestuarii* from the Çanakkale Strait

Seçil ACAR ^{1*} A. Suat ATEŞ ¹

^a Çanakkale Onsekiz Mart University Faculty of Marine Science and Technology, Department of Marine Biology, Çanakkale, Türkiye

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ABSTRACT

Lysosomal membrane stability and hemocyte composition are widely used biomarkers of physiological stress in marine crustaceans. This study investigated lysosomal destabilization dynamics and differential hemocyte distribution in 9 hemolymph-affected and 15 non-affected *Carcinus aestuarii* individuals exhibiting hemolymph alterations compared to non-affected crabs collected from the Çanakkale Strait. Neutral red retention time (NRRT) assays were conducted over a 30–180 min period, and repeated-measures mixed modeling was applied to assess temporal stability patterns. Healthy hemocyte percentages declined significantly over time ($p < 0.001$), and a significant time $\times$ hemolymph condition interaction ($p = 0.045$) indicated accelerated destabilization in hemolymph-affected individuals. Complementary stability descriptors showed consistent trends toward reduced membrane integrity in affected crabs. Multivariate analysis of hemocyte proportions revealed significant differences in overall cellular composition ($p = 0.029$), suggesting immune cell redistribution characterized by relative granulocyte dominance. Total hemocyte abundance did not significantly correlate with stability indices, indicating that cellular balance rather than cell density underlies the observed patterns. These findings demonstrate that hemolymph alterations in *C. aestuarii* are associated with coordinated changes in lysosomal stability dynamics and immune cell composition, highlighting potential early indicators of physiological disturbance in transitional marine systems.

Introduction

Parasitic and microbial infections represent a significant source of physiological stress in marine decapod crustaceans, imposing substantial energetic costs on the host and disrupting key homeostatic processes (Shields et al., 2000; Hatcher et al., 2012). Among the most widely documented biotic stressors in brachyuran crabs are dinoflagellate parasites of the genus *Hematodinium*, which cause progressive deterioration of host tissue, alteration of hemolymph biochemistry, and ultimately mortality in heavily infected individuals (Stentiford and Shields, 2005). Infections by *Hematodinium* and morphologically similar parasitic-like organisms have been reported across a broad range of decapod species, including *Carcinus maenas*, *Callinectes sapidus*, *Cancer pagurus*, and *Chionoecetes* spp., suggesting that biotic stress of parasitic origin is a recurrent and ecologically relevant phenomenon in coastal crustacean populations (Meyers et al., 1987; Stentiford et al., 2002; Davies et al., 2019). Hemolymph cellular alterations consistent with such infections — including the presence of intracellular or extracellular inclusion-like bodies in circulating hemocytes — provide a field-accessible indicator of parasitic burden, even in the absence of molecular confirmation.

The physiological consequences of such biotic stress are mediated primarily through the immune system of the host. Crustacean immunity is entirely innate and depends largely on circulating hemocytes and their associated cellular mechanisms. Hemocytes are central to key defensive processes, including coagulation, phagocytosis, encapsulation, melanization, and detoxification (Matozzo and Marin, 2010b; Maharajan et al., 2017). Because crustaceans possess an open circulatory system in which hemocytes freely circulate within

the hemolymph, changes in hemocyte abundance or composition can reflect shifts in immune activity and health status.

Total hemocyte count (THC) and differential hemocyte count (DHC) are widely used indicators of stress and physiological disturbance in crustaceans (Le Moullac et al., 1998; Lorenzon et al., 2008; Jussila et al., 2008). Hemocytes are typically classified into hyalinocytes, semigranulocytes, and granulocytes based on cytoplasmic granularity (Hose et al., 1990; Matozzo and Marin, 2010). Variations in the relative proportions of these cell types may indicate immune activation, redistribution processes, or altered physiological regulation in response to environmental or biological stressors.

Lysosomal membrane stability, commonly assessed using the neutral red retention time (NRRT) assay, is considered a sensitive biomarker of sublethal stress in marine invertebrates. Destabilization of lysosomal membranes reflects impaired cellular homeostasis and reduced physiological resilience. Importantly, when evaluated through repeated time-based measurements, NRRT provides insight into the dynamics of membrane integrity rather than static endpoints, allowing detection of subtle differences in stability trajectories that may not be evident from single time-point comparisons.

Carcinus aestuarii, a Mediterranean brachyuran species with wide ecological tolerance, plays an important role in transitional marine ecosystems. Due to its ecological plasticity, invasive potential in certain regions, and frequent use as bait in fisheries, monitoring the physiological condition and immune status of this species is relevant for both ecosystem functioning and resource management.

Despite the well-documented occurrence of parasitic and parasitic-like infections in brachyuran crabs, the coordinated cellular responses associated with such biotic stress remain insufficiently characterised at the level of individual

* Corresponding author

E-mail address: secilkolsal@comu.edu.tr (Seçil ACAR)

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biomarkers and their interactions. Previous studies have largely focused on parasite prevalence, pathological description, or isolated immunological parameters, without integrating time-resolved lysosomal stability dynamics with differential hemocyte profiling under natural field conditions. In particular, the application of repeated-measures NRRT analysis — capable of capturing destabilization kinetics rather than static endpoints — in the context of parasitic-like hemolymph alterations has not been reported for *C. aestuarii* or comparable Mediterranean brachyurans. Consequently, the degree to which parasitic-like infections reorganise the cellular immune landscape and compromise lysosomal membrane integrity in ecologically important transitional-zone crabs remains poorly understood.

Therefore, the aim of this study was to investigate whether *C. aestuarii* individuals exhibiting hemolymph cellular alterations consistent with parasitic-like infection differ from non-affected conspecifics in lysosomal membrane stability dynamics and hemocyte composition. By integrating repeated-measures NRRT analysis with total and differential hemocyte profiling under natural field conditions in the Çanakkale Strait, this study provides a comprehensive cellular-level framework for assessing physiological responses to biotic stress in a ecologically important transitional marine system.

Material and Methods

Sample collection

A total of 100 *C. aestuarii* specimens from Çanakkale Strait (Türkiye) were captured with crab traps in November 2021 and May 2022. The carapace length (CL) was measured using a digital caliper (0.01 mm) and the total wet body weight (TW) was measured with a digital scale (0.001 g). A total of 24 individuals were included in the physiological and immunological analyses. For each individual, carapace width (CW), carapace length (CL), and body weight were recorded. Sex was determined morphologically. Infection status was classified as infected or uninfected based on hemolymph smear examination.

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

Crabs were anesthetized on ice for 10 min before 500 µL 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 °C) in Eppendorf tubes, a manual count was performed on a Thoma slide to calculate the total number of hemocytes (THC) (Söderhall and 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. For differential hemocyte analysis, hemolymph smears were prepared and stained according to standard cytological procedures. Hemocytes were classified into three categories based on morphological characteristics: Hyalinocytes, Semigranulocytes, Granulocytes. For each individual, the relative proportion (%) of each hemocyte type was calculated by counting a minimum of 100 cells per slide. Since differential hemocyte components sum to 100%, the dataset was treated as compositional. To avoid spurious correlations caused by constant-sum constraints, proportions were transformed using the centered log-ratio (CLR) transformation prior to multivariate analyses. A small pseudocount was added where necessary to avoid zero values.

Hemolymph Examination and Classification of Infection Status

Hemolymph was extracted from the arthrodistal membrane using sterile syringes and examined immediately under light microscopy. Individuals were classified into two groups based on microscopic examination of hemolymph smears: Hemocyte samples showing abnormal intracellular or extracellular inclusion-like bodies were categorized as “hemolymph-affected” (hereafter referred to as “affected”). Individuals lacking such cellular alterations were classified as “non-affected”. Because no molecular (PCR-based) or histopathological confirmation was performed, the presence of parasitic infection could not be definitively diagnosed. Therefore, the term “infection” in this study refers to hemolymph cellular

alterations consistent with parasitic-like structures observed under light microscopy. This conservative classification was adopted to avoid overinterpretation of diagnostic status.

Lysosomal Membran Stability

Lysosomal membrane stability was evaluated using the neutral red retention time (NRRT) assay. Hemocytes were incubated with neutral red dye, and the percentage of dye-retaining (healthy) cells was recorded at 30, 60, 90, 120, 150, and 180 minutes for each individual. This time-based approach allowed assessment of the dynamics of membrane destabilization rather than a single endpoint measurement. To summarize temporal stability patterns in a biologically meaningful manner, three complementary descriptors were calculated for each crab: cumulative lysosomal stability across the 30–180 min period (area under the curve, AUC), the time required for healthy cell percentage to decline to 50% (t50), and the overall rate of decline in healthy cell percentage over time (slope). Higher AUC and t50 values indicate prolonged membrane integrity, whereas steeper negative slopes reflect faster destabilization. These parameters were used to compare lysosomal stability between non-affected and hemolymph-affected individuals.

Statistical Analysis

Lysosomal membrane stability was measured repeatedly within the same individuals, a linear mixed-effects model was applied to evaluate temporal dynamics while accounting for within-individual variability. Healthy cell percentage was modeled as a function of time, hemolymph condition, and their interaction, with individual identity included as a random effect. This approach allowed detection of differences in destabilization rates between groups rather than relying solely on single time-point comparisons. Differences in cumulative stability descriptors (AUC, t50, slope) were assessed using group comparisons. Differential hemocyte composition was analyzed using multivariate analysis of variance (MANOVA) after centered log-ratio (CLR) transformation of proportional data to account for compositional constraints. Total hemocyte counts were log10-transformed prior to analysis to meet assumptions of normality. Statistical significance was set at p < 0.05.

Results

Study Population and Morphometric Characteristics

A total of 100 *C. aestuarii* specimens were collected from the Çanakkale Strait across two sampling occasions (November 2021: n = 50; May 2022: n = 50). Of these, 24 individuals were selected for physiological and immunological analyses based on hemolymph smear examination results. Of these, 9 were classified as hemolymph-affected and 15 as non-affected based on microscopic examination of hemolymph smears. The sampled population comprised 35 females and 65 males. Mean carapace length was 45.38 ± 0.68 mm (range: 30.36–65.92 mm), mean carapace width was 37.59 ± 0.56 mm (range: 16.49–53.64 mm), and mean body weight was 29.77 ± 1.30 g (range: 10.59–88.19 g). Morphometric measurements by sex are presented in Table 1. Macroscopic examination of dissected individuals revealed evidence of tissue damage to the hepatopancreas and gills in a subset of specimens, consistent with physiological disturbance.

Time-Dependent Lysosomal Membrane Stability

Lysosomal membrane stability declined progressively over time in all

Table 1 Morphometric measurements in C. aestuarii

	N	Carapace length		Carapace width		Weight	
		CL (mm)	CL (min-max)	CW (mm)	CW (min-max)	W (g)	W (min-max)
Female	35	45.01±0.68	34.3-58.95	37.31±0.94	28.16-48.97	29.16±2.20	11.64-88.19
Male	65	45.38±0.85	30.36-65.92	37.59±0.69	16.49-53.64	29.77±1.62	10.59-88.19
Total	100	45.38±0.68	30.36-65.92	37.59±0.56	16.49-53.64	29.77±1.3	10.59-88.19

examined individuals (Figure 1). Healthy hemocyte percentages remained relatively high during the initial incubation phase (30–60 min), followed by a marked decline after 90 minutes that became more pronounced between 120 and 180 minutes. The linear mixed-effects model revealed a highly significant overall time effect ($p < 0.001$), confirming that lysosomal destabilization is a consistent and progressive cellular process across the full study population. Crucially, a significant time $\times$ hemolymph condition interaction was detected ($p = 0.045$), indicating that the rate of membrane destabilization differed between groups. Hemolymph-affected individuals exhibited a consistently steeper decline in healthy hemocyte percentage compared to non-affected crabs, with the separation between groups becoming most evident during the later time intervals (120–180 min).

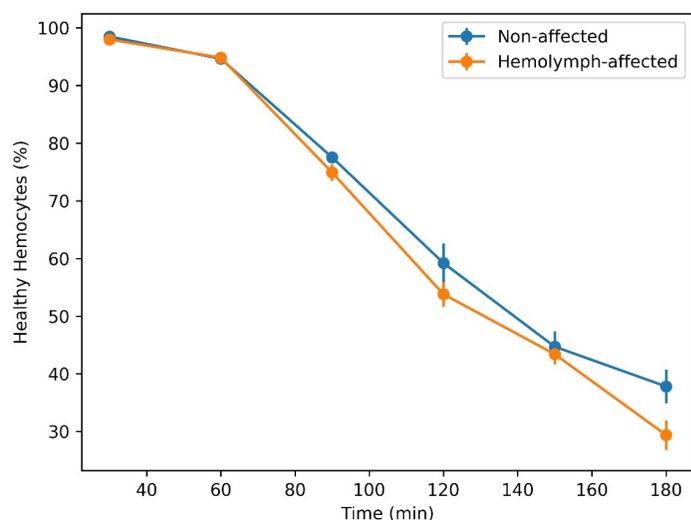


Figure 1 Mean ($\pm$ SE) percentage of healthy hemocytes over time in non-affected and hemolymph-affected *C. aestuarii*.

Individual Stability Patterns

Individual NRRT trajectories revealed considerable within-group variability in lysosomal stability (Figure 2). While all individuals demonstrated a time-dependent decline in healthy hemocyte percentage, hemolymph-affected crabs generally exhibited earlier onset and more abrupt reductions in cellular integrity. Several affected individuals reached markedly low stability values at 150–180 minutes, indicating substantially reduced cellular resilience compared to non-affected crabs. This pattern of accelerated destabilization in affected individuals was consistent across the repeated-measures framework and corroborated by the significant time $\times$ condition interaction identified in the mixed model.

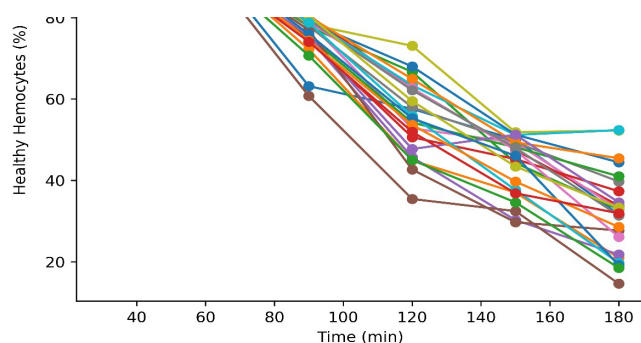


Figure 2 Individual NRRT trajectories showing time-dependent decline in healthy hemocytes

Cumulative Lysosomal Stability and Stability Descriptors

Cumulative lysosomal stability, expressed as the area under the curve (AUC) across the 30–180 min incubation period, tended to be lower in hemolymph-affected individuals compared to non-affected crabs (Figure 3). Although inter-individual variability within groups was considerable and group differences in AUC alone did not reach statistical significance,

the directional trend was consistent with the time-dependent findings. The time required for healthy hemocytes to decline to 50% (t50) was generally shorter in hemolymph-affected individuals, indicating earlier onset of membrane destabilization. As with AUC, within-group variability limited independent statistical separation of t50 values; however, the consistent shift toward lower t50 in affected crabs aligns with the accelerated destabilization dynamics identified in the longitudinal repeated-measures analysis. Considered together, AUC, t50, and slope descriptors all indicated a directional reduction in membrane integrity in hemolymph-affected individuals, supporting the overall pattern detected by the mixed-effects model.

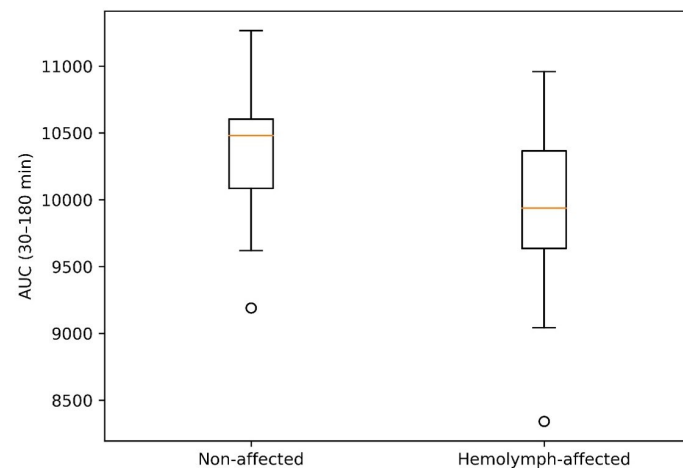


Figure 3 Boxplot of cumulative lysosomal stability (AUC) for non-affected and hemolymph-affected individuals.

Hemocyte Composition

Differential hemocyte analysis revealed a significant shift in cellular distribution associated with hemolymph condition (Figure 4). Hemolymph-affected individuals displayed higher relative proportions of granulocytes compared to non-affected crabs, suggesting immune cell redistribution rather than a simple change in total hemocyte abundance. Multivariate analysis of CLR-transformed hemocyte proportions confirmed a significant overall effect of hemolymph condition on cellular composition (MANOVA, $p = 0.029$), while effects of sex and the sex $\times$ condition interaction were not statistically significant. Total hemocyte count (THC) did not differ significantly between affected and non-affected individuals and showed no significant correlation with lysosomal stability indices, indicating that cellular balance rather than total cell density underlies the observed immunological differences.

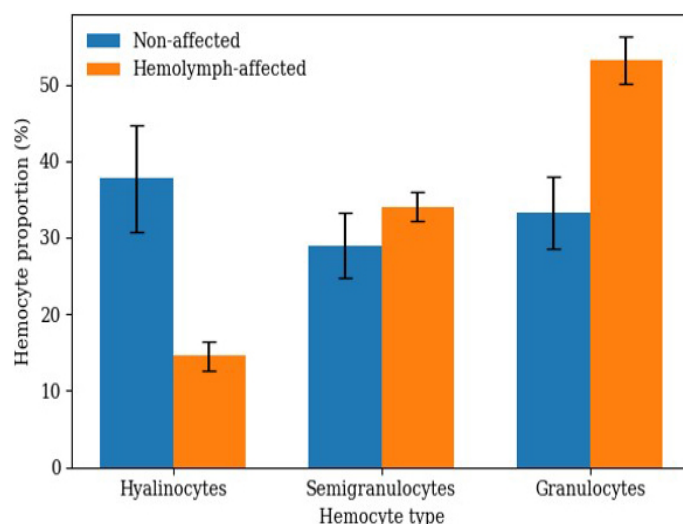


Figure 4 Differential hemocyte composition (% mean $\pm$ SE) in non-affected and hemolymph-affected *Carcinus aestuarii*.

Discussion

The present study demonstrates that individuals exhibiting hemolymph cellular alterations show coordinated changes in lysosomal membrane stability dynamics and hemocyte composition, providing a comprehensive cellular-level perspective on physiological stress responses in *C. aestuarii*. The time-dependent decline in healthy hemocyte percentage observed across all individuals confirms that lysosomal destabilization is a progressive cellular process. The significant time $\times$ hemolymph condition interaction detected in the mixed-effects model indicates, however, that destabilization proceeded at a faster rate in hemolymph-affected individuals, suggesting that cellular homeostasis and membrane integrity are more vulnerable in crabs displaying hemolymph abnormalities.

A key methodological contribution of this study is the application of a time-series approach to NRRT analysis. Whereas lysosomal membrane stability is conventionally assessed at a single time point, the repeated-measures framework employed here allowed the dynamics of destabilization — including its rate and severity — to be characterized rather than merely its presence or absence. By modelling the time $\times$ condition interaction within a mixed-effects structure, the analysis moves beyond static biomarker assessment towards a form of dynamic stress modelling, offering substantially higher resolution than single time-point comparisons.

Lysosomal membrane stability, as assessed by the NRRT assay, has been extensively applied as a biomarker of sublethal stress associated with heavy metal and chemical contaminant exposure in marine invertebrates (Wedderburn et al., 1998; Aguirre-Martinez et al., 2013). Similar lysosomal destabilization patterns have been reported in *C. aestuarii* from other Mediterranean lagoon systems following exposure to copper, supporting the broad applicability of NRRT as a sensitive indicator of cellular stress in this species (Aliko et al., 2015). However, the accelerated destabilization pattern observed in the present study cannot be attributed solely to exogenous chemical stressors. The earlier and more pronounced loss of stability in hemolymph-affected individuals suggests that internal biotic stressors — such as parasitic or microbial interactions — may exert effects on lysosomal integrity comparable in magnitude to those of environmental contaminants. These findings support the interpretation that NRRT dynamics can serve not only as an indicator of pollution-related cellular stress, but also as an early warning signal of endogenous biological stress processes within the organism.

A further notable finding is the absence of a significant relationship between total hemocyte count (THC) and lysosomal stability indices, contrasted with a significant shift in differential hemocyte composition. Although THC is frequently employed as a primary stress indicator in the crustacean immunology literature (Le Moullac et al., 1998; Lorenzon et al., 2008), the present results suggest that the immune response is shaped by qualitative cellular reorganisation rather than by quantitative changes in cell density. This apparent discrepancy is consistent with previous observations in *C. aestuarii*, where THC was found to exhibit considerable inter-individual variability even under controlled conditions, limiting its reliability as a standalone stress indicator (Matozzo & Marin, 2010a). The elevated proportion of granulocytes in hemolymph-affected individuals is consistent with the activation of active immune defence mechanisms — including melanisation and encapsulation — as described by Söderhall and Cerenius (1992). This pattern indicates that *C. aestuarii* exhibits immune plasticity under chronic stress conditions, functionally reorganising its existing cellular composition rather than simply increasing total hemocyte output.

The hemolymph cellular alterations described in this study, while not definitively diagnosed, are consistent with parasitic-like structures reported in decapod crustaceans, including Hematodinium-like infections documented in *Carcinus maenas* and other brachyurans (Stentiford et al., 2002; Stentiford & Shields, 2005). Such infections are known to impose substantial physiological costs on the host, diverting energetic resources away from growth and maintenance towards defence processes (Shields et al., 2000; Hatcher et al., 2012). This energetic reallocation may reduce the resilience of lysosomal membranes to environmental stressors, thereby providing a mechanistic explanation for the accelerated destabilization dynamics observed in affected individuals.

From an ecological perspective, the Çanakkale Strait should be regarded not merely as a sampling location, but as a natural experimental platform for investigating the intersection of biotic and abiotic stress. As a transitional marine system characterised by variable salinity, strong bidirectional currents, and the interaction of distinct water masses, the strait

subjects its resident organisms to simultaneous physical and biological pressures (Wahl, 1989). The cellular stress patterns documented here are therefore relevant not only to the local population of *C. aestuarii*, but may also serve as a generalizable model for ecophysiological responses in comparable estuarine and lagoonal systems under increasing anthropogenic and climate-driven pressures.

This study has several limitations that should be acknowledged. First, the classification of individuals as hemolymph-affected was based exclusively on light microscopy of hemolymph smears; without molecular (PCR-based) or histopathological confirmation, the precise identity of the causative agent — whether Hematodinium-like parasites, microbial pathogens, or another biotic source — remains unresolved (Stentiford & Shields, 2005). Second, the relatively small number of affected individuals ($n = 9$) limited the statistical power available for between-group comparisons of cumulative stability descriptors (AUC, t50), which may explain why these metrics did not reach significance despite the clear pattern detected by the repeated-measures mixed model. Future studies incorporating larger sample sizes, molecular diagnostics, and seasonal replication would help confirm and extend these findings.

Conclusion

This study demonstrates that hemolymph cellular alterations in *Carcinus aestuarii* are associated with coordinated and simultaneous changes in both lysosomal membrane stability dynamics and hemocyte composition, providing an integrated cellular framework for the assessment of physiological stress under natural field conditions. Time-series NRRT analysis revealed that lysosomal destabilization was initiated earlier and progressed more rapidly in hemolymph-affected individuals, confirming that lysosomal membrane integrity is sensitive not only to exogenous chemical stressors but also to endogenous biotic processes.

The finding that total hemocyte count did not constitute a reliable stress indicator, while differential hemocyte composition — particularly the relative dominance of granulocytes — more accurately reflected the organism's immunological status, underscores the importance of qualitative cellular profiling in crustacean health assessments. The observed immune cell redistribution suggests that *C. aestuarii* exhibits immune plasticity under chronic stress, reorganising its defensive capacity qualitatively rather than quantitatively.

The primary methodological contribution of this study lies in the transformation of NRRT from a static biomarker into a time-series-based dynamic stress indicator. Through repeated measurements and mixed-effects modelling, both the presence and the rate of change in lysosomal stability were characterised, offering a substantially higher-resolution physiological assessment than conventional single time-point approaches.

The hemolymph alterations documented here, while not definitively identified, are consistent with parasitic or microbial interactions reported in other decapod species, suggesting a biotic stress scenario in which energetic resources are redirected towards immune defence, thereby increasing cellular vulnerability to environmental perturbation. The combined assessment of lysosomal stability dynamics and hemocyte composition thus represents a sensitive and integrated biomarker approach for the early detection of sublethal stress in *C. aestuarii* and potentially in other ecologically important brachyuran species inhabiting dynamic transitional marine environments.

Future studies should prioritise the molecular and histopathological confirmation of the hemolymph alterations identified here, as well as the investigation of their interactions with environmental stressors across different seasons and population densities. Such work would substantially strengthen the ecological and ecotoxicological applicability of the integrated biomarker framework presented in this study.

Statements and Declarations

This study did not require approval from an institutional ethics committee, an invertebrate species and is not subject to animal experimentation ethics regulations.

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Declarations

(1998). Biomarkers of biochemical and cellular stress in *Carcinus maenas*: An in situ field study. *Marine Environmental Research*, 46(1–5), 321–324.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

S.A. designed the study, conducted the experiments, analyzed the data, and wrote the manuscript. A.S.A. contributed to study design and manuscript revision. Both authors approved the final version.

References

- Aguirre-Martínez, G. V., Buratti, S., Fabbri, E., Del Valls, T. A., & Martín-Díaz, M. L. (2013). Stability of lysosomal membrane in *Carcinus maenas* acts as a biomarker of exposure to pharmaceuticals. *Environmental Monitoring and Assessment*, 185, 3783–3793. <https://doi.org/10.1007/s10661-012-2827-2>
- Davies, C. E., Batista, F. M., Malkin, S. H., Thomas, J. E., Bryan, C., Crocombe, C., Coates, C., & Rowley, A. (2019). Spatial and temporal disease dynamics of the parasite *Hematodinium sp.* in shore crabs, *Carcinus maenas*. *Parasites & Vectors*, 12, 472. <https://doi.org/10.1186/s13071-019-3727-x>
- Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment*, 10(4), 186–194. <https://doi.org/10.1890/110016>
- Hose, J. E., Martin, G. G., & Gerard, A. S. (1990). A decapod hemocyte classification scheme integrating morphology, cytochemistry, and function. *The Biological Bulletin*, 178(1), 33–45. <https://doi.org/10.2307/1541535>
- Jussila, J., Henttonen, P., Huner, J. V., & Lindqvist, O. V. (2008). Hemolymph clotting time and its usability as a stress indicator in noble crayfish (*Astacus astacus*). *Freshwater Crayfish*, 16, 11–16.
- Le Moullac, G., Soyeux, C., Saulnier, D., Ansquer, D., Avarre, J. C., & Levy, P. (1998). Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. *Fish & Shellfish Immunology*, 8(8), 621–629. <https://doi.org/10.1006/fsim.1998.0166>
- Lorenzon, S., Giulianini, P. G., Libralato, S., Martinis, M., & Ferrero, E. A. (2008). Stress effect of two different transport systems on the physiological profiles of the crab *Cancer pagurus*. *Aquaculture*, 278(1–4), 156–163. <https://doi.org/10.1016/j.aquaculture.2008.03.011>
- Maharajan, A., Narayanaswamy, Y., & Ganapiriy, V. (2017). Haematological changes of fresh water crab, *Paratelphusa jacquemontii*, in response to the combination of chlorpyrifos and cypermethrin (Nurocombi) insecticide. *Annals of Aquaculture and Research*, 4(3), 1041.
- Matozzo, V., & Marin, M. G. (2010a). The role of haemocytes from the crab *Carcinus aestuarii* (Crustacea, Decapoda) in immune responses: A first survey. *Fish & Shellfish Immunology*, 28(4), 534–541. <https://doi.org/10.1016/j.fsi.2009.12.003>
- Matozzo, V., & Marin, M. G. (2010b). Bivalve immune responses and climate changes: Is there a link? *Invertebrate Survival Journal*, 7(2), 218–227.
- Meyers, T. R., Koeneman, T. M., Botelho, C., & Short, S. (1987). Bitter crab disease: A fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs *Chionoecetes bairdi*. *Diseases of Aquatic Organisms*, 3, 195–216. <https://doi.org/10.3354/dao003195>
- Shields, J. D., & Squyers, C. M. (2000). Mortality and hematology of blue crabs, *Callinectes sapidus*, experimentally infected with the parasitic dinoflagellate *Hematodinium perezii*. *Fishery Bulletin*, 98(1), 139–152.
- Söderhall, K., & Cerenius, L. (1992). Crustacean immunity. *Annual Review of Fish Diseases*, 2, 3–23. [https://doi.org/10.1016/0959-8030\(92\)90053-Z](https://doi.org/10.1016/0959-8030(92)90053-Z)
- Söderhall, K., & Smith, V. J. (1983). Separation of the haemocyte populations of *Carcinus maenas* and other marine decapods, and prophenoloxidase distribution. *Developmental and Comparative Immunology*, 7(2), 229–239. [https://doi.org/10.1016/0145-305X\(83\)90004-6](https://doi.org/10.1016/0145-305X(83)90004-6)
- Stentiford, G. D., Green, M., Bateman, K., Small, H. J., Neil, D. M., & Feist, S. W. (2002). Infection by a *Hematodinium*-like parasitic dinoflagellate causes pink crab disease (PCD) in the edible crab *Cancer pagurus*. *Journal of Invertebrate Pathology*, 79(3), 179–191. [https://doi.org/10.1016/S0022-2011\(02\)00028-9](https://doi.org/10.1016/S0022-2011(02)00028-9)
- Stentiford, G. D., & Shields, J. D. (2005). A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Diseases of Aquatic Organisms*, 66(1), 47–70. <https://doi.org/10.3354/dao066047>
- Wahl, M. (1989). Marine epibiosis. I. Fouling and antifouling: Some basic aspects. *Marine Ecology Progress Series*, 58, 175–189. <https://doi.org/10.3354/meps058175>
- Wedderburn, J., Cheung, V., Bamber, S., Bloxham, M., & Depledge, M. H.