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EVALUATION OF HAEMATOLOGICAL AND BIOCHEMICAL STRESS BIOMARKERS AND PHYSIOLOGICAL PARAMETERS IN *CARASSIUS GIBELIO* CAUGHT IN THE COASTAL ZONE OF THE CURONIAN LAGOON

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Abstract. This study aimed to assess the health status of *Carassius gibelio*, a comparatively tolerant fish species, in the largest coastal lagoon in Europe – the Curonian Lagoon. Haematocrit and glucose levels were evaluated as reliable biomarkers of stress responses in fish. Additionally, non-specific physiological biomarkers, including hepatosomatic index and condition factor, were utilized to assess the environmental quality of the Curonian Lagoon. Fish samples were collected from two sites: the southern part of Klaipėda port, known as Kiaulės Nugara Island, and the central part of the Curonian Lagoon near Ventė Cape. Based on the multi-biomarker responses of *C. gibelio*, differences were observed between the two sampling sites, but not statistically significant. Consequently, *C. gibelio* cannot be recommended as a suitable bioindicator of environmental pollution and is not recommended for biomonitoring surveys of the Curonian Lagoon.

Keywords: fish, lagoon, glucose, haematocrit, condition factor.

1. Introduction

The EU Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC) and the Water Framework Directive (WFD, 2000/60/EC) aims to protect the marine ecosystem and biodiversity and to achieve and maintain Good Environmental Status (GES) of marine and coastal waters. According to the WFD, transitional aquatic ecosystems like lagoons require preventive exploration and appropriate monitoring programs to ensure protection and restoration. Unfortunately, all Baltic lagoons have been identified as endangered biotopes in the HELCOM Red List (Baltic Marine Environment Protection Commission, 2013). The continuous rise in anthropogenic pressures has led to a significant decline in the quality of coastal lagoons, posing serious challenges to their resilience (Ligorini et al., 2022). Incidentally, it is pertinent to note that lagoons constitute an inherently unpredictable ecosystem characterized by conspicuous natural seasonal and diurnal variations in parameters (Voulvoulis et al., 2017).

It is well known that the use of ecotoxicological biomarkers at different levels of organism, organization allows for the prediction of the biological responses of organisms to environmental stressors, which is useful in environmental risk assessment (Pérez-Iglesias et al., 2023). For these reasons, ecotoxicologists suggest the use of effect-based approaches as tools for monitoring within the WFD (Milinkovitch et al., 2019).

Fish blood parameters are sensitive biomarkers of pollution-induced stress (Afiyah et al., 2023). Therefore, haematological parameters can be used as early-warning signals in aquatic ecosystem biomonitoring surveys (Witeska et al., 2022). Fish blood glucose level (GLU) is widely employed as a sensitive measure of environmental stress (Ohwofasa & German, 2023). As fish encounter stressful conditions, a physiological-biochemical adaptive mechanism is involved that enables the organism to cope with the disturbance and maintain homeostasis by mobilizing energy resources to facilitate the cellular and organismal response to physical threats or environmental challenges (Wu et al., 2017; Balasch & Tort, 2019). Haematocrit (HCT) is also regarded as an indicator of the secondary stress response and an excellent predictor of physiological changes and general health status in fish. The changes in HCT level depend on the type of stressor and stress duration to which the fish were exposed (Muñoz et al., 2018; Kim et al., 2019). Assessing stress responses is essential for understanding the impact of shifting environments on the health, performance, and welfare of fish, as well as how fish adapt physiologically to such changes (Makaras et al., 2022).

Organo-somatic and morphometric indices, such as condition factor (CF) and hepatosomatic index (HSI), are used as indicators of general fish health (Sadauskas-Henrique et al., 2021). The condition factor is used to assess the overall health and well-being of individual fish or all fish populations as well as partly reflecting feeding conditions. The HSI is the main indicator of metabolic activity in animals (Prakash & Verma, 2019). This index is widely known as a bio-indicator of contaminant exposure, which is expressed as liver size as a percentage of total body weight. The HSI is considered a sensitive indicator of fish's health status as it manifests changes at the tissue level (Gonino et al., 2019).

The Curonian Lagoon is the largest coastal lagoon in Europe. Unfortunately, it is known that the Curonian Lagoon is under high anthropogenic pressure (Jokšas et al., 2016). Due to low water exchange with the sea and high river discharge, the Curonian Lagoon is more vulnerable and susceptible to the effects of pollution (Vaikutienė et al., 2017). The main input of pollutants into the lagoon comes mainly from the Nemunas River, which provides more than 90% of the total water inflow (Manton et al., 2021). Agriculture is one of the biggest sources of pollution in the Nemunas River basin, accounting for 75–90% of total nitrogen (TN) and approximately 70% of total phosphorus (TP) runoff (Trehan, 2020). Also, sediment tests show polycyclic aromatic hydrocarbons, indicating low to moderate contamination near the Nemunas Delta. Not only is the Curonian Lagoon exposed to a potential risk of contamination from the Nemunas River basin, but it also suffers from anthropogenic sources from Klaipėda Port (Stakėnienė et al., 2019). The port houses several industrial facilities, including large stevedoring, shipbuilding, and ship repair companies. Intense industrial activities in the port could potentially have a significant impact on the ecological condition (Jokšas et al., 2019). The port impact zone was found to be contaminated with heavy metals (Jokšas et al., 2016), polycyclic aromatic hydrocarbons (Stakėnienė et al., 2016), organotin compounds (Raudonytė-Svirbutavičienė et al., 2023). Because of its importance to the Baltic Sea ecological status, the Curonian Lagoon is part of the state environmental monitoring program. Unfortunately, the Curonian Lagoon has not been comprehensively studied yet.

Fish are among the main target groups when conducting biomonitoring and ecological assessment of aquatic environments (Meng et al., 2023). The aim of this study was to evaluate the suitability of *Carassius gibelio* as a bioindicator species for biomonitoring in the Curonian Lagoon. To assess the health status of fish, haematological parameters (haematocrit), biochemical indicators (glucose), and physiological biomarkers (condition factor and hepatosomatic indexes) were evaluated. During this study, were selected two sites in the Curonian Lagoon. One research area was selected near Kiaulės Nugara Island, in the Klaipėda port impact zone and another area was in the central part of the Curonian Lagoon, near Cape Ventė, which is potentially affected by the Nemunas River and its pollution.

2. Materials and methods

Study area

The Curonian Lagoon, located in the southeast of the Baltic Sea, is a large (95 km in length and up to 48 km in width) shallow coastal body (mean depth of 3.8 m and maximum 5.8 m). It is the largest coastal lagoon in Europe, covering an area of about 1584 km^2. The Curonian Lagoon is connected to the sea by the narrow Klaipėda Strait and separated from the sea by the sandy Curonian Spit (Idzelytė et al., 2020). The locations of the study sites were chosen after an assessment of potential sources of pollution. One research area was selected near Kiaulės Nugara Island (55.640576, 21.150863), in the Klaipėda Port impact zone and another area was in the central part of the Curonian Lagoon, near Cape Ventė (55.346191, 21.193340), which is potentially affected by the Nemunas River and its pollution. The selected study locations are approximately 36 kilometers apart.

Sampling of fish

Carassius gibelio (Bloch, 1782) were collected using beach seine net in June 2023. At each research site, 11 fish were sampled, for a total of 22 fish. Immediately after capture, the fish blood samples were taken as described below and the fish were euthanized. All fish were measured to the nearest mm (total length) and weighed to the nearest gram (total weight). After this, fish were placed into a portable refrigerator with the ice, and transported to the laboratory, where dissection was performed. Fish liver weight was measured to the nearest decigram. Fish age was assessed based on growth curve presented by Cristina et al. (2016).

Haematological and biochemical biomarkers

Blood samples were taken from the caudal vein of fish using a disposable syringe (1 mL volume, washed with 3.8% sodium citrate) by drawing approximately 0.1 mL of blood and then transferring to blood collection tubes. The concentration of GLU level in fish blood was determined using the automatic Glucose analyser (EKSAN-Gm, Analita, Joint-Stock Company Ltd, Lithuania). The minimum detection limit of the blood GLU method is from 2 to 30 mmol/L and the error for repeated measurements (precision) is ≤5 %. The minimal blood sample volume per measurement is 50 µL.

Haematocrit (HCT) level (packed cell volume, PCV) was measured using a routine method by Svobodova et al. (1991). The blood-filled heparinized capillary tubes were centrifuged for 5 min at 100,000 rpm using Microhaematocrit Centrifuge (Haematocrit 210, Hettich Zentrifugen, Germany), after which HTC was determined. The percentage value obtained in this way was multiplied by a coefficient of 0.01, and the resultant value was the HTC in L L^{-1}. Samples for glucose concentration and HTC level were analysed immediately after the blood collection.

Physiological parameters measurement

Condition factor (CF) and hepatosomatic (HSI) index were computed using the following formulas (ICES, 2011): CF = [[body mass (g) / (total length)^3] *100]; HSI = [LW / (BW – LW) *100]; where LW= weight of liver (g) and BW – fish body weight (g).

Statistical analysis

The data were statistically analysed using R Studio (2022.07.2+576) (ggplot2 3.3.6; readxl 1.4.1.; DescTools 0.99.46; rstatix 0.7.0 and ggpubr 0.6.0 packages). The normality of variable distributions was assessed using the Shapiro–Wilk test ($p > 0.05$). The homogeneity of variance was assessed by Levene's test. The Wilcoxon test (Wilcoxon signed-rank test) was used to determine statistical significance.

3. Results

Fish and their general characteristics

Gross morphometric and biological parameters, haematocrit (HCT), glucose (GLU), hepatosomatic index (HSI) and condition factor (CF) of *Carassius gibelio* are presented in Table 1.

Blood glucose and haematocrit levels

HCT and GLU levels were found to be higher for fish caught at Ventė Cape station. However, statistical analysis of HCT and GLU parameters showed no significant differences when comparing different study sites (HCT ($p = 0.340$), GLU ($p = 0.606$)) as shown in Figure 1.

Table 1. Results of morphometric and biological parameters, haematological, biochemical, and other non-specific biomarkers of *C. gibelio* from different study sites (mean ± SD)

	Kiaulės Nugara Island	Ventė Cape
Morphometric and biological parameters		
Length, cm	17.30 ± 0.837	21.40 ± 4.164
Weight, g	95.43 ± 14.581	205.18 ± 116.835
Age	3.2 ± 0.4	3.8 ± 0.98
Biomarkers		
HCT, L-L^{-1}	0.25 ± 0.038	0.26 ± 0.040
GLU, mmol/L	5.30 ± 1.259	6.57 ± 2.883
HSI	3.33 ± 0.998	2.67 ± 0.830
CF	1.84 ± 0.151	1.92 ± 0.168

Condition factor and hepatosomatic index

For fish caught at Klaipėda port, near Kiaulės Nugara Island, study station were established higher HSI, but lower CF, compared to the fish caught in Ventė Cape station. The statistical analysis revealed no significant differences between the fish collected at different sites for both parameters: CF ($p = 0.243$) and HSI ($p = 0.151$), as depicted in Figure 2.

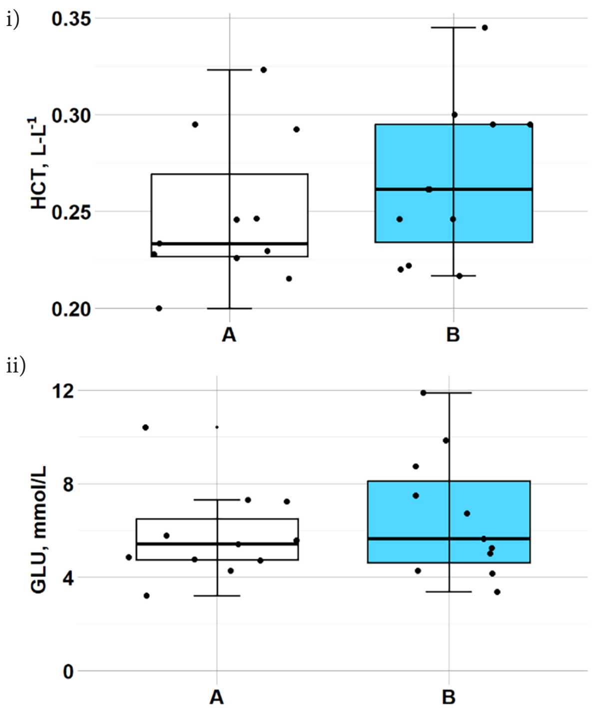

Figure 1. Box plots of *Carassius gibelio* haematological and biochemical parameters (minimum, 25th percentile, median, 75th percentile and maximum values, n = 11). i) haematocrit (HCT, L-L^{-1}); ii) glucose level (GLU, mmol/L). Letters indicate research sites near (A) Kiaulės Nugara Island; and (B) Ventė Cape

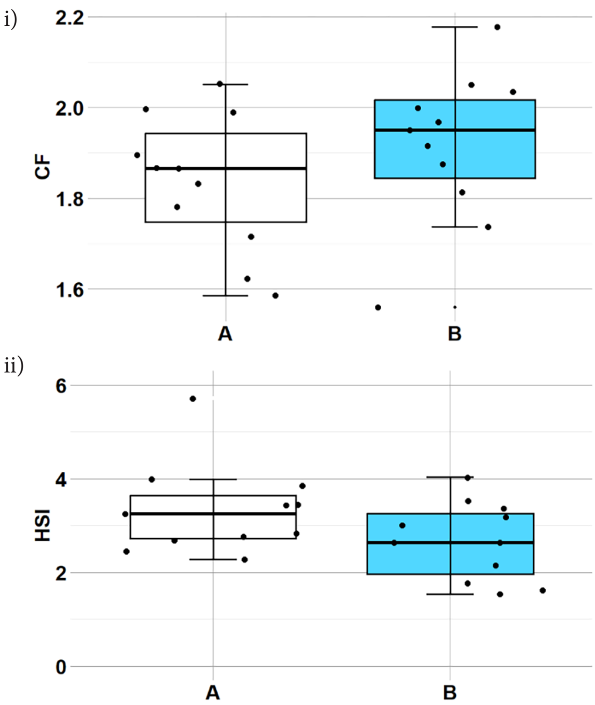

Figure 2. Box plot of *C. gibelio* physiological parameters (minimum, 25th percentile, median, 75th percentile and maximum values, n = 11). i) condition factor (CF); ii) hepatosomatic index (HSI) Letters indicate research sites near (A) Kiaulės Nugara Island; (B) Ventė Cape

4. Discussion

In the present study, the suitability of *C. gibelio* as a bioindicator in Curonian Lagoon biomonitoring surveys was evaluated using different (biochemical, haematological, physiological) biomarkers. Pollution from the Nemunas River basin and Klaipėda Port pose the greatest threat to the Curonian Lagoon (Stakėnienė et al., 2019), so study locations were chosen accordingly. Even though the fish were caught in study stations with different main sources of potential pollution, no statistically significant differences were found in any of the biomarkers studied between the two sampling stations. However, it is important to note that fluctuations in GLU and HSI biomarkers responses were observed, depending on the study location. Blood GLU level increases when fish are stressed by toxic substances or other stressors. For example, a change in glucose levels indicates that gluconeogenesis is occurring in the body, which leads to reduced glucose absorption and indicates liver dysfunction (Hamed et al., 2019). Previous studies have shown that normal blood GLU level in cyprinid fish ranges from 2 to 5 mmol/L mmol/L (Patriche, 2009). In this study, no statistically significant differences were found, but GLU concentrations higher than 5 mmol/L were detected in *C. gibelio* from both study stations, particularly close to Ventė Cape. The area closest to Klaipėda port had the highest average quantities of heavy metals. (Jokšas et al., 2016). Sediment tests reveal the presence of polycyclic aromatic hydrocarbons, indicating low to moderate pollution, as well as significant quantities of naphthalene, which has been linked to adverse biological impacts in the Nemunas Delta region near Ventė Cape (Stakėnienė et al., 2019).

According to the assessment of HCT levels, no differences were observed in fish from both research areas. The results are challenging to interpret due to the limited amount of research available. Previous research has indicated that *C. gibelio* had an average HCT value of 0.227 under laboratory settings (Nikolov & Boyadzieva-Doichinova, 2010). Environmental toxins can increase or decrease haematocrit levels in fish (Witeska et al., 2022), although these fluctuations are heavily influenced by factors such as water temperature, fish species, nutrition, weight, and activity (Seibel et al., 2021).

The HSI and CF are important indicators that help assess how species obtain resources from their habitat, their general well-being, and their adaptation to the environment. This is the first study to analyse HSI and CF in *C. gibelio* caught in a lagoon ecosystem. Based on data from other studies, in the autumn, the average HSI value for *C. gibelio* in a polluted river site was found to be 5.44, while in an unpolluted river, it was – 5.87. Correspondingly, these HSI values changed in the spring to 2.36 at the contaminated site and 1.44 at an unpolluted river site. These results show that the HSI value is significantly reduced during the autumn season, indicating site contamination (Mijošek et al., 2021). The HSI values obtained in this study are difficult to assess because it is known that HSI exhibits a clear seasonal pattern. In general, the HSI is higher in the summer than in other seasons because fish are actively feeding and metabolizing energy during this time. Further on, a higher CF value indicates without a doubt that the fish is in an improved condition. In a study conducted in uncontaminated lakes, CF values ranged between 1.61–1.64 for *C. gibelio* (Jelkić & Opačak, 2023). The mean CF values in this study did not show the potential impacts of environmental contamination. It is important to emphasize that HSI in fish from both study sites does not indicate the effects of chemical pollution and together with CF may indicate a good capacity of *C. gibelio* to cope with stress (Tenji et al., 2020).

Studies of different biomarker responses in *C. gibelio* show potential environmental pollution risks to fish health, but also demonstrate that *C. gibelio* is resilient to environmental contamination. The research results underscore the importance of studying the impact of Curonian Lagoon water quality on fish health. To comprehensively evaluate the status in the Curonian Lagoon, a wider variety of blood parameters must be evaluated.

Also, it is important to consider seasonal variations when evaluating biomarkers. Furthermore, *C. gibelio* is noted for its exceptional tolerance to environmental conditions. As a result, in addition to conducting biomonitoring surveys using various biomarkers, it is also necessary to analyse a wider range of fish species in order to gain a comprehensive understanding of potential environmental pollution risks and their impact on fish health and the ecosystem as a whole.

5. Conclusions

1. No significant differences were found in all biomarker responses of *Carassius gibelio* between two sites in the Curonian Lagoon.

2. Due to its high tolerance to environmental contamination, *C. gibelio* cannot be recommended as a suitable bioindicator and is not recommended for biomonitoring studies of the Curonian Lagoon.

3. It is important to identify reliable fish species for inclusion in pursuance of understanding, assessing, and predicting environmental impacts on lagoon ecosystems.

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CARASSIUS GIBELIO HEMATOLOGINIŲ IR BIOCHEMINIŲ STRESO BIOŽYMENŲ BEI FIZIOLOGINIŲ RODIKLIŲ ĮVERTINIMAS KURŠIŲ MARIŲ PAKRANTĖS ZONOSE

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Santrauka. Šiuo tyrimu siekta įvertinti *Carassius gibelio*, palyginti tolerantiškos taršai žuvų rūšies, sveikatos būklę didžiausioje Europos priekrantės lagūnoje – Kuršių mariose. Buvo vertinami hematologiniai (hematokrito kiekis), biocheminiai (gliukozės koncentracija), fiziologiniai (kepenų somatinis indeksas, įmitimo koeficientas) rodikliai kaip patikimi žuvų reakcijos į stresą biožymenys. Žuvys buvo gaudomos pietinėje marių pakrantėje ties Klaipėdos uostu, netoli Kiaulės nugaros salos, ir centrinėje Kuršių marių dalyje ties Ventės ragu. Vertinant biožymenų atsakus žuvyse reikšmingų skirtumų tarp tirtų stočių nenustatyta. Todėl nerekomenduojama *C. gibelio* rūšies naudoti kaip aplinkos taršos bioindikatoriaus.

Reikšminiai žodžiai: žuvys, marios, gliukozė, hematokritas, įmitimo koeficientas.