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Effect Of Parasitic Infection On The Enzymatic Function Of The Internal Organs Of Scomber Scombrus (Mackerel) And Euthynnus Alltteratus (Small Tuna) (Misrata, Libya)

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Abstract

The assessment of fish health has acknowledged the significance of helminthology and haematology characteristics as crucial instruments. The utilization of blood parameter measurement has been a longstanding practice in the field of fish health monitoring. The current study aimed to examine the impact of an indigenous infestation of parasites on various blood parameters, including activities of liver, kidney function tests, lipase, amylase, Creatine phosphokinase (CPK), and Lactate Dehydrogenase (LDH). The present study was undertaken to investigate the parasitic infection in a sample of 100 fish. The sample consisted of 50 of Euthynnus alletteratus, and 50 Scomber Scombrus. These fish were gathered from the waters of Misrata city, located in Libya. Results revealed the presence of statistically significant differences across various indices in both Euthynnus alletteratus and Scomber scombrus. A significant positive association was seen between urea values and many organs in Scomber scombrus, with the exception of the external body. Similarly, in Euthynnus alletteratus, a significant positive correlation was found between urea values and various organs, except for the external body and nostrils. The results of the study indicate that there is a positive link between creatinine levels and several internal organs in Euthynnus alletteratus, with the exception of external body tissues. A favorable correlation was seen between sodium levels and various organs in Scomber scombrus, with the exception of internal organs and the stomach. There was a positive correlation seen between potassium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), lipase, amylase, CPK, and total protein levels in several organs of both Euthynnus alletteratus and Scomber scombrus. A significant correlation was observed between LDH levels and several organs in both Euthynnus alletteratus and Scomber scombrus, with the exception of the external body in Scomber scombrus. In Euthynnus alletteratus, LDH levels were favorably connected with the nose, internal organs, and anterior intestine.

Keywords; *Hematological Evaluation, Fish Parasite, Euthynnus Alletteratus, Scomber Scombrus, Liver Function, Kidney Function Tests, Misrata.*

Introduction

Misrata, a seaside city in Libya, is situated at a geographical position of 32.8774 degrees North latitude and 13.1848 degrees East longitude [1]. It serves as a primary center for the fishing industry in the region. The copious marine fish stocks within the city's vicinity serve as a crucial resource for both the inhabitants of the area and the communities residing along the shore [2]. The aforementioned fisheries are actively involved in sustainable practices that depend on maintaining a fragile equilibrium between human exploitation and preservation of the marine ecosystem [3].

However, the delicate equilibrium is being jeopardized by the presence of environmental pollution, exemplified by the existence of parasitic worms [4]. The presence of these parasites presents a substantial risk to the commercially relevant fish species in the area, resulting in adverse effects on their physiological well-being and tissue condition [5].

Fish serve as a significant source of protein for a considerable number of individuals worldwide [6]. Moreover, the well-being of fish holds paramount importance, representing a serious concern that demands immediate attention. One of the primary concerns in aquatic ecosystems is the presence of parasitic worms, which have the potential to significantly impact the overall well-being of fish and the ecological systems in which they reside [7, 8]. The transmission of parasitic worm infections is significantly influenced by the dietary dependence of fish on crustaceans, mollusks, and gastropods, as these particular species serve as hosts for a wide range of parasitic worm types. Fish are susceptible to infections throughout their whole life cycle, and the parasites have the ability to endure until their adulthood [4]. Within aquatic ecosystems, these worms have the ability to elicit a range of symptoms that have deleterious effects on the behavior and overall well-being of fish. These symptoms encompass changes in movement, loss of appetite, physical abnormalities, and in severe instances, even death [9].

Parasitic helminths provide a significant source of health and environmental concerns in aquatic habitats [10]. The parasites exhibit varying effects, leading to physiological changes in the organ systems of marine fish. The aforementioned alterations have the potential to result in reduced fish populations and degraded ecosystem services, both of which play a crucial role in upholding biodiversity and sustaining local fisheries [11]. The mitigation of parasitic worms is of utmost importance in order to maintain the integrity of these aquatic ecosystems. Future research and management strategies should prioritize the development of sustainable solutions aimed at effectively managing the proliferation of these parasites. This is crucial to maintain the overall health of marine fish stocks, hence ensuring the environmental and economic well-being of communities that rely on these resources [12, 13].

The increasing environmental concerns highlight the importance of doing research focused on the functional dynamics of organs in aquatic organisms, namely fish, which play a crucial role in marine ecosystems. Enzymatic indicators, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP), play a crucial role in maintaining vital biological processes in these species [14]. The evaluation they provide is crucial for identifying physiological and biochemical changes resulting from exposure to environmental pollutants [15].

The presence of pollutants, particularly those of a parasitic type, heavy metals and other harmful substances, has a significant impact on the physiological functioning of fish organs. The immediate impact of their actions can have long-term consequences, as it disrupts the physiological balance and leads to a deterioration of the internal organ systems. Consequently, this phenomenon is observed as a decline in the overall well-being and vigor of fish. The pollutants have been observed to interact with the biological mechanisms of fish, resulting in the development of pathological conditions. This is evident by the detection of changes in enzyme activity levels and impaired functioning of organs, specifically the gills, liver, and kidneys. This research endeavor holds great importance as it seeks to clearly define and assess these impacts, thereby providing crucial insights that are vital in developing preventive measures for fish populations and preserving ecological integrity in aquatic systems [16].

Significance of the study

Coastal environments are widely acknowledged as areas characterized by significant ecological vulnerability, primarily attributed to intricate biogeochemical mechanisms and the extensive anthropogenic interventions. These factors collectively contribute to a substantial rise in the prevalence of both organic and inorganic contaminants within the surrounding ecosystem. Parasitic infection and heavy metals are prevalent contaminants in aquatic systems, and their presence can be attributed to either natural causes, such as weathering and erosion, or human activities, including mining, industrial processing, waste disposal, and agricultural practice. The accumulation of parasitic infection and heavy metals in the adipose tissues of organisms has the potential to adversely impact various physiological systems, including the digestive, cardiovascular, and central neurological systems. The primary route via which humans are exposed to pollutants is the consumption of fish, which can lead to the development of chronic or acute disorders.

Aim of the study

This study aimed to protocol for identifying suitable biomarkers to assess fish health in Misrata, a seaside city in Libya, using *Euthynnus alletteratus* and *Scomber scombrus* specimens as a case study.

Materials and methods

Fish Sample

100 fish were collected from various places, Misurata Fish Market, the sea fishing port, belonging to two species, which are caught from a number of areas in the marine waters of the coast of the city of Misrata, Libya, Figure (1). The fish were collected during the beginning of August until the beginning of November. They were examined to extract parasitic worms.

The fish targeted for study were collected from the sea fishing port of Qasr Ahmed in the city of Misrata, and the Misurata Fish Market, Libya, during the period between August and November, 2023. It included 50 carnivorous fish, represented by *Euthynnus alletteratus*, 4 times per month during the period. the study. Trawl nets are used to catch fish. And 50 samples of fish, *Scomber scomberus*, were treated in the same way as catching, and were examined to extract parasitic worms from them.

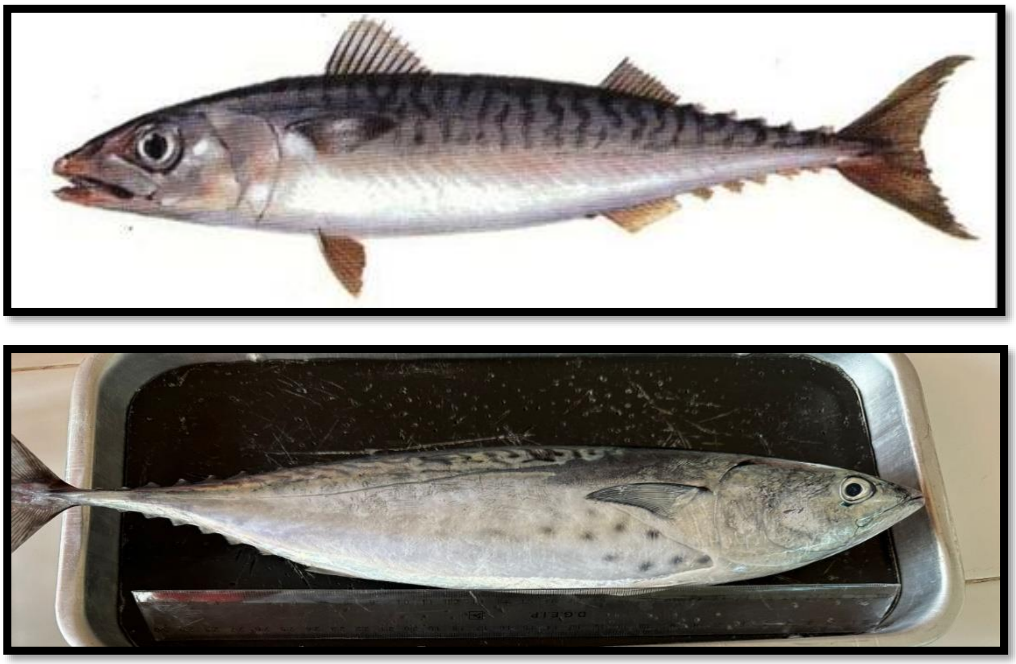

Figure 1. *Scomber scombrus* fish and *Euthynnus alletteratus* fish

- **Tools and materials required:**

1. Sample collection tools: clean, sharp knives, forceps, scissors, clean cutting board.
2. Sample containers: Petri tubes or dishes to store samples.
3. Fixation solutions: such as formalin preserved in water (4%) or absolute alcohol.
4. Ice: to cool the samples immediately.
5. Safety accessories: gloves, goggles, lab coat.

• Laboratory steps:

1. Collection and preparation:

• Collect fish ethically according to environmental standards.
• Preparing fish for blood draws and dissection of target organs.

• Samples:

• Draw blood samples using safe and effective methods.
• Extracting the target organs (liver, stomach, heart).

• Measurements:

Urea (Urea Assay Kit abcam; cat. No. ab83362/K375-100), Creatinine levels (Abcam's Creatinine Assay Kit (Colorimetric); cat. No. ab204537), Sodium (Sodium Assay Kit (Colorimetric), Abcam; cat. No. ab211096), Potassium (Potassium Assay Kit (Fluorometric), Abcam; cat. No. ab252904), ALT (ALT Activity Assay Kit (Colorimetric/Fluorometric), Abcam; cat. No. ab105134), AST (AST assay kit, Abcam; cat. No. ab105135), ALP (Alkaline Phosphatase Assay Kit (Colorimetric), Abcam; cat. No. ab83369), total protein (Total Protein Thiol Assay Kit (Colorimetric), Abcam; cat. No. ab219272), Lipase (Lipase Assay Kit, (Colorimetric), abcam; cat. No. ab102524), amylase (Amylase Assay Kit (Colorimetric), abcam; cat. No. ab102523), CPK (Creatine Kinase Activity Assay Kit, abcam; cat. No. ab155901), LDH (LDH Assay Kit (Colorimetric), abcam; cat. No. ab102526), were determined in accordance with the manufacturer's instructions.

Table 1. The importance of each measured enzyme

Enzyme/Component	Significance in Fish Health	Organ/System Typically Indicated
Urea	Waste product from protein metabolism; kidney function indicator	Kidney
Creatinine	Waste product from muscle metabolism; more specific for kidney function	Kidney
Sodium (Na+)	Electrolyte balance; critical for nerve and muscle function	General, Kidneys, Adrenal Glands
Potassium (K+)	Essential for cellular function and cardiac rhythm	General, Kidneys, Heart
Glutamic Oxaloacetic Transaminase (GOT/AST)	Enzyme release due to tissue damage; indicates liver or muscle injury	Liver, Muscle
Glutamic Pyruvic Transaminase (GPT/ALT)	Liver functionality; released in higher amounts when liver is damaged	Liver
Alkaline Phosphatase (ALP)	Bone and liver health; higher in cases of bile duct obstruction	Liver, Bone
Lipase	Fat metabolism; elevated levels may indicate pancreatic inflammation	Pancreas
Amylase	Starch digestion; elevated in pancreatic disorders	Pancreas
Creatine Phosphokinase (CPK)	Muscle damage; high levels often seen in muscular dystrophy	Muscle, Heart
Lactate Dehydrogenase (LDH)	Tissue damage; higher levels indicate cell damage or destruction	General (Many Organs)
Total Protein	Nutritional status and liver function; includes albumin and globulin levels	Liver, Nutritional Status

Statistical Analysis

Statistical analyses were performed to determine the outcomes, entailing the computation of the P-value by the utilization of the Chi-Squares test. The assessment of the significance of the measured data was conducted in the following manner: In the context of statistical analysis, a p-value greater than 0.05 is conventionally designated as non-significant (N.S), signifying a lack of substantial evidence to warrant the rejection of the null hypothesis. On the other hand, a p-value that is smaller than 0.05 is considered statistically significant, indicating substantial evidence to reject the null hypothesis.

Results

Figure 1 showed a significant increase in urea and creatinine levels in *Scomber scombrus* fish more than *Euthynnus alletteratus* fish. Figure 2 showed an increase in GPT, and ALK, levels in *Scomber scombrus* fish more than *Euthynnus alletteratus* fish, while there were decrease in GOT, and total protein levels in *Scomber scombrus* fish more than *Euthynnus alletteratus* fish. Figure 3 illustrated sodium levels were significantly increased in *Scomber scombrus* fish more than *Euthynnus alletteratus* fish, while there was non-significant change in potassium levels in *Scomber scombrus* fish and *Euthynnus alletteratus* fish. In figure 4 Lipase, and amylase levels were decreased in *Scomber scombrus* fish and *Euthynnus alletteratus* fish, while CPK levels were increased in *Scomber scombrus* fish more than *Euthynnus alletteratus* fish. The LDH levels in both types were non-significant.

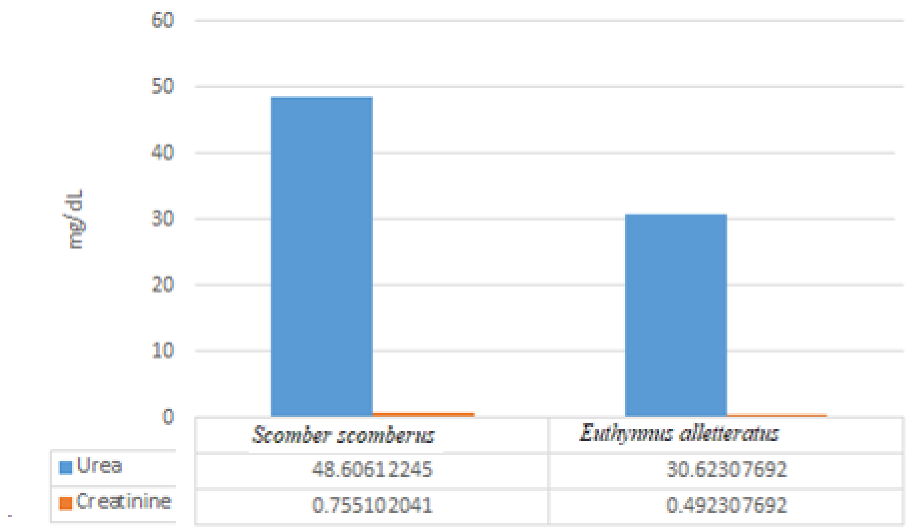

Figure 1. Urea and creatinine levels in *Scomber scombrus* fish and *Euthynnus alletteratus* fish.

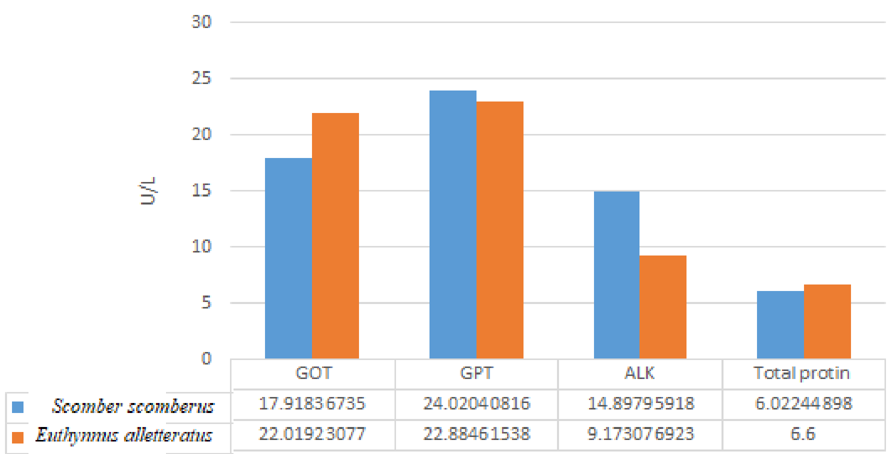

Figure 2. GOT, GPT, ALK, and total protein levels in *Scomber scombrus* fish and *Euthynnus alletteratus* fish.

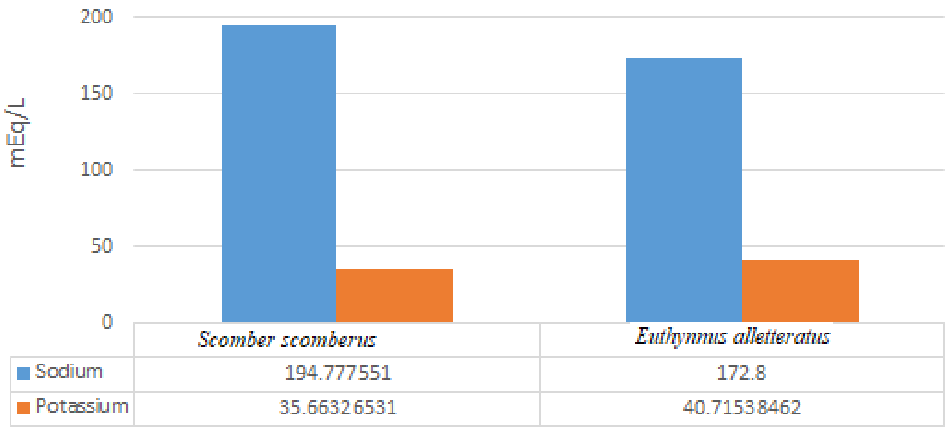

Figure 3. Sodium and potassium levels in *Scomber scombrus* fish and *Euthynnus alletteratus* fish.

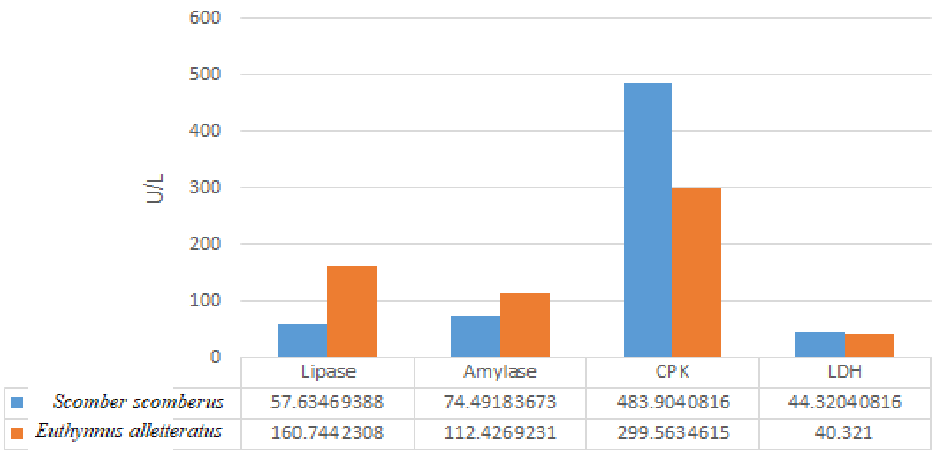

Figure 4. Lipase, amylase, CPK, and LDH levels in *Scomber scombrus* fish and *Euthynnus alletteratus* fish.

Table 2 showed there were a statistical significant differences seen most indices in both *Euthynnus alletteratus* and *Scomber scombrus.* There was a substantial positive correlation between urea values and several organs in *Scomber scombrus*, except for external body, and except for external body and nostrils in *Euthynnus alletteratus*. Creatinine showed positive correlation in relation to several organs except for external body in *Euthynnus alletteratus*. Sodium was positively correlated in relation to several organs except for Internal organs, and Stomach, in *Scomber scombrus*. Potassium, ALT, AST, lipase, amylase, CPK and total protein were positively correlated in relation to several organs in both *Euthynnus alletteratus* and *Scomber scombrus.* LDH was positively correlated in relation to several organs in both *Euthynnus alletteratus* and *Scomber scombrus* except for external body in *Scomber scombrus*, and in *Euthynnus alletteratus* in nostrils, internal organs, and anterior intestine.

Table 2. Pearson Correlation between different enzymes and each organ.

	Scomber scomberus		***Euthynnus alletteratus***	
	Pearson Correlation	**P value**	**Pearson Correlation**	**P value**
Urea				
External body	-0.210	0.160	-0.176	0.226
Nostrils	0.036	0.041	-0.060	0.68
Internal organs	0.003	0.035	0.035	0.045
Stomach	0.232	0.012	0.040	0.02
Anterior intestine	0.055	0.07	0.224	0.012
Hind intestine	0.098	0.05	0.071	0.031
Creatinine				
External body	0.212	0.006	0.156	0.128

Nostrils	0.158	0.03	0.200	0.018
Internal organs	0.842	0.04	0.127	0.038
Stomach	0.654	0.026	0.135	0.031
Anterior intestine	0.540	0.012	0.108	0.042
Hind intestine	0.025	0.034	0.152	0.029
Sodium				
External body	0.173	0.020	0.276	0.05
Nostrils	0.101	0.005	0.105	0.047
Internal organs	-0.148	0.326	0.160	0.027
Stomach	-0.179	0.234	0.069	0.026
Anterior intestine	0.297	0.045	0.137	0.034
Hind intestine	0.037	0.008	0.179	0.021
Potassium				
External body	0.032	0.060	0.112	0.042
Nostrils	0.127	0.023	0.055	0.001
Internal organs	0.160	0.011	0.184	0.021
Stomach	0.088	0.001	0.154	0.035
Anterior intestine	0.031	0.03	0.113	0.048
Hind intestine	0.158	0.04	0.081	0.05
ALT				
External body	0.298	0.031	0.158	0.048
Nostrils	0.078	0.011	0.137	0.05
Internal organs	0.040	0.027	0.039	0.021
Stomach	0.072	0.02	0.069	0.049
Anterior intestine	0.129	0.009	0.004	0.003
Hind intestine	0.062	0.031	0.101	0.025
AST				
External body	0.028	0.005	0.176	0.025
Nostrils	0.026	0.061	0.225	0.018
Internal organs	0.013	0.051	0.045	0.04
Stomach	0.118	0.024	0.016	0.026
Anterior intestine	0.191	0.031	0.033	0.032
Hind intestine	0.109	0.009	0.081	0.038
ALP				
External body	0.289	0.110	0.121	0.024
Nostrils	0.017	0.14	0.175	0.01
Internal organs	0.256	0.02	0.216	0.02
Stomach	0.174	0.034	0.341	0.043
Anterior intestine	0.062	0.07	0.090	0.015
Hind intestine	0.150	0.09	0.013	0.039
Total protein				
External body	0.116	0.009	0.100	0.026
Nostrils	0.114	0.037	0.010	0.068
Internal organs	0.102	0.037	0.018	0.021
Stomach	0.049	0.007	0.086	0.024
Anterior intestine	0.239	0.013	0.102	0.042
Hind intestine	0.206	0.036	0.048	0.011
Lipase				
External body	0.279	0.05	0.017	0.030
Nostrils	0.061	0.034	0.072	0.012
Internal organs	0.057	0.031	0.106	0.042
Stomach	0.154	0.023	0.288	0.010
Anterior intestine	0.055	0.005	0.216	0.004
Hind intestine	0.110	0.008	0.088	0.006
Amylase				

External body	0.084	0.004	0.108	0.021
Nostrils	0.073	0.035	0.022	0.028
Internal organs	0.208	0.010	0.160	0.015
Stomach	0.074	0.018	0.036	0.012
Anterior intestine	0.149	0.047	0.193	0.002
Hind intestine	0.048	0.039	0.051	0.011
CPK				
External body	0.105	0.01	0.035	0.008
Nostrils	0.022	0.024	0.134	0.006
Internal organs	0.064	0.005	0.202	0.015
Stomach	0.255	0.002	0.054	0.031
Anterior intestine	0.065	0.006	0.074	0.042
Hind intestine	0.068	0.036	0.121	0.051
LDH				
External body	0.248	0.19	0.2786	0.001
Nostrils	0.118	0.011	0.067	0.08
Internal organs	0.222	0.025	0.433	0.06
Stomach	0.066	0.032	0.146	0.008
Anterior intestine	0.122	0.043	0.239	0.09
Hind intestine	0.073	0.032	0.041	0.034

Discussion

During the course of this investigation, a correlation was observed between the pollution caused by parasitic infection and *Scomber scombrus*, and *Euthynnus alletteratus*. This finding is consistent with the findings of Gheorghiu et al. [17]. The current investigation revealed that the relation between different blood indices and different organs was found to be higher in the Misrata city, which is known to have a higher pollution level compared to other localities. This observation suggests that the increased incidence of protozoa infection in fish from the sea of misrata branch may be attributed to the detrimental effects of parasitic infection on the immune system of the exposed fish, rendering them more susceptible to such infections.

Khan and Thulin [18] conducted a study on the River Nile Branch exhibited the lowest incidence of parasitic crustacean infection, which coincided with an increase in pollution from heavy metals. This finding is consistent with the observations made by Galli et al. [19], who noted that the distribution of *Lamproglena pulchella* was restricted to river sectors that were either unpolluted or only slightly polluted. The observed negative relationship can be related to the detrimental impact of heavy metals on crustaceans, perhaps disrupting their life cycle. Ruben et al. [20] conducted a study, and showed that the activities of the enzymes AST and ALT, as well as the levels of Creatinine and Urea, were found to be higher in infected fish species (*Oreochromus niloticus* and *Clarias gariepinus*) with external parasites compared to non-infected fish in various locations. This suggests that the presence of external parasites leads to an increase in the activities of ALT and AST enzymes, as well as elevated levels of Urea and Creatinine. The findings of this study align with the research conducted by Younis [21], which reported a significant increase in AST, ALT, and urea levels in *O. niloticus* fish infected with external protozoa and monogenetic trematodes. In a study conducted by Osman et al. [22], it was observed that the activity of blood serum enzymes AST and ALT, as well as the levels of Creatinine and Urea, were found to be elevated in *Clarias gariepinus* infected with Trichodina. Regarding the impact of parasitic infection on the biochemical parameters of the fish species examined, in the context of heavy metal pollution, the findings of this study demonstrate that the activities of AST and ALT enzymes in the blood serum, as well as the levels of Creatinine and Urea, were significantly higher in both *Oreochromis niloticus* and *Clarias gariepinus* specimens obtained from the branch of the River Nile (which represents a more heavily polluted area with heavy metals), compared to the infected fish species collected from the drainage canal and fish farm (which are less affected by heavy metal pollution). According to Adams [23], it has been observed that the presence of heavy metals enhances the stimulation of ALT and AST enzyme activity in fish when they are exposed to parasite infection. The observed phenomenon can potentially be attributed to hepatic cell damage or an upregulation in the liver's enzymatic production [24]. The increase in urea levels seen in the infected fish could potentially be attributed to gill dysfunctions, as it is known that urea is primarily discharged through the gills [24]. Furthermore, it is plausible to link these findings to the inflammatory reactions and intoxications induced by the parasite in the fish under investigation.

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