Research Article

Hematological parameters of the European hake (*Merluccius merluccius*) in Toroneos Gulf, northern Greece: A case study

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Abstract

Background: The European hake (*Merluccius merluccius*) is a commercially valuable demersal species widely distributed in the Mediterranean Sea. Assessing the condition of fish populations in their natural habitats is challenging due to the lack of reliable reference points. **Objective:** This study aimed to utilize hematological analysis as an economical method to evaluate the physiological and health status of European hake, addressing the gap in hematological data for this species. **Methods:** Blood samples were collected from the caudal vein of 40 adult European hakes caught from the Toroneos Gulf (northern Greece) using a commercial bottom otter trawl. An automated hematological analyzer was used to assess hematological parameters alongside biometric and biological indices. **Results:** Female hakes showed significantly higher white blood cell (WBC) counts, thrombocyte (TC) counts, and red cell distribution width (RDW) than their male counterparts. Strong correlations were observed among various hematological parameters, notably between WBC and red blood cells (RBCs), hematocrit (Ht), and hemoglobin (Hb); between RBC and both Ht and Hb; between TC and both mean platelet volume and platelet distribution width (PDW); and between mean corpuscular Hb concentration and RDW. Significant differences were noted in RBCs, Hb, and Ht compared to data from wild-caught European hake populations in Argentina and Denmark. Both trawling depth and duration were found to significantly affect RBC, WBC, Hb, and Ht values, while having no notable impact on TC. Fish captured at an average depth of 80 m and with a trawling duration of 30 min exhibited significantly elevated hematological indices. **Conclusion:** This study demonstrates that hematological analysis is a valuable, cost-effective tool for assessing the physiological and health status of European hake populations in the Mediterranean. Notable differences in hematological parameters based on sex, as well as significant correlations among key blood metrics, underscore the importance of understanding species-specific hematological profiles. The influence of trawling depth and duration on certain blood parameters highlights the need for standardized sampling protocols in population health assessments. These findings contribute essential baseline hematological data for European hake, facilitating more informed fisheries management and conservation strategies.

Keywords: Hematology, Bottom otter trawl, Toroneos Gulf, Northern Greece

1. INTRODUCTION

The European hake (*Merluccius merluccius*), a species first formally described by Carl Linnaeus in 1758, is a bottom-dwelling species typically found on muddy substrates at depths ranging from 100 to 1000 m in the Mediterranean Sea [1]. As a member of a broader group of 14 demersal species collectively referred to as hakes [2], the European hake is expanding its range throughout the Mediterranean Sea and the southern Black Sea coast, as well as along the western coast of North Africa and the Atlantic coasts of Europe, extending north to Iceland and Norway, and south to Northwest Africa [3]. This species is highly sought after in

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the Aegean and Ionian Seas [4], and it faces significant fishing pressure across Western Europe and the Mediterranean basin.

Assessing the status of fish populations remains challenging due to the scarcity of reliable data from their natural habitats. The limited diagnostic methods available for aquatic species complicate evaluations of their clinical health. Despite advancements in fish veterinary medicine, the interpretation of hematological parameters remains rudimentary due to a lack of standardized collection and measurement methodologies [5]. Hematological analysis has emerged as a cost-effective [6] and reliable diagnostic tool for assessing the health and physiological condition of fish [7-11], widely utilized in clinical evaluations, prognostics, and diagnoses [5,12,13].

Hematological examinations have been employed for decades to evaluate various aspects of fish health [14], including nutritional status, maturation, immune function, endocrine and reproductive health, and are also applied in genetic studies [15]. Blood analysis offers a rapid, non-lethal sampling method for evaluating fish. Fish blood consists of a complex mixture of cell types, including erythrocytes or red blood cells (RBCs) [16], leukocytes or white blood cells (WBCs), and thrombocytes (TCs) [17], all serving functions similar to those of mammalian blood cells. Blood transports a variety of substances, including gases, waste products, hormones, and nutrients [18], making it a crucial component for studies of fish physiology.

Erythrocytes are crucial for oxygen transport to bodily tissues, and their efficiency is influenced by hemoglobin (Hb) concentration and the integrity of the oxygen exchange system [19]. Under increased metabolic demand, such as during physical exertion or stress, the spleen releases additional RBCs into circulation to meet oxygen requirements [20]. Although fish RBCs are nucleated and similar in size and shape to those of other vertebrates, automated hematology analyzers are typically calibrated for mammalian blood, leading to challenges in accurate result interpretation. The nuclei of lysed fish RBCs and TCs may be misclassified, resulting in inaccuracies in cell counts [11]. Moreover, distinguishing between WBCs and TCs can be challenging due to their morphological similarities, leading to confusion among researchers [21].

Leukocyte counts are often used as indicators of fish health, as they reflect immune system status and their involvement in non-specific defense mechanisms in marine species [23]. Hematocrit (Ht) levels, which are generally less variable than other blood parameters, should be interpreted in combination with Hb, RBC, and WBC counts [22,24,25]. Low Ht values may indicate physiological distress [26]. However, the predictive power of these hematological parameters is not yet fully utilized by aquatic practitioners, often due to difficulties in sample collection and the absence of reliable reference databases. These issues are compounded by the considerable intra- and interspecies variability in hematological parameters subject to intrinsic and extrinsic factors, such as age, gender, nutritional status, reproductive condition, seasonality, water quality, photoperiod, and temperature. Sampling stress can further affect the accuracy of results [27].

Hematological analysis in fish is influenced by a range of external and internal factors, including blood storage conditions [28], sampling methods [29], fish stress levels [28], analytical techniques (such as the choice of diluent [30] and anticoagulant use [31]), and cell classifications. Both the blood collection method and sampling site significantly impact analytical outcomes [13,29,32]. Notably, venous blood samples may yield higher Ht, Hb, and RBC levels compared to arterial ones [29], while another study has reported that Ht, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were higher in arterial blood samples, with RBC count being higher in tail venous blood [32].

Although routine hematological analyses are relatively straightforward and cost-effective, achieving reliable and reproducible results requires significant training and expertise. Recent automated hematological analysis aims to replace manual procedures [11,13,16,33-36]. However, adapting existing software for fish blood cell counts is essential for attaining accuracy in such applications [37].

Factors such as undernourishment [38], gender [39], environmental stress [40], fish size [41], reproductive efficacy, and seasonal variations [42] can significantly influence hematological parameters. Environmental factors have traditionally served as sensitive indicators of physiological and pathological changes in fish [43,44]. Despite advancements in establishing normal reference values for various aquatic species, hematological data of the *Merlucciidae* family remain limited. The individual fish's states, along with the conditions at the time of blood sampling, play a crucial role in the success of blood sampling and the accuracy of hematological analyses [6]. Hematological indices are increasingly employed to monitor changes in fish health and to evaluate the biological impacts of habitat modifications. Nevertheless, establishing reference intervals for key hematological parameters remains a challenge for many fish species across diverse environments [45].

Biometric parameters, such as condition factor (K), body weight, total length (TL), gonadosomatic index (GSI), and hepatosomatic index (HSI), are crucial for assessing how species obtain nutrients from their habitats. These parameters provide insights into specimens' health, fat deposition, gonadal development, and environmental adaptability [46].

Current data on the hematological parameters of *M. merluccius* are limited and outdated [47,48]. Therefore, this study aimed to provide new and contemporary insights into the hematological parameters of European hakes in northern Greece (Toroneos Gulf), alongside biometric parameters and biological indices, to evaluate their health status and the potential effects of sampling depth and time.

2. MATERIALS AND METHODS

2.1. Fish capture

The current research was conducted on a total of 40 adult European hakes (*M. merluccius*) caught from the Toroneos Gulf in northern Greece in November 2021. Fish were collected on the same day using a commercial bottom otter trawl at depths ranging from 80 to 120 m. The Toroneos Gulf, also known as Kassandra Gulf, is located in the Northern Aegean Sea, bordered by the Kassandra Peninsula to the west and Sithonia Peninsula to the east (Figure 1). The trawl used was of traditional Greek design, with a square mesh codend with a stretched mesh size of 28 mm, and was operated at a speed of approximately 3 knots. Four hauls were conducted, each lasting between 30 and 90 min, with geographical coordinates and depth at both the start and end of each haul.

2.2. Blood sampling

Blood samples were collected from the tail vein of all 40 fish immediately after capture using sterile syringes (2.5 mL) and heparinized needles (0.6 mm diameter). Prior to sampling, fish were externally examined for signs of health, ensuring the absence of lesions, skeletal abnormalities or infestations. Blood collection was performed within 5 min of capture to minimize stress. One aliquot of each blood sample was placed in a tube (Miniplast 0.6 mL; LP Italiana Spa, Italy) containing ethylenediaminetetraacetic acid (1.26 mg/0.6 mL) to prevent clotting. Due to the rapid activation of clotting factors upon contact with glass [49], Eppendorf tubes are recommended for fish blood collection [6]. Anesthesia was not employed, as it could have influenced the physiological responses of the fish, thereby affecting hematological indices [6,50-53]. Blood samples were gently mixed immediately after collection and stored at refrigeration temperatures until analysis by an automated hematology analyzer (MEK-6450, Nihon Kohden, Germany), which was performed within 24 h. Fresh blood samples from the target fish species were previously collected to establish reference values using manual methods. The analyzer was then calibrated for cell size, shape, and concentration to ensure its accurate differentiation of fish erythrocytes from other cells. Calibration was validated through comparison with known reference values.

The hematological indices measured included RBC count, WBC count, platelet count, Hb, Ht, RDW, mean platelet volume (MPV), and platelet distribution width (PDW). Additional secondary parameters, i.e., MCV, MCH, and MCH concentration (MCHC), were also measured, as these provide insights into RBC morphology [9]. MCV is the manifestation

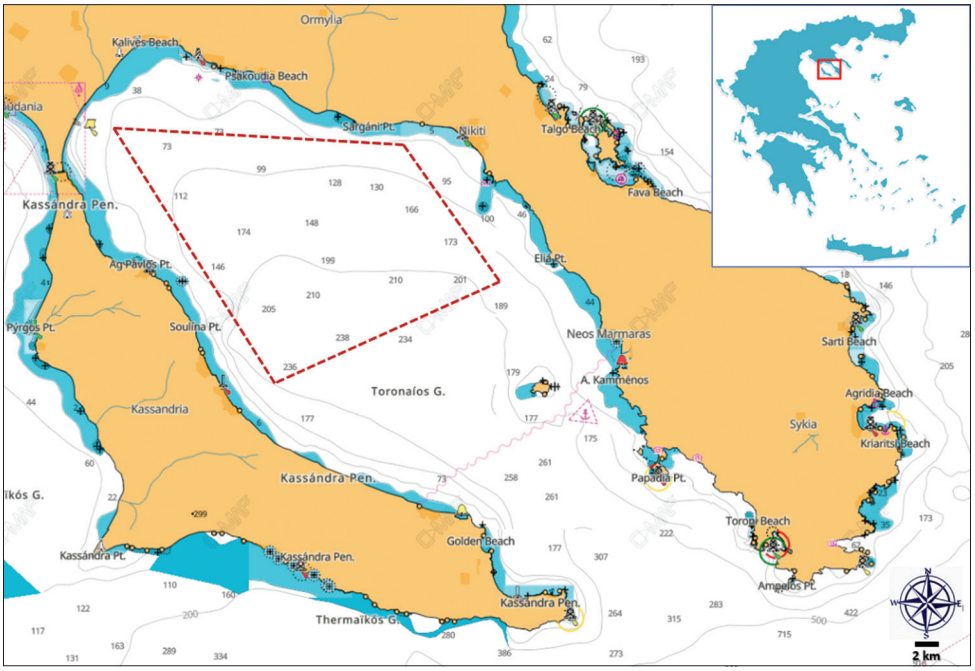

Figure 1. Study area

Notes: Haul 1: Approximate coordinates near the northern part of the quadrant (Latitude: 40.2850° N, Longitude: 23.5900° E, Depth: ~100 m); Haul 2: Towards the middle-left of the quadrant (Latitude: 40.2600° N, Longitude: 23.5700° E, Depth: ~90 m); Haul 3: Near the middle-right section of the quadrant (Latitude: 40.2700° N, Longitude: 23.6200° E, Depth: ~110 m); Haul 4: At the southern part of the quadrant (Latitude: 40.2450° N, Longitude: 23.6000° E, Depth: ~85 m).

of the individual RBC average volume, MCH is the average Hb content of a single RBC, and MCHC is the concentration of Hb in a volume of packed RBC [10]. Comparisons between manual and automated measurements for MCV, MCH, and MCHC indicated high consistency [54].

2.3. Fish measurements

After blood collection, fish were transported to the laboratory for weighing (±0.1 g using a Kern 440-49 N balance [Kern and Sohn GmbH, Germany]) and TL measurement (±1.0 mm using an ichthyometer). Sex and reproductive stages were determined macroscopically, following the classification system outlined in [46]: Stage I - immature (virgin), Stage II - developing (virgin), Stage III - developing, Stage IV - maturing, Stage V - mature, Stage VI - ripe (running), and Stage VII - spent. Liver and gonad weights were recorded to the nearest 0.1 g, and the stages of gonadal development for both males and females were documented. The study has been approved by the University of Thessaly Departmental Aquatic Animal Ethics Committee (DAAEC). The study was in strict accordance with the University of Thessaly's Ethics Committee on the Use of Animals in Research, which takes into account ethical, health, and safety considerations. Fish in this study were used in compliance with all relevant national and EU laws (Animal Scientific Procedures Act 1986/609/EEC and Directive (2010/63/EU) and institutional guidelines.

2.4. Statistical analysis

Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variance with Levene's test. One-way analysis of variance (ANOVA) was performed to test for significant differences among groups [55], with Welch's ANOVA employed when assumptions of homoscedasticity were violated [56]. ANOVA general linear model using least squares regression was further applied to explore spatial and temporal variations (depth and trawl time) [57]. Statistical analyses were conducted using Jamovi Software (2.3.28) with a significance level set at an alpha of 0.05.

Power analysis was performed to determine the required sample size for adequate statistical power to detect an effect [58]. The effect size was calculated using Cohen's d [59], expressed as:

$$D = \bar{x}1 - \bar{x}2 / S \quad \text{(I)}$$

where: $\bar{x}1$, $\bar{x}2$ are the means of groups 1 and 2, respectively, and S is the standard deviation.

The GSI was calculated according to [60]:

$$GSI = (\text{Wet gonad weight}/\text{Total wet body weight}) \times 100 \quad \text{(II)}$$

Fulton's condition factor (K) was determined using Equation III [61]:

$$K = (W/L^3) \times 10^N \quad \text{(III)}$$

where W is wet body mass (g), L is length (mm), and N is an integer to normalize the value of K near 1.

The HSI was calculated as [62]:

$$HSI = 100 \times (W_{liver}/W) \quad \text{(IV)}$$

where W_{liver} is the wet mass of the liver, and W is the wet body mass.

The Pearson's correlation coefficient was utilized to assess linear relationships among variables, calculated as follows:

$$r = [n(\sum xy) - \sum x \sum y] / \sqrt{n(\sum x^2) - (\sum x)^2][n(\sum y^2) - (\sum y)^2} \quad \text{(V)}$$

where *n* is the sample size, and Σ denotes the summation of all values.

3. RESULTS

3.1. Population composition

A total of 11 females (28%) and 29 males (73%) were analyzed, and they included 23 immature fish (6 females and 17 males, 57%) and 17 mature fish (5 females and 12 males, 43%). The hematological properties of the European hake population were assessed, as summarized in Table 1. The statistical power for all analyses conducted ranged from 0.80 to 0.94. Significant difference was observed in WBC count ($p < 0.05$), TC ($p < 0.001$), and RDW width ($p < 0.01$) between males and females, with females exhibiting significantly higher indices. However, no significant differences were found in the hematological properties between mature and immature individuals ($p > 0.05$).

Table 2 further illustrates significant differences in RBC, Hb, and Ht between the present study and indices from wild-caught European hakes from Argentina and Denmark.

The relationships among measured hematological properties of wild-caught European hakes are illustrated in Figure 2. Significant correlations were identified between WBC and RBC (correlation coefficient = 0.74, $p < 0.001$), Hb and Ht (correlation coefficient = 0.89, $p < 0.001$), Ht and RBC (correlation coefficient = 0.95, $p < 0.001$), Hb and RBC (correlation coefficient = 0.89, $p < 0.001$), Hb and WBC (correlation coefficient = 0.70, $p < 0.001$), Ht and WBC (correlation coefficient = 0.78, $p < 0.001$), MCHC and RDW (correlation coefficient = 0.44, $p < 0.01$), TC and PDW (correlation coefficient = 0.47, $p < 0.01$), as well as MPV and TC (correlation coefficient = 0.40, $p < 0.05$).

Figure 3 compares hematological parameters based on trawl time and sampling depths, highlighting statistically

Table 1. Biometric measurements (total length and total weight) and basic biological indices (Fulton's condition factor, gonad-somatic index, and hepatosomatic index) of wild-caught male and female European hakes (*Merluccius Merluccius*) in Toroneos Gulf, northern Greece

Physiological measurement	Sex	*n*	Mean	Median	SD	Minimum	Maximum
TL (cm)	F	11	29.4	30.8	4.72	21.49	36.56
	M	29	27.41	27.55	1.97	23.86	31.89
TW (g)	F	11	169.36	168.99	84.87	54.93	333.98
	M	29	118.14	116.94	30.46	70.49	195.8
FCF	F	11	0.62	0.62	0.06	0.54	0.73
	M	29	0.61	0.59	0.1	0.51	1.03
GSI	F	11	1.67	0.71	2.57	0.2	8.54
	M	29	0.72	0.66	0.38	0.19	2.04
HSI	F	11	1.82	1.7	0.59	0.92	2.78
	M	29	2.06	1.99	0.62	1.03	3.54

F: Female, FCF: Fulton's condition factor, GSI: Gonad-somatic index, HIS: Hepatosomatic index, M: Male, SD: Standard deviation, TL: Total length, TW: Total weight.

Table 2. Biometric indices (total length in cm and total weight in g), country of origin (Argentina, Denmark or Greece), and hematological properties of wild-caught European hakes (*M. Merluccius*)

Species	No	TL	TW	RBC	WBC	Hb	Ht	CO	Ref.
M. merluccius	25	NA	NA	1.80**	NA	6.4***	36**	AR	[47]
M. merluccius	16	Adult	Adult	NA	NA	5.2±1.6***	24.1±3.9***	DK	[48]
M. merluccius	40	27.96±3.05	132.22±55.22	1.64±0.34	21.68±9.62	8.07±1.80	40.33±8.91	GR	Cur. study
M. merluccius males	29	27.40±1.97	118.00±30.50	1.62±0.39	19.90±10.40	7.97±2.05	39.60±10.10	GR	Cur. study
M. merluccius females	11	29.40±4.72	169.00±84.90	1.68±0.16	26.40±5.15	8.35±0.84	42.30±4.04	GR	Cur. study
M. merluccius juveniles	23	27.70±2.98	131.00±55.40	1.65±0.25	21.70±9.43	8.25±1.40	41.20±6.60	GR	Cur. study
Species	**No**	**MCH**	**MCHC**	**TC**	**RDW**	**MCV**	**MPV**	**PDW**	
M. merluccius	25	36***	17.5***	NA	NA	200***	NA	NA	
M. merluccius	16	NA	21.5±6.0ns	NA	NA	NA	NA	NA	
M. merluccius	40	48.19±2.68	20.14±2.43	17.48±8.79	23.14±2.79	174.8±2.75	6.66±0.73	21.11±0.72	
M. merluccius males	29	48.10±2.77	20.30±2.77	14.30±6.59	22.30±2.70	174.7±2.56	6.49±0.85	21.20±0.63	
M. merluccius females	11	48.60±2.53	19.70±1.19	25.90±6.59	25.20±1.84	175.0±3.32	6.94±0.37	20.90±0.83	
M. merluccius juveniles	23	48.40±2.49	20.10±2.44	16.70±8.56	23.40±2.97	175.0±2.98	6.67±0.86	21.00±0.85	

Notes: Indices are presented as mean±standard deviation (SD), with significance levels indicated as ** $p<0.01$, *** $p<0.001$.
AR: Argentina, CO: Country of origin, Cur: Current, DK: Denmark, GR: Greece, Hb: Hemoglobin concentration (g/dL), Ht: Hematocrit (%), MCH: Mean corpuscular hemoglobin (pg), MCHC: Mean corpuscular hemoglobin concentration (h/dL), MCV: Mean corpuscular volume (fl), MPV: Mean platelet volume (fl), NA: Not available, No: Number, ns: Not significant, PDW: Platelet distribution width (%), RBC: Red blood cells (106/μL), RDW: Red cell distribution width (%), TC: Thrombocytes (103 μ/L), TL: Total length, TW: Total weight, WBC: White blood cells (103/μL).

significant differences in RBC, WBC, Hb, and Ht at the least depth and shortest trawling time ($p < 0.05$). Fish captured from an average depth of 80 m exhibited significantly higher indices than those from greater depths. Similarly, European hakes captured during a 30-min trawl showed significantly higher indices compared to those from longer trawls. However, no significant differences were noted in TC concentration across different trawling depths and times.

The comparative bar plots in Figure 3 illustrate the effects of sampling depth and trawl time on various blood parameters. As trawl time increased from 30 to 90 min, there was a general trend of decreasing RBC, WBC, Hb, and Ht levels, suggesting that prolonged trawling may induce physiological stress. While TC showed a slight increase with longer trawl time, the variation was less pronounced. Similarly, as the average depth decreased from 80 to 20 m, RBC, WBC, Hb, and Ht levels exhibited a modest decline, indicating that fish sampled at shallower depths may experience different environmental conditions or stressors.

4. DISCUSSION

Assessing the health of fish populations represents a significant challenge, primarily due to the limited availability of reliable data from their natural habitats. This study provided new insights into the hematological parameters of wild European hakes from the Toroneos Gulf, northern Greece, revealing notable variations compared to previous research. Our findings highlight the significant impact of sex on these hematological parameters, as well as the considerable effects of sampling depth and duration. These

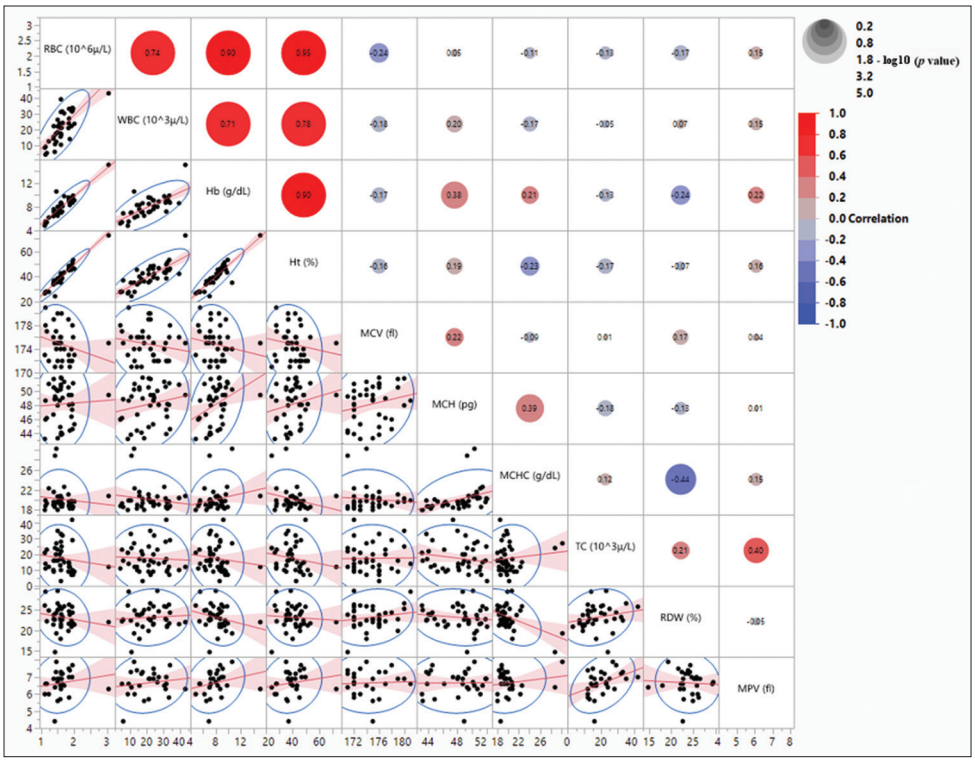

Figure 2. Scatterplot matrix of the relationships between hematological parameters. The lower left triangle shows scatterplots with fitted trend lines and blue ellipses, representing bivariate relationships and 95% confidence intervals, under the assumption of normal distribution. The upper right triangle features a heat map where the circle color indicates the strength of Pearson's correlations, and the circle size reflects the significance of these correlations, with larger circles representing more statistically significant relationships.

factors must be considered in future studies involving wild fish populations.

Hematological parameters in fish can be influenced by a wide array factors, including environmental conditions (such as water quality, dissolved oxygen levels, daily food intake, photoperiod, water temperature, salinity, and nitrate levels), as well as sampling procedures (including the type of anticoagulant used, the volume of blood collected, and the use of anesthesia). Furthermore, variations may arise from the measurement techniques, automated or manual, as well as the presence of pathological agents [13] and demographic factors such as sex [25], season [22] and age [63].

The erythrocyte (RBC) count, a crucial diagnostic indicator influenced by various environmental factors [64], can vary among populations of the same species [6]. In this study, RBC displayed a mean value of $1.64 \pm 0.34 \times 10^6/\mu L$, with ranges reported in literature spanning from 0.81 to $3.73 \times 10^6/\mu L$ [16]. Manually obtained RBC indices were comparable to those from automated measurements [54]. Prior research indicated that larger adult fish typically had higher erythrocyte levels compared to smaller, immature fish [65]. However, our study found the opposite trend, with immature fish exhibiting higher mean RBC count ($n = 23$, mean RBC = 1.65 ± 0.25) than mature European hakes ($n = 17$, mean RBC = 1.61 ± 0.44), although this difference was not statistically significant.

Significant differences in WBC were observed ($p < 0.05$), with females showing higher indices compared to males. WBC is a key factor in assessing the immunological state of vertebrates, and previous studies have reported a wide variability in WBC counts even within the same species [6]. In our study, the mean WBC count was $21.68 \pm 9.62 \times 10^3/\mu L$, with literature values for various species ranging from 9.41 to $829.33 \times 10^3/\mu L$ [16]. Factors such as sex, season, feeding behavior, stress, water pollution, and diseases can all influence leukocyte levels [66].

A significant difference was also observed in TC count ($p < 0.001$) between sexes, with females showing higher values. Although mature individuals had higher TC levels, this difference was not statistically significant. TCs, the second most abundant blood cells after erythrocytes, play vital roles in hemostasis and the immune response [67,68]. The maturity status, season, sex, age, and a spectrum of biotic and abiotic factors, including dissolved oxygen levels, pH, and water temperature, can significantly influence TC. In addition, factors such as feeding habits [66], stress [49], and disease [45] also play a critical part, with TC indices demonstrating considerable intra- and interspecific variability [49,66].

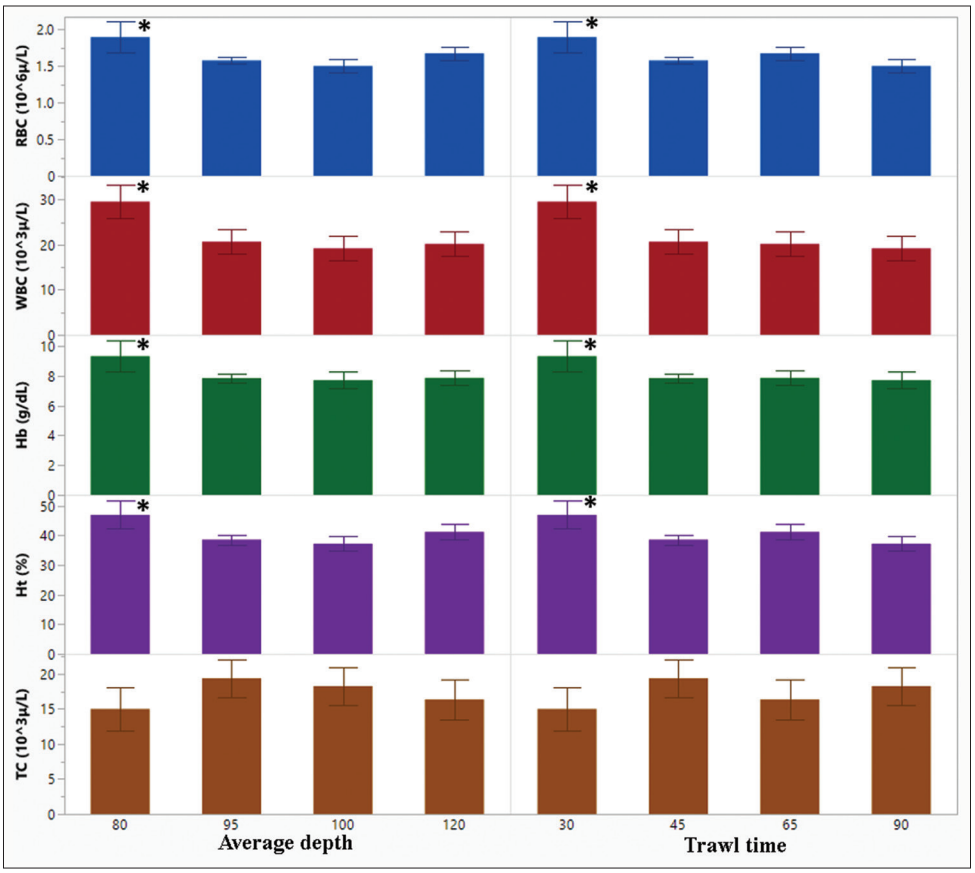

Figure 3. Comparative bar plot of red blood cells, white blood cells, hemoglobin concentration, hematocrit, and thrombocytes at different trawl times and sampling depths.
Notes: Bars represent the mean indices, with * denoting statistical significance ($p < 0.05$). Error bars indicate data variability, and the dotted red lines show reference values for comparison.

Ht, which measures the percentage of erythrocytes in blood volume [6], was estimated at 40.33 ± 8.91% in this study. This value falls within the reported range of 17.80% – 53.33% for various aquatic species [16]. Ht can be influenced by numerous factors, including medication, viral infections, and water quality conditions [69-71]. Meanwhile, Hb, a key biomarker of the secondary stress response [72-74], yielded a mean value of 8.07 ± 1.8 g/dL, which aligned with the established range for several aquatic species (4.70 – 16.6 g/dL) as determined by automatic analyzers [16]. Lower levels of Ht and Hb may indicate malnourishment, illness, or exposure to environmental pollutants [75]. Notably, we observed a significant correlation between Hb and Ht (correlation coefficient = 0.89, $p < 0.001$), corroborating previous findings in the literature [76]. While one study indicated a profound correlation between goldfish size with Hb and Ht [77], our results showed no significant correlation between TL or weight with Ht or Hb in European hake, similar to findings in common carp [78]. Interestingly, in our study, females exhibited higher Hb and Ht values compared to males, although this difference was not significant. This finding contradicts previous research on mature goldfish, which reported higher values of Ht and Hb in males than in females [77,79].

Temperature is a critical variable impacting hematological parameters, with seasonal changes likely linked to variations in water temperature and photoperiod [80]. For instance, during warmer periods, hematological parameters such as Hb, Ht, and RBC in rainbow trout tend to be lower compared to colder seasons [81], reflecting increased oxygen demand and carrying capacity. In addition, salinity affects hematological indices, as the hyperosmotic environment of the Nile is associated with decreased concentrations of Ht, Hb, and RBC in tilapia. These declines are likely due to changes in blood water content and osmoregulatory challenges [82-84]. Furthermore, a decrease in WBC counts may be linked to hemorrhagic damage caused by fluctuations in salinity [85].

After a viral infection in European sea bass, Hb and Ht decreased, while RBC and WBC increased. Granulocyte levels were elevated in fish infected with both parasites and viruses, whereas lymphocytes decreased, though this decline did not occur with viral infections alone [86]. Numerous studies have explored the relationship between fish size and hematological indices. Ht has been shown to either increase [41,87] or decrease [88] with size. In the current study, Ht showed a decreasing trend with size (length and

weight), although it was not significant. The only statistically significant relationship found was a rise in TC with fish size.

Our findings suggest that various factors, including season, sex, and maturity, influence WBC, TC and RDW, with females consistently exhibiting higher indices. Previous studies have documented variations in Ht and other blood indices between sexes, likely due to higher metabolic rates in males compared to females [89,90]. Notably, WBC counts were elevated in females during reproductive seasons, while males showed higher levels of Hg, Ht, and MCV over the year [22].

The GSI in this study was low (0.98 ± 1.41), possibly due to the high percentage of immature individuals (43%). A prior study in the Toroneos Gulf indicated a high abundance of young hake (age 0+), suggesting that this area serves as a nursery for juveniles [91]. The relationship between GSI and the distribution of gonadal maturity stages can help predict spawning seasons, as GSI is a reliable indicator of reproductive activity, peaking during the ripe stage and declining during spawning and spermiation [92].

Certain aquatic species utilize the liver for energy storage [62], and since liver function is influenced by dietary history [93], the HSI reflects hepatic involvement in vitellogenin production. In the present study, HSI was measured at 1.99 ± 0.61, which was lower compared to hakes from the Portuguese and Galician coasts [94,95] but comparable to juvenile hakes from the North Tyrrhenian and South Ligurian Seas [96]. This difference might be attributed to the smaller size of the sampled fish (27.96 ± 3.05 cm in TL). HSI has been shown to be a reliable indicator of energy storage in gadoids, reflecting both the quantity and quality of available food for growth [96]. In addition, the HSI value provides insight into fish health and the quality of their aquatic environment [97]. A higher HSI indicates that fish are growing rapidly and thriving in favorable conditions, while a lower HSI suggests the otherwise.

Fulton's condition factor, a common measure of fish health in fisheries, incorporates quantitative indicators that reflect the fish feeding conditions. It is utilized to assess the condition of individual fish. A fish with a higher K value is generally healthier than one with a lower one [98]. In the present study, K was low (0.61 ± 0.09), indicating that the fish might be in poor condition, possibly due to seasonal variability and/or being in early developmental stages. Variations in K reflect gonadal growth, fat store locations, and environmental adaptability. These factors can vary according to physiological influences and changes associated with developmental phases [99].

Despite advancements in fish veterinary medicine, interpreting hematological parameters remains a significant challenge due to a lack of standardized reference values methods. Blood indices provide crucial insights into fish health, but consistent and reliable data require careful attention and expertise. This study highlighted that trawling depth and duration significantly affected certain blood measurements in European hake, emphasizing the need to take these factors into consideration in future research. Variations in hematological parameters can be ascribed to environmental stress [40], influenced by trawling conditions. Although the parameters measured in this study were consistent with the findings of other studies, we advocate for the establishment of a hematological databank for various fish species to enhance the interpretation of hematological data in future studies.

5. CONCLUSION

The present work provided novel insights into the hematological parameters of the European Hake (*M. merluccius*) in the Toroneos Gulf, northern Greece, utilizing automated blood analysis. We identified significant variations in blood indices between sexes and pronounced effects of trawling depth and duration on hematological parameters, including RBC and WBC counts, Hb concentration, and Ht. These findings underscore the necessity of accounting for environmental and procedural variables in future research on wild fish populations. In addition, this work contributes to the limited hematological data available for European hake and suggests the establishment of a comprehensive hematological database for fish species to enhance diagnostic capabilities in fish health assessments. The results highlight the utility of automated hematological analyzers in fish research despite the challenges posed by species-specific blood characteristics. Furthermore, the biometric and biological indices, such as GSI and Fulton's condition factor, offer valuable insights into the health, reproductive state, and environmental adaptability of the fish population. Future studies should aim to expand reference ranges and improve calibration techniques for automated analyzers to support accurate interpretations of fish blood parameters across different species and environments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study has been approved by the University of Thessaly Departmental Aquatic Animal Ethics Committee (DAAEC). All fish in this study were used in compliance with all relevant national and EU laws (Animal Scientific Procedures Act 1986/609/EEC and Directive (2010/63/EU) and institutional guidelines.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

The datasets analyzed from the current study are available from the corresponding author upon reasonable request.

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