applied sciences

MDPI

Article

Assessment of Fish Health: Seasonal Variations in Blood Parameters of the Widely Spread Mediterranean Scorpaenid Species, *Scorpaena porcus*

Josipa Ferri [1,*], Sanja Matić-Skoko [2], Rozelindra Čož-Rakovac [3], Ivančica Strunjak-Perović [3], Blanka Beer Ljubić [4] and Natalija Topić Popović [3]

[1] Department of Marine Studies, University of Split, 21000 Split, Croatia
[2] Institute of Oceanography and Fisheries, 21000 Split, Croatia; sanja@izor.hr
[3] Laboratory for Aquaculture Biotechnology, Ruđer Bošković Institute, 10000 Zagreb, Croatia; rozelindra.coz-rakovac@irb.hr (R.Č.-R.); ivancica.strunjak.perovic@irb.hr (I.S.-P.); natalija.topic.popovic@irb.hr (N.T.P.)
[4] Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia; bljubic@vef.hr
* Correspondence: josipa.ferri@unist.hr; Tel.: +385-21558195

Abstract: The measurement of haematological and biochemical parameters is essential for monitoring the health status of wild fish. More specifically, blood parameters provide crucial information on the physiological changes that occur in fish in response to various fluctuations in their environment. This study presents reference ranges and seasonal variations for 15 blood parameters of the black scorpionfish, *Scorpaena porcus*, as a species of high value for ecosystem monitoring in the Mediterranean. The mean haematocrit (HCT) values differed significantly between seasons, with the highest value recorded in winter. In addition, six plasma parameters varied significantly during the year. Cholesterol (CHOL), non-esterified fatty acids (NEFA), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and superoxide dismutase (SOD) concentrations were notably higher during the spring/summer period and at their lowest levels in autumn/winter. On the contrary, levels of glucose (GLU) were highest during autumn and lowest during the spring season. The post hoc Tukey test revealed that there were significant differences in HCT values for winter vs. spring, in CHOL for spring vs. all other seasons, in NEFA for summer vs. autumn and summer vs. spring, in GLU for spring vs. summer, in AST for autumn vs. spring, in ALP for winter vs. all other seasons and in SOD for summer vs. all other seasons ($p < 0.05$ in all cases). A total of nine blood parameters showed a significant relationship with fish size throughout different seasons. Our results suggest that monitoring blood parameters may serve as a useful biomarker, and we provide a reliable basis for the future monitoring of the health status of the investigated *S. porcus*. Considering significant seasonal variations, the use of season-specific reference ranges is recommended for this scorpaenid species.

Keywords: *Scorpaena porcus*; blood parameters; reference ranges; health indicators; seasonal variations; Mediterranean

Citation: Ferri, J.; Matić-Skoko, S.; Čož-Rakovac, R.; Strunjak-Perović, I.; Beer Ljubić, B.; Topić Popović, N. Assessment of Fish Health: Seasonal Variations in Blood Parameters of the Widely Spread Mediterranean Scorpaenid Species, *Scorpaena porcus*. *Appl. Sci.* **2022**, *12*, 4106. https://doi.org/10.3390/app12094106

Academic Editor: Franz Jirsa

Received: 10 March 2022
Accepted: 18 April 2022
Published: 19 April 2022

1. Introduction

In constantly changing marine ecosystems, under conditions of increasing environmental and anthropogenic pressures, one of the greatest challenges for many organisms is coping with a variety of stressors, such as hypoxia, variations in pH, salinity and temperature, eutrophication, pollution, fishery and introduction of exotic species [1–3]. The response of an organism to a stressor is a dynamic process and the integrated result of two pathways: direct-acting, primarily through biochemical and metabolic processes, and indirect-acting, mainly through effects on the food chain, habitat availability and behavioural modifications of organisms [4]. To obtain information regarding the status

of stressed ecosystems and their interaction with aquatic organisms, the use of biomarkers, especially in fish, has been recognised as a good approach [5]. In this context, fish haematological and biochemical parameters have become promising stress biomarkers and therefore helpful diagnostic tools [6–14]. In general, they are essential for assessing both fish health status and the extent of stress-related responses. Blood parameters are species-specific; they depend on biometrics, sex, age, maturity and nutritional status and therefore undergo many changes during the fish life cycle [14,15]. All of this makes their evaluation difficult, so the prerequisite for the correct interpretation of blood parameters and their potential use as biomarkers is knowledge of reference values established on yearly cycles.

There are several studies describing the haematological and biochemical parameters of the black scorpionfish, *Scorpaena porcus* [16–19]. The activity of antioxidant blood enzymes was investigated in this species living in the Black Sea [18,19]. In the nearby Dardanelles, blood chemistry (lipoproteins and enzymes) was analysed during the summer months [16], and variations in blood glucose were evaluated on a monthly basis for one year [17]. However, there is no comprehensive published information about reference ranges for blood parameters of presumed healthy black scorpionfish. On the other hand, this species plays an important ecological role in the Mediterranean hard-bottom and seagrass habitats and has a high value for ecosystem monitoring and management [2,20,21]. Therefore, the development of reference ranges is crucial for the health assessment of the black scorpionfish. This is exactly what has increased our interest in establishing annual cycle variations in numerous blood parameters of *S. porcus*. During the present study, fish were collected in the northernmost part of the Mediterranean, and haematological and biochemical parameters, including protein, lipid, carbohydrate and enzyme profile parameters, were analysed in detail. Specifically, this work provided reference values for 15 key health indicators, and their relationship with fish size was established. Moreover, measured blood parameters were correlated with different seasons and could serve as biomarkers of the influence of seasons on the local community of the investigated species. Once baseline values and seasonal variations are established, blood parameters can be monitored and can provide important information on the responses of the black scorpionfish to changing marine ecosystems.

2. Materials and Methods

2.1. Fish Sampling and Blood Collection

Black scorpionfish specimens were collected during daylight hours using sets of trammel nets; 1.5 m wide and 32 m long trammel nets with inner layer mesh size of 28 mm and outer layer mesh size of 150 mm. Fish samples were captured in the nearshore coastal waters in the central-eastern Adriatic (northern Mediterranean) at depths ranging between 10 and 40 m. During a one-year study in 2008, a total of 96 black scorpionfish were collected, and they were divided according to the season in which they were caught (Table 1). Sampled fish did not manifest any external signs of trauma, injuries or infestation.

Table 1. Number, total length (TL) and weight (W) range, mean total length, weight and condition index (CI) with standard deviation (SD) of *Scorpaena porcus* during different seasons.

	Number	TL Range (cm)	W Range (g)	Mean TL ± SD	Mean W ± SD	Mean CI ± SD
Winter	21	14.9–25.9	59–446	18.0 ± 2.83	125.5 ± 89.14	1.91 ± 0.23
Spring	24	14.1–26.0	49–356	18.5 ± 3.78	131.2 ± 87.05	1.83 ± 0.19
Summer	34	14.4–23.1	55–246	17.3 ± 2.05	100.4 ± 40.22	1.85 ± 0.17
Autumn	17	14.0–20.8	50–189	17.1 ± 1.87	99.3 ± 38.93	1.87 ± 0.16

Once caught, fish were placed in a tank and immediately anaesthetised (MS-222 at 0.3 g L^{-1}) for 2–3 min until loss of coordination was visible [22]. Afterwards, blood samples were collected by caudal vein puncture using disposable sterile plastic syringes fitted with a needle. A part of each blood sample was transferred into microhaematocrit capillary tubes

and used for the haematological analysis of the field. The remaining blood was poured into tubes coated with anticoagulant lithium heparin and centrifuged at 12,000× *g* for 90 s. The resultant plasma was collected and stored at −20 °C for further biochemical analysis.

After blood sampling, the black scorpionfish were euthanised by severing the spinal cord; total length (TL, measured to the nearest 0.1 cm) and weight (W, measured to the nearest 1 g) were recorded, and sex was determined macroscopically by gonad observation. The fish condition index (CI) was calculated as $CI = (100 \times W)/TL^3$.

2.2. Haematological and Biochemical Analyses

Microhaematocrit capillary tubes were used for the haematocrit (HCT) determination. After sealing, tubes were centrifuged at 15,800× *g* for 120 s, and values were estimated using a microhaematocrit reader (final values are given as percentages).

Plasma samples were used for the analysis of blood biochemical parameters using a SABA-18 auto-biochemistry analyser (AMS Analyser Medical System, Rome, Italy). This analysis included (i) protein profile parameters: total proteins (TP; g/L), urea (UREA; mmol/L) and creatinine (CREA; mmol/L); (ii) lipid profile parameters: cholesterol (CHOL; mmol/L), triglyceride (TRIG; mmol/L) and non-esterified fatty acids (NEFA; mmol/L); (iii) carbohydrate profile parameter: glucose (GLU; mmol/L); (iv) enzyme profile parameters: aspartate aminotransferase (AST; U/L), alkaline phosphatase (ALP; U/L), gamma glutamyl transferase (GGT; U/L), lactate dehydrogenase (LDH; U/L) and creatine kinase (CK; U/L); and (v) antioxidative enzyme parameters: glutathione peroxidase (GPx; U/L) and superoxide dismutase (SOD; U/mL).

2.3. Statistical Analyses

Differences in the fish length, weight, condition index and blood parameter values between sexes and seasons were tested using one-way analysis of variance (ANOVA), followed by a post hoc Tukey test (statistical package STATISTICA, version 14.0.0.15). The statistical assumptions of the ANOVA were tested using Kolmogorov–Smirnov test of normality and Levene's test of homogeneity of variances, and when necessary, data were log(x + 1) transformed. Relationships between fish length and plasma parameters of the black scorpionfish were tested separately for each season using the Spearman rank correlation. A p value < 0.05 was considered significant, while a p value < 0.01 was considered highly significant.

3. Results

Ninety-six (96) fish were included in the data set from which the blood reference ranges were calculated, and their seasonal patterns were analysed. The total length and weight of all collected fish specimens ranged from 14.0 to 26.0 cm (mean ± SD TL = 17.7 ± 2.73 cm) and from 49.0 to 446.0 g (mean ± SD W = 113.4 ± 67.33 g), respectively. Table 1 shows the number of fish per season, as well as their size range (total length and weight) and the condition index. The largest individual (26.0 cm) was sampled during the spring; however, no significant differences in fish total length and weight ($p > 0.05$ for both measures) were observed among seasons. In addition, condition index values throughout the study period were between 1.45 and 2.57 (the highest mean value was estimated during the winter season; Table 1) but also depicted an insignificant difference between analysed seasons ($p > 0.05$).

As no significant differences were found in blood parameter values between females and males ($p > 0.05$ for all measured parameters), sexes were pooled together within each season. The results of the haematological analyses showed that the mean haematocrit values differed significantly between seasons, with the highest value recorded in winter (32.6% ± 10.05). Mean HCT values (±standard deviation) in other seasons, more precisely in spring, summer and autumn, were 24.5% ± 4.95, 28.6% ± 5.57 and 30.1% ± 7.76, respectively. The post hoc Tukey test revealed that there were significant differences in the HCT values for winter vs. spring.

Seasonal plasma biochemical parameters are presented in Table 2. The values of UREA and TRIG showed strong departure from normality, even when log(x + 1) transformed, so they were excluded from all statistical tests assuming normal data distribution. As shown in Table 3, six plasma parameters varied significantly throughout the seasons. CHOL, NEFA, AST, ALP and SOD concentrations were notably higher during the spring/summer period and at their lowest levels in autumn/winter (Figure 1). In contrast to that, levels of GLU were highest during autumn and lowest during the spring season. More precisely, the post hoc Tukey test revealed that there were only significant differences in CHOL for spring vs. all other seasons, in NEFA for summer vs. autumn and summer vs. spring, in GLU for spring vs. summer, in AST for autumn vs. spring, in ALP for winter vs. all other seasons and in SOD for summer vs. all other seasons ($p < 0.05$ in all cases).

Table 2. Seasonal variation in plasma biochemical parameters (TP—total proteins; UREA—urea; CREA—creatinine; CHOL—cholesterol; TRIG—triglyceride; NEFA—non-esterified fatty acids; GLU—glucose; AST—aspartate aminotransferase; ALP—alkaline phosphatase; GGT—gamma glutamyl transferase; LDH—lactate dehydrogenase; CK—creatine kinase; GPx—glutathione peroxidase; SOD—superoxide dismutase) of *Scorpaena porcus*.

	Mean ± SD			
	Winter	**Spring**	**Summer**	**Autumn**
Protein profile parameters				
TP (g/L)	29.88 ± 6.01	35.13 ± 7.58	35.25 ± 11.15	32.59 ± 9.93
UREA (mmol/L)	0.47 ± 0.39	0.84 ± 0.47	0.36 ± 0.33	0.49 ± 0.53
CREA (mmol/L)	44.71 ± 6.64	50.08 ± 25.83	47.37 ± 9.03	42.17 ± 13.52
Lipid profile parameters				
CHOL (mmol/L)	0.68 ± 0.39	1.55 ± 0.52	0.99 ± 0.33	0.83 ± 0.75
TRIG (mmol/L)	0.22 ± 0.15	0.69 ± 0.44	0.35 ± 0.18	0.50 ± 0.38
NEFA (mmol/L)	0.09 ± 0.01	0.10 ± 0.07	0.37 ± 0.30	0.06 ± 0.07
Carbohydrate profile parameter				
GLU (mmol/L)	8.07 ± 5.61	5.94 ± 6.76	9.86 ± 5.87	10.44 ± 6.93
Enzyme profile parameters				
AST (U/L)	9.07 ± 14.88	12.50 ± 10.14	10.47 ± 8.84	5.13 ± 2.85
ALP (U/L)	24.87 ± 13.30	68.07 ± 25.65	76.44 ± 20.12	47.42 ± 20.31
GGT (U/L)	2.64 ± 2.21	4.61 ± 10.66	3.32 ± 3.07	3.06 ± 1.47
LDH (U/L)	586.68 ± 561.25	881.68 ± 794.10	555.38 ± 586.37	422.80 ± 680.85
CK (U/L)	9.84 ± 6.91	16.94 ± 9.62	17.14 ± 18.34	11.38 ± 4.55
Antioxidative profile parameters				
GPx (U/L)	5474.33 ± 1960.31	4535.66 ± 1487.62	4446.46 ± 1342.74	4984.64 ± 2062.51
SOD (U/mL)	0.41 ± 0.14	0.41 ± 0.23	0.83 ± 0.19	0.32 ± 0.14

Table 3. Summary of ANOVA examining variations in plasma biochemical parameters (TP—total proteins; CREA—creatinine; CHOL—cholesterol; NEFA—non-esterified fatty acids; GLU—glucose; AST—aspartate aminotransferase; ALP—alkaline phosphatase; GGT—gamma glutamyl transferase; LDH—lactate dehydrogenase; CK—creatine kinase; GPx—glutathione peroxidase; SOD—superoxide dismutase) between seasons for *Scorpaena porcus* (SS—sum of squares; df—degrees of freedom; MS—mean square).

	SS	**df**	**MS**	**F**	***p***
TP (g/L)	0.065	3	0.022	1.630	0.188
CREA (mmol/L)	0.028	3	0.009	0.448	0.719
CHOL (mmol/L)	0.411	3	0.137	11.615	0.000
NEFA (mmol/L)	0.134	3	0.045	15.679	0.000
GLU (mmol/L)	1.235	3	0.412	3.068	0.033
AST (U/L)	1.086	3	0.362	3.842	0.013
ALP (U/L)	2.682	3	0.894	23.776	0.000
GGT (U/L)	0.085	3	0.028	0.385	0.764
LDH (U/L)	2.024	3	0.675	1.994	0.121
CK (U/L)	0.496	3	0.165	1.576	0.205
GPx (U/L)	0.052	3	0.017	0.640	0.591
SOD (U/mL)	0.211	3	0.070	26.007	0.000

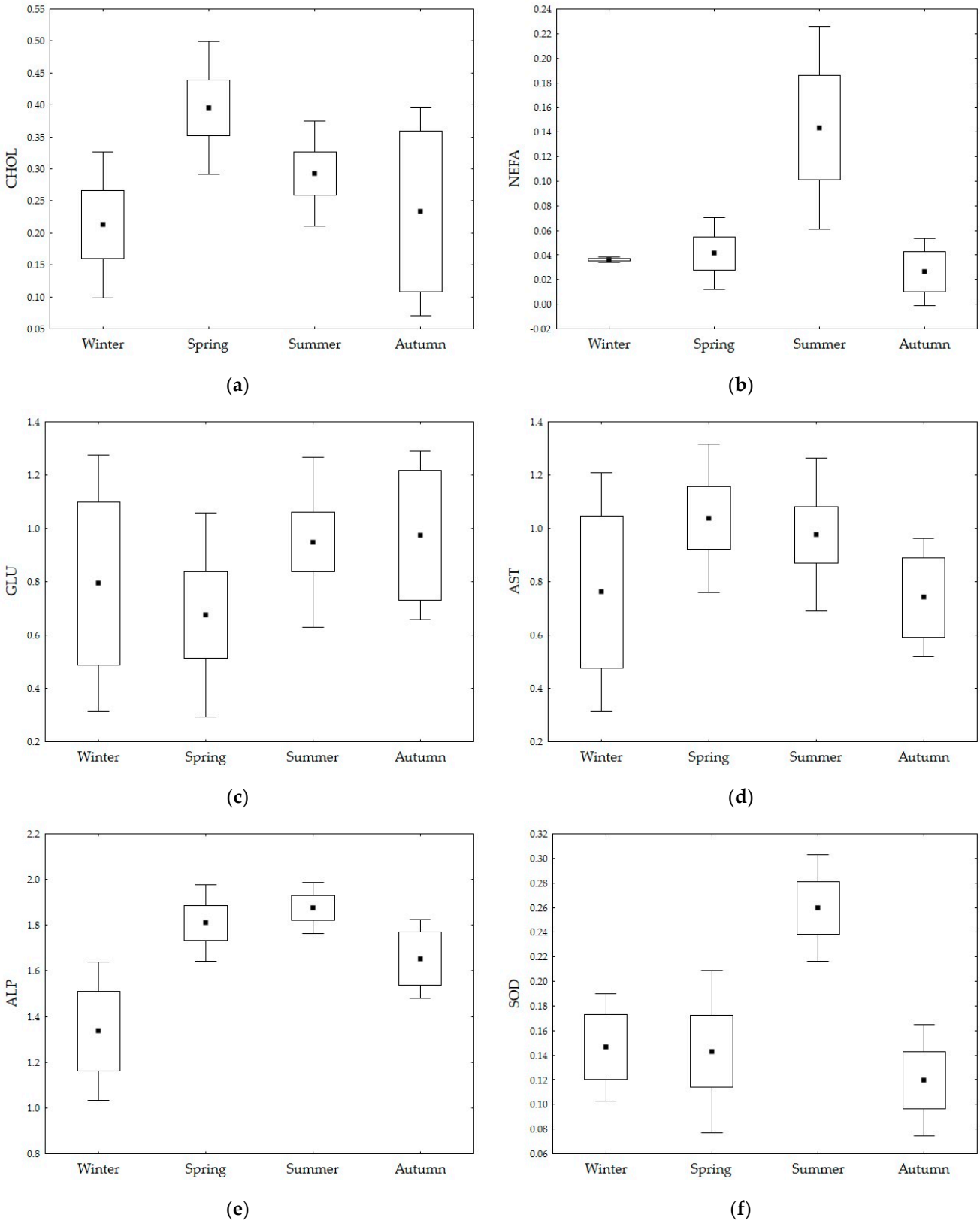

Figure 1. Mean values (±confidence intervals 95%) of plasma biochemical parameters: (**a**) CHOL—cholesterol; (**b**) NEFA—non-esterified fatty acids; (**c**) GLU—glucose; (**d**) AST—aspartate aminotransferase; (**e**) ALP—alkaline phosphatase; (**f**) SOD—superoxide dismutase (all displayed values are log(x + 1) transformed) of *Scorpaena porcus* that varied significantly throughout the seasons (whiskers denote +/− standard deviations).

Spearman rank correlation of fish length and plasma chemistry was conducted separately for each season (Table 4). We found that a total of nine parameters had a significant relationship with fish size throughout different seasons, and no such correlation was observed during summertime.

Table 4. Spearman rank correlation of fish length and plasma biochemical parameters (TP—total proteins, UREA—urea, CREA—creatinine, CHOL—cholesterol, TRIG—triglyceride, NEFA—non-esterified fatty acids, GLU—glucose, AST—aspartate aminotransferase, ALP—alkaline phosphatase, GGT—gamma glutamyl transferase, LDH—lactate dehydrogenase, CK—creatine kinase, GPx—glutathione peroxidase, SOD—superoxide dismutase) of *Scorpaena porcus* (* $p < 0.05$).

	TL	TP	UREA	CREA	CHOL	TRIG	NEFA	GLU	AST	ALP	GGT	LDH	CK	GPx
Winter														
TP	0.37													
UREA	0.02	−0.14												
CREA	0.23	0.57	0.02											
CHOL	0.46 *	0.26	0.47	−0.05										
TRIG	0.32	0.08	−0.14	−0.17	0.46									
NEFA	0.17	0.52	0.05	0.79	0.44	0.52								
GLU	−0.04	0.04	0.11	0.76 *	0.23	−0.01	0.19							
AST	0.53	0.45	−0.26	−0.08	0.05	0.03	0.14	−0.54						
ALP	0.22	−0.30	0.25	0.14	0.08	−0.05	0.49	0.12	0.01					
GGT	0.66 *	0.14	−0.24	0.26	0.09	0.22	0.71	−0.39	0.28	0.42				
LDH	−0.57 *	0.26	0.21	0.37	−0.17	−0.14	0.13	0.43	−0.30	−0.45	−0.53			
CK	−0.45	−0.20	−0.25	0.13	0.04	−0.13	−0.78	0.36	−0.33	−0.21	−0.28	0.16		
GPx	0.16	0.36	0.32	−0.07	0.18	−0.20	0.38	0.18	−0.05	−0.27	−0.10	0.11	−0.57	
SOD	−0.82 *	−0.31	−0.13	−0.12	−0.67 *	−0.50	−0.33	0.28	−0.45	−0.18	−0.70 *	0.45	0.22	0.00
Summer														
TP	0.61 *													
UREA	−0.53 *	−0.23												
CREA	−0.30	0.03	0.36											
CHOL	0.28	0.53 *	0.16	0.47 *										
TRIG	0.02	0.44 *	0.45 *	0.49 *	0.87 *									
NEFA	−0.32	−0.02	0.52 *	0.44	0.62 *	0.77 *								
GLU	0.02	0.01	0.41 *	0.55 *	0.65 *	0.62 *	0.52 *							
AST	0.24	0.21	−0.00	−0.08	−0.01	−0.00	−0.44	−0.12						
ALP	−0.60 *	−0.24	0.24	0.38	0.06	0.19	0.42	0.05	−0.18					
GGT	0.11	−0.02	−0.35	−0.17	−0.09	−0.08	−0.19	−0.10	0.14	−0.36				
LDH	−0.08	−0.01	0.35	0.43 *	0.28	0.42	0.07	0.35	0.34	−0.11	0.02			
CK	−0.29	−0.10	0.38	0.27	−0.21	0.20	0.29	−0.02	−0.29	0.08	−0.30	0.04		
GPx	0.61 *	0.59 *	−0.24	−0.16	0.40	0.41	−0.11	0.08	0.22	−0.21	0.32	0.03	0.00	
SOD	−0.20	−0.51 *	−0.25	−0.30	−0.84 *	−0.85 *	−0.50 *	−0.48 *	0.13	0.07	−0.12	−0.42	0.00	−0.50 *
Spring														
TP	0.22													
UREA	−0.14	−0.36 *												
CREA	0.16	0.52 *	−0.07											
CHOL	0.13	0.38	0.27	0.42 *										
TRIG	0.39	0.53 *	0.07	0.26	0.37									
NEFA	0.16	0.41	0.07	0.04	0.44	0.88 *								
GLU	0.15	0.31	−0.17	0.77 *	0.16	0.10	−0.10							
AST	−0.01	0.35	−0.01	0.28	0.10	0.46	0.37	0.30						
ALP	−0.07	0.24	0.28	0.76 *	0.30	0.23	0.16	0.56 *	0.13					
GGT	0.32	0.20	0.15	0.17	0.02	0.29	0.13	0.05	0.00	0.37				
LDH	−0.29	0.33	−0.22	0.11	−0.05	0.27	0.36	0.06	0.40 *	0.01	−0.16			
CK	−0.05	0.35	−0.17	0.41	0.38	0.54	0.45	0.24	−0.07	−0.01	0.00	0.41		
GPx	0.27	0.42	−0.19	−0.22	0.11	0.15	0.13	−0.29	−0.24	−0.27	0.01	0.14	−0.05	
SOD	−0.27	−0.40	0.24	−0.17	−0.18	−0.44	−0.29	−0.27	−0.02	−0.06	−0.11	−0.09	0.11	−0.47
Autumn														
TP	0.32													
UREA	−0.74	−0.36												
CREA	0.05	0.77 *	−0.29											
CHOL	0.58	0.87 *	−0.68	0.48										
TRIG	0.32	0.55	−0.34	0.37	0.63									
NEFA	0.73 *	0.68	−0.60	0.21	0.79 *	0.79 *								
GLU	−0.05	0.53	−0.11	0.73 *	0.33	0.55	0.14							
AST	0.22	0.70 *	−0.18	0.83 *	0.45	0.38	0.19	0.90 *						
ALP	0.49	0.55	0.00	0.48	0.38	0.48	0.71 *	0.28	0.55					
GGT	0.10	0.68 *	−0.40	0.45	0.77 *	0.70 *	0.50	0.15	−0.03	0.28				
LDH	0.08	0.60	−0.02	0.82 *	0.28	0.32	0.14	0.82 *	0.95 *	0.59	0.02			
CK	−0.80	0.30	0.00	0.80	−0.10	−0.70	−0.80	0.20	0.60	−0.20	0.20	0.90 *		
GPx	0.45	0.62	−0.38	0.25	0.77 *	0.48	0.28	0.10	0.15	0.13	0.41	0.10	−0.50	
SOD	−0.54 *	−0.50	0.70	−0.05	−0.82 *	−0.67 *	−0.53	−0.05	0.05	−0.11	−0.36	0.13	0.60	−0.71 *

4. Discussion

This study provides the first comprehensive data on haematological and biochemical reference values for *S. porcus* in the Adriatic Sea, and even in the entire Mediterranean, and is a valuable step towards using these parameters for the health monitoring of the investigated species. The analysed blood parameters covered a wide range of biological functions and metabolic processes, including the stress response (e.g., HCT), nutritional status (e.g., TP, CHOL, TRIG, NEFA and GLU), organ function (e.g., AST and ALP) and antioxidant defence system (e.g., GPx and SOD). Studies of blood parameters have proven to be a good approach for analysing fish health status. It is therefore not surprising that there has been a growing interest in such studies in recent years [23,24]. Most results showed that the baseline values of blood parameters are species-specific and were affected by numerous endogenous and exogenous factors, such as water temperature, food, and the age and sex of the fish [23]. The present study demonstrated the temporal variation in both the haematological and biochemical parameters of *S. porcus*, with non-significant alterations with respect to sex. Specifically, seven blood parameters showed significant seasonal differences, and while the values of CHOL, NEFA, AST, ALP and SOD were significantly higher during the warmer period (spring/summer), the levels of HCT and GLU were highest during colder seasons (autumn/winter). Elevated water temperatures during the spring and summer lead to an increase in fish metabolic rate; however, it should be emphasised that seasons in which most of the parameters increased (spring and summer) correspond to the spawning period of the investigated species (May–September) [25].

CHOL, NEFA and GLU, as contributors to the observed variations between seasons in *S. porcus*, can yield information about the fish nutritional status. Seasonal variation in the lipid profile analysis revealed that two fractions (CHOL and TRIG) were the highest in spring, probably in relation to the food availability and the feeding rate. Similar results were also reported in the past for other fish species in the Mediterranean and other areas [26–28]. On the other hand, it should be emphasised that the mean values of CHOL and TRIG for *S. porcus* throughout the year are lower than those reported for some Mediterranean species: *Oblada melanura* [11], *Scyliorhinus canicula* [27], *Sparus aurata* [29] and mullets [30]. The only similarity was noted with the wild *Dicentrarchus labrax* from the northern Adriatic Sea, whose TRIG values were 0.67 mmol/L [31]. The most metabolically active lipid form in the blood is NEFA, and it is indicative of the extent to which fish rely on lipid as a fuel [32]. However, as NEFA plasma levels are also affected by spawning in a way that causes concentrations to increase during this process, we believe that this is precisely the reason for the increased NEFA in *S. porcus* during the summertime. This peak associated with spawning is linked with gametogenesis and reflects the mobilisation of lipid reserves required for the development of gonads [33]. Seasonal variation in the blood GLU concentrations revealed a pattern different to that observed for the lipid profile parameters. Even though GLU is accepted as an indicator of fish nutritional status, this parameter is highly responsive to stressors, so its variations in the blood are more likely to be associated with other stress indices than with nutritional status [34]. In addition, in contrast to the results of the present study, *S. porcus* captured in the Dardanelles had the highest and the lowest GLU levels in spring and autumn, respectively [17]. These differences could be related to the sampling area and specific local conditions. In general, GLU concentrations vary considerably between species, which can be confirmed by a comparison with the results of research on blood parameters in other Mediterranean fish species. This carbohydrate profile parameter (mean values) varied from 2.1 mmol/L in captive *Acipenser naccarii* [35], 5.62 mmol/L in wild *Thunnus thynnus* [36] and 5.75 mmol/L in wild *O. melanura* [11] to 7.86 mmol/L in wild mullets [30], 11.11 mmol/L in captive *T. thynnus* [36] and 14.84 mmol/L in captive *S. aurata* [29].

Enzyme profile parameters have been widely used in fisheries science. AST is found in many fish tissues, and this enzyme represents a non-specific indicator of damage [37]. On the other side, ALP has no known function in the blood, but its plasma activities depend on the rate of leakage from cells and the rate of removal from the blood [37]. The

influence of water temperature on enzyme activities in fish blood is primary [38]; therefore, it is not surprising that values of both AST and ALP varied depending on the influence of seasons [28], as noted in our study. However, analyses of the annual dynamics of enzyme activities in the blood of several fish species showed that spawning is also one of the most important factors that influenced enzyme activities [38]. In the case of the investigated scorpionfish, spawning takes place during spring and summer seasons, so we believe that both temperature and spawning jointly affected the activities of AST and ALP. However, we observed that AST activities in this study during summertime were much lower than values reported for the same species caught in the same period in the Dardanelles (224.11 ± 14.09 U/L) [16], and we believe that such variations depend on the sampling technique, analysis methods, and fish age, diet and/or habitat. In addition, higher values of AST and ALP, compared to data from this study, were observed in *O. melanura* that aggregated around fish farm cages in the Adriatic [11]. On the contrary, wild *T. thynnus* caught in the Levantine Sea had lower ALP levels than the black scorpionfish [36].

Fish have an antioxidant defence system that is influenced by various factors, such as diet, behaviour, age, physiological status in general and chemical composition of water [19,28]. Antioxidant enzymes, including GPx and SOD investigated here, have an important role in protective mechanisms against reactive oxygen species [19]. Specifically, GPx protects proteins and nucleic acids from the action of oxidizing molecules, and SOD acts against free oxygen radicals [19,28]. Therefore, these blood parameters are often used as biomarkers for water pollution and ecological risk evaluation [19]. Unlike GPx activity, which showed insignificant seasonal variations, the plasma SOD level was significantly higher in summer, most likely because of changes in water temperature. In addition, nine blood parameters significantly changed with fish size, and this change was also influenced by seasons. Previous studies also found that fish body size influenced their blood profiles, and this should be considered when interpreting the results [11,14]. However, more research is needed to determine if these changes coincide with changes in some metabolic processes of the investigated species. In addition, to estimate and confirm the seasonality of the blood parameters in fish species, it is recommended to analyse two to three annual cycles [7].

5. Conclusions

In conclusion, the results of this study are the first comprehensive data on the baseline values of 15 blood parameters in *S. porcus*, which cover all seasons. Almost half of the analysed parameters show significant seasonal patterns, so the results clearly show that seasons have a pronounced effect on the investigated species. Therefore, as a starting point for future investigations, we recommend that seasons be taken into consideration when monitoring the health status of the investigated scorpionfish. Moreover, fish size should also be considered when interpreting the blood parameter values.

Author Contributions: Conceptualisation, J.F. and R.Č.-R.; methodology, J.F., B.B.L. and R.Č.-R.; software, J.F.; validation, I.S.-P., N.T.P. and B.B.L.; formal analysis, J.F. and B.B.L.; investigation, J.F., S.M.-S. and B.B.L.; resources, J.F., S.M.-S. and R.Č.-R.; data curation, J.F.; writing—original draft preparation, J.F. and S.M.-S.; writing—review and editing, J.F., S.M.-S., N.T.P., R.Č.-R. and I.S.-P.; visualisation, J.F., N.T.P. and I.S.-P.; supervision, S.M.-S., N.T.P., R.Č.-R. and I.S.-P.; funding acquisition, S.M.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Code of Ethics of the University Department of Marine Studies as well as in accordance with EC Directive 86/609/EEC for animal experiments. Fish were manipulated by competent authorised persons in accordance with the provisions of national legislation.

Data Availability Statement: Data from this study are available from the corresponding author upon reasonable request (J.F.: josipa.ferri@unist.hr).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Romão, S.; Donatti, L.; Fretia, M.O.; Teixeira, J.; Kusma, J. Blood parameter Analysis and Morphological Alterations as Biomarkjers on the health of *Hoplias malabricus* and *Geophagus brasiliensis*. *Braz. Arch. Biol. Technol.* **2006**, *49*, 441–448. [CrossRef]
2. Ferri, J.; Stagličić, N.; Matić-Skoko, S. The black scorpionfish, *Scorpaena porcus* (Scorpaenidae): Could it serve as reliable indicator of Mediterranean coastal communities' health? *Ecol. Indic.* **2012**, *18*, 25–30. [CrossRef]
3. Tenji, D.; Micic, B.; Sipos, S.; Miljanovic, B.; Teodorovic, I.; Kaisarevic, S. Fish biomarkers from a different perspective: Evidence of adaptive strategy of *Abramis brama* (L.) to chemical stress. *Environ. Sci. Eur.* **2020**, *32*, 47. [CrossRef]
4. Adams, S.M. Assessing cause and effect of multiple stressors on marine systems. *Mar. Pollut. Bull.* **2005**, *51*, 649–657. [CrossRef] [PubMed]
5. Viarengo, A.; Lowe, D.; Bolognesi, C.; Fabbri, E.; Koehler, A. The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2007**, *146*, 281–300. [CrossRef]
6. Roche, H.; Bogé, G. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Mar. Environ. Res.* **1996**, *41*, 27–43. [CrossRef]
7. Lusková, V. Factors Affecting Hematological Indices in Free-living Fish Populations. *Acta Vet. Brno* **1998**, *67*, 249. [CrossRef]
8. Ivanc, A.; Haskovic, E.; Jeremic Dekic, S.R. Haematological evaluation of welfare and health of fish. *Praxis Vet.* **2005**, *53*, 191–202.
9. Vázquez, G.; Guerrero, G.A. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). *Tissue Cell* **2007**, *39*, 151–160. [CrossRef]
10. Seriani, R.; Abessa, D.M.S.; Kirschbaum, A.A.; Pereira, C.D.S.; Romano, P.; Ranzani-Paiva, M.J.T. Relationship between water toxicity and hematological changes in *Oreochromis niloticus*. *Braz. J. Aquat. Sci. Technol.* **2011**, *15*, 47–53.
11. Ferri, J.; Topić Popović, N.; Čož-Rakovac, R.; Beer-Ljubić, B.; Strunjak-Perović, I.; Škeljo, F.; Jadan, M.; Petrić, M.; Barišić, J.; Šimpraga, M.; et al. The effect of artificial feed on blood biochemistry profile and liver histology of wild saddled bream, *Oblada melanura* (Sparidae). *Mar. Environ. Res.* **2011**, *71*, 218–224. [CrossRef] [PubMed]
12. Pradhan, S.C.; Patra, A.K.; Sarkar, B.; Pal, A. Seasonal changes in hematological parameters of *Catla catla* (Hamilton 1822). *Comp. Clin. Path A* **2012**, *21*, 1473–1487. [CrossRef]
13. Wade, N.M.; Clark, T.D.; Maynard, B.T.; Atherton, S.; Wilkinson, R.J.; Smullen, R.P.; Taylor, R.S. Effects of an unprecedented summer heatwave on the growth performance, flesh colour and plasma biochemistry of marine cage-farmed Atlantic salmon (*Salmo salar*). *J. Therm. Biol.* **2019**, *80*, 64–74. [CrossRef] [PubMed]
14. Casanovas, P.; Walker, S.; Johnston, H.; Johnston, C.; Symonds, J. Comparative assessment of blood biochemistry and haematology normal ranges between Chinook salmon (*Oncorhynchus tshawytscha*) from seawater and freshwater farms. *Aquaculture* **2021**, *537*, 736464. [CrossRef]
15. Jan, K.; Ahmed, I. The influence of sex and season on some hematological and biochemical parameters of snow trout *Schizothorax labiatus* in the Indian Himalayan Region. *Fish. Sci.* **2021**, *87*, 39–54. [CrossRef]
16. Çelik, E.S. Blood Chemistry (Electrolytes, Lipoproteins and Enzymes) Values of Black Scorpion Fish (*Scorpaena porcus* Linneaus, 1758) in the Dardanelles, Turkey. *J. Biol. Sci.* **2004**, *4*, 716–719.
17. Çelik, E.S. Çanakkale Boğazı'ndan Avlanan İskorpit Balığı (*Scorpaena porcus* Linnaeus, 1758)'nın Kan Glukoz Düzeyindeki Aylık Değişmeler. *Su. Ürünleri. Dergisi.* **2005**, *22*, 115–118.
18. Rudneva, I.; Skuratovskaya, E. Gender peculiarities of blood antioxidant enzyme activity of some Black Sea coastal fish species. *J. Ichthyol.* **2009**, *49*, 119–122. [CrossRef]
19. Rudneva, I.; Skuratovskaya, E.; Kuzminova, N.; Kovyrshina, T. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2010**, *151*, 229–239. [CrossRef]
20. Harmelin-Vivien, M.; Kaim-Malka, R.A.; Ledoyer, M.; Jacob-Abraham, S.S. Food partitioning among Scorpaenid fishes in Mediterranean seagrass beds. *J. Fish Biol.* **1989**, *34*, 715–734. [CrossRef]
21. Rudneva, I.I.; Shevchenko, N.F.; Zalevskaya, I.N.; Zherko, N.V. Biomonitoring of the coastal waters of the Black Sea. *Water Resour.* **2005**, *32*, 215–222. [CrossRef]
22. Topic Popovic, N.; Strunjak-Perovic, I.; Coz-Rakovac, R.; Barisic, J.; Jadan, M.; Persin Berakovic, A.; Sauerborn Klobucar, R. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *J. Appl. Ichthyol.* **2012**, *28*, 553–564. [CrossRef]
23. Muhammad, O.I.; Mahmoud, U.M.; Fazio, F.; Sayed, A. SDS-PAGE technique as biomarker for fish toxicological studies. *Toxicol. Rep.* **2018**, *5*, 905–909. [CrossRef] [PubMed]
24. Sheikh, Z.A.; Ahmed, I. Impact of environmental changes on plasma biochemistry and hematological parameters of Himalayan snow trout, *Schizothorax plagiostomus*. *Comp. Clin. Path* **2019**, *28*, 793–804. [CrossRef]
25. Ferri, J. Fishery–Biological and Ecological Characteristics of the Black Scorpionfish, *Scorpaena porcus* (Linnaeus, 1758) in the Eastern Adriatic Sea. Ph.D. Thesis, University of Split, Split, Croatia, 21 April 2011.
26. Kavadias, S.; Castritsi-Catharios, J.; Dessypris, A.; Miliou, H. Seasonal variation in steroid hormones and blood parameters in cage-farmed European sea bass (*Dicentrarchus labrax* L.). *J. Appl. Ichthyol.* **2004**, *20*, 58–63. [CrossRef]

27. Valls, E.; Navarro, J.; Barria, C.; Coll, M.; Fernandez-Borrais, J.; Rotllant, G. Seasonal, ontogenetic and sexual changes in lipid metabolism of the small-spotted catshark (*Scyliorhinus canicula*) in deep-sea free-living conditions. *J. Exp. Mar. Biol. Ecol.* **2016**, *483*, 59–63. [CrossRef]
28. Ihuț, A.; Răducu, C.; Cocan, D.; Munteanu, C.; Luca, I.D.; Uiuiu, P.; Lațiu, C.; Rus, V.; Mireşan, V. Seasonal variation of blood biomarkers in huchen, *Hucho hucho* (Actinopterygii: Salmoniformes: Salmonidae) reared in captivity. *Acta Ichthyol. Piscat.* **2020**, *50*, 381–390. [CrossRef]
29. Coz-Rakovac, R.; Smuc, T.; Topic Popovic, N.; Strunjak-Perovic, I.; Hacmanjek, M.; Jadan, M. Novel methods for assessing fish blood biochemical data. *J. Appl. Ichthyol.* **2008**, *24*, 77–80. [CrossRef]
30. Coz-Rakovac, R.; Strunjak-Perovic, I.; Topic Popovic, N.; Hacmanjek, M.; Smuc, T.; Jadan, M.; Lipej, Z.; Homen, Z. Cage culture effects on mullets (Mugilidae) liver histology and blood chemistry profile. *J. Fish Biol.* **2008**, *72*, 2557–2569. [CrossRef]
31. Coz-Rakovac, R.; Strunjak-Perovic, I.; Hacmanjek, M.; Popovic, N.T.; Lipej, Z.; Sostaric, B. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic Sea. *Vet. Res. Commun.* **2005**, *29*, 677–687. [CrossRef]
32. Hoar, W.S.; Randall, D.J.; Farrell, A.P. *The Cardiovascular System Part A, Fish Physiology Series Vol. XII*; Academic Press: San Diego, CA, USA, 1992; p. 340.
33. Farell, A.P.; Jones, D.R. The cardiovascular system. In *Fish Physiology, Part B*; Hoar, W.S., Randall, D.J., Farell, A.P., Eds.; Academic Press: San Diego, CA, USA, 1992; pp. 1–73.
34. Wagner, T.; Congleton, J.L. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* **2004**, *61*, 1066–1074. [CrossRef]
35. Cataldi, E.; Di Marco, P.; Mandich, A.; Cataudella, S. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): Effects of temperature and stress. *Comp. Biochem. Physiol. A* **1998**, *121*, 351–354. [CrossRef]
36. Percin, F.; Konyalioglu, S. Serum biochemical profiles of captive and wild northern bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean. *Aquac. Res.* **2008**, *39*, 945–953. [CrossRef]
37. Manna, S.K.; Das, N.; Bera, A.K.; Baitha, R.; Maity, S.; Debnath, D.; Panikkar, P.; Nag, S.K.; Sarkar, S.D.; Das, B.K.; et al. Reference haematology and blood biochemistry profiles of striped catfish (*Pangasianodon hypophthalmus*) in summer and winter seasons. *Aqua. Rep.* **2021**, *21*, 100836. [CrossRef]
38. Luskova, V. Annual cycles and normal values of hematological parameters in fishes. *Acta Sci. Nat. Brno* **2007**, *31*, 70–78.