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Stress related blood values in *Scyliorhinus canicula* as live-indicators of physiological status after bottom trawling capture activity

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ABSTRACT

The quantification of capture-related physiological stress is an important factor when assessing the potential for post-release survival in sharks that are incidentally captured. In the absence of these biological data and when the post-release fate is unknown, effective management plans cannot be formulated and may lead to highly susceptible shark populations being overfished. Here, we measured the levels of lactate, glucose, alanine amino transferase (ALT), aspartate amino transferase (AST), Ca^{2+}, Na^{+} K^{+},Cl^{-} Mg^{2+} and Pi in the plasma of mature and immature lesser spotted dogfish (*Scyliorhinus canicula*, herein dogfish) which were incidentally captured at two depths (shallow: 50-200 m, and deep: 201-500 m) by bottom trawl off the coast of southern Sicily. These values were used as biomarkers and physiological indicators of the secondary stress response associated with capture.

This study found that dogfish captured in deeper waters (below 200 m) had elevated levels of glucose, Na^{+}, Ca^{2+} and K^{+} compared to those inhabiting depths less than <200 m. We hypothesize that the elevated levels of physiological stress in dogfish captured at greater depths may be related to the prolonged duration of the interactions with the fishing gear in the area off southern Sicily. Our findings provide new data on the capture-related stress in dogfish and increase the understanding of the potential for post-release survival in sharks captured at two depths by bottom trawl, information that is important for improving the general management plans for the fishery. However, our PC Analysis results revealed that Maturity have a positive contribution from the sample weight, sample length, ALT, AST and a negative contribution from Pi.

1. Introduction

The lesser spotted dogfish (*Scyliorhinus canicula*, Linnaeus, 1758), the smallest representative of the genus *Scyliorhinus* (Compagno et al., 2005; Ebert et al., 2013; Weigmann, 2016) is widely distributed across the Atlantic Ocean ((Compagno, 1984; Henderson and Casey, 2001)and Mediterranean Sea (Capapé et al., 2000). Dogfish are eurybathic, as they are vertically distributed from the surface layer to approximately 400 m deep in the Atlantic Ocean (Compagno, 1984) and down to 800 m in the eastern Mediterranean Sea (Mytilineou et al., 2005).

Although lesser spotted dogfish are listed by the International Union for Conservation of Nature (IUCN) as a species of Least Concern at the global level (Ellis et al., 2018), recent reports indicate that there are declines in local populations in areas of the Wadden Sea and off Malta (Wolff, 2000). For this reason, it is becoming increasingly important to better understand the impact that both commercial landings and discards may have on local populations. The low commercial value of dogfish compared to other demersal species it in the Mediterranean Sea (Ragonese et al., 2013) commonly results in only specimens larger than 36 cm total body length (TL) being retained for human consumption, while all specimens <36 cm TL are discarded back to the sea (Abella and Serena, 2005) because it is estimated that *Scyliorhinus caniculas* could reach the first maturity after that body size (Bendiab et al., 2012). Many individuals that are discarded or incidentally captured (i.e., bycatch) are either already dead, experience post-release mortality, or suffer detrimental long-term sub-lethal effects as reported by (Falco et al., 2022).

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Dogfish are commonly captured with bottom trawl fishing gear off the coast of Sicily (Falco et al., 2022; Peristeraki et al., 2020; Ragonese et al., 2013). This fishing gear can subject the captured dogfish to cumulative physical trauma and physiological stress through: a) prolonged exposure (e.g., in excess of 60 mins) to compression from all the biomass accumulated in the cod end of the net with additional potential exposure to dangerous items (such as solid litter including metals); b) repeated attempts to escape through the mesh of the cod end of the net; c) marked changes to ambient temperatures, salinity and light intensity as the gear ascends from the deep waters to the surface; d) exposure on the deck of the boat to air and direct sunlight for extended periods of time; e) handling operations by the crew; f) exposure to different ambient conditions following discard back to the sea; and, finally, g) release into a new location that may not be as favorable to the dogfish as the original location of capture. Such varied and severe experiences during the trawling event can lead to deleterious consequences to the condition of the sharks when compared to the moment of capture (Skomal, 2007).

The life history of sharks is generally characterized by slow growth, late age of maturity, a long lifespan and low fecundity (Cortés, 2000). Because these life history characteristics appear to be more extreme in deep-sea sharks (e.g., *S. canicula)* (Simpfendorfer and Kyne, 2009*)* a better understanding of the physiology of these sharks species is essential to determine their overall fitness (Ricklefs and Wikelski, 2002; Wikelski and Cooke, 2006). This information can be used in the development of species-specific management and conservation plans that minimize the impact of bycatch by commercial fisheries (Wikelski and Cooke, 2006; Young et al., 2006).

The current study quantified how capture by bottom trawl affected the physiological stress levels in dogfish using the Fisheries Environmental and Physiological Stress Analysis (FEPSA) approach (Falco et al., 2022). The FEPSA approach suggests the use of specific physiological biomarkers to estimate the health status of exploited species and infer collateral fishing mortality after specimens are caught and discarded. This information can be used to improve fishing-related interactions and reduce overall fishing mortality, including direct (i.e., targeted capture) and indirect (i.e., non-targeted capture) mortality.

The stress response in fish is a disruption to homeostasis by intrinsic or extrinsic stimuli, which can elicit a behavioral or physiological compensatory mechanisms (Abdel-Tawwab et al., 2019; Burgos Aceves et al., 2019; Merola et al., 2022; Sehonova et al., 2018; Yalsuyi et al., 2021). Previous studies on sharks reveal that they respond differently to physiological stress than other species. Thus, it can be hypothesized that their reaction may be species-specific (Beerkircher et al., 2002; Gallagher et al., 2014; Hyatt et al., 2012; Jerome et al., 2018; Mandelman and Skomal, 2009; Marshall et al., 2012; Morgan and Burgess, 2007) and likely influenced by many factors including the method of capture, duration of struggle, respiratory mode and metabolic scope (Skomal and Mandelman, 2012). These responses are often measured through physiological changes in blood chemistry (e.g., changes in glucose, lactate, and acid–base status; (Skomal and Bernal, 2010) and electrolytes (Piiper et al., 1972) and have been used to determine the relative condition of the fish following a capture-event (Cliff and Thurman, 1984; Faggio et al., 2014; Fazio et al., 2013; Harrenstien et al., 2005; Wells et al., 1986). Additional work has also shown that a capture-related stress may result in a marked increase in the level of plasma enzymes (e.g., alanine aminotransferase, ALT and aspartate transaminase, AST) suggesting tissue-specific damage (Butcher et al., 2011; Morrissey et al., 2005; Rapp et al., 2012; Wells et al., 1986).

Interpreting the relative degree of physiological stress among sharks is challenging because the varied levels of stress arising from diverse types of capture, the difficulty in handling and processing specimens, and the inherent problems with establishing a realistic baseline (control) value for unstressed individuals. While a novel manner to collect samples underwater may help in the determination of unstressed physiological levels in sharks, the reality remains that most studies rely on the collection of blood-based parameters from sharks that have been captured and brought alongside the boat, some of which may even be considered to be moribund (Hight et al., 2001; Hutchinson et al., 2015; Moyes et al., 2006; Wosnick et al., 2017). In addition, there is a lack of understanding of how regional (i.e, oceanographic) conditions may affect the capture related stress responses in sharks, suggesting which advocates that caution should be taken to the overgeneralization of data for sharks that are often studied in distant regions and with markedly different gear types. For these reasons, establishing the regional-specific baseline values of physiological stress in vulnerable fish stocks can be used to inform fisheries managers on how to improve management guidelines and delineate new regulations. Recent declines in the population assessments of lesser spotted dogfish off the coast of Sicily warrant a closer look at the physiological stress response in sharks incidentally captured by bottom trawl and the potential viability of post-release survival as a management tool. The physiological stress repose in the blood of immature and mature dogfish captured by bottom trawl at two depths was assessed (below than 200 m and above than 200 m) in an important commercial fishing area off the coast of southern Sicily. This work can serve as an example, and the values of the main biomarkers applied to the *S. canicula*, could be used to be compare with the same species taken elsewhere.

2. Materials and methods

2.1. Study area and on board animal sampling

Dogfish were captured in the late spring, early summer of 2019 off southern Sicily (GSA 16) during the MEDITS survey programme provided by international bottom trawl surveys in the Mediterranen (https://www.sibm.it/SITO%20MEDITS/principaleprogramme.htm; (Spedicato et al., 2019) (Fig. 1). Bottom trawl hauls followed a depth stratified random design based on five strata: three on the outer shelf (51–200 m depth, 30 min haul time) and two on the upper slope (201–500 m depth, 60 min haul time) (see in Table 1 supplementary data). In an attempt to limit the chances of sharks having been recently captured specimens were collected from 12 hauls inside the area subjected to moderate commercial fishing activity. Hauls were completed using a commercial stern trawler (Pegaso S.B.- UE 7826) and an ad hoc designed trawl net (IFREMER reference GOC 73) with 20 mm mesh size (diamond stretched) at the cod-end. We had the opportunity to investigate any variation within the same specie were been influenced capture depth relationship with habitat, body size, maturation stage, sex, in order to better understand fisheries conservation and management strategies.

2.2. On board blood collection

Once the gear was hauled back and the catch was spilled on the deck, up to 10 dogfish were randomly selected and placed in an on-board tank (500 l,0.5m^3) with a black surface to minimize light penetration (Barkley et al., 2017). The water in the tank was recirculated using Jiasj electromagnetic air pump (30 L/min) for at least 30 min to guarantee an dissolved saturation. To determinate if the sharks were alive, fish were periodically agitated by stroking their tails or splashing the water in the tank. Only dogfish showing clear reactions to stimuli were immersed in a second water tank of 50 l for 10 min containing an anesthetic solution of 50 mg/l Eugenol (Álvarez-Perdomo et al., 2016). Blood was quickly collected from anesthetized specimens via the caudal vein using a 21gauge needle and a heparinized syringe to avoid clotting, and samples was then stored in 1 ml vials at (0–1 °C) inside in a container filled with ice (Lawrence et al., 2018). The samples were processed within half hours.

Plasma was separated from other cells by centrifugation at 10,000 (rpm) for 3 min and plasma samples were frozen in liquid nitrogen and transferred to the Department of Biology (University of Palermo, Italy), where they were stored at −80 °C until further analysis. Whole specimens were then frozen at −40 °C and transferred to the laboratory to

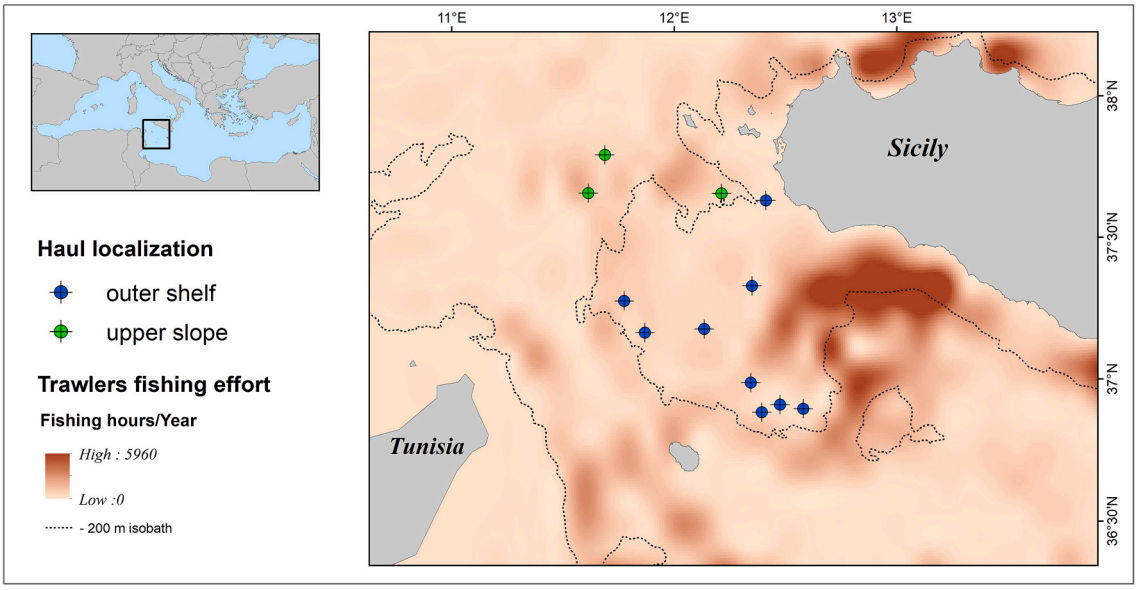

Fig. 1. Map showing the spatial distribution of hauls from which *Scyliorhinus canicula* (dogfish) were sampled and the fishing effort (hours) of commercial trawlers operating in the Mediterranean Sea during 2016. Outer shelf and upper slope refer to 50–200 and 201–500 m, respectively. Fishing effort data were downloaded from Global Fishing Watch (2021; https://globalfishingwatch.org/) at 100th degree resolution. Data were interpolated using a two-dimensional minimum curvature spline technique. Red colour gradient shows the intensity of fishing effort. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mean, morphometrics for dogfish (*Scyliorhinus canicula)* captured by bottom trawl.

			Total Length (cm)			Total Body Weight (g)		
Depth Level (m)	Sex	N.of specimens	Mean	Min	Max	Mean	Min	Max
	female (f)	20	40.6	30.5	55	206	75	330
Outer Shelf (50-200 m)	male (m)	23	41.9	24.5	48	244	40	350
	(f + m)	43	41	24.5	55	225	40	350
	female (f)	6	37.3	33	40	178.3	105	240
Upper Slope (201-500 m)	male (m)	16	41.8	36.5	47.5	219.1	145	300
	(f + m)	22	39.6	33	4758	198.7	105	300

collect data on morphometric and gonadal maturity.

Animals were kept and handled following the guidelines for experimental procedures in animal research of the Ethics and Animal Welfare Committee of European Union (2010/UE legislation).

2.3. Evaluation of morphometry and sexual maturity

Whole dogfish specimens were thawed overnight at an ambient temperature (21 °C) and morphometric (i.e., total length, TL, total body weight, TBW) and the stage of sexual maturity were determined. The stage of sexual maturity was macroscopically identified after dissection and separated into seven stages (0–VI) based on a universal scale for oviparous elasmobranchs (MEDITS-Handbook group, 2017, see Table 4 in (Follesa and Carbonara, 2019). Each stage was determined based on the size and condition of testes and claspers in males, and the size and condition and coloration of the ovaries, oocytes, oviduct glands, oviduct features (including the presence of egg cases) and uterus in females. Finally, juvenile/adult states were assigned based on stage 3a (mature) and stage 3b (mature/extruding active, i.e., active egg release) as discriminant stages both males and females. In this study immature and mature specimens were considered as those classified as stage 1_2 and 3_6, respectively.

2.4. Analysis of electrolytes, metabolites and enzymes in plasma

Plasma samples were defrosted at ambient temperature (about 20 °C for ten minutes) and immediately processed for automated spectrophotometry using an automatic analyser, Konelab 60 i (Thermo Electron Corporation) to determine the concentrations of alanine aminetransferase (ALT), aspartate aminotransferase (AST), glucose, lactate, inorganic phosphorus (Pi), calcium (Ca^{2+}), and magnesium (Mg^{2+}). An ISE (Ion-Selective Electrodes) module was used to measure chloride (Cl^{-}), potassium (K^{+}), and sodium (Na^{+}) using a potentiometric method.

Any values for lactate that fell below instrument measurement range (0.5 mmol/L^{-1}) and were not included.

2.5. Statistics

Our primary scope was to determine if there was a significant relationship between the level of plasma-based values and sexual maturity, sex, and depth using a three-way ANOVA analysis with interactions. Because the statistical analysis of all individual variables could be impaired by collinearity and the analysis could be flawed by a high family wise error rate due to the high number of variables (Hastie et al., 2009), we reduced the number of variables using a Principal Component Analysis (PCA) with (Jolliffe, 2002). The ANOVA analysis was then applied to the most representative PC's. For the ANOVA analysis we preferred the type II ANOVA because in our case we have an unbalanced design and because Type II is suitable for models with fixed cross factors (Doncaster and Davey, 2007). All the statistical analyses were carried out using the free statistical software R ver. 4.1.2 (R Core Team (2021).

Any p value <0.05 was considered statistically significant.

3. Results

Sixty- five dogfish specimens (43 from the outer shelf and 22 from the upper slope) were captured by 12 bottom trawl sets (Table 1) and blood plasma was used to determine the levels of lactate, glucose, AST, ALT, Ca^{2+}, Mg^{2+}, Pi, Na^{+}, Cl^{-}, and K^{+}. The concentrations of glucose ($p < 0.004$), Ca^{2+} ($p < 0.0024$), and Na^{+} ($p < 0.001$) were significantly different between specimens (where date was combined for both sexes). Conversaly, it was found that there was no significant change in Mg^{2+}, Pi, Cl^{-}, and K^{+} levels between the two depth groups ($p > 0.05$).

According to boxplot, Fig. 3, glucose levels were higher in male dogfish collected below 200 m than they were above 200 m ($P < 0.05$), when taking into account male and female (combined for both mature and immature) for each stratum. Specifically, glucose male mean values on deeper were (meanSD; number of specimens, 2,59 ± 0.47 mmol/l; $n = 16$); whereas glucose male mean values on shallower were (mean SD; number of specimens, 1,29 ± 1.01 mmol/l; $n = 23$).

Additionally, males caught at two different depths (below 200 m and above 200 m) also differed with Ca^{2+} and Na^{+} mean values ($P < 0.05$); male mean calcium values were (mean ± SD) and number of species, male (15.18 ± 2.13 mg/l, n = 16) and (mean ± SD and number of specimens, male: (12.29 ± 3.02 mg/l, n = 23) in deeper and shallower respectively, while male Na^{+} concentrations were (mean ± SD and number of species 'male: 288.9 ± 16.4 mmol/ (n = 16) (mean ± SD and number of specimen's male:267.59 ± 17.38 mmol/l, n = 23) and in deeper and shallower respectively.

When female (mature and immature combined) dogfish were compared between two strata, significant differences were also seen with Ca^{2+} and Na^{+}($P < 0.05$); female Calcium mean values were (mean ± SD and number of specimen's was (15.15 ± 2.86 mg/dl, n = 16) and (mean ± SD and number of specimen'(13.68 ± 1.58 mg/dl, n = 23) deeper and shallower respectively; while female sodium mean values were (mean ± SD and number of specimens, female, (290.3 ± 28.0 mmol/l n = 16) and (mean ± SD 272 ± 19.10 mmol/ ($n = 22$)deeper and shallower, respectively.

In each layer (below 200 m and above 200 m), there were no differences between the ALT and AST mean values between the sexes. However, there are some noticeable distinctions between mature and immature groups (where data was combined for both sexes), in each individual stratum or layer. In fact, the mean concentration of ALT and AST was greater in mature than immature (data were combined for both male and female) in the deeper layer ($p < 0.05$), although there was no discernible difference in ALT and AST values between mature and immature (sex combined) ($p > 0.05$) in the shallower layer, also confirmed by PC4 Analysis explain below. Table 2 summarises the information.

Considering the results of the PCA (see Supplementary Table 2 and 3), only the first five PC's, (PC1, PC2, PC3, PC4, PC5) were considered for the ANOVA analysis according to the Kaiser's rule (i.e. variance >1) (Jolliffe, 2002). The five PC's explained 28%, 18%, 14%, 11% and 8% of the overall variance, respectively, for a total of 79%. Accordingly, the ANOVA analysis was applied only to these five PC's.

The three-way ANOVA carried out for each PC's showed that there were only main effects on two of the five PC's. On PC1 there was a significant effect of the Double_layering and on PC4 there was an effect of Maturity.

Table 3 shows the loadings coming from PCA analysis, which represent in absolute value the contribution of the original variables on principal components. Positive/negative loadings indicate that a variable and a principal component are positively/negatively correlated. PC1 is mainly composed by variable related to the haul (deck temperature, haul time, bottom temperature and haul depth), which are clearly influenced by the Double_layering, i.e. depth, with a decreasing contribution from the plasma electrolytes and, with even smaller influence, from biomarkers. On the other hand, PC4 is mainly determined by the sample weight, sample length, P, GOT_AST, GTP_ALT, Mg and so on, see Table 3.

As can be seen in Fig. 4A, PC1 clearly separate the data between the outer_shelf and upper_slope with the values from outer_shelf mainly (89%) on the negative side of the PC1 axis, while the data from upper_slope are completely (100%) on the positive side of the PC1 axis. The length and the direction of the arrows represent the loadings of the original variables. For example, the bottom temperature (bot_temp) contribute negatively on PC1, i.e., when the bottom temperature was high, the PC1 values are shifted to the negative values of the PC1 axis. This is true also for the variable Pi. This means that data from outer_shelf have high values of bottom temperature and Pi. On the other hand, PC1 values on the positive side have high values of haul depth, deck temperature, K^{+}, Na^{+} and so on. In this figure there is no distinction of the two depths (outer_shelf and upper_slopre) with respect to PC4, the y axis, as the ANOVA analysis indicates that only the PC1 was affected by the Double_layering.

In Fig. 4B, the effect of Maturity on PC4 is shown. In this case, as indicated by the ANOVA analysis, we did not observe any effect of the factor Maturity on PC1. In this graph, the data for the Mature samples are mainly (84%) on the positive side of the PC4 axis, while the data for the Immature samples on the negative side (95%). As indicated by the arrows, data on the positive side (Mature) have a positive contribution from the sample weight, sample length, ALT, AST and a negative contribution from Pi, i.e. the Mature samples have lower values of P with

Table 2
Physiological parameters in the blood plasma of dogfish (*Scyliorhinus canicula*) captured by bottom trawl in waters deeper than 200 m (upper slope) and shallower than 200 m (outer Shelf), Glucose, Alanine amino transferase (ALT), Aspartate amino transferase (AST), as well as Potassium (K) Sodium (Na), inorganc Phosphorus (Pi), Chloride (Cl), and Magnesium (Mg) electrolyte concentrations in *S. canicula,* were express as, mean values (MV) ± Standard Deviation(SD), at various stages of development. In the first row, the number of specimens under research for each layer have been recorded as number (N). Lactate values below the instrument measurement range (0.5 mmol/L-1) were not included.

	Outer Shelf (50-200 m)				Upper Slope (201-500 m)			
	Female		Male		Female		Male	
N. valid specimens and maturity stage	N.8 Mature	N.12 Immature	N.16 mature	N.7 immature	N.4 mature	N.2 immature	N.13 mature	N.3 immature
	MV ± SD	MV ± SD	MV ± SD	MV ± SD	MV ± SD	MV ± SD	MV ± SD	MV ± SD
Glucose (mmol/l)	1.47 ± 1.64	2.38 ± 0.643	1.25 ± 1.069	1.43 ± 1.066	1.83 ± 1.30	2.78 ± 0.53	2.60 ± 0.49	2.35 ± 0.25
ALT (U/L)	9.88 ± 5.74	5.25 ± 3.28	10.10 ± 10.10	3.86 ± 4.78	7.00 ± 4.97	6.50 ± 0.50	5.85 ± 6.48	5.33 ± 3.2
AST(U/L)	8.88 ± 5.91	5.25 ± 3.22	10.80 ± 10.30	5.00 ± 5.63	6.50 ± 0.71	7.50 ± 0.50	7.08 ± 5.74	5.67 ± 3.06
Ca^{2+} (mg/dl)	13.8 ± 2.0	13.6 ± 1.3	12.0 ± 3.5	9.4 ± 6.3	14.5 ± 1.8	16.5 ± 3.65	15.3 ± 2.1	14.6 ± 2.8
K^{+}(mmol/L)	5.32 ± 0.56	4.64 ± 1.67	5.24 ± 0.79	5.81 ± 2.64	5.45 ± 0.67	5.25 ± 0.25	5.36 ± 1.68	5.40 ± 0.62
Na^{+} (mmol/L)	267 ± 22	253 ± 81	266 ± 19	234 ± 104	292 ± 32	286 ± 19	290 ± 18	285 ± 11
Inorganic phosphate (Pi) (mg/dl)	8.59 ± 1.39	10.20 ± 1.17	7.82 ± 3.42	7.53 ± 4.02	7.45 ± 0.64	8.15 ± 0.45	6.26 ± 3.44	8.20 ± 2.08
Cl^{-}(mmol/L)	222 ± 12	202 ± 65	228 ± 26	185 ± 82	236 ± 18	228 ± 8.25	189 ± 86	226 ± 9
Mg^{2+} (mEq/l)	2.98 ± 0.61	3.47 ± 0.55	2.82 ± 1.34	2.36 ± 1.50	2.62 ± 0.90	2.95 ± 0.25	3.20 ± 1.22	3.37 ± 0.57

Table 3
Loadings of the original variables for the selected five PC's. In PC 1 and PC4, the values that have a stronger correlation with the component values from the PCA analysis have been reported in bold.

PC1		PC2		PC3		PC4		PC5	
deck_temp	**0.44**	ALT	−0.45	haul_weight	0.47	**weight**	**0.57**	Cl^-	0.71
haul_time	**0.43**	AST	−0.44	Ca^{2+}	0.38	**LT**	**0.55**	Pi	−0.4
bottom_temp	**−0.43**	Na^+	0.37	K^{2+}	0.31	**Pi**	**−0.31**	Glucose	−0.3
Haul_depth	**0.42**	glucose	0.36	Pi	0.30	**AST**	**0.30**	K^+	0.23
K^+	0.27	Ca^{2+}	0.31	glucose	0.30	**AST**	**0.30**	haul_depth	−0.21
Na^+	0.21	Mg^{2+}	−0.27	Na^+	0.26	Mg^{2+}	−0.24	AST	−0.18
Pi	−0.19	LT	0.25	GOT_AST	0.24	bot_temp	−0.1	ALT	−0.18
Cl^-	0.17	K^+	−0.24	Cl^-	0.22	haul_depth	0.09	haul_time	−0.18
Ca^{2+}	0.15	weight	0.14	ALT	0.21	haul_time	0.09	Na^+	0.11
Mg^{2+}	0.14	deck_temp	−0.1	haul_depth	−0.2	Cl^-	−0.07	deck_temp	−0.1
weight	−0.14	haul_weight	−0.08	haul_time	−0.18	haul_weight	−0.07	Ca^{2+}	−0.09
LT	−0.1	bot_temp	0.07	bot_temp	0.16	deck_temp	0.07	LT	0.08
glucose	0.1	haul_depth	0.07	LT	0.16	Na	−0.06	Weight	0.08
haul_weight	0.08	haul_time	0.07	Mg^{2+}	0.11	glucose	−0.01	bot_temp	0.08
ALT	−0.03	Cl^-	−0.03	weight	0.03	Ca^{2+}	0.01	haul_weight	−0.08
AST	−0.004	Pi	0.004	deck_temp	0.02	K^+	0.001	Mg^{2+}	0.07

respect to the Immature, which are located on the negative side of the PC4 axis. This group, the Immature, have instead higher values Mg^{2+}, Cl^-, Na^+ and were taken from a higher bottom temperature common in shallower sea.

However, our finding had not evidenced any differences between mature and immature male, and mature and immature female for each stratum.

4. Discussion

Physiological biomarkers on dogfish (*Scyliorinus canicula)* that were captured using a bottom trawl were examined in this study. In addition, various context-sensitive elements were considered, such as how the connection varies with respect to sex, maturity, and body size (Cooke et al., 2013). Our findings show that the stress levels in the blood of dogfish captured by bottom trawls were elevated in both mature sharks and those captured at greater depths. Our funding showed that for sharks captured in deeper waters (below 200 m), glucose, Na^+, Ca^{2+} and K^+ values were higher than dogfish captured at shallower depths (above 200 m).

In the present study glucose concentration was greater in dogfish caught during experimental commercial bottom trawl in deeper water, with p value <0.05. According to our findings, Dogfish sharks (captured in deeper sea water below 200 m) may have experienced more physiological stress. To confirm our findings vave been compared with other shark and teleost fish species.

Recent investigations on longline-caught deep-sea sharks (200-2000 m depth) published by (Prohaska et al., 2021), discovered that the shark *Mustelus Canis* showed the highest glucose values (4.38 mmol/l) especially, in the in the samples captured in the depth range of about 270 m. (Talwar et al., 2017) found that sharks caught in the deep sea (456–846 m) are more likely to suffer significant physiological disturbances than shallower species with blood glucose levels (3.39 mmol/l). Moreover, this latter authors, also found that shorter overall length (58 cm) has been linked to an increased risk of death (Talwar et al., 2017). (Cain et al., 2004), showed that mean blood glucose concentrations in i.e., southern stingray *Hypanus americana,*captured from the trawl fishing were 2.2 mmol/l. Moreover, the glucose levels in dogfish captured in depeer depths in our study also were similar to those of other demersal elasmobranchs species captured by trawl (e.i in modified demersal longlines (*Squalus cubensis* (Prohaska et al., 2021)).

Glucose differences between dogfish captured below 200 m and those captured at shallower depths, match with different hook times: dogfish caught above 200 m experienced about 15 min of capture stress compared to 35 min for those caught deeper, suggesting that time under duress is more important than the type of duress. This latter was corroborated by (Jerome et al., 2018) in their studies on elasmobranch, showing that glucose levels increased significantly with hook time. Similarly, the increased levels of glucose in dogfish captured by trawls at depths in excess of 200 m likely reflect the utilization of hepatic glycogen (i.e., glycogen converted to glucose) to fuel muscle metabolism during the capture-related struggle (Hoffmayer and Parsons, 2001). Moreover, we found that glucose levels were 36% higher in mature dogfish captured in deeper waters compared to dogfish captured in mature shallower water (Table 2). Similarly (Prohaska et al., 2018), found only parameter that indicated any significant difference in the stress response in *Pristis pectinate*, captured between shallow and deep with longline was blood glucose, which indicated greater stress in deep longline caught individual. This again suggests that dogfish experiencing a longer duration of stress (i.e. higher hook time) mobilized glucose to support the exhaustive exercise associated with capture (Bouyoucos et al., 2019). This last data also has been corroborated by higher levels of K^+. This finding mirrors (Moyes et al., 2006), report that increasing plasma level of K^+ may be associated with the trans-membrane exchange of glucose during bouts of exhaustive exercise, as reported by (Alonge, 2013).

Potassium values reported in present study were greater in dogfish caught in deeper water, with p value <0.05. This results may be match with extended period of hook time (up to 2 times longer), that these specimens were trapped when compared to the other captured at shallower depths.

In general, capture-related elevated levels of K^+ have been reported in the plasma of various shark specie; for example Mandelman and Farrington (Mandelman and Farrington, 2007), published the results of a research on *Squalus acantia* taken with an otter trawl net. Their *S. acantia* sample were captured between 50 and 65 m of depth, observing higher K^+ levels (mean value of 5 mmo/l). This might be a case of bottom trawl-captured dogfish, according to our data, because dogfish treated to different hook time had the most disrupted plasma chemistry across all statistically significant criteria. This result might be attributable to the harsh character of the trawl capture experience when compared to other elasmobranch capture methods. Indeed, elasmobranchs exposed to longline (Brooks et al., 2012) and gillnet capture showed a similar result (Manire et al., 2001). Previous work on elasmobranchs suggest that the levels of stress indicators in plasma are affected by the method and duration of capture, respiratory mode and metabolism (Dapp et al., 2016; Guida et al., 2016; Mohan et al., 2020; Skomal, 2007); Indeed, rubbing during fishing activity: e.g. trawling or purse seining (Marcalo et al., 2006; Tenningen, 2012) or passive netting or trapping (Roth and Rotabakk, 2012) and purse seine, (Folkins et al., 2021), and crowding may be becoming hypoxic. Bottom trawl-caught dogfish, were subjected to physical compression, water pressure, and

physical trauma due to the time to retrieve the gear during ascent process, which can restrict ventilation and consequently gas exchange, much as gillnets and longlines can ((Manire et al., 2001); (Prohaska et al., 2021) showed that small shark species (*Centrophorus uyato)*, caught with chevron trap had higher K^+ concentrations than those caught in longline. This perhaps due to the larger body size of *C. uyato* species which may have bumped against the edges of the trap more frequently than other smallest shark species caught alongside them.

Previous work by Cliff and Thurman (Cliff and Thurman, 1984) investigating the physiology consequences of stress in in *Carcharhinus obscurus* during hand net captures, reported that potassium plasma values above 7 mmol/ l could generate myocardial disruption. Although, the potential levels of K^+ that may impact cardiac function in sharks remain unknown (Schwieterman et al., 2021) the dogfish captured in deeper waters in our study had elevated levels of K^+ an may have experienced a larger osmotic disruption than those captured in shallower waters as reported by (Skomal and Mandelman, 2012) (Whitney et al., 2021).

We know that ALT and AST are two transaminases of amino acid metabolism in teleost fish livers and are important indicators of cell membrane integrity (Cheng et al., 2017). However, ALT and AST presence in elasmobranchs is difficult to interpret given the lack of information about the functional significance and tissue specificity of each enzyme (Anderson et al., 2010). Similarly, our study could not definitively link these enzymes with a particular elasmobranch stress due to capture. While we believed ALT and AST levels may be impacted by capture stress, our results showed otherwise; indeed, PC Analysis was complicated by the fact that a greater concentration of ALT and AST was highly correlated with maturity, as shown in Fig. 4b. Our results were similar findings of Asadi et al. (Asadi et al., 2006), in that mature females had higher AST and lower ALT activity than immature females; and mature males had lower ALT activity than immature males (Asadi et al., 2006). It seems likely that the activity of the ALT and AST enzymes is not only related to capture stress, but also to maturity ((Rudneva et al., 2014) showed that ALT and AST may be employed as indicators of ecological condition, reproductive life cycles, and developmental stage). Perhaps, changes in AST and ALT levels reflect a physiological condition related to differences in stress reactions based on maturity level; likely, because these levels vary throughout the spawning cycles (Shahsavani et al., 2010).

Finally, differences in AST and ALT activity seem not influenced by different capture times (hook time) or deep stratum between two groups, on the contrary the difference in AST and ALT level between shallower and deeper samples may be based on maturity. Fig. 2 show different distribution with mature and immature between two different deep level, and seems that more mature are in shallow waters. We still maintain that the differences in physiological biomarkers may also be related to the prolonged exhausting struggle, hypoxia, and thermal shock brought on by trawl net capture.

5. Conclusion

The current study presents preliminary data on the physiological status of the lesser spotted dogfish (*Scyliorhinus canicula*) following capture during bottom trawl fishing in the waters off southern Sicily. By evaluating the effects of capture, we found differences in physiological values were highlighted, permitting a better understanding of theinfluence of trawl fishing on this elasmobranch species in relation to the depth of capture, as well as differences with respect to gender and maturity. The principal stress to which dogfish were subject was the trawl net and the resulting physiological stress due to struggle activity, especially an extensive duration and intensity of exhaustive exercise and hypoxia. To date, few studies have been done on the plasma biochemical parameters of this shark species and even fewer studies have been done on dogfish species, in particular. Direct comparisons with other studies were difficult due to differences in the fishing method, exposure time, and blood parameter used. This study used the FEPSA approach, which involves the use of specific physiological biomarkers related to stressors and their effects on physiology. Accurate estimates of hidden fishing mortality induced after the capture and release of fish (including escape through the mesh of the cod end) to will help generate more effective (vice nominal) survival probabilities of stressed specimens. Further studies are needed to better describe the physiological responses of these animals and their relationship to the subclass elasmobranch as a whole.

Ethical statement

Experiments on animals were performed in accordance with the guidelines and regulations set forth by committee of Animal care and Use EU Directive 2010/63/ EU for animal experiment http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

This work was carried out in the framework of the MEDITS project, all procedures were carried out with the approval by Ministry of Agricultural, Food and Forestry Policies and the European Commission,

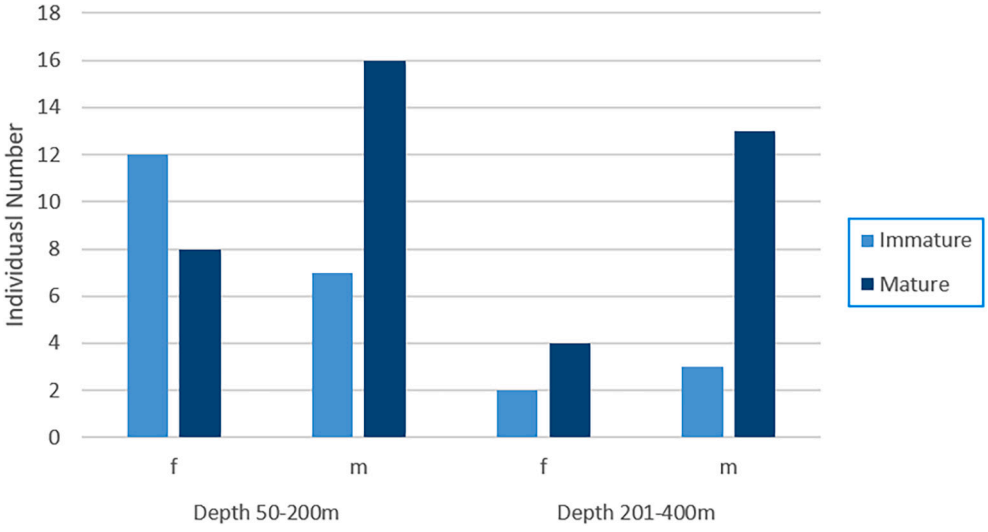

Fig. 2. Number of dogfish captured (female [f]and male[m]) mature and immature between two depths (outer shelf and upper slope); in blue and gray are reported both mature and immature female dogfish respectively; while in orange and in yellow are reported both mature and immature male dogfish; (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

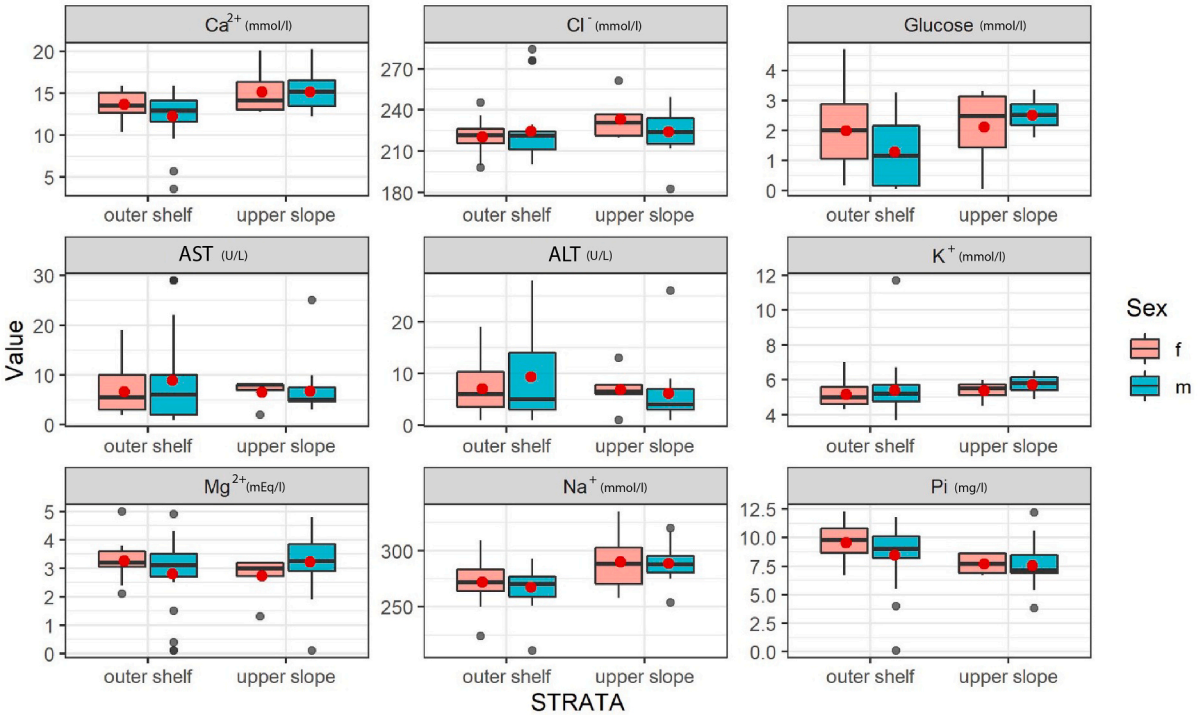

Fig. 3. Box and whisker plots for the plasma levels of Calcium (Ca^{2+}, mg/dl), Chlorum (Cl^{-}, mmol/L), Glucose (mmol/L), Aspartate aminotransaminase (AST, U/L), Aalanine aminotransferase (ALT, U/L), Potassium (K, mmol/L), Magnesium (Mg, mEq/l), Sodium (Na, mmol/L) and inorganic phosphate (Pi, mg/dl) in dogfish (*Scyliorhinus canicula*) specimens per sex (female [f], male [m]) (with data combined of mature and immature) and strata (outer shelf, upper slope). Upper and lower horizontal lines of the boxes represent the 25th and 75th percentiles, respectively; horizontal midlines represent the median; red dots represent the mean; upper and lower horizontal whiskers lines are the upper and lower limits; black dots are the outliers. In the text, statements about specific variables were discussed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

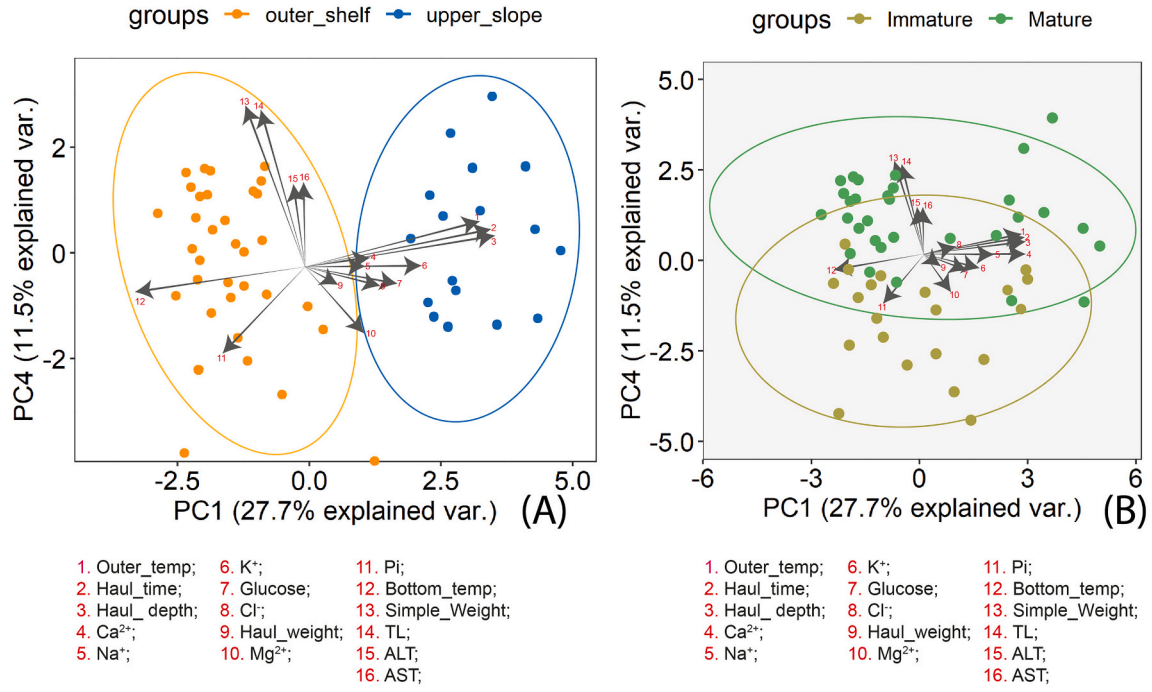

Fig. 4. Biplot showing PC1 and PC4 scores stratified for Double_layering) (A) and Maturity (B). The arrow direction and length represent the weight of the original variable, see legend.

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Data availability

All the relevant data related to this study are presented in tables and figures of the manuscript.

Additional data will be available on request to the corresponding author.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpb.2022.110802.

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