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Air Exposure in Catshark (*Scyliorhinus canicula*) Modify Muscle Texture Properties: A Pilot Study

Cristina Barragán-Méndez [1], Fini Sánchez-García [2], Ignacio Sobrino [3], Juan Miguel Mancera [1] and Ignacio Ruiz-Jarabo [1,*]

[1] Departament of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Av. República Saharaui s/n, E-11510 Puerto Real, Cádiz, Spain; cristina.barragan@uca.es (C.B.-M.); juanmiguel.mancera@uca.es (J.M.M.)

[2] Departament of Chemical Engineering and Food Technology, Faculty of Sciences, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Av. República Saharaui s/n, E-11510 Puerto Real, Cádiz, Spain; fini.sanchez@uca.es

[3] Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Cádiz, Puerto Pesquero, Muelle de Levante, s/n, P.O. Box 2609, E-11006 Cádiz, Spain; ignacio.sobrino@ieo.es

* Correspondence: ignacio.ruizjarabo@uca.es; Tel.: +34-615514755

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Abstract: Sharks are captured by tons for human consumption. Improving the quality of their meat will produce fillets that may have a higher economic value in the market, and thus be beneficial for the management of this fishery. In other animal species destined for human consumption, a negative relationship between pre-slaughtering stress and meat quality has been demonstrated. By studying the commercial small-spotted catshark (*Scyliorhinus canicula*), this work aimed at linking pre-slaughter handling of captured sharks and muscle fillets quality. An experimental group of adult and subadult living catsharks captured by hand and exposed to air (for 18 min, which is the minimum time this species is exposed to air in the fishing deck during fisheries procedures), and an undisturbed group, were evaluated. After air exposure, catsharks returned to water for recovery. Muscle lactate and water content were quantified after acute exposure (for 18 min), 5 h and 24 h. This challenge elicited stress responses in the muscle such as increased lactate levels and immediate dehydration, followed by recovery of lactate levels and overhydration. Muscle consistency, a relevant variable describing quality of seafood according to its ability to be swallowed by the consumer, paralleled muscle water content changes. The results indicated for the first time that handling alive sharks exposed to air results in muscle fillets with different texture properties. Whether these changes in muscle texture induce higher quality fillets has yet to be proven. Our recommendation is to minimize time of air exposure experienced by sharks when captured, including fast slaughtering instead of leaving them to die by asphyxia, as current on-board procedures.

Keywords: fisheries management; muscle texture; *Scyliorhinus canicula*; sharks; stress

1. Introduction

World consumption of elasmobranchs has increased during the last years, reaching 121,641 tons in 2011, which represents an increase of 42% by volume compared with 2000 [1]. According to the Food and Agriculture Organization of the United Nations, Spain is the world's third-largest producer of shark meat, and capture statistics show a strong upward trend in recent years [1].

The quality of the flesh in different species depends on the handling method of the fish before being slaughtered [2]. Improving the quality of seafood is a topical issue in the industry. Thus,

slaughter handling stress is important for the aquaculture industry because of its effects on fillet quality [3]. The degree of muscle texture degradation is related to the length of the pre-slaughter stress period in teleosts [4]. Moreover, stressful stunning methods also induce a higher deterioration rate in texture properties as seen in silver carp [4]. Fish flesh quality is a very complex concept that includes physico-chemical, biochemical, visual (color of the fillets) and microbiological attributes [5]. The use of texture analysis is widely extended in the food industry for the assessment of teleost fish fillet quality [3,6], as it provides more objective measurements [7]. Texture profile analysis test is based on the imitation of mastication or chewing process, and is employed for the evaluation of seafood, mostly teleost fish [8]. In this sense, texture consistency evidenced the water holding capacity of the fillet and reflects the difficulty of swallowing a piece of it [9]. However, despite the large volume of sharks captured for human consumption, little is known regarding their meat quality and texture properties. Stress in fish elicits physiological responses to cope with the environmental disturbance (presence of a predator, capture by a hook or a net, air exposure, etc.). These responses are broadly grouped as primary, secondary and tertiary [10]. Primary responses include the release of hormones to the blood, being cortisol for teleost fish and (presumably) 1α-hydroxycorticosterone in elasmobranchs [11–13]. Secondary responses are defined as the immediate actions and effects of these hormones [14], which basically make available to the cells an increased supply of oxygen and energy metabolites. These responses involve modifications in heart and breathing rates [15,16], changes in the dilation status of blood vessels [17], and rapid mobilization of glucose to meet energetic demands [18,19]. The onset of anaerobic glycolysis accumulates lactate in white muscle of teleosts [20]. As a side effect, dehydration processes occur in seawater elasmobranchs, enhancing plasma osmolality and sodium content after capture [21–23]. All these responses have consequences on the muscle texture properties of teleost fish [24,25], although no information is available regarding pre-handling stress and changes in muscle texture in elasmobranchs.

The small-spotted catshark (*Scyliorhinus canicula*) is a small demersal species inhabiting Atlantic waters from Norway to Senegal and the Mediterranean Sea, mostly captured by bottom trawl fisheries [26]. This and other catshark species are particularly appreciated in Europe [1]. In Spain, trawling time ranges from 1 h to 4 h [27], but this species may be exposed to air for 3 h before dying (personal observation). Thus, the objective of the present study was to use already established knowledge in sharks and teleost regarding stress reactions, and investigate if the relationship known from teleost, with flesh quality (based on muscle texture properties), also holds true in sharks. This information will be useful to examine the effects of exposure to air on the flesh quality (muscle texture) in the absence of the effects of prior fishing-related capture in the small-spotted catshark.

2. Results and Discussion

No mortality was observed in any group throughout the experimental period. All animals employed in this study (November 2016) evidenced signs of good health along the experimental time (from capture by a bottom-trawler vessel, to being euthanized at the end of the experiment), with normal behavior inside the tanks, and without external or internal evidence of disease. *P*-values resulting from two way-ANOVA of the different parameters assessed are presented in Table 1.

Table 1. *P*-values from two way-ANOVA from General Lineal Model (GLM) of parameters measured in muscle of *Scyliorhinus canicula* exposed to undisturbed control conditions or after an acute capture by hand processes followed by air exposure, and sampled at times 0, 5 and 24 h after the challenge.

Parameter	Group	Time	Group × Time
Lactate	<0.0005	<0.000001	<0.0001
Water content	<0.05	<0.000001	<0.00001
Consistency	0.7703	<0.0001	<0.0001
Cohesiveness	0.5715	0.6419	0.7598
Crispiness	0.7509	0.6896	0.6139
Firmness	0.6939	0.1156	0.8871
Work of penetration	0.6317	0.1452	<0.005
Springiness	0.1226	<0.0005	0.2542

2.1. Muscle Lactate

Scyliorhinus canicula when captured under normal bottom trawl fisheries conditions experienced periods of air exposure around 18 min or more [26,28]. Our study confirms that capture by hand followed by air exposure acutely stressed the animals as muscle lactate levels increased significantly (Figure 1), due to increased anaerobic energy metabolism [24,25]. In addition, our results are similar to those described for gummy shark (*Mustelus antarcticus*) captured by gill-net [29], with highest muscle lactate levels just after the stress situation induced by capture, decreasing to control values after 3–5 h.

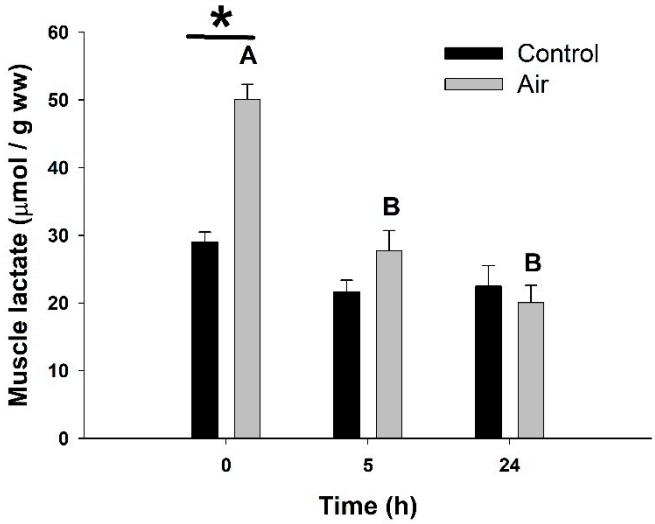

Figure 1. Muscle lactate levels (in µmol lactate per g of wet weight) in small-spotted catshark (*S. canicula*) controls (black bars) and after capture by hand and acute air exposure (gray bars). Data are expressed as mean ± standard error of the mean (SEM). No differences were described in the control group over time. Different letters indicate significantly different groups for the same treatment, while asterisks (*) indicate differences between control and air-exposure groups for the same time ($p < 0.05$, two-way ANOVA followed by a Tukey's post hoc test, $n = 7–8$).

2.2. Muscle Water Content

The percent of water in the muscle in this study ranged between 71.8% and 78.8% water (Figure 2A), in accordance with the water holding capacity (WHC) described for other shark species captured by longline (60–80% WHC) [30]. Figure 2A shows muscle dehydration after 18 min of capture by hand and air exposure (0 h). Muscle dehydration could be associated with a secondary stress response in fish maintained in seawater and it could be related to the described increase in plasma osmolality in *Solea senegalensis* after an acute air-exposure situation [31], or to the highest plasma

potassium concentration in Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks after gill-net capture [32]. This dehydration was described as a secondary stress response due to enhanced gill permeability, with an efflux of water to the hyperosmotic seawater environment. However, the apparent dehydration process in air-exposed catsharks 18 min after the challenge, sampled without being returned into the water, require further explanation. Thus, it was described that endurance exercise (similar to that performed by catsharks struggling for 18 min while exposed to air) induced a $\geq$2% body mass loss due to extracellular fluid volume contraction and, thus, muscle dehydration [33]. This body mass loss may be related to the reduction in muscle water content described in our study, with 74.5% and 73.6% muscle water content in the control and the stressed groups, respectively, at time 0 h after the stressful challenge. In this study, muscle water content enhanced in the experimental group after 5 h, suggesting the existence of a rebound effect due to the activation of the osmoregulatory system to recover the homeostatic balance after the stressful situation induced by air exposure. Several authors have studied the consequences of an acute stress on the osmoregulatory mechanisms in elasmobranchs and teleost fish showing a similar situation but different recovery times [31,34,35]. Our results showed that the small-spotted catshark was able to recover the muscle water content of the control-unstressed group within the first 24 h post-stress.

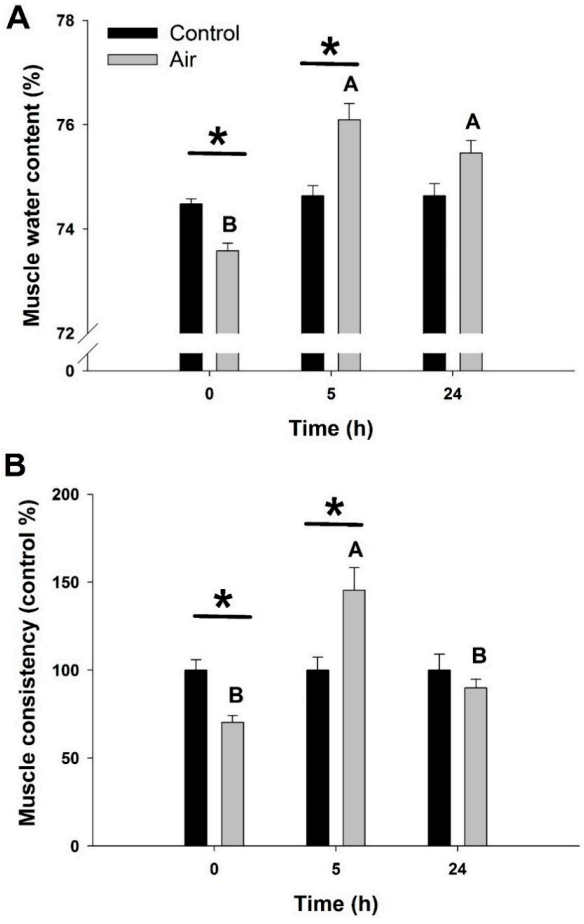

Figure 2. Muscle (**A**) water content (percent of total wet weight) and (**B**) consistency (as percent of variation compared to the control group at each sampling time) in small-spotted catshark (*S. canicula*) controls (black bars) and after capture by hand and acute air exposure (gray bars). Data are expressed as mean $\pm$ SEM. No differences were described in the control group over time. Different letters indicate significantly different groups for the same treatment, while asterisks (*) indicate differences between control and air-exposure groups for the same time ($p < 0.05$, two-way ANOVA followed by a Tukey's post hoc test, n = 7–8).

2.3. Muscle Texture

The most relevant result of the present study is that muscle consistency (Figure 2B), amongst all the texture-related parameters analyzed (Table 2), seems to be related to muscle water content. Fish muscle basically consists of connective tissues (including the extracellular aqueous matrix) and the intracellular contractile proteins. To determine consistency, the fillet has to be compressed producing a loss of intrafibrillar integrity, especially affecting actomyosin, which may influence the consistency/firmness values [36]. Muscle consistency roughly describes the capacity of the tissue to retain water after being compressed, and is useful to determine the swallowing properties of it [9]. It was described in salmon that muscle firmness was related not only to total collagen content but also to collagen stability [37]. As no differences were found in muscle firmness but in consistency in the present study, we can assume that both collagen content and stability were not affected by capture by hand and air exposure in *S. canicula*, being water content and the water holding capacity the most affected parameters during this study. Thus, major changes occurred in the extracellular matrix, while the structural integrity of the intracellular proteins is maintained after air exposure, as supported by the lack of differences in the analyzed texture parameters of this study (Table 2). The increased muscle consistency after 5 h recovery in this study may be related to an osmoregulatory rebound effect, but we were unable to find similar results in the literature. As far as we know, the shrinkage of shark fillets (due to denser myofibrillar and connective tissues) results in badly appreciated tough fillets [38]. Based on our results, if we considered that low muscle consistency is linked to dehydration and therefore tougher fillets, further studies should be conducted to elucidate if higher muscle consistency has better organoleptic properties. It should also be mentioned that freezing–thawing will itself affect muscle texture, but sometimes during commercial fisheries captured sharks are frozen aboard the vessel, and thus a possible bias of the results is assumed.

Table 2. Muscle texture profile analysis of small-spotted catshark (*S. canicula*) controls (Control) and after capture by hand and acute air exposure (Air). Data are expressed as mean ± SEM. The asterisk (*) indicate differences between control and air-exposure groups for the same time; no more differences have been described between groups or over time ($p < 0.05$, two-way ANOVA followed by a Tukey's post hoc test, $n = 7–8$).

Parameter	Group	0 h	5 h	24 h
Cohesiveness (N)	Control	−11.9 ± 2.6	−11.9 ± 4.0	−10.5 ± 1.1
	Air	−8.8 ± 2.0	−12.6 ± 1.6	−9.5 ± 2.7
Crispiness (no units)	Control	199 ± 3	195 ± 5	197 ± 4
	Air	197 ± 2	198 ± 5	193 ± 4
Firmness (N)	Control	244 ± 2	246 ± 1	246 ± 1
	Air	242 ± 1	247 ± 2	245 ± 2
Work of penetration (N mm)	Control	257 ± 7	228 ± 12	280 ± 15
	Air	234 ± 14	284 ± 14 *	260 ± 10
Springiness (mm)	Control	−38.4 ± 1.9	−36.2 ± 2.9	−28.1 ± 2.5
	Air	−40.4 ± 3.5	−29.0 ± 4.2	−20.9 ± 3.9

It was previously described in another shark species, *Mustelus lunulatus*, that postmortem changes such as loss of fluid and reduction of water holding capacity occurred in the muscle, but no changes in its texture were described [39]. The WHC has been reported as a good indicator for fish quality evaluation, being its reduction associated to tougher fillets and a loss of myofibrillar proteins [39]. Firmness of fish muscle is influenced by many factors [6,38], and the use of a texture profile analyzer seems to be useful when studying sensory conditions of raw fish fillets [40]. In this sense, further studies related to fillet firmness of elasmobranch fish including variables such as size, sex and fishing gear, amongst other variables, will be relevant to better describe their effects on the quality of the meat. Thus, Atlantic salmon fillets were firmer, paler, had a higher muscle pH and lower liquid loss in fish that

were not stressed during pre-slaughter handling [41]. Moreover, color variations in the meat serves as a quality standard for seafood products. Using this parameter, it was described that exhausted salmon [42] and stressed Bluefin tuna [2] showed changes in fillet color. Thus, it should be interesting to further analyze fillet color in future studies involving sharks employed for human consumption.

As this was designed as a pilot study, where we aimed at testing the effects of acute stress on the textural properties of the muscle using as few animals as possible, some flaws were evidenced. Future studies should be designed carefully and be focused in the individual stressful processes that sharks may experience when captured (such as muscle exhaustion after swimming, confinement, hypoxic conditions, blows, changes in the environmental conditions and, finally, air exposure alone), presumably including more animals per experimental group.

3. Material and Methods

3.1. Animal

Scyliorhinus canicula adults and subadults of both sexes (n = 26 males and n = 19 females, ranging from 169 to 512 g body wet weight and 41.5 to 57.0 cm total length) were obtained by bottom trawling in November 2016 in the Gulf of Cadiz (Spain). Healthy animals (with no external wounds, normal skin color and swim behavior in the onboard tanks employed to recover captured animals after trawling, active responses to external stimuli such as body movement when other catsharks touched them, and eyes clear and reactive) were transported immediately (transport process lasted around 8–12 h) to the fish husbandry facility of the Faculty of Marine and Environmental Sciences (Puerto Real, Cadiz, Spain) and acclimated to seawater (salinity 38 psu) until the beginning of the experiment. Fish were randomly divided into 6 bare-bottom, rectangular soft-edges tanks of 400 L (surface area of 0.72 m^2, 56 cm depth, and covered by a fine-mesh tissue to shade the aquarium) in a flow-through system under natural photoperiod (November; latitude 36°31′34″ N) and temperature (ambient temperature of approximately 19 °C, while temperature of the seawater when captured was 18.81–21.19 °C) and acclimated for 17 days. The number of males and females in each tank, though randomly distributed, included 4–5 males and 3–4 females per sampling group. Fish density per unit of surface was in accordance with previous studies conducted in *Squalus acanthias* and *Squalus suckleyi* [35,43]. Physical-chemical parameters were kept stable along the experimental time by means of the constant flow-through of well-seawater (constant 38 psu salinity, temperature 19.0 °C, >5 mg Dissolved Oxygen or DO L^{-1}, 0.1 ± 0.2 mg Total Ammonia Nitrogen or TAN L^{-1}, 0 ± 4 µg NH_3 L^{-1}, <0.1 mg nitrites L^{-1} and <4.1 mg nitrates L^{-1}). Salinity, temperature and dissolved oxygen levels were daily measured inside each tank. Temperature oscillated inside the tanks between 19.0 and 19.3 °C along the day. Oxygen levels were daily measured with an oximeter (Handy Polaris, OxyGuard, DK) and maintained above 5 mg L^{-1} (around 90% saturation) by an air diffusing stone inside each tank. Nitrogen compounds (TAN, NH_3, nitrites and nitrates) were analyzed once a week in the incoming well-water (which is also daily analyzed by the people in charge of the Fish Husbandry facility, without further differences along time) with commercial kits (Merck, Germany). Health of the animals was visually assessed, taking special interest in controlling that the body and eyes color, breathing rates and behavior of all sharks were normal. Fish were fed once a day at 20.00 UTC with unfrozen shrimps, prawns, sardines and anchovies to satiety. Red lights were employed for feeding in order to not disturb the animals during this process. As a curiosity, the animals remained inactive at the bottom of the tank most of the time but, after a few days, at the time of feeding began to actively look for the food offered, reaching to catch it directly from the hand of the caregiver with tranquility. This fact can be taken, with caution, as a sign that the animals were not specially stressed. Fecal matter was carefully removed each morning with a siphon under ambient darkened conditions. Animals were fasted 36 h before sampling to avoid osmoregulatory imbalances related to feeding, as has been described before in other catshark species [44]. All experimental procedures complied with the Guidelines of the European Union (2010/63/UE) and the Spanish legislation (RD 1201/2005

and law 32/2007) for the use of laboratory animals, and were approved by the local Committee of Ethics and Animal Experimentation.

3.2. Experimental Design and Sampling

Capture and air exposure are dramatic stressful events that catshark experience during fisheries procedures. Therefore, three tanks were selected as undisturbed controls, and catshark from the other three tanks were captured and exposed to the air. Animals were captured by hand and placed in similar dry tanks for 18 min, which is the minimum time this species experienced outside water during commercial bottom-trawl fishing conditions [26,28]. We considered unnecessary to fully expose the animals to the exact conditions after commercial fishing. Samples were collected at times 0 and 24 h after air exposure, consisting of 2–3 animals from each tank (in triplicate, n = 7–8 per group). The group sampled 0 h after the air exposure was not introduced back into the water tanks after the challenge. Moreover, an additional sampling point after 5 h was conducted to evaluate recovery responses according to previous studies in *S. canicula* and *Scyliorhinus stellaris* [45,46]. First sampling for both experimental groups was performed at 08:30–09:30 UTC. Catsharks were captured by hand and immediately anesthetized in 0.1% v/v 2-phenoxiethanol (P-1126, Sigma-Aldrich, St. Louis, MI, USA). Unless anesthesia has been shown to affect stress-related blood variables in sharks [47] we decided to anesthetize the animals and accept possible bias in the muscle parameters as part of this pilot study. Weight and length of the animals was measured. Euthanize was done by severing the head with a sharp knife. A 5-cm portion of the trunk, covering the area just after the first dorsal fin, was collected and immediately frozen at −20 °C. The process of freezing the trunk was previously described in similar studies [30,38] and resembles commercial activities for marketed *S. canicula* in the South of Spain. All procedures lasted less than four minutes per tank, aiming at minimizing secondary-stress responses due to fish manipulation [10].

3.3. Muscle Analysis

The samples were allowed to unfreeze overnight at 4 °C, the skin was removed, and the muscle fillets from both sides of the spine were separated by using a sharp knife. Previous to overnight unfreezing, muscle lactate was analyzed in 0.2 g subsamples (still at −20 °C) from these fillets as previously described [48]. In brief, frozen muscle was finely minced on an ice-cooled Petri dish, homogenized by ultrasonic disruption in 7.5 volumes ice-cold 0.6 N perchloric acid, neutralized using 1 M potassium bicarbonate, centrifuged (3 min at 10,000 g, Eppendorf 5415R), and the supernatant used to assay muscle lactate. Lactate was determined spectrophotometrically with a commercial kit adapted for 96-well microplates (ref. 1001330, Spinreact, St. Esteve de Bas, Girona, Spain).

Square pieces (3 cm × 3 cm) of each muscle fillet were cut and maintained on an ice-cold Petri dish for texture analysis. Instrumental texture analyses were performed on the 2 fillets per fish (in duplicate) using a texture analyzer TA1 (Lloyd-instruments, AMETEK GmbH, Meerbusch, Germany) equipped with an 80 N compression load cell controlled with the program Nexygen Plus 3.0 for Windows. A flat-ended cylindrical probe (12.5 mm diameter; type P/0.5) was used to analyze the consistency, cohesiveness, crispiness, firmness, work of penetration and springiness of the muscle fillets. Analyses were performed by pressing the cylinder into the muscle fillet at a constant crosshead speed of 4 mm s^{-1}. All samples were compressed twice to 50% of their original height. The parameters analyzed (and their Units) were cohesiveness (in N), consistency (in N mm), crispiness (no Units), firmness (in N), work of penetration (in N mm) and springiness (in mm). Cohesiveness describes how well a food retains its form between the first and second chew (is expressed as the area of work during the second compression divided by the area of work during the first compression). Consistency relates to the firmness of a semisolid, and is calculated as the breaking force in the first compression (N) multiplied by the deformation between first and second compression (mm). Crispiness or fracturability is the tendency of a material to fracture, crumble, crack, shatter or fail upon the application of a relatively small amount of force or impact. Firmness is related to the resistance

to deformation (given by the peak load of the initial compression). Work of penetration is described as the distance (in mm) necessary to give the maximum strength (in N) after the initial compression. Springiness is how well a product physically springs back after it has been deformed during the first compression and has been allowed to wait for the target wait time between strokes (expressed as the distance of the detected height during the second compression divided by the original compression distance). Muscle water content was analyzed by drying preweighted muscle at 65 °C until constant weight (circa 72 h), as previously described [49]. The percentage of water was calculated as the difference in weight between the fresh and the dry muscle divided by the fresh weight.

3.4. Statistics

Normality and homogeneity of variances were analyzed using the Shapiro–Wilk's test and the Levene's test, respectively. Differences between groups were tested using two-way ANOVA with group (control and capture by hand/air exposure) and time (0, 5 and 24 h) as the factors. When ANOVA yielded significant differences, Tukey's post-hoc test was used to identify significantly different groups. Statistical significance was set at $p < 0.05$. All results are given as mean ± SEM. All tests were performed using Statistica 7 software for Windows.

4. Conclusions

We have described, for the first time, that elasmobranch flesh texture conditions are affected by pre-slaughtering handling and/or air exposure of the animals. However, future studies are necessary to better characterize fillet quality in sharks, especially after fisheries procedures.

Author Contributions: I.R.-J., I.S. and C.B.-M. designed the experiment. I.R.-J., C.B.-M. and F.S.-G. conducted the animal maintenance and analysis. I.R.-J., C.B.-M., F.S.-G., J.M.M. and I.S. wrote the manuscript.

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