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Histomorphology of Trunk Muscles of Stargazer (*Uranoscopus scaber*, Linnaeus 1758)

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Abstract: Research was conducted on stargazer fish length 13-19 cm and body weight 70-115 g caught in the Novigrad sea area (Croatia). An average diameter of muscle fibers in trunk muscle dorsal of the horizontal septum (dorsal muscle) was slightly greater than in the trunk muscle ventral of the horizontal septum (ventral muscle). The greatest mean fiber diameter was measured in dorsal muscle of stargazer total length 19 cm and it was 112.33 μm and in ventral muscle 109.25 μm (diameters of muscle fibers in dorsal and ventral muscle were 41-150 μm). The smallest mean fiber diameter was measured in stargazer total length 13 cm in the dorsal muscle 70.27 μm and in ventral muscle 64.95 μm (diameters of muscle fibers in dorsal and ventral muscle were 11-120 μm). The statistical significant ($p<0.05$) differences were determinate for changes in diameter of muscle fibers in dorsal muscle between 15.5, 16, 17.5, 18, 18.5 and 19 cm total length. In the ventral muscle statistical significant ($p<0.05$) differences were determined only between 15.5, 16, 17.5 and 18 cm total length of examined fish. Very low activity of Succinic acid Dehydrogenase (SDH), Lactic acid Dehydrogenase (LDH) and alkaline stable Adenosintriphosphatase (ATP) moderate to high activity of acid stable Adenosintriphosphatase (ATP) and larger amount of glycogen suggests that muscle fibers are white fast twitch glycolytic fibers. The activity of LDH and SDH as well of the alcaline stable ATP was strong but activity of acid stable ATP was weak in red slow twitch oxidative muscle fibers. The red fibers were placed superficially at the lateral side of the body as dark muscles clearly distinguished from the white ones but in stargazer small red and intermediary muscle fibers runs as a band into white dorsal muscle. The intermediary (pink) oxidative glycolytic muscle fibers has got larger fiber diameter then red and moderate to strong activity of SDH, LDH, acid and alcaline stabile ATP. The total number of fibers of stargazer fish was increased (hyperplasia) till total length of fish reached 15.5 cm (77 g). In the some time muscle fiber diameter of dorsal muscle was progressive increased from 14 cm (74 g) to 19 cm (115 g) (hypertrophy) and very similar observation was in muscle fiber diameter of ventral muscle.

Key words: Histomorphology, muscles, stargazer (*Uranoscopus scaber*, Linnaeus 1758), fish, backbone, tissue

INTRODUCTION

Fish muscles are markedly segmented. They are structured of muscle fibers and some connective tissue. Body and tail muscles support the backbone from the left and right side and beneath and above the vertebrae and epibrachially and hypobrachially. There are separate muscles along the fins and head bones.

Major part of trunk and tail muscles is structured of white muscle fibers. Red fibers are placed superficially at the lateral side of the body as dark muscles clearly distinguished from the white ones. In some fish between the superficial red and deep white muscle fibers there is a layer of pink or intermediary, fibers (Chayen *et al*., 1993; Devicenti *et al*., 1998; Matsuoka, 1998). White muscle fibers, comprising majority of trunk and tail muscles are by their metabolic characteristics fast twitch glycolytic fibers. Being used for forceful and rapid contractions during hunting for prey or escaping from predators, they tire quickly (Rome *et al*., 1988; Altringham and Johnston, 1990; Van-Leeuwen, 1995).

According to their metabolic characteristics red fibers are slow twitch oxidative fibers, designed for slow repetitive contractions i.e., for slow swimming. Fin muscles are structured mostly of red fibers (Kronnie, 2000). Proportions of red fibers increase caudally. Intermediary fibers are multiply innervated and their proportions do not vary cranio caudally (Devincenti *et al*., 2000). Skeletal muscles in fish develop by hypertrophy and hyperplasia (Galloway *et al*., 1999).

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Both hyperplasia (genesis of new fibers) and hypertrophy (increase in fiber size) contribute to muscle growth. Biotic and abiotic factors are known to influence somatic growth. Somatic growth can be easily measured in the form of body weight (on carcass weight or length and/or condition factor) that gives only an indirect measure of muscle grow. Measurement of muscle fiber diameters in the area of lateral (trunk) muscle in fish of different size or condition is a method which provides useful quantitative data (Vegetti *et al*., 1990; Kiessling *et al*., 1991; Meyer-Rochow and Ingram, 1993; Rowlerson *et al*., 1995; Alami-Durante *et al*., 1997; Johnston *et al*., 1998; Valente *et al*., 1999). The diameters of the larger fibers provide an index of hypertrophic growth which continues until they reach the functional maximum value characteristic of the species. Fibers also grow in length. The presence of very small diameter fibers is often used as a measure of the appearance of new fibers and thus of hyperplasia. Presence of small fibers does not necessarily indicate fast growth because they are typical of fish size rather than growth rate and even some slow growing fish have muscle containing small diameter fibers.

Since, muscular system is commercially the most important part of the fish because it forms >50% of the body mass with help of morphological, histological and histochemical methods in the research we tried to gain insight into structural and metabolic characteristics of trunk muscles of teleost fish stargazer (*Uranoscopus scaber*, Linnaeus 1758) length 13-19 cm and weight 70-115 g (medium sized of stargazer fish is of 10-18 cm and they reach a maximum length of 30 cm and body mass of 0.46 kg). Also in the research we like to know possibility of relationship of length and body weight to muscle fibers of trunk muscles of stargazer and how muscle fibers influence on swimming of this fish.

MATERIALS AND METHODS

Researches were conducted on ten stargazer (*Uranoscopus scaber*, Linnaeus 1758) fish from family Uranoscopidae caught in the Novigrad sea area at the Adriatic coast (Croatia). Fishes were weighted and measured before samples were taken for histological analysis. From every fish sample of trunk muscles were taken in projection of the anal opening, dorsal (dorsal muscle) and ventral (ventral muscle) to the horizontal septum. Samples were frozen in a liquid nitrogen and cut to 10 μm thin slices which were then stained by Hematoxyline and Eosin (HE) (Romeis, 1968) and in which diameter of the muscle fibers was later measured by a microscale. For determination of metabolic activities of fibers, slices were subdued to procedures for showing: activity of Succinic acid Dehydrogenase (SDH) (Pearse, 1972), Lactic acid Dehydrogenase (LDH) (Pearse, 1972) and alkaline and acid stable adenosintriphosphatase (ATP) (Brooke and Kaiser, 1970). Van Gieson Method (Romeis, 1968) slices were stained to show connective tissue and by PAS Method (Romeis, 1968) to show glycogen. All calculations were processed with Statistica Release 8 software and t-test.

RESULTS AND DISCUSSION

An average length and mass of the fish and diameters of muscle fibers of trunk dorsally and ventrally to the horizontal septum are shown in the Table 1 and 2.

Table 1: Number and proportions of muscle fibre diameters of stargazer (13-19 cm total length) in trunk muscles taken in projection of the anal opening, dorsally to the horizontal septum (dorsal muscle)

Total length (cm)	Weight (g)	Total no. of fibers	Muscle fibre diameter (μm) 11-20	21-30	31-40	41-50	51-6	61-70	71-80	81-90	91-100	101-110	111-120	121-130	131-140	141-150
13	70	108	5	4	2	10	18	25	20	11	6	4	3			
			(4.62%)	(3.70%)	(1.85%)	(9.25%)	(16.66%)	(23.14%)	(18.51%)	(10.18%)	(5.55%)	(3.70%)	(2.77%)	-	-	-
14	74	97	-	2	2	4	3	7	15	17	21	15	8	3	-	-
			-	(2.06%)	(2.06%)	(4.12%)	(3.09%)	(7.21%)	(15.46%)	(17.52%)	(21.64%)	(15.46%)	(8.24%)	(3.09%)	-	-
t-test			-	0.204	-	0.257	0.394	0.326	0.090	0.134	0.322	0.334	0.271	-	-	-
15.5	77	123	-	2	3	5	-	18	24	10	15	20	12	14	-	-
			-	(1.62%)	(2.43%)	(4.06%)	-	(14.63%)	(19.51%)	(8.13%)	(12.19%)	(16.26%)	(9.75%)	(11.38%)	-	-
16	80	84	-	3	-	5	9	6	11	12	5	21	8	4	-	-
			-	(3.57%)	-	(5.95%)	(10.71%)	(7.14%)	(13.09%)	(14.28%)	(5.95%)	(25%)	(9.52%)	(4.76%)	-	-
t-test			-	0.125	-	-	-	0.295	0.226	0.057	0.295	0.015*	0.125	0.322	-	-
16.5	85	71	-	-	2	4	-	10	10	16	5	12	7	5	-	-
			-	-	(2.81%)	(5.63%)	-	(14.08%)	(14.08%)	(22.53%)	(7.04%)	(16.90%)	(9.85%)	(7.04%)	-	-
17	90	92	-	-	2	1	1	10	17	12	17	20	10	2	-	-
			-	-	(2.17%)	(1.08%)	(1.08%)	(10.86%)	(18.47%)	(13.04%)	(18.47%)	(21.73%)	(10.86%)	(2.17%)	-	-
t-test			-	-	-	0.344	-	-	0.161	0.090	0.317	0.155	0.111	0.257	-	-
17.5	97	90	-	-	6	5	3	-	14	10	17	13	8	8	6	-
			-	-	(6.66%)	(5.55%)	(3.33%)	-	(15.55%)	(11.11%)	(18.88%)	(14.44%)	(8.88%)	(8.88%)	(6.66%)	-
18	106	72	-	-	1	5	-	-	12	-	8	14	20	9	3	-
			-	-	(1.38%)	(6.94%)	-	-	(16.66%)	-	(11.11%)	(19.44%)	(27.77%)	(12.5%)	(4.16%)	-
t-test			-	-	-	-	-	-	0.048*	-	0.219	0.023*	0.257	0.037*	0.204	-
18.5	111	88	-	-	-	3	2	8	5	11	6	15	21	8	7	2
			-	-	-	(3.40%)	(2.27%)	(9.09%)	(5.68%)	(12.5%)	(6.81%)	(17.04%)	(23.86%)	(9.09%)	(7.95%)	(2.27%)
19	115	90	-	-	-	3	3	8	5	5	6	14	23	5	14	4
			-	-	-	(3.33%)	(3.33%)	(8.88%)	(5.55%)	(5.55%)	(6.66%)	(15.55%)	(25.55%)	(5.55%)	(15.55%)	(4.44%)
t-test			-	-	-	-	0.125	-	-	0.228	-	0.021*	0.028*	0.144	0.204	0.204

*Statistical significant p<0.05

Table 2: Number and proportions of muscle fibre diameters of stargazer (13-19 cm total length) in trunk muscles taken in projection of the anal opening, ventrally to the horizontal septum (ventral muscle)

Total lenght (cm)	Weight (g)	Total no. of fibers	Muscle fibre diameter (μm) 11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	101-110	111-120	121-130	131-140	141-150
13	70	107	5	5	5	11	26	24	12	9	6	3	1	-	-	-
			(4.67%)	(4.67%)	(4.67%)	(10.28%)	(24.29%)	(22.42%)	(11.21%)	(8.41%)	(5.60%)	(2.80%)	(0.93%)	-	-	-
14	74	99	2	3	1	5	8	15	19	19	11	10	5	1	-	-
			(2.02%)	(3.03%)	(0.10%)	(5.05%)	(8.08%)	(15.15%)	(19.19%)	(19.19%)	(11.11%)	(10.10%)	(5.05%)	(0.10%)	-	-
t-test			0.257	0.155	0.374	0.228	0.309	0.144	0.141	0.218	0.182	0.314	0.374	-	-	-
15.5	77	82	-	3	3	3	11	11	12	10	8	18	2	1	-	-
			-	(3.65%)	(3.65%)	(3.65%)	(13.41%)	(13.41%)	14.63(%)	(12.19%)	(9.75%)	(21.95%)	(2.43%)	(1.21%)	-	-
16	80	80	-	2	-	8	4	5	11	14	4	20	11	1	-	-
			-	(2.5%)	-	(10%)	(5%)	(6.25%)	(13.75%)	(17.5%)	(5%)	(25%)	(13.75%)	(1.25%)	-	-
t-test			-	0.125	-	0.271	0.277	0.228	0.027*	0.105	0.204	0.033*	0.385	-	-	-
16.5	85	88	-	1	2	3	5	4	13	16	15	16	9	4	-	-
			-	(12.5%)	(2.27%)	(3.40%)	(5.68%)	(4.54%)	(14.77%)	(18.18%)	(17.04%)	(18.18%)	(10.22%)	(4.54%)	-	-
17	90	87	-	3	8	3	3	14	13	10	12	11	6	4	-	-
			-	(3.44%)	(9.19%)	(3.44%)	(3.44%)	(16.09%)	(14.94%)	(11.49%)	(13.79%)	(12.64%)	(6.89%)	(4.59%)	-	-
t-test			-	0.295	0.344	-	0.155	0.322	-	0.144	0.070	0.116	0.125	-	-	-
17.5	97	87	-	1	6	5	1	-	6	16	21	17	6	6	2	-
			-	(1.14%)	(6.89%)	(5.74%)	(1.14%)	-	(6.89%)	(18.39%)	(24.13%)	(19.54%)	(6.89%)	(6.89%)	(2.29%)	-
18	106	66	-	-	2	1	3	-	8	12	17	19	-	3	1	-
			-	-	(3.03%)	(1.51%)	(4.54%)	-	(12.12%)	(18.18%)	(25.75%)	(28.78%)	-	(4.54%)	(1.51%)	-
t-test			-	-	0.295	0.374	0.295	-	0.090	0.090	0.066	0.035*	-	0.204	0.204	-
18.5	111	107	-	-	-	2	4	10	6	14	17	18	13	12	8	3
			-	-	-	(1.86%)	(3.73%)	(9.34%)	(5.60%)	(13.08%)	(15.88%)	(16.82%)	(12.14%)	(11.21%)	(7.47%)	(2.80%)
19	115	85	-	-	-	4	2	6	8	4	6	14	23	5	10	3
			-	-	-	(4.70%)	(2.35%)	(7.05%)	(9.41%)	(4.70%)	(7.05%)	(16.47%)	(27.05%)	(5.88%)	(11.76%)	(3.52%)
t-test			-	-	-	0.204	0.204	0.155	0.090	0.322	0.284	0.079	0.172	0.248	0.070	-

*Statistical significant p<0.05

Table 3: Mean value of fiber diameters (μm) in trunk dorsal and ventral muscle of stargazer total lenght 13-19 cm

Stargazer	Mean fiber diameter (μm)	
Total length (cm)	Dorsal muscle	Ventral muscle
13.0	70.27	64.95
14.0	79.89	75.40
15.5	87.96	82.50
16.0	89.52	84.31
16.5	90.56	85.97
17.0	91.90	86.61
17.5	94.00	88.10
18.0	96.67	94.02
18.5	107.44	102.53
19.0	112.33	109.25

Diameter of the muscle fibers is measuring on samples stained by hematoxyline and eosin (Fig. 1) by a microscale. According to the results, we determined that an average diameter of muscle fibers in the trunk dorsal muscle is slightly greater than in the ventral one (Table 3). Very low activity of Succinic acid Dehydrogenase (SDH), Lactic acid Dehydrogenase (LDH) (Fig. 2) and alkaline stabile Adenosintriphosphatase (ATP) (Fig. 3) moderate to high activity of acid stable Adenosinetriphosphatase (ATP) (Fig. 4) and larger amount of glycogen is found in all muscle fibers of dorsal and ventral trunk muscle (they are white fibers) except small red muscle fibers and intermediary muscle fibers which runs as a band only into the trunk dorsal muscle.

The activity of LDH (Fig. 2) and SDH as well of the alcaline stable ATP (Fig. 3) was strong in red muscle fibers but activity of acid stable ATP was weak (Fig. 4). The intermediary muscle fibers has got larger fiber diameter then red and moderate to strong activity of

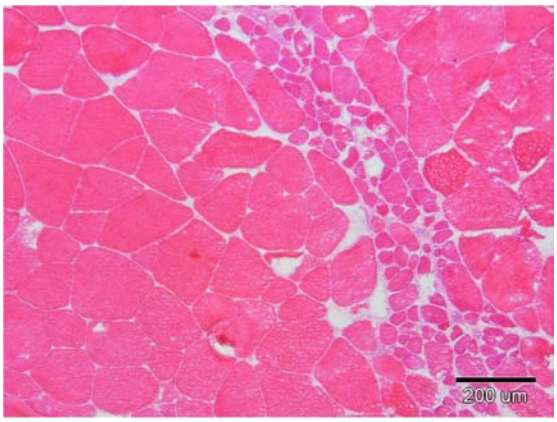

Fig. 1: Stargazer, muscle fibers in trunk muscle dorsal of the horizontal septum, hematoxyline and eosin, magnification 10x (further magnification)

SDH, LDH, acid and alkaline stabile ATP (Fig. 1-4). Larger amount of connective tissue in the investigated muscles was not found.

Fish muscles are known to be suitable for muscle fibers studying because they are distinctly separated which is not case with the muscles of higher vertebrates. They have a segmented structure and less connective tissue. Muscle segments (myomeres) are separated from each other by connective tissue septa and we can clearly observed so called white and red muscles. White muscles comprising majority of fish muscles contain exclusively white muscle fibers whereas red muscles, comprising markedly smaller portion of fish muscles and they have red fibers (Chayen *et al*., 1993; Coughlin, 2002). In the

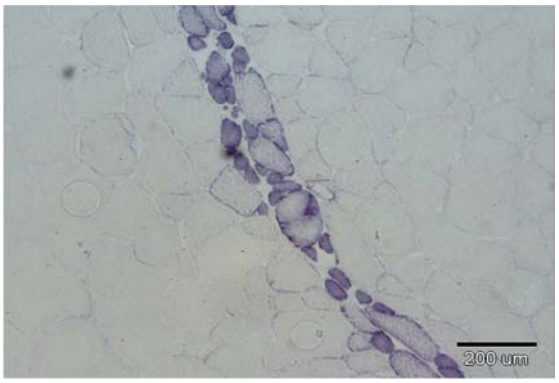

Fig. 2: Stargazer, muscle fibers in trunk muscle dorsal of the horizontal septum, activity of Lactic acid Dehydrogenase (LDH), magnification 10x (further magnification)

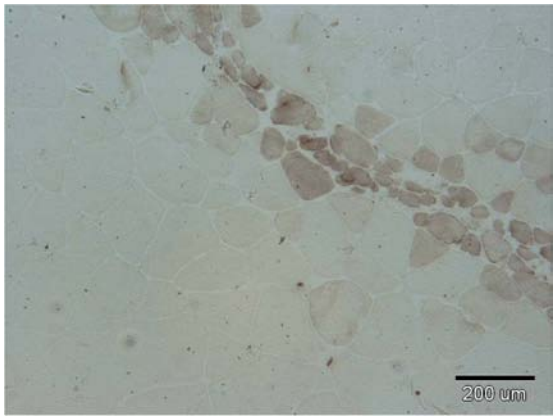

Fig. 3: Stargazer, muscle fibers in trunk muscle dorsal of the horizontal septum, activity of alcaline stable Adenosinetriphosphatase (ATP) 9.4, magnification 10x (further magnification)

research all investigated fish have red fibers which were placed superficially at the lateral side of the body as dark muscles clearly distinguished from the white ones. In stargazer small red muscle fibers and intermediary muscle fibers runs as a band into trunk muscle dorsally of the horizontal sepum. White muscle fibers are large in diameter i.e., larger than the red ones which is important for building up muscle weight (Spierts, 2000) and for rapid and forceful contractions especially during feeding i.e., catching prey or escaping from predators (Rome *et al*., 1988; Altringham and Johnston, 1990; Van-Leeuwen *et al*., 1995).

The muscles in hypobrachial tail myomeres are white i.e., they comprise exclusively white, rapidly contracting glycolytic muscle fibers. Somatic growth is generally

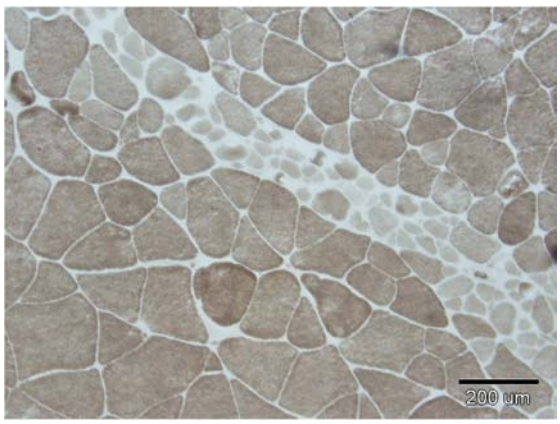

Fig. 4: Stargazer, muscle fibers in trunk muscle dorsal of the horizontal septum, activity of acid stable Adenosinetriphosphatase (ATP) 4.3, magnification 10x (further magnification)

viewed an increase in body size. Hence, the described relation between body weight and increased diameter of muscle fibers is referred to as hypertrophy. Johnston *et al*. (2000) describe the increase in fibers diameter during growth of premature and delayed maturity population of the Atlantic salmon (*Salmo salar* L.) in parallel with body weight. Nejedli *et al*. (2006) also describe the increase in fibers diameter during growth of European sea bass (*Dicentrarchus labrax*, L.).

In the investigations the greatest diameter of muscle fibers is measured in stargazer total length 19 cm (weight 115 g) and it is 112.33 μm in trunk dorsal muscle and 109.25 μm in trunk ventral muscle (diameters of muscle fibers in dorsal and ventral muscle are 41-150 μm). The smallest average diameter of fibers is measured in stargazer total length 13 cm (weight 70 g) in the dorsal muscle 70.27 μm and in ventral muscle 64.95 μm (diameters of muscle fibers in dorsal and ventral muscle are 11-120 μm). The statistical significant ($p<0.05$) differences were determined for changes in the number and diameter of muscle fibers between 15.5 cm (77 g) and 16 cm (80 g), 17.5 (97 g) and 18 cm (106) and 18.5 (111 g) and 19 cm (115 g) total length in dorsal muscle. In the ventral muscle statistical significant differences were ($p<0.05$) determined only between 15.5, 16, 17.5 and 18 cm total length of fish (Table 1 and 2).

According to the results of Succinic acid Dehydrogenase (SDH) activity and Lactic acid Dehydrogenase activity (LDH), very low activity was found in all white fibers, moderate to high activity of acid stable Adenosine Triphosphatase (ATP) and larger amount of glycogen which suggests that those muscle fibers are white fast twitch glycolytic fibers

(Carpene *et al*., 1982; Kilarski and Kozlowska, 1987; Martinez *et al*., 2000; Coughlin, 2002). White muscle fibers contract rapidly and tire faster and because of their large diameter they are very strong (Spierts, 2000) and thus capable of fast short and explosive swimming as in chasing prey or escaping predators. The activity of LDH and SDH as well of the alcaline stable ATP was strong but activity of acid stable ATP was weak in red slow twitch oxidative muscle fibers. The red fibers were placed superficially at the lateral side of the body as dark muscles clearly distinguished from the white ones but in stargazer small red and intermediary (pink) muscle fibers runs as a band only into white dorsal muscle. The intermediary oxidative glycolytic muscle fibers has got larger fiber diameter then red and moderate to strong activity of SDH, LDH, acid and alcaline stabile ATP.

Fast-growing fish generally show greater hyperplasia than slow-growing fish of same age (Kiessling *et al*., 1991; Meyer-Rochow and Ingram, 1993; Valente *et al*., 1999) but large part of this effect is related to the size reached at the time sampling (Kiessling *et al*., 1991).

CONCLUSION

The results showed that total number of fibers of stargazer fish was increased (hyperplasia) till total length of fish reached 15.5 cm (77 g). In the some time muscle fiber diameter of muscle (hypertrophy) in trunk muscle dorsal of horizontal septum was progressive increased from 14 cm (74 g) to 19 cm (115 g) and very similar observation was in muscle fiber diameter of ventral muscle.

The length of individual muscle fibers is linearly related to body length increasing from 13-19 cm total length. In the research we can concluded increase in fibers diameter parallel with body weight and that average diameter of muscle fibers in the trunk dorsal muscle is slightly greater than in the ventral one. Larger amount of connective tissue was not found.

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