



## Estimation of Gill Respiratory Area and Diameters of both Red and White Muscle Fibers in *Luciobarbus xanthopterus* (Heckel, 1843) and *Coptodon zillii* (Gervais, 1848) Local Bony Fish in Karbala City

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### ABSTRACT

Fish's activity can be influenced by several factors such as: Respiratory Area, and diameters of muscle fibers. This study is aimed at explaining some morphometric features of the gill and diameters of red and white muscle fibers in two local bony fish. Fifty specimens each of *Luciobarbus xanthopterus* and *Coptodon zillii* were collected from the Euphrates River at Karbala city in Iraq in September 2019. Histological examinations were performed on gills and muscle fibers based on standard method. The results showed a significant positive correlation between the length of gill filaments and the body length (0.9553 and 0.9881 in *L. xanthopterus* and *C. zillii*, respectively). It was revealed that the length of gill filaments was a direct effect on the total gill surface areas. Relationships between total gill surface area and body length were statistically significant. The correlation coefficients were positive and significant ( $P < 0.05$ ) with values that range from 0.9064 and 0.9757 in *L. xanthopterus* and *C. zillii*, respectively. Body length showed a reversible relationship with relative surface area (-0.964 and -0.975 in *L. xanthopterus* and *C. zillii*, respectively). However, a positive correlation was recorded, after the histological examination of *L. xanthopterus* and *C. zillii* muscle. Such a positive correlation was between the mean diameters of both the red and white muscle fibers and body length (0.9948 and 0.9886 in *L. xanthopterus* and *C. zillii*, respectively). There was a decreasing diameter of red and white muscle fibers toward the posterior region of the fish's body in both species.

**Keywords:** *Luciobarbus xanthopterus*, *Coptodon zillii*, Gill, Muscle.

### Introduction

The determination of the locomotor activity and fish growth depends on the gills' respiratory surface area.<sup>1</sup> This area is the point where gas exchange between the body of the fish and its watery environment takes place. Therefore, the efficiency of gas exchange largely relies on two factors. First, the effectiveness of the gills respiratory surface area,<sup>2,3</sup> and second the speed of water and blood flow through secondary platelets.<sup>4,5</sup> Due to their sensitivity, gills are crucial in the osmotic regulation process,<sup>6</sup> and function such as the changes in environmental factors like temperature, pH, and salinity. Also, The function of the gills is affected by all types of pollutants that affect the level of oxygen in the aquatic environment and the respiratory surface area. Consequently, they directly affect the normal respiratory function of the gills. Moreover, gills also evacuate nitrogenous waste.<sup>7</sup>

Normally, in a fish, muscle tissue constitutes the largest part (30 – 60%) of the body mass. It also represents the musculoskeletal system which is an important requirement of the locomotor system of fish due to the density of the aquatic environment. For rapid swimming, high

muscle tone requires sufficient energy.<sup>9</sup> In most fishes, two types of basic muscles are classified. The classification is decided in terms of color, location, blood supply, fiber diameter, speed and rate of muscle contraction, availability of myosin, in addition to the histochemical and biochemical properties.<sup>10-12</sup> The above-mentioned types are: 1- red muscle fibers or slow fibers and are specialized for continuous and slow swimming. These are arranged in the form of a thin lateral superficial sheet on the lateral surface of the fish trunk, and constitutes approximately 10% of the mass of the muscle tissue. It is also characterized by its small diameter, abundant blood supply, and high-density mitochondria, which has an aerobic function and high concentration of myoglobin and cytochrome.<sup>13-15</sup> 2- white muscle fibers or fast fibers, which are specialized for high swimming speeds. The largest mass of muscle tissue constitutes approximately 90-95% of the mass of the myotome. White muscle fibers are characterized by large diameters, having an anaerobic function, and containing low levels of myoglobin and small numbers of mitochondria.<sup>12,13</sup>

### Materials and Methods

#### Sampling

Fifty specimens of *Luciobarbus xanthopterus* (Heckel, 1834) belonging to the Cyprinidae family, and 50 specimens of *Coptodon zillii* (Gervais, 1848) which belongs to the Cichlidae family were collected from Shatt al-Hindi (Euphrates River) at Karbala city in Iraq between September 2019 and December 2019. In the laboratory, fish were separated into five different length groups ranging from 100 to 300 mm in total length and from 26.91 to 319.46 g in weight. To perform the tests associated with measurements of Gill Respiratory Surface Area and measured the Diameter of the red and white muscle

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fiber, samples were taken from two regions of the studied fish body: R1 represents the anterior region (trunk) and R2 is the posterior region (peduncle caudal).

#### Gill Respiratory Surface Area (GRSA)

Fifty gills from each species were collected, dissected and placed in 0.9% saline. Measurements were taken on one side of the gills. All measurements were made under a binocular microscope using a calibrated ocular micrometer (x10). In each gill arch, the total number of gill filaments was counted, and their lengths were measured. The previously mentioned procedures were applied to determine the average number of secondary lamellae on per 1 mm of the filaments. The lamellae surface area was determined by measuring the length and width using an ocular micrometer. The total gill surface areas were estimated following the equation below.<sup>16</sup>

$$A = Lfil \times 2Nlam \times Blam$$

(A) is the total gill respiratory surface area (mm<sup>2</sup>), (L) is the mean total length of gill filaments, (N) is the mean frequency Number of secondary gill lamellae on both sides of the filament per mm, and (Bl) is the bilateral surface area of a secondary lamella.

#### The Diameter of the Red and White Muscle Fiber

Muscle samples of approximately 1 cm<sup>3</sup> were taken from the R1 and R2 group of fish under investigation. Cross-sections of the red and white muscle fibers were taken with 5-micron thickness. Histological investigation of the muscle fiber was determined by inclusion technique in paraffin and stained with hematoxylin-eosin and mounted on microscope slides. For obtaining a better diameter of every measured fiber, the diameter of the fifty muscle fibers was measured at the widest and lowest points. Ocular micrometer and the calibration were used accordingly to measure the diameter of the fibers.<sup>17</sup>

#### Statistical analysis

Statistical analysis was carried out using SPSS 16. The significance was considered at the probability of P<0.05. Excel program was used to determine the correlation coefficient between variables in the sample of fish under investigation.

## Results and Discussion

#### Gill Respiratory Surface Area

The results of the gill morphological study showed a difference in the components of the respiratory surface area in the species. The length of gill filament in *L. xanthopterus* reached 1127.58 - 3408.65 mm as shown in Table 1, while in *C. zillii* they reached 3897.52 - 7670.21 mm (Table 2). The correlation coefficient showed that there was a positive correlation between the length of the gill filaments and the length of the fish in both species of fishes which were 0.9553 and 0.9881 for *L. xanthopterus* and *C. zillii*, respectively (Figure 1).

The results of the analysis showed significant differences (P<0.05) in the two species (Table 3). Besides, the results also showed the number of secondary gill lamellae (N) and area of the secondary gill lamellae (Bl). These ranged between 35.65 - 25.77 and 0.057- 0.067 mm<sup>2</sup> for N and Bl, respectively in *L. xanthopterus* (Table 1). Whereas, in *C. zillii* they ranged between 39.61-31.52 and 0.030 - 0.037 mm<sup>2</sup> for N and Bl, respectively (Table 2). Analysis of the correlation coefficient (r) between N and Bl showed that the correlation coefficient between the two fish species in N and Bl were -0.993 and 0.9731 in *L. xanthopterus* (Figures 2 and 3). While in *C. zillii*, the correlation coefficient was recorded as -0.951 and 0.981 for N and Bl, respectively (Figures 2 and 3).

The statistical analysis of N and Bl indicates differences (P > 0.05) between the two species (Table 3). This was shown as a clear difference in the mean values of the total gill respiratory surface area between both species. *L. xanthopterus* had the lowest value compared to *C. zillii*, with the values ranging from 6485.92 - 14042.49 mm<sup>2</sup> in *L. xanthopterus* (Table 1). While the total gill area ranged from 9237.32 - 17192.31 mm<sup>2</sup> in *C. zillii* (Table 2). The results of the correlation

coefficient showed a positive correlation between the total gill surface area and fish length in both species which were 0.9064 and 0.9757 in *L. xanthopterus* and *C. zillii*, respectively (Figure 4). The analysis indicates no significant differences (p > 0.05) (Table 3).

The current study showed a difference in the values of the relative gill respiratory surface area (mm<sup>2</sup>/gm) in the two fish species which ranged from 140.47- 44.04 mm<sup>2</sup>/gm in *L. xanthopterus* and 188.16 - 28.97 mm<sup>2</sup>/gm in *C. zillii* (Tables 1 and 2). A negative correlation was shown between the fish length and relative surface area in the two species; -0.964 and -0.975 in *L. xanthopterus* and *C. zillii*, respectively (Figure 5). However, these were not significant (P>0.05) (Table 3). Morphological examination of the fish gill showed differences in the average total length and abundance of gill filaments. The number of the secondary lamellae on the gill filaments and bilateral surface area varied with the body length of *L. xanthopterus* and *Cotodon zillii*. All of these components affected the values of the gill respiratory surface area which agreed with the work of Hughes.<sup>18</sup> In a specific manner, the average length of the gill filament influenced the total respiratory gill area in *L. xanthopterus* and *Cotodon zillii*.<sup>19</sup> The present study have shown an increase in gill filament number and length with increase in the fish length in both species. These results agree with the work of Mohammed (2018).<sup>20</sup> These increases are reflected in the activity and growth of fish, which leads to the growth of specialized respiratory structures which in turn lead to fast growing fish with longer bodies.<sup>21</sup> These findings also agree with the works of Satora and Wegner<sup>22</sup> and Mansour (2018).<sup>1</sup>

In the current study, parameters such as the number (N) and area of bilateral secondary lamellae (Bl) were estimated in both fish species. Results showed a negatives relationship between the fish length and N, while a significant positive relationship between average fish length and average Bl was shown. These findings are similar to those of Wegner<sup>23</sup> and Wilson and Laurent.<sup>24</sup> The current results indicated a significant positive relationship between the average fish length and the total gill surface area in both species and this agrees with the results of Mansour<sup>1</sup> and Mohammed.<sup>20</sup> Furthermore, we found small fishes showing a larger relative gill area as compared to bigger ones. Logically, this might be explained that the large gill surface area in the smaller fishes is due to a high metabolic rate and the high demand for respiratory requirements compared to large fishes.<sup>25,26</sup>

#### The Diameter of the Red and White Muscle Fiber

The current results showed differences in the diameters of red and white muscle fibers. The red muscle fibers were less in diameter as compared to the diameters of the white muscle fibers in both types of fish. Tables 4 and 5 showed the differences in the average diameters of red and white muscle fibers in the two fish species from regions R1 and R2. The diameters of red muscle fibers in R1 and R2 for both species ranged between 29.33 - 44.92 microns in R1 and 23.35 -38.48 in R2 for *L. xanthopterus* (Table 4), while they ranged between 27.02 - 45.50 microns in R1 and 18.64 - 38.33 in R2 for *C. zillii* (Table 5). However, the statistical analysis showed significant differences (p < 0.05) between the two species and in the two regions of the body (R1 and R2) (Table 6). The results of the current study demonstrated a decrease in the means of the diameters of the red muscle fibers in R2 in the two species (Tables 4 and 5). While the average diameters of the red muscle fibers increased with increase in the length of the fish in both fishes. Therefore, a positive correlation was shown between the diameters of the red muscle fibers and fish length in both species, which were 0.9948 and 0.9886 in *L. xanthopterus* and *C. zillii*, respectively (Figure 6). However, statistical analysis revealed no significant difference (p > 0.05) between *L. xanthopterus* and *C. zillii* (Table 7). The average diameters of the white muscle fibers ranged from 46.85 to 71.98 microns and from 42.28 to 66.42 microns in R1 and R2 in *L. xanthopterus* (Table 5). Whereas it ranged from 57.28 to 76.39 microns and from 51.36 to 70.32 microns for R1 and R2, respectively in *C. zillii* (Table 6). Nevertheless, the statistical analysis did not record differences (P > 0.05) between the two fish species and in the two regions of the body (R1 and R2) (Table 7). Moreover, the results of the study demonstrated a decrease in the average diameters of white muscle fibers in R2 in both fish species (Tables 4 and 5). But the average diameters of the white muscle fibers increased with

increase in fish length. Therefore, a positive correlation was demonstrated between the diameters of the white muscle fibers and the

fish length in the studied fish species, which were 0.9731 and 0.9886 in *L. xanthopterus* and *C. zillii*, respectively (Figure 7).

**Table 1:** Components of the gill respiratory surface area (total and relative gill area) in *L. xanthopterus*.

Length group (mm)	No. of fish	Total length (mm)	Fish weight (g)	Total length of gill filaments (mm)	No. of secondary lamellae	Bilateral secondary gill lamellae area (mm <sup>2</sup> )	Total gill surface area (mm <sup>2</sup> )	Relative gill area (mm <sup>2</sup> /g)
101 - 140	10	128.38 ± 1.61	26.91 ± 1.12	1127.58 ± 1.57	35.65 ± 1.34	0.057 ± 0.004	6485.92 ± 1.65	140.47 ± 1.50
141-180	10	168.31 ± 1.51	57.37 ± 1.53	1813.41 ± 1.58	32.31 ± 1.35	0.058 ± 0.001	6687.92 ± 1.73	118.21 ± 1.52
181 - 220	10	208.40 ± 1.54	97.07 ± 1.90	2243.46 ± 1.50	30.53 ± 1.34	0.061 ± 0.002	7553.77 ± 1.77	68.41 ± 1.55
221 - 260	10	236.89 ± 1.45	160.47 ± 1.56	2670.39 ± 1.51	28.91 ± 1.37	0.064 ± 0.003	10156.80 ± 1.63	63.79 ± 1.51
261 - 300	10	290.4 ± 1.67	319.46 ± 1.48	3408.65 ± 1.54	25.77 ± 1.35	0.067 ± 0.001	14042.49 ± 1.76	44.04 ± 1.56

Values are Mean ± Standard Deviation.

**Table 2:** Components of the gill respiratory surface area (total and relative gill area) in *C. zillii*

Length group (mm)	No. of fish	Total length (mm)	Fish weight (g)	Total length of gill filaments (mm)	No. of secondary lamellae	Bilateral secondary gill lamellae area (mm <sup>2</sup> )	Total gill surface area (mm <sup>2</sup> )	Relative gill area (mm <sup>2</sup> /g)
101 - 140	10	130.37 ± 1.66	52.31 ± 1.59	3897.52 ± 1.53	39.61 ± 1.36	0.030 ± 0.003	9237.32 ± 1.72	188.16 ± 1.57
141-180	10	160.35 ± 1.59	88.16 ± 1.93	4600.62 ± 1.58	37.29 ± 1.34	0.032 ± 0.001	10734.51 ± 1.76	124.14 ± 1.51
181 - 220	10	210.41 ± 1.67	224.86 ± 1.07	5388.91 ± 1.54	36.52 ± 1.32	0.034 ± 0.002	13219.81 ± 1.65	59.13 ± 1.51
221 - 260	10	240.49 ± 1.92	385.09 ± 1.56	6558.31 ± 1.55	33.32 ± 1.31	0.035 ± 0.001	15635.31 ± 1.67	39.71 ± 1.54
261 - 300	10	290.43 ± 1.71	620.33 ± 1.59	7670.21 ± 1.56	31.52 ± 1.36	0.037 ± 0.004	17192.31 ± 1.63	28.97 ± 1.56

Values are Mean ± Standard Deviation.

**Table 3:** Statistical analysis of the components of the gill respiratory surface area in *L. xanthopterus* and *C. zillii*.

The Studied Features	Calculated T -Value	P-Value	Significance level 0.05
The total length of gill filaments	4.330	2.446	Significant
No. of secondary lamellae	2.488	2.446	Significant
secondary lamellae area	14.111	2.466	Significant
Total gill surface area	2.135	2.466	Not Significant
Relative gill surface area	0.400	2.466	Not Significant

**Table 4:** the rate of fish length and body weight, and rate diameter of red and white muscle fibers at (R1, R2) in *L. xanthopterus*.

Length groups (mm)	No. of fish	Total length (mm)	Fish weight (g)	Diameters of red muscle fibers (microns)		Diameters of white muscles fibers (microns)		Total Diameters of Red Fibers (microns)	Total Diameters of white Fibers (microns)
				R1	R2	R1	R2		
101 - 140	10	128.38 ± 1.61	26.91 ± 1.12	29.33 ± 1.88	23.35 ± 1.96	46.85 ± 1.89	42.28 ± 1.74	25.65	43.56
141 - 180	10	168.31 ± 1.51	57.37 ± 1.53	33.68 ± 1.74	27.13 ± 1.78	52.63 ± 1.73	48.17 ± 1.85	29.54	47.42
181 - 220	10	208.40 ± 1.54	97.07 ± 1.90	37.61 ± 1.94	31.67 ± 1.84	58.11 ± 1.87	54.15 ± 1.76	33.64	53.13
221 - 260	10	236.89 ± 1.45	160.47 ± 1.56	40.41 ± 1.76	34.53 ± 1.94	65.03 ± 1.72	60.51 ± 1.86	37.47	61.65
261 - 300	10	290.41 ± 1.67	319.46 ± 1.48	44.92 ± 1.86	38.48 ± 1.93	71.98 ± 1.91	66.42 ± 1.97	41.71	67.21

Values are Mean ± Standard Deviation.

**Table 5:** Fish length and body weight, and diameter of the red and white muscle fibers at R1, R2 in *C. zillii*

Length groups (mm)	No. of fish	Total length (mm)	Fish weight (g)	Diameters of the red muscle fibers (microns)		Diameters of White muscles fibers (microns)		Total Diameter of Red Fibers (microns)	Total Diameters of white Fibers (microns)
				R1	R2	R1	R2		
101 - 140	10	128.38 ± 1.61	26.91 ± 1.12	27.02 ± 1.91	18.64 ± 1.74	57.28 ± 1.73	51.36 ± 1.87	21.73	53.54
141-180	10	168.31 ± 1.51	57.37 ± 1.53	31.44 ± 1.81	23.40 ± 1.98	61.43 ± 1.86	57.13 ± 1.96	27.54	59.13
181 - 220	10	208.40 ± 1.54	97.07 ± 1.90	36.82 ± 1.76	28.99 ± 1.75	66.51 ± 1.86	62.49 ± 1.86	31.94	63.34
221 - 260	10	236.89 ± 1.45	160.47 ± 1.56	40.62 ± 1.82	33.35 ± 1.84	71.68 ± 1.79	65.85 ± 1.92	35.76	67.63
261 - 300	10	290.41 ± 1.67	319.46 ± 1.48	45.50 ± 1.97	38.33 ± 1.74	76.39 ± 1.81	70.32 ± 1.87	41.64	71.35

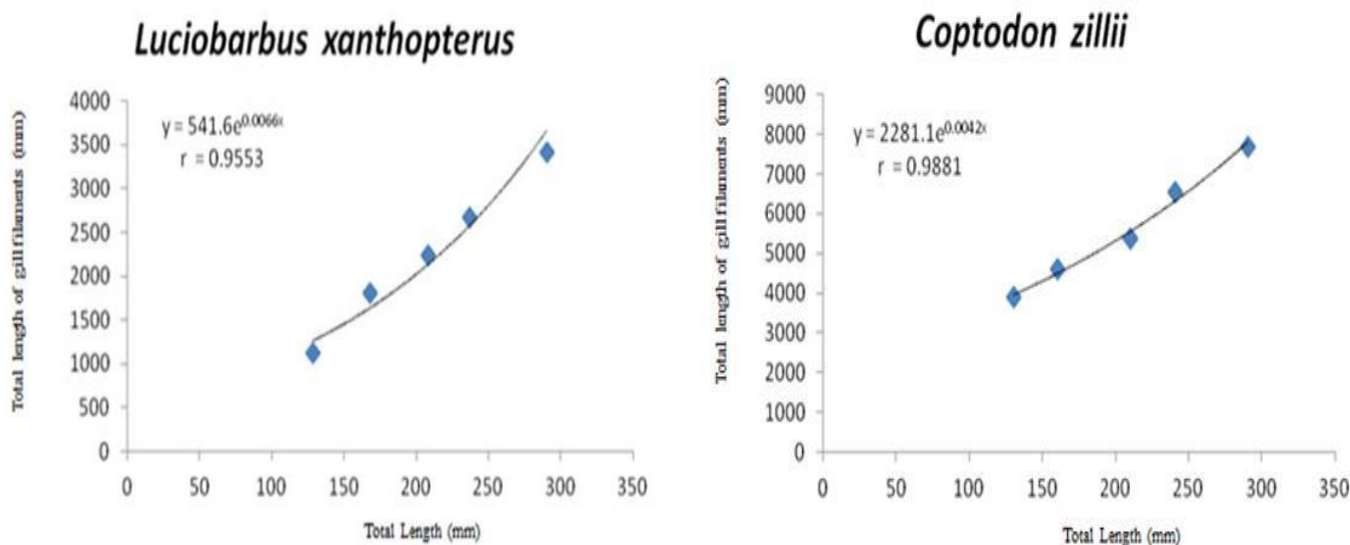
Values are Mean ± Standard Deviation.

**Table 6:** Correlation coefficients between rate diameter red and white muscle fibers at (R1, R2) for *L. xanthopterus* and *C. zillii*

The Studied Features	Region	Calculated T Value	P-Value	Statistical Differences
Diameters of red muscles Fibers (microns)	R1	2.054	1.441	Significant
	R2	1.478	1.441	Significant
Diameters of white muscles fibers (microns)	R1	1.374	1.441	Non-significant
	R2	1.333	1.441	Non-significant

**Table 7:** Statistical analysis of rate diameters red and white fibers in *L. xanthopterus* and *C. zillii*

The Studied Features	Calculated T Value	P-Value	Statistical Differences
Red muscles fibers diameters mean (microns)	0.345	1.441	Non-significant
White muscles fibers diameters mean (microns)	1.560	1.441	Significant

**Figure 1:** Relationship between the total length of fish and the total length of gill filaments in *L. xanthopterus* and *C. zillii*.

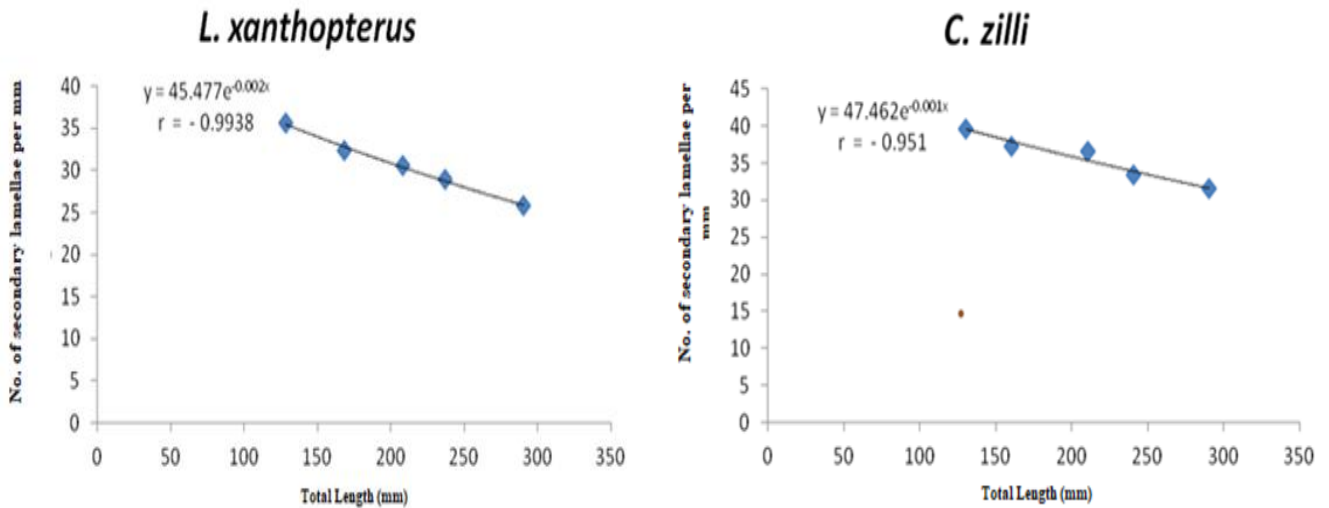


Figure 2: Relationship between total length and numbers of secondary lamellae in *L. xanthopterus* and *C. zillii*

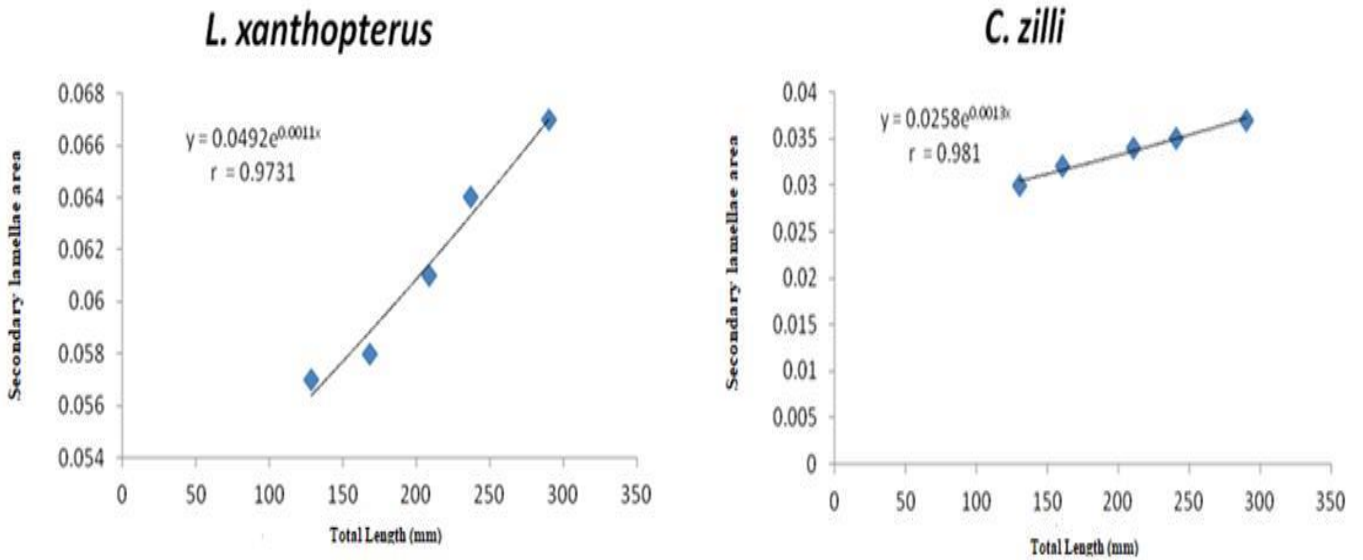


Figure 3: Relationship between total length and secondary lamellae area of *L. xanthopterus* and *C. zillii*.

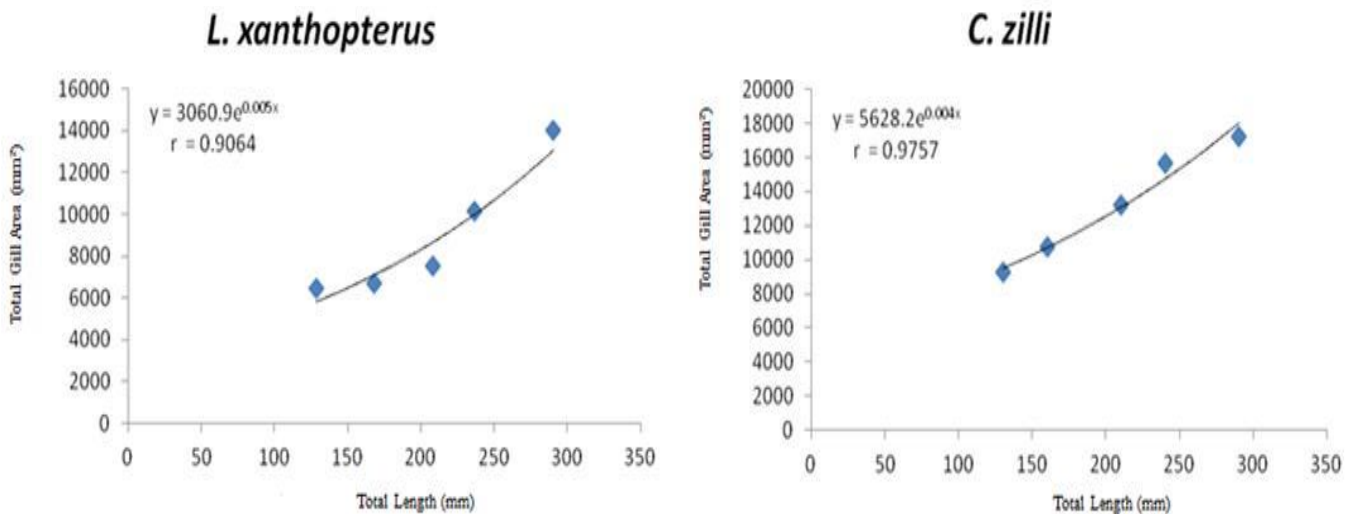


Figure 4: Relationships between the total gill area and total length in *L. xanthopterus* and *C. zillii*

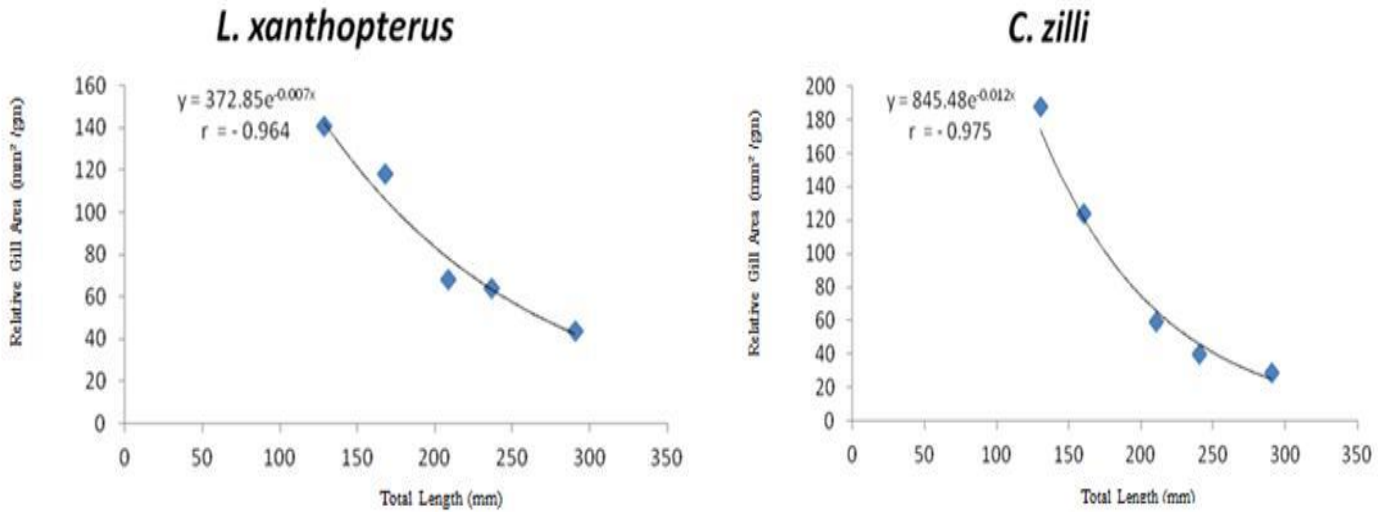


Figure 5: Relationship between total length and relative gill surface area in *L. xanthopterus* and *C. zillii*.

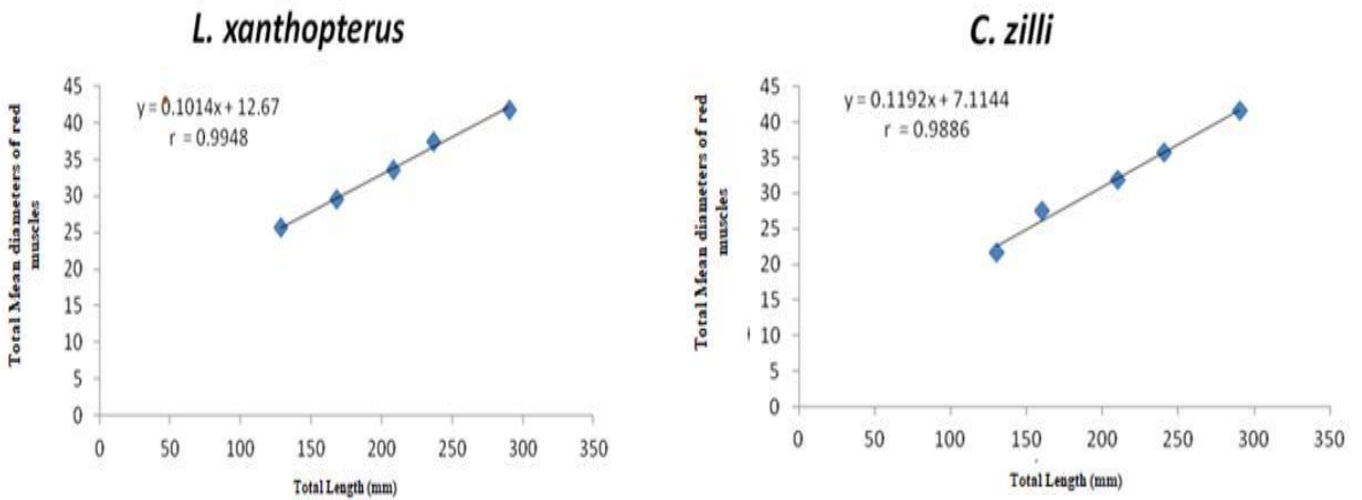


Figure 6: Correlation between fish length (mm) and diameter of red muscle fibers (microns) in *L. xanthopterus* and *C. zillii*

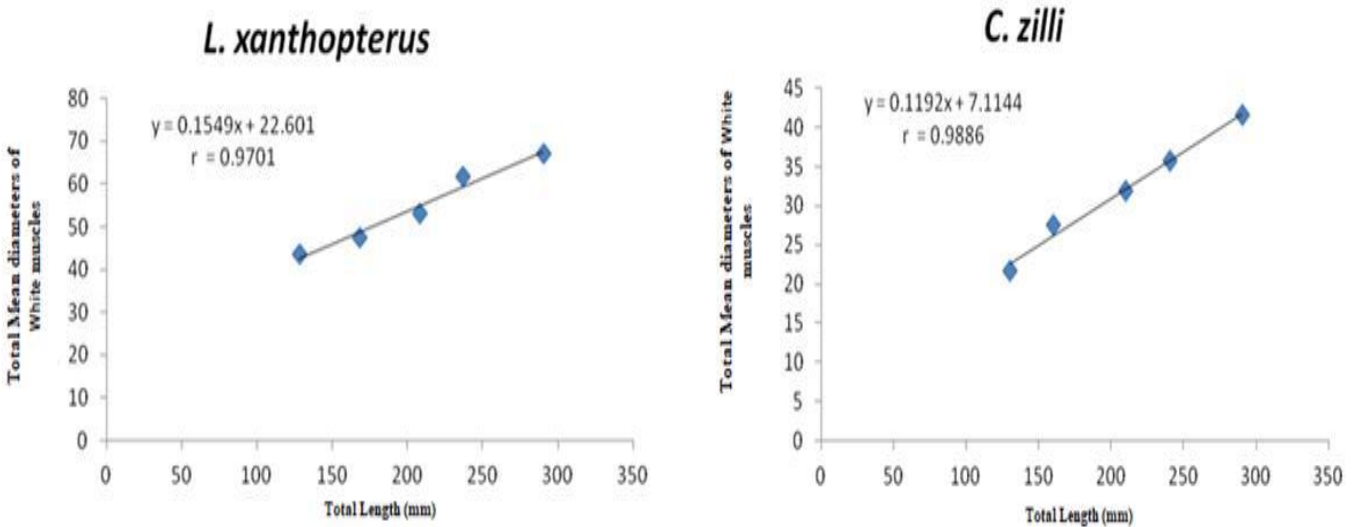


Figure 7: Correlation between fish length (mm) and diameter of white muscle fibers (microns) in *L. xanthopterus* and *C. zillii*.

In this study, we analyzed the diameters of red and white muscle fibers in two fish species. Results revealed that red muscle fibers have lower diameters than that of white muscle fibers. This corresponded with the findings of Wakeling<sup>9</sup> and Sanger and Stoiber.<sup>13</sup> Findings have shown differences in the diameters of red and white muscle fibers in the different body regions (R1 and R2) of the two fish species and a decrease in diameters of the red and white muscle fibers in R2 (caudal peduncle). These differences were due to increases in the number and size of small muscle fibers in the fish by the processes of hyperplasia and hypertrophy.<sup>12,27</sup> The difference in the diameter and size of muscle fibers in the posterior region of the fish reflects the importance of this region in fish movement.<sup>28</sup> The current study indicated differences in the diameters of the red and white muscle fibers which was associated with the length of the fish for both species. These results are consistent with the results of previous studies of Mansour and Al-Muhanna.<sup>29</sup> This variation in diameters is related to the activity and the metabolic pathways for the mode of life.<sup>12</sup>

### Conclusion

From the current study, the gill features were significantly affected by its body length, the length of the gill filaments has a direct effect on the total gill surface areas (mm<sup>2</sup>). The results of the study indicated an increase in diameters of the red and white muscle fibers with an increase in the body length. This variation is related to the growth of red and white muscle fibers.

### Conflict of interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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