RELATIONSHIP BETWEEN TOTAL LENGTH AND GILL SURFACE AREA IN ORANGE SPOTTED GROUPER, *EPINEPHELUS COIOIDES* (Hamilton, 1822)

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ABSTRACT

The present study deals with the estimation of gill surface area for orange spotted grouper (*Epinephelus coioides*) collected from Iraqi marine water, north west Arabian Gulf. In the laboratory, fishes were separated in to five different length groups ranging from 200-325 mm and weight range of 108.8-582.2g. Absolute and relative total gill surface area (GSA) were estimated for 33 fish from different lengths. Statistical analysis of results revealed that there were significant differences (P<0.05) between total length of gill filaments (TFL) in different length groups, while there were no significant differences (P>0.05) between secondary lamellae (Bl) in different length groups. Statistical analysis showed also that there were significant differences (P<0.05) between secondary lamellae numbers (N) in some length groups.Statistical analysis of results showed significant differences (P<0.05) between absolute surface area of most different length groups, and also significant differences (P<0.05) between relative surface area of different length groups except group of 276-300 mm with group of 301-325 mm. Results showed positive relationship between absolute surface area and length groups with significant correlation, and negative relationship between relative surface area and length groups with significant correlation (-0.989).It seemed that the factor L had a direct effect on the values of absolute respiratory area and Fish weight had an inverse effecte on the the relative area of the gills.

Keywords: fish, gill surface area, gill filaments, *Epinephelus coioides*

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العلاقة بين الطول الكلي والمساحة السطحية التنفسية في أسماك الهاموروالبرتقالي المرقط (Hamilton,1822) *Epinephelus coioides*

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المستخلص

تناولت الدراسة الحالية تقديرالمساحة السطحية التنفسية لخياشم أسماك الهاموروالبرتقالي المرقط *Epinephelus coioides* التي تم جمعها من المياه البحرية العراقية، شمال غرب الخليج العربي. تم فصل الأسماك في المختبر إلى خمس مجاميع طول مختلفة تتراوح بين 200–325 ملم ومدى وزن من 108.8–582.2غم. قدرت المساحة السطحية المطلقة والنسبية للخياشيم (GSA) لعدد (33) من الاسماك ذات الاطوال المختلفة. اظهر التحليل الاحصائي للنتائج وجود فروق معنوية (P <0.05) بين الطول الكلي للخيوط الخيشومية (TFL) في مجاميع الطول المختلفة، في حين لم تكن هناك فروق معنوية (P>0.05) بين الصفائح الثانوية (Bl) وأعداد الصفائح الثانوية (N) الا في بعض مجاميع الطول. كما اظهرالتحليل الاحصائي للنتائج وجود فروق معنوية (P<0.05) بين المساحة السطحية التنفسية المطلقة في مجاميع الطول المختلفة، وكذلك فروق معنوية (P<0.05) بين المساحة السطحية التنفسية النسبية لمجاميع الطول المختلفة باستثناء مجموعة الطول 276–300 ملم مع مجموعة الطول 301–325 ملم. بينت النتائج ايضا وجود علاقة ارتباط طردية بين المساحة السطحية التنفسية المطلقة ومجاميع الطول المختلفة، وعلاقة سلبية بين المساحة السطحية التنفسية النسبية ومجاميع الطول مع ارتباط معنوي 0.989–). تبين ان معدل الطول الكلي للخيوط الخيشومية له تاثير مباشرعلى قيم المساحة السطحية التنفسية المطلقة في حين ان وزن الاسماك لة تاثير عكسي على المساحة السطحية النسبية للخياشيم.

الكلمات المفتاحية: اسماك، المساحة السطحية التنفسية، الخيوط الخيشومية، *Epinephelus coioides*

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INTRODUCTION

Fish gills play a vital role in several physiological functions and their surface area is important for diffusion of respiration gases, release of some waste products and maintaining of acid-base level in the blood (13,16,30,33). Any change in the fine structure of fish gills indicates changes in the fish overall physiological condition (11). For this reason, examination of fish gills fine structure and function are important to understand all physiological processes and behavior characteristics of bony fishes (14). The overall gills structure and functional depends greatly on gill surface area. Moreover, the gills of fish contains several cell types that play an important function in ionic regulation and gill physiology of all fishes. (19). In general, the gills are covered by epithelium tissue which contains four types of cells; respiratory cells, mucous cells, pillar cells and chloride cells. (12,25). Variations in the fine structure of these components is related to fish activity. The actual number of fish filaments depends on a number of factors such as fish size, fish surface area and the general behavior of fish species (27). Total number of gill filaments varies between 300 in the arrow fish which is a benthic species to 2400 in mackerel which may reach 800 kg in weight. The number of gill lamellae on the both sides of the filaments ranges between 52 - 689. This number, however, varies depending on the total number of filaments and lamellae and also on fish size. The slow moving fish species possess 10 - 20 lamellae/millimeter while actively moving fish have 30-40 lamellae/millimeter. Most bony fishes have 15-30 lamellae/millimeter. It is, therefore, the study of fish gill surface area becomes important to determine the growth and activity of fishes (24) Groupers (family Serranidae) are considere as favorable fish in the Arabian Gulf region (8). They are sluggish fish and live mostly on rocky bottoms and coral reefs. The sluggish nature of these fishes keeps energy for growth (10).The fish under this study, orange spotted grouper (*Epinephelus coioides*) is a carnivore species widely distributed in the world and is economically important in many countries particularly in South East Asia (9).There are numerous studies on gills morphometry of Iraqi fish, for examples, (29) studied three species of cyprinid fishes, (28) investigated three species of finned black seabream (locally known as Shanek) and (20) who studied three marine species of the order Clupeiformis. Furthermore, (1) studied *Tenulosa ilisha*, *Nematolosa nausos* and *Ilisha elongata* (all belong to Clupeiformis) and the gill surface area of *Barbus sharpeyi* in Diwanyia River was investigated by Al-Hasnawi, and Al-Muhana, (4). A similar study on different length groups of *Aspius vorax* was made by Al-Muhana, *et al* (6) in Diwanyia River and Al-Muhana, *et al* (7) calculated the gill surface area of two fish species *Aspius vorax* and *Liza abu*. The present study aims to estimate the gill surface area of orange spotted grouper, *Epinephelus coioides* fish (Local name Hamour) which represent the first attempt to investigate the gill area of this species in the region

MATERIALS AND METHODS

A sample of 33 fish of orange spotted grouper was collected from its natural habitat in the north west Arabian Gulf. Fishes were kept in cool boxes, covered with crushed ice and transported to the laboratory. Fishes were weighted and total body length and standard length for all fish were measured. Fishes were separated into five different length groups ranging from 200-325 mm and weight range of 108.8-582.2 g. surface area of fishes gill was estimated following (17) To estimate the total gill surface area (GSA) whether absolute or relative, 33 fish, representing different length groups, were measured. Four gills are taken of left side of fish and washed with 0.9% normal saline then treated as the following according to Hughes (17): The gill arches were separated and placed in a Petridish conducted the measurements indicated below: Length of gill arch was recorded using a flexible metal line which was first wrapped around the gill arch and then stretched for correct length measurement.

1- Gill filaments were counted starting from its base at one side, to be multiplied by number 2. All counting was made under a dissecting microscope

2-If gill filaments number was less than 100 then length of filaments was measured from different locations along the gill arch. This is

because filaments length varies according to its position on the gill arch. If gill filaments number is more than 100, 20 filaments were measured. In all measurements, the average length of all counted filaments was calculated so that the result of all filaments of each gill arch is obtained.

3-The total gill filaments length (TFL) for each of the four gill arches is calculated according to the following equation:

TFL = average filament length x total number of filaments (L).

4-Dimension of one secondary lamella (SL) was determined by measuring the length and width by means of a graduated lens. Area was obtained by multiplying length by width if the lamellae shape is rectangular, otherwise area was calculated according to its geometric shape.

5-To determine the number of secondary gill lamellae (N), filaments from gill arches 2 and 3 were scrapped as these are the least exposed to external influences. The material was kept in 0.9% NaCl from which a sample was examined under a microscope to count secondary gill lamellae in 1 mm of a filament using a calibrated ocular micrometer (magnification 10X)

Estimation of gill surface area (GSA) (17):

GSA $(mm)^2$ **= TFL x N x Bl**

Where GSA: Absolute gill surface area (mm^2).

TFL: Total length of all filaments on both sides of the fish

N: Number of gill lamellae in 1 mm on both sides of the fish

Bl: dimension of one secondary lamella

By application of SPSS (version 18), the data were subjected to one-way analysis of variance (ANOVA) to determine the difference between the means and the significant differences were tested by LSD Test.

RESULTS AND DISCUSSION

Table (1) shows fish numbers, averages of total length, fish weights and all measurements of respiration biometry such as averages of total length for gill filaments (TFL), numbers of secondary lamellae (N) and their dimension (Bl) for orange spotted grouper. Results revealed that TFL values ranged between 3309 mm for length group (200-225) mm and 6153 mm for length group (301-325) mm, while B1 values ranges between 0.068 mm^2 for length group (200-225) mm and 0.076 mm^2 for length group (301-325) mm. The average numbers of secondary lamellae (N) was 25 for length group of (200-225) mm and 21 for length group of (301-325) mm. Statistical analysis revealed that there were significant differences between TFL in different length groups, while there weren't significant differences ($P>0.05$) between Bl in different length groups. Statically analysis showed also that there were significant differences ($P<0.05$) between N in length groups (200-225) mm and (226-250) mm, and also between these two groups and other length groups, while there weren't significant differences ($P>0.05$) between N in these other groups. Results appeared also positive relationship between total length and TFL with significant correlation (0.985) (Figure, 1), and negative relationship between total length and N with significant correlation (-0.902) (Figure, 2), while the correlation between length groups and B1 was 0.871 (Figure, 3). Table (2) showed absolute and relative surface areas for gills of different group lengths. Absolute surface area ranged between 5733 mm^2 for length group 200-225 mm and 10032 mm^2 for length group of 301-325 mm, while the value of relative surface area ranged between 91.77 mm^2/g for length group 200-225 mm and 22.08 mm^2/g for length group of 301-325 mm. statistical analysis of the results there were significant differences ($P<0.05$) between absolute surface area of different length groups except group of 251-275 mm with groups of 226 -250 mm and 276-300 mm. Statistical analysis of results showed also significant differences ($P<0.05$) found between relative surface area of different length groups except group of 276-300 mm with group of 301-325mm. Results revealed positive relationship between absolute surface area and length groups with significant correlation (0.975) (Figure, 4) and negative relationship.between relative surface area and length groups with significant correlation(-0.989) (Figure, 5).

Table1. Total length and weight groups and someof respiration measurements (TFL, N, Bl) of orange spotted grouper

Length Groups (mm)	Fish No.	Averages of Total Lengths (mm)	Averages of fish weighs(g)	Averages of total length for gill filaments (mm)	Averages of secondary lamellae numbers (N)	Averages of secondary lamellae dimension (Bilateral area mm2)
200-225	8	214.62± 8.65	108.8±11.67	3309.13±144.5 a	25.25±1.38 a	0.068±0.003 a
226-250	8	235.50±6.21	162.6±15.39	4291.87±174.8 b	23.13±0.64 b	0.069±0.006 a
251-275	7	264.85±6.51	226.7±18.30	4964.43±244.4 c	21.85±0.69 c	0.068±0.007 a
276-300	5	288.60±7.50	412.2±14.18	5311.40±161.1 d	21.40±1.14 c	0.072±0.008 a
301-325	5	314.40±8.14	582.2±10.28	6153.40±571.9 e	21.40±0.48 c	0.076±0.008 a

Different letters in one column is significantly different (P<0.05).

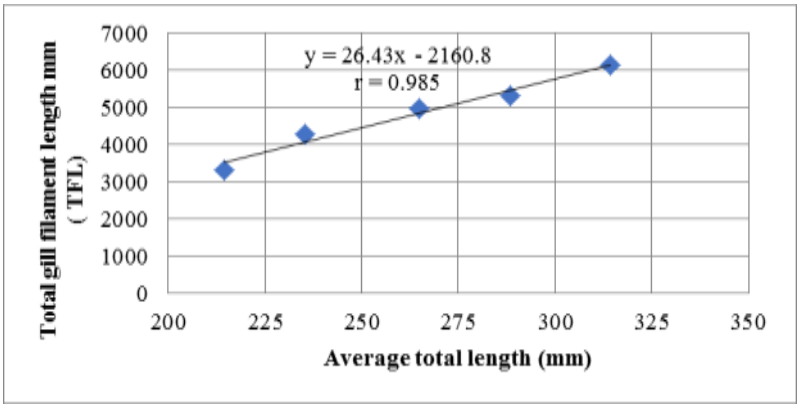

Figure 1. Relationship between total length and total length of gill filaments of orange spotted grouper

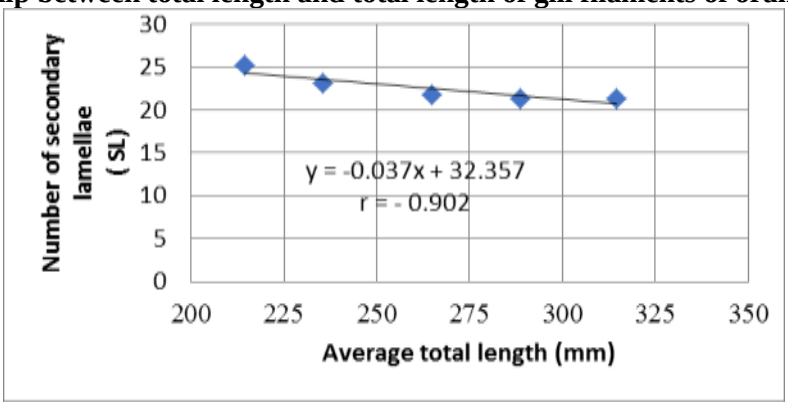

Figure 2. Relationship between total length and numbers of secondary lamellae of orange spotted grouper

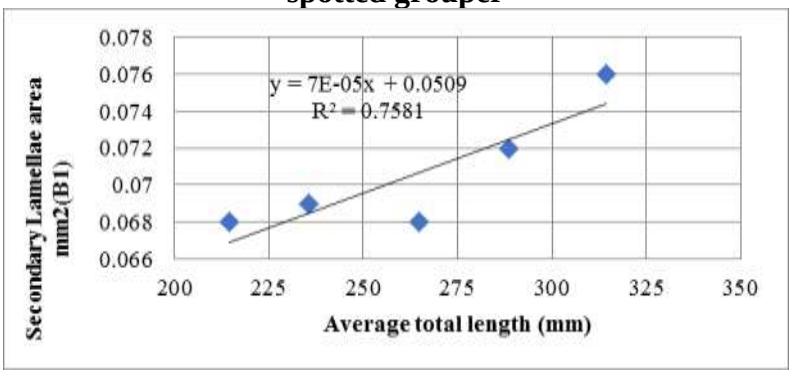

Figure 3. Relationship between total length and secondary lamellae area of orange spotted grouper

Table 2. Absolute and relative gill surface areas in different group lengths of orange spotted grouper

Length groups (mm)	Absolute surface areas for gills (mm^2)	Relative surface areas for gills (mm^2/g)
200-225	5733.97±349.25 a	91.77±19.09 a
226-250	6809.76±538.41 b	72.82±8.54 b
251-275	7436.23±829.97 bc	57.09±5.63 c
276-300	8159.74±824.28 c	30.97±3.24 d
301-325	10032.1±1797.8 d	22.08±1.86 d

Different letters in the same column are significantly different (P<0.05).

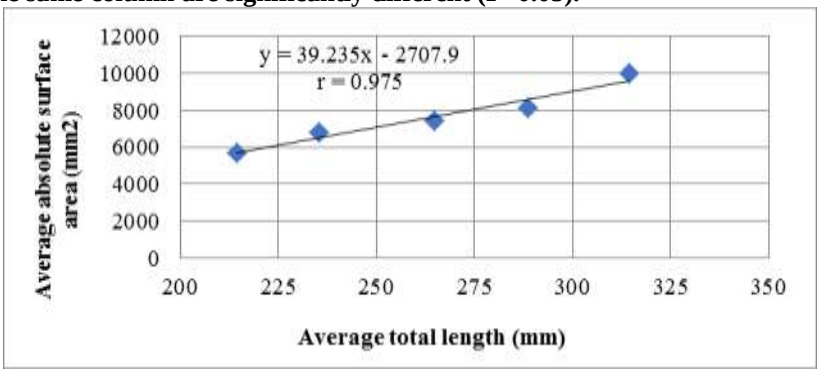

Figure 4. Relationship between total length and absolute gill surface area of orange spotted grouper

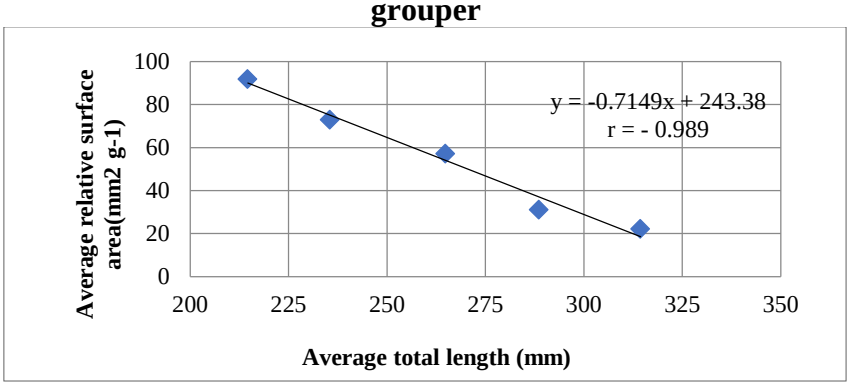

Figure 5. Relationship between total length and relative gill surface area of orange spotted grouper.

Table 3: Averages of total length for gill filaments (TFL) in fish from Roubal (1987)

fish species	Averages of total length for gill filaments (TFL)	Level of fish movement
Opsanus tau (toad)	923 – 8610	sluggish
Acanthopagrus australis (shank)	2414 – 15660	Intermediat
Thunnus sp (tuna)	15209 – 82435	Active

Fish are characterized by using gills as the primary organs of extracting dissolved oxygen from water and transferring it to the blood by a counter current mechanism. The efficiency of respiration in fish depends on gill surface area which is a permeable surface through which gas exchange occurs between blood in the fine vessels and water passing through surface of secondary gill lamellae which is full of fine blood vessels and respiratory cells and also covered with a one layered epithelium tissue (23). Therefore, the gills are considered as the main tool to measure fish physiological activity which is mostly related to oxygen consumption (26). The morphometry of gills in bony fishes (number, length of gill filaments, number and area of secondary gill lamellae) has a direct influence on gill surface area which also affects the overall activity of fish (18,20,27). In general, there are three major factors affecting gill surface area, these include the average length of gill filaments, number of secondary gill lamellae and the average surface area of secondary lamellae (18,27). Considering these three factors, on investigated fish in the present study exhibited different values for these components, although the average length of gill filaments

had the most influence (31). This may be attributed to the increased length of fishes which led to increment in the numbers and lengths of gill filaments in gill arches which in turn reflected the increase in the absolute gill surface area (32). This increase, however, was due to the fast growth of fishes which result in longer fish (15). Statistical analysis of the results obtained in the present study indicated a significant positive relationship between average fish length, average length of gill filaments and the absolute gill surface area. These results are in similar to the findings of (21) who investigated gill surface area of *Heteropneustes fossilis* and concluded that fish length and weight impose direct positive effect on the absolute gill surface area and negative inverse effect on the relative gill surface area (mm^2/g). The morphometry of fish gills are related to life mode and habits of fishes in the aquatic environment and to its metabolic requirements (2, 22,26). For this reason, active fishes have high metabolic rate accompanied with large gill surface area in comparison to less active fishes which possess smaller gill surface area and low metabolic rate (31). Gill surface area varies in fish species and different fish activity (26,27). The absolute gill area is also dependent on fish weight (mm^2/gill area/size or weight). The relative gill area is larger in small fishes because of its high metabolic rate (26,28). This means that the relative gill area estimated on the basis of fish weight decrease with increasing fish total length. This might be explained that large gill surface area in small fish is coming from the high demand of respiration requirements compared to large fishes (28,29). This condition relates to high movement activity and high growth rate in small fishes which are faster than big fishes and in turn require more oxygen consumption and higher metabolic rate (3). The groups of small fishes investigated in the present study showed larger relative gill area in comparison to bigger fishes. Based on previopus consideration, fish of the present study fall in the category of inactive species if compared to other species studied in the region regarding total length of gill filaments. For example, Salman *et al* (29) conducted a study on three cyprinid species, (28) on finned black seabreams, (20) on three species of Clupeiformis, (1) also on three species of Clupeiformis (*Tanualosa ilisha, Nematolosa nausos* and *Ilisha elongata*), (21) on *Heteropneus tesfossilis*., (5) on *Liza abu* (family Mugillidae, Hindyia River), (4) on *Barbus sharpeyi* (family Cyprinidae, Diwaniya River), (6) on *Aspius vorax* (Hindiya River) and Al-Muhana, and Mansour (7) investigated two different families, *Aspius vorax* (family Cyprinidae) and *Liza abu* (family Mugillidae). The results of the current study which etestimate the gill surface area for orange spotted grouper, have explained the factors influencing the area of the gills, which include (L, N, Bl). It seemed that a factor L had a direct effect on the values of absolute respiratory area. Fish weight had an inverse on the relative area of the gills. Fish of the current study are considered as inactive fish with low movement according to the activity groups of (27) due to short gill filaments and and their low numbers

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