Oxygen Consumption Rates of Hybrid Red Tilapia at Different Sizes during Challenge to Water Velocity

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ABSTRACT

This research was designed to determine the oxygen consumption (OC) rates of hybrid red tilapia at nine different fish sizes (fs 1-9), ranging from 110 g to 956 g, at five water velocities (wv) each with three replicates, ranging from 0 to 40 cm·s^{-1} using respirometers. A 5X9 factorial experiment, in randomized complete block design, showed highly significant ($p<0.01$) effects of both fish size and water velocity, as well as the interaction between them. The OC rates were highest in the first hour, then sharply decreased ($p<0.05$) by the second hour, and remained stable in the third hour. OC was significantly reduced as fish size increased. Additionally, average OC for all fish sizes was highest at water velocity of 0 cm·s^{-1} and decreased sharply at 10 cm·s^{-1}. Fish in size groups fs 1-2 had lowest OC (0.09-0.19 mg·l^{-1}·h^{-1}·100 g^{-1}) at 10-20 cm.s^{-1}, fs 3-4 had lowest OC (0.05-0.11 mg·l^{-1}·h^{-1}·100 g^{-1}) at 10-30 cm·s^{-1}, while fs 5-9 had lowest OC (0.03 - 0.08 mg·l^{-1}·h^{-1}·100 g^{-1}) at 10-40 cm·s^{-1}. An estimated simulation equation of oxygen consumption was best fitted in the power regression: OC = 0.686 – 0.002fs·wv + 1.732x10^{-5}fs^2·wv + 1.661×10^{-6}fs^2·wv^2 - 7.879×10^{-6}fs^3 - 3.985×10^{-8}fs·wv^3 + 8.686x10^{-7}wv^4, with R^2 = 0.874.

Keywords: Fish size, Hybrid red tilapia, Oxygen consumption rate, Water velocity

INTRODUCTION

Tilapia is one of the most commercially important freshwater species in the world (Dinesh *et al.*, 2017). Both production and economic importance of this fish are increasing rapidly (FAO, 2014; Gomna, 2011; Dinesh *et al.*, 2017). Tveteras (2013) reported that the total world tilapia production was less than 500 thousand tonnes before the year 1990; after that, production gradually increased to 3.9 million tonnes in 2014.

Intensive farming systems with optimal technology have become crucial in aquaculture to maximize productivity per unit area (Ayyat *et al.*, 2011). In these systems, several environmental factors must be controlled and well managed, one of the most important being water velocity, which affects both the amount of dissolved oxygen and the welfare of fish. Many researchers have reported the positive effects of appropriate water velocity on farmed fish growth, for example, Arctic charr (*Salvelinus alpinus* L.) (Christiansen *et al.*, 1989, juvenile salmonids (Jobling *et al.*, 1993), Atlantic salmon (*Salmo salar* L.) (Castro *et al.*, 2011), and juvenile qingbo (*Spinibarbus sinensis*) (Li *et al.*, 2016). Other studies examined the effects of water velocity on feed intake and feed-conversion ratios (Davison, 1997; Castro *et al.*, 2011). It can also reduce aggression behavior, cannibalism problems (Jobling *et al.*, 1993; Solstorm *et al.*, 2016) and continuous stress of fish (Woodward and Smith,

1985; Solstorm *et al*., 2015). In addition, some reports showed that water velocity can improve flesh texture (Bugeon *et al*., 2003; Li *et al*., 2016) by decreasing body fat (Watten and Johnson, 1990), increasing protein synthesis, glycogen and white muscle fibers (Leon, 1986; Ibrahim and Belal, 2008), resulting in higher quality and longer shelf life of fish meat (Takle *et al*., 2010). However, if water velocity level is too high, fish need to use more energy and oxygen to maintain their position in the water, which can lead to lactate accumulation in fish muscle due to anaerobic metabolism (Davison, 1997). This would eventually cause fish to become stressed, tired and reduce their growth (Solstorm *et al*., 2015; Solstorm *et al*., 2016). Therefore, water velocity has significant impacts on fish metabolism, growth, behavior and welfare (Palstra and Planas, 2011).

Hence, if we can determine the optimal water velocity for all sizes of tilapia under culture conditions, we should be able to maximize fish growth rates, get better consumption ratios, and higher quality of fish meat. The results will be useful not only for fish farmers but also for improving welfare of fish and benefit consumers.

However, the system of cage culture in ponds as used in this study (Hapas system) is quite new for hybrid red tilapia and some basic information, such as oxygen consumption rates at different water velocity levels, has not been determined. Most previous studies have dealt with tilapia oxygen consumption in lentic conditions; for example, Tran-Duy *et al*. (2008) studied the effects of oxygen concentration on growth parameters, and Fridman *et al*. (2012) studied the influence of salinity on embryo development and oxygen consumption. Therefore, the objectives of this work were to study 1) the effect of time length on oxygen consumption rates, and 2) the effects of fish size and water velocity on oxygen consumption rates. Results from these two consecutive studies could be further used to find the appropriate water velocity for each size of hybrid red tilapia, as well as the correlations between fish size, water velocity and oxygen consumption rate.

MATERIALS AND METHODS

Experimental Design

This research was designed to study the oxygen consumption (OC) of hybrid red tilapia at five water velocity (wv) levels (0, 10, 20, 30 and 40 cm·s^{-1}) for nine fish sizes (fs), based on body length. The experimental fish were bought from the same farm. The average total length and weight ranges for each fish size group were: 19.36 cm, 110-130 g; 23.22 cm, 253-267 g; 25.88 cm, 334-356 g; 28.35 cm, 440-450 g; 30.47 cm, 530-560 g; 32.13 cm, 620-650 g; 33.65 cm, 725-749 g; 35.03 cm, 814-840 g; and 36.33 cm, 934-956 g.

A 5X9 factorial experiment in randomized complete block design was used in three replications and OC was measured at 1, 2 and 3 hours in order to follow the response of the fish throughout the experiment. Fish were separated by size and conditioned in nine fiberglass tanks (1.5 x 2 x 1.0 m) with sufficient aeration (>6 mg·l^{-1}) for two weeks prior to the experiment. Ten fish of the same size were stocked in each tank. They were fed a commercial diet to satiation and then starved for 12 hours before starting the experiment. Nine modified respirometers were used for monitoring the OC of each fish size held at the same water velocity. One fish was put in each respirometer during experiments. Water temperature in the respirometer was controlled at 28.9-30.0 °C by a heater, and dissolved oxygen was added by use of an aerator in the reservoir tank, maintaining the concentration at more than 6 mg·l^{-1}. Water was continuously circulated through the system and supplied oxygen into the respirometers at all times.

Respirometer Design

A respirometer was designed to keep fish at the assigned water velocity without electricity; it was made from a 6-inch inner-diameter PVC pipe, 220 cm in length, with ball valves at the two ends, divided by a net into three parts: inlet, living and outlet areas (60, 100 and 60 cm long, respectively). Upstream of the inlet net and downstream from the

outlet net there were holes serving as dissolved oxygen (DO) check points. In the middle of the living area was a 10 x 80 cm fish gate and a window covered with an acrylic plate for observation, as illustrated in Figure 1.

Nine respirometers were connected and filled with water, which was circulated from a five-tonnes reservoir tank. A 0.5 hp submersible pump was used to push water into the respirometers, and a flow probe (Global water 800-876-112, Xylem Honkie Technology Hong Kong Ltd.) was used to measure velocity. Water velocity was adjusted with the ball valves at the inlet and outlet. Dissolved oxygen concentration was measured at the DO check points.

All measurements were carried out by a single researcher and were completed in one week to reduce time effect. Water quality tests were performed and parameters were adjusted as necessary before releasing each fish, so as to ensure optimal conditions and minimize experimental error. Water temperature, dissolved oxygen and pH levels were measured using a YSI Professional Plus device. Total ammonia nitrogen (TAN) and nitrite-nitrogen were analyzed by the modified indophenol method and colorimetric method, respectively (American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF, 2005)).

The experiment was performed in two trials. The first trial determined the length of time (measured at 1, 2 and 3 hours) the fish spent in respirometers at five water velocity levels before they settled down and consumed oxygen steadily. This result was used in the subsequent trial. The second trial studied the effects of fish size and water velocity on oxygen consumption.

Oxygen Consumption Measurement

Dissolved oxygen (DO; $mg \cdot l^{-1}$) was measured with a dissolved oxygen meter (YSI Professional Plus, YSI, USA) at the DO in and DO out check points. The average DO of these two points represented dissolved oxygen at that time. Oxygen consumption in 1 hour (OC_t; $mg \cdot l^{-1} \cdot h^{-1}$) was calculated by $OC_t = DO_i - DO_j$, where DO_i is DO at the start (0 hours), 1, or 2 hours, and DO_j is DO at 1, 2, or 3 hours and presented as OC per mass (100 g of fish), according to the method of Helfman *et al*. (2009).

Statistical Analysis

The average oxygen consumption rates of the nine fish sizes at the length of time (measured at 1, 2 and 3 hours) the fish spent in respirometers, at five water velocity levels were analyzed using one-way ANOVA followed by Duncan's new multiple range test at $p<0.05$. A two-way ANOVA was performed to investigate the effects of fish size, water velocity and their interaction. Duncan's new multiple range test was used to determine differences between water velocity groups for each fish size at $p<0.05$. If interaction among fish size, water velocity and oxygen consumption rate occurred, the correlations were analyzed using polynomial regression. All data analyses were performed with SPSS version 17.5.
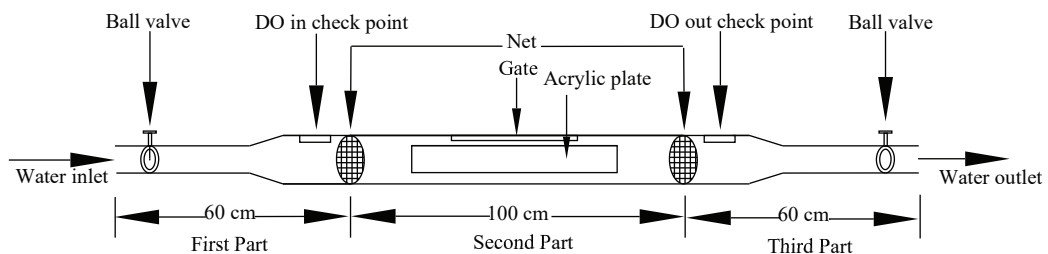

Figure 1. Design of respirometer modified from Carbonara *et al*. (2006).

RESULTS AND DISCUSSION

Water Quality

Average measured water quality parameters (temperature: 28.90±1.10 °C, dissolved oxygen: 6.73±0.53 mg·l^{-1}, pH: 7.39±0.49, total ammonia: 0.09±0.01 mg·l^{-1}, nitrite: 0.004±0.002 mg·l^{-1}) were all acceptable for fish culture (Alabaster and Lloyd, 1982; Boyd and Fast, 1992; Lawson, 1995) and standardized through the entire experiment.

Trial 1 - Effect of time length on oxygen consumption rate

A highly significant effect of time length after releasing the fish into the respirometer was found (p<0.01). Average OC for all fish sizes at five water velocity levels ranged from 0.05-0.71 mg·l^{-1}·h^{-1}·100 g^{-1}. The highest OC was in the first hour (0.11-0.71 mg·l^{-1}·h^{-1}·100 g^{-1}), but then sharply decreased in the second hour (0.06-0.36 mg·l^{-1}·h^{-1}·100 g^{-1}) and was lowest in the third hour (0.05-0.31 mg·l^{-1}·h^{-1}·100 g^{-1}). However, there were no significant differences between the average OC at any fish size between the second and third hours (p>0.05), as shown in Figure 2.

The high OC initially observed in this trial was due to the fish being frightened and disturbed. After the first hour, the fish adapted well to the system and settled down; as a result, optimal OC was reached for all fish sizes. Rankin and Jensen (1993) explained that fish experience stress when in a new environment, thus increasing their metabolism and oxygen consumption. Afterward, they try to adapt themselves to the new environment by reducing their activity, decreasing metabolism and oxygen consumption.

The average OC rates in the second and third hours for each fish size were not different, which means the fish had already settled down by the second hour, and therefore, the second hour was considered the ideal time to measure OC in Trial 2.

Trial 2 - Effect of fish size and water velocity on oxygen consumption rate

Oxygen consumption per fish mass, as shown in Table 1 and Figure 3, shows that in the same water velocity, increasing fish size resulted in decreasing fish OC rate. This means that smaller fish generally have a higher overall metabolic rate than larger fish, assuming other factors such as
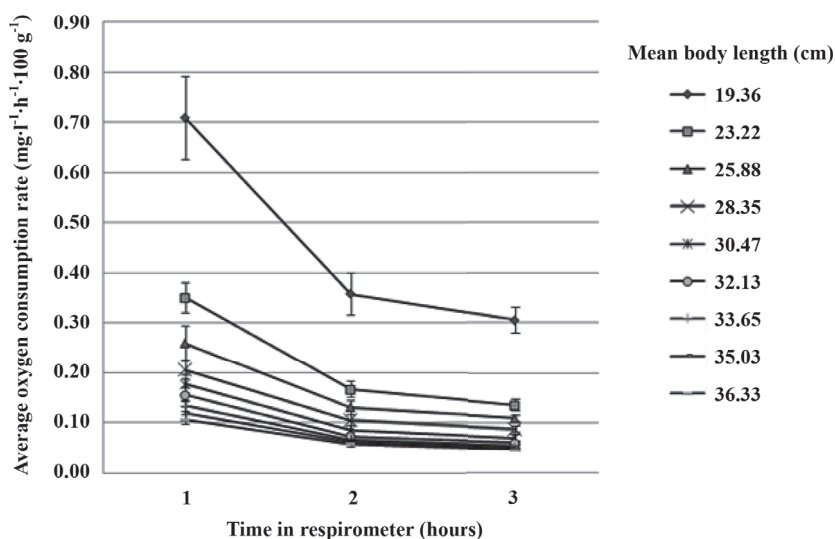

Figure 2. Effect of time in respirometer on average oxygen consumption per mass (mg·l^{-1}·h^{-1}·100 g^{-1}) of tilapia at nine sizes (body length, cm).

Table 1. Oxygen consumption rates per mass ($mg \cdot l^{-1} \cdot h^{-1} \cdot 100\ g^{-1}$) for each tilapia size group kept at five water velocities for two hours.

Fish size		Water velocity				
group no.	body length (cm)	$0\ cm \cdot s^{-1}$	$10\ cm \cdot s^{-1}$	$20\ cm \cdot s^{-1}$	$30\ cm \cdot s^{-1}$	$40\ cm \cdot s^{-1}$
1	19.36	0.77 ± 0.08^{c}	0.18 ± 0.02^{a}	0.19 ± 0.02^{a}	0.33 ± 0.04^{b}	0.38 ± 0.06^{b}
2	23.22	0.71 ± 0.04^{c}	0.09 ± 0.02^{a}	0.09 ± 0.02^{a}	0.15 ± 0.01^{b}	0.17 ± 0.01^{b}
3	25.88	0.65 ± 0.04^{c}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.11 ± 0.02^{ab}	0.13 ± 0.01^{b}
4	28.35	0.53 ± 0.02^{c}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.08 ± 0.02^{ab}	0.09 ± 0.01^{b}
5	30.47	0.45 ± 0.07^{c}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.08 ± 0.01^{ab}
6	32.13	0.40 ± 0.02^{c}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.07 ± 0.01^{ab}
7	33.65	0.35 ± 0.01^{c}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{ab}
8	35.03	0.32 ± 0.03^{b}	0.03 ± 0.01^{a}	0.04 ± 0.01^{ab}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
9	36.33	0.30 ± 0.01^{b}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}

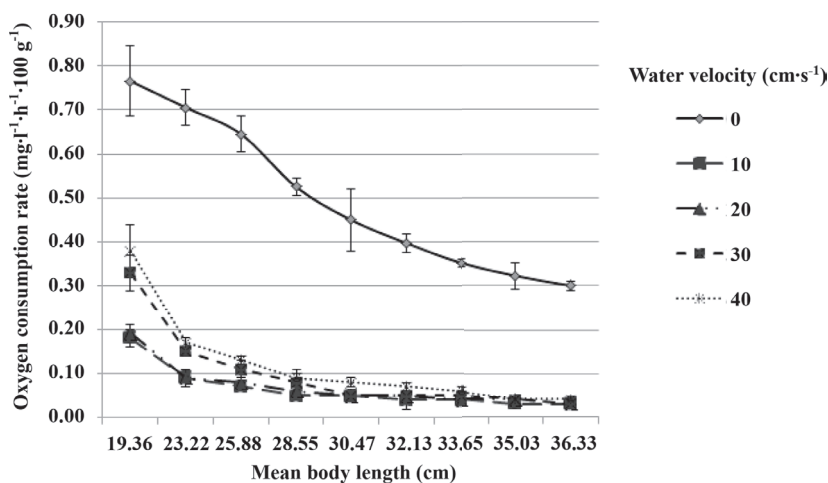Note: Values (mean±SD) within a row with different superscripts differ at $p < 0.05$.

Figure 3. Effect of water velocity on oxygen consumption per mass ($mg \cdot l^{-1} \cdot h^{-1} \cdot 100\ g^{-1}$) for red tilapia of different size groups (mean body length, cm).

activity are constant (Hoar et al., 1979). Therefore, the metabolic rate per unit of mass is higher for smaller fish (Helfman et al., 2009). The higher metabolic rate of small animals necessitates a greater delivery of oxygen to tissues around the body. Also, smaller animals have a greater surface area to volume ratio, so more heat is lost. Furthermore, fish gill surface area is allometrically related to body weight, and size and has a considerable effect on metabolism (Pauly, 1981; Post and Lee, 1996).

The average OC rates for all fish sizes were highest at water velocity of 0 $cm \cdot s^{-1}$, and ranged from 0.30- 0.77 $mg \cdot l^{-1} \cdot h^{-1} \cdot 100\ g^{-1}$. In this treatment, the water in the system was not moving; with no addition of oxygen into the water, the amount of oxygen in the water will decrease continuously. This causes stress in the fish and results in higher metabolic rates, which increases oxygen demand (Hoar et al., 1979). On the other hand, when having fast-moving water, more oxygen enters the system

continuously, which provides fish with sufficient oxygen. Observations from this study allowed us to compare rates of oxygen consumption for a range of fish sizes, and a range of water velocities. We found that fish sizes (fs) 1-2 consumed the lowest amount of oxygen (0.09-0.19 $mg \cdot l^{-1} \cdot h^{-1} \cdot 100 \ g^{-1}$) at 10-20 $cm \cdot s^{-1}$ water velocity ($p < 0.05$), while fs 3-4 consumed the least oxygen (0.05-0.11 $mg \cdot l^{-1} \cdot h^{-1} \cdot 100 \ g^{-1}$) at 10-30 $cm \cdot s^{-1}$ water velocity ($p < 0.05$), and fs 5-9 consumed the least oxygen (0.03-0.08 $mg \cdot l^{-1} \cdot h^{-1} \cdot 100 \ g^{-1}$) at 10-40 $cm \cdot s^{-1}$ water velocity ($p < 0.05$). Therefore, water velocity at 10-20 $cm \cdot s^{-1}$, 10-30 $cm \cdot s^{-1}$ and 10-40 $cm \cdot s^{-1}$ may be suitable for fish sizes of fs 1-2, fs 3-4 and fs 5-9, respectively. Ibrahim and Belal (2015) stated that at the optimum water velocity, growth parameters (weight gain, feed conversion rate, and the percentage of white muscle) of *O. niloticus* are improved, probably due to the gradual increase in feed intake and increase in muscle mass. When the water velocity exceeds the optimum level, the fish have to use energy from eaten feed to resist the higher water velocity. Thus, its growth parameters are gradually reduced. On the other hand, below the optimum water velocity, fish growth parameters are probably affected negatively due to lower feed intake and increased

CO_2 concentration from respiration, which causes lower water pH, resulting in a low growth rate.

Therefore, if we can determine the optimum water velocity, we may be able to maximize fish growth rates, maximize the feed conversion ratio and minimize body fat, which increases shelf life (Leon, 1986; Watten and Johnson, 1990).

Two-way analysis of variance showed highly significant effects for all factors: fish size, water velocity, blocks and interaction ($p < 0.01$), with $R^2 = 98.3\%$ (Table 2).

Simulated equations of oxygen consumption

Due to the interaction between fish size and water velocity, quadratic, cubic and power simulated equations were examined and are shown in Table 3. The quadratic equation of the studied factors gave R^2 of only 0.636, while the cubic equation gave R^2 of 0.688. The best equation for OC estimation was the power equation: OC = 0.686 - 0.002fs·wv + 1.732×10^{-5}fs^2·wv + 1.661×10^{-6}fs$^2 \cdot$wv^2 - 7.879×10^{-6}fs^3 - 3.985×10^{-8}fs·wv^3 + 8.686×10^{-7}wv^4, with $R^2 = 0.874$.

Table 2. Results of two-way analysis of variance with red tilapia of different sizes (mean body length, cm) held in different water velocities ($cm \cdot s^{-1}$).

	F-Ratio	Prob[1] > F
Replication (block)	29.641	0.0000
Fish size	206.398	0.0000
Water velocity level	1436.920	0.0000
Interaction	16.366	0.0000
Entire model; $R^2 = 0.983$		

Table 3. Polynomial regression of oxygen consumption simulated from a range of fish sizes and water velocities.

Simulated equations of oxygen consumption (OC)	R²
OC = 0.401 - 3.088×10^{-5}fs^2·wv + 5.975×10^{-6}fs^2·wv^2	0.636
OC = 0.438 - 4.465×10^{-5}fs^2·wv + 1.267×10^{-6}fs^2·wv^2 - 0.7214×10^{-9}fs·wv^3	0.688
OC = 0.436 - 5.383×10^{-5}fs^2·wv + 1.807×10^{-6}fs^2·wv^2 - 2.209 v 10^{-8}fs·wv^3 + 2.633×10^{-7}wv^4	0.725
OC = 0.478 - 0.001fs·wv - 2.476×10^{-5}fs^2·wv + 1.904×10^{-6}fs^2·wv^2 - 3.605×10^{-8}fs·wv^3 + 6.884×10^{-7}wv^4	0.800
OC = 0.686 - 0.002fs·wv + 1.732×10^{-5}fs^2·wv + 1.661×10^{-6}fs^2·wv^2 - 7.879×10^{-6}fs^3 - 3.985×10^{-8}fs·wv^3 + 8.686×10^{-7}wv^4	0.874
OC = 0.640 - 0.002fs·wv + 1.846×10^{-6}fs^2·wv^2 - 6.089×10^{-6}fs^3 - 3.906×10^{-8}fs·wv^3 + 7.880×10^{-7}wv^4	0.867

Remarks: fs = fish size (body length, cm), wv = water velocity level (0, 10, 20 , 30 and 40 $cm \cdot s^{-1}$)

CONCLUSION

1. Critically high oxygen consumption rates were observed for all fish sizes in the first hour (p<0.05) due to stress from handling during the transfer from the conditioning tank to the respirometer. The fish settled down and consumed oxygen at a normal rate in the second hour.

2. Oxygen consumption rate varied according to fish size and water velocity. It is apparent from the results of the second trial that water velocity for all fish sizes in pond culture should be maintained at approximately 10 cm·s^{-1}, since this velocity resulted the lowest oxygen consumption.

3. OC can be estimated by the power equation:

$$OC = 0.686 - 0.002fs \cdot wv + 1.732 \times 10^{-5} fs^2 \cdot wv + 1.661 \times 10^{-6} fs^2 \cdot wv^2 - 7.879 \times 10^{-6} fs^3 - 3.985 \times 10^{-8} fs \cdot wv^3 + 8.686 \times 10^{-7} wv^4$$

$$R^2 = 0.874$$

ACKNOWLEDGEMENTS

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