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RESEARCH ARTICLE

The Effect of Water Temperature on Standard and Routine Metabolic Rate in Two Different Sizes of Nile Tilapia [1]

Hakan TURKER *

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* Department of Biology, Abant Izzet Baysal University, TR-14280 Bolu - TURKEY

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Summary

The metabolic rate of fish is indirectly measured by their rate of respiration (oxygen consumption). Respiration provides oxygen for aerobic conversion of the energy contained in food. Respiratory data are important in the construction of bioenergetic models, indication of altered environment and estimation of carrying capacity in a rearing unit. In this study, standard and routine metabolic rates of 52.1±0.3 and 205.4±0.5 g Nile tilapia, *Oreochromis niloticus*, were determined with computerized intermittent flow-through respirometry at 19, 22, 25, 28 and 31°C water temperatures. The relationship between water temperature and oxygen consumption rate of Nile tilapia at two different body weights was found to best fit the exponential model.

Keywords: *Respirometer, Standard metabolism, Routine metabolism, Oxygen consumption, Temperature, Nile tilapia*

Su Sıcaklığının İki Farklı Büyüklükteki Nil Tilapyalarının Standart ve Rutin Metabolik Oranına Etkileri

Özet

Balıkların metabolik oranı dolaylı olarak solunum oranları (oksijen tüketimleri) ile ölçülür. Solunum besinlerin içinde yer alan enerjinin aerobik dönüşümü için oksijen sağlar. Solunum verileri biyoenerjetik modellerin kurulmasında, belirteç olarak değişmiş çevre koşullarında ve yetiştirme ünitelerinin taşıma kapasitesinin tahmininde önemlidir. Bu çalışmada, 52.1±0.3 ve 205.4±0.5 g ağırlığındaki Nil tilapyalarının, *Oreochromis niloticus*, standart ve rutin metabolik oranları 19, 22, 25, 28 ve 31°C su sıcaklıklarında bilgisayara bağlı zaman ayarlı sürekli akan solunum ölçer ile belirlenmiştir. İki farklı vücut ağırlığındaki Nil tilapyalarının, su sıcaklığı ve oksijen tüketim oranı arasındaki ilişki en iyi üssel model olarak ifade edilmiştir.

Anahtar sözcükler: *Solunum ölçer, Standart metabolizma, Rutin metabolizma, Oksijen tüketim oranı, Sıcaklık, Nil tilapyası*

INTRODUCTION

Oxygen consumption rate has become the conventional metabolic measure for fish since dissolved oxygen can be determined with relative ease and reliability. The sensitivity of metabolic rate measurements by direct calorimetry is less than the sensitivity of an indirect measure such as oxygen consumption rate [1]. Measurement of respiration is important in the construction of bioenergetic models for growth and reproduction [2,3]. The oxygen consumption rate is a sensitive indicator of physiological states for altered aquatic environmental conditions in teleosts [4]. The metabolic data is also used to establish a model for stocking density estimations in aquaculture [5]. Fry [6] categorized aerobic metabolism in fish as standard, the minimum metabolic rate for intact fish, and routine metabolism, the metabolic rate including spontaneous movements.

Measurements of oxygen consumption rate in different species of tilapias have been searched by various studies with varying levels of stress, types of equipment and experimental protocol [7-13]. Caulton [8,9] determined the oxygen consumption rate of red tilapia, *Tilapia rendalli*,

İletişim (Correspondence)
+ 90 374 2541223
h_turker@ibu.edu.tr

and Mozambique tilapia, *Oreochromis mossambicus*, related to water temperature using a constant flow (open) respirometer. However, this method bring along one significant disadvantage; in order to determine oxygen consumption by open respirometry it is crucial that the system is in steady state. This means that the oxygen content of the in-flowing and out-flowing water and the oxygen consumption of the fish have to be constant. If the oxygen consumption of the fish changes for some reason during the experiment, steady state does not exist for a while. Then, the computer controlled unit does not calculate the oxygen consumption rate until the system is not in steady state. The duration of the time lag depends on the relationship between the volume of respiration chamber and flow rate. Thus, open respirometry measurements have poor time resolution and are not suitable for determination of oxygen consumption on organisms with a highly variable respiration like fish.

The intermittent flow-through computer operated respirometer was suggested for measuring the oxygen consumption which combines the accuracy measurements in static systems with the benefit of flow through systems in long-term measurements over several days and provides controlled environmental conditions during the experiment [14]. The most important advantage is the great time resolution of this method. Oxygen consumption rates of fish can be determined for every 10th min over periods of hours or days, making our systems for automatic respirometry extremely suited for uncovering short term variations (minutes) in respiration. Standard and routine metabolic rates can be analyzed by computerized intermittent flow-through respirometry. The intermittent respirometry have been applied successfully with a high temporal resolution of the measurements of oxygen consumption rates over short time intervals for rainbow trout, *Oncorhynchus mykiss,* and shark-sucker, *Echeneis naucrates* [14], cod [15], horse mackerel, *Trachurus trachurus* [16], roach, *Rutilus rutilus* [17] and bream, *Abramis brama* [18].

Tilapias are thermophilic freshwater fish cultured intensively and extensively in warm waters almost all around the world. They are quite sensitive to cold [19] and probably suffer at low temperatures due to osmoregulatory failure. 26°C is reported to be the optimal temperature for growth, while 35°C is the upper lethal limit [20,21]. Tilapias become generally inactive about 16°C and death may occur below 12°C if exposure is maintained [22]. Acute changes in temperature occur in natural habitats, as well as in aquaculture facilities where environmental parameters cannot be entirely controlled. Metabolic costs in terms of oxygen consumption are affected by this environmental change. Therefore, the automatic respirometry, developed for prolonged and automatic measurements of oxygen consumption rate, was tested to determine the metabolic rate of Nile tilapia, *Orecohromis niloticus*, in the controlled laboratory environment.

The objective of the present study was to improve on the parameters for metabolism modeling of Nile tilapia determining the standard and routine metabolic rate for fingerling and adult Nile tilapia at five water temperatures.

MATERIAL and METHODS

Nile tilapias were randomly selected from the stock tank in the Fish Culture Laboratory at Abant Izzet Baysal University, Bolu, Turkey, and separated into two groups (n=6) as fingerling and adult. The fish were placed in a 1-m diameter another tank, maintained within a recirculating system and supplied with commercial food until running an experiment. Before each trial, the fish were left un-fed in the gut evacuation tank for 24 h prior to the start of each experiment to avoid elevated oxygen consumption due to specific dynamic action [16] and simultaneously acclimated to the experimental temperature. Each fish entered the chamber in the morning and acclimated for 4 h in the chamber. The metabolic rates were estimated after the fish acclimated to the chamber during the additional 48 h. All experiments were carried out in dim light to standardize activity levels and the apparatus was surrounded by black curtain to avoid visual disturbance.

Metabolic rates were measured using a DAQ-PAC-G4S four-chamber intermittent flow respirometry system and software (AutoResp®) from Loligo Systems (Tjele, Denmark). The respirometer system included four parallel transparent acrylic measuring chambers (0.98 and 2.28 L) for the measurements of fingerling (52.1±0.3 g, n=2) and adult (205.4±0.5 g, n=2), respectively. Three replicate trials were conducted per temperature for each size and naive fish were used in each trial. Dissolved oxygen inside the closed chamber was measured with a galvanic oxygen probe and recorded every second for 10 min before the system was open for a 4-min flush period followed by a 5-min lag between when the system closed, and metabolic recordings for the next loop began. The electrode was calibrated against air-saturated water at the experimental temperature and against a solution having an oxygen saturation of zero before stating each experiment. Two identical size tilapias from small and large group were anaesthetized mildly with benzocaine, weighed nearest 0.1 g and transferred into the experimental chamber. The respirometer system was cleaned and disinfected after each experiment. Water temperature was kept constant throughout the experiment by an aquarium heater (Eden®, 300W) and an external chiller (Resun®, CL-280) with a digital thermostat (±1°C). The water was fully oxygen-saturated by means of an airstone and diffuser in the outside of the chamber unit. Dissolved oxygen concentration and water temperature of the system was checked every minute with a polarographic oxygen meter (YSI®, 5000).

Standard and routine metabolic rates were statistically determined by data extraction method which will be commonly referred as the Fourth-Spread method [22]. Essentially, the boundaries of each of the quartiles in data set were identified, measured the fourth-spread which is the distance between the lower and upper quartiles, and set the upper and lower boundaries. From the definition of the routine and metabolic rate, the data were taken after upper boundary as routine due to increased oxygen consumption which result from spontaneous activity and lower boundary as standard metabolic rate due to resting metabolism. The parameters of metabolic rate formula were calculated by regression analysis with the oxygen consumption rates and water temperature using SPSS (Version 15, SPSS Inc., Chicago, IL, USA).

RESULTS

Metabolic rates of fingerling and adult Nile tilapia through oxygen consumption rates were measured by computerized intermittent flow-through respirometry with a high temporal resolution in this study.

High oxygen consumption rates were observed at the beginning of the each experiment after the fish transferred to the chamber due to stress. The adaptation phase was set to 4 h from preliminary experiments and then the fish became progressively calmer. In some measuring intervals, higher oxygen consumption rates occurred as a result of spontaneous activity. Those values have been automatically excluded from further data processing by the Fourth-Spread method. Separation between standard and routine boundaries for sample data with a 52.1 and 205.4 g tilapia at 25°C were seen in *Fig. 1*. Standard and routine metabolic rates were calculated for each fish at each water temperature regime after discarding the outliers from the data. The exclusion of the lowest and highest data caused an increase in the regression coefficients (r^2).

The relationship between the water temperature and metabolic rates of Nile tilapia in fingerling and adult size

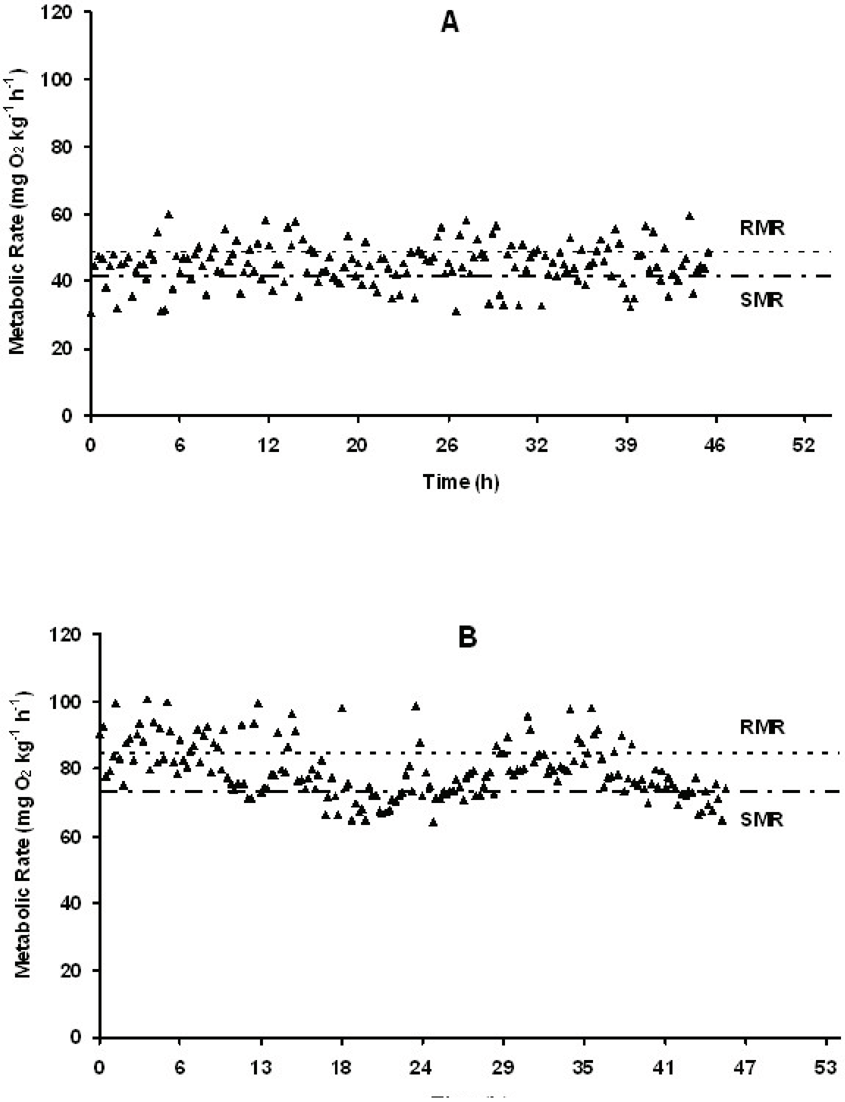

Fig 1. Oxygen consumption of a 52.1 (A) and 205.4 g (B) tilapia at 25°C. The upper and lower horizontal line indicates level considered to represent of standard (SMR) and routine metabolic rates (RMR), respectively

Şekil 1. 25°C sıcaklıkta 52.1 (A) ve 205.4 g (B) ağırlığındaki Nil tilapyalarının oksijen tüketim oranları. Sırası ile üst yatay çizgi standart (SMO) ve alt yatay çizgi rutin metabolik oranı (RMO) ifade eder appeared to be explained by exponential relationship (MR=a . $e^{b.T}$) *(Fig. 2)*. The standard and routine metabolism of fingerling and adult Nile tilapia under the condition of these experiments increased with water temperature. Using this model, water temperature determined 95% in both sizes tilapia for SMR, 0.95 and 0.90% of the variance in fingerling and adult tilapia for RMR *(Fig. 2)*. Metabolic rates of Nile tilapia for two different body weights at various water temperature conditions were summarized in *Table 1*. In both sizes, the standard and routine metabolism of Nile tilapia increase with increasing water temperature throughout the experiment.

Table 1. *The mean (mg O_2 kg^{-1} h^{-1}±SE) standard (SMR) and routine metabolic rates (RMR) with 52.1 and 205.4 g Nile tilapia at various water temperature regimes*

Tablo 1. *52.1 ve 205.4 g ağırlığındaki Nil tilapyalarının farklı sıcaklık rejimlerdeki ortalama (mg O_2 kg^{-1} h^{-1}±SE) standart (SMO) ve rutin metabolik oranları (RMO)*

Temperature (°C)	52.1±0.3 g		205.4±0.5 g	
	RMR	SMR	RMR	SMR
19	32.9±0.3	22.4±0.2	45.4±0.3	32.3±0.3
22	45.2±0.4	31.2±0.4	84.8±0.8	56.7±0.4
25	52.4±0.5	36.6±0.5	91.1±0.7	70.0±0.4
28	98.4±0.8	71.3±0.7	110.0±0.7	85.8±0.6
31	123.5±0.8	95.0±0.5	169.7±1.0	141.4±0.5

DISCUSSION

Outliers with abnormally high oxygen consumption values that could be clearly explained by spontaneous activity were identified and removed from the data according to outlier detection method of Devore and Farnum [22]. Hermann and Enders [16] also provided a similar procedure to separate between standard and metabolic rates when using intermittent flow respirometry. However, they recommended that their procedure should not be taken as a principal technique, because the details are very dependent on the data structure.

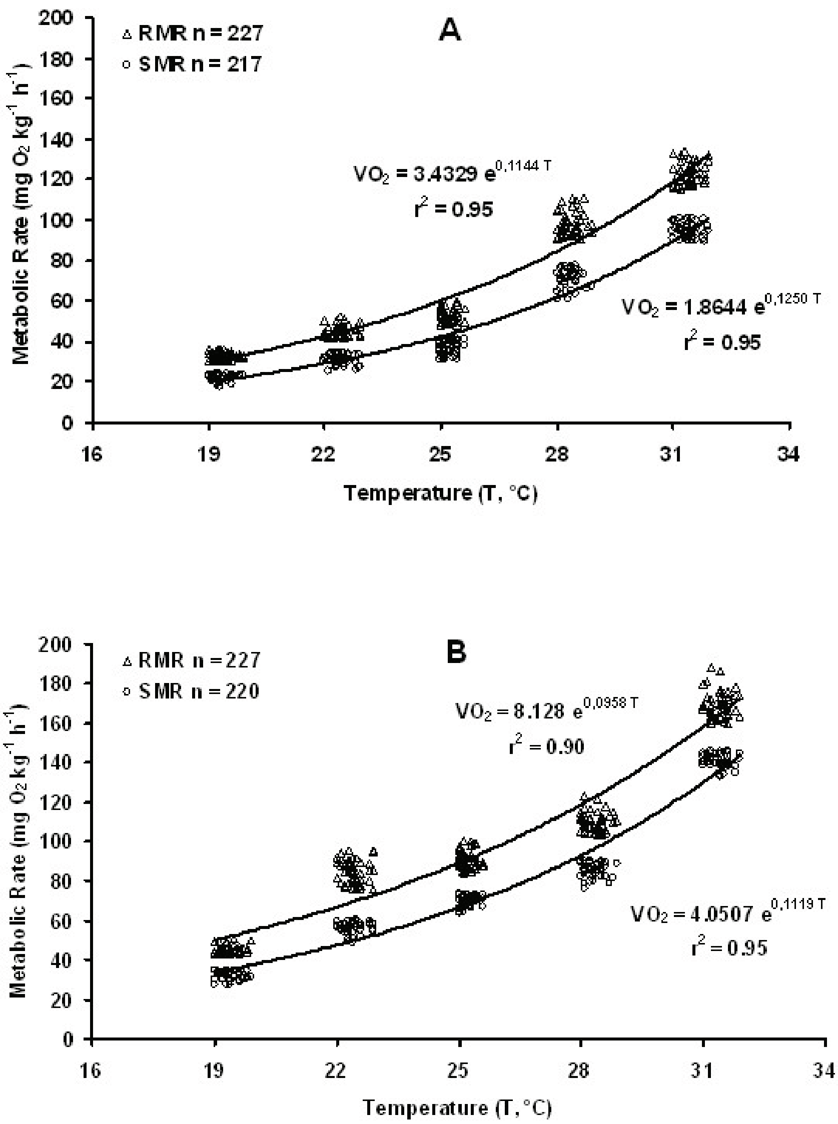

Fig 2. Relationship between water temperature and standard and routine metabolism after elimination of the outliers. The exponential regression lines represent best model for standard (SMR) and routine metabolic rates (RMR) with 52.1 (A) and 205.4 g (B) tilapia at various water temperature regimes

Şekil 2. Aykırı değerlerin atılımından sonra su sıcaklığı ile standart ve rutin metabolizma arasındaki ilişki. Üssel regresyon çizgileri 52.1 (A) ve 205.4 g (B) ağırlığındaki Nil tilapyalarının farklı sıcaklık rejimlerdeki standart (SMO) ve rutin metabolik oranlar (RMO) için en iyi modeli temsil eder

Measurements of oxygen consumption rate in tilapia have been estimated by various researchers. Estimates of Nile tilapia metabolic rate in terms of mg O_2 kg^{-1} h^{-1} can be calculated from the literature data. For example, estimated routine metabolic rates only for a 50 g fish at 25°C were 196 mg O_2 kg^{-1} h^{-1} by using a closed respirometer in Mozambique tilapia [23], 187.5 and 136 mg O_2 kg^{-1} h^{-1} in red tilapia and Mozambique tilapia [8,9] and 81 mg O_2 kg^{-1} h^{-1} in Nile tilapia with a constant flow respirometer [24]. One of the previous studies about on the metabolic rate of Nile tilapia was restricted to measurements of oxygen consumption for one temperature regime only [7]. In comparison, the standard and metabolic rates of 50 g Nile tilapia at the same water temperature used in this study were estimated as 36.6 and 52.4 O_2 kg^{-1} h^{-1} *(Table 1)*. There is a clear variation about reporting and describing the metabolic rates among the authors even in the same species, temperature and body weight for the respiratory data. Besides size and temperature, Ross and McKinney [10] indicated that a photoperiod mediated respiratory cycle in which respiration rate was higher during the light period than dark period. The discrepancies between the data of different researchers may be attributed by varying levels of stress, types of equipment and experimental protocol [10]. Respirometers vary considerably in size and overall design and there may be notable differences in results from closed or open-flow respirometers [9]. Apparently, it is very difficult to establish a true constant value without variation even at similar conditions. Therefore, a wide range of possible oxygen consumption rates could be calculated depending on the source data used in the planning of experimental conditions. In addition, an understanding of the factors affecting rates of oxygen consumption is required so that a comprehensive and applicable model can be used in a given species.

The water temperature is a strong and most important abiotic environmental factor which controls the metabolic rate in physiology of fishes [6]. One of the early studies by Ege and Krogh [25] showed that the metabolic rate of a goldfish increased exponentially as the ambient water temperature increased, providing a set of data that formed a basis for what is now known as "Krogh's standard metabolic curve" [25]. Respiratory data in this study confirmed Krogh's widely accepted standard metabolic curve idea relating to metabolic rate and temperature. This result corroborated the finding of Caulton [8,9] with red tilapia, *Tilapia rendalli*, and Mozambique tilapia, *Oreochromis mossambicus*, and Tucker and Robinson [24] with channel catfish, *Ichtalurus punctatus*. At higher water temperature, therefore, the metabolic rate of fish exponentially increases, unless they have some physiological mechanism to counter the temperature effect. As the increase in metabolic rate leads to the reduction in the amount of available energy for growth, for some species the increase in water temperature could slow down their growth, and thus make them less "fit" in the new environment.

Bioenergetic models have been demonstrated to be useful parameters to study the relationship between growth and food consumption in fish under different temperature regimes [26,27]. Therefore, the results of the present study provide parameters for a bioenergetic model for Nile tilapia. If it is assumed that the standard and routine metabolism are supported by aerobic metabolism and the oxidation of fat [28], then the oxycaloric conversion factor for fat from ELLIOTT AND DAVIDSON [29] of approximately 14 J mg O_2^{-1} can be used to calculate the energy requirement for the standard and routine metabolism of fingerling and adult Nile tilapia at given water temperature. In this approach the energetic costs, including basic metabolism and cost for locomotion, of Nile tilapia fingerling and adult exposed to acute changes in temperature was determined.

Respiratory responses have been used to monitor pollutant stress in fishes because of rapid and useful tool for identifying short pollution events [30]. Increasing demand on freshwater resources by industry and domestic use has led to the necessity for more efficient methods of detecting and monitoring pollutions in rivers and lakes. Since the changes in the aquatic environment cause the behavior of fish and breathing patterns to change, researchers have been using the breathing pattern of fish to detect industrial and agricultural spills in water supplies [4,31,32]. The oxygen consumption measurements were recommended as a robust indicator of whole fish stress and concomitant water quality [4]. Thus, Nile tilapia respiration rate models improved in this study can be successfully used in bioassay testing of industrial and municipal effluents before they are discharged into receiving waters.

Dissolved oxygen is one of the most important limiting factors in intensive fish culture. On the basis of measured metabolic data, maximum stocking densities of Nile tilapia under different water temperatures can be calculated. Increasing temperature in the culture system reduces dissolved oxygen and increases the metabolic rate, and then the aquaculturist can respond to this problem only by increasing oxygenation or decreasing stocking density to avoid a disaster.

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