



The effect of temperature on the standard and routine metabolic rates of young of the year sterlet sturgeon (*Acipenser ruthenus*)

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Abstract. This study examined the effect of temperature on the standard metabolic rate (SMR) and routine metabolic rate (RMR) of sterlet sturgeon (*Acipenser ruthenus*), acclimated at one of the five temperatures (10, 15, 20, 24 and 28°C, n = 6 for each tested temperature). The oxygen consumption rates were measured using intermittent flow respirometry systems (Loligo systems). The one-way Anova analysis revealed significant differences ($p < 0.05$) between the RMR and SMR values at all tested temperatures. Both SMR and RMR increased linearly with raising of temperature, from 68.61 ± 4.77 mg O₂ kg⁻¹ h⁻¹ for SMR and 92.08 ± 3.21 mg O₂ kg⁻¹ h⁻¹ for RMR to a maximum of 265.27 ± 12 mg O₂ kg⁻¹ h⁻¹ for SMR and 339.29 ± 11.65 mg O₂ kg⁻¹ h⁻¹ for RMR at 28°C. Overall, the results suggest that sterlet sturgeon is sensitive to temperature changes.

Key Words: sturgeon, oxygen consumption, respirometry, temperature.

Introduction. Environmental factors strongly impact the metabolic costs of animals (Keddy 2001). From all the factors affecting the metabolic rate of a fish (oxygen consumption), temperature has been coined as the “abiotic master factor” (Brett 1971). Since most fish are ectotherms, temperature plays a strong role in determining their distribution and abundance (Kieffer et al 2014). The impact of it upon the metabolic rates of a fish was widely studied through respiration measurements and the results have been well described (e.g. Fry 1971; Prosser 1991; Hölker 2003; Peck et al 2005). Also, it was noticed that the metabolic rate increases with the raise of temperature, typically in a linear or exponential fashion (e.g. Fry & Hart 1948; Brett & Glass 1973; Brett & Groves 1979; Carlson & Parsons 1999; Lee et al 2003; Kieffer & Cooke 2009). While there have been numerous studies on the effects of temperature changes on oxygen consumption in various species of fish (see above references), less is known about the effects of temperature changes on oxygen consumption rates in ‘primitive’ fish, such as sturgeon (Mayfield & Cech 2004).

The effects of temperature on the general metabolism of sturgeon species, however, are not well understood (Kieffer et al 2014). An understanding of the metabolism of sturgeon is particularly relevant now, due to the declining stocks and the natural and anthropogenic impacts, of most species in this group (Singer & Ballantyne 2004). Virtually all species are endangered or threatened (Birstein et al 1997). The sterlet sturgeon, *Acipenser ruthenus* (Linnaeus, 1758), is a freshwater fish that inhabits large rivers in Eurasia and is the only sturgeon native to both Asia and Europe (Bemis & Kynard 1997). The sterlet sturgeon is one of the smallest sturgeons, its standard length doesn’t go beyond 1 m and it weights up to 20 kg (Wegner et al 2009). Like all acipenseriformes members, it is classified by the IUCN Red List as endangered because the sterlet populations are seriously affected by the heavy pollution and anthropogenic activities (hydraulic constructions, dams, overfishing). Dams and other hydraulic

structures across rivers have substantial effects on fish physiology and spawning migration, and any acutely low or high temperature change is stressful (Mandal et al 2016). Low temperatures result from the hypolimnetic release of water from upstream reservoirs (Clarkson & Childs 2000) as well as from the use of ice prior to fish release by the aquaculture industry (Hyvarinen et al 2004). High temperatures are caused by thermal pollution in discharges from power plants and factories and the release of water from the epilimnion of reservoirs (Birtwell & Kruzynski 1989). Temperature change also accompanies the tidal cycle in estuarine reaches of rivers that discharge into the sea (Mandal et al 2016). Acute temperature change produced by anthropogenic activities is typically more severe than the routine change of temperature in nature (Mandal et al 2016). Thus, the acute temperatures resulting from human activity alter fish swimming behavior, muscle performance and metabolism more than what naturally occurs in temperature changes (Rome 1990).

Temperature has a profound effect on all physiological functions, including cardio-respiratory capacity, digestion and swimming performance (Fry 1971; Kieffer et al 1994; Luo & Xie 2008; Zeng et al 2009). Furthermore, oxygen availability also changes with temperature, which may profoundly affect metabolic rates in fish species (Pang et al 2011). Most of the studies have focused on the effect of temperature on swimming capability (Lee et al 2003; Deslauriers & Kieffer 2012; Cai et al 2014). There is little information available for the standard and resting oxygen consumption of sturgeon species in relation with temperature fluctuation (McKinley & Power 1992; Secor & Gunderson 1998; Wakefield et al 2004).

The objective of this study was to investigate the effect of acclimated temperature conditions (10, 15, 20, 24 and 28°C) upon the standard metabolic rate (SMR) and routine metabolic rate (RMR) for young of the year sterlet sturgeon. The temperatures used in this experiment replicate those that would normally meet by sterlet sturgeon in its natural environment. Examining the effects of acclimated temperature exposure is important because acclimated temperature exposure can be encountered in the natural habitat of a fish, due to both ordinary seasonal temperature fluctuations and anthropogenic influences that can alter water flows temperatures (Wakefield et al 2004).

Material and Method

Animal husbandry. Young of the year sterlet sturgeon (53.4±7.12 g) were achieved from Horia sturgeon farm, Tulcea county. Fish were kept in tanks (1 m diameter, water volume of 0.700 m³ each) for two weeks, at the Romanian Center for the Modeling of Recirculating Aquaculture Systems (MoRAS), facility of University "Dunărea de Jos", Romania; the experiment took place between January and March 2017. Tanks were constantly supplied with a flow through of fresh and aerated water with a temperature of approximately 20°C. The water temperature for the experiment was kept constant with the help of system incorporated heaters and chillers. The recirculating system characteristics have been described in other previous paper (Andrei et al 2017).

Water quality parameters were monitored daily with integrated sensors from the system and were kept in normal limits for the sturgeons (Mims et al 2002). Fish were fed once a day, at 12:00h, at a feeding intensity of 2% body weight with commercial pellets (2 mm, 50 % protein, 14% lipid). Prior to experimental protocol fish were not fed for 48 h to ensure a post absorptive state.

Experimental protocols. Rates of oxygen consumption (MO₂) were measured using intermittent flow respirometry systems (Loligo Systems, Denmark). The respirometry system is situated in a sound isolated room to diminish any disturbance of the fish. We used 3 resting chambers (Figure 1) with a water volume of 2.6 L, which were connected to a computer by an integration box whereby trials were monitored by a programmed computer (Loligo Systems, Denmark). The respirometry system contained a flush pump, re-circulation pump, oxygen probe and temperature electrodes. A single experimental fish was placed into each respirometer. Three resting chambers were submerged in an ambient tank filled with freshwater maintained at the established temperature. Water

was mixed and passed over the oxygen probe constantly by the recirculating pump. The water inside the resting chamber was replaced with the water from the ambient tank between the measurement phases; also, the oxygen content of the water was kept constant between 90 and 100% O₂ saturation using air stones.

The resting chambers and the body mass of the starlet sturgeons allowed a degree of natural activity of the fish (i.e. body undulation and small fin moves to maintain position). Measurements of MO₂ (mg O₂ kg⁻¹ h⁻¹) were carried out every 7 min using computerized intermittent flow respirometry. The repeated respirometry loops consisted of a 4 min flushing phase, 2 min waiting phase and 3 min measurement phase. The program calculates the mass-specific oxygen rates (mg O₂ kg⁻¹ h⁻¹) taking into account the automatic values acquired during the measurement phase.

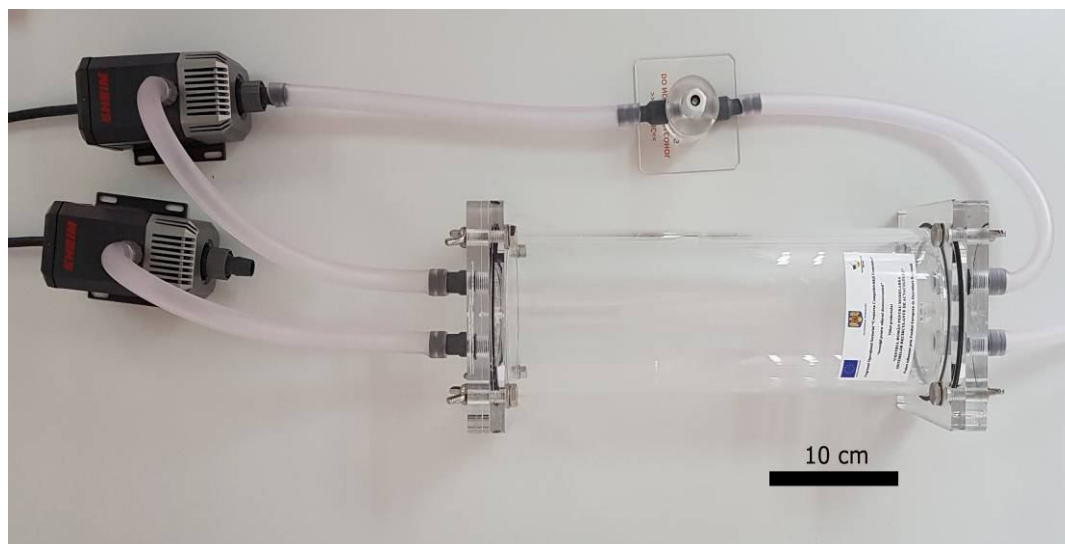


Figure 1. The static chamber used in the experiment.

Metabolic rate determinations. The young of the year sterlet sturgeon were accommodated for at least 10 days to one of the experimental temperatures (10, 15, 20, 24 and 28°C, n = 6 for each tested temperature) in the holding tanks which has incorporated chillers and heaters. On the measurement day, fish were arbitrarily chosen, quickly weighed and placed into the resting chambers. A black plexiglass covered the ambient tank.

After the fish were transferred to the resting chambers they were allowed to recover from the transfer for 4 h before the oxygen consumption started to be monitored. The recovered period was preferred to eliminate all the possible inaccuracies and to ensure the oxygen levels were stable, even if fish accommodated faster (aprox. 60-120 minutes) following moving stress (Kieffer et al 2001, 2014). After 4 h adaptation period, the oxygen was measured for 24 h. After 24 h, the experiment was stopped, fish were removed from the respirometer and placed in a separate holding tank, this way making sure we didn't pick the same fish for another experimental trial.

Calculations. Using the automatic values taken during each measuring phase, the software calculates the mass-specific oxygen rates (mg O₂ kg⁻¹ h⁻¹), according to the following equations:

$$MO_2 = ([O_2]_{in} - [O_2]_{out}) \times F/BW$$

where: F = water flow rate (L hour⁻¹);

[O₂]_{in} = oxygen content in water inflow (mg L⁻¹);

[O₂]_{out} = oxygen content in water outflow (mg L⁻¹);

BW = body weight of the fish introduced in the experiment (kg).

SMR in individual fish was calculated as the average of the lowest 10% from the measurements of MO_2 values collected over 24 h (Herrmann & Enders 2000; Rosewarne et al 2014).

RMR were calculated as the average of the data after the first 4h of accommodation was exceeded. The 4h accommodation period was chosen to assure that the fish's respiration is kept at a constant level (Kieffer et al 2014).

Statistical analysis. All the obtained data were analysed using Microsoft Excel 2010 for Windows and SPSS 21. The data are given as means \pm SD. One-way Anova was performed for the rate of oxygen consumption, calculated RMR and SMR for all six fish from each temperature. When significant differences were found, Tukey's test was applied. Results of all statistical tests were regarded as significant at $p < 0.05$.

Results and Discussion. The environmental factor which probably has the greatest effect on metabolic rate in ectotherms is temperature (Zhang & Kieffer 2017). According to some authors, temperatures beyond high and low thermal limits lead to reduced performance because tissue demands in oxygen exceed oxygen delivery (Sommer et al 1997; Pörtner & Zielinski 1998; Pörtner et al 2000; Chabot et al 2013). This phenomenon happens because of the slowing of circulation and ventilation in the cold, and their insufficient increase in the warm (Pörtner 2001).

The metabolic rates of young of the year sterlet sturgeon were measured by computerized intermittent flow-through respirometry. After all the automatic readings ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) were taken for each fish, the SMR and RMR metabolic rates were calculated according to the presented methodology. In Figure 2 are presented the average of the RMR ($n = 6$ fish) which were obtained during the measurement period and after the first four hours have been eliminated. From these values was determined the mean, minimum and maximum values.

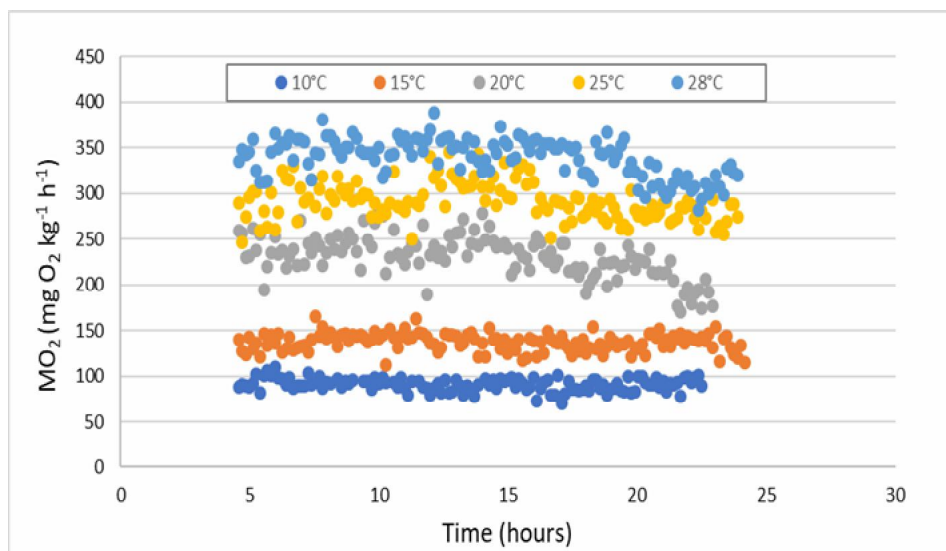


Figure 1. The average of RMR ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) after 4h accommodation period for the tested temperature ($n = 6$ fish).

Significant differences were found in the minimum, mean and maximum values of the RMR ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) between the tested temperatures. It can be observed an increasing trend of all values from 10 to 28°C (Figure 2).

A linear relationship was observed between the water temperature and metabolic rates of sterlet sturgeon (Figure 3). It can be observed that the temperature has a significant influence on the metabolic rate. The oxygen consumption increases rapidly being influenced by temperature, to a maximum of $265.27 \pm 12 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ for SMR and $339.29 \pm 11.65 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ for RMR in the case of 28°C. The one-way Anova analysis showed significant differences ($p < 0.05$) between the values of RMR and SMR

for the tested temperatures. In fact, the post hoc analysis Tukey revealed significant differences for RMR and SMR between all the tested temperatures.

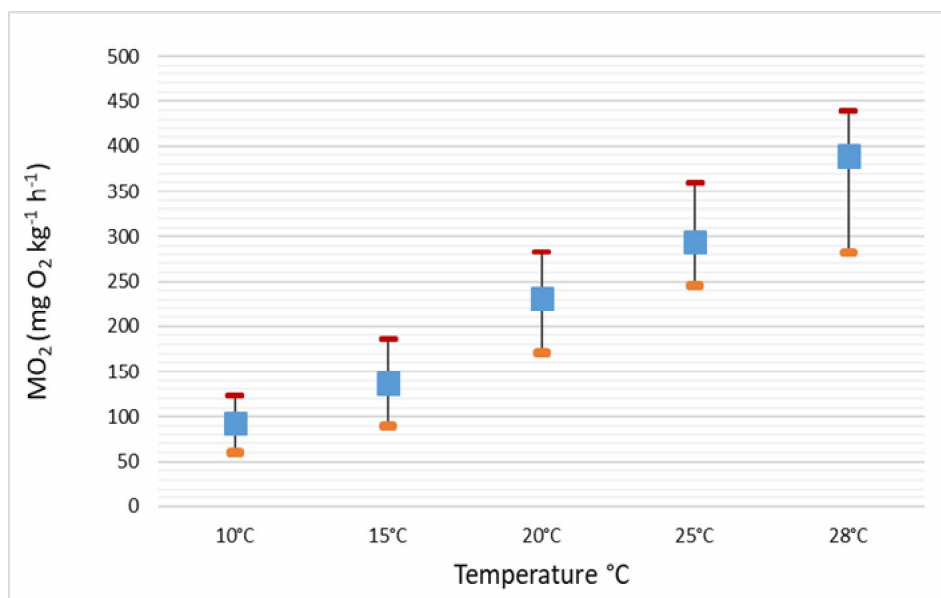


Figure 2. The minimum, average and maximum values of RMR (mg O₂ kg⁻¹ h⁻¹) obtained after 4h accommodation period for the tested temperature (n = 6 fish).

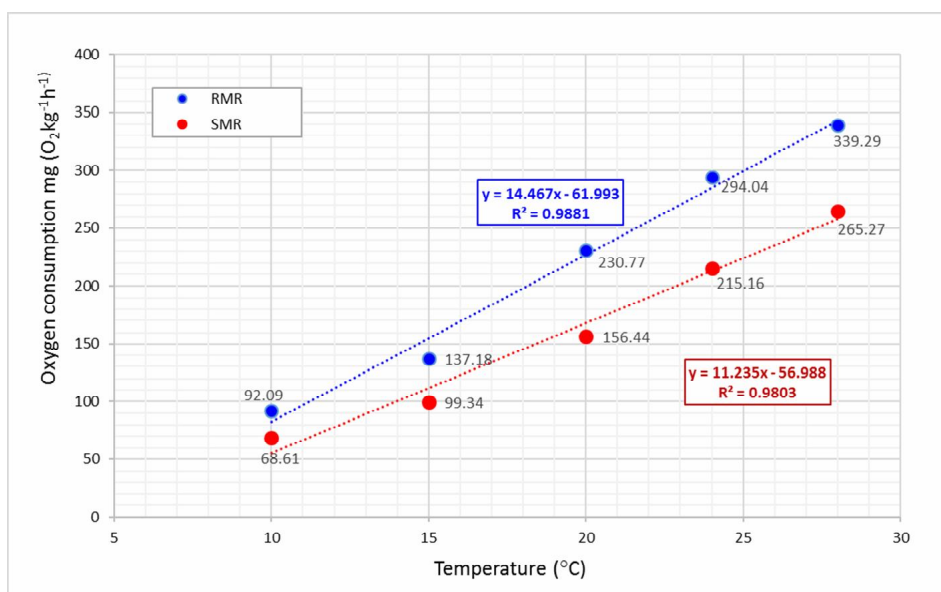


Figure 3. The relationship between water temperature and the SMR and RMR.

In our experiment, both SMR and RMR increased with increases in acclimation temperature. These findings are similar to other studies. For example, Zhang & Kieffer (2017) reported for 100 g shortnose sturgeon (*Acipenser brevirostrum*) a RMR of 127 ± 8.8 at 15°C, 199 ± 30 at 20°C and 253 ± 18 at 25°C, which are quiet similar to those obtained for the sterlet sturgeon, especially at 15 and 20°C. Also, an increase in metabolism can be observed in a study by Kieffer et al (2014), where 10 g shortnose sturgeon was used. The authors found a metabolic rate of $200 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 10°C and after exposure to 15°C, the metabolic rate increased significantly to $350 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$. This rate further increased to $389 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ after exposure at 20°C, and finally to $437.8 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 25°C. Also Mayfield & Cech (2004) showed that temperature beyond 19 to a limit of 24°C affects the MO₂ of 0+ green sturgeon (*Acipenser medirostris*), by obtaining a high Q₁₀ coefficient (Q₁₀ = 4.12). Also, green sturgeon exhibit higher metabolic rates compared with other sturgeons. This may happen because of

larger red muscle masses (Moyle 2002). Red muscle, with its higher mitochondrial density than white muscle, exhibits higher tissue MO_2s (Gordon 1968).

Even with these comparisons, other studies reported higher rates of metabolism at lower temperatures that were quite different from the present study. For example, Kieffer et al (2001) reported for *A. brevirostrum* and *A. oxyrhynchus* a RMR of $112 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 12°C . This shows that the acipenseridae members reveal a different temperature sensitivity.

This increasing tendency of RMR and SMR is also confirmed by our obtained results at the five tested temperatures. This increasing trend was predictable, since it was theorized that increases in temperature would have increased effects on metabolism, as has been noticed in other species of fish: salmonids (Lee et al 2003); largemouth bass (Cooke et al 2001); sharks (Carlson & Parsons 1999). According to Turker (2011), at higher water temperature, the metabolic rate of fish increases unless they have some physiological mechanism to counter the temperature effect.

Also, our results suggest a high sensitivity of metabolic rate related to temperature in this fish. The higher metabolic sensitivity means greater physiological variability with fluctuating temperature, as well as reduced viability upon exposure to extreme temperature (Hawkins 1995). Some fishes may migrate to cooler areas when water temperature is too high (Sogard & Olla 1998; Lafrance et al 2005; Morita et al 2010). Annual temperature range is -7 to 25°C in Volga, Ural and Danube systems, where sterlet sturgeon lives (IUCN Redlist). This shows that there is a large annual fluctuation in the metabolic rate. Our results suggest that changes in water temperature induced by different natural (i.e. global warming) or anthropogenic factors (i.e. dam construction, pollution, water released by hydroelectric power stations etc.) could have major negative impacts on the fitness of acipenseridae members. The increase in metabolic rate of individual fish would lead to an increase in the total energy demand of the population (Luo et al 2012).

Conclusions. Our study results are based on laboratory experiments performed with a respirometer. The findings from the present research suggest that the standard and the routine metabolic rate of the sterlet sturgeon are strongly impacted by temperature. This study may help with the conservation, ecology of the sterlet sturgeon and for a better design of fishways. Also, as various species of sturgeons are being raised in aquaculture, it is very important to understand the effects of temperature on the stress response. However, a more comprehensive analysis of the implication on the temperature on sturgeon physiology and biology is needed as they represent a vulnerable species in need of conservation and protection.

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