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Comparison of Metabolic Rates of Young of the Year Beluga (*Huso huso*), Sterlet (*Acipenser ruthenus*) and Bester Hybrid Reared in a Recirculating Aquaculture System

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Abstract: In the present study, oxygen consumption of two sturgeon species, beluga (*Huso huso*), sterlet (*Acipenser ruthenus*), and their hybrid reared in a recirculating aquaculture system were compared over body intervals from 54–107 g to determine the interspecific variation of metabolic rate. Metabolic rates were measured using the intermittent-flow respirometry technique. Standard oxygen consumption rates (SMR, mg O_2 h^{-1}) of sterlet were 30% higher compared with beluga and 22% higher compared with bester hybrid. The routine metabolic rate (RMR, mg O_2 h^{-1}) averaged 1.58 ± 0.13 times the SMR for *A. ruthenus*, 1.59 ± 0.3 for *H. huso*, and 1.42 ± 0.15 for the hybrid bester. However, the study revealed no significant differences ($p > 0.05$) between mean values of SMR and RMR for beluga and bester hybrid. The scaling coefficient reflected a closed isometry for the hybrid (b = 0.97), while for the purebred species the coefficient of 0.8 suggests a reduction in oxygen consumption with increasing body mass. These findings may contribute to understanding the differences in growth performances and oxygen requirements of the studied species reared in intensive aquaculture system.

Keywords: routine metabolic rate; standard metabolic rate; intermittent flow respirometry; sturgeons

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1. Introduction

Aquaculture is ultimately a multidisciplinary industry that benefits from several scientific disciplines, including biology, ecology, animal behavior, and engineering [1]. The fast development of highly intensive production systems such as recirculating aquaculture systems (RAS) was possible as a result of the common scientific effort of both engineering science, to optimize system design or waste management [2,3], and biological science, aiming to understand the physiological requirements of the cultivated species under intensive conditions. Considering the relatively high cost of water pumping or oxygen generation in recirculating aquaculture systems, it is important to understand the oxygen requirements of cultivated species under intensive conditions [4]. Chebanov et al., 2011 [5], suggests that the equipment for thermoregulation, water degassing, aeration and oxygenation, part of the rearing systems, should be incorporated at sufficient capacity, precalculated based on species's metabolic rates.

Standard metabolic rate (SMR) is defined for whole organisms exhibiting minimal functional activity, i.e., in the total absence of voluntary muscular movements and when no food was being digested or absorbed [6]. However, whenever subjects show some minor activity in a respirometer (swimming or maintaining position) many authors prefer the term routine metabolic rate (RMR), which includes a minor cost of the activity [7,8].

Generally, SMR is correlated with growth trajectories, maximum aerobic performance, and some behaviors [9,10] and has an important role in ecological fitness.

The metabolic rates of fish are influenced by multiple factors [11,12]. Between those factors, the most important are body weight and temperature [13]. In general, body weight increase leads to the resizing of the whole body. This change corresponds to the growth of body muscles, which efficiently transfer the energy, optimizing the locomotion and routine activity of the body [14]. On the other hand, body mass constrains the metabolic rate and assimilated energy, thus influencing the entire life cycle of a species, including their growth, reproduction, and survival [15–17].

In aquaculture, respirometry targets several purposes. Some examples may include the fish body's ability to interact with the husbandry conditions (e.g., diet, temperature, water quality, stocking density, and live transport) [5]. Others are related to the construction of the bioenergetic models, which are widely used as an analytical tool to address a broad range of questions in physiology, ecology, aquaculture, and fisheries management (e.g., feeding and growth of fish at different life stages, evaluation of the impact of invasive species on aquatic ecosystems, contaminant accumulation by fish, effects of climate change on foraging, growth, and mortality of fishes, etc.) [18–20].

It is well known that the Acipenserides are famous for their large size, superior meat quality, and their fish product, caviar [21,22]. That is why, in the last decade, the production of sturgeon in aquaculture has rapidly increased, aside to cover the market demands [23], but also to respond to the restocking purposes under international conservation programs [24]. Most of the respirometry studies of sturgeon species have followed also the relationships between MO_2 and body mass [25], temperature [26–28], or feeding management [29]. However, few species have been subjected to routine metabolic rate evaluation: *A. brevirostrum* [26,30], *A. medirostris* [31,32], *A. naccarii* [33], *A. oxyrinchus* [34] and currently large variability in the data exists due to different fish sizes analyzed, rearing conditions or methodology used. Regarding Ponto–Caspian sturgeon species or hybrids, there are no comparative studies about metabolic traits available according to our knowledge.

Sometimes in some farms, different species of sturgeon are bred in intensive conditions, within the same RAS, without considering different physiological requirements for oxygen or having sufficient insight into their metabolic rates. Hence, in the present research, we aimed to assess the interspecific variability of metabolic rates represented as Standard metabolic rate (SMR) and Routine metabolic rate (RMR) of beluga, sterlet, and their hybrid (*Huso huso* × *Acipenser ruthenus*) reared in the specific conditions of a RAS. Thus, measured metabolic rates serve for comparison and their relationship to body mass will be discussed within the framework of existing data available on other sturgeon species. Knowledge of oxygen consumption patterns of the three species will provide basic information for further studies on sturgeon physiology with higher applicability in RAS design for the aquaculture of sturgeon.

2. Materials and Methods

2.1. Fishes

Fishes were obtained from a private fish farm, Danube Research Consulting Company (DRC) from Tulcea County, Romania. Before the experiment, fishes were raised for 30 days in a recirculating aquaculture system, at the Romanian Center for the Modeling of Recirculating Aquaculture Systems (MoRAS, www.moras.ugal.ro, accessed on 5 June 2021), a facility of University Dunărea de Jos, Galați, România (Supplementary Figure S1).

During the accommodation period, fish were fed twice per day (at 8:00 and 16:00), at a feeding level of 2% body weight (% BW day^{-1}), with a commercial feed (1 mm diameter, 54% protein, 15% lipids, 0.5% fiber, ash, and 21.1 kJ g^{-1}; Alltech Coppens, Leende, The Netherlands). During the experiment duration, a 11:13 h light:dark cycle was maintained. All the fishes were growing in the same technological condition at a stocking density of 4.50 kg m^{-3}. At the start of the experiment, all the fishes were seven months old.

Additionally, during this period, water quality was monitored daily, with the help of the sensors integrated into the RAS, for temperature (22.04 ± 1.16 °C), dissolved oxygen (7.38 ± 1.09 mg L^{-1}), pH (7.53 ± 1.27 pH units), and ammonium (0.27 ± 0.2 mg L^{-1}). The concentrations of nitrite (0.03 ± 0.01 mg L^{-1}) and nitrates (33.45 ± 9.23 mg L^{-1}) were confirmed weekly with Spectrophotometer Nova 400, compatible with Merck kits (Merck, Darmstadt, Germany).

After one month, nine fishes from each species were randomly selected in such a manner to have more or less the same size range among species (Table 1).

Table 1. Mean ± standard error, minimum and maximum values for body mass and length of the tested fishes.

Species	Fish Weight (g)			Total Length (cm)		
	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum
Acipenser ruthenus	77.33 ± 5.36	54.00	99.00	28.2 ± 1.40	23.10	29.93
Huso huso	84.78 ± 4.42	67.00	107.00	30.2 ± 1.23	27.12	32.71
Bester	75.33 ± 2.50	65.00	89.00	27.9 ± 1.24	27.24	29.22

No significant differences (p >0.05) were recorded between individual weight and length of the three sturgeon species (ANOVA).

2.2. Respirometry Tests

The measurements of oxygen consumption MO_2 (mg O_2 kg^{-1} h^{-1}) were assessed through the intermittent-flow respirometry technique. Intermittent-flow combines the principles of closed-system and open-system respirometry [26,35,36].

The respirometer system (Loligo® Systems, Viborg, Denmark) contains four acrylic chambers with a volume of 2.6 L each, and was placed in an isolated room to diminish any fish disturbance which could lead to erroneous records of oxygen consumption. The chambers were submerged in a black tank with a volume of 200 L (Figure 1). Special barriers made from black plexiglass were placed to avoid visual contact between experimental fish in the chambers. Fish were placed in three chambers of the respirometer, while the fourth was left empty in order to measure the background respiration. The chambers were connected to a computer through a DAQ m instrument (Loligo® Systems, Viborg, Denmark) which is used for data acquisitions and relay controlling in combination with Loligo software (Loligo® Systems, Viborg, Denmark).

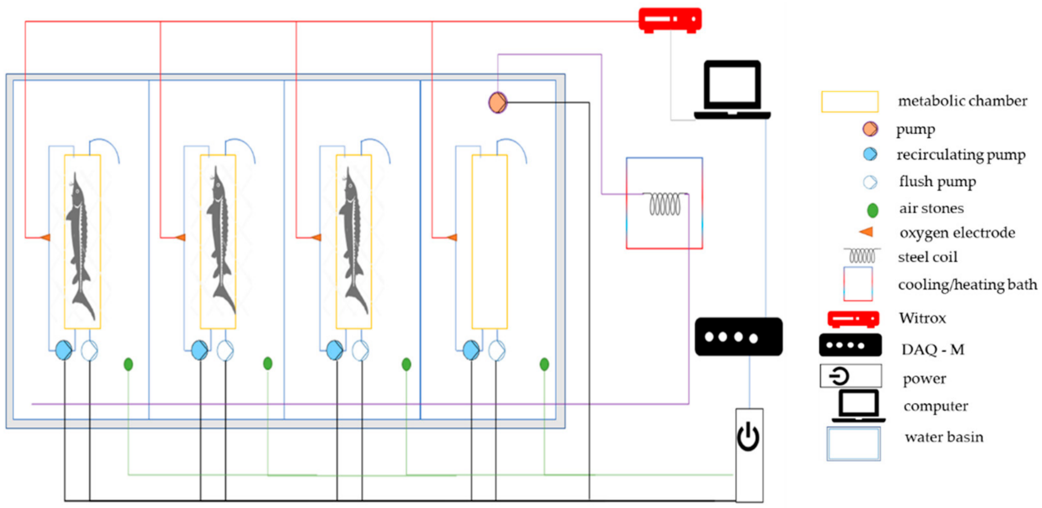

Figure 1. The diagram of the intermittent-flow respirometer and the experimental design.

The respirometry system comprised also a flush pump, recirculation pump, oxygen electrodes, temperature probe and a steel coil for water temperature control. The oxygen electrodes and temperature probe were connected to the Witrox equipment (Loligo® Systems, Viborg, Denmark). The recirculation pump runs continuously to ensure the proper mixing of the water inside the metabolic chambers and the passage of water through the oxygen electrode, while the flush pump exchanges water from inside the respirometer with water from the ambient tank between the measurement intervals. During the periods of oxygen consumption measurements, the flush pump was turned off while the recirculation pump remained on. After this interval, the flush pump turns on and flushes out the water from the respirometer, by replenishing it with ambient tank water [26]. One complete measurement cycle (loop) consists of an open-system flush period (F—120 s) and a closed-system, metabolism determination cycle, which itself is made up of a waiting period (W—60 s) and a measurement period (M—240 s) [36].

Oxygen content (% air saturation; O_2 saturation) of the water in the tank was controlled using air stones and the oxygenation level was kept over 95% O_2 saturation. During all respirometer experiments, the water temperature was 22.09 ± 0.54 °C. Before each test, the oxygen sensors from the respirometer system were calibrated under 100% oxygen saturated water and the chambers were cleaned and disinfected.

The fishes used in the respirometry experiments were in a postabsorptive state, feeding being stopped 24 h before testing. Before fish testing, the body weight and length were measured. All measurements were performed under anesthesia with phenoxyethanol (8 mL 40 L^{-1} of water for 5 min) [37] and all efforts were made to minimize suffering and reduce the number of fish used.

2.3. RMR and SMR Calculations

Using the automatic values taken during each measuring phase, the program calculates the mass-specific oxygen rates (mg O_2 kg^{-1} h^{-1}), according to the equations:

$$MO_2 = ([O_2]in - [O_2]\ out) \times F/BW \quad (1)$$

where: F = water flow rate (L h^{-1}); $[O_2]$ in = oxygen content in water inflow (mg O_2 L^{-1}); $[O_2]$ out = oxygen content in water outflow (mg O_2 L^{-1}); BW = body-weight of the fish introduced in the experiment (kg).

From all the obtained measurements only oxygen consumption rates with a regression coefficient greater than 0.95 [38,39] were taken into consideration to calculate the routine and standard metabolism. For each test group, the routine metabolic rates (RMR) were calculated as the average of the data after the first 4h of accommodation was excluded, while SMR was calculated as the average of the lowest 10% from the measurements of MO_2 values [27,40].

2.4. Statistical Analyses

All the obtained data were analyzed using SPSS 21 (SPSS Inc., Chicago, IL, USA). The values in tables are presented as an average ± standard error. The normality of the data used for statistical analysis was determined by Kolmogorov–Smirnov tests. One-way ANOVA was used to detect the differences in SMR and RMR mean values of different sturgeons. If differences among species were recorded, a Duncan test was performed. A *p*-value lower than 0.05 was considered statistically significant.

Slopes and intercepts of linear regressions describing the relationship of mass to respiration rate were assessed across the three species, after log10 transformation of the data. Regression analyses were used to generate parameters for predictive models of oxygen consumption, according to the equation:

$$SMR = a \times M^b \quad (2)$$

where “a” is the scaling coefficient (intercept) and “b” is the scaling exponent (slope).

3. Results

Each fish was kept in the respirometer for 24 h. Due to the stress installed during handling, after the transfer to the respirometer, the oxygen consumption of the fish was higher for about 3 h. However, for the validity of the measurements, the adaptation phase was set at 4 h. When the measurements were made, the fish were quiet, the oxygen consumption remaining constant during the respirometry tests. This fact indicates a rapid adaptation of fish to the metabolic chambers.

During the respirometry trial the background respiration measured in the empty chamber was insignificant (<5% of total measured metabolism). RMR was calculated as the mean of all the MO_2 measurements during the 20-h respirometry period (after removing the first four hours), and SMR was calculated by taking the mean of the lowest 10% of the MO_2 measurements. If abnormal outliers were identified (higher oxygen consumptions) due to the spontaneous activity, we removed these values from the calculations [7] (Figure 2).

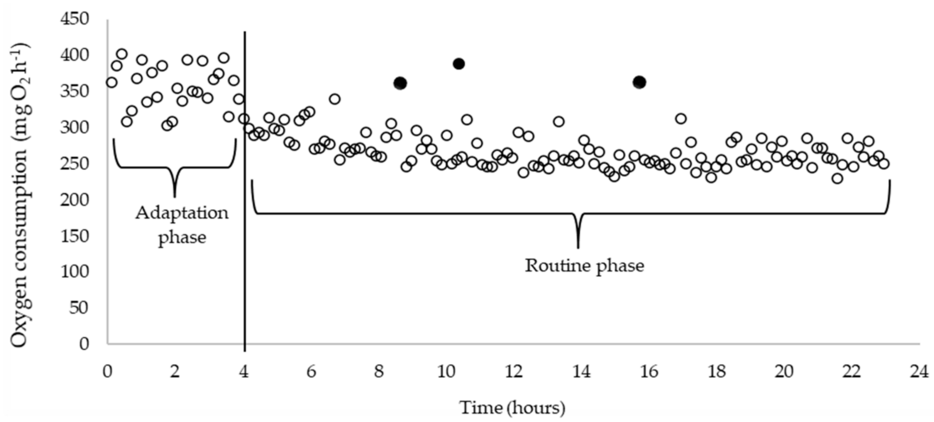

Figure 2. Example of oxygen consumption for beluga sturgeon (body weight 79 g). The first four hours and outliners "•" were excluded from the calculation of RMR and SMR.

The mean values of standard (SMR-mg O_2 kg^{-1} h^{-1}) and routine metabolic rates (RMR-mg O_2 kg^{-1} h^{-1}) for the experimental variants are presented in Figure 3. Mean SMR values were 276.49 ± 27.37 mg O_2 kg^{-1} h^{-1} for sterlet, 211.64 ± 17.26 mg O_2 kg^{-1} h^{-1} for beluga, and 226.9 ± 9.51 mg O_2 kg^{-1} h^{-1} for bester hybrid.

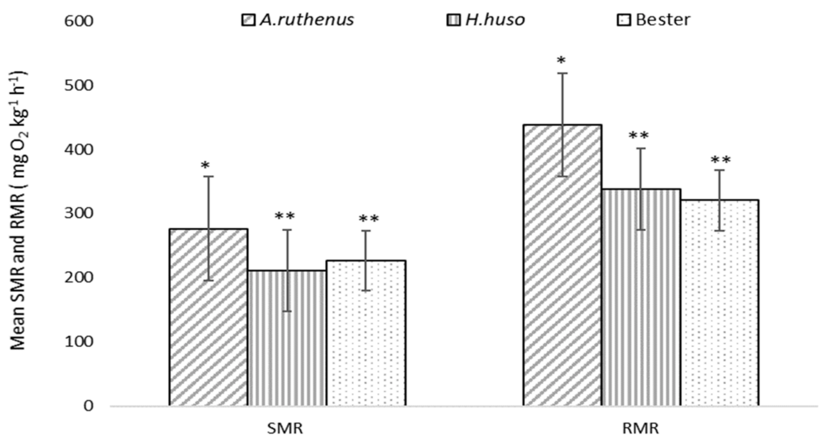

Figure 3. Mean SMR ± SE (mg kg^{-1} h^{-1}) and RMR±SE (mg kg^{-1} h^{-1}) values of sturgeon species during static respirometry trials. Different symbols */** indicate significant differences between values of SMR/ RMR of the studied species.

Mean RMR values were 437.49 ± 59.21 mg O_2 kg^{-1} h^{-1} for sterlet, 338.37 ± 71.38 mg O_2 kg^{-1} h^{-1} for beluga, and 320.35 ± 25.76 mg O_2 kg^{-1} h^{-1} for bester hybrid. The statistical analysis ANOVA showed significant differences ($p < 0.05$) between the obtained data for the RMR and SMR between the sterlet and the other two species (beluga and bester). No significant values ($p > 0.05$) were recorded between the obtained RMR and SMR of beluga and the hybrid.

The mean SMR values were around 17 mg O_2 h^{-1} for Beluga and hybrid while for Sterlet was approximately 20% higher. The routine metabolic rate averaged 1.58 ± 0.13 times the SMR for Sterlet, 1.59 ± 0.3 for Beluga, and 1.42 ± 0.15 for the hybrid (Table 2).

Table 2. Mean (±SE), minimum and maximum values for SMR and RMR (mg h^{-1}) of sturgeon species during static respirometry trials.

Species	SMR			RMR		
	Mean ± SE	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**
A. ruthenus	21.04 ± 0.96 **	17.33	24.51	33.06 ± 1.17 **	29.63	38.52
H. huso	17.78 ± 0.60 *	15.73	18.86	24.23 ± 1.32 *	20.28	31.25
Bester	17.03 ± 0.38 *	15.73	18.86	24.23 ± 1.32 *	20.28	31.25

*—No statistical differences ($p > 0.05$); **—Statistical differences ($p < 0.05$).

The relationships between standard metabolic rate and body mass for the three species are presented in Figure 4. All obtained data were log10 transformed and the regression analysis was used to describe the relationship between mass and fish respiration rate.

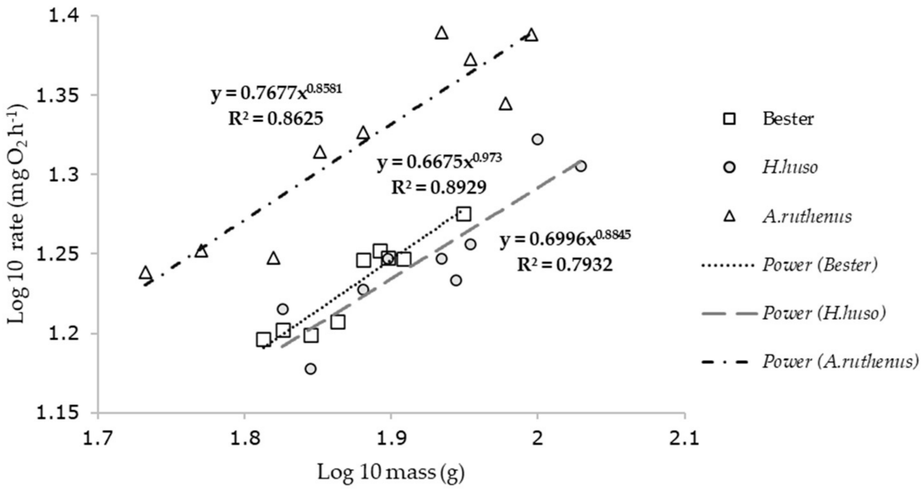

Figure 4. Relationships of log10 standard metabolic rate to log10 body mass for different sturgeon species during respirometry trials. Data are presented on $\log_{10}$ axes.

The scaling coefficients and the scaling exponents for all of these relationships are listed in Table 3. Slopes from the regressions were not statistically different between bester and beluga, but were statistically higher in the case of sterlet sturgeon. Additionally, it can be observed that large fish consume more oxygen, which is reflected in higher metabolic rates than small fish, but on a unit weight basis, small fish will consume more oxygen than larger fish [41].

Table 3. Relationship between oxygen uptake and mass of sturgeon species. β and r^2 were determined using data in Figure 4 (n = number of tested fish).

Species	Regression Equations		
	y = oxygen uptake (mg h^{-1}); x = mass (g)	**r^2**	***n***
Acipenser ruthenus	$y = 0.76x^{0.85}$	0.86	9
H. huso	$y = 0.69x^{0.88}$	0.78	9
Bester	$y = 0.66x^{0.9}$	0.89	9

4. Discussion

In recirculating aquaculture systems, due to high operational costs, oxygen supplementation must be judiciously estimated in order to cover both the physiological needs of the cultured species [42] and the chemical and biological consumption of oxygen due to the transformation processes of metabolites [43]. Understanding the metabolism of sturgeons is therefore relevant for improving RAS profitability.

The results of the present study revealed significant differences in the metabolism of the sterlet sturgeon, beluga, and the bester hybrid. Observations of higher locomotor activity of the sterlet were confirmed by a higher standard and routine metabolic rates measured during trials. The RMR (mg O_2 kg^{-1} h^{-1}) values obtained in our experiment are slightly higher compared with those reported by other authors for sturgeon species (Table 4). However, it is noteworthy that the used experimental conditions (fish weight, feeding regime, temperatures) were different than those from the present trial.

Among other authors quantifying RMR in sturgeons, Burggren and Randall [44] also reported RMR values around 270 mg O_2 kg^{-1} h^{-1} for the *Acipenser transmontanus*, with a body weight of 80 g. Similarly, Zhang et al. in 2017 [30] reported a routine metabolism of 253 ± 18 mg O_2 kg^{-1} h^{-1} for *Acipenser brevirostrum*, with a body weight of 100 g (Table 4).

The results of the present study, and other similar studies (Table 4), emphasize the lessening of metabolism with the increase of body weight. However, the metabolic rate variation does not depend only on weight but rather on of a series of intrinsic and extrinsic factors [41]. For example, some authors consider that a lower metabolic rate of some sturgeon species could partially be assigned to their depressed swimming ability [45]. The exposed aspects reinforce the idea that sturgeons represent a distinct group of fish, intermediates between sharks and more evolved bony fish (subclass Neopterygii), with special morphophysiology, which requires more complex studies [45]. The high diversity of data is reflected also by the high variability of measurement conditions (different sizes, temperatures, feeding regime) and therefore to the above-mentioned controversy.

Table 4. Mean routine metabolic rate RMR (mg O_2 kg^{-1} h^{-1}) of some sturgeon species.

Species	Body Weight (g)	RMR (mg O_2 kg^{-1} h^{-1})	Experimental Conditions					Reference
			T (°C)	Rearing System	Fasting Period before Trial (hours)	Photoperiod (hours)	Acclimatization Period before Trial (hours)	
A. brevirostrum	11	309 ± 33	25	FTS	24	natural	4	[26]
	100	127 ± 8.8	15	FTS	24	12 L/12 D	2	[30]
	100	199 ± 3	20	FTS	24	12 L/12 D	12	[30]
	100	253 ± 18	25	FTS	24	12 L/12 D	12	[30]
A. ruthenus	14.58 ± 2.81	approx. 320	20	FTS	48	natural	2	[46]
A. oxyrinchus	219.35 ± 2.2	217 ± 0.024	19.7 ± 0.05	RAS	12	natural	-	[34]
	approx. 69	200 to 300	19.26	RAS	12	natural	-	[34]
A. naccarii	198 ± 0.015	110 ± 9	23 ± 1	RAS	-	natural	10	[47]
	100	216 ± 25	23 ± 1	RAS	22	natural	22	[33]
A. sinensis	8.38 ± 0.27	266.6 ± 30.94	19.3–20.8	RAS	8	-	-	[48]
A. baeri	14.5 ± 0.8	168.29 ± 2.29	20 ± 0.5	RAS	48	natural	1	[49]
A. fulvescens	30.51 ± 1.21	88.44 ± 3.54	17 ± 1	FTS	48	12 L/12 D	20	[27]
A. medirostris	145.7 ± 3.8	63.36 ± 6.02	12 ± 0.01	RAS	72	natural	-	[32]
	30.3 ± 17.5	270	24	RAS	24	natural	6	[31]
A. schrenckii	32.7 ± 1.2	295.38 ± 10.42	20 ± 0.5	RAS	72	12 L/12 D	2	[50]
P. spathula	500 ± 130	157.49 ± 22.54	21 ± 0.3	P	72	natural	1	[51]

Legend: FTS—flow-through system; RAS—recirculating aquaculture system; P—ponds; 12 L/12 D—12 h light/12 h dark.

Regarding interspecific oxygen consumption, the higher oxygen consumption of sterlet compared to beluga and bester may illustrate a different allocation of energy in the body [52]. This hypothesis is supported by Biro and Stamps [53] who reported a positive relationship between metabolism and movement, explained by the fact that individuals that display intrinsically higher levels of activity may also develop an increased physiological capacity to facilitate the increased rate of movement. Sterlet, unlike anadromous migratory fish that perform long migrations a in limited time, is a potadromous species that resides in freshwater for its whole lifecycle, making only short migrations for spawning [54,55]. Thus, the higher swimming capacity of Danube sterlet could be regarded as a physiological adaptation to cope with the strong currents of the river. Beluga and bester hybrid showed a similar pattern of oxygen consumption, with no significant differences ($p > 0.05$) for both SMR and RMR. Therefore, the bester hybrid showed an apparent paternal inheritance of some physiological metabolic traits, demonstrated also for *Salmo salar* [56].

Because SMR is energetically expensive [15], a lower SMR could be considered more adaptive because the energy excess is directed for growth and reproduction. The lower SMR and RMR of beluga may be attributed to their flexible adaptation to environmental conditions during the nonmigratory phase, but this hypothesis should be confirmed by future studies.

The findings of the present study showed an inversely proportional relationship between the metabolic rate (SMR and RMR expressed as mg O_2 kg^{-1} h^{-1}) and body mass. This trend is also reported by other authors [4,57–59], which state that the oxygen absorption capacity per unit mass is lower in larger fish compared to smaller ones.

Large animals generally have higher absolute metabolic rates than small animals, but on a mass-specific basis, small animals usually out-pace larger animals in their rates of energy expenditure [60]. Metabolic rate (MR), therefore, changes allometrically with body mass (M) according to the equation $MR = a \times M^b$, where a is the scaling coefficient (intercept), and b is the scaling exponent. Usually, the value of scaling exponents, calculated for some species of bonefish varies between 0.4 and 1 [61]. According to Glazier 2005 [62], an organism with an isometric scaling coefficient ("1") benefits from a direct proportionality between body mass and different physiological processes, especially respiration, as the rate of oxygen consumption of an organism is an interaction between a multitude of processes (substrates that perform metabolic combustion, waste disposal, physical activity or cellular respiration at the level of various organs) [63]. The majority of the authors reported a scaling exponent for fish between 0.7 and 0.88 [64,65].

In this study, the values of the scaling coefficient are lower than those reported for other species. For example, Patterson et al., 2013 [51], which also reported the same trend of decreasing oxygen consumption with increasing body weight, shows a value of 0.91 calculated for the species *Polyodon spathula*. This aspect indicates a reduction in specific oxygen consumption, but it is not so pronounced as in the case of bony fish. For example, for flatfish, lower exponents (b between 0.5–0.7) were reported [66]. Mayfield and Cech [31], reported a value greater than 1 (1.1 to 11 °C) for *Acipenser medirostris*, which shows a proportional increase in oxygen consumption per measure unit area (grams). In our study, the scaling coefficient reflects a near isometry for the hybrid (b = 0.97) while for the purebred species the coefficient of 0.8 suggests a reduction in oxygen consumption with increasing body mass. Thus, there is an interspecific variability in the relationship between the standard metabolic rate and body mass, which is a variability also reported by other authors [64].

This finding may also have importance in understanding the fate of some sturgeon species in nature, in the context of climate change. Rising temperature is the main factor for oxygen limitation in aquatic ecosystems, and in this context, aerobic metabolism may be a fundamental mechanism driving the response of fish species to climate warming [67].

5. Conclusions

The present study focused on assessing metabolic traits of two different sturgeon species and their hybrid reared in recirculating aquaculture systems. The results emphasized that sterlet exhibited 22–30% higher oxygen consumption rates when compared with hybrid bester and beluga.

For RAS operation, the oxygen supply is correlated with the production cost and therefore producers have to be aware of the metabolic differences among species. In the purebred species, the scaling coefficient suggests a more pronounced reduction in oxygen consumption with an increase in body mass, while for the hybrid, for the body mass range tested here, a near isometry was recorded. These results also contribute to the knowledge of the physioecology of sturgeons, and bring new insights for the conservation of wild populations. Understanding hybrid's metabolic traits inheritance patterns is also important both for aquaculture and conservation ecology, since the presence of hybrids is also reported in natural waters.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fishes6040046/s1: Figure S1: The scheme of the recirculating aquaculture system.

Author Contributions: Conceptualization, M.C., R.-C.G. and L.D.; methodology, R.-C.G. and L.D.; formal analysis, R.-C.G.; investigation, R.-C.G., M.C. and M.-D.S.; writing—M.C., L.D. and R.-C.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The procedures presented in this study were approved by the Ethics Committee of the University in accordance with the Experimental Certificate of Animal Use (no. 200/14). The study was conducted according to the guidelines of the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Data Availability Statement: All the data are available from the first author, and can be delivered if required.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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