„Dunărea de Jos” University of Galaţi
Doctoral School of Industrial Mechanics and Engineering

# Ph.D. THESIS

## SYNOPSYS

# RESEARCH REGARDING THE EVALUATION OF METABOLIC RATES OF JUVENILE STURGEONS SPECIES INTENSIVELY REARED IN A RECIRCULATING SYSTEM

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# *Research regarding the evaluation of metabolic rates of juvenile sturgeons species intensively reared in a recirculating system*

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**Raluca-Cristina ANDREI (GURIENCU)**

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**Keywords:**
recirculating aquaculture systems, sterlet, Russian sturgeon, beluga, bester, bestbeluga, new diamond hybrid, static chambers, swimming tunnel, swimming performance

# INTRODUCTION

Aquaculture is considered a sustainable alternative to industrial fishing, being one of the fastest-growing sectors of the food industry in recent decades, and continues to be an important source of food and employment for many countries and communities.

The expansion of aquaculture has been facilitated by the continuous development of production practices and growing systems, which have led to efficiency in terms of costs and also of the quantity and quality of the finished product.

The global requirement for food safety, combined with an emphasis on environmental sustainability, has led manufacturers to implement new solutions that can maximize production and gather together the increasing demand requirements of the sales market, all this in terms of covering production costs.

Thus, in recent years, the development of intensive and super-intensive aquaculture has taken place, in particular, in recirculating systems, which, although more expensive, have stood out to the detriment of other growing systems, due to the multitude of advantages offered. The most important argument for approaching this type of aquaculture is the possibility of maintaining rigorous management of the water quality, which is reflected in the practice of high stocking densities, to which is added the possibility of obtaining final products in a continuous flow, throughout the year but also the ease of installation in locations where other aquaculture systems could not be implemented [1].

However, there are still many situations in which producers face problems related to the rapid deterioration of water quality or an increased incidence of diseases among fish, especially due to the high level of stress that leads to a decrease in their resistance. Aquaculturists often choose to use pharmaceuticals to resolve these problems, but their repeated use leads to adverse effects which are in contradiction with animal health and consumer safety policies and standards [2].

In general, stress results from the inability of an organism to adapt to an ecosystem, either natural or artificial. In aquaculture, the selection of biomass according to growth performance, accompanied by restricted activity and administration of diet with high energy content led to problems similar to those found in people with a sedentary lifestyle [3]. Among them, the most frequently reported issues to mention are the decrease of the body's immunity and the respiratory capacity, the presence of some dysfunctions at the cardiovascular level, or some malformations at the body level [4, 5, 6].

These functional deficiencies are symptoms of fish reduced capacity to adapt, being in contradiction with the strategies aiming at ensuring the well-being of the culture biomass.

There is a need to introduce new means of assessing fish stocks, updated criteria that emphasize the robustness or endurance of the body.

Thus, a new research direction has developed in recent years, especially on bony fish species and less on cartilaginous ones, research that focuses on improving the morphophysiological condition through non-invasive methods, following the evaluation of the metabolic rate and swimming performance of intensively farmed fish.

In this context, the research undertaken during the doctoral studies focused on these new directions of evaluation of organisms grown in the aquaculture environment, aiming to evaluate the metabolic rate and swimming performance of sturgeon, concerning the fluctuation of some environmental factors.

Through the approached topic, the doctoral thesis contributes to the development of the knowledge regarding the metabolic rate of some sturgeon species, being the first research carried out at the national level, which used the respirometry test in evaluating the metabolism of some juvenile sturgeons reared under the recirculating aquaculture systems conditions, offering also for the first time, on the international level, data regarding the oxygen consumption and swimming performance of Russian sturgeon and for the following hybrids bester, bestbeluga, and the new diamond hybrid. At the same time, the studies aim to provide preliminary data on the rearing technology of some sturgeon species (pure and hybrid) in recirculating systems and to complete the little information found in the literature related to the physiology of respiratory metabolism.

The results of these studies will contribute to the development of scientific knowledge, and can be useful to aquaculturists who want to implement new sustainable and environmentally friendly growth technologies with crop biomass, being especially useful to biologists and ecologists concerned with the conservation and recovery of rapidly declining sturgeon stocks.

Therefore, in close interdependence with the possibilities offered by the research infrastructure but also with the studies carried out so far, the general objectives of this doctoral thesis were to quantify the metabolic response (standard metabolic rate, routine, maximum, metabolic scope) and physiological performance (swimming, recovery capacity) in relation to a variety of factors such as body mass, temperature, and water velocity.

The secondary objectives but also the secondary activities that led to the fulfillment of the general objectives aimed:

- to complete the experimental design of the respirometry tests, by referring to the previous studies undertaken on the resident sturgeons of the North American and Asian rivers;
- to evaluate and control the technological water quality parameters within the recirculating system and metabolic chambers, in order to identify any potential disruptors of fish metabolism;
- to quantify the technological performance indicators of fish biomass, because metabolism is profoundly affected by pre-monitoring conditions, so that the reporting of metabolic parameters should be performed in conjunction with several descriptive factors of the environment of fish stocking conditions.

I emphasize that, prior to the start of the experiments, no data were found in the literature related to the metabolic rate, swimming, and behavior of Russian sturgeon or hybrids (bester, bestbeluga, and the diamond), while there is little information regarding the sterlet and beluga sturgeon.

Summarizing the obtained information, we can say that the tested sturgeons exhibit intraspecific peculiarities, are strongly affected by environmental conditions, and technological and operational management should place greater emphasis on the morphophysiological features of species reared in intensive aquaculture conditions. At the same time, a better knowledge of the biology of the species could contribute to the effort to restore and conserve sturgeon stocks.

We can conclude that assessing the metabolic rate and swimming characteristics of some species could open up new pathways for solving the fundamental problems faced by modern aquaculture, such as increasing the intensity of production while maintaining minimum stress among the biomass, or improving the health and growth performance of fish through the use of sustainable and environmentally friendly technologies.

The thesis is divided into two parts, as follows:

**I. STATE OF ART** includes two chapters which present in detail recent aspects regarding the research theme approached in this doctoral thesis. The first chapter presents a synthetic analysis regarding the evolution of sturgeon aquaculture on a national and international level, as well as the development perspectives of the recirculating aquaculture systems (RAS) and their territorial evidence in Romania. The second chapter includes information on the respiration physiology of fish, general considerations of fish metabolism and factors affecting it, techniques and equipment used to measure the metabolic rate of fish, and also a description of swimming types and morphophysiological features that influence the metabolic rate of fish.

**PART II. EXPERIMENTAL ACTIVITY** consists of five chapters, includes research conducted during the doctoral studies. These chapters evaluated the metabolic response (standard, routine, maximum metabolism rate, aerobic interval) and physiological performance (swimming and excess post-exercise oxygen consumption), in relation to the variation of some intrinsic (body mass) and extrinsic (water temperature and water velocity) factors.

The present Ph.D. thesis contains 186 pages, which includes 96 graphs and 22 tables.

The study of the literature represents a percentage of 13.5% and the experimental part a percentage of 86.5 %.

The research activities of the doctoral thesis were carried out using the infrastructure of the Romanian Center for the Modeling of Recirculating Aquaculture Systems (**MoRAS**) within the Faculty of Food Science and Engineering, University “Dunărea de Jos” from Galați.

# Chapter I.
# Bibliographic study

## 1.1. General framework for sturgeon aquaculture

Despite continuous changes in the socio-economic climate and environmental conditions, aquaculture has traditionally remained an important source of food, employment, and income in many countries and communities.

In recent decades, aquaculture has faced a rapid increase in production and economic impact, now providing a supply of 50% of total fish consumed worldwide. It is estimated that aquaculture will be the main supplier of fishery products by 2030, due primarily to high consumer requirements and secondly to the depletion of natural fish stocks [8].

There are two directions in sturgeon aquaculture: controlled propagation for release into the natural environment and commercial farming. The two approaches (commercial farming and restocking) were addressed in 2006 in the Ramsar Declaration on Sturgeon Conservation. While aquaculture aims to intensify and efficientize production (survival and high fish growth rate) [27], in the case of restocking programs, the main directions tend to aim the improvement of the physical condition of the young fish, to facilitate accommodation and survival in the natural environment [28].

## 1.2. Current status, perspectives of recirculating systems in aquaculture (RAS) and their territorial evidence in Romania

The increased emphasis on sustainability, traceability, food safety and affordability require continuous development of production technologies used in aquaculture. Traditional aquaculture has an impact on the environment, but modern aquaculture systems solve this problem, offering two major advantages: cost efficiency and reduced environmental impact.

Recirculating systems in aquaculture are an important alternative to traditional aquaculture, especially pond aquaculture. They are based on the reuse of water, previously restored to optimal parameters through a series of treatments applied in several stages (mechanical, chemical, and biological), with daily water losses not exceeding 10% of the total volume of the system [39]. Recirculation technology can be used for any species grown in aquaculture (fish, crustaceans, mollusks, plants, etc.), but is mainly used in fish farming.

Due to the complexity of the technological requirements and the constructive and functional diversity of the equipment used, it is not possible to speak about a standard configuration regarding the recirculating systems found in aquaculture, on the contrary, RAS design and operation requires a lot of knowledge [1]. In a recirculating system, the correlation between the carrying capacity and production capacity is indispensable. If the system is undersized or oversized, it becomes inefficient in the following aspects: low growth rate, low food conversion, high incidence of diseases and high mortality [1].

In a recirculation system, it is necessary that the water to be treated in a continuous flow to make possible the elimination of the excretion products as quickly as possible, and the water to be oxygenated to maintain the well-being of the fish [50].

The basic principle of recirculation is relatively simple, from the rearing units, water passes through filters that remove residual solids, oxidize ammonia and nitrites, remove carbon dioxide and aerate or oxygenate the water before returning to the growing units [1, 45].

The development of recirculation systems in aquaculture is promising for many countries that have limited access to some natural resources (water, land, unfavorable climate, etc.), because the development of aquaculture must be sustainable in the context of environmental policies becoming increasingly as restrictive as possible [34]. The development of RAS technology aims to obtain complete control over water parameters and better production concerning the impact on the environment and the cost of investment.

# Chapter II.
# General considerations regarding the metabolic rate of fish

## 2.1. The physiology of respiration

Respiration is a complex physiological process, which takes place cyclically and continuously, ensuring a two-way exchange of gases between the body and the external environment.

Simplified, the respiration process can be described as follows: the oral cavity together with the opercular structures acts as a hydraulic suction and pumping mechanisms so that by continuous movements it takes water from the external environment and send it to the gill region, at the level of the gill lamellae, which through the thin membranes, extract oxygen and finally direct it to the fish's circulatory system [61].

## 2.2. General considerations of the fish’s metabolism

Metabolism sums up the totality of physico-chemical processes and biochemical reactions of the transformation of nutrients, in close connection with the environment, to ensure the growth and development of the body, as well as the energy needed to maintain vital processes [64]. Metabolism is coordinated by the central nervous system, but involves all the body's systems.

Metabolism involves both the assimilation and introduction of nutrients into cells - processes characterized by chemical reactions generating energy and excreting unusable end products (catabolism), and growth, recovery, cell and tissue proliferation that ensures the development and survival of the body, energy consuming processes that lead to the synthesis of new substances, such as hormones or enzymes (anabolism).

Through metabolism, organisms undertake a continuous exchange of energy and information with the external environment, they are open systems from a thermodynamic point of view [64].

Since these chemical reactions release heat as a by-product, measuring it can directly estimate the metabolism of an organism, this method being called direct calorimetry. However, this direction can be difficult because the fish’s body, in comparison to mammals or birds, releases generally low heat production rates, which becomes difficult to quantify in the aquatic environment, which together results in limited measurement sensitivity [89]. As the energy produced by the body of fish is obtained aerobically and changes in the level of dissolved oxygen in water over time can be easily and safely determined, in recent years an indirect method of quantifying metabolism has become popular, namely indirect calorimetry or measurement of oxygen ($MO_2$) [72].

## 2.3. Metabolic rate in fish

According to the condition of the body, three types of metabolic rates are defined: Standard metabolic rate (SMR) which reflects the minimum energy required to ensure vital processes (cell proliferation, tissue repair and maintenance, body growth) [77, 78], is equivalent to the basal metabolic rate measured in birds and mammals. Routine metabolism rate (RMR) is measured in fish with a low level of activity, but including spontaneous movements necessary to maintain balance. The maximum rate of active metabolism (MMR), indicates the upper limit of aerobic metabolism / the maximum rate of oxygen transfer from the environment to the mitochondria, at a certain temperature [83]. The difference between MMR and SMR reflects the metabolic interval (AS) [87]. The metabolic interval is a determination of the amount of oxygen available for aerobic activities such as swimming, digestion, growth and reproduction, this concept being important in understanding the metabolic limits of an organism.

## 2.4. Environmental constraints on the metabolic rate

The metabolism of fish differs from other animals (birds, mammals), being extremely variable. This feature is known as plasticity and occurs in response to various factors that interact with the thermodynamic physiological processes in the body of fish [65].

Factors acting the metabolism are grouped into intrinsic factors (age, sex, feeding intensity, local adaptation, heritability, etc.) and extrinsic factors, which in turn are divided into abiotic (temperature, latitude and longitude, oxygen, carbon dioxide etc.), biotics (intra- and interspecific competition) [91, 92, 93, 106, 107].

## 2.5. Evaluation of metabolic rate

### 2.5.1. Equipment used for the evaluation of metabolic rate

The most common equipments are represented by the "swimming tunnel" and "static" respirometers. The swimming tunnel has a rectangular or rounded shape, it contains a metabolic chamber in which the fish is placed; it has a built-in propeller or rotor that directs the water in one direction, the water having a laminar flow [70]. The most common respirometers used to determine the active metabolic rates are Brett and Blazka.

Static respirometers are usually cylindrical or rectangular, being optimal for measuring the oxygen consumption of organisms at rest (standard or routine metabolic rate).

### 2.5.2. Techniques for the evaluation of the metabolic rate

Respirometry can be performed using three methods: closed respirometry, open respirometry, and intermittent respirometry [112].

According to the literature, the following general rules must be observed to determine the metabolic rate:

- ❖ The use of an appropriate respirometry system that includes the selection of the appropriate measurement technique, the correct sizing of the metabolic chamber (respecting the volume between the water and the body mass of the fish) and the use of oxygen sensors adapted to the experiment;

❖ Use of fish in a postprandial state (so that digestion and absorption of nutrients do not interfere with oxygen consumption) [74].

❖ Conducting proper acclimatization of the evaluated organisms through respirometric tests. It is necessary to report both the time and the method of acclimatization (how fast it was performed) [83].

❖ It is necessary that the reporting of the oxygen consumption of an organism to be carried out simultaneously with the body mass, the ontogenetic stage, or other extrinsic factors that could interpose with the values [74].

❖ Establishing an optimal measurement interval of at least 24 hours. The use of a shorter measurement time may include periods of unwanted activity, while the use of longer periods may induce stress on the body, which is reflected in inaccurate results [125].

## 2.6. Sturgeon's swimming and morphophysiological characteristics that influence the metabolic rate

In fish, for most species, swimming is closely linked to feeding type, habitat and environmental selection, social, reproduction and migratory behaviors [142, 218], being also the only defense mechanism against predators [82, 85].

Sturgeons, like most bony fish, use a subcarangiform swimming, the only difference being given by the caudal fin which is similar to that of sharks. They have a heterocircular tail, formed by two unequal lobes, the upper lobe being much longer than the lower lobe [134]. Also, the depth of the caudal is much lower in sturgeons compared to bony fish, which is most likely due to the low support capacity of the cartilaginous skeleton. In a comparative study, it was found that in the case of routine swimming, the caudal fin contributes only a third to the body's propulsion compared to that of a rainbow trout (*Oncorhynchus mykiss*) of the same size [134].

In general, swimming performance in the case of Acipenserides is low, this being attributed to the unique combination of physiological characteristics - slow metabolism [144] and morphological - the heterocircular swimming decreasing in speed to counteract the increased traction coefficient given by the elongated, fusiform body, covered with numerous bonny shields and thickened skin (Figure 2.1.) [134].

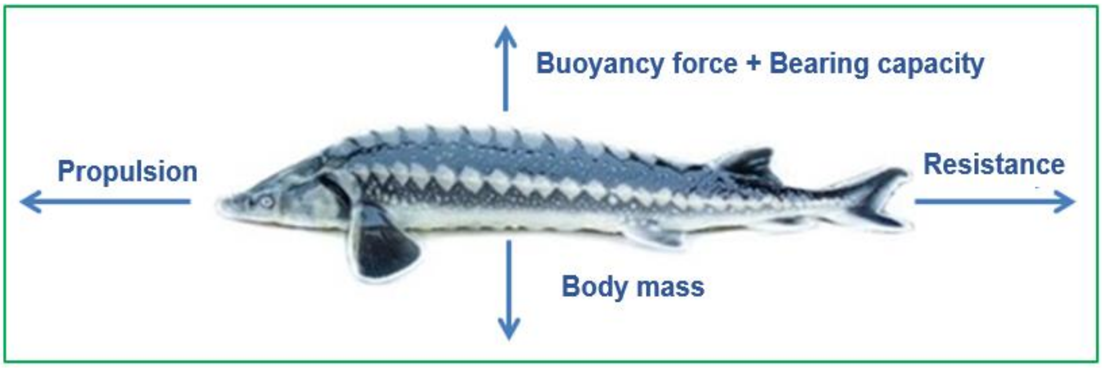


**Figure 2.1.** The forces affecting the fish's body during swimming [145]

# Chapter III.
# Materials, methods and investigation techniques

This chapter describes:

- ❖ the biological material used in the experimental studies, represented by three pure species (sterlet, Russian sturgeon and beluga sturgeon) and three hybrids: bester (*Huso huso* ♀ × *Acipenser ruthenus* ♂), bestbeluga (bester ♀ × *Huso huso* ♂), new diamond (*Acipenser gueldenstaedii* ♀ × *Acipenser ruthenus* ♂);
- ❖ infrastructure research (Pilot station - Recirculating aquaculture system and respirometry system configured for the measure of standard metabolic rates, routine metabolic rates and maximum metabolic rates);
- ❖ working methods and equipment used in determining the water quality parameters but also the technological requirements of sturgeons regarding the water quality in the rearing system;
- ❖ the calculation methodology for the technological performance indicators (growth rates, food conversion factor, specific growth rate, protein conversion factor);
- ❖ the calculation methodology for the evaluation of metabolic rate in fishes: standard metabolic rates (SMR), routine metabolic rates (RMR), maximum metabolic rates (MMR), aerobic interval (AS), excess post-exercise oxygen consumption (EPOC), critical swimming speed ($U_{crit}$), optimal swimming speed ($U_{opt}$), fish cost of transport in relation to swimming speed (COST), tail beat frequency (TBF) and opercular beat frequency (OBF);
- ❖ statistical methods for experimental data processing (through the following programs: Excel 2010 for Windows, SPSS 21.0 for Windows).

# Chapter IV.
# Standard metabolic rate (SMR) and routine metabolic rate (RMR) of sterlet (*Acipenser ruthenus* L., 1758), beluga (*Huso huso* L., 1758), bester hybrid (*Huso huso* ♀ × *Acipenser ruthenus* ♂ ) and bestbeluga hybrid (bester ♀ × *Huso huso* ♂), juveniles stage

## 4.1. Introduction

The main objective of this study was to evaluate and compare the standard (SMR) and routine (RMR) metabolic rates for sterlet (Ac - *Acipenser ruthenus*), beluga (H - *Huso huso*), bester (BE - *Huso huso* × *Acipenser ruthenus*) and bestbeluga hybrid (BB - bester × *Huso huso*), for different size classes at a temperature of 22±1.16 ºC.

## 4.2. Experimental design

The study took place during the August-October 2017, within the Romanian Center for Modeling Recirculating Aquaculture Systems - MoRAS, facility of the ,,Dunărea de Jos” University of Galați (Figure 4.1, Figure 4.2).

The experimental fish was reared for 30 days, at the end of the growth period the main parameters of technological performance were calculated and respirometric tests were started.

Three fish were selected daily, quickly weighed and measured (total length), being separated from the rest of the group. The rest of the fish were kept in parallel with the respirometric tests, under the same conditions, being separated periodically by three specimens, to ensure a continuous flow when testing oxygen consumption. The experiment with the respirometer ended when four classes of sizes were formed (Figure 4.2).

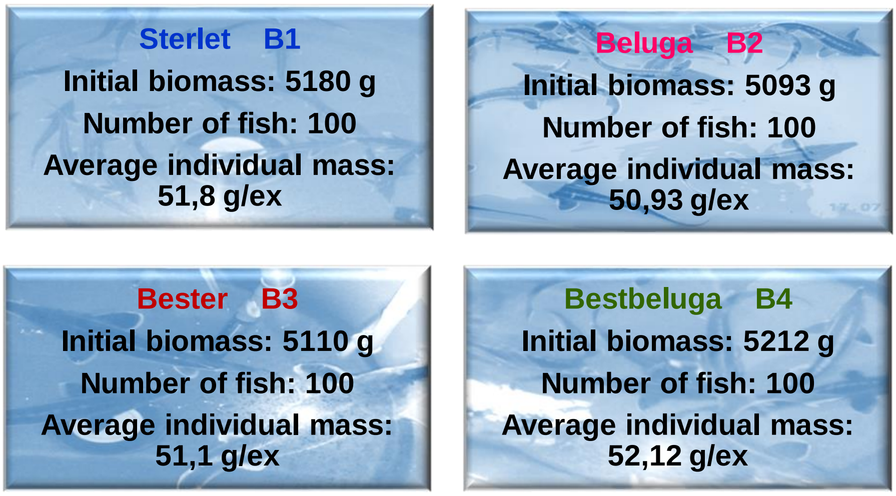


**Figura 4.1.** Distribution of experimental fish in the stocking units

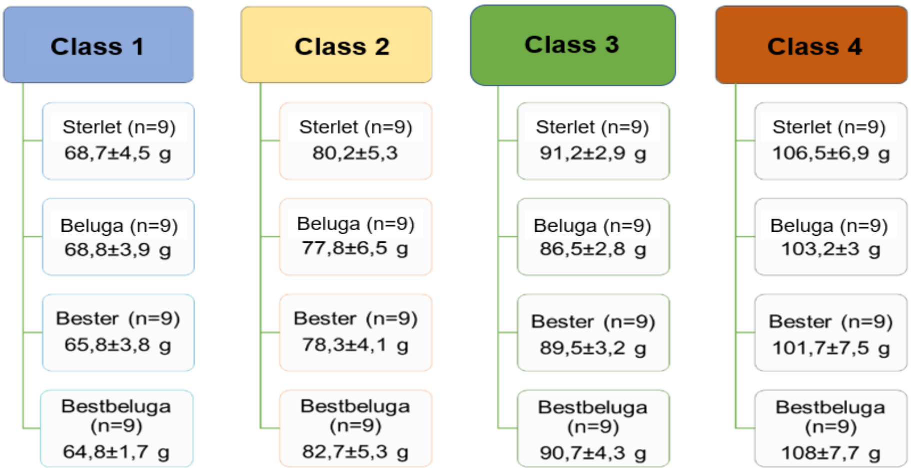


**Figure 4.2.** Design of the experiment carried out in the respirometer

## 4.3. Results and discussion

### 4.3.1. The rearing technology of the experimental fish, under the RAS conditions

The water quality parameters indicate that they were kept within optimal limits for sturgeon growth, and their evolution did not register significant oscillations that could have impacted their metabolic rate.

***Growth performance evaluation***

The technological parameters obtained during 30 days of growth outline a high potential to reach marketable size in terms of technological efficiency. Following the summary analysis of the technological indicators, similar values were observed, slightly higher in beluga and hybrids.

### 4.3.2. Evaluation of the metabolic parameters

The obtained data regarding the oxygen consumption for the juveniles of these species represent a first report for the literature, providing important information on the relationship between fish size and metabolic rate (Figure 4.3., Figure 4.4., Figure 4.5, Figure 4.6.).

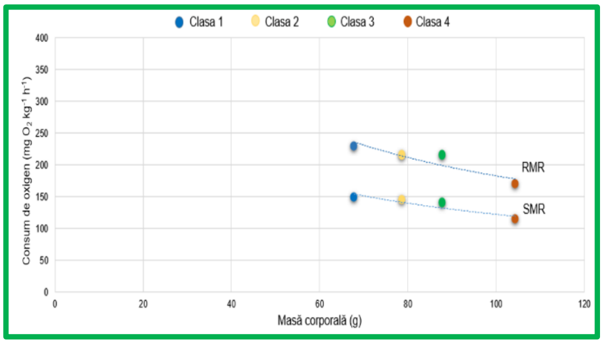


**Figure 4.3.** The relation between the SMR and RMR (mean values) at sterlet, for the four size classes

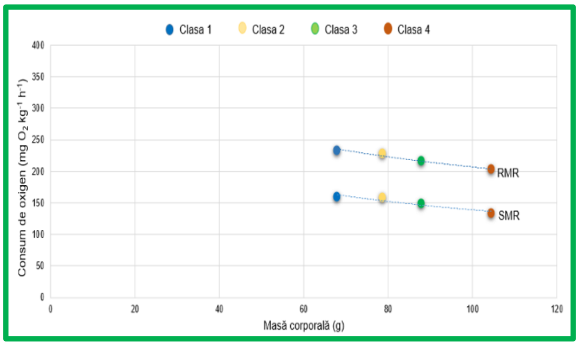


**Figure 4.4.** The relation between the SMR and RMR (mean values) at beluga, for the four size classes

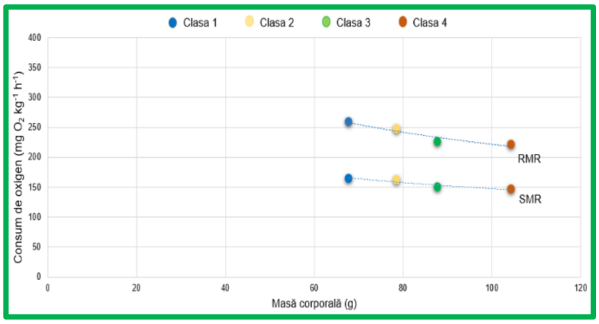


**Figure 4.5.** The relation between the SMR and RMR (mean values) at bester, for the four size classes

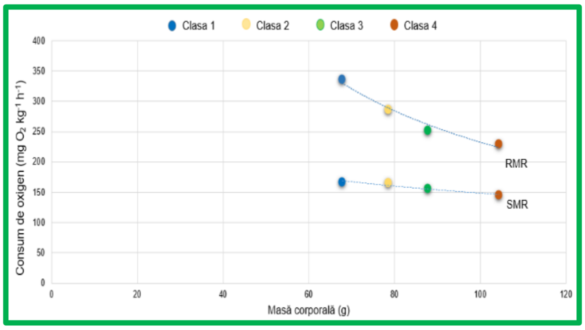


**Figure 4.6.** The relation between the SMR and RMR (mean values) at bestbeluga, for the four size classes

There is an inversely proportional relationship between the specific metabolic rate (SMR and RMR) and body mass (Figure 4.7, Figure 4.8). This trend is validated by other studies, being considered representative for fish, as the oxygen absorption capacity per unit mass is lower in larger fish compared to smaller ones [102, 216, 232, 233, 393 ].

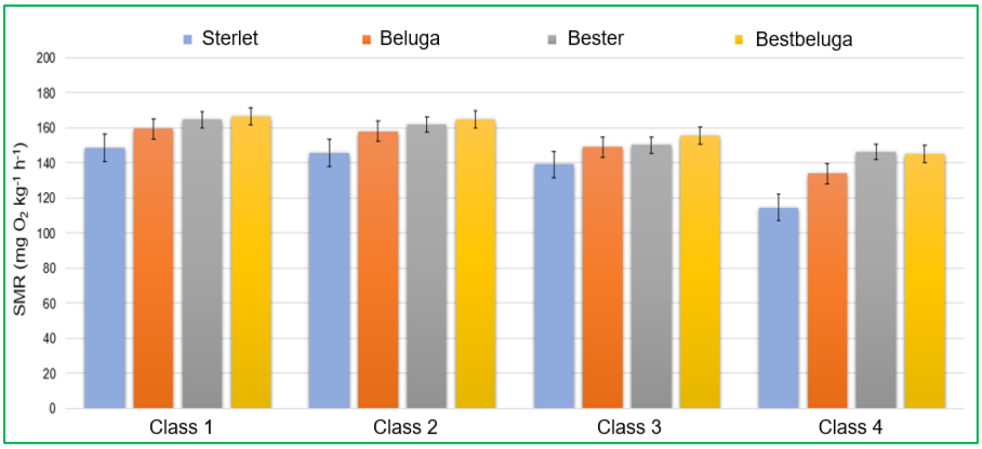


**Figure 4.7.** Evolution of SMR (mean ± SD) in the four sturgeon species

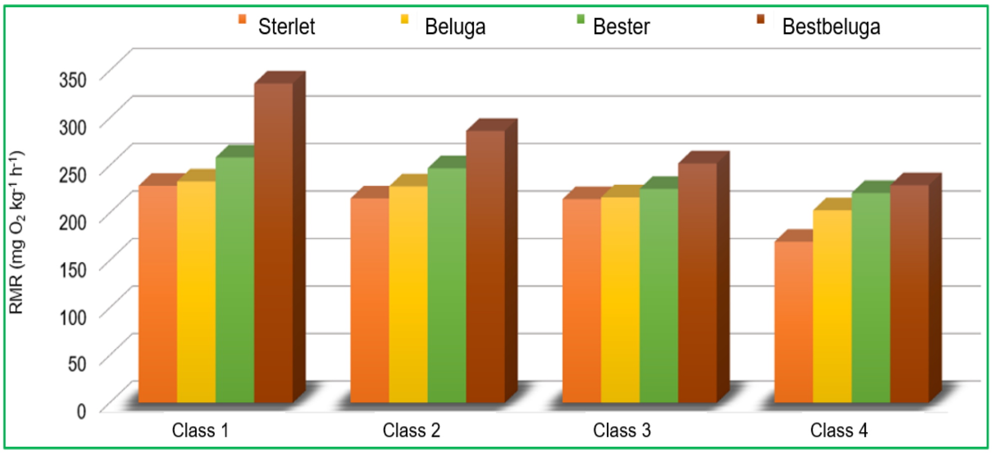


**Figure 4.8.** Evolution of RMR (mean ± SD) in the four sturgeon species

## 4.4. Conclusions

The main objective of this experiment was to evaluate and compare the standard and routine metabolic rates of four species of sturgeon (sterlet, beluga, and bester, bestbeluga hybrids) divided into four size classes.

The main conclusions of this study are the following:

- Regarding the physico-chemical indicators of the culture water, the pilot recirculation system in which the sturgeons were kept and reared ensured their keeping in an optimal technological interval for the species, without significant differences between the values recorded at the level of rearing units,
- the high survival rate of all species highlights a high degree of adaptation of fish biomass to the conditions offered by the recirculating system.
- the main growth performance indicators, calculated for the 30 days, outline, especially in the case of beluga and hybrids, a high potential to achieve a size that can be marketed in conditions of technological efficiency, respectively a lower technological yield, in case of sterlet;
- Regarding the influence of body mass on the SMR, the same trend is observed for all species: the SMR values for class 1 and class 2 are not statistically different ($p>0,05$), observing a significant decrease ($p<0,05$) for class 3 and size class 4, respectively.
- the interspecific comparison highlights lower values of oxygen consumption allocated to standard activities, in the case of sterlet in comparison with those obtained for beluga, bester,

and bestbeluga. The slightly higher values of SMR found in the case of hybrids, corroborated with the technological indicators obtained for these species, highlights a more intense metabolic activity, an aspect which is also underlined by the obtained technological indicators;

- Statistical tests showed significant differences in the case of RMR ($p>0,05$), both at the intraspecific level and in the case of interspecific comparison, the hybrids maintaining a higher oxygen consumption compared to pure species.

# Chapter V.
# Influence of water temperature on standard (SMR) and routine (RMR) metabolism rate in sterlet juveniles (*Acipenser ruthenus* L., 1758)

## 5.1. Introduction

This study aimed to evaluate the effect of temperature on standard and routine metabolic rate in sterlet juveniles (*Acipenser ruthenus*) and to contribute to the understanding of the ecological consequences of temperature variation on the ecology of the species.

## 5.2. Experimental design

The experimental activity took place between January and March 2017, at the Romanian Center for Modeling Recirculating Aquaculture Systems - MoRAS, a facility of the Lower Danube University, Galați (Figure 5.1.).

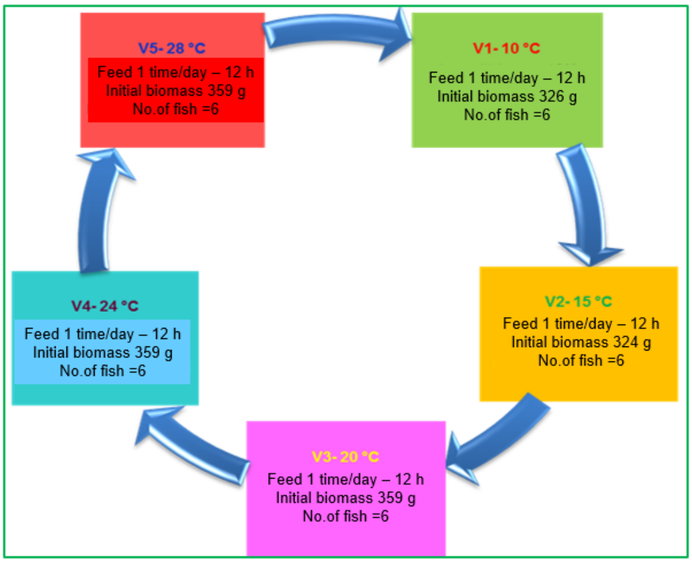


**Figure 5.1.** Distribution scheme for the experimental variants

## 5.3. Results and discussions

### 5.3.1. Water quality parameters from the stocking units

The values of the physico-chemical parameters of the water were maintained in the optimal range of the species, during the all experimental period, being similar to those recommended by the literature.

### 5.3.2. The evaluation of standard metabolic rate (SMR) and routine metabolic rate (RMR)

After the fish were transferred to the metabolic chambers, they had an accommodation period of about 3 hours. During this period, the oxygen consumption fluctuated, being higher. The chosen accommodation interval excluded the possible incorrect values of oxygen consumption, influenced by the stress induced by the handling of the fish [185, 242].

The standard metabolic rate (SMR) was determined for each fish monitored at the metabolic chambers, the average values being then calculated for each temperature (Figure 5.2.).

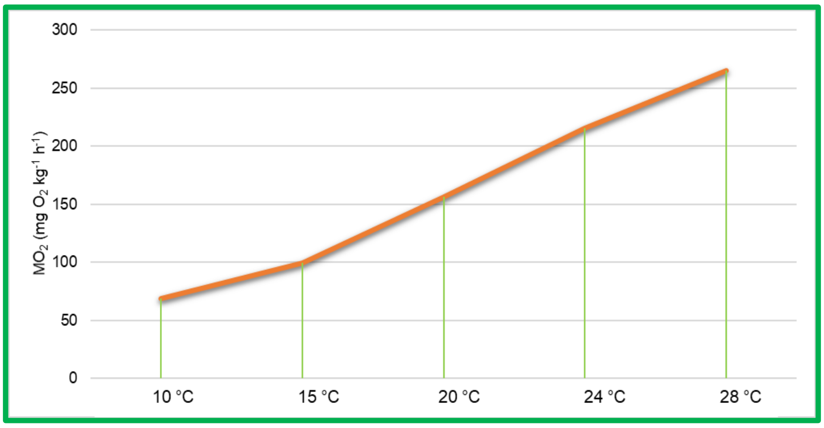


**Figure 5.2.** The mean values (± SD) of the SMR, obtained for the five tested temperatures

Because the Anova test showed significant differences between the experimental variants ($p < 0{,}05$), the post-hoc analysis of Tukey's B type was performed to determine the differences between the SMR values for the five temperatures tested. Thus, following the analysis, five sets of data were highlighted, the SMR values from each experimental variant being significantly different.

Regarding the rate of routine metabolism, a significant increase was also found, underlined by the statistical analysis that highlighted differences between all experimental variants (Anova, $p < 0{,}05$). In figure 5.3. is presented the evolution of RMR (mean values) for sterlet juveniles during 24 hours, for all temperatures tested, after excluding the three hours of accommodation.

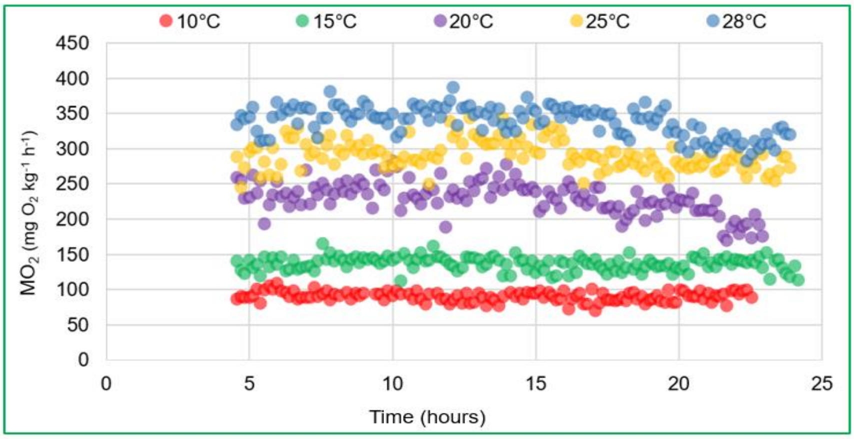


**Figure 5.3.** Evolution of the routine metabolism rate at the tested temperatures (after excluding the accommodation hours) [277]

A linear correlation was observed between water temperature and the metabolic rate of sterlet juveniles (SMR and RMR), described by the following equations (Figure 5.4.):

- $\mathbf{SMR_{cegă}} = 50{,}914x + 8{,}223$, ($r^2 = 0{,}99$)
- $\mathbf{RMR_{cegă}} = 65{,}128x + 23{,}292$, ($r^2 = 0{,}98$)

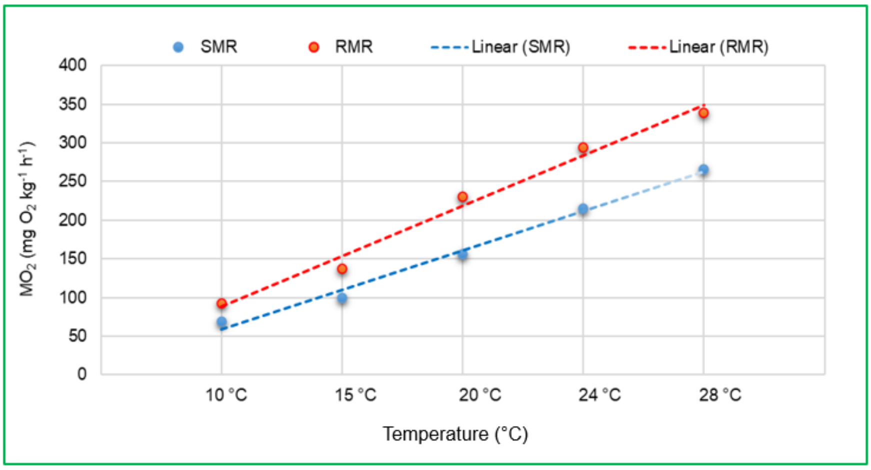


**Figure 5.4.** The relationship between water temperature and standard and routine metabolism rate (n = 6) of sterlet juveniles

The effect of temperature on the metabolic rate is also accentuated by the thermal coefficient ($Q_{10}$). The thermal coefficient $Q_{10}$ represents a measure of the sensitivity of a biological process to a temperature change, being useful in studying the physiological processes influenced by the temperature variation, in ectothermic organisms [270].

In this case, the coefficient $Q_{10}$ shows values higher than 1,5, which indicates a completely temperature-dependent reaction rate (Table 5.1.).

*Table 5.1. Thermal coefficient values ($Q_{10}$) for fish acclimatized to the 5 temperatures*

| | *Tested temperatures (ºC)* | | | |
|---|---|---|---|---|
| | **15 vs 10** | **20 vs 15** | **24 vs 20** | **28 vs 24** |
| ***Thermal coefficient* ($Q_{10}$)** | 2,10 | 2,48 | 2,22 | 1,69 |

### 5.4. Conclusions

The main objective of this study was to evaluate the standard and routine metabolic rate of sterlet juveniles gradually exposed to different temperatures (10 ºC, 15 ºC, 20 ºC, 24 ºC și 28 ºC).

Summarizing the above information we can conclude:

- following acclimatization to the tested temperatures, the biological material quickly adapted, maintaining a normal behavior throughout the experimental trial;
- the gradual increase of temperature involves a major intensification of the metabolic rate in the case of sterlet juveniles;
- the physiological response after exposure to different temperatures is not similar to that found for other sturgeon species, depending on the body-weight of the fish, but also the protocol, methods, and equipment used in respirometric tests;
- the obtained values in the case of the thermal coefficient ($Q_{10}$) indicate a completely temperature-dependent reaction of the metabolic rate, for all the experimental variants;
- the results suggest a high sensitivity of the metabolic rate to temperature, following a linear trend in this species. This finding may predict that changes in water temperature induced by various natural (such as global warming) or anthropogenic factors (pollution, water released by hydroelectric power plants, etc.) would have a major negative impact on sterlet populations from the natural environment.
- at the same time, rising temperatures would lead to a higher energy cost to maintain vital functions and a reduction in the energy allocated to feeding or avoiding predators, which at a long time can lead to declining of populations.
- on the other hand, from the perspective of fish growth in aquaculture, this aspect can be beneficial, because the temperature is known to influence both ingestion and metabolism, and will have direct implications on the growth rate of fish biomass. However, it is important to distinguish between the effect of temperature *per se* and the induced effect on fish growth, which is in close interaction with the other physico-chemical parameters of the water, or the availability of food.

# Chapter VI.
# Evaluation of the maximum metabolic rate (MMR) and the swimming performance of the sterlet (*Acipenser ruthenus* L., 1758), Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzenburg, 1833) and the new hybrid, *diamond* (*Acipenser gueldenstaedii* ♀ × *Acipenser ruthenus* ♂*)*, sturgeon juveniles reared under the RAS conditions

### 6.1. Introduction

The aims of this study were:

- ✓ performing a comparison of swimming performance for two size classes for the species *Acipenser ruthenus* (227.5±44.41 grames and 552.67±65.68 grames).

- ✓ evaluation of the water velocity effect on the metabolic rates in three species of sturgeon (*Acipenser ruthenus, Acipenser gueldenstaedtii* and their hybrid (*Acipenser gueldenstaedtii* × *Acipenser ruthenus*), with a body mass of approximately 540 grams, at a temperature of 22.2 ± 0.2 °C;
- ✓ monitoring the excess post-exercise oxygen consumption (EPOC);
- ✓ analyses of the fish swimming behavior;

## 6.2. Experimental design

The study took place between July and September 2017, at the Romanian Center for Modeling Recirculating Aquaculture Systems - MoRAS, a facility of the ,,Dunărea de Jos" University of Galați, comprised by 3 modules of 8 rearing units each (water volume of 700 liters).

Experimental fish was represented by a group of 216 sturgeon juveniles (sterlet, Russian sturgeon and the new hybrid, *diamond*), obtained by artificial reproduction, on the S.C. Kaviar House S.R.L farm, Tulcea county.

During the transfer to the recirculating system, the biological material was weighed and evaluated biometrically, the measurements being repeated in the middle (August 19) and the end of the rearing period (August 31). The intermediate weighing aimed to update the food needs, depending on the biomass existing at that time.

Each module was populated by a species (Module I - sterlet, Module II - Russian sturgeon, Module III - hybrids) (Figure 6.1.).

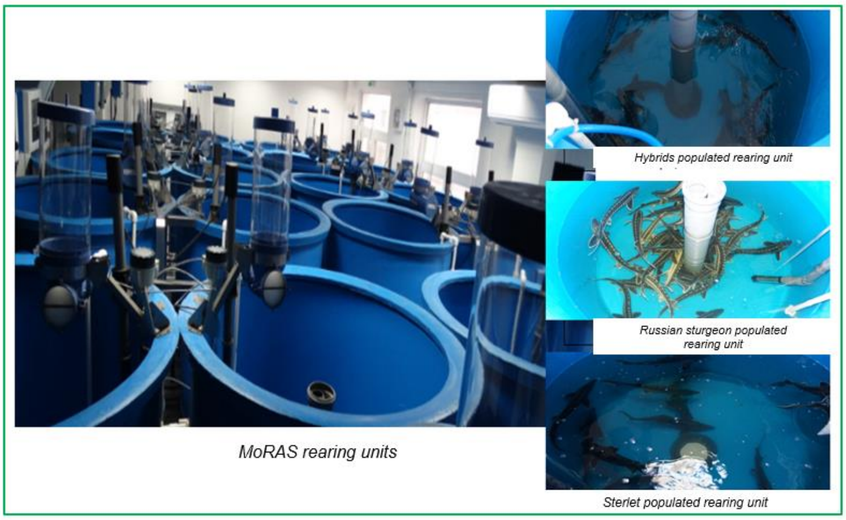


**Figure 6.1.** Pilot station - recirculating system populated with sturgeons (original photo)

The technique of intermittent respirometry was used (measurement - 4 minutes, recirculation - 3 minutes, waiting - 2 minutes), before the start of each test, the temperature and oxygen sensors being calibrated according to the manual (Figure 6.2.).

The water temperature inside the swimming tunnel was similar (ANOVA, $p < 0.05$) to the water in the rearing units (22.2 ± 0.2 ºC). During the swimming trials there was used dechlorinated fresh water, and after each tested fish, the solid suspensions were siphoned and about a third of the water was discharged and replaced. Aeration stones were constantly used, so that dissolved oxygen did not fall below 9 mg $L^{-1}$ [199].

**Figure 6.2.** Respirometry system - swimming tunnel (original photo)

**Figure 6.3.** Swimming monitoring via video recording

Quantification of the frequency of caudal fin movements was possible through observation of the upper lobe, a movement being considered complete when the upper lobe of the caudal fin completed the maximum amplitude and returned to the initial position (Figure 6.3).

## 6.3. Results and discussion

### 6.3.1. The rearing technology of the experimental fish, under the RAS conditions

The quality parameters of the water were maintained in the optimal interval for growth and development of the sturgeon juveniles, with no statistically significant differences ($p>0.05$) between the rearing units.

**Evaluation of the growth performance of juvenile sturgeons**

In order to obtain a high accuracy in the calculation of metabolic indices and to correctly assess the behavior of sturgeons subjected to different swimming speeds, a growth experiment was performed, which involved the administration of a commercial feed, specially designed for sturgeons.

Growth experiments began with homogeneous groups, tested by the Levene test ($p> 0.05$). The synthetic picture of growth performance indicators revealed better results in the case of hybrids, followed by Russian sturgeons. Following the technological experiment, it can be concluded that the diamond hybrid (*Acipenser gueldenstaedii* ♀ × *Acipenser ruthenus* ♂) can become a serious candidate for intensive sturioniculture, with a high feed conversion rate and an increased tolerance to the offered environmental conditions.

### 6.3.2. Influence of body mass on the maximum metabolic rate (MMR) and swimming performance of sterlet juveniles (*Acipenser ruthenus* L., 1758)

The gradual increase in swimming speed led to an increase in oxygen consumption ($MO_2$) (mg $O_2$ $kg^{-1}$ $h^{-1}$) for both $V_1$ sterlet and $V_2$ sterlet (Figure 6.4.), the resulting relationships being described most well by an exponential function ($r^2$ = 0.9396 - $V_1$, respectively $r^2$ = 0.9891 - $V_2$).

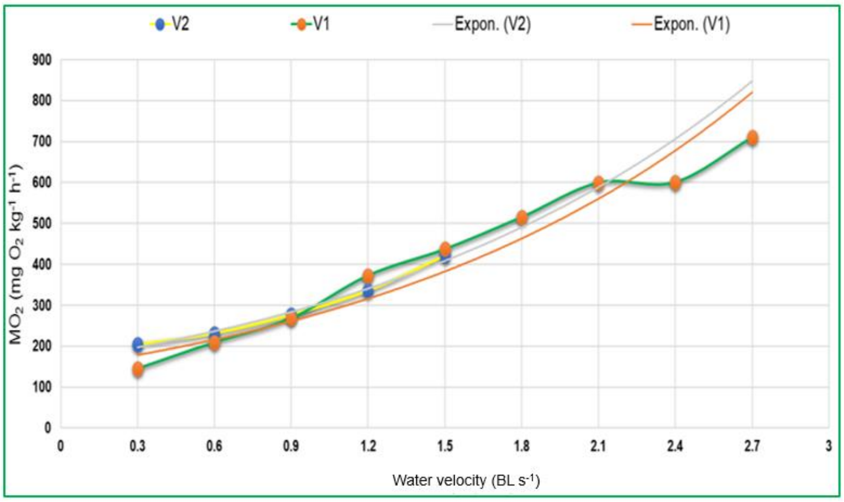


**Figure 6.4.** Relationship between water velocity and oxygen consumption rate ($MO_2$, mean ± SD) for both experimental variants, described by an exponential function

The standard metabolism rate (SMR) (Figure 6.5.) was not influenced by the two body weights (ANOVA, p> 0.05), but the sterlet from $V_1$ had slightly higher SMR values.

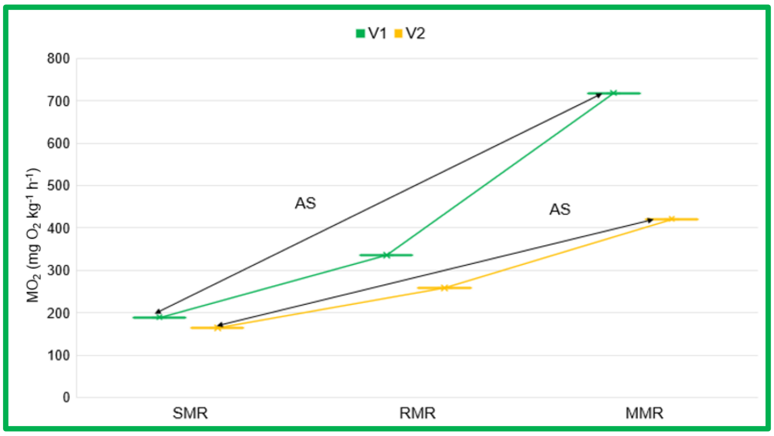


**Figure 6.5**. The values of SMR, RMR, MMR and AS interval for the two experimental variants

Routine metabolism rate (RMR) values were kept inversely proportional to the mass of the two tested groups, with smaller fish having higher oxygen consumption compared to larger fish.

However, statistical tests indicated, in this case, significant differences between groups (ANOVA, p <0.05), which points out a more intense metabolic activity in the case of smaller fish.

The maximum rate of active metabolism (MMR) (Figure 6.5.) shows major differences between the two tested groups (p<0.05), MMR values increasing by 3.79 times than SMR for variant $V_1$, respectively 2.55 times for variant $V_2$.

The value of the aerobic scope (AS) resulting from the difference between MMR and SMR, at 22.2 ± 0.2 ºC, remains relatively high for V1 compared to the sterlet from V2, indicating a longer metabolic interval dedicated to other routine activities and greater comfort among smaller sturgeons.

Swimming performance, lower at the sterlet from V2, leads to a decrease in aerobic range. Consequently, the metabolic costs allocated to breathing and swimming will become a priority, to the detriment of the energy allocated to the growth and development of the body. In time, a reduction of the metabolic interval can compromise the vital functions of the body, affecting both growth and survival [193].

The critical swimming speed (Ucrit) remained higher in smaller specimens, with about 69% higher (Figure 6.6.).

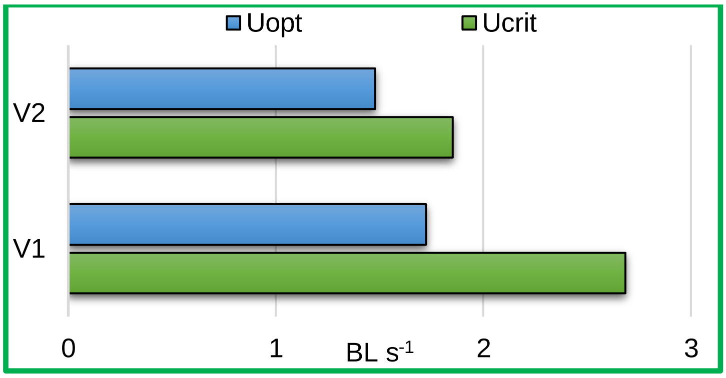


**Figure 6.6.** The values of the critical swimming speed ($U_{crit}$) and the optimal swimming speed ($U_{opt}$), for the two experimental variants

Regarding the swimming of the two groups, the sturgeons in $V_2$ approached a slower, stationary swimming, spending more time on the substrate, while the smaller sturgeons were more active. At speeds higher than 0.9 BL $s^{-1}$, the fish from both variants changed their swimming strategy, being positioned more in the front of the metabolic chamber, letting themselves be carried by the water current, returning to the initial position through sudden movements.

Depending on the unit of measurement used, the calculated optimum speed follows a directly or inversely proportional relationship with the total body length. Thus, expressed in cm $s^{-1}$, the optimal swimming speed maintains a positive relationship with the size of the fish. Instead, Uopt expressed in BL s-1 shows a negative relationship. The applied statistical tests revealed significant differences between variants (ANOVA, $p < 0.05$).

Following the analysis of the literature, no calculated values were found for any sturgeon species, in general very few references are available for this parameter.

By graphically representing the Uopt values obtained in this study as well as those in the literature, the following relation is obtained: $U_{opt}$ (BL $s^{-1}$) = 46,517 ($L_t$) -0.855 (BL $s^{-1}$), $R^2 = 0.9214$ (Figure 6.7.).

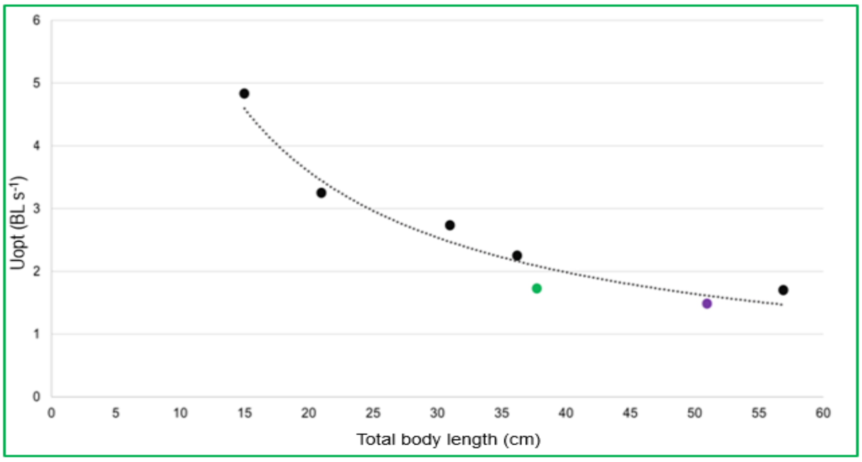


**Figure 6.7.** The relationship between $U_{opt}$ and total body length (•$V_1$, • $V_2$)

Regarding the values of the cost of transport (COST) among the tested variants, for both groups it can be observed a comfortable swimming range in terms of energy used (found between speeds 1-2 BL $s^{-1}$), as well as extremities that require higher energy costs (<1,> 2,5 BL $s^{-1}$) (Figure 6.8.). Statistical analysis indicates significant differences between the values calculated for the two variants (ANOVA, $p < 0.05$).

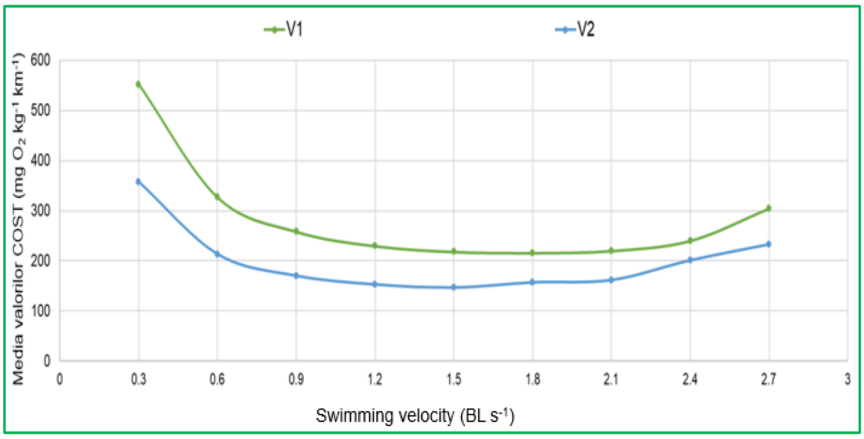


**Figure 6.8.** Mean values of COST for sterlet in $V_1$ and $V_2$

The only study showing COST data on a sturgeon species was conducted by McKenzie et al. 2001, on the Adriatic sturgeon (*Acipenser naccarii*), with a body mass of 279±17 g and a total length of 31.9±0.6 cm [244]. The results obtained in the case of sturgeons subjected to freshwater swimming indicate data similar to those obtained in this study, the COST of the Adriatic sturgeon being in the range 180-750 mg $O_2$ $kg^{-1}$ $km^{-1}$ (obtained between speeds 1-4 BL $s^{-1}$).

Following the the Ucrit protocol, each tested fish was further maintained in the metabolic chamber to measure excess post-activity oxygen consumption (EPOC).

Regarding the EPOC values, they were between 20.97-66.52 mg $O_2$ $kg^{-1}$ $h^{-1}$ in $V_1$, respectively 19.61-69.24 mg $O_2$ $kg^{-1}$ $h^{-1}$ in $V_2$.

### 6.3.3. Evaluation of the maximum metabolic rate (MMR) and the swimming performance of two pure species (*Acipenser ruthenus* L., 1758 and *Acipenser gueldenstaedii* Brandt & Ratzenburg, 1833) and their new hybrid, diamond (*Acipenser gueldenstaedii* ♀ × *Acipenser ruthenus* ♂)

Although the hybrids appear to have a more robust and thicker body at the trunk, the statistical analysis performed on the biometric characteristics did not indicate significant differences between the total length of the specimens (ANOVA, $p > 0.05$), the maximum body height and the trunk thickness (ANOVA, $p > 0.05$). As all 3 species were reared under the same conditions, no species showed any advantage that could influence swimming performance (Figure 6.9.).

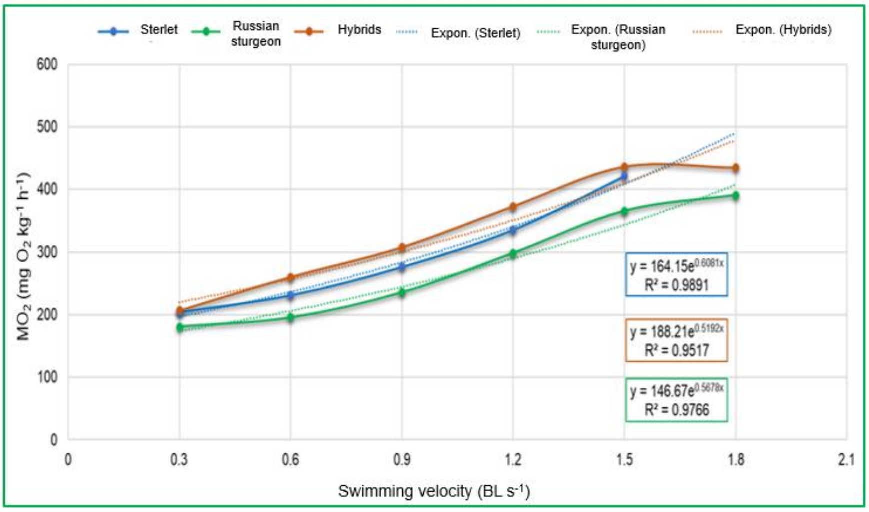


**Figure 6.9.** Oxygen consumption ($MO_2$) under the swimming speed for all the 3 studied species

Regarding the metabolic parameters, the statistical analysis did not indicate significant differences between the SMR, RMR and MMR values (ANOVA, p> 0.05), determined for the 3 species.

The highest SMR value (Figure 6.10.) was determined in the case of hybrids (approximately 10% higher). Routine metabolism rate (RMR) values ranged from 200 - 260 mg $O_2$ $kg^{-1}$ $h^{-1}$ for all three species. MMR values ranged from 400-450 mg $O_2$ $kg^{-1}$ $h^{-1}$, being 2.55 times higher than SMR. The highest oxygen consumption was also found in the case of hybrids, followed by Russian sturgeon and sterlet.

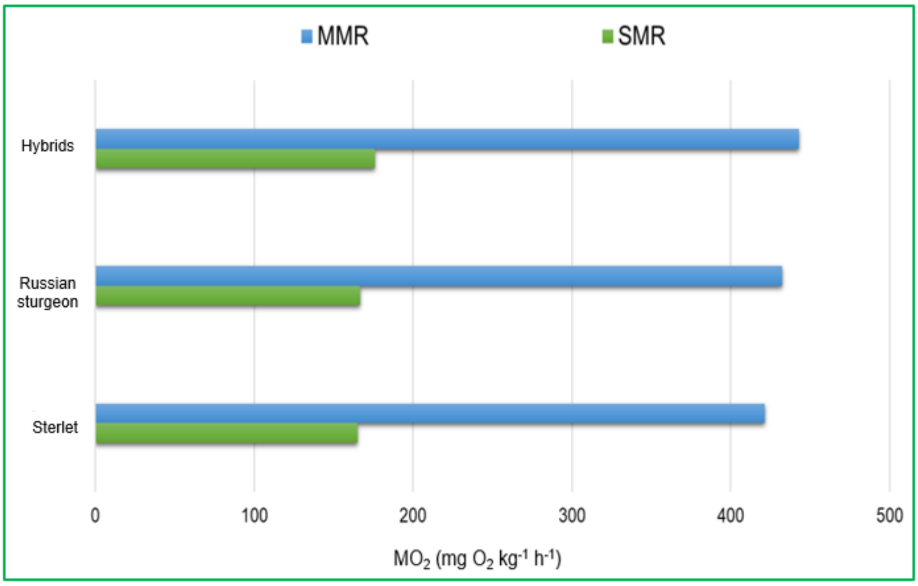


**Figure 6.10.** The values of SMR and MMR for the three species

The metabolic interval (AS) resulting from the difference between MMR and SMR did not show significant differences between the experimental variants (ANOVA, p>0.05).

Regarding the COST values, Tukey's B post-hoc analysis distributes the data sets as follows: the first set consists of sterlet, and the second set of Russian sturgeon and hybrids.

Statistical analysis indicates significant differences between EPOC values (ANOVA, $p < 0.05$), the Tukey's B test separating the values into 2 subsets: the first formed from sterlet and the second from Russian sturgeon, respectively hybrids.

Relatively low values, obtained in a short time, indicate a rapid post-exercise recovery/handling, in case of keeping the fish at a water speed of 0.3 BL $s^{-1}$. The calculated EPOC values are comparable to the data obtained for other sturgeon species, being much lower than those reported for other bone fish species [194].

Regarding swimming performance indices ($U_{crit}$ and $U_{opt}$), the ANOVA test identified significant differences between groups ($p<0.05$). Post-hoc analysis (Tukey's B) separated the corresponding $U_{crit}$ mean values into 2 subsets of data: the first set of Russian sturgeon, with a higher critical swimming speed, and the second set of sterlet and hybrids. High individual variability was observed in all tested groups.

Two possible reasons for why no distinct characters could be identified, resulting from hybridization could be due to the fact that the parents come from a similar environment (aquaculture) or that the degree of heterosis is not very pronounced in this juvenile stage [321] .

Sturgeons are generally considered to have a lower critical swimming speed than other bony fish species [134, 187, 194, 200, 372]. The low values of $U_{crit}$ are mainly due to the distinct morphology (presence of shields, presence of notochord or heterocircular caudal fin) [134, 241, 276]. In the case of the optimal swimming speed, the Tukey's-B test placed the sterlet in a distinct class, with Russian sturgeons and hybrids having similar values.

Ensuring an optimal swimming speed in aquaculture has been correlated by numerous studies with the reduction of stress and the improvement of technological indicators characteristic for the cultured biomass.

In order for aquaculture to benefit from the advantages of inducing swimming, several studies are needed that take into account the specificity of a species as well as the interaction between certain factors.

In sturgeons, for example, it must be worth noted that the aerobic scope is quite limited and that it often adopts a stationary behavior. Therefore, continuous swimming could lead to depletion or deformities of the skeleton, as noted in other species [386, 387]. On the other hand, intermittent swimming could be adopted in sturgeon culture, which has led to higher parameters, in the case of *Salmo salar*, bred under a hydraulic regime in the range of 0.7-1 BL $s^{-1}$ [314].

The results of this study need to be validated within a rearing system, where several parameters (storage density, feeding intensity, water indicators, ontogenetic stage, etc.) will be different from the experimental situation.

### 6.3.4. Behavior and kinematics of the tested species

Both the sterlet and the Russian sturgeon or hybrids quickly adapted to the offered conditions, a fact evidenced by the constancy of the resulting measurements. At low water speeds (<0.6 BL $s^{-1}$), they adopt a stationary behavior through slow movements of the pectoral and caudal fins. At intermediate speeds or higher speeds, sturgeons change their swimming, by alternating stationary behavior with "*cruising*" or "*burst*". This type of swimming has also been reported for other sturgeon species, being a method to save energy costs [337, 369, 389]. In terms of behavior, in most fish species, it is analyzed throught the following two parameters: tail beat frequency (TBF) and opercular beat frequency (OBF).

Following the statistical analysis, significant differences were found between TBF and OBF values for the 3 species (ANOVA, $p<0.05$). In the case of TBF, values higher than 1.2 BL $s^{-1}$ were similar for all species, with statistical differences (Tukey's B test) being reported for the first

3 swimming speeds (0.3 BL $s^{-1}$, 0.6 BL $s^{-1}$ and 0.9 BL $s^{-1}$) in the species *Acipenser gueldenstaeditii*.

The frequency of the opercular movement experienced interspecific variations, the OBF of the Russian sturgeons, at the level of speeds 0.3 BL $s^{-1}$ and 0.9 BL $s^{-1}$ being different from those of the sterlet and hybrids. At the same time, the Tuckey's B test identified significant differences in the case of the sterlet's OBF, corresponding to the swimming speeds of 0.9 and 1.2 BL $s^{-1}$, the rest of the values being similar between species.

The evaluation of TBF and OBF highlighted individual differences among the tested specimens. The general trend for both parameters is a positive one, but slightly lower values are observed, which can illustrate the stationary behavior adopted as a measure to optimize energy costs.

This is the first study to examine swimming performance and behavior in sterlet, Russian sturgeon and hybrid juveniles. Stronger correlations were obtained for the frequency of caudal movements, suggesting that the equations presented may be a model for estimating TBF in other sturgeon species as well. However, a lower $R^2$ value in the case of OBF, for all 3 species, cannot provide high accuracy. Additional tests are needed to refine a calculation pattern for these variables, so that they in turn become an estimation method of the oxygen consumption of a fish.

## 6.4. Conclusions

Following the experiments, a series of conclusions can be drawn, relevant both from a scientific point of view and from their practical applicability:

- both during the keep of the fish in the recirculating system and the metabolic chamber, the water quality parameters were maintained in the optimal interval for sturgeon, no significant differences were recorded that could have affected the metabolic rate;
- no biomass mortality was recorded throughout the experiments, which illustrates a high adaptability of biological material to the conditions offered by a RAS;
- at the intermediate weighing phase (after 25 days), the total growth increase of hybrids and Russian sturgeons was significantly different from that calculated for sterlet, so that the highest average body mass was obtained for hybrids (375±0,9 g), followed by Russian sturgeons (361±3.1 g) and sterlet (302±2.4 g), under the conditions of applying the same technological management;
- at the end of the growing period, major differences were found in the feed conversion factor (FCR) as well as in the protein conversion factor (PER), both parameters being higher in the case of hybrids and Russian sturgeons compared to those recorded in the case of sterlet.
- therefore, following the growth experiment, the synthetic table of growth performance indicators indicates better results in the case of hybrids, closely followed by Russian sturgeons. The higher indicators obtained for the two species may suggest an intensification of metabolic processes through a higher ability to retain proteins in feed. At the same time, following the growth experiment, a new potential candidate for intensive sturioniculture is emerging, namely, the new diamond hybrid;
- Regarding the metabolism parameters, there were no significant differences between the standard, routine and maximum metabolism rate, nor between the values of the aerobic interval for the three species, calculated for the same body mass (approx. 540 grams). The highest SMR value was recorded for hybrids, about 10% higher than for the other two species. The RMR followed another trend, being higher in the case of sterlet, followed by

hybrids and Russian sturgeons. The MMR ranged from 400-450 mg $O_2$ $kg^{-1}$ $h^{-1}$, being on average 2.55 times higher than the SMR. The metabolic scope calculated for the three species indicates a relatively low oxygen consumption, dedicated to routine activities.

- the values of SMR and RMR remained close even in the case of sterlet with a lower body mass, however, being observed a trend of inverse proportionality with the body mass of fish. Significant differences occurred at the level of MMR and AS, so that the sterlet with a lower body mass had a higher swimming performance, the MMR being 3.79 times higher than the SMR, which led to a longer aerobic scope.
- the swimming performance parameters ($U_{crit}$ and $U_{opt}$) presented different values. According to the Tukey's B test, the Russian sturgeons had a critical swimming speed, higher than the sterlet and hybrids, and in the case of the optimal swimming speed, the statistical analysis grouped the hybrids next to the Russian sturgeon, the sterlet having an optimal swimming speed, lower than the two species.
- Regarding the smaller sterlet, both the $U_{crit}$ and $U_{opt}$ values were significantly different, higher than those calculated for the sterlet with a higher body mass. Both $U_{crit}$ and $U_{opt}$ remained higher by about 69% and 15%, respectively, than larger fish.
- the excess post-activity oxygen consumption (EPOC) quickly returned to routine metabolism rate values after approximately 1 hour of monitoring. The values are comparable to those obtained for other sturgeon species, which strengthens the idea that sturgeons have an increased resistance to prolonged effort, recovering quickly, an essential feature imposed by their migratory and semi-migratory nature.
- the calculated cost of transport cost for both sturgeon sizes illustrates higher energy costs compared to other fish species. The data obtained illustrate a higher energy efficiency obtained by the sterlet, in the swimming range 1.2-1.8 BL $s^{-1}$, hybrids and Russian sturgeons preferring the interval of 1.5-2 BL $s^{-1}$. However, sturgeon swimming outside the indentified optimal range does not induce major changes in oxygen consumption, the COST showing an increase of only 10-15% (in the range between 0.6-1.2 BL $s^{-1}$ and 2-2.4 BL $s^{-1}$).
- the variables that characterize the swimming behavior (TBF and OBF) showed certain fluctuations, also described by the literature, being mainly due to the stationary behavior that characterizes sturgeons. The evaluation of TBF and OBF revealed a different behavior between individuals of the same species, however relatively strong correlations can be established in the case of TBF ($r^2$ approximately 0.87, 0.95, 0.84) which can provide an estimation model for TBF applicable to other sturgeon species.
- Regarding the behavior adopted by the tested specimens, it was found that sturgeons are generally docile and adapt quickly to the conditions offered by the metabolic chamber. At low water speeds it often adopts a stationary behavior, and at intermediate or high speeds, it alternates between stationary and "cruising" or "burst" swimming, this mechanism being described as a strategy to save energy costs.
- compared to other bony fish (especially salmonids), sturgeons have a lower swimming performance, with much lower critical swimming speeds, instead they have an increased resistance, which characterizes and supports migratory behavior.
- unlike the superior growth performance clearly observed in the case of hybrids, following the application of respirometric tests, no distinct features were found that could be attributed to the interspecific hybridization process, but rather outlined features close to the maternal part.

This study is a first time in literature evaluating the swimming performance in Russian sturgeons and the new diamond hybrid, being found only two studies on sterlet, but performed on much smaller body masses.

In conclusion, the results of the experimental study need applicability in a growth system, where technological management will be different from the conditions found in the metabolic chamber. In order to obtain positive results, the way in which swimming performance change according to the ontogenetic stage is an important condition to be considered in case of altering the hydraulic regime.

# Chapter VII.
# CONCLUSIONS, PERSONAL CONTRIBUTIONS, FUTURE RESEARCH DIRECTIONS

Thanks to the scientific research center "Romanian Center for Modeling Recirculating Aquaculture Systems - MoRAS" within the University "Dunărea de Jos" in Galați (UDJG), during the experimental studies, the experimental fish benefited by optimal conditions for development and growth due to advanced water quality control technologies.

Summarizing the information presented above, we can say that through the topic addressed, this paper opens new horizons in terms of the benefits of monitoring the metabolic rate of sturgeon species. Thus, following the studies carried out, a series of novelty information emerges:

- obtaining a complete picture of the parameters that describe the metabolic rate for different body masses encountered in the juvenile stage, in sterlet, beluga, bester and bestbeluga;
- contributes to the expansion of the information base by providing new information on the effects of changing the water temperature on the metabolism of sturgeon, or other sturgeon species, of the same size in general;
- for the first time, data for the oxygen consumption needs for carrying out standard and routine activities are presented, for sturgeon and three species of hybrids (bester, bestbeluga, diamond;
- for the first time, provides data on swimming performance and behavior for three native sturgeon species;
- Provides a model for estimating fish-specific oxygen consumption based on TBF quantification;
- It presents important aspects related to the technological and operational management of sturgeon juvenile reared under conditions of a recirculating aquaculture system and highlights a new potential candidate for intensive sturgeon farming, the new diamond hybrid (*Acipenser gueldenstaedii* ♀ × *Acipenser ruthenus* ♂).

The studies carried out in this doctoral thesis have treated only a small part of a vast topic regarding the monitoring of the metabolic rate in fish and its applicability. The results obtained from this series of experiments complete the profile of Danube sturgeon species and bring new information for three species of hybrids.

We can say that the observed trends for the tested species are somewhat comparable to those observed for other sturgeon species, especially those resident in North American rivers. However, there were also differences that can be attributed to the intraspecific variation or the measurement protocol used.

Therefore, in order to facilitate the comparison between sturgeon species but also to be able to provide a model for estimating metabolic rates, it is recommended to develop a standardized measurement protocol, applicable to a species.

However, many studies and research directions remain to be addressed:

- performing a comparison of metabolic parameters calculated following the application of different protocols (eg. $U_{crit}$ test versus endurance test);
- evaluation of the interaction between the size of the swimming tunnel and the metabolic parameters;
- evaluation of the effects produced by the application of feeding intensities or by the administration of feeds with different protein content, on the swimming performance;
- evaluation of the metabolic rate according to circadian rhythm, or other abiotic factors (pH, salinity, $O_2$ or $CO_2$ levels, food availability) or biotic (sex, intraspecific competition);
- inducing swimming in sturgeon culture in order to improve production (reducing cannibalistic or aggressive behavior, improving muscle mass and fitness in general) or to obtain vigorous fish, which can be successfully used in restocking programs;
- concomitant evaluation of the metabolic rates and of some oxidative stress biomarkers, in order to validate the respirometric tests as a non-invasive technique for monitoring the technological comfort state of the cultured biomass.

# LIST OF PUBLISHED AND PRESENTED ARTICLES AT SCIENTIFIC EVENTS

The results of the research activity undertaken during the doctoral studies were materialized by publishing or communicating scientific papers as follows:

## Scientific articles published in ISI PROCEEDINGS:

**1.** Crețu M., Dediu L., Docan A., **Andrei R.C. (Guriencu)**,''Effects of feeding levels on growth performance, and body composition of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), publicat în Scientific Papers-Series D-Animal Science (2019), Vol. LXII (2), pp. 341-347. WOS:000509121700054, ISSN 2285-5750. http://animalsciencejournal.usamv.ro/pdf/2019/issue_2/vol2019_2.pdf

**2.** Crețu M., **Andrei R.C. (Guriencu),** Dediu L., Docan A., Mocanu M., ''Effects of short-term starvation and different dietary protein level on leukocyte reaction in cultured rainbow trout Oncorhynchus mykiss (Walbaum, 1792)'', Scientific Papers-Series D-Animal Science (2020), Vol. LXIII (1), pp. 522- 527.
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**3.** Crețu M., Cristea V., Docan A., Dediu L., **Andrei R.C. (Guriencu)**, Cordeli (Savescu) A.N., ''Hematological profile of rainbow trout under different feeding intensities", Scientific Papers-Series D-Animal Science. Under publication.

## Scientific articles published in BDI journals:

1. **Andrei R. C. (Guriencu)**, Cristea V., Dediu L., Creţu M, Docan A.I., Grecu I.R., Coadă M.T., Simionov I.A., ''The influence of different stocking densities on growth performances of hybrid bester (*Huso huso* ♂ x *Acipenser ruthenus* ♀) in a recirculating aquaculture system'', articol publicat în Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society (2016), vol 9, Issue 3, pp. 541-549, http://www.bioflux.com.ro/docs/2016.541-549.pdf.
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4. **Andrei R. C. (Guriencu)**, Cristea V., Dediu L., Creţu M, Dediu L., Docan A.I.,'' The effect of feeding rate on growth performance and body composition of Russian sturgeon (*Acipenser gueldenstaedtii*) juveniles'', articol publicat în Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society (2018), vol 11, Issue 3, pp. 645-652, https://www.bioflux.com.ro/docs/2018.645-652.pdf.
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7. Crețu M., Cristea V., Docan A., Vârlan O.G., Dediu L., **Andrei R.C. (Guriencu)**, ''The effect of dietary supplementation with Sea-buckthorn (*Hippophae rhamnoides*) and Spirulina (Spirulina platensis) on the growth performance of some sturgeon hybrids'', articol publicat în Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society (2017), vol 10, Issue 5, pp. 1157-1163, http://www.bioflux.com.ro/docs/2017.1157-1163.pdf.
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### Others publications:

1. Lupoae P., Țupu E., Zlate E., Barbu A.V., Cimpoeru V., Lupoae M., **Guriencu R.C.**, Lificiu I.; Delectus Seminum – Grădina Botanică Galați, nr. 19/2019, nr. pag. 30; Editura Academica Galați, ISSN 1224-7006, ISSN-L 1224-7006.
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### Prizes obtained during the doctoral studies:

1. **Second place (II)** ,,Student award" ,, Aquaculture and Biosystems" section, for the paper ,,Oxygen consumption and swimming behaviour of Sterlet sturgeon (*Acipenser ruthenus*) in relation to water velocity" - **Raluca-Cristina Andrei (Guriencu)**, Victor Cristea, Mirela Crețu, Mogodan Alina, Lorena Dediu - "International Agricultural, Biological and Life Science Conference" AGBIOL Conference, 1-6 September 2018 Edirne, Turkey.

2. **Second place (II),** ,,Progress in science and engineering of food bioresources" section, for the paper **,,**Effects of dietary protein and meal frequency on growth performance of Nile tilapia (*Oreochromis niloticus*) reared in a recirculating aquaculture system" - **Raluca-Cristina (Guriencu) Andrei**, Victor Cristea, Lorena Dediu, Mirela Crețu, Ira-Adeline (Chihaia) Simionov – The 4th Edition of Scientific Conference of Doctoral Schools, Galați, Romania, 2-3 June 2016, Galati, Book of Abstracts pp. 91.

### Participation in research projects:

1. **INOVTEHNOSTUR** - ,, Genetic selection and breeding technology to increase the profitability of sturgeon aquaculture", Contract no. 53PTE/2016 – PN-III-P2-2.1-PTE-2016-0188.

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