fishes

MDPI

Article

A Study on the Metabolic Rate Change Pattern in F_2 Hybrid Sturgeon, the Bester (*Huso huso* × *Acipenser ruthenus*), during the Early Developmental Stage

Dong In Kim

Aquaculture Research Institute, Kindai University, Shirahama 3153, Nishimuro, Wakayama 649-2211, Japan; donginkim0508@gmail.com or dongin_kim@kindai.ac.jp

Abstract: The primary goal in aquaculture is to maximize the growth and survival of farmed fishes at a minimal cost, which requires an understanding of the changes in metabolism undergone by different fish breeds during development. In particular, highly intensive aquaculture production systems, such as recirculating aquaculture systems, require a better understanding of oxygen consumption. Ontogenetic phase shifts (i.e., sudden changes) in metabolism have been observed in several aquaculture species during early development, and such metabolic phase shifts may help to predict the oxygen consumption of aquaculture species during different stages of their development. Here, I analyzed the pattern of metabolic changes in the F_2 hybrid sturgeon, the bester (*Huso huso* × *Acipenser ruthenus*; hereafter, referred to as the F_2 bester), during its early development. I observed ontogenetic phase shifts in metabolism in the F_2 bester at body mass values of about 0.2 and 0.8 g. Thus, the F_2 bester undergoes ontogenetic phase shifts in metabolism during early development, which can help to characterize oxygen consumption at a specific developmental period. Therefore, oxygen can be appropriately adjusted and replenished during breeding.

Keywords: respirometry; oxygen consumption; hybrid sturgeon; aquaculture; resting routine metabolic rate; fish development

Citation: Kim, D.I. A Study on the Metabolic Rate Change Pattern in F_2 Hybrid Sturgeon, the Bester (*Huso huso* × *Acipenser ruthenus*), during the Early Developmental Stage. *Fishes* **2023**, *8*, 113. https://doi.org/10.3390/fishes8020113

Academic Editor: Gioele Capillo

Received: 9 January 2023
Revised: 4 February 2023
Accepted: 13 February 2023
Published: 15 February 2023
Corrected: 12 May 2023

1. Introduction

The ultimate goal of aquaculture is to maximize the growth and development of aquaculture species with minimal costs [1,2]. To this end, fishery-related industries have been developed using research and knowledge from fields including biology, ecology, animal behavior, and engineering [3–5]. The rapid development of highly intensive production systems, such as recirculating aquaculture systems (RASs), has resulted in the timely integration of engineering studies for optimizing waste management (including system design) and biological studies for understanding the physiological requirements of individual aquaculture species [3,4]. However, the water pumps and oxygen supply in RASs are very expensive; thus, determining the oxygen consumption of each aquaculture species is an essential aspect of cost-efficient aquaculture under intensive conditions [6–8].

Metabolism generally refers to the amount of energy per unit time that an organism requires to live and can be expressed as heat production; although, oxygen consumption is usually used as an indicator of metabolism [9,10]. Oxygen consumption varies depending on the condition of an organism; therefore, a defined metabolic rate is used for each organism according to its activity status at the time of measurement. For example, the standard metabolic rate (SMR) is defined by the oxygen consumption of an organism performing minimal functional activity, i.e., where voluntary muscle movement and activities related to food consumption and digestion are excluded [11]. However, when the organism exhibits some behavioral activity (e.g., during swimming or posture maintenance), the term routine metabolic rate is preferred; the definition of which incorporates oxygen consumption arising from some level of activity [11,12]. In addition, the maximum metabolic rate is defined

as the oxygen consumption of an organism performing an action for which maximum activity can be sustained for a limited time [13].

Although metabolism studies on aquaculture species are limited compared with those on other organisms, such as livestock, oxygen consumption has been used as an indicator of metabolism to examine the effects of breeding conditions, such as dissolved oxygen, on aquaculture species [7,8,14]. For example, low oxygen levels (hypoxia) are known to negatively affect the growth of aquaculture species [15,16], e.g., the growth rates of Atlantic salmon (*Salmo salar*), channel catfish (*Ictalurus punctatus*), Atlantic cod (*Gadus morhua*), and rainbow trout (*Oncorhynchus mykiss*) are known to decrease under hypoxic conditions [17–20]. Several studies have found that breeding under moderately high levels of dissolved oxygen (i.e., moderate hyperoxia) promotes the growth of organisms [18,19,21]; however, according to a study on Salmonidae, long-term exposure to hyperoxia could lead to problems such as oxidative stress and increased disease susceptibility [22]. These findings indicate that understanding the metabolic rate of aquaculture species is essential for establishing optimal breeding conditions that promote growth and survival under intensive production conditions, such as those in RASs, because the appropriate dissolved oxygen level (i.e., neither hypoxic nor hyperoxic conditions for each species) can only be predicted when the pattern of metabolic rate changes is clearly identified [3].

In addition, metabolism-related research on aquaculture species might provide clues for understanding the physiological, ecological, and morphological characteristics of each organism [23–26]. Intraspecific variations in metabolic rate are known to exist in aquaculture species during growth, including several distinct stages of metabolic change. For example, the metabolic rate of tiger puffer (*Takifugu rubripes*) changed rapidly from 0.002 to 0.01 to 0.1 g during the early developmental stages of the population, and this stepwise change in metabolic rate (i.e., an ontogenetic phase shift in metabolism) may be related to cannibalism, which is a problem encountered during tiger puffer breeding [23]. In the case of Japanese flounder (*Paralichthys olivaceus*), the metabolic rate of the population changed markedly from 0.002 to 0.01 to 0.2 g, and this stepwise change may be related to the development of the respiratory tract and other morphological changes [24,25]. Given that such research has improved our understanding of the unique characteristics of aquaculture species, further studies on stepwise changes in metabolism and the relationship of such changes to physiological, ecological, and morphological changes are warranted.

Acipenseridae species are becoming popular in the food industry because products made with the caviar, meat, oil, and air bladder have an exquisite taste [27,28]. However, demand has fallen short of supply, leading to indiscriminate overfishing, which has placed many Acipenseridae species in the danger of extinction [29]. Therefore, aquaculture research aimed at replenishing Acipenseridae numbers has been conducted over the past decade, and studies on preserving resources to increase wild populations of Acipenseridae are also considered important [29,30]. Regarding the sturgeon the bester, the F_2 hybrid is produced by recrossing the F_1 hybrid (*Huso huso* × *Acipenser ruthenus*; hereafter, referred to as the F_1 bester), which is an artificial hybrid between the natural purebred sterlet sturgeon (*Acipenser ruthenus*) and beluga sturgeon (*Huso huso*), produced to increase breeding efficiency [31]. This new breed has received attention in many countries, including Japan, because it is easy to raise, resistant to high oxygen levels during breeding, easily converted to the consumption of artificial feed, and can ingest all types of vegetable protein-based foods [31–35]. In addition, it can adapt to low feed levels and quickly adjust to its surroundings [31,34,35]. Therefore, the hybrid F_2 bester is an extremely important new fish breed in aquaculture.

Despite this fact, most studies on the metabolism of Acipenseridae have focused on fish in their juvenile or adult stages and/or have been limited to purebred fish [3]; thus, studies on artificial hybrids, such as the F_1 or F_2 bester, which are created to increase breeding efficiency are lacking, as are studies on their metabolic changes during early development when rapid physiological, ecological, and morphological changes occur. Considering that the breeding of larvae and fry is the most difficult hatchery process during the breeding

of Acipenseridae species [36], obtaining fundamental data that help breeders determine the appropriate oxygen demand and/or create an optimal breeding condition for this species are critical. Unfortunately, most Acipenseridae aquaculture farms, including those breeding hybrids such as F_1 and F_2 bester, typically conduct breeding under intensive conditions without considering the physiological requirements of the bred species for dissolved oxygen or their metabolic rate in reality.

Therefore, in the present study, I evaluated changes in the metabolic rate of the F_2 bester during its early development based on the resting routine metabolic rate (i.e., intermediate between the resting and routine activity states; [23–25]). In addition, the stepwise metabolic changes in the hybrid F_2 bester were compared with those of the naturally occurring purebred tiger puffer and Japanese flounder, and the relationships among their physiological, ecological, and morphological characteristics were analyzed based on previous studies. By identifying patterns of changes in metabolic rate during the early development of F_2 besters, this study will not only help predict the optimum oxygen demand in the operation of aquaculture systems but will also facilitate future research on the physiological, ecological, and morphological traits of this breed.

2. Materials and Methods

2.1. Fish Preparation

The Tsukuba Research Institute, Fujikin Inc. Ltd., in Tsukuba, Japan, generously provided the fish used in this study. Forty F_2 besters (*Huso huso* × *Acipenser ruthenus*) with wet body mass ranging from 0.0243 g (4-day-old) to 2.8647 g (65-day-old) were examined to determine resting routine metabolic rates. All fish were reared in a 150 L polycarbonate cylinder tank with a constant supply of fresh water regulated at 19 °C–21 °C. They were fed a synthetic compound diet consisting of brine shrimp (*Artemia* sp.) larvae enhanced with eicosapentaenoic acid and docosahexaenoic acid twice a day. Fish weighing between 0.0243 g (4-day-old) and 0.4631 g (38-day-old) were placed directly into a 30 L polycarbonate cylinder tank maintained at 20 °C for 5–12 h; fish weighing between 0.6481 g (40-day-old) and 1.6631 g (60-day-old) were kept at this temperature for 12–15 h; and fish weighing >2.0000 g were kept at this temperature for >24 h to adapt to the metabolic temperature. To obtain an exact measurement of the metabolic rate and body mass, all fish were fasted for 12–24 h or more prior to respirometry tests.

2.2. Respirometry Tests

Respirometry tests were performed using a semi-closed method under controlled conditions at a constant water temperature of 20 °C. The semi-closed method was used as previously described [23,37]. The following procedure was used to improve measurement accuracy. Fish were carefully transferred from the 30 L polycarbonate cylinder tank to 50 mL beakers, and obviously weak or dead individuals were carefully removed using a pipette. Only individuals that appeared to be healthy were immediately transferred to a respiration chamber and allowed to acclimatize for ~2 h to regain stability due to handling stress. The volume of the respiration chamber was adjusted based on the developmental stage of the fish (i.e., 60 mL for 4–44-day-old fish; 136 mL for 45–49-day-old fish; and 349 mL for 56–65-day-old fish). Subsequently, to set up the control group, the respiration chamber and a blank chamber were connected using a silicon tube. During this procedure, a pipette was often used to remove tiny air bubbles from the chambers and/or inside the silicon tube (length: 10–30 cm; inner diameter: 3 mm; and outer diameter: 5 mm) to ensure that the respiratory measurements were accurate. A small valve was then attached to the silicon tube to control the water flowing from the respiration chamber into the blank chamber. This procedure is essential for equalizing the oxygen concentration between the two chambers, i.e., the oxygen concentration in the blank chamber is appropriate for the control group because it is equivalent to the initial oxygen concentration in water. Both chambers were then sealed completely to prevent external water from entering, after which the breathing chamber, in which the oxygen concentration was reduced by fish respiration, was used

for the treatment group. On completion of this process, the oxygen concentration of the water in each chamber was measured using the Winkler titration method [37,38], and the calculated concentration difference was considered to be the oxygen consumption of the fish. Finally, the body mass of the fish in the breathing chamber was measured using an electronic balance that can measure up to five decimal places (ER-182A; A&D Company Ltd., Tokyo, Japan). The details of the calculation of the metabolic rate are described in detail in previous studies [23,24].

2.3. Data Analyses

Data analysis was performed using previously published statistical models to determine whether ontogenetic phase shifts in metabolism occurred [23,24]. Various negative allometric relationships were evaluated using two previous statistical models. Nearly linearly perpendicular relationship (i.e., 4–16-day-old) and/or transitional phases were excluded.

The first model is represented by Equation (1), which accounts for each incidence of negative allometry:

$$VO_2 = a_i M^{\hat{b}} \quad (1)$$

where a_i is an intragroup scaling constant of the ith group and $\hat{b}$ is the scaling exponent of each group.

The second model is represented by Equation (2), which accounts for the overall line (i.e., intergroup) constituting the total of each negative allometry:

$$VO_2 = aM^{\overline{b}} \quad (2)$$

where α is an intergroup of one of the groups and $\overline{b}$ is the overall scaling exponent.

Each model can be rewritten as Equations (3) and (4):

$$yij = \log \alpha_i + \hat{b}xij + \varepsilon ij, \quad (3)$$

$$yij = \log \alpha + \overline{b}xij + Eij, \quad (4)$$

where yij is log VO_2; xij is log M; and εij and Eij represent the random variation in the metabolism of the intragroup and intergroup, respectively.

Ordinary least-squares regression was used to estimate $\overline{b}$ and log α to minimize the sum of the μi squares, i.e., the vertical distance of the group mean $(\overline{x}_i, \overline{y}_i)$ from the overall line [39].

Given that $\log \alpha i$ can be expressed as $log\ \alpha + \mu i + \left(\overline{b} - \hat{b}\right)\overline{x}_i$, Equation (4) can be rewritten as follows [40]

$$yij = \log \alpha + \mu i + \left(\overline{b} - \hat{b}\right)\overline{x}_i + \hat{b}xij + \varepsilon ij \quad (5)$$

Statistical significance was determined using one-way ANCOVA by computing the sum of squares, degrees of freedom, and mean squares attributable to each term of Equation (5). All data were analyzed using Microsoft Excel for Office 365 (Microsoft Corp., Redmond, WA, USA).

3. Results

The results of regression analyses of oxygen consumption rates (VO_2 in μL of O_2 fish^{-1} min^{-1}) in proportion to changes in body mass (M in g) and mass-specific rates of oxygen consumption (VO_2/M in μL of O_2 g^{-1} min^{-1}) are shown in Figure 1. During the early prolarval stage (0.0243–0.0355 g; from 4–16-day old), no apparent allometric relationship was observed between VO_2 and M because body mass remained relatively constant (Figure 1). Although the log–log plot exhibited a linearly perpendicular relationship, the mass–metabolism relationship was not represented by the allometric equation

(i.e., $VO_2 = aM^b$) during the early prolarval stage (Figure 1); thus, this stage was excluded from the regression analysis.

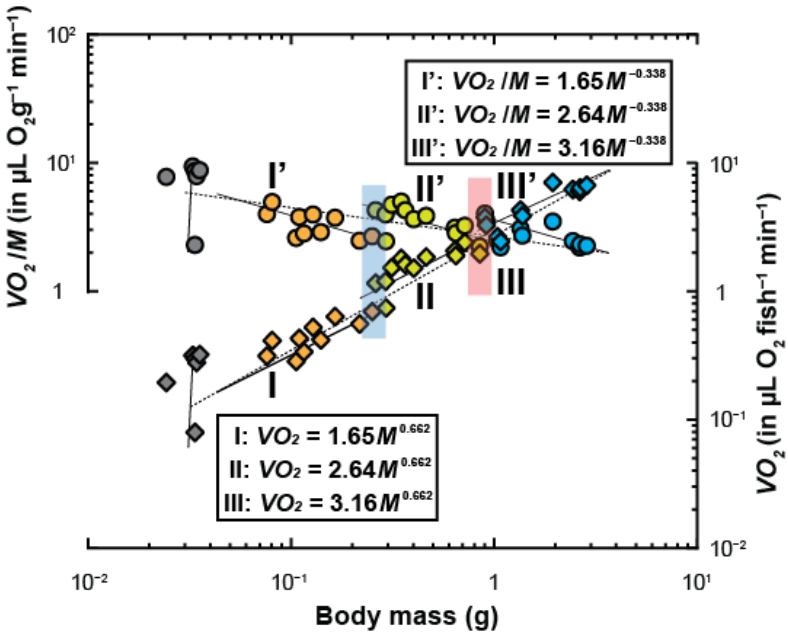

Figure 1. Changes in oxygen consumption (VO_2, closed rhombi) and mass-specific rates of oxygen consumption (VO_2 /M, closed circles) according to the body mass (M) of F_2 bester (*Huso huso* × *Acipenser ruthenus*) from the prolarval stage to early juvenile stage (from 0.0243 g [wet body mass, 4-day-old] to 2.8647 g [wet body mass, 65-day-old]; N = 40). Vertical black lines at about 0.03 g indicate VO_2 and VO_2 /M, which increased daily 4–16-day-old, despite almost no change in body mass. Intragroup phases of negative allometry are denoted by the ranges spanned by three solid black lines for VO_2 and VO_2 /M, respectively. The blue and red boxes indicate the intermediate phase shifting points, i.e., transitional phases with ranges covered by the overlapping solid lines, at about 0.2 and 0.8 g, respectively. Intergroup lines without transitional phases are shown as broken black lines for VO_2 and VO_2 /M, respectively.

The regression analysis results for each group (i.e., intragroup results) are shown in rows 1–3 of Table 1. The allometric relationships between VO_2 and M with growth from prolarvae (0.0761 g; 22 day old) to early juveniles (2.8647 g; 65 day old) exhibited three negative allometries, interposing two intermediate phase shifting points (i.e., transitional phases) at about 0.2 and 0.8 g, with a scaling exponent that was kept constant in each group (Figure 1). Each scaling exponent was significantly smaller than unity for any regression line ($P < 0.05$; two-tailed t-test). The slopes of the intraspecific lines ($\hat{b}$) in each of the three groups were statistically indistinguishable ($F_{2,\,28} = 0.22$; $P = 0.803$; one-way ANCOVA). The estimated intraspecific scaling exponent was $\hat{b} = 0.662 \pm 0.081$. The scaling constant of each group at $\hat{b} = 0.662$ had the following logarithm: $\log a_1 = 0.216 \pm 0.077$; $\log a_2 = 0.422 \pm 0.038$; and $\log a_3 = 0.499 \pm 0.030$. Each scaling constant was estimated as follows: $a_1 = 1.65$; $a_2 = 2.64$; and $a_3 = 3.16$. The results of intergroup regression analysis are shown in rows 4–6 of Table 1. The estimated interspecific scaling exponent was $\bar{b} = 0.911 \pm 0.014$.

Table 1. Summary of intragroup (rows 1–3) and intergroup (rows 4–6) regression analyses of the relationship between log VO_2 (VO_2 in μL of O_2 $fish^{-1}$ min^{-1}) and log M (M in g) in F_2 bester at 20 °C. N, number of individuals; P, scaling exponent difference from unity (two-tailed Student's t-test); R^2, squared correlation coefficient between log VO_2 and log M.

Group	N	Body Mass (g)	Scaling Constant	Scaling Exponent (x ± SEM)	P	R^2
1 [a]	10	0.0761–0.2511	1.50	0.616 ± 0.164	4.77×10^{-2}	0.637
2 [a]	12	0.2612–0.8507	2.54	0.613 ± 0.159	3.55×10^{-2}	0.597
3 [a]	12	0.9033–2.8647	3.06	0.727 ± 0.118	4.28×10^{-2}	0.792
1–3 [b]	34	0.0761–2.8647	$\alpha = 2.93$	$\hat{b} = 0.911 \pm 0.014$	2.38×10^{-7}	0.993
1–3 [c]	34	0.0761–2.8647	2.86	0.878 ± 0.035	1.37×10^{-3}	0.952
Total [d]	40	0.0243–2.8647	2.80	0.800 ± 0.034	1.03×10^{-6}	0.935

[a] Estimating parameters to minimize the sum of squares of εij in each group. [b] Estimating parameters to minimize the sum of squares of μi. [c] Estimating parameters to minimize the sum of squares of Eij. [d] Total individuals including those in the early prolarval stage (i.e., 0.0243–0.0355 g; from 4–16-day old).

The validity of Equation (5) was tested using one-way ANCOVA (Table 2). The μi values were not equal to zero ($F_{1,30} = 5.84$; $P = 2.19 \times 10^{-2}$; one-way ANCOVA), suggesting that the intragroup means were not on the intergroup (overall) line (Table 2). Furthermore, the intraspecific ($\hat{b} = 0.662$) and interspecific ($\overline{b} = 0.911$) scaling exponents differed significantly ($F_{1,30} = 8.12$; $P = 7.83 \times 10^{-3}$; one-way ANCOVA). These findings indicate that the intragroup scaling constant (a_i) rapidly increased from $a_1 = 1.65$ to $a_3 = 3.16$ during the early developmental stages (Figure 1). Consequently, the metabolic rate of F_2 bester can be defined as $VO_2 = a_i M^{0.662}$, with a_i increasing twice throughout the transitional phases.

Table 2. Summary of the one-way ANCOVA results for respirometric tests in F_2 bester using Equation (5). SS, sum of squares; DoF, degrees of freedom; MS, mean of squares; MSR, mean square ratio.

Term	SS	DoF	MS	MSR	p
$\log \alpha$	1.128248	1	1.128248	165	9.76×10^{-14}
μ_i	0.039872	1	0.039872	5.84	2.19×10^{-2}
$\left(\overline{b} - \hat{b}\right)\overline{x}_i$	0.055444	1	0.055444	8.12	7.83×10^{-3}
$\hat{b}x_{ij}$	5.993409	1	5.993409	878	8.94×10^{-24}
ε_{ij}	0.204753	30	0.006825		
Total (approximate mean)	6.293478	33			
Total (about zero)	7.421726	34			

4. Discussion

4.1. Importance of Metabolic Scaling Studies in the Aquaculture Industry

To breed the F_2 bester effectively using a RAS, its operational costs must be carefully considered, including the oxygen supplementation required to fulfill the breed's physiological needs as well its chemical and biological consumption of oxygen due to the transformation of metabolites [41,42]. To this end, the relationship between the body mass and metabolism of the F_2 bester at the early developmental stage was analyzed in the present study using regression analysis (Figure 1). I found that although the scaling constant (a_i) increased rapidly twice, the scaling exponent in each group was maintained at a certain level (i.e., $\hat{b} = 0.662$) (Figure 1 and Table 1). Therefore, during the early development of the hybrid F_2 bester, its metabolic rate exhibits a stepwise increase in the scaling constant (a_i), i.e., an ontogenetic phase shift in metabolism, similar to that of the naturally occurring purebred tiger puffer [23] and Japanese flounder [24,25], rather than exhibiting a nonlinear or curvilinear log–log metabolic scaling relationship with growth (i.e., with increasing body mass; Figure 1). Specifically, the scaling constant (a_i) rapidly increased with body masses of about 0.2 and 0.8 g (26 and 58 mm in total length; Kim, unpublished data), i.e., the starting

points of the transitional phases (Figure 1 and Table 1). Based on these results, it is thought that rapid metabolic changes can be predicted in the F_2 bester at the early developmental stage, which will be useful for determining the period during which the oxygen supply in the RAS should be adjusted.

Similarly, the ontogenetic phase shift could be used to understand the growth and survival of the aquaculture species from a metabolic perspective. In a study on the tiger puffer, for example, the scaling exponent tended to be maintained at a certain level (i.e., $\hat{b}$ = 0.795) at each stage, consistent with the F_2 bester in the present study, whereas the scaling constant (a_i) rapidly increased at body masses of about 0.002, 0.01, and 0.1 g during early development [23]. Surprisingly, cannibalism, which directly affects the survival of the tiger puffer during breeding, occurred most frequently during the transitional phase when a rapid increase in the scaling constant (a_i), i.e., an ontogenetic phase shift, occurred [23]. This indicates that metabolic phase shift occurs more rapidly in organisms with faster growth, and a higher consumption of oxygen is required during this process. Moreover, individuals that consume high levels of oxygen develop more favorable (e.g., strokes with high motility during swimming) morphology and behavior compared to those remaining in previous metabolic phases that consume less oxygen [23–25,43]. In particular, the pseudobranch of the tiger puffer reaches its complete form when the organism reaches a body mass of 0.01 g; thus, the visual system is highly developed [44]. In addition, a large amount of oxygen is supplied to the arterial blood in the developed first gill arch, and sufficient blood flow is supplied to the choroid of the eye through the pseudobranch [45], enabling cannibalism through the quick detection of underdeveloped individuals, i.e., individuals with slow growth [23].

Although data indicating the relationship between metabolic rate changes during early development and cannibalism in the F_2 bester were not obtained in the present study, cannibalism between F_2 bester individuals can be reasonably inferred based on previous studies in which similar developmental patterns were observed in many intergeneric hybrids [46,47]. In a study conducted by Oprea [36], three groups, Variants I–III, were divided into a 0.167 m^3 test water tank, and a 20-day breeding experiment was conducted with 300, 400, and 500 F_1 bester individuals, respectively, in the larval stage (body mass of about 0.14 g). The survival rate of Variant I, which had the lowest breeding density, was 70%, whereas that of Variant III, which had the highest breeding density, was only 48%. Furthermore, Variant I exhibited a growth rate of about 97.3%, whereas Variant III exhibited a growth rate of only 46.7%. Interestingly, the highest difference in growth was observed in the group with the highest breeding density, Variant III, suggesting that the growth difference among organisms was related to cannibalism, which was the major factor explaining the decline in survival [36]. Although the present study cannot provide evidence linking intense cannibalism to the transitional phase, i.e., the ontogenetic phase shift in metabolism, a rapid change in metabolic scaling according to growth observed during early development cannot be ruled out as a factor influencing the survival of the F_2 bester. Future studies should clarify the association between ontogenetic phase shift in metabolism and cannibalism through behavioral analysis.

4.2. Relationship between Changes in Metabolic Scaling and Development Trajectory

To date, studies on metabolic scaling have consistently found that the ontogenetic phase shifts in which the scaling constant (a_i) rapidly increases are related to the development trajectory of individual organisms. In Japanese flounder, the scaling constant a_i increases three times in a stepwise manner, i.e., it increases rapidly at body masses of 0.002, 0.01, and 0.2 g (~6, ~11, and ~28 mm in standard length, respectively), and this species undergoes a total metabolic rate change in four phases [24,25]. Interestingly, distinct changes in morphology and behavior were identified during the transitional phase when metabolic rate changed rapidly in this species [24,25], consistent with changes in the tiger puffer [23]. In particular, they exhibit characteristic morphological changes unlike those of ordinary fish [25,48]. For example, during metamorphosis, they transit from an

isomorphic three-dimensional form to a flat two-dimensional form [25,48], and these dramatic growth pattern changes occur at a body mass of about 0.01 g during the transitional phase, i.e., when the ontogenetic phase shift in metabolism occurs [24,25]. In addition, this transitional phase has been confirmed to coincide with the developmental period in which larvae transition to early juvenile stages [24,25,48]. Although the mechanism underlying metabolic scaling cannot be explained, as it depends on several internal and external factors, a statistical similarity was detected between the scaling exponents of the body surface and metabolism according to a comparison of the relationship between body mass and body surface based on developmental stage regression analysis with metabolic scaling in Japanese flounder [25]. This finding is consistent with those of many studies [49–51] and suggests that metabolic scaling may be affected by changes in body morphology [25]. In swimming activity, maximum swimming speed is higher in individuals with a body mass of 0.01 g, i.e., the starting point of the transitional phase, than in those remaining in the previous phase in this species [25]. Therefore, ontogenetic phase shift, during which the scaling constant (a_i) rapidly increases, appears to be closely associated with the development trajectory of fish.

Studies on the development trajectory of the F_2 bester in the early developmental stages are currently lacking. Nevertheless, based on studies wherein the developmental patterns of F_2 bester were similar to those of their grandparents (sterlet and beluga sturgeons) and parents (F_1 bester) [52], changes in metabolic scaling and its association with development trajectory can be inferred indirectly. According to a regression analysis of the relationship between the metabolism and body mass of the F_2 bester during the early developmental stage, an ontogenetic phase shift in metabolism was observed at a rapidly increasing scaling constant (a_i) at about 0.2 and 0.8 g (26 and 58 mm in total length; Kim, unpublished data; Figure 1 and Table 2).

The transitional phases (i.e., 0.2 and 0.8 g) of the F_2 bester were used to indirectly estimate their unreported development trajectory by referring to the early developmental patterns observed in the sterlet sturgeon, beluga sturgeon, and F_1 bester (Table 3). It was observed that the respiratory tract, including gills [53], and buccal cavity in front of the digestive tract [53,54] started developing in the sterlet sturgeon at ~0.2 g. The development of the respiratory and digestive systems, along with changes in swimming behavior, could improve survival by enabling individuals to settle on the bottom of the river where they can feed exogenously [53–55]. Such rapid morphological and behavioral changes were also observed in beluga sturgeon during this transitional phase [56,57]; the growth patterns of which included major changes in the development from an isomorphic three-dimensional form to an elongated one-dimensional form [57]. In terms of behavior, beluga sturgeon occasionally exhibit schooling as a means of optimizing survival when confronted by predators [57], similar to the survival mechanism of sterlet sturgeon, which settle at the bottom of the river to avoid predators in nature [55]. Therefore, after this developmental stage, severe cannibalism is frequently observed in aquaculture farms [57]. This tendency was also observed in the F_1 bester, a crossbreed of the sterlet sturgeon and beluga sturgeon, during this transitional phase, in which the morphogenesis of fins, including the fin ray, and the number and size of scutes increased markedly [28]. Such fin development is related to swimming and eating behavior and is thus critical to their survival. Scutes protect the body from external physical damage, improving survival in the face of predation [28]. As previously discussed, cannibalism often occurs during breeding when the stocking density is high in aquaculture farms [36]; therefore, individuals that have successfully undergone the metabolic phase shift at this time will be more likely to survive than those that have not.

Table 3. Summary of the changes in morphology and behavior associated with a phase shift in the scaling constant a_i during transitional phases.

Shifting Point (Body Mass: g)	Name of Fish	Morphological Changes	Behavioral Changes
*a*1–*a*2 (about 0.2)	Sterlet sturgeon (*Acipenser ruthenus*)	• Onset of development of respiratory organs, such as gills [53]. • Development of the buccal cavity [53,54].	• Onset of exogenous feeding signals [53,54]. • Larvae cease migration downstream and settle at the river bottom [55].
	Beluga sturgeon (*Huso huso*)	• Extreme changes in body shape and growth pattern [56,57]. • Elongation of the head and caudal area along with an increase in body depth [57].	• Onset of aggregating into schools and becoming positively rheotactic when facing the current and wiggling their tails [57]. • Occasions of high cannibalism [57].
	F_1 bester (*Huso huso* × *Acipenser ruthenus*)	• Onset of fin-ray morphogenesis [28]. • Increase in the number and size of the scutes up to this development stage [28].	• Increasing tendency for cannibalism at high stocking density [36].
*a*2–*a*3 (about 0.8)	Sterlet sturgeon (*Acipenser ruthenus*)	• Transition from late larvae to juvenile stage [53]. • Disappearance of the embryonic finfold and near completion of fin apparatus formation [53].	• Exhibiting a considerably high growth intensity by feeding on benthic invertebrates [53].
	Beluga sturgeon (*Huso huso*)	• Occurrence of most morpho-physiological changes, such as digestive system organization and eye and fin development [57].	• Onset of compound feeds [57].
	F_1 Bester (*Huso huso* × *Acipenser ruthenus*)	• Development of distal radials and fin rays [28]. • Mostly completed ossification of all scutes [28]. • Almost developing of dorsal rostral scales [28].	• Improvement of swimming ability and protection from external damage [28].

Rapid morphological and behavioral changes were also observed at ~0.8 g (Table 3) when the F_2 bester exhibited metabolic phase shifting (Figure 1 and Table 2). During this transitional phase, the fin apparatus of the sterlet sturgeon was almost fully developed [53], and metamorphosis from larvae to the juvenile stage had begun [53]. Such fin development

benefits swimming and eating behavior, enabling the consumption of benthic invertebrates in natural habitats [53]. In aquaculture farms, the growth rate also changes rapidly during this phase [53]. The beluga sturgeon was found to undergo rapid morphological and behavioral changes, and the development of the digestive system, eyes, and fins was evident [57]. These morphological changes enabled rapid digestion and absorption of compound feed in aquaculture farms; thus, a successful transition from live feed such as rotifer (*Brachionus* sp.) and/or brine shrimp (*Artemia* sp.) to compound feed is possible during this transitional phase [57]. Most fins and scutes of the F_1 bester had developed and the dorsal and/or rostral scales were prominent during this transitional phase [28]. These developments enabled high swimming capacity, which provided protection against physical damage from the external environment [28]. Although the present study cannot provide direct evidence supporting a relationship between the change in metabolic scaling and the developmental trajectory of the F_2 bester, this study is the first to suggest that metabolic shifts could be associated with changes in their developmental trajectory, even in hybrids. Future studies should directly examine morphological and behavioral changes during the early developmental stages of the F_2 bester to understand the relationship between metabolic scaling and development trajectory.

5. Conclusions

The results of this study revealed that the F_2 bester, a hybrid sturgeon, undergoes ontogenetic phase shifts in metabolism. Specifically, the F_2 bester underwent two stepwise increases in the scaling constant (a_i) during its early developmental stages at 0.2 and 0.8 g. During each phase, the scaling exponent was maintained ($\hat{b}$ = 0.662). These rapid, step-by-step changes in its metabolism can help to better understand the changes in the metabolism of aquaculture species, e.g., oxygen consumption rate. Such information is required to develop highly intensive aquaculture production systems. Considering that there is a strong association between rapid metabolic changes and morphological and behavioral changes, these results may also aid in understanding their developmental trajectory, even if it only indirectly infers the relationship of the F_2 bester based on previous data from their grandparents (i.e., Sterlet and Beluga sturgeons) and parents (F_1 bester). Further studies on the relationship between rapid metabolic changes and their associated developmental changes are required to improve the growth and survival of the breed. In addition, because this study analyzed only a small number of individuals, it is necessary to analyze more individuals from several bester F_2 families in future studies.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Institutional Review Board and Ethical Committee on Animal Experimentation at the Tsukuba Research Institute, Fujikin Inc. Ltd., Tsukuba, Japan. According to the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government, "experimental animal" refers to a mammal, bird, or reptile that is cared for or kept in the facilities of a research institution for the purpose of animal experiments, etc. (policy announced on 1 June 2006). Thus, in Japan, fish experiments do not require an approval code for review by the Animal Ethics Committee.

Data Availability Statement: Data generated in this study are available on request from the corresponding author.

Acknowledgments: I would like to thank Kiyoshi Hiraoka and his research group members at the Tsukuba Research Institute, Fujikin Inc. Ltd., (Tsukuba, Japan) for providing experimental animals and valuable suggestions while performing the experiments. I also thank Akane Ishida and Mikio Uchiba for their cooperation during the experiment. I sincerely thank the editor and reviewers for their careful reading of the manuscript and their constructive comments and insightful suggestions.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Pillay, T.V.R.; Kutty, M.N. *Aquaculture: Principles and Practices*, 2nd ed.; Blackwell Publishing: Hoboken, NJ, USA, 2005; pp. 7–41.
2. Rus, A.S.; Enjuto, C.; Morales, A.E.; Hidalgo, M.C.; García-Gallego, M. Description of a facility for studying energy metabolism in fish: Application to aquaculture. *Aquac. Eng.* **2000**, *21*, 169–180.
3. Crețu, M.; Guriencu, R.; Dediu, L.; Stroe, M. Comparison of Metabolic Rates of Young of the Year Beluga (*Huso huso*), Sterlet (*Acipenser ruthenus*) and Bester Hybrid Reared in a Recirculating Aquaculture System. *Fishes* **2021**, *6*, 46. [CrossRef]
4. Badiola, M.; Mendiola, D.; Bostock, J. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. *Aquac. Eng.* **2012**, *51*, 26–35. [CrossRef]
5. Xiao, R.; Wei, Y.; An, D.; Li, D.; Ta, X.; Wu, Y.; Ren, Q. A review on the research status and development trend of equipment in water treatment processes of recirculating aquaculture systems. *Rev. Aquac.* **2019**, *11*, 863–895. [CrossRef]
6. Helfrich, L.; Libey, G. *Fish Farming in Recirculating Aquaculture Systems (RAS)*; Department of Fisheries and Wildlife, Virginia Tech: Blacksburg, VA, USA, 1991.
7. Chebanov, M.; Rosenthal, H.; Gessner, J.; van Anrooy, R.; Doukakis, P.; Pourkazemi, M.; Williot, P. Sturgeon hatchery practices and management for release—Guidelines. *FAO Fish. Aquac.* **2011**, *110*, 570.
8. Cook, J.T.; McNiven, M.A.; Sutterlin, A.M. Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* **2000**, *188*, 33–45. [CrossRef]
9. Krogh, A. *The Respiratory Exchange of Animals and Man*; Longmans: London, UK, 1916.
10. Kleiber, M. Body Size and metabolism. *Hilgardia* **1932**, *6*, 315–353. [CrossRef]
11. Chabot, D.; Steffensen, J.F.; Farrell, A.P. The determination of standard metabolic rate in fishes. *J. Fish Biol.* **2016**, *88*, 81–121. [CrossRef]
12. Post, J.R.; Lee, J.A. Metabolic ontogeny of teleost fishes. *Can. J. Fish. Aquat. Sci.* **1996**, *53*, 910–923. [CrossRef]
13. Metcalfe, N.B.; Van Leeuwen, T.E.; Killen, S.S. Does individual variation in metabolic phenotype predict fish behaviour and performance? *J. Fish Biol.* **2016**, *88*, 298–321. [CrossRef]
14. Van Dam, A.A.; Pauly, D. Simulation of the effects of oxygen on food consumption and growth of Nile tilapia, *Oreochromis niloticus* (L.). *Aquac. Res.* **1995**, *26*, 427–440.
15. Martínez, M.L.; Landry, C.; Boehm, R.; Manning, S.; Cheek, A.O.; Rees, B.B. Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the Gulf killifish, Fundulus grandis. *J. Exp. Biol.* **2006**, *209*, 3851–3861. [CrossRef] [PubMed]
16. Chapman, L.J.; Chapman, C.A.; Brazeau, D.A.; McLaughlin, B.; Jordan, M. Papyrus swamps, hypoxia, and faunal diversification: Variation among populations of Barbus neumayeri. *J. Fish Biol.* **1999**, *54*, 310–327. [CrossRef]
17. Buentello, J.A.; Gatlin, D.M., III; Neill, W.H. Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (*Ictalurus punctatus*). *Aquaculture* **2000**, *182*, 339–352. [CrossRef]
18. Dabrowski, K.; Lee, K.; Guz, L.; Verlhac, V.; Gabaudan, J. Effects of dietary ascorbic acid on oxygen stress (hypoxia or hyperoxia), growth and tissue vitamin concentrations in juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2004**, *233*, 383–392. [CrossRef]
19. Bergheim, A.; Gausen, M.; Næss, A.; Hølland, P.M.; Krogedal, P.; Crampton, V. A newly developed oxygen injection system for cage farms. *Aquac. Eng.* **2006**, *34*, 40–46. [CrossRef]
20. Foss, A.; Evensen, T.H.; Øiestad, V. Effects of hypoxia and hyperoxia on growth and food conversion efficiency in the spotted wolffish *Anarhichas minor* (Olafsen). *Aquac. Res.* **2002**, *33*, 437–444. [CrossRef]
21. Hosfeld, C.D.; Engevik, A.; Mollan, T.; Lunde, T.M.; Waagbø, R.; Olsen, A.B.; Breck, O.; Stefansson, S.; Fivelstad, S. Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* **2008**, *280*, 146–153. [CrossRef]
22. Fridell, F.; Gadan, K.; Sundh, H.; Taranger, G.L.; Glette, J.; Olsen, R.E.; Sundell, K.; Evensen, Ø. Effect of hyperoxygenation and low water flow on the primary stress response and susceptibility of Atlantic salmon *Salmo salar* L. to experimental challenge with IPN virus. *Aquaculture* **2007**, *270*, 23–35. [CrossRef]
23. Yagi, M.; Kanda, T.; Takeda, T.; Ishimatsu, A.; Oikawa, S. Ontogenetic phase shifts in metabolism: Links to development and anti-predator adaptation. *Proc. R. Soc. B* **2010**, *277*, 2793–2801. [CrossRef]
24. Yagi, M.; Oikawa, S. Ontogenetic phase shifts in metabolism in a flounder *Paralichthys olivaceus*. *Sci. Rep.* **2014**, *4*, 7135. [CrossRef]
25. Kim, D.I. Ontogeny of the Respiratory Area in Relation to Body Mass with Reference to Resting Metabolism in the Japanese Flounder, *Paralichthys olivaceus* (Temminck & Schlegel, 1846). *Fishes* **2022**, *7*, 39. [CrossRef]
26. Glazier, D.S.; Butler, E.M.; Lombardi, S.A.; Deptola, T.J.; Reese, A.J.; Satterthwaite, E.V. Ecological effects on metabolic scaling: Amphipod responses to fish predators in freshwater springs. *Ecol. Monogr.* **2011**, *81*, 599–618. [CrossRef]
27. Nash, C.E.; Novotny, A.J. *Production of Aquatic Animals: Fishes—World Animal Science Series.*, 1st ed.; Elsevier: Seattle, WA, USA, 1995.
28. Zhang, X.; Shimoda, K.; Ura, K.; Adachi, S.; Takagi, Y. Developmental structure of the vertebral column, fins, scutes and scales in bester sturgeon, a hybrid of beluga *Huso huso* and sterlet *Acipenser ruthenus*. *J. Fish Biol.* **2012**, *81*, 1985–2004. [CrossRef] [PubMed]
29. Ludwig, A.; Lippold, S.; Debus, L.; Reinartz, R. First evidence of hybridization between endangered sterlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. *Biol. Invasions* **2009**, *11*, 753–760. [CrossRef]
30. Bronzi, P.; Rosenthal, H. Present and future sturgeon and caviar production and marketing: A global market overview. *J. Appl. Ichthyol.* **2014**, *30*, 1536–1546. [CrossRef]

31. Omoto, N.; Maebayashi, M.; Mitsuhashi, E.; Yoshitomi, K.; Adachi, S.; Yamauchi, K. Histological observations of gonadal sex differentiation in the F2 hybrid sturgeon, the bester. *Fish Sci.* **2001**, *67*, 1104–1110. [CrossRef]
32. Birstein, V.J.; Bauer, A.; Kaiser-Pohlmann, A. *Sturgeon Stocks and Caviar Trade Workshop*; ICUN: Gland, Switzerland, 1997.
33. Shivaramu, S. Hybridization of Sturgeons. Ph.D. Thesis, University of South Bohemia, Budejovice, Czech Republic, 2019.
34. Baradaran, N.S.; Bahmani, M.; Abdolhay, H.; Hosseini, M.R.; Chakmehduz, F.; Hallajian, A.; Darvishi, S.; Farabi, M.V. *Bester (Beluga ♀× Sterlet ♂) Production and Comparing Their Growth with Beluga in Iran*; Iranian Fisheries Science Research Institute: Tehran, Iran, 2009; 55p.
35. Arefjev, V.A. Cytogenetics of interploid hybridization of sturgeons. *J. Appl. Ichthyol.* **1999**, *15*, 277. [CrossRef]
36. Oprea, D.; Oprea, L. The effect of density on bester (*H. huso*× *A. ruthenus*) larvae reared in a superintensive system. *USAMV Iaşi.Lucr. Ştiinţifice Ser. Zooteh.* **2009**, *52*, 655–660.
37. Oikawa, S.; Itazawa, Y.; Gotoh, M. Ontogenetic change in the relationship between metabolic rate and body mass in a sea bream Pagrus major (Temminck & Schlegel). *J. Fish Biol.* **1991**, *38*, 483–496.
38. Helm, I.; Jalukse, L.; Vilbaste, M.; Leito, I. Micro-Winkler titration method for dissolved oxygen concentration measurement. *Anal. Chim. Acta.* **2009**, *648*, 167–173. [CrossRef] [PubMed]
39. Brownlee, K.A. *Statistical Theory and Methodology in Science and Engineering*; John Wiley & Sons: New York, NY, USA, 1956.
40. Feldman, H.A.; McMahon, T.A. The 3/4 mass exponent for energy metabolism is not a statistical artifact. *Respir. Physiol.* **1983**, *52*, 149–163. [CrossRef] [PubMed]
41. Summerfelt, S.T.; Vinci, B.J. Better management practices for recirculating aquaculture systems. In *Environmental Best Management Practices for Aquaculture*; Tucker, C.S., Hargreaves, J.A., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2008; Volume 297, pp. 389–426.
42. Seginer, I.; Mozes, N. A note on oxygen supply in RAS: The effect of water temperature. *Aquac. Eng.* **2012**, *50*, 46–54. [CrossRef]
43. Hare, J.A.; Cowen, R.K. Size, growth, development, and survival of the planktonic larvae of Pomatomus saltatrix (Pisces: Pomatomidae). *Ecol.* **1997**, *78*, 2415–2431. [CrossRef]
44. Suzuki, N.; Okada, K.; Kamiya, N. Organogenesis and behavioral changes during development of laboratory-reared tiger puffer, *Takifugu rubripes*. *Aquac. Sci.* **1995**, *43*, 461–474.
45. Harder, W. *Anatomy of Fishes*; Schweizerbart'sche Verlagsbuchhandlung: Stuttgart, Germany, 1975; pp. 1–612.
46. Yarmohammadi, M.; Shabani, A.; Pourkazemi, M.; Baradaran Noveiri, S. Identification of bester hybrids (female *Huso huso* Linnaeus, 1758 and male sterlet *Acipenser ruthenus* Linnaeus, 1758) using AFLP molecular technique. *Iran. J. Fish. Sci.* **2012**, *11*, 415–423.
47. Tranah, G.J.; Bagley, M.; Agresti, J.J.; May, B. Development of codominant markers for identifying species hybrids. *Conserv. Genet.* **2003**, *4*, 537–541. [CrossRef]
48. Okada, N.; Takagi, Y.; Seikai, T.; Tanaka, M.; Tagawa, M. Asymmetrical development of bones and soft tissues during eye migration of metamorphosing Japanese flounder, *Paralichthys Olivaceus*. *Cell Tissue Res.* **2001**, *304*, 59–66. [CrossRef]
49. Tan, H.; Hirst, A.G.; Glazier, D.S.; Atkinson, D. Ecological pressures and the contrasting scaling of metabolism and body shape in coexisting taxa: Cephalopods versus teleost fish. *Philos. Trans. R. Soc.* **2019**, *374*, 20180543. [CrossRef]
50. Hirst, A.G.; Glazier, D.S.; Atkinson, D. Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling. *Ecol. Lett.* **2014**, *17*, 1274–1281. [CrossRef]
51. Glazier, D.S.; Hirst, A.G.; Atkinson, D. Shape shifting predicts ontogenetic changes in metabolic scaling in diverse aquatic invertebrates. *Proc. R. Soc. B* **2015**, *282*, 20142302. [CrossRef] [PubMed]
52. Prokeš, M.; Baruš, V.; Mareš, J.; Peňáz, M.; Baránek, V. Growth of sterlet Acipenser ruthenus under experimental and farm conditions of the Czech Republic, with remarks on other sturgeons. *Acta Univ. Agric. Silvic. Mendel. Brun.* **2014**, *59*, 281–290. [CrossRef]
53. Abdali, H.; Eagderi, S. Ontogeny of gill structure in Sterlet, *Acipenser ruthenus* (Linnaeus, 1758). *Iran. J. Ichthyol.* **2015**, *2*, 87–92.
54. Rybnikár, J.; Prokeš, M.; Mareš, J.; Cileček, M. Early development and growth of sterlet (*Acipenser ruthenus*) in the Czech Republic. *Acta Univ. Agric. Silvic. Mendel. Brun.* **2014**, *59*, 217–226. [CrossRef]
55. Kalmykov, V.A.; Ruban, G.I.; Pavlov, D.S. Migrations and resources of sterlet *Acipenser ruthenus* (Acipenseridae) from the lower reaches of the Volga River. *J. Ichthyol.* **2010**, *50*, 44–51. [CrossRef]
56. Asgari, R.; Rafiee, G.; Poorbagher, H.; Agh, N.; Zadeh, H.E. Body shape changes during the early development of the Beluga (*Huso huso*). *Int. J. Aquat. Biol.* **2013**, *1*, 1–5.
57. Gisbert, E.; Asgari, R.; Rafiee, G.; Agh, N.; Eagderi, S.; Eshaghzadeh, H.; Alcaraz, C. Early development and allometric growth patterns of beluga *Huso huso* (Linnaeus, 1758). *J. Appl. Ichthyol.* **2014**, *30*, 1264–1272. [CrossRef]