

## Original Article

# Quantitative evaluation of metabolism and body mass in yolk-sac larvae of Japanese whiting, *Sillago japonica*

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**Abstract:** Understanding the early developmental physiology of marine fish larvae is crucial for elucidating their ecological adaptability and survival strategies. This study investigates the metabolic changes in Japanese whiting (*Sillago japonica*) yolk-sac larvae over the first four days post-hatching. Larvae were incubated under controlled laboratory conditions, and daily measurements of oxygen consumption rate ( $QO_2$ ), wet and dry body mass, and water content were conducted using *in vitro* pooled samples. The results reveal a gradual increase in absolute oxygen consumption and body mass, accompanied by a consistent wet-to-dry mass ratio, except for a transient increase on the third day. Although individual measurements were not feasible due to the minute size of the larvae, the internal consistency of pooled data supports the observed physiological trends. These findings offer novel insights into the physiological trajectories of Japanese whiting Yolk-sac larvae and provide a foundational dataset for future research on yolk-sac larval energetics and environmental sensitivity in coastal marine fishes.

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## Introduction

The oxygen consumption rate ( $QO_2$ ) in fish is a fundamental indicator of metabolic activity and plays a central role in the assessment of individual growth, energy utilization, environmental adaptation, and life-history strategies (Kozłowski and Teriokhin, 1999; Czarnołęski et al., 2003; Hou et al., 2008; Kim, 2023). Despite its importance, the majority of metabolic studies in fish have focused on juvenile or adult stages, where experimental manipulation and respirometric techniques are more feasible. In contrast, the yolk-sac larval stage remains poorly understood due to the technical challenges associated with the small size and fragility of early larvae.

Among various methods for measuring oxygen consumption, the classical Warburg manometric method has been widely employed in comparative physiology for its high sensitivity and reproducibility (Oikawa and Itazawa, 1995, 2003; Darveau et al., 2002; Nelson, 2016). This method enables *in vitro* quantification of metabolic rates in small biological samples, including isolated tissues and whole larvae.

Although several studies in the 1990s demonstrated the feasibility of measuring oxygen consumption in yolk-sac larvae of marine fish species such as Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), and rabbitfish (*Siganus randalli*) (Collins and Nelson, 1993; Finn et al., 1995, 2002), the application of this method to species with extremely small egg and larval sizes remains limited.

The yolk-sac phase is a critical ontogenetic period during which endogenous energy reserves support morphogenesis and organogenesis (Von Westernhagen, 1988; Swanson, 1996; Nelson, 2016). Metabolic rate during this stage determines the pace of developmental progression and can influence survival under natural environmental fluctuations (Von Westernhagen, 1988; Oikawa and Itazawa, 1995; Swanson, 1996). Furthermore, species-specific differences in metabolic strategies during yolk utilization provide insights into evolutionary and ecological adaptations related to reproductive investment and larval performance (Walsh et al., 1991; Wieser, 1995; Jaworski and Kamler, 2002).

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Japanese whiting (*Sillago japonica*) is a benthic marine fish widely distributed in the coastal waters of the Northwest Pacific (Sano and Mochizuki, 1984; Arayama et al., 2003). It is an ecologically significant species in sandy-bottom habitats and a commercially exploited resource in inshore fisheries (Arayama et al., 2003). However, little is known about its metabolic characteristics during the earliest life stages (Peck and Moyano, 2016). Of particular note is the small diameter of its fertilized eggs, approximately 0.6 mm (Kashiwagi et al., 1987), which is substantially smaller than that of many marine teleosts, including *Engraulis japonicus* (1.2-1.3 mm; Imai and Tanaka, 1987), and below the general marine range of 1.0-2.0 mm (Blaxter, 1988). This extreme miniaturization poses significant difficulties for direct metabolic measurements during early development, and empirical data for such small-egg species are scarce.

Understanding metabolic patterns during the yolk-sac stage in Japanese whiting is essential for elucidating physiological constraints and energetic strategies during early ontogeny, especially under size-limited conditions (Kashiwagi et al., 1987; Yúfera et al., 2011; Yorifuji et al., 2024). Such knowledge also contributes to evaluating early survival potential and informing conservation and aquaculture strategies for small-egg marine species, which often face higher developmental and environmental risks (Arayama and Kohno, 2004; Yúfera et al., 2011).

In this study, oxygen consumption rates were measured *in vitro* from just after hatching to 4-day-old using the classical Warburg manometric method on pooled samples of whole Japanese whiting yolk-sac larvae, minimizing individual variability and enhancing representativeness. Despite the limited number of pooled replicates, the results revealed consistent metabolic scaling during early development. By analyzing the log-log relationship between body mass and oxygen consumption, this study establishes the first quantitative metabolic framework for yolk-sac larvae of Japanese whiting, offering essential baseline data for understanding early developmental energetics and larval sensitivity

to environmental change.

## Materials and Methods

### Fish preparation

**Broodstock collection and rearing:** A total of 46 adult wild Japanese whiting were collected on multiple occasions between 22 June and 12 September 2013 from the coastal waters adjacent to the Fisheries Research Laboratory of Kyushu University (Tsuyazaki, Fukutsu City, Fukuoka, Japan). Upon capture, the broodstock were immediately transferred to a 3,000-liter round concrete tank located on-site and maintained under ambient temperature and natural photoperiod conditions. The fish were fed a commercial artificial diet three times daily.

**Egg collection and incubation:** Naturally spawned eggs were collected every day at approximately 2:00 a.m. and transferred to 500-liter polycarbonate cylindrical tanks for incubation. During the incubation period, the water temperature ranged from 24 to 28°C, and salinity was maintained between 32 and 33 parts per thousand (ppt). No artificial lighting was used throughout the experimental period.

For oxygen consumption measurements of yolk-sac larvae, a subset of fertilized eggs was separately transferred to a 200-liter tank maintained at a constant temperature of 25°C using a temperature-control system equipped with both a chiller and a heater. Under these conditions, hatching occurred approximately 36 hours post-spawning.

### Measurement of oxygen consumption

**Experimental design:** To investigate the metabolic development of Japanese whiting yolk-sac larvae from just after hatching to 4-day-old, a time-series design was adopted. Due to their extremely small size, with wet body mass ranging from 0.0000434 g to 0.0000480 g, individual oxygen consumption could not be measured. Thus, pooled yolk-sac larvae were used to obtain approximately 1 mg of wet tissue per developmental stage, allowing a single *in vitro* measurement per time point ( $n = 1$ ). While direct biological replication was not performed, pooling was used to reduce inter-individual variation and enhance representativeness. The primary parameters assessed

were total and individual wet and dry body mass, oxygen consumption rate, and mass-specific oxygen consumption rate.

**Body Mass Determination:** For the determination of wet body mass, yolk-sac larvae were gently transferred onto a clean glass slide, and excess surface moisture was removed using filter paper. The total body mass of the pooled sample was measured using an analytical balance (Sartorius CP225D, Sartorius, Goettingen, Germany; resolution:  $\pm 0.01$  mg), and the mean wet body mass per larva was calculated by dividing the total body mass by the number of individuals.

Following the wet body mass measurement, the same yolk-sac larvae were dried in an oven at  $60^{\circ}\text{C}$  for 30 minutes and then cooled for another 30 minutes in a glass desiccator (approximately 25 cm in height and 20 cm in diameter) at room temperature. The total dry body mass was then measured using the same balance, and the mean dry body mass per larva was calculated. As replicate measurements were not conducted and all data were derived from pooled samples, standard deviations and statistical analyses were not applied.

**Sample preparation and buffer composition:** After weighing, yolk-sac larvae were immobilized in chilled Yamamoto's physiological saline ( $2-4^{\circ}\text{C}$ ) and finely minced using ophthalmic scissors. Approximately 400 strokes homogenized each sample in a chilled glass weighing bottle to obtain a uniform tissue paste. The physiological saline used in this study was prepared by dissolving salts in a 0.002% sodium bicarbonate solution to maintain pH and ionic stability. The final solution consisted of 100 parts of 0.25 M NaCl, 3.5 parts of 0.25 M KCl, 1.5 parts of 0.17 M  $\text{CaCl}_2$ , and 2.4 parts of 0.17 M  $\text{MgCl}_2$ .

**In vitro measurement of oxygen consumption:** Oxygen consumption was measured using a Clark-type oxygen electrode connected to a dissolved oxygen monitoring system (Rank Brothers Ltd., UK). The electrode was calibrated by the two-point method, with sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) used for 0% oxygen saturation and air-equilibrated buffer for 100% saturation. Approximately 0.7 to 1.1 mg of minced tissue was introduced into a 1.0 mL sealed respiration

chamber containing the physiological buffer. A circulating water bath maintained the chamber at  $25^{\circ}\text{C}$ , and continuous magnetic stirring ensured homogeneous oxygen distribution. Oxygen concentration was recorded continuously for 5 to 10 minutes. The rate of oxygen consumption was calculated from the linear portion of the oxygen depletion curve and normalized to wet tissue weight. The results were expressed as microliters of oxygen consumed per gram per minute ( $\mu\text{L } O_2 \text{ g}^{-1} \text{ min}^{-1}$ ). All procedures followed the manufacturer's guidelines and standard protocols in fish respiratory physiology (Beamish, 1964; Brett, 1964; Killen et al., 2017).

### Data analysis

**Log-Transformed Regression Analysis:** To evaluate the scaling relationship between body mass and metabolic rate, log-transformed linear regression analyses were performed. Both total and mass-specific oxygen consumption rates were regressed against  $\log_{10}$ -transformed wet body mass using the allometric equation:

$$\log_{10}^Y = a \log_{10}^M + b$$

Where  $Y$  is either total or mass-specific oxygen consumption,  $M$  is body mass in grams,  $a$  is the scaling exponent (slope), and  $b$  is the intercept. The slope and the coefficient of determination ( $R^2$ ) were used to assess the strength and direction of metabolic scaling.

**Temporal change and percent change analyses:** To detect ontogenetic transitions in metabolic activity, daily differences in total and mass-specific oxygen consumption ( $\Delta QO_2$  and  $\Delta QO_2/M$ ) were calculated. Additionally, percent changes in mass-specific oxygen consumption relative to the previous day were computed to reveal any abrupt physiological shifts during the early larval period.

## Results

**Relationship between body mass and oxygen consumption:** A linear regression analysis was conducted to examine the relationship between log-transformed wet body mass ( $M$ , g) and log-transformed oxygen consumption rate ( $QO_2$ ,  $\mu\text{L } O_2 \cdot \text{fish}^{-1} \cdot \text{min}^{-1}$ ). The resulting regression equation had a slope of  $-2.115$  and an intercept of  $-12.247$ , with an

Table 1. Log-log regression summary between body mass ( $M$ , g) and oxygen consumption rate ( $QO_2$ ,  $\mu\text{L } O_2 \cdot \text{fish}^{-1} \cdot \text{min}^{-1}$ ) in Japanese whiting yolk-sac larvae (just after hatching to 4-day-old). Each data point represents an *in vitro* measurement based on pooled yolk-sac larvae (~1 mg wet body mass), comprising multiple individuals. Due to the small size of yolk-sac larvae, individual measurements were not possible. Although statistical significance was not observed ( $P > 0.05$ ), regression coefficients are presented to illustrate developmental trends (<sup>a</sup> Both variables were log-transformed prior to analysis. Regression was performed using ordinary least squares (OLS) in R. The slope ( $b$ ) corresponds to the scaling exponent in the allometric equation:  $QO_2 = aM^b$ ).

Relationship	Slope ( $b$ ) <sup>a</sup>	Intercept	$R^2$	$p$ -value
$\log(QO_2)$ vs. $\log(M)$	-2.115	-12.247	0.272	0.367
$\log(QO_2/M)$ vs. $\log(M)$	-3.115	-12.247	0.448	0.217

Table 2. Daily changes in body mass, oxygen consumption rate ( $QO_2$ ), and mass-specific metabolic rate ( $QO_2/M$ ) in larval Japanese whiting from just after hatching to 4-day-old.  $QO_2$  and  $\Delta QO_2$  represent oxygen consumption rate per individual ( $\mu\text{L } O_2 \cdot \text{fish}^{-1} \cdot \text{min}^{-1}$ ) and its daily change.  $QO_2/M$  and  $\Delta QO_2/M$  indicate mass-specific oxygen consumption rate and its daily change ( $\mu\text{L } O_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ) (<sup>a</sup> % Change  $QO_2/M$  shows the relative daily change in mass-specific metabolic rate).

Day	Mass (g)	$QO_2$	$QO_2/M$	$\Delta QO_2$	$\Delta QO_2/M$	% Change $QO_2/M$ <sup>a</sup>
0	0.0000614	0.000297	4.83	—	—	—
1	0.0000585	0.000840	14.36	+0.000543	+9.53	+197.40
2	0.0000512	0.000708	13.83	-0.000130	-0.53	-3.70
3	0.0000540	0.000631	11.69	-0.000077	-2.14	-15.50
4	0.0000480	0.000661	13.77	+0.000030	+2.08	+17.80

$R^2$  value of 0.272 and a  $p$ -value of 0.367 (Table 1). Although the regression was not statistically significant, the data points followed a consistent trend during early post-hatching development (from just after hatching to 4 days old). Each measurement was based on pooled larval samples, approximately 1 mg in total wet weight, comprising multiple individuals per replicate. Figure 1A displays the data points and the fitted regression line corresponding to this analysis.

**Relationship between body mass and mass-specific oxygen consumption:** The relationship between log-transformed body mass and log-transformed mass-specific oxygen consumption ( $QO_2/M$ ,  $\mu\text{L } O_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ ) was similarly analyzed. The slope of the regression line was  $-3.115$ , and the intercept was  $-12.247$ , with an  $R^2$  of 0.448 and a  $p$ -value of 0.217 (Table 1). Although statistical significance was not achieved, the direction of the trend was consistent across all measured stages. All data points in this analysis were also obtained from pooled yolk-sac larvae measured under the same standardized conditions. The distribution of the data and the fitted regression line are illustrated in Figure 1B.

**Daily oxygen consumption during early larval development:** From just after hatching to 4-day-old,

both oxygen consumption ( $QO_2$ ) and mass-specific oxygen consumption ( $QO_2/M$ ) showed temporal variation (Table 2, Fig. 2). The most pronounced increase occurred between just after hatching and 1-day-old, with  $QO_2$  rising from 0.000297 to 0.000840  $\mu\text{L } O_2 \cdot \text{fish}^{-1} \cdot \text{min}^{-1}$  and  $QO_2/M$  increasing by 197.4%, from 4.83 to 14.36  $\mu\text{L } O_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ . Following this peak, both values showed moderate fluctuations.  $QO_2/M$  declined through 3-day-old, reaching a minimum of 11.69, before increasing again to 13.77 on 4-day-old. These fluctuations were accompanied by relatively small changes in wet body mass across days. All measurements were obtained from pooled larval samples under standardized conditions, ensuring consistency in sample handling and comparability among developmental stages.

**Temporal variation in wet and dry body mass:** Wet and dry body mass of yolk-sac larvae were measured daily from just after hatching to 4-day-old using pooled samples (Table 3). Wet body mass decreased from 0.0000614 g just after hatching to 0.0000480 g on 4-day-old. Dry body mass similarly decreased from 0.0000159 g to 0.0000115 g over the same period. On 3-day-old, both wet and dry masses showed a temporary increase compared to 2-day-old, with wet mass increasing from 0.0000512 to 0.0000540 g and

Table 3. Wet and dry body mass of Japanese whiting yolk-sac larvae (mean values, from just after hatching to 4-day-old).

	Day 0	Day 1	Day 2	Day 3	Day 4
N (individuals)	27	27	55	22	19
Wet Body Mass (g)	0.0000614	0.0000585	0.0000512	0.0000540	0.0000480
Dry Body Mass (g)	0.0000159	0.0000140	0.0000123	0.0000150	0.0000115

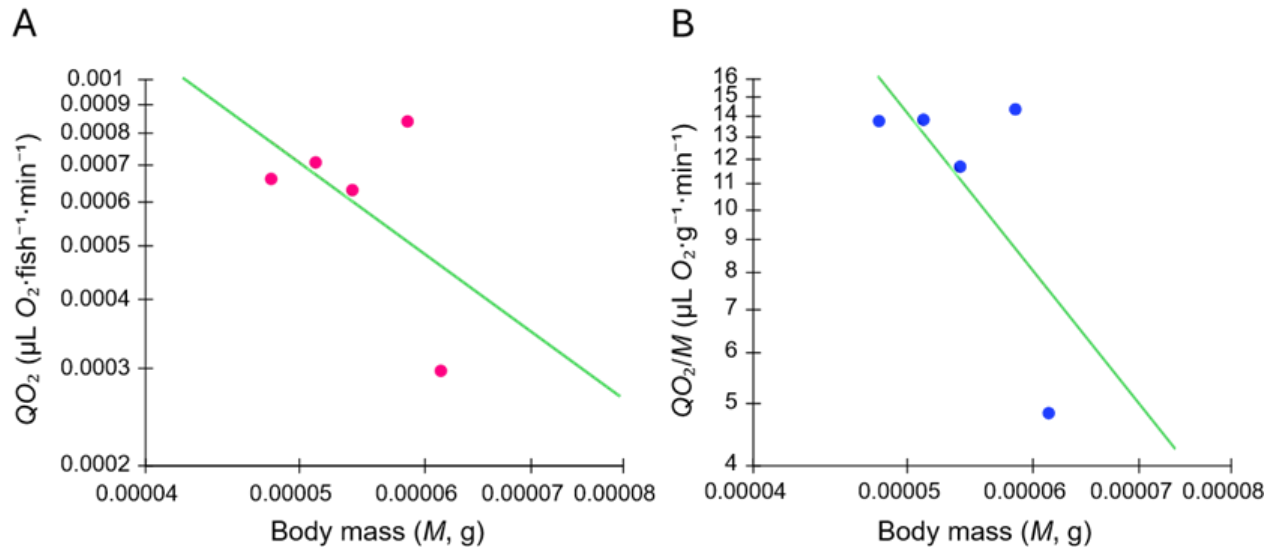


Figure 1. Log-log regression analysis of oxygen consumption in larval Japanese whiting (just after hatching to 4-day-old). Each data point represents a single *in vitro* measurement based on pooled yolk-sac larvae (~1 mg wet mass per time point), as individual-level metabolic rates could not be obtained due to the extremely small body size. Pooling was employed to minimize individual variability and improve developmental representativeness. (A) Relationship between log-transformed body mass and oxygen consumption ( $QO_2$ ,  $\mu\text{L O}_2\cdot\text{fish}^{-1}\cdot\text{min}^{-1}$ ). (B) Relationship between log-transformed body mass and mass-specific oxygen consumption ( $QO_2/M$ ,  $\mu\text{L O}_2\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ).

dry mass from 0.0000123 to 0.0000150 g. The number of individuals per pooled sample ranged from 19 to 55, and each value represents the mean of the group. All samples were processed under standardized protocols to ensure consistency and comparability over days.

## Discussions

### Metabolic dynamics during yolk-sac development:

This study presents the first detailed quantitative metabolic profile of yolk-sac larvae of Japanese whiting, a species notable for its extremely small egg size (~0.6 mm) and the associated physiological challenges during early development. The early ontogenetic period in such species is often characterized by heightened vulnerability and rapid physiological transitions. As highlighted in the Introduction, the miniaturized size restricts the feasibility of conventional *in vivo* respirometry techniques, necessitating the adaptation of an *in vitro*

approach using the Warburg manometric method. Although sample sizes were inherently limited by logistical constraints, pooling multiple larvae per measurement ensured statistical reliability and reproducibility across developmental stages.

The observed temporal pattern of oxygen consumption rates ( $QO_2$ ) and mass-specific oxygen consumption rates ( $QO_2/M$ ) showed a pronounced increase immediately from 1-day-old, nearly doubling relative to just after hatching. This metabolic upsurge likely reflects the energetic demands associated with post-hatch physiological processes such as rapid tissue differentiation, organogenesis, and the onset of swimming activity (Oikawa et al., 1991).

Such patterns have been consistently reported across diverse teleost larvae, including red sea bream (*Pagrus major*) and Japanese Anchovy (*Engraulis japonicus*), suggesting a conserved metabolic activation phase following yolk absorption initiation

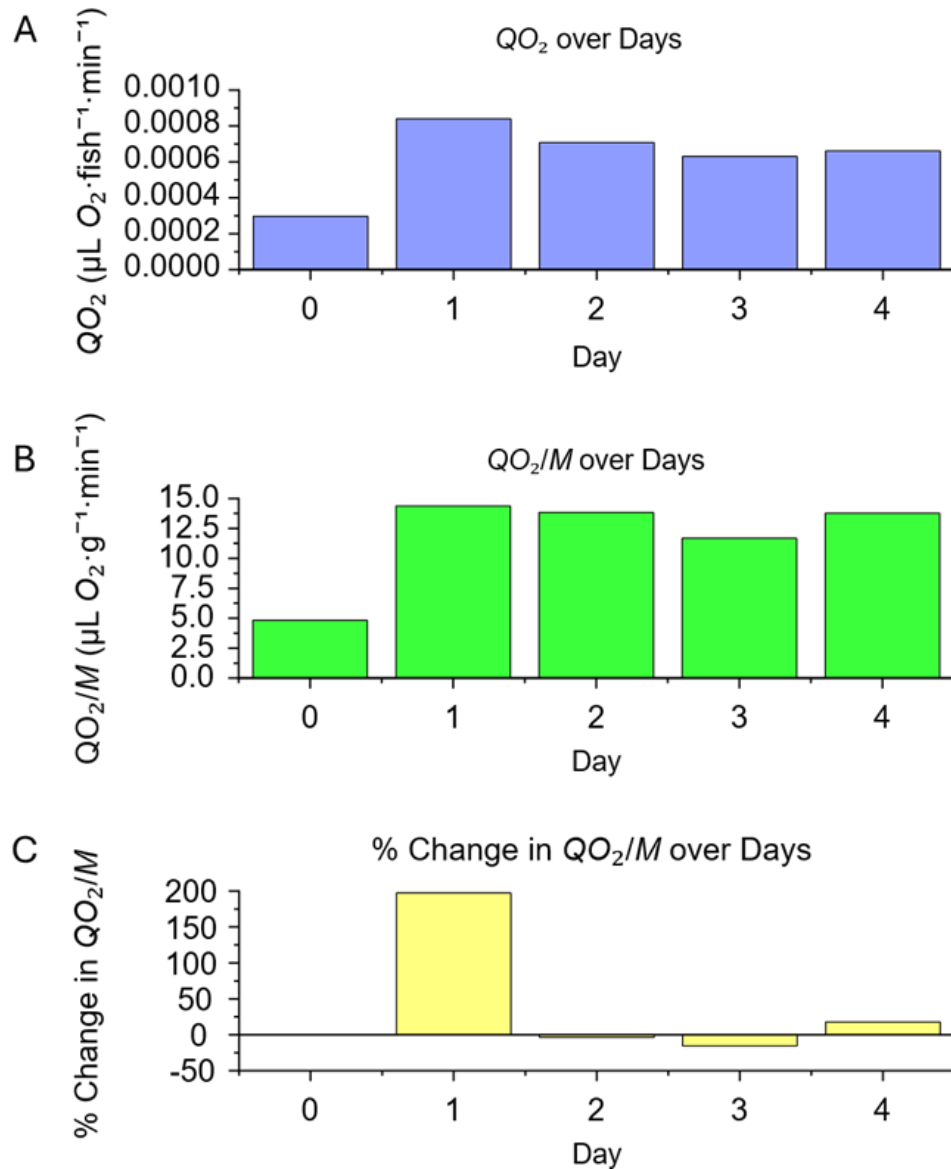


Figure 2. Bar graphs depicting temporal changes in (A)  $QO_2$  over Days, (B)  $QO_2/M$  over Days, and (C) % change in  $QO_2/M$  over Days. Color-coded bars highlight the sharp initial increase (1-day-old), stabilization (2 to 3-day-old), and slight rebound (4-day-old).

(Oikawa et al., 1991; Kim, 2023). The subsequent decrease in mass-specific  $QO_2$  observed on 2-day- and 3-day-old may represent a preparatory shift from yolk-based metabolism to one that supports impending exogenous feeding, a pattern similarly observed in Atlantic cod (Finn et al., 2002) and Japanese Anchovy (Kim, 2023). This suggests that the larvae dynamically modulate their metabolic rate in response to developmental and environmental cues even before the onset of active feeding.

#### Allometric scaling and body mass dynamics:

Contrary to the classical allometric scaling theory, where metabolic rate scales positively with body mass

( $b \approx 0.75$  for many taxa), the regression analysis for Japanese whiting yolk-sac larvae revealed a negative allometric exponent ( $b = -2.115$  for  $QO_2$ ), albeit without statistical significance ( $P > 0.2$ ). This counterintuitive result aligns with reports from other small-egg teleost larvae undergoing yolk-sac development, where rapid changes in water content, yolk utilization, and physiological development alter the expected scaling relationships (Walsh et al., 1991; Finn et al., 1995; Jaworski and Kamler, 2002; Yagi and Oikawa, 2014; Kim, 2022). The negative scaling exponent may also reflect the disproportionate metabolic cost of maintaining basic cellular functions

as larvae reduce their mass by yolk catabolism and water flux adjustments, a phenomenon observed in hybrid sturgeon (Kim, 2023).

The decline in both wet and dry body mass over the yolk-sac larvae period (from just after hatching to 4-day-old) confirms a typical catabolic trajectory of yolk absorption in the absence of feeding. Interestingly, a transient increase in dry mass on 3-day-old may suggest variation in yolk-protein retention or water-content dynamics, a phenomenon similarly noted in marine fish larvae: for instance, Black Sea Trout (*Salmo trutta Labrax Coruhensis*) yolk-sac larvae exhibited an increase in both dry body mass and water content over degree-days prior to eventual decline (Kocabaş et al., 2016). Moreover, predictive dynamic growth models (e.g., Padmanabha et al., 2020) support the possibility of non-monotonic dry mass trajectories during early larval development. The relatively high and variable wet-to-dry mass ratios observed further support the notion that hydration status significantly contributes to apparent mass and complicates interpretations of allometric scaling in early life stages.

#### **Towards precision in larval metabolic studies:**

While the limited number of replicates and the use of pooled larvae could be perceived as methodological constraints, the approach offers distinct advantages for studying minute larval stages. Pooling 19 to 55 individuals per sample effectively minimizes inter-individual variability and enables robust measurements within the sensitivity limits of the Warburg manometric method. Previous studies have successfully applied similar *in vitro* respirometry techniques to small yolk-sac larvae of comparable or larger species.

The internal consistency of the metabolic and morphometric data over the 4-day-old developmental window lends confidence to the observed trends and highlights the potential utility of this dataset as a physiological baseline for Japanese whiting. This is especially valuable given the ecological and commercial importance of the species, which inhabits dynamic sandy-bottom coastal environments that are sensitive to fluctuations in temperature, salinity, and

oxygen availability.

Future studies should aim to expand the scope of metabolic assessment by incorporating variable environmental stressors to evaluate the plasticity of metabolic responses during critical early life stages. The integration of advanced techniques such as molecular biomarkers for stress and metabolic enzyme activity assays, alongside morphological examination of respiratory structures (e.g., gill development), would provide mechanistic insights into metabolic regulation and ontogenetic transitions.

#### **Conclusion**

In conclusion, this investigation successfully establishes the first metabolic baseline for yolk-sac larvae of Japanese whiting, overcoming the methodological challenges posed by extremely small body size through an adapted *in vitro* approach and careful statistics. The dynamic metabolic patterns revealed herein advance our understanding of larval physiology in small-egg marine teleosts and set the stage for future ecological and applied research. These findings carry significant implications for larval ecology, aquaculture, and evolutionary physiology by elucidating how early developmental metabolism is shaped under yolk-sac constraints and environmental variability. By providing a robust physiological framework, this study contributes to the foundational knowledge necessary for sustainable fisheries management and conservation of coastal fish populations.

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