

Physiological and transcriptomic responses to fasting-refeeding in *Arapaima gigas*: Insights into compensatory growth for aquaculture production

T.H. Delgadín^a, L.E. Santos-Rojas^a, D. Medina-Herrera^a, N.M. Herrera-Castillo^d,
A.M. Alonso^{a,b,c}, A. Saavedra-Flores^a, E.A. López-Landavery^a, J.I. Fernandino^{a,b,c,*},
E. Zelada-Mázmela^{a,*}

^a Laboratorio de Genética, Fisiología y Reproducción, Facultad de Ciencias, Universidad Nacional del Santa, Chimbote, Peru

^b Laboratorio de Biología del Desarrollo - Instituto Tecnológico de Chascomús. INTECH (CONICET-UNSAM), Argentina

^c Escuela de Bio y Nanotecnologías (UNSAM), Argentina

^d Instituto de Investigaciones de la Amazonía Peruana, Pucallpa, Peru

ARTICLE INFO

Keywords:

Compensatory growth
FCR, fish
Hepatocyte
RNA-seq

ABSTRACT

Fish exhibit remarkably efficient feed conversion ratios (FCR), often outperforming homeothermic animals in terms of protein conversion efficiency — a feature that is increasingly relevant for sustainable aquaculture practices. *Arapaima gigas*, one of the largest freshwater fish species in the Amazon, is renowned for its exceptional FCR during the initial growth phase. The aim of this study was to evaluate whether a fasting-refeeding protocol could further enhance feed efficiency in *A. gigas*. The study examined its effects on growth performance, liver histology, and hepatic gene expression. The results of the study indicate that there is a loss of body weight after one to two weeks of fasting, as well as growth arrest in total length. In addition, there is liver shrinkage driven by hepatocyte size reduction. Following refeeding, liver and cell size recovered within a week, and fish resumed growth with significantly improved FCR. Treated fish demonstrated partial compensatory growth in both body weight and length, with sustained higher specific growth rates and feed intake relative to controls throughout the refeeding process. It is noteworthy that the refeed group consumed 51 % less feed over the course of entire trial, which highlights the significant potential for substantial reduction in feed cost. Transcriptomic analysis revealed significant metabolic changes: fasting increased the expression of genes involved in lipid catabolism (e.g., RXRA, ABCA1, ACOT2), while early refeeding (3 days) activated pathways associated with gluconeogenesis (*PPARGC1A*, *MDH1*), and later refeeding (2 weeks) upregulated genes involved in mitochondrial repair and tissue regeneration (*LIG3*, *A2MG*). These findings offer a valuable contribution to the development of sustainable aquaculture practices for this fast-growing and threatened Amazonian species.

1. Introduction

Growth is an integral biological process defined as a change in size over time (Beckman, 2011), and in fish, it is commonly assessed using both body weight (BW) and total length (TL), which reflect distinct but complementary aspects of somatic development. Understanding growth mechanism has broad implications, ranging from human growth-related disorders (Attanasio et al., 2004) to improvements in animal production through genetic modified and genome editing (Forabosco et al., 2013; Mokrani and Liu, 2024). In aquaculture, where maximizing biomass gain is a major goal, particular attention is placed not only on how much fish grow, but also on how efficiently they convert feed into growth.

Feed conversion ratio (FCR) is thus a key performance metric, quantifying the efficiency with which ingested feed is transformed into body mass (Bai et al., 2022). FCR is influenced by genetic, nutritional, health and environmental factors, and optimizing it is essential for sustainable aquaculture production (Doupé and Lymbery, 2003; Gjedrem et al., 2012; Mengistu et al., 2020; Rodde et al., 2020).

Poikilothermic animals, such as fish and insects, generally exhibit lower FCR values than homeothermic (Gjedrem et al., 2012). Technological advancements have reduced FCR values also in farmed fish, with values around 1.0 in salmon (Fry et al., 2018) and as low as 0.4 in turbot during the fast-growing larval stages (Árnason et al., 2009). These animals represent promising sources of sustainable protein. However, the

* Corresponding authors at: Laboratorio de Genética, Fisiología y Reproducción, Facultad de Ciencias, Universidad Nacional del Santa, Chimbote, Peru.

E-mail addresses: fernandino@intech.gov.ar (J.I. Fernandino), ezelada@uns.edu.pe (E. Zelada-Mázmela).

<https://doi.org/10.1016/j.aquaculture.2025.743267>

Received 29 July 2025; Received in revised form 1 October 2025; Accepted 1 October 2025

Available online 9 October 2025

0044-8486/© 2025 Elsevier B.V. All rights reserved, including those for text and data mining, AI training, and similar technologies.

extent to which their FCR can be further improved in production applications remains unclear.

Meeting future aquatic protein demands will require improved feed conversion efficiency through strategies such as optimized feed formulations, farm management, and selective breeding (National Research Council, 2011; de Verdall et al., 2018). Among the experimental approaches used to investigate growth and FCR dynamics, fasting–refeeding protocols are widely applied due to their simplicity, reproducibility, and ability to induce controlled growth arrest (Ali et al., 2003). While primarily used in research contexts, these protocols can offer valuable insights for aquaculture, particularly regarding compensatory growth potential and feed savings when appropriately adapted to production settings. Moreover, lowering FCR not only reduces production costs but also mitigates environmental impacts, including nutrient pollution from wastewater (Chávez-Crooker and Obreque-Contreras, 2010) and greenhouse gas emissions associated with feed production (MacLeod et al., 2020).

In addition to FCR, compensatory growth (CG) is a crucial factor in optimizing production. CG refers to accelerated growth following a period of growth arrest or depression (Ali et al., 2003). This phenomenon is driven by hyperphagia (Jobling and Koskela, 1996; Mglavys and Jobling, 1989; Wang et al., 2000; Tian and Qin, 2003; Álvarez, 2024) and/or improved FCR (Boujard et al., 2000). In aquaculture, CG is often induced through fasting–refeeding strategies (Ali et al., 2003), making it essential to understand how fasting influences growth efficiency and whether species-specific adaptations can enhance production outcomes.

Arapaima gigas, a bony tongue fish native to the Amazon Basin (Hrbek et al., 2005; Chu-Koo et al., 2009; Farias et al., 2019; de Oliveira et al., 2019), is one of the world's largest freshwater fishes, with the capacity to reach lengths of up to 2.5 m in length (Nelson, 2006). This species, also known as paiche or pirarucu, possesses several unique biological features, including obligate air-breathing via a vascularized swim bladder and a gland-like structure in the head for nursing fry (Pereira-Filho et al., 2003; de Oliveira et al., 2012; Val and de Almeida-Val, 2012). Notably, *A. gigas* has one of the lowest recorded FCRs among fish (FCR < 0.7, Du et al., 2019), making it a highly promising candidate for aquaculture. Despite its potential in aquaculture production, the extent to which its FCR can be further improved remains largely unexplored.

Considering the notably efficient feed conversion observed in *A. gigas* and its relevance for aquaculture, this study aimed to investigate whether its FCR can be further optimized under a controlled fasting–refeeding protocol. Specifically, we evaluated the capacity of this species' capacity for compensatory growth through this strategy, which is widely employed in fish to assess growth plasticity and feed efficiency (Ali et al., 2003). This approach allowed us to assess both growth performance and feed utilization efficiency following a period of fasting. By integrating growth and intake measurements with transcriptomic analysis of liver tissue, we aimed to better understand the molecular responses associated with fasting and refeeding, thereby gaining insight into the biological mechanisms that may underlie compensatory growth in *A. gigas*.

In order to address these aims, a two-phase experimental design was implemented: The first phase was a fasting phase, the purpose of which was to assess the effects of feed deprivation. The second phase was a refeeding phase, to evaluate compensatory growth responses. The concept of compensatory growth was determined based on treatment-related differences in growth rate during the refeeding process. A faster growth rate in the treated group compared to the control group was considered indicative of CG. If treated fish reached the same size as the controls by the end of the trial, growth was classified as fully compensatory; otherwise, it was classified as partial compensation.

Due to the biological and conservation constraints associated with *A. gigas*, the use of large sample sizes is not ethically sound or logistically viable. In order to overcome these limitations, a longitudinal design with repeated measures was employed, allowing each fish to serve as its own

control. This approach enhances statistical power by reducing inter-individual variability and maximizing the information extracted from each subject (Maxwell et al., 2017). It is particularly well-suited for aquaculture research on large-bodied or threatened species, where experimental replication is limited. When combined with appropriate hierarchical modelling, this design supports precise, time-resolved inferences about treatment effects and compensatory growth dynamics, even under sample size constraints.

2. Materials and methods

2.1. Animals

A total of 96 juvenile *A. gigas* were obtained from local farmers and subsequently quarantined for a period of one month at facilities in Pucallpa, Peru. Subsequently, the fish were then transferred to a recirculating aquaculture system (RAS) comprising twelve 500 L tanks, with eight fish randomly placed in each tank. During the acclimation period, the fish were fed to apparent satiation on three to four times a day. The mean water temperature was 29.35 ± 0.02 °C, the dissolved oxygen level was 4.58 ± 0.10 mg/L, pH level was 7.48 ± 0.2 and the nitrates were under the limit of detection. The experimental phase was conducted from May to June 2022, following the natural diurnal rhythm. During both acclimation and experimental phases, fish were fed with a commercial diet (PECES 42; Aquatech, Perú), formulated with 42 % minimum crude protein, 14–15 % minimum crude fat, and a minimum digestible energy content of 3400 kcal/kg.

2.2. Ethics statement

Fish were handled in accordance with the Universities Federation for Animal Welfare Handbook on the Care and Management of Laboratory Animals (<https://www.ufaw.org.uk>) and internal institutional regulations (Comité de Ética de Investigación de la UNS – CEIUNS - Oficio N° 203–2019-UNS-VRIN).

2.3. Fasting and refeeding

In order to assist in the reduction of pressure on wild *A. gigas* populations, we employed aquaculture-raised fish and utilized a longitudinal repeated-measures design as an alternative to a cross-sectional approach. This strategy reduced the number of fish required while maintaining, and potentially enhancing, statistical robustness (Maxwell et al., 2017). Following the acclimation period, six tanks were randomly assigned to the control group and the remaining six to the treated group, yielding six independent replicate tanks per treatment ($n = 6$). The experimental period lasted five weeks and was divided into two distinct phases: a two-week fasting period and a three-week refeeding period.

The control group was fed to apparent satiation four times a day throughout the entire experimental period. In contrast, the treated group underwent a complete fasting period during the initial two weeks, followed by refeeding to satiation during the subsequent three weeks.

All fish were individually measured for body weight and total length at six time points: at the beginning of the experiment (Week 0, W0), after 1 week of fasting (W1), at the end of the fasting phase (Week 2, W2), and during the refeeding phase at Week 1 (RW1), Week 2 (RW2), and Week 3 (RW3). These repeated measurements allowed for monitoring individual growth trajectories across both experimental phases.

2.4. Sampling

At each time point from W1 to RW3, one fish per tank was sampled for physiological and histological analyses. In addition, an extra sampling was conducted at 3 days of refeeding (RD3), specifically aimed at capturing the early hepatic transcriptional response to refeeding. The fish were anesthetized with eugenol and euthanized by decapitation.

The liver was then excised and weighed in order to calculate the hepatosomatic index (HSI), which was expressed as (liver weight / body weight) * 100. A portion of the liver was placed in 500 µL of RNAlater, kept at room temperature for one hour, stored at 4 °C for 24 h, and then transferred to −20 °C until RNA extraction. A further portion of the liver was fixed in Bouin's solution for subsequent histological analysis.

2.5. Histology

The liver samples were fixed in Bouin's solution for one hour at room temperature, followed by 16 h at 4 °C. The samples were then stored in 70° ethanol until histological processing. After graded alcohol dehydration, the liver samples were embedded in paraffin for 6 h, sectioned at a thickness of 7 µm and stained with hematoxylin-eosin (H&E). The nuclear and hepatocyte cross-sectional areas were subsequently calculated using Image-J software (NIH, USA).

2.6. Growth and nutritional parameters

The specific growth rate (SGR) was calculated on a separate basis for BW and total TL using the following formula: $SGR(X) = \ln(X_f / X_i) * t^{-1} * 100$, where X refers to either BW (in grams) or TL (in centimeters), X_i and X_f are the initial and final values for the measurement period, and t is the duration of the period in days. The FCR was calculated using the following formula: $FCR = \text{feed intake (g)} / \Delta BW \text{ (g)}$, where ΔBW corresponds to the net gain in body weight ($BW_f - BW_i$) during the same period. Feed intake was determined by calculating the difference between the total feed offered and the uneaten feed that was recovered from each tank.

2.7. RNA-Seq library preparation and sequencing

Total RNA was extracted from 50 mg of liver samples, which were homogenized in 500 µL of TRIzol reagent (Invitrogen) and stored at −80 °C. RNA extraction from the liver was performed following the manufacturer's protocol. The purified total RNA was eluted in 50 µL of elution buffer and quantified by absorbance using an Epoch spectrophotometer (BioTek, USA). To remove any residual DNA, 3 µg of total RNA from each sample was treated with DNase I (ThermoScientific). The DNase I to total RNA ratio was 1 U/µg of total RNA. RNA concentration was verified using a Qubit 3.0 fluorometer and the Qubit RNA Broad Range assay (Life Technologies, USA).

Libraries for RNA-Seq analysis were prepared using 1 µg of purified total RNA from each of the 4 biological replicates for each treatment. Libraries were prepared using the TruSeq® RNA Sample Prep v2 kit with IDT for Illumina DNA/RNA UD indexes (Illumina, San Diego, CA, USA). The library preparation process included mRNA purification and fragmentation, cDNA synthesis, end repair, adenylation, adapter ligation, PCR amplification, library validation, equimolar normalization, and pooling, following the detailed protocol in the TruSeq RNA-Seq Sample Prep v2 Guide (Illumina), using the Low Sample Protocol. Sequencing was performed on the Illumina NextSeq500 platform using the NextSeq500 System High-Output Kit, generating paired-end reads of 74 bp (2 × 74 bp). The raw RNA-seq data was deposited in BioProject with the Acc. No. is PRJNA1295878.

2.8. Transcriptome analysis

The liver transcriptome was assembled using Trinity v.2.12 (Haas et al., 2013). To reduce transcript redundancy, contigs with a similarity level of 95 % were subsequently clustered using CD-HIT-EST (Li and Godzik, 2006). The assembled contigs were subjected to a sequence similarity search against the UniProtKB/Swiss-Prot protein database using BLASTX (Altschul et al., 2009) with an E -value cutoff of 10^{-6} . Subsequently, the results were processed with Trinotate to retrieve Gene Ontology (GO) terms and metabolic pathways based on the Kyoto

Encyclopedia of Genes and Genomes (KEGG) database.

For differential expression analysis, the reads from each biological replicate were individually mapped to the assembled transcriptome and gene expression levels were quantified using the Expectation-Maximization method (RSEM, Li and Dewey, 2011). Additionally, transcript-level expression profiles across different samples were examined through hierarchical clustering. Differential expression analysis was performed using edgeR (Robinson et al., 2010). Pairwise comparisons between treatments and controls were conducted for each feeding week. Differentially expressed genes were identified using a minimum fold-change threshold of 2 and an adjusted p -value <0.01 for statistical significance.

Enrichment analysis of differentially expressed genes for each pairwise comparison was performed using the DAVID database. The analyses were conducted by contrasting the list of differentially expressed genes from each comparison against a reference list based on annotated transcripts. Statistical significance for enrichment was determined using Fisher's exact test with a significance threshold of 0.05.

2.9. Statistical analysis

All statistical analyses were performed using R version 4.4.2 (R Core Team, 2024), primarily relying on the nlme (Pinheiro and Bates, 2000), emmeans (Lenth, 2025), and robustlmm (Koller, 2016) packages. The study employed a hierarchical mixed-effects modelling framework tailored for longitudinal experimental designs. This approach, which contrasts with traditional cross-sectional analyses, enhanced statistical power and precision by leveraging the repeated measures structure while accounting for the nesting of individual fish within tanks, without increasing the number of animals used.

Each experimental phase—fasting and refeeding—was analyzed independently, with statistical models tailored to the outcome variables pertinent to each stage. All models included time and treatment (as well as their interaction) as fixed effects. When individual-level measurements were available across multiple time points—such as for BW, TL, and SGR—a hierarchical random effect was specified, with individual fish nested within tank. In these cases, a variance structure (varIdent) was incorporated to allow heterogeneous residual variances across treatment × time combinations. Furthermore, for variables with three or more time points (e.g., TL, BW, SGR), we evaluated and compared different within-subject correlation structures (AR(1), compound symmetry, and independence), selecting the best model using Akaike's Information Criterion (AIC).

For variables with only two time points, such as hepatosomatic index (HSI), hepatocyte area, and nuclear area during the fasting phase, the correlation structure could not be estimated and was therefore omitted. Nevertheless, the variance heterogeneity structure (varIdent) was retained, and tank was included as a random effect. Similarly, for variables measured at the tank level—such as feed intake, FCR, and percent body weight ingested—mixed-effects models without the fish-level nesting were used, but retained the same strategy of variance modelling and correlation structure evaluation.

Model diagnostics were conducted for all fitted models. Residuals were then inspected visually for homoscedasticity and normality using residual vs. fitted plots and Q-Q plots, respectively. The evaluation of autocorrelation was conducted using ACF plots of normalized residuals. In all cases, robust linear mixed-effects models were fitted using the `rlmer` function from the *robustlmm* package to evaluate the sensitivity of the main results to potential outliers and assumption violations.

Estimated marginal means (EMMs) were computed using the `emmeans` package, and *post hoc* comparisons were performed using pairwise contrasts between treatment groups at each time point, applying Sidak correction for multiple comparisons. Time is modelled as an ordinal factor. Linear and quadratic polynomial terms are included to describe the temporal growth trajectory with regard to body weight and total length during the refeeding and fasting phase. The linear term

captures the overall growth trend, while the quadratic term reflects curvature over time. In conjunction with the time-by-treatment interaction and SGRs, these components will facilitate the assessment of compensatory growth dynamics.

Finally, total feed intake per tank over the entire experimental period (W0 to RW3) and liver weight at the end of the experiment (RW3) were compared between groups using two-sample Student's *t*-tests. These tests were conducted only after verifying the normality and homogeneity of variances with the Shapiro–Wilk and Levene's tests, respectively. In all analyses, *p*-values < 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of fasting on growth parameters

During the fasting phase, BW (Fig. 1A) demonstrated a significant treatment × time interaction ($\chi^2 = 468.3$, $p < 0.001$). Polynomial contrasts revealed a strong linear effect of time in the control group ($t = 15.94$, $p < 0.001$), consistent with continuous weight gain. In contrast, fish subjected to fasting showed a significant negative interaction with the linear term ($t = -20.06$, $p < 0.001$), reflecting a progressive loss of BW over time. The estimated autocorrelation parameter (ϕ) of the AR(1) structure was 0.83, suggesting high within-individual temporal dependence. Post hoc comparisons confirmed that there were no significant differences in BW between groups at W0 ($p = 0.051$). However, treated fish exhibited significantly lower BW than the controls at W1 ($p < 0.001$; mean difference = 70.6 ± 13.9 g) and W2 ($p < 0.001$; 104.2 ± 14.3 g).

Total length (Fig. 1B) followed a similar pattern, with a significant treatment × time interaction ($\chi^2 = 705.3$, $p < 0.001$). In the control group, TL increased in a significantly linear ($t = 27.67$, $p < 0.001$) and quadratic ($t = -8.09$, $p < 0.001$) manner, suggesting deceleration

towards the end of the phase. The study revealed that fasting induced a marked growth arrest, as indicated by a significant negative interaction with the linear term ($t = -25.72$, $p < 0.001$), and a positive interaction with the quadratic term ($t = 6.05$, $p < 0.001$). The estimated ϕ was 0.96, indicating strong serial correlation in TL measurements. Pairwise comparisons revealed no significant difference in TL at W0 ($p = 0.49$), but treated fish were significantly shorter at W1 ($p = 0.0159$; 1.86 ± 0.64 cm) and W2 ($p = 0.0015$; 2.81 ± 0.65 cm) relative to controls.

The specific growth rate based on BW (Fig. 1C) showed a significant interaction between treatment and time ($\chi^2 = 15.9$, $p < 0.001$). While control fish maintained positive growth rates throughout the fasting period, SGR(BW) in the fasted group was reduced. Post hoc tests indicated highly significant differences at both W1 and W2 ($p < 0.001$). Similarly, SGR(TL) (Fig. 1D) exhibited a substantial treatment × time interaction ($\chi^2 = 52.3$, $p < 0.001$). The results showed that the controls had increased length, whereas the fasted fish had significantly lower values at both W1 and W2 ($p < 0.001$).

These somatic effects were accompanied by histological alterations. The hepatosomatic index (Table 1) was significantly reduced in fasted fish at W1 and W2 ($p < 0.001$). Representative liver sections (Fig. 1E–F)

Table 1

Hepatosomatic index (HSI) during the fasting and refeeding phases. Values are estimated marginal means ± SE. *p*-values indicate differences between treatment groups at each time point.

Phase	Week	Control	Treated	<i>p</i> -value
Fasting	W1	1.79 ± 0.09	1.01 ± 0.06	< 0.001
	W2 (RW0)	1.67 ± 0.08	0.92 ± 0.01	< 0.001
Refeeding	RW1	1.63 ± 0.08	1.38 ± 0.09	0.056
	RW2	1.55 ± 0.06	2.11 ± 0.06	0.001
	RW3	1.65 ± 0.05	2.00 ± 0.03	0.001

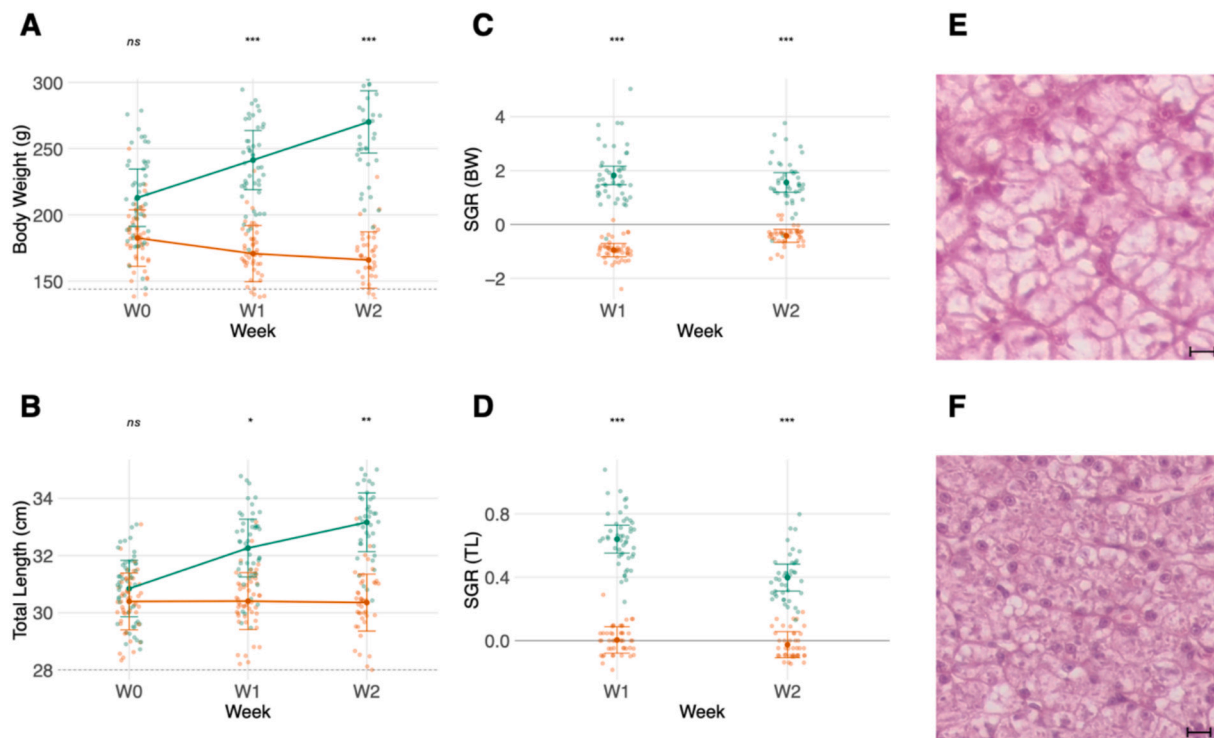


Fig. 1. Effect of a two-week fasting period on somatic growth and liver morphology in *A. gigas*. (A) Body weight, (B) total length, (C) SGR(BW), and (D) SGR(TL) in control (green) and fasted (orange) fish during the fasting phase (W0–W2). Data are presented as individual points and estimated marginal means ± 95 % confidence intervals (95 % CI) from mixed-effects models. (E–F) Representative liver histological sections stained with hematoxylin and eosin at the end of the fasting period (W2), showing the hepatic architecture of a control (E) and a fasted fish (F). Fasted livers exhibited marked cytoplasmic shrinkage and reduced nuclear size. Scale bar = 10 μm. *: $0.01 < p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

illustrate visible differences in hepatic architecture. Treatment resulted in a significant decrease in hepatocyte area (HA) and nuclear area (NA) were both significantly smaller in treated fish at W1 and W2 compared to the control group (Tables 2 and 3; all $p < 0.001$).

3.2. Effect of refeeding on growth parameters

During the refeeding period, body weight (Fig. 2A) demonstrated a significant treatment $\times$ time interaction ($\chi^2 = 24.1$, $p < 0.001$). Polynomial contrasts revealed a significant linear increase in body weight over time ($t = 24.55$, $p < 0.001$), with additional quadratic ($t = 8.38$, $p < 0.001$) and cubic ($t = -4.19$, $p < 0.001$) components indicating an accelerating then decelerating growth trend in control fish. Following the resumption of feeding, fasted fish demonstrated a clear trajectory. The interaction with the linear term was positive ($t = 4.31$, $p < 0.001$), as was the interaction with the quadratic term ($t = 3.70$, $p < 0.001$). The autocorrelation coefficient (ϕ) was 0.97, indicating strong temporal dependency in repeated measures. Estimated marginal means comparisons showed that the treated fish remained significantly lighter than controls throughout the refeeding period, with decreasing differences from RW0 ($\Delta = 106.6$ g, $p < 0.001$) to RW3 ($\Delta = 69.9$ g, $p = 0.0020$), reflecting partial catch-up growth.

Total length (Fig. 2B) exhibited a comparable trend, accompanied by a significant treatment $\times$ time interaction ($\chi^2 = 81.2$, $p < 0.001$). Control fish exhibited linear ($t = 36.47$, $p < 0.001$) and quadratic ($t = 5.71$, $p < 0.001$) trends. In treated fish, the significant interaction with the linear ($t = 5.12$, $p < 0.001$) and quadratic ($t = 7.94$, $p < 0.001$) terms indicated an accelerating length gain after refeeding. The autocorrelation estimate was once again high ($\phi = 0.97$). Comparisons of marginal means showed that fasted fish remained significantly shorter than controls at all time points (RW0: $\Delta = 2.89$ cm, $p = 0.0005$; RW3: $\Delta = 2.03$ cm, $p = 0.0081$), with the gap narrowing over time.

The specific growth rate based on body weight (Fig. 2C) displayed a significant treatment $\times$ time interaction ($\chi^2 = 40.4$, $p < 0.001$). Refed fish demonstrated a significantly higher SGR(BW) in comparison to the control group across all refeeding weeks (RW1–RW3; all $p < 0.001$), with the greatest difference at RW2 (Fig. 2C). In contrast, SGR based on total length (Fig. 2D) showed a higher difference at the end of the period. The interaction between treatment and time was found to be highly significant ($\chi^2 = 165.0$, $p < 0.001$). While no significant differences were observed at RW1 ($p = 0.26$), treated fish showed higher SGR(TL) than controls at RW2 ($p < 0.001$) and RW3 ($p < 0.001$).

Finally, liver metrics reflected the somatic trends. The hepatosomatic index (Table 1) remained lower in treated fish at RW1 ($p = 0.056$), but was significantly higher than in the control group at RW2 and RW3 ($p = 0.001$). Similarly, hepatocyte area (Table 2) was significantly smaller in the treated group at RW1 ($p = 0.0036$), but reached similar values at RW2 and RW3 ($p > 0.05$). The nuclear area (Table 3) showed no significant differences between the groups throughout the refeeding phase ($p > 0.05$). Representative liver sections (Fig. 2E–F) illustrate these morphological changes at the end of the refeeding period.

Table 2

Hepatocyte area (HA, μm^2) during the fasting and refeeding phases. Values are estimated marginal means $\pm$ SE. p-values indicate differences between treatment groups at each time point.

Phase	Week	Control	Treated	p-value
Fasting	W1	148.1 $\pm$ 3.2	67.2 $\pm$ 2.5	< 0.001
	W2 (RW0)	159.8 $\pm$ 2.9	68.2 $\pm$ 3.2	< 0.001
Refeeding	RW1	174.7 $\pm$ 11.0	129.7 $\pm$ 5.0	0.0036
	RW2	161.5 $\pm$ 4.5	167.0 $\pm$ 6.4	ns
	RW3	161.4 $\pm$ 10.2	172.6 $\pm$ 4.0	ns

Table 3

Nuclear area (NA, μm^2) during the fasting and refeeding phases. Values are estimated marginal means $\pm$ SE. p-values indicate differences between treatment groups at each time point.

Phase	Week	Control	Treated	p-value
Fasting	W1	16.3 $\pm$ 0.3	12.7 $\pm$ 0.1	< 0.001
	W2 (RW0)	17.0 $\pm$ 0.5	13.1 $\pm$ 0.3	< 0.001
Refeeding	RW1	15.8 $\pm$ 0.2	15.3 $\pm$ 0.4	ns
	RW2	15.2 $\pm$ 0.4	16.0 $\pm$ 0.4	ns
	RW3	14.9 $\pm$ 0.3	14.8 $\pm$ 0.4	ns

3.3. Effect of refeeding on FCR and feed intake

The next stage of the process was to evaluate the feed conversion ratio during the refeeding phase. The feed conversion ratio (Fig. 3A) during the refeeding phase demonstrated a significant effect of time ($\chi^2 = 23.29$, $p < 0.001$), while the treatment $\times$ time interaction was not significant ($\chi^2 = 3.75$, $p = 0.15$). The type I ANOVA confirmed a significant main effect of time ($F = 23.68$, $p < 0.001$) and treatment ($F = 11.99$, $p = 0.0061$), indicating that FCR changed over time and differed between groups.

As demonstrated in Fig. 3B, there was a significant interaction between treatment $\times$ time interaction ($\chi^2 = 84.31$, $p < 0.001$), indicating divergent temporal patterns between groups. Specifically, the control fish consumed more than the fish in RW1 ($p = 0.0436$), but not in RW2 or RW3 ($p > 0.05$). Conversely, feed intake relative to body weight (Fig. 3C) demonstrated a significant treatment $\times$ time interaction ($\chi^2 = 457.20$, $p < 0.001$). Subsequent, post hoc tests showed significantly higher feed intake (% BW) in treated fish compared to controls in RW2 and RW3 (both $p < 0.001$), while no discrepancy was observed at RW1 ($p > 0.05$). The results of the analysis indicate strong temporal dependence and marked heteroscedasticity. These findings are supported by the autocorrelation coefficient ($\phi = 0.77$) and the fitted varident structure.

It was observed that the fasted group exhibited a lower rate of cumulative feed intake during the refeeding period when compared to the control group. When the data was aggregated by group, the treated fish consumed a total of 2319.7 g, whereas the control fish consumed 4758.9 g. This represents a 51.3 % reduction in total feed use. At the tank level, mean total intake was significantly lower in treated tanks (386.6 ± 63.4 g) than in control tanks (793.2 ± 151.2 g), with a statistically significant difference confirmed by an unpaired two-tailed t -test ($t = 6.07$, $df = 10$, $p < 0.001$).

3.4. Liver transcriptome assembly and functional annotation

The liver transcriptome was assembled from 273.2 million clean reads, yielding a total of 118,810 transcripts and 97,454 genes. The GC content of the assembled transcriptome was 49.04 %, with a mean contig length of 902 bp and a median contig length of 440 bp. The final assembly consisted of 107,126,589 bases. To reduce transcript redundancy, contigs were clustered at 95 % similarity using CD-HIT-EST.

A sequence similarity search was performed against the UniProtKB/Swiss-Prot database using BLASTX with an E -value cutoff of 10^{-6} . The annotation process identified Gene Ontology (GO) terms and KEGG metabolic pathways using Trinotate (Fig. S1). Approximately 37 % of the assembled transcripts were successfully annotated.

3.5. Differential gene expression analysis

The mRNA expression levels for liver samples showed a similar profile for samples in the same treatment (Fig. 4A). Pairwise comparison between control and treated groups for each time point revealed 36 DEGs at W1, 334 DEGs at W2 of fasting and 15 DEGs at RD3 and 19 DEGs at RW2 weeks of refeeding (Fig. 4B).

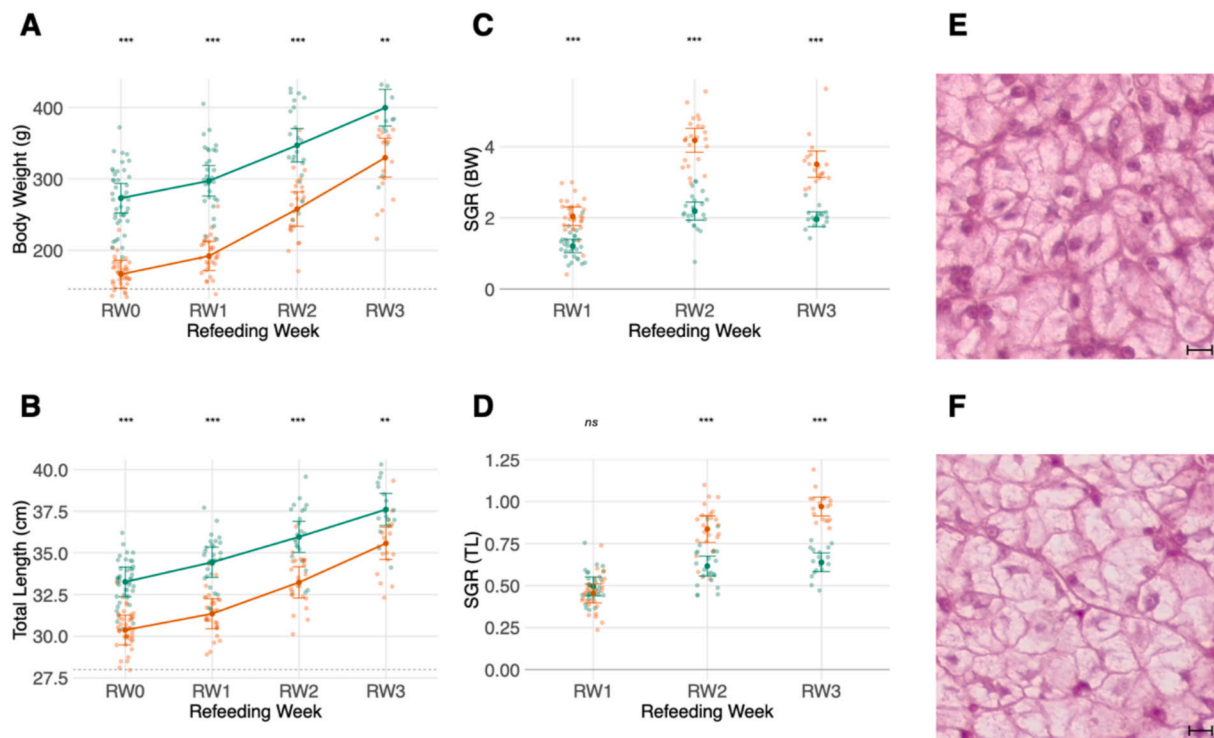


Fig. 2. Effect of a three-week refeeding period following fasting on somatic growth and liver morphology in *A. gigas*. (A) BW, (B) TL, (C) SGR(BW), and (D) SGR(TL) in control (green) and previously fasted (orange) fish during the refeeding phase (RW0–RW3). Data are shown as individual values with estimated marginal means $\pm$ 95 % CI from linear mixed-effects models. (E–F) Representative liver sections stained with hematoxylin and eosin from control (E) and refeeding (F) groups at RW3. Scale bar = 10 μ m. *: $0.01 < p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

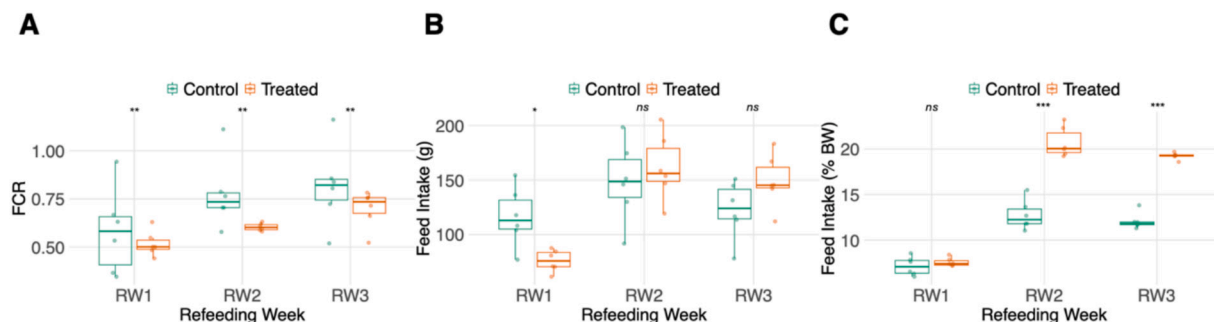


Fig. 3. Effect of refeeding on feed conversion efficiency and feed intake in *A. gigas*. (A) FCR, (B) feed intake (g), and (C) feed intake expressed as percentage of body weight (% BW) in control (green) and previously fasted (orange) fish during the refeeding period. Data are shown as boxplots overlaid with individual data points. *: $0.01 < p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p \leq 0.001$; ns: not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Functional enrichment analysis

Transcriptomic analysis of the *A. gigas* liver revealed significant changes in gene expression in response to fasting and refeeding (Fig. 4). Functional enrichment analysis using DAVID identified differentially expressed genes associated with metabolic pathways and biological processes relevant to energy homeostasis, lipid metabolism, and cellular repair (Table 4).

In the one-week fasting comparison (W1T vs. W1C), RXRA (retinoic acid receptor RXR- α) was upregulated (+41.1, $p = 1.10\text{E-}03$), along with ABCA1 (ATP-binding cassette transporter A1, +6.8, $p = 9.30\text{E-}03$). These genes are involved in lipid metabolism and cholesterol transport. In addition, PKHA7 (pleckstrin homology domain-containing family A member 7, +41.1, $p = 1.10\text{E-}03$) and SPAG8 (sperm-associated antigen 8, +35.5, $p = 1.40\text{E-}03$) were also differentially expressed.

After two weeks of fasting (W2T vs W2C), SLC13A5 and SLC22A7 (solute carriers involved in α -ketoglutarate transport) showed increased expression (+34.7, $p = 1.40\text{E-}03$). Genes associated with fatty acid metabolism, including ACOT2 (acyl-CoA thioesterase 2, +27, $p = 2.30\text{E-}03$) and SLC27A2 (solute carrier family 27 member 2, +27, $p = 2.30\text{E-}03$), were also upregulated. Additionally, MC4R (melanocortin receptor 4, +20.2, $p = 4.20\text{E-}03$) and AGRF5 (adhesion G protein-coupled receptor F5, +20.2, $p = 4.20\text{E-}03$) were differentially expressed, indicating involvement in energy regulation.

Following three days of refeeding (RD3T vs RD3C), PPARGC1A (peroxisome proliferator-activated receptor gamma coactivator 1- α) was highly upregulated (+171, $p = 5.90\text{E-}05$), along with BRD8 (bromodomain-containing protein 8, +171, $p = 5.90\text{E-}05$). These genes are involved in metabolic regulation and chromatin remodeling. Genes associated with gluconeogenesis, including MDH1 (Malate

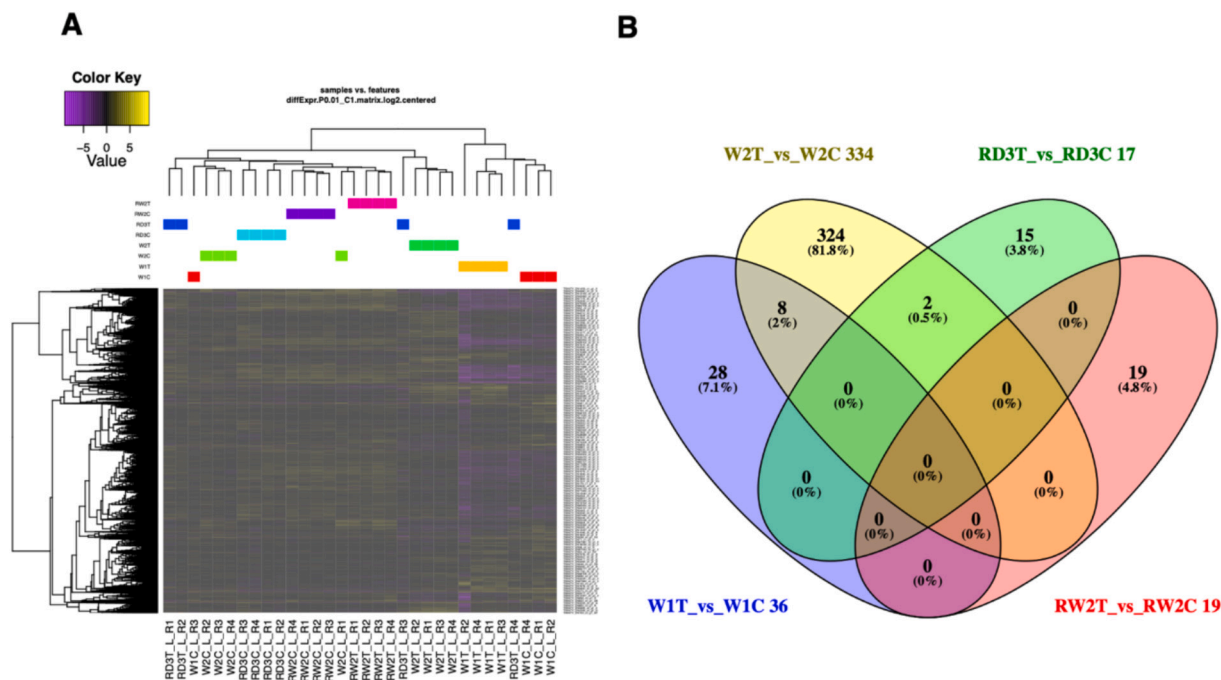


Fig. 4. A) Heatmap showing the hierarchical clustering of differentially expressed transcripts in the liver of juvenile *A. gigas* under a fasting–refeeding scheme. The colour scale represents expression levels, with yellow indicating upregulated transcripts and purple indicating downregulated transcripts. The clustering of biological replicates highlights the consistency of gene expression responses within treatments. The top annotation bar differentiates experimental groups, providing a visual overview of transcriptomic shifts across conditions. Differential expression was determined using an FDR threshold of 0.01 and a minimum twofold change in each pairwise comparison. B) Venn diagram illustrating the overlap of differentially expressed transcripts in the liver of juvenile *A. gigas* under fasting and refeeding conditions. The numbers indicate the count and percentage of transcripts exclusively differentially expressed in each condition or shared between conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4

Top three enriched biological processes in the liver of juvenile *A. gigas* under a fasting–refeeding scheme, identified through functional enrichment analysis using DAVID. The table presents differentially expressed genes, their associated biological processes, fold-change variation, and statistical significance (p-value) for each comparison: W1 (1-week fasting vs. control), W2 (2-week fasting vs. control), RD3 (3-day refeeding vs. control), and RW2 (2-week refeeding vs. control). Upregulated genes are indicated with positive fold-change values.

Week	Gene	Name	Biological Process	Change	p-value
W1	PKHA7_DANRE	Pleckstrin homology domain-containing family A member 7	Cardiac muscle cell differentiation	41.1	0.0011
	RXRA_MOUSE	Retinoic acid receptor RXR-alpha	Cardiac muscle cell differentiation	41.1	0.0011
	SPAG8_BOVIN	Sperm-associated antigen 8	Single fertilization	35.5	0.0014
	ABCA1_HUMAN	ATP-binding cassette transporter ABCA1	G protein-coupled receptor signaling pathway	6.8	0.0093
	THRB_HUMAN	Coagulation factor II, thrombin (F2)	G protein-coupled receptor signaling pathway	6.8	0.0093
W2	S13A5_HUMAN	Solute carrier family 13 member 5	Alpha-ketoglutarate transport	34.7	0.0014
	S22A7_BOVIN	Solute carrier family 22 member 5	Alpha-ketoglutarate transport	34.7	0.0014
	ACOT2_MOUSE	Acyl-CoA thioesterase 2 (Acot2)	Very long-chain fatty acid catabolic process	27	0.0023
	S27A2_RAT	Solute carrier family 27 member 2	Very long-chain fatty acid catabolic process	27	0.0023
	AGRF5_RAT	Adhesion G protein-coupled receptor F5	Energy reserve metabolic process	20.2	0.0042
	MC4R_DANRE	Melanocortin receptor 4	Energy reserve metabolic process	20.2	0.0042
	PRGC1_RAT	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A)	Cellular response to thyroid hormone stimulus	171	<0.0001
	BRD8_MOUSE	Bromodomain-containing protein 8	Cellular response to thyroid hormone stimulus	171	<0.0001
RD3	PRGC1_RAT	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A)	Gluconeogenesis	32.9	0.0016
	MDHC_HUMAN	Malate dehydrogenase 1	Gluconeogenesis	32.9	0.0016
	DCLK1_RAT	Doublecortin-like kinase 1	Prosencephalon development	28.8	0.0021
	A2MG_HUMAN	Alpha-2-macroglobulin	Embryonic liver development	378.1	<0.0001
	DNL13_HUMAN	DNA ligase 3	Mitochondrial DNA repair	236.3	<0.0001
RW2	A2MG_HUMAN	Alpha-2-macroglobulin	Response to prostaglandin E	210.1	<0.0001

dehydrogenase 1, +32.9, $p = 1.60\text{E-}03$), were also upregulated. Additionally, DCLK1 (Doublecortin-like kinase 1, +28.8, $p = 2.10\text{E-}03$) was differentially expressed.

After two weeks of refeeding (RW2T vs RW2C), genes related to liver regeneration and mitochondrial function were significantly

upregulated. A2MG (Alpha-2-macroglobulin) exhibited showed strong upregulation (+378.1, $p = 1.10\text{E-}05$), as did LIG3 (DNA ligase 3, +236.3, $p = 3.00\text{E-}05$), which is involved in mitochondrial DNA repair.

4. Discussion

The increasing demand for high-quality protein in the aquaculture industry has prompted the search for fish species that exhibit superior growth and feed conversion rates. The paiche is renowned for its exceptional feed efficiency (Du et al., 2019), yet scientific data supporting this claim remains limited. The study examined compensatory growth in *A. gigas* following fasting and refeeding, revealing key metabolic and morphological responses. To the best of our knowledge, this is the first experimental report documenting a feed conversion ratio as low as 0.518 in this species. While Du et al. (2019) reference a FCR of 0.7 for *A. gigas*, the source and empirical basis for this figure remain unclear, as no supporting data or citations are provided.

Although the FCR observed in this study was remarkably low, it is important to interpret such values with caution. In this study, FCR was calculated based on wet weight. While this conventional approach offers an operationally useful indicator of feed efficiency, we acknowledge that more precise estimations would require calculations based on energy content or dry matter, in order to exclude the confounding influence of tissue water content. Future studies incorporating energy-based assessments could help refine our understanding of feed conversion dynamics in *A. gigas*.

Interestingly, even under continuous feeding conditions, *A. gigas* exhibited a very low FCR, with control fish displaying values well below 1.0 throughout the refeeding period. This baseline efficiency reinforces previous claims regarding the species' exceptional feed utilization, although published evidence has been scarce (Du et al., 2019). One plausible explanation may lie in the species' unique respiratory physiology: *A. gigas* is an obligate air-breather that relies primarily on a highly vascularized swim bladder rather than gills for oxygen uptake. As a result, it may be less sensitive to fluctuations in dissolved oxygen (DO), which are known to strongly influence FCR in other species such as *Oreochromis niloticus* (Mengistu et al., 2020). This aerial respiration strategy could allow for more consistent internal oxygenation, possibly reducing the energetic cost of ventilation and supporting more efficient digestion and metabolism, although further physiological studies are needed to test this hypothesis.

Beyond this basal efficiency, the fasting-refeeding protocol induced an additional improvement in FCR, with treated fish reaching a remarkably low value of 0.518 during the first refeeding week. While this value should be interpreted with caution due to potential water content effects on wet weight calculations, it nonetheless suggests a highly efficient conversion of feed into biomass during early compensation. This phase coincided with a rapid restoration of liver condition: the hepatosomatic index increased markedly, and both hepatocyte and nuclear areas recovered to control levels. Such coordinated structural regeneration may reflect the reactivation of metabolic processes involved in nutrient assimilation, potentially contributing to the enhanced feed efficiency observed. Although these associations remain correlative, they point to a physiological context—combining efficient oxygen supply and rapid hepatic recovery—that may underpin the exceptional FCR dynamics observed in *A. gigas*.

Our results demonstrate that *A. gigas* exhibits a clear compensatory growth response following a fasting-refeeding cycle. Fasting for two weeks induced significant weight loss and growth arrest, followed by accelerated somatic growth during refeeding. This pattern was statistically supported by significant linear and quadratic coefficients of the time $\times$ treatment interaction for both BW and TL. Moreover, SGR provided further evidence of compensatory dynamics, with refeed fish displaying elevated SGR for BW throughout the refeeding period, and increased SGR for TL from the second week onwards. This recovery trajectory is consistent with compensatory growth observed in other teleosts, although its magnitude and timing vary across species (Tian and Qin, 2003; Montserrat et al., 2007; Fox et al., 2010; Peres et al., 2011; Fukada et al., 2012; Delgadin et al., 2015, 2018; Oh and Park, 2019; Álvarez, 2024).

The partial compensation observed in *A. gigas*—in which treated fish remained smaller than controls but exhibited sustained catch-up growth—differs from the complete compensation reported in other species, such as *Salmo salar* (Hvas et al., 2022), and underscores the species-specific nature of compensatory responses. For example, *Oreochromis mossambicus* shows incomplete recovery even after extended refeeding (Fox et al., 2010), whereas *Sparus aurata* achieves full compensation in body weight but not in total length (Bavčević et al., 2010). These interspecific differences may reflect variations in life-history strategies, metabolic flexibility and the specific conditions and durations of the experimental protocols employed.

An important aspect of the response in *A. gigas* was the parallel recovery of both weight and length, in contrast to other species where only one parameter may recover fully or growth decelerates over time (Delgadin et al., 2018). The persistence of elevated growth rates suggests that full compensation could be achieved with longer refeeding, a hypothesis worth testing in future studies.

From an aquaculture perspective, the most striking finding was the 51 % reduction in cumulative feed intake in treated fish, despite only partial recovery in BW and TL. This feed-saving effect implies that fasting-refeeding protocols may offer a cost-effective strategy for optimizing feed efficiency in *A. gigas* culture, although longer trials are needed to confirm the net production benefit. Additionally, it remains to be determined whether such feed-saving strategies may have downstream effects on fillet quality, a factor that merits consideration in future trials.

The hepatic response supported the somatic findings. Fasting led to reduced HSI and decreased hepatocyte size. While hepatocyte loss via apoptosis or necrosis cannot be completely ruled out (Karatas et al., 2021), the observed rapid recovery in hepatocyte and liver size suggests that cellular shrinkage was the dominant mechanism. Upon refeeding, HSI values surpassed control levels, a pattern also described in *Oryzias latipes* and other species (Picha et al., 2008; Delgadin et al., 2018; Jia et al., 2019), potentially reflecting hepatocyte hypertrophy or compensatory proliferation (Jia et al., 2019). Additionally, studies in other teleosts such as black carp (*Mylopharyngodon piceus*) have shown that hepatic glycogen stores are rapidly depleted during early fasting and restored during refeeding, suggesting that liver size recovery may also involve replenishment of energy reserves (Dai et al., 2024). Although we did not measure glycogen content directly, a similar mechanism may be operating in *A. gigas*, contributing to the observed HSI rebound and supporting the notion of metabolic compensation. Given the systemic nature of fasting responses, it is also plausible that other tissues, such as skeletal muscle or intestine, experienced structural and functional alterations (Zaldúa and Naya, 2014). Future studies could explore these additional targets to gain a more comprehensive understanding of whole-organism responses in *A. gigas*.

The compensatory growth response in *A. gigas* involved both enhanced feed conversion efficiency and hyperphagia—two well-established mechanisms in teleosts (Ali et al., 2003; Álvarez, 2024). Refed fish exhibited consistently lower FCRs than controls, and feed intake per unit body weight increased markedly from the second week of refeeding, suggesting that both increased appetite and metabolic efficiency contributed to catch-up growth. Interestingly, the absolute amount of feed consumed by refeed fish during refeeding was not higher than that of the control group, reinforcing the notion that the observed growth compensation occurred with a lower overall feed requirement.

These findings reinforce the exceptional growth efficiency of *A. gigas* and highlight its potential as a valuable reference species for understanding superior feed utilization strategies in teleosts. One key question raised by this work is whether the already remarkable FCR of *A. gigas* has reached a physiological threshold, or if it can be further improved through feeding strategies. Our results indicate that fasting-refeeding protocols can further optimize feed utilization, encouraging the exploration of such approaches in production systems. While compensatory growth mechanisms have been partially described in other teleosts

(Qian et al., 2016; Jia et al., 2019), they remain largely uncharacterized in *A. gigas*—a gap that this study begins to address. Future research will be essential to elucidate the molecular basis of this response.

It is important to note that this study also demonstrated the benefits of a longitudinal, within-subjects experimental design for detecting dynamic biological responses. By measuring the same individuals repeatedly, we were able to control for individual variability was controlled, thereby increasing the statistical power of the study (Maxwell et al., 2017). This approach enabled us to model both linear and nonlinear growth trajectories using hierarchical linear mixed models, with fish nested within tanks, heterogeneous variances (varIdent), and temporal autocorrelation (corAR1). In order to ensure the robustness of the process, we also employed an alternative estimation method using RLMER. The combination of these features resulted in a robust analytical framework, thereby enhancing the reliability of our findings and providing a valuable reference point for future aquaculture studies involving repeated measures and complex biological responses.

The number and overlap of differentially expressed genes (DEGs) across experimental comparisons revealed marked differences in the hepatic transcriptional response to fasting and refeeding. A greater number of DEGs was observed after two weeks of fasting than after one week, suggesting that extended nutrient deprivation amplifies the liver's adaptive response. The substantial overlap between DEGs in W1 and W2 indicates that many fasting-induced genes are persistently regulated over time, reinforcing the robustness of the metabolic adjustments initiated early during fasting. In contrast, there was limited overlap between fasting-related and refeeding-related DEGs, implying that refeeding elicits distinct regulatory programs rather than a simple reversal of fasting-induced expression patterns. Interestingly, only a small set of DEGs was detected in the early refeeding stage (RD3), which may initially suggest a limited transcriptional response. However, this apparent lack of differential expression likely reflects a normalization of gene activity following the pronounced changes induced by prolonged fasting. This points to a rapid reactivation of metabolic pathways and an early restoration of hepatic homeostasis, even before new transcriptional programs are initiated. By contrast, the broader transcriptional response observed after two weeks of refeeding (RW2T vs RW2C) may represent a later phase characterized by sustained metabolic reprogramming.

Fasting led to the upregulation of lipid metabolism genes (RXRA, ABCA1, ACOT2), indicating increased fatty acid oxidation and ketogenesis (Kersten et al., 1999; Lefebvre et al., 2006; Kersten, 2014). This is consistent with previous studies in other vertebrates, where fasting induces lipid mobilization as a primary energy source (Kersten, 2014). Prolonged fasting intensified these adaptations, with the upregulation of genes like SLC27A2 and MC4R suggesting shifts in energy utilization (Anderson and Stahl, 2013). Interestingly, during the early refeeding phase (RD3), genes involved in gluconeogenesis (e.g., PPARGC1A and MDH1) were upregulated. Although gluconeogenic activity is typically associated with fasting (Dai et al., 2024), this transient activation during refeeding is not entirely unexpected. It may reflect a carryover from the previous catabolic state or an acute metabolic response to rapidly meet glucose demands as feeding resumes. Notably, this response was not observed at RW2, suggesting that the effect is restricted to the initial transition phase. After two weeks of refeeding, gene expression patterns shifted towards tissue restoration and mitochondrial function, with strong upregulation of A2MG and LIG3, which are involved in liver regeneration and mitochondrial DNA repair, respectively. These findings highlight the dynamic and time-dependent nature of hepatic responses to nutritional status and reinforce the liver's central role in orchestrating energy homeostasis during fasting and refeeding cycles. Although growth is largely driven by increased protein synthesis, particularly in skeletal muscle, the liver also plays a central role in amino acid metabolism and systemic nutrient handling. Therefore, some transcriptional modulation of protein metabolism genes could be expected. The absence of significantly differentially expressed genes

related to protein synthesis in our data may reflect tissue-specific expression patterns, as well as the methodological focus on highly regulated transcripts. It is possible that genes involved in protein metabolism exhibited subtler changes not captured under our statistical thresholds, or that their expression remained stable despite their functional importance in the systemic growth response.

Our findings highlight *A. gigas* as a promising model for studying compensatory growth and feed efficiency in aquaculture. Its ability to rapidly recover from fasting, coupled with a low FCR, underscores its potential for sustainable fish farming. Further research should explore long-term refeeding effects and regulatory mechanisms underlying its exceptional growth performance, potentially leveraging omics approaches to refine aquaculture practices.

5. Conclusions

The present study demonstrates that the paiche exhibits one of the highest conversion efficiencies among fish species. Furthermore, experimental evidence indicates that these efficiencies can be enhanced through the implementation of a fasting and refeeding cycle. The incorporation of these modifications into feeding management strategies has the potential to reduce costs associated with paiche farming, while simultaneously minimizing the environmental impact. This is attributed to the fact that a higher conversion efficiency results in a reduced amount of feed necessary to increase biomass. The study's findings indicate that while paiche exhibits one of the highest conversion rates, this can be further enhanced by modifying feeding protocols. Furthermore, these rates could be further optimized by implementing a diet specifically formulated for this species and stage of development, which will positively impact paiche aquaculture.

CRedit authorship contribution statement

T.H. Delgadín: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **L.E. Santos-Rojas:** Methodology. **D. Medina-Herrera:** Methodology. **N.M. Herrera-Castillo:** Methodology. **A.M. Alonso:** Software, Formal analysis. **A. Saavedra-Flores:** Methodology. **E.A. López-Landavery:** Methodology. **J.I. Fernandino:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **E. Zelada-Mázmela:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Funding

This research was funded by Consejo Nacional de Ciencia y Tecnología CONCYTEC-PROCIENCIA: Semilla Project, Contract PE501078587–2022-PROCIENCIA “Análisis de la alta tasa de crecimiento somático del Paiche: una perspectiva genómica y experimental”.

This research was also funded by Consejo Nacional de Ciencia y Tecnología (CONCYTEC)-PROCIENCIA and the World Bank Project “Mejoramiento y Ampliación de los Servicios del Sistema Nacional de Ciencia Tecnología e Innovación Tecnológica” 8682-PE, Contract 018–2019-FONDECYT-BM-INC.INV.

Declaration of competing interest

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

Acknowledgements

The authors acknowledge the support from Universidad Nacional del Santa (UNS). They also thank to Luis Henostroza from Amazon Aquaculture SAC Company for their support during the development of the experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2025.743267>.

Data availability

No data was used for the research described in the article.

References

- Ali, M., Nieceza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. *Fish Fish.* 4 (2), 147–190.
- Altschul, S.F., Gertz, E.M., Agarwala, R., Schäffer, A.A., Yu, Y.K., 2009. PSI-BLAST pseudocounts and the minimum description length principle. *Nucleic Acids Res.* 37 (3), 815–824.
- Álvarez, D., 2024. Effects of compensatory growth on fish behavior. In: *Encyclopedia of Fish Physiology*, Second edition. Academic Press, pp. 403–409.
- Anderson, C.M., Stahl, A., 2013. SLC27 fatty acid transport proteins. *Mol. Asp. Med.* 34 (2–3), 516–528.
- Árnason, T., Björnsson, B., Steinarrson, A., Oddgeirsson, M., 2009. Effects of temperature and body weight on growth rate and feed conversion ratio in turbot (*Scophthalmus maximus*). *Aquaculture* 295 (3–4), 218–225.
- Attanasio, A.F., Shavrikova, E., Blum, W.F., Cromer, M., Child, C.J., Paskova, M., Shalet, S.M., 2004. Continued growth hormone (GH) treatment after final height is necessary to complete somatic development in childhood-onset GH-deficient patients. *J. Clin. Endocrinol. Metab.* 89 (10), 4857–4862.
- Bai, S.C., Hardy, R.W., Hamidoghli, A., 2022. Diet analysis and evaluation. In: *Fish Nutrition*. Academic Press, pp. 709–743.
- Bavčević, L., Klanjšček, T., Karamarko, V., Aničić, I., Legović, T., 2010. Compensatory growth in gilthead sea bream (*Sparus aurata*) compensates weight, but not length. *Aquaculture* 301 (1–4), 57–63.
- Beckman, B.R., 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. *Gen. Comp. Endocrinol.* 170 (2), 233–252.
- Boujard, T., Burel, C., Médale, F., Haylor, G., Moisan, A., 2000. Effect of past nutritional history and fasting on feed intake and growth in rainbow trout *Oncorhynchus mykiss*. *Aquat. Living Resour.* 13 (3), 129–137.
- Chávez-Crocker, P., Obreque-Contreras, J., 2010. Bioremediation of aquaculture wastes. *Curr. Opin. Biotechnol.* 21 (3), 313–317.
- Chu-Koo, F., Dugué, R., Alván Aguilar, M., Casanova Daza, A., Alcántara Bocanegra, F., Chávez Veintemilla, C., Nuñez, J., 2009. Gender determination in the Paiche or Pirarucu (*Arapaima gigas*) using plasma vitellogenin, 17 β -estradiol, and 11-ketotestosterone levels. *Fish Physiol. Biochem.* 35, 125–136.
- Dai, Y., Shen, Y., Guo, J., Yang, H., Chen, F., Zhang, W., Li, J., 2024. Glycolysis and gluconeogenesis are involved of glucose metabolism adaptation during fasting and re-feeding in black carp (*Mylopharyngodon piceus*). *Aquac. Fish.* 9 (2), 226–233.
- de Oliveira, E.G., Pinheiro, A.B., de Oliveira, V.Q., da Silva Júnior, A.R.M., de Moraes, M. G., Rocha, Í.R.C.B., Costa, F.H.F., 2012. Effects of stocking density on the performance of juvenile pirarucu (*Arapaima gigas*) in cages. *Aquaculture* 370, 96–101.
- de Oliveira, E.A., Bertollo, L.A.C., Rab, P., Ezaz, T., Yano, C.F., Hatanaka, T., Cioffi, M.D. B., 2019. Cytogenetics, genomics and biodiversity of the South American and African Arapaimidae fish family (Teleostei, Osteoglossiformes). *PLoS One* 14 (3), e0214225.
- de Verdal, H., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J.A.H., Vandeputte, M., 2018. Improving feed efficiency in fish using selective breeding: a review. *Rev. Aquac.* 10, 833–851.
- Delgadin, T.H., Pérez Sirkín, D.I., Di Yorio, M.P., Arranz, S.E., Vissio, P.G., 2015. GH, IGF-I and GH receptors mRNA expression in response to growth impairment following a food deprivation period in individually housed cichlid fish *Cichlasoma dimerus*. *Fish Physiol. Biochem.* 41, 51–60.
- Delgadin, T.H., Simo, I., Pérez Sirkín, D.I., Di Yorio, M.P., Arranz, S.E., Vissio, P.G., 2018. *Cichlasoma dimerus* responds to refeeding with a partial compensatory growth associated with an increment of the feed conversion efficiency and a rapid recovery of GH/IGFs axis. *Aquac. Nutr.* 24 (4), 1234–1243.
- Doupé, R.G., Lymbery, A.J., 2003. Toward the genetic improvement of feed conversion efficiency in fish. *J. World Aquacult. Soc.* 34 (3), 245–254.
- Du, K., Wuertz, S., Adolphi, M., Kneitz, S., Stöck, M., Oliveira, M., Scharl, M., 2019. The genome of the arapaima (*Arapaima gigas*) provides insights into gigantism, fast growth and chromosomal sex determination system. *Sci. Rep.* 9 (1), 5293.
- Farias, L.P., Willis, S., Leao, A., Verba, J.T., Crossa, M., Foresti, F., Hrbek, T., 2019. The largest fish in the world's biggest river: genetic connectivity and conservation of *Arapaima gigas* in the Amazon and Araguaia-Tocantins drainages. *PLoS One* 14 (8), e0220882.
- Forabosco, F., Löhms, M., Rydhmer, L., Sundström, L.F., 2013. Genetically modified farm animals and fish in agriculture: a review. *Livest. Sci.* 153 (1–3), 1–9.
- Fox, B.K., Breves, J.P., Davis, L.K., Pierce, A.L., Hirano, T., Grau, E.G., 2010. Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia. *Gen. Comp. Endocrinol.* 166 (3), 573–580.
- Fry, J.P., Mailloux, N.A., Love, D.C., Milli, M.C., Cao, L., 2018. Feed conversion efficiency in aquaculture: do we measure it correctly? *Environ. Res. Lett.* 13 (2), 024017.
- Fukada, H., Murashita, K., Furutani, T., Masumoto, T., 2012. Yellowtail insulin-like growth factor 1: molecular cloning and response to various nutritional conditions. *Domest. Anim. Endocrinol.* 42 (4), 220–229.
- Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. *Aquaculture* 350, 117–129.
- Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. *Nat. Protoc.* 8 (8), 1494–1512.
- Hrbek, T., Farias, L.P., Crossa, M., Sampaio, I., Porto, J.I., Meyer, A., 2005. Population genetic analysis of *Arapaima gigas*, one of the largest freshwater fishes of the Amazon basin: implications for its conservation. In: *Animal Conservation Forum*, Vol. 8, No. 3. Cambridge University Press, pp. 297–308.
- Hvas, M., Nilsson, J., Vågseth, T., Nola, V., Fjellidal, P.G., Hansen, T.J., Folkedal, O., 2022. Full compensatory growth before harvest and no impact on fish welfare in Atlantic salmon after an 8-week fasting period. *Aquaculture* 546, 737415.
- Jia, J., Qin, J., Yuan, X., Liao, Z., Huang, J., Wang, B., Li, W., 2019. Microarray and metabolome analysis of hepatic response to fasting and subsequent refeeding in zebrafish (*Danio rerio*). *BMC Genomics* 20, 1–13.
- Jobling, M., Koskela, J., 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. *J. Fish Biol.* 49 (4), 658–667.
- Karatas, T., Onalan, S., Yildirim, S., 2021. Effects of prolonged fasting on levels of metabolites, oxidative stress, immune-related gene expression, histopathology, and DNA damage in the liver and muscle tissues of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* 47 (4), 1119–1132.
- Kersten, S., 2014. Integrated physiology and systems biology of PPAR α . *Mol. Metab.* 3 (4), 354–371.
- Kersten, S., Seydoux, J., Peters, J.M., Gonzalez, F.J., Desvergne, B., Wahli, W., 1999. Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J. Clin. Invest.* 103 (11), 1489–1498.
- Koller, M., 2016. robustlmm: an R package for robust estimation of linear mixed-effects models. *J. Stat. Softw.* 75 (6), 1–24. <https://doi.org/10.18637/jss.v075.i06>.
- Lefebvre, P., Chinetti, G., Fruchart, J.C., Staels, B., 2006. Sorting out the roles of PPAR α in energy metabolism and vascular homeostasis. *J. Clin. Invest.* 116 (3), 571–580.
- Lenth, R., 2025. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.11.2. <https://rvinth.github.io/emmeans/>.
- Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinforma* 12, 1–16.
- Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22 (13), 1658–1659.
- MacLeod, M.J., Hasan, M.R., Robb, D.H., Mamun-Ur-Rashid, M., 2020. Quantifying greenhouse gas emissions from global aquaculture. *Sci. Rep.* 10 (1), 11679.
- Maxwell, S.E., Delaney, H.D., Kelley, K., 2017. Designing Experiments and Analyzing Data: A Model Comparison Perspective. Routledge.
- Mengistu, S.B., Mulder, H.A., Benzie, J.A., Komen, H., 2020. A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). *Rev. Aquac.* 12 (2), 524–541.
- Mglav, I., Jobling, M., 1989. Effects of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenile Arctic charr, *Salvelinus alpinus*, with particular respect to compensatory growth. *J. Fish Biol.* 34 (6), 947–957.
- Mokrani, A., Liu, S., 2024. Harnessing CRISPR/Cas9 system to improve economic traits in aquaculture species. *Aquaculture* 579, 740279.
- Montserrat, N., Gabillard, J.C., Capilla, E., Navarro, M.I., Gutiérrez, J., 2007. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* 150 (3), 462–472.
- National Research Council, Division on Earth, Life Studies, Committee on the Nutrient Requirements of Fish, & Shrimp, 2011. Nutrient Requirements of Fish and Shrimp. National academies press.
- Nelson, J.S., 2006. *Fishes of the World*, 4th edition. John Wiley & Sons, Nueva York.
- Oh, S.Y., Park, J., 2019. Effect of feed deprivation on compensatory growth in juvenile rock bream *Oplegnathus fasciatus*. *Fish. Sci.* 85, 813–819.
- Pereira-Filho, M., Cavero, B.A.S., Roubach, R., Ituassú, D.R., Gandra, A.L., Crescêncio, R., 2003. Cultivo do pirarucu (*Arapaima gigas*) em viveiro escavado. *Acta Amazon.* 33, 715–718.
- Peres, H., Santos, S., Oliva-Teles, A., 2011. Lack of compensatory growth response in gilthead seabream (*Sparus aurata*) juveniles following starvation and subsequent refeeding. *Aquaculture* 318 (3–4), 384–388.
- Picha, M.E., Turano, M.J., Tipsmark, C.K., Borski, R.J., 2008. Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *J. Endocrinol.* 199 (1), 81.
- Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-PLUS. Springer New York, New York, NY.
- Qian, B., Xue, L., Huang, H., 2016. Liver transcriptome analysis of the large yellow croaker (*Larimichthys crocea*) during fasting by using RNA-seq. *PLoS One* 11 (3), e0150240.
- R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26 (1), 139–140.
- Rodde, C., Chatain, B., Vandeputte, M., Trinh, T.Q., Benzie, J.A., De Verdal, H., 2020. Can individual feed conversion ratio at commercial size be predicted from juvenile

- performance in individually reared Nile tilapia *Oreochromis niloticus*? Aquac. Rep. 17, 100349.
- Tian, X., Qin, J.G., 2003. A single phase of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. Aquaculture 224 (1–4), 169–179.
- Val, A.L., de Almeida-Val, V.M., 2012. Fishes of the Amazon and their Environment: physiological and Biochemical Aspects, vol. 32. Springer Science & Business Media.
- Wang, Y., Cui, Y., Yang, Y., Cai, F., 2000. Compensatory growth in hybrid tilapia, *Oreochromis mossambicus* × *O. niloticus*, reared in seawater. Aquaculture 189 (1–2), 101–108.
- Zaldúa, N., Naya, D.E., 2014. Digestive flexibility during fasting in fish: a review. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 169, 7–14.