Fatter or Stronger: Resource Allocation Strategy and the Underlying Metabolic Mechanisms in Amphibian Tadpole

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Research

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Abstract

Background

Resource allocation trade-off between storage and somatic growth is an essential physiological phenomenon in animals. Revealing its patterns and underlying mechanisms are fundamental for behavior, evolutionary, and population ecological studies. Currently, our understanding of the real-time resource allocating patterns in animals is still limited, and the underlying metabolic mechanisms have been rarely investigated. The life strategy of amphibian larvae relies on precise coordination between storage and somatic growth, which makes them good model for studying this issue.

Results

Here, the resource allocation strategy was investigated for *Rana omeimontis* tadpoles, who exhibit prominent hepatic fat-accumulation. Results showed that their ontogenetic fat accumulation emerged when tadpoles grew to a body weight range of 30–50 mg, where their fat storage had a high priority in resource allocation. Beyond this range, the resource proportion for somatic growth increased, but continuous storage investment was likely maintained to kept higher body fat index in larger individuals. This seeming paradoxical allocation pattern could be explained by assuming a positive relationship between storage abundance and the investment to somatic growth. This speculation was supported by the observation that storage had the priority in resource allocation to reach a higher body fat index before increment in body weight when food level increased. Moreover, it was also supported by the metabolic pattern that presented lipid-based energy metabolism after ontogenetic fat accumulation, and activating the mobilization of fat storage in the liver can promote the somatic growth. In short, fat synthesis and fat accumulation in the liver may well modulated the resource allocation to somatic growth, and their liver likes a reservoir with valves to regulate energy flow for the downstream developmental processes.

Conclusion

In *Rana omeimontis* tadpoles, their hepatic fat level positively modulated the resource allocation to somatic growth through lipid-based energy metabolism. We reveal the real-time resource allocation pattern in an amphibian tadpole and explain it at molecular level. These results likely provide a new mechanistic insight into the resource allocation in animals.

Introduction

The trade-off between body traits in resource allocation influences fundamental physiological processes such as adaptation, survival, mating, and reproduction [1–3]. Among the various body traits of animals, energy storage and body size may be most essential for the fitness and success of individuals. For pre-reproductive and indeterminate growth animals, resource storage and somatic growth are always conflicting due to the usual limited resources [4–7]. The allocation to these two requirements show high plasticity in response to both intrinsic (e.g., developmental stage) and external factors (e.g., resource

abundance and predation pressure), to maximize the overall fitness of individuals [8, 9]. Revealing the resource allocation patterns and underlying mechanisms are fundamental for behavior, evolutionary, and population ecological studies [7, 10–12].

Although great progresses have been made in revealing the allocation patterns and explaining the observations based on mathematical models [13, 14], there were two major deficiencies in our current knowledge system. Firstly, the real-time allocation patterns have not been concerned. In fact, resource allocation to competing traits is an ongoing process at molecular level, whose patterns may not be accurately reflected by the quantitative relations of the final yield of traits. Secondly, the physiological or metabolic processes underlying trade-offs have rarely been illuminated [14], that limited the developing of mechanistic models for explaining and predicting physiological and ecological phenomena referring to allocation.

Organisms with several distinctly differentiated life stages possess particularly interesting and delicate strategies from the point of view of resource-allocation [15]. Amphibians are unique among tetrapods for their high degree of developmental plasticity which enables them to decouple growth and differentiation to a remarkable extent [16, 17]. Their early development (pre-metamorphosis and pro-metamorphosis) is devoted to the growing of body mass, while late stages, the metamorphic climax, is responsible for morphogenesis and organogenesis [18]. Meanwhile, fat accumulation during their early development is also required for fueling the late metamorphic climax or supporting their next life stage [1, 19–21]. Accordingly, the amphibian larvae are excellent models for investigating the resource allocation patterns and underlying mechanisms between storage and somatic growth. Additionally, these knowledges may also promote our understanding on life strategy of amphibians from an ecological perspective.

The liver of vertebrates plays a critical role in regulating somatic growth through both endocrine and metabolic approaches [22], and it has an additional function of fat storage in amphibians [19]. The metabolic pattern and the expression of critical regulation genes of lipid metabolism (e.g., peroxisome proliferator-activated receptors/PPARs) in the liver may give clues to the resource allocation mechanisms in amphibian tadpoles. *Rana omeimontis* tadpoles use their liver as the primary fat depot. Their hepatic energy storage is consumed rapidly during starvation and at the late metamorphic climax [19]. Fat accumulation in *R. omeimontis* tadpoles manifests obvious morphological change and size enlargement of the liver, and hepatocytes accumulated with fat are morphologically resemble the adipocytes, with enlarged intracellular lipid droplets and marginalized cell nuclei. Thus, the body fat index of *R. omeimontis* tadpoles can be evaluated expediently by the liver histological section, liver morphology, and hepato-somatic index (HSI). Their ontogenetic and food-driven variation of storage investment and somatic growth dynamics may provide critical clues to their ongoing resource allocation pattern.

In this study, the allocation trade-off between energy storage and somatic growth was investigated in *R. omeimontis* tadpoles. Interestingly, we observed a new resource allocation pattern, fat storage-depended somatic growth, of which the hepatic fat accumulation uncoupled the direct association between resource availability and somatic growth through lipid-based energy metabolism.

Materials And Methods

Animal and daily culture

Egg clutches of *R. omeimontis* were collected in October (in 2016, 2017, and 2018) at Anzihe Natural Reserve (E103.459885, N30.744614, 701 m) in Sichuan Province, China. These clutches (ranging from 400 to more than 1000 eggs) were hatched at 20 ± 0.5°C (water temperature, L: D = 12h: 12h) in aquatic containers (42 × 30 × 10 cm, water depth = 5 cm). Hatched larvae were fed with boiled chicken egg yolk (for the first two days) and spirulina powder (grinded in water, China National Salt Industry Corporation) once a day. Water was replaced daily. Tadpoles from the same clutch might be divided into several containers. Tadpoles cultured in the same container were defined as a population, and their developmental stages were identified according to the Gonser stages [23].

Observation on resource allocation pattern

Experiment 1 This experiment was conducted to observe the onset of ontogenetic fat accumulation in *R. omeimontis* tadpoles. Six tadpole populations (approximate 200 individuals for each) were fed with 0.15 g spirulina powder daily. Tadpoles were collected at 10, 14, 23, 32 and 43 days after hatching (d.a.h). At each timepoint, only relatively large individuals in a population were sampled, as small individuals might not grow well. In practice, 10-15 largest individuals were caught for each population, then 6-8 individuals with similar size were further screened by removing outlier one by one. After weighted for body mass and euthanized by MS-222, these tadpoles were dissected to observe their liver morphology or stored in 4% paraformaldehyde. Since a single liver was too small to measure accurately, 4-6 livers were weighted together and calculated for an average, and the corresponding body weight was also an average.

Experiment 2 This experiment was aimed to observe the liver and whole-body growth dynamics during the onset of ontogenetic fat accumulation at population level. Two tadpole populations (with 979 and 964, individuals, respectively) were chosen and fed with 0.7 g spirulina powder daily. Tadpoles were randomly collected (25-102 individuals for each timepoint and each population) at 10, 12, 14, 17, 22, 28, and 37 d.a.h to present the size structure at each timepoint. After anesthetized by MS-222, their body and liver mass were measured.

Experiment 3 This experiment was implemented to observe liver and whole-body growth dynamics in response to increased food abundance. Two tadpole populations (more than 900 individuals for each) were fed with 0.7 g spirulina powder daily until 95 d.a.h. Then, these populations were fed with sufficient spirulina powder to accelerate their growth. Tadpoles were collected randomly at -2, 0, 1, 3 and 5 days after sufficiently feeding (n = 47-64 for each population at each time point). After euthanized by MS-222, their body, tail, and liver mass were recorded.

Morphological observation on the liver

Variation of hepatic fat storage is accompanied by liver morphological change in *R. omeimontis* tadpoles (Figure S1). Liver lacking fat is in brown to black color. It looks like semitransparent gel-like tissue with

thick black pigment. Liver filled with fat is morphologically similar to adipose tissue in white to yellow color, without noticeable black pigment. Here, the liver morphology was used as a qualitative index to aid the identification of ontogenetic hepatic fat accumulation in *R. omeimontis* tadpoles.

Liver histological section

Liver samples were stored in 4% paraformaldehyde. After dehydration in a graded series of ethanol and transparency by xylene, livers were embedded in paraffin, and were sectioned in serial transverse sections (7 μm thick). Hematoxylin and eosin (H & E) stain was conducted [24].

Micro CT scanning

Samples fixed in 4% paraformaldehyde were stained in I_2 & KI water solution (1% & 2%, w/v) for 12 h. CT scan was conducted on Quantum GX Micro CT (PerkinElmer) with parameters as follow: scanning current, 70 eV; 10 μM; field of view: 36 × 36 mm for acquisition, 25 × 25 mm for reconstruction; scan duration, 15 min.

Liver transcriptome

Liver transcriptome before and after hepatic fat accumulation were compared. Tadpoles were collected from a population at 50 d.a.h. Livers before fat accumulation were collected from tadpoles at 40-60 mg, and proved by morphological traits. Livers after fat accumulation were collected from individuals at 80-125 mg. Three biological replicates were prepared for each group, and each replicate was prepared from 4-7 individuals. Total RNA extraction and cDNA library preparation was performed as described previously [25]. Sequencing were conducted on an Illumina HiSeq 4000 platform by Annoroad Gene Technology (Beijing, China), and paired-end reads were generated. Read filtering, assembly, annotation, and quantitation were also performed as described previously [25].

Whole blood metabolomics

Whole blood was collected from the heart and hepatic artery of tadpoles in stage 30-31 with 200-400 mg. Three blood samples/replicates were prepared, and each sample (approximate 25 μl) was prepared from 3 tadpoles. Equal volume methanol was added into the blood sample, followed by drying in oven at 60 ^{o}C. For each sample, 0.5 ml methanol: acetonitrile: water = 2:2:1 (v/v), followed by ultrasonication for 30 min × 2 and incubation at -20 ^{o}C for 1 h. After centrifugation at 13,000 for 15 min (4 ^{o}C), supernates were transferred into new tubes and freeze-dried. Samples were dissolved in 100 μL acetonitrile: water (1: 1, v/v) for analysis. Chromatographic analysis, metabolite identification and quantification were performed following the methods described previously [19]. The relative abundances/concentrations of metabolites were presented as the ion intensities of their molecular ion peaks (Supplementary material 1).

Drug treatment

Tadpoles with similar body mass were collected from the same population, and fed with sufficient spirulina powder for three days. Then, tadpoles were randomly divided into groups and kept in plastic containers (20 × 15 × 8 cm, with 600 ml water) containing DMSO (control), bezafibrate (BEZ, agonist of PPARβ; 55 μM), rosiglitazone (RSG, agonist of PPARγ; 40 μM), and pirinixic acid (PIR, agonist of PPARα; 60 μM), respectively (purchased from Selleck). Treatment was lasted for six days without food. Water and drugs were replaced every two days. High drug concentrations were maintained to offset the decreased drug intake due to the reduced swallowing behavior during food deprivation. Drug concentrations were selected according to the EC_{50} and cellular experiments [26-28].

To observe the effect of PIR on tadpole growth, tadpoles with similar body mass were treated with DMSO or PIR (2.5 and 5 μM), with the same condition mentioned above. Treatment was lasted for eight days, and food was provided sufficiently. Water and drugs were replaced every day.

Statistical analysis

HSI was calculated as the ratio of liver weight to body weight. To avoid the influence of intestine content on body weight, hepato-tail index (HTI) was also used to evaluate the relative size of liver. Basic statistical analyses were conducted using IBM SPSS v21.0 (IBM, Armonk, NY, USA). Curve-fitting was conducted on 1stOpt software (7D-Soft High Technology Inc, China). Distribution curve fitting was performed on normalmixEM (a function of R package mixtools), a Gaussian mixture model [29]. One-way ANOVA and Student-Newman-Keuls *post hoc* tests were conducted to analyzed the changes of whole-body mass, tail mass, liver mass, HSI and HTI with time, as well as the effects of drug treatment. DESeq (based on R) was used for identifying differently expressed genes (DEGs) in transcriptomes, with thresholds FDR < 0.05 (after Benjamini and Hochberg's correction). KEGG enrichment analyses were based on KOBAS 3.0 [30]. When focusing on the transcriptional pattern of a specific group of genes, independent sample T tests were conducted to compare the expression levels of individual genes. Graphs were created using Graphpad prism 5 or ggplot2, an R package [31].

Results

Ontogenetic fat accumulation and tadpole growth dynamics

In Experiment 1, tadpoles collected at 10, 14, 23, 32, and 43 d.a.h had body weight of 15-20, 20-30, 35-45, 70-90, 120-140 mg, respectively. At the first three timepoints, the livers of tadpoles presented as a brown gel-like tissue (Figure S2), and the relative growth of liver weight to body weight was low (Figure 1A). At 32 and 43 d.a.h, the livers presented as a yellow oil-like tissue (Figure S2), and it had grown remarkably in weight (Figure 1A). The fitting-curve between liver and whole-body weight indicated a dramatic increment of the relative liver growth rate (Figure 1A). To determine the initial timepoint of these changes, tadpoles ranging from 35 to 50 mg were collected at 32 d.a.h. These tadpoles had yellow oil-like liver (Figure S2), whose size was significantly larger than the liver of tadpoles collected at 23 d.a.h (Figure 1A-B) due to the enlarged hepatocyte and intracellular lipid droplets (Figure 1C) [19].

These phenomena were also observed at population level (Experiment 2). Tadpoles were randomly collected at 10, 12, 14, 17, 22, 28, and 37 d.a.h. The relation between their liver weight and body weight was studied by linear fitting and piecewise linear fitting (Figure 2A; detailed in Figure S3). The performance (R^2 value) of curve fitting models suggested an inflexion in the fitting curve of "liver weight *vs* body weight" at body weight of 30-40 mg. Correspondingly, tadpoles smaller than 30 mg always had brown gel-like livers, while those larger than 40 mg had yellow oil-like livers. The individual size variation increased with time for both populations, and their size structures shifted from normal to positive skewed or bimodal distributions gradually (Figure 2B). The non-normality of their distribution pattern could be explained by two mixed normal distributions (Gaussian mixture model; detailed in Figure S4). One of the normal distributions kept its peak within 30-40 mg, while the other one showed increased average value and relative proportion with time (Figure S4). Positive correlations ($p < 0.01$) between the body weight and HSI existed in tadpoles collected from the same populations (Figure 2C), as well as in tadpoles collected from the same population at each timepoint if their average body mass were larger than 35 mg (Figure 2D).

Food-driven fat accumulation and growth dynamics

In Experiment 3, the hepatic fat accumulation and somatic growth of two tadpole populations were observed at -2, 0, 1, 3 and 5 days after increasing the feeding intensity. Significant increment in the liver weight, HSI and HTI (one-way ANOVA, $p < 0.05$) was observed within 1-3 days after enhanced feeding, while significant increment in whole body and tail weight required at least five days (Figure 3).

Metabolic pattern after onset of ontogenetic fat accumulation

There were significant transcriptional variations between livers before and after ontogenetic fat accumulation (Figure S5 A-D; Figure 4D). The upregulated DEGs after fat accumulation were mainly enriched in cell proliferation (i.e., cell cycle and DNA replication) and lipid metabolism pathways (e.g., PPAR signaling pathway and fatty acid/FFA biosynthesis) (Figure S5 E), while those downregulated ones showed no significant enrichment ($q < 0.01$). Fat accumulation was associated with an overall upregulation of ribosomal components, aminoacyl-tRNA synthases, DNA replication components, and aerobic energy production (Figure S5 F-I). These "fatty" livers also had upregulated transcription of enzymes of FFA synthesis, fat storage, FFA elongation and desaturation, FFA activation, FFA beta oxidation, and ketogenesis (Figure 4A), which covered both lipid anabolism and catabolism. Meanwhile, the transcription of enzymes involved in amino acid catabolism (e.g., aminotransferases and arginase) and glycolysis (i.e., G6PI and G3PDH) were downregulated after fat accumulation, while three enzymes of pentose phosphate pathways (G6PDH, RPI, and UlaE) were upregulated (Figure 4B-C). In tadpoles after ontogenetic fat accumulation, their relative abundance of carbohydrate and unsaturated FFAs was much lower in the blood than in the liver, and their relative abundance of stearic acid and palmitic acid was much higher in the blood and tail than in the liver (Figure 4D & S6). The glucose level in these tadpoles were only 19.9 ± 4.8 mg/dl.

Hepatic fat accumulation was associated with transcriptional activation of three nuclear receptor factors (PPARα, PPARγ, and steroid hormone receptor ERR1) in the liver (Figure S7 A; $p < 0.05$, fold change > 2). Starved tadpoles showed accelerated hepatic storage mobilization when treated with PIR, but not for BEZ and RSG (Figure 4E & Figure S7 B-C; $p < 0.05$, one-way ANOVA). Fed tadpoles showed increased growth rate when treated with 5 μM PIR (Figure 4F; $p < 0.05$, one-way ANOVA).

Discussion

Resource allocation patterns in tadpoles

Fat storage of *R. omeimontis* tadpoles was not evenly accumulated with the growing of their body size, even the environmental food abundance was kept at constant level (Figure 1A & 2A). Instead, fat accumulation in these tadpoles initially emerged when they grew to a certain body weight range (e.g., 35-50 mg in experiment 1 & 30-40 mg in experiment 2; Figure 1). This ontogenetic shift in energy allocation resulted in an increased slope of the "liver weight *vs* body weight" curve (Figure 1A & 2A). From the perspective of population size structure, the maintenance of a distribution peak at 30-40 mg suggested retarded body growth for tadpoles at this body weight range (Figure 2B and S4). Taken together, these results suggested storage had high priority in resource allocation during the onset of ontogenetic fat accumulation. Meanwhile, tadpoles growing beyond this range likely had increased growth rate, as suggested by the forming of positive skewness in population size structure and the increased size variation between individuals (Figure 2B and S4). Since food was unchanged and distributed homogeneously in our study [32], the differentiation of growth rate between individuals was likely due to increased investment to somatic growth after initial ontogenetic fat accumulation. However, we also observed a positive correlation between HSI (body fat index) and body weight (range from 40 to 400 mg; Figure 2C-D & Figure 3). It suggested that larger individuals also kept higher hepatic fat storage. How did large individuals keep their size and storage superiority simultaneously? Although the investment to storage might decrease with the accumulation of fat storage, it was possible that the allocation proportion to storage was kept higher than their current body fat index (Figure S8). This allocation pattern could be explained by assuming a positive relationship between storage abundance and the investment to somatic growth.

This speculation was supported by the observations on resource allocation pattern in *Rana omeimontis* tadpoles provided with increased food. *Rana omeimontis* tadpoles can grow up (e.g., to 400 mg) with different HSI under different feeding intensity [19]. It suggested that it was unnecessary for these tadpoles to accumulate their body fat to a certain value before further growth in size. Since so, why a positive correlation existed between HSI and body weight? Food abundance is the major determinator of the growth rate of *R. omeimontis* tadpoles. In response to higher food abundance, liver growth preceded over somatic growth, and significant somatic growth was observed at least after five days of sufficiently feeding (Figure 3). It implied that a higher level of storage abundance was the precondition for increased investment to somatic growth.

Potential metabolic mechanisms underlying the allocation strategy

After ontogenetic fat accumulation, the liver of *R. omeimontis* tadpole possessed higher transcriptional level of genes involved in protein synthesis, DNA replication, and energy production (Figure S5 F-I), in consistent with its rapid increment in body size. These fatty livers showed simultaneous activation of lipogenic and lipid catabolic processes (Figure 4A). It suggested that the fat storage in the liver of *R. omeimontis* tadpoles was in a robust turnover, which was also supported by the transcriptional upregulation of PPARγ and PPARα (Figure S7 A), the key players in regulating hepatic lipogenesis and fat mobilization, respectively [33-37]. After fat accumulation, the hepatic carbohydrate flux was diverted from glycolysis to pentose phosphate pathways (Figure 4C), suggesting reduced contribution of carbohydrate to energy production. Similarly, the amino acid catabolism was also downregulated at transcriptional level (Figure 4B). These results suggested a metabolic switch from carbohydrate and amino acid to lipid to support the increased energy requirement in the liver.

Moreover, the liver and the blood had distinct profiles of metabolic substrates (Figure 4D). The high relative abundance of stearic acid and palmitic acid to carbohydrates in the blood suggested that lipids were likely exported from the liver preferentially. This was supported by the high abundance of stearic acid and palmitic acid in the tail. Early studies have reported that amphibians keep remarkably lower level of blood sugar (13.5 to 35 mg/dl) than other vertebrates (40 to more than 200 mg/dl) [38, 39], as they likely use alternate substrate as metabolic fuel [40]. For *R. omeimontis* tadpoles, their blood glucose level was as low as 19.9 ± 4.8 mg/dl, it is likely that the lipids were the major circulating metabolic fuel in these tadpoles, at least, after the onset of ontogenetic fat accumulation.

Activation of PPARα in fat depots was responsible for the fat mobilization as fuel in animals [41, 42]. Correspondingly, starved *R. omeimontis* tadpoles showed accelerated clearance of their hepatic fat when treated with PIR, an agonist of PPARα (Figure 4E & S4 B-C). When food was provided, PIR treatment promoted the growth of *R. omeimontis* tadpoles (Figure 4F). It indicated an association between circulating fat level and the somatic growth rate. This was reasonable, as the circulating nutrient level, along with the availability of anabolic substrates, were fundamental determinators of tissue growth [22, 43, 44]. Interestingly, hepatic fat accumulation could stimulate lipid mobilization [36], and the endogenic agonists of PPARα in the liver were mainly produced during de novo lipogenesis [45, 46]. It meant that a higher circulating lipid level could be expected in *R. omeimontis* tadpoles with more hepatic fat, and thus explained the resource allocation pattern in these tadpoles.

Accordingly, the dual roles of lipid in storage and circulating fuel in *R. omeimontis* tadpoles potentially constituted the metabolic basis for their resource allocation (Figure 5). Acquired resource might flow into anabolic substrates for macromolecule synthesis or fat storage. The equilibrium between substrate availability and energy level determined the resource allocation between somatic growth and energy storage. A higher fat abundance was required to support a higher level of systematic energy that required for more efficient transformation of resource to biomass. Overall, fat accumulation uncoupled the association between resource abundance and somatic growth from the aspect of energy supply, and

perhaps some types of anabolic components (e.g., sterol and phospholipid) (Figure 5). Consequently, tadpoles would not reach their maximal growth rate allowed by the nutrient level before adequate fat accumulation. This model should also be applicable to wild *R. omeimontis* tadpoles, as the tadpoles of wood frog mainly feed on algae in the field [47, 48]. This diet is not rich in lipids, and lipogenesis in their liver is likely a major resource of their circulating lipids.

Ecological significance of the resource allocation pattern

Storage-dependent somatic growth may be a proper selection for animal to coordinate the requirements of growth and storage. The energy storage has some priority of resource allocation. It enhances the capacity of tadpoles for surviving starvation during winter. More importantly, to maximize the overall fitness, the timing and body size of landing are highly flexible to environmental conditions in amphibians [16]. Such a developmental plasticity requires advanced preparation of storage for the non-feeding metamorphic climax [21, 49]. The priority of storage in resource allocation may be a physiological basis for their life cycle. Meanwhile, the priority of storage in resource allocation does not constitute a limitation to body growth. At environment rich in nutrition, tadpoles likely reach their metamorphic climax with abundant fat accumulation, which can improve the performance of juveniles [1, 50]. At environment deficient of nutrition, although their absolute growth rate was low, tadpoles can reach their maximal investment proportion to somatic growth at low storage abundance. It meant that more resource has been invested to somatic growth, which is beneficial to improve their capacity of resource acquirement.

At population level, the resource allocation strategy in *R. omeimontis* tadpoles can be a significant contributor to their within-cohort size variation, especially in the environment with low food abundance. Size variation may be beneficial to the survival and existence of the populations. For example, the winter mortality is always selectively against smaller individuals [51, 52]. Small within-cohort size variation may not allow any individuals to grow to the body size required for winter survival in environments with limited resources. Besides, variation in body size allows some individuals to reach their metamorphosis and niche shift more early, and thus reduce the overall intraspecies competition pressure [53, 54]. It is worthy to note that the amplification of size variation and the skewness of population body size structure has long been considered as a basic ecological phenomenon in growing tadpole populations [54]. Our results provided a new explanation for the origin of this phenomenon.

Conclusion

In this study, we revealed the resource allocation pattern between somatic growth and energy storage in *R. omeimontis* tadpoles. In the tadpoles, energy storage had the priority of resource allocation, as hepatic fat accumulation likely uncoupled the direct association between resource availability and somatic growth through lipid-based energy supply. Further investigation may focus on: (1) the hormone regulation of onset of genetic hepatic fat accumulation (e.g., the upstream signal of PPARs) and the accompanied metabolic reorganization; (2) the influence of environmental factors on ontogenetic fat accumulation;

and (3) the potential crosstalk between hepatic lipid metabolism and growth hormone-insulin-like growth factors axis in regulating somatic growth.

Declarations

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Availability of data and materials

The sequencing data from this study have been submitted to the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE147675. Other data are available from https://figshare.com/s/a023d8f0dba7b30be4de.

Authors' contributions

WZ, JJ and BW conceived and designed the study; WZ and LC performed the experiments, analyzed the data, and wrote the manuscript; GS assisted to performed the experiments; All authors helped to revise the manuscripts and approved the final manuscripts.

Ethics approval and consent to participate

All animal capture and handling protocols were approved by The Animal Care and Use Committee of Chengdu Institute of Biology (Permit No: CIBACUC20162110).

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Figures

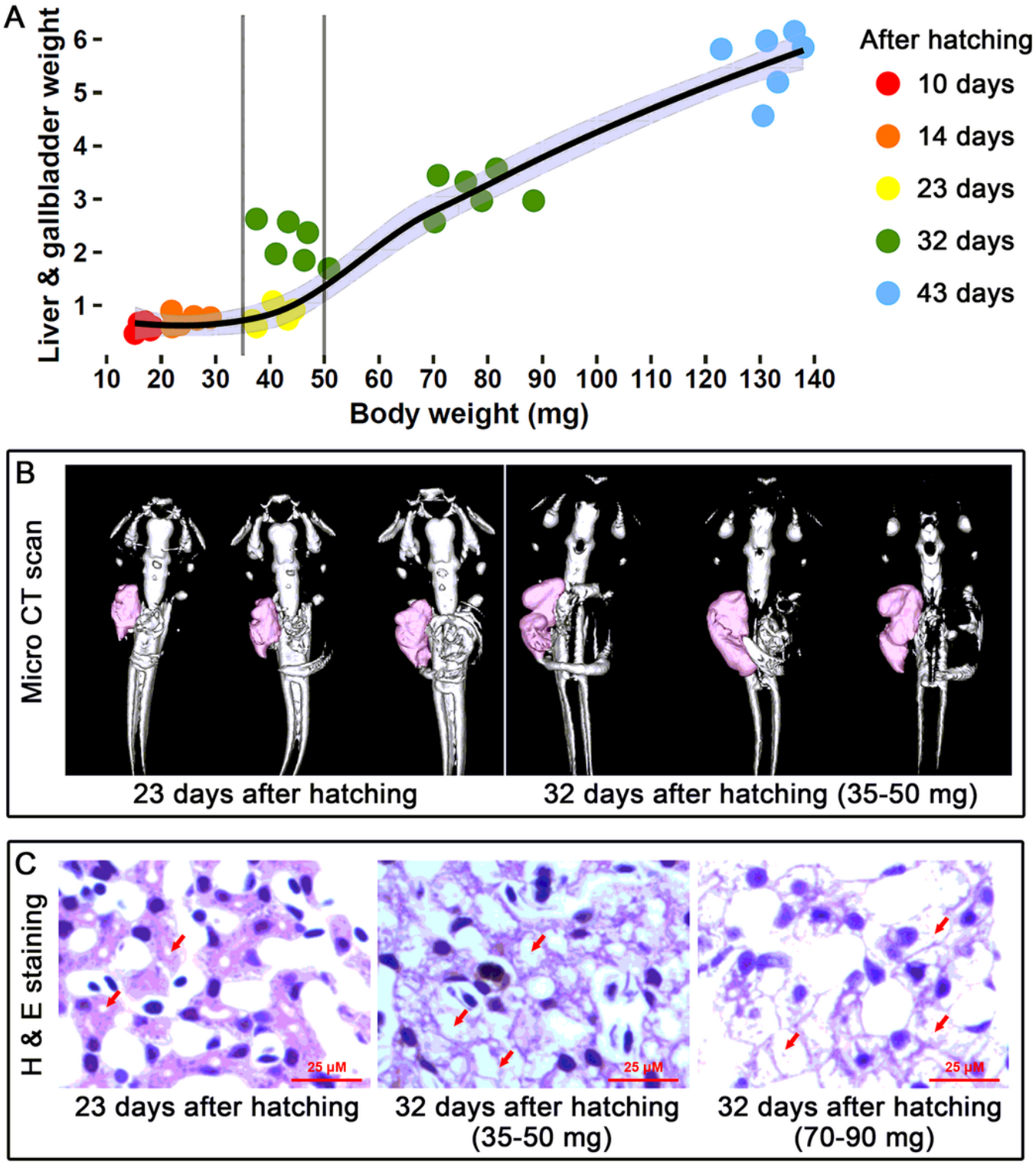

Figure 1

Ontogenetic hepatic fat accumulation in R. omeimontis tadpoles. (A) Variation of liver & gallbladder weight with whole-body weight in growing tadpoles. (B) CT images presenting the relative liver size. (C) Liver histological morphology (H & E stain). Red arrows indicate intracellular vacuoles, which are lipid droplets [19].

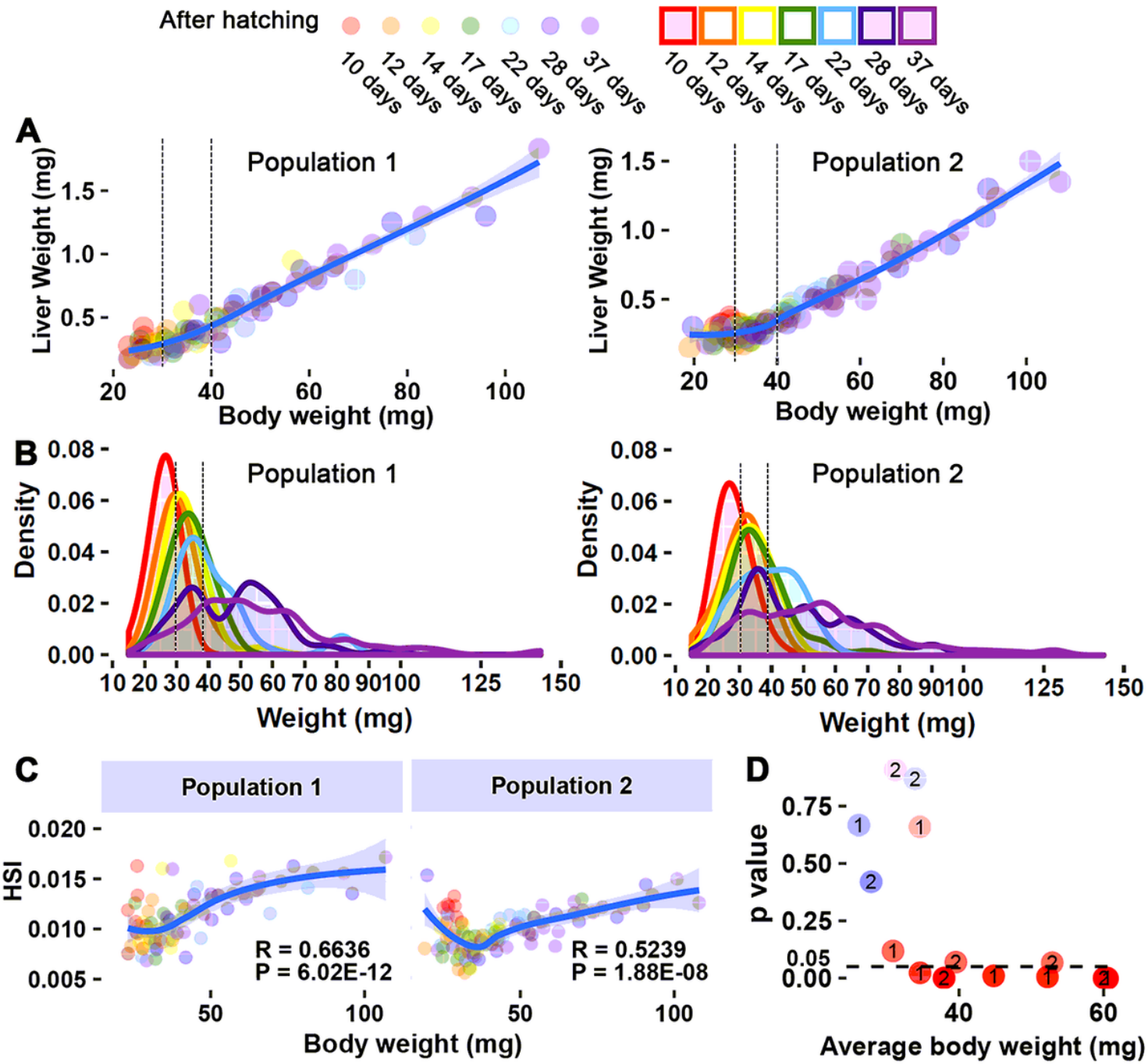

Figure 2

Growth dynamics of liver and whole-body at population level. (A) Variation of liver weight with whole-body weight in growing tadpoles. Dashed vertical lines denote the body weight ranges for the inflexions. (B) Variation of population size structure with time. (C-D) Relationship between HSI and body weight in tadpoles from the same population across the timepoints (C), or at each timepoint (D); different populations were denoted by numeric numbers.

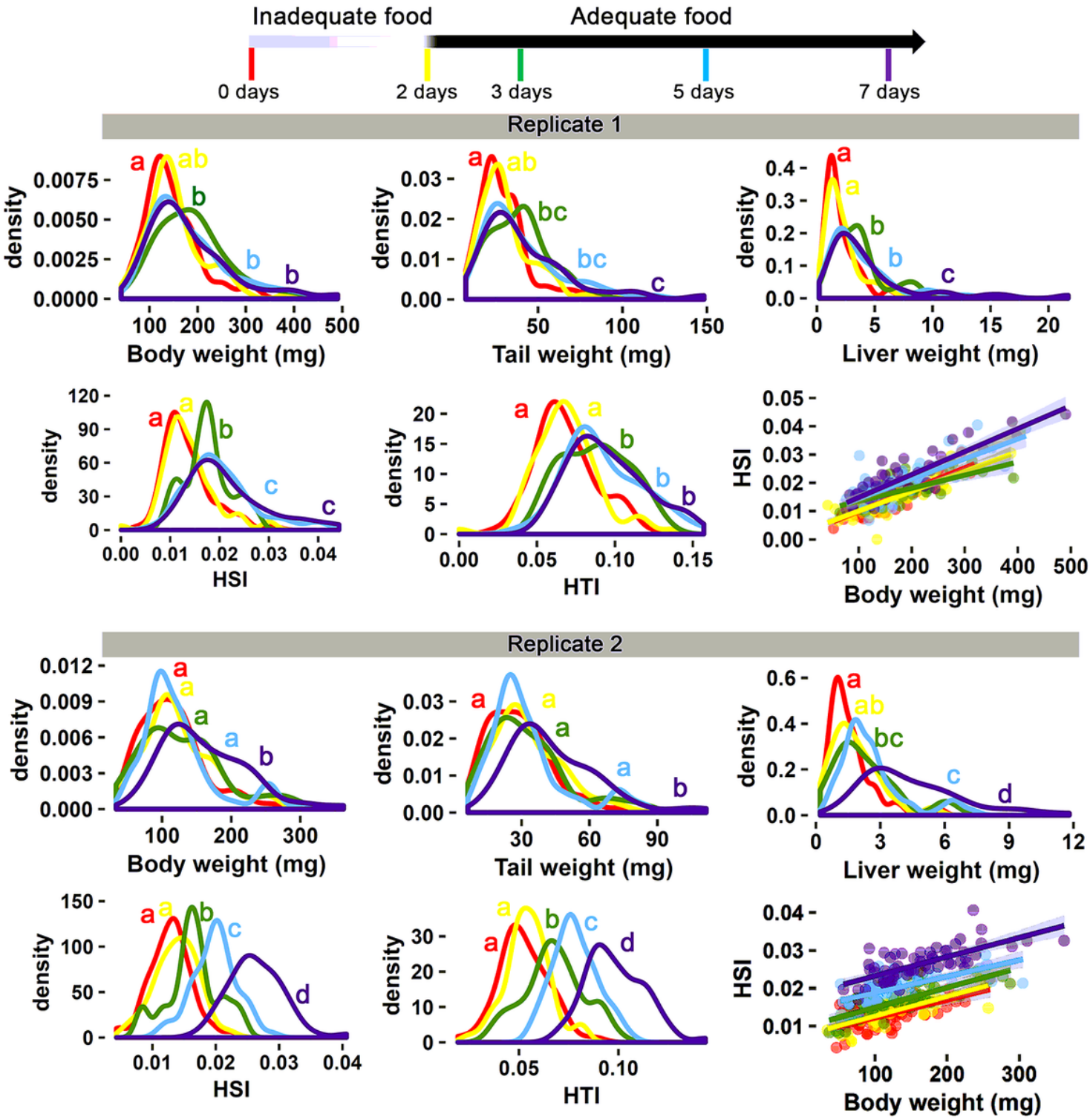

Figure 3

Growth dynamics of liver and whole-body in response to increased food abundance. Different letters (i.e., a-d) indicate significant differences between groups ($p < 0.05$), as shown by the Student Newman Keuls post hoc test after one-way ANOVA.

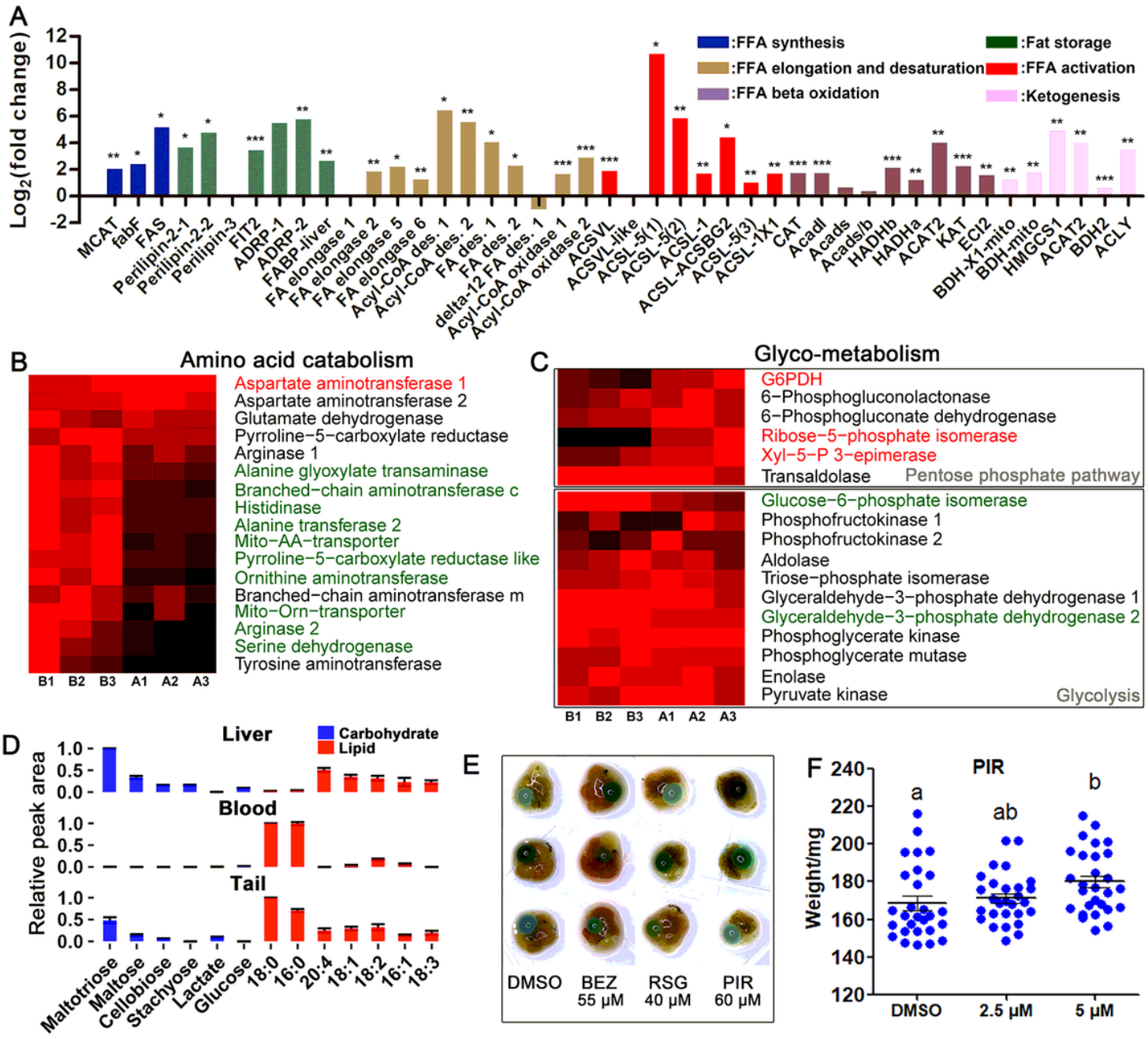

Figure 4

Metabolic pattern after ontogenetic fat accumulation. (A-C) Transcriptional changes of enzymes in lipid metabolism (A), amino acid catabolism (B), and glycol-metabolism (C) after fat accumulation. Annotations in red, green, and black colors denote increased, decreased, and unchanged, respectively, after fat accumulation. (D) Relative abundances of major lipids and carbohydrates in the liver, blood, and tail of tadpoles after ontogenetic fat accumulation (n = 3 for the blood; n = 7 for the liver and tail). The liver and tail data were adopted from our previously published metabolomes [19]. (E) The effect of PPAR agonists on hepatic fat mobilization in starved tadpoles. (F) The effect of PPARα agonist on the growth of fed tadpoles. Different letters indicate significant differences between groups ($p < 0.05$), as shown by the Student Newman Keuls post hoc test after one-way ANOVA. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ (T test).

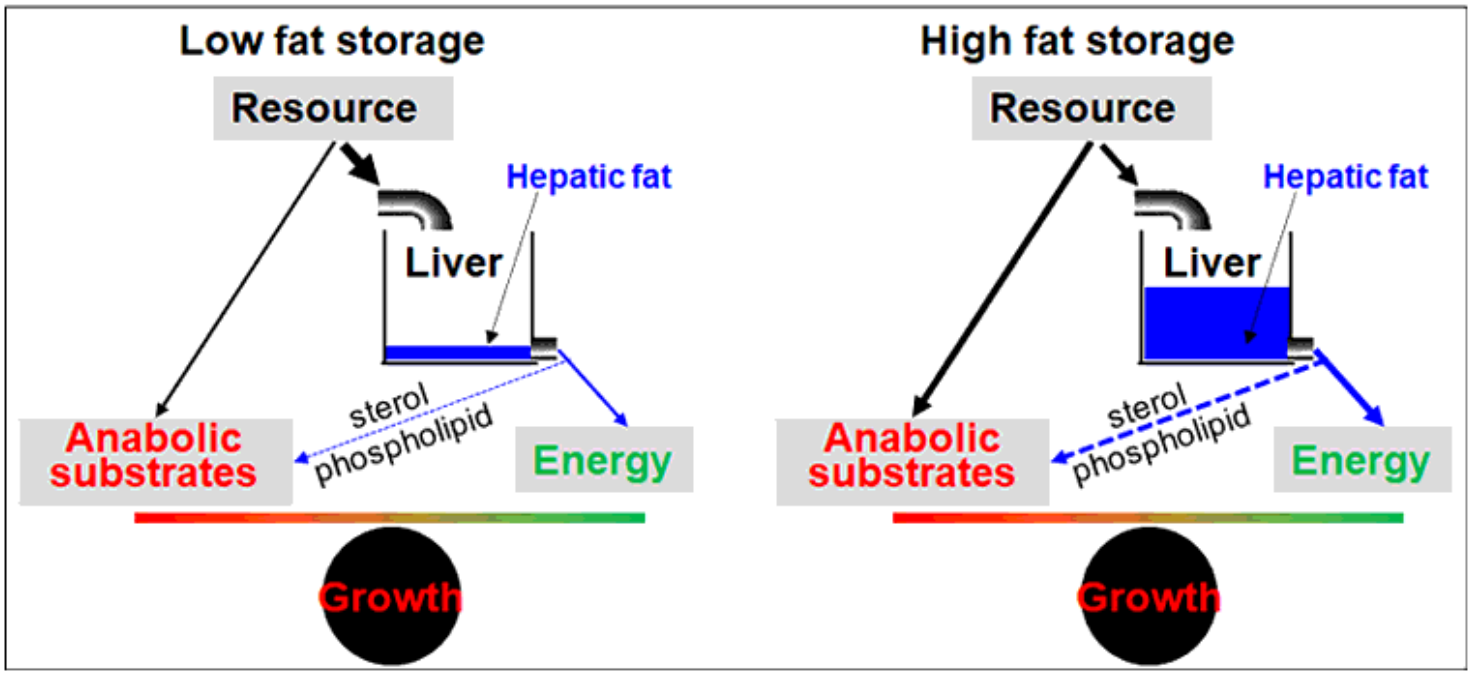

Figure 5

Deduced schematic diagram illustrating the resource allocation patterns and mechanisms in R. omeimontis tadpoles.

Supplementary Files

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