British Journal of Nutrition (2021), 126, 695–707 doi:10.1017/S0007114520004389
© The Author(s), 2020. Published by Cambridge University Press on behalf of The Nutrition Society

Effects of starvation and refeeding on growth performance, appetite, growth hormone–insulin-like growth factor axis levels and digestive function of *Acipenser dabryanus*

Xiao-yun Wu, Ye-yu Chen, Jian-sheng Lai, Ya Liu, Ming-jiang Song, Quan Gong* and Zhi-hai Long*

The Fishery Institute, The Sichuan Academy of Agricultural Sciences, Chengdu, 611730, People's Republic of China

(Submitted 17 June 2020 – Final revision received 28 October 2020 – Accepted 28 October 2020 – First published online 4 November 2020)

Abstract

The aim of this study was to explore the effects and mechanisms of different starvation treatments on the compensatory growth of *Acipenser dabryanus*. A total of 120 fish (60·532 (SEM 0·284) g) were randomly assigned to four groups (fasting 0, 3, 7 or 14 d and then refed for 14 d). During fasting, middle body weight decreased significantly with prolonged starvation. The whole-body and muscle composition, serum biochemical indexes, visceral indexes and digestive enzyme activities had been effected with varying degrees of changes. The growth hormone (GH) level in serum was significantly increased in 14D; however, insulin-like growth factor-1 (IGF-1) showed the opposite trend. The neuropeptide Y (*npy*) mRNA level in brain was significantly improved in 7D; peptide YY (*pyy*) mRNA level in intestine was significantly decreased during fasting. After refeeding, the final body weight, percentage weight gain, specific growth rate, feed intake, feed efficiency and protein efficiency ratio showed no difference between 0D and 3D. The changes of whole-body and muscle composition, serum biochemical indexes, visceral indexes and digestive enzyme activities had taken place in varying degrees. GH levels in 3D and 7D were significantly higher than those in the 0D; the IGF-1 content decreased significantly during refeeding. There was no significant difference in *npy* and *pyy* mRNA levels. These results indicated that short-term fasting followed by refeeding resulted in full compensation and the physiological and biochemical effects on *A. dabryanus* were the lowest after 3 d of starvation and 14 d of refeeding. Additionally, compensation in *A. dabryanus* may be mediated by appetite genes and GH, and the degree of compensation is also affected by the duration of starvation.

Key words: ***Acipenser dabryanus*: Compensatory growth: Appetite: Growth hormone–insulin-like growth factors**

In the natural environment, seasonal variations in food supply cause many species of fish to endure periods of starvation[1]. During food shortages and nutritional deficiency, fish may experience growth stagnation or even negative growth. However, when the food supply is adequate, the weight increases to different degrees and this phenomenon is called compensatory growth (CG). According to the degree of growth, CG can be divided into over, full, partial and no compensation (Fig. 1). CG is a special survival strategy adopted by animals to adapt to changes in the natural environment and has been extensively studied in many fish species, such as rainbow trout (*Oncorhynchus mykiss*)[2,3], Siberian sturgeon (*Acipenser baerii*)[4], channel catfish (*Ictalurus punctatus*)[5], Amur sturgeon (*Acipenser schrenckii*)[6], Persian sturgeon (*Acipenser persicus*)[7], hybrid striped bass (*Morone chrysops* ×*M. saxtilis*)[1], black sea bream (*Acanthopagrus schlegelii*)[8] and Dabry's sturgeon (*Acipenser dabryanus*)[9]. However, the mechanism of weight change in *A. dabryanus* is not clear.

Starvation and refeeding constitute an important experimental model to study CG in fish. The proper mode of the model can achieve full compensation or even overcompensation[4,6,10]. Most people believe that CG in fish is achieved mainly by increasing feed intake (FI) or improving feed utilisation[8,11], and it was reported that the appetite of fish significantly increased when feeding was resumed after starvation[8,12]. In fish, as in all vertebrates, the regulation of food intake is mediated by signals from neuropeptides via the central nervous system, which receive input regarding metabolic status and changes in energy homoeostasis as well as hunger and satiety signals from the digestive tract[13,14]. Neuropeptide Y (npy) plays an

Abbreviations: 0D, 0 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CG, compensatory growth; CHOL, cholesterol; FE, feed efficiency; FI, feed intake; GH, growth hormone; GLO, globulin; IGF, insulin-like growth factor; npy, neuropeptide Y; pyy, peptide YY; TP, total protein.

* **Corresponding authors:** Quan Gong, fax +86 28 87955508, email admiral671@163.com; Zhi-hai Long, email scyblzh@163.com

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

British Journal of Nutrition

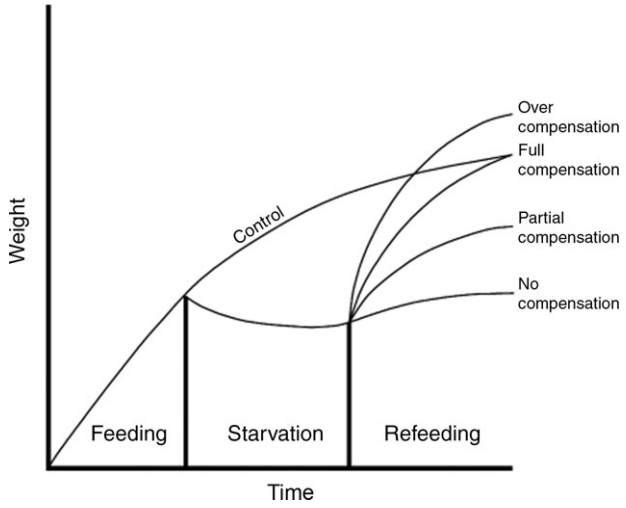

Fig. 1. Idealised patterns of growth compensation.

important role in the neural regulation of food intake in fish and acts as an orexigenic factor[15]. Peptide YY (pyy) has been shown to inhibit FI in goldfish (*Carassius auratus*)[16]. As reported in yellow catfish (*Pelteobagrus fulvidraco*)[17], grass carp (*Ctenopharyngodon idellus*)[18] and red-bellied piranha (*Pygocentrus nattereri*)[19], npy and pyy play major roles in the regulation of feeding and the expression of *npy* and *pyy* is changed by starvation and refeeding. Whether there are corresponding changes in *A. dabryanus* remains to be studied.

The growth hormone (GH) and insulin-like growth factor (IGF) axis plays a pivotal role in the neuroendocrine regulation of vertebrate growth[20]. A study in juvenile Atlantic salmon (*Salmo salar*)[21] showed that GH, IGF-1 and IGF-2 were regulated by nutritional regulation, which may indicate that changes in body weight are affected by fluctuations in GH–IGF. Whether this phenomenon is regulated by GH–IGF in the CG of *A. dabryanus* has not been reported.

Food deprivation and refeeding of fish cause changes in weight, body composition, organ mass, blood metabolites, etc.[7]. These aspects have been investigated in many fish to explore how physiological and structural responses mobilise to adapt to metabolism during fasting and refeeding[8,22–24]. The utilisation of food in fish is closely related to digestive capacity. The development of digestive tract tissues and the activity of digestive enzymes are important indicators of fish digestive capacity. In channel catfish[25], the visceral indexes are significantly decreased in fish during fasting, and after refeeding, the hepatosomatic values were recovered in Persian sturgeon[7]. These indicators are widely used in the study of nutrients to reflect the utilisation efficiency of fish feed and may be used to help us explore the mechanism of CG of *A. dabryanus*.

As a first-class protected animal in China, *A. dabryanus* shows extremely reduced numbers due to overfishing and water pollution. Therefore, the survival state of *A. dabryanus* in the wild is of great concern. Our purpose in this study was to simulate the effects of wild food shortage on the physiological and biochemical characteristics of *A. dabryanus* through starvation and to explore the possible mechanism of CG of *A. dabryanus* through a 2-week refeeding strategy.

Table 1. Nutrient content of diets

Ingredients	Content (%)
Amino acids	
Aspartic acid	3·87
Threonine	1·52
Serine	1·75
Glutamic acid	6·37
Glycine	2·20
Alanine	2·32
Valine	2·19
Methionine	0·68
Isoleucine	1·58
Leucine	3·42
Tyrosine	1·28
Phenylalanine	1·97
Lysine	2·48
Hlstidine	1·17
Arginine	2·41
Proline	2·12
Total amino acids	37·33
Nutrient content	
Crude protein	41·31
Crude lipid	10·00
Ash	13·51
Moisture	9·14

Materials and methods

Experimental design

The F2 generation of *A. dabryanus* (initial weight 60·532 (SEM 0·284) g), reproduced by The Fishery Institute of the Sichuan Academy of Agricultural Sciences, was used. Before the trial, the fish were acclimatised to the experimental conditions for 2 weeks. The trial lasted 28 d, including 14 d of refeeding after all fasting treatments. A total of 120 fish were randomly divided into four groups, and each group had three tanks (diameter of 1·5 m and water depth of 0·8 m): 0D: control group, 0 d of fasting and 14 d of refeeding; 3D: 3 d of fasting and 14 d of refeeding; 7D: 7 d of fasting and 14 d of refeeding and 14D: 14 d of fasting and 14 d of refeeding. The commercial feed contained 41·31 % protein, 10 % lipid, 9·14 % moisture and 13·51 % ash (Table 1). The fish were fed twice a day (08.00 and 18.00 hours). Uneaten feed was collected 30 min after feeding, dried and weighed to calculate FI. The water temperature was 18·5 (SEM 2)°C, dissolved oxygen content was 6·24 (SEM 0·53) mg/l, pH was 7·0 (SEM 0·5), NH_4^+-N content was 0·066 (SEM 0·003) mg/l and NO_2-N content was 0·001 (SEM 0·000) mg/l. The feeding trial was completed under a natural light and dark cycle. All experimental protocols were approved by the Animal Care Advisory Committee of the Sichuan Academy of Agricultural Sciences (20180929001A).

Sample collection

At the end of fasting and 14-d refeeding, the fish were anaesthetised by 3-aminobenzoic acid ethyl ester methanesulphonate (MS-222, 60 mg/l) and then counted, weighed and sampled (the samples of the control group were selected before and after refeeding). Three fish were randomly collected from each group (one fish per tank) and were stored at −20°C for the determination of body composition. Blood was collected from the tail vein

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

of three fish from each tank with a 2·5 ml syringe and placed in a centrifuge tube, which was placed at 4°C for 12 h. Then, the brain, muscle, liver, stomach and intestine were sampled for determination of muscle composition, visceral indexes, enzyme activity and quantitative tests, and the samples were stored at −80°C.

Biochemical analysis

The proximate compositions of the diets, fish carcass and muscle were analysed using standard methods of the Association of Official Analytical Chemists (Horwitz, 2000)[26]. Moisture was determined by drying the sample (105°C for 24 h) to a constant weight. The method of Kjeldahl nitrogen determination and Soxhlet extraction is used to measure crude protein and crude lipid, respectively. Ash was determined by incinerating the dried sample at 550°C for 12 h. The blood was centrifuged at 3000 rpm for 10 min, and the supernatant was collected and stored at −80°C for testing. The alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and the concentrations of total protein (TP), albumin (ALB), globulin (GLO), cholesterol (CHOL) and TAG in serum were analysed by a CL-8000 clinical chemistry analyser (Shimadzu). The GH, IGF-1 and IGF-2 levels were analysed by using fish ELISA kits (the Nanjing Jiancheng Bioengineering Research Institute), which were used in competitive method to detect the content of GH, IGF-1 and IGF-2 in samples. Stomach and intestine samples were each homogenised in ten volumes (w/v) of ice-cold physiological saline solution and centrifuged at 6000 ***g*** for 20 min at 4°C. The supernatant was collected for enzyme activity analysis. The protein content of all the tissues was determined by the Bradford method. The pepsin, trypsin, chymotrypsin, lipase and amylase activities were assayed as described by Jiang *et al.*[27].

RNA extraction and quantitative real-time PCR

The procedures of RNA isolation, reverse transcription and quantitative real-time PCR were similar to those previously described by Jiang *et al.*[28]. Total RNA was extracted from the brain and intestine using the RNAiso Plus Kit (TaKaRa) according to the manufacturer's instructions followed by DNAse I treatment. Samples were subjected to electrophoresis on 1 % agarose gels to confirm the integrity of the 28S and 18S rRNA bands, and RNA purity was assessed by the 260/280 nm absorbance ratio. Subsequently, 1 μg of total RNA was used to synthesise cDNA using the PrimeScript™ RT Reagent Kit (TaKaRa) according to the manufacturer's instructions. Specific primers for *npy* (in brain), *pyy* (in intestine) and *β-actin* were designed according to the unigene sequence obtained from transcriptome sequencing of *A. dabryanus* in our laboratory. All of the real-time PCR analyses were performed in a Bio-Rad CFX Connect System (Bio-Rad) (Table 2). The PCR mixture consisted of 2 μl of the first strand cDNA sample, 1 μl each of forward and reverse primers from 20 μM stocks, 6 μl of RNase-free dH_2O and 10 μl of 2× TB Green *Premix Ex Taq*II (TakaRa). The cycling conditions were 98°C for 2 min, followed by forty cycles of 98°C for 5 s, annealing at a different temperature (Table 2) for each gene for 10 s, and 72°C for 15 s. Melting curve analysis was performed following each real-time quantitative PCR assay to check and verify the specificity and purity of all PCR products. Each sample was amplified in triplicate. The target gene mRNA concentration was normalised to the mRNA concentration of the reference gene *β-actin*; for detailed methods, refer to Jiang *et al.*[28]. The target and housekeeping gene amplification efficiencies were calculated according to the specific gene standard curves generated from 10-fold serial dilutions. The expression results were calculated using the $2^{-\Delta\Delta CT}$ method after verification that the primers were amplified with an efficiency of approximately 100 %.

Table 2. Primer sequence, optimal annealing temperatures (OAT) of genes selected for analysis by real-time PCR

Name	Sequence (5′-3′)	OAT (°C)
npy-QF	TTGTGGCTGGCTACCGTG	60·4
npy-QR	GACCTCTTCCCATACCTCTGC	
pyy-QF	GCCTGAAGCCCTGGAGTGT	61·0
pyy-QR	GTGATGAGGTTGATGTAGTGCC	
β-actin-QF	GACCGAGGCACCCCTGAAC	54·9
β-actin-QR	GATGGGCACTGTGTGTGTGAC	

npy, neuropeptide y; *pyy*, peptide yy.

Statistical analysis

All data are presented as mean values with their standard errors. All data were subjected to a one-way ANOVA by Tukey's multiple range tests to determine significant differences among the treatments at $P < 0{\cdot}05$ with SPSS Statistics version 19.0 (SPSS Inc.).

Results

Growth performance, whole-body protein composition and protein retention value

The effects of fasting and refeeding on the growth parameters of *A. dabryanus* are given in Table 3. The middle body weight was higher in 0D than in the other groups ($P < 0{\cdot}05$). Percentage weight gain, specific growth rate, feed efficiency (FE) and protein efficiency ratio were highest in 0D and 3D, thereafter decreased ($P < 0{\cdot}05$). Final body weight and FI were the lowest in 14D, and there was no significant difference between 0D and 3D. The whole-body composition and correlation indexes during the fasting phase are displayed in Table 4. During fasting, the moisture content significantly improved from 0D to 14D. The protein content in 14D was the lowest among all the groups ($P < 0{\cdot}05$), and there was no difference among the other groups. The lipid content was highest in 0D and then significantly decreased. No significant difference was found in ash content. The whole-body composition and correlation indexes after the refeeding phase are displayed in Table 5. After 14 d of refeeding, the moisture content was minimal for fish in 0D ($P < 0{\cdot}05$) and there was no difference among the other groups. The protein content was significantly lower in 14D than in 0D and was not significantly different from that in the other groups. The maximum values of lipids, protein production value and lipid production value were observed in 0D, gradually declining thereafter ($P < 0{\cdot}5$). The opposite trend was observed for the ash and ash production value levels.

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

Table 3. Initial body weight (IBW, g/fish), middle body weight (MBW, g/fish), final body weight (FBW, g/fish), percentage weight gain (PWG), specific growth rate (SGR, %), feed intake (FI, g/fish), feed efficiency (FE, %) and protein efficiency ratio (PER) of *Acipenser dabryanus* during fasting and refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IBW	60·377	0·109	60·430	0·205	60·740	0·049	60·580	0·227
MBW	60·377^{c}	0·109	56·787^{b}	0·044	57·069^{b}	0·141	52·213^{a}	0·482
FBW	93·263^{c}	1·933	90·047^{c}	1·997	74·930^{b}	0·650	64·457^{a}	1·393
PWG†	54·478^{b}	3·408	58·566^{b}	3·454	31·304^{a}	1·455	23·493^{a}	3·350
SGR‡	3·103^{b}	0·157	3·289^{b}	0·154	1·945^{a}	0·079	1·502^{a}	0·194
FI§	39·668^{c}	1·591	40·778^{c}	0·890	30·917^{b}	0·063	26·301^{a}	0·246
FE‖	82·767^{b}	1·742	81·467^{b}	3·447	57·769^{a}	2·513	46·446^{a}	5·887
PER¶	2·004^{b}	0·042	1·972^{b}	0·084	1·399^{a}	0·061	1·124^{a}	0·143

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups.
† PWG (%) = 100 × (FBW (g/fish) − MBW (g/fish))/MBW (g/fish).
‡ SGR (%) = 100 × (ln FBW − ln MBW)/d.
§ FI (g/fish) = total dry feed intake (g)/number of fish.
‖ FE (%) = 100 × (FBW (g/fish) − IBW (g/fish))/FI (g/fish).
¶ PER = weight gain (g)/protein intake (g).

Table 4. Whole body composition (%, wet-basis) of *Acipenser dabryanus* during fasting*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Moisture	68·857^{a}	0·812	71·077^{a,b}	1·027	71·790^{a,b}	0·875	72·550^{b}	0·241
Protein	11·553^{b}	0·543	10·600^{b}	0·115	9·953^{a,b}	0·683	8·310^{a}	0·478
Lipid	1·500^{d}	0·010	1·143^{c}	0·080	0·650^{b}	0·050	0·347^{a}	0·019
Ash	2·803	0·029	2·550	0·093	2·770	0·524	3·100	0·311

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b,c,d} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

Table 5. Whole-body composition (%, wet-basis), protein production value (PPV), lipid production value (LPV) and ash production value (APV) of *Acipenser dabryanus* during refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Moisture	66·607^{a}	0·396	69·463^{b}	0·502	68·143^{a,b}	0·268	67·347^{a,b}	0·703
Protein	12·220^{b}	0·120	10·587^{a,b}	0·277	11·023^{a,b}	0·922	9·730^{a}	0·446
Lipid	3·447^{c}	0·003	2·870^{b}	0·166	2·430^{a,b}	0·180	1·923^{a}	0·056
Ash	2·223^{a}	0·072	2·160^{a}	0·095	2·930^{b}	0·197	3·263^{b}	0·153
PPV†	29·073^{b}	0·343	28·304^{b}	0·979	22·521^{a}	0·736	19·540^{a}	1·674
LPV‡	54·604^{c}	0·596	44·474^{b}	0·732	43·951^{b}	0·496	37·638^{a}	0·681
APV§	10·701^{a}	0·816	13·309^{a}	0·899	21·758^{b}	0·797	20·129^{b}	2·050

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.
† PPV = fish protein gain (g)/total protein intake (g) × 100.
‡ LPV = fish lipid gain (g)/total lipid intake (g) × 100.
§ APV = fish ash gain (g)/total ash intake (g) × 100.

Muscle composition

The effects of fasting and refeeding on the muscle protein composition of *A. dabryanus* are shown in Tables 6 and 7. During fasting, the levels of moisture and ash were significantly increased in 14D and no significant change was observed among 0D, 3D and 7D. The protein and lipid levels were maximum for fish in the control group and were significantly higher than those for fish in the 7D and 14D groups. During refeeding, the moisture and ash levels in 14D were higher than those in the control group ($P < 0·05$). The protein

British Journal of Nutrition
https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

Table 6. Muscle composition (%, wet-basis) of *Acipenser dabryanus* during fasting*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Moisture	67·210^{a}	1·641	68·330^{a,b}	0·365	68·882^{a,b}	1·073	74·460^{b}	2·600
Protein	13·097^{b}	0·445	11·560^{a,b}	0·768	10·880^{a}	0·270	11·103^{a}	0·367
Lipid	2·557^{b}	0·152	2·023^{b}	0·231	1·135^{a}	0·096	1·353^{a}	0·112
Ash	0·637^{a}	0·018	0·690^{a}	0·017	0·653^{a}	0·055	1·180^{b}	0·145

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

Table 7. Muscle composition (%, wet-basis) of *Acipenser dabryanus* during refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Moisture	65·848^{a}	0·710	67·380^{a}	0·160	67·547^{a,b}	0·232	68·970^{b}	0·499
Protein	13·150^{b}	0·267	11·560^{a,b}	0·723	10·880^{a}	0·257	10·940^{a}	0·308
Lipid	3·480^{b}	0·482	2·440^{a,b}	0·295	2·283^{a,b}	0·196	1·193^{a}	0·117
Ash	0·713^{a}	0·016	0·773^{a,b}	0·055	1·430^{a,b}	0·198	1·823^{b}	0·418

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

Table 8. Alanine aminotransferase (ALT, U/l), aspartate aminotransferase (AST, U/l), alkaline phosphatase (ALP, U/l), total proteins (TP, g/l), albumin (ALB, g/l), globulin (GLO, g/l), cholesterol (CHOL, mmol/l) and TAG (mmol/l) of *Acipenser dabryanus* during fasting*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ALT	1·667	0·667	2·333	0·333	2·333	0·333	1·667	0·667
AST	129·000	7·211	156·667	12·252	123·667	12·170	123·333	13·667
ALP	149·667^{b}	15·169	101·333^{a,b}	11·333	87·667^{a}	9·871	101·000^{a,b}	12·288
AST/ALT	59·777	38·978	69·000	7·000	53·667	2·901	59·777	38·978
TP	13·500^{b}	1·332	10·633^{a,b}	0·260	8·867^{a}	0·567	8·200^{a}	0·611
ALB	4·967^{b}	0·133	4·033^{a}	0·033	3·833^{a}	0·296	3·767^{a}	0·088
GLO	9·033^{b}	1·560	6·000^{a,b}	0·577	5·667^{a,b}	0·667	4·667^{a}	0·667
CHOL	1·720^{b}	0·065	1·487^{a,b}	0·090	1·267^{a}	0·124	1·160^{a}	0·064
TAG	4·717^{b}	0·800	3·620^{a,b}	0·290	2·573^{a}	0·061	2·313^{a}	0·337

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

and lipid levels in 14D were significantly decreased compared with the control group.

Serum biochemical indexes

The effects of fasting and refeeding on serum biochemical indexes are shown in Tables 8 and 9. During fasting, ALT, AST and AST/ALT were not significantly different among the groups ($P > 0·05$). The highest ALP value was observed in 0D, and the lowest was observed in 7D ($P < 0·05$). The maximum values of TP, ALB, GLO, CHOL and TAG were in the control group, and there was no statistically significant difference among the other groups. During refeeding, ALT, AST, ALP and AST/ALT were not significantly different among the groups. The minimum values of TP, TAG, ALB and CHOL were observed in 14D ($P < 0·05$), and there were no statistically significant differences in the other groups. The GLO value in 3D was higher than that in 7D and 14D ($P < 0·05$); however, there was no significant difference between 0D and 3D.

Concentrations of growth hormone, insulin-like growth factor 1 and insulin-like growth factor 2 in serum

The concentrations of GH, IGF-1 and IGF-2 in serum during fasting and refeeding are shown in Fig. 2(A) and (B), respectively. During fasting, GH was significantly increased in the 14D group compared with the control group and no significant difference was found in the other groups. The IGF-1 concentration significantly declined with prolonged starvation, and no obvious change was found among 3D, 7D and 14D. No significant change in IGF-2 concentration was found among the four groups

British Journal of Nutrition
https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

Table 9. Alanine aminotransferase (ALT, U/l), aspartate aminotransferase (AST, U/l), alkaline phosphatase (ALP,U/l), total proteins (TP, g/l), albumin (ALB, g/l), globulin (GLO, g/l), cholesterol (CHOL, mmol/l) and TAG (mmol/l) of *Acipenser dabryanus* during refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ALT	2·000	0·000	2·000	0·577	3·000	0·000	2·667	1·202
AST	98·500	0·577	104·667	5·457	117·500	9·500	96·667	7·881
ALP	63·500	4·500	62·000	6·429	73·000	13·000	66·667	5·175
AST/ALT	49·500	0·850	61·777	16·815	39·165	3·165	57·900	11·927
TP	13·950^{b}	0·400	13·767^{b}	1·135	9·700^{a,b}	0·600	7·067^{a}	1·272
ALB	4·800^{a,b}	6·500	4·667^{a,b}	0·384	5·500^{b}	0·500	3·200^{a}	0·416
GLO	7·500^{b,c}	0·500	9·433^{c}	0·555	5·150^{a,b}	0·150	3·533^{a}	0·593
CHOL	1·435^{b}	0·135	1·367^{b}	0·085	1·320^{b}	0·110	0·797^{a}	0·101
TAG	4·525^{b}	0·205	4·863^{b}	0·185	3·715^{a,b}	0·875	2·413^{a}	0·180

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

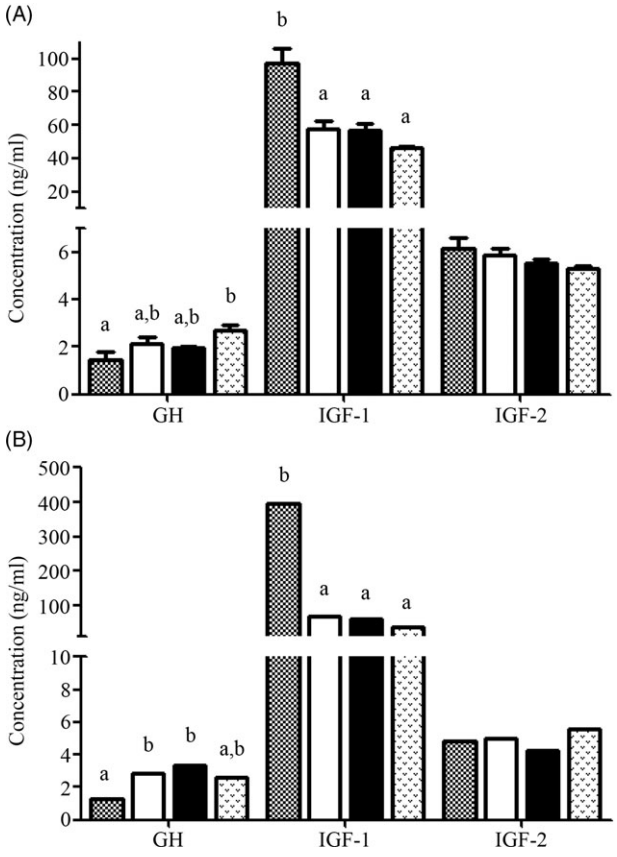

Fig. 2. Concentration of growth hormone (GH, ng/ml), insulin-like growth factor 1 (IGF-1, ng/ml) and insulin-like growth factor 2 (IGF-2, ng/ml) in serum of *Acipenser dabryanus* during fasting (A) and refeeding (B). Data are mean values with their standard errors, of six replicates. ^{a,b} Mean values with unlike letters are significantly different ($P < 0·05$). ▩, 0 d of fasting and 14 d of refeeding (0D); ▭, 3 d of fasting and 14 d of refeeding (3D); ■, 7 d of fasting and 14 d of refeeding (7D); ▩, 14 d of fasting and 14 d of refeeding (14D).

($P > 0·05$). During refeeding, the concentration of GH was significantly up-regulated in 3D and 7D and then declined slightly ($P > 0·05$). The highest IGF-1 concentration was observed in 0D, and no significant change was found in other groups. There was also no significant difference in the IGF-2 concentration.

Visceral index

The visceral indexes of fasting and refeeding in *A. dabryanus* are presented in Tables 10 and 11, respectively. During fasting, there was no significant difference in stomach weight with prolonged starvation. Stomach somatic index, liver weight, intestinal weight, intestinal length, intestinal somatic index and relative gut length exhibited the same trend, with the lowest values observed for 14D compared with the control group, and there was no difference between 3D and 7D. The liver somatic index in 0D and 3D was significantly higher than that in 14D. After 14 d of refeeding, the values of stomach weight, liver weight, liver somatic index, intestinal weight and intestinal somatic index in the 14D group were significantly lower than those in the other groups and the intestinal length and relative gut length in the 14D group were not significantly different from those in the control group. The lowest value of stomach somatic index was in 14D, and there was no significant change in other groups.

Digestive enzyme activities

The effects of fasting and refeeding on digestive enzyme activities of *A. dabryanus* are given in Tables 12 and 13. During fasting, the activity of pepsin was significantly decreased in 14D compared with 0D and 3D. Trypsin was the highest for fish in the control group, and there was no significant difference among 3D, 7D and 14D. Fish in 0D, 3D and 7D had significantly higher levels of chymotrypsin than those in 14D ($P < 0·05$). Lipase activities were decreased in 7D and 14D compared with 0D and 3D. The activity of amylase in 14D was lowest for fish in 3D ($P < 0·05$). After 14 d of refeeding, the activities of pepsin, trypsin and lipase in 14D were significantly lower than those in the control group and there was no significant difference between 3D and 7D. The chymotrypsin activity was significantly decreased in 7D and 14D. There was no significant difference in amylase activity.

Expression of the neuropeptide Y and peptide YY genes

Relative mRNA expression levels of *npy* and *pyy* during fasting and refeeding are displayed in Fig. 3(A) and (B). During fasting,

British Journal of Nutrition
https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

Table 10. Stomach weight (SW, g/fish), liver weight (LW, g/fish), intestinal weight (IW, g/fish), intestinal length (IL, cm/fish), stomach somatic index (SSI), liver somatic index (LSI), intestinal somatic index (ISI) and relative gut length (RGL) of *Acipenser dabryanus* during fasting*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SW	0·600	0·082	0·540	0·042	0·518	0·032	0·480	0·049
SSI	1·353^{b}	0·038	1·187^{a,b}	0·060	1·067^{a}	0·017	1·015^{a}	0·012
LW	0·767^{b}	0·257	0·643^{a,b}	0·154	0·535^{a,b}	0·070	0·226^{a}	0·040
LSI†	1·609^{b}	0·215	1·806^{b}	0·174	1·118^{a,b}	0·205	0·527^{a}	0·143
IW	1·415^{b}	0·258	0·910^{a,b}	0·123	0·838^{a,b}	0·042	0·650^{a}	0·135
IL	11·515^{b}	0·811	9·097^{a}	0·317	9·267^{a}	0·220	8·660^{a}	0·464
ISI‡	2·653^{b}	0·380	1·992^{a,b}	0·242	1·944^{a,b}	0·194	1·466^{a}	0·207
RGL§	57·114^{b}	5·270	46·195^{a,b}	2·707	47·436^{a,b}	1·475	43·266^{a}	2·088

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.
† LSI = wet liver weight (g)/wet body weight (g) × 100.
‡ ISI = wet intestine weight (g)/wet body weight (g) × 100.
§ RGL = whole intestine length (cm)/body length (cm) × 100.

Table 11. Stomach weight (SW, g/fish), liver weight (LW, g/fish), intestinal weight (IW, g/fish), intestinal length (IL, cm/fish), stomach somatic index (SSI), liver somatic index (LSI), intestinal somatic index (ISI) and relative gut length (RGL) of *Acipenser dabryanus* during refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SW	1·395^{b}	0·152	1·413^{b}	0·076	1·468^{b}	0·142	0·700^{a}	0·140
SSI	1·414^{b}	0·081	1·370^{a,b}	0·038	1·513^{b}	0·111	1·047^{a}	0·052
LW	3·208^{b}	0·363	3·087^{b}	0·209	2·734^{b}	0·414	1·213^{a}	0·390
LSI	3·257^{b}	0·231	2·995^{b}	0·164	2·729^{b}	0·111	1·733^{a}	0·322
IW	3·235^{b}	0·422	3·765^{b}	0·227	3·396^{b}	0·716	0·990^{a}	0·121
IL	11·400^{a,b}	0·586	12·583^{b}	0·352	11·940^{a,b}	0·707	9·600^{a}	1·137
ISI	3·267^{b}	0·262	3·666^{b}	0·220	3·296^{b}	0·391	1·938^{a}	0·169
RGL	48·233^{a,b}	1·766	52·139^{b}	1·358	48·360^{a,b}	2·180	43·042^{a}	3·297

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group.

Table 12. Activities of pepsin (U/mg protein), trypsin (U/mg protein), chymotrypsin (U/mg protein), lipase (U/g protein) and amylase (U/mg protein) of *Acipenser dabryanus* during fasting*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pepsin	20·896^{b,c}	2·263	26·108^{c}	2·276	15·228^{a,b}	2·927	10·174^{a}	0·821
Trypsin	2298·005^{b}	432·266	219·646^{a}	26·523	676·100^{a}	17·710	217·557^{a}	7·668
Chymotrypsin	26·667^{b,c}	3·169	27·938^{c}	2·848	16·744^{b}	2·271	4·210^{a}	0·681
Lipase	0·164^{b}	0·016	0·135^{b}	0·012	0·057^{a}	0·011	0·078^{a}	0·007
Amylase	0·316^{a,b}	0·010	0·384^{b}	0·020	0·281^{a,b}	0·003	0·271^{a}	0·041

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
^{a,b,c} Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group, while quadratic regressions were run with the triplicate data points.

the expression level of *npy* in 7D was significantly increased compared with that in 0D and 3D and was not significantly different from that in 14D. The *pyy* mRNA level was highest in 0D ($P < 0·05$) and was significantly down-regulated thereafter. During refeeding, no significant difference was found in *npy* and *pyy* mRNA levels.

Discussion

Growth performance

In the present study, a significant decrease in middle body weight was observed during constant starvation. Similar results have been found in channel catfish[25] and gilthead seabream[29]

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

Table 13. Activities of pepsin (U/mg protein), trypsin (U/mg protein), chymotrypsin (U/mg protein), lipase (U/g protein) and amylase (U/mg protein) of *Acipenser dabryanus* during refeeding*
(Mean values with their standard errors)

	0D		3D		7D		14D	
Items	Mean	SEM	Mean	SEM	Mean		Mean	SEM
Pepsin	15·621[b]	2·523	8·752[a,b]	0·928	8·280[a]	1·469	6·931[a]	0·686
Trypsin	2339·400[b]	146·610	215·015[a]	56·506	315·014[a]	67·602	246·387[a]	50·811
Chymotrypsin	39·695[b]	8·827	39·792[b]	4·386	1·868[a]	0·144	3·174[a]	1·050
Lipase	0·241[b]	0·017	0·128[a]	0·004	0·130[a]	0·025	0·109[a]	0·038
Amylase	0·324	0·025	0·276	0·005	0·292	0·026	0·266	0·016

0D, 0 d of fasting and 14 d of refeeding; 3D, 3 d of fasting and 14 d of refeeding; 7D, 7 d of fasting and 14 d of refeeding; 14D, 14 d of fasting and 14 d of refeeding.
[a,b] Mean values within a row with unlike superscript letters are significantly different ($P < 0·05$).
* Values are means with standard errors of three replicate groups with two fish in each group, while quadratic regressions were run with the triplicate data points.

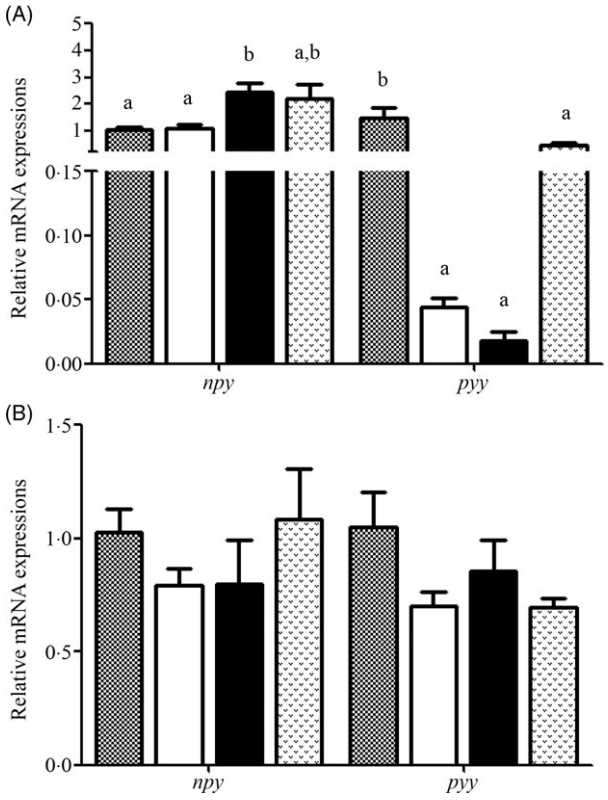

Fig. 3. Relative expression of neuropeptide Y (*npy*) in the brain and peptide YY (*pyy*) in the intestine of *Acipenser dabryanus* during fasting (A) and refeeding (B). Data are mean values with their standard errors, of six replicates. [a,b] Mean values with unlike letters are significantly different ($P < 0·05$). ▩, 0 d of fasting and 14 d of refeeding (0D); □, 3 d of fasting and 14 d of refeeding (3D); ■, 7 d of fasting and 14 d of refeeding (7D); ▨, 14 d of fasting and 14 d of refeeding (14D).

subjected to 21 and 28 d of fasting, respectively. It is remarkable that after 14 d of refeeding, the final body weight, percentage weight gain and specific growth rate in 7D and 14D were still significantly lower than those in the control group; however, during the 14 d of refeeding, the final body weight in 3D showed no significant difference compared with that in the control group. This result suggests that full compensation is evoked by 3-d starvation and 14-d refeeding, after which *A. dabryanus* exhibits partial CG.

Based on the above conclusion, we preliminarily explored the CG mechanism of *A. dabryanus*. At present, most scholars believe that CG is caused by the enhancement of food intake and food conversion efficiency, individually or collaboratively[30]. In our study, the FI and FE changed with our starvation–refeeding pattern and the trends were similar to those of Siberian sturgeon[4] and tongue sole (*Cynoglossus semilaevis*)[31]. The FI and FE during the 3-d starvation and 14-d refeeding were the same as those in the control group; however, even refeeding could not improve the values of FI and FE after 3-d starvation in *A. dabryanus*. Moreover, correlation analysis showed that percentage weight gain was positively related to FI (r 0·981, $P < 0·05$) and FE (r 0·980, $P < 0·05$). This may indicate that the increase in body weight (the compensation growth) in *A. dabryanus* during starvation and 14-d refeeding was due to the enhancement of FI and FE.

Appetite regulation

It has been reported that npy and pyy play key roles in the control of food intake and body weight[32,33]. In goldfish, it has been demonstrated that npy acts centrally to stimulate food intake[32] and pyy exhibits a wide presence and anorexigenic effects[16]. Hence, we speculated that npy and pyy might regulate the change in FI in *A. dabryanus*. Our results showed that during fasting, the expression of *npy* was significantly increased in 7D and then slightly decreased in 14D. The *npy* mRNA levels were similar to those in yellow catfish, which were markedly enhanced during 96 and 168 h of starvation[17]. The levels were significantly increased in grass carp[18] and goldfish[34] after 72 h of fasting. Fasting induced a significant decrease in *pyy* expression in the intestine of red-bellied piranha (*P. nattereri*)[19], and *pyy* mRNA levels in the brains of goldfish markedly declined after 3 and 7 d of starvation[16]. In our study, the expression of *pyy* in the intestine significantly declined during starvation, although it was slightly up-regulated in 14D, and there was no significant difference compared with the control group. We speculated that this phenomenon is coordinated with the slight down-regulation of *npy* mRNA levels. This suggested that the expression of *npy* was increased and that of *pyy* was decreased during starvation in *A. dabryanus*, and the appetite of fish increases when they are hungry.

British Journal of Nutrition
https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

After 14 d of refeeding, our results showed that the expression of *npy* and *pyy* was not significantly different among the groups. This may suggest that appetite regulation is rapid under the condition of refeeding and that the expression of *npy* and *pyy* remained stable after 14 d of refeeding. In yellow catfish, the *npy* mRNA levels returned to those in the control group after 3 h of refeeding[17]. The *npy* transcript was found to be dramatically decreased following refeeding in Argus snakehead (*Channa argus*)[35]. Similar results were also found at 47 d post-first feeding in Atlantic halibut (*Hippoglossus hippoglossus*)[36], and there was no significant response in the expression levels of *pyy* between before feeding and 1 and 3 h after feeding[36]. This implies that the recovery of food may lead to the rapid recovery of *npy* and *pyy* mRNA levels in other species; however, whether this phenomenon occurs in *A. dabryanus* needs further study.

Body and muscle components

Fish weight gain is primarily attributed to the accretion of protein and fat[37]. Starvation can induce metabolic depression[38]. After 14 d of starvation, the changes in the protein and lipid levels were, respectively, reducing 28·05 and 76·89 % in the whole body, and 15·22 and 47·09 % in the muscle. However, the opposite trends were observed for the levels of moisture and ash. Similar results were shown in channel catfish[25] and gilthead seabream[29]. It is said that under nutrient depletion, the lipids of fish degrade before the proteins[8,39]. This may be the reason for the decline rates of lipids in the whole body and muscle being higher than those of proteins. During refeeding, an obvious downward trend was observed, although the protein content in the whole body was not significantly different among the control group, 3D and 7D. However, our result is different from that for gilthead seabream[29], which showed no significant difference in protein, lipid, moisture and ash levels after refeeding for 10 weeks. In combination with the values of final body weight and FI, the decline in protein production value in 7D and 14D can be explained. In 0D and 3D, the value of protein production value showed no significant change. As protein synthesis is repressed in both skeletal muscle and liver after short-term fasting and is rapidly stimulated in response to feeding[40], refeeding can cause proteins in *A. dabryanus* to accumulate rapidly and reach the level in the control group after 3 d of starvation. However, there was a significant decrease in the lipid production value in the 3D group compared with the control group. This suggests that a large number of lipids are degraded during starvation and that our refeeding cycle is not enough to restore the lipid utilisation efficiency. On the other hand, the results for muscles seem to lag behind those for the whole body, which may be due to the order of different tissues in metabolism.

Serum biochemistry

The nutritional status of fish can be reflected by their serum biochemical indexes, which are closely related to the response of fish to environmental factors[41,42]. ALT and AST are tissue enzymes that may be enriched in blood as a result of leakage from cells in injured tissue. Because the liver is rich in ALT and AST and liver damage could result in the liberation of high levels of these enzymes into the blood, ALT and AST are used as indicators of liver dysfunction[43]. In the present study, we did not find any significant changes in ALT or AST activity or in the AST:ALT ratio in the starvation and refeeding groups. This suggests that the liver is not affected by starvation or refeeding. ALP is a phospholipase that can accelerate the uptake and transport of substances and is related to the metabolism of DNA, RNA, proteins and lipids[23]. In our study, the activity of ALP was significantly decreased in the 7D and 14D groups during fasting, similar to the results in Nile tilapia[24]. This suggests that the transport efficiency of serum substances is significantly decreased from 7D in *A. dabryanus*. ALB is an important protein in serum that can repair tissue and regulate plasma colloid osmotic pressure[44]. Our results show that the ALB content was significantly decreased at the beginning of starvation. GLO is mainly involved in the non-specific immune response and was decreased in 14D. The TP content includes ALB and GLO and was significantly decreased in 7D and 14D. The levels in yellow catfish[23] were reduced after 12 d of starvation, which is slightly different from our data. This may be caused by differences between the species. In general, these results may imply that starvation can rapidly reduce the abilities of tissue repair and plasma colloid osmotic pressure regulation, and long-term starvation may reduce fish immunity. The levels of TAG and CHOL in serum represent the levels that the body can ingest or synthesise, respectively. In the present study, the levels of TAG and GHOL were significantly decreased in 7D and 14D. These changes are similar to those observed in large yellow croaker[42], yellow catfish[23] and southern catfish[22]. During starvation, the lipid content in the whole body and muscle significantly declined, which may indicate that lipids were rapidly used by *A. dabryanus* to replenish energy. Nevertheless, lipid levels in serum were still lower than those in the control group, which may be closely related to the decrease in body metabolism. Overall, serological indicators also confirm that lipids are quickly used for energy under starvation conditions; however, the effect of short-term fasting (3D) on these indicators is not obvious. This may explain why refeeding after 3 d of starvation can fully compensate for growth.

During refeeding, the results show that no significant difference was observed in ALT, AST, ALP activity and AST/ALT among the four groups. This result suggested that the liver function and transport efficiency of serum substances in *A. dabryanus* could be retained under normal conditions under refeeding. The TP, TAG, ALB, GLO and CHOL levels were relatively stable in 3D and 7D but were significantly decreased in 14D compared with the control group. This result suggested that even refeeding did not reverse the declines in TP, TAG, ALB, GLO and CHOL levels, which were caused by the 14-d starvation. The results for TAG, TP and CHOL in grass crap[45] had the same trend as those observed in our data. However, the TAG and CHOL levels in Nile tilapia (*Oreochromis niloticus*)[24] were not significantly different after 14 or 21 d of refeeding, but the activities of ALT and AST significantly declined. These differences suggest that the effects of starvation on metabolism in different fish are diverse, which may be related to the nutrients accumulated by the fish and the ability to withstand hunger stress. In addition, long-term starvation slows down the ability of tissue repair, colloidal osmotic pressure regulation, and immune and fat metabolism in fish.

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

British Journal of Nutrition

Growth hormone–insulin-like growth factor axis

It is generally accepted that the GH–IGF axis plays a pivotal role in the neuroendocrine regulation of vertebrate growth[20]. Moreover, this progress is directly or indirectly affected by nutritional status. It was reported that 30-, 28-, 15- and 15-d fasting in rainbow trout[46], channel fish[47], Atlantic salmon[21] and black seabream[48] can significantly increase serum GH, respectively. These results are similar to our results, which exhibited a marked improvement in GH content after 14 d of fasting. However, the value for channel fish in Small's[47] research indicated little or no effect of 21-d fasting on plasma GH levels[49]. The reasons for the difference may be related to the species, and the fluctuation in GH levels in the body exhibits rebalancing over time[50]. The level of IGF-1 was significantly reduced in many teleost species, such as tilapia[51], Atlantic salmon[21] and channel fish[47]. It has been reported that fasting typically has the opposite effect on circulating concentrations of IGF-1 in fish and mammals[49]. The IGF-1 content in our study was significantly decreased by starvation, similar to the results in channel fish[49], Atlantic salmon[21], juvenile chum salmon[52] and tilapia[50]. The mechanism underlying the regulatory effect of fasting on GH and IGF in fish is not clear, but there are two points of view regarding the increased GH levels and decreased IGF-1 levels. One view supports the idea that the reduced hepatic binding capacity for GH during fasting was one of the mechanisms responsible for the decline in circulating IGF-1, and the other hypothesises that the observed increase in circulating GH after fasting was due to low plasma IGF-1 levels, resulting in reduced negative feedback effects on GH synthesis and release[49]. The level of IGF-2 showed no significant difference during starvation in our results; however, this is different from the results for rainbow trout and Atlantic salmon[21]. In juvenile Atlantic salmon[21], starvation for a period of 15 d caused a 19 % decrease in circulating IGF-2 levels, but there was no significant difference in 22-d starvation. In fact, our results for GH, IGF-1 and IGF-2 during the 14-d starvation are most similar to those observed in juvenile Atlantic salmon during a 22-d starvation[21,53]. Due to the lack of research on IGF-2, the mechanism is not clear.

During refeeding, the GH level was significantly increased in the 3D and 7D groups and there was no significant difference between the 14D and control groups. This suggests that the short-term food shortage caused a significant rise in serum GH after the 14-d refeeding; however, the GH content during long-term food shortage may slowly increase even when the nutrition becomes available again. The levels of IGF-1 were significantly decreased in the refeeding groups compared with the control group, but there was no significant difference among the refeeding groups. It may be inferred that refeeding cannot alleviate the effect of serum IGF-1 under our starvation conditions. These changes in GH and IGF-1 in 14D are still most similar to those observed in juvenile Atlantic salmon starved for 15 d and refed for 7 d[21]. However, it has been reported that the serum IGF-1 level and the expression of *igf-1* in the liver were restored to the levels in the control group with 14-d fasting and 14-d refeeding in gibel carp[54]. This may suggest that different fish may have different compensation mechanisms. In the present study, IGF-2 remained unchanged in the refeeding groups. It has been shown that the serum IGF-2 in *A. dabryanus* is not sensitive to changes in nutrients, but the underlying reason is unknown. On the other hand, full/partial CG was observed after 14 d of refeeding; however, the trend for IGF-1 did not seem to match this result. As proposed by Wilkinson *et al.*[21], the reason could be that the non-IGF-related GH activity promotes growth following the removal of GH resistance at the receptor level[21].

Digestive function

Rapid changes in both gut physiology and enzyme activity can be observed at the early stages of starvation[39]. In neon damselfish (*Pomacentrus coelestis*)[55], gut length was reduced after a short-term starvation period. In this trial, the effect of starvation on intestinal length was the most obvious in 3D. Luo *et al.*[25] reported a more linear decrease in VSI, hepatosomatic and IPR in channel catfish for up to 80 d of starvation. Similar results were observed in white sturgeon with 10 weeks of starvation[56]. Our results show that all the visceral indexes except stomach weight were markedly decreased in 14D. Although there was no significant difference in stomach weight, there was a downward trend. These results suggest that prolonged food deprivation can inhibit the growth of digestive organs.

Our study shows that the activities of pepsin, chymotrypsin and amylase, but not those of trypsin and lipase, decreased significantly in 14D. The activity of trypsin was rapidly reduced by 81·19 % in 3D, and the activity of lipase was reduced by 65·18 % in 7D. In Adriatic sturgeon, as previously reported, the activities of amylase and protease declined continuously throughout the 72 d of starvation[57]. This suggests that digestive enzyme activity was down-regulated by starvation. Interestingly, slight increases were observed in pepsin, chymotrypsin and amylase, but there were no significant differences compared with the control group. The mechanisms underlying these results are not clear.

After 14 d of refeeding, the growth and development of digestive organs, except intestinal length and RGL, still declined in 14D. However, hepatosomatic values in Persian sturgeon[7] and *A. dabryanus*[9] returned to the levels in the control fish after 4 weeks of refeeding[7]. This suggests that the effects of prolonged starvation on the digestive organs are enormous, and even refeeding takes a long time to reverse these effects. On the other hand, the effect of refeeding on digestive enzyme activities in *A. dabryanus* seemed weaker than those on the development of digestive organs. While there was no significant difference in amylase activity during refeeding, the activities of trypsin and lipase were still lower than those of the control group. As reported in Atlantic cod[58], the trypsin activity in D10f (10 weeks of food deprivation and 24 d of refeeding) did not show overcompensation, suggesting that this enzyme does not exert a substantial regulatory effect on CG[58]. This suggests that the effect of refeeding on the decrease in digestive enzyme activities caused by starvation was not significant, especially after long-term food deprivation, and that the CG in *A. dabryanus* is not closely related to digestive enzyme activity.

In general, we preliminarily explored the possible mechanism of compensation in *A. dabryanus* by starvation and refeeding (Fig. 4). Through further exploration, we showed that hunger stress can inhibit the growth of *A. dabryanus*; however, short-

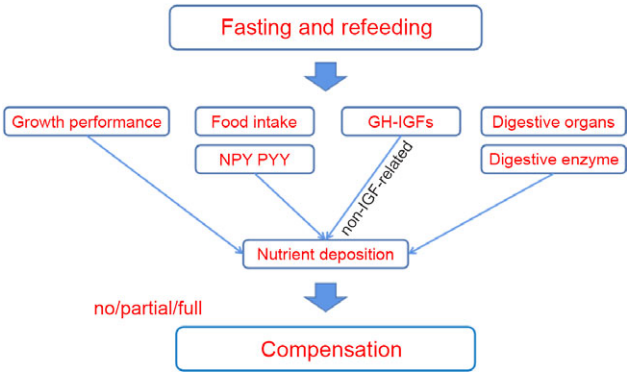

Fig. 4. The mechanism of compensatory growth during fasting and refeeding. NPY, neuropeptide Y; PYY, peptide YY; GH, growth hormone; IGF, insulin-like growth hormone.

term food deprivation (3 d in our study) has little effect on the physiological metabolism and digestive capacity of the fish. Finally, full CG of *A. dabryanus* was observed with 3-d fasting and 14-d refeeding. This may be caused by an increase in appetite, as reflected by the expression of *npy* and *pyy*, and another reason may be the non-IGF-related GH activity observed following the removal of GH resistance at the receptor level. Although the development of digestive organs was slowly restored, digestive capacity seemed to have no substantial effect on CG. On the other hand, this may suggest that the optimal growth benefit for *A. dabryanus* can be achieved by adjusting its dietary rhythm during the process of artificial propagation.

Acknowledgements

Thanks for the efforts by all of the members in our project team, as well as the assistance of the base colleagues.

This work was supported by Frontier project of Sichuan Academy of Agricultural Sciences (2019QYXK-21), Sichuan Science and Technology Program (2018JY0636), Sichuan Science and Technology Program (2016NYZ0047), the Project of Research on Large-scale Artificial Breeding and Breeding Technology of Triplophysa siluroides (2016TSCY-004) and National modern agricultural industrial technology System Sichuan Freshwater fish Innovation team.

X.-Y. W., Q. G. and Z.-H. L. designed the study; J.-S. L. and M.-J. S. conducted the trial; Y.-X. W., Y.-Y. C. and Y. L. performed the experiment and analysed the data; X.-Y. W. wrote the manuscript.

The authors declare that there are no conflicts of interest.

References

1. Turano M, Borski R & Daniels H (2008) Effects of cyclic feeding on compensatory growth of hybrid striped bass (*Morone chrysops* × *M. saxtilis*) food fish and water quality in production ponds. *Aquacult Res* **39**, 1514–1523.
2. Quinton JC & Blake RW (1990) The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. *J Fish Biol* **37**, 33–41.
3. Sevgili H, Hoşsu B, Emre Y, *et al.* (2013) Effect of various lengths of single phase starvation on compensatory growth in rainbow trout under summer conditions (*Oncorhynchus mykiss*). *Turk J Fish Aquat Sci* **13**, 465–477.
4. Chen X (2013) The compensation growth after starvation and physiology mechanism in juvenile Siberian sturgeon (*Acipenser baerii*). Master thesis, Ocean University of China.
5. Kim MK & Lovell RT (1995) Effect of restricted feeding regimens on compensatory weight gain and body tissue changes in channel catfish *Ictalurus punctatus* in ponds. *Aquaculture* **135**, 285–293.
6. Gao LJ, Chen LQ, Zhao XQ, *et al.* (2004) Starvation and compensatory growth of *Acipenser schrenckii* juveniles-effects on digestive organs structure and digestive enzymes activity. *J Fish Sci China* **11**, 29–35.
7. Yarmohammadi M, Shabani A, Pourkazemi M, *et al.* (2013) Effects of starvation and re-feeding on compensatory growth performance, plasma metabolites and IGF-I gene expression of Persian sturgeon (*Acipenser persicus*, Borodin 1897). *Iran J Fish Sci* **12**, 465–483.
8. Xiao JX (2010) Effect of cyclical starvation and refeeding on growth performance and biochemical indices of juvenile black sea bream, *Acanthopagrus schlegelii*. Master Thesis, Zhejiang University.
9. Yang S, He K, Yan T, *et al.* (2019) Effect of starvation and refeeding on oxidative stress and antioxidant defenses in Yangtze sturgeon (*Acipenser dabryanus*). *Fish Physiol Biochem* **45**, 987–995.
10. Whitledge GW, Hayward RS, Noltie DB, *et al.* (1998) Testing bioenergetics models under feeding regimes that elicit compensatory growth. *Trans Am Fish Soc* **127**, 740–746.
11. Shen WY & Shou JX (2003) Research progress of fish compensatory growth. *Fish Water Conserv* **23**, 40–42.
12. Oh SY, Noh CH, Kang RS, *et al.* Compensatory growth and body composition of juvenile black rockfish *Sebastes schlegelii* following feed deprivation. *Fish Sci* **74**, 846–852.
13. Volkoff H, Canosa LF, Unniappan S, *et al.* (2005) Neuropeptides and the control of food intake in fish. *Gen Comp Endocr* **142**, 3–19
14. Hoskins LJ & Volkoff H (2012) Daily patterns of mRNA expression of two core circadian regulatory proteins, Clock2 and Per1, and two appetite-regulating peptides, OX and NPY, in goldfish (*Carassius auratus*). *Comp Biochem Physiol A Mol Integr Physiol* **163**, 127–136
15. Zhou Y, Liang X, Yuan X, *et al.* (2013) Neuropeptide Y stimulates food intake and regulates metabolism in grass carp, *Ctenopharyngodon idellus*. *Aquaculture* **380**, 52–61.
16. Gonzalez R & Unniappan S (2010) Molecular characterization, appetite regulatory effects and feeding related changes of peptide YY in goldfish. *Gene Comp Endocr* **166**, 273–279
17. Cao L (2013) Cloning, expression and the relationship between *NPY* and somatostatin gene and growth traits in *Pelteobagrus fulvidraco*. Master Thesis, Huazhong Agricultural University.
18. Zhang F (2008) cDNA cloning of grass carp (*Ctenopharyngodon idellus*) neuropeptide tyrosing (*NPY*) and its expression in brain tissue in response to feed deprivation. Master Thesis, Southwest University.
19. Volkoff H (2014) Appetite regulating peptides in red-bellied piranha, *Pygocentrus nattereri*: cloning, tissue distribution and effect of fasting on mRNA expression levels. *Peptides* **56**, 116–124.
20. Duan C (2005) Insulin-like growth factor signaling in fish. *Int Rev Cytol* **243**, 215–285.
21. Wilkinson RJ, Porter M, Woolcott H, *et al.* (2006) Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

salmon and rainbow trout. *Comp Biochem Physiol A Mol Integr Physiol* **145**, 214–224
22. Chen XY (2000) Effects of starvation on the blood of southern catfish (*Silurus meridionalis*). *J Southwest Agric Univer* **22**, 167–169.
23. Sun HM (2004) Effects of starvation on the blood indices and immunity function of yellow catfish (*Pseudobagrus fulvidraco* Richardson). Master Thesis, Jilin Agricultural University.
24. Tian J, Tu W, Zeng LB, *et al.* (2012) The changes in growth, serum biochemical indices and GH/IGF-I/IN mRNA expression abundance of *Oreochromis niloticus* during fasting and re-feeding. *J Fish China* **36**, 900–907.
25. Luo Z, Tan XY, Wang WM, *et al.* (2009) Effects of long-term starvation on body weight and body composition of juvenile channel catfish, *Ictalurus punctatus*, with special emphasis on amino acid and fatty acid changes. *J Appl Ichthyol* **25**, 184–189.
26. Khan MA, Jafri AK & Chadha NK (2004) Growth, reproductive performance, muscle and egg composition in grass carp, *Ctenopharyngodon idella* (Valenciennes), fed hydrilla or formulated diets with varying protein levels. *Aquacult Res* **35**, 1277–1285.
27. Jiang J, Feng L, Tang L, *et al.* (2015) Growth rate, body composition, digestive enzymes and transaminase activities, and plasma ammonia concentration of different weight Jian carp (*Cyprinus carpio var.* Jian). *Anim Nutr* **4**, 373–377.
28. Jiang J, Wu XY, Zhou XQ, *et al.* (2016) Glutamate ameliorates copper-induced oxidative injury by regulating antioxidant defences in fish intestine. *Br J Nutr* **116**, 70–79.
29. 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**, 388.
30. Hang GQ, Li J, Tang X, *et al.* (2015) Research advance of compensatory growth in aquatic animals. *J Guangxi Acad Sci* **31**, 1–8.
31. Fang JH (2010) Effects of temperature and nutrition stress on the growth of tongue sole *Cynoglossus semilaevis* günther and eco-physiological mechanisms. PhD Thesis, Ocean University of China.
32. Aldegunde M & Mancebo M (2006) Effects of neuropeptide Y on food intake and brain biogenic amines in the rainbow trout (*Oncorhynchus mykiss*). *Peptides* **27**, 719–727.
33. Cristina V, Blanco AM, Suraj U, *et al.* (2008) The anorectic effect of central PYY 1-36 treatment in rainbow trout (*Oncorhynchus mykiss*) is associated with changes in mRNAs encoding neuropeptides and parameters related to fatty acid sensing and metabolism. *Gen Comp Endocr* **267**, 137–145.
34. Narnaware YK & Peter RE (2001) Effects of food deprivation and refeeding on neuropeptide Y (*NPY*) mRNA levels in goldfish. *Comp Biochem Physiol B: Biochem Mol Biol* **129**, 633–637.
35. Yang S, Wen ZY, Zou YC, *et al.* (2018) Molecular cloning, tissue distribution, and effect of fasting and refeeding on the expression of neuropeptide Y in *Channa argus*. *Gen Comp Endocr* **259**, 147.
36. Gomes AS, Jordal AEO, Olsen K, *et al.* (2015) Neuroendocrine control of appetite in Atlantic halibut (*Hippoglossus hippoglossus*): changes during metamorphosis and effects of feeding. *Comp Biochem Physiol A Mol Integr Physiol* **183**, 116–125.
37. Bureau D, Azevedo P, Tapiasalazar M, *et al.* (2000) Pattern and cost of growth and nutrient deposition in fish and shrimp: potential implications and applications. In *Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutricón Acuícola (Advances in Aquaculture Nutrition V. Proceedings of the Vth International Symposium on Aquaculture Nutrition)*, 19–22 November. Mérida, Yucatán, Mexico, pp. 111–140.
38. Hidalgo MC, Morales AE, Arizcun M, *et al.* (2017) Regional asymmetry of metabolic and antioxidant profile in the sciaenid fish shi drum (*Umbrina cirrosa*) white muscle, response to starvation and refeeding. *Redox Biol* **11**, 682–687.
39. Bar N & Volkoff H (2012) Adaptation of the physiological, endocrine, and digestive system functions to prolonged food deprivation in fish. In *Comparative Physiology of Fasting, Starvation, and Food Limitation*, pp. 69–89 [MD McCue, editor]. Berlin: Springer-Verlag Berlin Heidelberg.
40. Kimball SR, Jefferson LS, Nguyen HV, *et al.* (2000) Feeding stimulates protein synthesis in muscle and liver of neonatal pigs through an mTOR-dependent process. *AJP Endocr Metabol* **279**, E1080–E1087.
41. Jahanbakhshi A, Imanpoor MR, Taghizadeh V, *et al.* (2013) Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the great sturgeon (*Huso huso*). *Comp Clin Path* **22**, 1087–1092.
42. Zhang Z, Wan, Q, Kun YE, *et al.* (2016) Effect of fasting on body composition, muscle fatty acid profiles and serum biochemical parameters of large yellow croaker (*Larimichthys crocea*). *Shuichan Xuebao* **40**, 1440–1450.
43. Zaghloul KH, Hassan EM & Abdel Kader MH (2008) Hematoporphyrin toxicity in the cultured *Oreochromis niloticus*. *Egypt J Exp Biol (Zool)* **4**, 81–88.
44. Wang JY, Zhu SK, Xu CF, *et al.* (2004) *Biochemistry*, 3rd ed. Beijing: China Higher Education Press.
45. Li RX, Li HY, Tan BP, *et al.* Effects of starvation and refeeding on growth performance, glucose metabolism, and expression of glucose transporter 1 in *Ctenopharyngodon idellus*. *Zhongguo Shui Chan Ke Xue* **25**, 74–85.
46. Gabillard JC, Kamangar BB & Montserrat N (2006) Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (*Oncorhynchus mykiss*). *J Endocrinol* **191**, 15–24.
47. Small BC & Peterson BC (2005) Establishment of a time-resolved fluoroimmunoassay for measuring plasma insulin-like growth factor I (IGF-I) in fish: effect of fasting on plasma concentrations and tissue mRNA expression of IGF-I and growth hormone (GH) in channel catfish (*Ictalurus punctatus*). *Domest Anim Endocrin* **28**, 202–215.
48. Deng L, Zhang W & Lin H (2003) Effects of starvation on the contents of growth hormone and triiodothyronine in serum, and lipid and protein of white muscles and livers in black seabreams. *Zoo Res* **24**, 94–98.
49. Small BC (2005) Effect of fasting on nychthemeral concentrations of plasma growth hormone (GH), insulin-like growth factor I (IGF-I), and cortisol in channel catfish (*Ictalurus punctatus*). *Comp Biochem Physiol B Biochem Mol Biol* **142**, 223.
50. Fox BK, Breves JP, Hirano T, *et al.* (2009) Effects of short- and long-term fasting on plasma and stomach ghrelin, and the growth hormone/insulin-like growth factor I axis in the tilapia, *Oreochromis mossambicus*. *Domest Anim Endocrin* **37**, 11.
51. Fox BK, Breves JP, Davis LK, *et al.* (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 Endocr* **166**, 573–580.
52. Taniyama N, Kaneko N, Inatani Y, *et al.* (2016) Effects of seawater transfer and fasting on the endocrine and biochemical

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press

growth indices in juvenile chum salmon (*Oncorhynchus keta*). *Gen Comp Endocr* **236**, 146–156.
53. Dalbagni G (1996) Production of recombinant insulin-like growth factor-II in the development of a radioimmunoassay in rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocr* **104**, 156–167.
54. Shen WY, Ren G & Zhu YR (2012) Effects of compensatory growth on the levels of IGF-1, IGFBP-1 and expressions of *IGF-1* mRNA, *IGF-1R* mRNA in *Carassius auratus gibelio*. *Dongwuxue Yanjiu* **33**, 298–303.
55. Hall KC & Bellwood DR (2005) Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. *J Fish Biol* **47**, 438–454.
56. Hung SSO, Liu W, Li H, *et al*. (1997) Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture* **151**, 363.
57. Furné M, García-Gallego M, Hidalgo MC, *et al*. (2008) Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccarii)* and trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol A Mol Integr Physiol* **149**, 420–425.
58. Bélanger F, Blier PU & Dutil JD (2002) Digestive capacity and compensatory growth in Atlantic cod (*Gadus morhua*). *Fish Physiol Biochem* **26**, 121–128.

British Journal of Nutrition

https://doi.org/10.1017/S0007114520004389 Published online by Cambridge University Press