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Comparison of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*

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Abstract Haematological and serum biochemistry parameters were studied and compared between cultured and wild ecotypes of Dojo loach *Misgurnus anguillicaudatus* during the prime fishing season, i.e. May–August. Data were analysed for the impact of feeding regime and other ecological conditions on the physiology of fish. The results revealed that haemoglobin, cholesterol, total protein, creatinine and uric acid levels in the two ecotypes were significantly different ($n = 56$, $df = 54$, $P < 0.05$). In addition, red blood cell, glucose, triglyceride and urea nitrogen levels were significantly higher in cultured individuals ($n = 56$, $df = 54$, $P < 0.01$) than in their wild counterparts. In contrast, the white blood cell level in cultured fish was significantly ($n = 56$, $df = 54$, $P < 0.01$) lower than that in the wild ones. These differences can be attributed to the physiological acclimatization of the fish to their living conditions and feeding regime, which influences the energy metabolism and, consequently, the health of the fish.

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Introduction

The Dojo loach *Misgurnus anguillicaudatus* (Cobitidae, Cypriniformes) is a species endemic to Asia, where it inhabits mainly streams, ditches and rice paddy fields, preferably those with a soft muddy bottom (Man and Hodgkiss 1981). It is a popular traditional Chinese medicine in China and it is used in folk remedies for the treatment of hepatitis, osteomyeitis, carbuncles, inflammations and cancers as well as during recovery from debilities caused by various pathogens and ageing (Qin et al. 2002). However, the wild populations of *M. anguillicaudatus* have almost collapsed in recent years due to intensive fishing and water pollution, prompting the development of aquaculture facilities for the (commercial) culture of this species (Wang et al. 2007). A rearing system for the complete life cycle was developed in the 1990s, and, *M. anguillicaudatus* occupies a significant position in freshwater fish culture enterprises in China both for food and commercial purposes (Gao et al. 2007). The maximization of fish productivity is the aim of fishery management programmes, and an evaluation of the physiological status of the fish during rearing is necessary to achieve this.

The analysis of blood indices has proven to be a valuable approach for analysing the health status of

farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani et al. 2001). Exogenous factors, such as management (Svobodova et al. 2006), diseases (Chen et al. 2005) and stress (Cnaani et al. 2004), always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress (Arends et al. 1999; Kubokawa et al. 1999; Skjervold et al. 2001; Svobodova et al. 2006). The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions (Santos and Pacheco 1996; Svoboda et al. 2001; Lermen et al. 2004). Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters (Wood et al. 1960; Christofilogiannis 1993; Coz-Rakovac et al. 2005). Moreover, variations in the levels of blood corpuscles have been observed in cytological investigations (Bahmani et al. 2001; Nespolo and Rosenmann 2002). Accordingly, haematology and serum biochemistry data are of immense importance in monitoring the health status of aquatic organisms, especially in fisheries management programmes. To date, however, data reported in the literature refer mainly to acute stress status (Arends et al. 1999; Kubokawa et al. 1999; Skjervold et al. 2001) in different farmed fish species, and there have been an insufficient number of studies on the comparative haematological differences between cultured and wild types of fish that share the same ecological zone.

We have investigated various blood indices of cultured and wild loach during the prime fishing season, May–August, in order to (1) characterize normal variation in haematology and serum biochemistry of cultured and wild types of loach; (2) establish a relationship between blood indices of the two fish types; (3) assess the impact of different feeding regimes and other ecological conditions on fish health.

Material and methods

Fish sampling

Fish samples were collected from May to August in 2007/2008. Cultured loaches were obtained from a commercial fish farm while the wild individuals were netted from freshwater streams, all in the same area, near Xiantao city, Hubei province, China. The stocking density of reared fish was 5–10 kg m^{-3}. Fish were fed with commercial feed containing 12% moisture, 15% crude lipid, 45% crude protein, 18% carbohydrate and 10% ash, with a digestible energy of 3573.8 kcal kg^{-1}. We examined the physico-chemical factors of the two sampling ecosystems. The temperature, dissolve oxygen (DO) and pH were investigated using a digital water quality monitor (YSI610-D, USA), while NH_3-N, NO_3-N, NO_2-N and chemical oxygen demand (COD) were measured using an automatic ammonia nitrogen analyser (CN201, China) (Table 1). The sampled fish were immediately transferred to fish tanks at the College of Fisheries, Huazhong Agricultural University.

Previous studies have demonstrated a coexistence of populations of natural diploids and tetraploids in central China (Yu et al. 1989; Yin et al. 2005). To avoid the variance due to ploidy factor, the fish were screened for ploidy status using flow cytometer (FACScalibur; Becton Dickinson, Franklin Lakes, NJ). To accomplish this, 20 fish samples of each type were randomly selected and blood samples were prepared following Gao et al. (2007).

After confirming that both loach types were diploid, 30 disease-free individuals of medium size (wild fish with a total length of 16.3 ± 1.97 cm while cultured fish were 15.2 ± 1.68 cm) from each type were selected and maintained in tanks at a density of 2 kg m^{-3}. They were acclimatized for 15 days in a controlled flow-through water system under

Table 1 The physico-chemical factors of the sampling localities

Physico-chemical factor	Ecotype	
	Cultured population	Wild population
Temperature (°C)	24.1 ± 3.2	24.6 ± 3.7
DO (mg l^{-1})	5.95 ± 0.71	7.70 ± 0.63
NH_3-N (mg l^{-1})	0.102 ± 0.033	0.048 ± 0.019
NO_2-N (mg l^{-1})	0.07 ± 0.025	0.03 ± 0.016
NO_3-N (mg l^{-1})	3.9 ± 0.84	2.3 ± 0.49
COD (mg l^{-1})	12.50 ± 1.03	8.65 ± 0.82
PO_4^{-3}-P (mg l^{-1})	0.41 ± 0.09	0.27 ± 0.07
pH	7.13 ± 0.26	7.88 ± 0.32

DO, Dissolved oxygen; COD, chemical oxygen demand

environmental conditions of photoperiod and water temperature (20–25°C) and fed pelleted fish meal (35% crude protein) complemented with *Limnodrilus hoffmeisteri*. During the acclimatization and study period, the fish were observed daily. The individuals displaying any clinical signs of diseases, including a lack of appetite, increased opercular movements or visible lesions on the skin, tail and fins (Tavares-Dias and Moraes 2007), were discarded.

Haematological and biochemical analyses

After anaesthetizing the fish with MS-222 (200 mg l^{-1}), we collected two blood samples per fish by caudal venipuncture. The first sample was taken with a syringe containing heparin sodium (1%), while the second sample was taken without an anticoagulant.

The first samples were used for haematological examination. Red blood cells (RBCs) and white blood cells (WBCs) were counted using a Neubauer haemocytometer by Hendrick's dilution (Houston 1990). Blood smears, two for each fish, were prepared according to the method of Gao et al. (2007) for simultaneous differential leucocyte investigation and total blood cell count. In general, a total of 200 cells (RBCs + WBCs) per slide were counted twice under a light microscope equipped with a video camera linked to computer image analysis software (Motic Images Advanced 3.2, USA). Haematocrit was measured by the microhaematocrit method (Morris and Davey 1996), while haemoglobin concentration was determined using the cyanomethaemoglobin technique (Drabkin and Austin 1935). To evaluate the erythrocyte osmotic brittleness, one drop of blood was added to a series of 1 ml NaCl solutions, with concentrations ranging from 0.20 to 0.40%, and each solution successively varying by 0.02% (Xu 2004). After 2 h, these solutions were tested, and those with no obvious erythrocyte taken as the value of maximum erythrocyte resistance.

The second blood sample was left for 1 h on ice and then centrifuged at 3000 rpm for 10 min to isolate the serum, which was collected as a supernatant and stored in liquid nitrogen prior to further analysis. A suite of biochemical analyses for parameters, such as glucose, cholesterol, triglyceride, total protein, urea nitrogen, total bilirubin, alanine aminotransferase, direct bilirubin, creatinine and uric acid, were carried out using a Beckman CX5 automated analyzer (Beckman Instruments, Brea, CA) with a Beckman kit.

Statistical analysis

All statistical analyses were performed using the computer program SPSS ver. 12.0 (SPSS, Chicago, IL). Since data did not show a normal distribution (Shapiro–Wilk test), the Mann–Whitney nonparametric test (U) was applied to analyse the different levels of each parameter from two ecotypes. A P value < 0.05 was considered to be significant, while a P value < 0.01 was considered to be highly significant. As no significant difference was found among each sampling times, data obtained from different sampling times of cultured and wild types were combined, respectively.

Seven serum biochemical parameters showing significant differences were then subjected to stepwise discriminant-function analysis (SPSS subprogram DISCRIMINAT) to elucidate which combination discriminated best between ecotypes.

Results

Haematological analyses

The mean values, standard errors and ranges of the haematological parameters are summarized in Table 2. Statistical analysis revealed nonsignificant difference in haematocrit and erythrocyte osmotic brittleness between the two ecotypes. The erythrocyte volume of the fish ranged between 495.34 and 508.61 μm^3, with an average of 501.74 ± 4.97 μm^3 in the cultured fish and ranged from 493.88 to 506.54 μm^3, with an average of 498.17 ± 5.34 μm^3, in the wild type. The level of haemoglobin, and cell count of lymphocytes, thrombocytes and neutrophils between the two ecotypes of the fish were significantly ($n = 56$, $P < 0.05$) different. In addition, the RBC count in the cultured population was significantly higher ($n = 56$, $P < 0.01$) than that in the wild type. Conversely, the WBC count in cultured individuals was significantly lower ($n = 56$, $P < 0.01$) than that in wild loaches.

Serum biochemical analyses

As shown in Table 3, most of the serum biochemical parameters varied significantly, with the exception of serum concentrations of alanine aminotransferase, total bilirubin and direct bilirubin, which displayed

Table 2 Comparative haematological profiles of cultured and wild *Misgurnus anguillicaudatus*

Parameter	Cultured type			Wild type			*P*
	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	
RBCC (10^4 mm^{-3})	247.83 ± 28.27	219.36–271.59	28	221.61 ± 30.16	189.71–253.32	28	0.007**
WBCC (10^4 mm^{-3})	2.61 ± 0.29	2.29–2.95	28	2.78 ± 0.41	2.33–3.02	28	0.003**
Haemoglobin (g dl^{-1})	13.27 ± 1.56	11.31–14.83	28	11.26 ± 1.75	9.46–13.08	28	0.014*
Haematocrit (%)	0.41 ± 0.03	0.38–0.45	28	0.39 ± 0.03	0.36–0.43	28	0.059
Neutrophils (%)	39.46 ± 6.54	36.18–43.82	28	41.13 ± 7.62	38.81–44.63	28	0.036*
Lymphocytes (%)	24.83 ± 4.88	21.17–27.64	28	27.58 ± 5.26	24.36–30.98	28	0.021*
Thrombocytes (%)	25.18 ± 3.96	22.77–28.92	28	26.56 ± 4.27	24.33–28.59	28	0.044
Monocyte (%)	4.02 ± 1.77	3.27–5.84	28	4.95 ± 1.01	3.74–5.89	28	0.012*
Erythrocyte volume (μm^3)	501.74 ± 4.97	495.34–508.61	28	498.17 ± 5.34	493.88–506.54	28	0.213
Erythrocytes osmotic brittleness (g l^{-1} NaCl)	2.96 ± 0.05	2.91–3.02	26	3.12 ± 0.06	3.08–3.19	25	0.059

* Significant ($P < 0.05$), ** highly significant ($P < 0.01$)

RBCC, Red blood cell count; WBCC, white blood cell count; SD, standard deviation

insignificant variation between the two loach types. There were significant variations observed for the concentrations of glucose, cholesterol, triglyceride, total protein, urea nitrogen, triglyceride, creatinine and uric acid. The levels of glucose, cholesterol, triglyceride, total protein, urea nitrogen, creatinine and uric acid in cultured loach were significantly higher ($n = 56$, $P < 0.05$) than those in the wild loach. In contrast, the concentration of plasma total bilirubin in cultured individuals was lower than that in their wild counterparts.

Discriminant analysis

The result of the stepwise discriminant-function analysis showed that the seven significant parameters perfectly categorized 87.5% of the specimens as either cultured or wild individuals (Table 4, Subset 2), although only four (glucose, urea nitrogen, cholesterol and triglyceride) contributed significantly to the discrimination between cultured and wild ecotypes. These four variables produced a clear line of demarcation between cultured and wild types, as indicated by a final Wilks Lambda of 0.17 (χ^2 at 195.13; $df = 4$, $P < 0.01$) and a canonical correlation of 0.895, which correctly predicted group affiliation in 85.7% of the cultured types and 78.6% of the wild types (Table 4). The standardized and unstandardized canonical discriminant-function coefficients from the discriminant-function analysis are given in Table 5.

Discussion

Intensively cultured loaches are densely stocked. They are not allowed full exposure to the natural environment (e.g. sediment) and depend upon artificial feed. In addition, they frequently suffer physical stress during management. In contrast to the farmed loach, wild loaches feed on zooplankton and phytoplankton, there is a heterogeneous age structure in populations and the life history is relatively unknown (Coz-Rakovac et al. 2005). Such a gradient in ecological conditions induce significant fluctuations in the level of various blood indices.

Our findings show that there is a wide range in the levels of the haematological parameters between wild and cultured ecotypes, especially in terms of the RBC count and haemoglobin concentration. Since erythrocyte characteristics partly determine the efficiency of oxygen transport from respiratory systems to tissues (Nespolo and Rosenmann 2002), increments in their number could be related to the high respiratory demand in intensive living conditions (Shen et al. 1991). Nevertheless, other factors, such as food quality, DO concentrations and regular management also strongly influence the blood attributes (Xu and Cao 1989; Domezain et al. 1997; Bahmani et al. 2001). However, no significant differences in the values of erythrocyte volume between the two ecotypes were observed in this study, suggesting that, in the intensive culture environments, the

Table 3 Comparative biochemical analysis of serum parameters of cultured and wild *M. anguillicaudatus*

Parameter	Cultured type		Wild type		*P*
	Mean ± SD	Range	Mean ± SD	Range	
Glucose (mmol l^{-1})	5.14 ± 1.06	4.02–6.15	4.38 ± 0.85	3.54–5.37	0.003**
Cholesterol (mmol l^{-1})	4.72 ± 0.56	3.71–5.80	3.97 ± 0.54	3.39–5.14	0.034*
Triglyceride (mmol l^{-1})	3.21 ± 0.78	2.15–4.45	1.27 ± 0.27	0.88–1.81	0.000**
Total protein (g l^{-1})	43.37 ± 3.98	37.36–49.71	36.19 ± 4.85	27.13–44.52	0.031*
Urea nitrogen (mmol l^{-1})	4.99 ± 0.87	3.45–6.23	2.85 ± 0.52	1.97–3.70	0.009**
Total bilirubin (μmol l^{-1})	5.57 ± 0.94	4.15–6.63	6.89 ± 0.86	5.08–8.38	0.061
Alanine aminotransferase (IU l^{-1})	48.70 ± 4.52	39.00–65.00	61.39 ± 5.51	42.00–79.00	0.395
Direct bilirubin (μmol l^{-1})	3.70 ± 0.76	2.12–4.56	2.34 ± 0.58	1.36–3.36	0.116
Creatinine (μmol l^{-1})	13.96 ± 3.15	10.00–22.00	10.61 ± 2.55	8.00–16.00	0.024*
Uric acid (μmol l^{-1})	0.0386 ± 0.006	0.03–0.05	0.0222 ± 0.004	0.02–0.03	0.044*

* Significant ($P < 0.05$), ** highly significant ($P < 0.01$)

Table 4 Discrimination results of cultured and wild *M. anguillicaudatus* by discriminant-function analysis of serum biochemical parameters

Actual group of serum biochemical parameters	*n*	Predicted group membership		Percentage correct	Percentage correct averaged over the subset
		Cultured type	Wild type		
Subset 1[a]					
Cultured type	28	24	4	85.7	82.1
Wild type	28	6	22	78.6	
Subset 2[a]					
Cultured type	28	25	3	89.3	87.5
Wild type	28	4	24	85.7	

[a] Subset 1, Glucose, urea nitrogen, cholesterol and triglyceride; subset 2, glucose, cholesterol, triglyceride, total protein, urea nitrogen, creatinine and uric acid

Table 5 Standardized and unstandardized canonical discriminant-function coefficients from the stepwise discriminant-function analysis between cultured and wild *M. anguillicaudatus*

Parameter	Discriminant-function coefficients	
	Standardized	Unstandardized
Glucose	−1.501	−1.168
Urea nitrogen	−3.634	−3.356
Cholesterol	2.277	3.358
Triglyceride	2.988	5.147
Constant		3.462

elevated RBC counts and haemoglobin concentration are a response to the higher metabolic demand and have no impact on erythrocyte volume.

We also found that cultured individuals had a higher serum glucose concentration than their wild counterparts. An increase in the plasma glucose of teleosts (Hardy and Audet 1990; Torres et al. 1991; Cech et al. 1996; Santos and Pacheco 1996; Svoboda et al. 2001; Lermen et al. 2004) was believed to be caused by a wide range of environmental stressors (such as hypoxic environment, starvation and captivity). In the present study, it is very likely that the physical processes involved in transporting the fish, keeping them in tanks, and administering anaesthesia for blood sample collection caused physical stress and affected hormone and glucose levels in the plasma. In an attempt to neutralize this, we acclimatized the fish in large holding tanks for 15 days without disturbance and paid special attention to

maintaining a consistent capture protocol throughout the study in order to minimize these stress effects (Nespolo and Rosenmann 2002). Since glucose in serum is a major metabolite of carbohydrate metabolism (Artacho et al. 2007), the higher glucose concentration detected in cultured strains should be attributed, in part, to the higher glycogen reserves in artificially cultured fish than in their wild counterparts. Our findings confirm those of Coz-Rakovac et al. (2005) who reported a significantly elevated glucose level in farmed *Dicentrarchus labrax* as compared to the wild ecotypes.

The levels of total protein, cholesterol and triglyceride are considered to be major indices of the health status of teleosts. Increased concentrations of total protein can be caused by structural liver alterations reducing aminotransferase activity, with concurrent reduced deamination capacity (Burtis and Ashwood 1996) and impaired control of fluid balance (Coz-Rakovac et al. 2005). Elevated levels of cholesterol indicate disorders of lipid and lipoprotein metabolism, especially liver disease (Allen et al. 2005). Our results show that these indices were significantly ($P < 0.01$) higher in cultured fishes than in their wild counterparts, which is in line with the findings of Coz-Rakovac et al. (2005) for sea bass and Xu and Cao (1989) for grass carp.

The higher value of bilirubin in loach was a consequence of the increased level of haemoglobin. Bilirubin is a breakdown product of haemoglobin and can be logically expected to increase proportionally with increasing levels of haemoglobin (Grant et al. 1987; Mckenzie et al. 2002). Though a significant difference in haemoglobin levels existed between the two loach types, the difference in total bilirubin and direct bilirubin both was insignificant.

Fluctuations in serum urea indicate declining liver function or a failing of gill osmoregulatory capability in conjunction with creatinine (Walsh et al. 2003; Allen et al. 2005). Our results show that the mean levels of serum urea nitrogen and creatinine in cultured individuals were elevated relative to those in wild individuals. It is understandable that the content of ammonia-N tended to be higher in the intensively cultured loach ponds than in the wild environment (Table 1). This elevated ammonia-N level along with hypoxic conditions that cause an alterative osmotic pressure (Shen et al. 1991) could be responsible for the higher values of urea nitrogen and creatinine.

This study is the first to characterize and provide a comparative physiological account of loach in cultured and wild ecological systems. Based on our results, it appears that human manipulation has a physiological effect on the fish and that haematological parameters are useful tools for detecting this. We therefore suggest the introduction of regular checks of the blood profiles in cultured fish, since blood collection for analytical objectives need not kill the fish and can be applied repeatedly to the same individuals. This approach may provide a rapid means for determining the physiological status of the fish, thereby enabling changing conditions to be diagnosed early, which in turn would facilitate the implementation of remedial measures during culture operations.

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