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Reproductive characteristics of *Ancherythroculter nigrocauda*, an endemic fish in the upper Yangtze River, China

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Abstract Knowledge on the reproductive biology of fishes is critical for understanding a species' life history and devising appropriate management strategies. Anthropogenic forces, such as damming and overfishing, threaten fishes endemic to the upper Yangtze River. We conducted a study on the reproductive biology of an endemic species, *Ancherythroculter nigrocauda* in order to provide information on the life history of this species and to assist in its conservation. From July 2011 to June 2012, a total of 417 fishes were captured via monthly sampling by fishermen in the Longxi River, a tributary in the upper Yangtze River. Although the female-male ratio was 1:1.03, females were predominantly larger in body size. Gonad somatic indices and oocyte diameter distribution showed that the spawning period of *A. nigrocauda* in the Longxi River ranged from April to August, with the peak in April. Body length at 50 % sexual maturity of *A. nigrocauda* was estimated to be 125 and 106 mm for females and males, respectively. Absolute fecundity of *A. nigrocauda* varied between 11,300 and 504,630 eggs, with the mean of 162,377 eggs. In conclusion, *A. nigrocauda* mature early, spawn once, and are highly fecund, all of which are consistent with an r-selected life history. It is recommended that a moratorium on fishing this species be enacted and the culture of this species be continued.

Keywords *Ancherythroculter nigrocauda* · Endemic fish · Yangtze River · Sex ratio · Spawning season · Maturity · Fecundity · Life history

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Introduction

Knowledge on the reproductive biology of fishes is critical for understanding a species' life history and devising appropriate management strategies [1, 2]. Studies in reproductive biology mainly focus on sex ratio, spawning time, gonad development, length at maturity, and fecundity. Sex ratio, an important demographic parameter, and size structure constitute the basic information needed to assess the reproductive potential and estimate stock size of a population [3]. A balanced sex ratio is considered to be an optimal condition to produce offspring [4]. The sex ratio can be influenced by many factors, such as mortality, growth rate, longevity, sex reversal, and season [5–11]. Maturity size is one of the most important biological characteristics as it is usually reflects the species' life history strategy. Knowing this information can help establish conservation measures to maintain population sizes [12]. Another important aspect of a species' life history is timing of spawning activity. Timing of reproductive events reflects adaption to environmental conditions to maximize growth and development [13]. Generally, the spawning season of fish is determined by observing gonad development, calculating the gonad somatic index (GSI), and estimating the frequency distribution of oocyte diameter (OD) [14]. Fecundity, which refers to the number of eggs in a mature ovary [15], is another important life history characteristic. Fecundity in fish tends to be correlated with individual length and weight, rather than age [3]. Finally, understanding how a species' spawning time responds to environmental variation is necessary for the proper management of a species [16].

Ancherythroculter nigrocauda is a cyprinid fish endemic to the upper Yangtze River in China. This widespread species inhabited the main channel and tributaries of the upper Yangtze, such as the Chishui River, the Longxi River, and the Xishui River in the past. This species is an important commercial fish captured mainly by gillnets and longlines, and reaches a maximum weight and length of 1.5 kg and 50 cm, respectively. Over the past decades, *A. nigrocauda* abundance has declined and its distribution has shrunk, probably because of anthropogenic degradation, including deterioration of water quality, overfishing, and damming [17]. Like many other endemic fishes of the upper Yangtze, *A. nigrocauda* has been affected by environmental changes induced by the damming of the Yangtze River and its tributaries. In particular, the Three Gorges Dam (TGD) has had a strong negative impact on the endemic fishes of the upper Yangtze. In particular, its construction resulted in the alteration of habitats and spawning grounds of *A. nigrocauda* [18, 19].

While artificial propagation and the restocking of juveniles have been implemented to manage *A. nigrocauda*, very little is known about the life history of this species. Xiong et al. [20] documents the early ontogeny and the effects of delayed first feeding on the domestication and breeding of *A. nigrocauda*. However, the sustainability of wild populations still depends on natural reproduction. Thus, effective conservation and management measures should be implemented to ensure successful natural reproduction. Unfortunately, little is known about the reproductive biology of *A. nigrocauda*. The present study aims to provide basic information on the reproductive biology, such as sex ratio, spawning season, size at first maturity and fecundity, for *A. nigrocauda* in the Longxi River. The results will be helpful for the future assessment and management of this species.

Materials and methods

Study area

Fish were collected in the Longxi River, a tributary of the upper Yangtze River (Fig. 1). The Longxi River originates from Dengdong County and flows into the Yangtze River at Luzhou, Sichuan province, China. It is 97 km long and divided by 7 hydropower stations. Its drainage area is 512 km^2. The average flow of the river is 6.58 m^3/s. A waterfall, 40 m high, near the junction with the Yangtze River prevents the migration of fish from the Yangtze River to the Longxi River. Twenty-one fish species live in the Longxi River, however, the fish assemblage is dominated by *Rhodeus ocellatus*, *Megalobrama pellegrini*, *Hemiculter leucisculus* and *Culter alburnus* in addition to *A. nigrocauda*. Fish in the Longxi River are threatened by habitat disruption caused by pollution from agriculture and small-sized chemical factories, overfishing, and habitat degradation and loss as a result of damming [21].

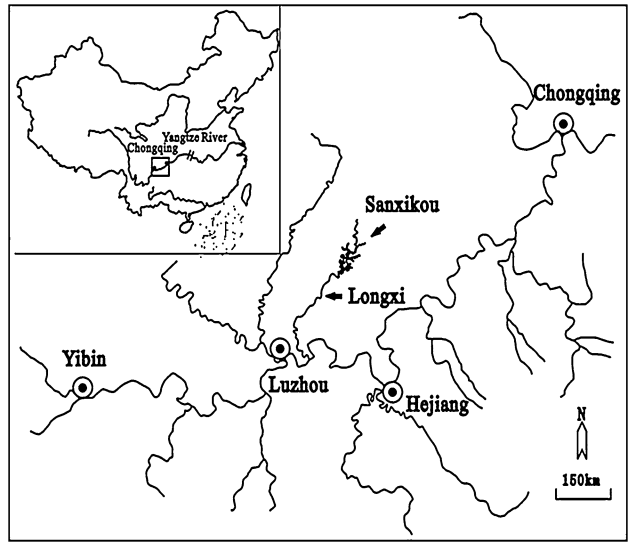

Fig. 1 A map showing the sampling area of *Ancherythroculter nigrocauda* in the Longxi River, China

Fish collection

Fish were collected from fishermen monthly from July 2011 to June 2012. Body length (the length from the tip of the snout to the posterior end of the last vertebra) was measured to the nearest 1 mm. Body, gonads and the gutted body were weighed to the nearest 0.1 g. Gonad development stages were identified with visual inspection. For each gonad, its maturity stage was classified as: (1) I, undeveloped; (2) II, early developing; (3) III, later developing; (4) IV, mature; (5) V, spawned; and (6) VI, spent [22]. The gonad development stages were distinguished according to gonad color, relative size and the presence of vitellogenic oocytes in females or milt in males [23–25] (Table 1). Females with vitellogenic oocytes were considered mature. These mature gonads were preserved in 10 % formalin for assessing the frequency distribution of oocyte diameter (OD) and fecundity of *A. nigrocauda*.

Table 1 Macroscopic maturity stages of *Ancherythroculter nigrocauda* in the Longxi River, China, July 2011–June 2012

	Classification	Macroscopic appearance
	Undeveloped	Sex cannot be identified macroscopically. Gonads are relatively tiny as two threadlike units
Female	Early developing	Gonads are translucent, small and reddish with visible vascularization. Oocytes not visually discernible
	Later developing	Increased in volume. Oocytes relatively small and clearly visible with naked eye. Oocytes couldn't be separated from ovaries
	Mature	Ovaries distinctly swelling, full of large mature oocytes, ovarian wall thin and flexible; oocytes run under moderate pressure
	Spawned	Ovaries soft, oocytes run under slight pressure or self-run with no pressure
	Spent	Ovaries significantly decreased in size, flaccid, shrunk, and bloodied, some scattered residual vitellogenic oocytes visible, ovarian wall thicker
Male	Early developing	Easily recognized testes. Thinner and longer than early developing ovaries. Increased volume, no visible vascularization, whitish in color and sometimes pinkish
	Later developing	Testes occupying about 15 % of the body cavity. No visible vascularization. Whitish in color
	Mature	Testes are large, plump, and occupy about 30 % of the body cavity. Whitish in color. Milt flowing when abdomen heavily compressed
	Spawned	Testes larger, occupying more than half of the body cavity. No visible vascularization. Whitish in color. Milt flowing freely when abdomen slightly compressed

Laboratory analysis

The preserved ovaries were used to estimate the frequency distribution of oocyte size and fecundity. A total of 66 ovaries (at least 4 ovaries per month) with oocytes from mature females were subsampled. Ovaries were weighed and all oocytes that had started vitellogenesis were counted as potentially ripe eggs. Oocyte diameter was measured with a calibrated eyepiece micrometer under a binocular microscope at 20× magnification. For each individual 100–150 oocytes were measured [26].

Data analysis

The overall sex ratio was expressed as female:male. The sex ratio was analyzed separately for month and size intervals of 10 mm. Deviations from a 1:1 sex ratio were tested using a Chi square analysis.

The spawning season was determined based on the monthly proportions of the maturity stages, monthly variations of the gonad somatic index (GSI) and oocyte diameter. The GSI was calculated as, $\mathrm{GSI} = (W_{\mathrm{g}}/W_{\mathrm{gt}}) \times 100$, where W_{g} is the gonad weight; W_{gt} is the gutted body weight.

Body length (L) at which 50 % of individuals were mature ($L_{\mathrm{m50\,\%}}$) is regarded as the mean size at first maturity [27]. $L_{\mathrm{m50\,\%}}$ was estimated based on the logistic model: $P = 1/(1 + e^{k \times (L_{\mathrm{tmid}} - L_{\mathrm{m50\%}})})$, which has been successfully used to estimate size at 50 % maturity for many species [28–31]. P is the proportion of sexually mature individuals in each size group (10 mm intervals), L_{tmid} is the mid-point of the body length class and k is a fitted parameter.

Absolute fecundity (AF) was calculated by a gravimetric method as follows: $\mathrm{AF} = n \times W/w$, where n is the number of oocytes in the subsample, W is the weight of the ovaries, and w is the weight of sub samples. Relative fecundity is expressed as the number of oocytes per gram of gutted body weight. Relationships among body length, body weight, ovary weight and AF of *A. nigrocauda* were studied by regression analysis [32].

Results

Sex ratio and size structure

Among the 417 individuals collected, 212 were identified to be male and 205 to be female, the sex ratio expressed by female:male was 1:1.03, there was no significant difference between the calculated sex ratio and the hypothetical distribution 1:1 ($\chi^2 = 0.118$, $df = 1$, $P > 0.05$). The preponderance of females over males was significant in September, December 2011 and March, 2012 ($P < 0.05$). In July 2011 and June 2012, the sex ratio showed a significant predominance of males ($P < 0.05$) (Table 2). When grouped by body size, males outnumbered females in the size range below 150 mm. Over 80 % of males were 125–155 mm in length while 74.2 % females ranged from 145 to 175 mm in length (Table 3).

Monthly gonad development

Gonads were assigned into stage II to stage VI because of an inability to distinguish male and female gonads in

Table 2 Monthly variations in sex ratio and Chi square (χ^2) values of *Ancherythroculter nigrocauda* in the Longxi River, China from July 2011 to June 2012

	Number		Sex ratio	Chi square
	Female	Male	Female: Male	
2011				
July	25	100	1:4	45.000*
August	56	55	1:0.98	0.009 ns
September	31	11	1:0.35	9.524*
October	19	9	1:0.47	3.571 ns
November	1	1	1:1	
December	15	1	1:0.07	12.250*
2012				
January	5	1	1:0.2	2.667 ns
February	4	1	1:0.25	1.8 ns
March	16	4	1:0.25	7.2*
April	10	5	1:0.5	1.667 ns
May	19	9	1:0.47	3.571 ns
June	4	15	1:3.75	6.368*
Total	205	212	1:1.03	0.118 ns

ns not significant ($P > 0.05$)

* Significant at $P < 0.05$

Table 3 Body length (*L*) frequency distribution (10-mm intervals) and the proportion of males and females in each size class of *Ancherythroculter nigrocauda* in the Longxi Rive, China in July 2011–June 2012

Range (mm)	Number	Mean *L* (mm)	(±)SD	Proportion (%)	
				Female	Male
90–100	1	97.0		0	100
100–110	2	107.5	3.54	0	100
110–120	16	117.2	2.37	18.75	81.25
120–130	38	125.8	2.37	18.42	81.58
130–140	86	136.0	2.37	29.07	70.93
140–150	106	145.0	2.37	44.34	55.66
150–160	74	155.3	2.37	55.41	44.59
160–170	39	165.8	2.37	89.74	10.26
170–180	26	175.3	2.37	80.77	19.23
180–190	12	185.3	2.37	91.67	8.33
190–200	9	195.8	2.37	88.89	11.11
200–210	3	203.0	2.37	100	0
210–220	2	211.5		100	0
220–230	2	222.5	0.71	100	0
230–240	1	235.0		0	100
Total	417				

stage I. For females, there was only one development stage (III) in November. The proportion of gonads that attained mature stages (stages IV–V) was higher than 50 % from

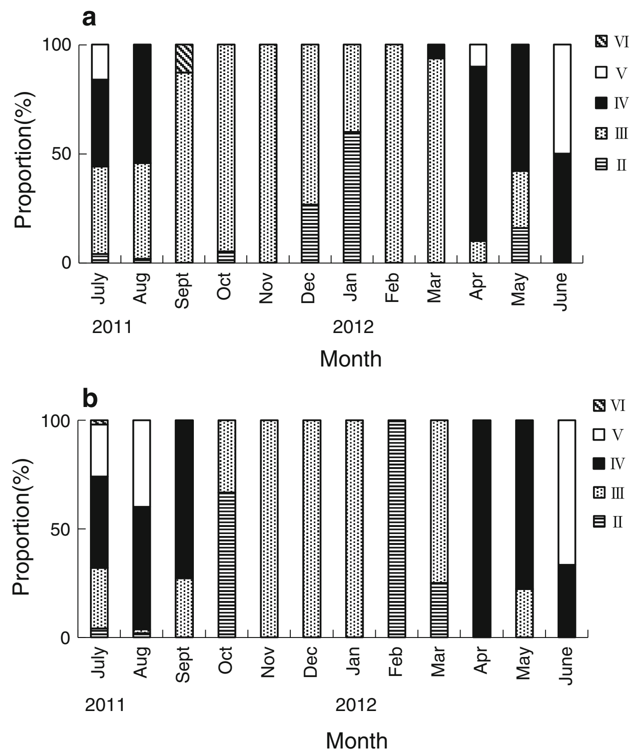

Fig. 2 Monthly maturity stages for female (**a**) and male (**b**) *Ancherythroculter nigrocauda* in the Longxi River, China. *Stages II* and *III* represent early stages of gamete development while *stages IV*, *V*, and *VI* represent stages containing mature gametes

April to August. For males, there was only one individual per month from November 2011 to February 2012. The variation in the proportion of mature gonads was similar for both females and males (Fig. 2). In May 2012, some female gonads were in stage II. The body size of these individuals ranged from 119 to 123 mm.

Monthly changes in GSI

Mean monthly GSI values were higher from April to August than in other months. It fluctuated between 0.9 and 11.7 for females and from 0.4 to 4.9 for males throughout the course of the year. During April to August, mean monthly GSI values ranged from 5.6–11.7 and 2.4–4.9 for females and males, respectively. Mean GSI reached a peak in April for both males and females (Fig. 3) and GSI decreased from April to August.

Size of oocyte

Egg sizes were measured from mature ovaries. For all 66 samples, oocyte diameter had a unimodal distribution. One sample per month in April, May, June, July and August were chosen to illustrate monthly variation in oocyte

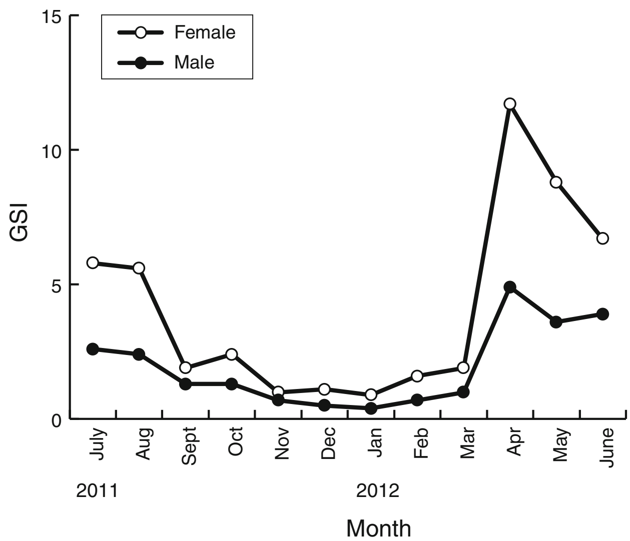

Fig. 3 Monthly changes in gonadosomatic index (GSI) of female and male *Ancherythroculter nigrocauda* in the Longxi River, China in 2011 and 2012

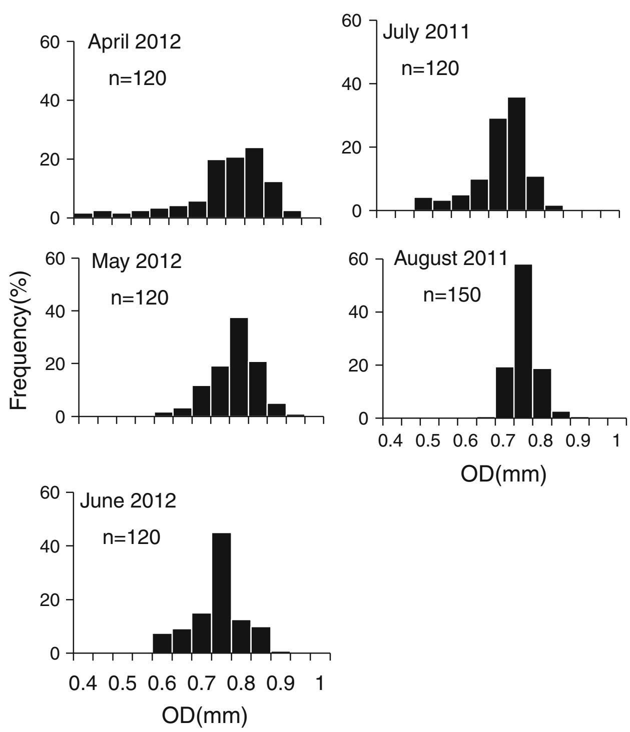

Fig. 4 Monthly size-frequency of oocyte diameter of *Ancherythroculter nigrocauda* in the Longxi River, China

diameter. These samples were from individuals belonging to the same body length group (170–190 mm). The mode of oocyte diameter was 0.85 mm in April. The oocyte diameter distribution showed a unimodal distribution (Fig. 4), suggesting that most oocytes mature and are spawned at the same time. Oocyte diameter continued to

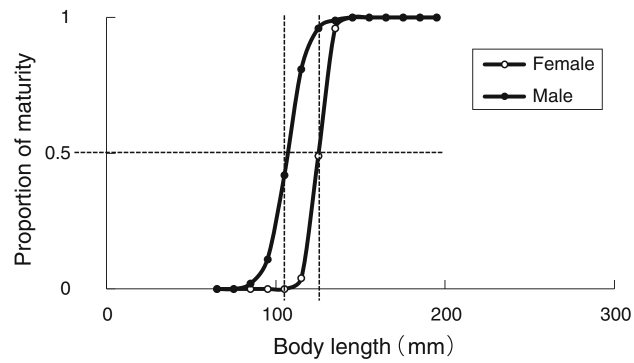

Fig. 5 Logistic functions fitted to percent mature by 10-mm intervals of *Ancherythroculter nigrocauda* in the Longxi River, China, showing the mean body length at sexual maturity

decrease from April to August. The pattern of decreasing oocyte diameter coincided with the decline in monthly GSI values.

Body length at 50 % sexual maturity ($L_{m50\ \%}$)

Size at first maturity showed clear difference between females and males. The smallest mature individual we sampled was 126 mm for females and 105 mm for males.

Body length at 50 % maturity ($L_{m50\ \%}$) was calculated by fitting the logistic function to the proportion (P) of mature individuals using the functions described as follows:

$$\text{Female}: P = 1\Big/\left(1 + e^{0.319\times(L_{tmid}-125)}\right)\ r^2 = 0.999,\ n = 205$$

$$\text{Male}: P = 1\Big/\left(1 + e^{0.176\times(L_{tmid}-106)}\right)\ r^2 = 0.989,\ n = 212$$

$L_{m50\ \%}$ of *A. nigrocauda* for females and males were estimated to be 125 and 106 mm, respectively (Fig. 5).

Fecundity

A total of 66 ovaries were used to estimate AF. The AF ranged from 11,300 ($L = 152$ mm) to 504,630 ($L = 196$ mm) eggs with a mean of 162,377 ± 110,640 eggs per fish. A significant linear relationship was found between AF and body length (L), body weight (W), and gonad weight (W_g). The linear relationships were as follows (Fig. 6):

$$AF = 3835.3L - 482069\ (r^2 = 0.55, P < 0.05, n = 66)$$

$$AF = 2801.4W - 30845\ (r^2 = 0.67, P < 0.05, n = 66)$$

$$AF = 22962W_g + 21022\ (r^2 = 0.93, P < 0.05, n = 66)$$

Relative fecundity ranged from 270.3 eggs/g to 5,193.8 eggs/g, with a mean value of 2,649.7 ± 1,160.1 eggs/g.

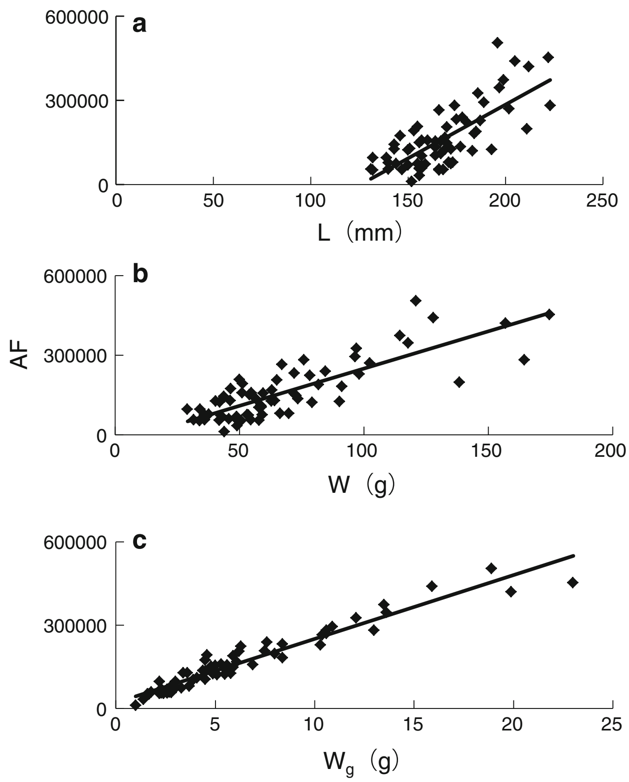

Fig. 6 Relationships between absolute fecundity (AF) and body length (*L*; **a**), body weight (*W*; **b**), and gonad weight (W_g; **c**) of *Ancherythroculter nigrocauda* from the Longxi River, China, showing the linear relationships of AF–*L*, AF–*W* and AF–W_g

Discussion

Sex ratio

Our results indicated that the population of *A. nigrocauda* had a balanced sex ratio in the Longxi River. However, females were more abundant at larger sizes. This phenomenon is found in other species, such as *Schizothorax o'connori*. Ma et al. [25, 33] showed that the greater longevity of females and high mortality of males may influence the sex ratio of *S. o'connori*. Other studies indicated that sex reversal, migration, season and differential growth rates also may be important factors influencing sex ratio as a function of size [34]. In the present study, the biased sex ratio at larger sizes in favor of female *A. nigrocauda* is thought to have resulted from the high mortality and low growth rate of males, rather than sexual reversal and migration [35]. However, the mechanism causing the biased sex ratio needs further research.

Spawning season

We concluded that *A. nigrocauda* spawns once a year and spawning activities occur from April to August. Based on the pattern of development and ovulation of oocytes, Tyler and Sumpter [36] defined two spawning patterns: single batch and multiple batches. For instance, golden catfish, *Horabagrus brachysoma*, was typical of single batch species due to its single peak of oocyte diameter distribution [23]. Likewise, *A. nigrocauda* has a unimodal distribution of oocyte diameter indicating that it is a single batch species. Observing gonad development, estimating GSI, and measuring oocyte diameter [37] were used to determine the time and duration of *A. nigrocauda* spawning. We observed mature gonads of females and males between April and August. Despite spawning in a single batch, many fish species like *A. nigrocauda* have a prolonged spawning season in the Yangtze River. For instance, *Megalobrama pellegrini* could spawn from April to July [38] and four major carps (grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix*, black carp *Mylopharyngodon piceus* and bighead carp *Aristichthys nobilis*) naturally spawn from April to July in the Yangtze River [39, 40]. This prolonged spawning season could maximize offspring survival during the early life history stages [41, 42].

Length at 50 % sexual maturity

Sexual maturity of fish is determined by genetic and environmental factors [43]. The present study indicates that the size at 50 % maturity of *A. nigrocauda* was 125 and 106 mm for females and males, respectively. Based on the von Bertalanffy growth function (Liu et al., unpublished data), it was estimated that males attained maturity within 1 year, and females attained maturity within 2 years. That males matured earlier than females may be attributed to the different growth rates [35] between females and males.

Opsariichthys bidens from the Qingyi River, China was reported to be an early maturing species. This small cyprinid species matured at a length of 118.1 and 126.5 mm for females and males, respectively. The authors conclude that early maturation is an adaptive response to variable and unpredictable environmental factors [44]. Likewise, *A. nigrocauda*, a medium-sized cyprinid, is an early maturing species which may utilize this life history strategy to adapt to an unstable environment and thereby maintain the population in the Longxi River.

Fecundity

Knowledge of fecundity is essential in understanding the life history strategy of a fish species. Fecundity research is usually conducted only for females possessing uterine eggs [45]. A previous study showed that the AF of *A. nigrocauda* in the Laixi River ranged from 4,541 to 114,356 eggs with a mean of 35,726.81 eggs. The relative fecundity of *A. nigrocauda* in the Laixi River ranged from 42.76 eggs/g to 601.72 egg s/g with a mean of 277.28 eggs/g [46]. Here we find that both the AF and the relative fecundity of *A. nigrocauda* in the Longxi River were higher than that in the Laixi River. Many factors could cause fecundity variation, such as the amount of rainfall, the concentration of dissolved oxygen, water temperature, food availability and fishing [47, 48]. This geographic variation in fecundity in *A. nigrocauda* may be a result of adaption to different ecological conditions [49].

The AF of *A. nigrocauda* increased with gonad weight. Larger sized individuals could devote more energy to gonad development, so that they might have a relatively higher AF. It is known that larger females tend to produce more eggs [50]; this is consistent with the studied population in that larger females had higher AF than those of a smaller size [51].

In conclusion, *A. nigrocauda* matures early, spawns in a single batch, and has a high fecundity which is typical of a species with an r-selected life history. The spawning season began and peaked in April and extended through August. At present, due to fishing, damming, and water quality degradation, *A. nigrocauda* is restricted to a few tributaries of the upper Yangtze River and its populations are vulnerable [52]. For the sustainability of the populations of this species, it is suggested that a moratorium on fishing this species be enforced and that restocking efforts be continued.

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