E-mail: tangfujiang@hrfri.ac.cn

Abstract

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

Cannibalism is considered one of the causes of intra-cohort size divergence in fish and is usually believed to result in increased fitness in terms of survival and reproduction, but direct evidence of this is lacking. Population demographics of the clearhead icefish (*Protosalanx* hyalocranius) from Lake Xingkai (Khanka) were investigated for one year. Size-frequencies exhibited a bimodal distribution from July through January, where the population diverged into an upper and a lower modal group based on size. Stomach content analysis confirmed the occurrence of cannibalism, where fish belonging to the larger, upper modal group preyed upon those of the smaller, lower modal group. We found P. hyalocranius does not spawn until all of the oocytes have reached maturity and then a single spawning event occurs although the oocytes may develope asynchronously in the ovary. Upper modal group females matured slightly earlier than those of the lower modal group, and reproductive investment was considerably greater in the upper modal group than the lower modal group. The lower modal males made up the majority of the population after the spawning period, which meant they may have few opportunities to participate in reproduction. Therefore, piscivory and cannibalism of *P. hyalocranius* appears to have increased fitness of the fish belonging to the upper modal group and reduced the fitness of those belonging to the lower modal group greatly.

41

42

40

- Key words: *Protosalanx hyalocranius*; clearhead icefish; cannibalism; size divergence;
- 43 reproductive investment; sex selectivity

Introduction

46

- 47 Many piscivorous fishes exhibit an ontogenetic dietary shift, transitioning from non-fish prey
- 48 to piscivory over time. An early transition to piscivory is thought to result in increased
- 49 growth and reproductive fitness, decreased mortality, and therefore enhanced lifetime fitness
- 50 (Werner and Gilliam 1984; Post et al. 1988). In some piscivorous fishes, young-of-the-year
- 51 (YOY) intra-cohort individuals exhibited differential timing to the onset of piscivory, driving
- 52 bimodal size frequency distributions and cannibalism in the age-0 group. In these cases, the
- larger, upper modal group (cannibals) increased their growth rate and decreased survival rate
- of the smaller, lower modal group (prey) (Buijse and Houthuijzen 1992; Frankiewicz et al.
- 55 1996; Post 2003; Huss et al. 2010; Heermann et al. 2013). However, direct evidence of
- increased reproductive fitness with the transition to piscivory is absent, although Post (2003)
- 57 suggested that an early transition to piscivory may result in higher lifetime fecundity since
- these fish have a larger relative size and potentially more spawning opportunities in a
- 59 lifetime.
- The clearhead icefish, Protosalanx hyalocranius (Abbott 1901), is a small, pelagic, euryhaline
- fish species of the family Salangidae that occurs in eastern Asia (Xie and Xie 1997;
- 62 Saruwatari et al. 2002). It is a short-lived semelparous fish, completing its lifecycle in about 1
- 63 year (Tang et al. 2012). Early-stage juveniles feed mainly on zooplankton, while larger
- 64 individuals in young and adult-stage could switch to fish and shrimp and feed primarily on
- conspecifics (Tang et al. 2013). Individuals tend to exhibit hierarchical growth (Zhu 1985).
- 66 Considering the unique biological characteristics of P. hyalocranius, we present evidence of
- 67 within-population size divergence, and the advantage of larger individuals over smaller
- 68 individuals in survival and reproduction. These findings provide direct evidence of
- 69 cannibalism, and its effects on reproductive fitness of both females and males.

71 Methods

- 72 Study area
- Lake Xingkai (Khanka) is a large, shallow lake (area, 4,190 km2; average depth, 4.5 m)
- 74 located in the upper reaches of the Ussuri River, a tributary of the Amur River. The lake lies
- between China and Russia, serving as a boundary. The northern part of the lake (28% of the
- total area) is located in China and the southern part (72% of the total area) is located in
- 77 Russia. Lake Khanka/Xingkai Natural Reserve is considered an important international

wetland with ecological protection in both China and Russia. All sampling for this project was conducted in China (Near the town of Dangbi, N 45°15′49.12″ - 45°15′8.62″, E 132°02′35.22″ - 132°01′7.73″, Fig.1).

Sampling procedure

Clearhead icefish (P. hyalocranius) were sampled monthly from March 2010 through March 2011 except for April 2010. Different sampling gear was used in winter and ice-free seasons to sample fish effectively. A cone trawler net (length, 3 m; diameter, 1 m; mesh size, 1 x 1 mm) was used for sampling fish in May, while a seine net (length, 200 m; width, 8 m; mesh size, 2 x 2 mm) was used for sampling from June through November 2010. For each sample, the seine net was drawn parallel to the shore where the water depth was less than 1.5 m, for about 50 m. Further, four gill nets (length, 30 m; depth, 1 m; mesh size, 10 mm, 15 mm, 20 mm, and 23 mm between opposite knots) positioned randomly were deployed in gangs, tied end to end perpendicular to the shore for 24 h per month during the freeze-up period (March 2010 and December 2010 to March 2011). For the reproductive biology and cannibalism experiments, we used two extra gill nets (length, 100 m; depth, 1 m; mesh sizes, 20 and 23 mm between opposite knots) in the freeze-up period to obtain adequate samples.

Life history traits analysis

The clearhead icefish died immediately after they were captured in nets. Over 100 specimens were caught in each monthly sample and were measured for standard length (SL; to the nearest 1 mm) and weight (WB; to the nearest 0.01 g). A row of scales typically

appears at the base of the anal fin of maturing males in mid-October, but no sexual

dissected gonads. Females have a larger ovary on the left of the ventral cavity and a smaller

dimorphism appears before that point, so the sex of each fish was determined by examining

ovary on the right side, while males have a single testis on the right of the cavity. Sex ratios

were determined, and their difference from parity was tested using a binomial test. Chi-

square tests were performed to assess differences in sex ratios among months.

The gonads of females sampled in November, December, and January were used to determine the gonadosomatic index (GSI) and maturity stage, and more than 50 specimens were dissected for reproductive biology studies each month. In P. hyalocranius, the left ovary is usually larger than the right one. The middle portion of the left ovary was selected for histological examination. Stages of maturity were classified into six phases according to Sun (1985). Both ovaries were removed and weighed to the nearest $0.0001~g~(W_G)$, and the GSI was calculated as follows:

 $GSI=100W_G/(W_{B^-}W_G)$.

The number of oocytes from dissected ovaries was counted in December and January.

Two sub-samples of tissue weighing approximately 0.1 g were taken from the middle section

of each ovary and preserved in 5% formalin. The oocyte counts in these two sub-samples

were determined using a dissecting microscope and were used to estimate fecundity if the

coefficient of variation of N per unit ovary weight was below 5% for the two sub-samples.

The total fecundity (F_A) and relative fecundity (F_R) were calculated as follows:

 $F_A = \sum C n W_O n / W_S n$ and $F_R = 100 F_A / W_B$,

where C_n is the counted number of eggs in sub-sample n, W_0 n is the corresponding ovary

weight, Wsn is the sub-sample weight and n is the number of subsamples.

To reveal the proportion of pre-vitellogenic oocyte change with overall gonad development, the most advanced oocyte diameters were determined by measuring the 10 largest oocytes under a dissecting microscope to represent the advancement of gonad development (West 1990). The proportion of pre-vitellogenic oocytes (P_p) can be described as:

 $P_{p} = 100(\text{Np} \times \text{N-1})$

The Mann-Whitney U test was used to compare the developmental stages of gonads between the upper and lower modal groups in the same cohort within the maturing season. The difference in GSI between November, December, and January, as well as the difference in fecundity between December and January, was tested using analysis of covariance (ANCOVA) with WB as the covariate. Differences in GSI, fecundity, and relative fecundity between the upper and lower modal groups in January were examined using one-way analysis of variance (ANOVA). The difference of Pp between December and January was compared using Mann-whitney U test. The relationship between Pp and the most advanced oocyte diameters was analyzed with general linear models. All statistical analyses were conducted using STATISTICA 12.0 (StatSoft, Tulsa, OK, USA). P < 0.05 was considered significant.

Analysis of cannibalism

P. hyalocranius are translucent, allowing for gross examination of gut contents and visual identification of cannibalism in individuals. We chose to dissect cannibals with visible prey in their guts. Cannibals and prey were examined for SL (to the nearest 1 mm). The prey to

cannibal size ratios in about 20 cases were calculated for each month from July through December.

Results

Growth and intra-cohort size divergence

Two cohorts of P. hyalocranius were identified during the sampling period.

Individuals collected in March 2010 belonged to the 2009 cohort and the individuals sampled after March 2010 belonged to the 2010 cohort. Samples collected in March 2010 ranged in SL from 81 to 225 mm and consisted of reproductive females and males from the 2009 cohort. P. hyalocranius juveniles belonging to the 2010 cohort were sampled from May 2010 onwards, and the LS distribution pattern was unimodal (range, 10-20 mm) until early July, when a bimodal length distribution began to emerge and lasted through January 2011. From November to January, the reproductive population could be divided into the lower modal group (SL < 120 mm) and upper modal group (SL ≥120 mm) on the basis of size (Fig. 2). The population sampled in February and March did not exhibit a bimodal distribution because the fish died after spawning.

Characteristics of cannibals and prey

Cannibalism was first observed in July. The SL of cannibals sampled during this month ranged from 65 to 91 mm, while those of prey ranged from 38 to 52 mm. Both cannibals and prey grew larger over time. The cannibals generally reached a SL of >120 mm by November, and the prey were usually smaller than 120 mm (Table 1). Given that the reproductive

population in November could be divided into two size groups by SL with 120 mm as the cutoff (Fig. 2), it is apparent that the upper modal group was comprised of cannibals and the lower modal group was comprised of the prey. The prey to cannibal size ratio ranged from 0.47 to 0.67 (Table 1).

Examination of sex ratio

The sex of all sampled individuals was determined from early October 2010 to early March 2011. Monthly observations of sex ratios (male to female) did not differ from parity (1:1) from October through January (P = 0.808, 0.410, 0.563, 0.621, respectively) or vary significantly among months (x2 = 1.499, df = 3, P = 0.683). However, the sex ratios in February and March were 13.4 and 18.1, respectively, due to natural mortality of females after spawning (Fig. 3). The remaining population mainly consisted of males in the lower modal group (Fig. 2). From October through January, sex ratios did not differ from parity in the upper modal group (P = 0.453, 0.450, 0.475, 0.527, respectively) or the lower modal group (P = 0.462, 0.409, 0.428, 0.635, respectively).

Gonad development and the differences between the two groups

Oocytes developed asynchronously in ovaries of P. hyalocranius; pre-vitellogenic (Phase III & II) oocytes and vitellogenic (Phase IV & V) oocytes existed in the same ovary. The gonads of female P. hyalocranius did not develop to stage II until early October, after which they developed quickly to stage III in the first half of October. In early November, individuals in stages III and IV of gonadal development accounted for 61.5% and 38.5% of the female population, respectively. A few oocytes developed to stage V at the beginning of

December, but all the oocytes in an ovary did not develop synchronously to the same stage. Most female gonads developed to stage IV or V and the fish could spawn in early January, but only a few post-spawning individuals were sampled in January and March (Fig. 4). The oocytes developed asynchronously, and oocytes of different developing phases may commonly occur in the same ovary of maturing females (Fig.5).

The Pre-vitellogenic oocytes occurred in samples from December and January (100%, n=50 and 22%, n=59, respectively). The proportion of pre-vitellogenic oocytes (Pp) in gonads sampled in January became significantly lower compared to those sampled in December (Z=-15.99, P=0.000, see Fig. 5). Linear regression indicated that the proportion of pre-vitellogenic oocytes declined significantly (F1,107 = 6.18, P=0.014, see Fig.6), as indicated by the observation of increased oocyte diameters in the developing gonads. This indicates that P. hyalocranius does not spawn until all of the oocytes have reached maturity and then a single spawning event occurs.

Most female gonads developed to stage III or IV in early November, and females in the upper modal group seemed to mature faster than those in the lower modal group, although this difference was not significant (Z=-1.117, P=0.239). In early December, females in maturity stage IV accounted for the largest proportion both in the upper (88.5%) and lower modal groups (81.5%), and the maturity stage distribution was not significantly different between the groups (Z=-1.401, P=0.161). In early January, almost all of the female gonads developed to stage IV or V, and no significant differences were observed in the developmental rates between the groups (Z=-1.934, P=0.053) (Fig. 7.).

Differences in reproductive investment between the groups

212 The GSI increased dramatically from November to January, ranging from 1.8 to 10.2% in November, 2.9 to 44.1% in December, and 7.6 to 76.4% in January (Fig. 8). Results of the 213 ANCOVA (BW as the covariate) indicated that the differences in GSI among the three 214 months were all significant (F2,161 = 185.3, P = 0.000). The GSI of fish in the upper modal 215 group was significantly higher than that of fish in the lower modal group in November 216 (ANOVA, F1,50 = 56.3, P = 0.000), December (ANOVA, F1,51 = 50.0, P = 0.000), and217 January (ANOVA, F1,57 = 95.1, P = 0.000) (Fig. 8). 218 Results of the ANCOVA (BW as the covariate) also revealed no significant difference in 219 220 fecundity between early December and early January (F2,110 = 3.4, P = 0.067). In other words, there was no down-regulation of fecundity during gonad development or 221 indeterminate spawning. Samples from January were used to analyze intra-population 222 divergence of fecundity. Fecundity ranged from 1,592 to 36,705 eggs, and relative fecundity 223 ranged from 500 to 1,646 eggs per gram BW. The relative and absolute fecundity were 224

significantly higher in females from the upper modal group than those in the lower modal

group (ANOVA, F1,57 = 121.5, P = 0.000 and ANOVA, F1,57 = 21.94, P = 0.000,

respectively) (Fig. 9).

228

229

231

232

225

226

211

Discussion

230 Piscivorous, cannibalism and size divergence

Size variation among clearhead icefish populations has been previously reported (Zhu 1985; Wang and Jiang 1992; Tang et al. 2003). Xie et al. (2001) noted two peaks within the

size frequency distribution of a reproductive population. However, this is the first report of within-cohort bimodal size distribution in *P. hyalocranius*. Bimodal size distribution is well documented in iteroparous fishes (Huss et al. 2010; Alejo-Plata et al. 2011), and studies on Percidae species revealed diet shift contributing to the size divergences in YOY fish, and that the extreme growth of the upper modal group was induced by piscivory (cannibalism) (Frankiewicz et al. 1996; Post 2003; Urbatzka et al. 2008). The synchronous investigation on diet composition showed that a few bigger individuals of *P. hyalocranius* began to feed on fish and cannibalism also occured in early July, and then piscivory and cannibalism accompanied the size bimodal pattern forming (Tang et al. 2013). And the victims of cannibalism accounted for the majority of the diet of cannibals in December and January (Tang et al. 2013).

Reproductive investment

The absolute and relative fecundity reported in the present study for a population of *P. hyalocranius* in Lake Khanka shows wider range (1,592 - 36,705 eggs, and 500 - 1,646 eggs/gram-BW) than populations from other locations (3,295 to 34,520 eggs, and 340 to 1,290 eggs/gram-BW) (Zhang et al. 1981; Xie et al. 2001). While there is no published information illustrating the difference in reproductive investment between the two modal groups of clearhead icefish, this investigation demonstrates that the reproductive investment of females belonging to the upper modal group(cannibals) was significantly higher than that of females from the lower modal group(victims) in terms of GSI, absolute fecundity, and total fecundity. In common, larger fish has higher absolute fecundity and lower relative fecundity

than the smaller conspecifics. While *P. hyalocranius* cannibals has both higher absolute fecundity and relative fecundity than the victims, which means piscivory and cannibalism increased reproductive fitness greatly.

Gonad development and spawning pattern

Researchers have offered multiple theories to describe the reproductive strategy of *P. hyalocranius*. Sun (1985) reasoned that *P. hyalocranius* spawn twice during their lifetime upon examination of their gonad histology, but Xu et al. (2000) determined that this species spawns once before death. Xie et al. (2001) theorized that *P. hyalocranius* can spawn once or multiple times. Our research supports the theory that *P. hyalocranius* exhibits a semelparous reproductive strategy, although oocytes developed asynchronously in the same gonad. This is different from other semelparous species such as chum salmon *Oncoryhchus keta* (Crozier et al. 2008).

Sex ratio and mate selectivity

Sex ratios did not differ from parity before spawning (October through December) or during spawning (primarily early to mid-January) in both the upper modal and lower modal groups. This is similar to reports for this species from other locations (Zhang et al. 1981; Sun and Zhou 1989; Xu et al. 2000; Xie et al. 2001). However, we found that the reproductive population was mainly composed of males in the lower modal group after January. We hypothesize that males in the lower modal group have a reduced opportunity to participate in spawning. The intra-cohort sex selectivity of *P. hyalocranius* may influence evolution

towards early birth and faster growth, although this theory requires further study.

Life history strategy

The *P. hyalocranius* is a small fish that matures early with a short life span and relatively high fecundity. This makes it an exception from the three-point life-history continuum model proposed by Winemiller and Rose (1992). Larger individuals (upper modal group) tend to exhibit an opportunistic life-history strategy, but characterized by relatively higher fecundity. An opportunistic colonization strategy facilitates range expansion of an invading species through early maturity in frequently changing or stochastic environments (Fox et al. 2007; Ribeiro et al. 2009). Exceptional higher fecundity would undoubtedly contribute to rapid increase in population size and spread of the opportunistic fish, *P. hyalocranius*.

Conclusion

Transition to feed on fish increased the investment of the female *P. hyalocranius* and may also increase the fitness of male in reproductive competing. This research provided a direct evidence that transition to be carnivorous is positively significant in evolution, although further study on increasing the fitness of male *P. hyalocranius* need to be conducted.

Acknowledgements

We wish to express our sincere gratitude to Zhang Xiaoguang and Zhou Dahai for their important support in specimens sampling, and Jennifer Bailey of US Fish and Wildlife Service for her help in manuscript preparation. This work was supported by the National Natural Science Foundation of China [grant number 31201993] and the National Basic

Research Project of China (973 program) [grant number 2009CB119200]. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

299

300

301

References

Alejo-Plata C, Díaz-Jaimes P, Salgado-Ugarte IH, 2011. Sex ratios, size at sexual maturity, and spawning seasonality of dolphinfish (Coryphaena hippurus) captured in the Gulf of Tehuantepec, Mexico. Fisheries Research 110: 207-216. Buijse AD, Houthuijzen RP, 1992. Piscivory, growth, and size-selective mortality of age 0 pikeperch (Stizostedion lucioperca). Canadian Journal of Fisheries and Aquatic Sciences 49: 894-902. Crozier LG, Hendry AP, Lawson PW, Quinn TP, Mantua NJ, Battin J, Shaw RG, Huey RB, 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications 2:252-270. DOI: 10.1111/j.1752-4571.2008.00033.x Fox MG, Vila-Gispert A, Copp GH, 2007. Life-history traits of introduced Iberian pumpkinseed Lepomis gibbosus relative to native populations. Can differences explain colonization success? Journal of Fish Biology 71(Suppl D):56-69. Frankiewicz P, Dabrowski K, Zalewski M, 1996. Mechanism of establishing bimodality in a size distribution of age-0 pikeperch, Stizostedion lucioperca (L.) in the Sulejow Reservoir, Central Poland. Annales Zoologici Fennici 33:321-327.

Heermann L, Scharf W, van der Velde G, Borcherding J, 2014. Does the use of alternative

food resources induce cannibalism in a size-structured fish population? Ecology of 321 Freshwater Fish 23(2):129-140. 322 323 Huss M, Kooten VT, Persson L, 2010. Intra-cohort cannibalism and size bimodality: a balance between hatching synchrony and resource feedbacks. Oikos 119:2000-2011. 324 325 Post DM, Kitchell JF, Hodgson JR, 1998. Interactions among adult demography, spawning date, growth rate, predation, overwinter mortality, and the recruitment of largemouth bass 326 in a northern lake. Canadian Journal of Fisheries and Aquatic Sciences 55:2588-2600. 327 Post DM, 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth 328 329 bass. *Ecology* 84:1298-1310. Ribeiro F, Collares-Pereira MJ, Moyle PB,2009. Non-native fish in the fresh waters of 330 Portugal, Azores and Madeira Islands: a growing threat to aquatic biodiversity. Fisheries 331 332 Management and Ecology 16:255–264. Saruwatari T, Oohara I, Kobayashi T, 2002. Salangid fishes: their past, present and future. 333 Fisheries Science 68(Suppl 1):71–74. 334 Sun G, 1985. On the maturity of the female gonads and the type of spawning of the ice fish. 335 Journal of fisheries of China 9:363-368. (In Chinese with an English abstract). 336 Sun G, Zhou Z, 1989. A preliminary study of ecology of *Protosalanx hyalocranius* in 337 Yangtze River estuary and nearby marine waters. Trans Ocean limin 4:76-79. (In Chinese 338 with an English abstract). 339 Tang F, Liu W, Wang J, Froese R, Xie S, 2012. Growth, length-weight relationship and 340 biological information on the clearhead icefish (Protosalanx hyalocranius Abbott, 1901) in 341

Lake Khanka (Xingkai). Journal of Applied Ichthyology 28:842-844.

- Tang F, Liu W, Wang J, Li Z, Xie S, 2013. Diet compositions and transition for clearhead
 icefish (*Protosalanx hyalocranius*) in Lake Xingkai. *Zoological Research* 24:493-498. (In
- 345 Chinese with an English abstract).
- Tang Z, Xie H, Xie Y, Li X, Cui S, 2003. Growth and population structure of *Protosalanx*
- 347 hyalocranius in Shuifeng Reservior, Liaoning, China. J Dalian Fish Univ 3:164-169. (In
- 348 Chinese with an English abstract).
- 349 Urbatzka R, Beeck P, vander Velde G, Borcherding J, 2008. Alternative use of food resources
- causes intra-cohort variation in the size distribution of young-of-the-year perch (Perca
- fluviatilis). *Ecology of Freshwater Fish* 17:475-480.
- Wang Y, Jiang Q, 1992. Research of growth properties of *Protosalanx hyalocranius* Abbott.
- *Journal of Lake Science* 1:56-62 (In Chinese with an English abstract).
- Werner EE, Gilliam JF, 1984. The ontogenetic niche and species interactions in size-
- 355 structured populations. *Annual review of ecology and systematics* 15:393-425.
- Winemiller KO, Rose KA, 1992. Patterns of life history diversification in North American
- fishes: implication for population regulation. Canadian Journal of Fisheries and Aquatic
- 358 *Science* 49:2196–2218.
- 359 Xie H, Tang Z, Xie Y, Li B, Li X, Yuan F, Duan K, Sun Y, 2001. Aspects of repoductive
- biology of large icefish (*Protosalanx hyalocranius*) in Shuifeng Reservoir. *J Dalian Fish*
- 361 *Univ* 2:79-86. (In Chinese with an English abstract).
- 362 Xie Y, Xie H, 1997. Classification, Distribution, and Population Ecology of Salangidae
- Fishes. *Chinese Journal of Fisheries* 10:11-19. (In Chinese with an English abstract).
- 364 Xu M, Wu H, Liu Y, Zhang L, 2000. Biological effect of transplanting *Protosalanx*

365	hyalocranius in Guandaohe reservoir (III): Propagation behavior. J Lake Sci 1:94-96 (In
366	Chinese with an English abstract).
367	Zhang K, Zhuang D, Zhang L, Gao C, Zhang J, Xu A, 1981. On the <i>Protosalanx</i>
368	hyalocranius and its propagation in Hongze Lake. J Fish Chin 1:29-39. (In Chinese with
369	an English abstract).
370	Zhu C, 1985. A preliminary study on growth and feeding habits of icefish in Taihu Lake.
371	Journal of Fisheries of China 3:275-287. (In Chinese with an English abstract).
372	
373	
374	
375	