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Who eats whom, when and why? Juvenile cannibalism in fish Asian seabass

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ABSTRACT

While juvenile cannibalism plays an important role in the evolution of organisms in natural populations, it is a serious problem in aquaculture. A number of genetic and environmental factors result in different rates of cannibalism. Whether there is kin recognition in juvenile cannibalism in fish is poorly understood. We studied cannibalism and kinship recognition in juveniles of Asian seabass using molecular parentage analysis with polymorphic microsatellites. In the three mass crosses, under an ordinary feeding scheme without size grading, the rate of juvenile loss due to cannibalism was 1.08% per day. In the group without feeding for 24 h, 2.30% ± 0.43% of offspring per day were lost within 24 h due to cannibalism. We detected that juveniles avoided cannibalizing their siblings when they were not hungry, whereas cannibalism among siblings increased when they were hungry. These data suggest that there is kin discrimination in fish cannibalism. Raising genetically closely related offspring in the same tanks and appropriate levels of feeding may reduce the rate of cannibalism. We hypothesized that the chemical cues for kin discrimination might be secreted by fish skins. To test this hypothesis, we analyzed gene expression profiles in the skins of juveniles under slightly and very hungry conditions using RNA sequencing and bioinformatics analysis. Genes differently expressed under slightly and very hungry conditions were identified. Among them, genes from the trypsin family were significantly down-regulated under starved conditions, suggesting that they may play a role in kin discrimination.

1. Introduction

Asian seabass (*Lates calcarifer*) is an important marine food fish species and has been cultured for over 30 years in Southeast Asia (Jerry, 2013; Yue, Li, Chao, Chou, & Orban, 2002) and recently in Australia (Domingos, Smith-Keune, & Jerry, 2014; Domingos et al., 2013; Qin, Mittiga, & Ottolenghi, 2004). Its annual production was over 60,000 tons in 2012 according to the FAO statistics (FAO, 2014). Selective breeding for some traits (e.g. growth and disease resistance) has been started in Singapore (Wang et al., 2008; Yue et al., 2002; Yue et al., 2009) and Australia (Domingos et al., 2013; Loughnan, Smith-Keune, Jerry, Beheregaray, & Robinson, 2016). A large number of DNA markers (Wang et al., 2011; Wang, Wan, Lim, & Yue, 2016; Wang et al., 2015; Zhu et al., 2010) and a molecular parentage method (Wang et al., 2008) have been developed, and used in analyzing genetic diversity (Yue et al., 2009), estimating heritabilities and parent contribution to offspring (Wang et al., 2008), mapping QTL (Liu, Wang, Wong, & Yue, 2016a; Liu et al., 2016b; Wang et al., 2011) and other genetic studies (Wang et al., 2016). All these efforts have contributed to the understanding of the biology and the genetic improvement of Asian seabass. However, in Asian seabass hatcheries, cannibalism causes severe loss of juveniles during the early stages of development (from 21 to 75 days post hatch (dph)) (Parazo & Avila, 1991; Qin et al., 2004). Most hatcheries use size grading to reduce the rate of cannibalism, which is very effective, but labor-intensive. Novel and simple approaches

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to minimize the rate of cannibalism are still limited in Asian seabass.

Cannibalism refers to the action of attacking and consuming a member of one's own species (Hecht & Pienaar, 1993). In aquaculture, intracohort cannibalism causes a serious problem in larval culture (Hecht & Pienaar, 1993). A number of genetic and environmental factors may influence the cannibalism. It is now clear that fish size and size variation (Kailasam, Thirunavukkarasu, Abraham, Chandra, & Subburaj, 2011; Xi, Zhang, Lü, & Zhang, 2016), feeding (Fibeiro & Qin, 2015; Kailasam et al., 2011), shelter (Moksnes, Pihl, & Van Montfrans, 1998), culture density (Sukumaran et al., 2011) and light intensity (Arockiaraj & Appelbaum, 2011; Qin et al., 2004) affect the rate of cannibalism. Genetic factors that cause cannibalism range from inherent differences in growth rates to a genetic predisposition for cannibalistic behavior (Baras et al., 2000; Hecht & Pienaar, 1993). Individuals expressing a cannibalistic phenotype exhibit a greater probability of cannibalistic behaviors (Baras & Jobling, 2002). A number of methods have been suggested to reduce the rate of cannibalism in hatcheries. These include supplying shelter (Moksnes et al., 1998), reducing light density (Qin et al., 2004), decreasing culture density (Sukumaran et al., 2011), feeding enough (Kailasam et al., 2011), and size grading (Loughnan et al., 2013). However, most methods are complicated and labor-intensive. Further studies on environmental and genetic factors influencing the rate of cannibalism may bring new methods and ideas in mitigating cannibalism. Although kin recognition has been studied in fishes (Pfennig, 1997; Smith & Reay, 1991) and there is evidence that kin selection plays a role in egg-cannibalism in fish (DeWoody, Fletcher, Wilkins, & Avise, 2001), whether kin recognition plays a role in fish cannibalism remains largely unknown.

The major purpose of this study was to examine whether kin recognition played a role in the rate of cannibalism in Asian seabass using molecular parentage to extend our knowledge about genetic factors affecting the rate of cannibalism. We found that juveniles avoided cannibalizing their siblings when they were not hungry, whereas cannibalism among siblings increased when they were hungry, suggesting that there is kin discrimination in fish cannibalism. We also tried to find the genes involved in kin recognition in cannibalism by sequencing RNA isolated from skins of juveniles at the age of 36 days post hatch (dph) using Hiseq 2000. RNA sequencing revealed that genes from the trypsin family were differentially expressed in skins of fish under normal and hungry conditions, suggesting that they play a role in kin discrimination in Asian seabass.

2. Materials and methods

2.1. Fish, sampling and DNA extraction

To study cannibalism in a hatchery of Asian seabass, three batches of offspring from natural spawning of three mass crosses in a breeding program were used. In each of mass crosses 1 (MC1) and 2 (MC2), 10 male breeding fish and 10 female breeding fish were used to produce progeny. In mass cross 3 (MC3), 15 male brooders and 15 female brooders were crossed to produce offspring. From each mass cross, 60,000 fertilized eggs collected on the same day were cultured in a tank (volume: 1 ton) following the standard culture protocol set by Marine Aquaculture Centre, AVA (Wang et al., 2008). From each cross, 400 fry at the age of 2 dph were collected to analyse parentage to examine the contribution of brooders to offspring. DNA from each fry was extracted on 96-well PCR-plates using a rapid and cost-effective method developed in our lab previously (Yue & Orban, 2005). All handling of fish was conducted in accordance with the guidelines on the care and use of animals for scientific purposes set up by the Institutional Animal Care and Use Committee (IACUC) of the Temasek Life Sciences Laboratory, Singapore. The IACUC has specially approved this study within the project “Breeding of Asian seabass” (approval number is TLL (F)-12-004).

2.2. Multiplex-genotyping of microsatellites and parentage analysis to determine the parental contribution to offspring

Parentage analysis was conducted for all three mass-crosses using nine microsatellites *Lca008*, *Lca020*, *Lca021*, *Lca058*, *Lca064*, *Lca069*, *Lca070*, *Lca074* and *Lca098*) (Liu et al., 2012; Zhu et al., 2010) in a multiplex-PCR using the software PAPA (Duchesne, Godbout, & Bernatchez, 2002) as described previously (Wang et al., 2008). Briefly, PCR was conducted for nine microsatellites in a multiplex PCR as described previously (Zhu et al., 2010). PCR was performed in a volume of 25 μL consisting of 10 ng of genomic DNA, 1 × PCR buffer with 1.5 mM $MgCl_2$, 0.05–0.8 μM of each primer of the nine microsatellites, 25 μM of each dNTP and 1 unit of Taq DNA Polymerase (Fermentas, PA, USA). The following PCR program was used to amplify the nine microsatellites simultaneously: 94 °C for 2 min followed by 36 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, then a final step of 72 °C for 5 min. Genotyping of microsatellites was carried out on a capillary DNA sequencer ABI 3730xl (Applied Biosystems, CA, USA). The Rox-500 size standard was used to estimate the allele size at each microsatellite using the software GeneMapper 5.0 (Applied Biosystems, CA, USA). Genotypes at the nine markers were exported for parentage analysis using PAPA. For parentage analysis, we use the most stringent parameters (i.e. error = 0) to ensure correct assignment.

2.3. Examining cannibalism and determining whether there is kin discrimination in cannibalism

The offspring of the three mass-crosses mentioned above were used to examine cannibalism and determine whether there is kin discrimination in fish cannibalism. The reason for the use of mass crosses to examine whether there is kin discrimination in cannibalism is that mass crosses reflect the real situation in the production of Asian seabass in hatcheries. At the age of 30 dph, 4800 juveniles at the same age from each mass cross were taken from the original tanks where the fish were cultured. These 4800 juveniles from each mass cross were split into two other tanks (volume: 1-ton). These 2400 juveniles/tank were fed on sized pellets (INVE, PHICHIT, Thailand) to satiation twice (9:00 a.m. and 5:00 p.m.) daily for six days. At 5:00 p.m. on the 6th day, due to loss of some juveniles by cannibalism and death, only 2000 fish were kept in each tank. At 36 dph, the fish in the two tanks were not fed. At 1:00 p.m., the fish (Group 1) in one tank were anaesthetized using Aqui-S (Aqui-S, Lower Hutt, New Zealand) following the supplier's instructions. The number of fish was counted. Fish eating other fish were checked by dissecting the stomachs of the predators. Fin clips of the predators were collected and stored in 75% ethanol. One prey in the stomach of each predator was collected by dissecting stomachs. To avoid potential contamination of the predators, we rinsed the collections from the stomachs using PBS buffer. After the rinse, we stored the collections from the stomachs in 75% ethanol. At 5:00 p.m., the fish (Group 2) in another tank were treated in the same way as in the first tank. Fin clips and prey in the stomachs of the predators were collected as described above. DNA was isolated as described previously (Yue et al., 2009).

Parentage assignment was conducted as described above. Percentages of full-sib, half-sib and non-kin cannibalisms under slightly (Group 1) and very hungry (Group 2) conditions were directly calculated based on the number of full-sib, half-sib and