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Aquaculture 247 (2005) 267–273

Aquaculture

www.elsevier.com/locate/aqua-online

Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci

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Received 15 February 2004; received in revised form 1 February 2005; accepted 1 February 2005

Abstract

Hybridization between silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) has been reported to occur fairly frequently in commercial aquaculture hatcheries in Bangladesh. The consequences of hybridization for broodstock purity had not previously been investigated. Allelic variation at three microsatellite DNA loci isolated from silver carp routinely distinguished between silver carp and bighead carp. These markers were used in the analysis of samples collected from hatcheries in different regions of Bangladesh. Of 422 hatchery broodstock that were morphologically identified as silver carp, 8.3% had bighead allele(s) at one or more of the three microsatellite loci, while 23.3% of the 236 fish morphologically identified as bighead carp had silver carp allele(s) at one or more loci. The results suggested that while some of these fish might be F_1 hybrids, others had more complex genotypes, suggesting further generations of hybridization or introgression between the species in hatcheries, with potentially damaging consequences for the integrity of these stocks and their performance in aquaculture.

Keywords: *Hypophthalmichthys molitrix*; *Aristichthys nobilis*; Microsatellite loci; Hybridization; Introgression; Broodstock

1. Introduction

Aquaculture accounts for nearly 40% of total fish production in Bangladesh (Hussain and Mazid, 2001), with carps, produced in polyculture systems, dominating. Silver carp (*Hypophthalmichthys molitrix*) and

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doi:10.1016/j.aquaculture.2005.02.018

bighead carp (*Aristichthys nobilis*) were introduced into Bangladesh for aquaculture (Hussain and Mazid, 2001). The vast majority of the seed for aquaculture in Bangladesh is produced in private hatcheries, which are estimated to number in excess of 700. There is much anecdotal evidence of genetic deterioration of carp hatchery stocks in Bangladesh, through inbreeding, negative selection and hybridization (Hussain and Mazid, 2001). Stocks of exotic (i.e., non-indigenous) species of carps are particularly vulnerable to such degradation, given that the opportunities to go back to wild populations for broodstock replenishment are very limited. Furthermore, anecdotal evidence suggests that hybridization between silver carp and bighead carp is common, at least partly due to a shortage of mature bighead carp males towards the end of the breeding season. Reported aquaculture production of the silver carp in Bangladesh in 2001 was 130,000 T, or 21.7% of freshwater aquaculture production (FAO, 2003), while there was no reported production figure for bighead carp. Bighead carp broodstock are present in many hatcheries, so presumably aquaculture production of bighead carp is present, but not high enough to be reported separately.

Several types of genetic markers have been developed which have potential application to fisheries and aquaculture. Used appropriately, these have the potential to differentiate between species, populations and individuals. Microsatellite DNA loci (Estoup and Angers, 1998) have a core of short, repeated units (generally 2–5 base pairs), flanked by unique sequence DNA. Primers for polymerase chain amplification (PCR) can be designed from the flanking DNA sequence, ensuring specific amplification of a single locus, with variation between alleles coming largely from variation in the number of repeat units in the central core region. Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent–offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for population genetics and phylogenetic studies (O'Connell and Wright, 1997; Estoup and Angers, 1998). Primers developed for one species will often cross-amplify microsatellite loci in closely related species (Estoup and Angers, 1998).

Microsatellite markers have been developed for silver carp from a stock held by the Northwest Fisheries Resource Development and Management Project (NFRDMP), Parbatipur, Bangladesh (J.B. Taggart et al., unpublished data). The stocks of silver and bighead carp held here originate from fairly recent (1994) direct importations from wild populations in China, and due to this and the management practices in this hatchery, are unlikely to have been hybridized. Preliminary screening showed that some of these loci could be used as species-specific markers to distinguish between silver and bighead carp. Although protein polymorphisms can be used to distinguish between these species (Valenta et al., 1990, 1991; Egenolf, 1996), and have continued to be used in recent years in hybridization studies in fish (e.g. Bruwer and van den Bank, 2002; Rognon and Guyomard, 2003; Costedoat et al., 2004), the simplicity of sample collection (biopsy – e.g., fin tissue or scales), preparation and transportation (fixation in 95% ethanol and transportation at ambient temperature) for PCR-based DNA techniques make sampling under field conditions much easier than for proteins. A variety of PCR-based DNA markers, including microsatellite loci (Docker et al., 2003), RAPDs (Elo et al., 1997), mtDNA (Docker et al., 2003; Rognon and Guyomard, 2003; Costedoat et al., 2004), several nuclear genes (Rubidge et al., 2001; Docker et al., 2003; Gross et al., 2004) and ribosomal DNA (Padhi and Mandal, 1997; Gross et al., 2004) have been used in studies on hybridization in fish.

A survey was designed in which fin samples and information about hatchery management techniques were collected from several hatcheries in each of five major hatchery areas in Bangladesh. This paper reports on the genetic analysis of samples from these hatcheries.

2. Materials and methods

Silver carp genomic DNA libraries were constructed, employing a microsatellite enrichment methodology (Kijas et al., 1994). This protocol uses biotinylated microsatellite motif sequences bound to streptavidin-coated magnetic particles as the basis for enrichment. Briefly, the libraries were constructed