

TOPIC AREA

QUALITY SEEDSTOCK DEVELOPMENT



GENETIC DIVERSITY OF STRIPED SNAKEHEAD (*CHANNA STRIATA*) IN CAMBODIA AND VIETNAM

ASIA PROJECT: CAMBODIA & VIETNAM

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Quality Seedstock Development/Study/16QSD01UC

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Objectives

1. To characterize and compare genetic diversity of (1) wild (non-domesticated) snakehead populations collected from different natural water bodies in Cambodia, and (2) Cambodia wild (non-domesticated) striped snakehead and Vietnamese domesticated striped snakehead (*Channa striata*) collected from different hatcheries in the Mekong Delta inferred from mitochondrial DNA markers.
2. To provide basic information and wise recommendations for (1) striped snakehead domestication and selection breeding and farming in Cambodia, and (2) possible exchange of snakehead genetic resources between Cambodia and Vietnam.

Significance

The striped snakehead (*Channa striata*) is one of the most widely distributed snakehead species with a native range covering southern China, Pakistan, most of India, southern Nepal, Bangladesh, Sri Lanka and most of the Southeast Asian countries (Froese and Pauly, 2010). It is well-represented in the ditches, swamps, lakes, paddy fields, irrigation canals, small streams, mining pools, and old ponds, showing the highest preference for muddy stagnant waters. Striped snakehead is often utilized in the biomedical field in many local Asian communities (Mat Jais et al., 1994; Baie and Sheikh, 2000). The striped snakehead is an economically important species in both culture and capture fisheries throughout Southeast Asian countries including Cambodia and Vietnam.

This species is being extensively farmed, particularly in the Lower Mekong River basin of Cambodia and Vietnam. In Cambodia wild snakeheads are generally cultured in smaller cages and ponds. Feed represents more than 70% of the total operational cost and the main type of feed for snakehead culture is small-sized or low value fish, representing 60 to 100% of the total feed used depending on feeding strategies adopted by different farmers (So et al., 2005). During the dry season (October to May), the most important source of feed is freshwater small-sized fish, while more marine small-sized or low-value fish species are used during the rainy season (June to September) (So et al., 2005). Importantly, the snakehead production contributes more than 70% of total aquaculture production in Cambodia and one of

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the major aquaculture fish species in Mekong Delta of Vietnam, which has been farmed since the 1990s (So Nam, 2009; Sinh et al., 2012) due to its popularity as food being found in most Cambodian and Vietnamese dishes at all wealth class levels (i.e., from poor, medium to rich people) and high market and trade demand.

The government of Cambodia put a ban on snakehead farming in September 2004 by the Announcement No. 4004 kor.sor.ko.sor.chor.nor. The reason for this was the potential negative impacts on wild fish populations from wasteful snakehead seed collection and on other fish species diversity, particularly the freshwater small-sized fish used as feed for snakehead aquaculture, and also potential negative effects on poor consumer groups from decreased availability of small-sized fish due to dependency of snakehead aquaculture on small-sized (So et al., 2007). In order to remove this ban, the same Announcement mentioned that successful technologies of domesticated breeding, weaning and rearing/growing-out of snakeheads using formulated diets should be developed and applicable in on-station and on-farm levels in Cambodia. In April 2016, the Government of Cambodia lifted the decade-old ban on snakehead fish farming following a request from the Ministry of Agriculture to allow farmers to fish. This project will support the development of sustainable snakehead aquaculture in Cambodia through genetic research for snakehead domestication and selective breeding and farming in Cambodia.

During the second phase of AquaFish CRSP (2009-2011), the wild striped snakehead broodstocks were successfully developed, matured and semi-artificially induced spawning using the hormone HCG on-station in Cambodia (So et al., 2011). The striped snakehead aged 30 days old after hatch could gradually and successfully accept AquaFish CRSP Snakehead Formulated Feed developed by AquaFish CRSP project (Hien & Bengtson, 2009; 2011) in replacement of small-sized fish in the rate of 10% every three days for a period of 30 days of feeding (So et al., 2011).

Under third research phase funded by AquaFish Innovation Lab, the Inland Fisheries Research and Development Institute (IFReDI) at Cambodia's Fisheries Administration further set out (1) to compare performance of domesticated (Vietnamese) vs. non-domesticated (Cambodian) striped snakehead with regard to weaning performance and grow-out on pellet feed; (2) To assess economic efficiency of experimental grow-out of the two types of snakehead on different diets; and (3) To assess product quality of the two types of striped snakehead. The study concludes that both Vietnam hatchery snakehead (domesticated) and Cambodia wild snakehead (non-domesticated) can accept formulated feed, with similar product quality. However, Viet Nam hatchery snakehead show higher survival rate, growth rate and profit than Cambodia indigenous wild snakehead because Vietnam hatchery snakehead has been undergone domestication and selection breeding for more than 20 years (Nen Phanna et al., 2015). To release the current ban on snakehead farming, which has now been in force for 10 years and achieve sustainable development of snakehead aquaculture in Cambodia, the study recommended that genetic diversity of striped snakehead collected from different natural water bodies should be assessed for further success in conducting domestication breeding, weaning and growing-out programs in Cambodia. Now that the snakehead ban is released and if the wild snakehead fishery is sustainably managed in Cambodia, there is good potential to increase trade and marketing of fresh and processed forms of snakehead in Cambodia as well as with other Mekong riparian countries such as Lao PDR, Thailand and Viet Nam, and other countries in Asia, Europe, America and Australia, and to enhance investment of snakehead aquaculture in Cambodia.

Genetic diversity plays important roles in wild and captive populations, indicating ability of a population to adapt to changing environments and the potential of sustained genetic improvement (Allendorf & Luikart, 2007). In the wild, low genetic diversity can cause risks of endanger or extinction of a population under severe environments (Frankham, 2005). Genetic diversity of wild populations can be threatened by over-exploitation, habitat fragmentation, introduced species, and also by interbreeding with escaped hatchery-bred individuals (Hutchings & Fraser, 2008; Laikre et al., 2010). In aquaculture, low genetic

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diversity of broodstock can result in bad seed quality such as high mortality and susceptibility to diseases, or low growth rates. Long-term domestication can result in low levels of genetic diversity of hatchery-bred fish populations due to small population sizes and inappropriate broodstock management (Tave, 1999; Hallerman, 2008). Further genetic improvement of such populations requires genetic information of these populations and other possible sources for genetic exchange. On the other hand, in the process of domestication of potential cultured species, evaluating genetic diversity of different sources is the first and important step to establish good base populations.

This study will focus on (1) characterization and comparison of genetic diversity of both Cambodia wild and Vietnamese hatchery striped snakehead collected from each sampling locality inferred from mitochondrial DNA marker; and (2) provision of basic information and wise recommendations for striped snakehead domestication and selection breeding and farming in Cambodia and for possible exchange of genetic resources of this species between Cambodia and Vietnam.

Quantified Anticipated Benefits

This research will provide basic information on genetic diversity of both Cambodia wild and Vietnamese hatchery striped snakehead for supporting domestication breeding, weaning and growing-out in Cambodia in order to inform snakehead culture in Cambodia. The following are quantifiable anticipated benefits:

- At least 20,000 farmers in Cambodia will benefit from this Investigation by restarting their snakehead farming leading to increased household income and improved snakehead fish market and trade.
- 250 scientists, researchers, government fisheries officers/managers and policy makers, extension workers, NGO staff, and private sector working on the issues of snakehead aquaculture in Cambodia and Vietnam as well as in other Mekong riparian countries will be better informed about research methods and findings, and have better recommended policies and strategies for sustainable snakehead aquaculture in the region.
- Two (under)graduate students will be supported and trained by this investigation through their BSc/MSc thesis research.
- At least 1,000,000 indirect beneficiaries in Cambodia and Vietnam as well as other Mekong riparian countries who consume snakehead fish in their protein diets leading to improved household food security and nutrition.
- Benefits to the US include improved information and knowledge on population genetic diversity of snakehead for domestication and sustainable aquaculture in Cambodia and Vietnam and this aquaculture is considered as a climate change adaptation measure.

Research Design and Activity Plan

Location

Fish samples of the striped snakehead (i.e., fin clips) will be collected in both Cambodia from the wild and Vietnam from hatcheries where snakehead breeders are also collected for condition and domestication at the hatchery of Freshwater Aquaculture Research Center (FARDeC), Cambodia (See Figure 1). DNA lab work and analysis of Cambodia wild (non-domesticated) snakehead populations will be conducted at Inland Fisheries Research and Development Institute (IFReDI), Cambodia. Similar work of Vietnam wild and cultured snakehead populations will be conducted at College of Aquaculture and Fisheries, Can Tho University (CTU), Vietnam.

Two on-the-job/site trainings on (1) basic fish population genetics and (2) DNA lab work, genetic data analysis, and reporting provided by Can Tho University (CTU) members will be conducted at CTU and at IFReDI, respectively. Genetic data generation, analysis and reporting will be jointly carried out by IFReDI and CTU researchers at IFReDI.

Methods

Fish sampling: Sampling locations are designed based on So Nam and Sam Narith (2011) and key criteria including geographic distance, minimal human disturbance and high abundance of wild snakehead populations, and the popularity and scale of hatchery snakehead stocks/populations (Figure 1). In Cambodia, wild (non-domesticated) snakehead samples will be collected from five provinces in floodplains of Tonle Sap Lake: Battambang (BB), Siem Reap

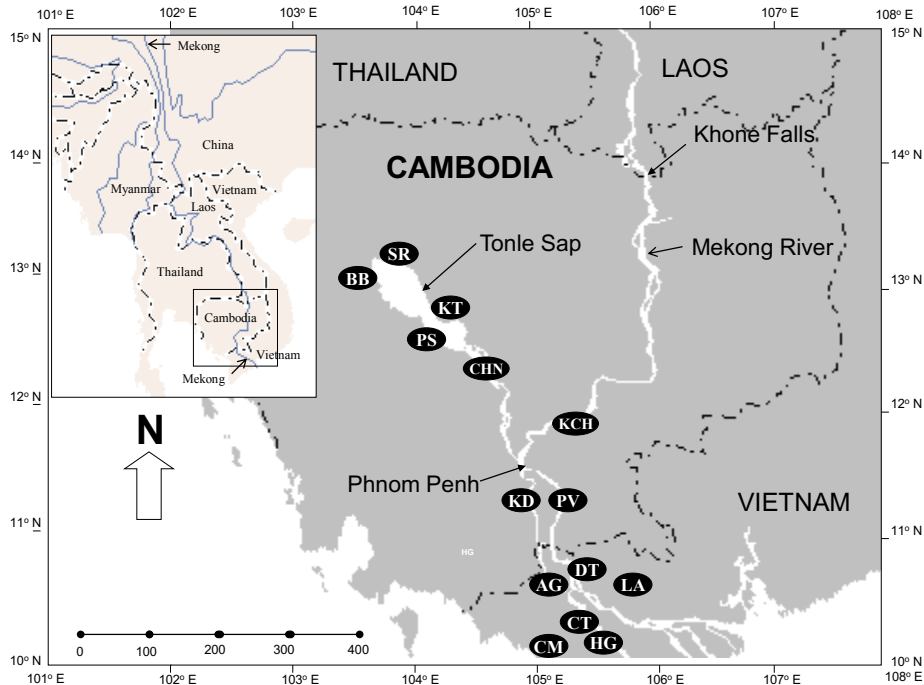


Figure 1: Location map showing sampling locations of the striped snakehead *Channa striata* in Cambodia and Vietnam. BB: Battambang province, SR: Siem Reap province, PS: Pursat province, KT: Kampong Thom province, CHN: Kampong Chnang province, KCM: Kampong Cham province, KD: Kandal province, PV: Prey Veng province, AG: An Giang province, DT: Dong Thap province, CT: Can Tho province, HG: Hau Giang, CM: Camau, and LA: Longan province. Source: Modified from So et al., (2006).

Fin clips from (SR), Pursat (PS), Kampong Thom (KT), and Kampong Chnang (CHN) provinces were collected for the study by So Nam and Sam Narith (2011) and stored at room temperature for the past six years causing the samples to degrade and become unviable for this study. In addition, fin clips will be also collected from other three provinces in floodplains of the upper and lower Mekong River (Kampong Cham, KCH and Prey Veng, PV, provinces) and Bassac River (Kandal, KD, province) in order to have all different populations of the striped snakehead from all geographical areas of Cambodia. In Vietnam, hatchery (domesticated) snakehead samples will be collected from three hatcheries in Dong Thap, An Giang and Can Tho provinces, where most snakehead fingerlings are produced and supplied for other provinces in the Mekong Delta, Vietnam. In addition, wild fish will be collected at three populations from Lang Sen conservation park (Longan province), U Minh conservation park (Camau province), and rice-fields in Haugiang province. These wild populations are hypothesized to be exposed to different levels of human disturbance and be genetically different. At least 50 fish samples will be collected from each sampling location. Fin clips of all collected individuals will be preserved in 100% ethanol and stored in refrigerator for DNA analyses.

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DNA extraction: DNA will be extracted using a commercial kit (e.g. AQUAGENOMIC™kit, MultiTarget Pharmaceuticals, Salt Lake City, Utah 84116) according to the manufacturer's protocol. DNA extracts can be checked for quantity and quality by using agarose gel electrophoresis.

mtDNA marker selection and polymerase chain reaction (PCR) optimization: Cytochrome b gene from mitochondrial DNA (mtDNA) will be selected for PCR amplification. This gene has been used in other genetic studies on genus *Channa* (Abol-Munafi et al., 2007; Adamson et al., 2010) and other fish species (Nazia et al., 2010; Sousa-Santos et al., 2014). Cytochrome b exhibits higher levels of genetic divergence within fish species (Abol-Munafi et al., 2007) compared to other mtDNA genes (Adamson et al., 2010), and it is also a powerful marker for phylogeographic studies (Gaither et al., 2011; Hulsey & García-de-León, 2013). PCR conditions will be optimized based on previous studies and PCR products will be visualized on 1.7% agarose gels stained with ethidium bromide and purified (QIAGEN Sciences, Maryland 20874, USA) according to the manufacturer's instruction. Purified products will be sent for DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia).

Data analysis: Multiple cytochrome b sequences will be aligned and all unambiguous operational taxa units will be compiled for editing using ClustalW implemented in MEGA v. 6.0 (Tamura et al., 2013). DNA sequences will be translated into protein to ensure accurate alignment and detection of nuclear mitochondrial DNA (numt), if present. The aligned sequences will be exported to Collapse v. 1.2 (Posada, 2004) to construct a haplotype datasheet. Three estimates of genetic diversity measurement to describe DNA polymorphism at each sampling location will be calculated using Arlequin (Excoffier & Lischer, 2010). The first, haplotype/gene diversity (Hd), measures the probability of uniqueness of a haplotype in a given population. The second, nucleotide diversity (p), is the mean number of pairwise nucleotide differences among individuals in a sample. The third, theta S (qs) (Watterson, 1975), is a measure of the number of segregating sites among haplotypes in a sample. Phylogeographic structure of wild snakehead populations (in Cambodia and Vietnam) will be analyzed using several programs including MEGA v. 6.0 (Tamura et al., 2013) and NETWORK (Bandelt et al., 1999).

Trainings and Deliverables

The deliverables of this investigation will include (1) final technical report, including policy recommendations, and (2) factsheet.

Short-term training

Two short-term trainings will be carried out to improve capacity of IFReDI staff members on (1) basic fish population genetics and (2) practical skills on DNA lab work, genetic data analysis, and reporting.

Long-term training

Two graduate (MSc) students will be partially supported for their thesis work under this investigation.

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Schedule

The duration of implementation of this proposed investigation will be 24 months, starting from 1 March 2016 till 28 February 2018.

Activity	Beginning	Ending
Collection of fish samples from the wild in Cambodia	March 2016	April 2016
Collection of fish samples from hatcheries in Viet Nam	March-2016	April-2016
Two-week Training on “Basic fish population genetics at Can Tho University, Viet Nam (provided by CTU)	April-2016	April-2016
Set up DNA lab at IFRaDI and hand-on training on DNA analysis (provided by CTU)	May 2016	June 2016
DNA lab work and analyses	July 2016	September 2017
Data analyses and reporting (IFRaDI and CTU team members jointly work together at IFRaDI)	October 2017	December 2017
Consultation and dissemination workshop on research findings	January 2018	February 2018
Finalization of final technical report	January 2018	February 2018