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CENTER FOR TROPICAL AND SUBTROPICAL AQUACULTURE

Letter from the Director

Aloha,

As I mentioned last month, CTSA's development process is undergoing some improvements this year. The first step in our annual development cycle is to determine aquaculture priority areas that potential projects should address. This determination has historically been reserved for our internal committees. However, this year we have developed a survey to gather input from a broader range of industry stakeholders.

The survey takes about ten minutes to complete. If you are in the CTSA region and interested in taking the survey, please contact Meredith at mbrooks@oceanicinstitute.org.

The feature in this month's issue of e-Notes is an update on the CTSA Tilapia DNA project, which has recently completed Year 1 activities. As always, if you have any questions, concerns, or comments, please feel free to share them with our team.

Mahalo,

Cheng-Sheng Lee
Executive Director, CTSA

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DNA-based Identification of wild and captive tilapia species in Hawaii

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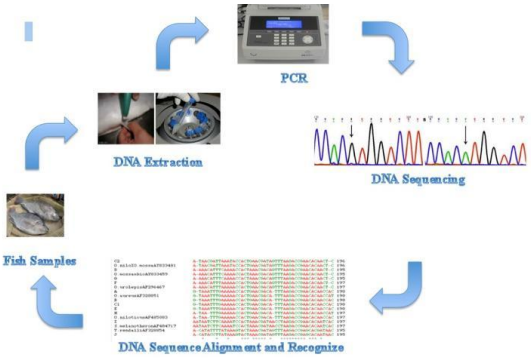
Introduction

Figure 1. Local tilapia

With easy breeding and high survival rate, tilapia family of the Cichlidae has been an excellent species for aquaculture. It is one of the most widely farmed fish in the world with primary production from developing countries in Asia and Africa. In these countries, tilapia aquaculture not only provides dietary sources of protein and minerals for millions of impoverished families, but also is an important means of the economic and social empowerment. While tilapias are widely used for freshwater aquaculture, several strains and hybrids of tilapia tolerate a wide range of water salinity. For example, Mosambique tilapia (O. mossambicus) and its hybrids can tolerate high salinity and are increasingly used for co-culture with marine shrimp. Strains of tilapia such as O. aureus, O. mossambicus and S. melanotheron were introduced to Hawaii several decades ago. In 1950s and 1960s, O. mossambicus and T. melanopleura and T. Melanopleuron were imported to Hawaiian Islands to control vegetation and used as baitfish for the tuna fishery. Taiwan and Florida red hybrid tilapias

were imported in the early 1980s. Backcrossing and hybridization of the red tilapia with other stocks had been tested. It is believed that most imported tilapia had entered the fresh and marine environment along the recreational beaches as these tilapias have been observed in local rivers, reservoirs and brackish water in Hawaii.

The traditional distinction of species within the tilapia (Figure. 1) depends on the differences of appearances, such as the body size, shape, color, number of anal spines, shape of fins, color of the head, and so on. However, the introduction of alien species and the hybridization between different species have made it more complicated to identify tilapia species by morphological distinctions. Compared with the appearance observation and description, DNA-based approach is practical and accurate (Figure 2). Several fish genomic marker systems have been reported for species identification including microsatellite markers, 45s and 5s rDNA, mitochondrial cytochrome c oxidase I (COI) and the control region of the mitochondrial DNA (mtDNA CR). Those molecular methods depend on the polymerase chain reaction and sequencing technology. Specific DNA sequences or variations associated with tilapia species and strains provide the basis for tilapia classifications and identifications.



MtDNA sequence alignment analysis is one of the more effective approaches to predict and recognize different species or populations. Usually, mtDNA is inherited from the mother and does not undergo male and female DNA recombination. The DNA sequence variations of the mtDNA is very useful for tracking the descendents from a particular maternal individual within a population. To compare the sequence differences between species, geneticists pay more attention to mtDNA control region (mtDNA CR), which located between the tRNA-glu gene and the tRNA-phe because of its more rapid rate of evolutionary change compared to the rest of the mitochondrial genome. The function of mtDNA CR is to regulate the replication and transcription of the mitochondrial genome. It is also the most variable part of the mtDNA and evolves three to five times more rapidly compared with the rest of the mitochondrial genome. Sequence variation/changes of the mitochondrial control region may lead to length differences in mitochondrial genomes.

Until now, the information about the genetic diversity and various types of tilapia species and hybrids existing in Hawaii is unavailable. The state law currently controls the import of genetically improved niloticus tilapia to Hawaii. In the mean time, local tilapia farms are in need of tilapia broodstock with better growth performances and production. It is important to initiate the investigation of tilapia species classification and identification for broodstock breeding and wild fish managements in Hawaii. To start the investigation, we employed mtDNA sequence variations and comparison with published data to identify and classify wild and captive tilapia strains and hybrids in Hawaii.

Tilapia fin clip sampling and DNA extraction

Tilapia fin clip samples were collected from ten populations (sites), including two wild populations in Oahu islands and eight aquaculture and experimental facilities. Fin clip samples from 30-50 fish were collected from each site, and possible strain identifications or pictures of the tilapia fish were taken at the time of sampling. All the tilapia fin samples collected are preserved in 100% ethanol. Genomic DNA was isolated from caudal fin clips. Briefly, tissue samples were digested with 0.5 mg/ml proteinase K at 55°C overnight. The resulting solution was centrifuged; supernatant was extracted by phenol-chloroform and precipitated in ethanol and dissolved in 1X TE buffer. The quality and concentration of DNA are assessed by spectrophotometer and agarose gel electrophoresis, and DNA samples have been stored at 4°C until use. The method of genomic DNA isolation was established in the laboratory.

PCR Reaction and DNA sequencing

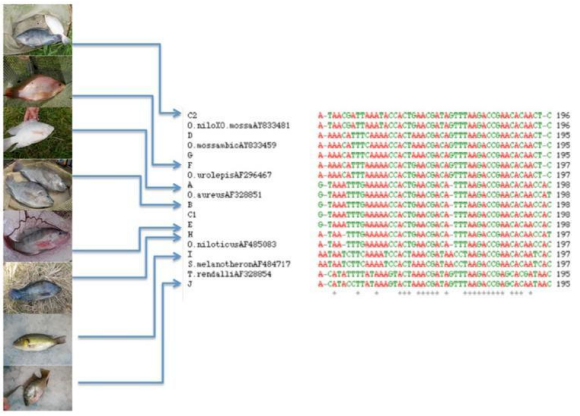


Figure 3. Tilapia species recognition by DNA sequence

The control region (CR) of the mitochondrial (mt) DNA sequence is known to have more rapid mutations over the course of species evolution than other part of the mt DNA sequences. Using the DNA sequence from mtDNA CR may be easily used for identifications of most tilapia species existing in Hawaii. We developed PCR and DNA sequencing protocol of mtDNA CR. PCR for mtDNA CR were carried out using the primer: ORMT-F, 5'-CTAACTCCCAAAGCTAGGAATTCT-3'; ORMT-R, 5'-CTTATGCAAGCGTCGATGAAA-3'. The total reaction volume was 25μL in a micro PCR tube, including 2.5μL 10 × PCR buffer, 0.5 μL of each primer (0.01 mM), 0.5μL of dNTPs (10 mM), 0.2μL of Taq DNA polymerase (5U/μL), and 1.0μL of template DNA. The PCR program consisted of a denaturation step for 3 min at 94°C, followed by 35 cycles of 94°C for 30s, 54°C for 40s and72°C for 40 s; and a final extension step of 72 °C for 10 min. Negative controls were used in all PCR reactions to make sure that no contamination took place in reaction system. During PCR reaction, the specific DNA fragment (approximately 500 bp) between the two primer locations (ORMT-F and ORMT-R) is produced in millions of copies. PCR products were separated on 1.5% agarose gels for checking the presence of the 500 bp fragment. Images were photographed under UV light with imaging system. The agarose gel contained DNA bands was cut off and the DNA bands were recovered with the DNA purification kit. After purification, amplified PCR products were sequenced by the ABI PRISM 377 sequencer. Sequence alignments are presented in Figure 3 for MtDNA CR.

DNA Sequences Data Analysis

The sequences are aligned by clustalW2 program. All the sequences have been analyzed and compared with published tilapia species data including the O. aureus, S. melanothern, T. rendalli, O. mossambicus, O. urolepis reported by Nagl, S et al (Genbank Accession #AF328851, AF328854, AF328851, AF328843, AF296493, AF328843), S. melanothern, O. niloticus reported by Falk (Genbank Accession # AF484717, AF485083), O. mossambicus and Hybrid tilapia reported by D' Amato, M.E (Genbank Accession #AY833459, AY833481). Representative sequence alignments are presented in Figure 3 for mtDNA CR. Neighbor-joining (NJ) tree are constructed with Kimura Two-parameter distance model by MEGA version. All transitions and transversions are calculated in the tree. Genetic distances are quantified within and among species using the Kimura two-parameter (K2P) distance model by MEGE version 4. The relationships between the populations are analyzed with MEGE 4. Preliminary identifications of ten populations are presented in Table 1.

Table 1. Identifications of the tilapia samples by DNA sequence.

Population (Site)	Number of fish	Claimed species	Genetic composition
	Aquaculture Sites		
A	51	Unknown	O. aureus
B	34	O. Aureus	O. aureus
C	30	Unknown	O. niloticus × O. mossambicus and O.aureus
D	102	Unknown	O. mossambius
E	50	Unknown	O. aureus
F	50	Unknown	O.urolepis
G	50	Unknown	O.mossambicus
H	29	Unknown	O. niloticus
	Wild/Feral Sites		
	44	Unknown	S. melanotheron
J	44	Unknown	T.rendali

Primary Results and Conclusion

MtDNA CR sequence analysis identified all the sampled tilapia. Based on the ten populations or sites, there are seven distinct tilapia species or hybrids, including O. aureus, hybrids from O. niloticus X O. mossambicus x O. Aureus, O. mossambius, O. urolepis, O. niloticus, O. melanotheron, T. rendali. The species of O. aureus are present in three populations. The species O. mossambicus are present in two populations. O. niloticus is present in one of the aquaculture farming site. The two wild sites are located in Oahu island, having two original imported tilapia species S. melanotheron and T. rendali. These results provide strong support for mtDNA CR sequencing for classification and identification of tilapia species. Of all the populations examined, 7 populations showed some degree of introgression and hybridization. Some hybrid populations included the genes both of O. niloticus and O.aureus probably related to the hybrids known to be imported years ago. Based on the genetic diversity and phylogenetic tree analysis, it was found that the population C has a high average number of nucleotide differences and showed characteristics of O. niloticus, probably from hybrid O. mossambicus x O. niloticus. In conclusion, this is the first report of the DNA-based identification of wild and captive tilapia species in Hawaii, and the DNA sequence comparisons of mtDNA control region appears a valid method for the species identification.

CTSA Video Project: Reaching Beyond our Region

CTSA Cyanotech Video Featured at USA Science & Engineering Festival

CTSA's recent Island Farmer Spotlight video on Cyanotech will be featured at the Phycological Society of America booth at the 2nd annual USA Science & Engineering Festival. The festival will be held in Washington D.C., April 28-29, 2012. Click here to watch the video.

CTSA Micronesian Pearl Video Featured at the Swiss Gemmological Society Conference in April

Masahiro Ito (CTSA's Pacific Islands Extension Agent and P.I. of the Black Pearl projects in Micronesia) is teaming up with a sustainable pearl farming initiative to promote his CTSA-funded work. In April, the CTSA video profiling the pearl project will be shared with jewelers from across the world at the Swiss Gemmological Society Conference. Click here to watch the video.

Pacific Island Spotlight: Asia-Pacific Tropical Sea Cucumber Aquaculture Proceedings Available Online

Proceedings from the ACIAR-SPC Asia-Pacific Tropical Sea Cucumber Aquaculture Symposium held in February 2011 in Noumea are available for download (free) or hardcopy (for purchase).

These proceedings contain the most current information on research in this exciting and expanding field. The collection of manuscripts feature detailed information on sea cucumber culture protocols and current activities across the Pacific, including in CTSA's region.

Click here to visit the ACIAR website for more information.

AquaClip: Kampachi Farms' "Velella" Research Project Celebrates Successful Harvest

By Natalia Real, FIS - www.fis.com. March 2, 2012.

Kampachi Farms has announced the successful final harvest from the "Velella" Research Project raising fish for the first time in US federal waters.

This harvest has completed the grow-out cycle of sashimi-grade kampachi fish (a tropical yellowtail) in the open ocean, 3-75 mi off the Big Island of Hawaii in the Pacific, since last summer.

"This final harvest far surpassed our expectations," said Neil Anthony Sims, Co-CEO of Kampachi Farms. "The fish thrived in the research net pen far from shore, with phenomenal growth rates and superb fish health... and without any negative impact on water quality, the ocean floor, wild fish or marine mammals."

The fish were raised in a single unanchored, submersible net pen linked to a manned sailing vessel, in water up to 12,000 ft deep.

Click here to read the full article.

FYI, next month's issue of e-Notes will feature an Island Farmer Spotlight video profiling Kampachi Farms. Be sure to check it out!

The Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers in the United States established and funded by the U.S. Department of Agriculture's National Institute of Food and Agriculture (NIFA) under grants 2007-38500-18471, 2008-38500-19435, and 2010-38500-20948. The regional aquaculture centers integrate individual and institutional expertise and resources in support of commercial aquaculture development. CTSA was established in 1986 and is jointly administered by the Oceanic Institute and the University of Hawaii.