

D-08: Preliminary studies towards amplification of a cytochrome b region and a 12S RNA region of *Amblygaster sirm* mitochondrial DNA

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Amblygaster sirm is an important food fish in Sri Lanka providing over 20% to the total fresh fish production in the country. This resource is exploited extensively in the west and south coasts of the country. The management of this resource needs information on the radiation of the exploited population. A possibility exists for fish with pelagic larvae or adult migration habits to be distributed over wide ocean ranges. Therefore, it is essential to know the population structuring of this species around the Indian Ocean region. The present study was initiated to study the *Amblygaster sirm* population around Sri Lanka based on molecular markers. Analysis of mitochondrial DNA (mtDNA) provides some important information on population structuring due to the maternal inheritance of this DNA and the ability of mtDNA to evolve faster than the nuclear DNA. The present investigation was carried out to make preliminary preparations towards preparing DNA templates from cytochrome b and 12S RNA regions of the mtDNA for cycle sequencing.

Optimum conditions and molar ratios required to amplify double stranded cytochrome b and 12S RNA regions from total DNA is presented here. The primers used were Cytochrome b L14841, H 15149, 12S RNA L1091 and

H1478 (known sequences). The conditions required for single stranded band amplification for the same regions using double stranded PCR product is also given. Overcoming the problems associated with non specific band amplifications is also discussed. A trial sequencing of double stranded amplified product of 12S RNA using thermostable fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP and cyanine labeled forward primer is also reported.