

## ABSTRACT

### **Molecular Genetic Analyses of the Four-wing Flyingfish, *Hirundichthys affinis*, in the Central Western Atlantic and Their Implications for Fisheries Management**

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The four-wing flyingfish, *Hirundichthys affinis*, is an epipelagic, open-water species found throughout the tropical (central) Atlantic. In the central western Atlantic it supports commercially important fisheries in three geographically distinct subregions: the eastern Caribbean islands; the southern Netherlands Antilles; and northeast Brazil, although the resource remains unmanaged in all three areas. The stock structure remains unresolved and there is no information on the flyingfish genome.

In this study, mitochondrial DNA markers (restriction fragment length polymorphisms (RFLPs) of the D-loop region) and genomic DNA markers (randomly

amplified polymorphic DNAs (RAPDs)) were identified and used to examine intra- and inter-population (population being defined in this study as a single sample of fish taken from a specific location (country) at a specific time (month)) genetic variation and therefore stock structuring of the flyingfish resource within the central western Atlantic. A genetic database for flyingfish was also established through restriction site mapping of the mtDNA molecule and DNA sequencing of the D-loop region.

For stock structure analysis, a total of 360 spawning flyingfish were sampled from 6 populations across the central western Atlantic (Barbados (January and May), Dominica and Tobago (April), Curaçao (July), and Brazil (August)) between January and August 1995. Mitochondrial DNA RFLP markers were identified by amplification of the D-loop region using the polymerase chain reaction (PCR) and two primers (ProL1 and H16498) followed by restriction enzyme digestion with five restriction enzymes (*HinP*II, *Hha*I, *Mbo*I, *Mse*I and *Rsa*I). RAPD markers were obtained by PCR amplification of genomic DNA with three arbitrary primers and paired combinations of these primers.

Results of analyses of both mtDNA and genomic DNA markers indicated significant genetic diversity in flyingfish. Analysis of the mtDNA RFLP markers detected distinct composite mitotypes (genotypes defined by all five restriction enzymes together) for each of the three geographical subregions, indicating no significant gene flow between these areas and the existence of at least three unit stocks (genetically discrete groups) of flyingfish in the central western Atlantic. The results of cluster analyses of composite mitotype sequence divergence and population

sequence divergence, and parsimony analysis of composite mitotypes were entirely consistent with this 3-stock model. Moreover, cluster analyses of similarity and percent match indices obtained from analysis of genomic DNA RAPD markers, and a comparison of  $\phi_{st}$  (an index of genetic diversity) and  $N_m$  (an index of gene flow) values obtained from RFLP and RAPD marker analyses supported the three separate stocks hypothesis.

Genetic diversity was also detected among eastern Caribbean populations by both mtDNA and genomic DNA analyses, indicating restricted gene flow even within a subregion, and emphasising the need for more detailed studies of behaviour patterns associated with flyingfish spawning activities. These results contrast with the typically low levels of genetic variation reported for oceanic pelagic species (blackfin tuna, swordfish, striped marlin, blue marlin and sailfish), and for other marine species (several reef fish species, queen conch and spiny lobster) in the Caribbean and indicate that basic life history characteristics and major ocean current patterns are not good predictors of gene flow for all species.

The implications of these results for management of the flyingfish resource in the central western Atlantic are that three independent stock assessments and management strategies would be appropriate at the subregional level. This stock structure indicates that a subregional-level, shared-stock approach to management would be appropriate for the eastern Caribbean, since the eastern Caribbean islands appear to share a common unit stock. It also suggests that national-level management of a single unit stock would be appropriate for flyingfish in the southern

Netherlands Antilles (which share a common national Government) and for the flyingfish unit stock off northeast Brazil

For a more detailed examination of the organisation of nucleotide sequences in the mitochondrial genome, flyingfish were sampled from the Barbados (January) population (for restriction site mapping) and from the Barbados (May) population (for DNA sequencing). The relative positions of six restriction enzyme recognition sites (*ApaI*, *BglII*, *EcoRI*, *HindIII*, *SalI* and *XbaI*) and the D-loop region were successfully mapped on the mtDNA molecule. The nucleotide sequence of the D-loop region (comprising approximately 480 bp) was successfully resolved and its identity was confirmed through alignment with D-loop sequences of 61 other fish species. This information has allowed initiation of a genetic database for flyingfish and is expected to provide valuable reference material for future research on *H. affinis* as well as on other closely related species.