Stock identification and length-length relationships of frigate tuna, bullet tuna and kawakawa populations of Sri Lankan waters

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Abstract

Understanding the origin of different populations is useful when managing the stocks of a species. Kawakawa (*Euthynnus affinis*), frigate tuna (*Auxis thazard*) and bullet tuna (*Auxis rochei*) are very important neritic tuna species found in Sri Lankan waters. Stock identification studies for these 3 species were carried out by using morphometrics as well as by using molecular techniques. The use of two or more methods for the identification of stocks makes a stock identification study more accurate and reliable. Samples were collected from the commercial fishers that operated in the Southern, Southwestern, Western, Northwestern and the Eastern coastal areas of Sri Lanka from August 2015 to July 2018. The morphometric analysis involved recording 22 morphometric measurements for each fish. The length-length relationships determined using the total length, fork length and the standard length revealed that the 3 species are showing healthy growth patterns. The Principal Component Analysis performed with all 22 morphometric measurements to determine the origin of the stocks, showed for all 3 species that different clusters contained individuals from all provinces indicating that the populations of different coastal areas have originated from one common ancestor and that they have evolved as one stock. The stock structure

analysis of the 3 species involving phylogenetic trees constructed with the sequences of the mitochondrial COI gene and the mitochondrial D-loop region support the finding of the morphometric analysis by showing that the populations of these species across different coastal areas of Sri Lanka form a single stock. This means that the populations are genetically similar and likely belong to the same genetic pool, corroborating the morphometric analysis results. Therefore, when management plans need to be implemented for these species, a unified strategy could be implemented throughout the studied coastal areas for each species. Moreover, it is recommended to conduct similar studies that combine samples from different nations. This approach would provide a comprehensive understanding of the status of fish populations across the region.

Keywords: frigate tuna, bullet tuna, morphometric, molecular, stock structure

Introduction

The fishery industry is an important sector of the economy of Sri Lanka and provides almost 65% of the total protein requirement of the people. The fishery sector contributes approximately 2.7 % to the Gross Domestic Product (GDP) of the country. The marine fishery constitutes more than 85% of the total fisheries sector and the tuna fishery contributes nearly 75% to the marine fishery. Neritic tuna fishery constitutes approximately 4% of the tuna fishery (Rathnayake and Perera, 2023). The neritic tuna fishery of Sri Lanka includes frigate tuna (*Auxis thazard*), bullet tuna (*Auxis rochei*) and kawakawa (*Euthynnus affinis*) and the tuna-like species narrow-barred Spanish mackerel, (*Scomberomorus commerson*) and Indo-Pacific king mackerel (*Scomberomorus guttatus*). In the neritic tuna fishery, frigate tuna (*Auxis thazard*) dominates the catch by constituting almost 40% of the catch (Dalpathadu *et al*., 2019).

E. affinis is a pelagic fish distributed throughout the warm waters of the Indo-West Pacific region (Ahmed *et al*., 2014). It grows to a maximum fork length of 100 cm The species belonging to the genus *Auxis* are the smallest species in the tribe thunnini. And they are highly migratory and are found in the Indian, Atlantic, and the Pacific Oceans. *A. thazard* and *A. rochei* are indeed very similar morphologically, which often leads to confusion and misidentification. *A. rochei* has a vertical dorsal marking pattern while *A. thazard* has a slanted marking pattern (Relini *et al*., 2009).

Due to this confusion, the catches are sometimes referred to as *A. thazard* - *A. rochei* mixed catches.

Limited information is available on the biology and stock structures of these neritic tuna species in the Indian Ocean. Mudumala *et al* (2011) have carried out DNA barcoding and phylogenetic analysis on 4 coastal tuna species including *E. affinis*, *A. thazard* and *A. rochei*. The fishery, biology, growth and stock structure of *E. affinis* has been studied in Pakistan by Ahmed *et al*., (2014). The fishery, biology and genetics of *A. thazard* have been studied by some surrounding Indian Ocean countries such as India (Ghosh *et al*. 2012, Kumar *et al*. 2012a). Indonesia (Noegroho *et al*., 2013; Hamidi & Rizal, 2018,) and Tanzania (Johnson *et al*., 2015) have also carried out biological and genetic studies on *A. thazard*. Population genetic structures of different tuna species have been studied using various molecular markers such as, mitochondrial COI (Kumar and Kocour, 2015) and mitochondrial D-loop sequencing (Habib and Sulaiman, 2017). Johnson *et al*., (2015) have stated that a single stock of *A. thazard* exists in the Northern waters of Tanzania. Some studies have been carried out on the biology of frigate tuna and bullet tuna from Sri Lanka but genetic studies have not been carried out. Therefore, studying the stock structure of *A. thazard* is very important for this species as this information can be compared with the information available for the region for the purpose of managing the stocks of this important resource. Therefore, the aims and objectives of the study were to study the stock structure of the neritic tuna species *A. thazard* and *A. rochei* by comparing their morphometric features and by using the molecular markers mitochondrial COI and the mitochondrial D-loop region.

length-length relationships are fundamental in fish biology and fisheries science, providing crucial information for understanding growth patterns and assessing the health and condition of fish populations (Moutopoulos and Stergiou, 2002). LLRs for *A. thazard* of Sri Lankan waters have been SL = 0.853TL (R^2 =0.955), FL = 0.918TL (R^2 =0.974) and SL = 0.928FL (R^2 =0.972) for the overall catches of the species (Bandaranayake *et al*., 2015). The R^2 values indicate that the correlations between the different length parameters have been good. This study describes the phylogenetic relationships between the neric tuna species *E. affinis*, *A. thazard* and *A. rochei* and the length-length relationships of these 3 species.

Methodology

Samples of *E. affinis*, *A. thazard*, and *A. rochei* were collected from 6 different locations in the western, southern, southwestern and northwestern coastal areas of Sri Lanka. The samples were transported on ice to the laboratory for further analysis. 22 morphometric measurements of each fish were taken for the morphometric analysis and tissue samples from each fish were preserved in 95% ethanol for the molecular analysis.

Molecular analysis

DNA of the samples taken from each fish was extracted using the phenol chloroform method and the quantity of DNA in the extracted samples was determined using agarose gel electrophoresis. The mitochondrial COI region was amplified using the FishF1/R1 universal fish identification primers (Ward et al., 2005) and the mitochondrial D-loop region was amplified using primers specific to neritic tuna species (NTFP and NTRP) (Kumar *et al*., 2012). The PCR products were then sent to Macrogen Inc., Korea for sequencing. The sequences were analyzed to obtain the consensus sequences using Bioedit (version 7.2.6) software program (Hall., 1999) and these sequences were submitted to the NCBI (National Center for Biotechnology Information) GenBank database. The sequences resulting from the use of the two markers mitochondrial COI and the mitochondrial D-loop region were used for the phylogenetic analysis with the aid of Mega-X program (Kumar *et al*., 2018) using the Maximum likelihood method based on Tamura-Nei Model (Tamura and Nei, 1993) and Population parameters were determined by using the DnaSP (version 6.12.01) software program (Rozas *et al*., 2017).

Morphometric analysis

The weight (in g) and 22 other morphometric measurements were recorded for each fish: total length, fork length, standard length, head length, post-orbital distance, pectoral fin length, base length of 1st dorsal fin, base length of 2nd dorsal fin, length of finlets, anal fin length, pelvic fin length, snot length, snout to origin of dorsal fin, snout to origin of anal fin, snout to origin of pectoral fin, origin of anal fin to insertion of pelvic fin and caudal peduncle length were measured to the nearest 0.1 cm, while the head depth, body depth, eye diameter, origin of dorsal to insertion of pelvic fin and the caudal peduncle depth were measured to the nearest 0.001 cm. The 22 morphometric measurements of all the fish analyzed, were used in R studio software program by

using Principal Component Analysis (PCA) to detect the variation that exists within the different populations. Using the total length, fork length and the standard length, the length-length relationships between these 3 main lengths (TL vs. SD, TL vs. FL and SD vs. FL) were also calculated and plotted for each species.

Results and Discussion

Molecular analysis

The number of samples sequenced for each species with the 2 different markers is given in Table 1. These sequences were multiple aligned and used to construct phylogenetic trees for the 2 markers using the maximum likelihood method, taking a related species as an outgroup and with a bootstrap value of 1000.

Table 1: Number of samples sequenced for the mitochondrial COI and Mitpchondrial D-loop for the 3 species.

	E. affinis	***A. thazard***	***A. rochei***
Mitochondrial COI	112	95	72
Mitochndrial D-loop	112	84	81
Total	224	179	53

These sequences were multiple aligned and used to construct phylogenetic trees for the 2 markers using the maximum likelihood method, taking a related species as an outgroup and with a bootstrap value of 1000. Sequences of mitochondrial COI and mitochondrial DL of that particular species of India retrieved from the NCBI database were also included in the phylogenetic analysis (Fig. 1) when available. A third phylogenetic tree was drawn for the alignment of the combined sequences of the Mt COI and Mt DL sequences of each of the samples. All 3 phylogenetic trees showed the populations being mixed up in the different clades indicating that the populations of the different regions have one single origin.

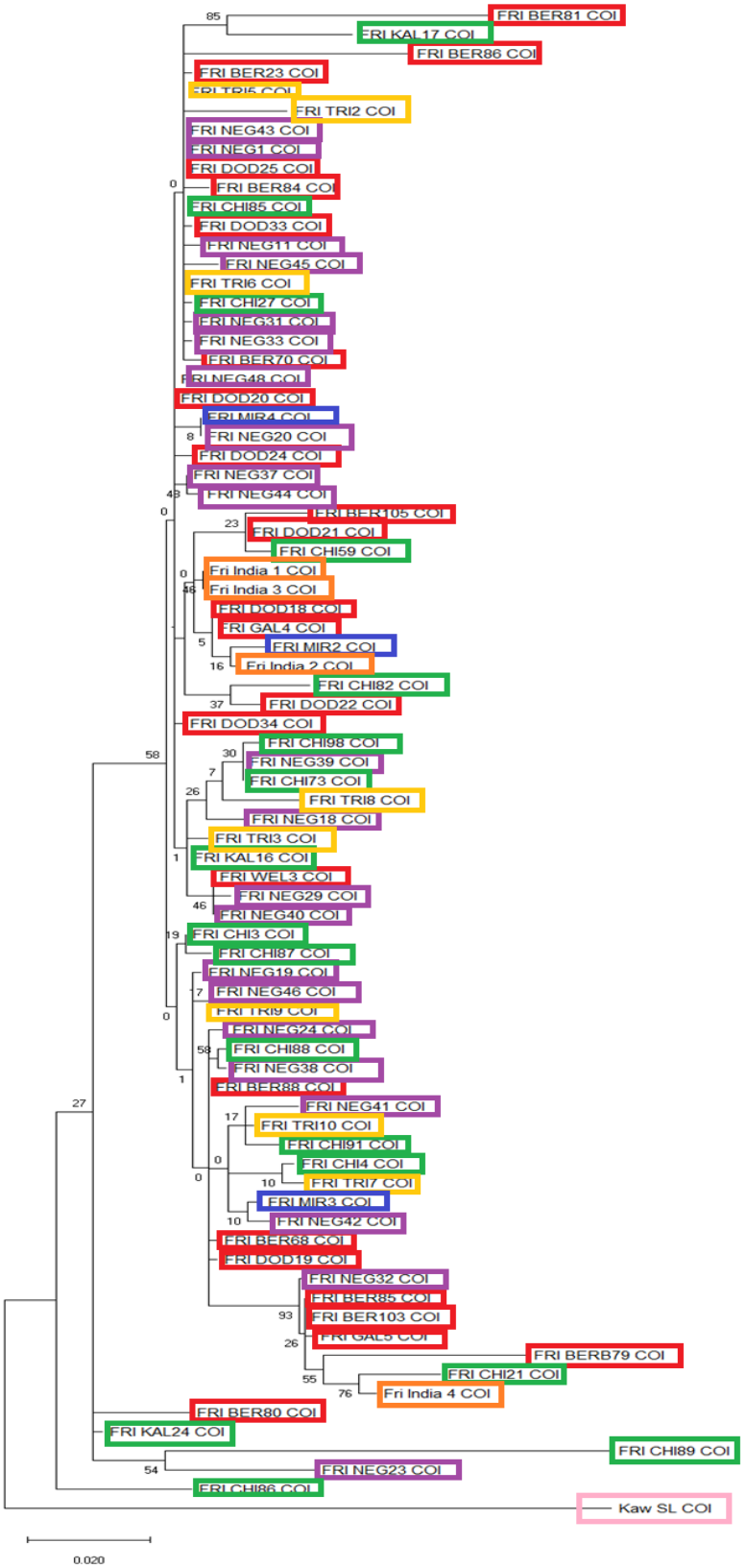

Fig. 1: Phylogenetic tree for mt COI sequences of *A. thazard*

When population parameters were considered for the species, haplotype diversities were high and the nucleotide diversities were low. Table 2 gives values for haplotype diversity and nucleotide diversity of *A. thazard*. The highest F_{ST} value for *A. thazard* has been obtained for the southwestern and northeastern provinces. These 2 provinces are geographically far apart so interbreeding would be the least among all the populations.

Table 2: Population parameters for *A. thazard* combined mt COI and mt DL sequences for the overall population and for the individual populations.

	Overall	North-western	Western	South-western	Southern	North-eastern
Number of sequences	70	15	23	19	4	8
Number of haplotypes	50	15	17	14	4	7
Haplotype diversity (Hd)	0.97272	1.000	0.95257	0.94737	1.000	0.96429
Nucleotide diversity (Pi)	0.01660	0.02698	0.00913	0.01795	0.01596	0.01210

For the species *A. rochei* the phylogenetic tree showed the clades of the sequences of the samples obtained from different provinces being mixed together. A special point to note is that the Mt COI sequences of India downloaded from NCBI are seen separately in the bottom clade of the tree (Fig. 2). This result could mean that the *A. rochei* stocks of the different provinces of Sri Lanka belong to one stock but they are of a different origin from the *A. rochei* found along the Indian coast. The F_{ST} values for the populations of *A. rochei* ranged from 0.011 to 0.098. These relatively low F_{ST} values together with the mixed clades in the phylogenetic tree could mean that the *A. rochei* populations around Sri Lanka form a single genetic stock with only slight differentiation among provinces.

The haplotype diversity of the combined sequences of *A. rochei* was 1.00 for all provinces. This shows that all sequences belong to different haplotypes. For *A. rochei* the highest F_{ST} value is shown between Southern and western provinces. As these are smaller fish their migration rate is limited and therefore migration could be less than for larger species. Therefore, similar to A. *thazard*, the *A. rochei* populations also show that they belong to one single stock.

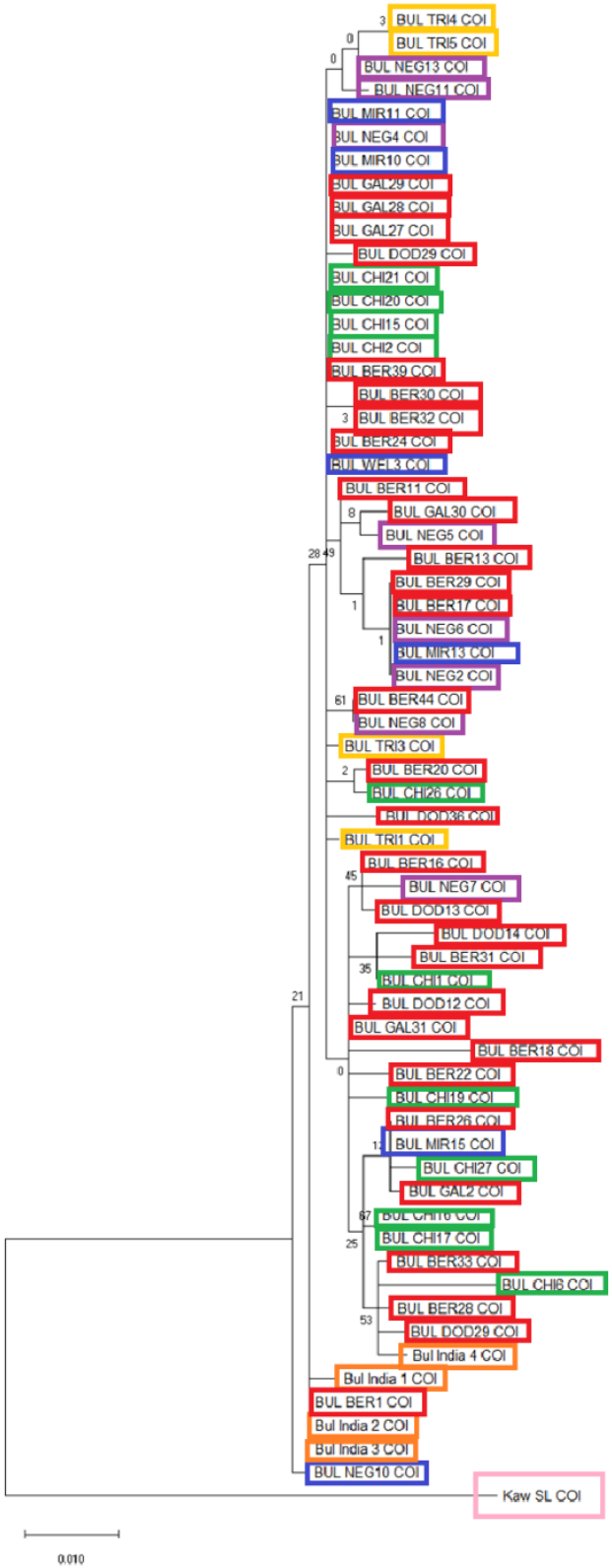

Fig. 2: Phylogenetic tree for mt COI sequences of *A. rochei*

Morphometric analysis

Stock identification

The 22 morphometric measurements of 488 *E. affinis* samples, 374 *A. thazard* samples and 200 *A. rochei* samples were recorded and analyzed using R studio software program to detect the variation that exists within the different populations. The analysis of the variables obtained for all 3 species indicate that there are no distinct clusters for this species. The PCA analysis biplot (Fig. 3 and Fig. 4) shows that although there are 4 groups, each group contains individuals of all the provinces which indicates that the stocks are all of one origin. Therefore, it can be concluded that the populations of the different provinces are all of one single origin, complementing the results obtained in the molecular study for all 3 species.

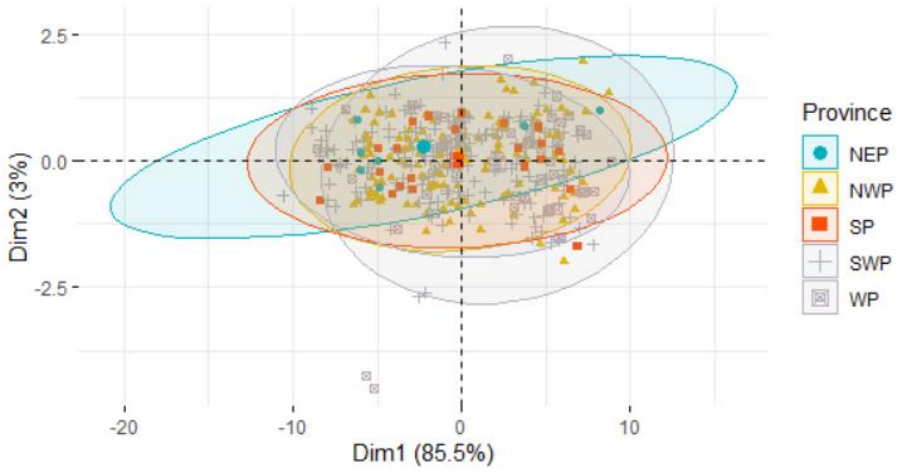

Fig. 3: Principal component analysis biplot for *A. thazard*

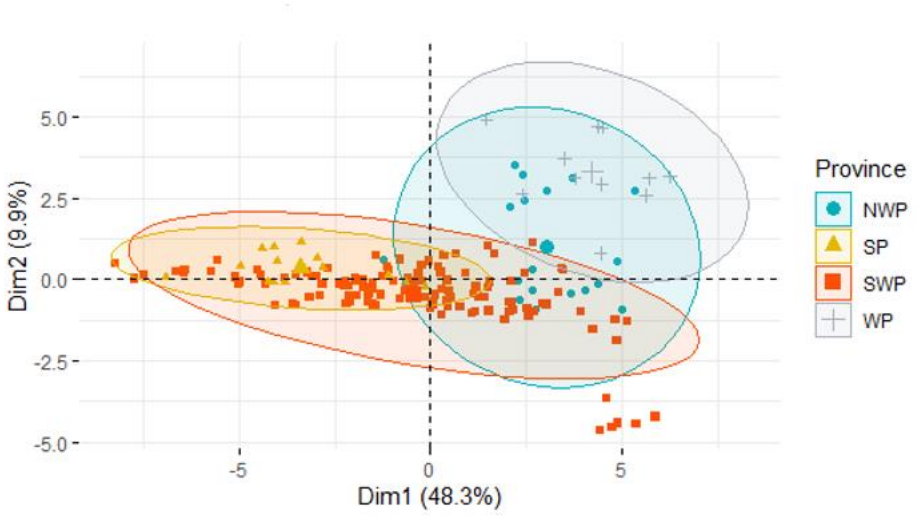

Fig. 4: Principal component analysis biplot for *A. rochei*

Length-length relationships

The length-length relationships for the 3 species show similar values for all 3 species (Table 2). The relationships between the different lengths for all three species show R^2 values of more than 0.9 (Fig.5), indicating high correlation between the different lengths. The length-length relationship equations and R^2 values are given in Table 5.

Table 2: Length-length relationships for *E. affinis*, *A. thazard* and *A. rochei*

	E. affinis	***A. thazard***	***A. rochei***
Sample no. (n)	488	374	200
FL= a + bTL	FL=-0.041 + 1.006TL R^2=0.997	FL= - 0.046+1.015TL R^2=0.996	FL= - 0.020+1.000TL R^2 =0.969
SL= a + bTL	SL= -0.087 + 1.024TL R^2=0.994	SL= - 0.070+1.019TL R^2=0.989	SL = -0.003+0.977TL R^2 =0.933
SL= a + bFL	SL=-0.046 + 1.019FL R^2=0.996	SL= - 0.023+1.004FL R^2=0.993	SL = 0.018+0.975FL R^2 =0.961

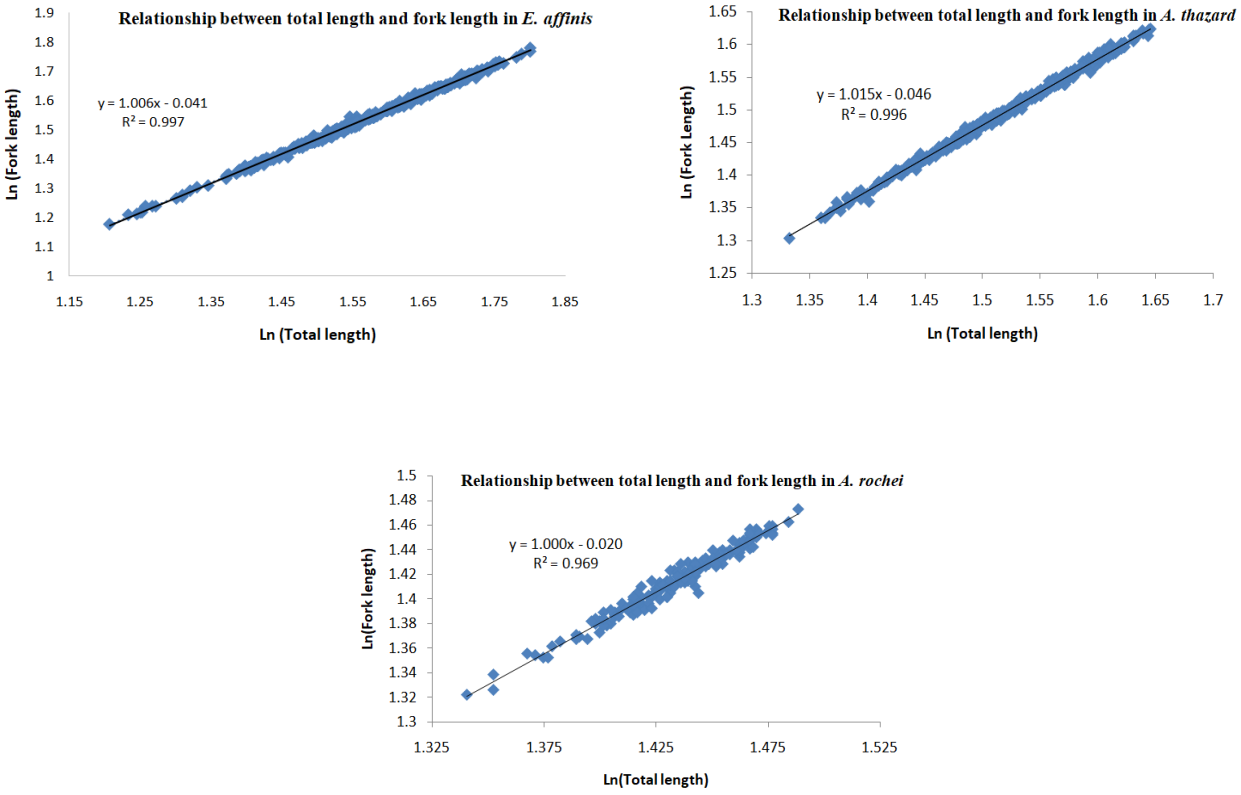

Fig. 5: Relationship between total length and fork length for E. *affinis*, *A. thazard* and *A. rochei.*

The R^2 values being close to 1 indicates that the relationship between the 2 length variables is very strong and that the growth of the fish is at an optimal level. The relationship between the length parameters were all highly significant ($p<0.001$) for the pooled samples of the 3 species. Length-length relationships are important for comparative growth studies in fisheries management issues. Therefore, this data is very important in the management of these important neritic tuna resources of Sri Lanka and in the Indian Ocean region in the future.

Conclusion

The phylogenetic analysis of *A. thazard*, *A. rochei* and *E. affinis* revealed that for all 3 species, one single stock exists along the coast of Sri Lanka. It could also be observed from the PCA analysis

of the variables of the different morphometric features, that the stocks of the different provinces all clustering together for both species, confirming the results of the molecular analysis.

The study indicates that collaborative studies involving other Indian Ocean countries where the populations of frigate tuna, bullet tuna and kawakawa of the waters of those countries could be compared as they are migratory species. This would make it possible to draw conclusions regarding the relationship between these populations, thereby aiding in the implementation of future management plans

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