Albacore Tuna Larval Occurrence in the Southwest Indian Ocean and Associated Species

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ABSTRACT

Improving our knowledge about the reproductive biology of albacore tuna (*Thunnus alalunga*) is critical for the stock assessment of this species. While most of the stock assessment data are from adults landed by the commercial longline fisheries, here we present preliminary results of the distribution and abundance of larva albacore tuna from the southwestern Indian Ocean near Reunion Island. Surface and subsurface plankton tows were conducted in the spawning habitat during peak spawning period of albacore and the collected tuna larvae were quantified, measured, visually identified, and genetically confirmed using multiplex PCR using primers for six tuna species: kawakawa (*Euthynnus affinis)*, skipjack (*Katsuwonus pelamis*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*), bigeye (*Thunnus obesus*), and Atlantic bluefin tuna (*Thunnus thynnus*). Albacore larvae were the most numerous and most abundant in our collection (N = 214, i.e. 89.17% of total tuna larvae collected and identified to species). Using the reported age-length relationship for *T. alalunga* larvae from the Mediterranean Sea, our specimens are estimated to be 0–6 days post hatch. Further early life history research is needed to determine larval indices to better estimate larval survival and their recruitment to fisheries.

KEYWORDS: Albacore tuna, larval distribution, southwest Indian Ocean, tropical tuna, Reunion, Tromelin Exclusive Economic Zone.

1. Introduction

The identification of spawning and nursery habitats for marine species is essential to define spatialized management measures. Indeed, it is during these younger stages that most natural mortality occurs (greater than 99%; Hjort, 1914). In the case of albacore tuna *Thunnus alalunga* (Bonnaterre, 1788), larval survival is particularly critical as these species have high fecundities (millions of eggs per female) and thus small variations in the percentage of larval survival will have a significant impact on the abundance of the next cohort.

This pelagic fish species represents a significant part of the catches of the Reunion Island longline fishery with ~ 300 t landed annually (~ 15% of total longline catch in Reunion; (Serazin *et al.,* 2021). Assessments of this stock show current or future problems with exploitation levels (IOTC, 2019) as the stock status is subject to overfishing. Moreover, there is a large uncertainty about this stock due to the lack of knowledge on key biological and ecological characteristics, including knowledge of its early life history.

The Working Party on Temperate Tuna species (albacore and southern bluefin tuna) at IOTC has established research priorities to enable a sustainable exploitation of these species. Among these priorities, the identification of reproductive areas, spawning seasons, and the biological parameters (growth, maturity) has been defined as a high priority. Indeed, to date, little information is available for albacore (the southern bluefin tuna stock is managed by another RFMO: CCSBT).

Some spawning areas have been identified through international research projects, including an French Research Institute for Exploitation of the Sea (IFREMER) project in the southwest Indian Ocean (FEP GERMON, Programme Pêche Palangrière) but these need to be further developed for a more complete understanding of the spawning grounds. These projects found that the spawning areas are likely to be located between 10°S and 30°S in the Indian Ocean with a main spawning season in the austral summer between October and March (Nikolic *et al*., 2014).

Scientific campaigns to capture tuna larvae and swordfish were carried out in the 1960s and 1970s (Fig. 1) and allowed the identification of certain areas in the Indian Ocean (IO) where albacore larvae were observed (Ueyanagi, 1969; Fig. 2). However, this larval sampling was not exhaustive, especially in the potential breeding areas of albacore and the areas where larval net hauls were carried out do not cover the potential breeding area (between 10°S and 30°S of the IO, Nishikawa *et al*., 1985; Fig. 3).

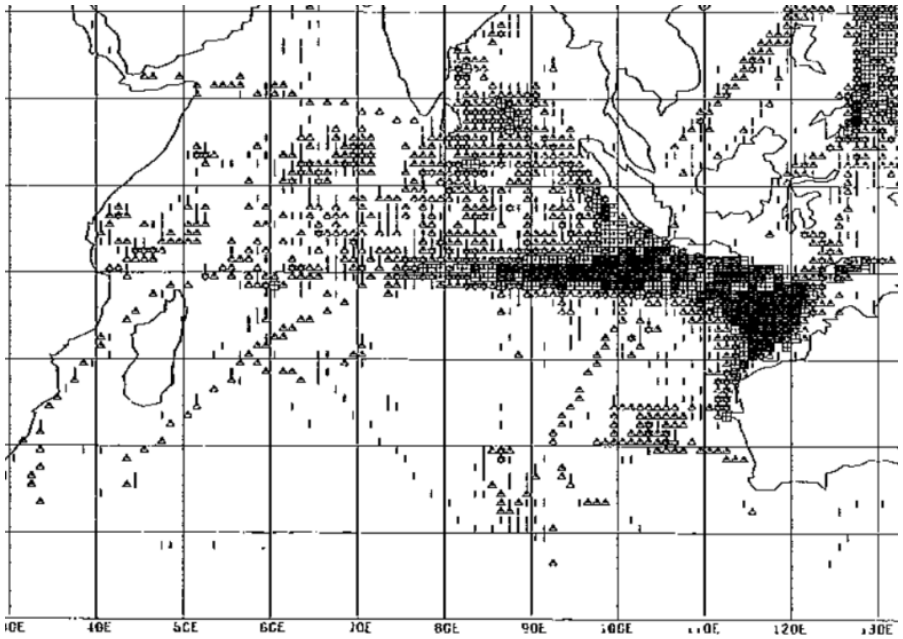

Figure 1: Sampling effort of the Japanese scientific larval surveys during the 1960s and 1970s (from Ueyanagi, 1969).

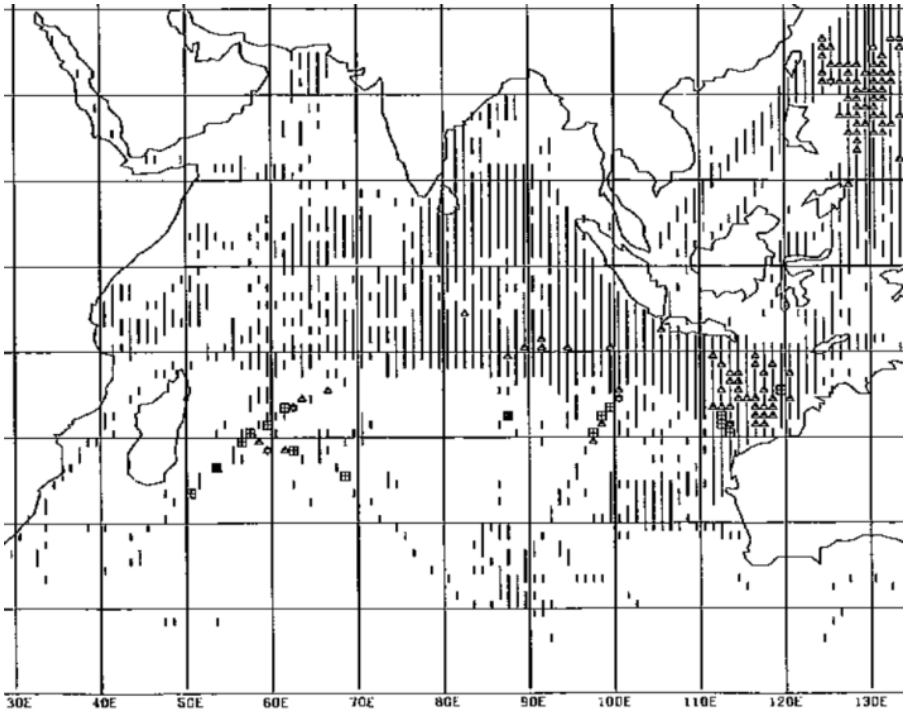

Figure 2: Results of the Japanese scientific larval surveys for albacore during the 1960s and 1970s. Vertical bars indicate no catch (from Ueyanagi, 1969).

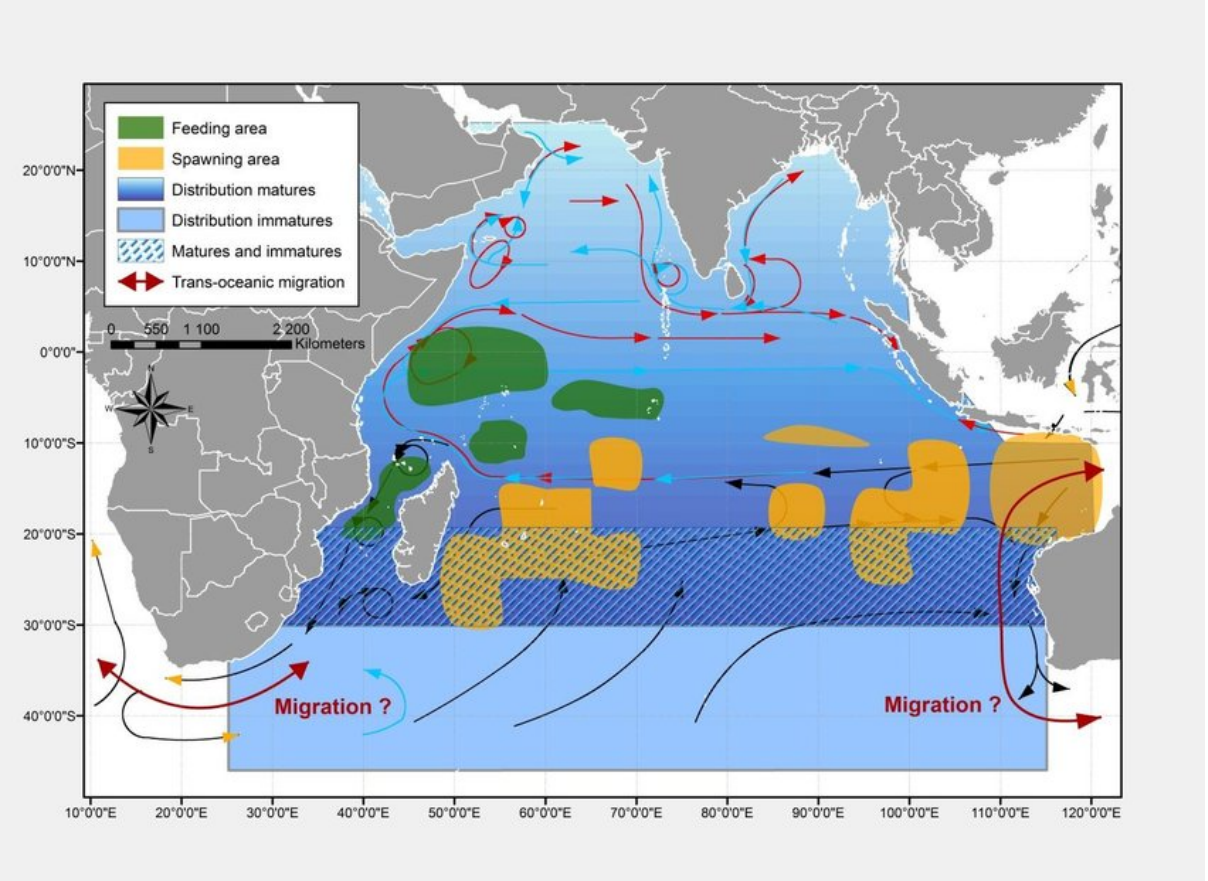

Figure 3: The presumed spawning and feeding areas of albacore in the Indian Ocean (from Nikolic *et al.*, 2014).

Here we present the results from a larval survey that was carried out in January and February 2022 in the southwest IO. The scientific survey aimed at identifying the spawning ground of large pelagic species using plankton nets and by deploying pop-up satellite tags on adult fish caught by a longline. eDNA samples have been taken by filtering seawater at the same depth of larval tuna occurrence but we only report results from the plankton net tows in the present document.

2. Materials and Methods

2.1. Station locations and global cruise description

The scientific survey took place from January 17th to February 2nd, 2022, onboard a French surveillance vessel, OSIRIS II, which is a 55.5 m long longliner that has been converted to fisheries control and scientific activities. Over the 2.5 weeks at sea, the daily sampling was split into longline fishing at night to target large pelagic species and catch individuals alive to tag them with satellite tags. During the day, plankton nets were towed to target tuna and billfish larvae. Seawater samples were collected to use eDNA methods and identify the presence of large pelagic species. The OSIRIS II left Le Port in Reunion Island and headed towards Tromelin Island. The sampling strategy was driven by the longline activities and plankton net tows were set opportunistically two or three times a day.

Environmental conditions were used as cues for the potential presence of tuna and billfish species, e.g. sea temperature fronts and sea level anomalies (Copernicus Marine Service, 2022).

A total of 36 stations were sampled starting from the south of Tromelin EEZ and ending close to the Reunion Island coast (Fig. 4). Date and time, GPS location, depth of the net, volume filtered, and other information were recorded at each station (see next section).

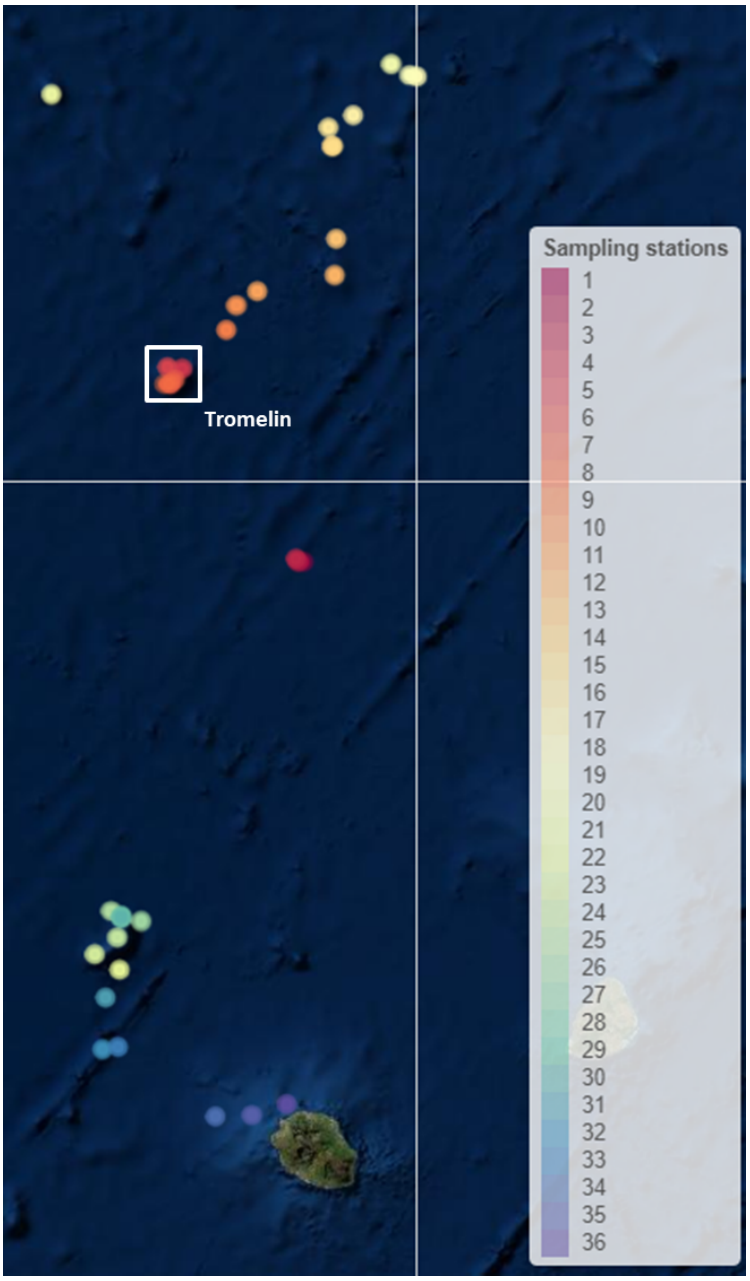

Figure 4: Location of the larval net tow stations.

2.2. Ichthyoplankton sampling

Larval samplings were conducted near longline stations that were approximately 770 km north (Sta. 1—21, 14º S, 55.5º E) and 170 km northwest (Sta. 22—36, 20º S, 54º E) of Reunion Island (Fig. 4). Since the target species are known to dwell in the upper 25 m of the water column during the

day (Habtes *et a*l., 2014; Llopiz and Cowen, 2008; Llopiz *et al*., 2010), larval sampling took place opportunistically during daytime at around 11:00, 15:00, and before sunset at around 18:00, local time. A 60 cm bongo frame equipped with 500 µm mesh nets (333 µm at northern stations due to damaged equipment) with codends and mechanical flowmeters (438 110, Hydro-Bios Kiel) were towed against the current for 10 minutes at a speed of approximately 3.5 knots, from the starboard side, off 1.5 m long davit. For surface neuston sampling, a 5 kg weight was added to keep the net stable during the tow and a 40 kg weight was added to submerge the gear for an undulating subsurface tow down to target depth of 25 m (Habtes *et al*., 2014). A 10-minute quadruple oblique tow was made for most stations. Depth was estimated using line out and line angle during the tow and the maximum depth and tow profile were recorded by a custom-made electronic sea turtle tag specifically adapted for that purpose (Gogendeau *et al*., 2022).

2.3. Visual identification, sample sorting, and preservation

Samples from the left nets were immediately preserved in 95% EtOH and stored in an air conditioned room. Samples from the right nets were sorted live on board for tuna and billfish larvae under a dissecting microscope (MZ12.5, Leica Microsystems), following live sorting methods by Shiroza *et al*. (2021). Tuna and billfish larvae were visually identified following references (Nishikawa and Rimmer, 1987; Okiyama, 2014; Richards, 2005) and larvae were measured, photographed, and tissue samples were removed for genetic analysis. The eyeball was primarily used as a tissue sample (right eyeball if both were intact) or the tail was severed with a sterilized scalpel if both eyes were missing (Richardson *et al*., 2007). Tissue samples were isolated in individual vials with 200 µl of 95% EtOH and kept frozen at -20 °C until a batch was collected for an onboard DNA extraction. The remainder of larvae were individually preserved in vials with 95% EtOH and the sorted zooplankton samples were also preserved in jars with 95% EtOH and stored in an air conditioned room. If more than 10 larvae were found from a live sorting, subsequent sampling followed five nautical miles away in four directions, in an attempt to locate the center of the larval patch. The initial preservative was changed 24 hours post-collection to compensate for dilution due to sample dehydration.

Back in the lab, all samples were sorted to separate fish larvae, and tuna and billfish larvae were visually identified to the lowest possible taxon. All other larvae were identified to family level using references (Leis and Carson-Ewart, 2000; Okiyama, 2014; Richards, 2005). Tuna and billfish larvae from unsorted left bongo samples and those missed in initial onboard live sorting were genetically identified.

2.4. Genetic identification

2.4.1 DNA extraction

The DNeasy Tissue Kit (Qiagen, Hilden, Germany) was used to make the DNA extraction, as per the manufacturer's instructions, except for the elution step, the same 100 µL of elution buffer (EB) was applied twice on the column to concentrate the DNA whereas they recommend to put 200 µL of EB. The extracted DNA's concentration was measured using an Invitrogen™ Qubit™ 4 Fluorometer (Waltham, Massachusetts, United-States)

2.4.2 Species-specific multiplex primer

PCR primers used during the experiments were based on a literature review. Six species-specific pairs were found to amplify different genes from mitochondrial DNA sequences. Ga-Young *et al*. (2022) designed five species-specific pairs of primers for bigeye (*Thunnus obesus*), skipjack (*Katsuwonus pelamis*), Atlantic bluefin tuna (*Thunnus thynnus*), albacore (*Thunnus alalunga*), and yellowfin tunas (*Thunnus albacares*). The last pair was designed by Weng-Feng *et al*. (2007) for *Euthynnus affinis*. The six original primer pair sets for multiplex PCR are shown in Table 1.

It has conventionally been assumed that though they may be found in the IO as adults, due to their strong homing behaviors (Block *et al*., 2005; Rooker *et al*., 2008; Wilson *et al*., 2015) Atlantic bluefin tuna do not spawn in the IO.. However, there have been reports of spawning outside of their primary spawning grounds in the Mediterranean Sea (MED) and Gulf of Mexico (McGowan and Richards, 1989; Muhling *et al*., 2011; Lamkin *et al*., 2015; Richardson *et al*., 2016), thus, we included the primers to detect *T. thynnus* as well.

Table 1: Species-specific primers pairs for multiplex PCR, their sequences, the lengths of PCR amplicons, the accession number, and the bibliographic reference.

Target Species	Target Gene	Primer Name	Sequence (5' —> 3')	Amplicon Size (bp)	Accession No.	Reference
Thunnus obesus	ATP6	Obe-F	ACT TGC ATT CCC CCT ATG	270	KY400011.1	Ga-Young et al. (2022)
		Obe-R	GCT GTT AGG ATT GCC ACA G			
Katsuwonus pelamis	Cytb	Kat-F	GGT CCT AGC TCT TCT TGC A	238	NC_005316.1	Ga-Young et al. (2022)
		Kat-R	TGC AAG TGG GAA GAA GAT G			
Thunnus thynnus	NADH5	Thy-F	AAC TCT TTA TCG GGT GGG AG	200	KF906720.1	Ga-Young et al. (2022)
		Thy-R	AGC GGT TAC GAA CAT TTG CTT C			
Thunnus alalunga	Cytb	Ala-F	GTT TCG TGA TCC TGC TAG TG	178	NC_005317.1	Ga-Young et al. (2022)
		Ala-R	CCT CCT AGT TTG TTG GAA TAG AT			
Thunnus albacares	NADH4	Alba-F	CAT GAT TGC CCA CGG ACT TA	127	KM588080.1	Ga-Young et al. (2022)
		Alba-R	TGT TGT TAT AAG GGG CAG C			
Euthynnus affinis	Cytb	EA401-L	GGG GGA GAA AAG TGC TAA GG	398	/	Weng-Feng et al. (2007)
		EA777-H	CTA GTG ATG ATA ACT GCC TTC G			

2.4.3 PCR amplification

For multiplex PCR, a mix of forward and reverse primers were prepared by adding 2 µL of each primer for a total volume of 20 µL. The final volume of PCR reaction was 20 µL, containing 4 µL of extracted DNA, 1 µM of each primer (from mix), 800 µM of dNTP, 2.5 U of Taq DNA Polymerase (Qiagen, Hilden, Germany), QIAGEN PCR buffer and complete with Milli-Q Water. Multiplex PCR was carried out in an Applied Biosystems 2720 Thermal Cycler (Waltham, Massachusetts, United-States) using the following program: 94°C for 2 minutes, followed by 35 to 50 cycles (depending on the DNA extracted concentration) of 30 seconds at 94 °C, 30 seconds at 55 °C, 30 seconds at 72 °C. The final extension step at 72 °C was performed for 3 minutes or longer.

2.4.4 DNA electrophoresis

Ten microliters of PCR product and 2 µL of DNA-dye Non-Tox were mixed and loaded onto a 3% agarose gel containing 8 µL of Midori Green. The electrophoresis was running in the TE buffer at 100 V for 90 minutes. The DNA bands were observed under ultraviolet light and photographed using InfinityCapt (Vilber Lourmat Sté, France, Fig. 5).

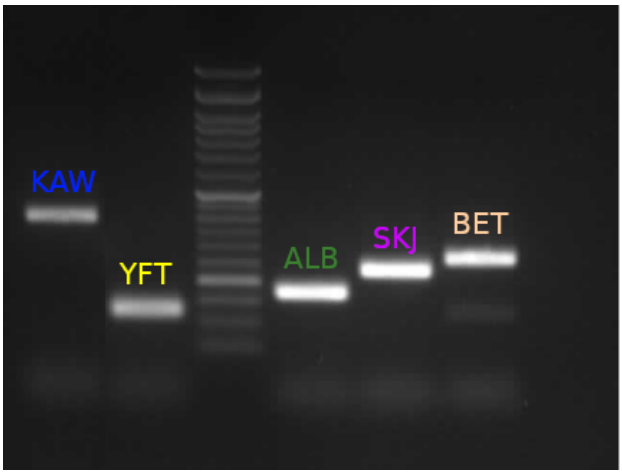

Figure 5 : Specificity of multiplex PCR under UV light (from left to right): *Euthynnus affinis* (KAW, 398bp), *T. albacares* (YFT, 127bp), *T. alalunga* (ALB, 178bp), *K. pelamis* (SKJ, 238bp), and *T. obesus* (BET, 270bp).

2.5. Data analysis

Two-way analysis of variance (ANOVA) was run to check the influence of categorical independent variables, depth and site, on larval tuna densities using R (R Core Team, 2022). Density data were log+1 transformed to compensate for stations where larvae were absent, prior to linear model fitting.

3. Results

3.1. Larval tuna assemblage and distribution

From 65 bongo tows (30 surface, 35 subsurface tows), 2258 ichthyoplankton representing more than 35 families of fish were collected and of these, 330 (14.61% of total ichthyoplankton) were tuna larvae. Most other ichthyoplankton were larvae of mesopelagic fishes (72.93% of total ichthyoplankton) such as Myctophidae, Gonostomatidae, Gempylidae, Chiasmodontidae, Stomiidae, and Phosichthyiidae (49.00%, 13.25%, 5.54%, 1.86%, 1.15%, and 0.93% of total ichthyoplankton, respectively). Larvae of some pelagic families were also abundant, such as Hemiramphidae and Exocoetidae (5.94% and 1.28% of total ichthyoplankton).

3.1.1 Larval tuna morphological identification

In total, 330 larval scombrids were collected and 329 were morphologically identifiable at least to genus and further species identification was confirmed via genetic methods (Table 1). Since small 60 cm bongo nets were used, collected larval scombrids were mostly underdeveloped, small preflexion stage larvae (average SL = 3.50 ± 0.73 mm, Fig. 6), which made visual identification difficult

as some key differences appear further on in growth, at about 4–5 mm SL (Nishikawa and Rimmer, 1987). Of the 240 morphologically identified that were also genetically confirmed to species, five could not be identified to genus, three were misidentified at genus level, 12 were misidentified at species level, 131 were correctly identified to genus but could not be identified to species, and only 89 larvae were correctly identified to species (2.08%, 1.25%, 5.00%, 54.55%, and 37.08%, respectively).

Table 2: Larval tuna species metrics (N = 329, excluding one unidentified scombrid larva) showing individuals collected (N), mean density per tow ± SD (ind./ 1000 m^3), ratio represented in total density within depth or site, and mean standard length (SL) ± SD. Most of the larvae were collected from the subsurface tows (71.08% N) and, on average, more abundant (17.10 ± 11.92 ind./ 1000 m^3). Generally, more tuna larvae were found from the subsurface layers of southern stations. *T. alalunga* was the most abundant species collected from this survey (68.21%), followed by *K. pelamis* (4.45%). Mean SL was under 4 mm for all species. *Thunnus* sp. includes 73 larvae that were frozen and isolated for another project that were not genetically identified, but were morphologically identified to genus.

Tuna species	*E. affinis*	*K. pelamis*	*T. alalunga*	*T. albacares*	*T. obesus*	*Thunnus* sp.	Total
Surface							
N	1	3	50			6	60
Density	0.12 ± 0.68	0.45 ± 1.83	7.70 ± 18.32			0.79 ± 4.35	8.18 ± 20.65
%	100.00%	35.18%	38.88%			11.37%	28.92%
SL (mm)	3.30	2.70 ± 0.57	3.56 ± 0.65				3.51 ± 0.67
Subsurface							
N		11	164	3	8	83	269
Density		0.72 ± 2.02	10.37 ± 7.93	0.19 ± 0.63	0.52 ± 1.83	5.31 ± 6.98	17.10 ± 11.92
%		64.82%	61.12%	100.00%	100.00%	88.63%	71.08%
SL (mm)		2.94 ± 0.40	3.55 ± 0.75	3.57 ± 1.34	3.30 ± 0.43	3.43 ± 0.77	3.49 ± 0.74
North							
N	1	13	87	2	8	14	125
Density	0.10 ± 0.61	0.98 ± 2.44	7.01 ± 9.98	0.12 ± 0.54	0.48 ± 1.76	0.87 ± 2.09	3.29 ± 4.11
%	100.00%	96.01%	44.86%	73.26%	100.00%	15.82%	37.88%
SL (mm)	3.3	2.85 ± 0.42	3.24 ± 0.73	4.04 ± 1.52	3.30 ± 0.43	3.46 ± 0.84	3.23 ± 0.73
South							
N		1	127	1		75	204
Density		0.06 ± 0.30	12.13 ± 17.41	0.06 ± 0.33		6.54 ± 8.47	7.56 ± 8.01
%		3.99%	55.14%	26.74%		84.18%	61.82%
SL (mm)		3.41	3.75 ± 0.66	2.64		3.29 ± 0.35	3.73 ± 0.66
Total							
N	1	14	214	3	8	89	329
Density	0.06 ± 0.46	0.60 ± 1.92	9.14 ± 13.68	0.10 ± 0.47	0.28 ± 1.36	3.22 ± 6.29	12.98 ± 16.99
%	0.43%	4.45%	68.21%	0.74%	2.09%	24.08%	100.00%
SL (mm)	3.30	2.89 ± 0.43	3.55 ± 0.73	3.57 ± 1.34	3.30 ± 0.43	3.43 ± 0.77	3.50 ± 0.73

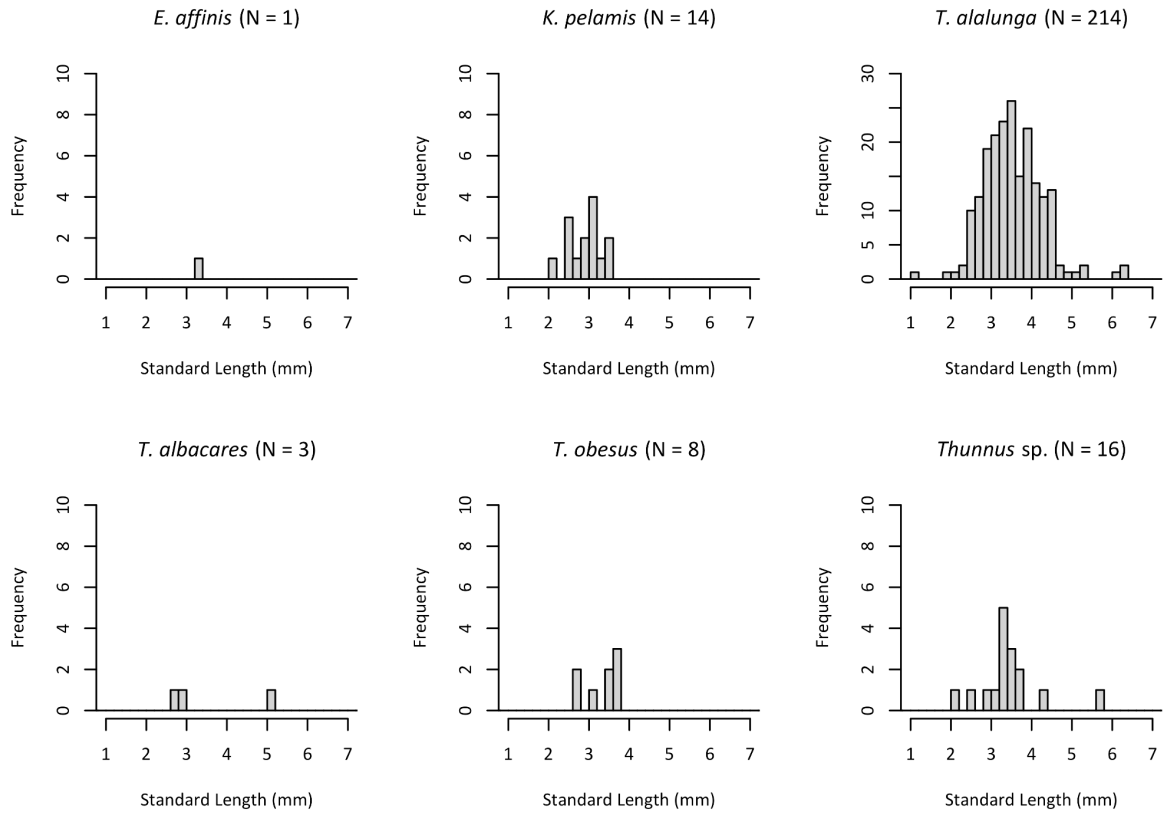

Figure 6: Histogram of tuna species genetically identified by standard length (mm) preserved in EtOH (N = 256), excluding one unidentified scombrid larva and 72 *Thunnus* spp. larvae that were frozen and not genetically identified. See Table 2 for mean SL ± SD for each species.

3.1.2 Larval tuna abundance and distribution

269 tuna larvae were collected from the subsurface tows (Table 2). Larval densities were generally higher in the subsurface layer than the surface for all species except the highest density recorded was from a surface tow for *T. alalunga* (at Sta. 32, 87.56 ind./ 1000 m^3). The most abundant species in both layers were *T. alalunga* (average density = 7.70 ± 18.32 and 10.37 ± 7.93 ind./ 1000 m^3 for surface and subsurface layers, respectively).

The southern stations had the highest density of larval tuna (Sta. 22—36, Fig. 7), mainly comprised of *T. alalunga* (average density = 12.13 ± 17.41 ind./ 1000 m^3, Table 2). *K. pelamis*, *T. albacares*, and *T. obesus* were more abundant at the northern stations though *T. alalunga* was the most abundant species at the northern stations as well. A single *E. affinis* larva was collected from the northern station.

Two-way ANOVA run for two species that were collected in both depths (surface and subsurface) and sites (northern and southern) shows that site was a significant factor ($F = 4.25$, $P < 0.05$) for *K. pelamis*, and depth was a significant factor for *T. alalunga* ($F = 13.39$, $P < 0.001$) (Table 3).

Table 3: Two-way ANOVA table for log transformed densities of *K. pelamis* and *T. alalunga*. Factors are depths (surface and subsurface) and sites (northern and southern stations). For *K. pelamis*, site

was a significant factor (F = 4.25, P < 0.05) and depth was a significant factor for *T. alalunga* (F = 13.39, P < 0.001).

		df	F	P
K. pelamis	Depth	1	0.83	0.37
	Site	1	4.25	0.04
	Depth x Site	1	0.19	0.66
	Residuals	61		
T. alalunga	Depth	1	13.39	5.23E-04
	Site	1	3.03	0.09
	Depth x Site	1	0.04	0.84
	Residuals	61		

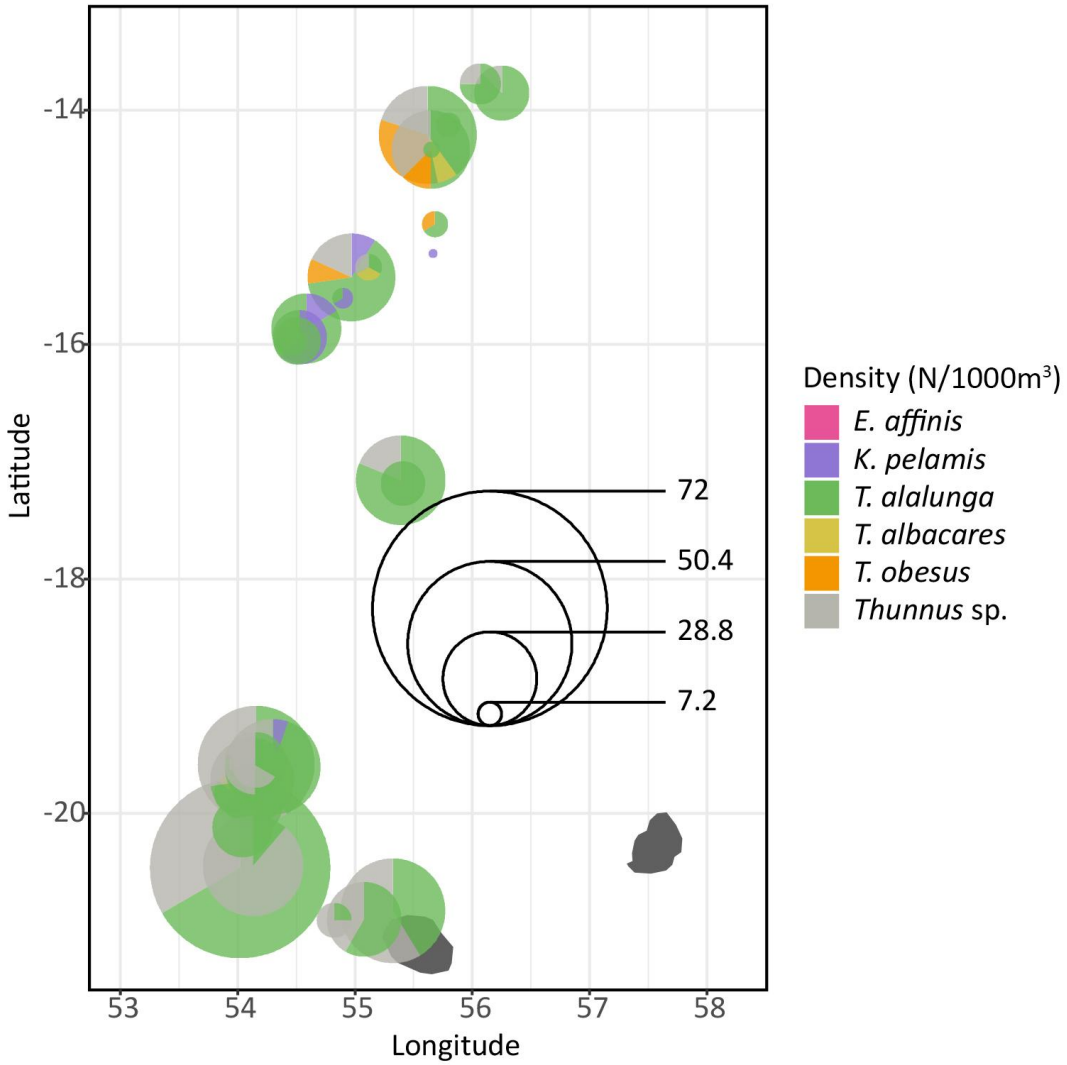

Figure 7: Larval tuna density (ind./ 1000 m^3) from subsurface 60 cm bongo tows. Highest tuna density was found from the southern stations.

3.2. Genetic identification

As shown in Figure 8 for *T. alalunga* and *T. obesus*, the six species-specific pairs of PCR primers succeeded in identifying larvae with a multiplex PCR approach.

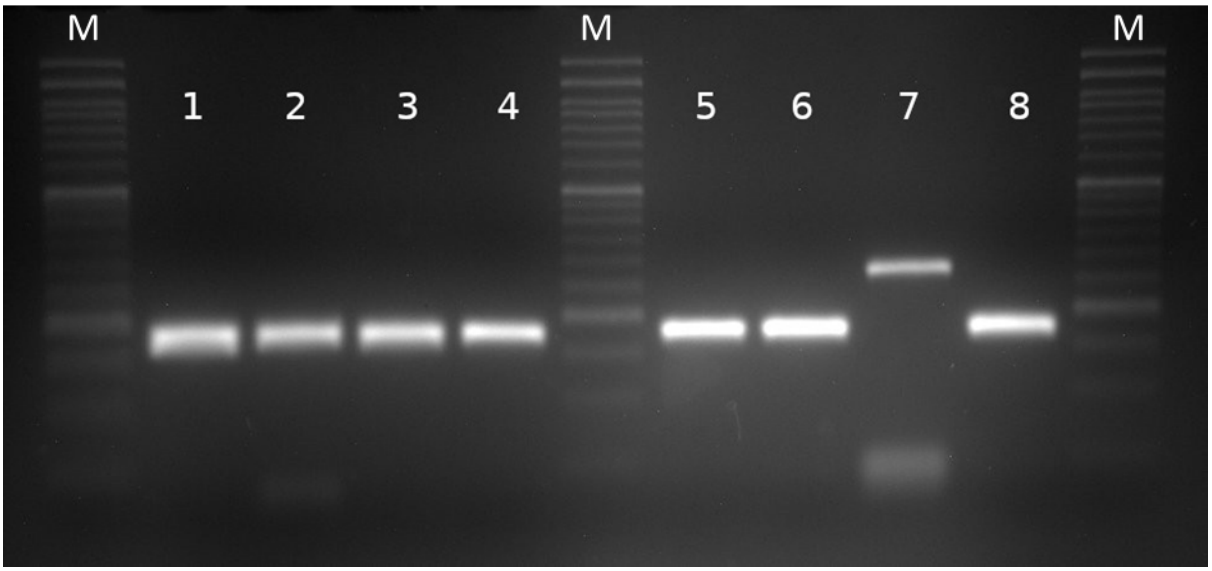

Figure 8 : Sample image of a result from the multiplex PCR: ladder (M), *T. alalunga* (lanes 1–6 and 8), *T. obesus* (lane 7).

A total of 257 tuna larvae were identified by genetics to verify visual identification (Table 4). *T. alalunga* was the most abundant species genetically-identified with a total of 214 larvae (83.15%) followed by *K. pelamis* with 14 larvae (5.45%) and eight larvae for *T. obesus* (3.11%). No *T. thynnus* larvae were identified from this survey. However, 17 larvae were not genetically-identified and classed as *Thunnus* sp. from visual identification.

Species	N	%N
T. alalunga	214	83.27
T. albacares	3	1.17
T. thynnus	0	0
T. obesus	8	3.11
K. pelamis	14	5.45
E. affinis	1	0.39
Thunnus sp.	16	6.23
Scombridae	1	0.39
Total	257	100

Table 4: Number of genetically identified tuna larvae (N) and their proportion (%N).

4. Discussion

4.1. Larval tuna assemblage and distribution

4.1.1 Larval tuna identification

We were only able to collect small larval tuna that were mostly under 4 mm SL because our net mouth was too small to effectively collect larger, older, and faster swimming larvae capable of escaping the 60 cm bongo nets. Larval tuna can swim up to 2 body lengths per second (Sabate *et al*., 2010), therefore, larger mouth nets such as 90 cm bongo, 2x1 neuston frame, 1 m MOCNESS, and larger are conventionally used for sampling larval tunas at older larval growth stages (Habtes *et al*., 2014; Llopiz *et al.*, 2010; Shiroza *et al*., 2021).

Older, larger larvae are also generally easier to visually identify than younger, smaller larvae because some key identification characteristics appear later on in growth. This is especially true when differentiating between lightly pigmented *Thunnus* species (ex. lower jaw pigment on the underside of jaw ca. 4 mm TL, *T. albacares*; lower jaw pigment appears later than ca. 8 mm TL, *T. alalunga*) (Nishikawa and Rimmer, 1987; Okiyama, 2014).

Initially, 180 *Thunnus* spp. were visually unidentifiable to species due to their small size, but also due to peculiar pigment patterns; i.e. an unpigmented tail except for a point of pigment in the ventral caudal fin area. These individuals were genetically identified as mostly *T. alalunga* (125 out of 137 genetically identified). This phenotype is not observed in the MED population and it would be interesting to investigate the genetic differences between the southwest IO and the MED *T. alalunga*. The 89 larvae that only identified to genus were *T. alalunga* and *Thunnus* spp. that were frozen for other studies and failed to be genetically identified.

4.1.2 Larval tuna distribution

Generally, tuna larvae were more abundant in subsurface tows, which agrees with other larval tuna abundance and distribution reported from other parts of the world. Surface current runs westward in the study area and finding larval tuna at our sampling area agrees with feeding and spawning migration schematic drawn by Nikolic *et al*. (2014). One consecutive subsurface bongo tow sets were conducted after station 27, where we identified more than 10 *T. alalunga* from onboard sorting. As such, generally, there were more tuna larvae in the southern site.

Origin of the spawning aggregation can be back calculated by using larval growth curve and particle distribution models but no growth curve is currently available for *T. alalunga* from the southwest IO. García *et al*. (2006) provides age-length relationships for frozen *T. alalunga* larvae from the MED and frozen measurements can be estimated from samples preserved in EtOH using conversion equation by Satoh *et al*. (2008) from *T. orientalis* larvae with similar body shape. Applying those measurements to the age-length relationship, mean age of our larvae are estimated to be 2.67 ± 1.97 and 4.10 ± 1.84 days post hatching (dph) for the northern and southern sites, respectively (removed two negative values for age from both sites). Hatch time for tuna larvae are within 24–48 hours but according to the aquaculture data for *T. thynnus*, hatching time can be reduced with higher temperature (Gordoa and Carreras, 2014). SST data was obtained from Optimum Interpolation SST (Reynolds *et al*., 2007) and mean SST for the northern and southern sites during our sampling were 28.88 ± 0.22 °C and 27.95 ± 0.17 °C, respectively, with slightly higher temperature observed from the northern site (Fig. 9). SST where *T. alalunga* larvae spawned were between 27.5–28.5 °C in the MED (García *et al*., 2006), similar to the SST range in our study area. Accounting for those factors, our *T. alalunga* larvae are assumed to be in the range of 0–5 and 3–6 dph for the northern and southern

sites, respectively. Despite age length relationship from different waters was used—with different prey availability and physical environment that would influence growth—because our larvae were mostly young preflexion larvae that had undergone only few feeding cycles (first feeding of *Thunnus* larvae 2 dph), and *Thunnus* eggs and yolk-sac larvae length at hatching are similar, this age estimate would be fairly reasonable. This is only a preliminary estimation of the origin of spawning area and age-length relationships need to be created for *T. alalunga* in the IO for further comparative analysis of growth between IO and MED and between western and eastern spawning grounds in the IO.

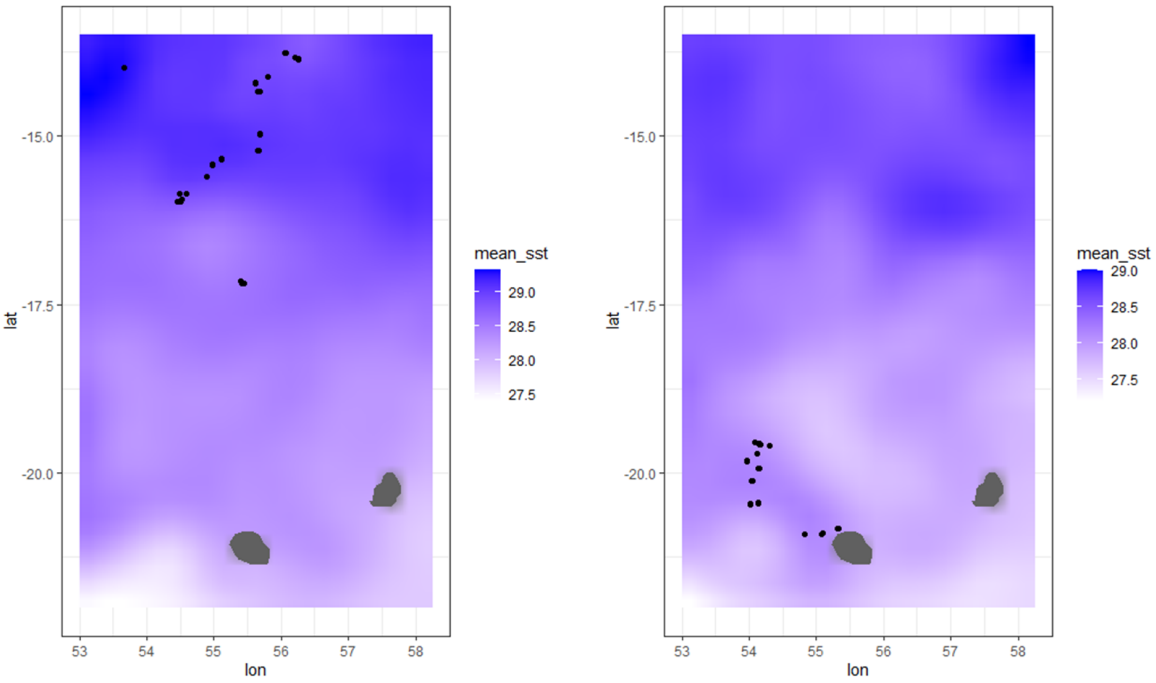

Figure 9: Mean sea surface temperature (SST) for the northern stations between January 17−25, 2022 with a mean of 28.88 ± 0.22 °C, (left) and the southern stations between January 28th to February 2nd, 2022 with a mean of 27.95 ± 0.17 °C (right).

4.2 Genetics approach

The primers used during the campaign allowed us to identify almost all the tuna larvae to species. The multiplex PCR/electrophoresis approach, however, has a major drawback; the visualization of the bands on agarose gel cannot be done onboard—the genetic identification must be done in the lab. For future surveys it is envisaged to replace the multiplex PCR by a multiplex qPCR, deleting the procedure using the agarose gel. The qPCR would allow faster identification (results in about six hours including DNA extraction) and can be directly done on board. This would be useful in confirming the species for targeted sampling of tuna larvae where visual identification is difficult. For larvae that currently remain unidentified with PCR, it is necessary to sequence the extracted DNA. The sequencers will be the GridION or MinION from Oxford Nanopore Technologies (Oxford, United Kingdom).

4.3 Future direction

This was a preliminary ichthyoplankton study done in the area and further systematic, stratified sampling using multi nets, such as Multiple Opening and Closing Nets Environmental Sampling System (MOCNESS), would unveil the larval assemblage and distributions of other tuna species in southwestern IO. Finding the spawning area and season for these highly migratory and economically important tuna species through larval sampling is important for protecting spawning stock for sustainable fisheries.

Environmental factors affecting feeding, growth, and mortality affect larval survival and recruitment into fisheries. Because there are no recent reports on feeding, growth, and mortality of larval tunas from southwestern IO, creating larval tuna indices through ichthyoplankton studies would benefit the fisheries management. Comparative early life history study in feeding and growth between the southeastern IO larvae that spawn around northwestern coast of Australia and other parts of the world (i.e. Mediterranean for *T. alalunga*) would reveal environmental factors that are advantageous/disadvantageous to those spawning grounds and how they may ultimately affect survival and recruitment of the species.

Furthermore, combined with adult tissue samples from commercial fisheries, periodical targeted larval sampling combined with genetic data would create data bank for use in Close-Kin Mark Recapture (CKMR) studies to estimate absolute abundances of stocks that were proven to be successful for bluefin tuna species such as *T. maccoyii* (Bravington *et al*., 2016) and *T. thynnus* (McDowell *et al*., in review).

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Raw genetic data :

https://docs.google.com/spreadsheets/d/1gQAfRaF1lexosIYWvEdCzOSqOq-m2GKR/edit?usp=sharing&ouid=117034914595674675623&rtpof=true&sd=true

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