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Understanding the role of insular African mangroves as nursery areas for the early life stages of fish

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DEDICATION

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RESUMO

As florestas de mangal têm vindo a ser mundialmente reconhecidas como importantes zonas de viveiro para larvas de peixe e juvenis. Apesar da reconhecida importância destes sistemas para a gestão de recursos pesqueiros, o conhecimento quanto às comunidades de larvas de peixe que existem em mangais insulares é, ainda, escasso, e consequentemente o potencial das florestas de mangal na ilha de São Tomé como zonas de viveiro continua desconhecido. Durante quatro semanas entre os meses de outubro e novembro de 2020, as comunidades larvares de peixes de duas florestas de mangal da ilha de São Tomé foram amostradas com recurso a armadilhas de luz, redes de cerco e redes de plâncton usando uma abordagem multi-habitat. Para suplantar a falta de informação necessária para a identificação das larvas, um conjunto de métodos morfológicos e moleculares foram utilizados para a identificação dos exemplares. Um total de 4 010 larvas foram recolhidas, pertencentes a 16 famílias e contabilizando 27 espécies. Um pequeno número de espécies dominou a comunidade de ictioplâncton, em que as famílias Cichlidae (47%), representada pela invasora *Oreochromis mossambicus*, e Gobiidae (43%) com sete espécies encontradas, totalizaram cerca de 90% da comunidade de larvas capturadas.

As restantes 14 famílias representaram apenas 10% da comunidade, sendo que três espécies são novos registos para os habitats de mangal na ilha e três espécies são novos registos para a ilha de São Tomé. A riqueza e diversidade de espécies variou consoante o método de amostragem. A maior riqueza específica foi registada no mangal de Malanza (27 espécies) enquanto que a Praia das Conchas (9 espécies) não revelou valores semelhantes de biodiversidade. Foram encontradas diferenças nas comunidades larvares entre diferentes locais em cada um dos dois mangais, revelando uma forte influência do tipo de habitat. Os padrões espaciais de diversidade revelaram uma fraca influência por parte de variáveis ambientais como a temperatura e oxigénio dissolvido. Onze espécies encontradas em forma larvar ou juvenil têm interesse comercial e a sua presença numa fase de vida precoce nos mangais de São Tomé reforça a necessidade de conservação destes ecossistemas e com implicações diretas para a sustentabilidade das pescarias locais.

PALAVRAS-CHAVE: LARVAS DE PEIXE, JUVENIS, SÃO TOMÉ, OESTE AFRICANO, CITOCROMO OXIDASE I (COI)

ABSTRACT

Mangroves have been recognized worldwide as crucial nursery areas for fish larvae and juveniles. Although critical for managing coastal fish stocks, information about larval fish communities in African island mangroves is scarce and these potential nursery areas in São Tomé Island have remained understudied. Fish larvae were collected over four weeks from October to November 2020 using light traps, passive plankton tows and seine nets in a multi-habitat approach. To overcome species identification constraints, both morphology and molecular analysis were taken under consideration for specimen identification. A total of 4,010 larvae were caught across all methods belonging to 16 families or 27 species. Few species dominated the ichthyoplankton community of which, the most abundant families were Cichlidae – the invasive *Oreochromis mossambicus* (47%) - and Gobiidae (43%), constituted by seven *taxa*. The remaining 14 families only accounted for about 10% of total larvae captured, with three new species were recorded for the first time in these mangroves and three more species are new records for São Tomé Island. Taxa composition and richness varied considerably between sampling techniques. The highest taxa richness and diversity were recorded in the Malanza mangrove (27 species) while Praia das Conchas (9 species) was not able to sustain similar levels of biodiversity. Differences on fish larvae composition were found within the studied mangroves, depicting a strong influence by habitat type and a relative position within each system. These community composition patterns were marginally influenced by environmental variables such as water temperature and dissolved oxygen. Overall, a total of eleven taxa have commercial interest and their presence as juveniles and larvae in São Tomé mangroves reinforces the need for conservation of these ecosystems with direct implications for the sustainability of the local fisheries.

Keywords: fish larvae; juvenile; São Tomé; West Africa; cytochrome c oxidase I (COI)

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1. Introduction

Mangrove forests are unique coastal ecosystems, typical of tropical and subtropical latitudes, where a well-adapted plant community embraces the interface between terrestrial, estuarine and marine ecosystems (Polidoro *et al.,* 2010). Here, mangrove trees act as a "founding species", forming an often dense and monospecific community that regulates the entire ecosystem from energy and nutrient fluxes to food webs and biodiversity (Ellison *et al.,* 2005). Mangrove trees provide a plethora of conditions such as abundance of food, structural complexity and turbid waters that diminish predator foraging efficiency and, consequently, provide shelter. Therefore, mangrove forests are globally recognized as key nursery areas (Parrish, 1989; Nagelkerken *et al.,* 2001; Cocheret de la Morinière *et al.,* 2003). While nursery areas were originally linked to high abundances of juvenile fishes, this concept has evolved to take in consideration the contribution in juveniles of a given species to the adult population on a per-unit-area basis allowing its comparison to other nursery areas such as coral reefs or marine prairies (Kimirei *et al.,* 2013; Nagelkerken *et al.,* 2015; Cravo *et al.,* 2021). Moreover, mangroves are a necessary link in a chain of habitats that some species are dependent on and thus, can have cascading impacts in adjacent areas and ultimately influence coastal stocks. Naylor et al. (2000) stated that one third of all commercial fishes are mangrove dependent and in southeast Asia it has been reported that mangrove-related species contribute to 30% of fish harvests (Rönnbäck, 1999).

Their role as nursery areas of commercially important fish is often mentioned as the most relevant ecosystem service provided by mangrove forests, however there is poor understanding about the spatial patterns and processes influencing the species recruitment. The knowledge gap is spatially variable, being higher in African mangroves, especially lacking in insular mangroves (Félix *et al.,* 2017). In recent years, however, some studies have started to tackle this issue and some publications on São Tomé and Príncipe mangrove fish communities have emerged (Félix *et al.,* 2017; Afonso *et al.,* 2021; Cravo *et al.,* 2021; Heumüller, 2021; Afonso *et al.,* 2022). These studies have created baseline information about the composition of fish assemblages, the adult and juvenile stages that occur in these brackish systems, their habitat usage, functional diversity and, even assessing some ecosystem services provided by small mangrove forests, typical of insular areas.

Nevertheless, there is an urgent need for information given that both agriculture and fisheries make an important contribution to the São Tomé economy (14% of GDP according to the World Bank) and are the main source of income for coastal communities, employing over 5,000 people (4.6% of the population). Thus, accessing the significance of the island mangroves to the maintenance of fish communities and understanding how environmental variables may influence fish larvae assemblages through space is of key importance to identify species dependent on mangrove ecosystems and, consequently provide suitable management measures for its protection.

Afonso et al. (2022) identified 12 mangrove forests for São Tomé Island, being only three (Malanza, Praia das Conchas and Praia Quinze) included in the nation's only natural park of Obô (PNOST) that covers 30% of the island. Since 2009, these areas were classified with the intent of protecting "particular species or habitats", but the only management measures included were deforestation prevention and tourism fomentation (Cesarino & Albuquerque, 2009). Since 2014, a participative management plan of Malanza and Praia das Conchas has been in development. A report on the mangroves biophysical and socio-economical characteristics was first published in 2015 (Pisoni *et al.,* 2015) with an initial assessment of several abiotic parameters and faunal communities followed by publications on habitat usage and alfa diversity descriptions (Félix *et al.,* 2017) and, on the phytoplanktonic communities and their relationship with anthropogenic pressures (Brito *et al.,* 2017). However, there is still a knowledge gap regarding the earlier and more vulnerable life stages of fish that need these habitats as refuge and

feeding grounds to maximize their survival, reach adulthood and contribute to the spawning biomass of their populations (Ramos, 2007; Whitfield, 2016).

Given its location, Sao Tomé fish fauna is of particular interest. Here, the seasonal equatorial countercurrents and the subsurface equatorial under-current link the eastern and western Atlantic with known species from both faunal regions coexisting (Wirtz *et al.*, 2007). Yet, very few studies have been conducted on fish larvae in west Africa with most of the existing ones focusing on the Benguela current (Olivar *et al.*, 1993) and a small portion carried in continental mangroves (Vidy, 2000; Sloterdijk *et al.*, 2017). Until presently, there is no available information on fish larvae communities' composition in insular mangroves, even more so in São Tomé waters making this work the first of its kind for the region.

The study of the early life history of fishes is characterized by the specific eco-morpho-physiological features of early life stages that require specific sampling methodologies and taxonomic information for taxa identification (Catalán *et al.*, 2020). While there are well developed methods for fish larvae and egg sampling in open waters and oceanic environments, these are poorly suited to shallower systems and prop-root dominated mangroves (Brogan, 1994). Most studies regarding fish larvae in mangroves often use horizontal bongo net tows (e.g., Barletta & Barletta-Bergan, 2009; Ooi & Chong, 2011; Silva-Falcão *et al.*, 2013) although this is not always the most effective sampling method for a given system (Neal *et al.*, 2012), few incorporate alternative methodologies such as light traps (Dennis, 1992), tide trap nets (Barletta-Bergan *et al.*, 2002) and small-seine nettings (Faunce & Serafy, 2006) according to field conditions but none of these have been widely used. Consequently, sampling properties and limitations are not fully known, thus, complicating method selection (Dennis, 1992).

For taxa identification, morphological and meristic characters have been the most common approach of species identification (Strauss & Bond, 1990; Ko *et al.*, 2013). However, during the early stages of development several species often share the same morphology and measures are not often useful to differentiate species apart. Moreover, numerous phenotypic changes typically occur in this phase from preflexion larvae to postflexion to the pre-juvenile stage (Ko *et al.*, 2013; Shirak *et al.*, 2016). Because of this, species are hard to distinguish and inconsistent identifications are often reached when considering individuals at different larval stages or even by different taxonomists with different capabilities (Ko *et al.*, 2013). Adding to this problematic, larval descriptions in tropical marine and coastal waters are rare and for most species non-existent while these waters have a high species richness (Chu *et al.*, 2019). However, with the appearance of molecular techniques, DNA barcodes and other molecular markers have provided new perspectives in fish systematics and diversity studies (Shirak *et al.*, 2016). Genetic data overcomes some limitations of morphologic information, mainly as molecular identities of adults are enough to identify the remaining life stages using available DNA barcodes (Hubert *et al.*, 2014).

The main objectives of the current study were to provide the first inventory of fish larvae present in two of São Tomé's mangroves, paving the way to establish the role of the island mangroves as nursery areas and serving as a reference for future larvae related studies. Secondly, to assess differences between mangroves and to determine how environmental conditions influence the spatial distribution of larvae in these ecosystems.

2. Methods

2.1 Study area

São Tomé (Figure 1.1) is a volcanic island formed around 13 million years ago, it is located in the Gulf of Guinea between 1˚42N and the equator, about 241 km off the west African coast (Maia, 2018). The island is influenced by the Benguela current and the Gulf of Guinea current, both converging in this region and, consequently, increasing the probability of occurrence of amphi-Atlantic species (Maia, 2018).

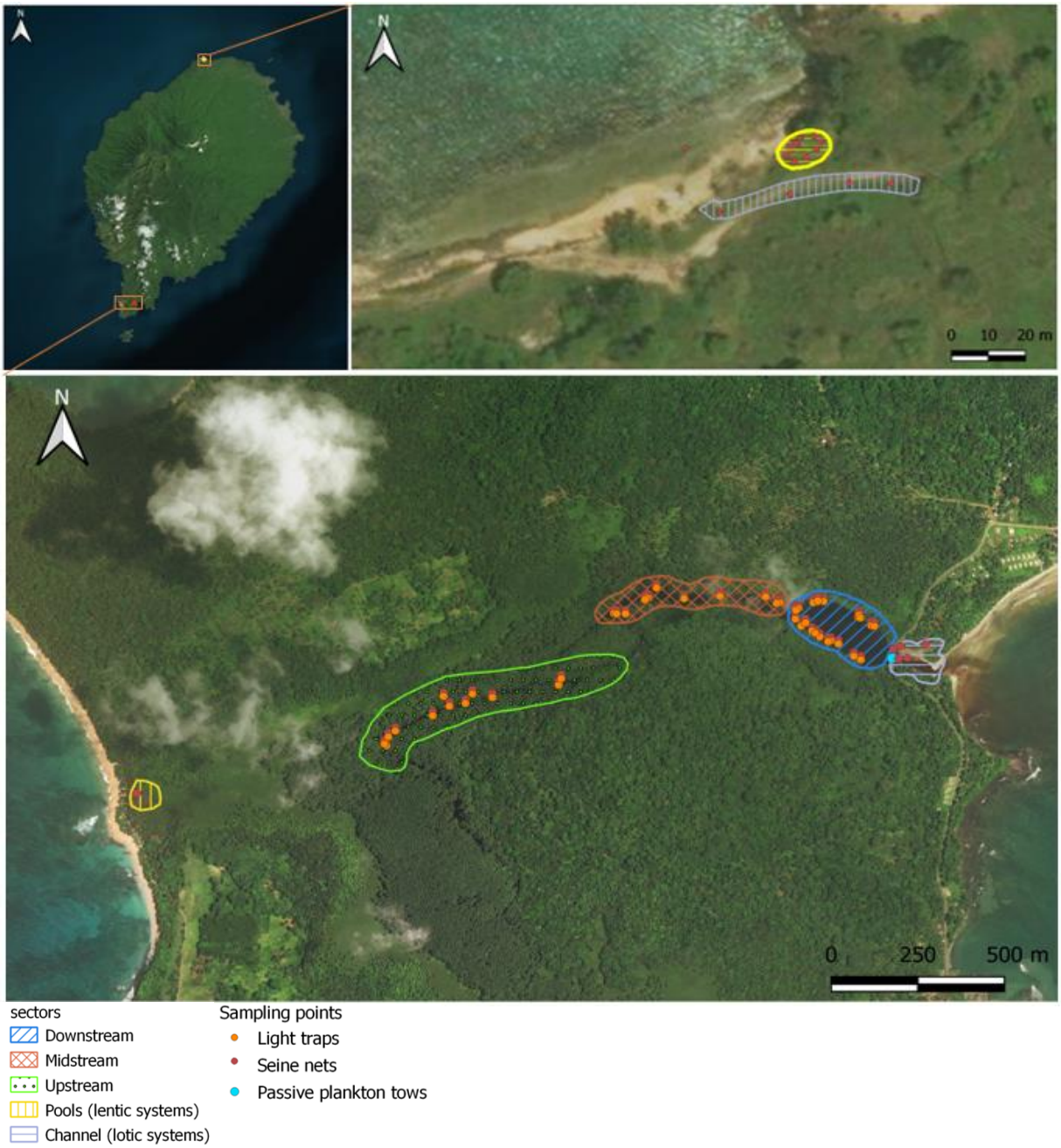


Figure 1.1 - Location of both studied mangroves in São Tomé Island (upper left panel); Praia das Conchas mangrove (upper right panel) with the different areas: in yellow lentic isolated pool, in blue a stream that crosses the mangrove); Malanza mangrove (lower panel) with different areas: in yellow is Jalé, in light blue the channel portion of the mangrove downstream of the bridge, the green, orange and cyan areas correspond to the upstream, midstream and downstream sectors of the mangrove, respectively; red points represent sampling points where seine nets were used, orange corresponds to light trap locations and light blue points represent the location where all passive plankton tows were done.

The climate is typically considered as monsoonal, the island has two rainy seasons, one between February and March and the second between October and December (Makowski, 2018; Costa, 2021). However, pluviosity is not homogeneous throughout the territory, the south of the island receives around 7,000 mm $year^{-1}$ of rain while the northeast is considerably dryer with an average 1,000 mm $year^{-1}$. Despite this, the majority of people live in the north (Água Grande district mainly) (Costa, 2021). São Tomé has a population of 215,056 people, two thirds of which are considered poor and one third below the poverty line (World Bank, 2019).

Mangroves exist in the north and east shorelines of the island, in exposed coasts, gulfs and inlets, coastal lagoons, marshes and estuaries or river mouths (Makowski, 2018). Two genera of mangrove trees dominate these forests, black mangroves (*Avicennia germinans* (L.) Stearn (1958)) and red mangroves (*Rhizophora mangle* L. (1753) and *R. racemosa* G.Mey (1818)). Twelve mangrove forests have been identified in São Tomé and this study was carried in two: Malanza and Praia das Conchas (Figure 1.1), both belonging to the Ôbo National Park (Makowski, 2018).

Malanza is located in the south of the island (Figure 1.1), in the Caué district. This mangrove has an estimated area of 0.70 km^2 and a maximum depth of 3.5 m, making it the most extensive mangrove in the country with a hydrographic basin of 7.40 km^2 consisting mostly of secondary forest and farmland (Heumüller, 2021). A bridge built near the mangrove mouth to connect two villages, limits its connection to the sea to two floodgates which increase the upstream water mass and alter the flow of sediments, species, waves, and tides (Félix *et al.*, 2017). The system is connected to the ocean on the east, while on the west end it approaches the sea again forming a small, closed lagoon (Jalé beach) with periodical saltwater intrusions through an over wash phenomena and percolation, establishing more lentic characteristics with shallow still waters, differentiating it from the remaining of the mangrove forest. The mangrove supports a small tourism operation of guided tours by local fishermen (Afonso, 2019).

On the northern side, Praia das Conchas (Figure 1.1) has an area of 0.01 km^2, located in the north - Lobata district. The mangrove consists mainly of a small river and a shallow enclosed pool, usually isolated and with occasional saltwater intrusions through over-wash in spring tides. The system is highly modified, its drainage area of 13.31 km^2 with a land use dominated by agroforest and includes several villages and the district's landfill. A road built across the mangrove restricts the water flow preventing the existence of a brackish waterbody (Félix *et al.*, 2017). It is mainly used as a water source for irrigation, personal hygiene and laundry.

2.2 Sampling

Field work was conducted in four weeks between October and November of 2020, using three methods to collect fish larvae: light traps, passive plankton tows and seine nets (Anex 3). Twelve light traps were built adapting the model put forward by Kissick (1993), these were used in deep mangrove areas wherever it was possible to keep the traps floating and flooded at all times. Only in Malanza their deployment was possible (Table 1). Within the four-week sampling period, the traps were deployed each week, being set before sunset and collected at dawn. Trap locations were never repeated to ensure independence between samplings. The mangrove was divided into three sectors: a) Downstream or water mirror, b) Midstream channel and c) Upstream channel (Figure 1.1). By sampling event, six traps were deployed in the Downstream sector and three traps were deployed in each of the remaining sectors, given their smaller areas as to maintain a similar relation between traps deployed and sector area. Once a trap was set or collected, environmental parameters (temperature, oxygen, salinity and conductivity) were registered using a multiparametric sonde (YellowSpring Inc, Model YSI Multi-parametric), water velocity was measured using a flow meter (GEOPACKS), water transparency was recorded with a Secchi disk, depth was recorded with a portable sounder and other abiotic variables such as weather (cloudiness), moon stage and tide were documented through tide and weather charts (Table 1.2). At collection, traps contents were washed into the collection cup, where they were preserved with 96% alcohol.

At the mangrove mouth of Malanza, the intake constriction caused by the bridge allowed the use of a passive plankton tow (Ø: 40 cm, 500 μm of mesh size) to sample water mass exchanges and sample larvae moving between the mangrove and the ocean and vice versa (Figure 1.1). At night, the plankton net was placed in the floodgates (waterflow area) for 30 minutes at a time at flood tide. A flowmeter (Hydro-bios Kiel, mechanical flowmeter) was attached to the net at all times recording the water volume that passed through it, environmental parameters were recorded roughly every 7 minutes. After the 30 min sampling time, the net was retrieved, washed into a collection cup and the content was preserved in 96% alcohol. This procedure was repeated three times per sampling night, resulting in three sampling tows. Due to field conditions and flood events, only three sampling events were possible, one of which in draining waters from the mangrove while the other two were with flooding oceanic waters, one event could only produce two sampling tows instead of the intended three.

To allow the sampling of low depth areas during the day, seine nets were used as a sampling tool. A 500 μm mesh-size net with two by three meters was used as a seine net. Two operators drag this seine net across transects of variable distance and depth according to location. Seines were performed in Malanza only downstream of the bridge, identified as channel and in Jalé (Figure 1.1). This method also allowed the sampling of Praia das Conchas mangrove (Figure 1.1). For this sampling method, each mangrove was sub-divided into two system types, lotic like systems with moving water masses and permanent ocean connectivity and lentic systems, nearshore confined areas with occasional sea water intrusions. After each seine, the net was inspected for captures and then washed before used again, all samples were preserved in 96% alcohol.

Table 1.1 – Sampling techniques used in each Mangrove/Sector and number (N) of samples collected in each of those sites

Mangrove	Sector	Technique	N
Malanza	Channel	Passive Plankton tows	8
	Up-Mid-Downstream	Light traps	48
	Channel	Seine nets	18
	Jalé	Seine nets	12
Praia das Conchas	Channel	Seine nets	9
	Pool	Seine nets	4

Table 1.2 - Mean values (± St. dev) of the obtained environmental parameters in each sampling event, considering the respective technique. Moon (as % area to full moon); Cloudiness (as % of the sky covered by clouds); Tide level (m); Distance to the sea (m), Temperature (ºC); Total suspended solids (mg/L); Conductivity (µS/Cm)*; Salinity; Oxygen concentration (mg/L); Oxygen saturation; Maximum depth (m) and Turbidity (cm).*

	System	**Malanza**			**Malanza-Jalé**	**P. Conchas**	
Env. Variables	Technique	P. P. Tows	Light traps	Seine nets	Seine nets	Lent	Lot
Moon	Moon	73.75±15.52	60.10±23.01	75.56±14.55	66.25±18.24	68.33±24.62	70±20
Cloudiness	Clou.	0.54±0.47	0.50±0.38	0.17±0.26	0.84±0.30	1±0	1±0
Tide level (m)	Tide	1.49±0.21	1.78±1.02	1.13±0.40	1.13±0.34	1.23±0.41	1.28±0.45
Distance to the Sea (m)	Dist-Sea	0±0	632±514.78	150±15.89	74±0	26±0	32±0
Temperature (ºC)	Temp.	26.52±1.02	25.61±0.38	26.11±0.52	28.47±2.54	29.97±0.81	25.32±0.49
Total suspended solids (mg/L)	Tss	16.35±12.61	1.301±0.94	7.24±5.75	10.55±7.68	35.16±3.37	0.24±0.001
Conductivity (µS/Cm)	Cond.	24.83±21.04	2.18±1.65	12.40±10.81	17.45±12.88	56.3±2.87	0.38±0.01
Salinity	Sal.	16.45±12.86	1.08±0.77	6.96±6.37	9.77±7.28	33.73±1.51	0.18±0
Oxygen concentration (mg/L)	O2c	4.68±1.58	3.94±0.94	7.46±1.39	5.15±1.38	6.47±0.93	7.12±0.16
Percentage of oxygen (%)	O2p	65.81±26.55	48.08±12.30	67.83±17.25	69.05±20.45	102.53±15.20	86.75±1.1
Maximum depth (m)	Depth_max	0.5±0	1.48±0.49	0.18±0.13	0.5±0	0.072±0.03	0.1±0
Transparency (cm)	Turb.	0±0	55.52±20.76	17.22±12.15	5±0	0.072±0.03	0.1±0

2.3 Laboratorial work

In the laboratory, fish larvae and juveniles were first separated from plant material and other zooplankton using a stereo microscope (LEICA MZ125, X1.0). In each sample, individuals were then separated by morphotype and identified to lowest possible taxon. Larvae identification followed an initial identification to the Family level, and posteriorly was identified to a genus or species level considering the species occurrences with the larval identification guides (FAO, 1990; Fahay, 1996; Moser, 1996; Waldman, 2012; FAO, 2016; Nelson, 2016). All larvae were screened and identified to the lowest taxonomic level using morphological characteristics, when a species level identification could not be reached, a morphotype was attributed and all morphologically similar specimens were classified as such. The number of individuals per taxon was counted from all samples and fish densities were calculated based on captures per units of effort, that vary between the methods used: individuals per hour of deployment for light traps, individuals per m^3 of water for plankton tows and number of larvae per area for seine nets. All samples were kept in 96% alcohol to allow molecular identification.

2.4 Genetic analysis

2.4.1 DNA extraction, COI amplification and sequencing

Subsamples were selected from each species/morphotype previously identified based on morphological characters. The selected larvae were representative of each species/morphotype and include different larvae stages of the same putative morphotype. The number of individuals selected per morphotype ranged from one to 16, in order to correspond with the total number of each species/morphotype. In total 77 individuals were selected with 73 being successfully sequenced.

DNA was extracted from tissue from the selected samples using an E.Z.N.A.® Tissue DNA Kit from Omega bio-tek. The 5′ region of the COI gene was selected as the basis for the DNA barcoding, approximately 655 bp were amplified using a cocktail of four primers following the procedures used by Ward *et al.* (2005) and Ivanova *et al.* (2007) (Table 1.3).

Table 1.3 - PCR primer used to amplify COI. M13 tails are highlighted (indicates original reference for the untailed version of each primer), reference used.

Primer	Sequence	Reference
VF2_t1	5'TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC3'	Ward *et al.*, 2005
FishF2_t1	5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC3'	Ward *et al.*, 2005
FishR2_t1	5'CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA3'	Ward *et al.*, 2005
FR1d_t1	5'CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA3'	Ivanova *et al.*, 2007

PCR reaction mixes were prepared with a volume of 15 µL that included 3 µL of 10xPCR buffer, 1.2 µL of MgCl2 (50 mM), 0.3 µL of each primer (0.01 mM), 0.6 µL of dNTPs (0.05 mM), 0.09 µL of Taq polymerase, and an 8.91 µL mixture of pure water (15MΩ) and DNA template to a 5 ng/µL concentration.

Amplifications were performed using a PCR machine with the thermal regime consisting of an initial step of 2 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 54°C, and 1 min at 72°C, followed by 10 min at 72°C and then held at 12°C until being preserved at -20°C. PCR products were visualized on 1.2% agarose gels. For each sample, DNA concentration was validated using a NanoDrop 1000. Samples were then purified using ExoSAP-IT™ (PCR Product Cleanup) and sent to an external laboratory for sequencing (STABVIDA, sanger sequencing).

2.4.2 Sequence alignment and analysis

For each sample, the sequenced COI PCR chromatogram was first analyzed through BioEdit v7.0.4.1 (Ibis Therapeutics, CA., USA) to access the prevalence of impurities that could interfere with future procedures and identifications, samples with high frequencies of double peaks (deemed contaminated) were discarded. All samples were then align using ClustalW (10000 bootstraps) and both ends of each sequence were trimmed. A program was used to assess the best base substitution model (HKY85; proportion of invariable sites = 0.550; number of substitution rate categories =4; Gamma shape parameter = 0.7160) for the obtained sequences, it was then used to elaborate a phylogenetic tree using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) with 10000 bootstraps to compare with the taxonomically identified morphotypes.

2.4.3 Taxonomic identification

Each valid sequence was used as the query sequence for BLASTN search in the BOLD system and in GenBank. The top 25 results were analyzed to determine the most probable identification when accounting for the taxonomic identification, their geographic distribution, and related taxonomic groups. Similarity thresholds were defined as >98% for species level, 90-98% to genus level, 85-90% for family level and 80-85% for order level following Juhel *et al.* (2020).

To overcome gaps in the data bases, 35 samples from previous campaigns of morphologically identified adult fishes were also sequenced following the same procedures as described for the fish larvae samples. The sequences were then integrated in the phylogenetic trees to provide known identifications to the species level. Species distribution data was included in the analysis as to reach the most trustworthy results possible by accounting for the absence of regional species from the genetic data bases.

2.5 Data analysis

Fish larvae abundance per species was corrected and updated after the integration of the molecular and morphological identifications. Fish larvae communities and environmental conditions were analyzed at several levels, always considering sampling events as replicates. The obtained data was tested for normality and homogeneity of variances and failed to satisfy these assumptions; thus, only non-parametric tests were used. To compare mangroves only seine net captures were analyzed throughout the application of a Kruskal-Wallis test on relative abundances (nr. of larvae/m^2). Within this data set, lentic and lotic systems in both mangroves were also compared, specifically for Praia das Conchas mangrove: channel vs pool; and for Malanza mangrove: channel vs Jalé (see Figure 1.1). Light traps samples were used to compare larval fish composition between sectors and between sampling events,

this allowed to assess how distributions shift within the mangrove, thus, representing any significant temporal variation. For passive plankton tows, larval movements were compared between water currents moving into and out of the mangrove. For each sampling event, Shannon's diversity and Pielou's evenness were calculated (Shannon & Weaver, 1963; Pielou, 1966). Comparisons between mangroves, techniques, sectors, and sampling events were carried out using Kruskal-Wallis tests to compare abundances and diversity values (Zar, 1999). The correspondent environmental matrices were also compared, using the same factors, with PERMANOVA (Permutational analysis of variance) (Anderson *et al.*, 2001).

PERMANOVAs were used to test the differences between the sampled mangroves, lentic and lotic systems, mangrove sectors of Malanza. The extent of any significant differences produced by this test were determined using the R-statistic value (Clarke, 1993; Clarke *et al.*, 2014), which can range from +1 if all the samples from one group are more similar to each other than to other samples down to -1 when the opposite occurs. For these analyses, a dummy variable was included to allow the inclusion of sampling events with zero catches. Whenever an analyzed factor presented more than two levels in PERMANOVA (e.g. sectors in light traps data) (Clarke *et al.*, 2014) and differences were statistically significant, levels went through a pairwise comparison following the same criteria as before described. PERMANOVA comparisons used 10000 permutations and simultaneously Monte Carlo tests were employed to compensate for when permutations were limited (Possible permutations <100) and provide a significance value for the obtained results (Anderson *et al.*, 2001). Abundance data was log (X+1) transformed and Bray-Curtis similarities coefficient was used as a resemblance measure. Environmental data was normalized, and the Euclidean distance used as a resemblance measure. Each environmental variable was plotted against each other using a Draftsman's plot and the correspondent correlations matrix was analyzed to detect redundant variables.

The RELATE procedure (Clarke *et al.*, 2014) was used to test the significance of the relationships between the larval fish densities of the taxa (larval fish resemblance matrix) and the environmental variables data (environmental resemblance matrix). When a significant match was found between matrices, the BEST (BIOENV) procedure using all possible combinations was used to determine which subset of environmental variables provided the best correlation with the larval fish matrix.

A Canonical Correspondence Analysis (CCA) was performed to determine the relationships between the abundance of total fish larvae and environmental variables using the VEGAN package of R functions (Oksanen *et al.*, 2005). In each CCA, the environmental variables incorporated in the analysis were selected as to not discriminate a priori between sampling locations and dates, thus weather, tide, moon stage, distance to the sea and depth were not considered; when two or more variables displayed a high degree of correlation ($r>0.7$), one was chosen to represent that relation (e.g. salinity and conductivity). Four environmental variables were used for the CCA analysis for seine nets and three for light traps, fish species were ordinated to indicate the relative strengths among those associations.

For all mentioned statistical tests, results were considered significant when $p<0.05$ (Zar, 1999).

3. Results

3.1 Species assignment: Morphological and molecular identification

A total of 4,010 fish larvae were collected from a sum of 99 sampling events (2,581 from Malanza and 1,429 from Praia das Conchas). Morphological identification classified the sampled larvae into 14 families, to which corresponded 6 identifications to the species level, 6 to the family and 12 potential morphotypes from two families: Eleotridae (4) and Gobiidae (8) (Table 1.4; Figures 1.2 & 1.3). For DNA barcoding, four samples failed to produce any amplified PCR products or were contaminated. Consequently, these four samples were excluded from subsequent analysis. The remaining 73 samples were successfully sequenced and the results were interpreted alongside the 35 barcodes from adult specimens and compared to the previous morphological identifications (Table 1.4, Annex II). As a final result, 27 possible species from 21 genera were identified representing 16 families (Table 1.4). From these 27 *taxa*, 22 were assigned to species, three to the genus with similarities <98% and two to the family based on similarity levels <90%. One individual was morphologically assigned to the family Blenniidae as its molecular analysis failed to provide reliable results. *Gobionelus occidentalis* and *Porogobius schlegelii* were also identified by its morphology as the specimens were only identified after the molecular oriented *taxa* revision of the collected individuals. Taxonomic and molecular identification of fish larvae produced different classifications when comparing both methods. Some families of fish larvae were only identified using DNA barcode, namely Sciaenidae (Table 1.4). On the other hand, molecular results of 24 samples identified 7 species whose distribution is not reported to São Tomé or even the East Atlantic, some with similarity levels >98%. These were then crossed with genetic results from identified adult specimens and matched based on a phylogenetic analysis to reach a final identification, if possible, if no adult sequences were available, the sample identification fell to the genera level. Additionally, molecular identification was not able to provide an identification to a lower taxonomic level than Actinopterygii for 40 larvae that morphologically corresponded to four different morphotypes of the Gobiidae family. Overall, the family Gobiidae was the richest family with seven different species while the second richest family was Mugilidae with three species (Table 1.4).

Gobionelus occidentalis, *Citharichthys* sp. and *Microdesmus* sp. have not yet been reported to occur in São Tomé. In addition to these three, an *unidentified pleuronectiform*, *Sardinella maderensis* and an *unidentified Blenniidae* are new reports for the island mangroves.

Figure 1.2 - General morphology of Gobiidae and Eleotridae larvae collected at São Tomé Island mangroves. Molecular identification resulted in Bathygobius soporator (A, 7 mm); unidentified Gobiidae sp*2 (B, 11 mm); Awaous lateristriga (C, 22 mm); unidentified Gobiidae* sp*.1 (D, 8 mm); Eleotris annabonensis (E, 17 mm) and Sicydium bustamantei (F, 28 mm).*

Figure 1.3 – General morphology of fish larvae collected at São Tomé Island mangroves. Oreochromis mossambicus (A, 23 mm); Unidentified Pleuronectiform (B, 9 mm); Citharichthys sp. *(C, 16 mm); Monodactylus sebae (D, 4 mm); Microphis aculeatus (E, 82 mm); Elops senegalensis (F, 36 mm).*

Table 1.4 - Fish larvae samples used for molecular identification, with the attributed Code, Morphological Identification (ID), top Molecular match via Blast (ID), Molecular identification correspondence Match (Via Blast) and Final Identification considering both results and species distribution areas (See Annex 1 for code correspondence, identification and image of the fish larvae).

Code	Morphological ID	Molecular ID	Match	Final Identification (Family > Genus > Species)
D3	Morph. G2	*Awaous banana*	98.38	Gobiidae > *Awaous* > *Awaous lateristriga*
D1	Morph. G2	*Awaous banana*	98.38	
F6	Morph. G6	*Awaous banana*	98.38	
K10	Ophichthidae	*Dalophis imberbis*	93.21	Ophichthidae > Ophichthinae > *Dalophis cephalopeltis*
K6	Lutjanidae	*Lutjanus agennes*	99.65	Lutjanidae > Lutjanidae > *Lutjanus agennes*
A5	*Elops senegalensis*	*Elops hawaiensis*	99.69	Elopidae > Elops > *Elops senegalensis*
K4	Syngnathidae	Syngnathidae	84.58	Syngnathidae > *Microphis* > *Microphis aculeatus*
B2	Perciform	*Pseudotolithus brachygnathus*	99.67	Sciaenidae > *Pseudotolithus* > *Pseudotolithus senegallus*
K1	*Caranx*	*Caranx bartholomaei*	100	Carangidae > *Caranx* > *Caranx* sp2
A10	*Citharichthys*	*Citharichthys*	88.99	Cyclopsettidae > *Citharichthys* > *Citharichthys* sp.
A3	*Eucinostomus melanopterus*	*Eucinostomus melanopterus*	100	Gerreidae > *Eucinostomus* > *Eucinostomus melanopterus*
A8	Clupeidae	*Sardinella maderensis*	99.83	Clupeidae > *Sardinella* > *Sardinella maderensis*
A9	Clupeidae	*Sardinella maderensis*	100	
A7	Clupeidae	*Sardinella maderensis*	99.83	
B3	Cynoglossidae	Actinopterygii	99.41	Unidentified Pleuronectiform
B4	Cynoglossidae	Actinopterygii	99.41	
A1	*O. mossambicus*	*Oreochromis mossambicus*	99.85	Cichlidae > *Oreochromis* > *Oreochromis mossambicus*
A2	*O. mossambicus*	*Oreochromis mossambicus*	99.11	
A6	Mugilidae	*Liza grandisquamis**	99.83	Mugilidae > *Parachelon* > *Parachelon grandisquamis*
A4	*Monodactylus sebae*	*Monodactylus argenteus*	87.5	Monodactylidae > *Monodactylus* > *Monodactylus sebae*

K3	Mugilidae	*Mugil bananensis*	100	Mugilidae> *Mugil* > *Mugil bananensis*
G7	Blenniidae	*Mugil* sp.	98.32	Blenniidae > Unknown > Unknown
K2	Mugilidae	*Mugil curema*	100	Mugilidae> *Mugil* > *Mugil curema*
K5	Lutjanidae	*Epinephelus aeneus*	100	Serranidae > *Epinephelus* > *Epinephelus aeneus*
F10	Morph. G4	*Eleotris picta*	91.83	Eleotridae > *Eleotris* > *Eleotris vittata*
G5	Morph. E2	*Eleotris pisonis*	98.72	Eleotridae > *Eleotris* > *Eleotris annabonensis*
G4	Morph. E2	*Eleotris pisonis*	98.72	
G3	Morph. E1	*Eleotris pisonis*	98.72	
G1	Morph. E1	*Eleotris pisonis*	98.72	
G2	Morph. E1	*Eleotris pisonis*	98.72	
F8	Morph. G7	*Millerigobius macrocephalus*	86.23	Gobiidae > Unknown > Gob sp2
F9	Morph. G7	*Millerigobius macrocephalus*	86.23	
F7	Morph. G6	*Millerigobius macrocephalus*	86.23	
D2	Morph. G2	Parasicydium	99.35	Gobiidae > Sicydiinae > *Sicydium bustamantei*
G6	Morph. E4	*Wheelerigobius maltzani*	98.59	Gobiidae > Gobiinae > *Wheelerigobius maltzani*
D4	Morph. G3	*Bathygobius soporator*	100	Gobiidae > Gobiinae > *Bathygobius soporator*
D6	Morph. G3	*Bathygobius soporator*	99.83	
D5	Morph. G3	*Bathygobius soporator*	99.83	
D7	Morph. G3	*Bathygobius soporator*	99.83	
C2	Morph. G1	Unknown	87.33	Gobiidae > Unknown > Gob sp1
C3	Morph. G1	Unknown	87.33	
C9	Morph. G1	Unknown	87.33	
B5	Morph. G1	Unknown	87.33	
B9	Morph. G1	Unknown	87.33	
C7	Morph. G1	Unknown	87.33	
C4	Morph. G1	Unknown	87.33	
C8	Morph. G1	Unknown	87.33	
C1	Morph. G1	Unknown	87.33	
E5	Morph. G4	Unknown	87.33	
C5	Morph. G1	Unknown	87.33	
F5	Morph. G5	Unknown	87.33	
C10	Morph. G1	Unknown	87.33	
C1	Morph. G1	Unknown	87.33	
D8	Morph. G4	Unknown	87.33	
E6	Morph. G4	Unknown	87.33	
E10	Morph. G4	Unknown	87.33	
E2	Morph. G4	Unknown	87.33	
K9	Morph. G8	Unknown	87.33	

F3	Morph. G4	Unknown	87.33	
B8	Morph. G1	Unknown	87.33	
D9	Morph. G4	Unknown	87.33	
E3	Morph. G4	Unknown	87.33	
B10	Morph. G1	Unknown	87.33	
F1	Morph. E1	Unknown	87.33	
E1	Morph. G4	Unknown	87.33	
E4	Morph. G4	Unknown	87.33	
C6	Morph. G1	Unknown	87.33	
E7	Morph. G4	Unknown	87.33	
E9	Morph. G4	Unknown	87.33	
E8	Morph. G4	Unknown	87.33	
D10	Morph. G4	Unknown	87.33	
F2	Morph. G4	Unknown	87.33	
L1	*Gobionelus occidentalis*	NA	NA	Gobiidae > *Gobionelus* > *Gobionelus occidentalis*
L2	*Porogobius schlegelii*	NA	NA	Gobiidae > Gobiinae > *Porogobius schlegelii*

* Synonym of *Parachelon grandisquamis*

3.2 Variation in taxonomic richness and abundance

The fish larvae communities were dominated by two families that represented 90.9% of all individuals: Cichlidae, encompassing a single species but accounting for 47.4% of captures and Gobiidae with 43.5% of captures from seven different species (Table 1.5). The remaining 14 families only represent 9.1% of all captures, from these, Carangidae (2.2%), Gerreidae (1.8%) and Mugilidae (1.2%) were the most abundant (Table 1.5). Mugilidae was the second most diverse with three identified species (*Mugil curema*, *Mugil bananensis* and *Parachelon grandisquamis*) followed by Eleotridae with two species. Twelve families were only represented by one species (Table 1.5). From all caught *taxa*, two species accounted for 82% of the total registered larval abundance, the Mozambique tilapia (*Oreochromis mossambicus*) was the most numerous *taxon* followed by the Gobiidae morphotype sp. 1 (Table 1.5). From all 27 species, 9 were recorded in both mangroves (Malanza and Praia das Conchas), namely *Oreochromis mossambicus*, *Eucinostomus melanopterus*, *Parachelon grandisquamis*, *Microphis aculeatus*, *Caranx* sp. 2, *Awaous lateristriga*, Gobiidae morphotype sp1, *Eleotris annabonensis* and *Eleotris vittata*, while the remaining 18 were only found in Malanza.

Between the two mangroves, Malanza recorded the highest diversity overall with 27 species being detected, while in Praia das Conchas only 9 were found. Following these results Malanza's mangrove exhibited the highest diversity (H = 1.528) and evenness (J = 0.474) of the two mangroves (for PC; H = 0.208 and J = 0.095).

The employed methodologies resulted in different captures, the highest taxonomic richness was accounted for in passive plankton tows (21) while the lowest was in light traps (11), with seine nets in-between (13). Richness varied significantly across the three methods (PERMANOVA, $p= 8.4e^{-7}$). In seine nets, light traps and passive plankton tows diversity varied significantly as well (PERMANOVA, $p=6.3e^{-6}$), with higher diversity values in light traps (1.265) while Seine samples presented the lowest (0.599) and plankton tows was 0.825. Evenness followed the same pattern to diversity with the highest value occurring in light traps (0.527), followed by plankton tows (0.285) and then by seine nets (0.234).

The use of different methodologies resulted in differences in species sample composition. Seine nets were the most effective method, capturing a total of 2,154 fishes across all locations, being *O. mossambicus* the most captured species, consisting in 87.8% of the catches (1,891 specimens). Other seven families were collected with seine nets, including Gerridae (2.9%), Mugilidae (2.3%), and Carangidae (4.1%) as the most abundant families (Table 1.5). *Mugil curema*, *M. bananensis*, *P. grandisquamis*, *Caranx* sp. 2, and *Citharichthys* sp. were the *taxa* only captured using seine nets. Passive plankton tows captured a total of 1,740 individuals, corresponding to 12 different families, being Gobiidae the most abundant family (92.2% of the total catch) and the richest (seven species). *Porogobius schlegelii* was the biggest contributor to this dominance accounting for 79.7% of total catch with passive tows (Table 1.5). Excluding gobiids, the most common species were *E. annabonensis* (2.6%), *Pseudotolithus brachygnathus* (1.0%) and *S. maderensis* (0.8%). Eleven species were only collected with passive plankton tows, namely *P. brachygnathus*, *S. guineense*, *Dalophis cephalopeltis*, *Elops senegalensis*, *Lutjanus agennes*, *Epinephelus aeneus*, *Sicydium bustamantei*, *S. maderensis*, *G. ocidentalis*, *Wheelerigobius maltzani* and an unidentified Blennid. Finally, light traps resulted in the capture of 116 fish larvae spanning across 6 families of which Gobiidae was the most represented, composing 78.4% of captures, and the most diverse (four species). The most abundant taxon was an Unidentified Gobiidae species, classified as morphotype sp.1 (N=80), the second most abundant species was *O. mossambicus* (N=9), followed by *E. annabonensis* (N=7) (Table 1.5).

Significant differences in fish larvae abundance were identified between the two sampled mangroves (Table 1.6) using seine nets, with Praia das Conchas lentic pools having a higher larvae abundance (12.48 ind. $\cdot$ m^{-2} $\pm$ 17.35) relatively to Jalé (Malanza's lentic zone) (3.01 ind. $\cdot$ m^{-2} $\pm$ 4.91) while the lotic habitats displayed lower abundances (Malanza - 0.34 ind. $\cdot$ m^{-2} $\pm$ 0.43; Praia das Conchas – 0.6 ind. $\cdot$ m^{-2} $\pm$ 0.3). In both mangroves the most abundant species was *O. mossambicus* being found in average densities of 2.9 to 8.7 ind. $\cdot$ m^{-2} between mangroves but only in lentic habitats.

Regarding seine net captures, the community composition varied between the two mangroves (Table 1.6), where in Malanza six families were detected covering ten species. Similar richness levels were found in PC, with nine species present, six of which occurring in both mangroves. In Malanza, eight species were found occupying the lotic habitats whereas only two occurred in the lentic. For Praia das Conchas, richness values were more similar between habitat type (lentic vs lotic), with five species each, two of which in common. Nevertheless, lotic habitats displayed a higher diversity relatively to the lentic habitats, with H'=1.416 vs H'=0.122 in Malanza, and H'=1.020 vs H'=0.039 in PC, for lotic and lentic respectively.

Table 1.5- Composition of larval fish assemblages in Malanza and Praia das Conchas collected per sampling method: PPT – Passive Plankton Tows (nr. larvae per 100 m^3), LT – Light Traps (nr. larvae per 10hours of illumination), SN – Seine Nets (nr. larvae per 100 m^2), lot – lotic habitats, lent – lentic habitats, with average CPUE ± SD, and total number of captured larvae between brackets. Dashes represent zero catches. Total species richness, average Shannon's diversity index and average Pileou's evenness.

				Malanza		**P. Conchas**	
Species		PPT	LT	SN - lot	SN - Jalé (lent)	lot	lent
***Oreochromis mossambicus* (Peters 1852)**	Omos	-	0.109±0.660 (9)	-	2.919±4.948 (515)	-	1262.4+2204.9 (1376)
***Eucinostomus melanopterus* (Bleeker 1863)**	Emel	0.034±0.047 (10)	0.121±0.083 (1)	9.07±18.3 (60)	-	-	5.56+13.33 (3)
***Parachelon grandisquamis* (Valenciennes 1836)**	Pgra	-	-	1.08±3.567 (5)	-	0.67+1.33 (2)	-
***Mugil curema* Valenciennes 1836**	Mcur	-	-	4.63±12.74 (18)	-	-	-
***Mugil bananensis* (Pellegrin 1927)**	Mban	-	-	8.52±26.3 (24)	-	-	-
***Microphis aculeatus* (Kaup 1856)**	Macu	-	0.040±0.159 (3)	-	-	3.25+4.56 (3)	-
***Caranx latus* Agassiz 1831**	Clat	-	-	7.8±31.756 (84)	-	1.67+3.33 (4)	-
***Citharichthys* sp. (Cyclopsettidae)**	Cith	-	-	0.463±1.38 (2)	-	-	-
***Pseudotolithus brachygnathus* Bleeker 1863**	Pbra	0.051±0.079 (17)	-	-	-	-	-
Unidentified Pleuronectiform	Uple	0.023±0.052 (6)	-	-	-	-	-
***Monodactylus sebae* (Cuvier 1829)**	Mseb	0.011±0.021 (4)	0.024±0.115 (2)	-	-	-	-
***Dalophis cephalopeltis* (Bleeker 1863)**	Dcep	3.201E-03±9.054E-03 (1)	-	-	-	-	-
***Elops senegalensis* Regan 1909**	Esen	6.95E-03±0.014 (2)	-	-	-	-	-
***Lutjanus agennes* Bleeker 1863**	Lage	6.95E-03±0.014 (2)	-	-	-	-	-
***Sardinella maderensis* (Lowe 1838)**	Smad	0.044±0.068 (14)	-	-	-	-	-
***Awaous lateristriga* (Duméril 1861)**	Alat	0.026±0.038	0.067±0.200	0.093±0.393	-	11+17.83	0.56+1.66

		(5)	(5)	(1)		(32)	(1)
***Gobionellus occidentalis* (Boulenger 1909)**	Gocc	4.869E-03±0.014 (1)	-	-	-	-	-
Gob sp**p.1 (G1)**	Gob1	0.442±0.687 (153)	1.01±2.46 (80)	-	0.1±0.169 (14)	-	0.278+0.83 (1)
***Bathygobius soporator* (Valenciennes 1837)**	Bsop	0.151±0.106 (47)	0.028±0.192 (2)	2.22±9.43 (2)	-	-	-
***Porogobius schlegelii* (Günther 1861)**	Psch	4.19±4.99 (1387)	0.036±0.246 (3)	-	-	-	-
Gob sp**p.2 (G7)**	Gob2	0.041±0.001 (8)	0.015±0.102 (1)	-	-	-	-
***Eleotris annabonensis* Blanc. Cadenat & Stauch 1968**	Eann	0.163±0.186 (46)	0.094±0.298 (7)	-	-	1.92+2.41 (5)	0.28+0.83 (1)
***Eleotris vittata* Duméril 1861**	Evit	2.421E-03±6.849E-03 (1)	0.038±0.191 (3)	-	-	-	0.28+0.83 (1)
Blenniidae	Blen	0.005±0.013 (1)	-	-	-	-	-
***Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817)**	Eaen	4.84E-03±0.014 (2)	-	-	-	-	-
Sicydium bustamantei (Greeff, 1884)	Sbus	4.85E-03±0.014 (2)	-	-	-	-	-
***Wheelerigobius maltzani* (Steindachner 1881)**	Wmal	2.422E-03±6.849E-03 (1)	-	-	-	-	-
Total captured individuals	N	1740	116	196	529	46	1383
Total Richness	S	18	11	8	2	5	6
Shannon's diversity index	H'	1.749±0.511	0.178±0.364	0.028±0.106	0.153±0.277	0.443	0.017
Pielou's evenness	J	0.622±0.313	0.095±0.297	0.181±0.345	0.147±0.282	0.275	0.0095

In Malanza's mangrove, light traps allowed larval fish composition comparison within the mangrove. A total 116 fish larvae were caught, 71 larvae in the most downstream sector belonging to eight *taxa*, 11 larvae in the second sector (midstream) from five *taxa* and 34 larvae in the upstream sector belonging to four *taxa* (Figure 1.4). Overall unidentified morphotype Gobiidae sp.1 was the most common *taxon* across all sectors. Shannon Wiener's diversity was the highest in the midstream sector (H'=1.468), while the downstream and upstream sector displayed more similar diversities (H'=0.940 and H'=0.983, respectively). The fish larvae assemblage did not vary significantly across sectors (Table 1.6).

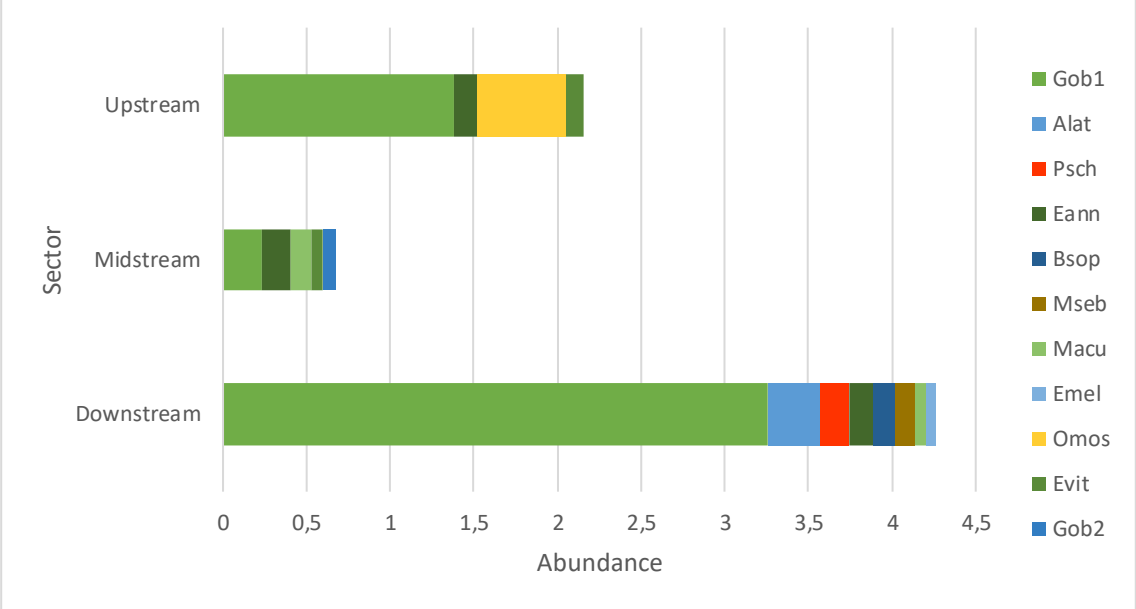


Figure 1.4 – Fish larvae abundance (larvae/100 hours) between mangrove sectors in Malanza. Species abbreviations: Gob1 - Gobiidae morphotype sp*1; Alat - Awaous lateristriga; Psch - Porogobius schelegi; Eann - Eleotris annabonensis; Bsop - Bathygobius soporator; Mseb - Monodactylus sebae; Macu - Microphis aculeatus; Emel - Eucinostumos melanopterus; Omos - Oreochromis mossambicus; Evit - Eleotris vittata; Gob2 - Gobiidae morphotype* sp.*2*

Table 1.6- Permutational Analysis of Variance results resulting from larval community comparison, per applied methodology (Technique), mangrove comparison (Mangroves), lentic vs lotic systems (Systems), between downstream, midstream, and upstream sector of Malanza (Sectors) and between sampling each week (Sampling events). Displaying for each analysis; df - degrees of freedom, SS - single squares, MS - multiple squares, F - F value, Pr(>F) - significance, Permutations - number of permutations and P(MC) Monte Carlo test results.

	PERMANOVA						
	df	SS	MS	F	Pr(>F)	Permutations	P(MC)
Technique	2	53609	26805	7.3124	0.0001	9895	0.0001
Seine net							
Mangroves	1	6085.7	6085.7	1.932	0.0184	9902	0.0344
Systems	1	24314	24314	7.7188	0.0001	9907	0.0001
Light traps							
Sectors	2	215.13	107.57	1.7299	0.1326	9937	0.1422
Samp. events	3	136.83	45.611	0.7299	0.639	9945	0.6209

3.3 Relationship between environmental variables and community structure

Distribution of larval fish, captured by seine net, varied among mangroves and such patterns are observable in the Canonical Correspondence Analysis. For seine nets, sampling sites were separated in two groups, lentic and lotic systems, this grouping was confirmed to be statistically significant (PERMANOVA, p=0.0001, Table 1.6). Overall, lentic sites tended to have lower oxygenation levels and higher water temperatures (Figure 1.5). This ordination analysis showed little explanatory power for samples form lentic systems, but sites from lotic systems have higher oxygenations and lower temperatures overall. Lotic systems display a high range of salinities and total suspended solids concentrations. Lentic systems were dominated by *O. mossambicus* only coexisting with few additional species (one species in Malanza and four species in Praia das Conchas) that appeared as rare occurrences, usually Gobiidae morphotype sp1. In lotic systems a more diverse community was often found, these sites displayed a higher variety of taxa, with the presence of larvae from the families Gobiidae, Eleotridae, Syngnathidae, Carangidae, Mugilidae, Paralichthyidae and Gerreidae. Despite the existence of variation among mangroves, no significant difference was found between the fish larvae communities from Malanza and Praia das Conchas mangroves when only considering larvae caught using seine nets (PERMANOVA, p = 0.404).

For light traps (Figure 1.6), the distribution of sample scores was not able to separate samples between each sector within the Malanza mangrove. The downstream sector of Malanza (S1) has more sites positively correlated with oxygen in comparison with the midstream (S2) and upstream (S3) sectors and both temperature and salinity do not contribute to explain the distinction between sectors, but rather indicate a high variability within each, considering these two variables. A pairwise permutational analysis of variance of the larvae abundance data, indicated that there are no significant differences between the downstream and upstream sectors of the mangrove (PERMANOVA, p = 0.4405) or between the midstream and downstream sectors (PERMANOVA, p = 0.0906). However, these differences were significant between the midstream and upstream sectors (PERMANOVA, p = 0.0475). Further away from the sea, in poorly oxygenated waters fewer species were found, with *O. mossambicus* and *E. vittata* appearing only on the upstream, while in the downstream parts of the mangrove more species were present. The patterns displayed in the CCA underlined a negative correlation between the Mozambique tilapia, an invasive species, and oxygen concentration, typically in association with fewer species and only cohabitating with larvae from the families Gobiidae and/or Eleotridae. Locations with higher hydrodynamics, resultant from the ocean-mangrove interface are often inhabited by a more diverse larval community, composed by Mugilids, Carangids, Gerrids and several Perciforms.

The RELATE function, used to test the correspondence between the observed patterns in environmental and the larval community, revealed a significant relationship ($p<0.05$), although with a low goodness of fit (Rho = 0.142) for seine net captures while it was not significant for light trap captures (p=0.70; Rho = -0.046). BEST (BIOENV) revealed that distance to the sea and salinity were the most relevant variables in distinguishing the larval assemblages at sites represented by seine nets.

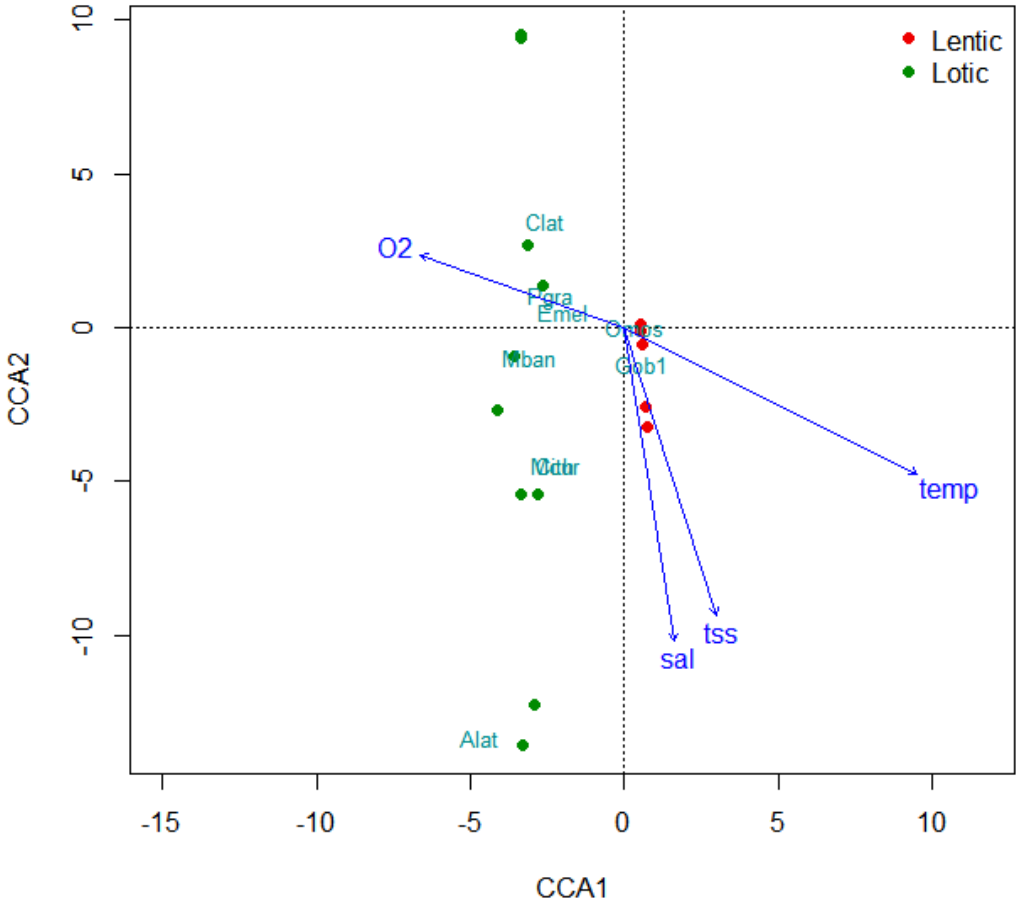


Figure 1.5 – CCA ordination triplot diagram showing samples from seine nets representing lotic end lentic areas from the Malanza mangrove, taxa identified and explanatory environmental variables (vectors). Alat - Awaous lateristriga, Mban - Mugil bananensis, Emel - Eucinostumos melanopterus, Clat - Caranx latus, Omos - Oreochromis mossambicus, Gob1 – Gobiidae morphotype sp.*1, Pgra - Parachelon grandisquamis, Cith - Citharichthys* sp.*, Mcur - Mugil curema; sal – salinity, temp – temperature, tss – total suspended solids, O2 – dissolved oxygen; Eigenvalue and percentage of explained variance of site distribution extracted for the first ordination axis: 0.5712, 11.13%; Eigenvalue and percentage of explained variance of site distribution extracted for the second ordination axis: 0.1332, 2.595%*

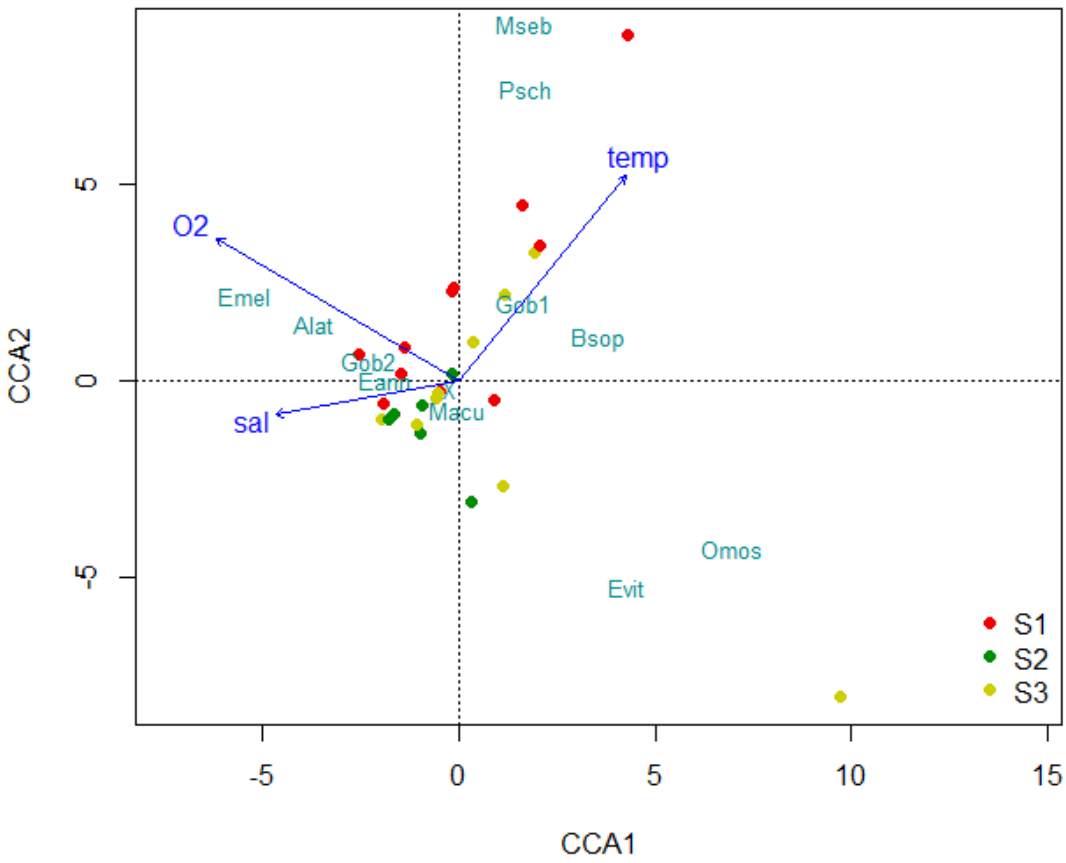


Figure 1.6 – CCA ordination triplot diagram showing samples from light traps representing sectors of the Malanza mangrove, taxa identified and explanatory environmental variables (vectors). In red are samples from the downstream sector (S1); in green are samples from the midstream sector (S2); and in yellow are from the upstream sector (S3). Mseb - Monodactylus sebae, Psch - Porogobius schelegi, Emel - Eucinostomus melanopterus, Gob1 – Gobiidae morphotype sp.*1, Alat - Awaous lateristriga, Bsop - Bathygobius soporator, Gob2 - Gobiidae* sp.*2, Eann - Eleotris annabonensis, Macu - Microphis aculeatus, Omos - Oreochromis mossambicus, Evit - Eleotris vittata; Sal – salinity, temp – temperature, O2 – dissolved oxygen; Eigenvalue and percentage of explained variance of site distribution extracted for the first ordination axis: 0.04835, 4.537%; Eigenvalue and percentage of explained variance of site distribution extracted for the second ordination axis: 0.02911, 2.731%*

4. Discussion

Mangrove forests are unique estuarine like systems with a widely recognized role as nursery areas for larvae and juvenile fish. Despite this, mangroves still lack considerable information about its nursery role, especially when it comes to African mangroves and more so regarding insular African mangroves. This study moves a step forward, assessing the larval fish composition in São Tomé mangroves, existing only one previous study focused on one single species (Batista *et al.*, 2020). Six new species were identified in its larval stage, when compared to previous studies on adult and juvenile for the same mangroves, where 26 fish species were identified (Félix *et al.*, 2017; Heumüller, 2021). The present study provides a first record on the use of São Tomé's mangroves by fish larvae, and the description of the abiotic parameters with putative influence on the spatial patterns of fish larvae community along these systems. Despite the small area of the island mangroves, these habitats encompass a relatively high richness of fish in its early life stages including species that do not occur in this system in subsequent life stages, corroborating the hypothesis of Malanza as a nursery area (Félix *et al.*, 2017). Diversity was found not to be uniform throughout the mangroves with communities differing between habitats. The use of several methodologies adapted to the distinct habitats, allowed an integrative view of these communities. Additionally, this work used a combination of molecular and morphological tools that enabled the identification of the sampled taxa, in an area that lacked larval fish identification keys.

In this work, 27 species were identified using the combination of morphology and molecular techniques, three of which were recorded for the first time in São Tomé's mangroves (*S. guineense, S. maderensis* and an unidentified Blenniidae) and three others are new occurrences in the island (*G. occidentalis*, *Citharichthys* sp. and *Microdesmus* sp.). The morphological identification of fish larvae was made until the family level to 89.5% of the samples, while 1% went up to the genus level and 9.5% were identified to the species in light of very distinct meristic characteristics. Most of the morphological mismatches were on larvae from eleotrids that belonged to the same suborder of Gobioidei, which in very early stages are indistinguishable. As for the molecular results, 48.6% of samples reached a species-level identification, while 2.8% were identified to the genus, and 48.6% were "unidentified." According to Ko et al. (2013) the accuracy rates of identification of fish larvae average on 80.1% to the family level, 41.1% for the genus and 13.5% for the species. In relation to our final identifications, morphological identification misidentified 8.2% of the samples to a family level whereas all species and genus level identifications were accurate, molecular identification produced a total of 17.6% misidentified results to the species level (47.2% of species-level identifications) where the genus was always correct, but not the species geographic distribution (Indian, Pacific, Western-Atlantic). Molecular mismatches and the high prevalence of "unidentified" specimens are the result of still very incomplete data bases, especially for the west African region. The majority of the problems faced during larvae identification in this work were related to species without economic value for which there is little or no data. This not only makes morphologic identification difficult but delays their integration in the COI databases (Ko *et al.*, 2013).

The accurate identification of fish larvae can be a difficult and strenuous task (Neira *et al.*, 2015). Traditionally, this has been achieved through morphology but in the last years DNA barcoding has surged as an efficient and objective method to confirm and identify *taxa* (Yao *et al.*, 2009; Dentinger *et al.*, 2010). However, both methods have their own shortcomings. Morphological identification is dependent on frequently non-standardized morphometric characters that are especially disadvantageous when comparing meristic characters across several development stages, and in similar species near impossible to identify when there is no previous reference for the early stages of a *taxa* (Ko *et al.*, 2013; Shirak *et al.*, 2016; Azmir *et al.*, 2017). Thus, molecular identification is susceptible to a wrong indexing of the barcode, due to varying species delimitation thresholds between taxonomic groups (Krishnamurthy & Francis, 2012). Moreover, molecular identification is dependent on previous

knowledge from confirmed specimens and robust data bases such as BOLD and GenBank that are not yet complete and are prone to erroneously match a miss identified collection specimen to a barcode (Collins & Cruickshank, 2013; Azmir *et al.,* 2017). Thus, the best approach has been to combine both to help enhance taxonomic research findings (Baldwin & Johnson, 2014).

Both Malanza and Praia das Conchas displayed a heterogeneity of habitats that led to a multi-habitat approach and required the combined use of several sampling techniques for an effective assessment of biodiversity from root dense areas, where nets were ineffective, to shallow sand/mud pools and fast current channels where light traps could not be set. The fish larvae community varied across habitats, and there is evidence that for other estuarine type systems some fish species show preference for a type of habitat independently of being of marine or estuarine origin (e.g. Edworthy & Strydom, 2016). It was observed in Malanza through light traps, that the community composition shifted between the downstream mangrove area in comparison to the mid and upstream mangrove, where estuarine species (Gobiidae sp.1, *E. annabonensis, E. vittata*) dominated the mangrove while the number of marine species increased with the proximity to the sea (e.g. *P. grandisquamis, E. melanopterus*). It has also been suggested that some species favor habitats that offer protection directly through the habitat as vegetation or behaviorally for predator avoidance (Weinstein & Brooks 1983, Orth et al. 1984), as observed with the occurrence of *M. aculeatus* in the midstream section of the mangrove.

Mangroves tend to show high larval fish diversities (Nagelkerken *et al.,* 2002; Tse *et al.,* 2008), as it was observed in Malanza and Praia das Conchas. However, diversity varies between habitats inside the mangrove, as more complex habitats such as root dominated streams have shown high diversities whereas sand habitats tend to only support specialists such as some gobiids and flatfishes (Edworthy & Strydom, 2016) on par with the sampled lentic habitats that exhibited lower diversities. Besides varying between habitats, species vary in between sampling methods as these display some degree of selectivity. Seine nets actively catch larvae whose taxonomic composition depends on mesh size and towing speed (Carassou *et al.,* 2009), passive plankton tows are more efficient with larvae of smaller sizes (McLeod & Costello, 2017) that depend on tidal current strength. Light traps only target species that display attraction to light and can actively move towards it (Doherty, 1987). These characteristics of each method are evident in our results, light traps caught the least taxa, only targeting species at a mobile stage with phototaxis, seine nets caught more species of larger size and were able to capture pleuronectiforms (e.g. *Citharichthys* sp.) at a post-flexion stage and the plankton tows were the most generalist but had a high affinity for pre-flexion larvae, once more mobile stages were able to avoid the net. Therefore, the usage of multiple techniques showed the importance of adapting procedures to field conditions as was done in this work (Dennis, 1992; Barletta-Bergan *et al.,* 2002; Faunce & Serafy, 2006; Neal *et al.,* 2012). The use of active sampling techniques, such as towing a bongo net from a motorboat, could help sample the inner parts of the mangrove but the depth and vegetation in these mangrove systems precluded it. Thus, the use of tidal currents from bridges or piers (Ribeiro *et al.,* 2015) provides an additional source of information. Unfortunately, climate conditions during the first two weeks ended up exerting an additional influence on the sampling, once high pluviosity hampered the first week of passive tows and hindered the completion of the second. Consequently, these first samples were only used for taxa inventory and not integrated in statistical analysis. Nevertheless, an extension of the sampling period to the dry season would be ideal to fully understand annual recruitment dynamics in both mangroves but it was not possible in this thesis.

São Tomé Mangroves presented different larval fish compositions and structures that are related with the respective mangrove dimensions. Malanza as the most extensive mangrove forest of the country is able to sustain higher levels of diversity (H'=1.58) than Praia das Conchas (H'=0.127). Several factors influence the distribution of larvae and juveniles between nursery sites. Differences in physical factors, structural heterogeneity and differences in productivity/food availability have been pointed out as some

of the major stressors between larvae abundance and diversity across sites (Robertson & Duke, 1987), which may explain why Malanza has higher diversities and abundances in comparison to the smaller and more degraded mangrove in Praia das Conchas. Other authors have pointed water clarity has a major influence on habitat choice, as turbid waters provide better cover from predators (Blaber & Blaber, 1980) while others have suggested low salinity outflows are potential cues to attract early life stages to inshore habitats (Robertson & Duke, 1987). Both these factors were relevant in Malanza's mangrove, where deeper and more turbid waters in a mangrove with a larger drainage area, bare the greatest potential as a nursery area. While fish species richness varies between mangroves, larvae communities appear to be comparable between similar habitats. For both mangroves, the sampled communities were characterized by a Gobiidae larvae dominance, with the exception of lentic habitats that were dominated by the invasive cichlid, *O. mossambicus*. These diversity patterns are consistent with other works on fish larvae from mangroves and other estuarine environments, where few species are usually found in greater abundances and where Gobiidae are often mentioned group to dominate the communities (Powell *et al.*, 1989; Tzeng & Wang, 1992; Barletta-Bergan *et al.*, 2002; Ooi & Chong, 2011; Ai Lin, 2012). The dominance of few and more abundant tolerant species are typical patterns for biological communities within brackish systems with high natural variability, often smaller or with constricted openings (Tzeng *et al.*, 2002; Félix *et al.*, 2013). Gobiid larvae dominated all the mangrove sectors, contributing to it is the elevated species richness of the family and their relatively long larval stage (Thresher, 1984; Nelson, 2016).

In Malanza, the distribution of fish larvae revealed that the number of species decreased from downstream to upstream, in similarity to other estuarine systems (Tzeng & Wang, 1992), due to fish larvae entering from coastal waters, consistent with the high *taxa* richness observed on the passive plankton tow samplings. This is a regular pattern in estuaries worldwide, as the predominant functional group is comprised of marine species, either estuarine-dependent or estuarine-opportunist species associated with the presence of some tolerant species that can colonize the remaining system (Elliott *et al.*, 2007; Selleslagh & Amara, 2008). Another factor, not considered in this work, is predation risk and food accessibility that, apart from environmental factors, can influence the distribution of fish larvae and juveniles (Ooi & Chong, 2011). As an example, the distribution and abundance patterns of *O. mossambicus* may be restricting the distribution of other fish larvae to occupy the upstream areas of Malanza's mangrove. This observed pattern is consistent, with studies in Australia where *O. mossambicus* occupies closed estuaries and coastal lakes, similar to Jalé and Praia das Conchas lentic pools, where salinity is more conservative (Russel *et al.*, 2012), despite being reported to tolerate a wide range of salinities (Costa-Pierce & Riedel, 2000). Since only the rainy season was sampled, when high volumes of water are retained due to the constrained connectivity to the sea from the existing bridge in the mangrove entrance (Félix *et al.*, 2017), the patterns observed in this study are prone to change in the dry season as freshwater input to the mangrove is reduced. In similar ecosystems the number of species and their abundance appears to be related to variations in salinity (Albaret & Ecoutin, 1990; Plumstead, 1990; Barletta-Bergan *et al.*, 2002) which, in turn, is influenced by freshwater inflow that varies seasonally (Barletta-Bergan *et al.*, 2002). In this work, in both light trap (LT) and passive plankton tow (PPT) captures, there was a difference in larvae abundances and richness between the first two weeks of sampling, that had high pluviosity (n = 30 in LT, ~n = 7 in PPT), and the remaining two, without rain (n = 86 in LT, ~n = 347 in PPT). The correlation between abiotic factors and fish larvae distribution and abundance was not significant, which can be a result of the short sampling period and low number of samples, associated with narrow environmental range of the studied variables. These factors did not allow the full observation of temporal patterns such as the peak in the number of species that tends to occur in temperate and subtropical estuaries in spring and summer, due to annual patterns in temperature (Neira *et al.*, 1992). Despite this, community patterns in fish larvae are similar to those found with adults

and juveniles in previous works (Félix *et al.*, 2017; Heumüller, 2021) and high diversity of environmental conditions was as well found to occur, fortifying the hypothesis that heterogeneity is one of the most significant characteristics of Malanza.

Despite the comparisons made between both mangroves, Praia das Conchas is highly impacted by human activity and is extremely small, the individuals there captured were mainly juveniles with the only caught larvae belonging to *O. mossambicus and E. melanopterus*. Due to its smaller size, Praia das Conchas mangrove is more susceptible to further degradation by anthropogenic action. However, the consequences of habitat degradation in Malanza should have a higher impact on biological communities, with potential consequences to nearby ecosystems and fish stocks.

Malanza, in contrast to Praia das Conchas displayed a high potential as nursery area. Several species now reported at a larval stage had been previously reported (Félix *et al.*, 2017, Heumüller, 2021) to inhabit the mangrove as juveniles (e.g. *E. melanopterus, M. sebae, M. bananensis, P. grandisquamis, O. mossambicus*) and adults (e.g. *E. annabonensis, E. vittata, P. macrolepis, B. soporator, E. senegalensis, L. agennes*) displaying the importance of this ecosystem during different ontogenetic stages for several species. The conservation of these habitats is vital to maintain their unique biodiversity and maintain their provisioning of ecosystem services and local fisheries as several commercially relevant species were found either at a larval or a juvenile stage, such as *Caranx* sp., *L. agennes, E. aeneus., P. brachygnathus, E. senegalensis, S. maderensis*. The high abundances of the cichlid *O. mossambicus*, an invasive species with generalist feeding habits and highly tolerable also raises concerns as its unknown habitat use in these systems might result in competition or direct predation on native larvae reducing the nursery potential. The results of the present work, helped to identify which fish species use the São Tomé Mangroves as nursery areas, enabling us to improve fisheries management in this region. As such, this study findings on Malanza's and Praia das Conchas biodiversity and recruitment processes of early life stages of fishes, provides a baseline information important to understand the relevance of these ecosystems to an array of species, some of which relevant to fisheries, and the need to prioritize management and conservation practices to ensure fisheries sustainability for future generations.

5. References

Afonso F, Félix PM, Chainho P, Heumüller JA, de Lima RF, Ribeiro F, Brito AC (2022) Community perceptions about mangrove ecosystem services and threats. Reg Stud Mar Sci 49:102114. doi: 10.1016/j.rsma.2021.102114

Afonso F, Félix PM, Chainho P, Heumüller JA, de Lima RF, Ribeiro F, Brito AC (2021) Assessing Ecosystem Services in Mangroves: Insights from São Tomé Island (Central Africa). Front Environ Sci 9:1–16. doi: 10.3389/fenvs.2021.501673

Albaret J-J, Ecoutin J-M (1990) Influence des saisons et des variations climatiques sur les peuplements de poissons d'une lagune tropicale en Afrique de l'Ouest. Acta Ecologica 11:557–583.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. doi: 10.1111/J.1442-9993.2001.01070.PP.X

Baldwin CC, Johnson GD (2014) Connectivity across the Caribbean Sea: DNA Barcoding and Morphology Unite an Enigmatic Fish Larva from the Florida Straits with a New Species of Sea Bass from Deep Reefs off Curaçao. PLoS One 9:e97661. doi: 10.1371/JOURNAL.PONE.0097661

Baptista V, Dias E, Cruz J, Branco M, Vieira S, Teodósio MA (2020) Feeding Ecology of Sicydium bustamantei (Greeff 1884, Gobiidae) Post-Larvae: The "Little Fish" of São Tomé Island. Ocean 2020, Vol 1, Pages 300-310 1:300–310. doi: 10.3390/OCEANS1040020

Barletta M, Barletta-Bergan A (2009) Endogenous Activity Rhythms of Larval Fish Assemblages in a Mangrove-fringed Estuary in North Brazil. Open Fish Sci J 2:15–24. doi: 10.2174/1874401X00902010015

Barletta-Bergan A, Barletta M, Saint-Paul U (2002) Community structure and temporal variability of ichthyoplankton in north Brazilian mangrove creeks. J Fish Biol 61:33–51. doi: 10.1006/jfbi.2002.2065

Blaber SJM, Blaber TG (1980) Factors affecting the distribution of juvenile estuarine and inshore fish. J Fish Biol 17:143–162. doi: 10.1111/J.1095-8649.1980.TB02749.X/FULLPDF

Brito AC, Silva T, Beltrán C, Chainho P, de Lima RF (2017) Phytoplankton in two tropical mangroves of São Tomé Island (Gulf of Guinea): A contribution towards sustainable management strategies. Reg Stud Mar Sci 9:89–96. doi: 10.1016/J.RSMA.2016.11.005

Brogan MW (1994) Two methods of sampling fish larvae over reefs: a comparison from the Gulf of California. Mar Biol 118:33–44.

Carassou L, Mellin C, Ponton D (2009) Assessing the diversity and abundances of larvae and juveniles of coral reef fish: A synthesis of six sampling techniques. Biodivers Conserv 18:355–371. doi: 10.1007/s10531-008-9492-3

Catalán IA, Reglero P, Álvarez I (2020) Research on early life stages of fish: A lively field. Mar Ecol Prog Ser 650:1–5. doi: 10.3354/meps13491

Chu C, Loh K, CC N, Ooi A, Konishi Y, Huang S, Chong V (2019) Using DNA Barcodes to Aid the Identification of Larval Fishes in Tropical Estuarine Waters (Malacca Straits, Malaysia). Zool Stud 58:e30–e30. doi: 10.6620/ZS.2019.58-30

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143. doi: 10.1111/J.1442-9993.1993.TB00438.X

Cocheret de la Morinière E, Nagelkerken I, van der Meij H, van der Velde G (2003) What attracts juvenile coral reef fish to mangroves: habitat complexity or shade? Mar Biol 2003 1441 144:139–145. doi: 10.1007/S00227-003-1167-8

Collins RA, Cruickshank RH (2013) The seven deadly sins of DNA barcoding. Mol Ecol Resour 13:969–975. doi: 10.1111/1755-0998.12046

Costa Resende Ferreira N, Martins M, da Silva Tavares P, Chan Chou S, Monteiro A, Gomes L, Santana A (2021) Assessment of crop risk due to climate change in Sao Tome and Principe. Reg Environ Chang 21:1–12. doi: 10.1007/S10113-021-01746-6/FIGURES/5

Costa-Pierce BA, Riedel R (2000) Fisheries ecology of the tilapias in the subtropical lakes of the United States. In 'Tilapia Aquaculture in the Americas' World Aquac Soc Bat Rouge, LA 2:1–20.

Cravo M, Almeida AJ, Lima H, Azevedo e Silva J, Bandeira S, Machava-António V, Paula J (2021) Fish Assemblages in a Small Mangrove System on Príncipe Island, Gulf of Guinea. Front Mar Sci 8:1–12. doi: 10.3389/fmars.2021.721692

Dennis GD (1992) Island mangrove habitats as spawning and nursery areas for commercially important fishes in the Caribbean. In: Proceedings of the Gulf and Caribbean Fisheries Institute, 41. AquaDocs & Gulf and Caribbean Fisheries Institute Proceedings, pp 205–225

Dentinger BTM, Margaritescu S, Moncalvo JM (2010) Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. Mol Ecol Resour 10:628–633. doi: 10.1111/J.1755-0998.2009.02825.X

Doherty PJ (1987) Light-Traps: Selective but Useful Devices for Quantifying the Distributions and Abundances of Larval Fishes. Bull Mar Sci 41:423–431.

Edworthy C, Strydom N (2016) Habitat partitioning by juvenile fishes in a temperate estuarine nursery, South Africa. Sci Mar 80:151–161. doi: http://dx.doi.org/10.3989/scimar.04333.01B

Elliott M, Whitfield AK, Potter IC, Blaber SJM, Cyrus DP, Nordlie FG, Harrison TD (2007) The guild approach to categorizing estuarine fish assemblages: A global review. Fish Fish 8:241–268. doi: 10.1111/J.1467-2679.2007.00253.X

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak W V, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486. doi: 10.1890/1540-9295(2005)003[0479:LOFSCF]2.0.CO;2

Moser, H. (1996). The early stages of fishes in the California current region.

FAO (2009) Integrated Mariculture. A Global Review. Food and Agriculture Organization of the United Nations, Rome

FAO (2016) The Living Marine Resources of the Eastern Central Atlantic. Food and Agriculture Organization of the United Nations, Rome

Faunce CH, Serafy JE (2006) Mangroves as fish habitat: 50 Years of field studies. Mar Ecol Prog Ser 318:1–18. doi: 10.3354/MEPS318001

Félix P, Chainho P, Costa J, Correia M, Chaves M, Medeiros J, Cabral H, Wouters N, Bernardo J, Costa A, Cristo M, Silva G, Azeda C, Tavares P, Costa M, Cancela Da Fonseca L (2013) Short-term versus long-term changes in the benthic communities of a small coastal lagoon: implications for ecological status assessment. Vie Milieu 63:11–22. http://hdl.handle.net/10174/10450

Félix PM, Chainho P, Lima RF, Costa JL, Almeida AJ, Domingos I, Brito AC (2017) Mangrove fish of São Tomé Island (Gulf of Guinea): New occurrences and habitat usage. Mar Freshw Res 68:123–130. doi: 10.1071/MF15392

Heumüller JA (2021) Fish Diversity in Mangroves of São Tomé Island (Gulf of Guinea). Master Thesis. University of Lisbon, Faculty of Sciences

Hubert N, Espiau B, Meyer C, Planes S (2014) Identifying the ichthyoplankton of a coral reef using DNA barcodes. Mol Ecol Resour 15:57–67. doi: 10.1111/1755-0998.12293

Ivanova N V., Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548. doi: 10.1111/j.1471-8286.2007.01748.x

Juhel J-B, Utama RS, Marques V, Vimono IB, Sugeha HY, Kadarusman, Pouyaud L, Dejean T, Mouillot D, Hocdé R (2020) Accumulation curves of environmental DNA sequences predict coastal fish diversity in the coral triangle. Proc R Soc B Biol Sci 287:20200248. doi: 10.1098/RSPB.2020.0248

Kimirei IA, Nagelkerken I, Trommelen M, Blankers P, van Hoytema N, Hoeijmakers D, Huijbers CM, Mgaya YD, Rypel AL (2013) What Drives Ontogenetic Niche Shifts of Fishes in Coral Reef Ecosystems? Ecosyst 2013 165 16:783–796. doi: 10.1007/S10021-013-9645-4

Kissick L (1993) Comparison of Traps Lighted by Photochemicals or Electric Bulbs for Sampling Warmwater Populations of Young Fish. North Am J Fish Manag 13:864–867. doi: 10.1577/1548-8675(1993)013<0864:cotlbp>2.3.co;2

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ, Chen WY, Shao KT (2013) Evaluating the Accuracy of Morphological Identification of Larval Fishes by Applying DNA Barcoding. PLoS One 8:3–9. doi: 10.1371/journal.pone.0053451

Krishna Krishnamurthy P, Francis RA (2012) A critical review on the utility of DNA barcoding in biodiversity conservation. Biodivers Conserv 2012 218 21:1901–1919. doi: 10.1007/S10531-012-0306-2

Maia HA, Morais RA, Quimbayo JP, Dias MS, Sampaio CLS, Horta PA, Ferreira CEL, Floeter SR (2018) Spatial patterns and drivers of fish and benthic reef communities at São Tomé Island, Tropical Eastern Atlantic. Mar Ecol 39:1–16. doi: 10.1111/maec.12520

Makowski C, Finkl CW (2018) Threats to Mangrove Forests: Hazards, Vulnerability, and Management. Springer, Boca Raton, Florida

McLeod LE, Costello MJ (2017) Light traps for sampling marine biodiversity. Helgol Mar Res 71:1–8. doi: 10.1186/s10152-017-0483-1

Nagelkerken I, Kleijnen S, Klop T, Van Den Brand RACJ, Cocheret De La Morinière E, Van Der Velde G (2001) Dependence of Caribbean reef fishes on mangroves and seagrass beds as nursery habitats: A comparison of fish faunas between bays with and without mangroves/seagrass beds. Mar Ecol Prog Ser 214:225–235. doi: 10.3354/MEPS214225

Nagelkerken I, Roberts CM, van der Velde G, Dorenbosch M, van Riel MC, Cocheret de la Morinière E, Nienhuis PH (2002) How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. Mar Ecol Prog Ser 244:299–305. doi: 10.3354/meps244299

Nagelkerken I, Sheaves M, Baker R, Connolly RM (2015) The seascape nursery: a novel spatial approach to identify and manage nurseries for coastal marine fauna. Fish Fish 16:362–371. doi: 10.1111/FAF.12057

Neira FJ, Perry RA, Burridge CP, Lyle JM, Keane JP (2015) Molecular discrimination of shelf-spawned eggs of two co-occurring Trachurus spp. (Carangidae) in southeastern Australia: a key step to future egg-based biomass estimates. ICES J Mar Sci 72:614–624. doi: 10.1093/ICESJMS/FSU151

Neira FJ, Potter IC, Bradley JS (1992) Seasonal and spatial changes in the larval fish fauna within a large temperate Australian estuary. Mar Biol 1992 1121 112:1–16. doi: 10.1007/BF00349721

Nelson JS, Grande TC, Wilson MVH (2016) Fishes of the World: Fifth Edition. Wiley Blackwell

Oksanen J, Kindt R, O' B, Maintainer H (2005) The vegan Package: Community Ecology Package. 1–88.

Olivar, M. & Shelton, Peter. (1993). Larval Fish Assemblages of the Benguela Current. Bulletin of Marine Science. 53. 450-474.

Ooi AL (2012) (2012) Fish communities of mangrove estuary. PhD thesis. University of Malaya

Ooi AL, Chong VC (2011) Larval fish assemblages in a tropical mangrove estuary and adjacent coastal waters: Offshore-inshore flux of marine and estuarine species. Cont Shelf Res 31:1599–1610. doi: 10.1016/j.csr.2011.06.016

Orth RJ, Heck KL, van Montfrans J (1984) Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 1984 74 7:339–350. doi: 10.2307/1351618

Parrish JD (1989) Fish communities of interacting shallow-water habitats in tropical oceanic regions. Mar Ecol Prog Ser 58:143–160.

Pielou EC (1966) The measurement of diversity in different types of biological collections. J Theor Biol 13:131–144. doi: 10.1016/0022-5193(66)90013-0

Plumstead EE (1990) Changes in ichthyofaunal diversity and abundance within the Mbashe Estuary, Transkei, following construction of a river barrage. South African J Mar Sci 9:399–407. doi: 10.2989/025776190784378817

Polidoro BA, Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, Farnsworth EJ, Fernando ES, Kathiresan K, Koedam NE, Livingstone SR, Miyagi T, Moore GE, Nam VN, Ong JE, Primavera JH, III SGS, Sanciangco JC, Sukardjo S, Wang Y, Yong JWH (2010) The Loss of Species: Mangrove Extinction Risk and Geographic Areas of Global Concern. PLoS One 5:e10095. doi: 10.1371/JOURNAL.PONE.0010095

Powell AB, Hoss DE, Hettler WF, Peters DS, Wagner S (1989) Abundance and distribution of ichthyoplankton in Florida Bay and adjacent waters. Bull Mar Sci 44:35–48.

Ramos S (2007) Ichthyoplankton of the Lima estuary (NW Portugal): Ecology of the early life stages of Pleuronectiformes. PhD thesis. Universidade do Porto

Ribeiro F, Hale E, Hilton EJ, Clardy TR, Deary AL, Targett TE, Olney JE (2015) Composition and temporal patterns of larval fish communities in Chesapeake and Delaware Bays, USA. Mar Ecol Prog Ser 527:167–180. doi: 10.3354/meps11265

Robertson AI, Duke NC (1987) Mangroves as nursery sites: comparisons of the abundance and species composition of fish and crustaceans in mangroves and other nearshore habitats in tropical Australia. Mar Biol 205:193–205. doi: 10.1007/bf00427019

Russell DJ, Thuesen PA, Thomson FE (2012) Reproductive strategies of two invasive tilapia species Oreochromis mossambicus and Tilapia mariae in northern Australia. J Fish Biol 80:2176–2197. doi: 10.1111/J.1095-8649.2012.03267.X

Selleslagh J, Amara R (2008) Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (eastern English Channel). Estuar Coast Shelf Sci 79:507–517. doi: 10.1016/J.ECSS.2008.05.006

Selleslagh J, Blanchet H, Bachelet G, Lobry J (2015) Feeding Habitats, Connectivity and Origin of Organic Matter Supporting Fish Populations in an Estuary with a Reduced Intertidal Area Assessed by Stable Isotope Analysis. Estuaries and Coasts 38:1431–1447. doi: 10.1007/S12237-014-9911-5

Shannon CE, Weaver WW (1963) A Mathematical Theory of Communication. Univ Illinois Press Urbana 0–177. doi: 10.1002/J.1538-7305.1948.TB01338.X

Shirak A, Dor L, Seroussi E, Ron M, Hulata G, Golani D (2016) DNA barcoding of fish species from the Mediterranean coast of Israel. Mediterr Mar Sci 17:459–466. doi: 10.12681/mms.1384

Silva-Falcão EC, Severi W, Araújo ME De (2013) Spatial–temporal variation of Achirus larvae (Actinopterygii: Achiridae) in mangrove, beach and reef habitats in north-eastern Brazil. J Mar Biol Assoc United Kingdom 93:381–388. doi: 10.1017/S0025315411001706

Sloterdijk H, Brehmer P, Sadio O, Müller H, Döring J, Ekau W (2017) Composition and structure of the larval fish community related to environmental parameters in a tropical estuary impacted by climate change. Estuar Coast Shelf Sci 197:10–26. doi: 10.1016/j.ecss.2017.08.003

Strauss RE, Bond CE (1990) Taxonomic Methods: Morphology. In: Moyle P, Schreck C (eds) Methods for Fish Biology. American Fisheries Society, pp 109–140

Thresher RE (1984) Reproduction in Reef Fishes. Reprod Reef Fishes 114:343–388.

Tzeng W, Wang Y, Chang C (2002) Spatial and temporal variations of the estuarine larval fish community on the west coast of Taiwan. Mar Feshwater Res 53:419–430. doi: 10.1071/MF01136

Tzeng WN, Wang YT (1992) Structure, composition and seasonal dynamics of the larval and juvenile fish community in the mangrove estuary of Tanshui River, Taiwan. Mar Biol 113:481–490. doi: 10.1007/BF00349175

Waldman J (2012) Ecology of Estuarine Fishes: Temperate Waters of the Western North Atlantic By Kenneth W. Able and Michael P. Fahay. Baltimore (Maryland): Johns Hopkins University 566 p.; ill.; index. ISBN: 978-0-8018-9471-8. 2010. Q Rev Biol 87:381–381. doi: 10.1086/668172

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Weinstein M, Brooks H (1983) Comparative ecology of nekton residing in a tidal creek and adjacent seagrass meadow, community composition and structure. Mar Ecol Prog Ser 12:15–27. doi: 10.3354/MEPS012015

Wesley Neal J, Adelsberger CM, Lochmann SE (2012) A comparison of larval fish sampling methods for tropical streams. Mar Coast Fish 4:23–29. doi: 10.1080/19425120.2011.651521

Whitfield AK (2016) The role of seagrass meadows, mangrove forests, salt marshes and reed beds as nursery areas and food sources for fishes in estuaries. Rev Fish Biol Fish 27:75–110. doi: 10.1007/s11160-016-9454-x

Wirtz P, Ferreira CEL, Floeter SR, Fricke R, Gasparini JL, Iwamoto T, Rocha L, Sampaio CLS, Schliewen UK (2007) Coastal fishes of São Tomé and Príncipe islands, Gulf of Guinea (Eastern Atlantic Ocean) - An update. Zootaxa 1–48. doi: 10.11646/zootaxa.1523.1.1

World Bank Group (2016) Africa's economic growth - Taking off or slowing down? World Bank 1–28. doi: 10.2861/443750

Yao H, Song JY, Ma XY, Liu C, Li Y, Xu HX, Han JP, Duan LS, Chen SL (2009) Identification of Dendrobium Species by a Candidate DNA Barcode Sequence: The Chloroplast psbA-trnH Intergenic Region. Planta Med 75:667–669. doi: 10.1055/S-0029-1185385

Zar JH (1999) Biostatistical analysis, 4th ed. Prentice Hall, Upper Saddle River N.J.

ANNEXES

Annex A – Photographic records of all species

Annex A.1 – Photographic records for each individual used for molecular identification with associated traits used for species morphological identification

Figure A.1.1 - Dalophis cephalopeltis (K10)

Identifying characteristics: Ophichthidae

- Elongated eel/snake like
- Nostrils widely separated
- Caudal fin absent
- Lateral line complete, with well-developed pores

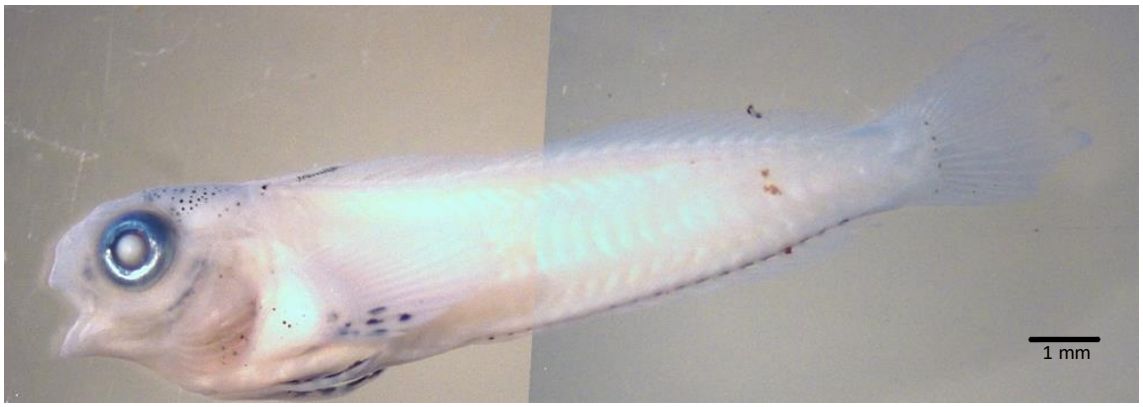


Figure A.1.2 - Blenniidae *(G7)*

Identifying characteristics: Blenniidae

- Dorsal and anal fins long
- Pelvic fins anterior to pectoral fins
- rounded head
- pigment at base of anal-fin pterygiophores
- pigmented pectoral fins

Figure A.1.3 - Caranx sp. 2 *(K1)*

Identifying characteristics: Carangidae

- scutes along the side of the caudal peduncle
- 2 detached spines ahead of the anal fin
- Two separated dorsal fins
- Elongated pectoral fin

Figure A.1.4 - Oreochromis mossambicus (A1)

Figure A.1.5 - Oreochromis mossambicus (A2)

Identifying characteristics: Cichlidae

- One nostril on each side of the head
- Dark spot on the dorsal fin identical to those found in adults of the same species

Figure A.1.6 - Sardinella maderensis (A7)

Figure A.1.7 - Sardinella maderensis (A8)

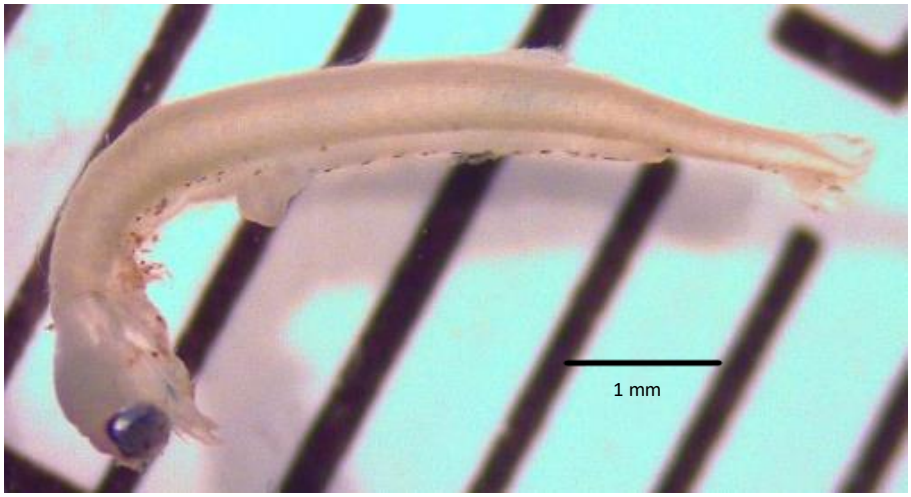


Figure A.1.8 – Sardinella maderensis (A9)

Identifying characteristics: Clupeidae

- Tubular shape body
- Long gut

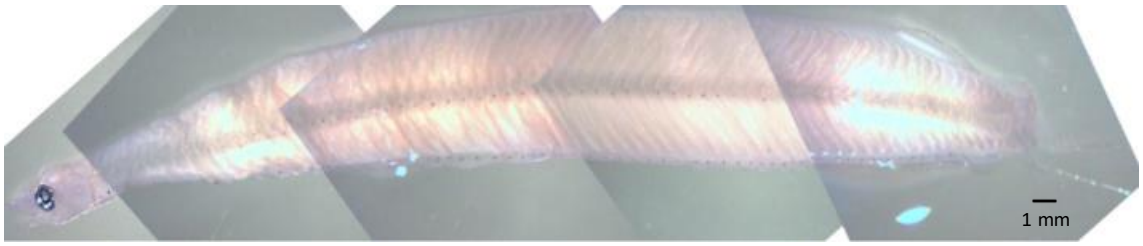


Figure A.1.9 - Elops senegalensis (A5)

Identifying characteristics: Elopidae

- Leptocephali
- Anal fin starts before the dorsal fin

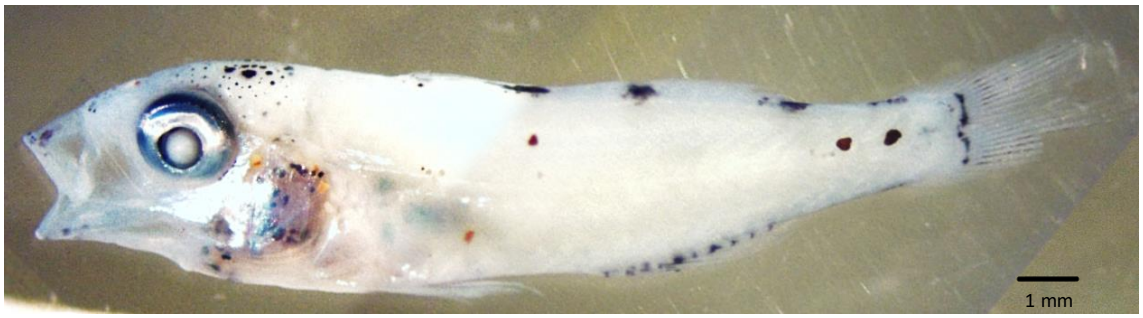


Figure A.1.10 - Eucinostomus melanopterus (A3)

Identifying characteristics: Gerreidae

- highly protrusible snout, pointing downward when extended
- single dorsal fin with a black tip typical of the black fin mojarra
- head pigmentation

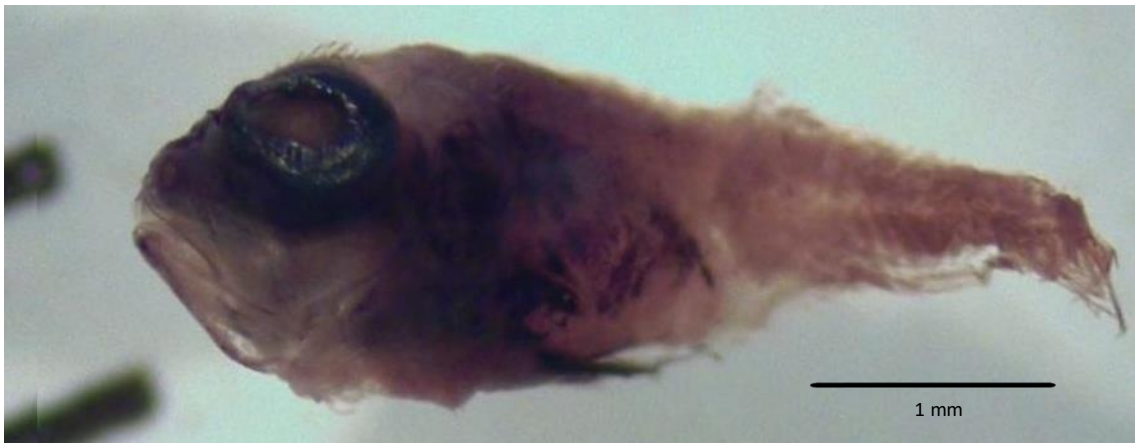


Figure A.1.11 - Monodactylus sebae (A4)

Identifying characteristics: Monodactylidae

- Five spines on the margin of the preopercle
- projecting spines on the supraorbital, on the preopercle and on the upper part of the opercle
- body depth about 48% of body length

Figure A.1.12 - Epinephelus aeneus (K5)

Figure A.1.13 - Lutjanus agennes (K6)

Identifying characteristics: Serranidae & Lutjanidae

- Preopercle has many spines
- Anal fin with three spines
- Pointed snout and terminal mouth

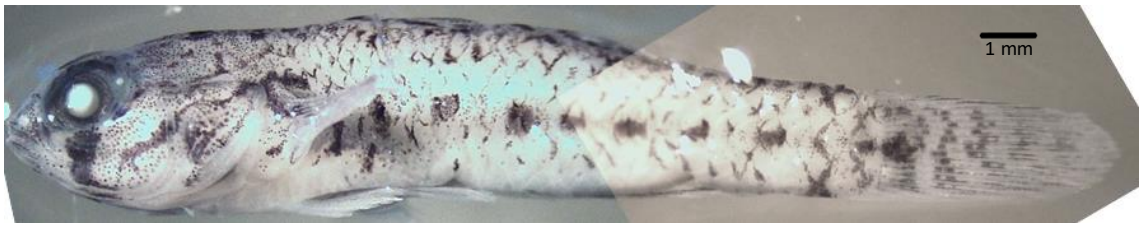


Figure A.1.14 - Gob sp1 (*B5)*

Figure A.1.15 - Not sequenced (B6)

Figure A.1.16 - Not sequenced (B7)

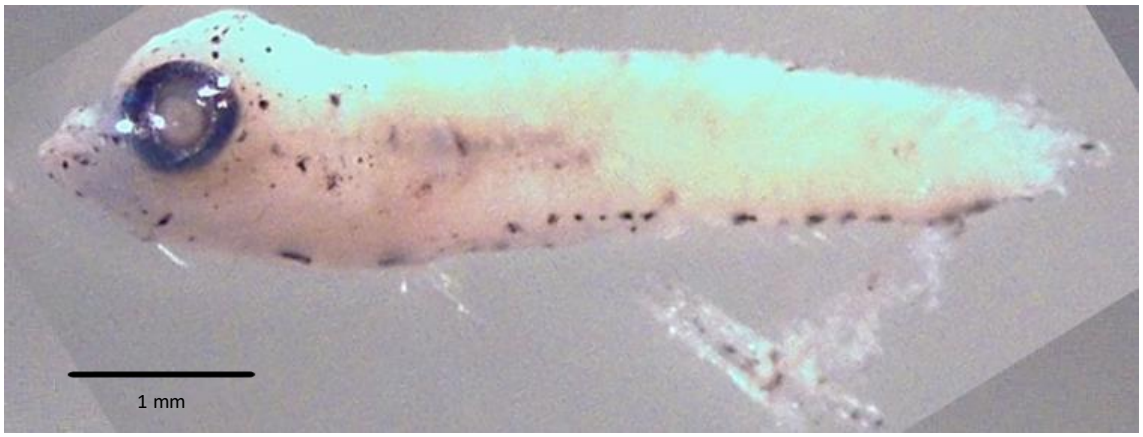


Figure A.1.17 - Gob sp. 1 *(B8)*

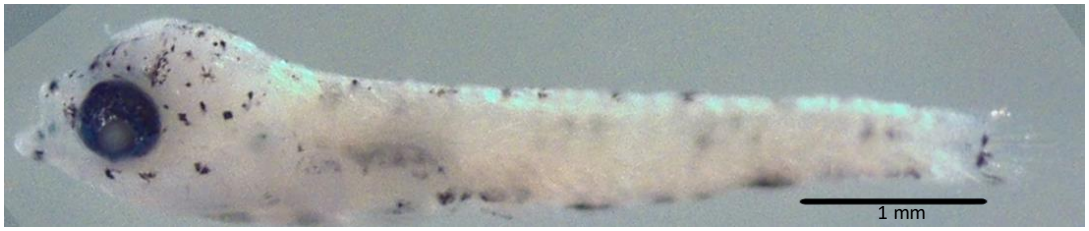


Figure A.1.18 - Gob sp. 1 *(B9)*

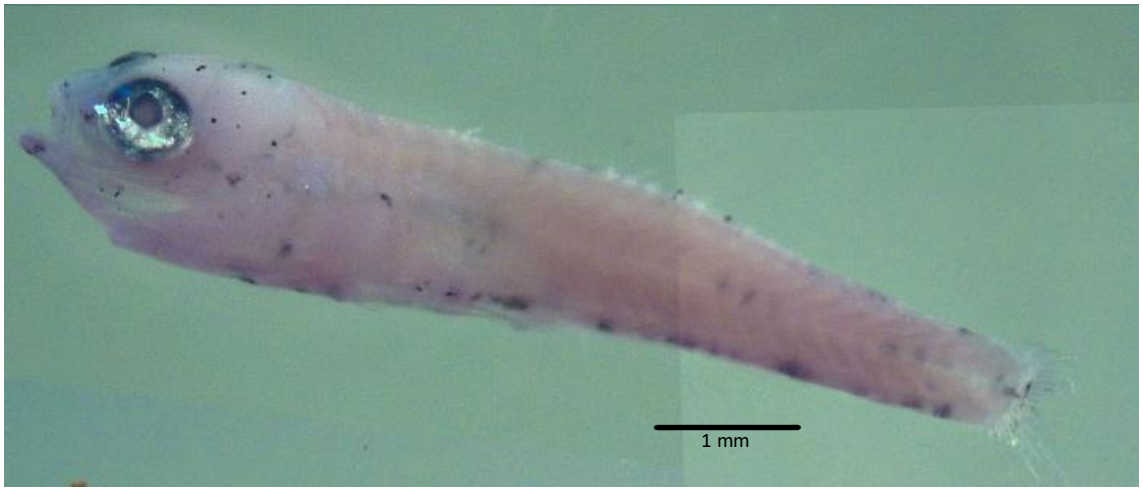


Figure A.1.19 - Gob sp. 1 *(B10)*

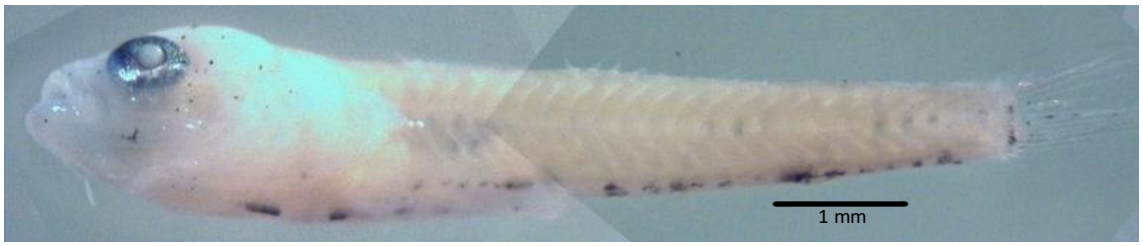


Figure A.1.20 - Gob sp. 1 *(C1)*

Figure A.1.21 - Gob sp. 1 *(C2)*

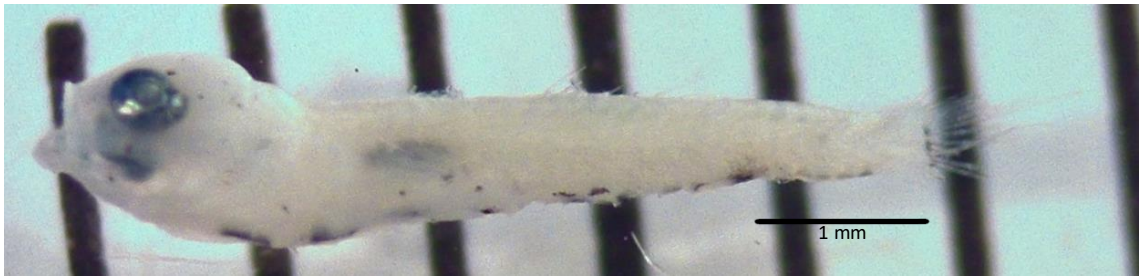


Figure A.1.22 - Gob sp. 1 *(C3)*

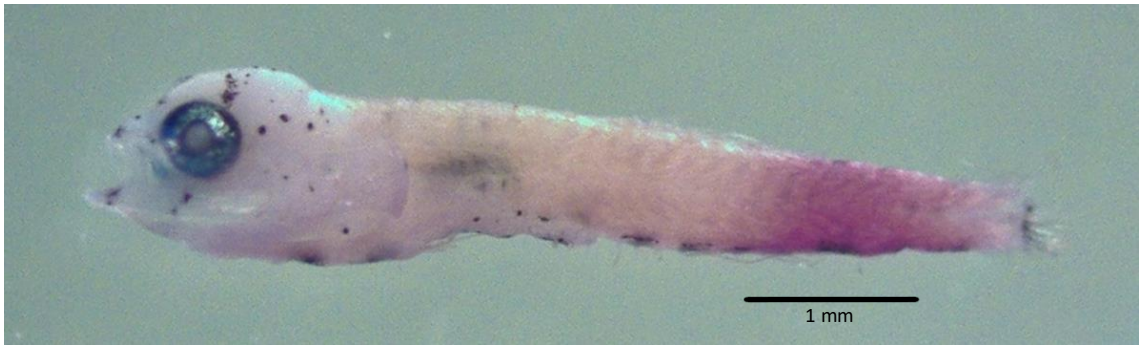


Figure A.1.23 - Gob sp. 1 *(C4)*

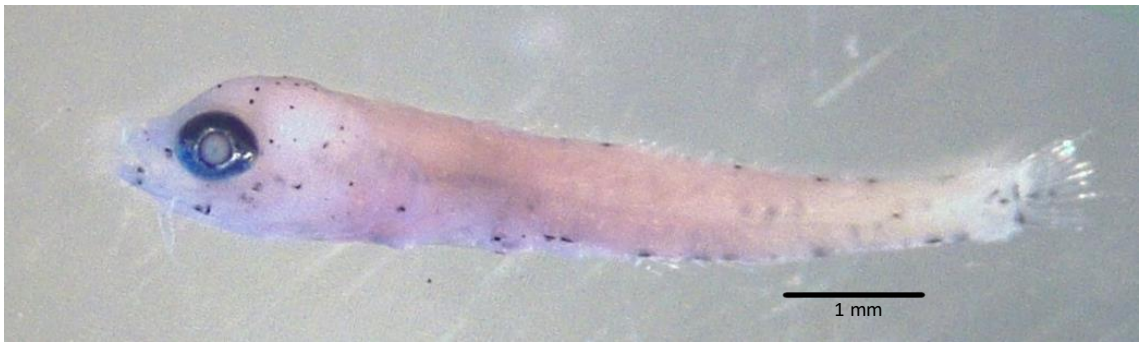


Figure A.1.24 - Gob sp. 1 *(C5)*

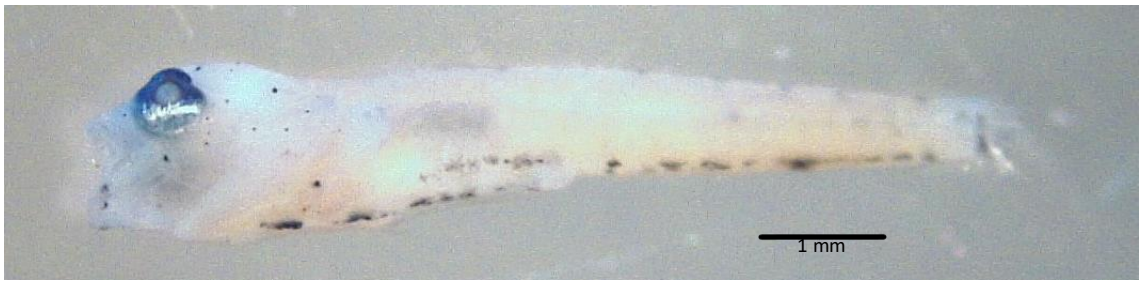


Figure A.1.25 - Gob sp. 1 *(C6)*

Figure A.1.26 - Gob sp. 1 *(C7)*

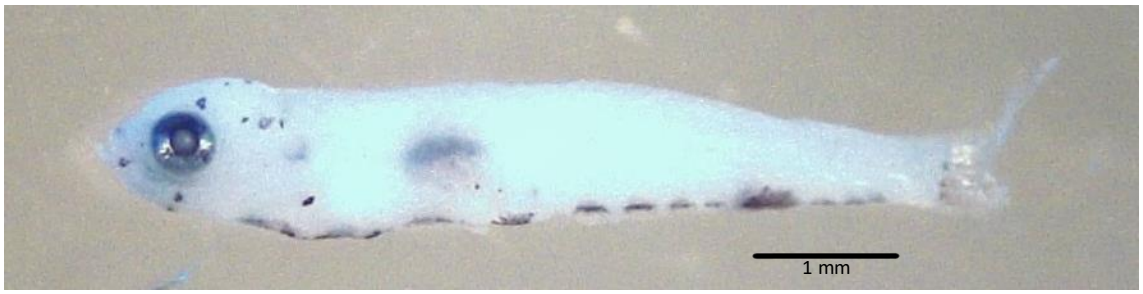


Figure A.1.27 - Gob sp. 1 *(C8)*

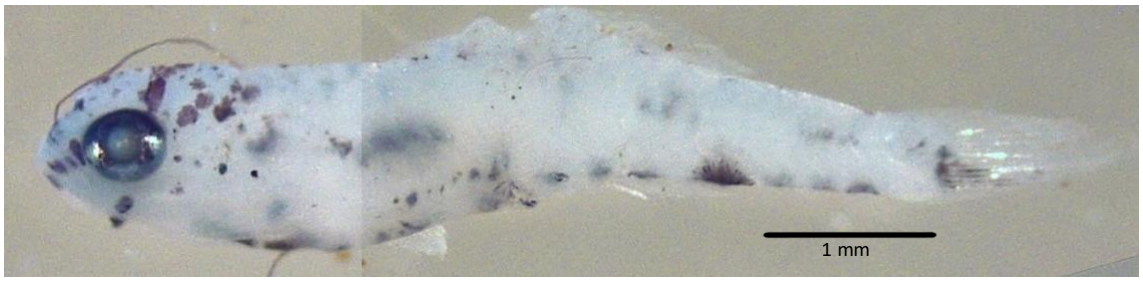


Figure A.1.28 - Gob sp. 1 *(C9)*

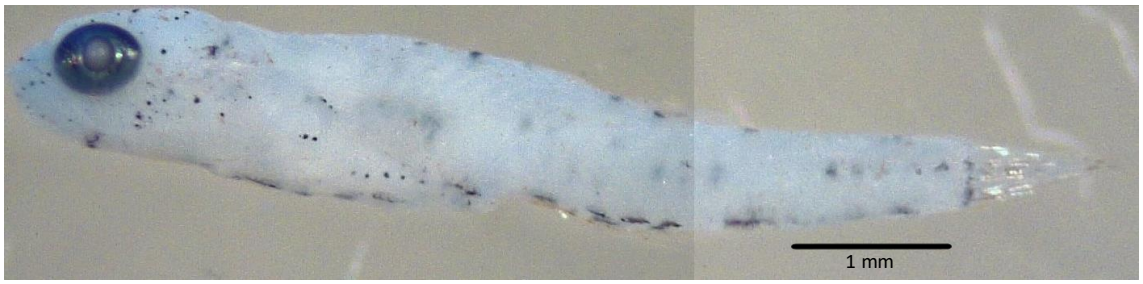


Figure A.1.29 - Gob sp. 1 *(C10)*

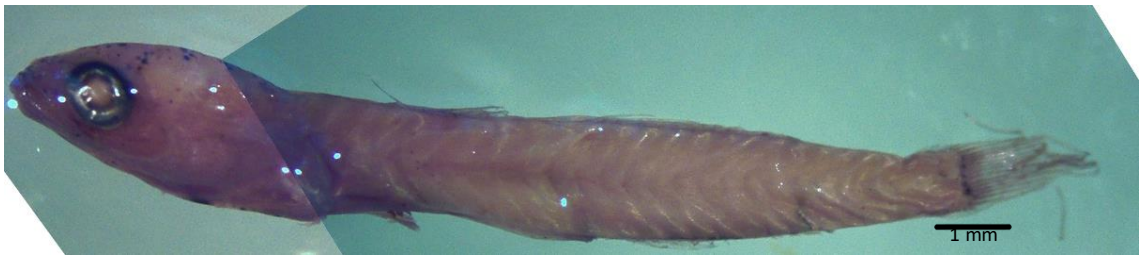


Figure A.1.30 - Awaous lateristriga (D1)

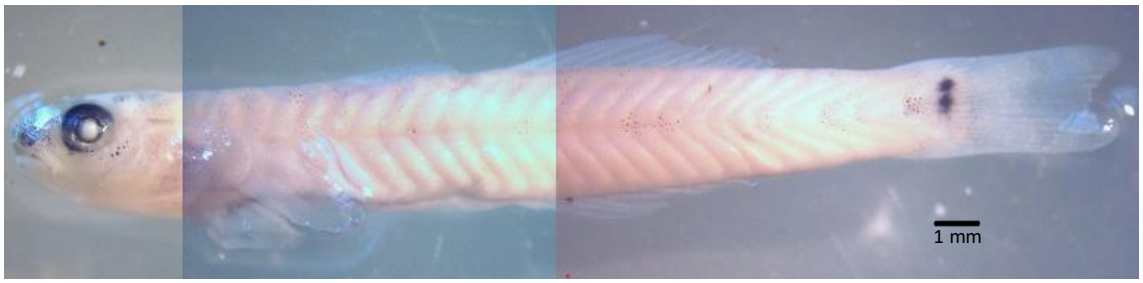


Figure A.1.31 - Sicydium bustamantei (D2)

Figure A.1.32 - Awaous lateristriga (D3)

Figure A.1.33 - Bathygobius soporator (D4)

Figure A.1.34 – Bathygobius soporator (D5)

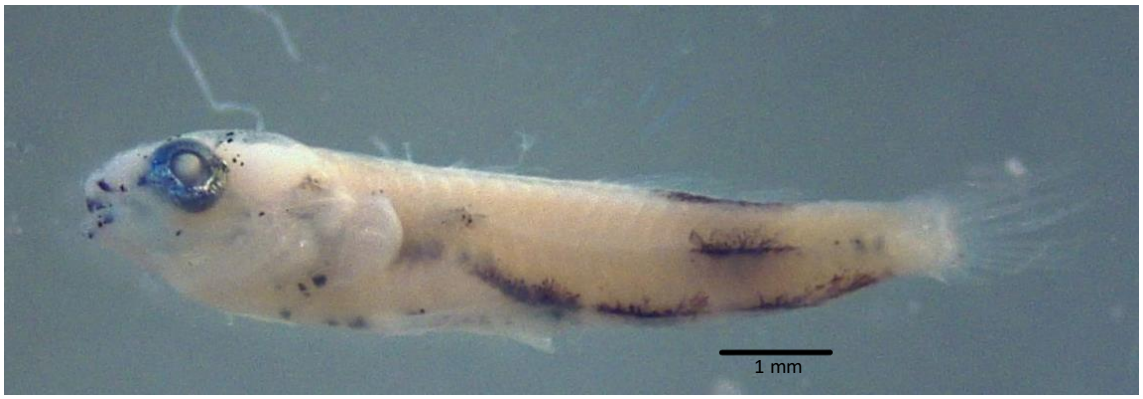


Figure A.1.35 - Bathygobius soporator (D6)

Figure A.1.36 - Bathygobius soporator (D7)

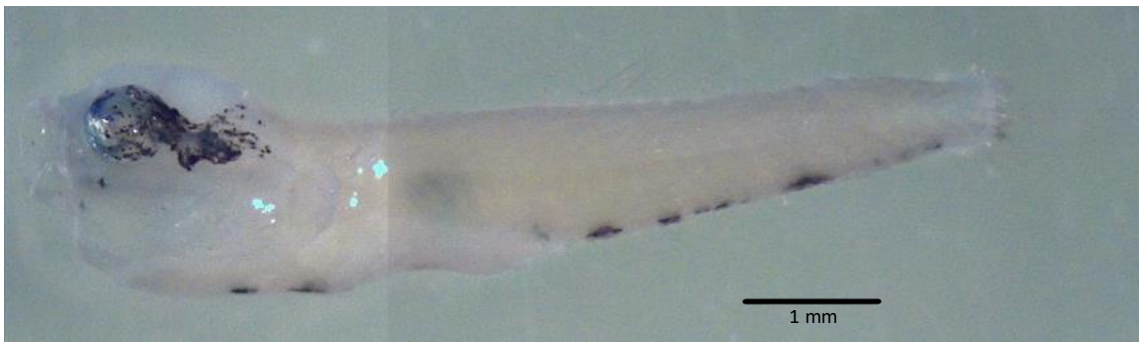


Figure A.1.37 - Gob sp. 1 *(D8)*

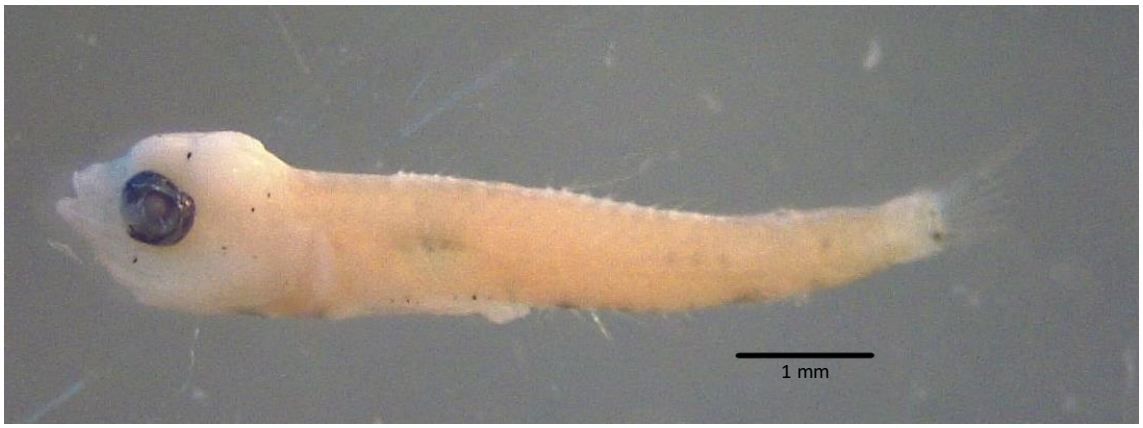


Figure A.1.38 - Gob sp. 1 *(D9)*

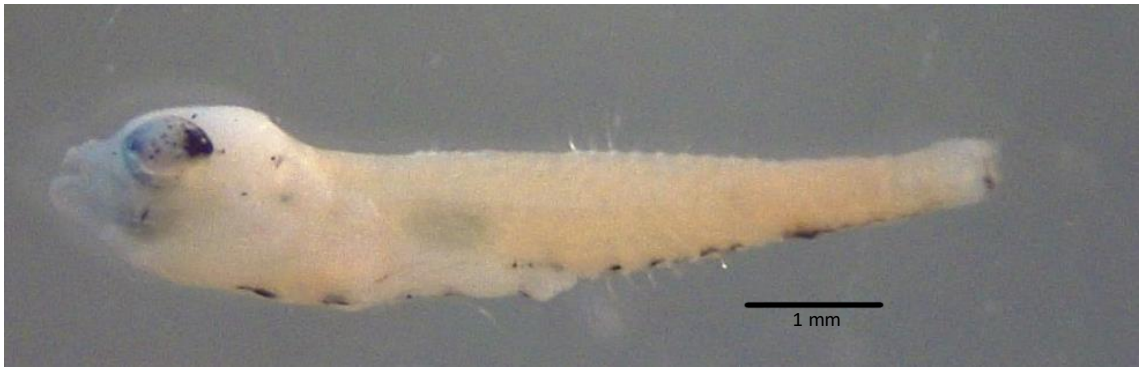


Figure A.1.39 - Gob sp. 1 *(D10)*

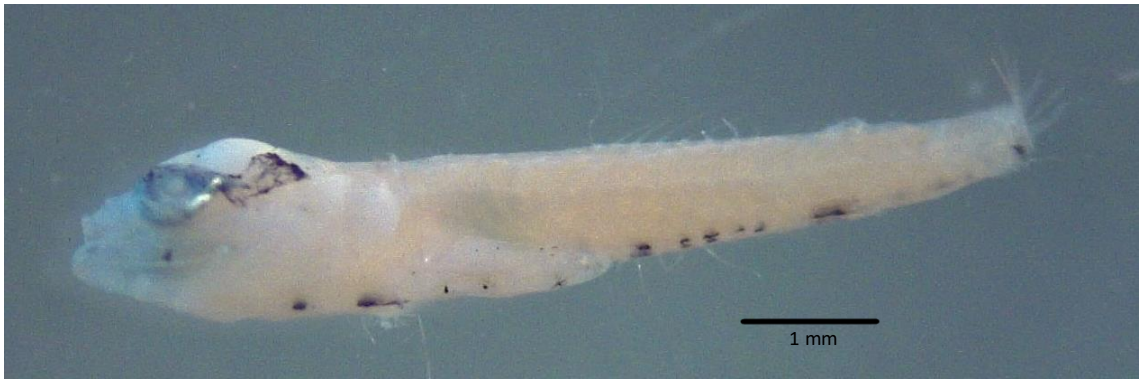


Figure A.1.40 - Gob sp. 1 *(E1)*

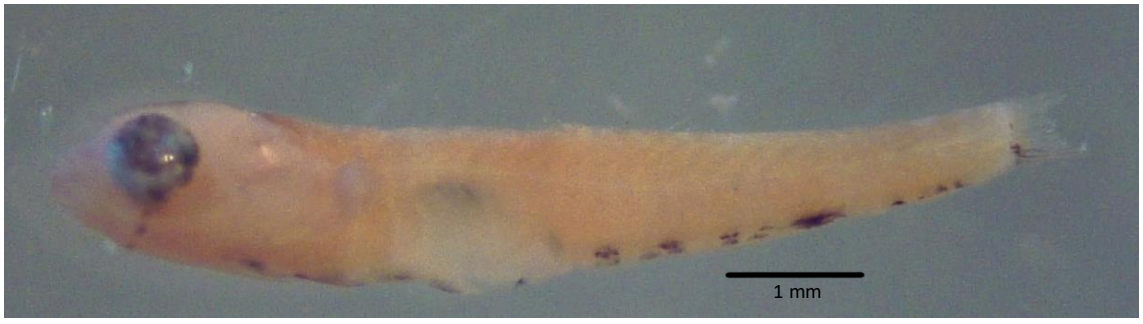


Figure A.1.41- Gob sp. 1 *(E2)*

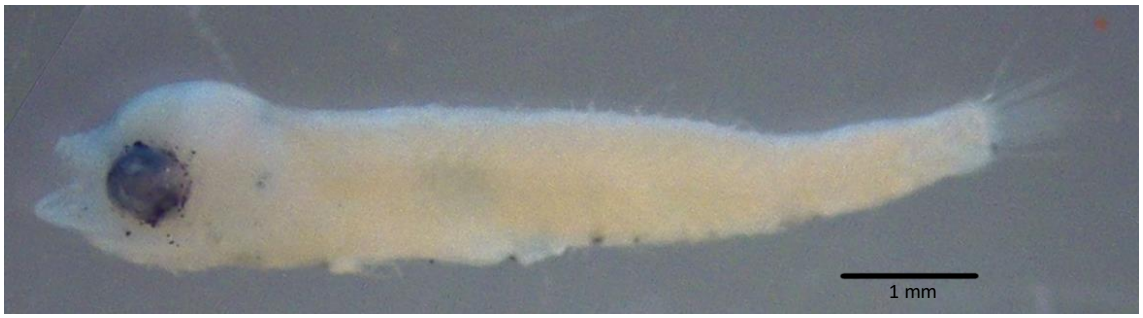


Figure A.1.42 - Gob sp. 1 *(E3)*

Figure A.1.43 - Gob sp. 1 *(E4)*

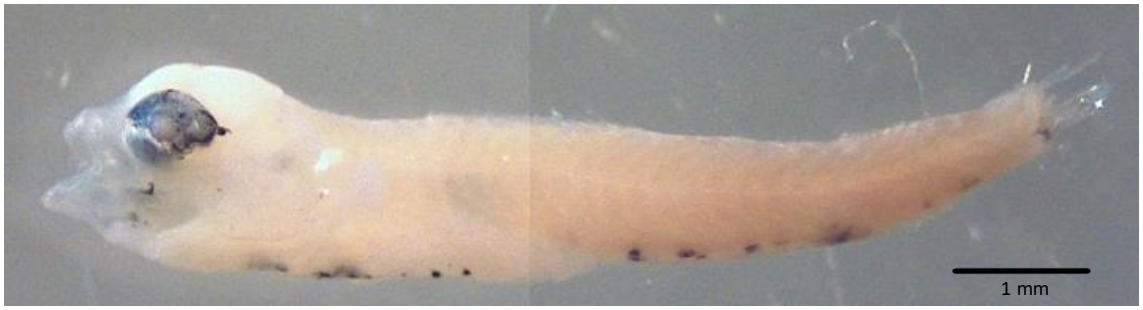


Figure A.1.44 - Gob sp. 1 *(E5)*

Figure A.1.45 - Gob sp. 1 *(E6)*

Figure A.1.46 - Gob sp. 1 *(E7)*

Figure A.1.47 - Gob sp. 1 *(E8)*

Figure A.1.48 - Gob sp. 1 *(E9)*

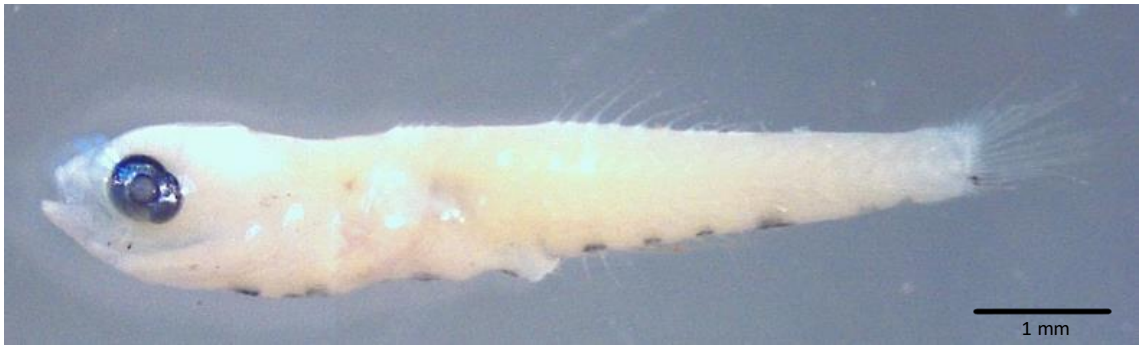


Figure A.1.49 - Gob sp. 1 *(E10)*

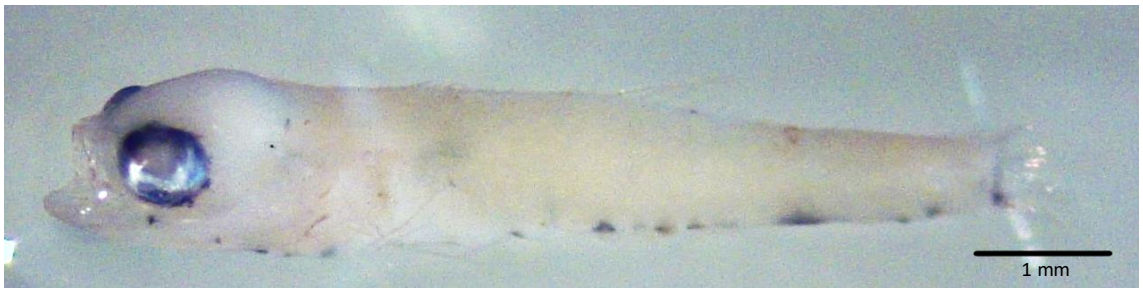


Figure A.1.50 - Gob sp. 1 *(F1)*

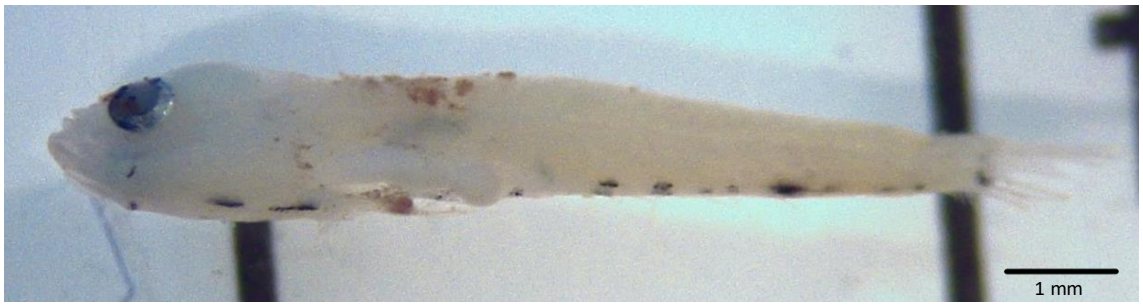


Figure A.1.51 - Gob sp. 1 *(F2)*

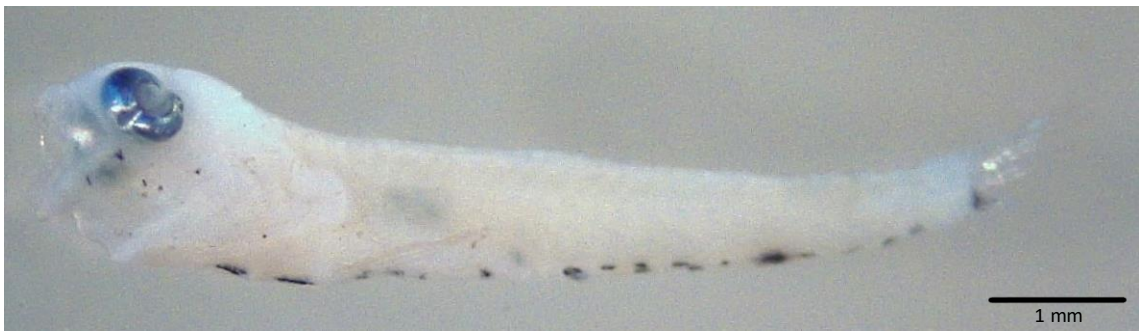


Figure A.1.52 - Gob sp. 1 *(F3)*

Figure A.1.53 – Not sequenced (F4)

Figure A.1.54 - Gob sp. 1 *(F5)*

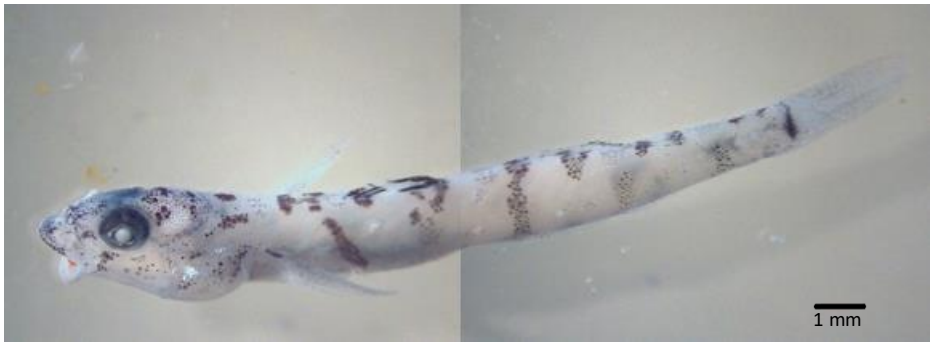


Figure A.1.55 - Awaous lateristriga (F6)

Figure A.1.56 - Gob sp. 2 (*F7)*

Figure A.1.57 - Gob sp. 2 (*F8)*

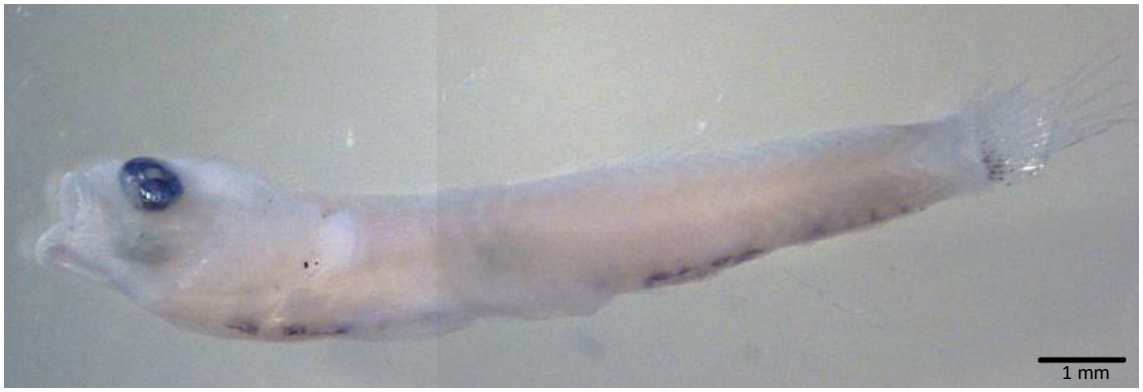


Figure A.1.58 - Gob sp. 2 (*F9)*

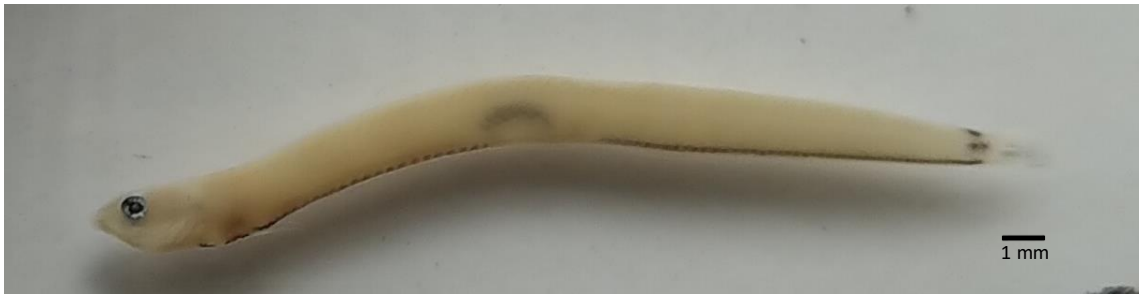


Figure A.1.59 - Microdesmus sp. (*K8)*

Figure A.1.60 - Gob sp. 1 *(K9)*

Identifying characteristics: Gobiidae

- Pelvic fins united
- Six flexible spines on first dorsal fin
- Large gas bladder

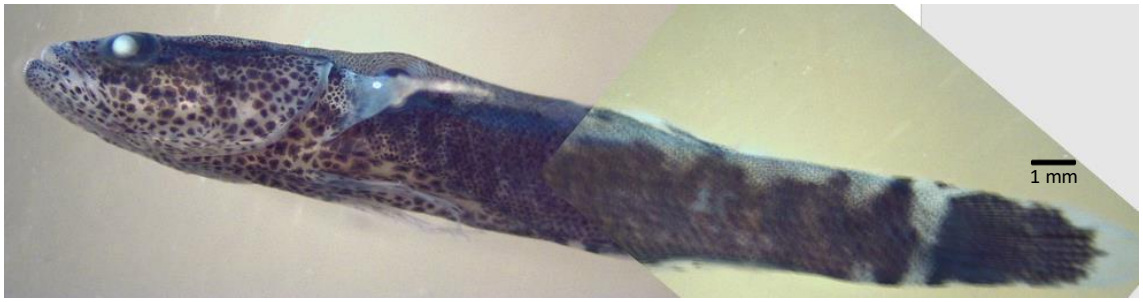


Figure A.1.61 - Eleotris vittata (F10)

Figure A.1.62 - Eleotris annabonensis (G1)

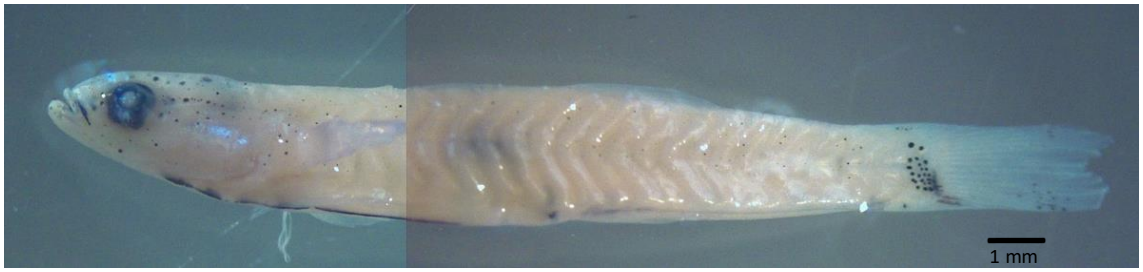


Figure A.1.63 - Eleotris annabonensis (G2)

Figure A.1.64 - Eleotris annabonensis (G3)

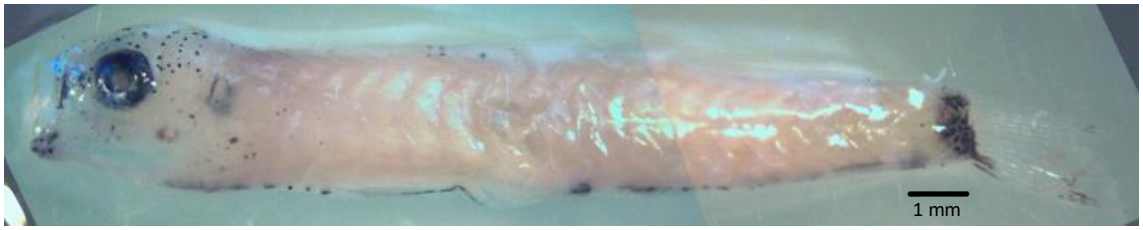


Figure A.1.65 - Eleotris annabonensis (G4)

Figure A.1.66 - Eleotris annabonensis (G5)

Figure A.1.67 - Wheelerigobius maltzani (G6)

Identifying characteristics: Eleotridae

- Pelvic fins separated
- Second dorsal fin with 1 spine
- One spine on anal fin

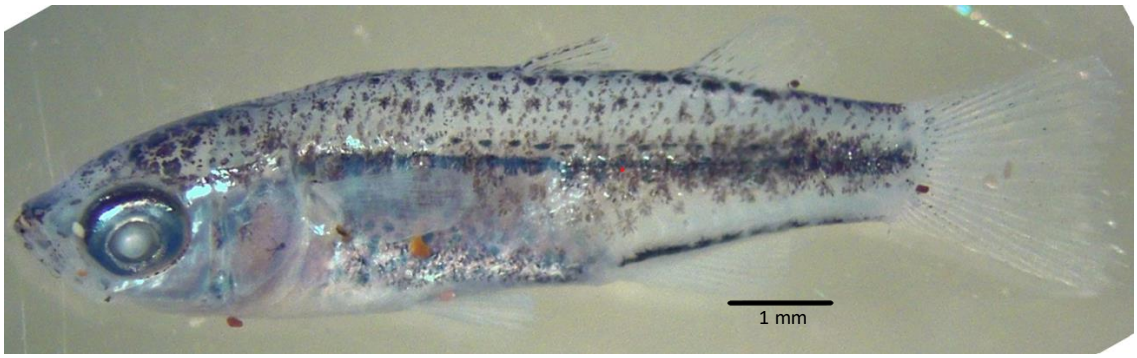


Figure A.1.68 - Parachelon grandisquamis. (A6)

Figure A.1.69 - Mugil curema (K2)

Figure A.1.70 - Mugil bananensis (K3)

Identifying characteristics: Mugillidae

- Adipose eye fold
- Two short dorsal fins well separated
- Quilled mouth
- Pectoral fins high on body
- Flanks silvery

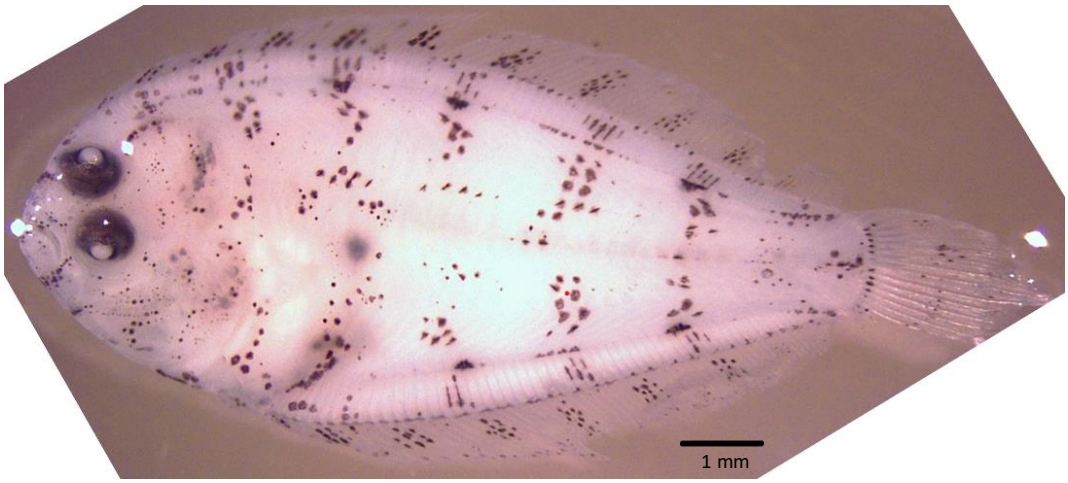


Figure A.1.71 – Citharichthys (A10)

Identifying characteristics: Paralichthyidae
- eyes on the left side
- dorsal and anal fin not attached to caudal fin
- elongated 1st fin ray on the earlier stages

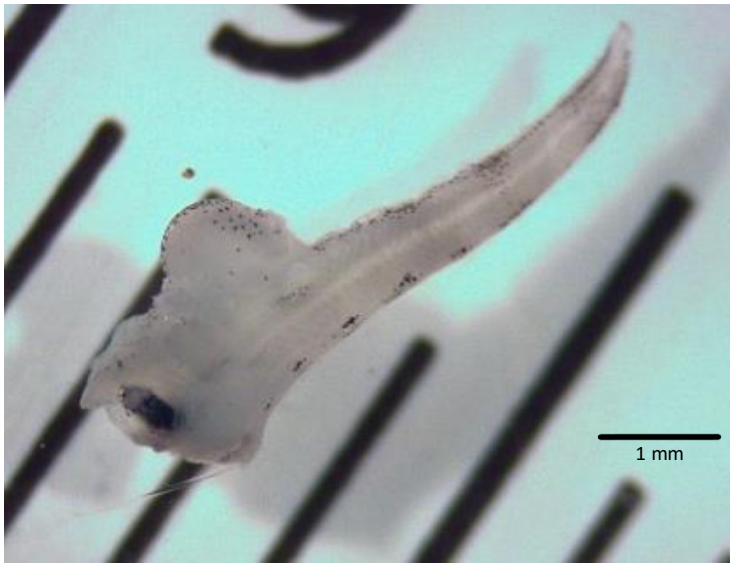


Figure A.1.72 – Not sequenced (B1)

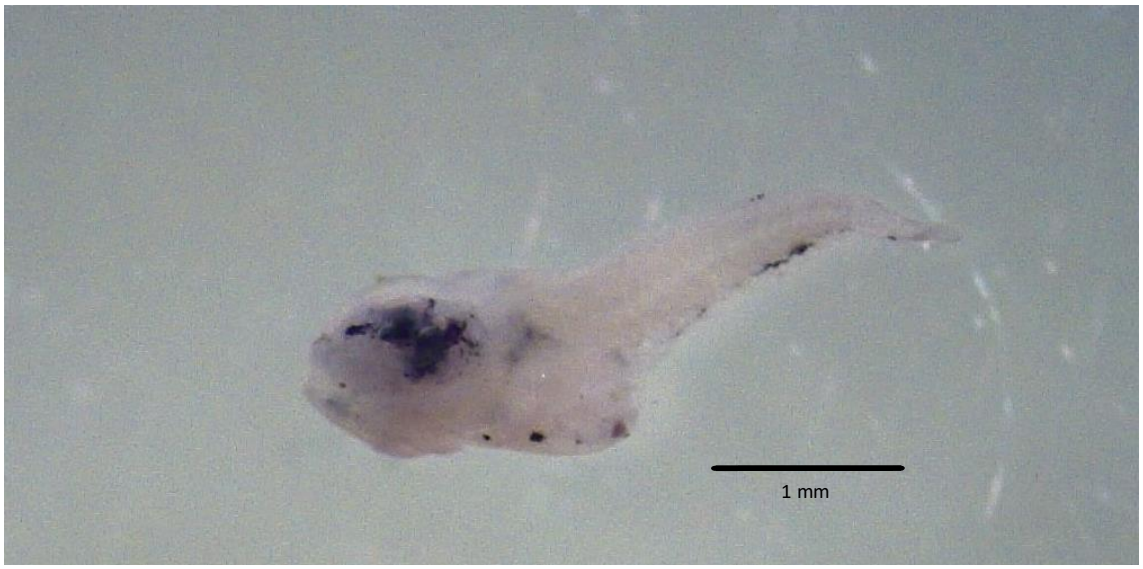


Figure A.1.73 - Pseudotolithus senegalensis (B2)

Identifying characteristics: Serranidae
- dorsal and anal fin not fully developed
- elongated 1st fin ray on the earlier stages

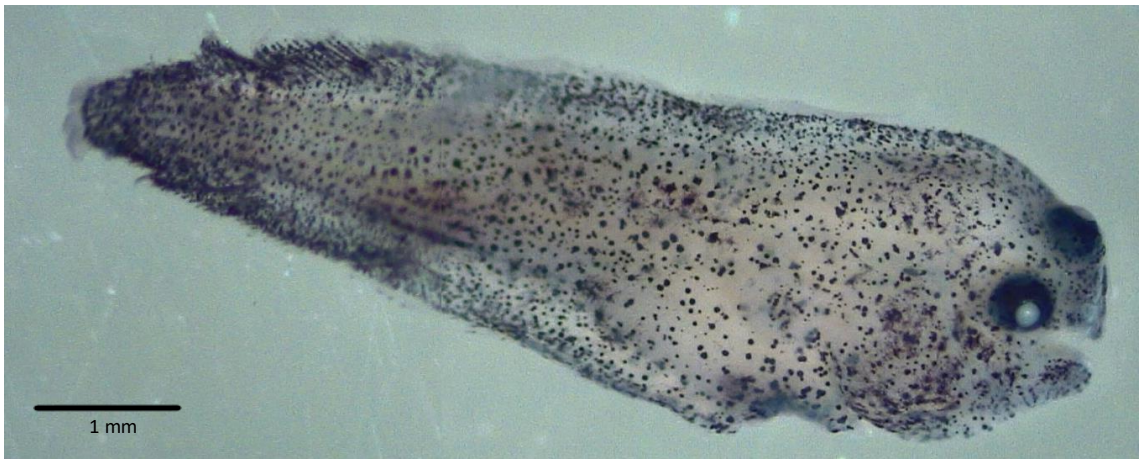


Figure A.1.74 – Unidentified pleuronectiform (B3)

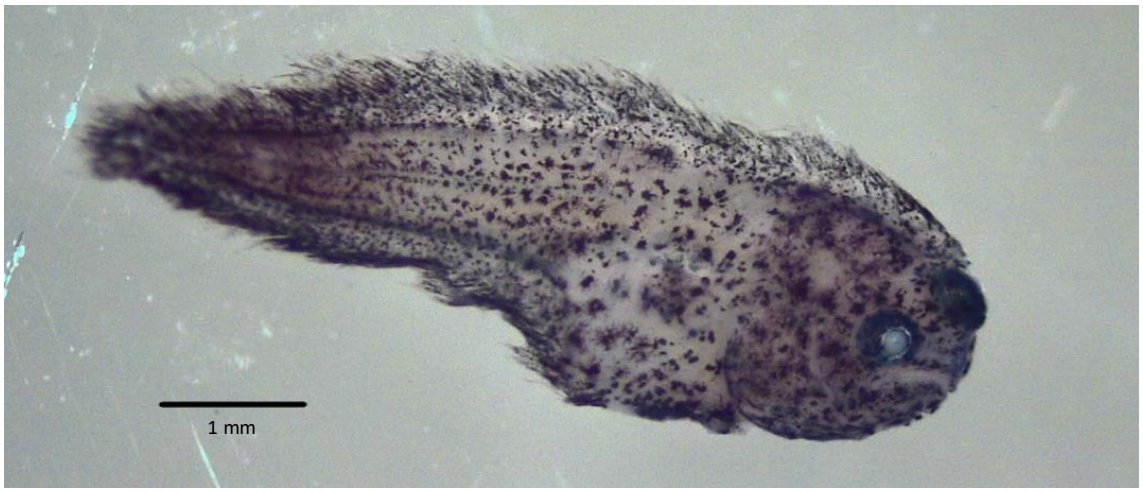


Figure A.1.75 – Unidentified pleuronectiform (B4)

Identifying characteristics: *Unidentified pleuronectiform*
- rostral hook bellow mouth
- dorsal, anal, and caudal fin confluent
- pectoral fin absent

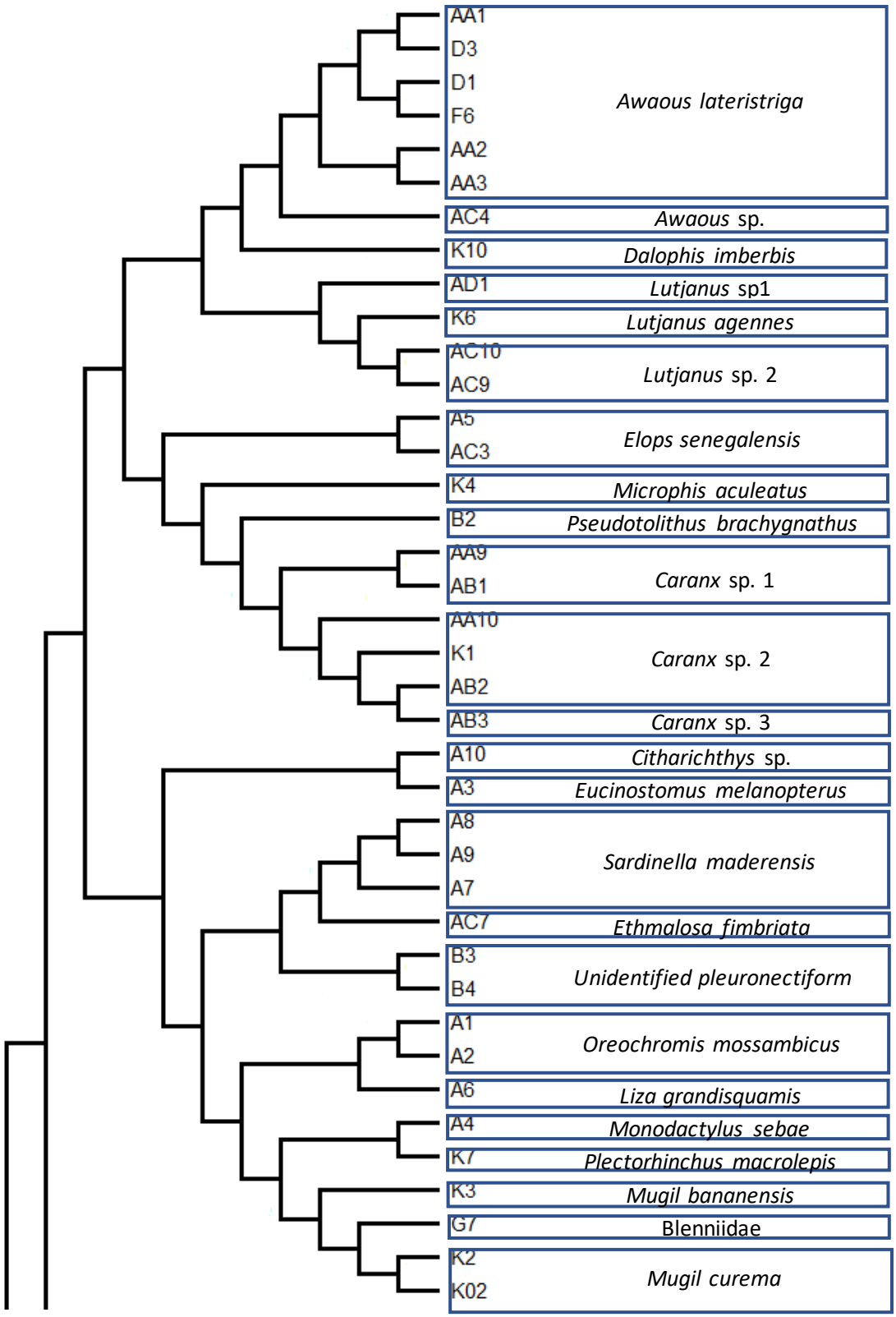
Figure A.1.76 - Microphis aculeatus (K4)

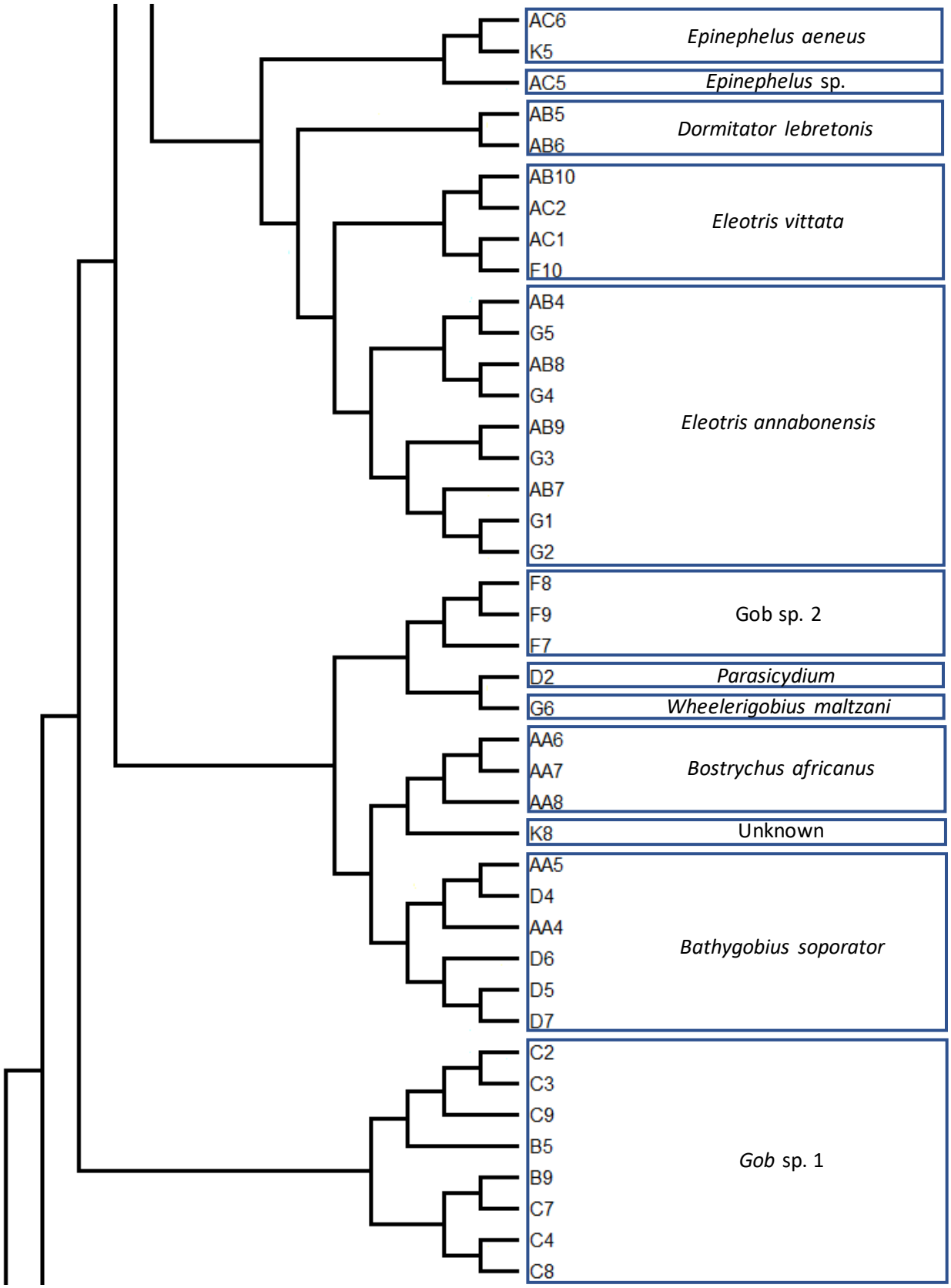
Identifying characteristics: Syngnathidae
- body extremely elongated, encased on a bony armor
- mouth small, toothless, placed at the end of a tubular snout

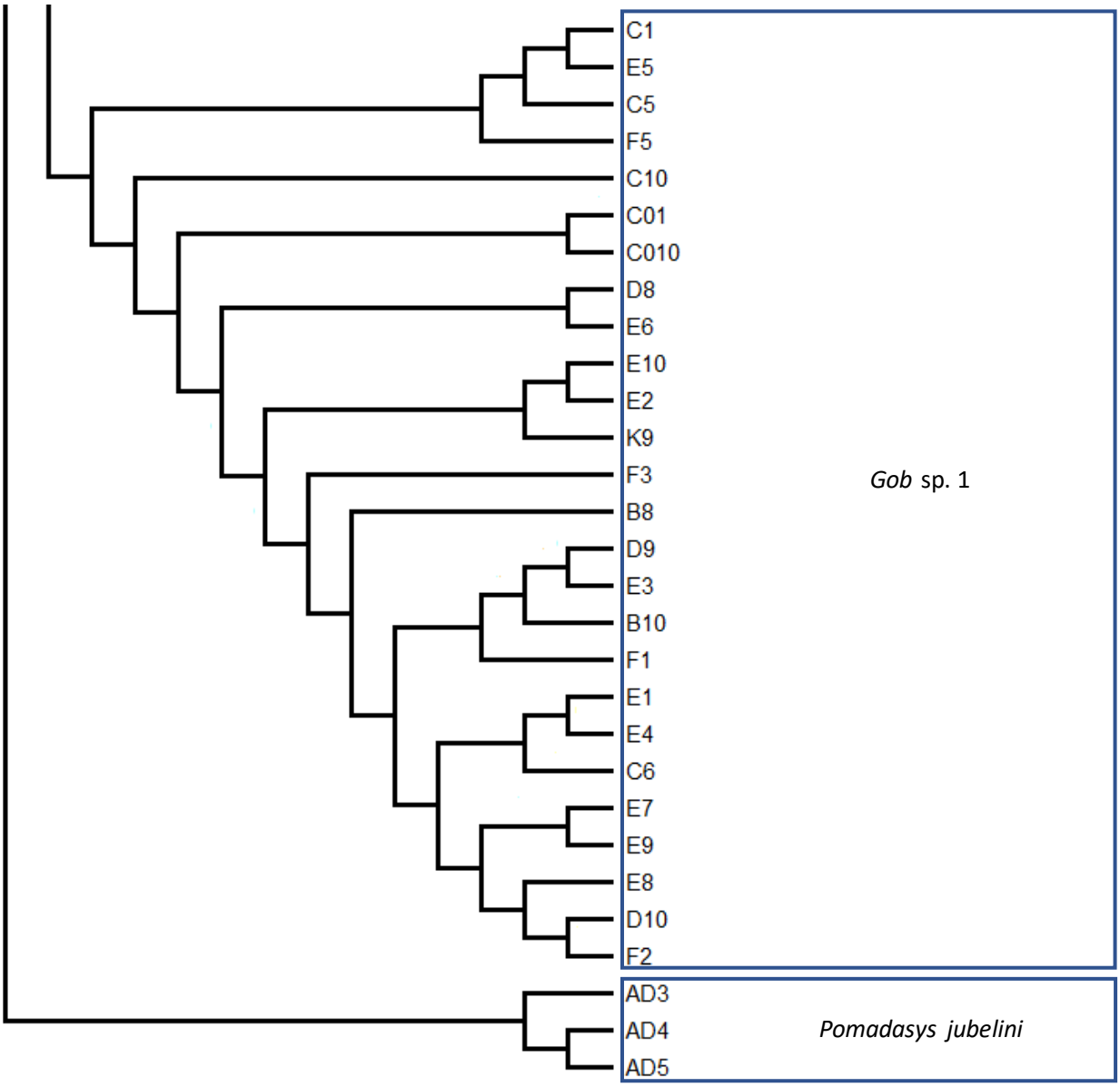
All specimen's resultant from this work will be deposited in **MUNHAC** – Museu Natural da História Natural e da Ciência

Annex B – Phylogenetic tree of the molecular results

Annex B.1 Phylogenetic tree of all larvae (single letter codes) and adult fish (double letter codes) samples using the program PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) with 10000 bootstraps.




AC6
K5
Epinephelus aeneus
AC5
Epinephelus sp.
AB5
AB6
Dormitator lebretonis
AB10
AC2
AC1
F10
Eleotris vittata
AB4
G5
AB8
G4
AB9
G3
AB7
G1
G2
Eleotris annabonensis
F8
F9
F7
Gob sp. 2
D2
Parasicydium
G6
Wheelerigobius maltzani
AA6
AA7
AA8
Bostrychus africanus
K8
Unknown
AA5
D4
AA4
D6
D5
D7
Bathygobius soporator
C2
C3
C9
B5
B9
C7
C4
C8
Gob sp. 1


C1
E5
C5
F5
C10
C01
C010
D8
E6
E10
E2
K9
F3
B8
D9
E3
B10
F1
E1
E4
C6
E7
E9
E8
D10
F2
Gob sp. 1
AD3
AD4
AD5
Pomadasys jubelini

Annex C – Sampling methods

Annex C.1 Light traps based on the model of Kissick 1993, that were built during the course of this thesis. Slight adaptations were done with LED strips with 15 LEDs in each trap.

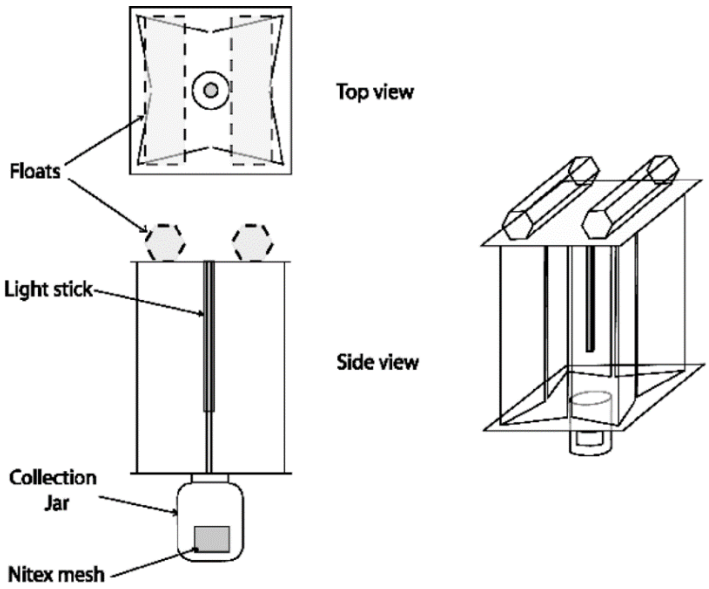


Figure C.1.1 - Light trap scheme from Kissick (1993)

Figure C.1.2 – Light traps in assembly (upside down) with collection jar not attached while being built in São Tomé

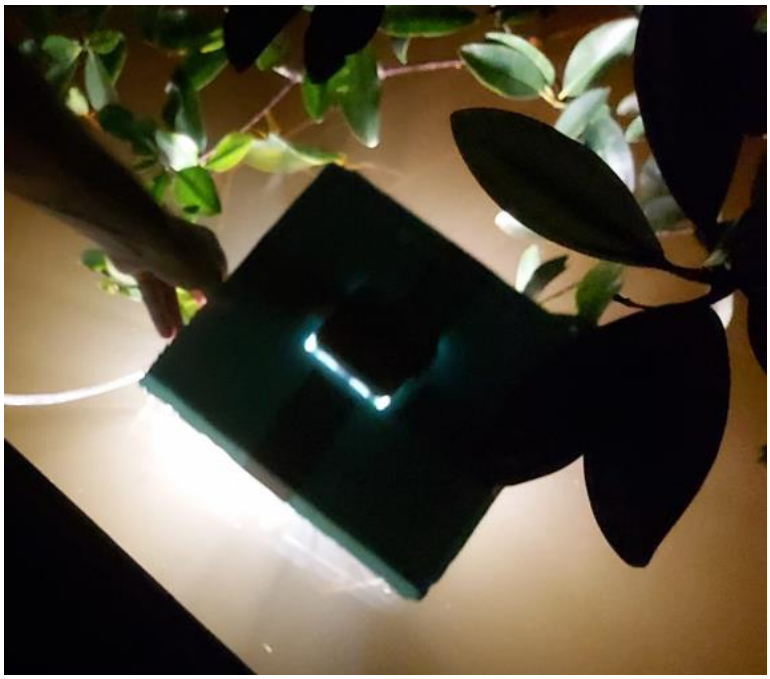


Figure C.1.3 – Light trap deployed in Malanza

Annex C.2 Seine nets

Figure C.2.1 – Seine net example (from https://www.beachseines.com/)

Annex C.3 Passive plankton tows

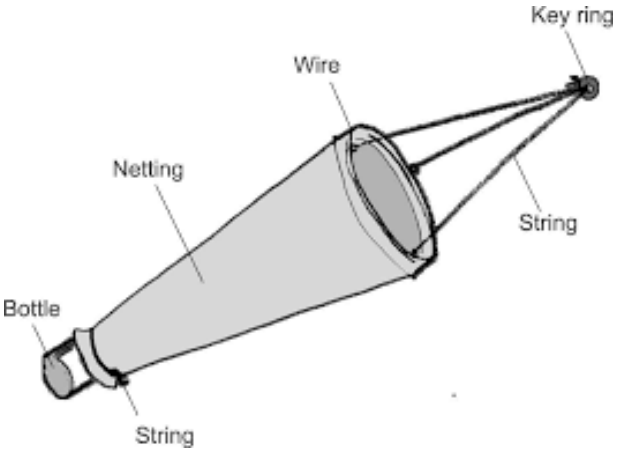
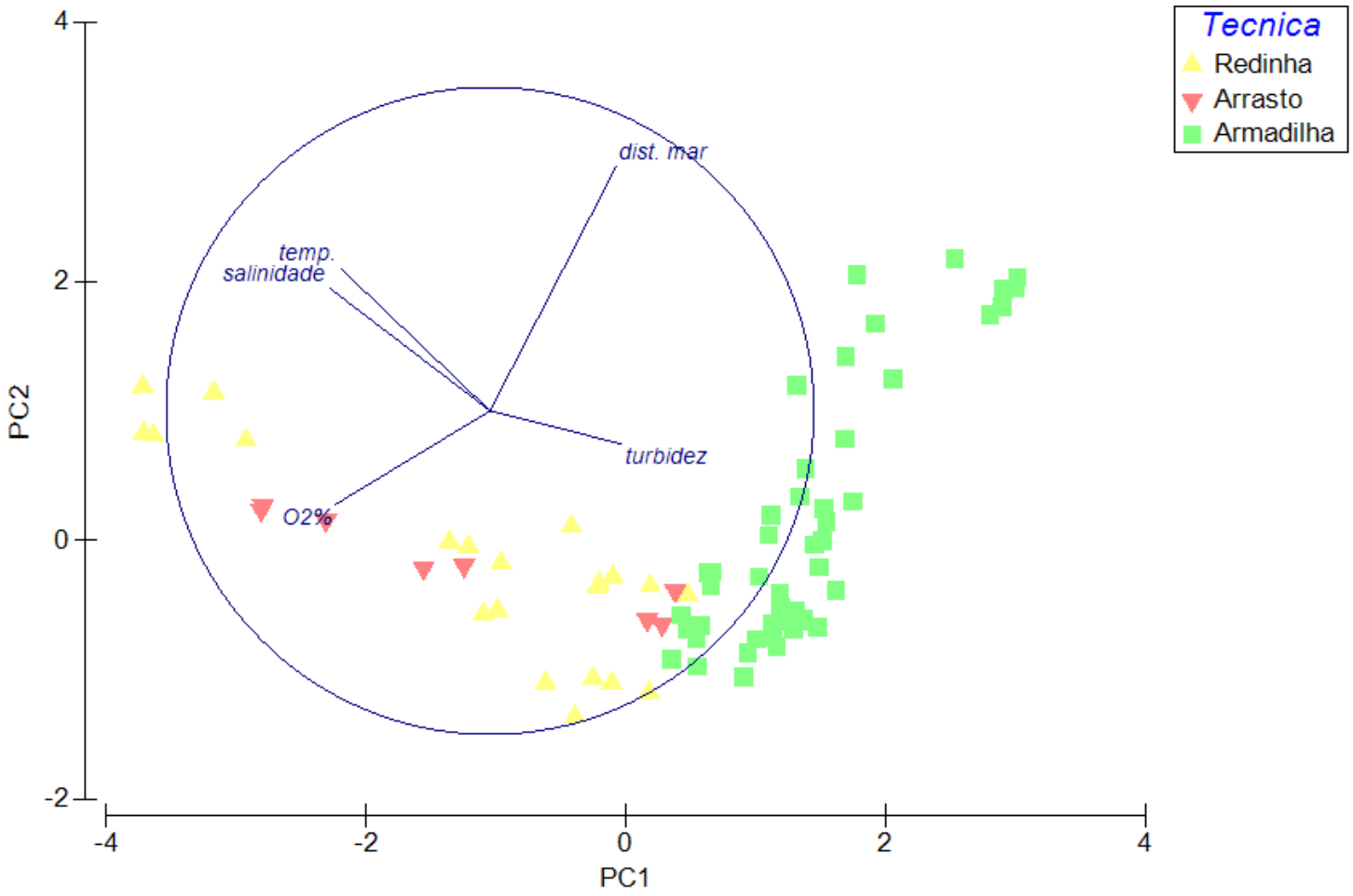


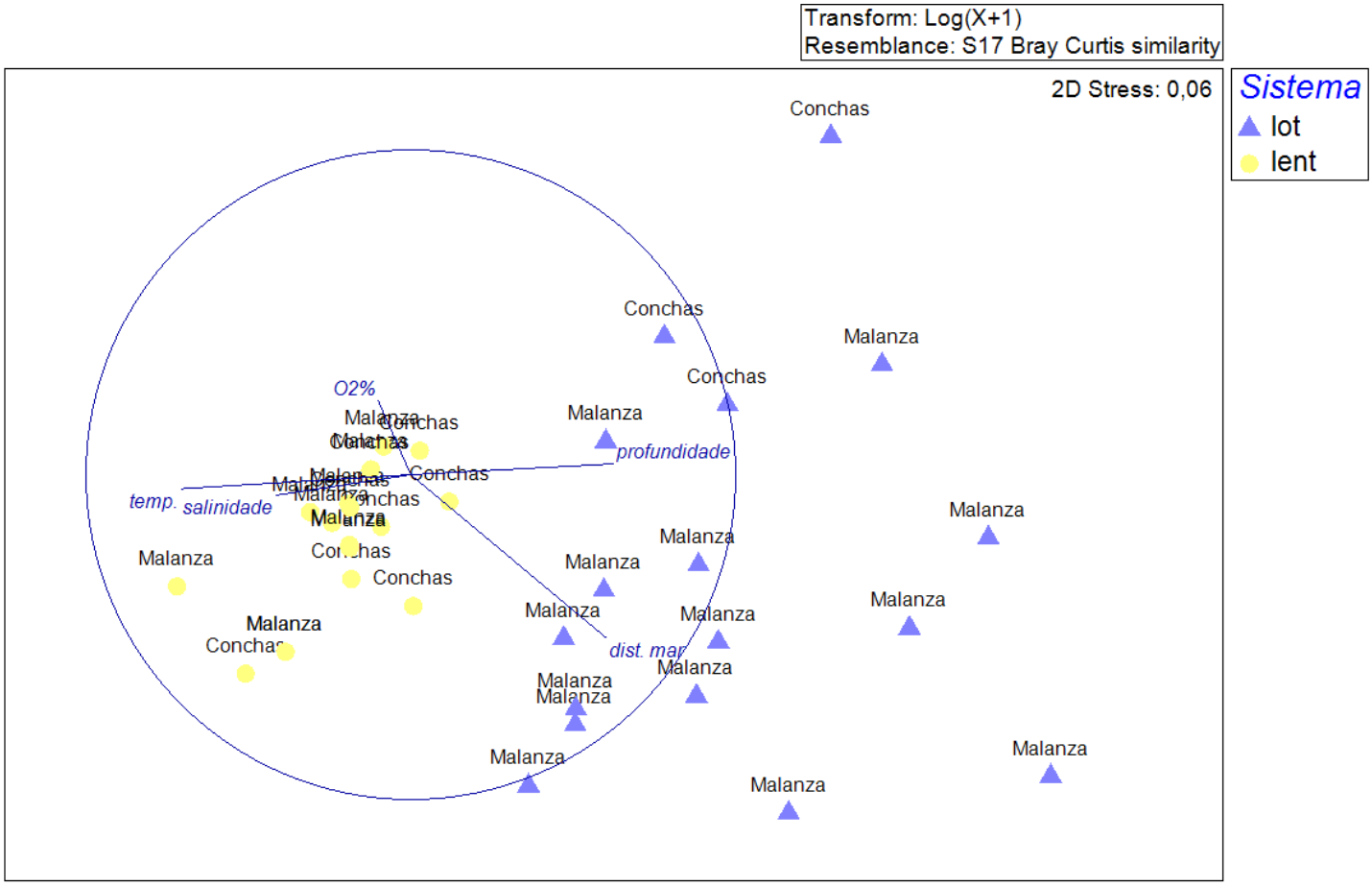
Figure C.3.1- Plankton net scheme (from https://noaateacheratsea.blog/tag/plankton/)

Annex D – PRIMER analysis outputs

Annex D.1 Principal component analysis for all samples represented by method of capture with an overlay of environmental variables as vectors, where Redinha - seine nets, Arrasto – Passive plankton tows, Armadilha – Light traps; salinidade – salinity; turbidez – turbidity; dist. Mar- distance to the sea; temp. – temperature; O2% - oxygen



Annex D.2 Multi Dimensional scaling of seine net captures represented by sampling system, either lotic (lot) or lentic(lent) and labeled by mangrove origin, either Malanza or Praia das Conchas; with salinidade – salinity; O2% - oxygen; dist. Mar – distance to sea; temp. – temperature; Salinidade – Salinity; profundidade - depth



Annex D.3 Multi Dimensional scaling of Light trap captures represented by sampling sector of Malanza, either downstream (Jusante), Midstream (Medio) or Upstream (Montante); with salinidade – salinity; O2% - oxygen; dist. Mar – distance to sea; temp. – temperature; Salinidade – Salinity; profundidade – depth; luan – moon stage; clima – cloud coverage; turbidez - turbidity