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DETERMINING THE POPULATION DYNAMICS AND REPRODUCTIVE LIFE HISTORY OF COMMERCIALLY IMPORTANT TUNAS IN THE GULF OF MEXICO

by

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ABSTRACT

The Gulf of Mexico (GOM) is an important spawning ground for many commercially important fish species including yellowfin, skipjack, and blackfin tunas. In particular, it is an essential spawning habitat for Atlantic bluefin tuna, whose stock status is of great international concern. It is vital to the appropriate conservation management of these species to determine both the location of their spawning grounds and the genetic diversity found within them. Fish eggs are a fisheries-independent source of spawning information that can provide more accurate estimates of spawning sites compared to larvae. This study describes population diversity and reproductive life histories in *Thunnus* and *Katsuwonus* using fish eggs that were collected during the National Marine Fisheries Service's (NMFS) 2011 spring larval survey in the northern Gulf of Mexico. Of 12,731 eggs surveyed genetically from 121 different sites, 38 *Thunnus thynnus*, 49 *Thunnus albacares*, 192 *Thunnus atlanticus*, and 15 *Katsuwonus pelamis* were identified. *T.thynnus* and *K.pelamis* had very high haplotypic diversity (h=.995 and h=.946, respectively), while *T.albacares* and *T.atlanticus* had relatively lower haplotypic diversity (h=.288 and h=.454). Phylogenetic and molecular diversity analyses for each species revealed that bluefin and skipjack tunas display aggregate spawning at relatively few sites, while yellowfin and blackfin individuals spawn independently at multiple sites. All species were found throughout the GOM apart from bluefin tuna which was only identified along the northern-most edge of the GOM within the continental shelf and slope.

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CHAPTER 1

DETERMINING THE POPULATION DYNAMICS AND REPRODUCTIVE LIFE HISTORY OF COMMERCIALLY IMPORTANT TUNAS IN THE GULF OF MEXICO

1.1 INTRODUCTION

Atlantic bluefin tuna (*Thunnus thynnus*) are one of the most highly sought after and valued marine fish and their stock status is of great international concern. This concern has prompted petitions to list bluefin tuna under CITES appendix I, which would prohibit international trade, and as an endangered species under the US Endangered Species Act. Much of the modeling work conducted to determine whether bluefin populations met CITES or ESA listing criteria suggested either that the stock has been reduced to a small fraction of historical levels or that the stock could decline to fewer than 500 individuals by 2030, a number that would threaten the existence of the stock and raise the potential for inbreeding depression and genetic drift. Furthermore, the loss of several migratory contingents (*sensu* Hjort 1914, Secor 1999) that once occurred in Norwegian waters and off the coast of Northern Brazil poses the potential for additional loss of genetic diversity. Thus, it is critical that material be collected and analyzed to determine the baseline genetic diversity of the population in its current reduced state. The presence/absence of fertilized bluefin tuna eggs in ichthyoplankton surveys can be used to identify spawning habitats of tunas in the Gulf of Mexico (GOM). This method of spawning ground definition is independent of the tuna fishery and can be used in conjunction with hydrogeographic models of GOM to ‘back calculate’ the location of spawning aggregations. Physical

properties of these presumed spawning locations (e.g., temperature, turbidity, presence/absence of gyres, etc.) can then be defined. In addition, genetic sequence data of fertilized eggs can be used to estimate mitochondrial DNA (mtDNA) control region sequence variation in *Thunnus* eggs to determine the haplotype diversity in the spawning population as well as the genetic variation within and between spawning sites in the Gulf of Mexico.

Investigating the genetic diversity of commercially important tunas in the Gulf of Mexico will further understanding of spawning aggregations and population dynamics and provide for more effective species management. The methodology developed in this project can be applied to any species, allowing researchers to improve management of many of the species on which world economies rely. For example, in bluefin tuna it is presently unknown if there is a single spawning population located primarily in the western Gulf or if there are two separate spawning populations in both the eastern and western Gulf. Routine ichthyoplankton surveys in the GOM suggest that there is one spawning stock primarily in the western GOM based on data collected from the National Oceanic and Atmospheric Administration (NOAA) and National Marine Fisheries Service (NMFS) joint annual spring larval surveys geared specifically towards collecting bluefin tuna eggs and larvae (Atlantic Bluefin Tuna Status Review Team 2011). On the other hand, tagging studies have suggested that there may be two stocks; one in the eastern GOM and one in the western GOM (Hazen et al. 2016; Wilson et al. 2015). There are different management strategies that would be most effective for one large population versus two smaller populations and data from this study will allow managers the ability to choose more effectively from these strategies.

1.1.1 LIFE HISTORY

The Gulf of Mexico (GOM) is a shallow, warm sea bordered by Mexico to the West, the United States to the North and Cuba to the Southeast. It is an important spawning ground for many commercially important species including flounder (*Paralichthys albigutta; P.lethostigma*), sheepshead (*Archosargus probatocephalus*), and red snapper (*Lutjanus campechanus*) (Carter 1986, Collins 1996, and Render 1992). Despite the importance of the GOM as a spawning ground for commercially valuable fish species, there is limited understanding about the precise spawning locations, the number of eggs spawned, and other important metrics that would be useful for fishery managers. It is particularly important to have a complete understanding of spawning for each fish species because this information can provide insight into the current and future stock health. The GOM is the spawning ground for many tunas including: yellowfin (*Thunnus albacares*), blackfin (*Thunnus atlanticus*), and skipjack tunas (*Katsuwonus pelamis*). The GOM is an especially important spawning ground for North Atlantic bluefin tuna who spawn exclusively in the GOM and the Mediterranean (Fromentin and Powers 2005). Because bluefin tuna are commercially important and only spawn in these two locations, their population status is heavily monitored. As part of that monitoring effort, the National Marine Fisheries Service (NMFS) in conjunction with the National Oceanic and Atmospheric Administration (NOAA) conducts annual spring larval sampling cruises throughout the GOM (since 1982) to characterize that year's bluefin tuna breeding population.

Atlantic bluefin tuna are large, highly migratory pelagic fish that are known to spawn in the Gulf of Mexico (western Atlantic BFT) and the Mediterranean Sea (eastern

Atlantic BFT). Western Atlantic bluefin tuna spawn primarily from April-May and reach reproductive maturity between ages 8-12 years (Block et al. 2005; Boustany et al. 2008) which is much older than eastern Atlantic tuna who are believed to begin spawning at 3-4 years old. Bluefin tuna are multiple batch spawners, producing 5 million eggs (5 yr old tuna) to 45 million eggs (15-20 yr old tuna) (Fromentin and Powers 2005) and spawning approximately every 1.02 days (Medina et al. 2002) during breeding season. Their average fecundity is about 93 oocytes per gram of body mass. Fertilized eggs hatch 24-36 hours after spawning. The larvae are pelagic and reabsorb their yolk sac within a few days after hatching (Fromentin and Powers 2005). Bluefin tuna grow relatively rapidly; fish born in May attain lengths of 30-40 cm by September, growing approximately 1 mm a day (ABFT Status Review Team 2011).

Yellowfin tuna are a globally distributed, highly migratory species. They inhabit temperate to tropical waters in the Atlantic, Pacific, and Indian Oceans. Yellowfin tuna have an average lifespan of six to seven years, reaching reproductive maturity at two years. They are a highly sought-after fisheries resource and are harvested on both large and small scales. They are predominantly fished by purse seines and longlines (Itano 2000). Like bluefin tuna, yellowfin tuna are multiple batch spawners, producing a range of between .97 million and 4.69 million oocytes and spawning about every 1.52 days (Sun et al. 2005).

Blackfin tuna are caught mainly as bycatch in yellowfin tuna fisheries. They are smaller than yellowfin tuna weighing between 1-5 kg for females and 1.5 to 8.4 kg for males (compared to yellowfin's maximum weight of 200kg) (Colette et al. 2011; Schaefer 1998). Relatively little is known about blackfin tuna's reproductive life history because, as species that are not highly sought-after, they have not been as heavily researched.

Skipjack tuna are found in tropical and warm waters across the world's oceans and are one of the most abundantly fished tunas, constituting approximately 40% of the world's annual tuna catch (Ely et al. 2005). Skipjack tuna spawn on average every 1.18 days during spawning season (Hunter et al 1986). Bluefin, yellowfin, and skipjack tuna all play important roles in the global fishing economy, and require proper management, especially an understanding of their reproductive life history and genetic diversity, to ensure long term sustainability.

1.1.2 EGGS VS. LARVAE

Larval fishes are known to be an important source of fishery-independent data that are essential to informed management decisions in *Thunnus* and other commercially important species (Ingram et al. 2010; Muhling et al. 2010). While larvae provide valuable fishery data, there are certain limitations associated with their use. For many fish, larvae can be nearly as difficult to identify conclusively as eggs, making them inaccessible for use in spawning stock biomass (SSB) calculations. Correct identification is especially critical for imperiled species like the Atlantic bluefin tuna (*Thunnus thynnus*). If the distribution, abundance and relatedness of larval fishes is a rich source of these data, then certainly similar surveys of eggs have the potential to contribute equally, perhaps more so, to informed management decisions. The distribution and abundance of the eggs of commercially important species might, in some cases, provide more valuable information than larvae. Since many marine fish in the GOM spawn small, predominately spherical eggs that hatch relatively quickly (usually within 24 hours), eggs are comparatively closer to the source of spawning than larvae that might be several days to weeks removed from their source depending on size, age and oceanographic conditions.

Eggs can more precisely indicate spawning areas because they are completely planktonic and oftentimes only hours instead of days or weeks away from the initial spawning event. Therefore, eggs allow for less complicated hindcasting based on location overlaid on comprehensive physical models. Similarly, rapid DNA-based identification of fertilized eggs might be useful for ‘adaptive’ sampling, that is, corrections or additions to sampling schemes, in near-real-time, that target spawning areas of commercially important species. Finally, fertilized eggs might represent an additional fisheries-independent source of abundance data with which to inform indices of spawning stock biomass, particularly if the distribution of eggs and larvae are spatially heterogeneous and their co-occurrence is uncorrelated. Eggs have not been utilized thus far because as difficult as larvae are to identify morphologically, eggs are more so.

1.1.3 DNA BARCODING

Accumulating over the past several decades have been molecular genetic surveys that have contributed to comprehensive fisheries management, and, in many cases, altered our understanding of the dynamics of marine fish populations (Hauser and Carvalho 2008). This is especially true for highly migratory species such as bluefin tuna (*Thunnus thynnus*) wherein significant amounts of population structure have been determined despite life-histories conducive to high gene flow (Riccioni et al. 2010, Boustany et al. 2008, Hauser and Carvalho 2008). However, the estimation of among-population genetic diversity in fish does not have to address population differentiation directly to have an important impact on the refinement of fisheries practices. For example, many genetically accessible population-level parameters are essential components of properly managed fisheries such as whether populations are expanding or contracting (due to recent management practices and/or over-

exploitation), the number of contributing individuals (especially females if sperm limitation is not an issue) to yearly spawning events, and the maintenance of genetic diversity across annual spawning events. Currently, fisheries themselves supply the majority of the data for stock assessment, sometimes resulting in biased or inaccurate data. To avoid bias, management decisions should be supplemented with fishery-independent approaches such as molecular genetic data and tagging techniques. These fishery-independent approaches allow for the construction of more comprehensive models for managing exploited species and populations (Riccioni et al. 2010).

This project uses a molecular genetic approach to quantify bluefin tuna eggs and cost-effectively characterize the spawning females that contribute annually to the western Atlantic bluefin tuna population in the northern Gulf of Mexico (GOM). By genetically analyzing sampled fertilized fish eggs collected during the Spring NMFS larval fish cruise in May 2011 we can estimate the frequency, abundance and location of fertilized bluefin, yellowfin, blackfin, and skipjack tuna eggs. We can also estimate the haplotype diversity of these eggs, which represents the probability that two randomly sampled alleles are different (Nei 1987).

Surveys of reproductively mature fish in the family Scombridae are known to have high haplotype diversities. Populations of adult wahoo (*Acanthocybium solandri*) demonstrate haplotype diversity ranging from .918 to .999 (Theisen et al. 2008; Garber et al. 2005). Likewise, Spanish mackerel (*Scomberomorus commerson*), the common mackerel (*Scomber scombrus*), and the chub mackerel *(Scomber japonicus*) had similarly high haplotype diversities of .91, .97, and .99 (Sulaiman and Ovenden 2010; Zardoya et al. 2004). A population of Atlantic bonito (*Sarda sarda*) in the Northern Mediterranean was

found to have a haplotype diversity of .993 (Vinas, Alvarado-Bremer, and Carles 2004). This appears to be especially true in tuna where estimates of population parameters in adult tunas have been found to have extremely high levels of haplotypic diversity: bigeye tuna have been found to have haplotype diversities ranging from .998 to .999 (Martinez 2006; Chiang 2008); yellowfin displayed a haplotype diversity of .997 (Ely et al. 2005); and bluefin tuna have been found to have a range of haplotypic diversity from .991-.998 (Ely et al. 2001; Carlsson 2006; Boustany, Reeb, and Block 2008; Carlsson 2004).

Elevated levels of haplotype diversity in adults results in the probability of randomly sampling two individuals with the same haplotype being very low; in other words, for bigeye tunas there is a 97% chance that two randomly sampled individuals would be genetically different (Nei 1987). Diversity in the mtDNA control region of bluefin, yellowfin, and skipjack tunas is so high that essentially every individual in the population is unique. Based on this, we can quantify the number of females contributing to any given sample. If two eggs have identical mtDNA control region haplotypes, then based on the extremely high haplotype diversity of Scombridae adults, it can be assumed that the two eggs came from the same female. Alternately, if two eggs have different mtDNA control region haplotypes, then it can be assumed that they were spawned from different females. Using this information we can then determine the minimum number of spawning females for a given sample.

I hypothesize that bluefin spawning is not evenly distributed across the Gulf of Mexico, and that the largest component of genetic variation in bluefin eggs within years lies within sampling locations (ie, eggs collected in the same location are the products of multiple females). This hypothesis is supported by NOAAs observation that the vast

majority of the larvae have been found in the western GOM. However, tagging studies suggest that there are two spawning populations in the western and eastern Gulf. Alternatively, eggs may be produced in both portions of the Gulf of Mexico, but preferentially advected into the western portion (Oey, Ezer and Lee 2013).

1.2 MATERIALS AND METHODS

1.2.1 EGG COLLECTION

The NOAA vessel Gordon Gunter collected complex mixtures of eggs from 121 stations in the northern Gulf of Mexico from May 3, 2011 to May 27, 2011. A Spanish neuston net with a .505mm mesh netting attached to a 1x2 m pipe frame was used to collect the eggs (Figure 1.1). The net was towed in an oscillating pattern from just below the surface to a depth of 10m for ten minutes at a speed of two knots (Habtes et al. 2014). A total of 49,808 eggs were collected and preserved in 95% ethanol prior to DNA extraction. All eggs utilized in genetic analyses were photographed using a Leica EX4D digital microscope. A scale was included in each image in order to convert the diameter of the eggs from pixels to the closest hundredth of a millimeter. Of the 49,808 eggs collected, 9,331 eggs from all 121 stations were extracted via a plate extraction method. Additionally, in a previous study 3,400 eggs from sixty-eight sampling stations that yielded greater than fifty eggs were analyzed by pooling fifty eggs into one DNA extraction procedure (Quattro, unpublished data).

1.2.2 DNA BARCODING

Pooled Extraction Method

Total DNA was extracted from eggs using Qiagen DNeasy columns following the manufacturer's instructions, except that DNA was eluted in a final volume of 50 µl. PCR reaction volumes (25µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.1% Tween 20, 5% DMSO, 200 mM each dNTP, 10 pmol of each primer (either Scombrid specific or *Thunnus* specific sets) and one unit of Taq DNA polymerase. Cycling conditions were: initial denaturation at 94°C for 4 minutes, followed by 40 cycles of a denaturation at 94°C for 1 minute, an annealing at 48°C for 1 minute, and an extension at 72°C for 1 minute, followed by a final extension of 72°C for 6 minutes. Amplification success was confirmed by agarose gel electrophoresis. Positive *Thunnus* amplifications were confirmed by DNA sequencing using the Big Dye Terminator 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosciences) in both the forward and reverse directions using the respective amplification primers. Cycle sequencing products were analyzed on an Applied Bioscience 3130 automated sequencer. Sequence trace files were edited manually using Sequencher (Applied Biosciences) and visually compared to known *Thunnus* sequences.

Plate Extraction Method

Total DNA was extracted from individual eggs using a plate extraction method developed as part of this study. Each egg was placed in a single well within a 96-well PCR plate. Then, 47.5 µl of lysis buffer (10mM Tris, 10mM EDTA, 10mM NaCl, 0.5% Sarcosyl, distilled H_2O) was placed into the wells, followed by 2.5 µl of proteinase k. The

eggs then lysed at 55□ for at least three hours or until all of the tissue had been lysed (up to twelve hours). After lysing, 150µl of precipitation buffer (100% EtOH, 5M NaCl stock) was added to the wells. The samples incubated at room temperature for 30 minutes and then were centrifuged at 1500g for 45 minutes. The plates were inverted and lightly tapped to remove the supernatant. 150µl of wash buffer (70% EtOH) was then added to the plates, which were centrifuged at 1500g for 15 minutes. Once again, the supernatant was decanted by turning the plates over and lightly tapping. This step occurred three times. After the final wash, the plates incubated in a thermocycler with the lid off at 37□ until all of the ethanol had evaporated. The DNA was then re-suspended in 50µl of molecular grade water.

Amplification

After extraction, amplification via the Polymerase Chain Reaction (PCR) was performed using the 'universal' 16S rRNA primers, 16sar and 16sbr (Palumbi 1996). This PCR was used as a positive control to ensure that the DNA extraction was successful. To determine if any of the *Thunnus* species are present, another PCR was performed using *Thunnus* specific primers (Thunnus171F and Thunnus 349R,16S, Quattro unpublished). Unfortunately, this locus is not a reasonable choice to estimate maternal contribution to the egg pool because it is unable to unambiguously identify yellowfin versus blackfin tuna. Additionally, the sequence is not variable enough among individuals to determine maternal contribution. However, a variety of 'hypervariable' primer sets useful for assaying mtDNA variation in fishes have been published including several for tunas – most sets overlap to a large extent and therefore are not exclusive. We employed primers used described in Jones and Quattro (1999 – PRO-LO and TCSBR, dloop)– these primer pairs have been successfully applied to a wide variety of animals including flounders (Jones and Quattro

1999), pygmy sunfishes (Quattro et al. 2001), sturgeons (Quattro et al. 2002), and more recently tunas and billfishes (both larvae and eggs; Quattro, unpublished data). All samples positive for *Thunnus* were subsequently amplified and sequenced at the mtDNA control region in order to differentiate between *Thunnus* species.

Reaction volumes for all three PCRs (Universal 16S, *Thunnus* specific 16S, and dloop) (25µl) contained: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.1% Tween 20, 5% DMSO, 200 mM each dNTP, 10 pmol of each primer and one unit of *Taq* DNA polymerase. Cycling conditions consisted of an initial denaturation at 94°C for 4 minutes followed by 40 cycles of a denaturation at 94°C for one minute, an annealing at 48°C for one minute, an extension at 72°C for one minute, followed by a final extension of 72°C for six minutes. Amplification success was confirmed by 1.5% agarose gel electrophoresis. Amplification products were sequenced using the Big Dye Terminator 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosciences) in both the forward and reverse directions using the respective amplification primers. Cycle sequencing products were then visualized using a commercial service (Functional Biosciences; Madison, Wisconsin). Sequence trace files were edited manually using Sequencher (v4.1.4; Gene Codes Corp.), exported and parsed into the programs MEGA7 (Molecular Evolutionary Genetics Analysis version 7.0; Kumar, Stecher, and Tamura 2015), DNASP (Librado and Rozas 2009) and Arlequin (Excoffier and Lischer 2010) for subsequent phylogenetic and molecular analyses.

1.2.3 EGG IDENTIFICATION

The software package SAP (Statistical Assignment Package; Munch et al. 2008) was used to assign taxonomic identity to the samples. SAP assigns samples to a taxonomic group by querying GenBank, the NCBI online genetic database, for homologues. SAP returns the 100 most similar sequences from GenBank that have a sequence identity of greater than 0.90. SAP aligns these sequences using ClustalW2 (Larkin et al. 2007) and creates phylogenetic trees from the sequences using a Markov chain Monte Carlo simulation and then uses a Bayesian approach to calculate the probability of assignment to individual taxonomic categories. Taxonomic assignment is based on probability of placement of the sample sequence (i.e., individual egg) against homologues within the resultant trees. Taxonomic assignments with probabilities below 0.95 were considered to be ambiguous matches and the next most proximal taxonomic category (e.g., family if genus was ambiguous) was used for identification. Taxonomic assignments, even with high probabilities, were assumed to be unreliable if sequence identity values between the queried sample sequence and the most similar homologue were less than 0.95. In such cases, the sample was assigned the next most proximal taxonomic category.

1.2.4 STATISTICAL ANALYSES

MEGA7 (Molecular Evolutionary Genetics Analysis version 7.0; Kumar, Stecher, and Tamura 2015) was used to align sequences using Muscle (Edgar 2004) and to create neighbor joining trees for each species. MEGA files were then imported into DNASP (Librado and Rozas 2009) to determine haplotypes. The haplotypes were then used to

create Arlequin files to compute haplotype diversity, nucleotide diversity, Tajima's D, AMOVA, FST, and the number of polymorphic sites.

1.3 RESULTS

Of the 49,808 eggs collected during NMFS's 2011 Spring larval cruise, a total of 12,731 eggs were surveyed and 12,658 were successfully extracted in this study. 3,400 eggs were extracted by the pooled extraction method and 9,331 eggs were individually extracted using the plate extraction method. A total of 12,658 eggs were successfully extracted and amplified resulting in a failure rate of 0.573%. The sequence analyses revealed that there were 38 bluefin (*Thunnus thynnus*), 49 yellowfin (*Thunnus albacares*), 192 blackfin (*Thunnus atlanticus*), and 15 skipjack (*Katsuwonus pelamis*) tuna (Table 1.1).

PCR amplifications using the pooled method yielded ten stations with positive *Thunnus* specific and Scombrid specific amplifications. DNA sequencing determined that four of the sampled stations contained at least one bluefin tuna (*T. thynnus*). Three stations contained at least one blackfin or yellowfin tuna (*T. atlanticus or T. albacares*; which cannot be unambiguously identified using the currently analyzed locus). And one station contained at least one *T. thynnus* and at least one *T. atlanticus or T. albacares*. PCR amplifications using the plate extraction method yielded thirty-five stations with positive *Thunnus* and *Katsuwonus* specific amplifications. Three stations contained at least one bluefin tuna (*T. thynnus*), 14 stations contained at least one yellowfin tuna (*T. albacares*), 26 stations contained at least one blackfin tuna (*T. atlanticus*), and five stations contained at least one skipjack tuna (*K. pelamis*). (Table 1.1)

The geographic location of identified *Thunnus* eggs was distributed throughout the northern Gulf of Mexico (Figure 1.2). Bluefin tuna were found in both the eastern and western portions of the GOM on the continental shelf and slope. Yellowfin tuna were found throughout the eastern and western GOM, primarily on the continental shelf and slope, but some were also present in deeper waters off of the slope. Blackfin tuna were found across the GOM in both the shallower waters of the shelf and deeper waters off the slope. Blackfin tuna were the most widely distributed of the species sampled. Skipjack tuna were found in sites on the continental slope in both the eastern and western GOM.

Thirty-one haplotypes were identified for 33 *Thunnus thynnus* individuals from a total of three stations. All but two haplotypes (Tthy_CR2 and Tthy_CR4) had a single individual with the haplotype. Tthy_CR2 and Tthy_CR4 had two individuals each. Site 154 had a single individual, site 226 had twenty-five individuals with 23 haplotypes, and site 227 had seven individuals with seven different haplotypes (Table 1.2). Twenty haplotypes were identified for 45 *Thunnus albacares* individuals from 14 different stations. All haplotypes had a single individual except for Talba_CR4 (5 individuals), Talba_CR8 (2 individuals), Talba_CR14 (15 individuals), Talba_CR18 (2 individuals), and Talba_CR19 (6 individuals). In most cases, individuals collected from the same site shared a haplotype (Table 1.3). Sixty-one haplotypes were identified for 188 *Thunnus albacares* individuals from a total of 26 stations. Similar to *Thunnus albacares*, in most cases, individuals collected from the same site shared a haplotype (Table 1.4). Twelve haplotypes were identified in 15 *Katsuwonus pelamis* individuals from a total of five stations. Nine out of the twelve haplotypes were present in a single individual. Three haplotypes were present in two individuals (Kpela_CR2, Kpela_CR3, Kpela_CR4) (Table 1.5).

A total of 405 base pairs (bp) of the nucleotide sequence of the mitochondrial DNA (mtDNA) control region were determined for 33 bluefin tuna. For all samples, there were 83 polymorphic sites (Table 1.6) that defined 31 haplotypes (h=0.995) and resulted in a nucleotide diversity (π) of 2.23% (Table1.7). Haplotypic diversity ranged from a low of 0.993 to a high of 1.00 for individual sites (Table 1.8). A total of 404 bp of the nucleotide sequence of the mitochondrial DNA (mtDNA) control region were determined for 45 yellowfin tuna. For all samples, there were 75 polymorphic sites (Table 1.9) that defined 20 haplotypes (h=0.288) with a nucleotide diversity (π) of 1.47% (Table1.7). Haplotypic diversity for individual sites ranged from 0.125 to 1.00 (Table 1.8). A total of 400 bp of the nucleotide sequence of the mitochondrial DNA (mtDNA) control region were determined for 188 blackfin tuna. For all samples, there were 309 polymorphic sites (Table 1.10) that defined 61 haplotypes (h=0.454) and a nucleotide diversity (π) of 2.26% (Table1.7). Haplotypic diversity for individual sites ranged from 0.378 to 1.00 (Table 1.8). A total of 401 bp of the nucleotide sequence of the mitochondrial DNA (mtDNA) control region was determined for 15 skipjack tuna from five sites. For all samples, there were 71 polymorphic sites (Table 1.11) that defined 12 haplotypes (h=0.946) and a nucleotide diversity (π) of 6.41% (Table1.7). Haplotypic diversity was only calculated based on one site because the other four sites only had a single individual (Table 1.8). Bluefin tuna had the highest haplotypic diversity followed by skipjack tuna while yellowfin and blackfin tuna had comparatively lower values of haplotypic diversity. Skipjack tuna had the highest level of nucleotide diversity being over twice has high as the other three species'.

These patterns of similarities between the species are also reflected in the phylogenetic analyses which resulted in neighbor-joining trees with similar topologies for

bluefin tuna and skipjack tuna, while yellowfin and blackfin trees had similar topologies to each other but not to bluefin tuna and skipjack tuna. The trees for yellowfin and blackfin tuna contain clades defined primarily by sampling site, with little variation within clades (Figure 1.5, Figure 1.6). On the other hand, the phylogenetic trees for bluefin tuna and skipjack tuna contain clades that are not defined primarily by sampling site and contain considerably higher relative diversity within clade (Figure 1.3, Figure 1.4). When comparing the haplotypic diversity in the eggs sampled in this study to the haplotypic diversity of adults sampled in previous studies (Ely et al. 2001, Ely et al. 2005) bluefin tuna (egg, h=0.995; adult, h=0.997) and skipjack tuna (egg, h= 0.946; adult, h=0.999) had very high haplotypic diversity at both egg and adult stages (Figure 1.7). However, the haplotypic diversity of adult yellowfin tuna (h=0.997) is much higher than in eggs (h=0.289). Previous research has not been conducted on the haplotypic diversity of adult blackfin tunas, likely because they are not as important commercially and can be hard to distinguish morphologically from yellowfin tunas. However, based on the similarity of blackfin and yellowfin tuna it is likely that blackfin tuna may also have a very high haplotypic diversity in adults compared to the relatively low haplotypic diversity found in eggs in this study (h=0.454).

An Analysis of Molecular Variance (AMOVA; Excoffier, Smouse, and Quattro 1992) was conducted on bluefin tuna, yellowfin tuna, and blackfin tuna populations to determine the diversity among versus within populations (sampling sites). An AMOVA was not run for skipjack tuna because only one site had more than one individual. All variation in bluefin tuna occurred within populations (100%) (Table 1.12). Yellowfin tuna had 75.2% variation among populations and only 24.8% within populations (Table 1.13).

Blackfin tuna's variance was distributed more evenly with 51.19% variation among populations and 48.81% variation within populations (Table 1.14).

1.4 DISCUSSION AND CONCLUSION

The ability to accurately identify eggs of commercially important species is a critical component of comprehensive fisheries management. This information can be used to make hindcast predictions about location of spawning grounds, increase our understanding of reproductive life history, and determine population diversity within species (Muhling et al. 2011). Despite the myriad of information that eggs can provide, planktonic surveys of fish eggs are uncommon. This is because eggs are extremely difficult to identify to species using a purely morphological approach. Fortunately, the advent of innovative technologies has made it possible to analyze large quantities of eggs in a relatively rapid and cost-effective manner. To this end, using a plate extraction method, two-step PCR protocol, and sequencing analyses we have identified the abundance of *Thunnus* and *Katsuwonus* in 12,658 eggs collected from 121 stations in the Gulf of Mexico during the Spring 2011 NMFS larval fish survey.

Of the 12,658 eggs, there were 38 bluefin (*T. thynnus*), 49yellowfin (*T. albacares*), 192 blackfin (*T. atlanticus*), and 15 skipjack (*K. pelamis*) present. Overall, there were 2.20% *Thunnus* and 0.119% *Katsuwonus*. Bluefin tuna composed 0.3% of the total population surveyed. These abundance results are consistent with results from the genetic analysis of eggs from the Gulf of Mexico sampled via CUFES (Continuous Underway Fish Egg Sampler), in which *Thunnus* composed a similar percent of the surveyed population (Quattro, unpublished).

The geographic distribution of identified *Thunnus* eggs is not consistent with the known spawning biology of tunas. Black and yellowfin tuna are smaller than bluefin and were believed to spawn in shallower areas; these results are not congruent with this trend as their eggs were identified in the shallower waters of the continental shelf as well as deeper waters on and past the slope. Similarly, bluefin tuna tend to be larger and are thought to spawn in deeper waters (Teo et al. 2007) but in this study, bluefin tuna eggs were found in the relatively deeper waters of the continental slope, but also in waters on the continental shelf. Bluefin tuna eggs were found in the western GOM and the frontal zone of the loop current in the central and eastern GOM. These results are consistent with the results from prior tagging studies on mature bluefin tuna (Teo et al. 2007). Yellowfin, blackfin, and skipjack tuna eggs were found to be evenly distributed across the eastern and western Gulf of Mexico.

Tuna possess a hypervariable mtDNA control region; this region is so variable that nearly every tuna analyzed at this locus has been genetically unique (Menezes et al. 2012; Wenink et al. 1993). This fact enables us to compare haplotypes of eggs and approximate how many females are contributing to a given spawning effort. If two eggs have the same haplotype, then it can be presumed that they derive from the same mother. If two eggs have different haplotypes, then they have different mothers. Here, haplotype analyses revealed 31 different haplotypes for 33 bluefin tuna from only three sites. This indicates that there were only two pairs of eggs where each pair was spawned by the same female, while the rest were all spawned from different females. However, these thirty one individuals all are likely to have spawned in only three locations. From this we can infer that bluefin tuna appear to spawn in aggregations at a select number of sites. Haplotype analyses for skipjack

tuna showed similar patterns, with twelve haplotypes present for fifteen eggs from five sites, meaning three sets of eggs shared the same mother, while all other eggs were spawned from different females. Again, this indicates that females are spawning in aggregations or are drawn to spawn at the same location. This pattern was more striking in bluefin tuna than in skipjack possibly due to the patterns of collection because fewer skipjack were sampled than bluefin overall, but from more sites. Twelve individual eggs were all found at a single site, with eight different haplotypes. Meaning that eight of the twelve skipjack tuna females spawning all went to a single site, while the other four females all went to different sites. Yellowfin and blackfin tuna haplotype analyses support more individualized spawning patterns. Yellowfin tuna had 20 haplotypes present for 45 eggs from 14 sites, while blackfin tuna had 61 haplotypes for 188 eggs from 26 sites. For both species, many eggs with identical haplotypes, indicating a single spawning female, were found at several sampling sites; indicating that both yellowfin and blackfin tuna spawned more independently and at a variety of sites than did bluefin and skipjack tunas.

Haplotypic diversity was very high for bluefin tuna (h=0.995) and skipjack tuna (h=0.946). On the other hand, haplotypic diversity was low for yellowfin tuna (h=0.288) and blackfin tuna (h=0.454). Haplotype diversity represents the probability that two randomly sampled alleles are different (Nei 1987). Yellowfin and blackfin tuna had a much lower haplotypic diversity, meaning that the populations were less diverse than the bluefin and skipjack tuna populations.

AMOVA estimates population differentiation directly from molecular data (Excoffier, Smouse, and Quattro 1992). In this study, results from AMOVA analyses revealed that genetic variation within bluefin tuna in the GOM was found primarily within

sampling sites, while yellowfin and blackfin tuna's genetic variation existed primarily among sample sites. High overall variance within populations and low variance among populations indicates that populations are not homogenous. Alternately, low overall variance within and high variance among populations would indicate that individual populations are homogenous, but vary from other populations. This study reveals that individual bluefin tuna sample sites are not genetically homogenous, while yellowfin and blackfin tuna populations can be homogenous. The AMOVA results further support that yellowfin and blackfin tuna spawn independently at multiple sites throughout the GOM, while bluefin tuna spawn in aggregations at a few sites.

Our results suggest that bluefin tuna spawn in both the eastern and western portions of the Gulf of Mexico and that, overwhelmingly, genetic variation is concentrated within sites rather than between, indicating that multiple bluefin tuna females spawn in a given location. Larvae may be found predominantly in the western GOM due to currents advecting eggs into the western Gulf where they accumulate due to eddies formed from the loop current (Muhling, Lamkin, Roffer 2010). This suggests that it is essential to monitor both eastern and western portions of the GOM. However, because genetic diversity was found within sites and not among sites, that indicates that there are not two genetically differentiated spawning populations of bluefin tuna in the eastern vs. western GOM.

This study demonstrates the utility of eggs as a tool for fisheries management. We successfully genetically analyzed eggs in a rapid and cost-effective manner to estimate spawning grounds and determine genetic diversity in spawning females. Eggs provide a more accurate source of spawning ground information than larvae, which are typically used for spawning location analyses. Eggs provide not only a more accurate estimate of

spawning ground locations, they also provide insight into the life history of tunas. For example, are tunas spawning in aggregations or individually? Our results suggest that bluefin and skipjack tuna spawn in large aggregations, while yellowfin and blackfin tuna spawn individually or in small groups. This information is useful to conservation managers because they can then determine which regions need to be afforded additional protections during spawning seasons. The methodology developed in this project can easily and affordably be applied to other commercially important species where reproductive life histories are poorly understood but where monitoring spawning to calculate spawning stock biomass is critical for management.

1.5 TABLES AND FIGURES

Table 1.1. Sampling stations, counts of eggs sampled via Spanish neuston nets during the Spring 2011 NMFS larval fish cruise, counts of positive *Thunnus* and *Katsuwonus* identified by DNA sequence analysis.

Station #	SEAMAP #	# Eggs Surveyed	#Successfully Extracted	# Positive *Thunnus thynnus*	# Positive *Thunnus albacares*	# Positive *Thunnus atlanticus*	# Positive *Katsuwonus pelamis*
126	41252	45	45	0	0	0	0
127	41256	45	45	0	0	0	0
128	41260	74	74	0	0	0	0
129	41262	93	81	0	0	0	0
130	41267	93	92	0	0	0	0
130*	41267*	50	50	0	0	0	0
131	41269	93	87	0	0	0	0
131	41269	50	50	0	0	0	0
132	41273	93	93	0	0	17	0
133	41277	32	32	0	0	0	0
134	41279	9	9	0	0	0	0
135	41281	14	14	0	0	0	0
136	41283	18	18	0	0	0	0
137	41287	45	45	0	0	0	0
138	41291	93	79	0	0	0	0
138*	41291*	50	50	0	0	0	0
139	41295	93	93	0	0	0	0
140	41297	93	93	0	0	0	0
140*	41297*	50	50	0	0	0	0
141	41301	93	93	0	0	0	0
141*	41301*	50	50	0	0	0	0
142	41303	93	93	0	1	8	1
142*	41303*	50	50	0	1^{+}	1^{+}	0
143	41307	93	93	0	0	0	0
143*	41307*	50	50	0	0	0	0
144	41309	93	84	0	0	0	0
145	41313	93	93	0	0	1	0
145*	41313*	50	50	0	0	0	0
146	41315	93	89	0	0	0	0
146*	41315*	50	50	0	0	0	0
147	41320	186	186	0	0	0	0
147*	41320*	50	50	0	0	0	0
148	41322	186	186	0	1	1	0
148*	41322*	50	50	0	1^{+}	1^{+}	0
149	41324	93	93	0	0	0	0
149*	41324*	50	50	0	0	0	0
150	41334	186	186	0	0	0	0
150*	41334*	50	50	0	0	0	0

151	41336	93	93	0	0	0	0
152	41338	93	93	0	1	0	0
152*	41338*	50	50	0	0	0	0
153	41342	48	48	0	0	0	0
154	41344	48	48	1	0	0	0
155	41347	186	186	0	0	0	0
155	41347	50	50	0	0	0	0
156	41351	186	186	0	0	0	0
156	41351	50	50	0	0	0	0
157	41353	186	186	0	7	5	0
157*	41353*	50	50	0	1^+	1^+	0
159	41356	186	186	0	0	0	0
159	41356	50	50	0	0	0	0
161	41361	93	93	0	0	0	0
161*	41361*	50	50	1*	0	0	0
162	41363	93	93	0	0	0	0
163	41368	93	93	0	1	7	0
163*	41368*	50	50	0	0	0	0
164	41369	48	48	0	0	0	0
165	41374	93	93	0	0	0	0
165*	41374*	50	50	0	0	0	0
166	41380	10	10	0	0	0	0
167	41385	93	93	0	3	7	11
167*	41385*	50	50	0	0	0	0
168	41386	93	93	0	1	0	0
168*	41386*	50	50	1*	0	0	0
169	41391	93	90	0	0	2	0
169*	41391*	50	50	1*	0	0	0
170	41395	93	93	0	0	1	0
170*	41395*	50	50	0	0	0	0
171	41397	93	93	0	0	0	0
171*	41397*	50	50	0	0	0	0
172	41401	93	93	0	0	0	0
172*	41401*	50	50	0	0	0	0
173A	41403	93	93	0	0	0	0
173A*	41403*	50	50	1*	1^+	1^+	0
173B	41409	93	93	0	0	4	0
173B*	41409*	50	50	1*	0	0	0
173C	41410	69	67	0	0	0	0
173C*	41410*	50	50	0	0	0	0
173D	41411	93	93	0	0	2	0
173D*	41411*	50	50	0	0	0	0
173E	41412	93	93	0	0	0	0

173E*	41412*	50	50	0	0	0	0
174	41416	93	93	0	0	0	0
174*	41416*	50	50	0	0	0	0
175	41418	93	93	0	0	0	0
175*	41418*	50	50	0	0	0	0
176	41422	93	93	0	0	0	0
176*	41422*	50	50	0	0	0	0
177	41424	93	93	0	0	0	0
177*	41424*	50	50	0	0	0	0
178	41428	93	93	0	0	0	0
178*	41428*	50	50	0	0	0	0
179	41430	93	91	0	0	0	0
179*	41430*	50	50	0	0	0	0
180	41434	93	93	0	3	8	0
180*	41434*	50	50	0	0	0	0
181	41436	93	93	0	0	0	0
181*	41436*	50	50	0	0	0	0
182	41440	93	93	0	0	0	0
182*	41440*	50	50	0	0	0	0
183	41442	93	92	0	0	0	0
184	41446	93	93	0	0	1	0
184*	41446*	50	50	0	0	0	0
185A	41448	38	38	0	0	0	0
185B	41449	29	29	0	0	0	0
185C	41450	25	25	0	0	0	0
185D	41451	24	24	0	0	0	0
185E	41452	50	50	0	0	0	0
186	41456	93	93	0	0	0	0
187	41458	93	93	0	16	0	1
187*	41458*	50	50	0	0	0	0
188	41462	93	93	0	0	0	0
188*	41462*	50	50	0	0	0	0
189A	41464	93	93	0	0	0	0
189A*	41464*	50	50	0	0	0	0
189B	41465	93	93	0	1	6	1
189B*	41465*	50	50	0	0	0	0
189C	41466	21	21	0	0	0	0
189C*	41466*	50	50	0	0	0	0
189D	41467	93	93	0	0	2	0
189E	41468	48	48	0	0	4	0
189F	41469	93	93	0	0	0	0
189F*	41469*	50	50	0	0	0	0
190A	41471	93	91	0	0	0	0

190A *	41471*	50	50	0	0	0	0
190C	41473	21	21	0	0	0	0
190D	41474	57	57	0	0	0	0
190D *	41474*	50	50	0	0	0	0
190E	41475	45	45	0	0	3	0
190E*	41475*	50	50	0	0	0	0
191	41479	22	22	0	0	0	0
192	41481	93	93	0	1	8	0
192*	41481*	50	50	0	0	0	0
193	41485	32	30	0	0	1	0
194	41487	50	50	0	0	0	0
194*	41487*	50	50	0	0	0	0
195	41491	29	28	0	0	1	0
196	41493	93	93	0	0	0	0
196*	41493*	50	50	0	0	0	0
197A	41497	49	49	0	0	10	0
197B	41499	45	45	0	0	0	0
199	41503	21	21	0	0	0	0
200	41505	45	45	0	0	0	0
201	41509	93	93	0	0	17	0
201*	41509*	50	50	0	0	0	0
202	41516	93	93	0	0	10	1
203	41525	93	93	0	0	0	0
203*	41525*	50	50	0	0	0	0
204	41532	93	91	0	0	0	0
204*	41532*	50	50	0	0	0	0
205	41536	22	22	0	0	0	0
206	41537	93	93	0	2	0	0
206*	41537*	50	50	0	0	0	0
207	41539	93	93	0	6	0	0
207*	41539*	50	50	0	0	0	0
208	41540	93	93	0	0	1	0
209	41541	31	31	0	0	0	0
210	41545	6	6	0	0	0	0
211	41547	34	34	0	0	0	0
212	41551	38	38	0	0	0	0
213	41553	93	93	0	0	60	0
214	41557	48	48	0	0	0	0
215	41559	45	45	0	0	0	0
216	41563	12	12	0	0	0	0
217	41565	27	27	0	0	0	0
218	41567	45	45	0	0	0	0
219	41571	43	43	0	0	1	0

220	41575	60	60	0	0	0	0
221	41577	45	45	0	0	0	0
222	41581	93	93	0	0	0	0
222*	41581*	50	50	0	0	0	0
223	41585	93	90	0	0	0	0
224	41586	93	93	0	0	0	0
224*	41586*	50	50	0	0	0	0
225	41592	93	92	0	0	0	0
225*	41592*	50	50	0	0	0	0
226	41593	93	93	24	0	0	0
226*	41593*	50	50	0	0	0	0
227	41597	93	93	8	0	0	0
227*	41597*	50	50	0	0	0	0
228	41598	93	90	0	0	0	0
228*	41598*	50	50	0	0	0	0
229	41604	93	89	0	0	0	0
229*	41604*	50	50	0	0	0	0
230	41608	93	92	0	1	0	0
230*	41608*	50	50	0	0	0	0
231	41609	93	93	0	0	0	0
231*	41609*	50	50	0	0	0	0
	TOTAL	12731	12658	38	49	192	15

Table 1.2. *Thunnus thynnus* haplotypes		
Haplotype	# of Individuals	Sample ID
Tthy_CR1	1	154-KCH-44
Tthy_CR2	2	226-KCH-01, 226-KCH-05
Tthy_CR3	1	226-KCH-02
Tthy_CR4	2	226-KCH-03
Tthy_CR5	1	226-KCH-04, 226-KCH-28
Tthy_CR6	1	226-KCH-06
Tthy_CR7	1	226-KCH-07
Tthy_CR8	1	226-KCH-08
Tthy_CR9	1	226-KCH-09
Tthy_CR10	1	226-KCH-17
Tthy_CR11	1	226-KCH-20
Tthy_CR12	1	226-KCH-25
Tthy_CR13	1	226-KCH-28
Tthy_CR14	1	226-KCH-48
Tthy_CR15	1	226-KCH-54
Tthy_CR16	1	226-KCH-60
Tthy_CR17	1	226-KCH-62
Tthy_CR18	1	226-KCH-64
Tthy_CR19	1	22 s6-KCH-72
Tthy_CR20	1	226-KCH-77
Tthy_CR21	1	226-KCH-79
Tthy_CR22	1	226-KCH-88
Tthy_CR23	1	226-KCH-89
Tthy_CR24	1	226-KCH-93
Tthy_CR25	1	227-KCH-20
Tthy_CR26	1	227-KCH-22
Tthy_CR27	1	227-KCH-26
Tthy_CR28	1	227-KCH-39
Tthy_CR29	1	227-KCH-47
Tthy_CR30	1	227-KCH-48
Tthy_CR31	1	227-KCH-66
TOTAL	33	

Table 1.3. *Thunnus albacares* haplotypes

Haplotype	# of Individuals	Sample ID
Talba_CR1	1	142-KCH-18
Talba_CR2	1	148-KCH-03
Talba_CR3	1	152-KCH-81
Talba_CR4	5	157-KCH-09, 157-KCH-35, 157-KCH-59, 157-KCH-73, 157-KCH-87
Talba_CR5	1	157-KCH-72
Talba_CR6	1	157-KCH-84
Talba_CR7	1	163-KCH-38
Talba_CR8	2	167-KCH-12, 167-KCH-45
Talba_CR9	1	167-KCH-40
Talba_CR10	1	168-KCH-48
Talba_CR11	1	180-KCH-01
Talba_CR12	1	180-KCH-63
Talba_CR13	1	180-KCH-77
Talba_CR14	15	187-KCH-01, 187-KCH-05, 187-KCH-08, 187-KCH-11, 187-KCH-14, 187-KCH-23, 187-KCH-24, 187-KCH-25, 187-KCH-26, 187-KCH-40, 187-KCH-46, 187-KCH-63, 187-KCH-65, 187-KCH-68, 187-KCH-71
Talba_CR15	1	187-KCH-53
Talba_CR16	1	189B-KCH-61
Talba_CR17	1	192-KCH-56
Talba_CR18	2	206-KCH-02, 206-KCH-64
Talba_CR19	6	207-KCH-11, 207-KCH-26, 207-KCH-33, 207-KCH-51, 207-KCH-91, 207-KCH-93
Talba_CR20	1	230-KCH-44
TOTAL	45	

Table 1.4. *Thunnus atlanticus* haplotypes

Haplotype	# of Individuals	Sample ID
Tatla_CR1	9	132-KCH-02 132-KCH-04 132-KCH-15 132-KCH-34 132-KCH-37 132-KCH-45 132-KCH-71 132-KCH-75 132-KCH-83
Tatla_CR2	4	132-KCH-05 132-KCH-41 132-KCH-77 132-KCH-90
Tatla_CR3	1	132-KCH-06
Tatla_CR4	3	132-KCH-17 132-KCH-70 132-KCH-86
Tatla_CR5	4	142-KCH-15 142-KCH-23 142-KCH-49 142-KCH-58
Tatla_CR6	2	142-KCH-27 142-KCH-50
Tatla_CR7	1	142-KCH-59
Tatla_CR8	1	142-KCH-65
Tatla_CR9	1	145-KCH-54
Tatla_CR10	1	148-KCH-35
Tatla_CR11	2	157-KCH-34 157-KCH-92
Tatla_CR12	1	157-KCH-57
Tatla_CR13	1	157-KCH-65
Tatla_CR14	1	157-KCH-67
Tatla_CR15	1	163-KCH-10
Tatla_CR16	3	163-KCH-15 163-KCH-57 163-KCH-58
Tatla_CR17	2	163-KCH-46 163-KCH-69
Tatla_CR18	1	163-KCH-47
Tatla_CR19	4	167-KCH-08 167-KCH-18 167-KCH-26 167-KCH-86
Tatla_CR20	1	167-KCH-16
Tatla_CR21	1	167-KCH-53
Tatla_CR22	1	167-KCH-73
Tatla_CR23	64	169-KCH-40 173B-KCH-52 173B-KCH-93 195-KCH-26 213-KCH-01 213-KCH-03 213-KCH-04 213-KCH-05 213-KCH-06 213-KCH-07 213-KCH-08 213-KCH-14 213-KCH-15 213-KCH-21 213-KCH-22 213-KCH-23 213-KCH-25 213-KCH-26 213-KCH-27 213-KCH-28 213-KCH-29 213-KCH-30 213-KCH-31 213-KCH-32 213-KCH-36 213-KCH-37 213-KCH-38 213-KCH-39 213-KCH-42 213-KCH-43 213-KCH-44 213-KCH-45 213-KCH-46 213-KCH-47 213-KCH-48 213-KCH-52

		213-KCH-53 213-KCH-54 213-KCH-55 213-KCH-58 213-KCH-61 213-KCH-62 213-KCH-67 213-KCH-68 213-KCH-69 213-KCH-70 213-KCH-74 213-KCH-75 213-KCH-76 213-KCH-77 213-KCH-78 213-KCH-81 213-KCH-82 213-KCH-83 213-KCH-84 213-KCH-85 213-KCH-86 213-KCH-87 213-KCH-88 213-KCH-89 213-KCH-90 213-KCH-91 213-KCH-92 213-KCH-93
Tatla_CR24	1	169-KCH-44
Tatla_CR25	1	170-KCH-23
Tatla_CR26	2	173B-KCH-50 173B-KCH-73
Tatla_CR27	1	173D-KCH-03
Tatla_CR28	1	173D-KCH-04
Tatla_CR29	2	173B-KCH-50 173B-KCH-73
Tatla_CR30	1	180-KCH-31
Tatla_CR31	1	180-KCH-51
Tatla_CR32	1	180-KCH-59
Tatla_CR33	1	180-KCH-67
Tatla_CR34	1	180-KCH-75
Tatla_CR35	1	180-KCH-93
Tatla_CR36	1	184-KCH-84
Tatla_CR37	2	189B-KCH-19 189B-KCH-69
Tatla_CR38	1	189B-KCH-34
Tatla_CR39	2	189B-KCH-50 189B-KCH-53
Tatla_CR40	1	189B-KCH-64
Tatla_CR41	1	189D-KCH-39
Tatla_CR42	1	189D-KCH-59
Tatla_CR43	2	189E-KCH-14 189E-KCH-36]
Tatla_CR44	2	189E-KCH-16 189E-KCH-37]
Tatla_CR45	1	190E-KCH-78
Tatla_CR46	1	190E-KCH-82
Tatla_CR47	1	190E-KCH-87
Tatla_CR48	1	192-KCH-02
Tatla_CR49	1	192-KCH-06
Tatla_CR50	6	192-KCH-22 192-KCH-46 192-KCH-48 192-KCH-51 192-KCH-66 192-KCH-88
Tatla_CR51	1	193-KCH-23

Tatla_CR52	3	197A-KCH-09 197A-KCH-26 197A-KCH-33
Tatla_CR53	6	197A-KCH-19 197A-KCH-20 197A-KCH-22 197A-KCH-25 197A-KCH-30 197A-KCH-35
Tatla_CR54	1	197A-KCH-23
Tatla_CR55	10	201-KCH-06 201-KCH-14 201-KCH-21 201-KCH-24 201-KCH-26 201-KCH-27 201-KCH-34 201-KCH-67 201-KCH-70 201-KCH-76
Tatla_CR56	7	201-KCH-31 201-KCH-46 201-KCH-50 201-KCH-59 201-KCH-84 201-KCH-89 201-KCH-90
Tatla_CR57	8	202-KCH-07 202-KCH-16 202-KCH-43 202-KCH-46 202-KCH-53 202-KCH-59 202-KCH-70 202-KCH-76
Tatla_CR58	1	202-KCH-54
Tatla_CR59	1	202-KCH-67
Tatla_CR60	1	208-KCH-60
Tatla_CR61	1	219-KCH-33
TOTAL	188	

Table 1.5. *Katsuwonus pelamis* haplotypes

Haplotype	# of Individuals	Sample
Kpela_CR1	1	142-KCH-86
Kpela_CR2	2	167-KCH-07, 167-KCH-78
Kpela_CR3	2	167-KCH-10, 167-KCH-13
Kpela_CR4	2	167-KCH-11, 167-KCH-39
Kpela_CR5	1	167-KCH-14
Kpela_CR6	1	167-KCH-15
Kpela_CR7	1	167-KCH-17
Kpela_CR8	1	167-KCH-54
Kpela_CR9	1	167-KCH-56
Kpela_CR10	1	187-KCH-57
Kpela_CR11	1	189B-KCH-43
Kpela_CR12	1	202-KCH-82
TOTAL	15	

Table 1.6. Mitochondrial DNA control region haplotypes of bluefin tuna (*Thunnus thynnus*) observed in this study. Dots indicate identity to the reference sequence (haplotype Tthy_1) and dashes indicate insertion/deletion events. For clarity, only variable sites are shown; numbered positions are relative to haplotype Tthy_1.

Haplotype	Position	Site 154	Site 226	Site 227
	11111111111111112222222222222222222222233333333333			
	22346889900124566667999990000011111224455778999900001277899			
	26313120108004404786234590357912349355656143146715672378117			
Tthy_1	C-GCATTAGTTTTTCTTATACAAAACCAATATATTGCCCATCTTTGCACAACGGTATAA	1		
Tthy_2	.T...C.........C................C...................A......		2	
Tthy_3	.........C..........T...GT......C............A.....T.......		1	
Tthy_4	...T..................G.........C.......C...........A......		1	
Tthy_5	.........C..............G.......C..........C...............		2	
Tthy_6	................................T........T.................		1	
Tthy_7	......................G.G.......C..........C...............		1	
Tthy_8	........A.......................C..........................		1	
Tthy _9	.......................GG......CT..A.................A.....		1	
Tthy _10	...............C................C..A......C................		1	
Tthy _11	............C..CC.C......T......C..........C...............		1	
Tthy _12	..........C............GG.......TC...................A...G.		1	
Tthy _13	.......G....C.............T.....C.....................C....		1	
Tthy _14	..............T..........T......T......G....C..............		1	
Tthy _15	......C.........................C.......C..C...G...T...G...		1	
Tthy _16	............C..................CC..........................		1	
Tthy _17	.........C..............GT......C..AT........A.....T.......		1	
Tthy _18	.......................G........C..........................		1	
Tthy _19	............C..C................C..A......C................		1	
Tthy _20	....G..G....C....G........T.....C..........................		1	
Tthy _21	............C.......T...G...G...C.......................C.G		1	
Tthy _22	T.......A......................CC..............G.........G.		1	
Tthy _23	...........C.................C.CT...T...C........G.........		1	
Tthy _24	...........................G..G.C........................G.		1	
Tthy _25	.........C..........T...G.......C............A.............		1	1
Tthy _26	.....CC.........................C.......C..C...GG.GT...G...			1
Tthy _27	...............C........GT......C......................G...			1
Tthy _28	.....C........T.................T..........................			1
Tthy _29	.............C..C..........G....C.....T.C..................			1
Tthy _30	...........................G..G.C..........................			1
Tthy _31	.......GA............G.......C.CC.C.T.........T............			1

Table 1.7. Summary of the mean statistics for the mtDNA control region (dloop) of *Thunnus thynnus, Thunnus albacares, Thunnus atlanticus,* and *Katsuwonus pelamis*.

Species	N	# Haplotypes	*h*	# polymorphic sites	π	FST	Tajima's D
Thunnus thynnus	33	31	0.995	83	0.022	-0.017	-1.441
Thunnus albacares	45	20	0.288	75	0.015	0.752	-0.416
Thunnus atlanticus	188	61	0.454	309	0.026	0.512	0.477
Katsuwonus pelamis	15	12	0.946	71	0.064	--	0.317

N, Number of sequences; h, haplotypic diversity; π, nucleotide diversity

Table 1.8. Summary of the statistics for the mtDNA control region (dloop) of *Thunnus thynnus, Thunnus albacares, Thunnus atlanticus,* and *Katsuwonus pelamis*.

Species	Site	N	# Haplotypes	*h*	π	# polymorphic sites	Tajima's D
Thunnus thynnus	226	25	23	0.993 (±0.0134)	0.020 (±0.0105)	51	-1.584
	227	7	7	1.00 (±0.0764)	0.025 (±0.0149)	32	-1.298
Thunnus albacares	157	7	3	0.524 (±0.2086)	0.024 (±0.0145)	25	-0.219
	167	3	2	0.667 (±0.3143)	0.030 (±0.0232)	18	0
	180	3	3	1.00 (±0.02322)	0.030 (±0.0232)	18	0
	187	16	2	0.125 (±0.1064)	0.004 (±0.0030)	14	-2.276
	206	2	1	0	0	0	0
	207	6	1	0	0	0	0
Thunnus atlanticus	132	17	4	0.669 (±0.0913)	0.029 (±0.0153)	30	1.185
	142	9	5	0.806 (±0.1196)	0.020 (±0.0118)	21	0.245
	157	5	4	0.900 (±0.1610)	0.025 (±0.0159)	20	0.154
	163	7	4	0.810 (±0.1298)	0.026 (±0.0156)	24	0.366
	167	7	4	0.714 (±0.1809)	0.021 (±0.0128)	22	0.250
	213	64	1	0	0	0	0
	173B	4	2	0.667 (±0.2041)	0.020 (±0.0141)	12	2.233
	173D	2	2	1.00 (±0.500)	0.035 (±0.0363)	14	0
	180	6	6	1.00 (±0.0962)	0.032 (±0.0193)	33	-0.761
	189B	6	4	0.867 (±0.1291)	0.026 (±0.0161)	23	0.245
	189D	2	2	1.00 (±0.500)	0.038 (±0.0387)	15	0
	189E	4	2	0.667 (±0.2041)	0.020 (±0.0141)	12	2.233
	190E	3	3	1.00 (±0.2722)	0.023 (±0.0185)	14	0
	192	8	3	0.464 (±0.200)	0.019 (±0.0110)	23	-0.827
	197A	10	3	0.600 (±0.1305)	0.013 (±0.00779)	11	1.511
	201	17	2	0.515 (±0.0592)	0.023 (±0.0126)	18	2.902
	202	10	3	0.378 (±0.1813)	0.011 (±0.0068)	17	-1.119
Katsuwonus pelamis	167	11	8	0.946 (±0.0535)	0.064 (±0.0346)	71	0.317

N, Number of sequences; h, haplotypic diversity; π, nucleotide diversity

Table 1.9. Mitochondrial DNA control region haplotypes of yellowfin tuna (*Thunnus albacares*) observed in this study. Dots indicate identity to the reference sequence (haplotype Talba_1) and dashes indicate insertion/deletion events. For clarity, only variable sites are shown; numbered positions are relative to haplotype Talba_1.

Haplotype	Position	Site													
		142	148	152	157	163	167	168	180	187	189	192	206	207	230
	111111111111111122222222222222222222222222333333333333333														
	268888899123456667779999900001112234445555677789999000234566779999														
	520123909965383789046234502591230143450356001353679026221101570245														
Talba_1	CACTCTCGTTTTCGTCCATGTAAATCTCCATCAAGCCCCAACATTTTAGTAACCGATTTGAAAATT	1													
Talba_2	....C........		1												
Talba_3	......AA...A..C.....CG...............TT...........G.T.....GA......			1											
Talba_4	.......A.C....C.......G..............TT....C....A...T.........G..C				5										
Talba_5	T....A.A......C.A.....G....T.....G...T.C...CCC.....GTT.C.....G..C.				1										
Talba_6	T....A........C.A...C.G.......C....T.TTC...CC.A..C.GTTAC.....G..C.				1										
Talba_7	T....A........C.A.....G....T..C.....TTTC...CC......GTT.C.....G..C.					1									
Talba_8	.GT...G....CT.CT...A......CT....G....TT.C......GA........C......C.						2								
Talba_9	..........A.T.C..........T.....TG....TTG.T......A...T.............						1								
Talba_10	..........A.T.C..........T.....TG....TTG.T......A...T.......GG....							1							
Talba_11	...C........T.C.......G..............TTG..G.....A...T.............								1						
Talba_12	.......A.C....C...C...G..............TT....C....A...T.........GGCC								1						
Talba_13	...C..A.....T.CT............A.C......TT.........A.................								1						
Talba_14	....T...C...T...........C........GA..TTG........A...T.............									15					
Talba_15	......T.......C..............G.......T..................C.........									1					
Talba_16	..............C.............T.C......T........C.A........C...G...C										1				
Talba_17	T.A..A........C.AG....GG...T..C......TTT...CC.C....GTT.C.....G..C.											1			
Talba_18	.....A.......AC.A.....T....T.........TTC...CC........T.C.....G..C.												2		
Talba_19	T....A.A......C.A.....G....T.....G...T.C...CC......GTT.C.....G..C.													6	
Talba_20	.....A........C.A...C.G.......C....T.TTC...CC.A..C.GTTAC.....G..C.														1

36

Table 1.10. Mitochondrial DNA control region haplotypes of blackfin tuna (*Thunnus atlanticus*) observed in this study. Dots indicate identity to the reference sequence (haplotype Tatla_1) and dashes indicate insertion/deletion events. For clarity, only variable sites are shown; numbered positions are relative to haplotype Tatla_1.

```
Haplotype                 Position                                                                    Site
                                                                                                        1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2
                                                                                                        3 4 4 4 5 6 6 6 7 7 7 8 8 8 8 8 9 9 9 9 0 0 0 1 1
                                                                                                        2 2 5 8 7 3 7 9 0 3 3 0 4 9 9 9 0 2 3 7 1 2 8 3 9
                                                                                                                  B D     B D E E     A

                     1111111111111111111122222222222222222222222222222222222222223333333333333333
          1234567888999901345666677789999999900000000111222222334455556788999990000002257789999
          912009001202779538378906931234578901345789012016890457012372160146701347836278115 6

Tatla_1   TCACAATCTCGCTCTTCACCCATTAACAGACTATCTAACCTCAACAATAGAGTCTAAA-TTCTCGAAAACACTGTCCATATC 9
Tatla_2   ................G.A...C....A.T......G.T.T..T....A...A....-..TCTA...GT.T...TT..... 4
Tatla_3   ..G.......A......G.A...CG...A.T......G.T.T......GA...A....-C.TC.A....T.....TT..G.. 1
Tatla_4   .T......C.....C..G.........A.T...........GT....A.......-..T..A....T.....TT..... 3
Tatla_5   ................G.A......T.A.T..CT....T.T.G.....AG..A....-..TCTA..........TT.....  4
Tatla_6   ................G.A........A.TC.......T.T..T.G..A...AC..G-..TCTA....T.T...TT.....  2
Tatla_7   ................G.A...C....A.T........T.T.......A...A....-..TCTA...GT.T...TT.....  1
Tatla_8   ................G.A........A.T..........T.GT....A...A....-...CTA....T.T...TT.....  1
Tatla_9   ................G.A........A.T........T.........A...A..G.-..TC.A..GGT.....TT.C...   1
Tatla_10  ................G.A.......GA.T........T.T.GT....A..CA.-..-..TC.A....T.....TT.....    1
Tatla_11  ................G.AT.......A.T..........TGGT....A...A....-..TCTA....T.T...TT.....     2
Tatla_12  ................G.A..........T..C.....T.T.......A........-..A..A..G.T.....TT...C.     1
Tatla_13  .........A......G.A..C..G..A.T.......T..TG......A...A....-..T..A....T.....TT.....     1
Tatla_14  ......T.........G.A...C....A.T.......TT.T..T....A...A....-..TCTA....T.T...TT.....     1
Tatla_15  ................G...........T........T....T....A....C...-..T.TA....T....CTT..G..      1
Tatla_16  ..................A.........T........T...G......A...T....-..T..A.G..T.....TT....T      3
Tatla_17  .........A......G.A........A.T...AC...T.T...G...A........-..TC.AG...T.....TT.....      2
Tatla_18  ................G.A.....G..A.T........T.T.G.....A...A....-..TC.A..G.T...A.T...GC.      1
Tatla_19  ................G.A........A.T........T.T.G.....A...A....-...CTA....T.T...TT.....       4
Tatla_20  ................G.A.......G..T.GC.....TCT.GT.G..A........-..A..A..G.T.....TT...C.       1
Tatla_21  ......T........AGTA........A.T..C.....T.T..T....A........-..A..A..G.T.....TT...C.       1
Tatla_22  ................G.A......T.A.T..C.....TCT.G.....A...A.-..-...C.A..........TT....T       1
Tatla_23  ................G.A........A.T..C.....T.T.GT....A...A.-..-..TC.A..........TT.....        1                    53
Tatla_24  ................GTA......T.A.T..CT....T.T.G.....AG..A....-..TC.A..........TT..G..        1
Tatla_25  ................G.A..........T..C.....TCT.......A...G....-..A..A..G.T.....TT...C.         1
Tatla_26  ................G.A........A.T..C.....T...GT....A...A.-..-..TC.A..........TT.....          2
Tatla_27  ................G.AT.......A.T........T.T..T....A...AC...-..TCTA....T.T...TT.....           1
```

```
Tatla_28 .........T.......G.A.........T..C.....T.T...G...A........-..A..A..G.T.....TT...C.     1
Tatla_29 ..G.............G.A...CG...A.T.......T.T.......A...A....-C.TC.A....T.....TT..GC.     2
Tatla_30 ................G.AT....G..A...G.....T.T..T....A...A....-..TC.A.........TT..G..     1
Tatla_31 ..........................C.T.......T....T.......A....-..T..A..........TG....     1
Tatla_32 ................G.A.......GA.T..C.....T.T.GT....A...A.-..-..TC.A.........TT.....     1
Tatla_33 ................G.A........A.TC.......T.T..TGG.GA...A....-..TCTA....T.T...TT.....     1
Tatla_34 C............C..G.........A.T.......T..G.T....A........-.CTC.A....T.....TT.....     1
Tatla_35 ...............TG...G........T.......T....T....A........-..T..A..G.T......T....T     1
Tatla_36 ...........C....G.A........A.T..C.....T.T.GT....A...A.-..-..TC.A.........TT.....     1
Tatla_37 ................G.A.....G..A.T.......T.T.G.....A...A....-..TC.A..G.T.....TT..GC.     2
Tatla_38 ................G.A........A.T...TC...T....T.G..A...A...G-..TCTA....T.T...TT..G..     1
Tatla_39 ................GTA......T.A.T..CT....T.T.G.....AG..A....-..TC.A.........TT.....     2
Tatla_40 ......C...A....C.G.A.........T.......T.T.T.....A...A....-...CTA....T.T...TT.....     1
Tatla_41 .....T.......T...G.A..................T.T.......A........A..TC.A..G.T.....TT...C.     1
Tatla_42 ................G.A........A.T..........TGGT....A...A....-..TCTA....T.T...TT..G..     1
Tatla_43 ................G.A.......G..T..C.....T.T..T.G..A........-..A..A..G.T.....TT...C.     2
Tatla_44 ................G.A.......GA.T........T...GT....A..CA.-..-..TC.A....T.....TT.....     2
Tatla_45 ................G...........T.......T...GT....A........-..T..A....T.....TT.....     1
Tatla_46 ................G.A........A.T..C.....T.T.GT....A...A.-..-..TC.A.......C..TT.....     1
Tatla_47 ...............TG.A.........T.......TT.........A...AC-..-..TC.A....T.....TT.....     1
Tatla_48 ................G..........A.T.......T....T....A........-..T..A.G..T.....TT.....     1
Tatla_49 ...T............G.A......T.A.T..C.....T.T...........A.-..-...C.A........A..T.....     1
Tatla_50 .............T...G.A...C...G..T..C.....T.T..T....A........-..A..A..G.TG....T...G..     6
Tatla_51 ................GTA.......G..T..........T..T.G..A........-..A..A....T.....TT...C.     1
Tatla_52 ................G.A........A.T...TC...T.T.G..G..A...A...G-..TCTA.G..T.T...TTG.G..     3
Tatla_53 ....T......A.....G.A........A.T...T....T.T.C.....A...A....-..TCTA..........TT.....     6
Tatla_54 ...........A.....G.A........A.T...T....T.T.C.....A...A....-..TCTA..........TT.....     1
Tatla_55 ................G.A.......G..T..C.....TCT.GT.G..A.......G-..A..A..G.T.....TT...C.     10
Tatla_56 ...........................CT.......GT.T.G...C..........-..T..A..........T.....T     7
Tatla_57 ..................A.........T.......T.........A...T....-..T..A.G..T.....TT....T     8
Tatla_58 ................G.A......T.A.T..C.....TCT.G.....A...A.-..-..TC.A.........TT.....     1
Tatla_59 ................GTA........A.T.......T.T......GA.A.A....-..TC.A..G.T.....TT.....     1
Tatla_60 ................G..........A.T.....G..T....T....A........-..T..A..G.T.....T...G..     1
Tatla_61 ..........A......G.A........A.T.......T.........A........-..T..A....T.....TT.....     1
```

Table 1.11. Mitochondrial DNA control region haplotypes of skipjack tuna (*Katsuwonus pelamis*) observed in this study. Dots indicate identity to the reference sequence (haplotype Kpela_1) and dashes indicate insertion/deletion events. For clarity, only variable sites are shown; numbered positions are relative to haplotype Kpela_1.

Haplotype	Position	Site				
		142	167	187	189B	202
	1111111111111111111222222222222222222222222222222222222223333333333333					
	889111334455555668999999000000011111112224455555677788889999999900133477779999					
	5653565809246746902458902467891246789345890378996896789023456788912883467123					
Kpela_1	CACTT-CTCCTCGCAATCCGTACCACATACCATTAAGTGGAGATGTTTTCTCCCGTAGTTCCAATTCTCGATCTTA	1				
Kpela_2	T....AT..T......C.....T.......T....G..AA....A.......T....ACCTT.....C........		2			
Kpela_3	T...CAT..TC....G..T..G..G....TTG.CGGA.A..T..AC......T..C..CCTT.....CT.......		2			
Kpela_4	.....AT..T......C.T...T.......T....G..A.....A...C...T...GACC.T.....C..G.T...		2			
Kpela_5	T...CAT..TCT.T...TTACGT.G.TACAT.......AA...CACG.C...T..C.AC.TAGGCC..........		1			
Kpela_6	T.T..ATA.T......C.T..GT.G.T...T.C.G.ACA.GAG..CG.....T....AC..AG....CTTGCG...		1			
Kpela_7	T...CAT..TC..........TTGT...T.G...G..A.....A..............CTT.....CT.......		1			
Kpela_8	T.TC.AT.TT.TA.T..AT.C.TTG...........ACAA.A..ACG..TCT..A..AC.TAG...T..A..T.C.		1			
Kpela_9	T...CAT..T.T......T.....G...G.T....G..A.....AC......TT.C.A..TT.............G		1			
Kpela_10	.GT..AT..TCT..C..A..CGTTGT...........CA..-..ACG...C.T..C.AC.TGG..C......T.C.			1		
Kpela_11	T.T..AT..T..A...........G.....T.......A.....AC......T..C.A.CTT.....C.....C..				1	
Kpela_12	T.T..AT.TTCT..T..AT..GT.G...........ACAA.A..ACGC.TC.T..C.AC.TA...C..........					1

Table 1.12. Analysis of Molecular Variance of mtDNA control region nucleotide sequence data in bluefin tuna (*Thunnus thynnus*), yellowfin tuna (*Thunnus albacares*), and blackfin tuna (*Thunnus atlanticus*).

Species	% var. among populations	% var. within populations
T. thynnus	0	100
T. albacares	75.2	24.8
T. atlanticus	51.19	48.81

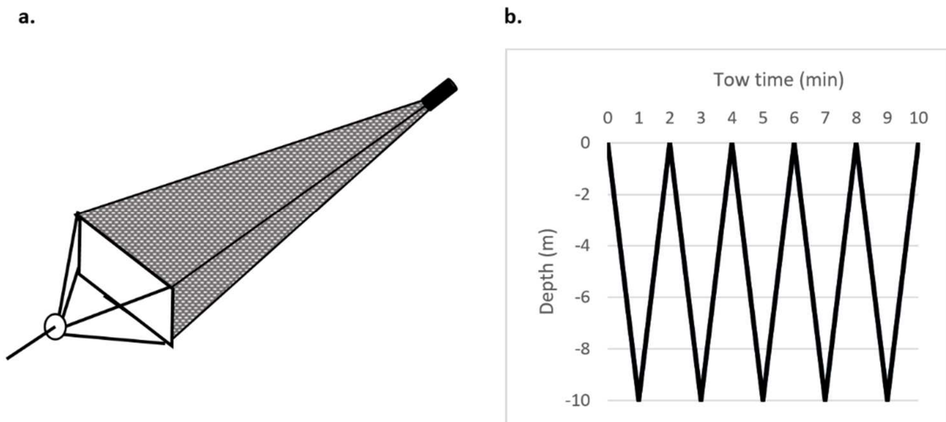

Figure 1.1. a. Schematic of Spanish neuston net. b. Idealized depth profile of Spanish neuston net tow.

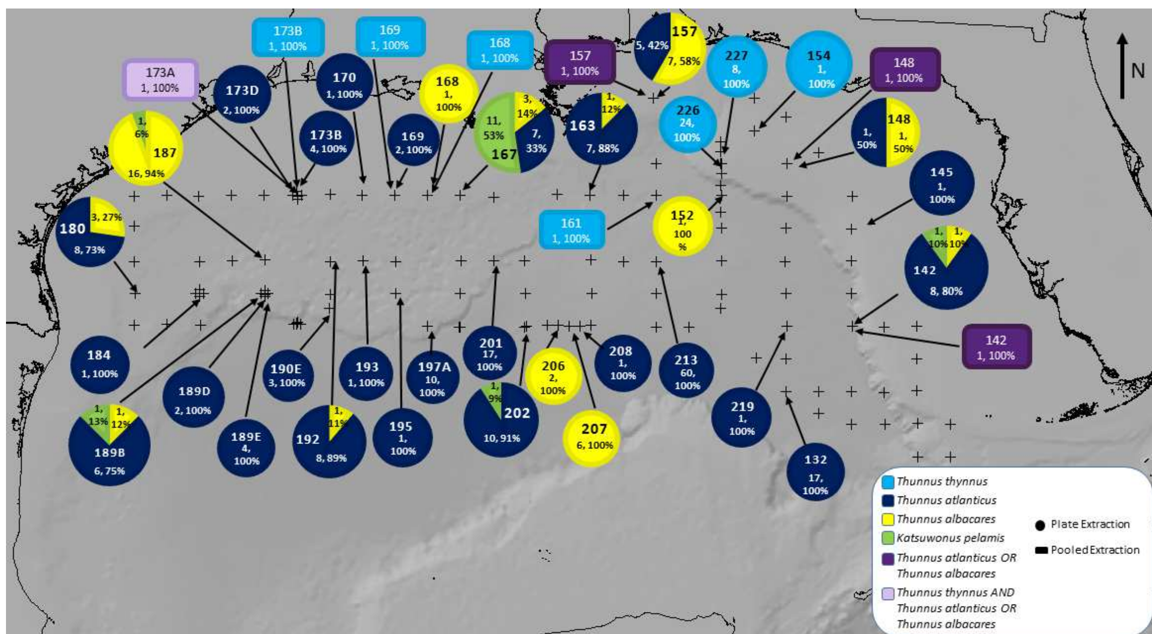

Figure 1.2. Species ratio map showing stations at which *Thunnus* and *Katsuwonus* were present.

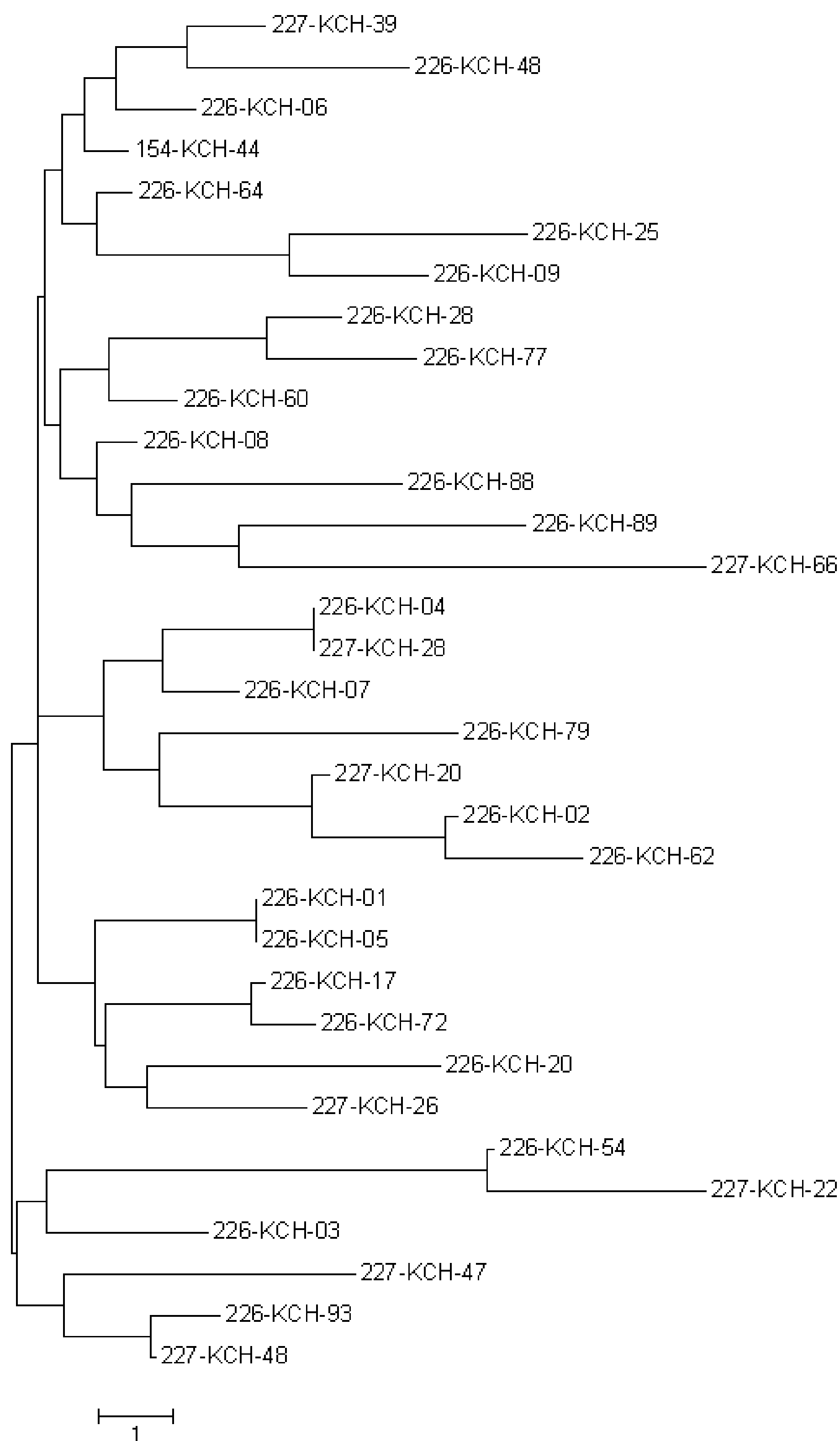

Figure 1.3. Neighbor-joining tree showing the relationship of 33 bluefin tuna (*Thunnus thynnus*) with 31 haplotypes.

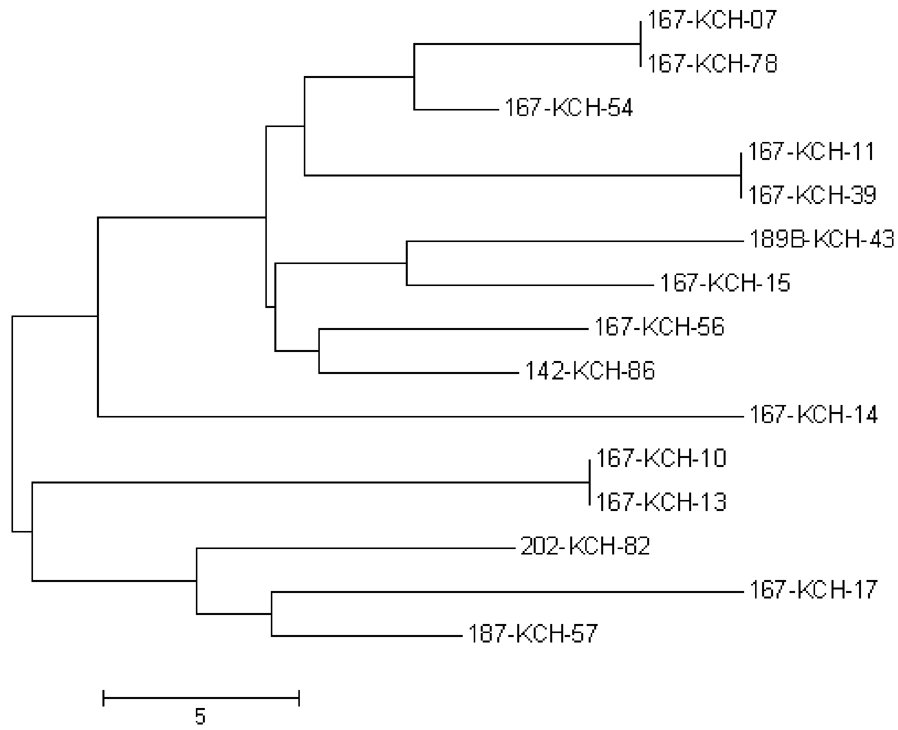

Figure 1.4. Neighbor-joining tree showing the relationship of 15 skipjack tuna (*Katsuwonus pelamis*) with 12 haplotypes.

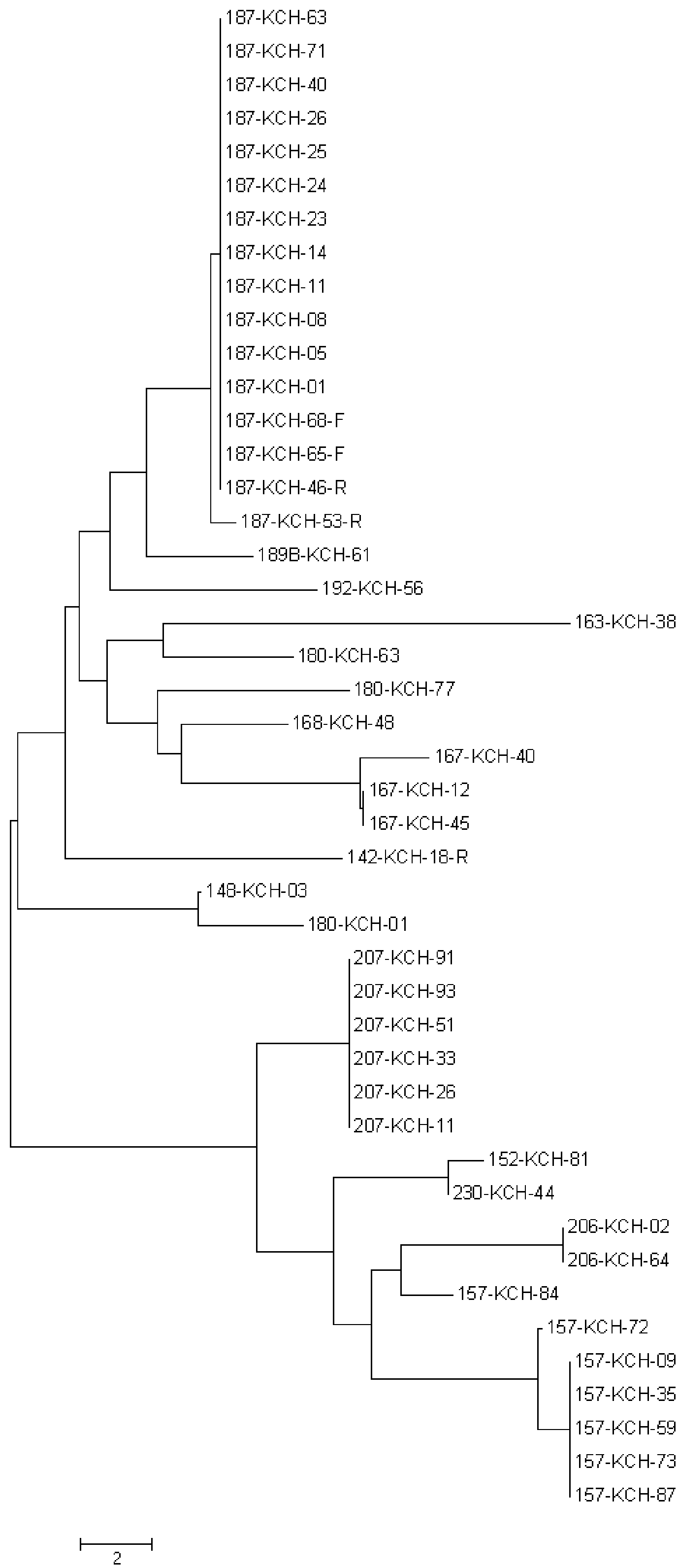

Figure 1.5. Neighbor-joining tree showing the relationship of 45 yellowfin tuna (*Thunnus albacares*) with 20 haplotypes.

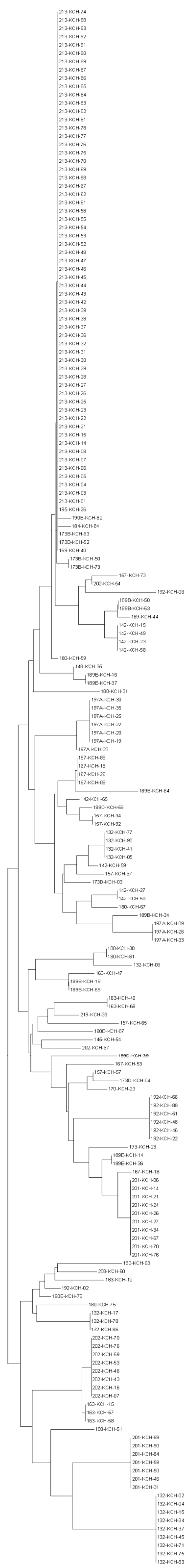

Figure 1.6. Neighbor-joining tree showing the relationship of 188 blackfin tuna (*Thunnus atlanticus*) with 61 haplotypes.

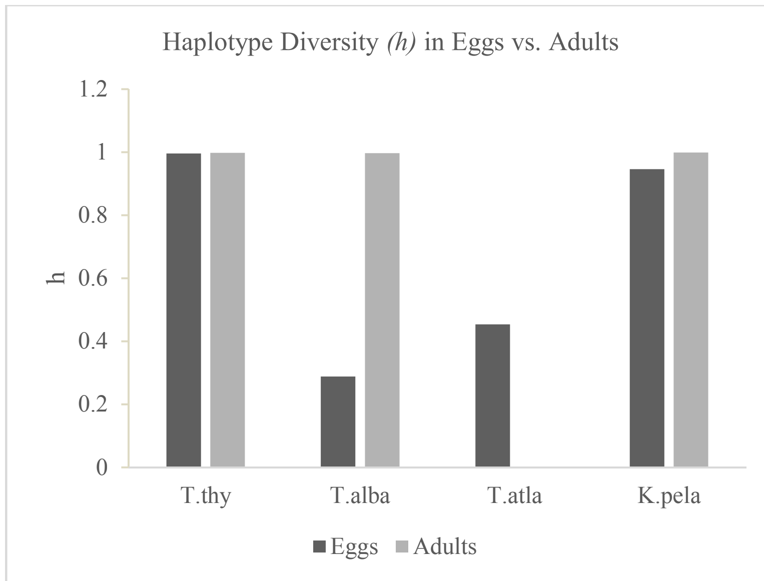

Figure 1.7. Haplotype Diversity in eggs vs. adults of *T.thynnus, T. albacares, T. atlanticus,* and *K. pelamis*

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