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Physical and Biological Characteristics of Billfish Spawning Habitat in the Straits of Florida

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UNIVERSITY OF MIAMI

PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF BILLFISH SPAWNING
HABITAT IN THE STRAITS OF FLORIDA

By

David Earl Richardson

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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the requirements for the degree of
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PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF BILLFISH SPAWNING
HABITAT IN THE STRAITS OF FLORIDA

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The objective of this dissertation was to examine sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*) spawning in the Straits of Florida, with a specific focus on 1) the physical and biological characteristics of the spawning environment, and 2) the role of the region within the broader spawning patterns of these two species. In order to accomplish these objectives, two years of monthly ichthyoplankton collections and physical measurements across the Straits of Florida were combined with a finer-scale Lagrangian study. Additionally, a molecular species-identification methodology was developed that was both high-throughput and suitable for use with a broad taxonomic range of species.

An initial analysis considered the diversity, assemblages and associated habitat of the larvae of large and medium size pelagic species. In total 36 species and 14,295 individuals were collected during this study, with the highest diversity occurring during the summer, and in the western frontal region of the Florida Current. Sailfish were included in an assemblage with *Auxis rochei*, *A. thazard* and *Euthynnus alleterattus*, all species found in highest abundance during the summer along the western edge of the Straits of Florida. Blue marlin grouped most closely with *Thunnus atlanticus*, *Ruvettus pretiosus* and *Lampris guttatus*, all summer spawners, whose larvae tended to occur further offshore. The primary environmental factors associated with these assemblages

were SST (highest summer-early fall), day-length (highest early summer), thermocline depth (shallowest on the Florida side) and fluorescence (highest on the Florida side).

A Lagrangian sampling effort was then used to more specifically evaluate the role of frontal zones in sailfish spawning. The results of this sampling indicated that the highest levels of sailfish spawning occurred in a frontal zone associated with the formation of a submesoscale frontal eddy. This spawning resulted in the first-feeding larvae occupying an area rich in prey items. Given the small spatial-scale of the front, and the distribution of the eggs of adult prey items, the results of this work would suggest that sailfish are actively targeting features for spawning that are favorable to the growth and survival of their larvae.

Finally the relative importance of the Straits of Florida as a spawning ground was evaluated by calculating the annual egg production of both sailfish and blue marlin within this region. In total it was estimated that 2.1% of western Atlantic sailfish spawning and 1.6% of Atlantic wide blue marlin spawning occurs in the SF. Pop-up satellite tags deployed on sailfish at the start of the spawning season revealed their short residency times in the SF, suggesting that a large ($\approx 10\%$) transient portion of the sailfish population is responsible for the SF egg production. These results indicate that the SF is a migratory bottleneck for sailfish.

In conclusion the results of this study indicate that a hierarchy of physical and biological processes influence the distribution of billfish spawning in space and time. The results provide insights into the movement patterns and life history strategies of these species, and ultimately may aid in the development of the spatially explicit ecosystem based management approaches that are currently being advocated.

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1. GENERAL INTRODUCTION AND SCOPE OF DISSERTATION

Six billfish species inhabit the western North Atlantic, four in the family Istiophoridae (blue marlin, *Makaira nigricans*, white marlin, *Tetrapturus albidus*, longbill spearfish, *T. pfluegeri*, roundscale spearfish, *T. georgii*, and sailfish, *Istiophorus platypterus*) and one in the family Xiphiidae (broadbill swordfish, *Xiphias gladius*). These top trophic level species are targeted by sport fisheries, regional artisanal fisheries, and directed commercial fisheries in the case of swordfish. However, for the istiophorid billfishes the highest levels of mortality occur due to incidental take in longline fisheries targeting other pelagic species. Though a matter of much uncertainty, it is now estimated that this exploitation has driven the biomass of these species to around 10% of pre-industrial levels (Myers and Worm 2003), with overfishing continuing to push these declines further in some species (ICATT 2003).

The implementation of ecosystem-based management (EBM) approaches in place of the traditional single-species management is currently being advocated as one means of slowing or reversing further population declines in fished species (Pauly et al. 2002; Browman and Stergiou 2004; Pikitch et al. 2004). The advocates of this approach argue that the declines in many marine populations, and their reduced resiliency, have resulted from a lack of consideration of predator-prey relationships and habitat integrity in fisheries management. However, the implementation of these ecosystem-based approaches is not without severe challenges. Many of the world's fisheries operate in a data poor environment that precludes predictions about how different management actions will affect individual species and the ecosystem as a whole (Pikitch et al. 2004).

Additionally, in the face of this uncertainty, it is difficult to develop a plan that has sufficiently broad stakeholder support to make its implementation and enforcement likely to succeed. Addressing both of these concerns requires research directed at a comprehensive understanding of the interaction of species, their environment, and fishing operations.

Often coupled to the call for EBM is the recognition that the spatial structure of marine populations must be more explicitly accounted for in management regulations (Babcock et al. 2005). Increasingly, there is a realization that the ocean is an extremely spatially heterogeneous environment (e.g. McGillicuddy et al. 2007), and that management based on the assumption of spatial homogeneity of processes across populations is inherently flawed (Sharp 2001). One spatial management option that has received extensive support in recent years is the establishment of marine protected areas. For species with restricted adult movements, this option is backed up by strong theoretical underpinnings and extensive field studies (Russ 2002). However, the extension of a marine protected area approach to the diversity of highly migratory pelagic species has not been well validated as a management tool either in field or theoretical work. This is not to question its use for isolated species of concern, such as bluefin tuna, that have been shown to congregate in specific areas during specific seasons (Block et al. 2005). Rather, it is to raise the possibility that the benefits of open-ocean protected areas, in terms of the conservation of the entire pelagic community, may be disproportionately small relative to their socioeconomic impacts. In such a case, the increased implementation and enforcement of established management procedures would be preferred.

Currently, much of what is known about the movement, distribution, and life histories of billfish and other large pelagic species has come from either recreational or commercial fisheries. While these fishery-dependent data are undeniably valuable, their interpretation is confounded by spatial and temporal changes in the catchability of fish (Sharp 1978; Hampton et al. 2003). For example, it has been found that in areas with dense prey aggregations, catch rates of longline gear decrease due to competition between the bait and natural prey resources (Betrand et al. 2002). Additionally, changes in the degree to which individuals aggregate in a location readily identified by fishers will affect catch rates and may incorrectly suggest changes in the regional abundance of that species. Further constraining the use of fisheries data are the poor spatial resolution or species identification in much of these data (ICCAT 2003) and the lack of concurrently collected environmental information with most fishing operations (Sharp 2001).

Over the past decade, advances in the electronic tagging of large pelagic species have considerably improved our understanding of the movement patterns of individual species and their interactions with their environment (Block et al. 1998; Block et al. 2005). The most rapid increase in knowledge, and the most readily applicable to management, has concerned vertical habitat use by these species (Prince and Goodyear 2006). The understanding of horizontal movement patterns has been slower to develop, due in part to the cost of electronic tags, and the limitations in the degree to which they resolve horizontal positions (Sibert et al. 2003). There are also limits in the extent to which behavior, such as spawning, can be inferred from the information provided by these tags. The result is that electronic tagging data have proven to be revolutionary in terms of addressing the large-scale movements of certain pelagic species. At the same

time they have left open many questions concerning finer scale horizontal processes, and pelagic community structure.

Ichthyoplankton collections, combined with oceanographic surveys, provide an alternate valuable tool to address many questions about the life history strategies of pelagic species. Their broader use has included the estimation of the number or biomass of spawning individuals (Hunter and Lo 1993; Scott et al. 1993; Ralston et al. 2003), the back calculation of spawning sites and times (Hare and Cowen 1991; Hare and Cowen 1996; Hare et al. 2002; Govoni et al. 2003; Serafy et al. 2003) and the comparison of reproductive and early life history strategies across a suite of species (Boehlert and Mundy 1993; Cowen et al. 1993; Miller and McCleave 1994; Cowen and Sponaugle 1997; Grothues and Cowen 1999; Miller 2002; Sponaugle et al. 2003). Previous ichthyoplankton work with billfish has determined that spawning occurs in a number of different areas of the western North Atlantic, including Exuma Sound, the Mona Passage, Barbados and the Straits of Florida (Serafy et al. 2003; Luthy 2004; Prince et al. 2005; Cowen unpubl. data). These collections have only been cursorily put into an oceanographic context, and have primarily been focused on single-species. With the exception of one limited sampling effort (Prince et al. 2005), the broader linkage of these spawning grounds to the individual adult movement patterns is unknown, nor is it known how important each of these spawning grounds are to the species.

The goal of this dissertation was to investigate the physical and biological characteristics of billfish spawning habitat in the Straits of Florida, and the regional importance of this spawning ground. The first chapter of this work was the development of a molecular methodology for species identification across a range of species. This

allowed the remaining work to be targeted at the species level and at the earliest developmental stages, including eggs and yolk-sac larvae. In the second chapter an assemblage approach is used to examine the larval distributions of a suite of 36 large and medium size pelagic species collected during two years of monthly sampling in the Straits of Florida. The oceanographic characteristics associated with larval distributions, and between-species similarities in spawning patterns were addressed with this approach. The third chapter analyzed sailfish spawning relative to a submesoscale frontal eddy, and the community characteristics of their spawning location. Finally, in the fourth chapter the relative importance of the Straits of Florida spawning ground was examined through both an analysis of the egg production of blue marlin and sailfish, and their movement patterns. In all, this dissertation is intended to provide unique insights into the spawning dynamics of billfish, with the aim of furthering our understanding of the processes involved in the movement and recruitment of these species.

2. HIGH-THROUGHPUT SPECIES IDENTIFICATION: FROM DNA ISOLATION TO BIOINFORMATICS

Ichthyoplankton collections provide a valuable means to study fish life histories. However these collections are greatly underutilized, as larval fishes are frequently not identified to species due to their small size and limited morphological development. Currently there is an effort underway to make species identification more readily available across a broad range of taxa through the sequencing of a standard gene. This effort requires the development of new methodologies to both rapidly produce and analyze large numbers of sequences. The methodology presented in this paper addresses these issues with a focus on the larvae of large pelagic fish species. All steps of the methodology are targeted towards high-throughput identification using small amounts of tissue. To accomplish this, DNA isolation was automated on a liquid handling robot using magnetic bead technologies. PCR and a unidirectional sequencing reaction followed standard protocols with all template clean-up and transferring also automated. Manual pipetting was thus reduced to a minimum. A character-based bioinformatics program was developed to handle the large sequence output. This program incorporates base-call quality scores in two types of sample to voucher sequence comparisons and provides suggested identifications and sequence information in an easily interpreted spreadsheet format. This technique when applied to tuna and billfish larvae collected in the Straits of Florida had an 89% success rate. A single species (*Thunnus atlanticus*) was found to dominate the catch of tuna larvae, while billfish larvae were more evenly divided between two species (*Makaira nigricans* and *Istiophorus platypterus*).

Introduction

Nearly all marine teleost fishes possess a pelagic larval stage that is morphologically distinct from the adults and many orders of magnitude smaller (Kendall *et al.* 1984). Understanding the processes affecting both the survival and transport of this stage is one of the principal challenges in marine fish ecology, as these processes will influence the spatial distribution, population dynamics, migration strategies and evolution of a species (Bakun 1996; Cowen *et al.* 2006). While many indirect methods have been developed over the years to evaluate larval ecology and transport (e.g. modeling, recruitment/environmental time series, otolith-based studies on juvenile fishes), a comprehensive understanding of these issues still requires the sampling of eggs and larvae in their natural environment. In addition, ichthyoplankton collections are a powerful tool for addressing many other important questions in fish ecology and fisheries management including the identification of spawning locations and seasons of migrating species (Schmidt 1922; McCleave 1993; Govoni *et al.* 2003; Serafy *et al.* 2003), the quantification of population levels or biomass of fished species (Hunter and Lo 1993; Scott *et al.* 1993; Ralston *et al.* 2003), and the determination of the distribution and diversity of rare and cryptic species (Smith 2002; Richardson and Cowen 2004; Wouthuyzen *et al.* 2005).

Despite considerable effort (e.g. Moser 1996; Leis and Carson-Ewart 2000; Richards 2006a) larval identification remains one of the major factors limiting the questions that can be addressed by field-based ichthyoplankton studies. These limitations are especially acute within certain regions, for certain taxonomic groups and during the earliest stages of larval development (Kendall *et al.* 1984). For example, Kendall and

Matarese (1994) calculated that a description of any stage of larval development existed for only 10% of Indo-Pacific fish species. Similarly, problems remain in identifying the larvae of many well studied and economically valuable species such as grouper (Richards *et al.* 2006), tunas (Richards 2006b) and billfish (Richards and Luthy 2006). While increased effort in developing morphological identification systems will likely be successful for many taxonomic groups of fishes, it appears that the underlying limits have been reached for many others. Notably, similar limitations of morphological identification systems for early life stages are widespread in the animal kingdom, occurring with a variety of marine and terrestrial invertebrates (Medeiros-Bergen *et al.* 1995; Kiesling *et al.* 2002) and amphibians (Vences *et al.* 2005).

Over the past years molecular means of identification have increased in use in response to the limitations of morphological identification. For fishes, these techniques have most frequently involved the use of the Polymerase Chain Reaction (PCR) followed by a restriction enzyme digestion (McDowell and Graves 2002; Chow *et al.* 2003; Luthy *et al.* 2005a) or the more efficient utilization of species-specific primers in a single PCR step (Rocha-Olivares 1998; Shivji *et al.* 2002; Chapman *et al.* 2003; Hyde *et al.* 2005). Each of these methodologies has been successfully used in ichthyoplankton studies, with the latter technique even being implemented with basic laboratory equipment on board a research vessel (Hyde *et al.* 2005). While designed for ease of use in modestly equipped lab settings, these techniques face a critical drawback of only being suitable for discriminating a limited number (generally 5-15) of species determined at the time of development. Because of this, these two methodologies are generally appropriate for

studies targeted at one or two low diversity taxonomic groups, but are not applicable for the identification of a wide diversity of species.

It has been proposed that the sequence of a single gene may be suitable for discriminating most animal species (Hebert et al. 2003a; Hebert et al. 2003b). This concept, termed DNA barcoding, has been tested with North American bird species (Hebert *et al.* 2004), Australian fish species (Ward *et al.* 2005), and a variety of invertebrate taxa (Barrett and Hebert 2005; Janzen et al. 2005; Smith et al. 2005). The conclusions of these studies, using the cytochrome oxidase subunit 1 (*cox1*) gene, are that sufficient inter-specific differences exist for species identification. While most of the focus to date has been on developing the foundations of DNA barcoding, the technique has proven useful in a number of studies. For example, ant diversity in Madagascar was evaluated through DNA barcoding, an otherwise tedious and difficult task using traditional morphological identification methods (Smith *et al.* 2005). Sequencing has also been used in ichthyoplankton work to verify the identity of morphologically distinct larval types (Hare *et al.* 1994). The primary appeal of using a sequence-based identification approach, in these and a variety of other ecological studies, is that a single methodology using a limited amount of tissue can be used to discriminate a broad range of species. Historically the drawback to the large scale implementation of sequence based identification has been the time and cost requirements per sample.

Here we present a methodology that capitalizes on recent advances in molecular techniques and equipment to make sequence-based identification suitable for a large-scale larval fish study. Specifically this methodology is: 1) high throughput (>800 individuals/week), 2) highly automated, 3) suitable for work with small amounts of

tissue, 4) easily modified for use across a broad taxonomic range of species and 5) provides results readily interpreted with a simple bioinformatics approach. We show the utility of these techniques for a few taxa of great economic importance throughout the tropics, tunas (family: Scombridae) and billfish (family: Istiophoridae), and present preliminary data on the species composition of these taxa in the Straits of Florida. This paper supports the scaling up of barcode databases, and demonstrates that sequence based identification is sufficiently advanced to be applied in ecological studies requiring the identification of large numbers of individuals.

Materials and methods

Collections of larvae and voucher tissue

Larval fishes used in this study were collected in the Straits of Florida as a part of a project focused on the larval ecology and spawning strategies of billfishes and other pelagic fish species. Larvae were collected with a coupled asymmetrical MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System) equipped to simultaneously trigger a 4-m² 800 µm mesh net and a 1-m² 150 µm mesh net (Guigand *et al.* 2005) and a combined neuston net (1x2 m -1000 µm mesh net attached to a .5x1 m 150 µm mesh net). Samples were immediately fixed in 95% ethanol. They were placed in 70% ethanol for long term storage 2-10 days following collection. Samples were genetically identified 1-3 years after collection.

All larval fish were separated from the remaining plankton and identified morphologically to the lowest possible taxonomic category. These morphological identifications generally ranged from family to genus level. Larval fishes were measured

using either the Image Pro Analysis software (Media Cybernetics, Silver Spring, MD) or an ocular micrometer, and ranged in size from 2.5 mm to 13 mm SL. A majority of these were at the lower end of the size range, reflective of the typical pattern of abundance at size for fishes collected in ichthyoplankton studies. The eyeball and/or tail was removed from each larval fish for genetic analysis. Tissue removal sought to avoid damage to the otoliths (ear bones located in the skull) and guts of the fish, so that each individual could also be used in growth and diet studies.

Voucher sequences for each species were obtained from adult tissue from a variety of sources. Istiophorid voucher specimens (4 species: *Istiophorus platypterus*, *Makaira nigricans*, *Tetrapturus albicans*, *T. pfluegeri*) were collected from fishes tagged by the NOAA Southeast Fisheries Science Center billfish tagging program in the western North Atlantic. *Thunnus* adult voucher specimens (5 species: *T. atlanticus*, *T. albacares*, *T. thynnus*, *T. obsesus*, *T. alalunga*) were collected by NOAA fishery observers stationed onboard longline vessels in the Gulf of Mexico. Additional sequences (Finnerty and Block 1995; Chow et al. 2003; Hyde et al. 2005) of all these species were obtained from GenBank for the cytochrome-b (*cytb*) gene used in this study. These additional sequences resulted in inter-ocean differences being included in the voucher sequences for those species occupying both oceans. For each species 2-5 sequences of newly collected specimens from the western North Atlantic study region and 1-5 additional GenBank sequences were used to create the voucher sequences. Sequences (Ward *et al.* 2005) of the *cox1* gene were also obtained from Genbank for comparative purposes. For each species a single sequence containing polymorphic sites was generated from all the voucher sequences of that species.

DNA isolation

All reactions were performed in 96-well plates. Tissue was not added to three of the wells to serve as negative controls for the DNA isolation. These negative controls also functioned as secondary identifiers of the orientation and numbering of the plates. An additional two wells were not included in the isolation procedure, and instead were used as a positive and negative control for the PCR reaction.

DNA isolation was automated on the Evolution P³ liquid handling robot (Perkins Elmer, Wellesley, MA, USA) and used the Genfind kit (Agencourt, Beverly, MA, USA). This kit functions by the absorption of DNA to magnetic beads in the presence of the provided buffers, followed by the removal of contaminants, and then the release of DNA from the beads in the presence of aqueous buffers. Prior to isolation, tissue samples were placed in a 30 μ l lysis buffer (containing 24 μ l Genfind lysis buffer, 3 μ l 18 mg/ml proteinase-K and 3 μ l 1 M DTT) and were shaken for 6-16 hours at 57° C and 1400 rpm on a Thermomixer (Eppendorf, Hamburg, Germany). Convex domed plastic tops (Bio-Rad, Hercules, CA, USA) were used to seal each well individually and prevented cross-contamination unlike other sealing methods. After the samples were digested, the plates were centrifuged for 5 min at 4000 rpm to prevent cross-contamination during the removal of these tops.

The DNA isolation protocol for the liquid handling robot can accommodate up to 8 plates (768 samples) at a time. It requires approximately 1.5 hours of unattended robotic handling time and 30 min of manual labor (excluding the placing of tissue samples in the plates) regardless of the number of plates. The first step in the protocol was the addition to each well of 30 μ l of Genfind lysis buffer followed by 30 μ l of

Genfind binding buffer (containing the magnetic beads) and the transfer of the plate to the stacker for 5 min. The plates were then moved to a magnet on the work platform for an additional 5 min and the liquid was removed, leaving the DNA bound to the beads in each well. The following steps were then repeated twice: 1) the plate was removed from the magnet, 2) 200 µl of Genfind protein wash was added, 3) the plate remained off the magnet for 5 minutes before being transferred to the magnet for an additional 5 min, and 4) the liquid was removed. Further purification was achieved (with the plate on the magnet) by washing the DNA bound to the beads four times with 100 µl of 70% EtOH. The beads were then allowed to dry for a 15 min period before the DNA was eluted in 50 µl of 0.5mM Tris pH 7.5. During this elution the plate was removed from the magnet for 10 min and then placed on the magnet for 5 min before the purified DNA was transferred to a new 96-well plate.

In order to test the effectiveness of the automated DNA isolation procedure, the yield and quality of DNA obtained from using this technique was compared to a standard ammonium acetate DNA isolation (Sambrook and Russell 2001) across a wide size range of larvae. From 20 larval tuna DNA was isolated from one eyeball using the automated technique and the other eyeball using the ammonium acetate DNA isolation. Yield and quality of DNA were evaluated spectrophotometrically and by gel electrophoresis respectively.

PCR and sequencing reactions

PCR amplification of a 715-bp fragment of the *cytb* gene used the primer pair CBF-A (5'-CCC TCT AAT ATC TCQ GTC TGA TGA AA-3') and CB3-3 (5'-GC GTA

GGC AAA TAG GAA RTA TCA YTC-3' (modified from Palumbi 1996). The reactions had a final volume of 50 μ l and contained 12 μ l of the DNA template, .2mM dNTP, 20 pmoles of each primer, 0.4ul of Taq, and a reaction buffer (final concentrations: 50 mM tris HCl pH 9.2, 16mM (NH₄)₂SO₄, 2.25 mM MgCl₂, 2% DMSO, 0.1% Tween 20) (Paschall *et al.* 2004). Template DNA concentrations ranged from not detectable (<1 ng/ μ l) for the smallest larvae to approximately 100 ng/ μ l for the largest. Manual pipetting was used to make and aliquot the PCR mix, and then the liquid handling robot was used to transfer DNA to the PCR plate. The thermocycling protocol was 50 cycles of [94° C for 15 s, 55° C for 30 s and 72° C for 1 min] followed by 5 min at 72° C and utilized a DNA tetrad Thermocycler (Bio-Rad, Hercules, CA, USA). Samples were electrophoresised on a 1% agarose gel to verify results. PCR products were then purified using the standard AmPure kit (Agencourt, Beverly, MA, USA) protocol on the liquid handling robot, but with a 1 to 1 ratio of magnetic beads to PCR reaction volume. For non-voucher specimens, sequencing was unidirectional using 3.2 pmol of CBF-A primer, 0.5 μ l of BigDye Terminator (Applied Biosystems, Foster City, CA, USA), reaction buffer diluted to 1x and 2 μ l of template in a 10 μ l reaction. The sequencing thermocycling protocol consisted of 40 cycles of [94° C for 15 s, 50° C for 20 s and 60° C for 4 min]. For voucher specimens both primers were used in bidirectional sequencing in order to increase the reliability of the voucher sequences. Sequences were purified on the liquid handling robot using the standard CleanSeq kit protocol (Agencourt, Beverly, MA, USA) and then run on a 3730 xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence analysis

Sequence analysis was performed in MATLAB (MathWorks, Natick, MA, USA) using procedures in the publicly available MBEToolbox (Cai *et al.* 2005) and a custom written script (available from drichardson@rsmas.miami.edu). A single run of this script produces the identifications and statistics for an entire 96-well plate. The input files are the *.phd.1 files from the sequencer. One critical component of these files is an estimated probability of error of each base-call in the sample sequence. This value is termed the Phred score and is defined by the expression $q = -10 \cdot \log(p)$, where p is the probability of error (e.g. a Phred score of 30 corresponds to a probability of error of 10^{-3}). A variety of parameters in the trace data of the DNA sequencer are used to calculate the Phred score, with empirical tests revealing that these scores are very accurate in predicting the probability of base-call errors (Ewing and Green 1998).

The script for species identification allows the user to choose a set of voucher sequences (e.g. all istiophorids, all scombrids), based on the taxonomic resolution of the morphological identification of the larvae. Sample sequences in groups of 8 and all the voucher sequences are aligned using alignSeqFile procedure in the MBEToolbox. Sample nucleotides that do not align with nucleotides in the voucher sequences are removed. Additionally, only non-polymorphic sites in the voucher sequences are used in all of the comparisons. The Phred score assigned to each sample nucleotide is the lower of the nucleotide specific Phred score and a 5 nucleotide moving average Phred score.

Two sequence comparisons are used for species identification. The first is a full sequence length comparison to each voucher sequence. As a measure of reliability, the number of correctly and incorrectly paired nucleotides are determined in each of three

Phred score categories (1-20, 21-40 and 41+) for each voucher sequence to sample sequence pair. The second comparison uses only informative diagnostic characters. To obtain these informative sites, voucher sequences from each possible species pair are compared to extract the nucleotide positions that differ between the two species. At these informative sites the sample sequence (Phred score > 20) is compared to both of the voucher sequences. The number of nucleotides in the samples sequence that matches each of the voucher sequences at these informative sites is quantified. The end result of this step of the script is an $n \times n$ (n =number of voucher sequences) matrix of the number of matching diagnostic nucleotides obtained from each comparison between voucher sequences.

The results from these two comparisons are used to generate a most likely identification of the sample. Criteria for species identification (across the portion of the sample sequence with phred scores >20) are: 1) >300 bp in length 2) less than a 5% difference with the voucher sequence and 3) >80% of informative nucleotides matching the identified species for every possible comparison with another species. These criteria are readily changed in the script, and can be tailored to different identification needs. For this specific study, more stringent values were chosen for the informative nucleotide comparison than the total sequence match criteria. This was done because each larva could be morphologically identified to a narrow set of species, all of which were included in the voucher sequence file. The implementation of more conservative total sequence match criteria would be desired in instances where the unknown sequence may represent a species not included in the voucher sequence file.

The final output of the script is a comma delimited text file containing the results of these informative nucleotide and total sequence match comparisons for all 96 sequences. This file can be readily opened in Microsoft EXCEL and other spreadsheet programs. This allows for the manual inspection of sequence quality, comparison data, and suggested identifications. An example of this output from the larvae of two closely related species is shown in Table 2.1.

Results

A total of 493 tuna and billfish larvae were subjected to sequence analysis, with 438 being successfully sequenced and identified on the first try. The overall success rate for identifying these samples was 89%. This success rate did not vary between the billfish and tunas (Chi-test $p=.54$). The size of the larvae analyzed did not play a role in the success rate of the technique (Chi-test $p=.40$). Low quality sequences were obtained for the blanks from one of six plates. These sequences matched a taxonomic group not included on the plate, indicating that one of the reagents used in the technique was contaminated, but that well to well contamination did not occur. All of the other 20 blanks did not produce any sequence.

Total DNA yields ranged from not measurable (< 50 ng) to 9834 ng for the 20 larval tuna eyeballs used to test the magnetic bead extraction technique. In all but 1 of the 20 paired eyeball comparisons the amount of DNA yielded using the GenFind kit automated on the liquid handling robot was lower than the amount yielded using the manual ammonium acetate DNA isolation. On average the GenFind kit yielded 43% as much DNA as the ammonium acetate DNA isolation. The quality of the DNA yielded by

the two types of extractions appeared similar on an agarose gel. The extent to which the automated DNA yields were reduced relative to the ammonium acetate DNA yields did not appear to vary across the size range of larvae used, nor did it negatively impact the identification rate of these larvae.

The use of this technique resulted in high quality sequences. On average, 355(+/- 93) base calls had a Phred score >40, 449 (+/- 82) had a Phred score >30 and 563(+/- 61) had a Phred score > 20 in successfully identified samples. Low quality Phred scores generally occurred at the beginning of the sequence, the end of the sequence and at two locations within the sequence (Figure 2.1).

Interspecific differences in the *cytb* gene were sufficient for the identification of all of the species we tested. The number of diagnostic characters available to separate *Thunnus* species were low (3-15) but sufficient for species level identifications (Table 2.2). For the billfishes many more diagnostic characters (15-25) existed to separate species. Polymorphic sites ranged from 3-13 for billfish and 2-11 for the *Thunnus* species. These values included inter-ocean differences for most species. As a 655 bp portion of the *cox1* gene has been sequenced for the *Thunnus* species it was possible to make a between gene comparison of the number of diagnostic characteristics separating each species pairs. For all but one comparison the *cytb* gene contained more diagnostic characteristics than the *cox1* gene (Table 2.2). These differences were generally relatively small. Polymorphic sites in the *Thunnus cox1* gene based on 5 specimens for each species were lower (0-3) than in the *cytb* gene.

Versus morphological identification techniques, the larval composition of large pelagic species in our study area was more finely resolved with the molecular

methodology (Table 2.3). In addition to pinpointing which species were most abundant as larvae in the Straits of Florida, the samples identified to date also indicate that some species which occur as adults in the region do not occur as larvae. This latter conclusion could not be reached with morphological identification techniques due to the large numbers of unidentifiable larvae. For the istiophorids, morphological and molecular techniques indicated a much different species composition. This is explained by the fact that sailfish can be morphologically identified to species over a much broader size range than blue marlin, and are thus overrepresented in the species composition values.

Discussion

The inability to identify individuals to species is one of the major factors limiting the questions that can be addressed in many ecological studies, including those focused on the early life stages of fishes. In this paper we have demonstrated a high-throughput species identification technique that should prove to be a critical tool in elucidating the life-history strategies and interactions of a wide range of species. To make this technique both time and labor efficient, we automated DNA isolation and other laboratory steps and reduced manual pipetting to the making and aliquoting of 3 master mixes (Lysis Buffer, PCR mix, sequencing mix). This automation allows multiple plates to be run simultaneously with only minimal increases in total time requirements, while in turn reducing the potential for pipetting errors and the misplacement of samples. In practice, the factor limiting the number of samples that can be identified, and the most labor intensive portion of the technique, is the placement of tissue samples in individual wells. Importantly the DNA isolation technique we used, while not as efficient in yielding DNA

as traditional isolation techniques, did produce enough DNA for multiple reactions from even the smallest tissue samples. This makes the technique suitable for identifying small organisms, such as larval fishes, without compromising the integrity of the sample for use in additional studies.

In addition to requiring minimal labor, this technique was also very successful. The 89% success rate of the technique on the first try with larvae was higher than the 68% rate for billfish identification using an RFLP technique (Luthy *et al.* 2005a), but lower than the 95% rate using a species-specific multiplex PCR assay (Hyde *et al.* 2005). Preservation methods used with the larvae may be the primary sources of these differences, as Luthy *et al.* (2005a) included some larvae preserved in BHT buffered ethanol, while Hyde *et al.* (2005) worked with larvae just after their collection. This 89% success rate was also equal to or better than 5 different DNA isolation methods evaluated using fish tissue samples (Hajibabaei *et al.* 2005). Within plate cross-contamination found in early runs was eliminated by switching to convex domed plastic tops during the lysis buffer digestion and centrifuging the samples to remove condensation on the tops prior to their removal. However an undetermined contaminated reagent was used in one plate of reactions. This resulted in low quality sequences of a non-target species in the blank wells. These contaminant sequences were readily identified during analysis. Highlighted by this particular contaminated run is the importance of the extensive control process used with this methodology.

The bioinformatics approach we used proved effective at species identification and reduced the time requirements of the technique in two different ways. The first is that the preprocessing of the sequencer data is not required; rather a single run of the

MATLAB script automatically inputs all the files from a run of a 96-well plate and provides a readily interpreted output. The second is that by incorporating Phred scores into the analysis, less accurate, but more efficient unidirectional sequencing can be used. Assuming a comprehensive sequence library has been developed, misidentifications from the method described here, would require multiple sequencing errors at informative sites, an unlikely scenario given the Phred score cutoff of 20 ($p=.01$ of an error for each nucleotide) used in the program. As with any analytical approach for species identification, sources of errors or ambiguities in identification are most likely to result from an insufficient number of individuals being used to develop a representative sequence for a species and closely related co-occurring species not being sampled. For the specific application described here neither of these concerns appeared to be an issue.

Unlike most other studies that use distance or tree-based method to match sample sequences to species, this study utilized a character based method. De Salle et al. (2005) argued for the use of character based methods for a number of reasons including the similarity to classical taxonomic methods and the ease versus distance methods in establishing a fail-to-identify criteria. Additionally, character-based methods are very suitable for use with sequences containing errors, whereas tree-based methods will often group sample sequences containing random errors on more distant branches than species closely related to the one being identified. While this may not affect the suggested identification of tree-based programs, it will make the visualization of these results more ambiguous than the visualization of diagnostic characters. On the other hand, the character based program used in this study is not designed for use with large voucher sequence databases as are most of the distance methods. This is not a factor in this

specific application, as all the larvae can be morphologically identified to at least family level, but will be an issue to address in future modifications of the program.

The development of this methodology was focused on supporting a comparative study of the spawning strategies of large and medium size pelagic fishes. Because of this, voucher specimens were collected from only a limited number of families, and the degree to which this technique is useful for other species was not established. This issue is ultimately dependent upon the universality of primers being used and the extent of between-species differences in sequences. Both these issues were addressed in the sequencing of the *cox1* gene from 207 Australian fish species (Ward *et al.* 2005). In that study, inter-specific differences were sufficient for the identification of all the species, while combinations of two forward primers and two reverse primers were sufficient for successful PCR amplification. Of all the genera that Ward (2005) considered, *Thunnus* contained the least between-species differences, and thus represents a critical test of the suitability of a gene for identification purposes. Our comparison indicates that the *cytb* gene provides slightly more characters for species identification than the *cox1* gene, but also contains more polymorphic sites. Despite these differences both genes can equally well be applied to species identification in the taxa considered here. Currently the *cox1* gene is being set as the standard for species identification with large sequence databases being generated. Future applications of our methodology with additional species should capitalize on these efforts by switching to the *cox1* gene.

The development of a large sequence database as part of the barcode of life project, and more readily utilized sequencing techniques, such as the one described here, will greatly expand the questions that can be addressed through ichthyoplankton studies.

Currently, the limitations of established identification systems necessitates that many studies use only family or genus level data, despite consistent indications that the ecology and distribution patterns of fish early life stages differ between even closely related species. As an example, within the wrasse (Labridae) family between-species differences exist in the timing and nature of spawning events (Robertson 1991), pelagic larval durations (Victor 1986) and temporal patterns of settlement (Sponaugle and Cowen 1997). Likewise, highly-migratory pelagic species spawn at times and in locations that reflect a unique tradeoff between the habitat preferences of adults and the need to place larvae in an area suitable for their survival. The species composition data presented in this study reflects this as some species present in the region as adults were not represented in the larval catches. A move towards species-level identifications in ichthyoplankton studies, especially in the tropics, will undoubtedly reveal many interesting strategies for spawning and surviving the larval stage that had previously been obscured by the available coarse resolution identification methods.

Over the long term, one of the primary rationales for developing sequence databases and making identifications more readily available is that these tools will enable us to more comprehensively understand and assess biodiversity (Hebert et al. 2003b; Blaxter 2004). Large-scale ichthyoplankton surveys, which commonly collect hundreds of thousands to millions of fishes, may have a unique role in accomplishing this goal. It is well recognized that many traditional means of quantifying diversity in the marine environment greatly underestimate species occupying cryptic habitats (Ackerman and Bellwood 2000) or deep-water environments (Grassle and Maciolek 1992). As with nearly all teleost fishes, most species occupying these cryptic and deep-water habitats

have a pelagic larval stage that occupies the upper couple hundred meters of the water column and is readily collected in net tows. For Elopomorph fishes (true eels, bonefish, tarpon etc.) which possess a leptocephalus larval stage that can be identified to species, larval collections have proven to be a strong tool in assessments of regional diversity (Miller 1995; Minagawa et al. 2004; Richardson and Cowen 2004; Wouthuyzen et al. 2005), and have indicated that many species of fishes remain to be collected and described as adults (Bohlke 1989; Smith 2002). Biodiversity assessments of many other high-diversity taxonomic groups with similarly cryptic species would greatly benefit from the use of plankton tows and a readily utilized means of species identification.

Ultimately an effective sequence-based identification system will require a well developed database of high-quality sequences matched to voucher specimens, as well as a rapid means of generating identifications from unknown samples. Here we have demonstrated laboratory techniques that will make populating these databases and generating identifications from unknown samples more efficient. Additionally, we have shown that once quality voucher sequences are established, it is possible to use rapid analytical techniques that also reduce the amount of required laboratory work by allowing for unidirectional sequencing. When combined, these laboratory and analytical techniques make sequence-based identification time and cost effective for use in large-scale ecological studies.

Table 2.1. Output of the bioinformatics script for identifying individuals to species. For each sample two comparisons are made. The portion on the left contains the results of the comparison of the entire sample sequence against each of the voucher sequences, with the results (# wrong/total) broken down by Phred scores. The right box contains the results of comparisons at informative nucleotides. In parentheses is the total number of informative nucleotides that separate the two species. The first number is the percentage of informative nucleotides in the sample sequence matching the species in the row heading versus the species in the column heading.

Sample: G2_C10 **Most Likely ID: TALB**

Species	Phred Score				Species	<i>Tatl</i>	<i>Talba</i>	<i>Tthy</i>	<i>Tobe</i>	<i>Tala</i>
	0-20	21-40	41+	Total(>20)						
<i>Tatl</i>	23/62	3/179	4/431	7/610	<i>Tatl</i>	-	0%(3)	56%(9)	60%(5)	77%(13)
<i>Talb</i>	23/56	0/135	0/434	0/569	<i>Talb</i>	100%(3)	-	100%(7)	100%(5)	100%(11)
<i>Tthy</i>	24/69	4/201	7/434	11/635	<i>Tthy</i>	44%(9)	0%(7)	-	25%(4)	67%(15)
<i>Tobe</i>	23/56	0/134	6/428	6/562	<i>Tobe</i>	40%(5)	0%(5)	75%(4)	-	78%(9)
<i>Tala</i>	24/69	5/200	10/431	15/631	<i>Tala</i>	23%(13)	0%(11)	33%(15)	22%(9)	-

Sample: G2_D10 **Most Likely ID: TATL**

Species	Phred Score				Species	<i>Tatl</i>	<i>Talba</i>	<i>Tthy</i>	<i>Tobe</i>	<i>Tala</i>
	0-20	21-40	41+	Total(>20)						
<i>Tatl</i>	19/49	2/119	0/503	2/622	<i>Tatl</i>	-	100%(3)	89%(9)	100%(5)	100%(13)
<i>Talb</i>	19/47	0/74	5/503	5/577	<i>Talb</i>	0%(3)	-	71%(7)	60%(5)	91%(11)
<i>Tthy</i>	20/51	5/146	7/506	12/652	<i>Tthy</i>	11%(9)	29%(7)	-	25%(4)	73%(15)
<i>Tobe</i>	19/47	0/74	6/496	6/570	<i>Tobe</i>	0%(5)	40%(5)	75%(4)	-	89%(9)
<i>Tala</i>	19/51	6/146	13/502	19/648	<i>Tala</i>	0%(13)	9%(11)	27%(15)	11%(9)	-

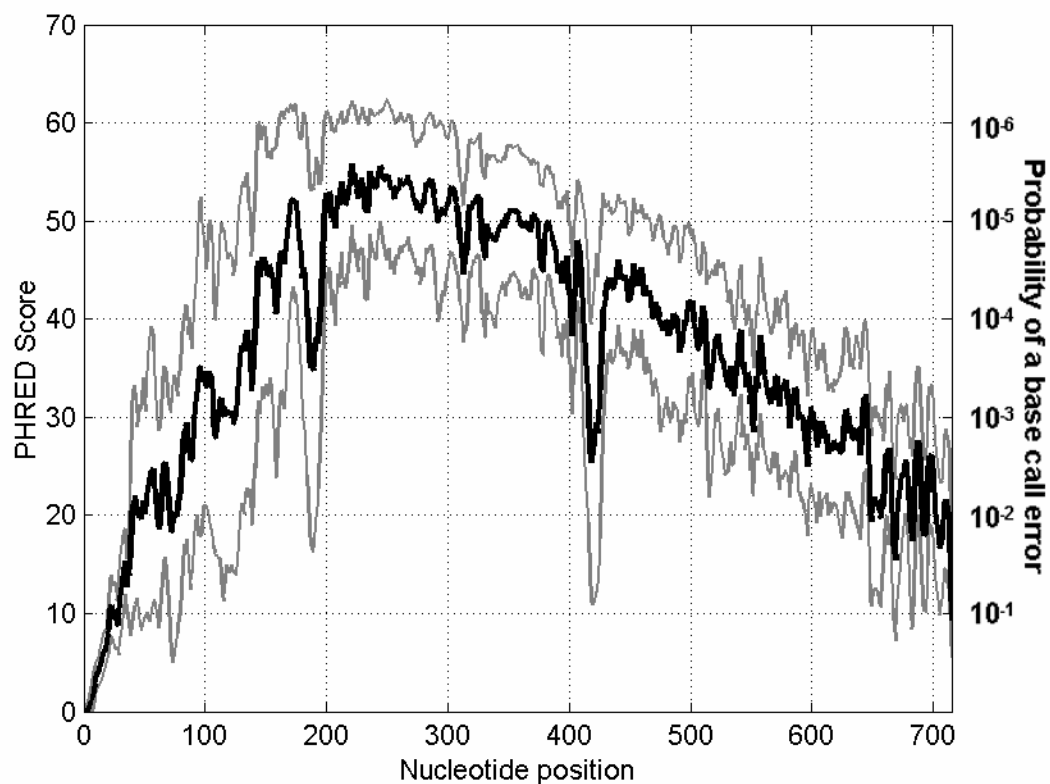
Table 2.2. Number of diagnostic characteristics separating Atlantic *Thunnus* species with the cytochrome-*b* gene sequenced in this study (above the diagonal) and the cytochrome oxidase subunit 1 gene (below the diagonal). Both sequences are similar in length

	<i>T. atlanticus</i>	<i>T. albacares</i>	<i>T. obesus</i>	<i>T. alalunga</i>	<i>T. thynnus</i>	
<i>T. atlanticus</i>		3	5	13	9	<i>cytb</i>
<i>T. albacares</i>	2		5	11	7	
<i>T. obesus</i>	4	2		9	4	
<i>T. alalunga</i>	9	9	9		15	
<i>T. thynnus</i>	6	4	4	9		
	cox1					

Table 2.3. Species composition of billfishes and tuna in the Straits of Florida obtained using morphological identification techniques versus the molecular methodology presented in this study. The molecular identification numbers represent only those individuals which could not be identified to species through morphological means.

Taxonomic group	Morphological identification only	Molecular identification on remaining
Istiophoridae unknown	46%	12%
<i>Istiophorus platypterus</i>	42%	18%
<i>Makaira nigricans</i>	12%	69%
<i>Tetrapturus albidus</i>	-	1.3%
<i>Tetrapturus pfluegeri</i>	-	0%
Scombridae		
<i>Thunnus</i> unknown	100%	10%
<i>T. atlanticus</i>	-	81%
<i>T. albacares</i>	-	12%
<i>T. thynnus</i>	-	0.8%
<i>T. obsesus</i>	-	0%
<i>T. alalunga</i>	-	0%

Figure 2.1. Average (black) +/- standard deviation (grey) of a 5 bp moving average of Phred scores and the associated probabilities of base call errors over the 715 bp sequence of successfully identified *Thunnus* and istiophorid larvae (n=311).



3. LARVAL ASSEMBLAGES OF LARGE AND MEDIUM SIZED PELAGIC SPECIES IN THE STRAITS OF FLORIDA

Critical gaps in our understanding of the distributions, interactions, life histories and preferred habitats of large and medium size pelagic fishes severely constrain the implementation of ecosystem-based, spatially-structured fisheries management approaches. In particular, spawning distributions and the environmental characteristics associated with the early life stages are poorly documented. In this study we consider the diversity, assemblages, and associated habitat of the larvae of large and medium sized pelagic species collected during two years of monthly surveys across the Straits of Florida (SF). In total, 36 taxa and 14,295 individuals were collected, with the highest diversity occurring during the summer and in the western frontal region of the Florida Current. Only a few species (e.g. *Thunnus obesus*, *T. alalunga*, *Tetrapturus pfluegeri*) considered for this study were absent. Small scombrids (e.g. *T. atlanticus*, *Katsuwonus pelamis*, *Auxis* spp.) and gempylids dominated the catch and were orders of magnitude more abundant than many of the rare species (e.g. *Thunnus thynnus*, *Kajikia albida*). Both constrained (CCA) and unconstrained (NMDS) multivariate analyses revealed a number of species groupings including: 1) a summer Florida edge assemblage (e.g. *Auxis* spp., *Euthynnus alleterattus*, *Istiophorus platypterus*), 2) a summer offshore assemblage (e.g. *Makaira nigricans*, *T. atlanticus*, *Ruvettus pretiosus*, *Lampris guttatus*), 3) an ubiquitous assemblage (e.g. *K. pelamis*, *Coryphaena hippurus*, *Xiphias gladius*), and 4) a spring/winter assemblage that was widely dispersed in space (e.g. trachipterids). The primary environmental factors associated with these assemblages were sea-surface temperature (highest in summer-early fall), day length (highest in early summer),

thermocline depth (shallowest on the Florida side) and fluorescence (highest on the Florida side). Overall, the results of this study provide insights into how a remarkable diversity of pelagic species spatially and temporally partition spawning within a region characterized by dynamic oceanography and strong habitat gradients.

Introduction

Large (>50 kg) and medium (1-50 kg) sized pelagic teleosts support many important fisheries around the world. Included within this group are species notable for their high annual yields (e.g. skipjack tuna), high price per unit weight (e.g. bluefin tuna), and high value in recreational fisheries (e.g. blue marlin), as well as several rarely encountered enigmatic species (e.g. louvar). During the past 60 years, the overcapitalization of fishing fleets and technological changes in fishing methods (reviewed in Sharp 2001) have led to the depletion of this group as a whole and the reduction of many individual species to <10% of their pre-exploitation biomass (Sissenwine et al. 1998; Myers and Worm 2003; Ward and Myers 2005). In response to these system-wide population declines, and due to the recognition that the effects of overfishing extend beyond the targeted species, there have been a number of recent calls for ecosystem-based management approaches within pelagic fisheries (Botsford et al. 1997; Pauly et al. 2002; Pikitch et al. 2004). However, for such management approaches to be effective critical gaps must be filled in our understanding of the distributions, interactions, life histories, and preferred habitats of these difficult to study species.

The study of the habitat associations and distributions of migratory pelagic fishes has traditionally focused on three main explanatory factors: 1) the distribution of forage

species, 2) regional hydrographic properties, and 3) species-specific physiological capabilities (Sharp 1978; Brill 1994; Sharp 2001). Elevated concentrations of forage often exist at frontal zones and have been shown to support a higher abundance, and on a global scale, a higher diversity of apex pelagic predators (Worm et al. 2005). Similarly, locations where a vertical concentration of organisms exists also tend to support higher densities of pelagic species, both in the open ocean (Betrand et al. 2002) and where the depth range of vertically migrating mesopelagic species is compacted by bathymetry (Weaver and Sedberry 2001). Within any one location, the accessibility of forage to a pelagic species is controlled by a combination of the species-specific physiology and the temperature, dissolved oxygen, and light levels both in the surface layer and vertically in the water column (Brill 1994; Betrand et al. 2002). This interaction of forage distribution, physiology, and water column properties, is considered to be primarily responsible for the prominent ocean-basin scale distributional differences among tuna species (Fonteneau 1997; Worm et al. 2005).

With some exceptions, the influence of reproduction on the distribution of pelagic species has received less attention than the influence of feeding. Spawning habitat quality in all species is determined by how favorable a location is to the survival of larvae (a function of the availability of prey, the abundance of predators and the proximity to favorable juvenile habitat) and how spawning in that area fits into the energy budget of the adult (a function of the feeding opportunities on the spawning ground and the energy costs associated with migrating to and from it). For species such as the bluefin tuna, where long-distance migrations are made to spawning grounds and residency times on the spawning ground are short (Block et al. 2005; Teo et al. 2007), specific oceanographic

features appear to be chosen that are favorable to the survival of the larvae (Bakun 1996). At the other extreme are species such as skipjack tuna that spawn across their entire range during long spawning seasons, with spawning appearing to occur in any area occupied by the adults with a sea surface temperature (SST) above a minimum level (Schaefer 2001; Lehodey et al. 2003). As fish movement models advance and fish tagging data becomes more sophisticated, gaps in the understanding and parameterization of spawning habitat characteristics have become more apparent and critical to fill.

In addition to influencing the movement patterns of individuals, the oceanographic conditions within the primary spawning area of a species or population can strongly impact the recruitment dynamics of that species or population. These effects occur over a range of temporal scales, but are most dramatically illustrated by the synchronous multi-species fluctuations in recruitment over decadal time scales. For example in the Pacific, population cycles of skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) tend to fluctuate synchronously and in opposition to albacore tuna (*T. alalunga*). It is thought that multi-year climatic changes that positively effect the survival of early life stages in the shared skipjack and yellowfin tuna spawning habitat negatively affect survival in the albacore spawning habitat (Bakun and Broad 2003; Lehodey et al. 2003). Similar environmental controls on recruitment are ubiquitous in pelagic fish populations, and have led some to conclude that the “environment-free” population models commonly used in fisheries management are inherently destined to fail (Sharp 2001).

The collection and identification of the early life stages of fishes is one of the most tractable ways to obtain information on spawning patterns and recruitment

dynamics. Such collections have been used to identify spawning areas for a range of large pelagic species including, bluefin tuna, swordfish, blue marlin and white marlin (Richards 1976; Govoni et al. 2003; Serafy et al. 2003; Prince et al. 2005), and on a more limited basis have been used to identify oceanographic features associated with higher larval abundances (McGowan and Richards 1989). To date, most studies have focused on either a single species or have made comparisons within a single family. This contrasts notably with the multi-species research on adult pelagic species (Block *et al.* 2003) and the long standing tendency in early life history research to consider larval assemblages, or groups of co-occurring species (e.g. Cowen et al. 1993; Grothues and Cowen 1999; Hare et al. 2001). Work on larval assemblages specifically has been useful in determining commonalities in the distribution of early life stages that reflect similarities in spawning strategies and in the processes that drive successful recruitment.

Here we consider the diversity, assemblages, and associated habitat of the larvae of large and medium size pelagic fishes collected during two years of monthly surveys in the Straits of Florida (SF). The dynamic oceanography within this region, the strong spatial gradients in key habitat features (e.g. thermocline depth, productivity), and the seasonal changes in other characteristics (e.g. SST and wind patterns) make it a particularly suitable region to explore the influence of the environment on the spawning and early life history strategies of pelagic fish. This information will complement further analyses of these collections targeted at understanding the trophic environment of these larvae, the factors influencing their growth and survival, and the biomass of spawning individuals.

Materials and Methods

Study location

The Straits of Florida (SF) encompasses the narrow (70-150 km) region between Florida and Cuba to the south, Cay Sal Bank (CSB) to the southeast and the Great Bahama Bank (GBB) and Little Bahama Bank (LBB) to the east (Fig. 3.1). Maximum water depths decrease from about 2000 m along the southern SF to 800 m in the northern SF. Steep topography characterizes the Cuba and Bahamas side of the SF, whereas a comparatively more gradual slope occurs along the Florida edge of the SF.

Flow in this region is dominated by the Florida Current (FC) which links the Loop Current in the eastern Gulf of Mexico to the Gulf Stream to the north. Maximum current speeds within the FC are generally about 2m/s (Leaman et al. 1987). On the Florida side of the FC, the presence and absence of cyclonic gyres accounts for much of the variability in patterns of flow. When the Loop Current penetrates far into the Gulf of Mexico, the current tends to overshoot the entrance to the SF, resulting in the formation of cyclonic gyres along the Florida edge of the current (Lee et al. 1994). These gyres range in size from 100-200 km and tend to remain in the southern SF for many months before moving downstream and decreasing in size due to bathymetric constraints. Alternatively, when the Loop Current in the Gulf of Mexico sheds an anticyclonic ring, the current tends to flow directly from the Yucatan Channel to the entrance of the SF, in turn shifting the core of the current towards the Florida edge of the SF and hindering the development of mesoscale cyclonic features.

Oceanographic processes are generally less well explored on the eastern and southern edge of the SF than along the Florida side. Repeated surveys in the Santaren

Channel (separating CSB from the GBB) and Northwest Providence Channel (separating GBB from the LBB) have revealed that these channels on average contribute 1.8 and 1.2 Sv, respectively, of the ≈ 29 Sv of total flow exiting the SF (Leaman et al. 1995). During certain times reverse flow does occur along portions of these channels (Leaman et al. 1995). Drifter tracks (<http://www.iaslinks.org/>) and surface radar measurements (<http://iwave.rsmas.miami.edu/wera/>) also indicate that anticyclonic eddies can occur in all of these areas, though the persistence and frequency of these features is unknown.

In addition to the differences in current patterns, the SF contains strong gradients in other environmental characteristics. Isopleths of temperature and oxygen slope strongly downward from Florida to the Bahamas and Cuba. One result of this is the presence of oligotrophic waters off the Bahamas in contrast to the nearshore more productive waters off Florida (Hitchcock et al. 2005). Frontal zones form along all portions of the SF, though the most persistent ones occur along the Florida side of the current and are characterized by sharp density gradients and strong current shear. In the center of the SF a frontal zone can form where the main current, that passes between CSB and Florida, converges with flow from the Santaren Channel. Along the eastern edge of the SF a frontal zone can form where water flowing off the Bahamas Banks converges with offshore waters.

The seasonal cycles in atmospheric conditions in the Straits of Florida have a notable effect on the regional oceanography. Prevailing winds shift from the southeast in the summer to the northeast in the fall, and the east-northeast in the winter. Wind intensity also increases from summer to winter, as does the variability in both intensity and direction due to the increased passage of cold fronts. These shifts in wind direction

and intensity ultimately impact the current patterns and zones of downwelling and upwelling in the SF, effects that are especially prominent in the nearshore environment (Lee and Williams 1999). Additionally, the summer months are characterized by increased precipitation and higher air temperatures which in turn drive maxima in sea surface temperatures and stratification and minima in coastal salinity during these months.

Sampling protocol

During 2003 and 2004, surveys were performed near the beginning of each month along a 17-station, 80 km long transect crossing the Straits of Florida at 25°30' N (Fig 3.1). The R/V F.G. Walton Smith was used during all cruises. Stations were more tightly spaced along both edges of the transect than in the center. Biological sampling occurred during the day using a combined MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System) equipped to simultaneously sample a 4-m² 1000 µm mesh net and a 1-m² 150 µm mesh net (Guigand et al. 2005) and a combined neuston net (1x2m 1000 µm mesh net attached to a 0.5x1 m 150 µm mesh net). The MOCNESS system contained temperature, salinity, fluorometry and light sensors, and measured the volume of water sampled by each net. Individual nets were triggered in 25 m depth intervals, and the entire system was deployed to 100 m at all but the first station where shallow bathymetry limited the tows to 50 m depth. The neuston net was towed with half of the frame out of the water, and the volume of water filtered was measured with a General Oceanics flowmeter.

At each station physical measurements were made through the entire depth of the water column using a CTD equipped with Seabird sensors measuring temperature, salinity, fluorescence, oxygen, beam transmission, and light levels. Surface temperature, salinity, and fluorescence were measured continuously at <60 s intervals during the entire duration of each cruise via a flow through system. Continuous measurements of the currents were also made using both a 600 kHz (2 m depth bins from 4-40 m) and a 150 kHz (8 m depth bins from 14-200 m) RDI Acoustic Doppler Current Profiler (ADCP).

Generally the entire transect was sampled within a 48-hour period. The most common pattern of sampling involved net sampling along the western half of the transect during the first day, followed by CTD casts along the second half of the transect during the night. For the second day of sampling the boat proceeded in the opposite direction, sampling the second and first portions of the transect with the MOCNESS and CTD respectively. This sampling strategy was extremely time efficient, but as with any ship-based sampling did not present a synoptic picture. Of particular note are that 1) near the center of the transect adjacent stations were sampled 24-hours apart, and 2) depending on the station, CTD casts and net tows were separated by 1-48 hours. Because of the latter issue, if possible, physical data from the MOCNESS or the underway systems, rather than the CTD, were used when analyzing larval distributions.

For 21 of the 24 monthly cruises, biological sampling was successfully performed at ≥ 16 of the 17 stations. The exceptions were the December 2003 cruise (10 stations sampled), the January 2004 cruise (10 stations sampled), and the November 2004 cruise (7 stations sampled). Suspension of sampling during each of these three cruises was weather related. For 12 additional stations a single depth bin of the large-mesh nets was

not successfully collected, and the station was omitted from the analyses. In total, all depth bins at 371 stations were successfully and completely sampled for this study.

Environmental data processing

The environmental parameters used in the analyses were: 1) sea surface temperature (SST), 2) 100 m integrated fluorescence (a proxy for chlorophyll), 3) depth of 20° C isotherm, 4) depth of 3 ml/l oxygen isopleths, 5) day length, 6) sea surface density gradient ($\Delta \sigma_t$), and 7) horizontal current shear. At each station SST was measured with the MOCNESS using values in the 3 m bin to ensure the sensor had equilibrated and to avoid the warmer surface skin that occurs during the day in low wind conditions. Integrated fluorescence values were also calculated from the MOCNESS. For the first 5 cruises the MOCNESS did not contain a fluorometer, and thus CTD fluorescence measurements, that were not collected concurrently with the plankton tows, were used. Analyses of the larval fish distribution data both with and without these non-synoptic values determined that this had little effect on the results. All fluorescence values were standardized to the CTD units of measurements using calibrations derived from stations where the CTD and MOCNESS were towed sequentially.

Both the 20° C isotherm and 3 ml/l oxygen isopleths were derived from CTD measurements. At the westernmost shallow (≈ 70 m) station, the 3ml/l oxygen level was frequently not reached and thus the bottom depth was used in the analyses. Day length was determined using the time of sunrise and sunset on the U.S. Naval Observatory website (http://aa.usno.navy.mil/data/docs/RS_OneYear.html). Sea surface density gradients were determined from the underway measurements using the Webster method

of detecting discontinuities (Legendre and Legendre 1998). Measurements were first averaged within 0.001° longitude (≈ 100 m) bins. The gradient value was then calculated as the difference between the average value in 2 km windows on either side of the point.

Horizontal current shear was calculated using the 6 m depth bin from the 600 kHz ADCP. One-minute average files were created using the WINADCP software.

Subsequent processing removed data with Percent Good-4 (percentage of measurements with four beam solutions) values below 80%, or with boat speeds < 2 m/s. Data collected when boat speeds were slow and the ship was on station tended to have higher errors, possibly due to the erratic cruise track during these times. Data were then averaged in 0.01° longitude (1 km) intervals, and smoothed using a 0.04° longitude moving average. Horizontal current shear in the along-Straits direction was calculated as the difference in current speed in locations on 2 km of either side of the station.

Sample processing and larval identification

The targeted species for this study were pelagic species that exceed 1 kg in maximum adult weight (See Table 3.1). This criterion was relaxed to include species belonging to families with large and medium size pelagic species (e.g. Gempylidae and Scombridae). Overall, the targeted species included most of the teleost fish caught in the pelagic longline fishing operations and a number of other smaller species. Excluded from the study were sphyraenids and carangids, which occupy both pelagic and coastal habitats, and often recruit to inshore waters as juveniles.

Sample processing began with the removal of all of the larval fish from the 1000 μm mesh samples. The finer mesh samples were not considered for this portion of the

project. All individuals were then identified morphologically following Richards (2006a), generally to the family level, though if possible, the pelagic taxa were identified to species. Taxa considered in this study that could not be identified morphologically to species-level were identified molecularly following Richardson et al. (2007). This sequence-based identification methodology is based in concept on the barcode of life project (Hebert et al. 2003a). More specifically, a high degree of automation is achieved in its use through a magnetic bead-based extraction procedure and a MATLAB character-based bioinformatics script. Both the cytochrome-*b* primers described in Richardson et al. (2007) and the cytochrome oxidase subunit 1 primers listed in Ward et al. (2005) were used.

The extent to which molecular identification was employed varied by taxon. Molecular identification was used for all istiophorids not identifiable using lower jaw pigment patterns or snout length measurements (Luthy et al. 2005a), and coryphaenids not identifiable using characters in Ditty (2006). *Thunnus* larvae were only identified molecularly because of uncertainty in whether external morphological characteristics are in fact diagnostic (Richards et al. 1990). Due to their high abundance, a subsampling approach was instituted for stations with >5 *Thunnus* larvae. At these stations, five larvae plus an additional 10% of the remaining larvae were identified molecularly. For the *Auxis* larvae, individuals <5 mm SL that lacked midlateral pigment in the caudal region required molecular identification. The same subsampling approach used for *Thunnus* larvae was applied to these larvae. *Thunnus* and *Auxis* larvae not molecularly identified were assigned a species randomly based on the observed ratio of molecularly identified individuals at that station. No attempts were made to molecularly identify a

number of less common or less economically valuable families to species. These included trachipterids, trichiurids, bramids, alepisaurids, and a fifth grouping composed of lophotids and regalicides.

Abundance calculations

Abundances (number of larvae per 10,000 m²) of each taxon were calculated for each station. Abundance calculations assume that the entire depth range of each taxon was sampled. For most taxa this assumption appears to be valid. However, >20% of the individuals of three taxa, Alepisauridae (5 of 6), Trichiuridae (36 of 56), and *Diplospinus multistriatus* (158 of 311), occurred in the deepest depth bin (75-100 m) that was sampled. The reported abundances of these taxa are considered to be underestimates though they were retained in the subsequent analyses nonetheless. The overall average abundance values reported for each species were based on a weighted mean in order to account for the influence of station spacing and cruise completeness. Within each cruise, each station was weighted based on the spacing between it and adjacent stations. Subsequently, each cruise was given the same weight to obtain an average abundance value.

Larval assemblage analysis

Species distributions and environmental data were evaluated using both indirect and direct gradient analysis. These approaches are complementary, as the unconstrained indirect ordination allows all the variability in species composition to be evaluated, including that not explained by the environmental factors. On the other hand, the

constrained direct gradient analysis restricts the ordination to axes that are a linear combination of the predetermined set of environmental variables, and thus only identifies variability associated with them. Concordance between the two approaches is indicative of strong support of species groupings and their relationship to measured environmental variables (Leps and Smilauer 2003).

The indirect gradient analysis followed the methods outlined in Field et al. (1982) and the application of these methods to larval fish in Hare et al. (2001). Taxa were included in this analysis if ≥ 20 individuals were collected at ≥ 10 stations. Stations were excluded from the analysis if all of the depth bins were not completely sampled. This resulted in the inclusion of 26 species at 371 stations. The analysis of larval fish assemblages used relativized larval fish abundances, a Bray-Curtis dissimilarity matrix, and hierarchical clustering using the weighted pair group method (WPGMA). Species were also ordinated using non-metric multidimensional scaling of this matrix with a designated two-dimensional solution.

For the direct gradient analysis, a Canonical Correspondence Analysis was performed using the CANOCO software package (version 4.5, Microcomputer Power, Ithaca, NY) following the methods outlined in Grothues and Cowen (1999). All eight environmental variables were used in this analysis. All taxa were included in this analysis, though rare species were downweighted. Larval abundances ($\text{ind}/100 \text{ m}^2$) were $\ln(x+1)$ transformed. Biplot scaling with a focus on interspecies distances was used. Environmental variables were included if their sequential addition was significant at $p < 0.01$ (4999 Monte Carlo simulations).

Diversity calculations

Measures of species density were calculated using sample-based rarefaction curves (species accumulation curves). This analytical technique provides a means to standardize species counts to a given level of sampling effort. Rarefaction curves were computed in the Estimate-S software (Colwell 2006) which implements the algorithms of Colwell et al. (2004) rather than sub-sampling methodologies. For each calculation, stations were used as the unit of sampling-effort. A rarefaction curve was first calculated for the entire sampling effort in the Straits of Florida. Subsequently, seasonal patterns of species-density were evaluated, with stations from both years for each month pooled. We report results for the number of species per 20 stations. Alternative standardizations of 5, 10, and 15 stations were qualitatively similar and are not reported here.

Cross-Straits patterns of diversity were also evaluated using rarefaction, with the number of species standardized to 20 stations reported. Alternative standardizations to 5, 10, and 15 stations (not reported here) were not qualitatively similar to the 20 station standardization. Because of this, an additional analysis was used to consider the persistence of spatial patterns of diversity over time. For this analysis, taxon counts at each station were transformed into z-scores (Legendre and Legendre 1998) by subtracting the cruise mean from the species count at each station and dividing by the cruise standard deviation. These z-scores were then averaged at each station across cruises. Excluded from the spatial diversity comparisons, were the three cruises with <16 stations sampled, all the stations at which a single depth-bin was not successfully sampled, and the westernmost station at which a lesser volume of water was sampled due to the shallow depth of the tows.

Results

Environmental data

Consistent seasonal and spatial patterns were evident in most of the measured environmental characteristics (Fig. 3.2). Day length varied by approximately 3.5 hours over the year, with peak SST trailing day length by about 2 months (Fig 3.3: bottom panel). Peak SST ranged from 28-30° C and extended from early June through early November. Low SSTs of 25-26° C occurred from January to April, and coincided with increased spatial differences in SST. The depth of the 20° C isotherm and the 3 ml/l oxygen isopleths were consistently shallower on the Florida side of the transect. For the 20° C isotherm this depth difference averaged 150 m, whereas for the 3 ml/l oxygen isopleth this difference was approximately 350 m. Increased stratification on the western side of the transect during the summer months resulted in a shallower thermocline than during the winter months. Fluorescence measurements were consistently higher on the Florida side of the transect, though patches of higher fluorescence also occasionally occurred offshore.

Steep gradients in sea surface density and northward current shear tended to occur most frequently on the western side of the transect. This was most notable for the current shear, which except in the case of an offshore shift of the current axis was always very high on the Florida side of the transect and low elsewhere. Most density fronts coincided with gradients of sea surface temperature. The exception to this occurred during some cruises in August to October during which a shallow band of lower salinity (34.8-35.8)

water occurs over the western half of the transect, resulting in salinity-driven surface density fronts on both sides of the low salinity band.

Taxonomic composition

A total of 14,295 individuals representing at least 36 species of large or medium size pelagic fishes were collected over the two years of sampling (Table 3.1). These individuals made up 8% of the 166,903 larval fishes of all taxa collected in the 1000 μm mesh neuston and MOCNESS nets. Five of the smaller scombrid species (*Thunnus atlanticus*, *Katsuwonus pelamis*, *Euthynnus alleteratus*, *Auxis thazard* and *A. rochei*), three of the smaller gempylid species (*Nesiarchus nasutus*, *Diplospinus multistriatus*, and *Gempylus serpens*) and unidentified bramids dominated the catch. Abundances of the larvae of large pelagic species, such as yellowfin tuna (*T. albacares*), bluefin tuna (*T. thynnus*), blue marlin (*Makaira nigricans*), sailfish (*Istiophorus platypterus*) and swordfish (*X. gladius*), were at least an order of magnitude lower than abundances of these smaller species. In aggregate, larvae of large pelagic species accounted for only 4.3% of the total abundance of pelagic species considered for this study. Absent from the samples were five species: albacore tuna (*T. alalunga*), bigeye tuna (*T. obesus*), longbill spearfish (*Tetrapturus pfluergeri*), roundscale spearfish (*Tetrapturus georgii*) and sharptail mola (*Masturus lanceolatus*), that would have been readily identified either molecularly or morphologically.

Larval assemblages

The cluster analysis and ordination identified four primary assemblages (I to IV) with the largest of these assemblages (IV) further dividing into a number of additional groupings (Fig. 3.4a). Species clustered based on the relative abundance of larvae at stations nearest to the Florida coast versus offshore stations (Fig. 3.5) and the seasonality of spawning (Fig. 3.3). A single species, *Scomber colias*, was a distinct outlier from the remainder of the species (Fig. 3.4a,b), as its larvae were caught only in the winter in nearshore waters. The first assemblage (I) encapsulated three taxa, *Neolatus tripes*, Regalicidae/Lophotidae and Trachipteridae that primarily spawn during the winter and spring. The larvae of these taxa were primarily caught along the western half of the transect. Assemblage II contained only two taxa, Trichiuridae and *Neopinnula americana*, both of which had their highest abundance along the eastern half of the transect, and tended to occur year round with a fall to winter peak in abundance. The ordination supported these two species as distinct from most other species, but did not strongly support their grouping (Fig. 3.4b). Assemblage III consisting of *Lampris guttatus*, *Ruvetus pretiosus*, and *Promtichthys promethius* encompassed species collected in the summer at offshore locations.

The remaining 17 species grouped into assemblage IV and could be further subdivided. Within this assemblage, *Istiophorus platypterus* clustered (IVc1) distantly with the strongly supported grouping of *Auxis thazard*, *Euthynnus allutteratus*, *A. rochei*, and *Scomberomorus cavalla* (IVc2). These species were all summer spawners. Larvae of the latter four were almost exclusively found on the Florida side of the transect, while larval *I. platypterus* abundance was highest on both edges of the transect.

Acanthocybium solandri and *T. albacares* clustered together, and in the ordination diagram were close to the previous assemblage (IVa). These species were also summer spawners, with a majority of individuals collected on the western half of the transect, though not exclusively in the nearshore station as were members of the previous group.

The final grouping (IVb) within assemblage IV encompassed the much more spatiotemporally ubiquitous species. *Diplospinous multistriatus*, Bramidae, *Gempylus serpens*, *Coryphaena equiselis*, *Katsuwonus pelamis* and *C. hippurus* larvae occurred year round and were spread out across the transect (Group IVb3). *Xiphias gladius* (IVb1) similarly was found in all seasons and along all portions of the transect. Finally, *T. atlanticus*, *Nesiarchus nasutus* and *Makaira nigricans* larvae were spread across the transect, but were restricted to the summer months (IVb2).

Species environment relationships

The first two canonical axes of the CCA explained 16.8% of the total variation in the distribution of species, with the third and fourth axes contributing an additional 3% combined (Table 3.2). The eigenvalues, which provide an indication of the influence of each axis on the ordination, dropped off over four-fold between the second and third axis. The stepwise forward selection indicated that all 7 of the environmental variables contributed significantly ($p=0.01$, Monte Carlo permutation test) to explaining the species distributions. The first two canonical axes of the CCA explained 81.5% of the total variation in the environment-species relationship. Environmental gradients in the depth of the 20° C isotherm, 3 ml/l oxygen isopleths, and fluorescence, and to a lesser extent current shear and Δ sigma-t explained much of the variation along the first canonical axis.

SST and daylength contributed largely to the second canonical axis (Table 3.2). A moderate correlation between SST and daylength ($r^2=0.45$), a strong correlation between the 20° C isotherm and 3ml/l oxygen isopleth ($r^2=0.91$), and a moderate negative correlation between these two variables and fluorescence ($r^2=-0.50$ and -0.45 respectively) were found (Table 3.3).

The broad grouping of taxa formed by the indirect NDMS analysis tended to also occur in the CCA analysis (Fig. 3.6). *Scomber colias* was again an outlier in the CCA biplot. Group I in the cluster analysis and ordination also grouped together in the CCA. Species that occurred primarily on the western edge of the transect, and were contained in groups IVc and IVa in the cluster analysis, tended to separate out from the other species along the first axis. Along the second axis, year-round spawners (groups II, IVb1, IVb3) tended to separate out from the summer spawners (IVb2 IVa) and spring spawners (II). As with the NMDS ordination, the CCA revealed few clear cut discontinuities, but rather a gradient of species. Additionally, some clusters tended to break down in the CCA, such as the separation of the Cluster III from cluster IVb2. Rare species not included in the indirect analysis occurred in a variety of locations in the CCA plots.

Species diversity and abundance

Sample rarefaction curves calculated across the entire dataset indicated a strong leveling off of taxon-counts between 100 and 200 samples (Fig. 3.7a). Seasonally, species density reached a maximum in June, remained high through October, and then reached a minimum in January (Fig. 3.7b). Spatially, rarefaction of species counts to 20 stations did not reveal cross-straits patterns in diversity (Fig. 3.7c), however the

alternative measure of diversity did reveal a strong spatial pattern (Fig. 3.7d). Diversity using standardized z-scores was highest 3-5 stations offshore of the westernmost station and reached a minimum along the eastern half of the stations.

Discussion

Spawning strategies

Our aim was to determine the environmental properties associated with spawning and larval distributions across the spectrum of large and medium size pelagic fishes. While numerous studies have done this for individual species or small sets of species, no previous work has taken such a comprehensive approach. The results of both types of multivariate analyses (CCA and NMDS) revealed a continuum of patterns of larval distribution in space and time rather than clear discontinuities in species groupings. Additionally the concordance of these analyses indicated that between species distributional differences were well explained by the seven environmental variables we used. In evaluating these results, it is important to note that comparisons of the relative importance of different strongly correlated environmental variables in observational data of this sort are not robust, nor do these correlations necessarily imply a causal relationship (Leps and Smilauer 2003). On the other hand, when viewed in the context of previous work on these species, these results make it possible to infer how spatial and temporal patterns of spawning reflect the habitat preferences of adults and the suitability of an area for the survival of their offspring.

The seasonal component of spawning in fishes is most often related to photoperiod and temperature cues, a factor supported in this study by the strong role both

day length and SST have in explaining the distribution of larvae. In more temperate latitudes, spawning in many species, while cued to these factors, may ultimately be timed to seasonal cycles of productivity (Cushing 1975). This is likely not the case for pelagic species in the SF and other subtropical locations, where specific oceanographic features (e.g. cyclonic eddies) rather than seasonal cycles most strongly influence patterns of productivity (Falkowski et al. 1991; McGillicuddy and Robinson 1997; Hitchcock et al. 2005; McGillicuddy et al. 2007), and summer and year-round spawning dominates despite higher productivity in the winter (Hitchcock unpubl). The importance of daylength and SST in the timing of spawning may thus be attributed to their more direct effects on rates of larval growth and development. Many of the taxa considered in this study exhibit extremely rapid larval growth (De Vries et al. 1990; Govoni et al. 2003; Luthy et al. 2005b; Sponaugle et al. 2005a), a strategy that is likely adapted to minimize the duration of the high mortality larval stage. Spawning in higher temperature waters will positively affect rates of larval growth and development, assuming food is not limiting (Houde 1989; Pepin 1991). Additionally, daily food intake increases with daylength (Llopiz and Cowen, in review), with energetics models in turn suggesting that small increases in food intake have a disproportionate affect on growth rates (Feeley 2006). On a basinwide scale, a spawning strategy maximizing larval growth can account for the reduced seasonality of spawning (i.e. more protracted) in some tuna species at lower latitudes (Schaefer 2001; Margulies et al. 2007). One further prediction of this spawning and life history strategy is that in regions and at times where both daylength and SST are maximized (i.e. summer in the subtropics) higher growth rates, and possibly elevated spawning will occur.

Spatial patterns of spawning and larval distribution in pelagic species have often been evaluated in the context of the distance larvae occur from the shore. For example, in sampling around French Polynesia (Leis et al. 1991) and Hawaii (Boehlert and Mundy 1994) high abundances of *Thunnus* and *Auxis* larvae occurred at nearshore stations, with *Katsuwonus pelamis* larvae occurring farther offshore in the latter study. The highest abundances of black marlin, blue marlin, and sailfish larvae were also found in sampling just offshore of the Great Barrier Reef (Leis et al. 1987). Boehlert & Mundy (1994) attributed the nearshore distributions of pelagic taxa around islands to the enhanced feeding opportunities for the adults and larvae in these locations. In our study, proximity of spawning to a coastline or shallow bank cannot be evaluated. The shallow bathymetry on all sides of the SF, combined with the estimated age range of the majority of larvae (4-15 days based on scombrid and istiophorid measurements) would allow individuals spawned in both nearshore and offshore waters to occur along any portion of our transect. This is reflected in the consistently high abundances of both reef fishes and mesopelagic larvae along all portions of the sampling transect. However, consistent spatial patterns in the collection location of many larvae indicate that other spatially varying environmental characteristics are important in spawning site selection. Importantly, the decoupling between spawning and larval collection sites in the values of many of the other variables considered for this study (20° C isotherm, 3 ml/l oxygen isopleth and fluorescence) is likely minimal due to the major currents following their isopleths.

The three correlated environmental characteristics, 20° C isotherm, 3 ml/l oxygen isopleth, and fluorescence, that accounted for much of the spatial variability of larval distribution in the SF have also been suggested to structure the distribution of adult

pelagic fishes (Sharp 2001). The distribution of these variables in the SF present a unique tradeoff to pelagic species. In comparison to the eastern side, the western SF is more productive but also characterized by vertical profiles of temperature and oxygen that should restrict the depth range of pelagic species and limit their access to deeper food resources. For many species, such as blue marlin, the benefits of being able to forage at deeper depths along the eastern edge of the SF may offset the presumably higher food levels at the surface along the western edge of the SF (Prince and Goodyear 2006). Conversely, restriction to the western edge of the SF of the larvae of a number of smaller scombrid species (e.g. *Auxis* spp.), may be due to the high energy lifestyle of adults and their shallow distribution regardless of water column properties. Support for a similar mechanism controlling the distribution of tuna is provided by a consideration of ocean basin scale fisheries data (Sharp 2001), and a more comprehensive study over a large (16° latitude x 20° longitude) area of the Pacific Ocean (Betrand et al. 2002). In the latter study, differences in regional abundances of tuna could be accounted for by a combination of species-specific physiological capabilities and the vertical profiles of oxygen, temperature and acoustically measured forage concentrations. Across the SF, variability in the vertical profiles of temperature and dissolved oxygen exceeds that found in Betrand et al. (2002) and approaches longitudinal differences across ocean basins (Fonteneau 1997). This makes the SF and other western boundary currents a unique place to apply more process-oriented approaches to studying the combined effects of productivity and vertical habitat characteristics in structuring adult distributional differences. Such studies are necessary to verify the potential mechanism proposed here to account for the between-species differences in the spatial distribution of spawning.

Two other environmental variables that were also strongly patterned in space, density gradient and current shear, had a comparatively small impact in explaining larval fish distributions. High current shear and steep density gradients are associated with convergence of the currents. Areas of high current shear are also associated with the generation of frontal eddies. As such, high current shear and strong density gradients generally lead to biological enrichment, and are predicted to be areas where spawning would occur and/or larvae would accumulate (Bakun 2006). The relatively minor impact attributed to these two variables in the analysis thus runs counter to the general biological importance attributed to these features. Methodological concerns associated with station spacing ($\approx 3\text{-}5$ km) and the narrowness of fronts in the region (often <1 km) may explain some of the ambiguity that resulted from this analysis. Possibly more important is the relatively crude measure (association between larval density and $\Delta\sigma\text{-}t$ or current shear) that was used in an attempt to characterize what is a rather complex and time-varying biophysical process. In this region, multiple types of density fronts occur, each differing in their vertical extent, the mechanism that generated them, and their temporal evolution. A combination of issues such as these led Bakun (2006) to conclude that the systematic sampling design that characterizes the majority of ichthyoplankton studies is poorly suited for addressing issues related to mesoscale and submesoscale features such as fronts and eddies. This assertion is supported by the contrast between the results of this sampling and finer-scale Lagrangian-based sampling around a Florida current frontal eddy which demonstrated elevated sailfish spawning in the front separating the interior of the eddy from surrounding waters (Chapter 4). Illustrated by this contrast is the

importance of recognizing the limitations of any single sampling strategy, and the need to integrate multiple forms of sampling into a study.

Regional diversity

Nearly all previous analyses of patterns of abundance and diversity of predatory oceanic species have relied on catch data from commercial fisheries. Ichthyoplankton studies such as this one, while only characterizing the reproductively active component of the community, provide an alternative means of assessing these patterns. One remarkable result of this sampling is that the larvae of only 5 of 41 possible Atlantic pelagic taxa considered were not collected (*Thunnus alalunga*, *T. obesus*, *Tetrapturus pfluergi*, *T. georgeii* and *Matsurus lanceolata*). Unfortunately, direct comparisons of this diversity to other studies are not possible as this study has not been matched in the fine resolution of species identifications or the magnitude and temporal and vertical completeness of the sampling. Yet, even without a basis for comparison, the results of this study strongly suggest that the Straits of Florida is unique in the diversity of reproductively active large and medium size pelagic species.

Numerous factors may drive the high diversity of larvae collected in the SF including 1) the western Atlantic bathymetry which makes the SF a bottleneck for species migrating between the Atlantic and the Gulf of Mexico or western Caribbean, 2) the fast regional currents that may transport larvae spawned in distant locations to the SF, 3) the persistence and predictable location in the SF of a strong frontal zone and cyclonic eddies, both features considered favorable for spawning, and 4) the presence of strong environmental gradients in both space and time allowing species with different optimum

spawning habitats to occur in a constrained area. The importance of the last two factors is supported by the time-averaged peak in diversity in the most common location of the western FC front. The assemblage analysis suggests that this peak in diversity may result from two faunas mixing at the frontal zone, though it is also likely that some species are specifically spawning at the front. Similarly, on a seasonal basis, the SF supports species with different optimum temperatures for spawning, a factor which likely elevates larval diversity in this area above that in more temperate or tropical latitudes. Similar subtropical peaks in diversity have also been found in an analysis of commercial fishing data (Worm et al. 2005). However in contrast to our study, in which diversity peaked through the summer months at SST > 28.5° C, that study found a peak in adult diversity at intermediate SST of about 25° C. This likely reflects the tendency of many species to spawn at the higher SST portion of their adult range.

One of the contentious debates with respect to pelagic fisheries concerns the extent to which industrialized fishing has resulted in a community-wide shift from large pelagic species, such as blue marlin, swordfish and bluefin tuna, to the smaller components of the community including bramins, *Auxis* spp., and snake mackerels (Ward and Myers 2005). Myers and Worm (2003) asserted that 50 years of fisheries data indicate over 90% declines in large predators, a contention that has been strongly questioned (Hampton et al. 2005). The essence of this debate concerns the analysis of fisheries dependent data, and how to account for the changing catchability of different species in light of constant changes in gear, techniques, targeted species and the spatial allocation of fishing operations. Highlighted by this debate is the undeniable need for fisheries-independent data in assessing fish stocks, especially as more multi-species

management approaches are considered. Ichthyoplankton studies such as ours can fulfill this need, due to their minimal species level bias and their readily quantifiable results. On a community wide basis, the results of our study indicate that medium size pelagic species are many times more abundant than large pelagic species. This may reflect the sampling location, but for many of the large pelagic species (e.g. swordfish, blue marlin) the abundances collected in the SF were as high as or higher than have been reported elsewhere. Unfortunately, while ichthyoplankton samples have been collected in the SF going back decades (Richards 1976), the data on the full diversity of pelagic species remain unpublished, compromising our ability to address whether the regional species composition has changed.

One large pelagic species for which past ichthyoplankton sampling does allow decadal scale comparisons to be made in the SF is bluefin tuna. Richards (1976) collected 123 bluefin tuna larvae while sampling (with a 333 μm mesh 1 m plankton net towed at the surface for 10 minutes) three stations on 31 different days from early April to early July of 1969-1971. Additionally, a five station cross-Straits transect, at nearly the same latitude sampled by this project, was also sampled 11 times in 1975. This sampling used a surface net of the same dimensions and mesh as used in this project. In 55 tows, 38 bluefin larvae were collected. Bongo net tows to 200 m depth at these same stations yielded an additional 23 bluefin larvae. Both the 1969-1971 and the 1975 set of net tows had a similar magnitude of sampling during the bluefin spawning season as this study. Our collection of only 3 larvae thus stands in sharp contrast to these much higher numbers 30 years prior. It cannot be ruled out, but is considered unlikely, that the higher bluefin tuna numbers in the older studies resulted from morphological misidentifications.

Specifically, the morphological identification criteria of Richards (2006b), the same author of this previous study, were applied in our study prior to molecular identification, without incorrectly identifying bluefin tuna. Such a contrast between the historical numbers and the more recent sampling thus likely reflects the precipitous decline in the western North Atlantic bluefin tuna spawning stock that has been seen across multiple datasets and is reflected in the stock assessments (Scott et al. 1993; Sissenwine et al. 1998). Interestingly, a similar comparison between historical swordfish collections and the recent sampling in the Straits of Florida does not show such a precipitous drop. Grall et al. (1983) reported a catch rate of 138 swordfish larvae at 263 stations in the Straits of Florida during sampling from 1953-1972. These numbers are comparable to the catch rate of 198 larvae at 371 stations in this study, though the early studies used such a range of gear (e.g. dip nets, conical nets, neuston nets) that more detailed comparisons are not possible. An extension of comparisons, such as these for bluefin tuna and swordfish, to components of the pelagic community that are not assessed but are taken in fisheries (e.g. opah, escolar), or are not targeted by fisheries but may have a substantial ecological role (e.g. *Auxis* spp.), could provide substantive evidence for or against broader community-wide changes in species abundance.

Conclusions

The dataset described herein provides the most complete description to date of the patterns of spawning across the suite of large and medium-sized pelagic species. Most previous studies have constrained their efforts to a select few targeted species, which while very useful, run in sharp contrast to the multi-species nature of pelagic fisheries,

and by extension, the need for multi-species approaches in their management.

Importantly, the benefits of this type of analysis will expand as more comparative and similarly comprehensive datasets from different regions become available. At the same time, it is important to recognize the limitations of the correlational approach with respect to addressing the critical questions concerning patterns of the movement and recruitment in pelagic species. Ongoing analyses of these collections with a focus on the feeding ecology (Llopiz and Cowen in review) and growth rates (Sponaugle et al. 2005a) of larvae are better suited to exploring the mechanisms that underlie recruitment variability. Additionally, sampling focused on the temporal development of submesoscale features (Chapter 4) and the tagging of adult fish during the spawning season (Chapter 5) have provided additional information on finer-scale spawning habitat associations and individual-level spawning related movements. In aggregate, these approaches can identify the mechanisms that link environmental conditions to the behavior and population dynamics of predatory pelagic species. This ultimately will provide useful information for the management of these species, and will represent a critical step towards predicting how these species will respond to changes in climatic conditions over the next decades.

Tables

Table 3.1. Larval abundances in the large mesh nets. Code corresponds to the identifier used in the figures. Maximum weights are based on either the record size fish caught in the Atlantic recreational fishery (www.igfa.org) or Fishbase (Froese & Pauly 2000). Abundance values indicated with a star are considered underestimates because of the likely occurrence of some individuals below the deepest sampled depth. The method used to identify each taxa is also indicated (Morp=Morphological, Molec=Molecular, Sub=Subsampled ID: see text). Relatively higher N values than abundance values occur for species frequently collected in the surface net tows.

Species	Common name	Family	Code	Max. Weight (kg)	Abundance (N/10,000 m ²)	Positive stations	Identification method			
							N	Morph	Molec	Sub
<i>Katsuwonus pelamis</i>	Skipjack tuna	Scombridae	Kpel	19	1216.08	298	2130	2128	2	-
<i>Thunnus atlanticus</i>	Blackfin Tuna	Scombridae	Tatl	20	1105.04	213	3383	-	1037	2346
<i>Nesiarachus nasutus</i>	Black gemfish	Gempylidae	Nnas	3	925.19	202	1515	1515	-	-
<i>Diplospinus multistriatus</i>	Striped Escolar	Gempylidae	Dmul	1	*695.65	199	1002	1002	-	-
<i>Euthynnus alletteratus</i>	Little Tunny	Scombridae	Eall	12	517.46	69	1705	1705	-	-
<i>Gempylus serpens</i>	Snake Mackerel	Gempylidae	Gser	3	301.72	208	651	651	-	-
Bramidae	Pomfrets	Bramidae	Bram		266.27	199	436	436	-	-
<i>Auxis thazard</i>	Frigate Mackerel	Scombridae	Atha	1	218.71	97	549	456	63	30
<i>Auxis rochei</i>	Bullet Mackerel	Scombridae	Aroc	1	181.30	79	586	176	196	214
<i>Coryphanea hippurus</i>	Dolphin	Coryphaenidae	Chip	40	127.65	193	477	400	77	-
<i>Coryphanea equiselis</i>	Pompano dolphin	Coryphaenidae	Cequ	5	67.17	129	204	166	38	-
<i>Thunnus albacares</i>	Yellowfin tuna	Scombridae	Talb	176	66.71	46	181	-	84	97
Trichiuridae	Cutlassfish	Trichiuridae	Tric		*60.41	52	97	97	-	-
<i>Neopinnula americana</i>	American sackfish	Gempylidae	Name	1	45.66	47	83	83	-	-
<i>Ruvettus pretiosus</i>	Oilfish	Gempylidae	Rpre	45	44.85	32	79	79	-	-
<i>Makaira nigricans</i>	Blue Marlin	Istiophoridae	Mnig	636	44.48	85	249	59	190	-
<i>Istiophorus platypterus</i>	Sailfish	Istiophoridae	Ipla	61	43.57	92	310	240	70	-
<i>Acanthocybium solandri</i>	Wahoo	Scombridae	Asol	67	28.03	36	56	56	-	-
<i>Scomber colias</i>	Atlantic Chub Mackerel	Scombridae	Scol	1	27.53	14	99	99	-	-
Lophotidae/Regalecidae	Unicornfish/Oarfish	Order:Lampridiform	Lamp		26.75	35	48	48	-	-

Table 3.1. continued

Species	Common name	Family	Code	Max. Weight (kg)	Abundance (N/10,000 m ²)	Positive stations	N	Identification method		
								Morph	Molec	Sub
<i>Xiphias gladius</i>	Swordfish	Xiphiidae	Xgla	650	26.08	117	198	198	-	-
<i>Scomberomorus cavalla</i>	King Mackerel	Scombridae	Scav	42	24.14	35	69	68	1	-
<i>Lampris guttata</i>	Opah	Lamprididae	Lgut	270	18.16	24	29	29	-	-
<i>Nealotus tripes</i>	Black Snake Mackerel	Gempylidae	Ntri	1	17.50	24	32	32	-	-
Trachipteridae	Ribbonfish	Trachipteridae	Trac		17.24	29	35	35	-	-
<i>Prometichthys prometheus</i>	Roudi escolar	Gempylidae	Ppro	1	15.84	21	29	29	-	-
<i>Lepidocybium flavobrunneum</i>	Escolar	Gempylidae	Lfla	45	12.14	11	15	15	-	-
Alepisauridae	Lancetfish	Alepisauridae	Alep	5	*5.09	6	7	7	-	-
<i>Ranzania laevis</i>	Slender mola	Molidae	Rlae	20	5.00	8	9	9	-	-
<i>Scomberomorus regalis</i>	Cero	Scombridae	Sreg	8	3.86	7	8	8	-	-
<i>Mola mola</i>	Ocean sunfish	Molidae	Mmol	2000	2.15	2	2	2	-	-
<i>Epinnula magistralis</i>	Sackfish	Gempylidae	Emag	1	1.74	2	5	5	-	-
<i>Thunnus thynnus</i>	Bluefin tuna	Scombridae	Tthy	678	1.31	1	3	-	3	-
<i>Luvarus imperialis</i>	Louvar	Luvaridae	Limp	150	1.26	3	4	4	-	-
<i>Kajikia albida</i>	White marlin	Istiophoridae	Kalb	82	1.25	7	9	-	9	-
<i>Scomberomorus maculatus</i>	Spanish mackerel	Scombridae	Smac	5	0.14	1	1	1	-	-
TOTAL							14295	9838	1770	2687

Table 3.2. Summary statistics for the canonical correspondence analysis (CCA) of 36 taxa and 371 stations

	AX1	AX2	AX3	AX4
Eigenvalue	0.234	0.204	0.046	0.031
Species-environment correlation	0.834	0.849	0.514	0.487
Cumulative percentage variance				
of species data	9.0	16.8	18.6	19.8
of species-environment relation	43.6	81.5	90.1	95.9
Inter-set correlations of environmental variables with axes				
SST	0.14	-0.79	0.10	-0.09
20° C isotherm	-0.70	-0.25	-0.02	0.19
3 ml/l oxygen isopleth	-0.60	-0.28	0.01	0.29
day length	0.37	-0.49	-0.30	0.15
fluorescence	0.52	0.40	0.20	0.15
Δ sigma-t	0.26	0.00	-0.05	0.06
current shear	0.38	0.05	0.14	0.01

Table 3.3. Correlations of environmental variables used in the CCA

	<i>SST</i>	<i>20° C isotherm</i>	<i>3 ml/l oxygen isopleth</i>	<i>day length</i>	<i>fluorescence</i>	<i>Δ sigma-t</i>
20° C isotherm	0.07	-	-	-	-	-
3 ml/l oxygen isopleth	0.06	0.91	-	-	-	-
day length	0.45	-0.06	0.07	-	-	-
fluorescence	-0.25	-0.50	-0.45	-0.12	-	-
Δ sigma-t	-0.04	-0.21	-0.21	0.08	0.14	-
current shear	-0.05	-0.35	-0.30	0.00	0.22	0.11

Figures

Figure 3.1. Straits of Florida and adjacent regions with sampling transect and primary flow pattern. Abbreviation on figure: SF: Straits of Florida, FC: Florida Current, LC: Loop Current, YS: Yucatan Strait, GOM: Gulf of Mexico, CAR: Caribbean Sea, CSB: Cay Sal Bank, GBB: Great Bahama Bank, LBB: Little Bahama Bank, SC: Santaren Channel, NPC: Northwest Providence Channel.

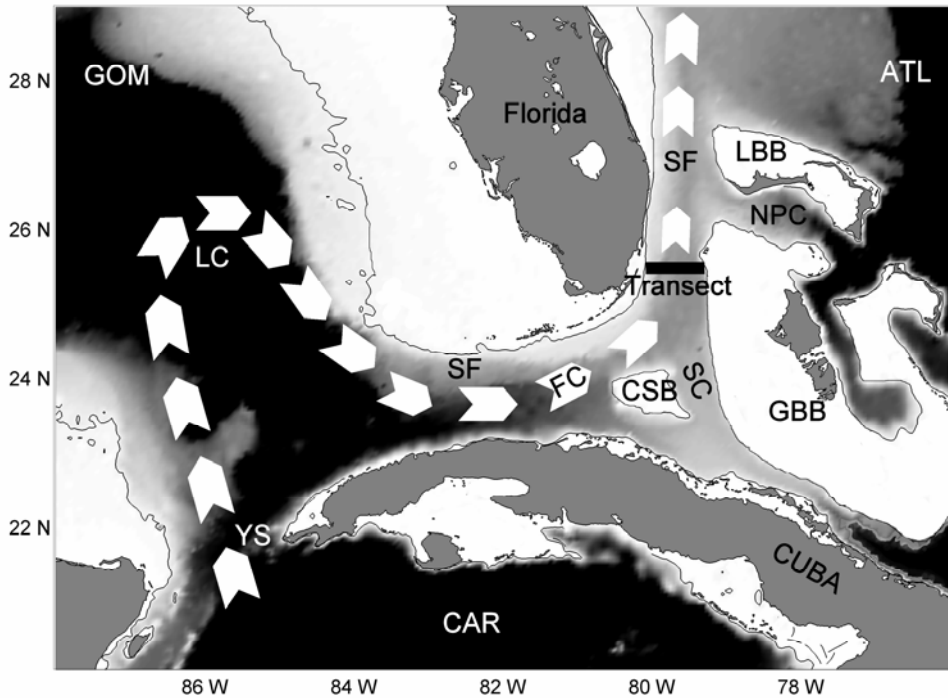


Figure 3.2. Spatial and temporal patterns of environmental variables used in the canonical correspondence analysis. Longitude of sampling is indicated on the x-axis, month of sampling along the y-axis. Stations sampled with the MOCNESS are indicated with an X. Gaps in the data indicate stations that were not sampled with either the MOCNESS or CTD. Fluorescence is the 100 m integrated measurements (V) from the CTD.

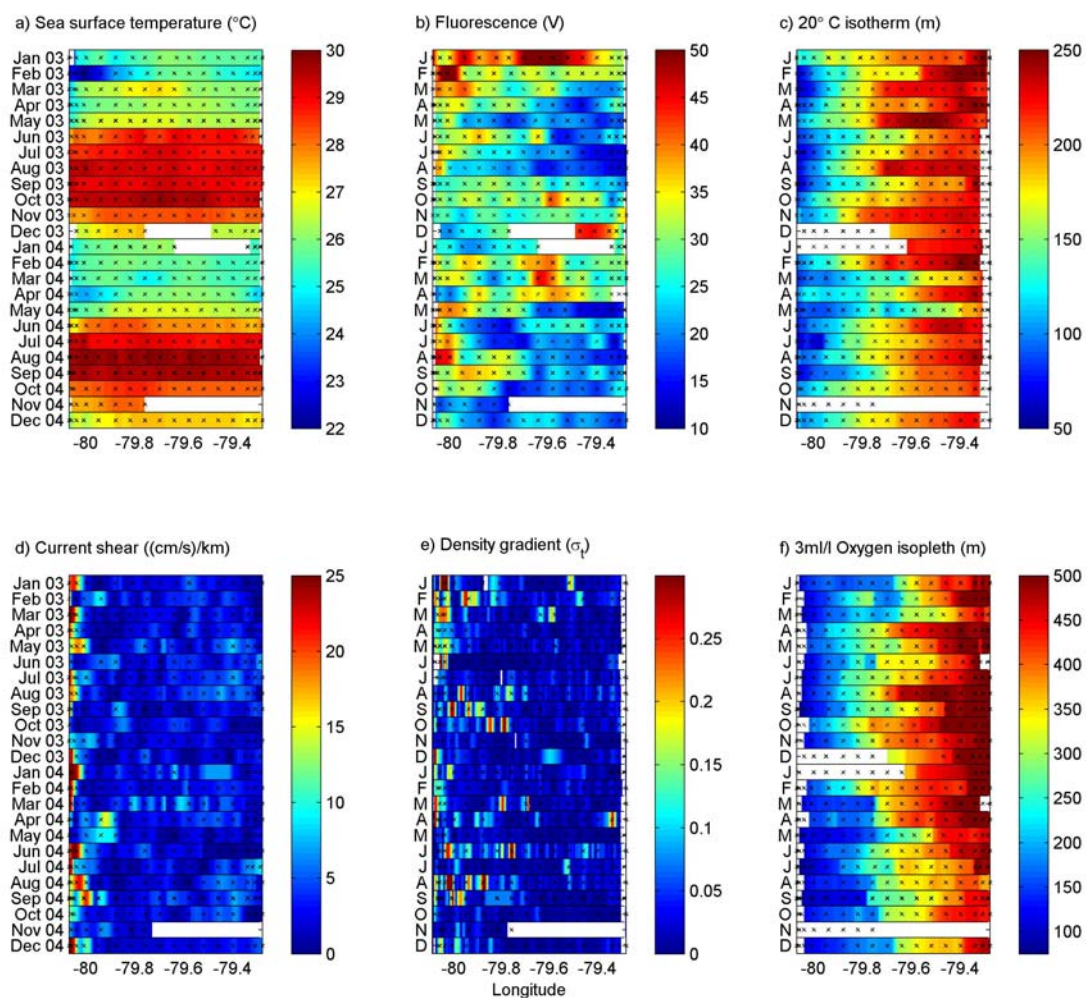


Figure 3.3. Temporal patterns of larval occurrence of pelagic species during the two years of monthly sampling in the SF. Width of band is scaled to the maximum abundance of that species collected on any one cruise. Day length and SST mean and range for each cruise are plotted at the bottom of the figure. Species codes used in the labeling are in Table 3.1. Species are grouped and labeled based on the results of the cluster analysis.

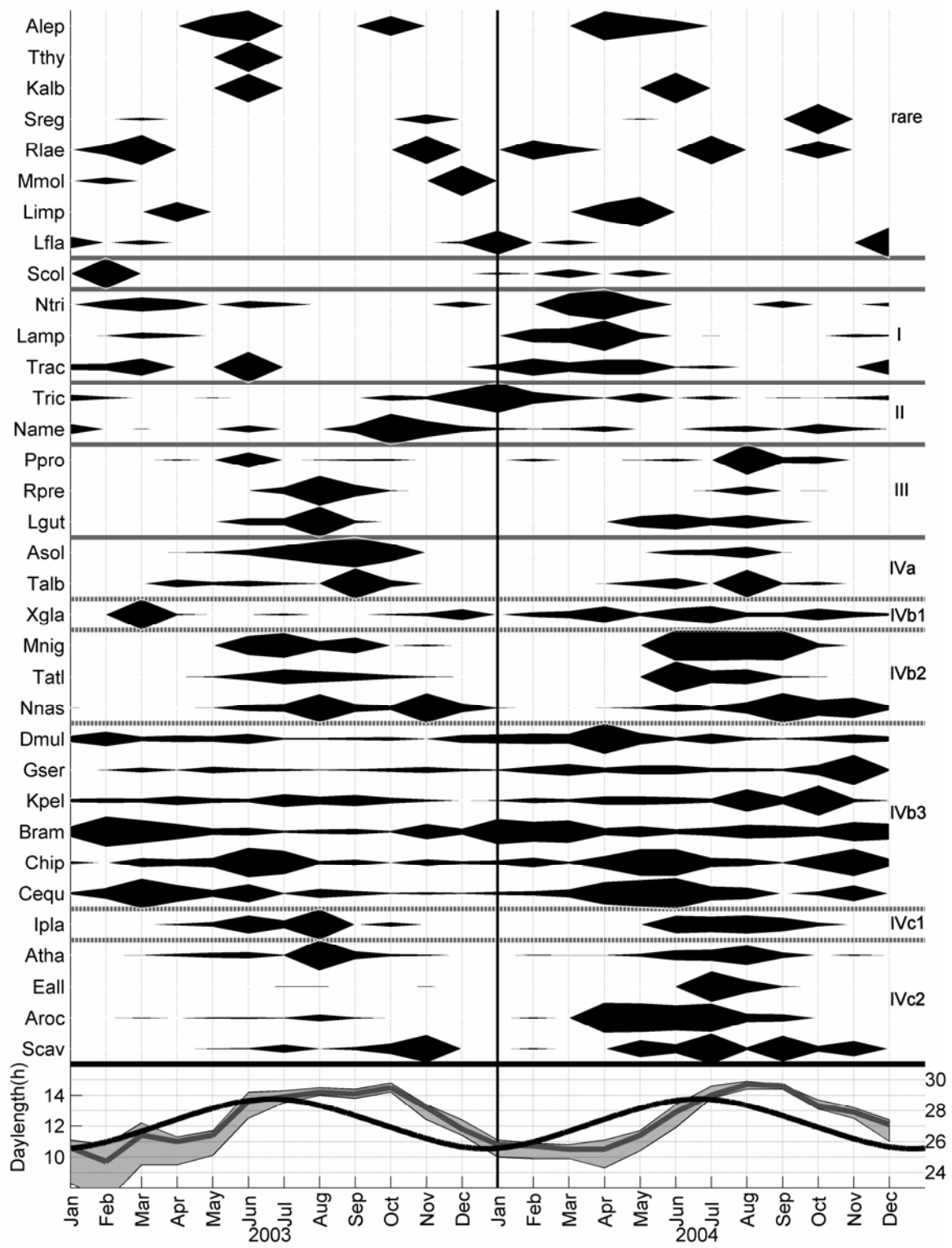


Figure 3.4. Classification of larval fish assemblages using relativized larval fish concentrations and a Bray-Curtis similarity matrix. a) Results of the WPGMA clustering. Four main assemblages, and a number of groups and subgroups within assemblages were defined. b) Non-metric multiple dimensional scaling ordination of the same matrix with assemblages and groups noted. Species codes used in the labeling are in Table 3.1.

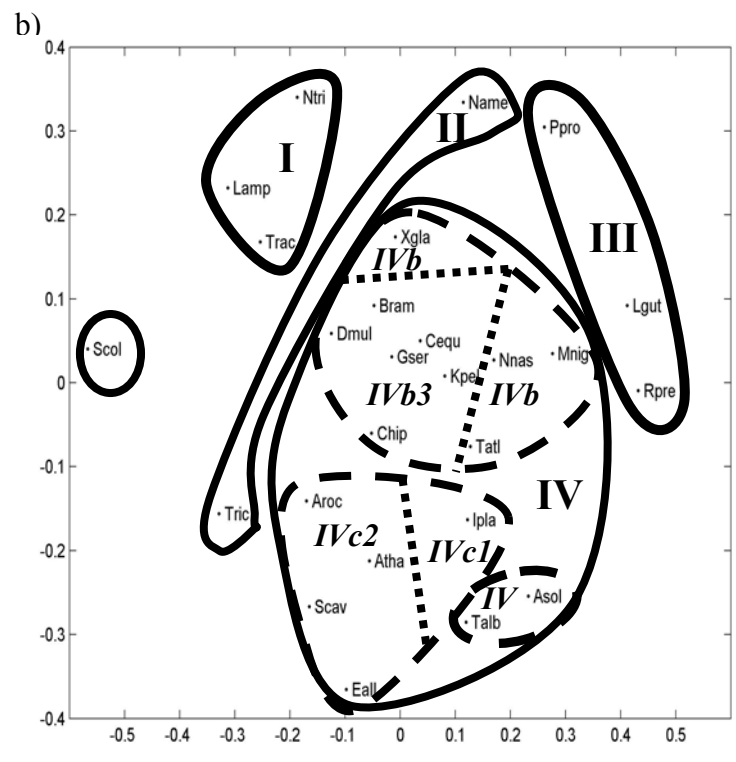
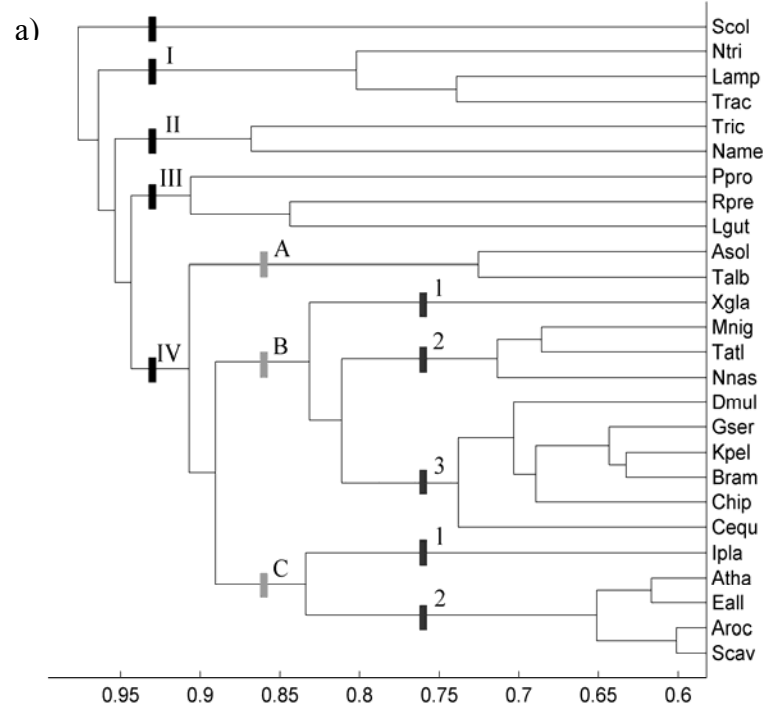


Figure 3.5. Cross-straits patterns of larval occurrence of pelagic species during the two years of monthly sampling in the SF. Width of band is scaled to the maximum abundance of that species at a station averaged across cruises.. Species codes used in the labeling are in Table 3.1. Species are grouped and labeled based on the results of the cluster analysis.

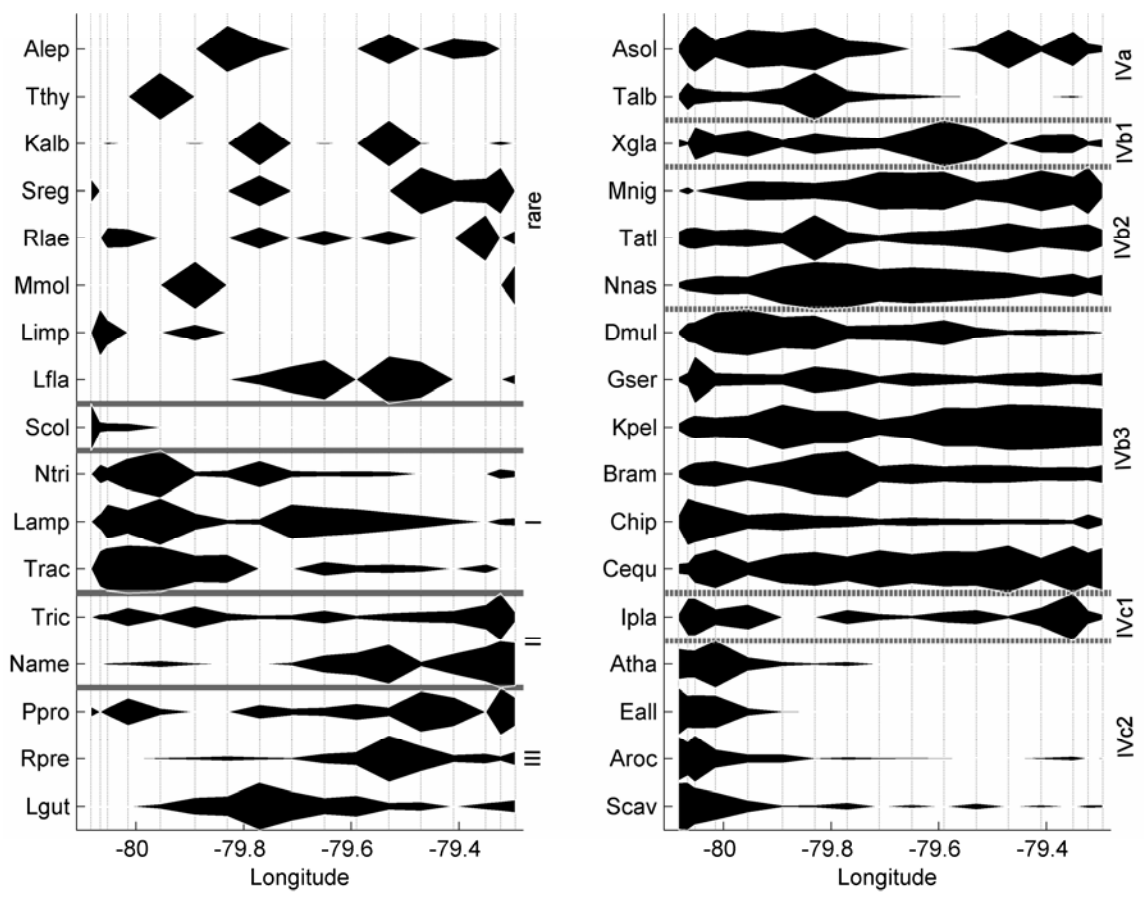


Figure 3.6. CCA ordination of taxonomic groups and environmental variables. Species groupings from the cluster analysis are indicated. Species codes used in the labeling are in Table 3.1.

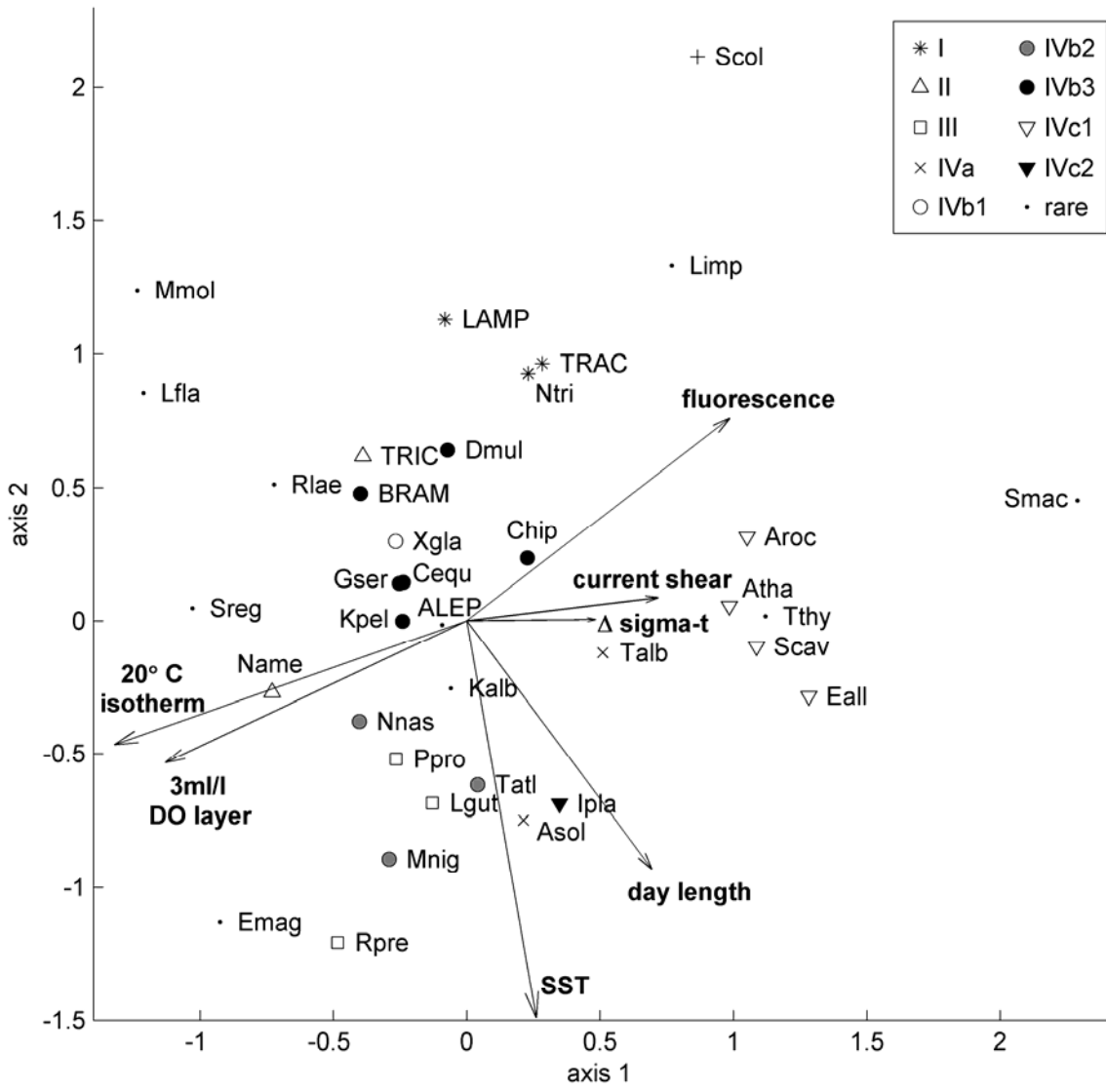
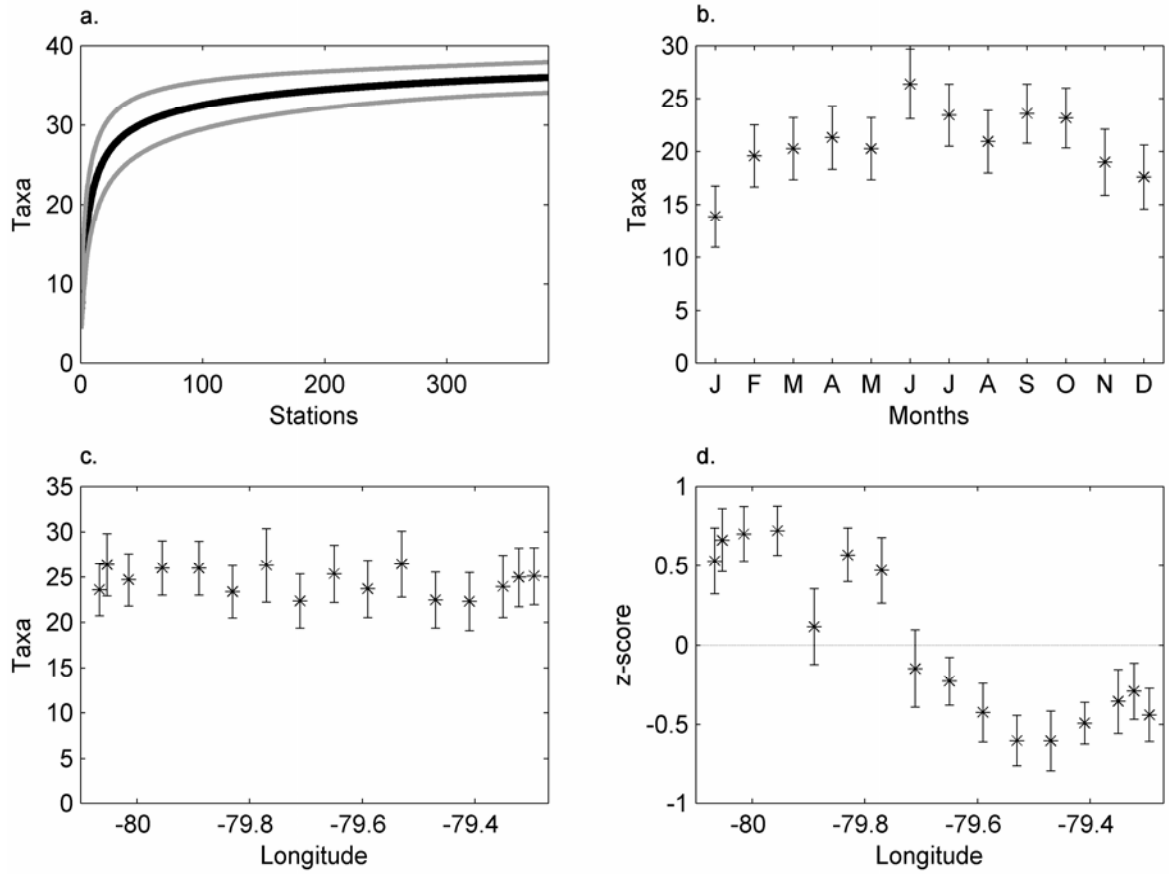


Figure 3.7. Patterns of diversity. A) Rarefaction curve for entire sampling effort, with mean and 95% CI, B) Monthly rarefied diversity standardized to 20 stations (\pm SE), C) Cross-straits rarefied diversity standardized to 20 station (\pm SE). D) Cross-straits diversity z-scores (\pm SE).



4. SAILFISH (*ISTIOPHORUS PLATYPTERUS*) SPAWNING AND LARVAL ENVIRONMENT IN A FLORIDA CURRENT FRONTAL EDDY: RESULTS FROM A LAGRANGIAN STUDY

Fronts and eddies are widely hypothesized to be critical spawning habitat for large pelagic species, though the processes driving spawning at these features is debated. Under one scenario, adult fish target features that will provide a favorable feeding environment for their early life stages, whereas under the alternative scenario spawning occurs haphazardly at oceanographic features that support the feeding requirements of adults. This study examines these two alternatives with respect to sailfish spawning in and around a cyclonic sub-mesoscale Florida Current frontal eddy. Three satellite tracked drifters were deployed within this eddy, and non-linear least squares fitting of their positions was used to determine the size ($\approx 11 \times 7$ km), period (≈ 12 h), translation speed (≈ 80 cm/s) and orientation of the eddy over three days of plankton sampling. Additionally continuous measurements of salinity, temperature and fluorescence, each higher in the eddy interior, allowed for the assigning of the ship's position to one of three zones: eddy interior, eddy frontal zone, and eddy exterior. A well defined peak in larval sailfish densities ($n=2435$, stations=49), composed primarily of yolk-sac or first feeding larvae (1-3 days old), occurred at the eddy frontal zone. Based on an analysis of satellite imagery and the age distribution of larvae, spawning likely occurred during the formation of the eddy. A comparison between the distribution of these larvae and similar age scombrid larvae indicated that spawning directly at the front, rather than convergence, was responsible for this peak in larval density. The first-feeding prey items of larval sailfish (*Farranula* and *Corycaeus* copepods) also had a peak in overall density at the frontal zone. The distribution of the primary prey items of adult sailfish (e.g. *Auxis* spp.,

Euthynnus alletteratus, small carangids) relative to the eddy, were indirectly assessed by examining the distribution of their eggs. *E. alletteratus* and *Auxis spp.* eggs were in highest abundance outside the eddy, while the eggs of small carangids were in highest abundance at the eddy frontal zone. Overall, this small-scale study on the physical and biological features of the environment that drive sailfish spawning site selection, can aid in the understanding of larger scale patterns of movement and recruitment.

Introduction

The factors driving spawning site selection in pelagic species not only influence their movement patterns, but also their patterns of recruitment. For tuna species, a strong contrast exists between the temperate species (bluefin and albacore) that spawn in a restricted portion of their adult range over short seasons, and the tropical species (yellowfin and skipjack) that spawn across much of their adult range over extended spawning seasons (Schaefer 2001). This has led to both the conjecture that species target spawning at locations that provide a favorable feeding and predation environment for their larvae (Bakun 1996), and that species spawn haphazardly within areas that support the feeding requirements of the adults (Lehodey 2001). These two strategies can have profoundly different effects on the population dynamics and movement patterns of a species. This should be particularly true when these species are confronted with fisheries or climate related changes in the ocean environment, such as the collapse of the adult forage base on a spawning ground. Addressing this adult/larval habitat tradeoff in spawning site selection is thus fundamental to developing a predictive capability for the movement and recruitment of these species.

Two related features in the ocean environment, fronts and eddies, are widely hypothesized to be critical spawning habitat for large pelagic species (Bakun 2006). These features have been shown to aggregate species when considered across a range of spatial scales (Royer et al. 2004, Schick et al. 2004, Worm et al. 2005). However, fine-scale (<5 km) spawning directly at fronts or eddies has not been verified. One reason for this is that ichthyoplankton collections, commonly used to determine the spawning locations of highly migratory species, most often focus on larger individuals, generally spawned >5 d before their collection (e.g. Leis et al. 1987, Govoni et al. 2003, Serafy et al. 2003). During the early development of the larvae there can be a decoupling of features of the larval and spawning environments, either by the concentration of individuals at a front, or the dispersing of individuals spawned into a front as the feature breaks up. A second reason for the lack of a strong evidence of a link between spawning and fronts is that most sampling programs are designed to cover large areas, resulting in stations being too widely spaced to resolve finer-scale processes (Bakun 2006). For oceanographic studies in general, resolving the temporal evolution of mesoscale (50-200 km) and sub-mesoscale (10s km) processes often requires a Lagrangian approach to sampling (Hitchcock & Cowen 2007). This approach has not been applied to resolving the fine-scale spawning habitat of large pelagic species.

The Atlantic coast of South Florida and the Florida Keys provides a unique area to address the biophysical linkage between spawning in pelagic species and specific oceanographic features. The offshore environment in this region is dominated by the Florida Current, a portion of the North Atlantic western boundary current system that links the Loop Current in the Gulf of Mexico to the Gulf Stream further north. The

waters of the Florida Current are separated from coastal waters by a persistent frontal zone along which mesoscale and submesoscale eddies are consistently generated (Lee 1975, Lee et al. 1995, Haus et al. 2000). An economically important recreational fishery concentrates their fishing effort at this frontal zone, specifically targeting sailfish (*Istiophorus platypterus*) during much of the year. Throughout the summer months, concentration of larger (>3.5mm and >5 days) sailfish larvae occur in the frontal region (Luthy 2004), though the precise environmental characteristics associated with spawning are not known.

The goal of this study was to determine, relative to a submesoscale frontal eddy, the fine-scale distribution of sailfish spawning. Furthermore we sought to determine the characteristics of the feeding environment at the spawning location for both adult and larval sailfish. Supporting the high resolution nature of this work was 1) an integrated analysis of the physical environment that used drifter tracks, remote sensing, and continuous shipboard measurements of surface properties, and 2) biological sampling and processing focused on fish eggs and the earliest (<5 d old) larval stages of sailfish.

Materials and methods

Prior to the start of sampling, the objectives of this study were to 1) locate a patch of sailfish larvae and mark it with drifters, 2) define the size and shape of the patch, 3) track the advection and diffusion of this patch, and 4) track the growth of larvae within the patch. Subsequently, during the sampling, it was recognized that the drifters, and patch of larvae, were entrained in a frontal eddy. Furthermore, in processing the samples, it was determined that yolk-sac billfish larvae, not identifiable during the cruise,

comprised a large portion of the total sailfish larvae collected during the sampling. This dataset thus provided an additional unique opportunity to address sailfish spawning in and around a sub-mesoscale frontal eddy.

Shipboard sampling

All sampling for this study occurred from 25-28 June 2004 onboard the R/V F.G. Walton Smith. Plankton samples were collected with a 2x1 m 330 μ m mesh neuston net towed with half the frame out of the water. This net was sampled while the ship was turning to keep the net out of the ship's wake. This also resulted in the samples being collected from a narrow point (100-150 m diameter) location versus the long transects (>1 km) of most plankton tows. The volume sampled by the neuston net was calculated using a General Oceanics flowmeter. Previous sampling had established that sailfish larvae concentrate at the surface during the day, and disperse through the water column at night. Thus results from only the daytime surface sampling are reported here, though a limited number of deeper tows with a combined MOCNESS (Guigand et al. 2005) were made at night.

A continuous flow through system measured surface temperature, salinity (both at \approx 10 s time intervals), and fluorescence (at 1-2 s intervals) throughout the cruise. The ship was also equipped with two RD Instruments Acoustic Doppler Current Profilers (ADCP). The 600 kHz system was used to measure shallow currents (4-30 m in 2 m depth bins) while a 150 kHz system measured deeper currents (14-200 m in 8 m depth bins). Vertical profiles of eddy properties (e.g. temperature, salinity, fluorescence, dissolved oxygen) were made with five CTD casts.

Plankton sampling commenced during the morning of 25 June 04 in the region 10 to 25 km offshore of the lower Florida Keys (see timeline: Fig. 4.1a). Previous work had established this location as a probable source area for sailfish larvae collected farther downstream. Samples were immediately and partially sorted for billfish larvae on board the ship, and were subsequently fixed in 95% ethanol. On the tenth sampling station on 25 June, a high density sample of billfish larvae was collected. After five additional stations of variable larval densities, a single satellite-tracked drifter (Technocean), drogued with a Holey sock at 15 m, was deployed just prior to sunset. A shipboard receiver for the Argos signal from the drifter allowed it to be tracked in real time. During the first night of drifter tracking, MOCNESS tows were made in proximity to the drifter. In the surface net sampling the following morning (26 June) it was confirmed that the drifter had been tracking a patch of billfish larvae. Two additional drifters were subsequently deployed, ≈ 12 h after the first drifter deployment.

Following the deployment of all three drifters, sampling proceeded during the day with the neuston net. Most samples were collected within a 3 km radius of the drifters, though a limited number were taken at farther (5-7 km) distances away. Five CTD casts were made in rapid succession on the morning of 27 June. Five rapid cross-shore transects were made on 28 June to characterize the surface properties of the eddy, and the vertical current structure (Fig. 4.1a). Each of these transects was performed in the same geographic location, with the translation of the eddy past that location ensuring that all portions of the eddy were sampled. The tracking of the drifters with the ship finished during the afternoon of 28 June, 65 h after the initial drifter deployment. In total, 49 stations were sampled with the neuston net during this study.

Drifter data processing

A modification of the drifter feature model (Glenn et al. 1990, Glenn & Ebbesmeyer 1994b) was used to determine the eddy dynamics over the course of the sampling. This approach requires two steps. First, it is necessary to define an equation and parameters characterizing the shape, position, and translation of the eddy and the drifter positions within the eddy. Second, to estimate these parameters, a non-linear least-squares method is used to fit the observed drifter positions at time to the modeled positions. This second step, when repeated across time, allows for a characterization of the temporal evolution of the eddy.

For this application of the model we assumed that the eddy was an ellipse oriented in the same direction as it was translating. As degrees of latitude and longitude are not equivalent in distance, all equations were written with positions defined in a kilometer coordinate system and were subsequently converted back to latitude and longitude. Parameters defined the eddy center (x,y), translation speed (s), orientation (=translation direction, θ), and the change in orientation over time ($d\theta/dt$). A parameter for the period (T) of the eddy was set as a constant (12.2 h) based on an analysis of the periodicity of cross-shore and alongshore velocities of the drifters. For the first drifter, parameters for a long axis (a_1), short axis (b_1) and the angular frequency of the drifter orbit (Φ_1) were defined. It is important to note that this methodology determines the long and short axis of the eddy at the position of the drifters and not at the eddy edge. For both the second and third drifters a long axis (a_2, a_3) and angular frequency of the drifter orbit ($\Phi_2 \Phi_3$) were also defined. It was not necessary to include a parameter for the short axis at these

two drifters, as this value could be calculated from the aspect ratio of the eddy (a_1/b_1) and the long axis at that drifter. Other characteristics of the eddy, such as its area, were calculated from this parameter set.

The implementation of the non-linear least squares method used the *lsqnonlin* function in the MATLAB optimization toolbox. This is an iterative function that progressively minimizes the sum of the squared difference between observed and modeled values (i.e. drifter positions in this application). Prior to implementation of this function the drifter position data were resampled at 15 min time intervals using a cubic-spline function. The least-squares function was also set to calculate the full set of parameters at 15 min intervals over the course of the sampling.

One critical issue in the application of this method was the width of the time window of observed values used in the estimation of the eddy parameters at each time step. In initial tests, the use of a short (< 12 h or 1 orbit of the drifters) time window resulted in an erratic pattern to the results due to the inability of the model to properly partition the effects of different parameters on the drifter data. Alternatively, too long of a time window results in a high degree of smoothing of the data and can obscure temporal changes in the eddy dynamics. To balance these two issues, a 15 h time window of drifter position data was used. Furthermore, the difference between the model and true position at each time step was weighted directly proportional to their temporal proximity to the time step being estimated (i.e. a weight of 0.5 for drifter positions 3.75 h before and after the time step being estimated). This weighting of values is conceptually equivalent to a linearly weighted moving average. Finally, in order to resolve the initial eddy parameters, and to account for the deployment of only a single drifter for the first 12

h, the least-squares approach was modified for a mix of complete data from a single drifter and partial data from two other drifters. This approach is considered less reliable than the full three drifter approach.

To independently validate the fit of the eddy feature model, the predicted currents along the cruise track were calculated using the model parameters. These predicted currents were then compared to the measurements of the currents at 14 m from the 150 kHz ADCP. A vector coefficient of determination (ρ_v^2 : analogous to the scalar coefficient of determination, r^2 , except measured on a 0-2 scale) was computed for this comparison (Crosby et al. 1993). The computation of ρ_v^2 included: 1) only portions of the cruise track that the model predicted occurred within the ellipse traced by the drifters, and 2) the time period when cross-eddy transects were made, in order to ensure that portions of the eddy distant from the drifters were included in the calculation.

Underway data processing

The underway data (surface fluorescence, salinity, temperature) were used to visually evaluate the fit of the modeled eddy and to characterize more precisely the eddy frontal zone. For the latter objective, the entire time series of data was manually screened to determine when the ship crossed the eddy front, as indicated by a concurrent change in temperature, salinity, and fluorescence. Furthermore, front crossings were used in subsequent analyses only if: 1) they occurred during a relatively straight portion of the cruise track, 2) were not interrupted by sampling, and 3) occurred in proximity to the expected front from the drifter based eddy model (to exclude inshore front crossings). In total, all of these criteria were met for 34 crossings of the eddy front.

To define the environmental characteristics of the three zones of the eddy (interior, exterior and frontal), the Webster method of detecting discontinuities (Legendre & Legendre 1998) was applied to the salinity measurements from all of the front crossings. Surface fluorescence and temperature measurements were not included in the quantitative evaluation of the front, as they undergo a diel cycle and are not comparable between day and night sampling. The specific utilization of the Webster method involved calculating the mean of the five salinity values (50 s at 10 s intervals along the cruise track) preceding and following a point in the time-series of data. The point at which the absolute value of the difference between these two means (i.e. the sharpest discontinuity) reached a maximum was considered the center of the front. The two locations where the gradient in salinity first reached 20% of the maximum gradient at the center of the front, were considered to be either edge of the front. The inner and outer boundaries of the eddy frontal zone were defined as the mean salinity value at each front edge from the 34 front crossings.

The width, in meters, of the front was estimated using the salinity-based definition of the boundaries of the frontal zone. In determining the front width, the eddy feature model was used to correct for the translation speed and direction of the eddy and the angle at which the boat crossed the front. With respect to this latter factor, crossings of the front that were $>45^\circ$ from perpendicular were excluded from the analysis.

Satellite imagery

Satellite imagery was used to characterize the timing of eddy formation, and the oceanographic setting within which the eddy formed. Processed SEAWIF satellite

images were downloaded from the University of South Florida Institute for Marine Remote Sensing (IMaRS) website (<http://imars.usf.edu/>). Sea surface temperature images were also considered, but were not found useful due to the small temperature gradients between onshore and offshore waters during this season. Gulf of Mexico sea surface height images (Naval Research Laboratory Ocean Dynamics and Prediction Branch: <http://www7320.nrlssc.navy.mil>) were used to assess the position of the Loop Current upstream of the sample transect.

Sample processing

Neuston samples were sorted for all fish larvae, and sailfish larvae were subsequently identified. Based on previous aging work (Sponaugle, unpubl. data), six developmental stages of sailfish larvae were defined. Yolk-sac stage larvae were defined by the presence of unpigmented eyes as the yolk-sac often does not survive collection and preservation in ethanol. Five other stages were defined based on preopercular spine length, a characteristic in the early stage larvae that provides as precise of an indication of larval age as notochord length (NL). This character can be more accurately and rapidly measured using an ocular micrometer than NL. Preflexion larval sailfish were grouped as: Stage I-no preopercular spines, Stage II-0-0.2mm, stage III-0.2-0.75 mm and stage IV 0.75-1.25 mm. Larvae with spine length >1.25 mm (NL > \approx 4.5 mm) were all grouped together. Species-level identification of post-yolk sac larvae used lower jaw pigment patterns (Luthy et al. 2005a). The identity of a small subset of yolk sac larvae was confirmed through molecular identification.

A determination of the availability of first-feeding prey for larval sailfish followed methods described in Llopiz & Cowen (in review). Each sample was standardized to 1 l volume. Subsequently, two 5 ml subsamples were collected with a Hensen-Stempel pipette. *Farranula* spp. and *Corycaeus* spp. copepods, and the cladoceran *Evadne* were quantified in the first of these subsamples. In instances in which *Farranula* counts were <100 individuals, the second subsample was processed. This sample processing occurred for a subset of 41 stations for *Farranula* and *Evadne* and 37 stations for *Corycaeus*. Generally, a 150 µm mesh net is used to estimate the abundance of these zooplankton taxa. Unfortunately, the frame holding this net was damaged early in the study, and only samples from the 330 µm mesh size net were available. It is expected that some extrusion of each of these zooplankton taxa will occur through the 330 µm mesh size. To minimize the effects of this, only female *Farranula*, the larger of the two sexes, were considered in the analysis.

Egg densities of small pelagic species, including *Auxis* spp., *Euthynnus alleteratus*, and scads (*Selar crumenophthalmus* and *Decapterus punctatus*) were used to indirectly measure the relative abundance of prey items of adult sailfish. This methodology secondarily focused on the distribution of sailfish eggs. The quantification and identification of fish eggs required an extensive subsampling approach. From the plankton sample, standardized to 1 l volume, 10 ml subsamples were obtained with a Hensen-Stempel pipette. Eggs were counted in these subsamples, and subsampling proceeded for a minimum of 3 subsamples and until at least 100 eggs were counted or the eggs had been removed from 10% of the sample. These eggs were then grouped into the following size classes: <0.7 mm, 0.7-0.9 mm, 0.9-1.1 mm, 1.1-1.3 mm and >1.3 mm.

The selection of eggs for molecular identification was focused on both characterizing the diversity of species in the samples and obtaining an accurate abundance estimates for the more prevalent species. This selection process occurred within the inherent cost and time limitations of the molecular methodology. The selection of eggs was as follows: 1) for the most abundant ($>1 \text{ m}^{-3}$) sample/size class combinations at least 10 eggs were selected for identification, 2) for the remaining sample/size class combinations $\approx 10\%$ of the eggs were selected, 3) all eggs from the 0.9-1.1 mm size class (which contains sailfish eggs) were selected with the exception of one high abundance sample that was subsampled, 4) a number of eggs $> 1.1 \text{ mm}$ were subsampled. In total ≈ 600 eggs were chosen for molecular identification.

Molecular identification

Molecular species identification relied upon the amplification and sequencing of a ≈ 600 bp portion of the cytochrome oxidase 1 (COI) gene in accordance with the standardized approach being advocated by the Consortium for the Barcode of Life (Hebert et al. 2003). The laboratory methodology followed that outlined in Richardson et al. (2007). Briefly, DNA was extracted from the complete egg or yolk sac larvae. The Polymerase chain reaction (PCR) was performed using the COI primers (FishF1, FishF2, FishR1 and FishR2) listed in (Ward et al. 2005). Combinations of these primers have been shown to successfully amplify a majority of fish species (Ward et al. 2005). For our study, primer pairs were tested sequentially (F1xR1 \rightarrow F2xR2 \rightarrow F1xR2 \rightarrow F2xR1) until a successful amplification was verified on an agarose gel. Our previous work had

confirmed the adequacy of these primers for identifying scombrid and istiophorid species collected in our area (Chapter 3).

To link individual sequences to species, the bioinformatics approach described in Richardson et al. (2007) was first used with the available scombroid voucher sequences. The remaining unidentified sequences were queried against the barcode of life database (Ratnasingham & Hebert 2007). This database is constantly being expanded, and at the time of the study had 4189 fish species barcoded, including a majority of the pelagic species found in the waters off Florida. The identities from the database were used in instances where the samples had a >98% match to a sequence in the database. For one common sequence, a \approx 95% match was obtained to *Selar crumenophthalmus* specimens from the Indian Ocean. No Atlantic specimens for this species exist in the database. All species from the Florida area that are closely related to *S. crumenophthalmus* are present in the database, and all of these species had a lower percent match than the Indian Ocean specimens. For that reason we report these eggs as *S. crumenophthalmus*. However it seems likely that the Atlantic and Indian Ocean *S. crumenophthalmus* are in fact two different species. No attempt was made to speculate on the species level identity of other sequences that did not have a direct match in the database, though in most cases benthic taxa (e.g. scorpaeniformes) were the best match to these sequences.

Biological data analysis

For each plankton tow, an average salinity, fluorescence, and temperature were determined from the underway data. Each station was assigned to one of three zones: the eddy interior, eddy exterior or frontal zone, based on its average salinity value and the

defined frontal boundaries. A one-way ANOVA was used to compare densities of larval sailfish, *Corycaeus* spp. and *Farranula* spp. between zones. Natural-log transformed densities were used in the *Corycaeus*, and *Farranula* comparisons. For sailfish, a single sample contained 0 individuals, and thus a $\ln(x+1)$ transformation was used. A Tukey's test was used to make pairwise comparisons between eddy zones. *Farranula* undergo a reverse diel vertical migration (Paffenhofer & Mazzocchi 2003), resulting in a near absence of *Farranula* in early morning samples regardless of the location of the sample. For that reason, stations from the first 2 h of sampling post-sunrise were excluded from the *Farranula* comparisons.

The extensive subsampling of eggs resulted in low numbers of most taxa being molecularly identified. Because of this, only three taxa were statistically analyzed for differences in egg densities between zones. Two of these taxa, *Auxis* spp. and scads (*Decapterus punctatus* and *Selar crumenophthalmus*), represented a pooling of two species, while the third, *Euthynnus alleteratus*, was a single species. Qualitative comparisons revealed no differences between species in the taxa that were pooled. Egg densities did not conform to the assumptions of the ANOVA tests due to the presence of numerous samples with 0 densities. As an alternative means of analyzing data with large numbers of 0 density samples, the delta method utilizes both frequency of occurrence and the abundance of individuals in positive samples (e.g. Serafy et al. 2007). However, this method was not used in this analysis, because the subsampling procedure resulted in very different probabilities of detecting species between samples. For that reason, a Kruskal-Wallis test was used to compare the zone-specific abundance of each taxa. A Tukey test was used to make pairwise comparisons when results were significant.

Two processes can result in the concentration of larvae at a frontal zone: convergent flow into the front and spawning at the front. Comparisons between the densities of sailfish larvae and scombrid larvae were used to test these alternatives with respect to the distribution of sailfish larvae at the front. It is expected that if physical processes (i.e. convergence) were primarily responsible for the distribution of sailfish larvae, then scombrid larvae of a similar age would have a similar distribution pattern. This assumes that the vertical distributions and behavior of the eggs and early larval stages of both taxa are similar. Only scombrid larvae <2.5 mm on the first full day of sampling the eddy (26 June) were used in this comparison. A linear regression was used to compare the densities of each taxa.

The spawn dates of the sailfish larvae sampled in this study were assigned using the date of capture and the distribution of ages from otolith readings for each larval stage. No sailfish larvae from this collection were aged. Alternatively a total of 66 sailfish larvae from other collections in stage I-IV had previously been aged using established techniques (Luthy et al. 2005b, Sponaugle et al. 2005a). To validate the age of first increment formation, the otoliths of further set of 8 yolk-sac sailfish were measured, and where possible, aged. The otolith width of these yolk-sac larvae were compared to the width of the otolith at initial increments in later stage larvae. For each sample the age composition of larvae was determined by assigning ages to larvae in direct proportion to the age distribution for that larval stage (Table 4.1).

Results

Drifter tracks

The drifter trajectories showed a repeatable pattern of 1) minimal or upstream movement when located near to shore, 2) slow offshore and downstream movement, 3) rapid downstream movement when located farther from shore, and 4) slow onshore and downstream movements (Figs. 4.1b,c, 2). For each drifter, the entire cycle took between 12-12.5 h. For the first drifter (D1) this cycle repeated five times, for the second (D2) and third drifter (D3), deployed 12 h later, it repeated four times. This pattern stopped when the drifters reached about 25.4° N, just before sampling ended (Fig. 4.2).

The cyclic pattern of the drifters velocities is evident when they are broken into alongshore and cross-shore components. Maximum and minimum alongshore velocities ranged from 1.6-1.8 m/s and -0.2-0.0 m/s respectively (Fig. 4.1b). Maximum and minimum cross-shore velocities were approximately 0.5 and -0.5 m/s, with some difference between drifters and over the course of the sampling (Fig. 4.2c).

Drifter feature model

The drifter feature model indicated that the aspect ratio (long/short axis) of the eddy ranged from 1.3-2.5 over the course of the sampling (Fig. 4.1d). During the first portion of the eddy model, resolved with only a single drifter track, it is difficult to determine changes in the aspect ratio. However, the low minimum (-0.3 m/s) in the first alongshore drifter speed, and the low first maximum (0.3 m/s) in cross-shore speed would suggest a relatively more elongate eddy, than occurs farther along in the study (Fig.

4.1b,c). From about 26 June 2100z to 28 June 0100z there is a gradual widening of the eddy (i.e. towards a more circular form). This is followed by a rapid elongation towards the end of the drifter tracking (Fig. 4.1d,f, 4.2). The area of the eddy, calculated at the position of the drifters, remained at $\approx 60 \text{ km}^2$ through most of the study (Fig. 4.1e), though at the beginning, when only a single drifter was in use, estimates of the area are erratic. Towards the end of the track the area of the eddy at the drifters increases, likely corresponding to the release of the drifters from the primary circulation of the eddy.

The statistical model estimated an initial translational speed of 0.45 m/s, then an 8 h period of acceleration, followed by a stasis of the translational speed at around 0.8 m/s (Fig. 4.1g). This initial slower translation speed, estimated in the model from a single drifter, was further validated by considering that drifters track. During minima and maxima of cross-shore current speeds, which occurred when the drifter was at the leading and trailing edge of an eddy, the alongshore speed of the drifter should correspond to the translation speed of the eddy. For the first minimum of the drifter cross-shore speed, the drifter alongshore speed was $\approx 0.4\text{-}0.5 \text{ m/s}$ (Fig. 1b, c). Additionally, the first minimum in the alongshore drifter speed was lower than during most of the rest of the track suggesting a slower eddy velocity. Towards the end of the track, coincident with the elongation of the eddy, there is a small reduction in the eddy speed.

The orientation of the eddy proceeded from a generally northeasterly to northerly direction (Fig. 4.2), with the most distinct turn in heading coinciding with the rapid elongation of the eddy and the reduction in translation speed (Fig. 4.1g, h). The root mean square error in fitting the eddy model was generally 200-300 m. However, this error increased substantially at the time period when the eddy turned rapidly and

elongated. The error also increased substantially at the end of the study when the drifters were likely released from the eddy.

The relative positions of the drifters to each other changed over the course of the deployment. Drifter 1 was located at a more interior position of the eddy at both the start and end of the tracking. During the middle of the track this drifter was roughly equal distance from the eddy center as the other two drifters. Drifter 1 also tended to precede the other drifters in its rotation around the eddy. Drifter 2 and 3 tend to converge towards each other both in terms of distance from the center and rotation around from the center (Fig. 4.1b, c, f).

The correlation between the predicted currents and the measured currents was high ($\rho_v^2=1.727$) for the repeated crossings of the eddy (Fig. 4.3). This ρ_v^2 was only slightly below the correlation between the 150 kHz ADCP and the 600 kHz ADCP at 14 m ($\rho_v^2=1.8441$). Additionally this calculation of ρ_v^2 was based on data from the time period of the study when the RMS error of the modeled eddy reached its first maximum (Fig. 1a,i).

Eddy properties

Salinity, temperature, and fluorescence were all positively correlated, and were all higher in the interior of the eddy (Fig. 4.4). A crossing of the shoreward front also indicated that these measurements were higher in coastal waters. Salinity at the center of the front averaged 36.486. On either edge of the front the salinity averaged 36.452 and

36.520 (Fig. 4.5a). The estimated width of the front from the series of front crossings ranged from 270-1700 m, with most of the estimates between 400-1000 m (Fig. 4.5b).

Vertical profiles of the currents indicated that circulation associated with the eddy penetrated to ≈ 40 m (Fig. 4.6a,b). This depth corresponds with the top of the thermocline and halocline and the depth of the deep chlorophyll maximum (Fig. 4.6 c,d,e). The five CTD casts were all clustered around the eddy edge, and thus did not allow for an analysis of the cross-eddy differences in the vertical distribution of eddy properties.

Satellite image characterization

In late May and early June a well formed Tortugas Gyre (TG) off of the lower Florida Keys (FK) was clearly visible in ocean color satellite imagery (Fig. 4.6a). During this same time period sea surface height imagery indicates that the Loop Current in the Gulf of Mexico was beginning to pinch off a warm-core ring. By 24 June this ring was nearly completely free of the Loop Current, resulting in the Yucatan Current taking a nearly direct route into the southern Straits of Florida. Much of the satellite imagery in mid-June was obscured by cloud cover, but by 18 June the TG had broken up and the remnants were spread along the FK (Fig. 4.7b). On 23 June cloud cover obscured the satellite imagery of the upper and middle FK, but in the lower FK no remnants of the TG or any other sub-mesoscale features are visible (Fig. 4.7c).

On 24 June 1819 GMT the remnants of the TG are visible off of the upper FK (Fig. 4.7d). Additionally, an eddy was present between the lower and middle Florida

Keys that is marked by chlorophyll levels much higher than in the TG and the sub-mesoscale features that it generated. This first eddy will subsequently be referred to as SME-1 (submesoscale eddy -1).

While partially obscured by cloud cover, the image on 25 June, 1721 GMT depicts two SMEs (Fig. 4.7e). The first of these, SME-1 is a distance of 57 km downstream of its location in the previous image suggesting a translation speed of 0.69 m/s. The distance between SME-1 and the second eddy SME-2 is 41 km. Importantly, 9 h after this image, the first of the drifters was deployed in a frontal eddy (SME-3) in proximity to the location of SME-2 in this image. In the 25 June satellite image, cloud cover obscures the upstream location where SME-3 would likely be located.

On 26 June 1802 GMT the ocean color satellite image indicates the presence of four and possibly five distinct SMEs (Fig. 4.7f). Between this image and the previous image, SME-1 and SME-2 moved downstream 68 km and 61 km, suggesting translation speed of 0.76 m/s and 0.69m/s respectively. The distance separating SME-1 and SME-2 was estimated at 46 km. SME-3, the eddy in which the drifters were deployed, was 27 km upstream of SME-2. Based on a subjective chlorophyll level defining the eddy edge, the dimensions of SME-3 were estimated at 13.5x7.2 km. SME-4 is 25.2 km upstream of SME-3. Additionally, though cloud cover partially obscured the area, it appears that a fifth eddy, SME-5, was 25 km upstream of SME-4.

Another cloud-free satellite image of the FK does not occur until 30 June, well after the drifters have exited the SF. In this image, one SME is clearly visible and two or three more are likely present, though these are partially obscured by cloud cover (Fig. 4.7g). Based on the time interval between images and the translational speed of the

eddies, it is clear that these eddies do not correspond to any of the eddies in the previous images. Additionally on 6 July four similar size SME are visible in the imagery (Fig. 4.7h). By late-July/ early-August it appears that a TG is developing in the far western portion of the Straits of Florida, and similar chains of SMEs are no longer present.

Sailfish densities

In total, 2435 sailfish were collected during the 49 neuston tows sampled before and after the drifter deployment. Of these, 261 had spine lengths >1.25 mm, corresponding to larvae approximately >4.5 mm NL and >5 d old. This largest size class of larvae was excluded from subsequent analyses and will not be discussed further. For the remaining larvae, densities peaked at the intermediate salinity values of the eddy frontal zone (Fig. 4.8a). ANOVA indicated that the between zone differences in sailfish densities were significant (Fig. 4.8b). Pairwise comparisons indicated that outside the eddy had significantly lower sailfish densities than the frontal zone or inside the eddy, but that the difference between the frontal zone and inside the eddy was only marginally significant ($p=0.052$).

The composition of different stages in the catches changed over the course of the sampling. On 25-26 June, yolk-sac larvae composed 41.5% and 55.0% respectively of the total sailfish catch versus 7.0% and 2.8% of the catch on 27-28 June (Fig. 4.10a). Stage I sailfish were in highest abundance on three of the four days of sampling, with the 25 June catch largely driven by a single high density sample. The proportion of stages II-IV larvae in the samples increased during the last two days of sampling.

On the first day of sampling, larvae spawned on the 23 June and the 22 June (mostly contained in a single sample) were dominant (Fig. 4.10b). The middle two sampling days had corresponding spawn dates (23 June and 24 June) dominating the catch. By the final day, a larvae with an estimated 25 June spawn date were also present.

Larval feeding environment

The density of *Evadne* cladocerans was negligible and thus they were not considered further. Densities of both *Farranula* and *Corycaeus* copepods peaked at the frontal zone (Fig. 4.8c,e). For both species between zone differences in the densities were significant (Fig. 4.8d,f). The pairwise comparisons indicated that the differences in density between outside the eddy and the other two zones were significant for both species. The differences between the eddy front and eddy interior were not significant for either species ($p > 0.05$).

Egg densities

In total, 450 eggs were molecularly identified, representing 38 different species (Table 4.2). Only the pelagic species are considered here. The six most abundant species, *Auxis rochei*, *Euthynnus alleteratus*, *Selar crumenophthalmus*, *Decapterus punctatus*, *A. thazard*, and *Caranx crysos*, are all small to medium sized schooling pelagic fishes. In aggregate, these species comprised 81% of the molecularly identified eggs. The subsampling procedure for molecular identification was disproportionately focused on certain samples and size classes of eggs. When the effects of the subsampling

procedure are accounted for, it is estimated that 87.7% of the total eggs in all the samples were one of these six small to medium size pelagic species.

Significant between zone differences in egg density were found for each of the three taxa for which comparisons were made. Densities of *Euthynnus* sp. eggs were significantly higher outside the eddy than in either the frontal zone or inside the eddy (Fig. 4.8g,h). Densities of *Auxis* spp. egg were significantly lower inside the eddy than either outside the eddy or the eddy frontal zone (Fig. 4.8i,j). The density of scad eggs was highest in the frontal zone, intermediate inside the eddy, and lowest outside the frontal zone (Fig. 4.k,l). Only the comparison between outside the eddy and the frontal zone was significant.

Only five sailfish eggs were identified from three different stations. Sailfish egg densities were low at all of these stations. Two of the stations were outside of the eddy and a third was within the front. For one station the sailfish egg was collected 1-2 h after sunrise, and the developing larva extended $\approx 40\%$ around the diameter of the egg. For the other two stations, the eggs were collected 6-9 h after sunrise and the developing larvae extended $\approx 70-90\%$ around the diameter of the eggs.

Discussion

Frontal eddy dynamics

Biological processes are well known to change over time and vary spatially within individual eddies, and differ among eddies (Bakun 2006, McGillicuddy et al. 2007). This is most evident and well documented for the distribution and growth of the lowest trophic

levels (Sweeney et al. 2003), however, it also occurs with more complex processes, such as the recruitment of coral reef fishes (Sponaugle et al. 2005b, D'Alessandro et al. 2007). Because of this, the development of a broader understanding of the relative importance of eddy-associated processes to biological communities is highly dependent on field studies capable of resolving not just the presence of an eddy, but also its dynamics. For this study, three types of information were used to describe the eddy: satellite imagery, a drifter feature model, and continuous underway measurements of surface properties and subsurface currents. Each of these data sources provided information of progressively higher resolution, but across smaller space/time scales. This nested analytical approach allowed for a detailed understanding of the eddy within its broader oceanographic context.

Much of the work to date on eddy dynamics along the Florida Keys has focused on the Tortugas Gyre (TG), a mesoscale (100-200 km) cyclonic eddy that can persist in the southern SF for time periods ranging from 50-140 days (Lee et al. 1994, Fratantoni et al. 1998). The displacement of this feature can result from either the entrance into the SF of a Loop Current frontal eddy, that replaces the established TG (Fratantoni et al. 1998), or the shedding, whether permanent or temporary, of a large anticyclonic ring by the Loop Current (Lee et al. 1995). This latter process results in a northward shift in the FC axis in the southern SF, as the Loop Current takes a more direct path from the Yucatan Channel to the SF. This shift in the FC then displaces the TG, and initiates the process of it being advected downstream, elongated, and sheared apart (Lee et al. 1995). In the weeks prior to this study, the Loop Current temporarily shed a ring of sufficient size to displace the TG. One day prior to the drifter deployment the final stages of the breakup

of the TG were clear in the satellite imagery, with its remnants present off of the upper Florida Keys.

One of the unique aspects of the sampled submesoscale eddy (SME) is that rather than being an isolated feature, it was the third SME in a continuous chain of similar sized SMEs visible in satellite imagery. These SMEs first appeared on 24 June, and continued to appear for at least 13 days. They were generated at a frequency of about 1-2 per day, suggesting that ≈ 20 eddies were generated during this time period. This chain of eddies was also generated in a single region of the lower FK over time, rather than synchronously across the entire SF at one point in time. While a comprehensive time-series analysis of submesoscale eddy formation has not been performed, events such as this have not previously been recorded, and are, at the very least, not a common occurrence. This raises the question of what made this specific time period particularly favorable for eddy formation in the southern SF.

The formation of submesoscale eddies along the high current shear boundary of a front is a common process that occurs throughout all oceans, and in particular, along the edges of the strong western boundary currents (Lee 1975). The process of formation of these frontal eddies is initiated by instabilities in the front, that lead to a filament of offshore water protruding in and wrapping around onshore water. This onshore water can ultimately pinch off, at which point the background flow will advect the resulting eddy (Lee et al. 1981). Two factors may have contributed to unusually high horizontal current shear in the specific region where this chain of eddies formed. The first, well supported by the satellite imagery, is a shoreward shift of the FC axis in response to the loss of the LC and TG. The second, and more speculative, involves westward flow, counter to the

direction of the FC, in the coastal waters onshore of the frontal zone. Previous work has established that 1) there is a net average outflow from Florida Bay to the Atlantic coastal waters through a number of Florida Keys tidal channels, 2) a large amount of variability in this outflow is associated with local wind events and the position of the Loop Current, and 3) the largest portion of net transport occurs through Long Key Channel (Lee & Smith 2002). Outflow from Long Key Channel (see Fig. 2a for location) would be expected to turn right and run counter to the Florida Current. This would increase current shear in the region directly upstream of this channel, where these eddies formed. Unfortunately, no measurements of outflow from Florida Bay are available from the time period these eddies were formed. However, net outflow seems likely given the large volume of onshore water these eddies are transporting offshore and ultimately out of the system.

The exact process and timing of formation of the specific eddy sampled in this study was not directly or remotely sampled, however, evidence from both satellite imagery and the eddy feature model indicate that the eddy formed within the 2-d period prior to the deployment of the first drifter. The absence of the eddy in the 23 June satellite image, despite the lack of cloud cover where it likely would have occurred, indicates the earliest time that this eddy could have been formed. Also indicating the timing of eddy formation is the acceleration in the eddy translational speed early in the sampling. This acceleration would occur with an eddy being picked up by the background flow after it fully releases from the frontal zone. Additionally, its initial offshore movement and shortening towards a more circular form, are likely indicative of

its transition from an elongate filament still attached to the coastal waters to a detached eddy.

During the downstream progression of the eddy, the eddy feature model provides a highly resolved means of evaluating its characteristics and temporal changes in its dynamics. Previous work in a Gulf Stream frontal eddy used a variety of means to assess the performance of this analytical approach. This included a comparison between the modeled eddy and XBT estimates of the structure of the eddy and its center location (Glenn & Ebbesmeyer 1994b). For this study, the continuous measurements of the currents verified that the model was highly accurate in capturing the eddy dynamics. Relative to other methods used to sample eddies, this model was capable of detecting rapid changes in the eddy properties, such as the doubling of its aspect ratio over a 6-8 h period coincident with a sharp turn northward in the eddy. This follows previous observations of eddy elongation in this region associated with the narrowing of the SF, and the convergence of the strong flow of the FC with the sharp topography off of the upper Florida Keys (Lee et al. 1995).

For most biological processes, the critical factors in an eddy are associated with the processes of divergence and convergence, parameters not estimated in this study. For cyclonic eddies in general, the early stages of formation will be marked by upwelling at the core of the eddy and a convergent frontal zone around the outer edge. Over time, as these cyclonic eddies reach a more mature stage and begin to spin down, convergence will cease along the eddy edge, and ultimately will shift to the center of the eddy (Bakun 2006). The exact evolution of convergence and divergence in this eddy is unknown, though it is likely that the initial spin-up of the eddy was the period with the most intense

convergence at the eddy front. It is also possible, given the ephemeral nature of the eddy and its topographically induced breakup, that it never reached a state where convergence shifted to the core. Sampling revealed that the frontal zone around the eddy 1) was relatively narrow ≈ 1 km with differences in width likely associated with position along the eddy at which the front was sampled, and 2) was marked by a minimal density gradient, but distinct differences in salinity and temperature. This latter characteristic reflects the switch during this period from winter, higher density nearshore waters that are both saltier and colder than offshore waters to the summer, lower density nearshore waters that are both warmer and less saline.

Distribution of sailfish spawning

This study demonstrated a peak in the density of early stage larval sailfish in a frontal zone surrounding a submesoscale frontal eddy. These collections included the highest larval sailfish densities collected in ≈ 450 plankton tows made with a fine mesh surface net as part of the broader sampling effort in the Straits of Florida. Two challenges exist in evaluating this dataset with a focus on the spawning strategies of adult sailfish. The first is to determine at what stage of eddy formation sailfish spawning occurred. The second is to determine whether this peak in density resulted from sailfish spawning directly at the front, or alternatively from larvae being advected into this frontal zone from spawning over a broader area.

The ages and timing of collection of larvae in this study indicated that a majority of the spawning occurred on 23-24 Jun. The presence of a sizable 22 June cohort on the first day of sampling was driven by high abundances in a single sample. Additionally,

the presence of a 25 June cohort on the last day of sampling, but not before then, is likely an artifact of not directly aging the larvae, but rather relying on a larval stage-based age model. It can also be speculated that the spawning associated with each of these cohorts occurred during the period around sunset on each of these 2 d, a common spawning strategy for many pelagic species. Supporting this is the collection of mid-development sailfish eggs in the early morning, late stage eggs in the early afternoon, and the fact that egg development times are most often <24 h for pelagic teleosts at these warm water temperatures (Houde 1989, Margulies et al. 2007). The highest levels of spawning thus occurred when the eddy was being formed, with much lower levels of spawning occurring after the eddy was fully formed and advecting downstream. This temporal distribution of spawning is similar to the expectations for the temporal patterns of convergence rates at the eddy front.

Addressing the spatial distribution of spawning relative to the frontal zone is much more difficult than addressing the timing of spawning. In a region rich in frontal activity, the temporal evolution of a patch of depth-regulating organisms will be structured by the rates of both convergence and diffusion (Olson & Backus 1985). Changes in the distribution of organisms will be dominated by diffusion, when the organism has a narrow initial distribution centered on the front, and will be dominated by convergence when the organisms have a widespread initial distribution. After a period of time, regardless of the initial distribution, patches of organisms will reach the same steady-state distribution at which point the rates of diffusion and convergence are in balance (Olson & Backus 1985). The implications of this for ichthyoplankton studies is

that larval distributions can only be used to distinguish spawning directly at a front from spawning in proximity to a front for a set period of time post-spawning.

The physical data collected in this study, and an understanding of the physical processes in the region, are by themselves not sufficient to eliminate the possibility that the sailfish larvae were transported to the front. The strong peak in larval abundance found at the front in this study is driven primarily by individuals spawned in the 24-72 h period before collection. This time period is much less than the 4 week time period that Olson and Backus (1985) calculated was required to concentrate a mesopelagic fish at the edge of a warm-core Gulf Stream ring. However, peak convergence rates estimated for that ring ($4.5 \times 10^{-6} \text{ s}^{-1}$) are much less than have been measured with surface current radar in isolated areas of the Florida Current front ($>1 \times 10^{-4} \text{ s}^{-1}$; Lane et al. 2003). The rate at which convergence related aggregations of organisms form scales directly to the convergence rate. As such it is possible that under certain conditions in the SF, convergence can result in notable levels of aggregation of organisms over a several day time-scale.

The biological data point much more conclusively to spawning distribution rather than larval transport as the primary determinant of the distribution of larval sailfish at the front. Specifically, if transport was the primary determinant of the sailfish larval distribution, it would be expected that the larvae of other taxa of a similar age, would also be concentrated at the front. In fact, no correlation exists between the distribution of early stage scombrids and early stage sailfish larvae. Vertical behavioral differences can affect the distribution and accumulation of taxa around a front (Franks 1992), but are not expected to account for the differences observed here. Specifically, scombrids and

billfish share a positively buoyant egg stage, yet the horizontal distribution of their yolk sac larvae (structured primarily by transport during the egg stage) still differs.

Additionally, there is a consistency across all stages (eggs, yolk-sac larvae, first feeding larvae) in the distribution of scombrids, with a similar consistency across stages (yolk-sac larvae, first feeding larvae) also occurring in the distribution of billfish larvae.

Sailfish spawning environment

Most of the work to date describing the spawning environment of large pelagic species has focused on the physical traits of the environment, or basic biological characteristics such as fluorescence levels (e.g. Chapter 3). In contrast, this study explicitly addressed the biological community at the frontal zone where the sailfish were spawning. This was done with an emphasis on the feeding environment for both adult and larval sailfish. The front around the eddy was found to be an area of enhanced biological activity, as would be predicted for a convergent front. However, as with previous sampling (Limouzy-Paris et al. 1997), this front did not just contain a concentration of taxa that occurred elsewhere, but rather was unique in the composition of the community.

The plankton sampling verified the expectation that higher densities of both first-feeding prey items of larval sailfish would occur at the front. However, contrary to expectations, the comparisons in densities between the front and eddy interior were not significant for either taxa. This lack of significance may be in part due to the nature of sampling the eddy interior. Because the eddy size and position were not resolved during the cruise, most of the eddy interior samples were taken near the periphery of the eddy,

rather than near its core. It is at the core of an upwelling cyclonic eddy that zooplankton densities are expected to be lower. Despite this limitation in the sampling, the results of the study still suggest that sailfish spawning occurred in a location and at a time that resulted in their larvae occupying a favorable feeding habitat.

The evaluation of egg densities, as an indication of the prey environment of adult billfishes, indicated that the eddy and area around the eddy contained high abundances of small pelagic fishes, the primary prey items for adult sailfish. Specifically, six species, three small scombrids and three small carangids, were estimated to comprise $\approx 88\%$ of the total collected eggs. Each of these species is common in the diets of adult sailfish, and in aggregate they compose a majority of the gut contents of adult sailfish caught off Florida (Jolley 1977). Spawning by an additional important prey, beloniform fish (exocoetids, hemiramphids, belonids) was not assessed as these species spawn eggs that attach to floating material.

The species-specific distribution of these eggs, relative to the eddy, indicates these species do not all follow the same spawning strategy. Scad eggs occurred largely at the front, and sporadically within and outside the eddy. *Auxis* spp. eggs occurred primarily outside the eddy and within the eddy front. *E. alleteratus* eggs occurred primarily outside the eddy. *Caranx crysos* eggs, while present only in a limited number of samples, appeared to be most abundant outside the eddy. These contrasting egg densities indicate that the frontal zone does not necessarily attract all species equally for spawning. These results would also suggest that a favorable feeding environment for adult sailfish may occur over a broader area than the narrow front at which spawning occurred. However, one possibility that cannot be eliminated, for all of the species,

including sailfish, is the occurrence of sub-daily movements of the adults relative to the eddy or other frontal zone. This could result in the separation of spawning and feeding areas, and could confound the use of egg surveys to explore the fine spatial scale overlap of predators and prey during feeding periods. Other billfish species have sustained swimming speeds of ≈ 0.5 body lengths s^{-1} (Block et al. 1992) which would allow an adult sailfish to cross the entire 1 km front in ≈ 20 min. For the smaller pelagic species, longer time periods would be required to cross the front, though sub-daily front crossings are still reasonable. This uncertainty about small-scale adult movements, combined with the different distributions of adult prey items, results in some ambiguity in defining the best feeding location for adult sailfish.

Overall this study strongly supports targeted rather than haphazard spawning by sailfish at a front. The direct result of this targeted spawning is that the first-feeding larvae occupy a favorable feeding habitat. Haphazard spawning in an area favorable to the feeding requirements of adults is not supported, as this likely would have included higher levels of spawning outside the eddy. Additionally, if movement patterns leading up to spawning were not directed, the combination of the width of the front, and the swimming speed of the adults would be expected to result in a much broader spawning distribution for sailfish than the ichthyoplankton data indicate.

Implications for sailfish recruitment and migration

Many of the most widely accepted hypotheses concerning recruitment variability in marine fishes (e.g. critical period, match-mismatch, stable-ocean) are based on the assumption that the feeding environment will dictate survival rates of the early life stages

and ultimately year class strength (Hjort 1914, Cushing 1975, Lasker 1978). All of these hypotheses have been developed with a focus on small to medium size temperate species. The relatively lower swimming ability of many temperate species, in comparison to sailfish, will result in a reduced ability to rapidly adjust their spawning distributions to local conditions. Additionally, the long egg and yolk-sac stage development times of temperate species (Houde 1989), relative to sailfish, further limits the extent to which the spawning adult directly influences the environment experienced by the early stage larvae. This raises the important question of whether targeted spawning in sailfish minimizes some of the random component of larval feeding success that is hypothesized to underlie recruitment variability in more temperate species. The demonstration of consistently high feeding rates in a broad survey of larval sailfish and blue marlin would suggest that this is the case (Llopiz & Cowen in review). Alternatively, recruitment success could be driven by one or a combination of other factors. These include predation rates on the early life stages, and factors controlling the condition of adults (e.g. Golet et al. 2007) and in turn the quantity and quality of eggs produced during spawning.

The strong association between fronts and spawning in sailfish also has distinct implications for the movement patterns of this species. Sailfish tagged in the SF have been found to disperse over a much wider area during the spawning season. This includes transits to the Eastern Gulf of Mexico, the Mid Atlantic Bight, and the western Sargasso Sea (Chapter 5). Each of these areas is characterized by strong frontal zones and cyclonic eddies (Vukovich & Maul 1985, Halliwell et al. 1991). One question raised by this study is whether the movement patterns of sailfish relative to the SF are in part driven by the regional patterns of formation of mesoscale and sub-mesoscale eddies.

This would occur in the simple scenario of unfavorable small-scale conditions initiating larger-scale movements.

While an analysis has not been done in the SF, in locations along the Gulf Stream, the patterns of frontal eddy formation shifts between two dominant and persistent modes (Glenn & Ebbesmeyer 1994a); very different frontal eddy characteristics define these two eddy formation modes. Furthermore, at an even longer temporal scale, paleoceanographic records indicate that the frequency of occurrence of the mesoscale Tortugas Gyre in the SF, can be strongly influenced by climatic events, such as the Little Ice Age 200 years ago (Lund & Curry 2006). Observations of this sort provide a critical link between fine scale-studies, such as this one, and the development of a predictive capability for recruitment and migration over much longer temporal scales. Specifically, these studies would indicate that smaller-scale oceanographic processes are in fact predictably driven by larger scale oceanographic changes. In turn, these small-scale processes may be the most critical ones controlling various aspects of recruitment and migration in large pelagic species. This would suggest that it is critical to understand oceanographic coupling across spatial-scales, and to resolve the fine-scale interaction of species with the environment. Such coupling of scales would expand the approach of much of the research exploring climatic affects on large pelagic species beyond the current focus on larger scale patterns of temperature and productivity.

Conclusions

Recent observations in tropical and subtropical waters have determined that mesoscale features, such as eddies, have a disproportionate and previously unrealized

effect on larger scale ocean processes (McGillicuddy et al. 2007). Highly targeted spawning in sailfish, demonstrated in this study, indicates that these smaller-scale oceanographic features may also have a disproportionately large effect on the life histories of top predators. Further support for this concept requires the incorporation of higher resolution sampling of the biological and physical environment than generally occurs in most field studies. Ultimately, an understanding of how these fine-scale processes affect the survival of larvae and the behavior of adults may provide insights that can further the goal of developing a predictive capability for larger-scale patterns of recruitment and migration.

Tables

Table 4.1. Distribution of ages for each larval sailfish stage. The yolk-sac (YS) stage was defined based on the presence of an unpigmented eye. The other stages were defined based on preopercular spine length. This age distribution was used to assign spawn dates to the larvae.

Age (d)	Stage				
	YS	I	II	III	IV
1-2	1	1			
2-3		15	9	1	
3-4		4	5	11	4
4-5				2	8
5-6				1	2
6-7					3

Table 4.2. Results from the molecular identification of eggs. The number of each species identified and the average egg diameter are indicated. The estimated percentage of total eggs accounts for the extensive subsampling approach used in selecting eggs for molecular identification. The other categories are composed entirely of benthic species, or sequences that did not have a match in the barcode of life database.

Species	Family	Common name	Avg. diameter (mm)	N	Est. Percent of total eggs
<i>Auxis rochei</i>	Scombridae	Bullet mackerel	0.74	128	35.5%
<i>Euthynnus alletteratus</i>	Scombridae	Little tunny	0.79	95	28.5%
<i>Selar crumenophthalmus</i>	Carangidae	Bigeye scad	0.58	50	8.0%
<i>Decapterus punctatus</i>	Carangidae	Round scad	0.60	50	4.3%
<i>Auxis thazard</i>	Scombridae	Frigate mackerel	0.77	24	7.9%
<i>Caranx crysos</i>	Carangidae	Blue runner	0.63	19	3.7%
<i>Scomberomorus cavalla</i>	Scombridae	King mackerel	0.84	14	2.7%
<i>Seriola rivoliana</i>	Carangidae	Alamaco jack	0.98	10	2.0%
<i>Thunnus atlanticus</i>	Scombridae	Blackfin tuna	0.77	9	≈1%
<i>Acanthocybium solandri</i>	Scombridae	Wahoo	0.92	5	≈1%
<i>Istiophorus platypterus</i>	Istiophoridae	Sailfish	1.06	5	<1%
<i>Echeneis naucrates</i>	Echeneidae	Sharksucker	2.15	3	<1%
<i>Trachinotus falcatus</i>	Carangidae	Permit	0.85	2	≈1%
<i>Coryphaena hippurus</i>	Coryphaenidae	Dolphinfish	1.25	1	<1%
<i>Scomberomorus maculatus</i>	Scombridae	Spanish mackerel	0.90	1	<1%
<i>Sardinella aurita</i>	Clupeidae	Spanish sardine	0.93	1	<1%
Other <0.7 mm		5 species		6	≈1%
Other 0.7-0.9 mm		5 species		8	≈1%
Other 0.9-1.1 mm		4 species		4	<1%
Other 1.1-1.3 mm		3 species		4	<1%
Other >1.3 mm		5 species		11	≈1%
Total		38 species		450	

Figures

Figure 4.1. Temporal distribution of sampling and eddy parameters. a) The timeline of sampling indicates when physical measurements of the environment were made, the timing of drifter deployment (Δ) and the timing of neuston tows (\bullet). The estimated timing of spawning for the two dominant cohorts, and daylight and nighttime (gray shading) hours are also indicated. Speed of all three drifters in the b) alongshore and c) cross-shore directions. From the drifter feature model the estimated d) aspect ratio, e) area of the eddy at each drifter, f) short-axis length at each drifter, g) translation speed, h) change in heading, and i) the Root Mean Square (RMS) error of the model fit.

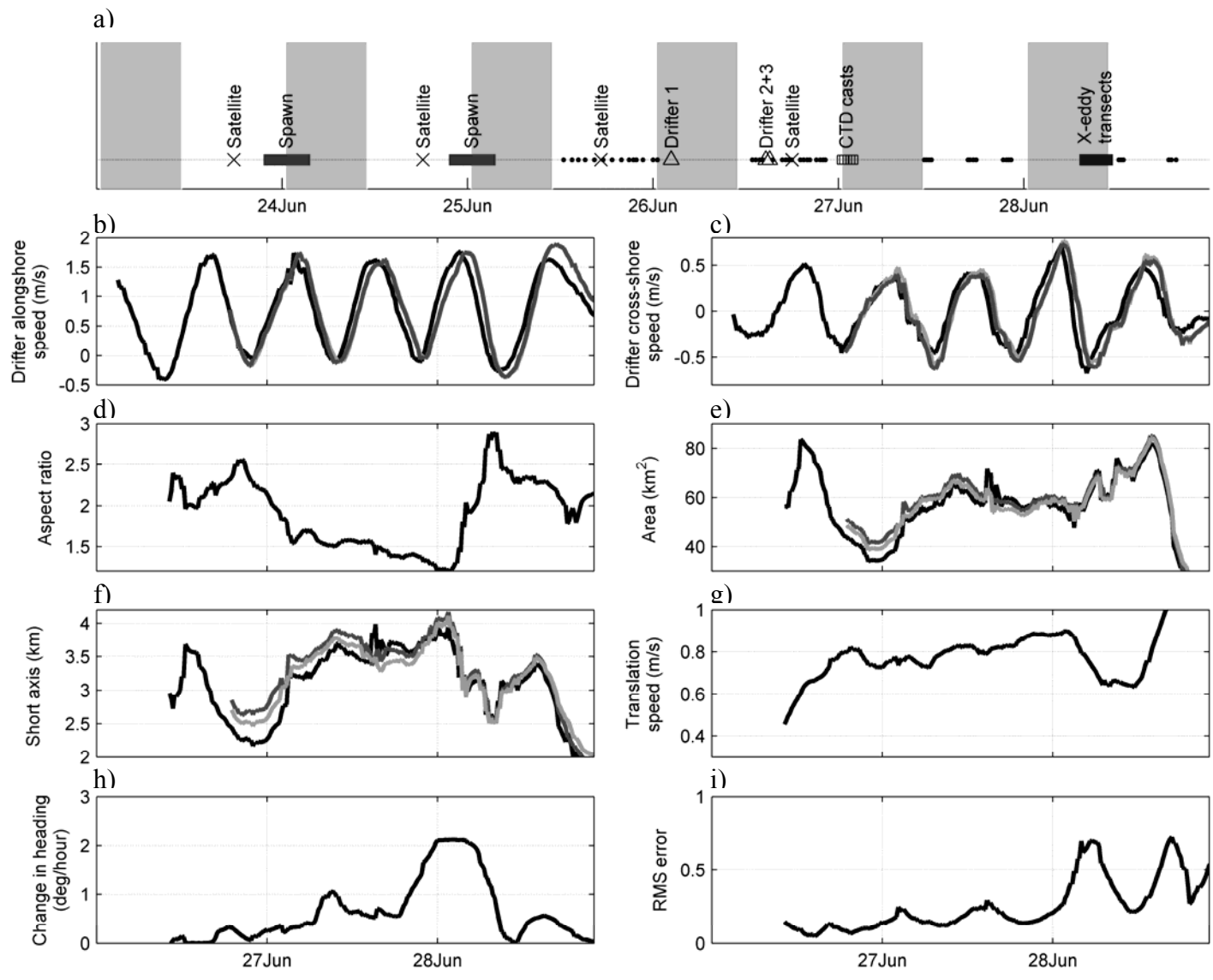


Figure 4.2. Drifter tracks and depiction of the eddy from the eddy feature model. a) The eddy depiction at 6 h time intervals through the point at which the drifters were ejected from the primary circulation of the eddy. Long Key Channel (LKC) is marked on the plot. b) A depiction of the rapid elongation and turn in the eddy over a 9-h period when the eddy was located offshore of the upper Florida Keys. Drifter positions are plotted at 30 min intervals. Filled markers denote the drifter positions at the four time periods that the eddy is plotted.

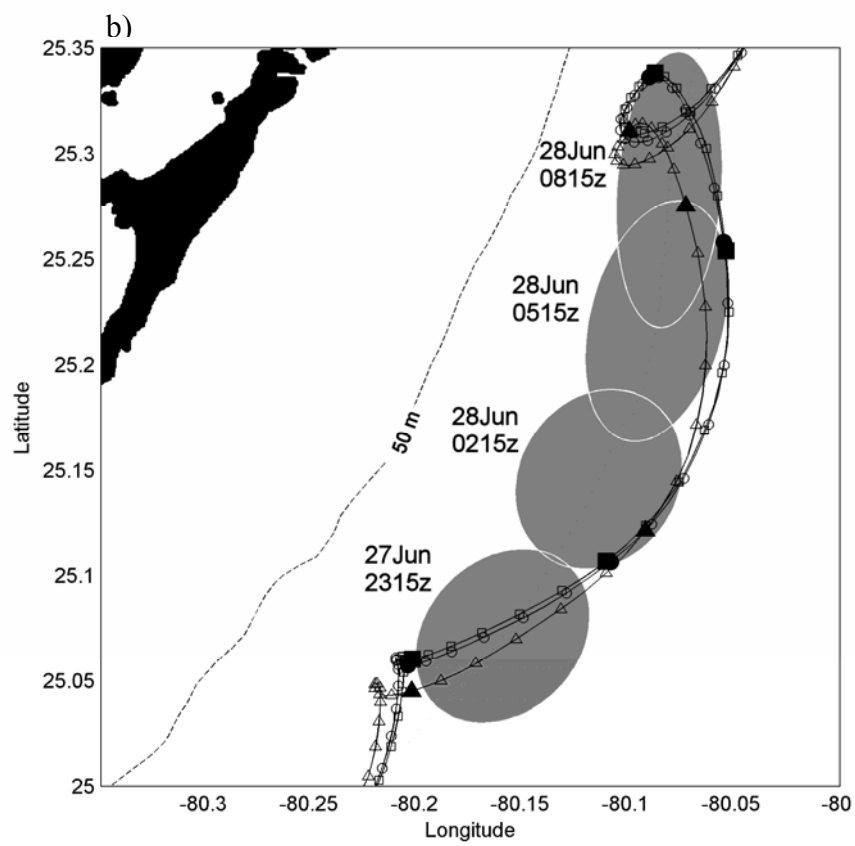
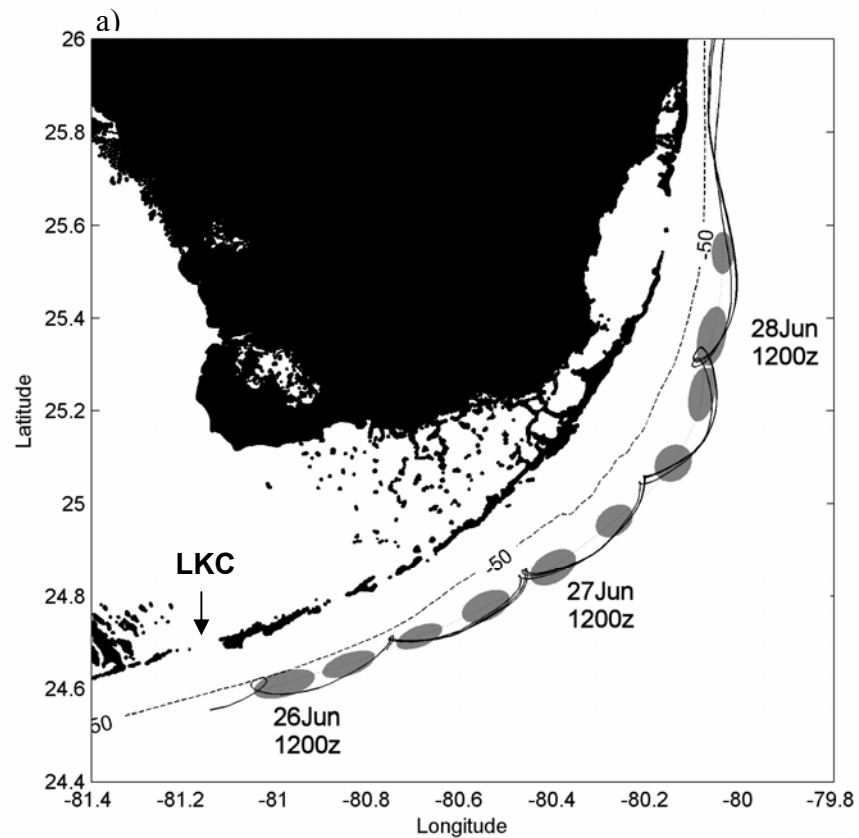


Figure 4.3. Currents during the series of cross-eddy transects on 28 Jun. These repeated measurements were made along the same transect line as the eddy passed through. The x-axis and y-axis are the distance of the ship from the center of the eddy as estimated from the drifter feature model. During the ≈ 6 h time period these measurements were made, the eddy was undergoing distinct changes in shape and orientation. The plotted eddy indicates the shape and orientation of the eddy during the mid-point of the transects. a) The absolute current velocities as measured at the 14 m depth bin by the 150 kHz ADCP. b) Measurements of the eddy component of the currents calculated by subtracting the translation velocity of the eddy obtained from the eddy feature model. The translation velocity of the eddy at the midpoint of the sampling is indicated in red. c) Estimated currents calculated using the eddy feature model parameters. d) Estimated eddy component of currents calculated using the eddy feature model parameters. The vector correlation coefficient between the currents in plots a and b is 1.727.

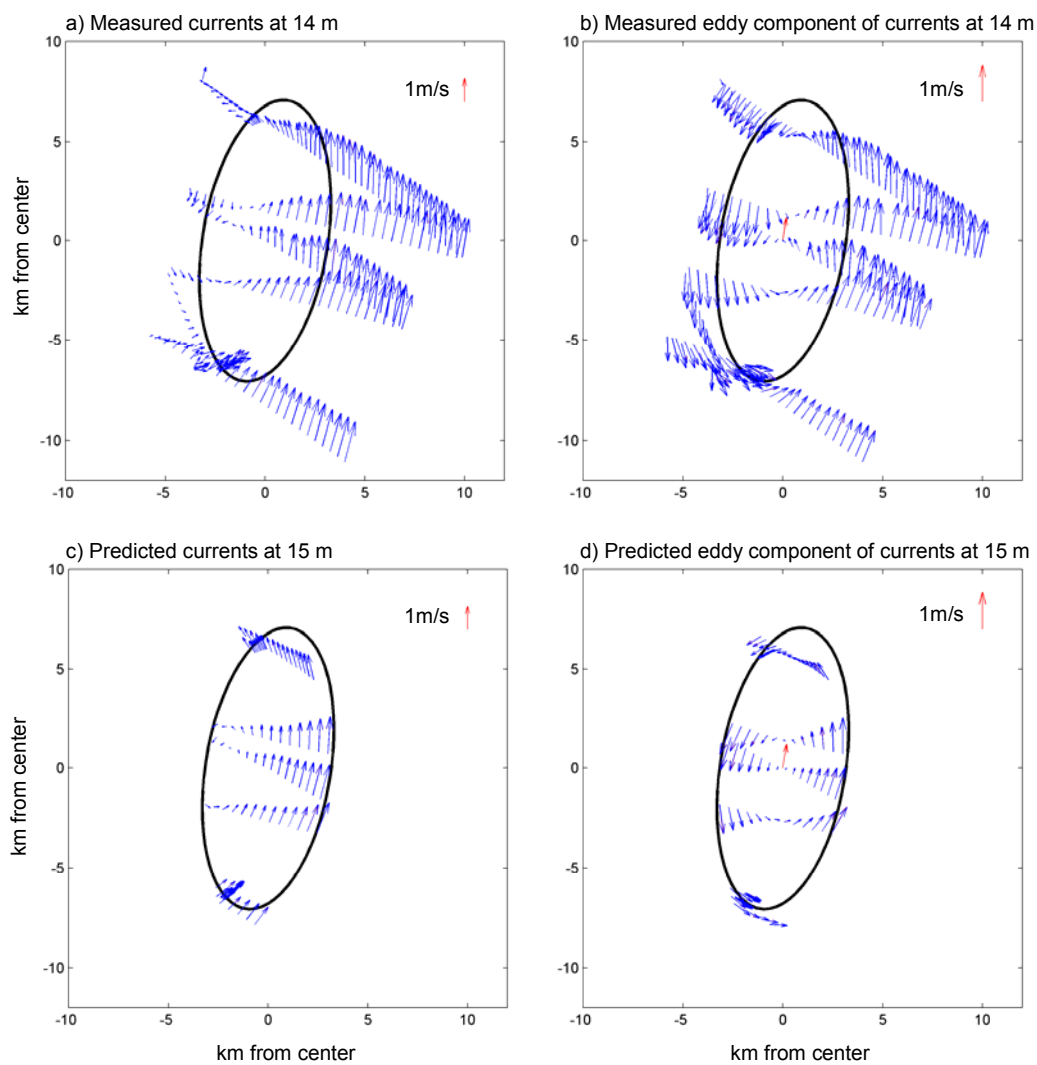


Figure 4.4. Surface measurements of a) salinity, b) temperature, and c) fluorescence from the series of cross-eddy transects on 28 Jun. The eddy is depicted in the same manner as in Fig. 4.3.

Figure 4.5. a) Measurements of surface salinity at the outside edge, center and inside edge of the front from the series of front crossings. b) Estimates of the width of the eddy front from the same series of front crossings.

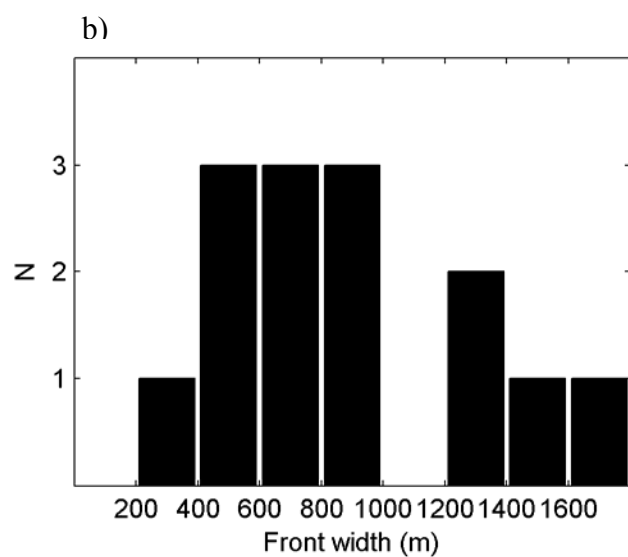
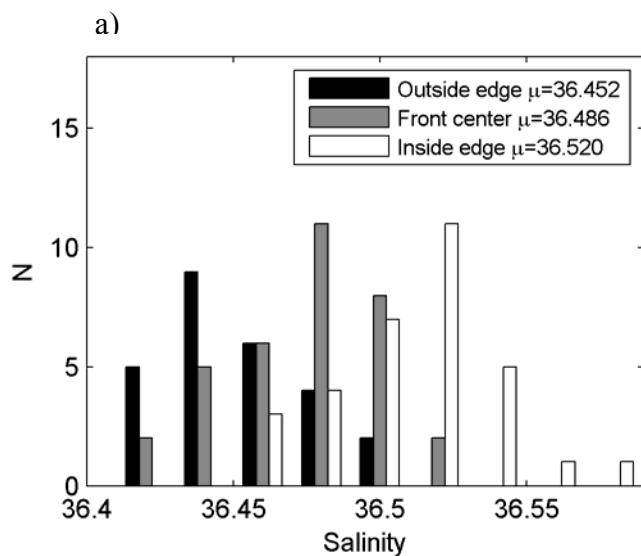


Figure 4.6. Vertical properties of the eddy. Cross-eddy measurements of the alongshore current from a combination of the 600 kHz ADCP (for the shallow currents) and 150 kHz ADCP (for the deep currents). These currents are for the a) transect just north of the eddy center as depicted in Fig. 3 and b) through the eddy center. The translation velocity of the eddy has been removed from these measurements. Vertical profiles of c) temperature, d) salinity and e) fluorescence from the series of five CTD casts, all in proximity to the edge of the eddy.

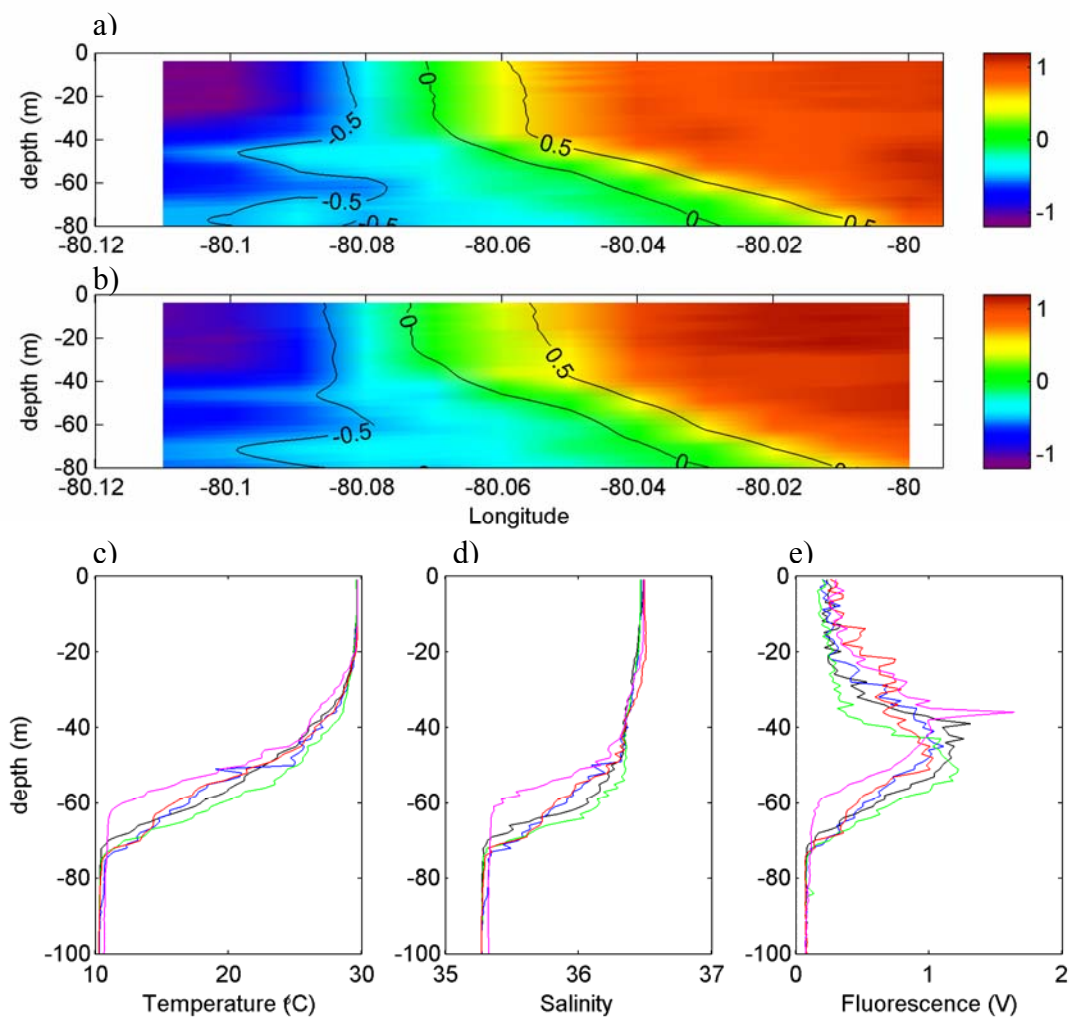


Figure 4.7. Seawifs satellite imagery during the time period of the study. a) Tortugas Gyre (TG) in the southern Straits of Florida 1-month prior to the study. b) By 18 June 04 this gyre has begun to be sheared apart and the remnant (TG-rem) are visible in satellite imagery. c) On 23 June no sub-mesoscale features are present in the southern SF. d) On 24 June the remnants of the TG are evident off the upper Florida Keys, and a higher-chlorophyll submesoscale eddy (SME) has developed off the lower Keys. e) On 25 June two SMEs are visible in the satellite imagery. The estimated location of the sampled eddy (3) is indicated but is obscured by cloud cover. f) Five SMEs are visible in the image including eddy 3 which was being sampled at that time period. g, h) 2 and 8 days after the end of the shipboard sampling finished a chain of SMEs is still present in the satellite imagery.

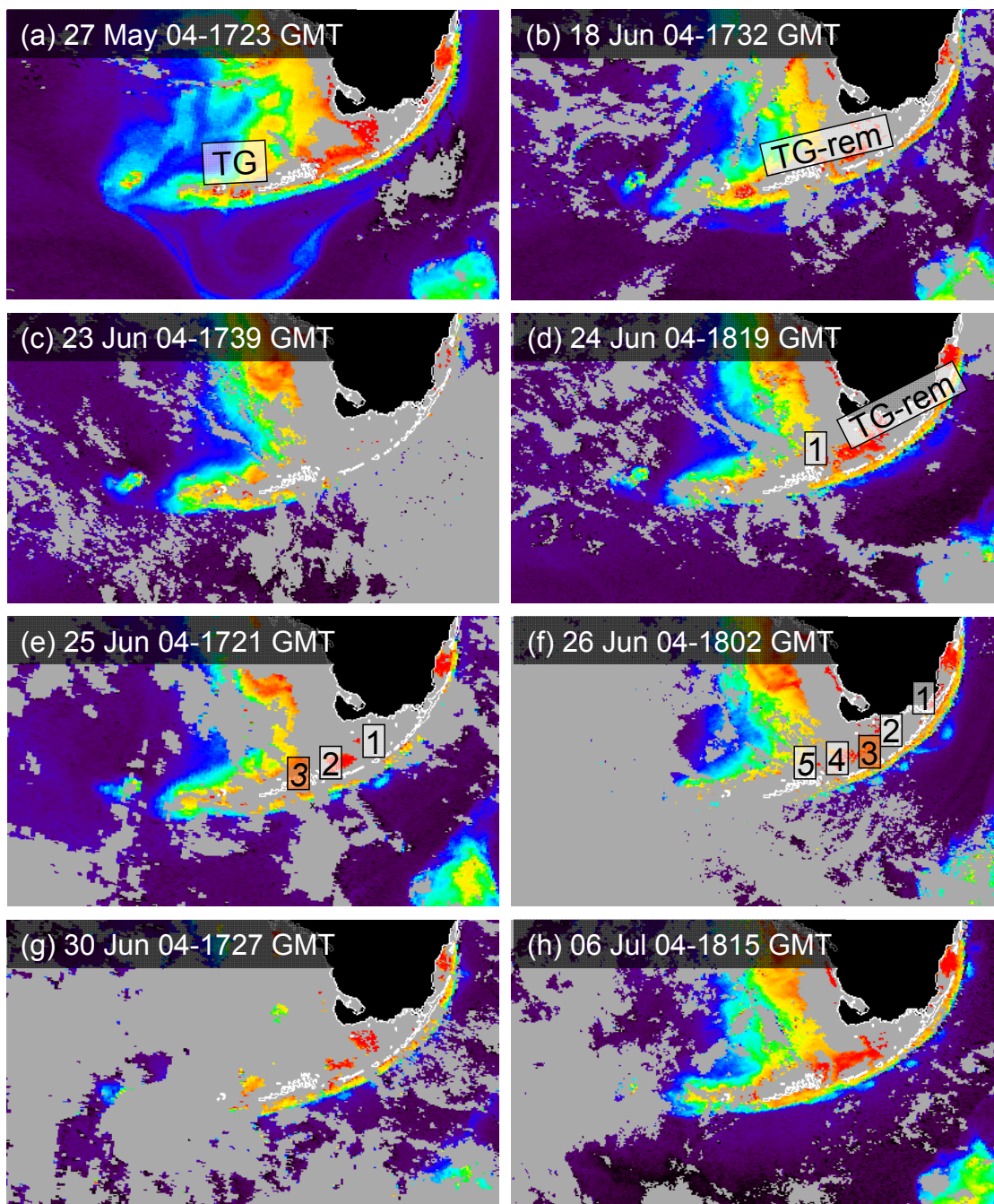


Figure 4.8. The distribution relative to salinity and the eddy front of a,b) billfish larvae, c,d) *Corycaeus* copepods, e,f) *Farranula* copepods, g,h) *Euthynnus alleteratus* eggs, i,j) *Auxis* spp. eggs, and k,l) scad (*Selar crumenophthalmus* and *Decapturus punctatus*) eggs. The boundaries of the eddy front are indicated in the plots on the left side. These salinity values are used to group stations in the bar graphs on the right side. The comparison between eddy zones for billfish and the two copepods species used an ANOVA. The comparisons between zones for the fish eggs used a Kruskal-Wallis test. If the eddy zones share a letter (a or b) they were not significant in the pairwise comparisons.

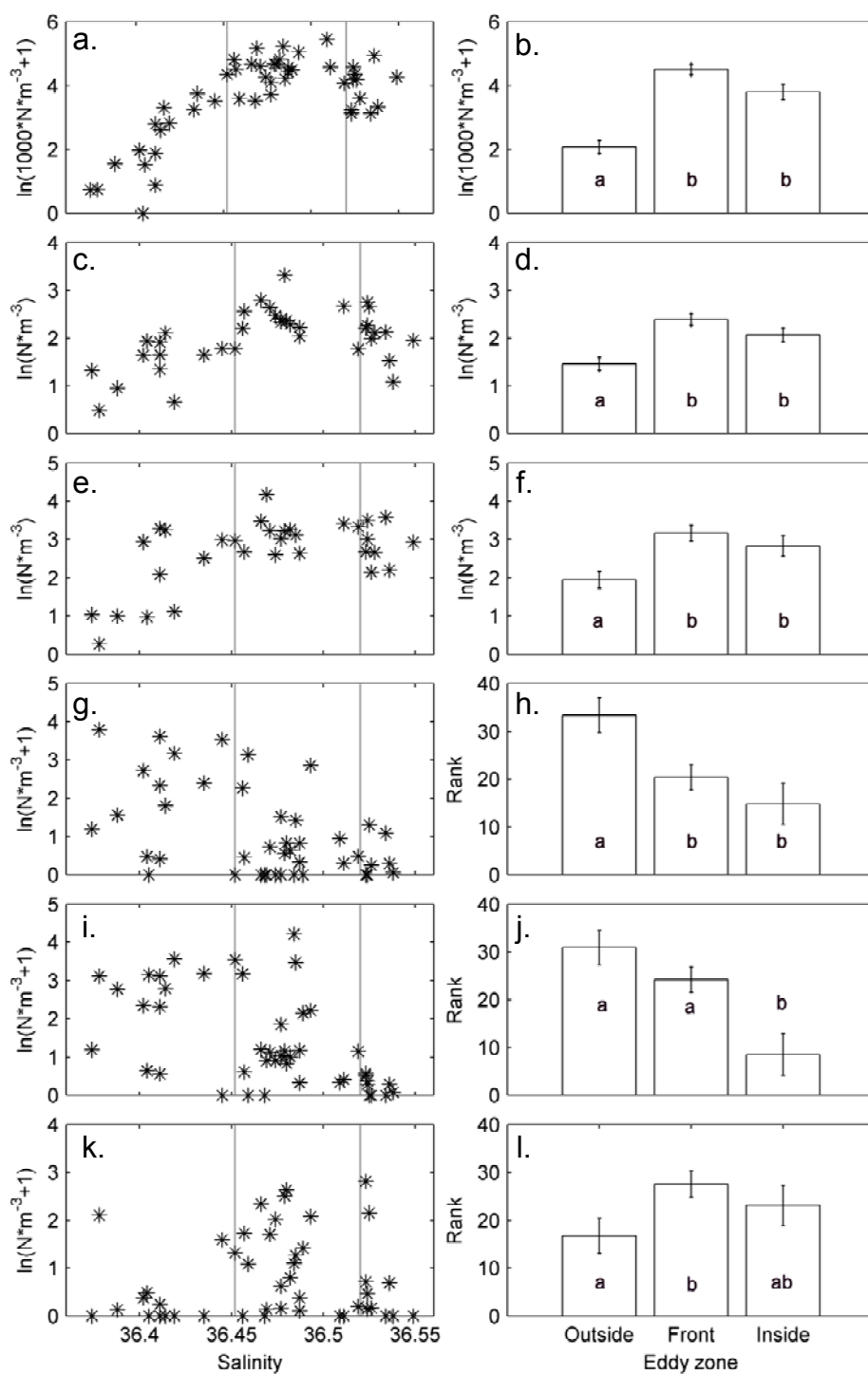


Figure. 4.9. Comparison between the distribution of early-stage (<2.5 mm) scombrid larvae and early stage sailfish larvae on the first complete day (26 Jun) of sampling around the drifters. a) The distribution of each taxon relative to the salinity measurements at the sample station. b) The relationship between scombrid densities and sailfish densities.

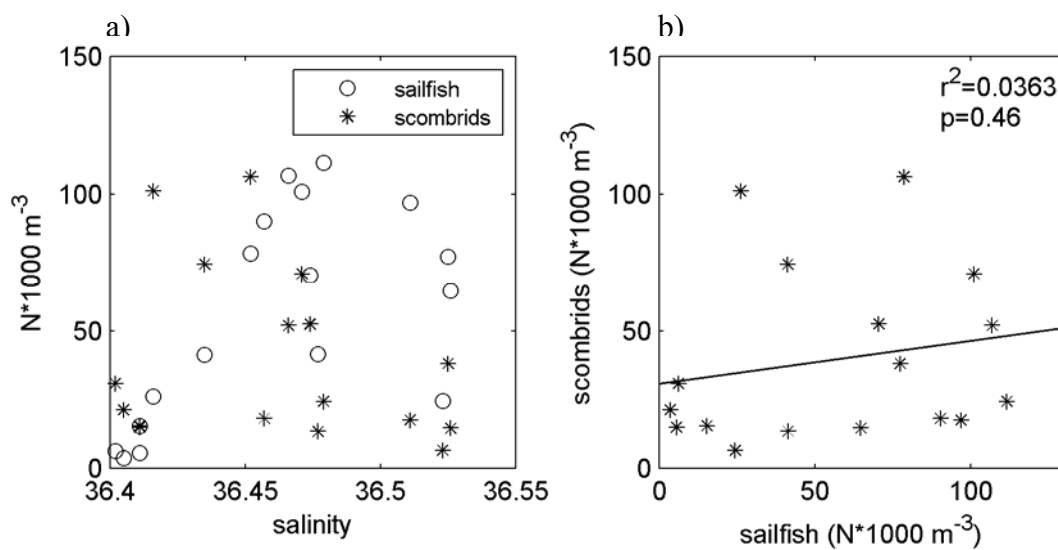
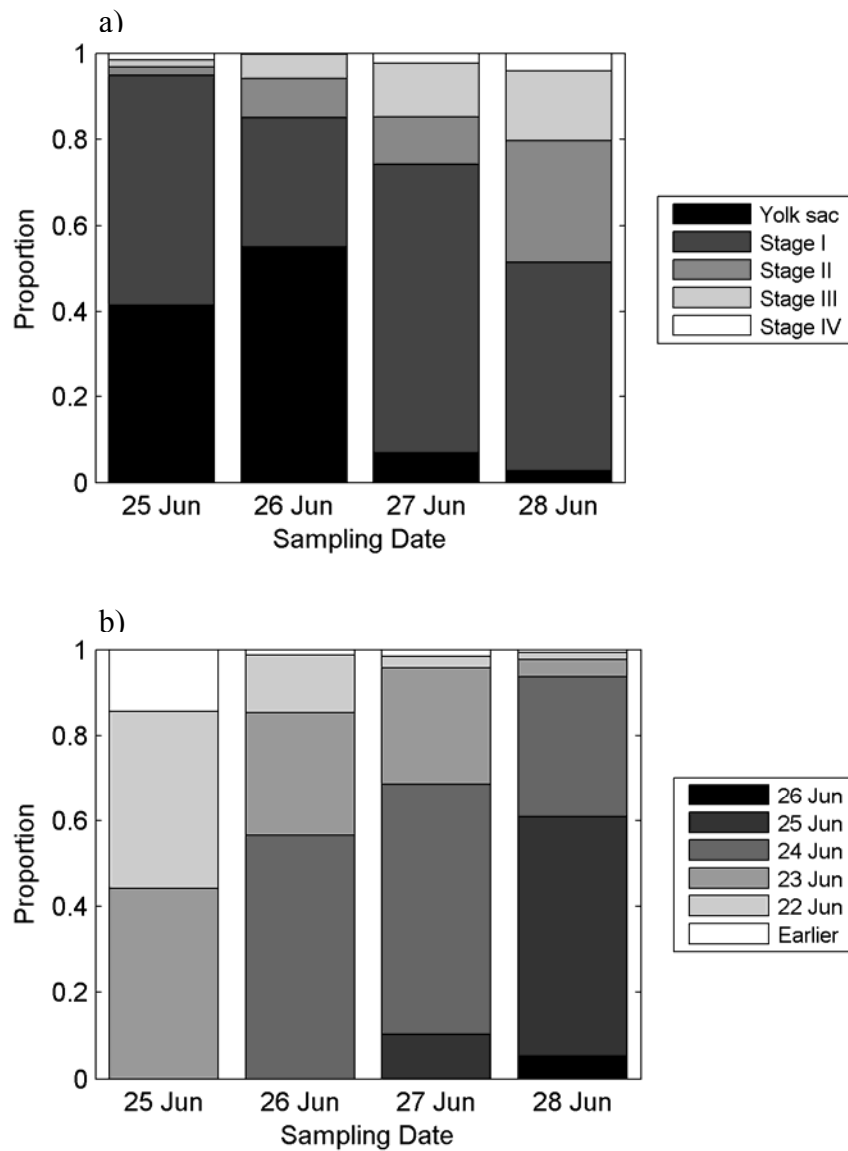


Figure. 4.10. Relative abundance of a) sailfish larval stages, and b) calculated spawn dates for larvae on the four days of sampling.



5. IMPORTANCE OF THE STRAITS OF FLORIDA SPAWNING GROUND TO ATLANTIC SAILFISH (*ISTIOPHORUS PLATYPTERUS*) AND BLUE MARLIN (*MAKAIRA NIGRICANS*)

Much of the uncertainty in managing highly migratory pelagic species results from the scarcity of fisheries-independent data relevant to determining long-term trends in abundance, migratory movements, and the relative importance of different spawning grounds. To address these issues, this study used an ichthyoplankton-based method to quantify the overall level of spawning of sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*) in the Straits of Florida (SF). It was estimated that during the two years (2003-2004) of the study 3.87×10^{11} sailfish eggs and 5.00×10^{11} blue marlin eggs were produced on an annual basis in this region. These egg production values, when combined with estimates of annual fecundity for each species, and the most recent stock assessment estimate of total biomass, indicate that about 2.1% of western Atlantic sailfish spawning and 1.6% of Atlantic wide blue marlin spawning occurs in the SF. Additionally, pop-up satellite tags deployed on sailfish at the start of the spawning season revealed their short residency times in the SF suggesting that a large ($\approx 10\%$) transient portion of the sailfish population is responsible for the SF egg production. Overall, this study provides a critically needed fisheries-independent method of quantifying spatial and temporal trends in the abundance of highly migratory species. The application of this methodology in the SF indicated that elevated levels of sailfish and blue marlin spawning occur in this area, and possibly more importantly, that the SF is a migratory bottleneck for these species.

Introduction

Two of the fundamental goals of fisheries science are to delineate stock structure, and to assess the abundance of individuals within and among different stocks (Begg et al. 1999). Highly-migratory pelagic species present a unique challenge to accomplishing these goals. For these species, the isolation of stocks may occur during just one portion of their annual movement, most commonly during spawning activity (Block et al. 2001; Magnuson et al. 2001; Block et al. 2005). Understanding the degree to which spawning grounds are isolated in space and/or time, and conversely, the degree to which individuals from the same spawning area overlap in different feeding areas, is thus critical for the management of these species (Lutcavage et al. 1999; Magnuson et al. 2001). However, for nearly all large pelagic species spawning grounds are poorly delineated and individual-level migratory movements are unknown. Additionally, due to the wide spatial distribution of highly-migratory species, their stock assessments almost exclusively rely on fisheries-dependent data. This runs counter to the well recognized need to incorporate fisheries-independent data, collected through a coordinated sampling effort, into stock assessments (NRC 1998). The result is that there are high levels of uncertainty and limited consensus about the status of most highly-migratory stocks (e.g. Myers and Worm 2003; Hampton et al. 2005), and a minimal ability to devise spatially-structured management approaches.

Ichthyoplankton surveys have long been used in the identification and assessment of fish stocks (Hare 2005; Stratoudakis et al. 2006). Their use in stock identification has primarily focused on the determination of spatially and temporally isolated spawning grounds. This is one key component of a multi-faceted approach to stock identification

that also requires individual-level information on adult movements (Hare 2005).

Ichthyoplankton studies also have been used to calibrate stock assessments by providing either an index of population trends (e.g. Scott et al. 1993) or through the more data intense method of estimating the absolute abundance of spawning fish (Hunter and Lo 1993; Pepin 2002; Ralston et al. 2003). The latter approach, in addition to requiring the sampling of the early life stages, also requires knowledge of the reproductive parameters of adults, and for migratory species the turnover of individuals on the spawning ground.

Blue marlin and sailfish are two large pelagic species that are critically important in a diversity of tropical and subtropical fisheries (Brinson et al. 2006). Though uncertain, the stock assessments indicate that blue marlin are overexploited and sailfish are fully exploited (ICCAT 2002; Restrepo et al. 2003; Die 2006; ICCAT 2007). Currently blue marlin is managed as a single Atlantic stock while sailfish is managed as a western and eastern Atlantic stock. Spawning has been documented for both species in a number of locations throughout the western North Atlantic (e.g. Serafy et al. 2003; Luthy 2004; Prince et al. 2005), though the relative importance of each of these spawning grounds, and the full spatial extent of spawning is unknown. Tagging studies on these species have revealed both long distance movements and a certain level of site fidelity (Ortiz et al. 2003; Prince et al. 2005; Prince et al. 2006), but with a few exceptions (e.g. Prince et al. 2005) most of this work has not been able to link these movement patterns to spawning. This scarcity of data useful to addressing stock structure and population trends, severely constrains the available management options for blue marlin and sailfish (Die 2006).

Previous work has determined that the Straits of Florida (SF) is spawning ground for sailfish and blue marlin (Luthy 2004). This relatively narrow (70-150 km) passage links the Gulf of Mexico and Caribbean Sea to the broader North Atlantic Ocean. Current patterns within this area are dominated by the Florida Current within which current speeds can exceed 2 m s^{-1} . The goal of this study was to quantify the importance of the SF spawning ground for sailfish and blue marlin. This work encompassed three specific tasks: 1) using larval surveys to estimate the annual egg production for both species in the SF, 2) using pop-up satellite tagging of adult sailfish to determine their residency times on the spawning grounds and interregional movement patterns, and 3) combining these two pieces of information with published adult fecundity estimates and stock assessment data to evaluate the number of individuals spawning in the SF and the relative importance of SF spawning ground.

Materials and Methods

Assessment approach

A modification of the larval production stock assessment method was utilized in this study to estimate the annual egg production (P_a) for sailfish and blue marlin in the SF as well as two additional measures of the relative importance of this spawning ground (Fig. 5.1). Traditionally, the larval production method has two components to the field work and data analysis. The first component, fully implemented in this application of the methodology, is a comprehensive ichthyoplankton study used to estimate P_a . The second component, for which we relied upon published values, is an adult reproductive study used to determine egg production per female or per unit of biomass. Within this second component we also incorporated an estimate of the total stock size, to calculate the total

stock egg production, and ultimately, the percentage of total stock spawning that occurs in the SF. Additionally, because of the highly migratory nature of these species, a third adult tagging component was added to this study. Logistical constraints restricted this third component to only sailfish. This adult tagging component was used to quantify the turnover of individuals on the spawning ground, and ultimately, the percentage of the stock that likely migrates through the SF during the spawning season. A comprehensive evaluation of the precision of the estimate of SF P_a (component 1) was performed; however the nature of the available data prevented an assessment of the error of values associates with the other two components.

Ichthyoplankton sampling and processing

Chapter 3 contains a detailed description of the sampling procedure. Briefly, ichthyoplankton was sampled over a 2-3 d period along a 17 station transect crossing the SF at 25°30' N (Fig. 2a) on a monthly basis from January 2003-December 2004. At the latitude of the sampling, current flow is nearly unidirectional (Fig. 2b). Two net systems were used to sample ichthyoplankton. The first, a combined neuston net, consisted of a 1x2 m 1000 μm mesh net attached to a 0.5x1 m 150 μm mesh net. This system was deployed with half of the frame out of the water and the volume of water filtered was measured with a General Oceanics flowmeter. The second, a combined MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System) contained a 4 m² 1000 μm mesh net and a 1 m² 150 μm mesh net (Guigand et al. 2005). This system allows for the precise opening and closing of individual nets at discrete depths with continuous measurements of the volume of water filtered. It was sampled from 100 m to

the surface with nets triggered in 25 m intervals. Current velocities concurrent to the collection of larvae were obtained from a 150 kHz Acoustic Doppler Current Profiler (ADCP).

Billfish were removed from the plankton samples and were identified either morphologically following Luthy et al. (2005a) or molecularly following Richardson et al. (2007). Standard length (SL) measurements were made using a dissecting microscope equipped with a digital camera and the Image Pro Plus image analysis software. For the samples collected in 2003 all of the nets were processed for billfish larvae. Within these samples $\approx 0.5\%$ of billfish larvae occurred in samples collected deeper than 25 m. For the 2004 samples, all of the larger mesh nets were processed, as were the fine-mesh net samples collected at the surface and in the upper 25 meters. Due to time constraints the fine mesh samples collected deeper than 25 m were not processed for billfish larvae. To account for the effects of net extrusion, all analyses were run using only data and individuals from the fine mesh nets for the <4 mm length classes.

A total of 121 sailfish larvae and 187 blue marlin were aged using the protocol outlined in Sponaugle et al. (2005a) and Luthy et al. (2005b). Measured and aged otoliths from genetically identified yolk-sac billfish and published information on egg durations (≈ 1 d) of scombroid larvae at $>26^\circ$ C (Margulies et al. 2007) verified that the otolith-based ages accurately estimated time since spawning and did not require a correction factor.

Regional egg production

In general two sampling criteria must be met to properly assess annual egg production (P_a), using the larval production stock assessment: 1) sampling must occur across the entire spatial extent occupied by the larvae of the assessed species or population, and 2) sampling must occur frequently enough in time to model the seasonal cycle of egg production (Pepin 2002; Ralston et al. 2003). For wide ranging species such as blue marlin and sailfish that spawn in fast currents, the requirement of sampling the entire spatial extent occupied by their larvae is not achievable. As an alternative, we considered the flux of larvae across the sampling transect, eliminating the need to sample a large two-dimensional grid of stations. This in turn allowed sampling to occur more frequently in time, and along a closer spaced set of stations.

The use of daily flux across a transect requires that an alternate means be used to estimate the area over which P_a is calculated. Both the regional current patterns and the age range of larvae used in the flux calculations will determine the size and shape of this area. Additionally, the area of egg production will not contain hard boundaries, but rather there will be locations upstream of the transect where only a portion of the egg production will cross the transect in the defined period of time. These issues were addressed using a larval transport model (Cowen et al. 2006). The specific implementation of the model used the $1/12^\circ$ (≈ 9 km) resolution HYCOM model with a particle tracking code to simulate larval dispersal. Particles ($n=100$) were released from a grid of 319 locations upstream of the SF on a bi-weekly basis from 15 May- 9 Oct for the 2003 and 2004 model year runs. Particles remained in the upper layer of the model (5 m) consistent with the vertical distribution of billfish larvae and were passive. From each

release location the percentage of particles crossing the transect in a 3-12 day period was quantified.

The implementation of the larval production method was a four step procedure: 1) the age of each larva was estimated from a length-at-age regression, 2) an apparent mortality rate (incorporates mortality and increasing net avoidance with age; Houde et al. 1979) was calculated using an abundance-at-age regression, 3) for each cruise, a daily egg production was calculated based on the age-specific flux of larvae across the transect and the apparent mortality rate, and 4) a non-linear regression of daily egg production versus ordinal day of year was used to calculate the annual egg production (Fig. 5.1, Table 5.1). A standard exponential model (Eq. 1,2, Table 5.1) was used for both the growth and mortality regressions (Houde 2002; Sponaugle et al. 2005a), with the calculation of the mortality rate restricted to larvae ages 3-12 d. Additionally a Gaussian curve (Eq. 4, Table 5.1) was fit to the seasonal cycle of egg production (Ralston et al. 2003). This non-linear least-squares regression was performed using the *nlinfit* function in the MATLAB statistics toolbox.

The one novel step in this four step process was the methodology used to calculate the daily egg production value (Eq. 3, Table 5.1). To calculate daily egg production it was first necessary to determine the age-specific larval flux at each station, a product of the density of larvae in each sampling depth bin (ind m^{-3}) and the concurrent transport across that depth bin ($\text{m}^3 \text{sec}^{-1}$). Transport rates were determined from the ADCP measurements. A transect-wide age-specific larval flux rate was then calculated for each cruise using a linear interpolation between stations. This age-specific flux of larvae was converted to the corresponding equivalent level of eggs using the apparent

larval mortality rate (z). These values were summed across all of the age classes. A 3 d minimum age was used in this calculation based on the minimum age of larvae considered to be accurately quantified. The 12 d maximum age was set to ensure that each age class had an adequate sample size, and to restrict the area over which the egg production estimates were calculated. Finally, a constant ($86,400=60*60*24$) was used to convert this flux rate value from the units of egg s^{-1} to eggs d^{-1} .

The development of confidence intervals for the estimate of P_a used a Monte Carlo simulation approach (Fig. 5.1). This approach accounts for the error propagation that is inherent in the multiple step calculation of P_a . More specifically, this approach arrives at a final confidence interval for P_a by considering the error in estimating L_0 and G_L from the age-length regression, z from the abundance-at-age regression, and P_a from the final daily egg production to day-of-year regression. For each species, 10,000 Monte Carlo simulations were performed. First, a L_0 and G_L value were sampled from their respective distribution (t-dist; sailfish df: 119, blue marlin df: 185). Subsequently, the abundance-at-age regression was performed and a mortality rate (z) and corresponding error were calculated. From the mortality rate distribution (t-dist: df: 8) a value was randomly selected. Using this set of parameters, a daily egg production value for each of the cruises was calculated, and the non-linear regression was performed. The MATLAB statistics toolbox *nlinparci* function was then used to obtain the 95% confidence intervals of the non-linear regression parameters. A final P_a value for that simulation run was randomly selected from its distribution. Additionally, to evaluate the contributions of each individual parameter to the final error in estimating P_a , a new set ($n=2000$) of simulations was run that only allowed a single parameter to vary from its median value.

Unique to the calculation of confidence intervals for this study is the consideration of a single transect as a unit of sampling. The extensive spatial autocorrelation of larval flux within transects does not allow for the use of standard techniques to estimate variance based on a station as a unit of sampling (Legendre and Legendre 1998), while at the same time, the number of stations per cruise (17) and the non-stationarity of the data are not conducive to the use of standard geostatistical techniques, especially within a simulation framework. The occurrence of temporal autocorrelation in the flux estimates was not expected considering the ≈ 30 d period separating cruises.

Reproductive and stock assessment parameters

An assessment of relative importance of the SF as a spawning area was made by comparing the estimates of P_a in the SF versus across entire western Atlantic sailfish stocks and Atlantic-wide blue marlin stock. To estimate total stock P_a it was necessary to consider the average annual female fecundity and the number of females in the stock (Eq. 7; Table 5.1).

Three parameters were required to estimate annual female fecundity: batch fecundity, interval between spawning, and the length of the spawning season (Eq. 5, Table 5.1). The spawning season duration was defined as the time period during which 95% of spawning occurs and was calculated based on the σ value from the regression of daily egg production versus day-of-year. The only comprehensive reproductive study of sailfish (Chiang et al. 2006) and blue marlin (Tseng 2002) is based on sampling the waters offshore of Taiwan. The latitude (22-24° N), habitat (western boundary current),

and spawning season length (≈ 5 months) around Taiwan are similar to the SF. For blue marlin, the exact values of the parameters obtained in the Pacific study were used. On the other hand, Pacific sailfish tend to be larger than Atlantic sailfish. Because of this, the average batch fecundity data for western Atlantic sailfish was estimated using an approximated scaling factor of 70% of the average Pacific sailfish batch fecundity. This scaling factor is based on difference (19 vs. 28 kg) in average size between western Atlantic (Jolley 1974) and western Pacific females (Chiang et al. 2006).

A calculation of the number of females in the stock (Eq. 6, Table 5.1) depended upon estimates of three parameters: stock biomass, female proportion of stock biomass, and average female weight. The stock biomass values were obtained from the most recent stock assessments (ICCAT 2002; ICCAT 2007). These assessments are sensitive to the weighting of various CPUE indices, and specific model parameters, and as such, they provide a number of different total biomass estimates. For both blue marlin and sailfish, the median of these estimates was used in our analysis. Importantly, the best available sailfish biomass estimates are based on model runs that were considered unsatisfactory by the assessment working group, and thus must be viewed with caution (ICCAT 2002). No estimates of female proportion of the biomass exist and thus this value was set at 0.5. In general, studies have found a higher proportion of males in spawning areas (Erdman 1968), though this is likely offset by the higher weight of females. Average female weight was obtained from Tseng (2002) for blue marlin and Jolley (1974) for sailfish.

Adult tagging and analysis

The adult tagging component of the study was designed to address: 1) the residency time of individual adult sailfish in the SF, and 2) the interregional movement patterns of adult sailfish during the spawning season. Twenty Wildlife Computers PAT-4 pop-up satellite tags were deployed on adult sailfish in the Straits of Florida between 25 Apr and 11 May, 2005 (Table 5.2). These tags were programmed to record temperature, depth and light levels at 30 s intervals for deployment duration of 120 days coinciding with the majority of the spawning season. The location (lower Florida Keys) at which most of the tags were deployed approximated the center of the spawning area that was assessed with the ichthyoplankton work. One additional tag used in this study was deployed offshore of Miami (25.68 N 80.16 W) on 30 Apr 03 for a 60 day duration.

Sailfish were caught using standard recreational fishing techniques. Those fish that were in poor condition after capture or were considered unlikely to be reproductively mature based on their size (<15 kg) were released without a tag. Fish handling, tag rigging and tag attachment followed an established protocol (Prince et al. 2005). The function of PSAT tags is described in detail elsewhere (Block et al. 1998). Briefly, after their programmed deployment duration the tags are designed to release from the fish, float to the surface, and transmit data summaries via the ARGOS satellite system. Position estimates are made using light-based geolocation, a methodology that inherently has large latitudinal and somewhat smaller longitudinal errors (Sibert et al. 2003). To partially account for this, the position data were processed using the manufacturer provided software, a sea-surface-temperature corrected Kalman filter (Nielsen et al. 2006), and a bathymetry filter (Hoolihan and Luo in press). Sailfish residency times in

the Straits of Florida were estimated primarily based on the longitudinal portion of their track, which has smaller estimation errors. The reliability of these residency time estimates is positively affected by the fact that the longitudinal band associated with the SF occurs in deep (> 25 m) water only in the SF and south of Cuba. The turnover of individuals on the spawning ground was based on the ratio of the spawning season duration to the residency times of adult sailfish (Eq. 9; Table 5.1).

Composite analysis

The final stage of the analysis involved uniting the three components of the broader methodology. The percentage of spawning estimated to occur in the SF (Eq. 8, Table 5.1) was determined from the estimate of SF total egg production (ichthyoplankton component) and total stock egg production (adult reproductive component). This, in turn was combined with the estimates of turnover of adults in the SF (adult tagging component) to calculate the percentage of the stock that likely passes through the SF during the spawning season (Eq. 10; Table 5.1)

Results

Egg production estimates

In total 648 blue marlin larvae (2.3-23.0 mm SL) and 684 sailfish larvae (2.3-22.8 mm SL) were collected. For both sailfish and blue marlin the correlation coefficients of the age-length regressions were high ($r^2=0.884$ and $r^2=0.943$ respectively; Fig. 5.3a,d). Length-at-hatch values were similar for both species, though the growth rates for sailfish were higher, as were the standard errors for both parameters (Table 5.3). Calculated

instantaneous larval mortality rates for sailfish (median: 0.413) were slightly higher than those for blue marlin (median: 0.377) (Fig. 5.3b,e; Table 5.3). Using the median values of L_0 and G_L , the correlation coefficients of the mortality rate regressions (sailfish $r^2=0.910$; blue marlin $r^2=0.961$) were also high. In the simulations, the mortality rate distribution was much broader for sailfish (95% CI: 0.30-0.62) than blue marlin (95% CI:0.29-0.47) with sailfish mortality exhibiting a notable skew towards higher values (Fig. 5.4a,c). The simulations also revealed a slight positive relationship between the growth and mortality rates, indicating that on average an underestimate of the true growth rate from the age-length regression would result in a calculation of a lower apparent mortality rate (Fig. 5.5a).

The regression of daily egg production versus day of year indicated that the peak egg production day occurred on 17 July for sailfish and 19 July for blue marlin. The σ value quantifies the length of the spawning season, specifically indicating with the median estimate that 95% of spawning will occur between May 14-Sept 18 for sailfish and May 4-Oct 2 for blue marlin (Fig 5.3 c,f).

The P_a estimates were higher for blue marlin (median: 5.00×10^{11}) than sailfish (3.87×10^{11}), though the CI on the sailfish estimates were larger (Fig. 5.4 b,d; Table 5.3). A strong skew in the P_a distribution towards higher values was evident. Between simulation variability in the P_a values resulted from an interaction of the errors associated estimating L_0 , G_L , z and P_a . As such it is important to consider how each of these variables contributes to the error in the final calculation. When the simulated values for each parameter were plotted against the resulting P_a estimate, a strong exponential relationship is evident between z and P_a (Fig. 5.5b). A weak positive relationship exists

between the G_L and P_a (Fig. 5.5c), likely due to the weak positive relationship between G_L and z . No trend was evident in the relationship between L_0 and P_a (Fig. 5.5d).

Simulations, run while holding three of the parameters constant at their median value and letting the fourth vary, revealed that the error in estimating z was the dominant source of error in the P_a estimates (Table 5.3). For blue marlin, only allowing the mortality rate to vary resulted in a CI for the estimate of P_a that was 78.3 % of the width of the CI when all parameters were allowed to vary. For sailfish, this value was 47% for the mortality rate, with the estimate of L_0 also having a notable affect at 31.6%. Finally, when the estimates of L_0 , G_L , and z were kept constant, the CI in the final P_a parameter estimate was relatively small (sailfish: 13.1%; blue marlin: 24.9 %) in comparison to the complete model CIs.

Area of egg production

The area of egg production estimated from the model encompassed most of the southern Straits of Florida, and to a lesser extent portions of the Santaren Channel (Fig. 5.6). The distances upstream that contributed notable levels to the calculated egg production were shorter along the edges of the SF than in the center. Only a limited amount of spawning in the Gulf of Mexico was expected to result in larvae that cross the sampled transect in the designated 3-12 d period.

Adult movements

Data for the entire 120 day deployment duration were obtained from 8 of the 20 tags deployed in 2005, and for a single 60 d deployment in 2003. One of the 120 day

deployment tags was recovered 7 months after deployment still attached to the fish, having not successfully released at the programmed time. Of the remaining 12 tags, 3 released prematurely (<10 days) and reported by satellite, and one was recovered, damaged and without data, shortly after deployment.

Over the tagging period all of the fish moved away from the SF, including transits to the west into the Gulf of Mexico, to the north into waters off the southern U.S. Atlantic States, and to the east into the Sargasso Sea (Fig. 5.7). The maximum distance a fish moved away from the SF was ≈ 1500 km. Estimates of residency time ranged from 5 to 43 days and averaged 24 days. Residency times were not estimated for the fish tagged off Miami in 2003. Residency occurred only at the start of the tagging period for all other individuals, with two exceptions (Fig. 5.8). One fish made a rapid 5 d transit between the Gulf of Mexico and the east coast of Florida at the end of the tagging period. For a second fish, the track produced using the Kalman filter suggests a two week residency in the southwestern portion of the SF during August. This fish occupied the eastern Gulf of Mexico for the period preceding and following this SF residency.

Composite analysis

Annual fecundity was estimated at 61×10^6 eggs and 334×10^6 eggs for sailfish and blue marlin respectively (Table 5.4). The total stock biomass value used in the analysis was the median of three model runs (7348, 11390, 12590 mt) for sailfish and five model runs (7920, 11228, 19244, 45418, 222690 mt) for blue marlin from their most recent stock assessments (ICCAT 2002; ICCAT 2007). These values were used to estimate a western Atlantic stock of 299,700 female sailfish producing 18.3×10^{12} eggs and an

Atlantic-wide stock of 96,200 female blue marlin producing 32.1×10^{12} eggs (Table 5.4). Based on the calculated egg production value for the SF, 2.13% (80% CI: 1.3-4.0%; 95% CI: 1.0-7.9%) and 1.56% (80% CI: 1.1-2.3%; 95% CI: 0.8-3%) of the total stock egg production for sailfish and blue marlin, respectively, occurs in the SF (Fig. 5.9). Given an estimated turnover of sailfish on the spawning ground of 5.3 times per season, the resulting percentage of the sailfish stock that moves through the SF is estimated at 11.3% (Fig. 5.9, Table 5.4).

Discussion

Fisheries-independent index

In reviews of the current state of Atlantic billfish stock assessments, Restrepo et al. (2003) and Die (2006) noted that a high level of uncertainty exists with respect to our understanding of the trends and abundances of these stocks. Specifically for blue marlin, low and in some cases negative correlations exist between the nine regional and oceanwide fisheries-dependent indices used in the assessment (Die 2006). This results in the stock assessment being highly dependent upon the weight (e.g. equal weight, proportional to catch) assigned to each index (ICCAT 2007). At this time little evidence exists to evaluate the relative merit of each of these indices. As in nearly all pelagic fisheries, much of the uncertainty in developing billfish CPUE indices results from the misreporting or underreporting of catch, temporal changes in fishing methods and targeted species, and a poor understanding of the spatial allocation of fishing effort versus the population distribution (NRC 1998).

To date, most of the effort aimed at improving billfish stock assessments has focused on revising analytical methods and improving the quality of data reported by the fisheries (Restrepo et al. 2003). While these advances are undoubtedly necessary, the current state of the stock assessment process as a whole, points to a clear and overwhelming need for the development of fisheries-independent indices. This study outlines an ichthyoplankton-based methodology that can be used to calculate the annual egg production and spawning stock biomass of billfish in the Straits of Florida. While the latter measure provides information in the standard unit of measure used in fisheries applications, the former, egg production, is quantified using a reduced parameter set and more limited sampling requirements, and thus, in practice, is a more suitable index to use in evaluating population trends.

Relative to other stock assessment methodologies, ichthyoplankton-based methods of assessing abundance are generally considered to consist of simple calculations based on readily measured parameters (Hunter and Lo 1993). This holds true for the methodology considered in this paper which requires the estimation of only 4 parameters using well established regression techniques. For the parameters derived from the age-length and abundance-age regressions, the exponential models used to fit the data were appropriate and the associated correlation coefficients were high. However, even with these high correlation coefficients, the estimates of larval mortality contributed heavily to the ultimate uncertainty in the estimates of annual egg production. One reason for this is associated with the high billfish larval mortality rates. Specifically, as the mortality rates used in the calculation increases, so does the impact of even small errors in their estimation on the calculation of annual egg production. In contrast for a

temperate rockfish species, Ralston et al. (2003) estimated a mortality of $z=0.11 \pm 0.011$ that contributed minimally to the final error in the egg production calculation. To narrow the confidence intervals associated with the mortality term in our study an increase in the number of individuals in each age class would be required.

One of the underlying assumptions in the estimate of annual egg production is that a Gaussian distribution is appropriate for modeling the seasonal component of spawning. Ralston et al. (2003) urged caution in applying this model due to a lack of studies with sufficient temporal resolution and coverage to verify its appropriateness. Indeed, the seasonal larval abundances of a set of 34 other pelagic species collected during this same project (Chapter 3) clearly indicates that this model is not universally applicable, as some species exhibit no discernable seasonal spawning cycle. However our frequent sampling demonstrated that the Gaussian curve was a good fit for the seasonality of spawning of sailfish and blue marlin.

In order for any fisheries-independent index to be usable in the stock assessment process it must be scaled across multiple years and preferably multiple decades, criteria not met by the two years of data used in this sampling. As a whole, the development of long-term ichthyoplankton datasets usable in stock assessments has been minimal within the tropical and subtropical waters where most large migratory pelagic species spawn. Exceptions to this occur for bluefin tuna (Scott et al. 1993) and king mackerel (Gledhill and Lyczkowski-Shultz 2000) in the Gulf of Mexico. Much of the reason for the absence of long-term ichthyoplankton-based fisheries-independent indices is likely methodological. Specifically, the cost and time associated with collecting ichthyoplankton is high, while morphological characters often only allow genus or family

level identification of larvae (Kendall and Matarese 1994). Recent advances in optical sampling techniques appear promising in terms of addressing the former issue (Cowen and Guigand in review), while molecular identification methods have advanced to such a level that the latter constraint should no longer be a major issue (e.g. Richardson et al. 2007). Within that context, an increased role for applied ichthyoplankton work seems timely.

In devising long-term sampling strategies and locations for fisheries independent monitoring, the merits of the approach outlined here are numerous and include the rapid survey time due to a natural bounding of the sampling domain and the high diversity of economically important pelagic and reef associated species spawning in the region (Chapter 3). The use of larval flux rates in this approach avoids the often unmet assumption that the entire spatial extent occupied by the larvae was sampled, and allows for an increased temporal component to the sampling. The drawback of the flux rate approach is that oceanographic models are required to estimate the area over which the egg production is calculated. These models have become one of the most powerful tools to address larval dispersal (e.g. Cowen et al. 2006), however, they likely overestimate rates of transport in the more complex nearshore currents. This will affect the delineation of the area of egg production. More specifically, our assessment may not have quantified spawning across a majority of the nearshore areas of the Florida side of the SF as the model depicts (Fig 6), but rather a more restricted portion of this area. For sailfish, which spawn at the Florida Current front close to shore, egg production within the geographic boundaries of the SF may be higher than is suggested by this study. The increase in oceanographic model resolution over time will allow this issue to be addressed.

Importance of the SF spawning area

One of the consistent challenges in studying migratory pelagic species has been to assess the relative importance of spawning areas. Billfish provide an excellent example of these issues. Serafy et al. (2003) reviewed the larval catch rates of blue marlin and other billfish in published studies, and noted the difficulty in making between-area comparisons due to differences in how data were collected and reported. The result is that our understanding of the spatial distribution of spawning in blue marlin and other billfish species is only slightly better than presence/absence data in a select few locations rather than the desired location specific productivity data.

The larval survey data presented in this study provides the critical information necessary to classify the relative importance of spawning grounds. Specifically, highly productive habitats are those that contain high levels of reproduction (P_a), growth (G_L), and survival ($1-z$), all parameters estimated in this analysis. When evaluated in the simulation framework described here, the importance of a composite approach to estimating these parameters is highlighted. Specifically, in this analysis the calculated mortality and growth rates were correlated as were mortality rates and egg production estimates. As a result, the error in estimating the quality/productivity of a spawning area will actually be less than the error in estimating individual parameters. That is, within the simulations for a single spawning area, high estimates of egg production, an indication of a productive habitat, tend to occur when mortality rates are also on the higher end of the estimated range, an indication of a low productivity habitat. Future comparisons between

spawning areas would thus benefit from a comprehensive consideration of the simulation output in the three-dimensional space of G_L , z , and P_a .

While no comparable billfish early life history studies exist in other locations, it is possible to make rough comparisons to blue marlin larvae collected in mid-July of 2000-2002 around the outer Bahamas (OB) (Serafy et al. 2003, Serafy unpubl. data). These larvae were found to have higher growth rates ($G_L=0.125\pm 0.004$; Sponaugle et al. 2005) than the SF larvae aged as part of this project ($G_L=0.114\pm 0.002$). Furthermore, for the comparable net (1x2 m 1000 μm neuston net), the OB sampling collected a higher density of blue marlin larvae (1.55 ind/1000 m^3 , 173 stations) than tows during the July and early August SF sampling (0.76 ind/1000 m^3 , 64 stations). Both of these factors suggest that the OB may be a more critical spawning area for blue marlin than the SF. Unfortunately, as with many other billfish-specific ichthyoplankton studies, the OB collections did not sample below the upper half meter, precluding calculations of water column integrated abundance (ind. m^{-2}), mortality rates, and regional egg production.

The incorporation of adult fecundity, movement, and stock assessment data into the analysis provides a means of evaluating the importance of the SF spawning area that is not dependent on the existence of comparable ichthyoplankton datasets. This can be done by addressing two questions: 1) what percentage of spawning in the stock occurs in the SF? and 2) for sailfish, what percentage of the stock transits through the SF during the spawning season? The answers to both these questions should be viewed only as rough estimates due to the use of stock assessment estimates of biomass, the limited number of adult sailfish used to determine residency time, assumptions about the female proportion of the stock biomass, and the use and modification of fecundity data from studies of

Pacific billfish. Currently, the most critical of these uncertainties is the sailfish stock biomass estimate, which was based on a model not accepted by the ICCAT billfish working group (ICCAT 2002). On the other hand the eggs/female versus number of females spawning curves (Fig. 5.9) provide an easy means of visualizing deviations in any of these parameters from the assumed values, and make it possible to modify these estimates as additional information becomes available.

The estimated percentage of spawning that occurs in the SF for both sailfish (2.13%) and blue marlin (1.56%) reveals two aspects of the broader spawning patterns of the species. The first is that spawning levels for both species are about 10-fold higher within the SF than would be expected if spawning occurred randomly across their range. More specifically, the area over which this egg production is calculated is only about 0.3% of the area occupied by the sailfish stock and 0.15% percent of the area occupied by the blue marlin stock. The second is that the SF is likely one portion of a larger spawning area complex. The larvae of both species have been collected in numerous locations throughout the Atlantic (e.g. blue marlin: outer Bahamas, Mona Passage, Gulf of Mexico, South Atlantic offshore of Brazil; sailfish: Barbados, Gulf of Mexico, outer Bahamas) and in most instances the larval catch rates in these locations were comparable or lower than in the SF (Bartlett and Haedrich 1968; Houde et al. 1979; Serafy et al. 2003; Prince et al. 2005). Adult reproductive studies also indicate that many more spawning grounds likely exist (e.g. Luckhurst et al. 2006). Pelagic species are generally considered to range in patterns of spawning from the high spatially and temporally restricted spawning areas of bluefin tuna, to the much more broadly distributed spawning of skipjack tuna (Schaefer 2001). While insufficient data exist to fully characterize the spawning

distribution of sailfish and blue marlin, this and other studies suggest that they exhibit a somewhat intermediate, if not more broadly distributed, regional spawning pattern.

Unlike the percentage of spawning that occurs in the SF, the percentage of the stock that transits through the SF during the spawning season would suggest that this area is critical in life history of sailfish. The short residency times of sailfish in the SF relative to their 4-5 month spawning season, indicates that a larger number of transient individuals, rather than a smaller number of resident individuals, must have accounted for the total egg production in the area. Assuming an average 24 d residence, or about 19% of the spawning season, the percentage of the western Atlantic sailfish stock that transits through the SF during the spawning season would be $\approx 11\%$. Higher values would be obtained from shorter residency times and *vice versa*. Addressing this issue in more detail would require a larger dataset of tagged fish, including fish tagged elsewhere for which their entire movement through the SF is recorded in the tag data.

Implications for stock identification and management

The minimal and haphazard spatial structuring of management approaches is one issue consistently cited as requiring improvement in fisheries management (Babcock et al. 2005). Currently, for most highly migratory species, the lack of understanding of migratory movements, the relative importance of different spawning grounds, and the degree to which isolated stocks are present, hampers the development of spatial management options. This study revealed that individual adult sailfish tagged at the start of the spawning season in the SF, a restricted area known to support spawning, moved extensively over a much wider area ($\approx 2500 \times 1200$ km) over the course of the season.

This occurred to such an extent that all tagged individuals were absent from the SF during the peak of the spawning season. Absent from the data was any movement south of the Yucatan Straits into the Caribbean Sea, an observation that in a larger dataset would suggest stock structure, but could simply be an artifact of sample size in this dataset. As a whole, the tagging data would suggest that the SF is one portion of a much wider spawning area extending from the Gulf of Mexico to the Sargasso Sea.

Interestingly, habitat features associated with billfish spawning, specifically strong frontal zones containing cyclonic eddies (Chapter 4), are consistently present within both the eastern Gulf of Mexico/Loop Current (Vukovich and Maul 1985) and the southwestern Sargasso Sea/subtropical convergence zone (Halliwell et al. 1991; Weller 1991) both areas occupied during the spawning season by adult sailfish tagged for this study.

To date most of the discussion with respect to spatial management options for pelagic species has focused on the utility of closing areas to fishing for certain portions of the year. Supporting this has been research using fisheries data (Goodyear 1998; Worm et al. 2003) or, more recently, archival tag data (Block et al. 2005). These studies have sought to determine hotspots of abundance of species of concern or diversity as a whole. The approach used in this study can contribute to this goal of determining hotspots in a quantified fashion, particularly when the full diversity of species collected in the sampling is considered (e.g. Chapter 3). Additionally, this approach can be useful in delineating migratory bottlenecks which are commonly referred to in studies of migratory birds, but less commonly used in the marine literature. Migratory bottlenecks are restricted areas through which a notable portion (often set at >5%) of a population or

species passes in a designated period of time, regardless of the abundance of individuals in that area at any one time (Birdlife International 2004). The data presented here strongly suggest that the SF is a migratory bottleneck for sailfish, and likely also for blue marlin, if their residency times are comparable. This designation is not surprising given the narrowness of the SF and its position between the much larger expanses of the Gulf of Mexico, Caribbean, and North Atlantic Ocean.

One question raised by this study is what management strategies are best suited for migratory bottlenecks that do not support a high abundance of individuals at any one time, but over time do contain a notable portion of the population. One of the primary motivations for time-area closures, particularly for bycatch species, is to reduce effort in areas with high CPUE levels (Goodyear 1998; Worm et al. 2003). Bottleneck sites may not fall into this category, and thus would not be included in most of the current designations of critical habitat. On the other hand, in migratory birds, migratory bottlenecks and stopover sites are recognized to be critically important in assuring the success of the migration, and the arrival of individuals in good condition at the end of their migration (Moore et al. 2005). Preserving the integrity of the ecosystem processes and the presence of a suitable forage base at these sites is critical in the management of the species that pass through them (Moore et al. 1995; Newton 2006), possibly more so than eliminating directed take of individuals. The importance of migratory bottlenecks in the broader lifecycle of any highly migratory pelagic fish species has not been demonstrated. However, in concept, it is clear that the abundance-based designation of conservation hotspots needs to be broadened to include categorizations for high turnover areas.

Conclusions

The absence of high quality data, rather than the inadequacies of analytical procedures, is considered the primary underlying factor constraining marine fish stock assessments (NRC 1998). For highly migratory species the uncertainties in single species stock assessments and management are particularly severe, while at the same time there is an increasing push to develop multi-species spatially-informed management procedures that incorporate environmental variability (Pauly et al. 2002). For such management approaches to be successful there is an overwhelming need for the development of high quality fisheries-independent datasets. Our study demonstrates that an ichthyoplankton methodology can be used to quantify the inter-annual and spatial trends in the distribution of highly-migratory species. Its application over two years in the SF provided a measure of the relevance of this spawning ground to sailfish and blue marlin. The expansion of this approach over multiple years and in other areas would undoubtedly provide new insights into the population trends and spatial dynamics of these species. Such information is critical to the development of the more holistic management strategies currently being advocated.

Tables

Table 5.1. Equation and parameter list. The equation numbers are provided and referred to in the text and the broad outline of the methodology in Fig. 1.

#	Equation	Parameters	Parameter Name/Description
[1]	$L_{age} = L_0 e^{(age * G_L)}$	L_{age} L_0 G_L	Length at age (d) Length at hatch (mm) Instantaneous growth rate (mm/day)
[2]	$N_{age} = N_0 e^{(-z * age)}$	N_{age} N_0 z	Abundance of larvae at age Abundance of newly spawned eggs Apparent mortality rate (day ⁻¹)
[3]	$P_d = C \sum_{age=3}^{12} Flux_{age} * e^{(z * age)}$	P_d C $Flux_{age}$	Daily egg production Seconds per day constant= 86,400 Age specific flux of larvae across transect
[4]	$P_d(t) = \frac{P_{a,SF}}{\sigma \sqrt{2\pi}} e^{-\frac{(t-\mu)^2}{2\sigma^2}}$	$P_d(t)$ $P_{a,SF}$ μ σ	Daily egg production at time t Annual egg production, Straits of Florida Peak day of egg production Standard deviation in days
[5]	$F_a = \left(\frac{BF}{I} \right) S$	F_a BF I S	Annual fecundity Batch fecundity Spawning interval Spawning season duration (d)
[6]	$N_F = \frac{B_{stock} * PB_F}{W_F}$	N_F B_{stock} PB_F W_F	Number of females in stock Total biomass (kg) Female proportion of biomass Average female weight (kg)
[7]	$P_{a,stock} = N_F * F_a$	$P_{a,stock}$	Total stock annual egg production
[8]	$\%P_{a,SF} = \frac{P_{a,SF}}{P_{a,stock}}$	$\%P_{a,SF}$	Percent of stock egg production that occurs in the SF
[9]	$T = \frac{S}{R_{SF}}$	T R_{SF}	Turnover on spawning ground during spawning season Residency time in SF (d)
[10]	$\%Transit_{SF} = \%P_{a,SF} * T$	$\%Transit_{SF}$	Percent of stock transiting through the SF during the spawning season

Table 5.2. Data from successful tag deployments on adult sailfish. Track color corresponds to Figures 6&7. Residency times are best estimates based on the fish tracks. SS=Sargasso Sea; SAB=South Atlantic Bight; NB=Northern Bahamas; EGOM=Eastern Gulf of Mexico; WGOM=Western Gulf of Mexico.

Fish #	Track color	Estimated size (kg)	Deployment			Pop-off			Estimated Residency Time (d)	Other regions visited
			Date	Lat	Lon	Date	Lat	Lon		
40606	Pink	18	4/30/2003	25.70	-80.17	7/1/2003	26.6	-73.67	-	SS; SAB
57176	Purple	18	4/25/2005	24.43	-81.92	8/23/2005	33.44	-78.55	6	SS; NB; SAB
57177	Brown	27	5/11/2005	24.50	-81.55	N/A			7	NB; SAB
57178	Yellow	20	4/28/2005	24.43	-81.89	8/26/2005	27.00	-79.97	43	SS; NB; SAB
57179	Blue	25	5/11/2005	24.49	-81.54	9/9/2005	27.44	-78.60	40	NB
57180	Red	18	5/3/2005	24.46	-81.69	8/31/2005	27.69	-95.26	13	EGOM; WGOM
57181	Green	20	5/8/2005	24.45	-81.76	9/5/2005	25.70	-79.97	34+5	EGOM
57182	Turquoise	18	5/8/2005	24.44	-81.80	9/5/2005	36.33	-72.21	5	SAB
57184	Light Blue	18	5/10/2005	24.00	-81.00	9/7/2005	24.79	-84.55	25+14	EGOM
									Mean = 24 d	

Table 5.3. Estimated parameters from egg production analysis. 95% confidence interval corresponds to the complete set (n=10,000) of simulations with all parameters allowed to vary. Effect of error on P_a corresponds to the final egg production estimate when only the specified parameter was allowed to vary. The 95% CIs are in parentheses, and the magnitude of this error relative to the total model error is given.

Parameter	Parameter name	Sailfish			Blue Marlin		
		median	95% CI	effect of error on P_a	median	95% CI	effect of error on P_a
G_L	Growth rate	0.130	1.218-1.387	11.0% (3.2-4.6x10 ¹¹)	0.1142	.110-.118	21.2% (4.2-5.7x10 ¹¹)
L_0	Length at hatch	1.966	1.707-2.294	31.6% (3.0-7.0x10 ¹¹)	1.98	1.917-2.060	16.6% (4.12-5.34x10 ¹¹)
z	Apparent larval mortality rate	0.413	.299-.618	46.7% (1.8-7.7x10 ¹¹)	0.377	.286-.471	78.3% (3.3-9.0x10 ¹¹)
P_a	Annual egg production	3.87x10 ¹¹	1.76-14.4x10 ¹¹	13.1% (2.7-4.5x10 ¹¹)	5.00x10 ¹¹	2.58-9.92x10 ¹¹	22.5% (4.43-6.08x10 ¹¹)
μ	Mid point of spawning season	199 (17-Jul)	185-206		201 (19-Jul)	194-208	
σ	StDev of seasonal egg production	32.3	11.9-47.8		38.6	31.1-46.3	

Table 5.4. Estimated reproductive and stock parameters used to calculate the measures of the relative importance of the SF spawning ground.

	Sailfish	Blue Marlin
Reproductive Characteristics		
Batch fecundity	0.91 x 10 ⁶	5.4 x 10 ⁶
Spawning interval	1.89	2.44
Spawning season duration	127	151
Annual female fecundity	61 x 10 ⁶	334 x 10 ⁶
Stock Assessment Values		
Total biomass	11,390 mt	19,244 mt
Female proportion of biomass	0.5	0.5
Average female weight	19 kg	100 kg
Total females	299,700	96,220
Composite Values		
Annual egg production, Stock	18.3 x 10 ¹²	32.1 x 10 ¹²
Annual egg production, SF	0.387 x 10 ¹²	0.500 x 10 ¹²
% of stock egg production that occurs in the SF	2.13%	1.56%
Residency time in SF (d)	12	-
Turnover on spawning ground during spawning season	5.3	-
% of stock transiting through the SF during the spawning season	11.29%	-

Figures

Figure 5.1. Outline of the three main components of the larval production methodology. Parameters are enclosed in circles and equations are enclosed in brackets, with details on both listed in Table 5.1. For the ichthyoplankton component of the study, the final product is the SF annual egg production. The Confidence Intervals of this value are determined using Monte Carlo simulations that take into account the error from the parameters in black circles. Parameters in grey for the adult reproductive component of the study were taken from the published literature.

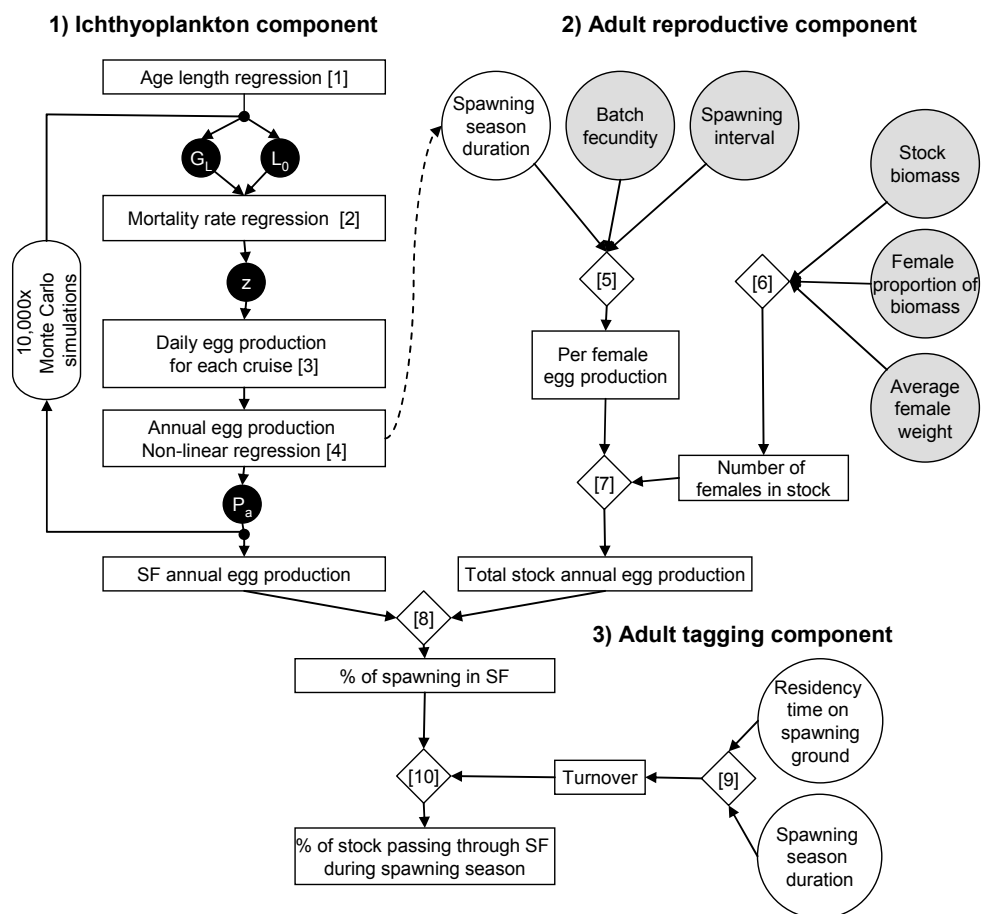


Figure 5.2. Location of sampling transect and current structure across the transect (a) Sampling stations (Δ) along the transect are indicated, (b) average north component of the current across the SF sampling transect.

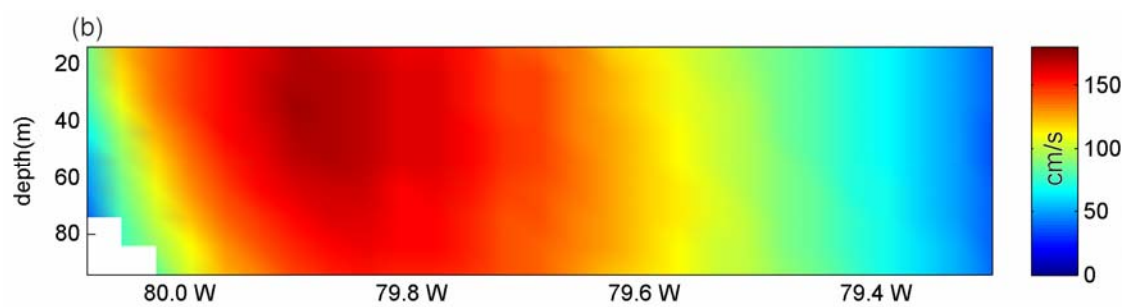
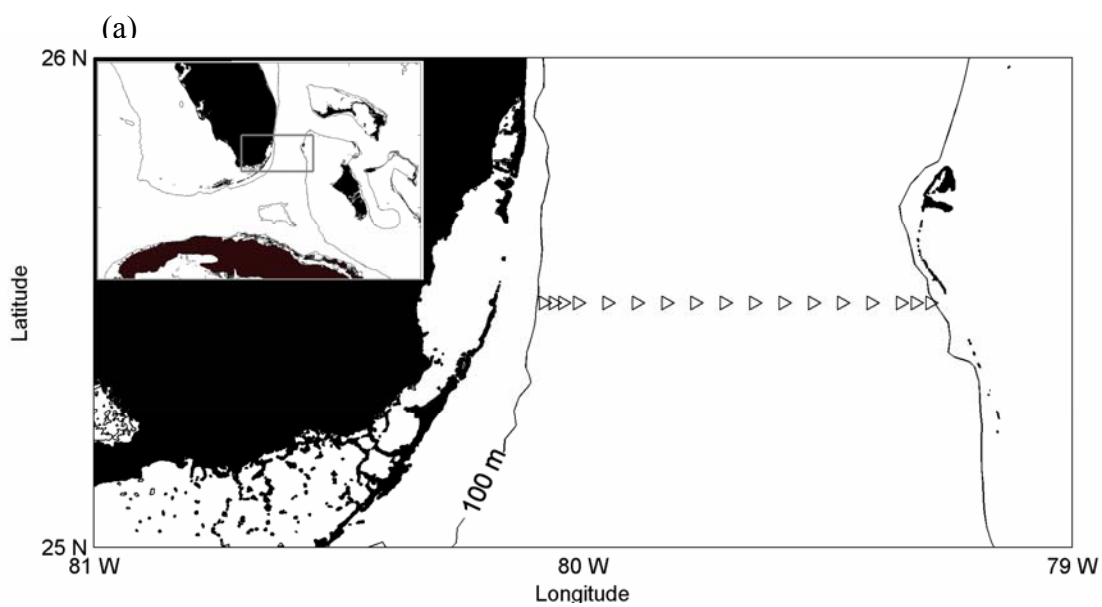


Figure 5.3. Regressions of (a,d) length vs. age, (b,e) abundance vs. age, and (c,f) daily egg production vs. day of year for both sailfish and blue marlin. The latter two regressions assume median parameters from the previous regressions. The peak day of egg production for sailfish (199) and blue marlin (201) corresponds to 17 July and 19 July, respectively.

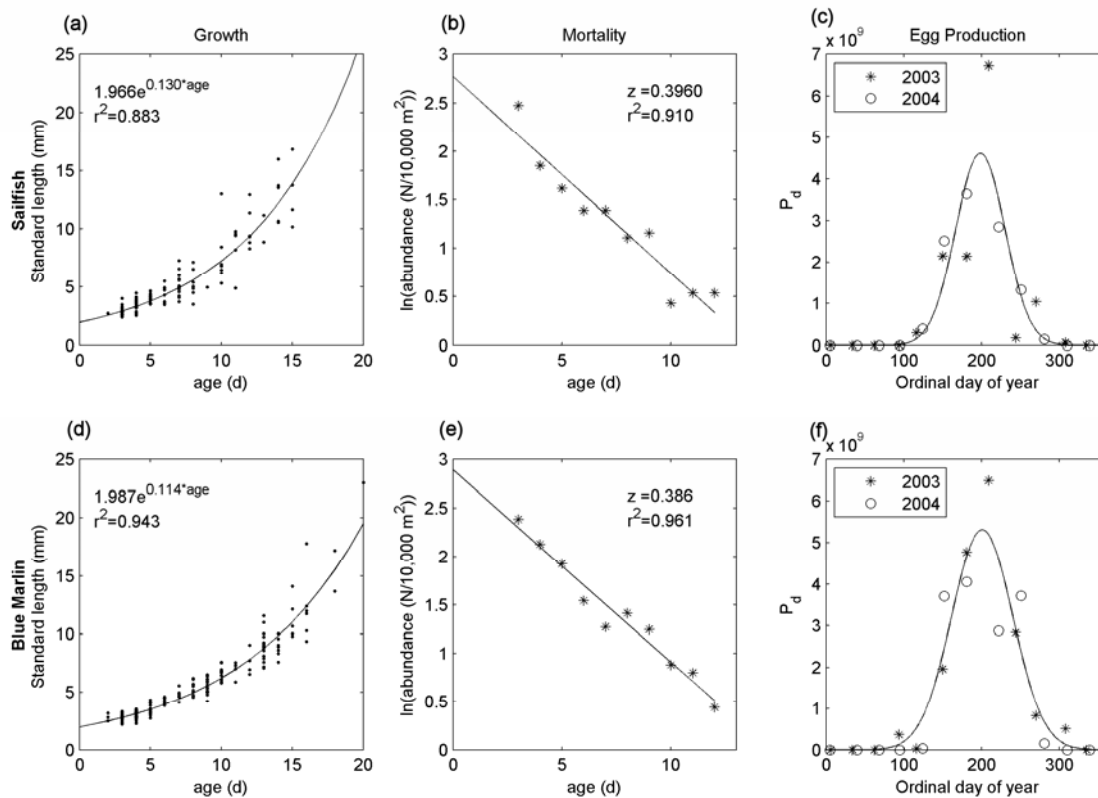


Figure 5.4. Distribution of mortality rate and annual egg production estimates from the Monte Carlo ($n=10,000$) simulations for (a,b) sailfish and (c,d) blue marlin.

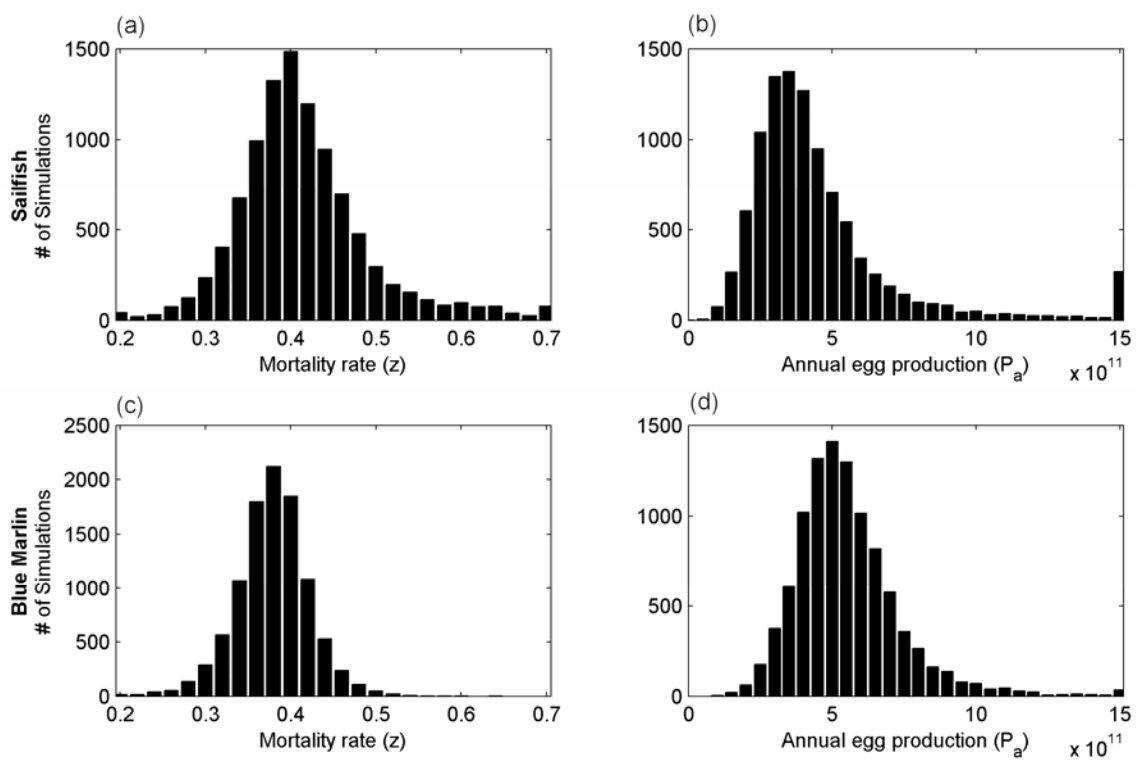


Figure 5.5. Relationship between parameters values from the 10,000 Monte Carlo simulations for blue marlin.

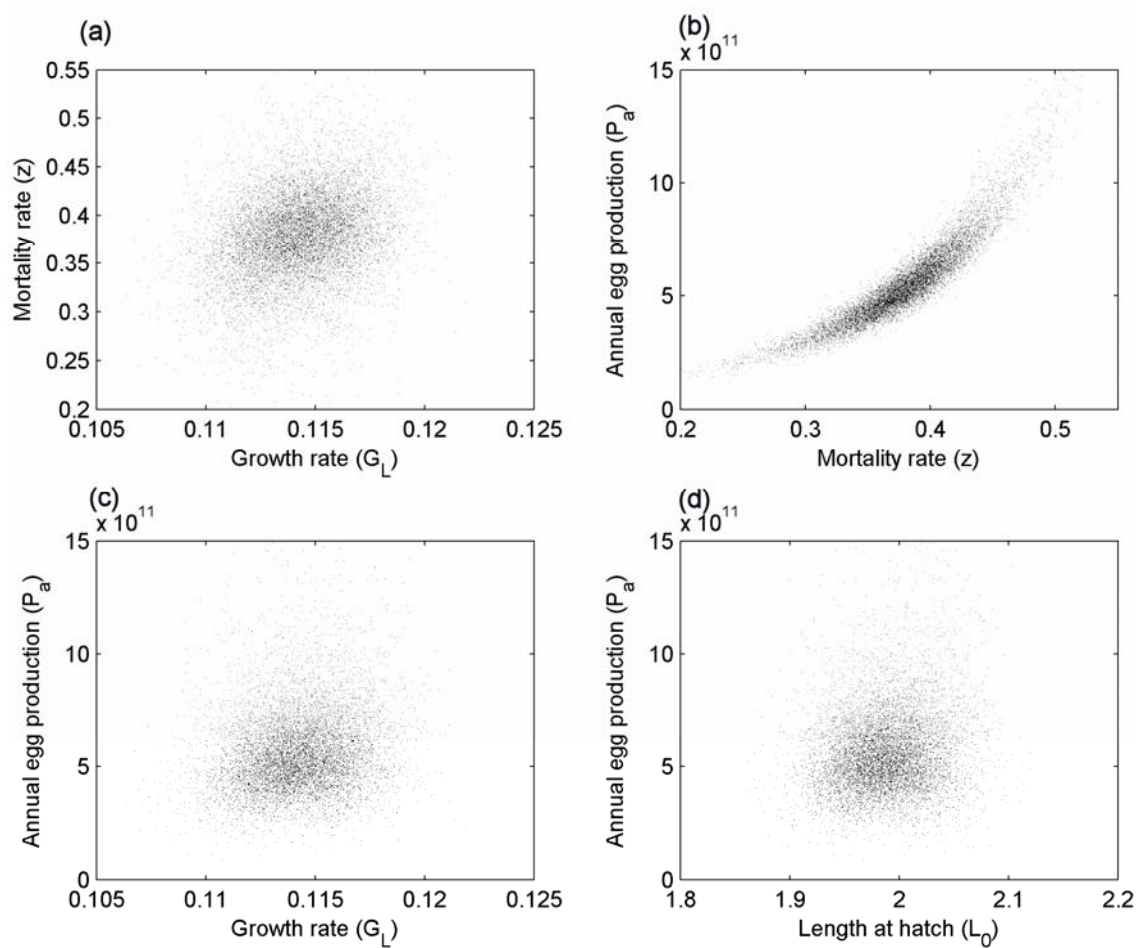


Figure 5.6. Area over which egg production estimate was calculated. The use of larval flux rates, versus the sampling of a two-dimensional horizontal grid results in a lack of hard boundaries to the area being assessed. The colorscale indicates the percentage of spawning at that location that would be expected to contribute to the calculated annual egg production in this study. That is, these values represent the percentage of virtual larvae in the transport model that cross the transect in a 3-12 day period of time, corresponding to the age range of larvae used in this assessment. The 319 particle release locations (x) are indicated.

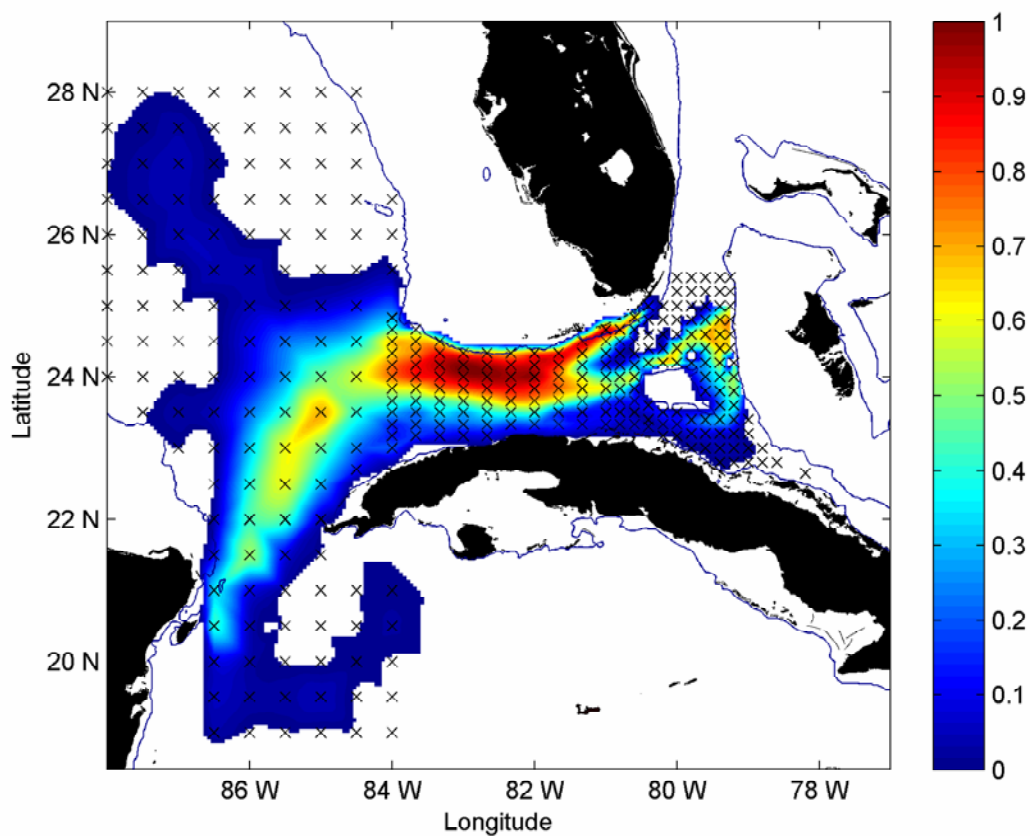
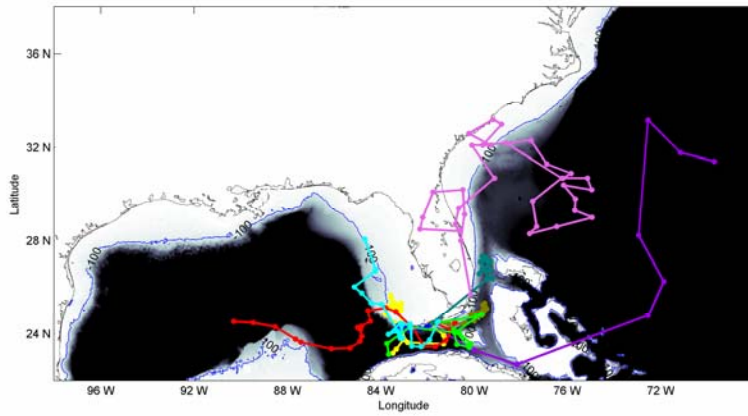
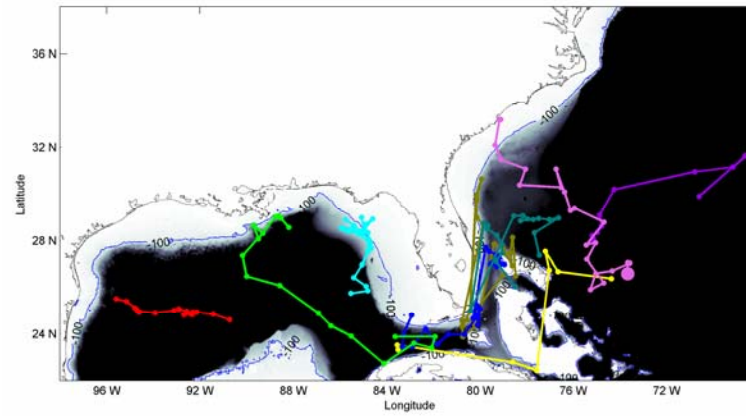


Figure 5.7. Tracks of the nine PSAT tagged sailfish by month. Tag pop-off locations are indicated with a large circle. Position estimates were processed with a Kalman filter and a bathymetry filter.

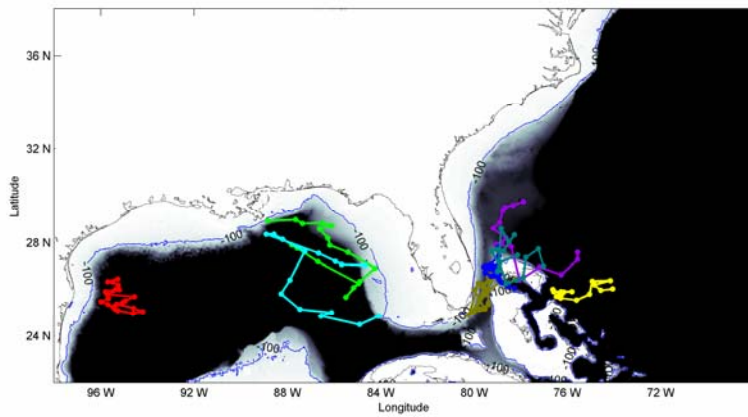
a) April-May



b) June



b) July



b) Aug-Sep

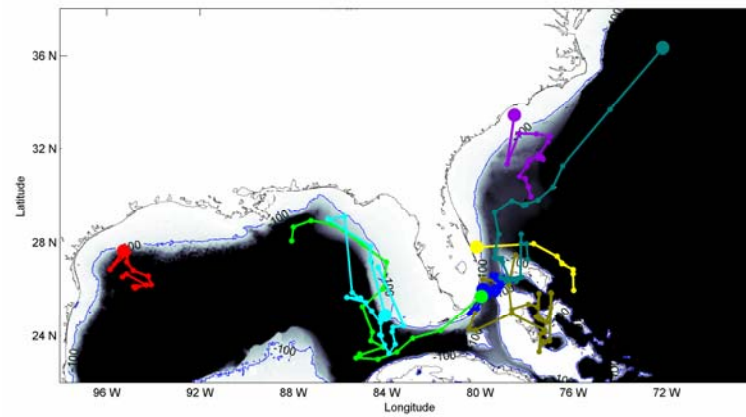


Figure 5.8. Longitude estimates of each tagged sailfish versus time. The Straits of Florida band of longitude is shaded. Estimated residency times for each fish are indicated in color on the right side of the figure. The shaded normal curve corresponds to the spawning season as indicated by the ichthyoplankton sampling.

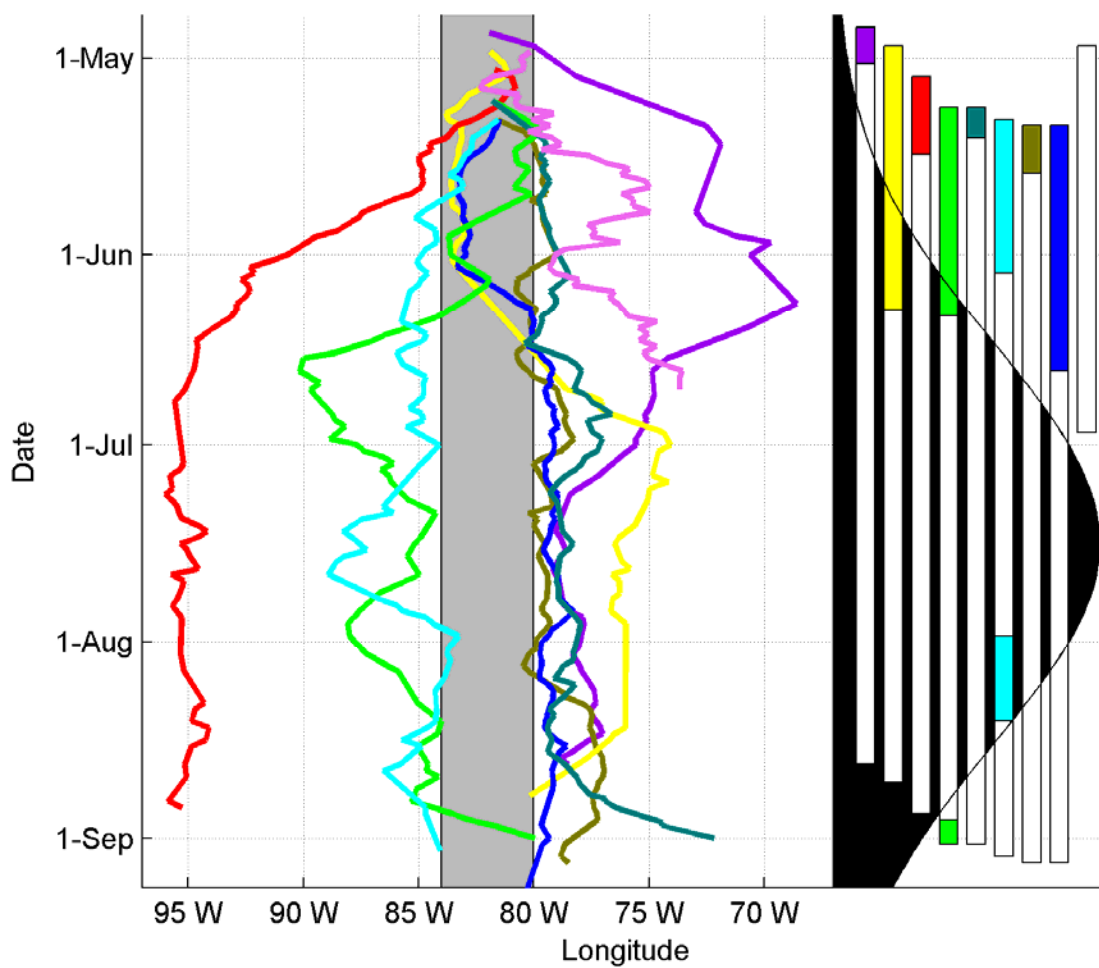
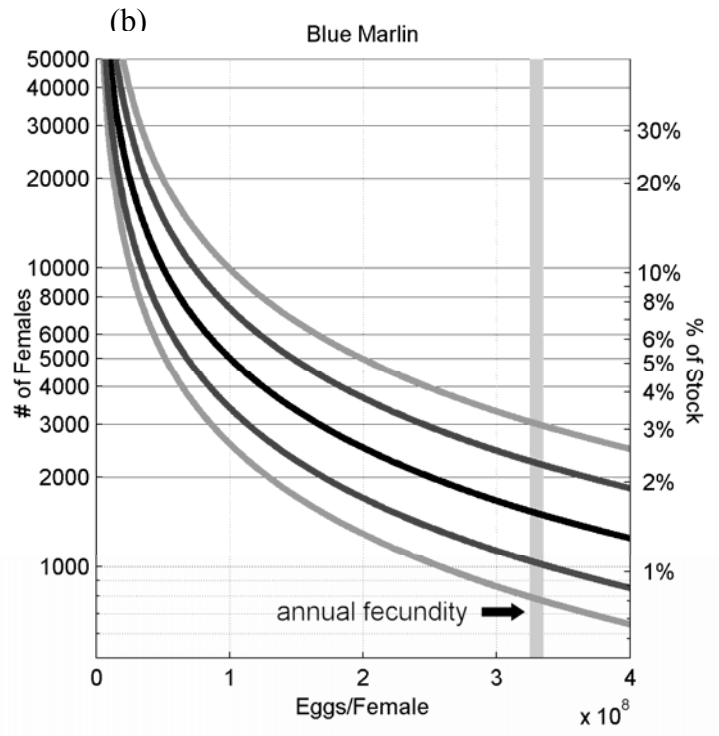
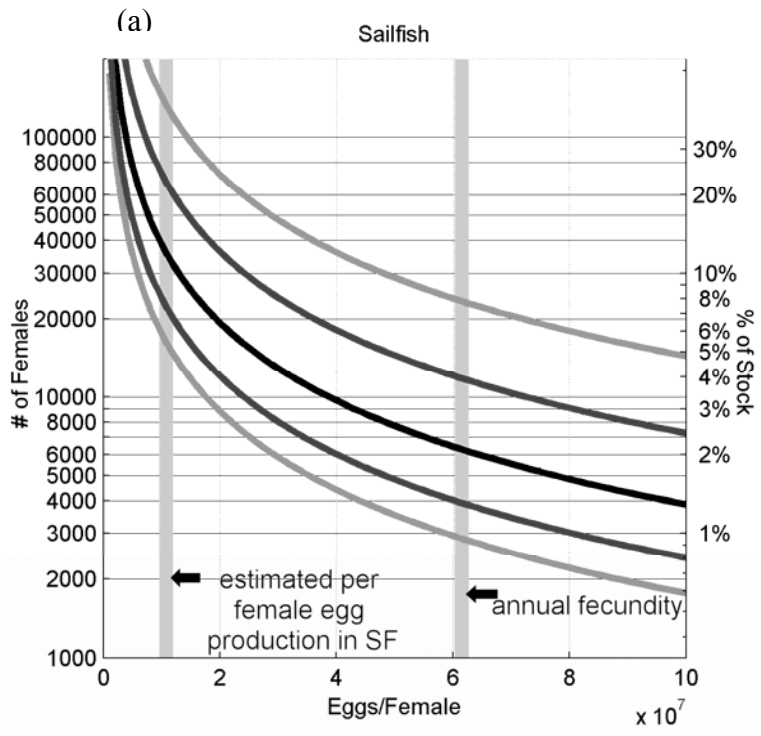


Figure 5.9. Estimated number of individuals spawning assuming different per female annual egg production values. The right y-axis represents these values as a percentage of the spawning stock. Values are plotted for the median, 80 percentile and 95 percentile values of the estimated SF annual egg production. Estimated annual female fecundity is indicated for both (a) sailfish and (b) blue marlin. (a) includes a second estimate for sailfish, incorporating the turnover rate of adults on the spawning ground.



6. GENERAL CONCLUSIONS

The study of spawning site selection in large pelagic fishes provides a critical link between two often separated fields, one focused on the processes that control recruitment and the other focused on the processes that control migration. Research done prior to this study provided a relatively ambiguous picture of spawning in billfish species. In general, the focus of these earlier studies was on the regional occurrence of larvae, with some basic estimates of the spawning locations that would account for the observed larval distributions. This dissertation sought to address the oceanographic characteristics of billfish spawning sites, and to provide a perspective of spawning site selection at multiple spatial scales.

Most of the broad questions addressed in this dissertation are not new, and it is likely that they will continue to be the important questions in research on pelagic species over the coming decades. What distinguishes this work is largely related to the methodologies that were employed. One critical aspect of this dissertation is the sheer magnitude of data collection and processing. This was in large part enabled by a redesign of the MOCNESS (Guigand et al. 2005). Additionally, it resulted from a novel molecular identification method (Chapter 2) that capitalized on an increasingly standardized approach to species identification (Hebert 2003a). In terms of describing the physical environment of spawning, this project integrated many existing technologies for data collection (ADCP, continuous shipboard measurements, satellite imagery, oceanographic drifters, electronic tagging of adult sailfish) and data analysis and visualization (Monte Carlo methods, drifter feature model, larval transport model). In large part, the broader

applicability of this dissertation, beyond research on large pelagic species or the Straits of Florida, is tied to the integration of these methodological aspects.

The objective of Chapter 3 was to resolve the seasonal cycle and spatial patterns of larval occurrence of pelagic species, and ultimately, to link these to the regional oceanographic conditions. The striking results of this portion of the study were that the larvae of nearly all of the species considered occurred within the Straits of Florida. Diversity peaked on the Florida half of the transect during summer. These species grouped into a number of distinct assemblages associated with specific environmental characteristics. These included 1) a summer Florida edge assemblage (e.g. *Auxis* spp., *Euthynnus alleterattus*, *Istiophorus platypterus*), 2) a summer offshore assemblage (e.g. *Makaira nigricans*, *Thunnus atlanticus*, *Ruvettus pretiosus*, *Lampris guttatus*), 3) an ubiquitous assemblage (e.g. *Katsuwonus pelamis*, *Coryphaena hippurus*, *Xiphias gladius*) and 4) a spring/winter assemblage that was widely dispersed in space (e.g. trachipterids). The primary environmental factors associated with these assemblages were SST (highest summer-early fall), day length (highest early summer), thermocline depth (shallowest on the Florida side) and fluorescence (highest on the Florida side).

The results of the assemblage analysis provided unique insights into the commonalities and differences in spawning and larval distributions among species. At the same time this work had some distinct limitations characteristic of studies of this nature. The broad correlative nature of an assemblage analysis makes it difficult to determine the relative importance of different environmental parameters on larval distributions. These results provide only a coarse resolution of the processes associated with spawning site selection as is reflected in the ambiguity within the analysis of the role

of fronts in structuring larval distributions. The second question left unanswered by an analysis of this sort, and to date a question that has not been answered in any billfish study, is the relative importance of the regional spawning ground for each of these species. These two major issues were addressed in the subsequent two chapters.

The results of Chapter 4 pointed strongly to the importance of small-scale processes in sailfish spawning site selection. Spawning in billfish was determined to be highly targeted at a frontal zone associated with the formation of a submesoscale eddy. This spawning strategy resulted in first-feeding sailfish larvae occupying a favorable feeding habitat. At the same time, large numbers of eggs of the prey items of adult sailfish were found in these samples, though interestingly, the eggs of only one taxa, scads, were found to occur predominantly at the front. Overall, the implications of these results are that the small-scale active targeting of a front by sailfish for spawning is likely driven by the availability of prey for their larval stages. At larger spatial scales the prey items of adults may play an important role. This structuring of spawning site selection at fine spatial scales is believed to have important implications for recruitment and migration in these species. Specifically, for recruitment-related analyses, these surveys indicate that sailfish larvae are not experiencing the average prey concentrations of the area, but rather occur in those narrow areas where convergence has served to concentrate organisms. Similarly for migration work, the consistency and persistence of small scale features may be driving the larger scale migration patterns of the species.

The broader inter-regional focus of Chapter 5 addressed the relative importance of the SF spawning ground. This type of question, while widely recognized as being critically important, has been nearly completely unanswered in billfish studies until now.

The evaluation of the importance of the SF was focused on two specific questions: 1) what percentage of spawning occurs in the SF? and 2) what percentage of fish migrate through the SF during the spawning season? The answer to the first question, using the best available stock assessment information, was that $\approx 2\%$ of spawning in the Atlantic blue marlin and western Atlantic sailfish stocks occurs in the SF. This number does not assign an overwhelming importance to SF spawning, but does indicate that spawning is much higher than would be expected if it were randomly distributed. The movement data on sailfish corroborates this conclusion by indicating that sailfish move over large areas during the spawning season. Accompanying these results is the indication that a much larger percentage ($\approx 10\%$) of the sailfish stock likely moves through the SF during the spawning season. This may suggest that this location is a migratory bottleneck for sailfish. Migratory bottlenecks are among the critical areas for conservation, and particularly measures directed at maintaining ecosystem integrity and a suitable forage base.

Recommendations for future research

The study of spatial and temporal processes in ecological research often focuses on two different factors, termed the grain and extent (Petersen et al. 2003). Grain refers to the smallest process that can be resolved, analogous to the pixel size of a picture. Extent refers to the length over which the study was conducted. For studies on spawning in large pelagic fishes there is an overwhelming need to both reduce the grain of the studies and increase their extent. Breakthrough technological advances (e.g. Cowen and Guinand in review) have the ability to do both, as has occurred with various physical

measurements through remote sensing. However, until these new technologies are readily available, and even when they are, there must be a more explicit consideration of 1) the scales associated with the most important questions concerning recruitment and migration in large pelagic species, and 2) the sampling approaches best suited to address these scales.

Many of the critical scientific questions pertinent to the management of large pelagic species concern large temporal and spatial scale processes. The development of electronic tags a decade ago provided the major technological advance necessary to address many of these larger spatial-scale questions (Block et al. 1998). It is now timely to consider how such results from tagging studies can begin to be integrated into a more comprehensive understanding of pelagic species. The results of Chapter 5 and Prince et al. (2005) illustrate one means of integrating tagging and early life history studies. The results provided by these studies can feed directly into the conceptual framework of migratory connectivity. This framework is focused on determining the relative importance of different feeding and breeding grounds, and the degree to which individuals from the same breeding area migrate to the same non-breeding area and *vice versa* (Webster et al. 2002). For species such as sailfish, for which little is still known about regional patterns of spawning, it will be critical to allow tagging studies to guide ichthyoplankton work. That is, a first step to choosing a location for ichthyoplankton work in the future should be an analysis of tagging data with a focus on where individuals are aggregating during the spawning season.

One of the most glaring problems with respect to research on the early life stages of large pelagic species is the near absence of a long time series of collections. This

problem is not unique to this system, nor is it unrecognized. This dissertation outlined sampling and analytical methods that could serve as the foundation for long-term monitoring of pelagic ecosystem structure. A resampling of this transect on a periodic basis (e.g. every 5 years) using the same equipment and approach should be considered.

At smaller spatial scales, there is an increased need for further process oriented and feature-targeted studies. Specifically, the role of convergence in plankton dynamics needs to be further studied. To date there has been some high quality conceptual work (Olson and Backus 1985; Franks 1992), and a limited amount of field work at river plumes (Govoni et al. 1989; Govoni and Grimes 1992). However, there is still great uncertainty about how different species respond to convergence. This includes the behavioral aspect of direct spawning at convergence zones. It also includes the behavior of planktonic organisms, and the extent to which they exhibit strong depth-regulating behavior that will allow them to concentrate in these zones (Genin et al. 2005). It can be hypothesized that a pelagic species will not spawn at a front if the prey item of their larval stages does not exhibit strong enough depth regulating behavior to be concentrated at a front. The collections around the eddy provide a means to address this hypothesis for certain species (e.g. scombrid larvae and their first feeding prey items, larvaceans).

The means to study convergence has benefited from the recent availability of high spatial and temporal resolution 2-dimensional maps of surface currents from HF radar systems (Shay et al. 1998; Lane et al. 2003). This capability, if integrated with an appropriate biological sampling strategy, could allow for a comparison of the predicted level of aggregation of organisms (given the observed temporal progression of convergence) and the observed aggregation. The field-validated understanding of

convergence that this would allow, would be a critical step in understanding fine-scale structure in the ocean.

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