Larval Billfish Dataset

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Product # 3 in support of using a 20-year time series to understand larval habitat and seasonality of four billfish species in West Hawai`i's 'Kona Hotspot'

Executive Summary

Understanding the habitat requirements of species throughout their life history provides insight into the environmental drivers that regulate their distribution and abundance. Identifying essential habitats used by larval and juvenile fishes has become a critical component of Ecosystem-Based Fisheries Management (EBFM) especially for commercially important fisheries species. Billfishes (Blue Marlin *Makaira nigricans*, Striped Marlin *Kajikia audax*, Shortbilled Spearfish *Tetrapturus angustirostris*, and Swordfish *Xiphias gladius*) are popular sportfishing targets in West Hawai'i Island. Despite their commercial and recreational value, there is currently no published information on larval distribution and habitat requirements for billfishes in Hawaiian waters. To address this knowledge gap, here we develop a new 20-year (1997-2018) dataset of neustonic ichthyoplankton collections from West Hawai'i and beyond focusing on four species of billfishes. We compiled observations of 1963 larvae over this 20-year period (from 998 discrete ichthyoplankton tows in West Hawaii), and provide specimen information and species level identifications for 1109 larvae using both existing and new genetic assignments. Additionally, we filtered these data to tows with consistent methodologies revealing 1761 larval billfishes (from 771 discrete surface tows in West Hawaii). We also constructed a thorough dataset of environmental variables (temperature, salinity, chlorophyll) associated with each occurrence of larval billfish and all absences using oceanographic data collected in-situ during cruises and satellite data. We present the first complete multispecies larval billfish dataset with coupled environmental data in the Pacific Islands Region ready for statistical analysis. We also provide the first high level analysis of these dataset to explore products that are now possible. This dataset will be critical for improving our understanding of how oceanographic features and environmental changes will affect larval growth, survival, and ultimately population replenishment.

Introduction

For many highly mobile pelagic fishes, including marlins and swordfish, preferred habitat can often be defined by water masses that are characterized by temperature, salinity, and dissolved oxygen (Braun et al. 2015, Rohner et al. 2022). However, this habitat can vary seasonally, annually, and across life stages. Adults may forage in water masses differing from their spawning locations (Suca, *pers. comm.,* Young et al. 2003). This is particularly true for species that develop endothermic capabilities such as bluefin tuna, swordfish, and lamnid sharks, where young life stages may not be able to withstand cold temperatures prior to endothermic

development, requiring adults to reproduce in warm waters (Suca et al. 2018, Braun et al. 2015). As billfish eggs and larvae are primarily neustonic, understanding the water masses in which they occur (Werner 2002, Boehlert and Mundy 1994) will improve our understanding of their distribution through space and time. From a management lens, knowing in which habitats larvae are found will also allow for understanding how larval mortality and thus populations might change with changes to their environment (Houde 2002) This in turn might influence our understanding of their recruitment patterns (Rutherford 2002). The leeward side of Hawaii Island near Kailua-Kona (hereafter referred to as West Hawai'i) is considered a billfish hotspot (Shomura and Williams 1972, Ditton and Stoll 2003). Since 1959 the Hawaiian International Billfish Tournament has been held almost annually in Kona, owing to consistent catches of marlin in the area (Naughton 1973). The mechanisms underlying this high concentration of billfish are incompletely understood. Eddies are common off the leeward side of Hawai'i Island, and these features are believed to drive efficient foraging (Arostegui et al. 2022, Seki et al. 2002) and likely create beneficial nursery conditions for billfish larvae (Hyde et al. 2006, Richardson et al. 2009, Bakun 2006) and juveniles. The presence of larvae in the same area where fishing occurs suggests spawning takes place nearby (Hyde et al. 2006, Serafay et al. 2003, González-Armas et al 2006).

Recent NOAA policy directives about Ecosystem-Based Fisheries Management (EBFM) have included goals which include "[b]uild[ing] habitat…mapping and designation capacity…with the goal of enhancing fish productivity and recovering protected resources"(NMFS Procedure 01-120-01, 2016). As such, identifying essential habitats used by larval and juvenile fishes, especially commercially and recreationally important fisheries species, falls within EBFM. With increased interest in billfish species being managed as a fishery as opposed to as by-catchmore formal designations of billfish habitat, including those of early life history stages, could become a part of science supporting management action.

In order to examine larval habitat and year-to-year patterns of variability in larval abundance and distribution for four billfish species in the Main Hawaiian Islands, we compiled all existing ichthyoplankton samples from surface tows and corresponding cruise data within the Hawai'i EEZ from 1997-2018. We then attempted to genetically identify larvae to species and compile and relevant biological data for all surface tows in our dataset. Finally, we used the cruise metadata to pair in situ and remotely sensed environmental data with larval occurrences and absences. We expect that this amalgamated larval and environmental dataset will serve the billfish community in a variety of research endeavors.

Metadata Compilation

We compiled all data from Pacific Islands Fisheries Science Center cruises (previously the Honolulu Lab of the Southwest Fisheries Science Center) from 1997 to 2018 that conducted neustonic plankton tows off the coast of West Hawai'i island (where billfish larvae are known to occur). We digitized both the narrative cruise reports and paper datasheets for cruises where digital data did not exist. We compiled all metadata available and filled gaps in missing information wherever possible. To characterize the physical oceanographic features at each of sites (tow) we compiled all associated in-situ environmental data including CTD casts,

thermosalinograph measurements, and chlorophyll fluorometry. A summary of the cruise information is presented below.

From 1997-2002, surveys were conducted on the Townsend Cromwell, after which surveys switched to the Oscar Elton Sette from 2003-present. Collectively, from 1997-2011, 861 tows were conducted, the majority of those tows (761) were ~60-minute surface tows using the 6'IKMT (505 um mesh or graded 5mm to 505um) done during daylight hours. About 10% of those tows were done during crepuscular hours (51) or at night (28). Surveys across all years were conducted between the months of March and November, which is the normal field season in our region due to scheduled ship downtime for repairs during the winter months, which also have less favorable weather conditions. From 2016-2018, a dedicated 3-year Slick Nurseries sampling campaign consisted of three cruises, two from the Sette in 2016 and 2017 and one shore-based field mission in the summer of 2018. These surveys are described in detail in Gove and Whitney et al. (2019); Whitney et al. (2021); and Smith et al. (2021), but generally surface tows were conducted using a 1m Neuston net (1-m diameter, 4.5-m length, 335-µm mesh, 300-µm mesh soft cod ends; Gove et al. 2019) and 6' IKMT (as described above).

The metadataset consisted of a combined 998 unique tows across the aforementioned sampling efforts. After we filtered the data to only include surface tows with relevant gear types (6' IKMT and 1m Neuston net), the final dataset consists of 770 samples from 19 research expeditions spanning 1997 to 2018 across the MHI. A total of 1891 billfish larvae were collected as a part of this sampling effort, of which 729 had been previously identified to species while 1162 were simply listed as "unknown Istiophoridae". The identification of these unknown specimens is detailed in the Specimen Biological Data Compilation section below. A github repository contains the code to conduct the merging of the two main datasets and is available on github: https://github.com/a-larval-schmidt/billfish_2024

The deliverable for the first phase is a single spreadsheet (30 columns) with all relevant metadata for 770 surface tows (described in the Header tab). This data has been compiled from many sources including paper datasheets that were not yet digitized. Paper data sheets were transcribed and merged with existing digital spreadsheets from archives. Extensive quality assurance/quality control has been done on these data to fill in gaps and ensure accuracy, fixing errors in data recording and investigating outliers. There were a few (~50) coordinate points which were flagged as inaccurate, these have been noted and fixed. The distance between start and end coordinates (when available) was used to calculate the distance that the 6'IKMT net was trawled and the corresponding volume of water sampled (when not measured with flow meters). Metrics such as tow length and volume filtered permit us to standardize the catch across samples, cruises, and years (Buenafe et al. 2022). Finally, a subset of draft available environmental variables were also included (i.e., temperature, salinity and chlorophyll α concentration values taken at 1m below the surface). A complete set of environmental variables associated with these plankton tows are presented in the Environmental Variable Compilation section below.

Graphical and tabular summaries of the data are presented below and on the following pages.

Table 1: Number of tows and cruises by month and year conducted from the Townsend Cromwell (1997-2002) and Oscar Elton Sette (2003-2011).

Month	Number of tows	Number of cruises	Years
March	29	1	2005
April	90	4	1998, 2006, 2009, 2017
May	72	3	2000, 2002, 2005
June	41	3	1998, 2001, 2005
July	42	2	2000, 2004
September	110	3	2004, 2006, 2011
November	33	1	1999

Table 2: Summary of Cruise Effort from 1997-2011 conducted from the Townsend Cromwell (1997-2002) and Oscar Elton Sette (2003-2011).

Cruise	Year	Month	Days	6'IK tows (N)
TC9703	1997	April	5	55
TC9804	1998	April	2	17
TC9806	1998	June	3	32
TC9911	1999	Nov	4	46
TC0006	2000	May	2	28
TC0008	2000	July	4	43
TC0106	2001	June	4	44
TC0203	2002	May	4	45
SE0303	2003	May	5	1
SE0409	2004	July	3	33
SE0412	2004	Sept	7	86
SE0503	2005	March	5	52
SE0507	2005	May	5	52
SE0605	2006	April	5	44
SE0610	2006	Sept	6	59
SE0903	2009	April	9	56
SE1106	2011	Sept	11	68

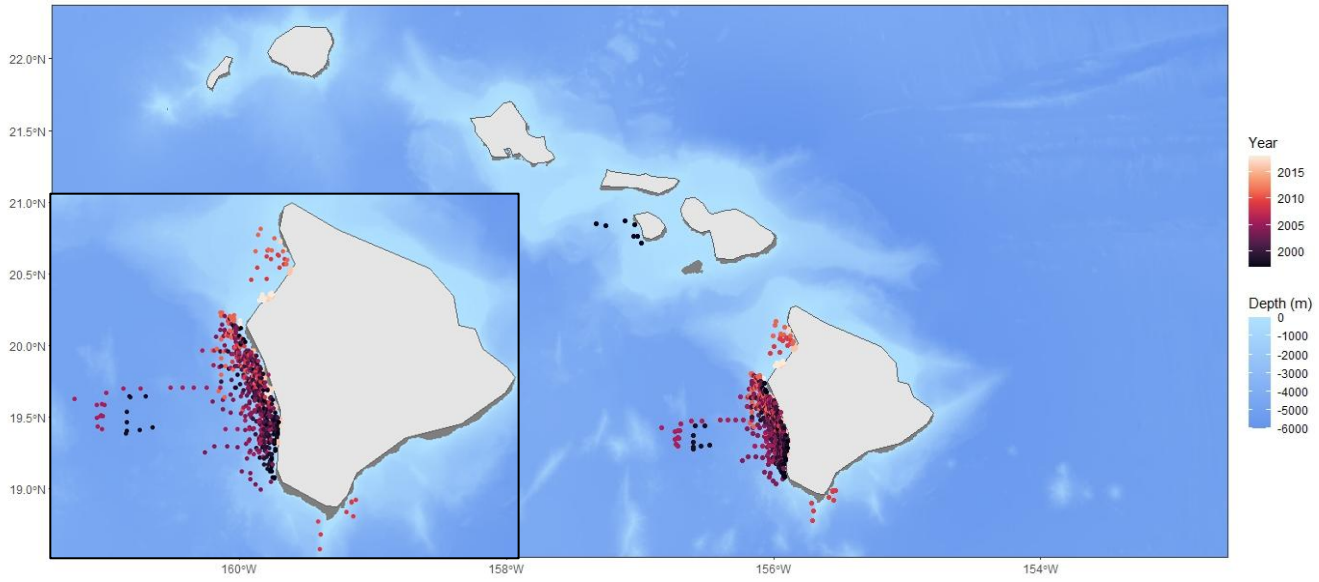


Figure 1. Map of survey effort with each point representing a single trawl (using starting coordinate points in the Tow Metadata dataset) colored by year across the Main Hawaiian Islands and Hawai`i Island specifically (inset). The majority of surface tow sampling was focused on West Hawai`i with overlapping spatial coverage between years.

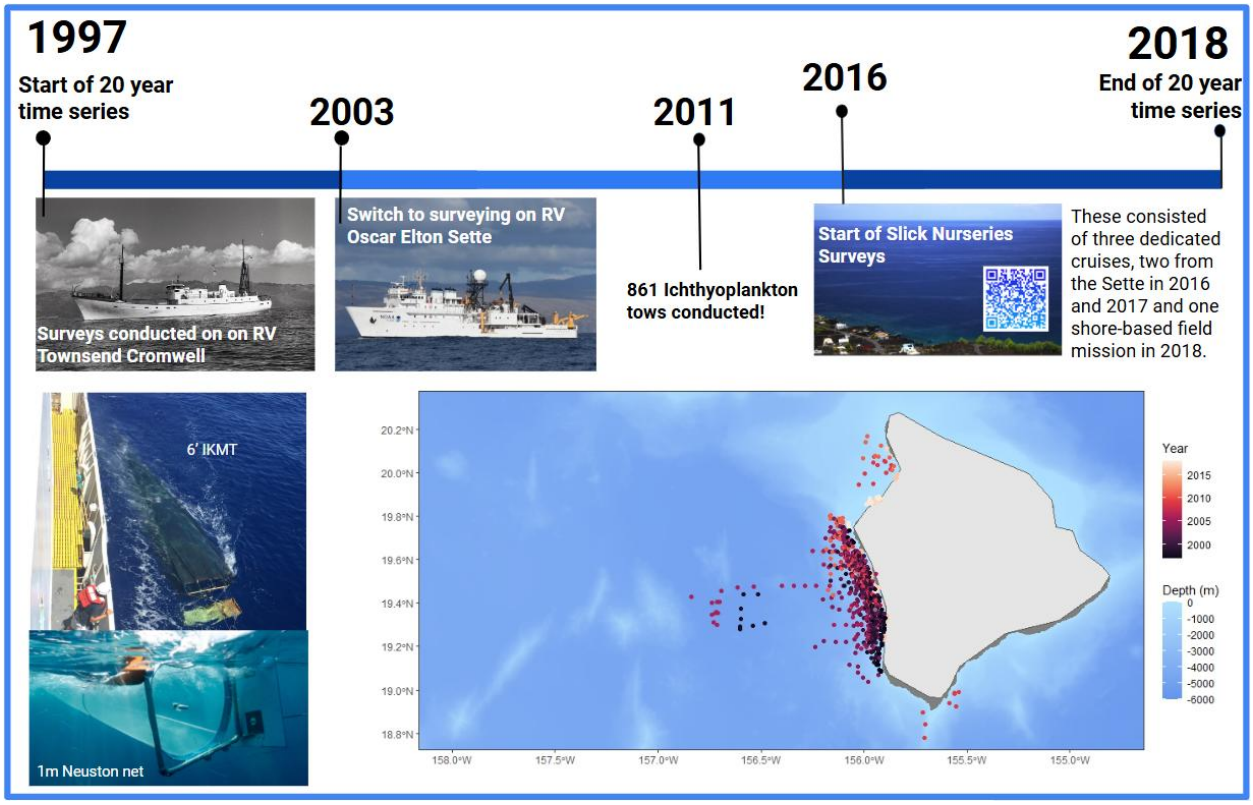


Figure 2. Graphical summary of survey effort broadly reflecting the West Hawaii Ichthyoplankton Time Series from 1997-2018 used in this effort.

Specimen Biological Data Compilation

Species level identifications are integral to understanding larval billfish habitat. Adult billfish vary in their thermal preferences, for example the range of water temperatures where *Kajikia audax* spend most of their time is 6°C greater than the range of temperatures where *Tetapterus angustrirostris* were observed (Arostegui et al. 2019). *Makaira nigricans* are observed most frequently between 22 and 27°C, with excursions to temperatures as low as 17°C (Block et al. 1992). To what extent the variability in thermal niches observed across adults extends to their larvae remains to be seen. Furthermore, larvae identified to species level associated with a specific point in space and time can be tracked back using ocean modelling to predict where spawning might have occurred (e.g., Hernández et al., 2019). This is valuable as spawning behavior is known to vary by species.

We utilized a suite of genetics methods to identify available larval billfishes to species that were inventoried from the wet archive at PIFSC (Table 3). Our first investigation identified observations of 1888 larvae, of which 726 (38%) were already identified to the species-level. Through the inventory effort a total of 2048 larvae observations (records of larvae both identified

and not identified to species) were compiled (including the addition of 160 specimens that were discovered after our initial assessment of 1888). Of those 2048, 1322 had unknown identities (i.e., unknown Istiophoridae). Physical specimens were located for 1137 of 1322 larval records still to be identified, 81 of those were too damaged, desiccated or had too little tissue to be extracted. The remaining 1056 larvae with physical specimens were the focus of our genetic efforts and specimen examination. We physically examined, measured larval length to the nearest 0.5mm of 1395 larvae (some of these already had been identified) and noted the developmental stage of 949 larvae (preflexion, flexion or post flexion, see Figure 3).

We extracted DNA (eyeballs, tail tissue or entire specimens) for all 1056 larvae utilizing the HOTSHOT protocol (following Truett et al. 2000 and outlined in Appendix 1).
Species-specific multiplex PCR-based genetic assays (as described in Hyde et al. 2005) were conducted multiple times on 110 of these larvae. Despite months of troubleshooting, we ultimately found this methodology to be troublesome and assay performance was below our standards. For example, amplification success (i.e., the presence of bands) was lower than anticipated. This was optimized extensively to improve amplification (which it did), however the core problem was the inconsistency in bands that would lead to incorrect species identification. During our experiments, we tested each assay in a singleplex (each species-specific primer separate) compared to the multiplex (pools of primers together), and found that multiplex results did not always align with the more reliable single plex assays. We tested numerous combinations of primers (e.g., leave one out design) to identify troublesome primers and attempt to improve multiplex performance to a sufficiently high level of confidence. Ultimately we abandoned multiplex reactions in favor of more reliable singleplex reactions for all larvae (see Appendix 1 for PCR protocols and troubleshooting). *Makaira nigricans* and *Tetrapturus angustirostris*is are the two most common species we would expect to see, based on the ratios of the existing species-level identifications. These two species became the focus of high-throughput screening. The *M. nigricans* singleplex primers yielded relatively consistent results, thus 1040 larvae were first screened with a *M. nigricans* singleplex PCR and from that 401 larvae were positively identified as this species (Table 4). Some of those larvae that were not identified with the *M. nigricans* screen (n=319) were assayed with a *Tetrapturus angustirostris* single-plex PCR leading to 25 positive identifications. Unfortunately, despite much troubleshooting the *T. angustirostris* primers yielded double bands consistently, which were difficult to interpret and so were not utilized to screen all 639 remaining non-*M. nigricans* larvae. While we were constantly striving to improve species assignment success rate, our success rate is comparable to the noted 57% success rate of species-level identifications by Lynn and Musyl in their 2001 Cruise report (see Appendix 1). This low success rate of the method was not reported in the publication of those results (Hyde et al. 2005), which only reports those that were successfully genetically identified.

For 210 of the larvae remaining unidentified (i.e., not identified in *M. nigricans* and *T. angustirostris* singleplex assays) we extracted DNA a second-time from 210 larvae utilizing a different technique (DNEazy Blood and Tissue Kit, QIAGEN, Germantown MD) and then barcoded using COI specific primers (after Ward et al. 2005). We selected larvae that were 5mm total length or longer in an attempt to increase the chance of successful extractions. Gel

electrophoresis revealed bands in 84 of these larval extracts, which were Sanger sequenced at the Center for Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at University of Hawai'i at Mānoa. Sequences were then trimmed of N-heavy ends, aligned in Geneious (Geneious Prime 2023.2.1,(https://www.geneious.com)) using the MUSCLE alignment, and ambiguities annotated. Cleaned sequences were BLASTed against NCBI's nucleotide (nt) database and all pairwise identities 98% or higher were kept as positive identifications (98.7% of successfully sequenced larvae had pairwise identities greater than 99%).

In short, all inventoried larval specimens were handled at least once throughout this process (minimally: to note preservation state of larva, maximally: hotshot and kit extracted, multiplexed, singplexed and COI barcoded, sequenced). The attached datasheet consists of a line of information for each individual larva. The individual specimen information is associated with a Site number (a unique tow identifier), which allows the specimen information dataframe to be easily joined with the matrix of mean environmental data values across all sites and years. The deliverable for this phase is a single spreadsheet (7 columns) with all relevant specimen data for 2048 larvae (described in the Header tab). This data has been compiled from many sources, most notably from handling the specimens themselves, but also including paper datasheets. Graphical and tabular summaries of the data are presented below and on the following pages.

Table 3: Number of larvae processed at each stage of the identification work-flow

Processing Stage	**Number of larvae**	**Notes**
Original larvae total	1888	Larval records from digital inventory
Larvae already ID'd before 2024	729	~38 % of original total larvae anything not labelled "unk. Istiophoridae"
Unknown Istiophoridae Larvae inventoried	1159	
Excluded larval specimens	96	Larvae that were too damaged (desiccated) or had too little tissue.
Unknown larvae subjected to genetic study	1063	90% of all inventoried larvae
Larvae multiplexed	110	Many of these larvae multiplexed multiple times
Larvae subjected to *Makaira nigricans* singleplex assay	1040	
Larvae identified to *Makaira nigricans*	400	
Larvae identified to *Tetrapterus angustirostris*	25	25 of 319 larvae tested with *Tetrapterus angustirostris* singleplex assay
Larvae re-extracted using DNEazy kit	210	Mix of early on opportunism and then filtered dataset by larval length to increase likelihood of yield
Larvae amplified at COI gene for Sanger Sequencing	210	All re-extractions using DNEasy kits were amplified at COI (PCR) and inspected on gel
Larvae sent for Sanger sequencing	85	85 of 210 samples showed positive bands at COI gene on gels.
Larvae with IDs now	1109	

A

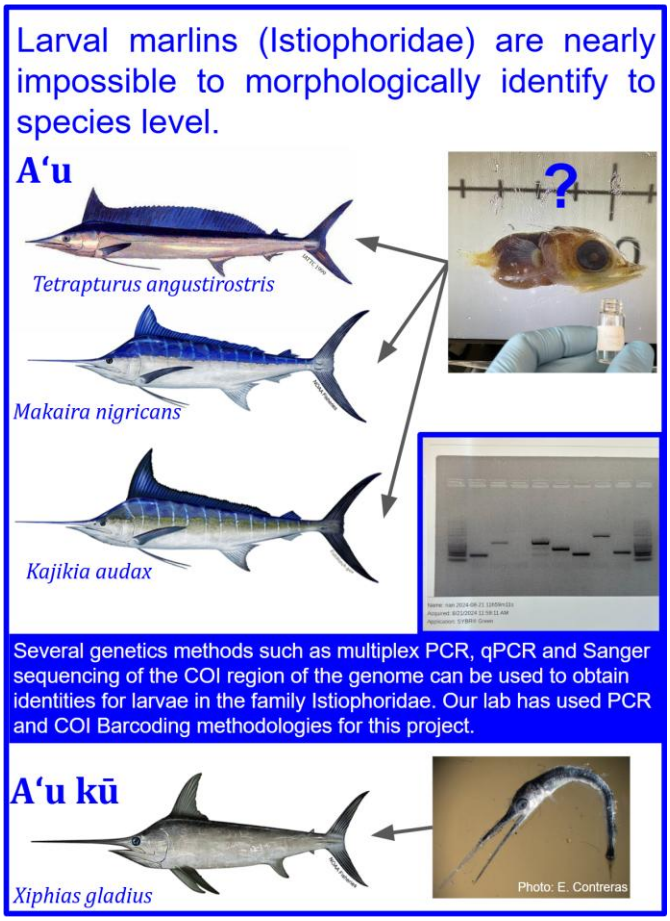
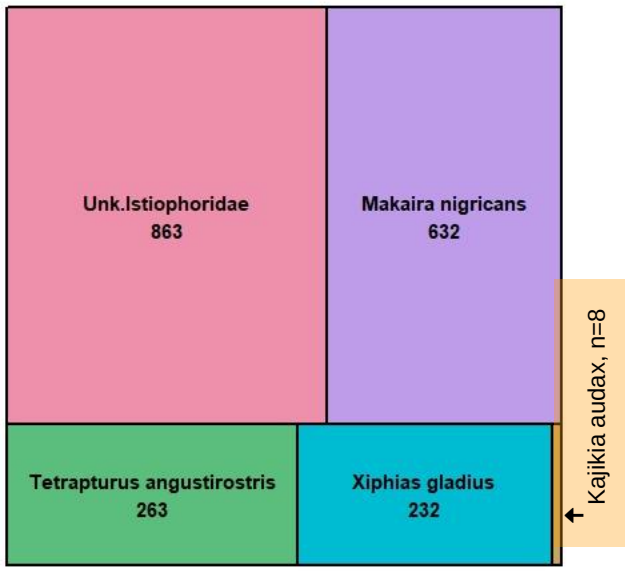


Figure 5. A) Graphical summary of efforts to identify 4 locally-occurring billfish species B) Proportions of species identities out of all larvae considered in this effort. Larvae identified only to the family level (Istiophoridae) are denoted by "Unk. Istiophoridae", all other identities are to the species level

B



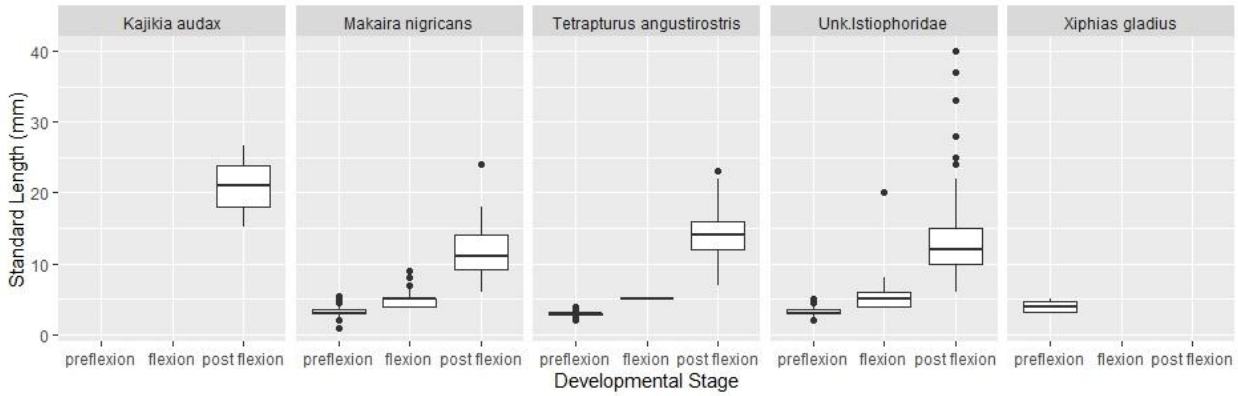


Figure 3: Larval developmental stages of four billfish species and those that remain unidentified (Unk. Istiophoridae). Note that data for Kajikia audax and Xiphias gladius are mostly missing. The previously identified Kajikia specimens were unable to be located during the specimen inventory and their lifestages were only noted when clearly discernible from photos in Hyde et al. 2006.

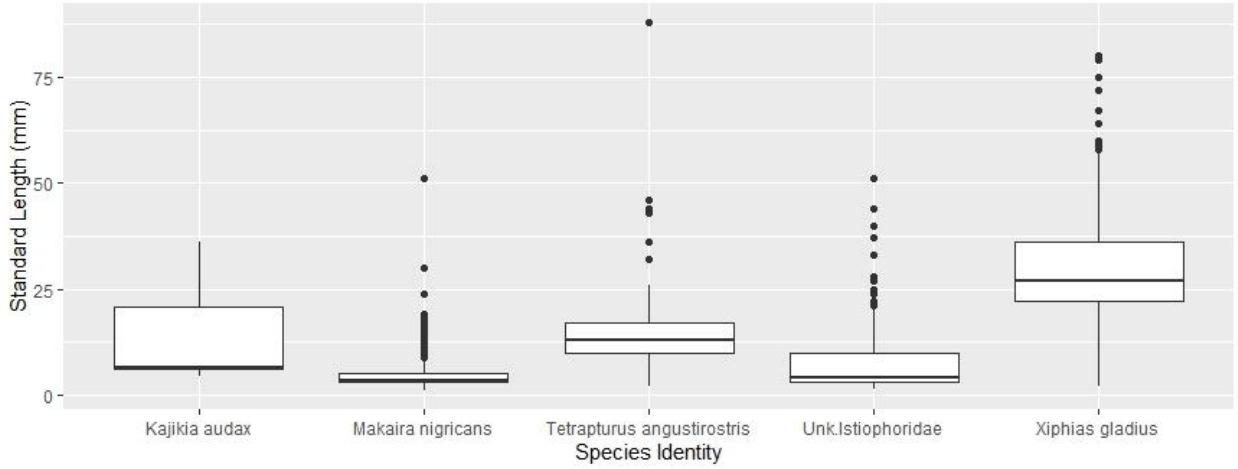


Figure 4: Boxplots of larval standard length across four billfish species and those remaining unidentified (Unk. Istiophoridae). Despite missing life stage data across taxa, length data are available for many specimens. Based off the clear divisions of life stages by length (Figure 3), life stage could be extrapolated for these specimens.

Table 4: Species identities and life stage data for all larvae across Procut 3's dataset.

Species	Total	Life Stage	Subtotals
Tetrapturus angustirostris	263	preflexion	25
		flexion	2
		post flexion	49
		unknown	187
Makaira nigricans	632	preflexion	262
		flexion	53
		post flexion	49
		unknown	268
Xiphias gladius	232	preflexion	6
		flexion	0
		post flexion	0
		unknown	226
Kajikia audax	8	preflexion	0
		flexion	0
		post flexion	2
		unknown	6
Unknown Istiophoridae	863	preflexion	312
		flexion	61
		post flexion	134
		unknown	356

Environmental Variable Compilation

During a given research cruise several instruments were employed to record temperature, salinity, and depth, including a Thermosalinograph (TSG) and a Conductivity, Temperature, Depth sensor (CTD). The CTD also measured fluorometry and sometimes oxygen.
Since the majority of the plankton tows of interest for billfish were neuston tows, the TSG data, which are measured using the ship's flow-through system from surface water, were used for *in situ* measurements. We compiled all available TSG data, united the column names, column

orders and formats and bound them together as a single csv file containing data from the years 1999-2011. TSG data were filtered for only those that occurred during plankton tows and were matched accordingly. Any measurements recorded after the start or before the end of a given plankton tow were kept and the average of all these values at that site was taken.

After matching in this fashion, 86.5% of the TSG data had a match within the tow metadata. We used remotely sensed satellite data products to fill in gaps where TSG data are not available. ERDAPP and COPERNICUS were utilized as hubs to pull relevant temperature, salinity and chlorophyll data. Remotely sensed data for all points in the metadata set are included as well as only those without a TSG match. Finally, those tows which had both TSG data and remote sensed data available were compared to assess the utility of remotely sensed data in this sort of work. Below specifics about the data source of a given environmental variable and the caveats needed to be considered are shared.

Table 5: Proportion of Stations with matched in situ thermosalinograph (TSG) data. Only 10 tows (from three cruises) out of 771 considered in this work had any missing TSG data at all.Therefore, we provide TSG data for 98.7% of all tows. Temperature and salinity data for these 10 missing tows are supplemented with remotely-sensed data.

Cruise	Year	Number of Stations Missing TSG Data	Number of TSG Files availible	Percentage of Stations with TSG Data
SE0412	2004	8	77	90.6
SE0605	2006	1	43	97.7
TC9703	1997	1	53	98.1
MP1812	2018	0	47	100.0
SE0303	2003	0	1	100.0
SE0409	2004	0	33	100.0
SE0507	2005	0	13	100.0
SE0610	2006	0	59	100.0
SE0903	2009	0	58	100.0
SE1106	2011	0	55	100.0
SE1606	2016	0	46	100.0
SE1704	2017	0	41	100.0
TC0006	2000	0	28	100.0
TC0008	2000	0	21	100.0
TC0106	2001	0	44	100.0
TC0203	2002	0	44	100.0
TC9804	1998	0	17	100.0
TC9806	1998	0	32	100.0
TC9911	1999	0	45	100.0

Temperature

Daily Sea Surface Temperature (SST) data were obtained in July 2024 from NOAA's Coral Reef Watch (CRW) at 5km spatial extent (ERDAPP dataset id: noaacrwsstDaily). Although the daily values are coarser than the *in situ* data, which capture variations between cruises on the same day, it can still be useful for considering across days within a cruise (which had durations up to two weeks) and most certainly between years.

Salinity

Sea surface salinity (SSS) was sourced from the GLORYS12V1 product. This product is a global ocean eddy-resolving (1/12° horizontal resolution, 50 vertical levels) reanalysis. This is hosted and created by the E.U. Copernicus Marine Service https://doi.org/10.48670/moi-00021. We chose to use the GLORYS reanalyzed SSS product as it includes values from the start of the time series in 1997. Due to remotely sensed salinity data not being available before 2011 (Lagerloef 2012), the use of modelled salinity values allows us to work with the entirety of the 20- year time series. Other working groups have utilized this same GLORYS12v1 product to look at, amongst other phenomena, "the [impact of] variability of the Iberian Current System…on the dispersion, growth, and survival of fish eggs and larvae of small pelagic fish" over the same time period used in this present study (IMPA, n.d.). Since the plankton tows were neustonic we felt that sea surface data from satellites were appropriate. Other remotely sensed data sources include ARGO floats but we chose satellite data over these for the same reasons we chose TSG over CTD data. The intersection of temperature and salinity was an explanatory variable for larval tuna distributions around O'ahu, another island of Hawai'i pae 'āina (Boehlert and Mundy 1994) and we think it is worth investigating for larval billfish as well.

Chlorophyll- a

Chl-a data was an eight-day composite which came from the European Space Agency's Ocean Colour Climate Change Initiative (OC-CCI) version 5 (Sathyendranath et al. 2021).
We chose to use the 8-day composite to maximize our chl-a data coverage over almost the entire twenty year time series (data only goes back to September 1997 which means we do not have coverage for 1997 as that cruise occurred in April). Daily measurements for a certain spatial extent are usually collected at the same time each day and cloud coverage or other disturbances can block the ocean from the satellite and make data unavailable for a given day. Utilizing an 8-day composite makes these chl-a data best for inter-month or inter-annual comparisons. Furthermore, water depth can influence ocean color and thus chla-a values which are derived from ocean color. J. Perelman created a mask for measurements taken at the 30m isobath in order to further increase the reliability of these measurements. Chl-a concentrations and phytoplankton particle size have been shown to be potential predictors for tuna recruitment (Woodward-Jefcoats and Wren 2020). Furthermore, since eddies are likely important for larval nurseries (Hyde et al. 2006, Richardson et al. 2009, Bakun 2006), chl-a is especially important

to consider as its concentrations are significantly influenced by eddies around the main Hawaiian islands (Gaube et al. 2014, Seki et al. 2001).

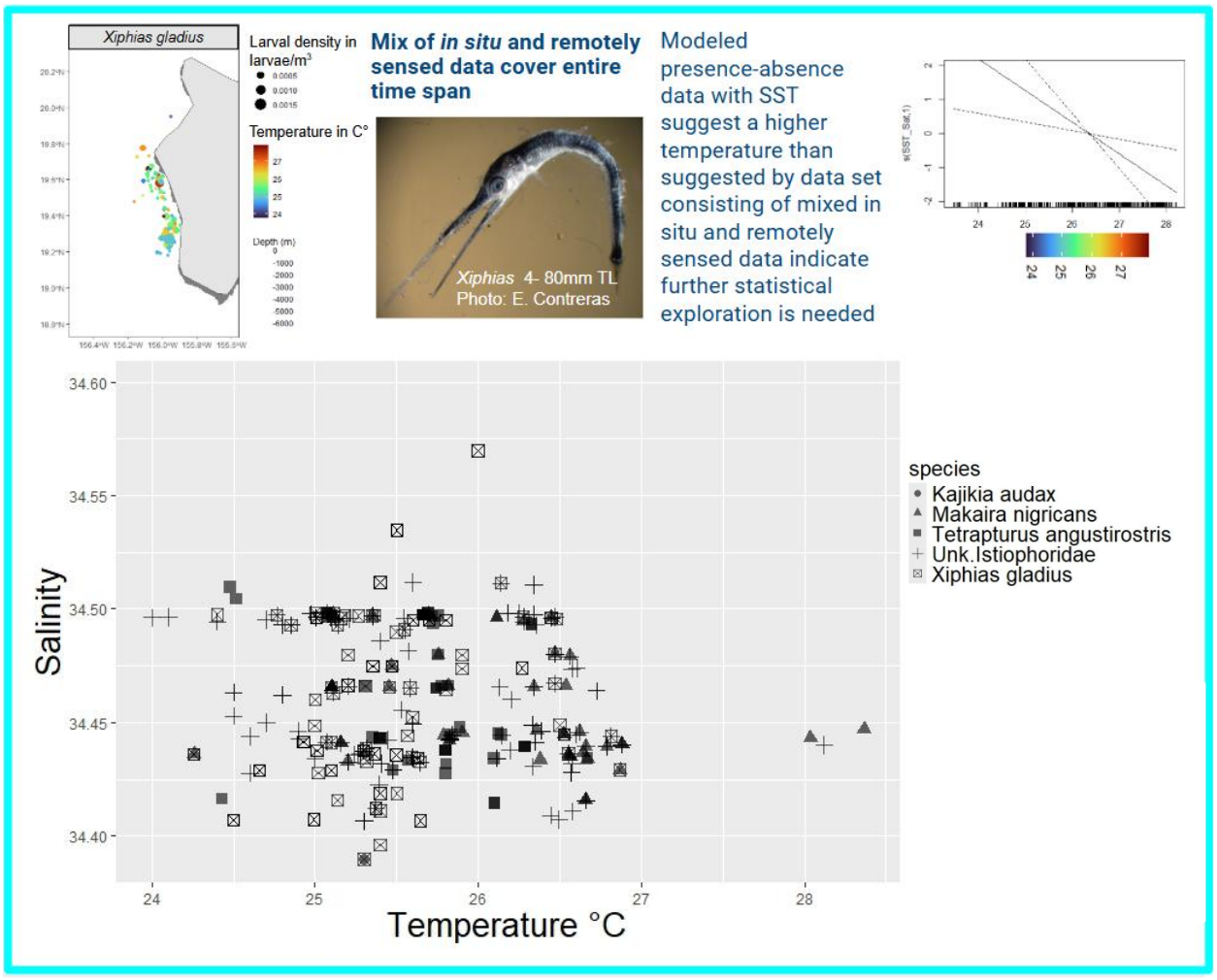


Figure 5. Graphical summary of billfish species occurrences across temperature-salinity space for four billfish species and those remaining unidentified (Unk. Istiophoridae).

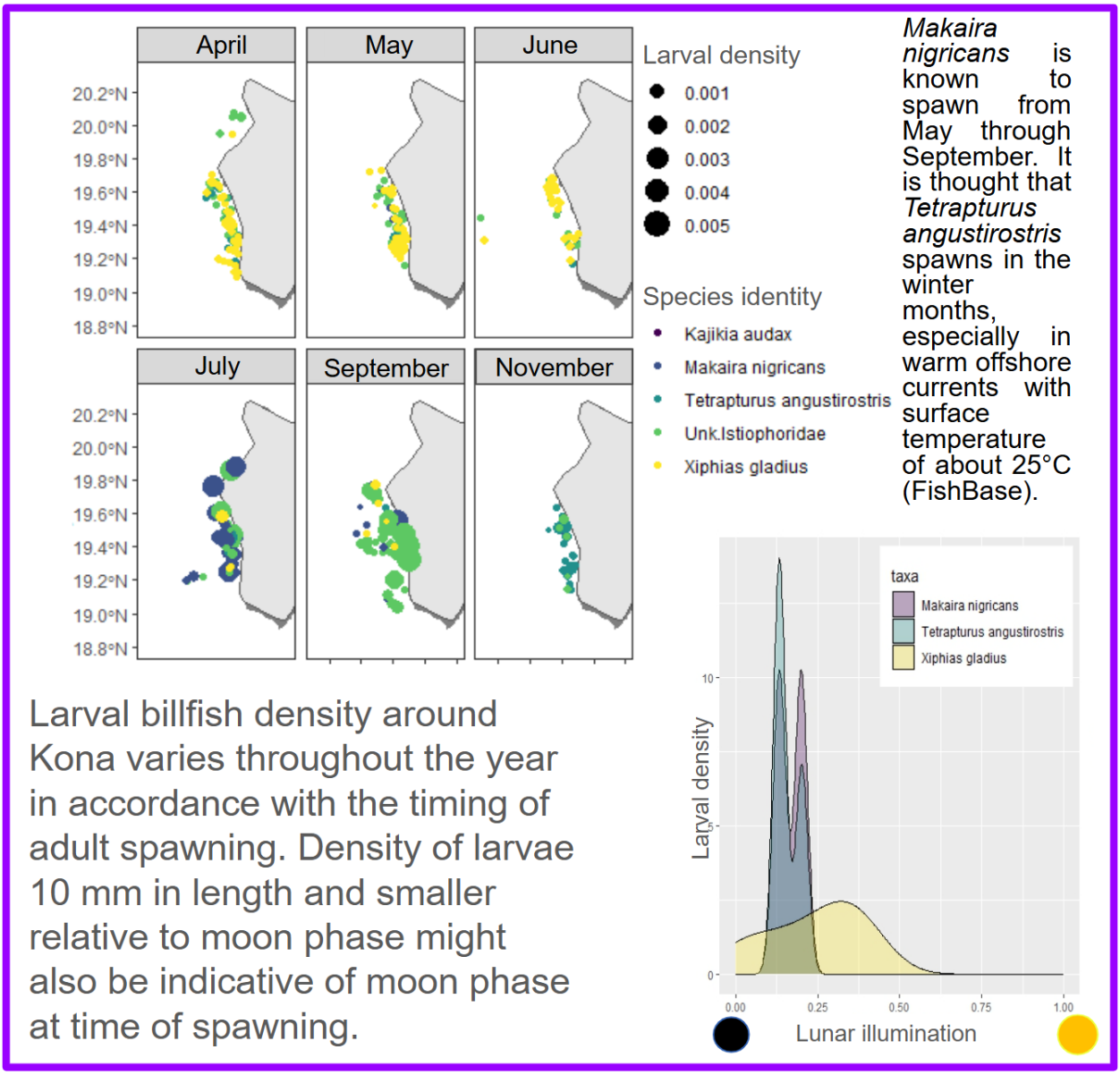


Figure 6. Graphical summary of larval billfish density by season and lunar illumination.

Citations

Arostegui MC, Gaube P, Woodworth-Jefcoats PA, Kobayashi DR, Braun CD. (2022). Anticyclonic eddies aggregate pelagic predators in a subtropical gyre. Nature 609, 535–540. https://doi.org/10.1038/s41586-022-05162-6

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Appendix 1

HOTSHOT Extractions

The following is our specific way of following UConn Health's HOTSHOT Protocol (here). Petridishes, forceps and scissors that had been previously cleaned with 10% bleach solution and then subsequently rinsed thrice in deionized water were used to extract a 1mm or smaller piece of tissue from the specimen. Tissue was placed directly into a sterile 1.5ml eppendorf OR the sterile well of a 96-well plate. The well-plate sat upon ice and the tissue was either left for several hours to air dry with a loose lid and/or excess EtOH was removed with a 20µl pipettetip. Each well was visually checked for ethanol globules before adding 50 µl Alkaline Lysis Reagent (from a stock of 25 ml water NaOH at a final concentration of 25 mM and disodium EDTA at a final concentration is 0.2 mM. Plate was incubated at 95°C for one hour in a thermocycler, then kept at 4°C for at least 15 minutes before adding 50 µl Neutralization Reagent (stock of 24ml of water Tris-HCl at a final concentration of 40 mM). We used a multichannel pipette with filter tips for each sample. Solution was mixed up and down thrice. For samples with a large amount of particulate matter remaining in the well, plate was spun down and 50 µl of extract was transferred to a new 96-well plate.

Multi-plex Protocol and troubleshooting

Protocols

MASTER MIX for multiplexing

reagent	volume	reagent concentration	final rxn concentration uM	[unit]
UniversalF	**4**	10	0.5	uM
IplatypterusR	0.5	10	0.5	uM
MindicaR	0.5	10	0.5	uM

MnigricansR	0.5	10	0.5	uM
TangustirostrisR	0.5	10	0.5	uM
TaudaxR	0.5	10	0.5	uM
XgladiusR	0.5	10	0.5	uM
cequiselis	0.5			
hhippurus	0.5			
immomix	**10**	2		
MgCl2	0	50	3.5	mM
BSA	**0.5**	0.5	0.5	mg/ml
MM total	**18.5**			
DNA	**1.5**			
rxn total	**20**			

MASTER MIX FOR SINGLE PLEXING

reagent	volume	reagent concentration	final rxn concentration uM	[unit]
UniversalF	**1.2**	10	0.5	uM
MnigricansR	1.25	10	0.5	uM
immomix	**10**	2		
BSA	**0.5**	0.5	0.5	mg/ml
H2O	1.5			
MM total	**16**			
DNA	**45**			

THERMOCYCLER PROTOCOL for HYDEUNivF Reactions

temp(°C)	time(min:sec)	cycles
95	10:00	1
94	0:10	
56.6	0:30	35
72	0:30	
72	3:00	1

MASTER MIX FOR COI BARCODING (After N. Himmelsbach)

reagent	Volume (1x reaction)
WardF	**1**
WardR	1
2x immomix red	**10**
BSA	**0.5**
H2O	5.5
MM total	**18**
DNA	2

temp(°C)	time(min:sec)	Number of cycles
95	2 s	1
94	00:30	35
54	00:30	
72	01:00	
72	10:00	1
10	infinite	hold

Selected page from the Narrative Report of research cruise TC0106

During this cruise, one of the genetics personnel from the La Jolla Laboratory (Eric Lynn) was brought out on the cruise along with all the required lab equipment and reagents to test whether this technique could be adapted to work in the field onboard a research ship at-sea. During these trials, Lynn was assisted by Mike Musyl who has had previous research experience using similar molecular techniques. Our main objective was to first determine whether the PCR and restriction fragment length polymorphism (RFLP) protocols used in the laboratory environment could be duplicated out at sea, and, if so, whether unidentified billfish larvae could be identified in near real-time (~1 day). All of the PCR protocols were conducted in the ship's Wet Lab using the desk as a bench top for the various lab equipment. The RFLP and electrophoretic gel runs were conducted in an air-conditioned environment (Electronics Room) on a section of the aft bench top.

Over the course of the 4-day period that this molecular technique was tested, a number of significant accomplishments were made. Lynn and Musyl demonstrated the successful 1) extraction of genomic DNA from both eyeballs and body tissue, 2) purification of genomic DNA, 3) amplification (PCR) of genomic DNA, 4) restriction enzyme digestion of PCR products, 5) process in the gel electrophoresis of restriction fragments, 6) process in photo-documenting the gel-run restriction fragments, and 7) molecular identification of larvae at-sea in near real-time (1 day). Lynn and Musyl were able to identify 27 of 47 istiophorid larvae (57%) to species; the remainder yielded either too little or poor quality PCR amplified genomic DNA and could not be further analyzed.