Spring 5-14-2022

TROPHIC ECOLOGY OF SAILFISH LARVAE IN THE GULF OF MEXICO: AN ANALYSIS OF DIET, PREY AVAILABILITY, PREY QUALITY, AND INFLUENCES ON LARVAL GROWTH

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by

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A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Ocean Science and Engineering
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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May 2022

Published by the Graduate School

ABSTRACT

The Gulf of Mexico is an important early life habitat for billfishes, whose larvae experience high growth rates despite having highly specialized diets, limited foraging abilities, and oligotrophic offshore habitats. Variability in the recent growth of sailfish larvae was compared to environmental, spatial, diet, prey availability, and fatty acid metrics using data collected from near-surface waters in the northern Gulf of Mexico (2017 - 2019). Larvae exhibited a highly selective feeding strategy in early development and expanded their feeding niche to include larger prey items (calanoid copepods, larval fish) once they reached flexion. Sailfish larvae showed significant positive selection for *Evadne* and female *Farranula*. To examine quality of preferred prey and condition of sailfish larvae, fatty acid concentrations were examined as fatty acid methyl esters. Results indicated that total fatty acid concentration of preferred prey was highest closest to shore with a trend for lower total fatty acid concentration further offshore. Additionally, *Evadne* had lower DHA% and higher AA% than did *Farranula* or *Corycaeus*. While DHA%, EPA%, and AA% increased in the tissue of sailfish larvae with ontogeny, total fatty acid remained consistent. Recent larval growth was estimated by otolith increment analysis and was higher when more prey were consumed, when dissolved oxygen concentrations were greater, and when larvae were found in anticyclonic boundary regions. This is the first study to examine and report sex-specific selection of zooplankton prey by sailfish larvae, and to examine the fatty acids of sailfish larvae and their preferred prey.

ACKNOWLEDGMENTS

The completion of this thesis was made possible by the kindness, knowledge, and assistance of many individuals at the Gulf Coast Research Laboratory. Firstly, I would like to thank my advisor, Dr. Frank Hernandez for his continual guidance and support from helping me develop the research questions that informed this project, all the way through to writing the final thesis. I would also like to thank my committee members, Dr. Chet Rakocinski and Dr. Simon Geist for their support and for challenging my ideas to encourage me to think more deeply.

This project relied heavily on the technical support of Jason Tilley who provided equipment and protocols for lipid extraction and fatty acid composition analysis as well as Patrick Graham who helped develop protocols for otolith increment analysis and who served as a second reader for age estimation based on otolith increments. They were both integral to the success of this project and volunteered their time and expertise generously. I would also like to thank Kevin Dillon who allowed me to use his facilities and equipment for portions of the fatty acid analyses. I was lucky to be part of the Fisheries Oceanography and Ecology lab and received support and/or assistance from the many members (Carla Culpepper, Carley Zapfe, Verena Wang, Courtney Stachowiak, Eric Haffey, Glenn Zapfe, Emily Gipson, Cindy Gavins, Angie Hoover, Anna Millender, and Luciano Chiaverano).

Lastly, I would like to acknowledge the financial support provided by the NOAA Restore Science Program (Award # NA17NOS4510099), the Mississippi Chapter of the American Fisheries Society, and the PADI Foundation.

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LIST OF ABBREVIATIONS

GoM	Gulf of Mexico
FAs	Fatty acids
PUFA	Polyunsaturated fatty acid
EFA	Essential fatty acid, 20:5(n-3)
DHA	Docosahexaenoic acid, 22:6(n-3)
EPA	Eicosapentaenoic acid
LN_2	Liquid nitrogen
DO	Dissolved oxygen
chl	Chlorophyll
CPUE	Catch per unit effort
HSD	Honestly significant difference
RelGCW	Relative gut content weight
GFDW	Gut-free dry weight
relTOD	Relative time of day
FAME	Fatty acid methyl esters
BHT	Butylated hydroxytoluene
AA	Arachidonic acid, 20:4(n-6)
DRG	Detrended recent growth
k	Daily instantaneous growth rate
VIF	Variance inflation factor
AIC	Akaike information criterion

CHAPTER I – TROPHIC ECOLOGY OF SAILFISH LARVAE IN THE GULF OF MEXICO: AN ANALYSIS OF DIET, PREY AVAILABILITY, PREY QUALITY, AND INFLUENCES ON LARVAL GROWTH

1.1 Introduction

Billfishes (families Istiophoridae and Xiphiidae) are pelagic marine fishes that are of global economic, social, and ecologic importance. Although the only significant harvest in the northwest Atlantic Ocean is from longline fishery bycatch (Kitchell et al. 2006), billfishes have long been a commercial target of other nations, such as Japan (de Sylva & Breder 1997). There is also a valuable catch and release fishery that supports tourism economies in the Atlantic waters of the U.S., Puerto Rico, Costa Rica, and Mexico (Carter et al. 2002). The sportfish fishery for billfishes is valued at $200 million in the U.S. alone (Ditton & Stoll 2003), with the primary species being Atlantic Sailfish *(Istiophorus platypterus)*, Blue Marlin *(Makaira nigricans)*, Swordfish *(Xiphias gladius)*, and occasionally White Marlin *(Kajikia albida)* and Longbill Spearfish *(Tetrapturus pfluegeri)*.

The Gulf of Mexico (GoM) is recognized as an important habitat for billfishes. Specifically, Atlantic sailfish are abundant and broadly distributed in the GoM, where the warm waters promote fast growth and high biomass production (Simms et al. 2010). Longline bycatch data reveals that catch rates of Atlantic sailfish are two times higher in the GoM than in any other area of the northern Atlantic (de Sylva & Breder 1997). Although the GoM is an important environment for billfishes, human impacts due to harvesting and climate change may threaten current populations. For example, adult billfish are experiencing vertical habitat compressions in the eastern tropical Pacific and

western north Atlantic due to increasing hypoxia in the oxygen minimum zones which limit foraging depths for these apex predators (Prince et al., 2010). While Atlantic sailfish and swordfish are not currently listed or considered vulnerable, blue marlin and white marlin populations are in decline, and are categorized as vulnerable according to IUCN (Collette et al. 2011). These declines are thought to be attributed to by-catch of commercial fisheries for tuna and swordfish (Cox et al. 2002).

1.1.1 Larval fish feeding

The greatest source of natural mortality for billfishes occurs during the egg and larval stages well before they are susceptible to longline and recreational fisheries (Houde 2008). Variability in the growth and survival of larval fishes is largely the result of variability in their biotic and abiotic environments. The early life history of most marine fishes represents a vulnerable stage where mortality rates approach 100% and small variations in mortality rates or growth rates in early life can translate to large fluctuations in recruitment (Houde, 1987). Prey quality, prey availability, and prey density have demonstrated effects on the survival of larval fish (Robert et al. 2009, Paulsen et al. 2014a). Starvation is thought to be an important source of mortality for many larval fishes but is difficult to observe in field studies since starving fish larvae are quickly removed from the population via predation. However, lab studies indicate that first feeding larvae must grow quickly to escape the danger zone of low Reynolds numbers where viscous forces, like drag, dominate over inertial swimming forces. Larvae may experience “hydrodynamic starvation” because feeding performance may be mechanistically limited by low Reynolds numbers. (China & Holzman 2014). There is a critical period for poorly developed, recently hatched fish larvae, where food capture is

crucial. If these larvae fail to ingest prey after the endogenous nutrition from their yolk sac has been exhausted, they reach the "point of no return" where death due to starvation becomes inevitable (Hjort 1914). This is especially true for low latitude fishes that hatch at small body sizes and have high metabolic rates due to high water temperatures. Larvae that are larger at hatch will have a greater window of opportunity to initiate successful feeding (Miller et al., 1988) and they will become less susceptible to starvation as they accrue body mass. The risk associated with weight-specific metabolism is reduced in larger larvae because tissue such as muscle can serve as an energy store to stave off starvation (Fuiman 2002).

Much of the work concerning larval fish and preferred prey availability has been informed by Cushing's match/mismatch hypothesis on high latitude, temperate fishes (Cushing 1969). High latitude fishes often have fixed spawning times that sometimes coincide with the primary production cycle and result in a strong year class for the fishery. At other times, poor recruitment can result when the off-set seasonal production cycle does not line up with the fixed spawning time. This hypothesis generally does not apply to low latitude fishes, like most billfishes, because their environment does not experience distinct seasonal pulses of primary production that occur somewhat predictably in higher latitudes (Cushing 1990). Furthermore, billfishes are highly fecund batch spawners whose females may broadcast spawn up to four times a year throughout a protracted spawning season (de Sylva & Breder 1997). Without a seasonal nutrient pulse, the early life history of billfish presents an apparent paradox where eggs are spawned and hatch in oligotrophic offshore waters that are thought to contain relatively low food concentrations. This strategy may offer larvae some protection from predators, while

allowing successful feeding and growth in convergent zones where food is concentrated in high enough densities for successful prey capture (Bakun & Broad 2003).

The feeding success of billfish larvae is likely a consequence of a directed spawning strategy where eggs are spawned into mesoscale oceanographic features, quickly hatch, and are entrained with their potential prey items (Richardson et al. 2009b). Previous field studies have found larval billfishes aggregated at frontal features in the GoM. For example, Rooker et al. (2012) observed a negative relationship between the abundances of larval Atlantic sailfish, blue marlin, and swordfish and sampling distance from the Loop Current during several summer sampling seasons (2006-2008). Larvae that encounter patches of high prey densities aggregated along ocean convergence features may be afforded high growth rates for extended periods, even if prey densities are diminished, or if they are transported to an area of lower prey density, because they can rely on storage lipids (triacylglycerols) when food is scarce (Fraser 1989, Pepin et al. 2015).

1.1.2 Zooplankton Prey Nutritional Quality

Prey availability for larval fish has been a major focus of recruitment hypotheses for more than a century (Hjort 1914, Cushing 1969, Lasker 1981). While these hypotheses focus on prey abundances, they often fail to take prey quality into account. Fish larvae encounter a broad range of potential prey which differ in quality. The nutritional differences between prey items may be compensated for if predators feed on a wide variety of prey species. However, because billfishes have a narrow feeding niche, they can be vulnerable to changes in the quality of their preferred prey.

Selective feeding presents a trade-off for organisms by reducing the encounter and ingestion rate-per-unit of foraging area. Studies of optimal foraging often use energy content as a surrogate for fitness, but increased energy intake does not always translate to improved fitness or growth if there is a deficiency in any required nutrient not met by the combination of prey items ingested (Simpson et al. 2004, Raubenheimer et al. 2009). Fatty acids (FAs) are important components for development of the retina, brain, and spinal cord in larval fish (Sargent et al. 1999). Polyunsaturated fatty acids (PUFAs) are key components of cellular membranes and fish can use these lipids as energy stores in the form of triglycerides. Because FAs comprise the majority of lipids found in all organisms, are diverse, biochemically restricted, and sometimes have unique origins, they are useful for investigating both organismal nutrition and trophic relationships (Budge et al. 2006). Fatty acids that are not biosynthesized by animals and must be acquired via ingestion are termed essential fatty acids (EFAs). Many lab experiments have demonstrated the negative effects of low levels of dietary FAs on the development and growth of larval fish (Bell et al. 1995, Cutts et al. 2006, Copeman & Laurel 2010, Perez & Fuiman 2015). Researchers have also demonstrated that high levels of dietary EFAs, especially docosahexaenoic acid (DHA), are associated with high levels of growth in fish larvae (Paulsen et al. 2014a).

Fatty acid composition of zooplankton prey will allow both top-down and bottom-up qualitative inferences about trophic relationships. Fatty acid biomarkers allow basic inferences about the contributions of diatoms, flagellates, and bacteria in the prey's diet (Nichols et al. 1986, Budge & Parrish 1998, Lee et al. 2006). Most copepods cannot biosynthesize long-chain, unsaturated FAs *de novo*. Because poly unsaturated FAs are

obtained exclusively from dietary consumption (except in rare instances), they can be used to reconstruct feeding histories (Budge et al. 2006, Malzahn et al. 2007, Jónasdóttir 2019). Some marine organisms show an increase in growth rates when their prey have high DHA concentrations and a decrease in growth when their prey contains high levels of eicosapentaenoic acid (EPA) (Thompson & Harrison 1992, St. John et al. 2001). An increase in some FA concentrations in prey, especially PUFAs, may improve the ecological performance of larval fish which may be translated to faster growth or better nutritional condition. While many lab studies have addressed the effect of dietary FA concentrations on the development, growth, and survival of larval fish, few field studies address the effects of FA concentrations of prey items on larval fish (Paulsen et al. 2014a).

Fatty acids are largely synthesized by primary producers (except in the case of cyanobacteria and some chlorophytes) and transferred up to higher trophic levels. Therefore, the base of the food chain will largely determine the amount and ratio of EFAs that larval fish consume. Environmental conditions affect FA compositions, however they contribute very little variability compared to phylogeny so the community composition or the diet of larval fish prey will largely determine the FA composition (Galloway & Winder 2015). Although there is a large body of work on lipids of calanoid copepods that dominate the zooplankton biomass in many marine systems, there is very little information on “microcopepods”, like *Farranula* , that often dominate in terms of copepod abundance (Dalsgaard et al. 2003) in offshore waters, including in the GoM.

1.1.3 Larval Billfish Feeding Ecology

Billfishes hatch with a body length between 2 – 4 mm, but grow exponentially throughout the larval phase (Yasuda et al. 1978, Govoni et al. 2003, Luthy et al. 2005a, Sponaugle et al. 2005). To support this rapid growth, larval billfish must have high encounter rates with prey that meet their nutritional requirements, as well as the ability to successfully capture the prey they encounter. Previous studies of istiophorid larvae suggest that diets are defined by successful feeding, (characterized by a high feeding incidence, typically ≥ 0.90), a high degree of gut fullness, an initial zooplankton feeding phase consisting mainly of adult copepods and cladocerans, and a switch to piscivory that begins between 5-7 mm standard length (SL) (Lipskaya & Gorbunova 1977, Uotani & Ueyanagi 1997, Llopiz & Cowen 2008). These larvae do not appear to feed regularly at night, but typically have two peaks of intense feeding during daylight hours. Diel collections and gut evacuation rates indicate that larval istiophorids occupy surface waters and feed during daylight hours with a period of peak feeding just before sunset. After sunset they occupy subsurface waters and cease feeding (Lipskaya & Gorbunova 1975, Llopiz & Cowen 2008). While there is consensus that small istiophorid larvae occupy subsurface waters at night, larger larvae were occasionally collected in surface night tows which may suggest an ontogenetic component involved in the diel vertical migration where older larvae inhabit surface waters at night (Bartlett & Haedrich 1968). Swordfish larvae (Xiphiidae) feed mainly on *Corycaeus* copepods (Order Cyclopoida) and cladocerans (Order Cladocera) of the genus *Evadne*, until they switch to piscivory between 10-12 mm SL. Studies on larval swordfish water column position are equivocal, with some reports that larval swordfish may sink into subsurface waters at night and

midday, and other reports that large larvae inhabit surface waters day and night (Gorbunova 1969, Govoni et al. 2003).

Earlier studies of larval billfish gut contents (prior to 2003) reported mainly qualitative measures of gut contents, which can only be described in general terms. One study of 14 sailfish larvae collected from the Pacific Ocean and Indian Ocean, found that in addition to the main prey items discussed above, some Pontellidae nauplii were consumed (Lipskaya & Gorbunova 1977). A second study of 145 blue marlin larvae from the Indian Ocean and Pacific Ocean found that zooplankton prey were almost exclusively Corycaeidae copepods (Lipskaya & Gorbunova 1975). More recently, a study from the Indo-Pacific reported that *Corycaeus* copepods, *Evadne* cladocerans, and fish larvae made up nearly 100% of the diets of 1104 blue marlin larvae and 427 shortbill spearfish larvae (Uotani & Ueyanagi 1997). Swordfish larvae from the Atlantic, Pacific, and Indian oceans were found to have similar diets consisting of *Corycaeus*, *Evadne*, and larval fish (Gorbunova 1969). Copepods in the genera *Corycaeus* and *Farranula* are morphologically similar and belong to the same family, Corycaeidae, but they have been reported as cyclopoid copepods in earlier studies.

Although there have been previous efforts to describe the diets of larval billfish, many studies in the western Atlantic are constrained by either low sample sizes or limited geographic scope. Two studies reported the number and composition of prey items extracted from the stomachs of istiophorid larvae over the spatially confined regions of a sampling corridor in the northern GoM (Tidwell et al. 2008) and the Straits of Florida (Llopiz & Cowen 2008). A third study provided the number and type of prey items extracted from swordfish larvae collected over the broad, spatially undefined regions of

the northwest Atlantic (Govoni et al. 2003). Therefore, the feeding ecology of larval billfish has been understudied in large areas of the GoM. And, although gut contents of larval istiophorids have been described for the northern GoM shelf waters, the diets of only five swordfish larvae from the GoM have been reported (Govoni et al. 2003).

Prey selectivity of larval billfish has been analyzed only in the Straits of Florida region for larval blue marlin and sailfish. Larvae with a higher proportion of copepods in their guts than cladocerans exhibited faster growth (Sponaugle et al., 2010). However, billfish larvae consumed cladocerans in higher proportions than were available in their environment, and copepods in lower proportions than were available (Llopiz and Cowen, 2008). Of the 452 larvae examined, no calanoid copepods were consumed despite being three times more abundant than preferred prey, *Evadne* and *Farranula* (Llopiz & Cowen 2008). This paradox may suggest there is something about the behavior, physiology, or nutritional content of prey items that make copepods in the family Corycaeidae and *Evadne* cladocerans more suitable than calanoid copepods as prey for billfish larvae. Potential driving factors of increased suitability of preferred prey items include enhanced visibility of copepods in the family Corycaeidae due to their enlarged and ventral eyes, smaller size of Corycaeidae copepods compared to most calanoids collected in the samples, and the different modes of swimming exhibited by these copepods. Landry et al. (2019) noted that both cladocerans and poecilostomatoid copepods swim with jerky strokes while calanoid copepods have appendages that allow them to glide smoothly through the water. Jerky swimming patterns may enhance the visibility of prey by giving away or drawing attention to their location in the water column to visual predators.

1.1.4 Objectives

The overall goals of this project are to describe the trophic ecology of larval sailfish collected across environmentally variable open water habitats in the GoM, and to examine how prey selection and prey quality impact larval sailfish growth and condition. To address these goals, my specific objectives are to:

1. examine the patterns underlying sailfish abundance and distribution in the northern GoM
2. quantify larval sailfish diets using gut content and prey selectivity analyses;
3. estimate the nutritional "quality" of larval sailfish zooplankton prey using analyses of essential fatty acids (DHA, EPA, AA) and total lipid/dry weight;
4. estimate larval sailfish growth using otolith increment analysis; and
5. examine the spatial relationships and associated variability among environmental conditions, zooplankton prey quality and abundance, and larval sailfish feeding and growth.

Data for this project were collected in the GoM as part of a NOAA RESTORE project examining *Sargassum* communities and adjacent (open water) habitats. Four cruises were conducted aboard the *R/V Point Sur* during the late spring/early summer of 2017, 2018, and 2019. Sampling locations varied among cruises, but plankton net collections were primarily in oceanic surface waters adjacent to *Sargassum* patches. Among the billfish species collected, sailfish larvae were the most abundant and widespread in distribution, and therefore were selected for most analyses. Data from all four cruises will be used for analyses of larval sailfish diets (Objective 2, in part), growth (Objective 4), and environmental-spatial patterns (Objective 5). Due to variability in

sample preservation and net sampler mesh sizes during each cruise, prey selectivity analyses (Objective 2, in part) and fatty acid analyses (Objective 3) will be limited to a subset of samples collected during a single cruise in 2019. Although fewer in number, brief descriptions of diets are also reported for blue marlin, white marlin, and swordfish larvae (Objective 2).

1.2. Materials and Methods

1.2.1 Study Region

Larval fish were collected in the GoM as part of a NOAA RESTORE project examining *Sargassum* communities and adjacent (open water) habitats. Sampling locations varied among cruises, but the area surveyed encompassed the oceanic northeastern region of the GoM (Figure 1.1). Plankton net collections were primarily in oceanic surface waters adjacent (within 1 km) to *Sargassum* patches and ranged from 24 km to 377 km offshore (Table 1.1, Figure 1.1).

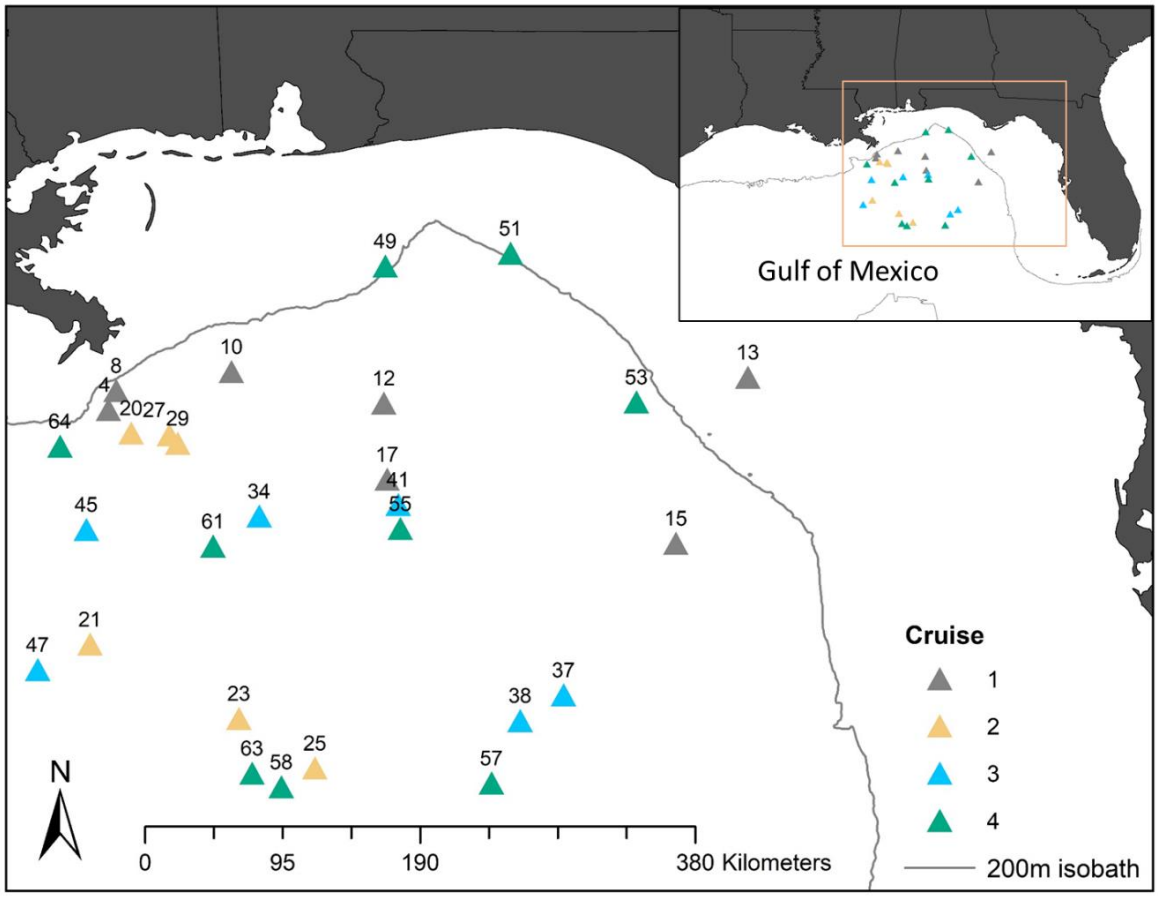

Figure 1.1 Open water stations sampled during four research cruises in the northern Gulf of Mexico. Numbers next to symbols denote unique station identification numbers; associated station data are provided in Table 1.1.

Table 1.1 Station data and biological and physical observations associated with each plankton sampling site during four research cruises in the northern Gulf of Mexico (2017-2019). Plankton sampling locations are depicted in Figure 1.1. Plankton tow numbers (Tow ID) identify neuston (N) and double neuston (D) samplers. Variables include distance from shore (km), mesoscale circulation features (common water (CW), anticyclonic boundary (AB), anticyclonic region (AR), temperature (°C), salinity, dissolved oxygen (DO, mg/L), sea surface chlorophyll a concentration (CHLA, mg/m^3). Billfish densities include blue marlin, white marlin, and sailfish.

Cruise	Date	Station	Tow	Distance from Shore	Meso. feature	Temp.	Salinity	DO	CHLA	Sailfish/ 1000m^2	Billfish/ 1000m^2
1	7/21/2017	4	N4	35.8	CW	29.9	34.8	2.8	11.0	0.0	0.0
1	7/22/2017	8	N7	24.0	CW	29.7	34.9	2.7	11.3	0.0	0.0
1	7/22/2017	8	N8	24.0	CW	29.7	34.9	2.7	11.3	0.0	0.0
1	7/23/2017	10	N10	71.6	CW	29.1	35.1	6.0	13.2	8.9	8.9
1	7/23/2017	10	N11	71.6	CW	29.1	35.1	6.0	13.2	0.0	0.0
1	7/24/2017	12	N13	165.5	CW	29.5	34.7	2.7	1.3	0.0	0.0
1	7/24/2017	12	N14	165.5	CW	29.5	34.7	2.7	1.3	0.0	0.0
1	7/25/2017	13	N15	82.1	CW	30.0	33.0	2.4	0.4	0.0	0.0
1	7/26/2017	15	N17	202.7	CW	30.9	36.3	2.6	0.1	0.8	0.8
1	7/26/2017	15	N18	202.7	CW	30.9	36.3	2.6	0.1	4.0	4.0
1	7/27/2017	17	N20	189.5	CW	29.5	36.0	2.9	0.1	0.8	0.8
2	5/30/2018	20	N21	54.6	CW	28.2	36.1	6.1	0.2	0.0	0.0
2	5/31/2018	21	N22	192.6	CW	28.4	36.4	6.0	0.1	0.0	0.0
2	6/1/2018	23	N24	261.7	CW	28.4	36.4	6.0	0.1	11.3	11.3
2	6/2/2018	25	N27	310.7	AB	28.3	36.5	6.0	0.1	0.0	0.0
2	6/2/2018	25	N28	310.7	AB	28.3	36.5	6.0	0.1	27.5	27.5
2	6/3/2018	27	N30	66.8	CW	28.5	36.2	6.0	0.2	0.0	0.0
2	6/4/2018	29	N33	74.4	CW	28.4	36.0	6.0	0.1	0.0	0.0
3	7/10/2018	34	N42	144.8	CW	30.2	32.0	6.3	0.3	0.0	0.0
3	7/11/2018	37	N44	321.2	AB	30.0	36.2	5.9	0.1	0.0	0.0
3	7/11/2018	38	N45	345.8	AB	30.0	36.2	5.9	0.1	0.8	1.6
3	7/13/2018	41	N48	204.4	CW	29.8	35.5	6.0	0.2	2.5	3.1
3	7/15/2018	45	N55	114.1	CW	30.1	33.6	6.1	0.2	1.6	1.6
3	7/16/2018	47	N57	209.6	CW	30.4	35.9	6.0	0.1	1.5	1.5
4	5/28/2019	49	N59	82.9	CW	28.5	29.9	5.4	1.9	0.0	0.0
4	5/29/2019	51	N62	85.2	CW	28.7	32.5	4.9	0.6	2.4	2.4
4	5/29/2019	51	D1	85.2	CW	28.7	32.5	4.9	0.6	3.1	3.1
4	5/29/2019	51	D2	85.2	CW	28.7	32.5	4.9	0.6	0.0	0.0
4	5/30/2019	53	D3	114.4	CW	28.4	36.5	3.9	0.1	0.0	0.0
4	5/30/2019	53	D4	114.4	CW	28.4	36.5	3.9	0.1	2.5	2.5
4	5/30/2019	53	N64	114.4	CW	28.4	36.5	3.9	0.1	0.0	0.0
4	5/31/2019	55	D5	215.0	AR	28.4	33.9	4.1	0.1	0.0	0.0
4	5/31/2019	55	N66	215.0	AR	28.4	33.9	4.1	0.1	0.0	0.0
4	6/1/2019	57	D6	377.2	AB	28.9	36.4	3.9	0.1	2.3	2.3
4	6/1/2019	57	D7	377.2	AB	28.9	36.4	3.9	0.1	21.1	25.4
4	6/1/2019	57	N69	377.2	AB	28.9	36.4	3.9	0.1	0.9	0.0

Table 1.1 continued

Cruise	Date	Station	Tow	Distance from Shore	Meso. feature	Temp.	Salinity	DO	CHLA	Sailfish/ 1000m^2	Billfish/ 1000m^2
4	6/2/2019	58	D8	314.7	AR	28.7	36.2	4.4	0.1	0.0	0.0
4	6/2/2019	58	N70	314.7	AR	28.7	36.2	4.4	0.1	0.0	0.0
4	6/3/2019	61	D11	146.0	CW	29.8	36.3	4.0	0.1	17.8	20.9
4	6/3/2019	61	D12	146.0	CW	29.8	36.3	4.0	0.1	3.6	3.6
4	6/3/2019	61	N74	146.0	CW	29.8	36.3	4.0	0.1	0.0	0.0
4	6/2/2019	63	D9	299.1	AR	28.9	36.2	3.2	0.1	3.1	3.3
4	6/2/2019	63	D10	299.1	AR	28.9	36.2	3.2	0.1	0.0	6.2
4	6/4/2019	64	D13	55.5	CW	28.6	36.6	4.0	0.1	0.8	0.8
4	6/4/2019	64	D14	55.5	CW	28.6	36.6	4.0	0.1	0.0	0.0

1.2.2 Data Collection

1.2.2.1 Field Data Collection

During each cruise, a standard 1 x 2 m neuston frame fitted with a 505 µm mesh net was towed for 10 minutes at a speed of approximately 2 kt (~ 617 m transects) at each station. During the fourth cruise, a "double" neuston net sampler was used (in addition to the standard neuston sampler) to collect zooplankton prey (150 µm mesh net) concurrently with fish larvae (505 µm mesh net) (Figure 1.2). At least one net tow was collected at each station; in some instances, additional tows were collected if time allowed. All standard neuston net samples were sieved and preserved at sea in 95% ethanol; ethanol was replaced after 24 hours.

The 505 µm and 150 µm plankton samples from double neuston net tows were immediately transferred to volumetric pitchers in an ice bath for sorting. The plankton samples from the 505 µm net were quickly sorted for larval billfishes (all species) under low magnification. Identified billfish were placed in 1 ml cryovials and frozen in liquid nitrogen (LN_2) for lipid analysis. A zooplankton aliquot was taken from each sample collected in the 150 µm net, transferred to 5 ml cryovials, and frozen (LN_2) for sorting

and lipid analysis. The remainder of each plankton sample was preserved in 95% ethanol; ethanol was refreshed after 24 hours. At the end of the cruise, all LN_2 samples were transferred to a −80 ºC freezer for storage until analysis, and all ethanol-preserved samples were transferred to 85% buffered ethanol in glass jars for long term storage.

A suite of environmental observations was recorded at each station. Water depth (m) and location (latitude and longitude, decimal degrees) were recorded from the vessel's navigation instrumentation package. A SBE 09 Plus CTD (SBE 11 deck box) was used to collect near-surface (4.5 m depth) observations of temperature (°C), salinity, and dissolved oxygen (DO) (mg/L) (Table 1.1). Sea surface chlorophyll (chl) *a* concentration estimates (mg/m^3) were derived from satellite data and were provided by collaborators at the University of South Florida's Optical Oceanography Laboratory (https://optics.marine.usf.edu/). Station distance from shore (km) was estimated using the proximity tool in ArcGIS which accounts for the curvature of the earth and calculates the shortest distance between a coordinate (station) and vector (shore outline). Each station was also categorized by its associated surface water mesoscale activity (common water, cyclonic region, anticyclonic region, cyclonic boundary region, or anticyclonic boundary region) following the methodology of Domingues et al. (2016), which was implemented using R code from, https://github.com/rtleaf/Mesoscale-Circulation-Tracking. Mesoscale circulation classifications were calculated with satellite altimetry data and therefore do not distinguish between currents and eddies. Because the Loop Current flows in an anticyclonic loop toward the Florida Straits, stations sampled on the loop current were classified as anticyclonic.

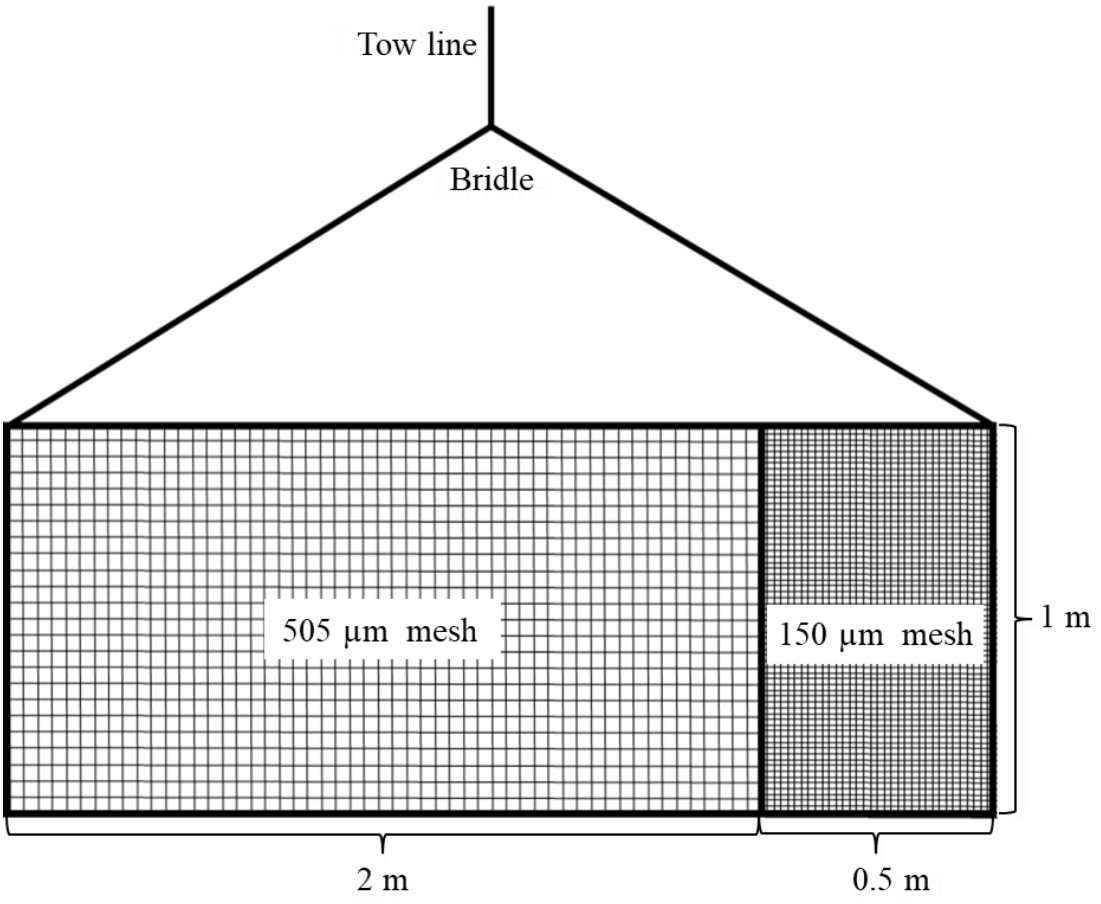

Figure 1.2 Diagram of the "double" neuston net sampler used for simultaneous larval fish (505 µm mesh) and zooplankton prey (150 µm mesh) collection.

1.2.2.2 Plankton Sorting, Identification, and Measurements

All larval fishes were sorted and enumerated from each ethanol-preserved sample. Billfish larvae (all species) were identified to species by morphometrics and lower jaw pigment patterns following Luthy et al. (2005a), and confirmed with DNA barcoding conducted by colleagues at the Marine Genomics Laboratory, Texas A&M University-Corpus Christi. Larval billfish body lengths were measured to the nearest 0.001 mm using a Zeiss dissecting microscope with a digital camera and iSolution Lite imaging software (IMT iSolution, Inc., 2018, Vancouver, BC). Standard lengths were measured

from the tip of the snout to the end of the notochord for pre-flexion and flexion larvae and from the tip of the snout to the posterior edge of the caudal peduncal for post-flexion larvae. Larvae stored in ethanol were measured after they had been preserved in 85% buffered ethanol for at least a week since any shrinkage happens in the first few days of preservation (Fey 1999).

1.2.3 Data Analysis

1.2.3.1 Billfish Abundance and Distribution

Catch per unit effort (CPUE) was calculated for billfish larvae by standardizing each neuston trawl to 10 minutes. Because the speed for each trawl was held constant at 2 knots, it was assumed that each standardized 10-minute neuston trawl covered the same area: 1,235 m^2 for the two meter wide net and 309 m^2 for the half meter wide net. Larval CPUEs were then converted to larval density per 1,000 m^2 to allow for comparisons with previous studies. A PCA was conducted using a scaled correlation matrix of five environmental variables and sailfish CPUE where variables were scaled proportional to eigenvalues. Environmental variables included temperature (°C), salinity, DO (mg/L), sea surface chl *a* (mg/m^3), and distance to shore (km).

1.2.3.2 Diet Analysis

The alimentary canal of each larval billfish was removed and transferred to a drop of glycerin on a glass slide for microscope examination. Gut contents were removed and identified to the lowest possible taxonomic resolution. Larvae with damaged guts were removed from analyses (4%). Individual prey items were enumerated for each prey group, and undamaged prey items were photographed and measured for length (prosome length for copepods; capapace length for cladocerans; standard length for larval fishes).

For all billfish species, feeding incidence (the proportion of larvae with prey in the alimentary canal) and incidence of piscivory (the proportion of larvae with larval fish prey in the gut) were used to describe larval billfish feeding success. Prey data are reported as the percentage of each prey group by the total number of prey extracted (%N).

Multiple one-way ANOVAs were conducted to determine if the total number of prey ingested differed by species. Because larvae of the four billfish species were not sampled evenly across all size classes and gut capacity increases with larval length, data were binned to take advantage of size class overlap between the species where it existed. Tukey's honestly significant difference (HSD) tests were used to test the difference between the mean number of gut contents for each species when three or more species were being compared.

For the numerically dominant sailfish larvae, the relationship between prey and predator length was analyzed using a linear mixed effects model with larval fish specimen number included as a random variable to control for multiple prey measurements associated with each individual larva. Gut fullness was decribed volumetrically for larvae that contained only *Evadne* and *Farranula* (n = 124) using relative gut content weight as a proxy (RelGCW). The sailfish guts examined in this study contained little to no mucus or unidentifiable organic matter, so was determined to be an acceptable proxy for gut fullness (Buckland et al. 2017). RelGCW was calculated as prey dry weight as a percentage of predator gut-free dry weight. Weights for preferred prey (*Evadne, Farranula*) were derived from previously published length:weight relationships for zooplankton collected from the Straits of Florida (Llopiz 2008):

Evadne tergestina: (Ln W= 2.77 In L - 17.27, $r^2 = 0.99$)

Farranula gracilis: (Ln W= 2.88 In L - 17.39, $r^2 = 0.99$)

When consumed prey were too damaged for accurate length measurements, they were assigned the mean length of consumed prey from all sailfish larvae caught in the associated net tow. Larval sailfish gut-free dry weight (GFDW) was derived using a length-GFDW relationship previously established for larval sailfish collected from the Straits of Florida (Llopiz & Cowen 2008):

$$GFDW = 0.005\ BL^{2.62},\ n = 69,\ r^2 = 0.91$$

RelGCW was compared to the percentage of daylight elapsed relative to the total daylight (relTOD) and larval standard length. Larvae were grouped into 10% relTOD bins and 1 mm length class bins. Larvae collected after sunset were included in the analysis with reTODs > 100%. A multi-way ANOVA assessed cruise, year, and station as random factors with RelGCW.

1.2.3.3 Prey Selectivity

To assess the prey field available to larval billfish, zooplankton abundance was determined for each sample collected with the 150 µm mesh net of the double neuston following the methods outlined in Harris et al. (2000). In brief, a displacement volume for each sample was recorded; if the displacement volume exceeded 100 ml, the sample was split to no less than 25 % of the original sample with a Folsom plankton sub-sampler. An aliquot of zooplankton from each sample was taken by mixing the entire sample (for samples with displacement volumes < 100 ml) or a known proportion (sample aliquot) of the sample (for samples with displacement volumes > 100 ml) in a beaker with a small aerator. An aliquot was then extracted from the uniform mixture with a Hensen-Stempel

pipette. The mixture of zooplankton and water from which the aliquot was taken had a volume (count volume) between 300 ml and 510 ml per sample. One ml aliquots were taken from this uniform mixture consecutively until 200 copepods and 100 non-copepods were identified or until 1500 copepods were identified even if the 100 non-copepod threshold was not met. Zooplankton counts from the aliquot were then standardized as zooplankton concentration (number/m^2) for the entire sample using the equation:

$$\text{Zooplankton CPUE} = \frac{\text{Taxon raw count} * (\text{count volume}/\text{aliquot volume})}{\text{Sample aliquot} * \text{Swept area } (m^2)}$$

For the numerically dominant larval sailfish, prey selectivity was calculated by the selection ratio,

$$w_i = o_i / \pi_i,$$

where the proportion of each prey type *i* ingested (o_i) is divided by the proportion of prey type *i* in the environment (π_i) (Manly et al. 2002). A selection ratio > 1 represents a positive selection for a prey type and a ratio < 1 indicates selection against a prey type in relation to the prey available in the environment; a selection ratio ≈ 1 indicates consumed prey proportions match the prey proportions available in the environment. Prey items were assigned to categories based on taxonomy, sex, and size. Because selectivity ratios can be sensitive to rare prey items (Confer and Moore 1987), selectivity calculations included sailfish larvae that had consumed only the dominant zooplankton prey *Farranula*, *Evadne*, or *Corycaeus* (83% of total sailfish larvae). Selection ratios were calculated for every sample collected with the double neuston where sailfish larvae were present. The gut contents of all sailfish larvae in each sample were pooled. Bonferroni-adjusted confidence intervals (SE(w_i)) were used to test for significant prey selection,

$$SE(w_i) = \sqrt{[o_i (1 - o_i) / (u_+ \pi_i^2)]},$$

where u+ is the total number of prey items consumed (Llopiz 2008). Selection for or against a prey type was determined when confidence intervals did not overlap with 1.

Farranula copepods are sexually dimorphic. Measurements of *Farranula* taken from surface waters of the northern GoM during the fourth cruise in this study indicated that the dry weight of females was approximately 250% greater than that of males, and female carapace length was approximately 80% greater than male carapace length. Therefore, *Farranula* copepods were further classified by sex as separate prey items. Each of the four preferred prey categories (female and male *Farranula*, *Corycaeus*, *Evadne*) were divided into size classes based on the range of prey lengths available in the environment. Female and male *Farranula* exhibited relatively consistent lengths across all environmental samples compared to *Corycaeus* and *Evadne* which exhibited a much larger range of lengths. Therefore, female, and male *Farranula* were divided into three sizes classes for the prey size selection analyses, and *Corycaeus* and *Evadne* were divided into four size classes.

1.2.3.4 Lipid Analysis

Lipid analyses were conducted on the four preferred prey categories identified for the selectivity analyses. Zooplankton collected with the 150 µm mesh net and preserved in LN_2 were sorted for female *Farranula*, male *Farranula*, *Corycaeus*, and *Evadne* until the required bulk sample weight for FA analysis was met (~1 mg per replicate). The relative concentration of the FAs of zooplankton prey was measured as fatty acid methyl esters (FAME). Lipid extraction, trans-esterification, and gas chromatography analysis of FAME was performed according to the methods outlined by Budge et al. (2006). In short,

total lipids were extracted by placing freeze-dried tissue samples in a 2:1 ratio of chloroform:methanol containing 0.005% butylated hydroxytoluene (BHT) for at least 48 hours. Two phase separations were performed, and the resulting extraction was dissolved in chloroform, flushed with nitrogen gas, and stored at -20 ^{o}C. Samples were then transmethylated with sulfuric acid and hexane. A tricosanoic acid standard (NU-Chek, Tritricosanoic, T-185) was added at this step to allow quantification of FAs (Peters et al. 2006). FAME samples were run on a gas chromatograph (SHIMADZU GC- 2010 Plus) and a marine oil standard was used to identify peaks for 20 common marine FAs (RESTEK Marine Oil Fame Mix). The internal standard allowed for the relative quantification of fatty acid masses. Individual FAs were standardized as % of total FA by dividing the area under each chromatogram peak by the total area under all peaks. FA data was analyzed to determine significant differences in the response variables: total FA, DHA %, EPA %, and arachidonic acid (AA) %, with one-way ANOVAs and Tukey's HSD post hoc. Predictor variables included prey type, station, and shore proximity. Prey were pooled for each station to make spatial comparisons of FAs. The response variables were also examined for larval sailfish muscle tissue. Predictor variables included station, shore proximity, and ontogenetic stage.

1.2.3.5 Otolith Microstructure Analysis

Larval sailfish otoliths were removed from collected specimens, prepared for microscopy, and examined following the mehods outlined by Luthy et al. (2005). Heads were removed from fish larvae and placed in a drop of immersion oil to clear the tissue. Lower jaw and branchiostegal rays were removed to allow an unobstructed ventral view of otolith arrangement. Otoliths were then extracted and transferred to a microscope slide

with a clean drop of immersion oil. Otoliths were left to soak in immersion oil for several days prior to reading to enhance the clarity of the daily growth increments. Otoliths were then imaged with a digital camera mounted to a compound microscope under high oil immersion magnification (600x - 1000x). Saggital otolith increments were enumerated and measured (to the nearest 0.1 µm) with iSolution Lite imaging software along the longest growth axis where all increments were visible from the primordium to the outer edge.

Although it has not been directly validated, daily increment deposition has been assumed in previous studies of sailfish and blue marlin (Luthy et al. 2005, Sponaugle et al. 2005, Simms 2009) based on previous studies of fish larvae with similar life histories to billfishes (e.g., bluefin tuna) (Itoh et al. 2000). To estimate larval sailfish age, sagittae were blind coded and examined by two separate readers. If the increment counts of the two readers matched exactly, that count was taken for the age estimate. If the increments counts did not match, a third read was conducted and if it matched one of the previous two reads, that count was taken for the age estimate. If none of the three increment counts matched, the larva was excluded from the analysis.

Larval sailfish growth was examined using a detrended growth index which takes into account increased growth and variance of older larvae compared to younger larvae (Pepin et al. 2001):

$$DG_{ij} = (G_{ij} - G_j)SD_j^{-1}$$

where DG_{ij} is the detrended growth of individual i at age j, G is the otolith increment width, and SD is the standard deviation of all increments measured at age j. This index provides a measure of growth independent of age and therefore allows comparisons of

the effects of biotic and abiotic factors on larval growth (Baumann et al. 2003, Robert et al. 2009, Sponaugle et al. 2010). Detrended recent growth (DRG) was estimated by the sum of the last three complete detrended otolith increments (DGij), excluding the outermost partial increment in contact with the otolith edge since it doesn't represent a full day of growth. Exponential regressions were used to describe the relationships between larval sailfish standard length and estimated age as well as standard length and otolith diameter. A homogeniety of slopes test was conducted to determine if the daily instantaneous growth rate (*k*) of larval sailfish differed bewteen years and one-way ANOVAs followed by Tukey's HSD were used to look at interanual and intermonthly differences in age.

1.2.3.6 Multi-way ANOVA

To maximize sample size, FA and prey availability data were analyzed separately from all other predictor variables to examine the effects of environmental conditons, diet, and spatial variation on growth of sailfish larvae with DRG data (n = 112) The environmental variables that were considered are temperature, salinity, DO, and surface chl *a*. Diet variables included total number of prey in gut, relGCW, and the proportions of each preffered prey type found in gut. Spatial variables included distance from shore (km), depth (m), and surface mesoscale circulation classification. Age, developmental stage, and standard length were not considered because detrended growth is independent from age. To reduce the liklihood of model overfitting, the number of potential predictor variables was decreased by examining the relationship of each variable with mean DRG individually with one-way ANOVAs. Predictor variables were considered to be potentially significant if p values were less than 0.05. Once predictor variables were

selected, they were tested for multicolinearity with the varience inflation factor (VIF). If multicolinearity was determined, seperate multi-way ANOVA models were built to split up multicolinear variables. A final model was selected by comparing the F-statistics and the Akaike information criterion (AIC) of these ANOVA models. Insignificant interaction terms were then removed from the final model. The final model selected included only variables with VIF scores < 4, significant main effects, and significant two-way interaction terms. Post- hoc tests were then conducted.

The process above was then repeated for the subset of fish collected in 2019 that had accompanying prey availability data (n = 57). The variables included all of the variables from the first analysis above plus prey availability variables which inclided total zooplanktion CPUE, individual preffered prey CPUEs, and total preffered prey CPUE.

A third analysis was conducted for a subset of the 2019 sailfish that also had accompanying FA data. FA variables included total FA (mg/g), DHA %, EPA %, and AA % for each preffered prey category and for sailfish muscle tissue. (n = 35).

1.3 Results

1.3.1 Environmental Conditions

Data were collected at 28 sampling stations during 2017 (n = 7), 2018 (n = 12) and 2019 (n = 9) (Table 1.1; Figure 1.1). Station locations ranged from approximately 24 km to 377 km from shore at water depths from 95 m – 3156 m. With respect to oceanographic features, most stations were positioned in common waters (n = 22) that lacked any distinguishable mesoscale circulation features; relatively few stations were in anticyclonic regions (n = 3) or anticyclonic boundary regions (n = 3). Temperatures at the

stations ranged from 28.2 – 30.9 °C, salinities from 29.9 – 36.6, DO concentrations from 2.4 – 6.3 mg/L, and chl *a* concentration from 0.055 – 13.172 mg/m3.

During Cruise 1 (July 2017), all stations sampled were in common water, and relatively little variability was observed in near surface temperature (29.1 – 30.9 °C) and salinity (33.0 – 36.3) across stations (Table 1.1). However, stations sampled nearest the birdsfoot delta (Stations 4, 8, 10) were characterized by relatively high chl *a* concentrations (11.0 – 13.2 mg/m^3) relative to stations further offshore and east of the delta (Stations 12, 13, 15, 17), where chl *a* concentrations were orders of magnitude lower (0.1 – 1.3 mg/m^3) (Table 1.1; Figure 1.1). Although not hypoxic, relatively low DO concentrations (2.4 – 2.9 mg/L) were observed at all stations except Station 10 (6.0 mg/L).

During Cruise 2 (May – June 2018), stations were sampled in both common water and anticyclonic boundary regions. There was very low variability in near surface temperature (28.2 – 28.5 °C), salinity (36.0 – 36.5), chl *a* (0.1 – 0.2 mg/m^3) and DO (6.0 – 6.1 mg/L) across stations (Table 1.1).

During Cruise 3 (July 2018), common water and anticyclonic boundary regions were sampled. Variability in environmental conditions was relatively low across sampled stations with the largest deviations driven by the stations closest to shore and south of the birdsfoot delta (Stations 34, 45), where salinity was lower (32.0 – 33.6), DO was slightly higher (6.1 – 6.3 mg/L) and chl *a* was slightly higher (0.2 – 0.3 mg/m^3), than stations farther offshore (salinity = 35.5 – 36.2; DO = 5.9 – 6.0 mg/L; chl *a* = 0.1 – 0.2 mg/m^3) (Table 1.1; Figure 1.1). Temperature also showed little variation (29.8 – 30.4) across stations and did not vary predictably with distance from shore (Table 1.1).

During Cruise 4 (May – June 2019), stations were sampled in common water, anticyclonic regions, and anticyclonic boundary regions. Although there was relatively low variability in near surface temperature (28.4 – 28.9 °C) for all sampled stations, neritic stations (Stations 49, 51) showed slightly elevated levels of DO (4.9 – 5.4 mg/L) and chl *a* (0.6 – 1.9 mg/m^3) as well as lower salinities (29.9 – 32.5) relative to oceanic stations which had higher salinities (36.2 – 36.6), lower DO (3.2 – 4.4 mg/L), and lower chl *a* (0.1 mg/m^3) concentrations (Table 1.1, Figure 1.1).

1.3.2 Abundance and Distribution

Of the 188 billfish larvae collected, 146 were identified as sailfish (*I. platypterus*), 20 were identified as swordfish (*X. gladius*), 11 were identified as white marlin (*K. albida),* 9 were identified as blue marlin (*M. nigricans*), and 2 remained unidentified. The density of billfish larvae ranged from 0 – 27.5 larvae/1,000 m^2 for an individual neuston tow (Figure 1.3) and the average density per station ranged from 0 – 13.75 larvae/1,000 m^2. The overall average density of billfish for all tows in this study was 2.58 ± 0.87 larvae/1000m^2 (Table 1.2). A PCA biplot of environmental variables and sailfish CPUE indicated that sailfish abundance was inversely related to chl *a* concentration and was positively correlated with salinity and distance from shore (Figure 1.4). Larval sailfish abundance also differed among mesoscale circulation features (one-way ANOVA, df = 2, F = 4.322, p = 0.024). Specifically, anticyclonic boundary regions had higher sailfish abundance than anticyclonic regions (Tukey HSD, p = 0.046) or common water (Tukey HSD, p = 0.025). (Figure 1.5). There were no significant differences in larval sailfish CPUE among years (one-way ANOVA, df = 2, F = 0.381, p = 0.687) or cruises (one-way ANOVA, df = 3, F = 1.012, p = 0.404).

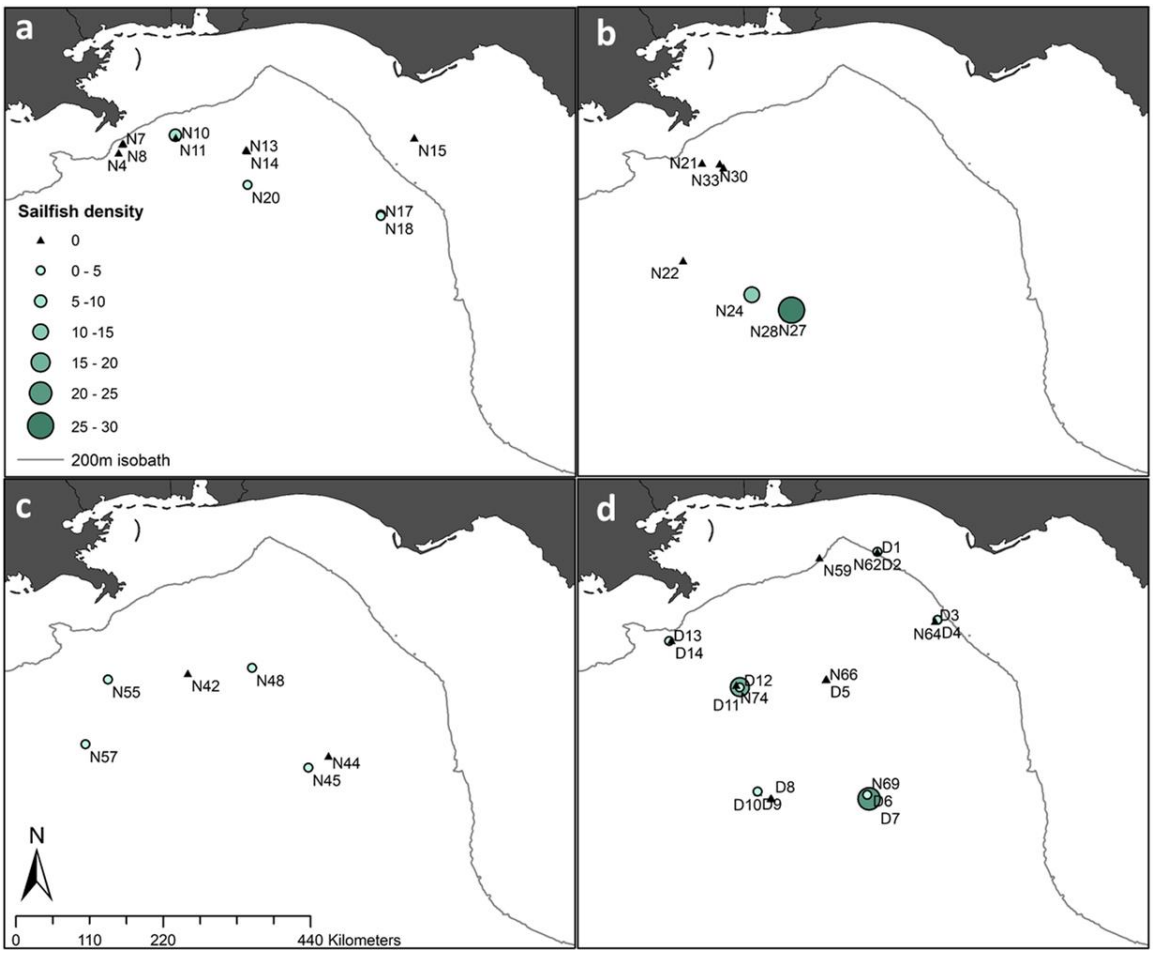

Figure 1.3 Larval sailfish density (larvae/1000 m^2) from each cruise: July 2017 (**a**), May-June 2018 (**b**), July 2018 (**c**), and May-June 2019 (**d**). Each symbol denotes a unique sample; associated sample data are provided in Table 1. Neuston net samples (N) were collected during all four cruises (**a**-**d**); duel neuston net samples (D) were only collected in May-June 2019 (**d**).

Table 1.2 Total catch, frequency of occurrence (percent of tow that yielded ≥ 1 larvae), mean density (± standard error [SE]), and maximum density of larval sailfish sampled during 4 cruises in the northern GoM. The number of tows for each cruise is represented by n.

Cruise	n	Sailfish Catch	Frequency of Occurrence (%)	Mean density (±SE) (larvae/1000m^2)	Maximum density (larvae/1000m^2)
July 2017	11	17	36	1.25 (0.85)	8.9
May-June 2018	7	40	29	5.54 (3.99)	27.5
July 2018	6	8	67	1.03 (0.38)	2.3
May-June 2019	21	80	48	2.74 (1.25)	21.1
Total	45	146	44	2.58 (0.87)	

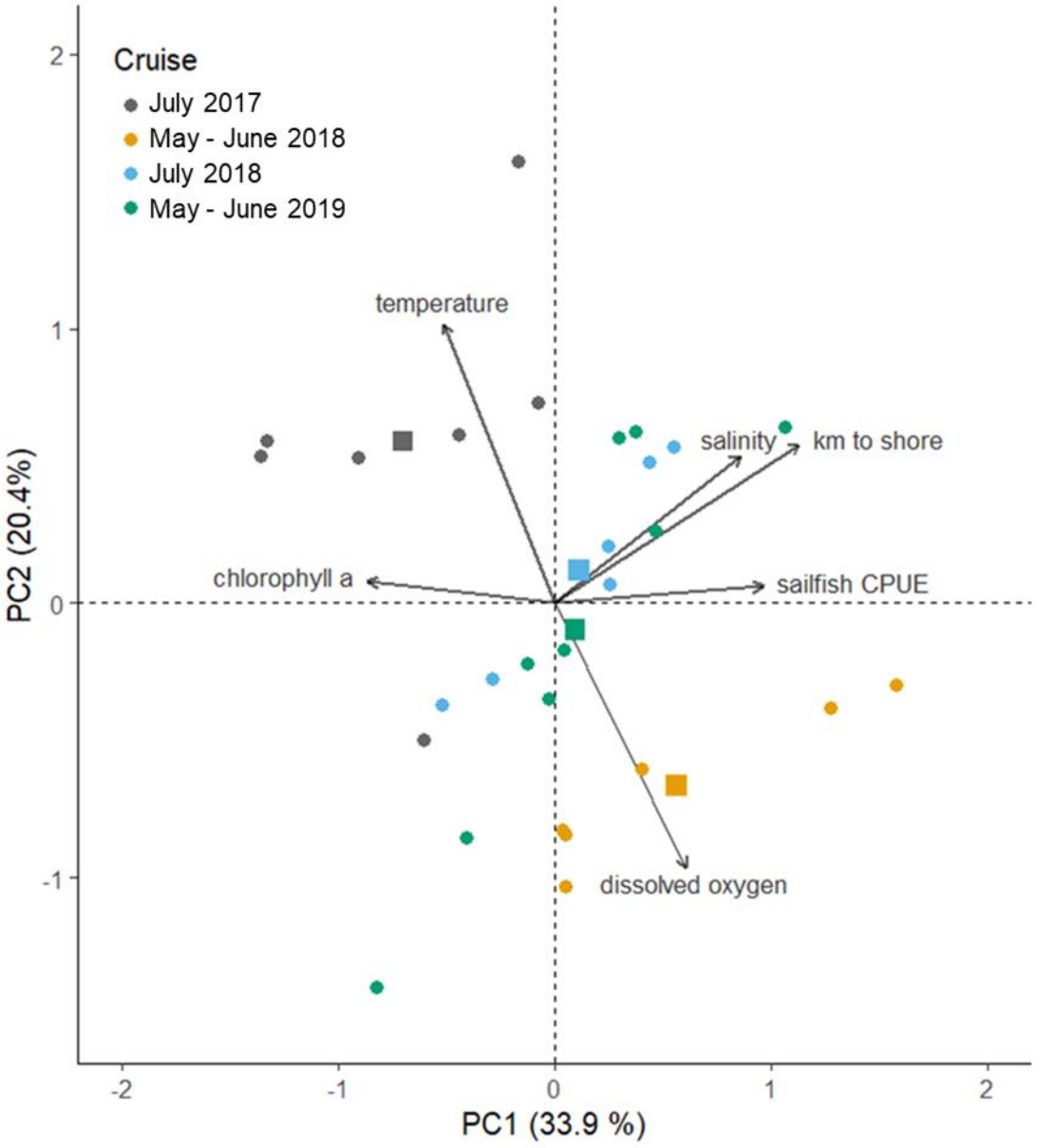

Figure 1.4 PCA biplot based on a correlation matrix of environmental variables (near-surface temperature, salinity, and dissolved oxygen; surface chl *a*; distance in km from shore) and larval sailfish CPUE for each open water station sampled. Arrows depict the loading on each variable. Arrow length approximates the variance associated with each variable, and smaller angles between each variable represent stronger correlations. Circles represent individual stations and squares represent cruise centroids. PC1 explains 33.9 % of the variance in the data. PC2 explains 20.4% of the variance.

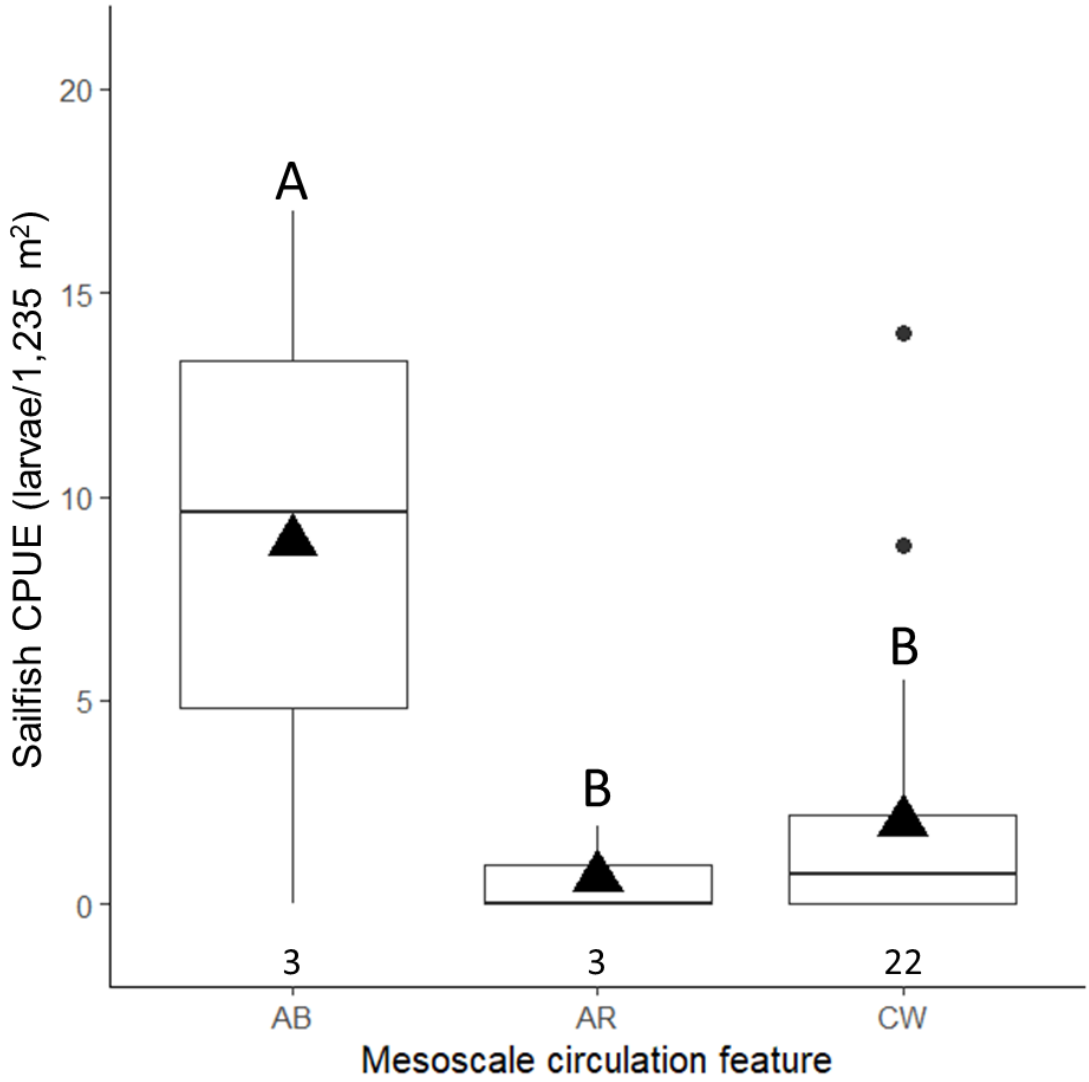

Figure 1.5 Boxplots of larval sailfish abundance (CPUE) collected in different mesoscale circulation features: anticyclonic boundary region (AB), anticyclonic regions (AR), and common water (CW). The bold line within each box indicates the sample median and the upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the mean sailfish CPUE at each mesoscale feature and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. The number of stations included in each mesoscale circulation feature is indicated at the bottom of each plot.

1.3.3 Diet Analysis

A total of 1224 prey items were excised from guts of 179 billfish larvae (all species). Billfish larvae exhibited high feeding incidence; intact guts from open water stations contained at least one prey, and as many as 28 prey items were found in a single gut (Fig 6a). At smaller sizes (3 – 5 mm), the number of prey items observed in guts did not vary among larval sailfish, blue marlin, and white marlin (one-way ANOVA: df = 2, $F = 3.074$, $p = 0.051$, Figure 1.6b). The average number of prey found in the guts of larvae that ranged from 3 – 5 mm in length was 4.5. Larger larvae showed species-specific differences in the number of prey found in their guts. Differences in total gut contents were found between billfish species captured in the 5 – 7 mm size bin (one-way ANOVA: df = 3, $F = 3.733$, $p = 0.016$); larval blue marlin had significantly more prey items in their guts than sailfish (Tukey's HSD, $p = 0.025$) and swordfish (Tukey's HSD, $p = 0.018$) (Figure 1.6c). The average number of prey consumed for all billfish species between 5 and 7 mm was 8.8. In the 5 – 7 mm size class, blue marlin had an average of 14.5 prey/gut, white marlin had an average of 10.3 prey/gut, sailfish had an average of 8.4 prey/gut, and swordfish had an average of 6.7 prey/gut (Figure 1.6c). In the 7 – 11 mm size bin, larval swordfish had significantly more prey in their guts than did sailfish (one-way ANOVA, df = 1, $F = 13.93$, $p < 0.001$) (Figure 1.6d). For all larvae between 7 and 11 mm, an average of 11.6 prey were found in the gut. Swordfish had an average of 16.6 prey/gut and sailfish had an average of 6.1 prey/gut in the 7 – 11 mm size bin.

The main prey item for all billfish species was *Farranula* copepods, which made up over 68% of the gut contents for sailfish, and between 80% and 90% of the gut contents for blue marlin, white marlin, and swordfish (Table 1.3). For all billfish species,

female *Farranula* were three times more abundant in guts than male *Farranula* and comprised 59% of total gut contents for all species of billfish combined, relative to 16% of total gut contents comprised by male *Farranula*. Greater consumption of female *Farranula* than male *Farranula* was observed for all billfish species at each developmental stage (i.e., preflexion, flexion, postflexion) (Table 4). *Evadne* cladocerans were the second most abundant prey item for sailfish and blue marlin at 17% and 11% of total gut contents, respectively, and *Corycaeus* copepods were the second most abundant prey for white marlin and swordfish at 6 % of total gut contents for both species. Prey items represented infrequently in the diets included calanoid copepods, *Limacina* gastropods, and larval fish (Table 1.3). Because of the limited sample size of swordfish (n = 19), blue marlin (n = 9), and white marlin (n = 11), all remaining analyses include only sailfish larvae.

Gut content analyses of larval sailfish indicated that diet varied with ontogeny. Recently hatched sailfish larvae in the 2 – 3 mm size class contained few prey items, and the number of prey consumed increased with larval length until the 5 – 6 mm size class where number of prey began to decrease with larval size (Figure 1.6a). This decrease coincided with the inclusion of piscivory which began at approximately 5.0 mm standard length. The onset of piscivory coincided with upward flexion of the notochord tip which also occurred at 5.0 mm. The 5 mm size class (n = 37) had an incidence of piscivory of 0.16 which increased with larval sailfish length and reached 1.0 for all sailfish > 8 mm standard length (n = 6). *Farranula* and *Evadne* comprised over 88% of the diet for preflexion sailfish larvae. Dominant prey for postflexion sailfish larvae were more diverse, and included *Evadne* (40.2 %), *Farranula* (21.7 %), larval fish (19.6 %),

Corycaeus (13.0 %), and calanoid copepods (5.4 %) (Figure 1.7). In addition to changes in the number of prey consumed and the type of prey consumed throughout larval development, the size of prey also increased with sailfish standard length. (Linear mixed effects model, df = 96.8, F = 23.5, $p < 0.001$); this relationship is largely driven by the inclusion of larval fish prey in the diets of larger larvae (Figure 1.8).

The relative gut content weight (relGCW) of individual sailfish larvae ranged from 1.33% to 17.6% (mean = 8.65% ± 0.39%). When larvae were binned by relTOD, mean relGCW ranged from 6.47% ± 0.72% for the larvae collected earliest after sunrise to 16.71% ± 0.89% for larvae collected latest after sunrise (Figure 1.9a). When larvae were divided into 1-mm size classes, the relGCW size class mean was lowest (4.69% ± 1.65%) for the larvae in the smallest size class (2 – 3 mm). Larvae in the 4 – 5mm size class had the highest relGCW size class mean (9.94% ± 0.95%) (Figure 1.9b). However, there were no significant differences in relGCW between cruises, years, or stations (Table 1.5).

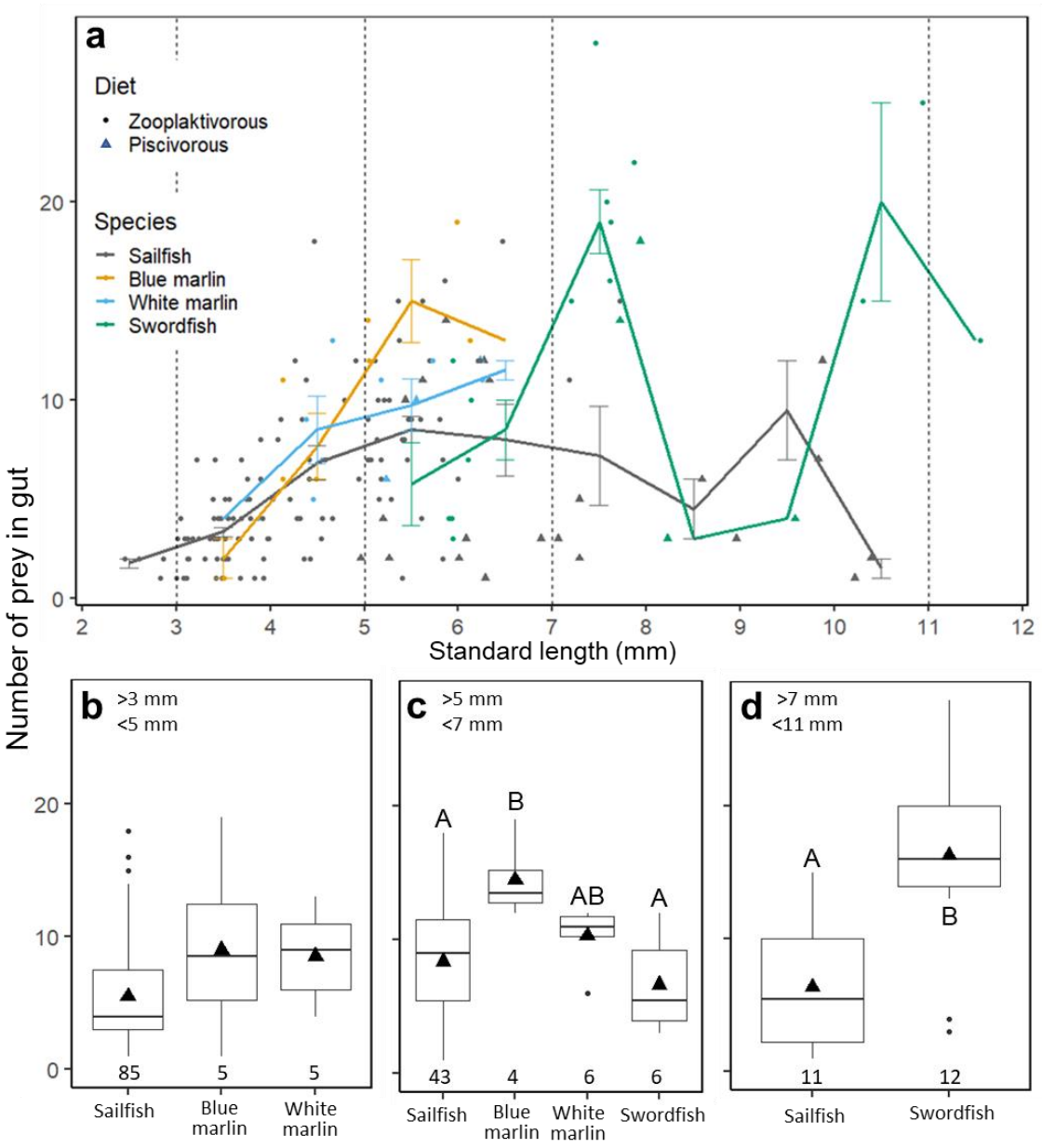

Figure 1.6 Relationships between the number of prey per gut and larval billfish standard length. Top panel (**a**): small symbols represent individual larvae and denote zooplanktivorous (circle) or piscivorous (triangle) diet. Each line represents taxon-specific size class means; error bars represent the standard error around the mean. Bottom panels (**b-d**): boxplot comparisons of the number of prey found in larval billfish guts in three size categories: (**b**) 3-5 mm, (**c**) 5-7 mm, and (**d**) 7-11 mm. The bold line within each box indicates the sample median and the upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the number of prey prey gut and letters indicate significant differences between species as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot and size class ranges are shown on the top of each graph.

Table 1.3 Number of prey items (N) and percentage of overall diet (%) of zooplankton prey consumed by larval billfishes collected in the Gulf of Mexico (2017-2019). The total number of larvae examined for each species is reported in parentheses (n).

Prey Category	**Sailfish** (n = 140)		**Blue Marlin** (n = 9)		**White Marlin** (n = 11)		**Swordfish** (n = 19)	
	%	**N**	**%**	**N**	**%**	**N**	**%**	**N**
Cladocera								
Evadne	17.1	134	10.6	9	3.0	3	2.0	5
Copepoda								
Cyclopoida								
Farranula (♀)	56.1	441	62.4	53	61.0	61	69.0	173
Farranula (♂)	13.2	104	20.0	17	27.0	27	17.9	45
Corycaeus	7.6	61	2.4	2	6.0	6	6.0	15
Calanoida	0.8	6	0	0	0	0	0.4	1
Gastropoda								
Thecosomata								
Limacina	1.0	8	0	0	0	0	0	0
Larval fish	3.7	29	0	0	3.0	3	1.6	4
Thaliacea								
Doliolida	0.1	1	0	0	0	0	0	0
Crustacea								
Unidentified	0.1	1	4.7	4	0	0	3.6	9
Unidentified	0.1	1	0	0	0	0	0	0

Table 1.4 Numerical percentage of prey found in larval billfish gut contents by species and ontogenetic stage. Prey items include female *Farranula* (FF), male *Farranula* (MF), *Corycaeus* (Cor), unidentified poecilostomatoid copepods (Poe), *Evadne* (Ev), calanoid copepods (Cal), larval fish (LF), and *Limacina* (Lim). The number of fish larvae included in each group is represented by n.

Species	**Stage**	**n**	**FF**	**MF**	**Cor**	**Poe**	**Ev**	**Cal**	**LF**	**Lim**
Sailfish	preflexion	96	56.7	14.4	7.3	0.2	19.6	-	-	1.6
Sailfish	flexion	30	68.6	14.9	7.1	-	4.7	-	4.3	0.4
Sailfish	postflexion	14	18.5	3.3	12.0	-	39.1	6.5	19.6	-
Blue marlin	preflexion	7	55.2	20.7	3.4	6.9	13.8	-	-	-
Blue marlin	flexion	2	77.8	18.5	-	-	3.7	-	-	-
White marlin	preflexion	6	57.1	34.7	2.0	-	6.1	-	-	-
White marlin	flexion	5	64.7	19.6	9.8	-	-	-	5.9	-
Swordfish	preflexion	5	86.0	14.0	-	-	-	-	-	-
Swordfish	flexion	10	68.4	11.8	9.9	5.9	1.3	0.7	2.0	-
Swordfish	postflexion	4	56.1	36.8	-	-	5.3	-	1.8	-

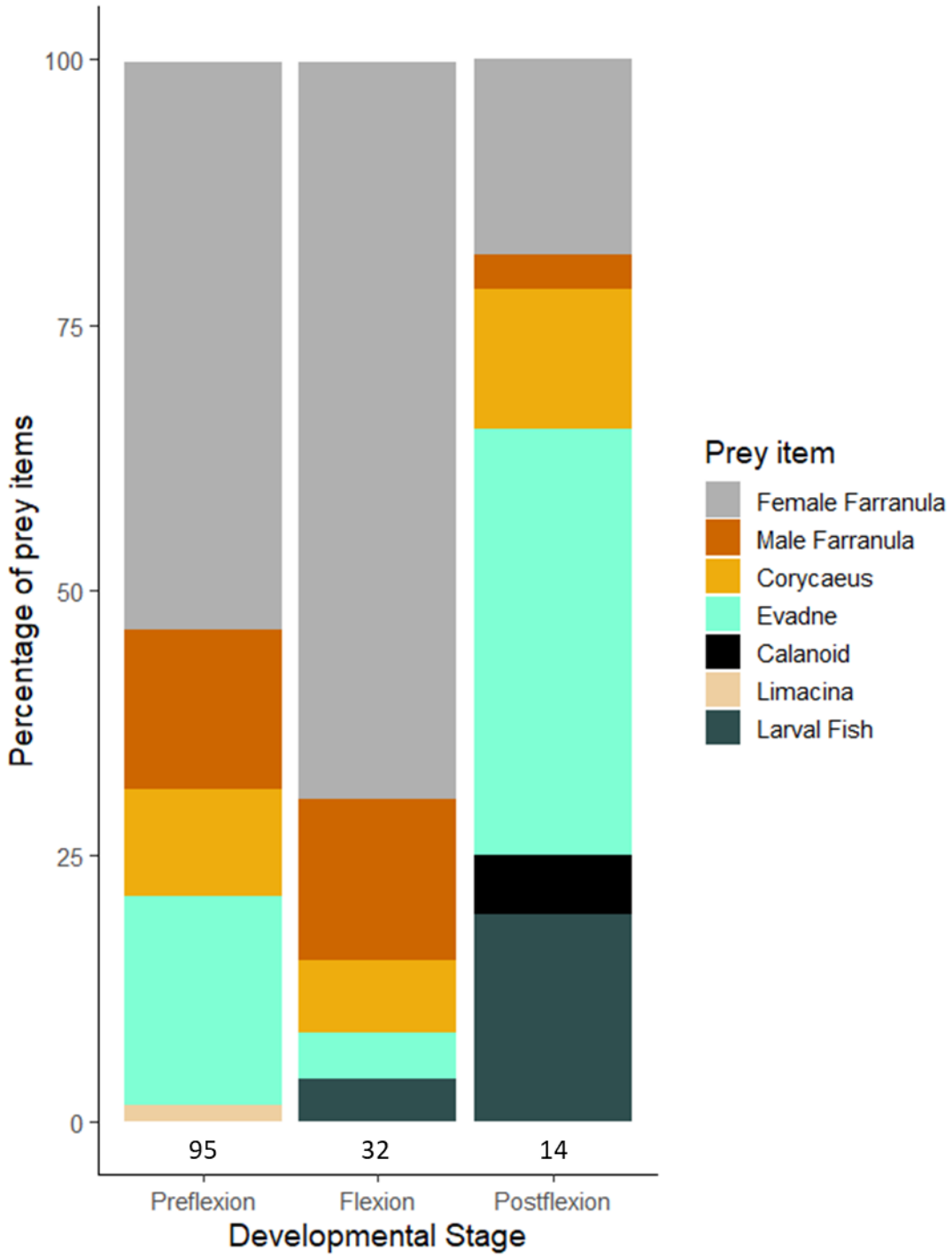

Figure 1.7 Numerical percentages of diet items for preflexion, flexion, and postflexion sailfish larvae. Prey items that contributed <1% of the total diet per developmental stage were excluded. The number of larvae for each developmental stage is indicated at the bottom of each plot.

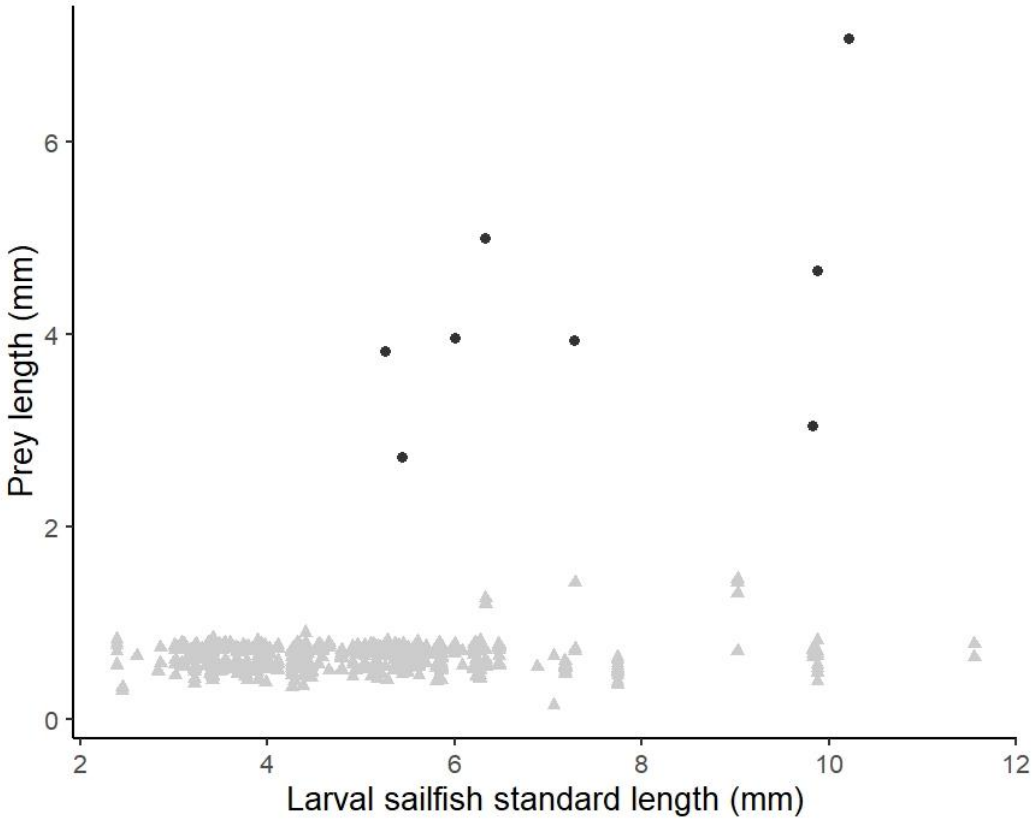

Figure 1.8 Relationship between larval sailfish size (SL, mm) and ingested prey size (prosome length for copepods; carapace length for cladocerans, mm). Grey triangles represent zooplankton prey and black circles represent larval fish prey.

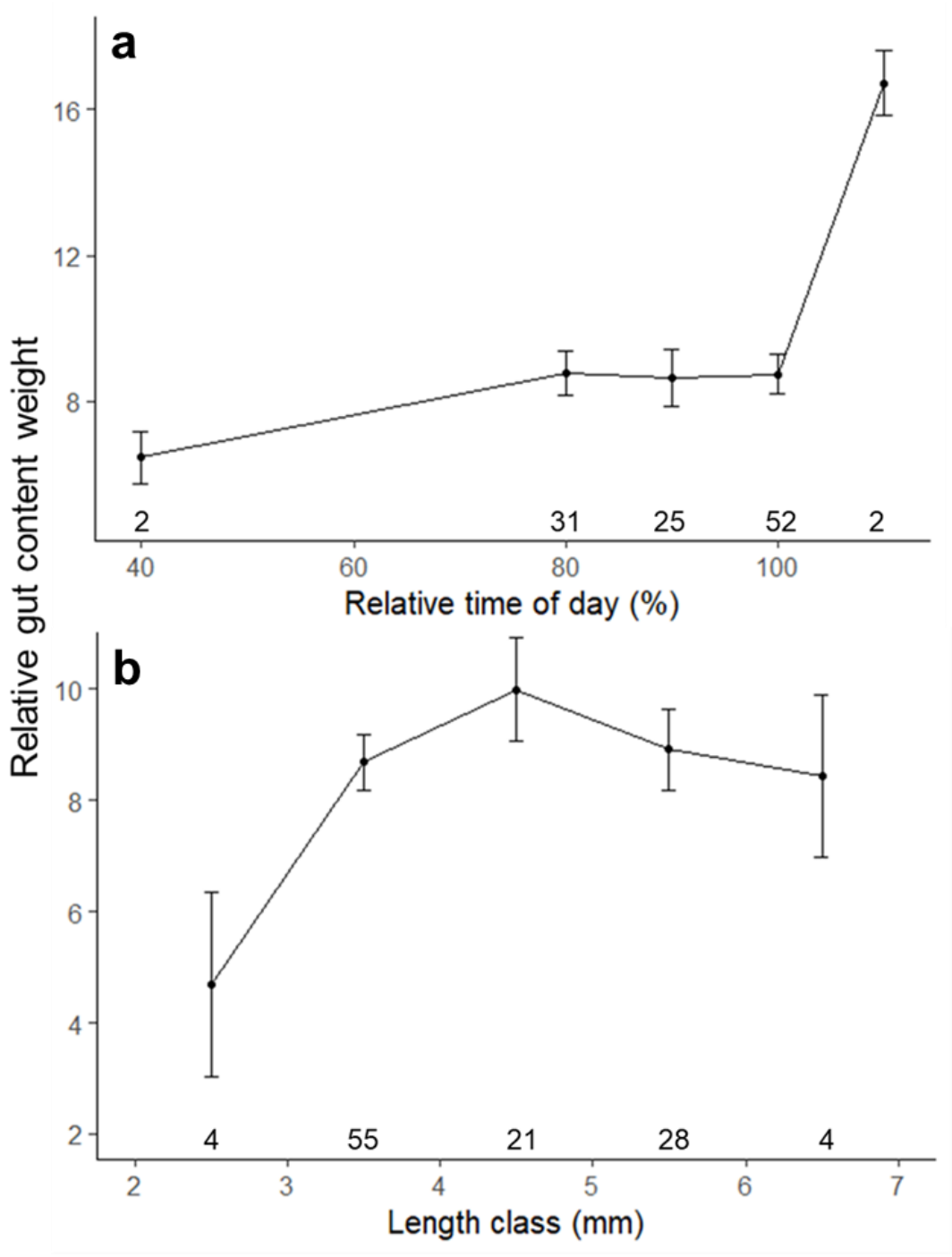

Figure 1.9 Mean (±SE) relative gut content weight for larval sailfish vs. (**a**) relative time of day (% of daylight elapsed since sunrise; values >100 are indicative of larvae caught after sunset) and (**b**) size (SL, mm). Only zooplanktivorous larvae < 7 mm were included in analysis (n = 112). The number of larvae in each size bin are indicated at the bottom of each plot.

Table 1.5 ANOVA results examining the variability of gut fullness with random factors: cruise, year, and geographic station.

Source	df	MS	*F*	p
Cruise	1	6.58	0.21	0.64
Year	1	2.0	0.07	0.80
Station	1	95.40	3.11	0.08
Residuals	111	30.72		

1.3.4 Prey Selectivity

A trend toward positive selection for *Evadne* was observed for all tows where *Evadne* was available in the prey field, with significant selection observed in all but one of these tows (86% of tows with *Evadne* present). The majority (79%) of non-piscivorous sailfish larvae collected in the double neuston sampler (with associated prey) were from two tows (D7 and D11). Prey selectivity at these stations showed similar trends with positive selection for *Evadne* and female *Farranula*, and negative selection for male *Farranula* and *Corycaeus* (Table 1.6).

Larval sailfish consistently exhibited positive selection for smaller, female *Farranula* (0.601 – 0.677 mm size class), neutral selection for medium sizes (0.678 – 0.754 mm size class), and negative selection for larger sizes (0.755 – 0.83 mm size class). Larvae also showed consistently positive selection for male *Farranula* in the large size class (0.579 – 0.638 mm), neutral selection for the medium size class (0.518 – 0.578 mm) and selected no prey from the small size class (0.457 – 0.517 mm). Larvae did not select any *Evadne* from the largest two size classes available (0.682 – 0.846 mm, and 0.847 – 1.011 mm) or *Corycaeus* from the two largest size classes (0.907 – 1.238 mm, and 1.239 – 1.569 mm) available in the prey field (Figure 1.10, Table 1.7).

Table 1.6 Prey selectivity ratio by net tow for non-piscivorous sailfish larvae sampled with a double neuston in 2019. Sailfish larvae and prey were pooled by standardized 10-minute net tow. Prey items include female *Farranula* (♀*Farr.*), male *Farranula* (♂*Farr.*), *Corycaeus* (*Cory.)* and *Evadne* (*Evadne).* Significant selection ($p < .05$) for ($w_i > 1$) or against ($w_i < 1$) a prey type is in bold. The number of sailfish included in prey selectivity calculations for each tow is represented by n.

		w_i			
Tow	**n**	***♀Farr.***	***♂Farr.***	***Cory.***	***Evadne***
D1	1	-	-	**0.09**	**21.85**
D2	1	-	-	1.20	-
D4	3	0.62	-	1.25	6.23
D6	3	**0.38**	**1.40**	**2.16**	**8.32**
D7	26	**1.11**	**0.81**	**0.52**	**7.91**
D9	4	**0.71**	**0.20**	**0.34**	**290.89**
D11	22	**1.98**	**0.66**	**0.22**	**24.50**
D12	3	1.29	1.04	-	**25.37**

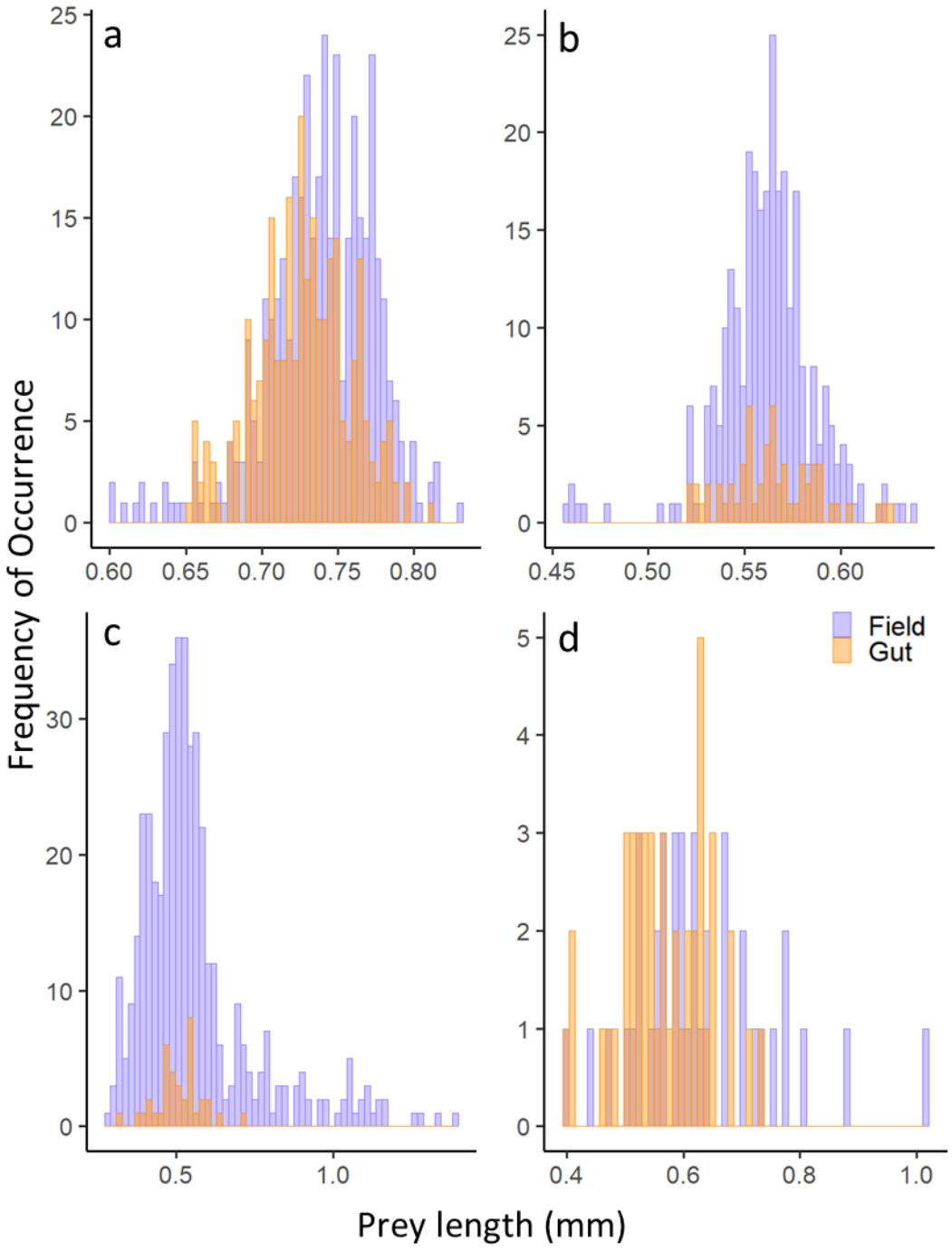

Figure 1.10 Length frequency distributions of preferred zooplankton prey species collected in near-surface waters (purple bars) and in the guts of non-piscivorous, larval sailfish (orange bars) collected concurrently in the northern Gulf of Mexico (June 2019): (**a**) female *Farranula*; (**b**) male *Farranula*; (**c**) *Corycaeus*; and (**d**) *Evadne*.

Table 1.7 Size selection of the dominant prey types of sailfish larvae sampled with a double neuston in 2019. Sailfish larvae and prey were pooled by standard 10-minute net tow. Selectivity ratios were calculated individually as size class proportions for each unique prey type. Significant selection ($p < .05$) for ($w_i > 1$) or against ($w_i < 1$) a prey type is in bold. Prey items include female *Farranula* (♀*Farr.*), male *Farranula* (♂*Farr.*), *Corycaeus* (*Cory.)* and *Evadne* (*Evadne).* Zeros represent instances where no prey were consumed by sailfish but were available in the environment and dashes represent instances where prey were not found in the environment or in sailfish guts. The number of sailfish included in prey selectivity calculations for each tow is represented by n. Sailfish larvae selected no *Corycaeus* or *Evadne* from size classes 3 and 4 (not shown here).

		w_i									
		♀***Farr.*** [1]			♂***Farr.*** [2]			***Cory.*** [3]		***Evadne*** [4]	
Tow	**n**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**1**	**2**
D1	1	-	-	-	-	-	-	2.66	-	1.18	0.79
D2	1	-	-	-	-	-	-	**0.39**	**2.42**	-	-
D4	3	0	1.39	0	-	-	-	**2.88**	1.23	1	-
D6	3	**3.46**	0.64	0	0	1.11	0	-	-	-	-
D7	26	**4.61**	0.98	0	0	1.0	**1.48**	**3.88**	**0.63**	-	-
D9	4	**3.43**	0.45	-	0	1.04	0	2.79	-	0.67	-
D11	22	-	1.02	**0.55**	-	0.97	**1.32**	**0.40**	**1.74**	**4.5**	0.75
D12	3	-	1.04	0	-	1.08	0	-	-	-	-

[1] Size classes in mm: 1 = 0.601 – 0.677, 2 = 0.678 – 0.754, 3 = 0.755 – 0.83
[2] Size classes in mm: 1 = 0.457 – 0.517, 2 = 0.518 – 0.578, 3 = 0.579 – 0.638
[3] Size classes in mm: 1 = 0.243 - 0.575, 2 = 0.576 - 0.906, 3 = 0.907 – 1.238, 4 = 1.239 – 1.569
[4] Size classes in mm: 1 = 0.350 - 0.515, 2 = 0. 0.516 - 0.681, 3 = 0.682 – 0.846, 4 = 0.847 – 1.011

1.3.5 Lipid Analysis

The ten most abundant FAs separated from the lipid extracts of sailfish larvae were C16:0 (34.2%), C18:0 (21.2%), C22:6n3 (DHA) (20.4%), C20:5n3 (EPA) (4.8%), C18:1 oleate (3.6%), C24:1 (2.7%), C20:0 (2.2%), C:14 (2.0%), C20:3n3 (1.8%), and C18:3n3 (1.6%) which accounted for ~ 94% of all FAs detected (Table A.1). The most plentiful polyunsaturated fatty acid (PUFA) was docosahexaenoic acid (DHA; 22:6n-3), followed by eicosapentaenoic acid (EPA; 20:5n-3).

The percentage of DHA differed among preferred prey types (one-way ANOVA, df = 3, F = 14.77, $p < 0.001$) with copepods *Farranula* and *Corycaeus* having greater DHA% than the cladoceran *Evadne* (Tukey HSD, $p < 0.001$). The percentage of AA also differed among preferred prey (one-way ANOVA, df = 3, F = 4.234, p = 0.032). Specifically, AA % was found to be greater in *Evadne* than in male *Farranula*, but this result was only based on one *Evadne* sample and should be interpreted with caution (Tukey HSD, $p < 0.001$). (Figure 1.11d). Total FA (mg/g) and EPA % did not differ between preferred prey types (Fig 11a & c) (Table 1.8a).

Preferred zooplankton prey collected at station 64 (closest to shore) had higher total FA than prey collected at Station 63 (Tukey HSD, p = 0.033) (Figure 1.12a). An ANOVA indicated that there were significant differences between prey EPA% across stations, but this was not supported by a post-hoc test (Tukey's HSD, $p > 0.05$). The percent contribution of DHA, EPA, and AA to the total analyzed FAs was not significantly different for preferred prey across stations (Table 1.8a).

Total FA of larval sailfish muscle tissue also differed between stations (Table 1.8b) with higher FA concentrations found in the larva from station 64 (closest to shore) than in larvae from station 57 (Tukey HSD, p = 0.026). Total FA of sailfish muscle tissue did not differ among ontogenetic stages (Figure 1.13a; Table 1.8b), however the percentages of DHA, EPA and AA varied across larval sailfish developmental stages (one-way ANOVA; DHA%, df = 2, F = 4.999, p = 0.014; EPA%, df = 2, F = 3.428, p = 0.047; AA%, df = 2, F = 4.84, p = 0.024). Postflexion larvae had higher DHA% and EPA% than preflexion larvae (Tukey HSD; DHA%, p = 0.012; EPA%, p = 0.038)

(Figure 1.13b & 1.13c), and preflexion larvae had lower AA% than both flexion (Tukey HSD, p = 0.039) and postflexion larvae (Tukey HSD, p = 0.045) (Figure 1.13d).

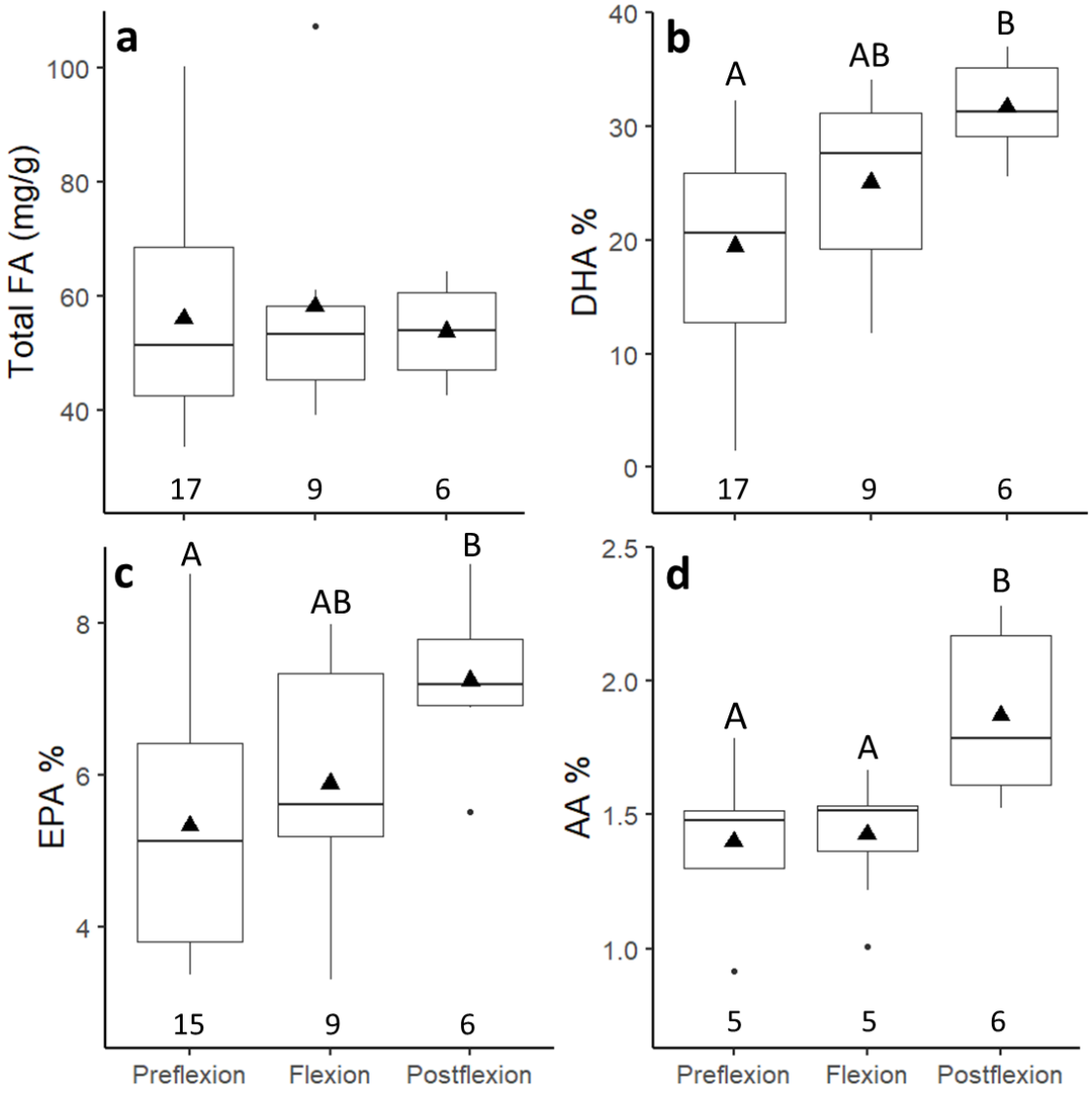

Figure 1.11 Total fatty acid concentration (**a**) (mg of fatty acid per gram of larval sailfish muscle tissue dry weight) by developmental stage. The percentage of DHA (**b**), EPA (**c**), and AA (**d**) from total analyzed fatty acids for each developmental class. The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

Table 1.8 Results of one-way ANOVAs for preferred zooplankton prey of sailfish larvae (a) and larval sailfish muscle tissue (b) with total fatty acid (FA) concentration (mg of fatty acid per gram of larval sailfish muscle tissue dry weight, DHA %, EPA %, and AA%. Significant results ($p < .05$) are bold.

a. Preferred zooplankton prey				**b**. Larval fish muscle tissue			
Total FA				**Total FA**			
Factor	*df*	*F*	*P*	Factor	*df*	*F*	*P*
Station	4	3.083	**0.033**	Station	4	2.729	**0.05**
Prey group	3	0.234	0.872	Developmental stage	2	0.053	0.948
DHA %				**DHA %**			
Factor	*df*	*F*	*P*	Factor	*df*	*F*	*P*
Station	4	0.866	0.497	Station	4	1.584	0.207
Prey group	3	14.77	**<0.001**	Developmental stage	2	4.999	**0.014**
EPA %				**EPA %**			
Factor	*df*	*F*	*P*	Factor	*df*	*F*	*P*
Station	4	2.957	**0.04**	Station	4	0.993	0.429
Prey group	3	2.205	0.111	Developmental stage	2	3.428	**0.047**
AA %				**AA %**			
Factor	*df*	*F*	*P*	Factor	*df*	*F*	*P*
Station	3	1.899	0.188	Station	4	1.139	0.381
Prey group	3	4.234	**0.032**	Developmental stage	2	4.84	**0.024**

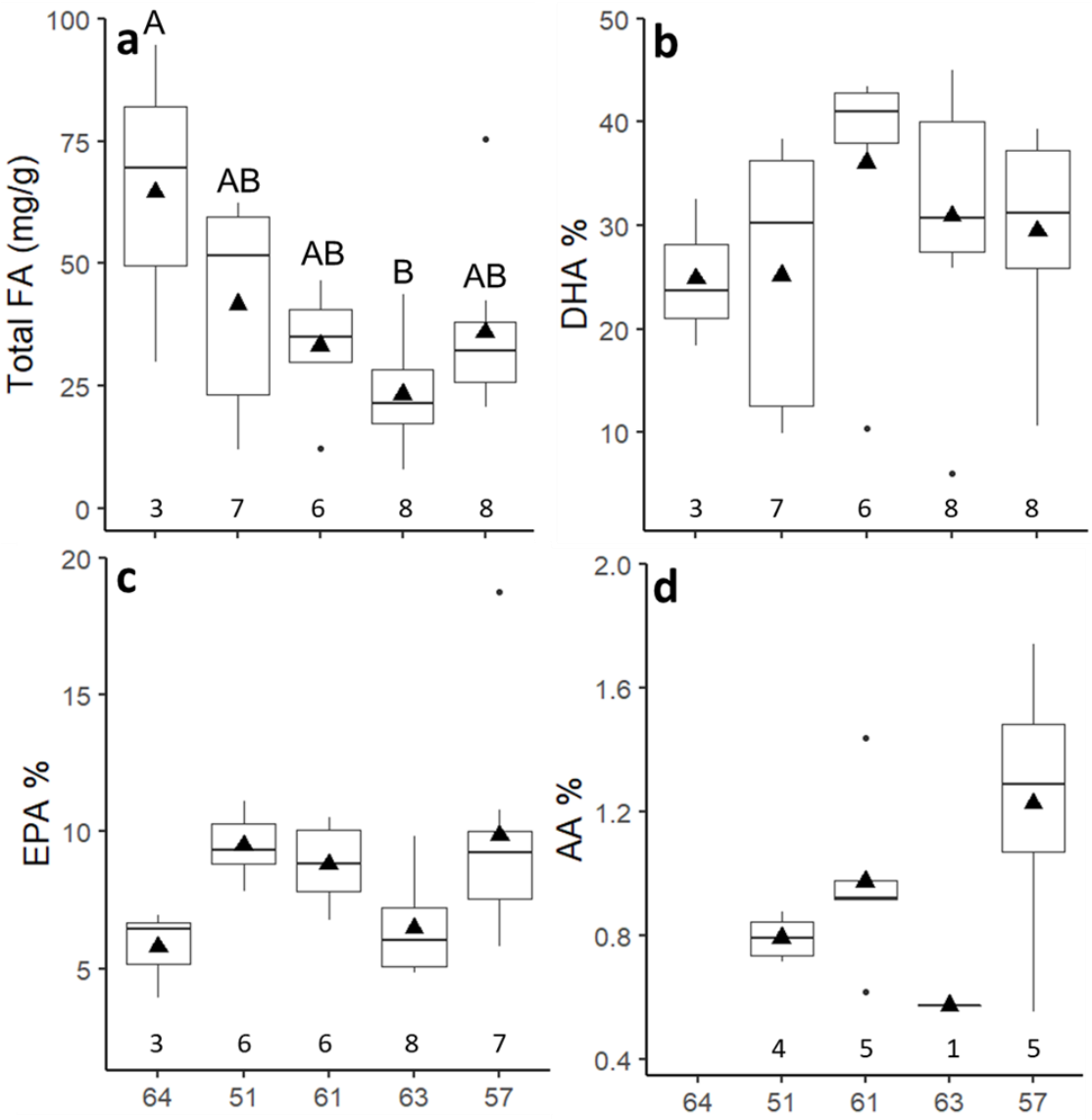

Figure 1.12 Total fatty acid (FA) concentrations (**a**) and the percentages of DHA (**b**), EPA (**c**), and AA (**d**) relative to total FA of the combined preferred zooplankton prey of sailfish larvae (*Corycaeus* spp., *Evadne* spp., female *Farranula* spp., and male *Farranula* spp.) pooled by station. The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot. Stations are arranged from left to right by proximity to shore.

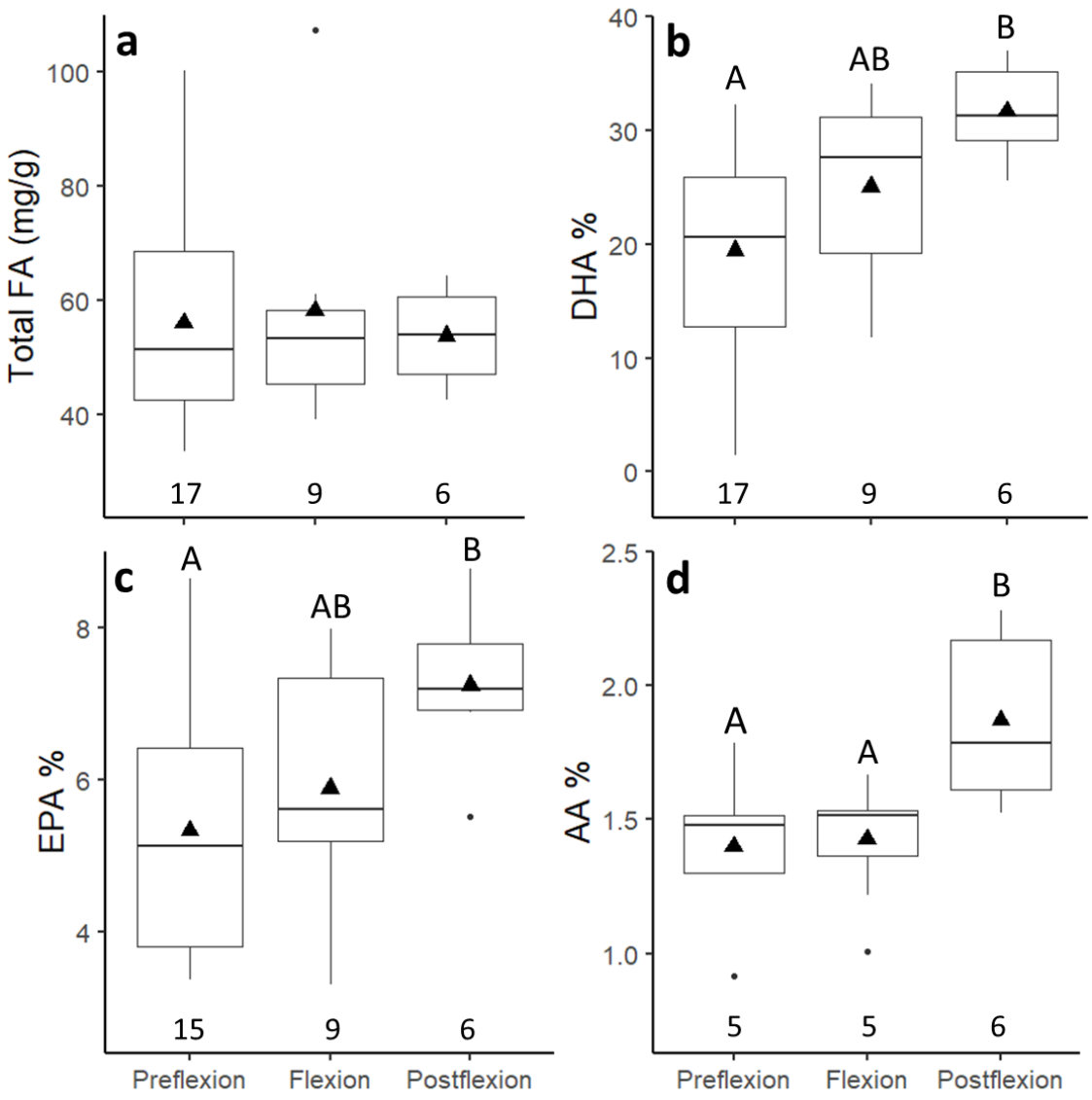

Figure 1.13 Total fatty acid concentration (**a**) (mg of fatty acid per gram of larval sailfish muscle tissue dry weight) by developmental stage. The percentage of DHA (**b**), EPA (**c**), and AA (**d**) from total analyzed fatty acids for each developmental class. The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

1.3.6 Otolith Microstructure Analysis

Linear regression analysis indicated larval sailfish standard length was positively related to otolith radius ($R^2 = 0.79$, $p < 0.0001$). Regressions of otolith radius-at-age residuals vs. standard length-at-age residuals were also significant ($R^2 = 0.21$, $p < 0.0001$). The daily instantaneous growth rate (*k*) was highest for sailfish larvae collected in 2018, however there was no significant difference in *k* for larvae collected in 2017 (Figure 1.14a), 2018 (Figure 1.14b), and 2019 (Figure 1.14c) (homogeneity of slopes test: $p > 0.05$).

The estimated age of larval sailfish in this study ranged from one to twelve days post-hatch. Most larvae (81.3 %) were captured when they were four to seven days post-hatch (Figure 1.15). Sailfish larvae collected in 2017 were significantly older (one-way ANOVA, df = 2, F= 7.177, p = 0.001) than larvae collected in 2018 (Tukey's HSD, p <0.001) or 2019 (Tukey's HSD, p = 0.007) (Figure 1.16). Larval age also differed with cruise (one-way ANOVA, df = 3, F= 10.79, $p < 0.001$) and month (one-way ANOVA, df = 1, F = 22.81, $p < 0.001$). Younger larvae were collected in late May/early June (grouped as May) during cruise 1 and 3 and older larvae were collected in July during cruises 2 and 4 (Figure 1.17). The width and variance of otolith increments trended upward with age (Figure 1.18a). When otolith increments were detrended, age was independent from the DG_{ij} ($r^2 = 0.001$, $p = 0.34$) (Figure 1.18b).

To examine the effects of gut content composition on recent growth, sailfish were sorted into groups based on percentage of prey type consumed. No significant changes in growth rate were detected when the percentages of *Farranula* (df = 2, F= 0.107, p = 0.745) or *Evadne* (df = 2, F = 0.234, p = 0.792) found in sailfish guts differed. However,

when percentages of consumed *Farranula* were analyzed separately by sex they exhibited opposite patterns with respect to recent growth rate. When mean DRG was compared to the proportions of male *Farranula* found in gut, sailfish larvae that consumed < 33.3% male *Farranula* had the highest average mean DRG (Figure 1.19a). Conversely, mean DRG was highest when sailfish consumed > 66.6% female *Farranula* (Figure 1.19b). While these trends were not significant at alpha = 0.05, a trend toward significantly higher growth was detected sailfish with > 66.6% female *Farranula* in their guts (one-way ANOVA, df = 1, F = 2.876, p = 0.093).

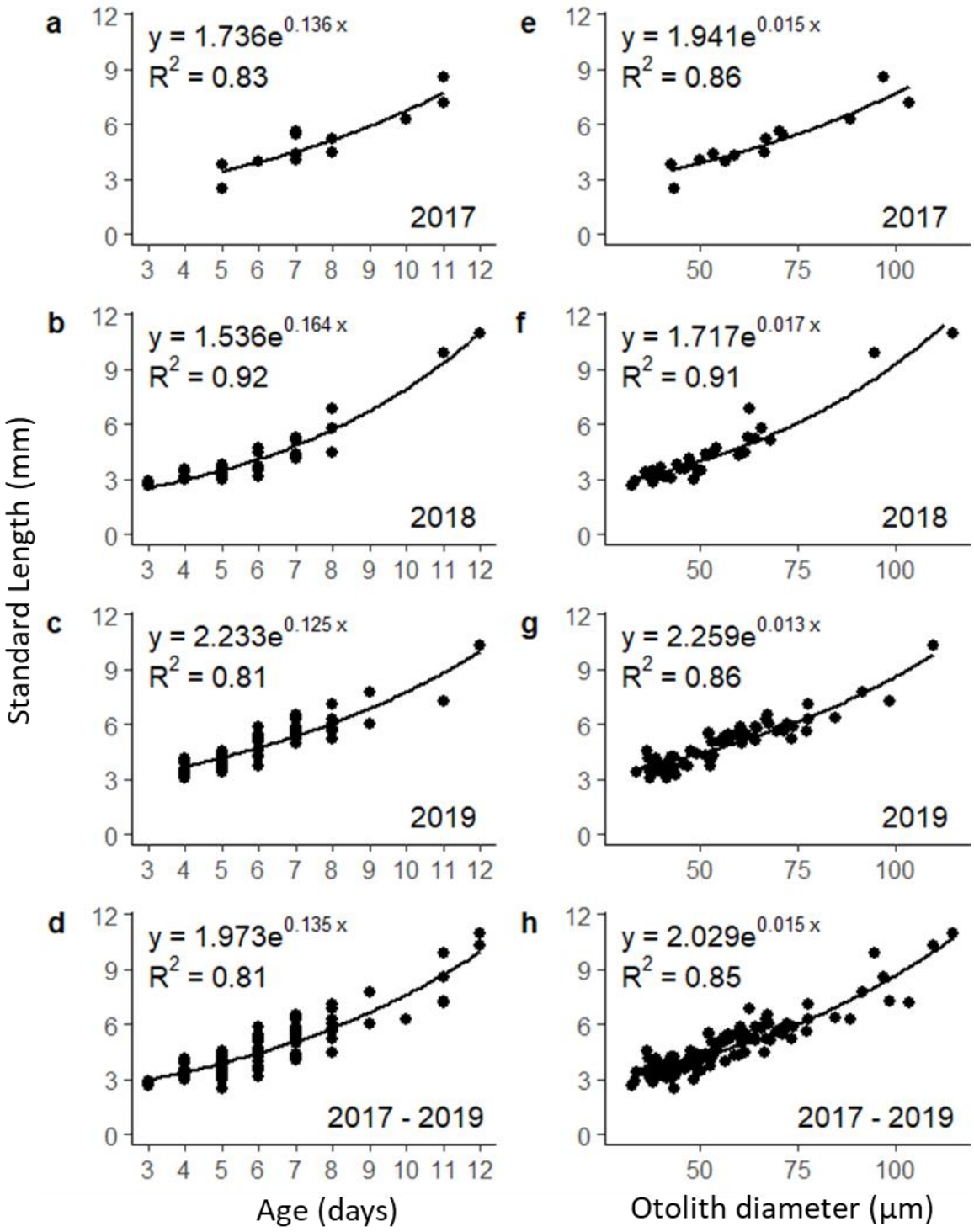

Figure 1.14 Relationships between standard length (mm) and estimated age (days) and standard length (mm) and otolith diameter (µm) for larval sailfish collected in surface waters of the northern GoM in 2017 (**a, e**), 2018 (**b, f**), 2019 (**c, g**), and pooled for all three years (**d, h**). Data were fit with exponential regression models.

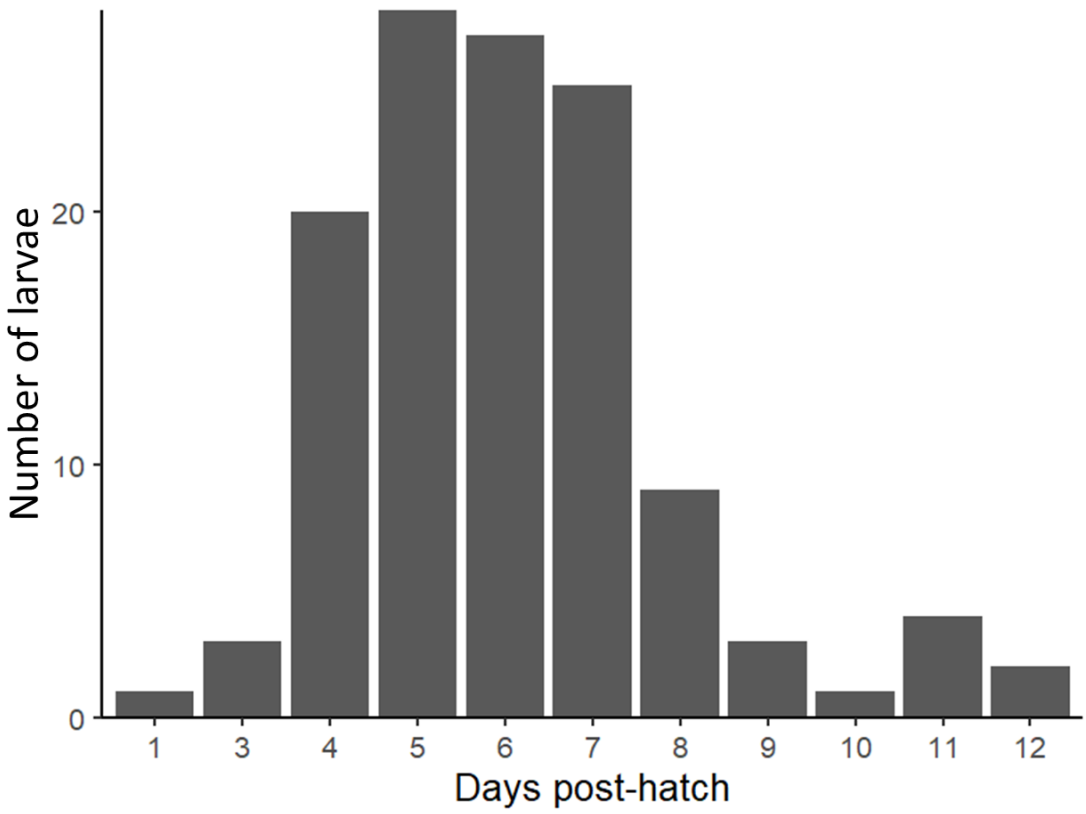

Figure 1.15 Age distribution of larval sailfish (n = 123) collected during four research cruises in the northern Gulf of Mexico (2017-2019).

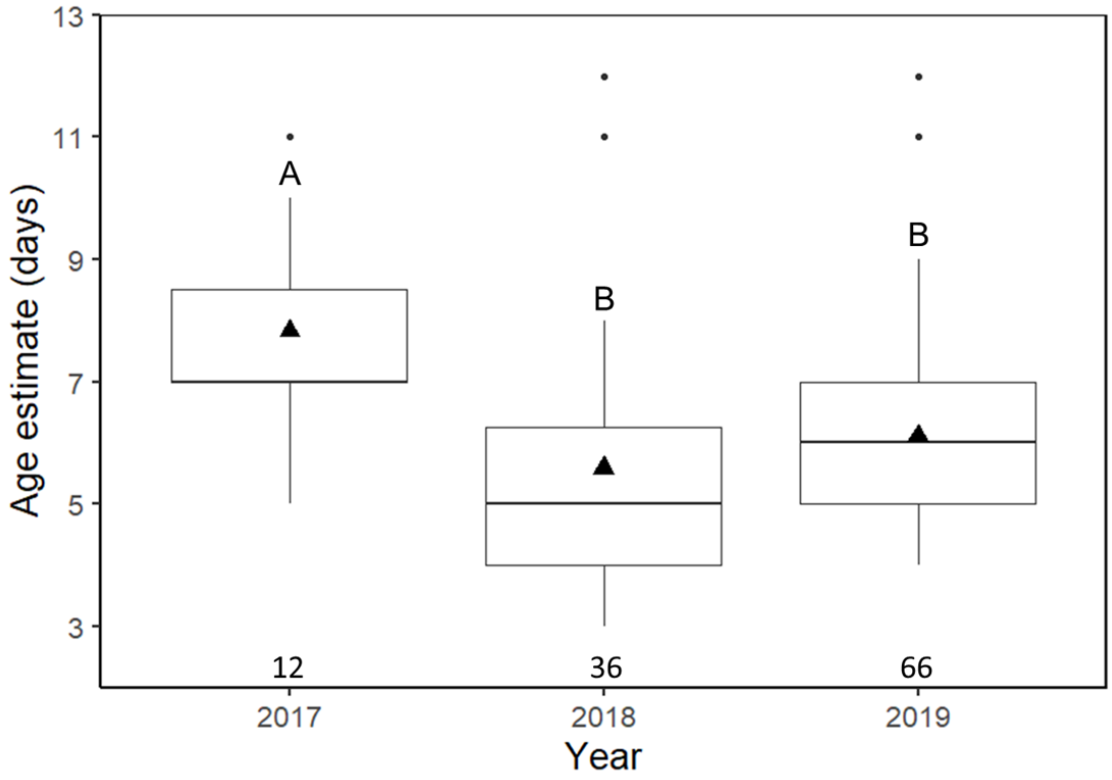

Figure 1.16 Age estimates of sailfish larvae by year. The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

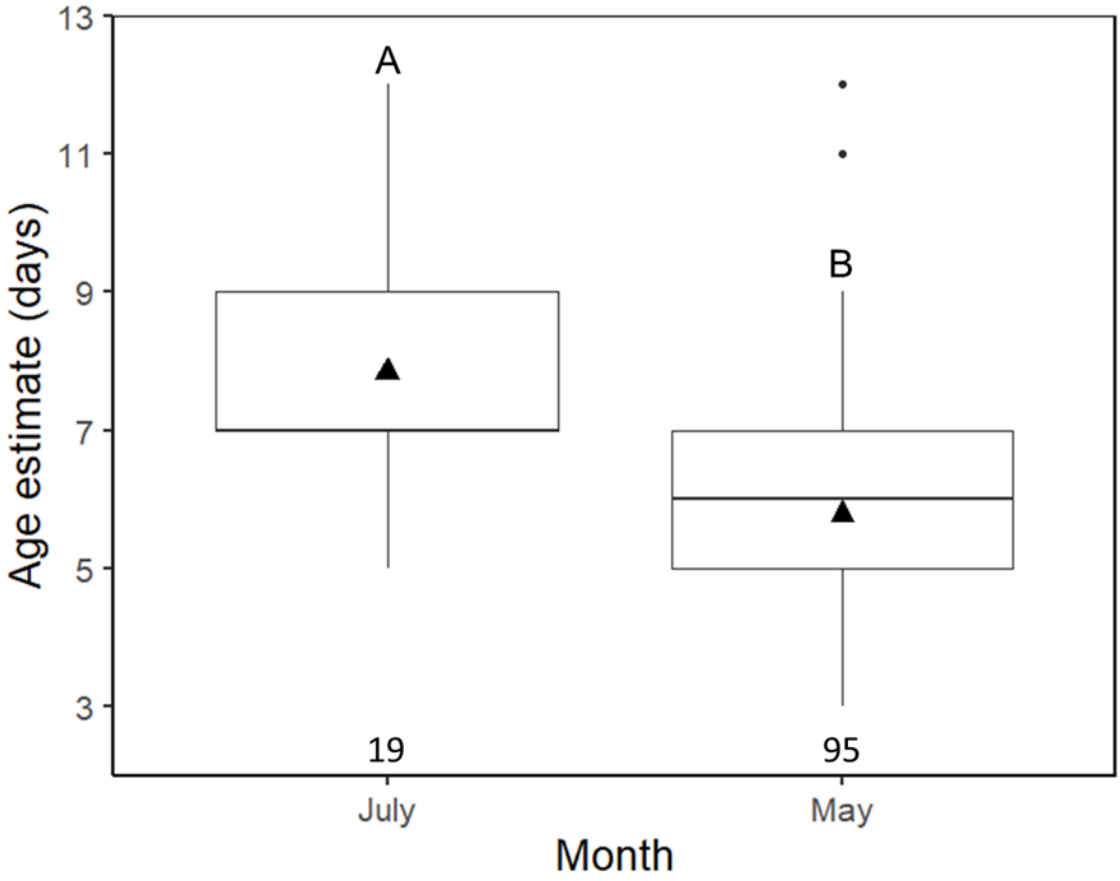

Figure 1.17 Age estimates of sailfish larvae by month. The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters indicate significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

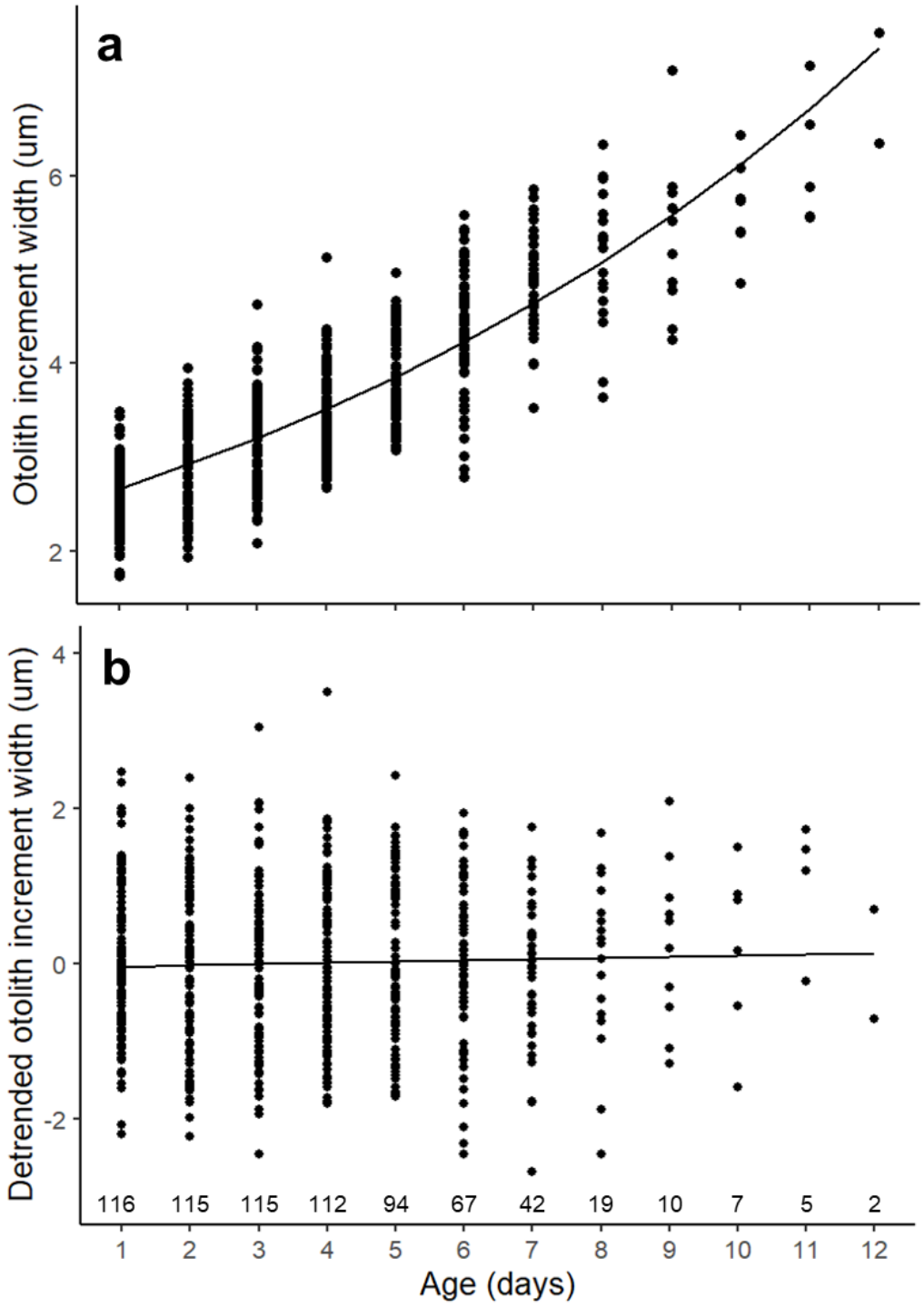

Figure 1.18 Scatterplot of otolith increment widths (**a**) and detrended otolith increment widths (**b**) with estimated age. Exponential regression equation: increment width = $2.432e^{0.092(Age)}$ ($r^2 = 0.73$, $p > 0.0001$, $n = 123$). No significant relationship was found between detrended otolith increment width and age ($r^2 = 0.001$, $p = 0.34$, $n = 123$). Sample sizes for each increment are indicated at the bottom of each plot.

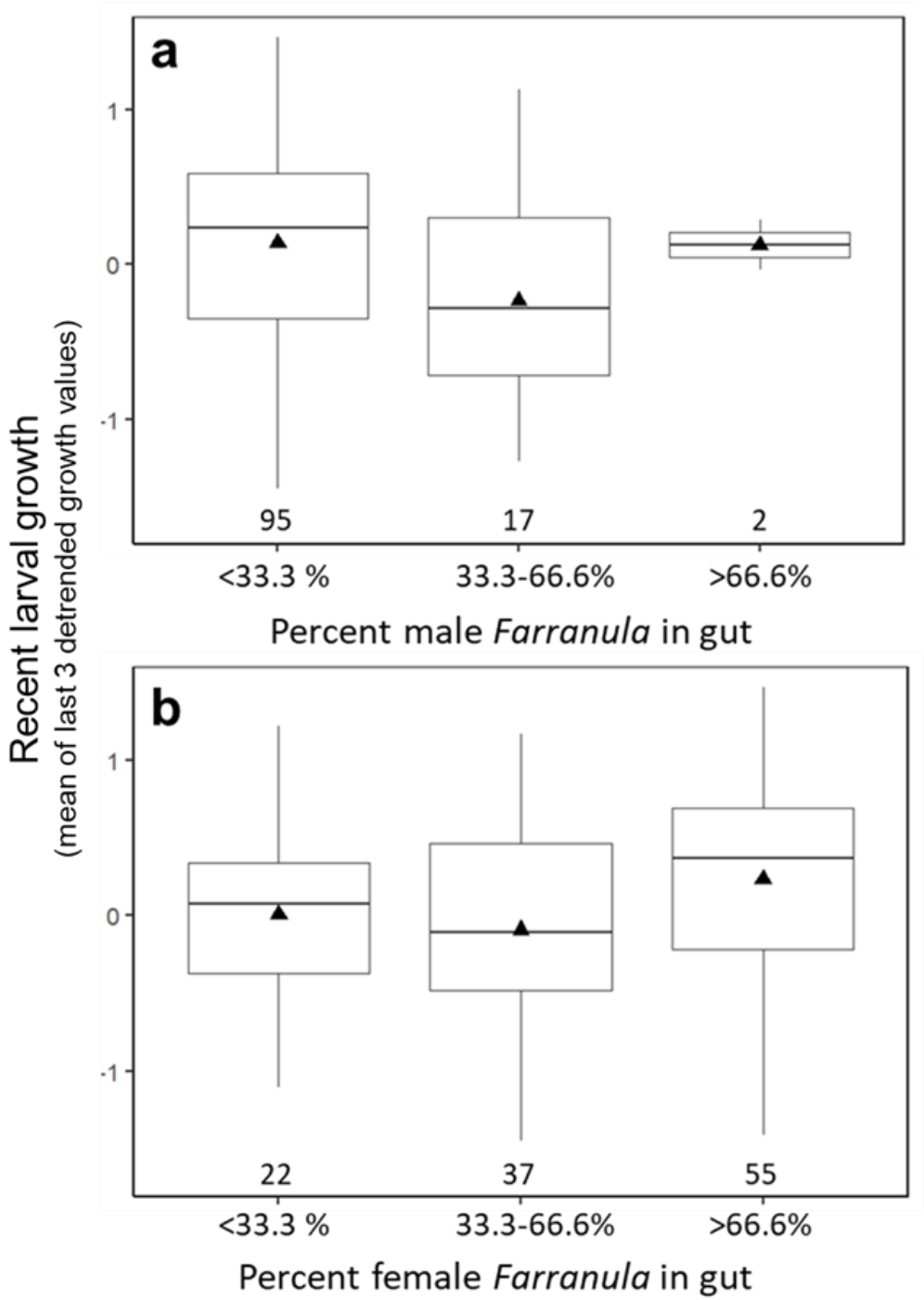

Figure 1.19 Mean DRG of sailfish larvae according to percentage of prey consumed for (**a**) male *Farranula* copepods and (**b**) female *Farranula* copepods. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and sample sizes are indicated at the bottom of each plot.

1.3.7 Multi-way ANOVA

Using data from all four cruises (2017-2019), multiple one-way ANOVAs were conducted using mean DRG as the dependent variable and a suite of dietary, environmental, and spatial predictor variables indicated significant effects related to number of prey in larval guts (diet variable), distance from shore (spatial variable), and type of mesoscale circulation feature (spatial variable) (Table 1.9a). The significant spatial variables had VIFs > 4, indicating collinearity. AIC indicated that mesoscale circulation feature (F = 4.159, AIC = 243.689) improved model fit over distance from shore (F = 3.231, AIC = 251.964), therefore distance from shore was eliminated from the final model. All two-way interactions were insignificant and therefore removed from the final model. The resulting multi-way ANOVA model included total prey in larval guts and mesoscale circulation feature as significant main effects with VIFs < 4 (Table 1.9b) and had a F-statistic of 6.929 on 3 and 108 DF and a p-value of <0.001. Mean DRG was positively correlated with the total number of prey in gut, although the relationship was relatively weak (Figure 1.20). Higher mean DGR was observed in in anticyclonic boundary regions relative to common water (Tukey's HSD, p = 0.036) or anticyclonic regions (Tukey's HSD, p = 0.002). Mean DRG was also higher for larvae captured in common water than it was for larvae from anticyclonic regions (Tukey's HSD, p = 0.029) (Figure 1.21).

Using data from the 2019 cruise only, a second multi-way ANOVA model using mean DRG as the dependent variable and a suite of prey availability, dietary, environmental, and spatial predictor variables indicated potentially significant effects related to nine different variables (Table 1.10a). Significant prey availability variables

(total zooplankton, preferred prey CPUE, male *Farranula* CPUE and female *Farranula* CPUE) were strongly correlated (range of Pearson's r = 0.89 - 0.99, p <0.001); therefore, preferred prey CPUE was selected to represent the "prey availability" category of predictor variables since it had the greatest impact on DRG (Table 1.10a). Within the significant environmental variables, surface chl *a* was significantly correlated with DO (Pearson's r = 0.6, p <0.001) and temperature (Pearson's r = -0.822, p <0.001); therefore, surface chl *a* was removed from the final model. Among the significant (and colinear) spatial variables, mesoscale circulation feature was selected since it had the lowest p-value (one-way ANOVA, df = 3, F = 15.53, p = 3.28e-06, Table 1.10a). All two-way interactions between the four selected predictor variables were not significant, so they were removed from the final model. The main effects of preferred prey CPUE and temperature were also not significant, and they were removed from the final model. The resulting multi-way ANOVA model included mesoscale circulation feature and DO as significant main effects for 2019 data with VIFs < 4 (Table 1.10b) and had a F-statistic of 13.4 on 3 and 62 DF and a p-value of < 0.001. Mean DRG was significantly lower for larvae captured in waters with relatively low DO (2.5 – 3.5 mg/L) than it was for larvae captured in waters with higher DO (3.5 – 4.5 mg/L, Tukey's HSD, p = 0.004; 4.5 – 5.5 mg/L, Tukey's HSD, p = 0.005; Figure 1.22). The 2019 DRG trends relative to mesoscale features were the same as those reported above for the entire data set (2017 - 2019), with higher mean DRG in anticyclonic boundary regions than either anticyclonic regions (Tukey's HSD, p <0.001) or common waters (Tukey's HSD, p <0.001) and higher mean DRG in common waters than in anticyclonic regions (Tukey's HSD, p = 0.018) (Figure 1.23).

Using data from the 2019 cruise only, a third multi-way ANOVA model using detrended recent growth (DRG) as the dependent variable and a suite of fatty acid variables for zooplankton prey and larval sailfish indicated potentially significant effects related to ten different zooplankton fatty acid variables (Table 1.11a). Significant zooplankton prey FA predictor variables were strongly correlated with each other, and with mesoscale circulation feature since they were all collected at the tow or station level rather than the individual fish level and VIFs were > 4 when they were used in a model. When mesoscale circulation feature and total FA of sailfish were used in a multi-way ANOVA, the main effect of sailfish total FA became insignificant. Therefore, a multi-way ANOVA was not able to explain the effects of FA variables of larval sailfish mean DRG.

Table 1.9 Results of one-way ANOVAs for environmental, diet, and spatial predictor variables with mean DRG for sailfish larvae collected 2017- 2019 (**a**). Main effects of a multi-way ANOVA examining relationships with DRG (**b**). Prey items include female *Farranula* (♀*Farr.*), male *Farranula* (♂*Farr.*), *Corycaeus* (*Cory.)* and *Evadne* (*Evadne).* Significant results ($p < .05$) are bold.

Predictor Variable	**n**	***df***	***F***	***P***
a. Environmental				
Dissolved oxygen	112	1	1.721	0.192
Temperature	112	1	0.042	0.838
Salinity	112	1	0.773	0.381
Surface chlorophyll	112	1	2.822	0.096
Diet				
Total prey in gut	112	1	4.321	**0.040**
RelGCW	92	1	0.007	0.932
Proportion ♀ *Farr.* in gut	112	1	2.98	0.087
Proportion ♂*Farr.* in gut	112	1	1.21	0.274
Proportion *Cory.* In gut	112	1	2.72	0.102
Proportion *Evadne* in gut	112	1	0.296	0.588
Spatial				
Distance from shore (km)	112	1	7.751	**0.006**
Depth (m)	112	1	0.666	0.416
Mesoscale circulation feature	112	2	8.013	**0.0006**
b. Final Model				
Total prey in gut	112	1	4.278	**0.041**
Mesoscale circulation feature	112	2	7.959	**0.0006**

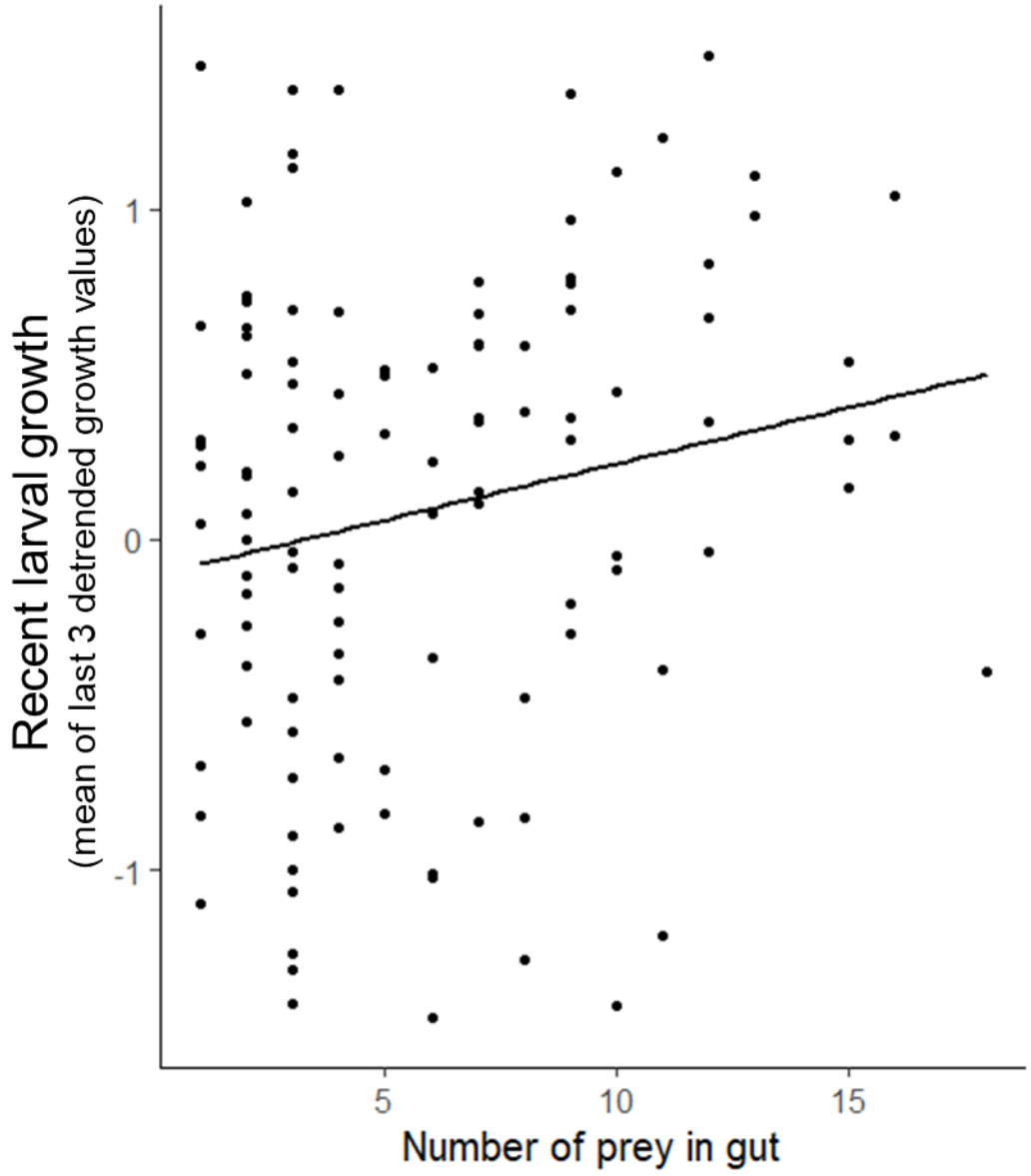

Figure 1.20 Mean DRG of 2017 – 2019 sailfish larvae relative to the number of prey observed in larval guts (n = 112).

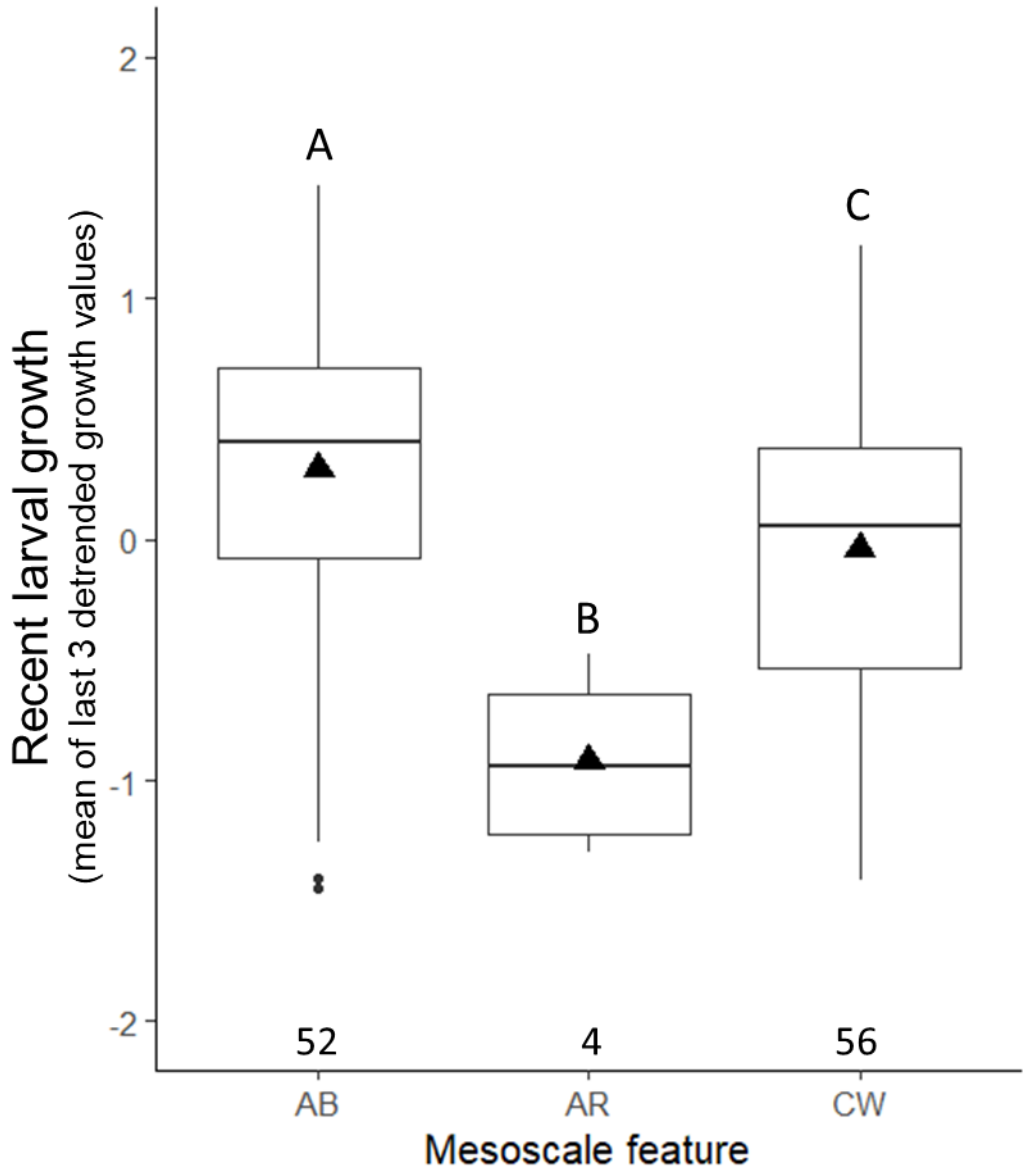

Figure 1.21 Mean DRG of 2017 – 2019 sailfish larvae and mesoscale circulation feature including anticyclonic boundary region (AB), anticyclonic region (AR), and common water (CW). The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters signify significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

Table 1.10 Results of one-way ANOVAs for prey availability, environmental, diet, and spatial predictor variables with mean DRG for sailfish larvae captured in 2019 (**a**). Main effects of a multi-way ANOVA examining relationships with mean DRG (**b**). Prey items include female *Farranula* (♀*Farr.*), male *Farranula* (♂*Farr.*), *Corycaeus* (*Cory.)* and *Evadne* (*Evadne).* Significant results ($p < .05$) are bold.

Predictor Variable	**n**	***df***	***F***	***P***
a. Prey Availability				
Total zooplankton CPUE	66	1	9.704	**0.003**
Preferred Prey CPUE	66	1	15.65	**0.0002**
♀ *Farr.* CPUE	66	1	13.06	**0.0006**
♂*Farr* CPUE	66	1	15.04	**0.0003**
Cory. CPUE	66	1	2.104	0.152
Evadne CPUE	66	1	0.535	0.467
Environmental				
Dissolved oxygen	66	1	7.674	**0.007**
Temperature	66	1	7.081	**0.009**
Salinity	66	1	0.925	0.34
Surface chlorophyll (mg/m^3)	66	1	7.395	**0.008**
Diet				
Total prey in gut	66	1	3.892	0.053
RelGCW	54	1	0.267	0.608
Proportion ♀ *Farr.* in gut	66	1	3.802	0.056
Proportion ♂*Farr.* in gut	66	1	0.8	0.374
Proportion *Cory.* in gut	66	1	3.202	0.078
Proportion *Evadne* in gut	66	1	2.607	0.111
Spatial				
Distance from shore (km)	66	1	9.06	**0.004**
Depth (m)	66	1	1.095	0.299
Mesoscale circulation feature	66	2	15.53	**3.28e-06**
b. Final Model				
Mesoscale circulation feature	66	2	14.625	**6.267e-06**
Dissolved oxygen	66	1	6.439	**0.014**

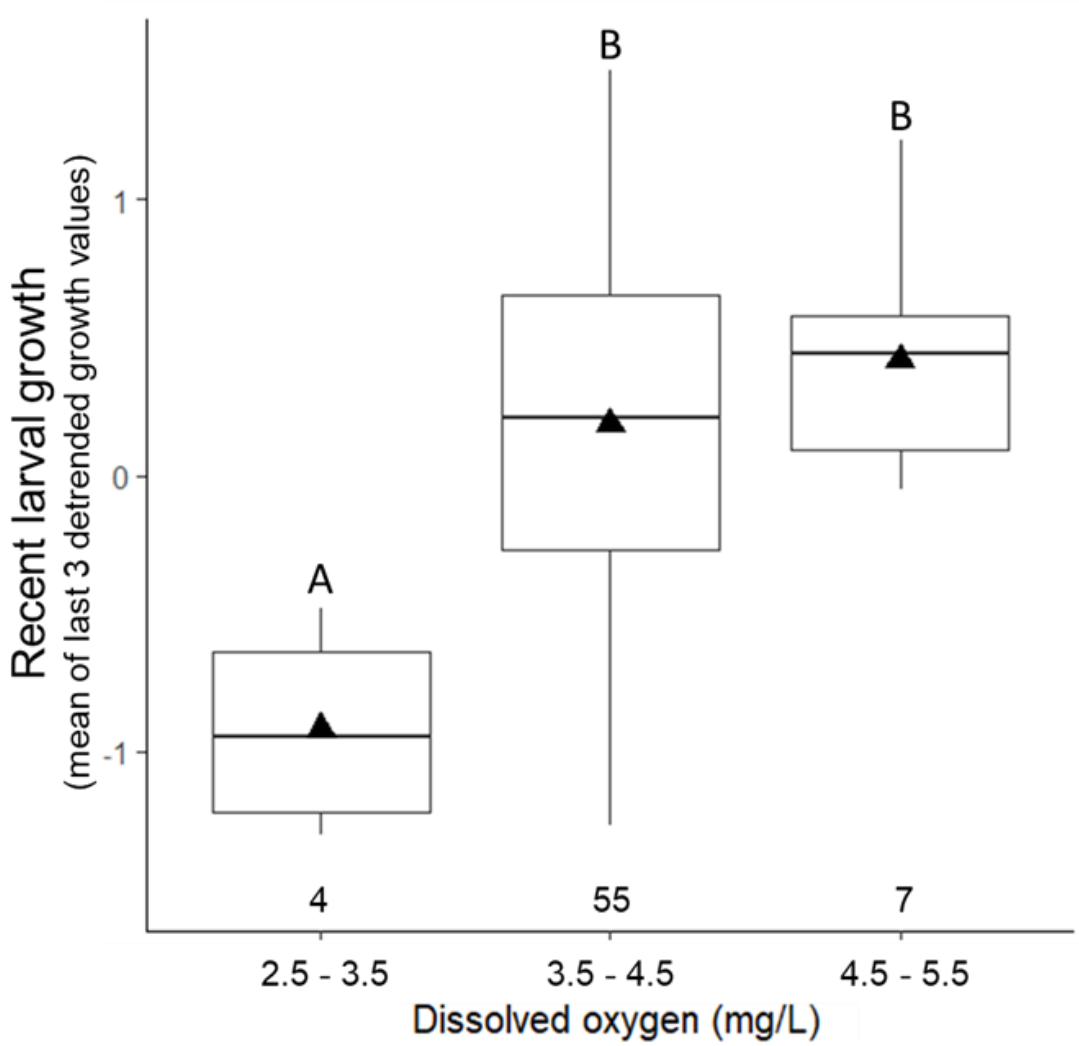

Figure 1.22 Mean DRG of 2019 sailfish larvae and dissolved oxygen concentration (mg/L). The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters signify significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

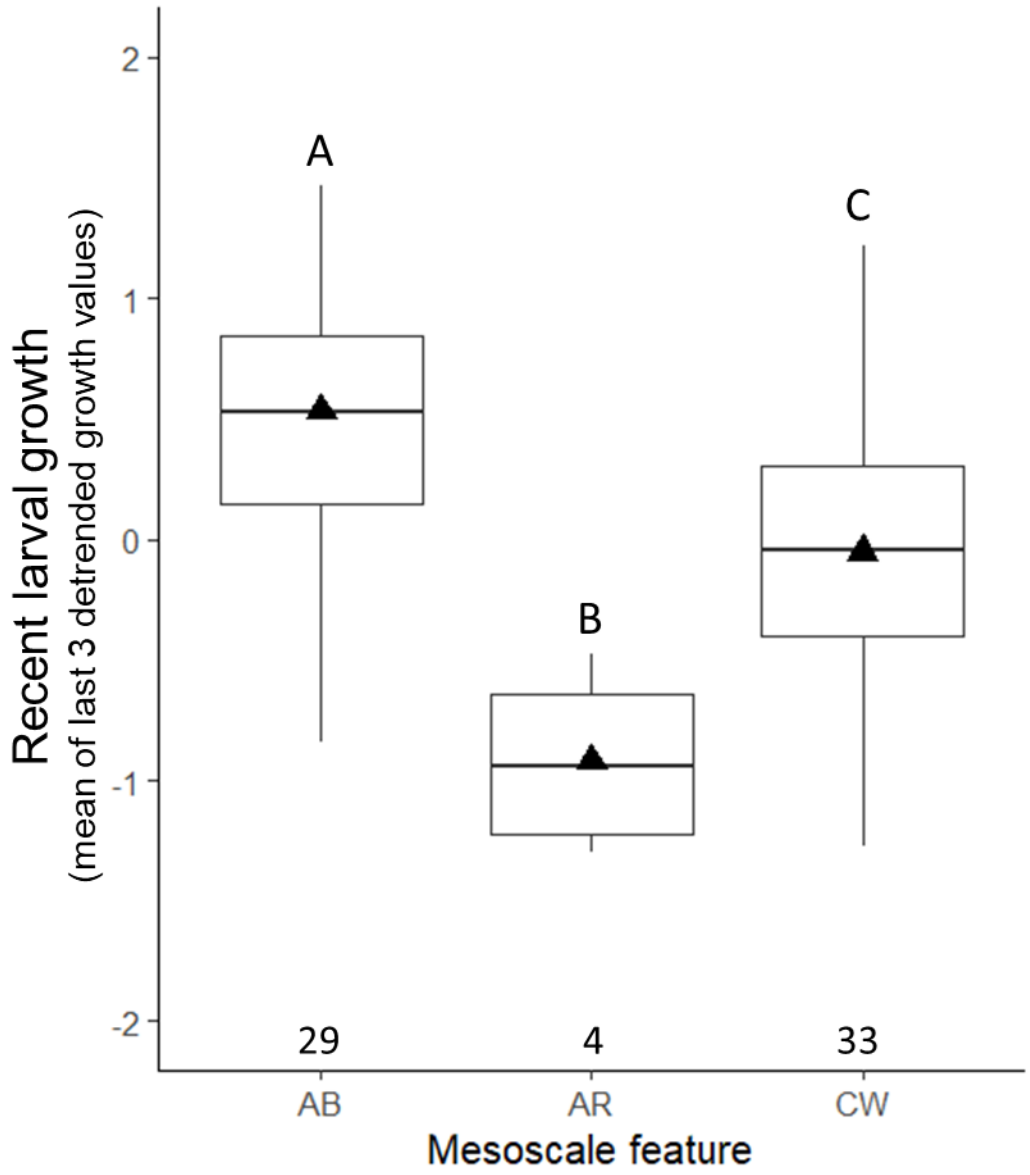

Figure 1.23 Mean DRG of 2019 sailfish larvae and mesoscale circulation feature including anticyclonic boundary region (AB), anticyclonic region (AR), and common water (CW). The bold line within each box indicates the sample median. The upper and lower bounds of each box represent the first and third quartile respectively. Triangles represent the means for each group and letters signify significant differences between groups as indicated by Tukey's HSD post hoc test. Sample sizes are indicated at the bottom of each plot.

Table 1.11 Results of one-way ANOVAs for FA predictor variables with mean DRG for sailfish larvae. Main effects of a multi-way ANOVA examining relationships with DRG. Prey items include female *Farranula* (♀Farr.), male *Farranula* (♂Farr.), *Corycaeus* (Cory.) and *Evadne* (Evadne). Significant results ($p < .05$) are bold.

Predictor Variable	n	df	F	p
Prey FA data				
♀ *Farr*. total FA	63	1	8.66	**0.005**
♂*Farr.* total FA	55	1	11.85	**0.001**
Cory. total FA	63	1	0.011	0.916
Evadne total FA	35	1	0.229	0.635
♀ *Farr* DHA %	63	1	32.58	**3.45e-07**
♂*Farr.* DHA %	55	1	9.61	**0.003**
Cory. DHA %	63	1	4.37	**0.041**
Evadne DHA %	35	1	0.462	0.501
♀ *Farr* EPA %	63	1	11.81	**0.001**
♂*Farr.* EPA %	55	1	16.37	**0.0002**
Cory. EPA %	63	1	32.94	**6.21e-08**
Evadne EPA %	35	1	0.469	0.498
♀ *Farr.* AA %	63	1	0.012	0.914
♂*Farr.* AA %	49	1	20.1	**4.56e-05**
Cory. AA %	60	1	20.74	**2.69e-05**
Fish FA data				
Sailfish total FA	34	1	4.638	**0.039**
Sailfish DHA %	28	1	4.094	0.053
Sailfish EPA %	26	1	2.444	0.131
Sailfish AA %	15	1	2.86	0.115

1.4 Discussion

In early life stages, fishes experience mortality rates that approach 100%, and numerous abiotic and biotic factors have been proposed as determinants of larval fish survival. Resolving the relative contributions of these factors *in situ* is challenging, however, as the local oceanographic and biological conditions encountered by larvae during the pelagic phase vary temporally and spatially across a range of scales. In this study, my research focused on the variability in larval sailfish feeding environments,

which has been shown to impact larval growth rates (and ultimately, survival); my research also includes the first assessment of essential fatty acid content in a larval fish and its zooplankton prey in the GoM. Among the major findings, my results suggest that variation in larval sailfish abundance and growth is related to meso-scale features, with higher abundances and growth rates observed in frontal areas associated with anticyclonic boundary regions. As in previous studies, the diets of larval sailfish were found to be highly specific, with a preference for relatively few zooplankton taxa (*Farranula*, *Corycaeus*, and *Evadne*); however, my findings suggest even greater feeding specificity based on sex-based size differences in dominant prey, with larval sailfish preferring larger, female *Farranula* over male *Farranula* and other taxa. In addition, I found that essential fatty acid content varied among preferred zooplankton prey, and that the corycaeid copepods *Farranula* and *Corycaeus* had significantly higher concentrations (relative to *Evadne*) of at least one EFA (DHA) known to enhance larval fish growth. Collectively, these and other findings provide new insights into larval sailfish trophic ecology and planktonic food webs in the seldom-studied, oceanic waters of the GoM.

1.4.1 Abundance and Distribution

Several studies have previously reported on the abundances and distribution of sailfish larvae in the Gulf of Mexico (e.g., Tidwell et al. 2008; Simms et al. 2010; Rooker et al. 2012). Notably, these studies were based on larger sampling efforts that were conducted along standardized survey lines. In contrast, station selection in my study was not determined *a priori*, but based on the distribution of nearby (within 1 km) *Sargassum* habitats (the primary goal of the research cruises). Despite these differences, the mean overall abundance (2.6 larvae/1000 m^2), mean range in abundance per cruise (1 - 5.5

larvae/1000 m^2), and maximum abundance recorded in a single net tow (27.5 larvae/1000 m^2) of sailfish larvae in my study were of the same order of magnitude as reported in the larger surveys. For example, Simms et al. (2010) reported a mean overall larval abundance of 1.5 larvae/1000 m^2, a mean range of 0.6 - 2.1 larvae/1000m^2, and a maximum abundance of 51.4 larvae/1000 m^2 during their offshore transect surveys in 2005-2006. In 2006-2008, Rooker et al. (2012) surveyed the same transect region and reported a mean overall larval abundance of 0.96 larvae/1000 m^2, a mean range of 0.37 - 1.99 larvae/1000m^2, and a maximum abundance of 22.09 larvae/1000 m^2. Overall percent frequency of occurrence of sailfish larvae was similar in my study (44%) relative to previous studies in the GoM (45%, Simms et al. 2010; 37.5%, Rooker et al. 2012), and to previous studies in the Florida Straits for all billfish larvae (41.1 %, Luthy 2004).

Variations in larval abundances and frequency of occurrence among studies is expected due to a variety of reasons, including temporal patterns in adult spawning behaviors and biases related to sampling distribution and effort. For example, my samples were collected over a relatively short portion of the spawning period (May – July), and likely prior to peak spawning in the GoM for sailfish (de Sylva & Breder 1997, Richardson 2007), in contrast to the larger sampling efforts in the GoM, which included May – September (Simms et al. 2010). Also, my study encompassed 28 sampling stations, which is far fewer in number than the transect survey studies (e.g., 288 sampling stations; Simms et al. 2010). Overall, the environmental conditions encountered across the 28 stations had relatively low variability, so my assessment of the effects of environmental variability on the abundance and distribution of sailfish larvae was limited. A PCA indicated that sailfish CPUE was positively correlated with salinity and distance from

shore, but negatively correlated with surface chl *a* concentration. Similarly, using a generalized additive modeling approach, Rooker et al. (2012) reported higher larval densities at stations with higher salinity values, relatively low-moderate sea surface temperature, and either low or high *Sargassum* biomass association. Combined, the findings of both studies indicate that higher densities of larval sailfish are found in offshore waters and not associated with coastal waters characterized by low salinity and high chl *a* concentrations.

A major determinant of larval sailfish abundance and distribution was the presence of mesoscale oceanographic features. Specifically, larval sailfish abundance was highest in anticyclonic boundary regions. This observation supports the previous findings from the region of elevated sailfish density in the Loop Current boundary (Rooker et al. 2012) and frontal regions in the Florida Straits (Llopiz & Cowen 2008). Previous studies on tunas in the GoM also found similar patterns of distribution and abundance where larval densities were highest in anticyclonic boundary regions (Lindo-atichati et al. 2012). Physical and biological processes may work in concert to support higher densities of sailfish larvae in frontal zones. These processes include targeted spawning into frontal regions by adult sailfish (Richardson et al. 2009b), physical concentration and/or retention of young larvae and planktonic prey due to hydrodynamic convergence in these zones, and the subsequent higher survival probabilities due to elevated prey availability (Bakun 2006). It has been previously hypothesized that anticyclonic eddies set up an ocean triad sequence once they drift from their original formation location, where an initial period of enrichment is followed by horizontal particle convergence and concentration which then retains the initial products of enrichment within the eddy along with any fish larvae that are spawned within the region (Bakun 2006). It is therefore likely that sailfish and other offshore, pelagic species that spawn in oligotrophic waters seek out these enriched habitats to enhance larval survivorship.

1.4.2 Diet

Literature reviews on larval fish feeding ecology have documented several latitudinal trends as they relate to feeding success, diet breadth, diet shifts, and other trophic variables. In general, compared to higher latitude larvae, larvae from lower latitudes feed on more diverse taxa, have higher feeding incidences, have a narrower niche breadth, have more consistent prey preference and diet composition across season, are more likely to be piscivorous, and are less likely to exhibit ontogenetic diet shifts (Llopiz 2013, Robert et al. 2014). As reported in previous studies of larval sailfish, I found that diets were relatively narrow consisting almost entirely of *Farranula* spp., *Corycaeus* spp., and *Evadne* spp. in early stages until they began incorporating larger prey (larval fish, calanoid copepods) into their diets after flexion of the notochord (Llopiz & Cowen 2008, Tidwell 2008). Interestingly, higher percentages of corycaeid copepods in larval sailfish guts have been reported for the GoM (76.9 %N, this study; 71.1 %N; Tidwell 2008) than for the Florida Straits (%N = 39.6; Llopiz & Cowen 2008), where diets were numerically dominated by *Evadne* cladocerans (%N = 53.3). This regional difference in diet may reflect lower concentrations of *Evadne* in the GoM since they were relatively rare in our analysis of prey field availability. Llopiz and Cowen (2008) reported that the trend for higher consumption of *Evadne* by sailfish larvae was not consistent across their sampling transect, and diets consisted of a greater number of corycaeid copepods in the western end of their transect (closer in proximity to the GoM).

Feeding incidence in my study was 100%, which is in agreement with previous studies that reported relatively high values (79%, Tidwell 2008; 94%, Llopiz & Cowen 2008). Feeding incidence was likely higher in my study than previous studies because all but one sample was collected close to sunset (after 80% of the daylight had elapsed), which is the daily peak feeding time for young billfish larvae (Llopiz & Cowen 2008). Sailfish gut

fullness exhibited the same pattern shown previously in the Florida Straits in relation to time of day and larval length. Relative gut fullness was low in small larvae, peaked at the 4 – 5 mm size class just before notochord flexion, and decreased for larger, zooplanktivorous larvae (this study; Llopiz & Cowen 2008). Similar to the observations of Llopiz & Cowen (2008), relative gut fullness remained low throughout the day; gut fullness peaked just after sunset in my study, just before sunset for larvae in the Florida Straits. Sailfish larvae exemplify many of the traits that are typically observed in low-mid latitude larvae including high feeding success, a relatively narrow diet, consistent prey preference, and piscivory.

1.4.3 Prey Selectivity

Because sailfish larvae reside in oligotrophic oceanic waters with relatively low prey densities, they should have a generalist foraging strategy according to optimal foraging theory (Pyke 1984). However, sailfish larvae are highly selective and consume very few prey types despite residing in relatively scarce but diverse prey environments. Of the preferred prey of sailfish larvae, *Evadne* were consistently the rarest in the environment, but were always selected for when available. *Evadne* are commonly selected for by larval fish (Llopiz & Cowen 2008, Robert et al. 2009, Shiroza et al. 2021) including sailfish larvae from the Florida Straits (Llopiz & Cowen 2008). In their study, Llopiz & Cowen (2008) acknowledged that selection for the smaller *Evadne* runs counter to optimal foraging theory, and proposed that *Evadne* may not actually be selected for over *Farranula* since *Farranula* was included in larval billfish diets over a broad range of abundances relative to *Evadne.* The authors further suggested that the discrepancies between consumed and ambient proportions of each prey type may be due to other factors, such as low prey volumes encountered by larvae, higher capture success or

detection of *Evadne* over *Farranula*, or congregations of *Evadne* at the air-sea interface. This suggestion is supported by Shiroza et al. (2021) who reported that larval bluefin tuna also showed intense selection for *Evadne* even when environmental proportions were low and theorized that the explanation may be due to lower escape abilities of *Evadne* compared to other prey.

While Llopiz and Cowen (2008), did not look at sex specific selection of *Farranula* or selection for *Corycaeus*, they did observe exclusively negative selection for *Farranula*. Conversely, larvae from our study showed significant selection for female *Farranula* in plankton tows where larval densities were highest; sailfish exhibited diametrically opposing sex specific selection for *Farranula* in three of the four plankton tows where significant selection was detected in relation to *Farranula*. This pattern did not hold up in the fourth tow (D9) because *Evadne* made up > 40 % of gut contents for sailfish in this tow despite being extremely rare in the environment and representing only 0.1% of the available preferred prey. This extreme selection for *Evadne* obscured any sex specific selection patterns regarding *Farranula*. The preference for larger *Farranula* females over males is in line with expectations of optimal foraging theory, supporting our observations of sex-specific selection.

Preferred zooplankton prey of sailfish larvae share two common attributes that may make them desirable prey; low activity and high visibility. Weak swimming abilities or low general activity may reduce the likelihood of zooplankton prey to escape predation attempts. Cyclopoid copepods exhibit relatively long periods of inactivity and have lower overall activity than calanoid copepods (Buskey et al. 1993) and *Evadne* are regarded as slow and weak swimmers compared to copepods (Bainbridge 1958). High activity has

typically been regarded as an attribute that would increase selectivity of zooplankton prey since it increases visual detection by predators and has been proposed as the reason why other fish larvae may select against cyclopoids (Hillgruber et al. 1995, Robert et al. 2008). However, the preferred zooplankton prey of sailfish larvae have physical attributes that contribute to their high visibility even when they are stationary. Specifically, *Evadne* have red pigment throughout their bodies and have a large darkly pigmented compound eye (Wong et al. 2008). Additionally, *Corycaeus* and *Farranula* possess well developed large paired eyes which may enhance their visibility in the plankton (Selander et al. 2017).

Larval sailfish also appeared to exhibit size-specific selection of prey, with selection for the largest size class of male *Farranula*, and the smallest size class of female *Farranula* which encompassed roughly the same size range (0.579 – 0.677 mm). This indicates that there may be an optimal prey size for detection or handling. No prey were selected from the largest two size classes of *Corycaeus* and *Evadne* available in the environment. The genus *Corycaeus* represents a wide range of species with differing morphologies measuring from 0.243 mm to 1.569 mm for prosome length in the environment. *Evadne* cladocerans also cover a wide range of sizes (0.359 mm to 1.011 mm) from small neonates to brooding females over three times larger. These larger size classes may be larger than the maximum prey size available to the larvae captured based on predator mouth gape. Sailfish larvae show nearly exclusive selection for a narrow pool of preferred prey despite residing in a varied prey environment. Furthermore, zooplanktivorous sailfish regardless of size (2.6 - 7.7 mm SL) exhibit relatively narrow size selection within their already limited preferred prey pool. Overall, sailfish larvae

displayed high trophic specialization characteristic of low latitude scombrid fish larvae (Llopiz & Hobday 2015).

1.4.4 Lipid Analysis

The nutritional and physiological condition of fish larvae affect their growth and survival and therefore has deterministic effects on recruitment (Suthers 1988). Lipid analysis was employed in this study because previous studies have shown elevated levels of some EFAs have been associated with higher growth and better prey quality (Paulsen et al. 2014a b). However, studies of fatty acid concentrations have widespread uses from examining nutritional requirements of organisms, to predicting population dynamics of fishes, to studying trophic transfer within food webs (Parrish 2008). The food-web pathways that support low latitude scombrid fish larvae are still poorly understood (Landry et al. 2019), and this study represents the first fatty acid analysis of sailfish larvae and their prey and therefore provides valuable baseline data for subtropical oligotrophic food webs.

Larval sailfish EFAs were variable between regions and across developmental stages. Total FA (2.9 – 10.7 % dry mass) of the sailfish larvae analyzed did not increase with age. Similar total FA values were observed for two species of Hake from the Benguela current (3.5 – 10.3% dry mass for *Merluccius paradoxus*) (6.3 – 7.9% dry mass for *Merluccius capensis*) where total FA also showed no variability with age through the larval stage (Grote et al. 2011). Despite stable total FAs through early larval development, EFA percentages (DHA, EPA, AA) all increased significantly with larval development. Similar results were observed for anchovy larvae from the Gulf of Cadiz. Despite a decrease in total FA with size for anchovy larvae, DHA %, EPA %, and AA % were significantly higher for larger anchovy larvae than for smaller larvae (Teodósio et al. 2017). In this study, total FA was highest for zooplankton prey at the station closest to shore. Teodósio et al. (2017) found that FA

concentrations increased with proximity to the coast which also had a positive relationship with both chl *a* concentrations and temperature. Chl *a* concentrations were correlated with distance to shore, but low variability in observed chl *a* concentrations (0.1 mg/m^3 at stations where sailfish larvae were collected and analyzed for FAs) precluded significant relationships with sailfish FA concentrations.

Additionally, Corycaeidae copepods had significantly greater DHA % than Evadne cladocerans which may be the underlying cause of any growth benefit afforded by diets with higher proportions of *Farranula* as shown by (Sponaugle et al. 2010) for blue marlin larvae from the Florida Straits.

1.4.5 Otolith Microstructure Analysis

Many biotic and abiotic variables have been shown to affect the growth rates of larval fish, including temperature, prey availability, and density of conspecifics (Rilling & Houde 1999, Wexler et al. 2007). Scombrid larvae and specifically sailfish larvae have very fast growth compared to other fish larvae which is reflected by larval growth rates that are an order of magnitude higher in sailfish than in high latitude fish like Arctic gadids (Pepin et al. 2015). However, even when sailfish larvae are compared to other subtropical pelagic fish larvae, they still exhibit more rapid growth. For example, Atlantic bluefin tuna larvae from the GoM have a reported growth rate of 0.67 mm d^{-1} and sailfish larvae from our study have an average growth rate of 0.83 mm d^{-1} over the same age range (Malca et al. 2017). Significant positive relationships were found between larval sailfish standard length and otolith radius as well as between otolith radius-at-age residuals and standard length-at-age residuals. These relationship indicate that otolith increment data from GoM sailfish larvae can effectively describe larval somatic growth

as was similarly demonstrated for larvae from the Florida Straits (Hare & Cowen 1995, Sponaugle et al. 2010). Daily instantaneous growth coefficients showed no significant interannual differences in our study. Daily growth was higher (k = 0.125 – 0.164) than reported previously for sailfish in the GoM (k = 0.113 – 0.127; Simms et al. 2010), and similar for sailfish in the Straits of Florida (k = 0.130 to 0.146; Luthy et al. 2005b, Richardson 2007, Richardson et al. 2009b, Sponaugle et al. 2010). Additionally, daily instantaneous growth rates (overall k = 0.135) were nearly equivalent to those reported from two studies of sailfish larvae captured in the Florida Straits (overall k = 0.134; Sponaugle et al. 2010) (overall k = 0.137; Luthy et al. 2005b) showing similar growth patterns between these adjacent regions. These similarities are not surprising since environmental conditions were similar between regions.

Interannual differences in larval sailfish age at collection were observed with older larvae collected in 2017 than in 2018 and 2019. This interannual age discrepancy was likely caused by exclusive sampling of common water features in 2017 in contrast to substantial inclusion of anticyclonic features in 2018 (31% of stations) and 2019 (43% of stations). Older larvae are more likely to reside in common waters because they have swimming capabilities that allow them to effectively influence their own distribution (Downie et al. 2020) and can therefore resist the hydrodynamic convergence that concentrates younger larvae in frontal regions when they are spawned nearby (Bakun 2006, Richardson et al. 2009a). Larvae collected earlier in the year were also younger than those collected later in the year. This is likely because the early cruises took place at the beginning of the spawning season for sailfish (de Sylva & Breder 1997, Richardson 2007) and older larvae were not yet present.

1.4.6 Multi-way ANOVA

Identifying the biological and oceanographic factors that determine larval survival is a major goal of fisheries oceanography. Using a multi-tiered approach to my analysis (to account for non-uniform data collection across sampling stations and larval specimens), several trends related to larval sailfish growth were identified. Notably, the type of mesoscale feature sampled was a significant predictor variable for larval growth in all model runs. Larval sailfish collected in anticyclonic boundary regions were significantly more abundant and had significantly faster recent growth rates relative to larvae collected in anticyclonic regions or common waters. Similar observations of elevated abundance associated with boundaries of mesoscale features have been reported for larval sailfish in the GoM and Florida Straits (Tidwell 2008, Sponaugle et al. 2010, Rooker et al. 2012), which suggests frontal zones are an important habitat for sailfish early life stages. Frontal features have been demonstrated to show elevated primary productivity relative to anticyclonic regions in the Gulf of Mexico which have been shown to be depleted in nitrate, and to have extremely low chlorophyll, primary productivity, and zooplankton biomass (Biggs 1992). Sailfish have successfully exploited loopholes in the pelagic environment to thrive in oligotrophic offshore waters by benefiting from the hydrodynamic convergence and retention generated by frontal zones that results in the concentration of larvae with their zooplankton prey (Bakun & Broad 2003). The ephemeral and turbid nature of these zones may create the necessary conditions for sailfish to survive past the “point of no return” during their very sensitive first feeding stages (Hjort 1914) where they have limited swimming capabilities and face challenges like “hydrodynamic starvation” due to the difficulties of maneuvering at low

Reynold's numbers (China & Holzman 2014). Because these pockets of productivity are relatively short lived and have wavering spatial and temporal bounds, there may be a lower predation risk for larvae than there would be in areas with a more constant and predictable nutrient pulse.

Gut content analyses have been used previously to infer larval condition, and gut fullness has been positively correlated with recent growth for larval fish (Sponaugle et al. 2009). In my study, total number of gut contents was a significant predictor of mean DRG for sailfish larvae, however as in a previous study (Sponaugle et al. 2010), no relationship was observed between gut fullness and recent growth. Although not statistically significant, the proportions of female *Farranula* and *Corycaeus* consumed trended positively as predictor variables for mean recent growth. Although percentages of female and male *Farranula* in sailfish diets were not significant predictors of mean DRG, consumption each sex reflected diametric trends on sailfish mean DRG with higher proportional consumption of females reflecting higher mean DRG and lower proportional consumption of males reflecting higher mean DRG. This study shows that pooling sexually dimorphic prey types together in analyses of diet can obscure underlying patterns and reduce predictive power of models that are based on prey consumption, especially when predators exhibit consistent sex-specific selection. Sponaugle et al. (2010) also found no significant effect of consumed prey composition on the recent growth of sailfish larvae but did find that higher consumption of *Farranula* was a significant predictor of faster recent growth in blue marlin larvae, suggesting sailfish may be less constrained to the specific type of prey they consume in their early development than are blue marlin. Sailfish larvae have been found to inhabit wider ranges of

environmental conditions compared to other species of billfish (Rooker et al. 2012). The combination of low variability in observed environmental variables, relatively wide habitat tolerances for sailfish larvae, and the serial correlation of environmental conditions and otolith increment width (Pepin et al. 2001) makes it difficult to observe the effects of environmental conditions on larval growth.

It is well understood that the respiration rate of ectothermic poikilotherms increases with rising temperatures within each organism's thermal range. Few studies have shown deleterious effects of low oxygen on pelagic organisms in the field (Ekau et al. 2010). However, the physiological effects of low DO have been well studied under laboratory conditions where fish growth has been demonstrated to decline under low DO (Bejda et al. 1992, Chabot & Claireaux 2008). Fishes have also been shown to be more sensitive to low DO than crustaceans or mollusks (Vaquer-Sunyer & Duarte 2008). Aquaculture studies have shown inverse relationships between food conversion ratios and DO level where fish convert a higher proportion of their food into weight gain when oxygen levels are higher (Tsadik & Kutty 1987). DO was retained in the final model for 2019 sailfish larvae as a significant predictor of mean DRG which was higher for larvae collected in waters with higher DO. Larval sailfish may be particularly vulnerable to the deleterious effects of low DO concentrations since they reside in warm waters where their oxygen demand is high and where effects of low DO have been shown to be more severe for some organisms (Roman et al. 2019).

Small sample sizes precluded significant effects for prey FA metrics with larval sailfish mean DRG. While not retained in a final model, there was a negative relationship between sailfish tissue total FA and mean DRG. This is likely due to the wide sampling

range and the positive associations between FA with shore proximity where sailfish larvae are less likely to reside. However, it may be possible that lower FA content was found in muscle tissue of fish larvae with higher mean DRG because FAs were used as energy to fuel high growth rates rather than being stored in the muscle tissue.

Previous studies have shown the positive effects of high DHA prey on larval fish growth in the field (Paulsen et al. 2014a), and it has been shown that larval blue marlin exhibit faster growth when their diets are dominated my Farranula copepods (Sponaugle et al. 2010). This study demonstrated that sailfish larvae exhibit sex-specific selection for *Farranula* females that contain on average higher percentages of the three EFAs examined in this study (DHA, EPA, AA) than do males of the genus, but no direct link between dietary EFAs and recent sailfish growth was able to be inferred in our study.

1.5 Conclusion

The goals of this study were to describe diet composition and prey selectivity of larval sailfish, describe the total FA content and EFA concentrations of sailfish and their zooplankton prey, describe larval growth, and examine the abiotic and biotic variables that affect larval sailfish recent growth in the field. Larval sailfish had similar diets to those previous described for the species. This study was the first to reveal sex-specific selection in line with optimal foraging theory for a copepod prey of sailfish larvae. Total FA of sailfish prey varied spatially and EFA concentrations varied between prey type. Additionally, EFA concentrations of sailfish larvae increased with ontogeny. Growth rates were similar or slightly higher than those previously described for sailfish larvae and recent growth varied with number of prey consumed, DO, and mesoscale circulation feature. Recent growth of sailfish was higher when they consumed more prey and when

they were collected in waters with higher levels of DO. This study supports previous evidence that suggests a strong connection between boundary regions and larval sailfish abundance (Simms et al. 2010) and demonstrates faster recent growth within anticyclonic boundary regions relative to anticyclonic regions and common waters. Future studies to investigate the role of spatial, biological, and environmental variables on larval fish would benefit from finer scale measurements that align more closely to the scale experienced by the larvae especially in dynamic environments like frontal regions where relatively large-scale sampling can obscure the variability experienced by fish larvae especially in regards to small prey patches that may allow some larvae to survive in otherwise oligotrophic low prey density environments.

APPENDIX

Table A.1 Mean (± standard error) of total fatty acid (FA) and fatty acid concentrations (% of all FAs detected) of sailfish larvae by developmental stage.

	Preflexion larvae *n* = 19	Flexion larvae *n* = 15	Postflexion larvae *n* = 5
FA			
Total FA	45.4 ± 3.9	48.7 ± 5.4	42.9 ± 6.8
14:0	2.8 ± 0.2	2.4 ± 0.2	1.4 ± 0.2
14:1	0.5 ± 0.3	0.1 ± 0.1	-
16:0	35.2 ± 1.7	35.3 ± 2.5	30.1 ± 4.1
16:1	0.2 ± 0.2	0.6 ± 0.2	0.9 ± 0.1
18:0	22.0 ± 1.3	20.4 ± 1.5	20.5 ± 3.7
18:1(oleate)	4.6 ± 0.4	4.7 ± 0.3	5.8 ± 0.1
18:0(vaccinate)	0.1 ± 0.1	0.8 ± 0.2	1.5 ± 0.4
18:2(n-6)	-	0.6 ± 0.1	1.2 ± 0.1
18:3(n-3)	5.6 ± 0.5	2.8 ± 0.8	0.7 ± 0.3
20:0	4.9 ± 0.5	3.0 ± 0.5	0.6 ± 0.1
20:1(n-9)	0.2 ± 0.2	-	-
20:2(n-6)	0.1 ± 0.1	-	-
20:3(n-3)	3.0 ± 0.2	2.3 ± 0.3	1.1 ± 0.3
20:4(n-6)	0.3 ± 0.1	1.5 ± 0.1	2.0 ± 0.2
20:5(n-3)	5.2 ± 0.4	6.2 ± 0.4	7.0 ± 0.6
22:0	-	0.1 ± 0.1	0.5 ± 0.1
22:1(n-9)	2.0 ± 0.1	0.6 ± 0.2	-
22:6(n-3)	19.1 ± 2.1	25.8 ± 2.0	32.8 ± 2.3
24:0	1.9 ± 0.1	1.6 ± 0.1	1.3 ± 0.2
24:1	4.6 ± 0.6	3.1 ± 0.9	2.6 ± 2.0

Table A.2 Mean (± standard error) of total fatty acid (FA) and fatty acid concentrations (% of all FAs detected) of the preferred prey of sailfish larvae. Prey items include female *Farranula* (♀*Farr.*), male *Farranula* (♂*Farr.*), *Corycaeus* (*Cory.)* and *Evadne* (*Evadne)*.

	♀ *Farr.* *n* = 9	♂*Farr.* *n* = 11	*Cory.* *n* = 12	*Evadne* *n* = 6
FA				
Total FA	39.1 ± 2.7	41.5 ± 16.9	37.5 ± 5.9	42.6 ± 16.1
14:0	5.7 ± 0.5	3.8 ± 0.3	4.8 ± 0.5	3.4 ± 0.3
14:1	0.4 ± 0.1	-	0.5 ± 0.1	-
16:0	27.8 ± 1.1	35.1 ± 2.8	33.0 ± 3.5	30.0 ± 3.1
16:1	1.3 ± 0.2	0.6 ± 0.0	1.6 ± 0.2	1.8 ± 0.1
18:0	9.2 ± 0.7	19.1 ± 2.4	15.1 ± 1.6	18.6 ± 3.0
18:1(oleate)	2.6 ± 0.2	3.6 ± 0.8	2.9 ± 0.2	4.5 ± 0.9
18:0(vaccinate)	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	-
18:2(n-6)	1.2 ± 0.1	1.1 ± 0.1	2.7 ± 1.2	-
18:3(n-3)	0.8 ± 0.2	3.6 ± 0.5	0.7 ± 0.2	4.2 ± 2.2
20:0	1.4 ± 0.6	5.0 ± 0.8	2.2 ± 0.5	7.2 ± 1.8
20:1(n-9)	-	2.0 ± 0.1	0.9 ± 0.3	-
20:2(n-6)	-	-	0.2 ± 0.0	-
20:3(n-3)	1.5 ± 0.7	3.0 ± 0.7	1.5 ± 0.7	5.7 ± 1.2
20:4(n-6)	0.9 ± 0.1	1.4 ± 0.4	1.1 ± 0.1	-
20:5(n-3)	9.9 ± 0.5	5.9 ± 0.5	7.9 ± 0.5	8.7 ± 2.1
22:0	-	-	0.4 ± 0.0	-
22:1(n-9)	0.8 ± 0.3	-	-	-
22:6(n-3)	36.6 ± 2.1	27.3 ± 4.3	32.0 ± 2.7	10.8 ± 2.1
24:0	1.2 ± 0.3	4.6 ± 1.2	1.9 ± 0.3	11.9 ± 6.5
24:1	0.4 ± 0.0	1.5 ± 0.0	0.9 ± 0.0	-

REFERENCES

Bainbridge V (1958) SOME OBSERVATIONS ON EVADNE NORMDMANNI LOVEN. J Mar Biol Assoc United Kingdom 37:349–370.

Bakun A (2006) Fronts and eddies as key structures in the habitat of marine fish larvae: opportunity, adaptive response and competitive advantage. Sci Mar 70:105–122.

Bakun A, Broad K (2003) Environmental 'loopholes' and fish population dynamics: Comparative pattern recognition with focus on El Niño effects in the Pacific. Fish Oceanogr 12:458–473.

Bartlett MR, Haedrich RL (1968) Neuston Nets and South Atlantic Larval Blue Marlin (Makaira nigricans). Copeia 1968:469–474.

Baumann H, Pepin P, Davidson FJM, Mowbray F, Schnack D, Dower, F. J (2003) Reconstruction of environmental histories to investigate patterns of larval radi- ated shanny (Ulvaria subbifurcata) growth and selective survival in a large bay of Newfoundland. ICES J Mar Sci 3139:211–223.

Bejda AJ, Phelan BA, Studholme AL (1992) The effect of dissolved oxygen on the growth of young-of-the-year winter flounder,Pseudopleuronectes americanus. Environ Biol Fishes 34.

Bell M V., Batty RS, Dick JR, Fretwell K, Navarro JC, Sargent JR (1995) Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (Clupea harengus L.). Lipids 30:443–449.

Biggs DC (1992) Nutrients, plankton, and productivity in a warm-core ring in the western Gulf of Mexico. J Geophys Res 97:2143–2154.

Buckland A, Baker R, Loneragan N, Sheaves M (2017) Standardising fish stomach content analysis: The importance of prey condition. Fish Res 196:126–140.

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Mar Mammal

Sci 22:759–801.

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547–1559.

Buskey EJ, Coulter C, Strom S (1993) LOCOMOTORY PATTERNS OF MICROZOOPLANKTON : POTENTIAL EFFECTS ON FOOD SELECTIVITY OF LARVAL FISH. Bull Mar Sci 53:29–43.

Carter AL, Mackesey B, Chaibongsai P, Cox A, Peel E (2002) Caribbean Pelagic Recreational Fishing, Economic Growth, Poverty Alleviation, and Food Security.

Chabot D, Claireaux G (2008) Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, Gadus morhua. Mar Pollut Bull 57:287–294.

China V, Holzman R (2014) Hydrodynamic starvation in first-feeding larval fishes. Proc Natl Acad Sci 111:8083–8088.

Collette BB, Carpenter KE, Polidoro BA, Boustany A, Die DJ, Elfes C, Fox W, Graves J, Harrison LR, Mcmanus R, Nelson R (2011) High Value and Long Life — Double Jeopardize Tuna and Billfishes. Science (80-) 333:291–292.

Copeman LA, Laurel BJ (2010) Experimental evidence of fatty acid limited growth and survival in Pacific cod larvae. Mar Ecol Prog Ser 412:259–272.

Cox SP, Essington TE, Kitchell JF, Martell SJD, Walters CJ, Boggs C, Kaplan I (2002) Reconstructing ecosystem dynamics in the central Pacific Ocean, 1952-1998. II. A preliminary assessment of the trophic impacts of fishing and effects on tuna dynamics. Can J Fish Aquat Sci 59:1736–1747.

Cushing DH (1990) Plankton production and year-class strength in fish populations: An

update of the match/mismatch hypothesis. Adv Mar Biol 26:249–293.

Cushing DH (1969) The Regularity of the Spawning. ICES J Mar Sci 33:81–92.

Cutts CJ, Sawanboonchun J, Mazorra De Quero C, Bell JG (2006) Diet-induced differences in the essential fatty acid (EFA) compositions of larval Atlantic cod (Gadus morhua L.) with reference to possible effects of dietary EFAs on larval performance. ICES J Mar Sci 63:302–310.

Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340.

Ditton RB, Stoll JR (2003) Social and economic perspective on recreational billfish fisheries. Mar Freshw Res 54:545.

Domingues R, Goni G, Bringas F, Muhling B, Lindo-Atichati D, Walter J (2016) Variability of preferred environmental conditions for Atlantic bluefin tuna (Thunnus thynnus) larvae in the Gulf of Mexico during 1993-2011. Fish Oceanogr 25:320–336.

Downie AT, Illing B, Faria AM, Rummer JL (2020) Swimming performance of marine fish larvae : review of a universal trait under ecological and environmental pressure. Rev Fish Biol Fish 30:93–108.

Ekau W, Auel H, Ṗortner HO, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosciences 7:1669–1699.

Fey DP (1999) Effects of preservation technique on the length of larval fish: Methods of correcting estimates and their implication for studying growth rates. Arch Fish Mar Res 47:17–29.

Fraser A (1989) Triacylglycerol Content as a Condition Index for Fish, Bivalve, and Crustacean Larvae. Can J Fish Aquat Sci 46:1868–1873.

Fuiman LA (2002) Special Considerations of Fish Eggs and Larvae. In: *Fishery Science: The Unique Contributions of Early Life Stages*. Fuiman L, Werner R (eds) Blackwell Science, p 1–32

Galloway AWE, Winder M (2015) Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS One 10:1–23.

Gorbunova NN (1969) Breeding grounds and food of the larvae of the swordfish (Xiphias gladius). J Ichthyol 9:375–387.

Govoni JJ, Laban EH, Hare JA (2003) The early life history of swordfish (Xiphias gladius) in the western North Atlantic. Fish Bull 101:778–789.

Grote B, Hagen W, Lipinski MR, Verheye HM, Stenevik EK, Ekau W (2011) Lipids and fatty acids as indicators of egg condition, larval feeding and maternal effects in Cape hakes (Merluccius paradoxus and M. capensis). Mar Biol 158:1005–1017.

Hare JA, Cowen RK (1995) Effect of age, growth rate, and ontogeny on the otolith size - fish size relationship in bluefish, Pomatomus saltatrix, and the implications for back-calculation of size in fish early life history stages. Can J Fish Aquat Sci 52:1909–1922.

Harris, R., Wiebe, P., Lenz, J., Skjoldal, H. R., & Huntley M (ed) (2000) ICES Zooplankton Methodology Manual. Elsevier.

Hillgruber N, Haldorson LJ, Paul AJ, Hillgruber N, Hal LJ (1995) Feeding selectivity of larval walleye pollock Theragra chalcogramma in the oceanic domain of the Bering Sea Feeding selectivity of Theragra chalcogramm of the Bering Se. 120:1–10.

Hjort J (1914) Fluctuations in the great fisheries of northern europe viewed in the light of biological research. Rapp procès-verbaux 20:237.

Houde ED (2008) Emerging from Hjort's shadow. J Northwest Atl Fish Sci 41:53–70.

Itoh T, Shiina Y, Tsuji S, Endo F, Tezuka N (2000) Otolith daily increment formation in laboratory reared larval and juvenile bluefin tuna Thunnus thynnus. Fish Sci 66:834–839.

St. John MA, Clemmesen C, Lund T, Köster T (2001) Diatom production in the marine environment: Implications for larval fish growth and condition. ICES J Mar Sci 58:1106–1113.

Jónasdóttir SH (2019) Fatty acid profiles and production in marine phytoplankton. Mar Drugs 17:1–20.

Kitchell JF, Martell SJD, Walters CJ, Jensen OP, Kaplan IC, Watters J, Essington TE, Boggs CH (2006) Billfishes in an ecosystem context. Bull Mar Sci 79:669–682.

Landry MR, Beckley LE, Muhling BA (2019) Climate sensitivities and uncertainties in food-web pathways supporting larval bluefin tuna in subtropical oligotrophic oceans. ICES J Mar Sci 76:359–369.

Lasker R (1981) The role of a stable ocean in larval fish survival and subsequent recruitment. Mar fish larvae Morphol Ecol Relat to Fish:79–87.

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306.

Lindo-atichati D, Bringas F, Goni G, Muhling B, Muller-karger FE, Habtes S (2012) Varying mesoscale structures influence larval fish distribution in the northern Gulf of Mexico. Mar Ecol Prog Ser 463:245–257.

Lipskaya NY, Gorbunova NN (1975) Feeding of larvae of the blue marlin (Makaira nigricans)(Pisces, Istiophoridae). J Ichthyol 15:95–101.

Lipskaya NY, Gorbunova NN (1977) Feeding of Sailfish larvae. Oceanology 17:0–4.

Llopiz JK (2013) Latitudinal and taxonomic patterns in the feeding ecologies of fish larvae: A literature synthesis. J Mar Syst 109–110:69–77.

Llopiz JK (2008) The tropic ecologies of larval billfishes, tuna and coral reef fishes in the straits of florida: piscivory, selectivity, and niche separation. University of Miami

Llopiz JK, Cowen RK (2008) Precocious, selective and successful feeding of larval billfishes in the oceanic Straits of Florida. Mar Ecol Prog Ser 358:231–244.

Llopiz JK, Hobday AJ (2015) A global comparative analysis of the feeding dynamics and environmental conditions of larval tunas, mackerels, and billfishes. Deep Res Part II Top Stud Oceanogr 113:113–124.

Luthy SA (2004) Billfish larvae of the Straits of Florida. University of Miami

Luthy SA, Cowen RK, Serafy JE, McDowell JR (2005a) Toward identification of larval sailfish (Istiophorus platypterus), white marlin (Tetrapturus albictus), and blue marlin (Makaira nigricans) in the western North Atlantic Ocean. Fish Bull 103:588–600.

Luthy SA, Serafy JE, Cowen RK, Denit KL, Sponaugle S (2005b) Age and growth of larval Atlantic sailfish, Istiophorus platypterus. Mar Freshw Res 56:1027–1035.

Malca E, Muhling B, Franks J, García A, Tilley J, Gerard T, Ingram W, Lamkin JT (2017) The first larval age and growth curve for bluefin tuna (Thunnus thynnus) from the Gulf of Mexico: Comparisons to the Straits of Florida, and the Balearic Sea (Mediterranean). Fish Res 190:24–33.

Malzahn AM, Aberle N, Clemmesen C, Boersma M (2007) Nutrient limitation of primary producers affects planktivorous fish condition. Limnol Oceanogr 52:2062–2071.

Miller TJ, Crowder LB, Rice JA ME (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can J Fish Aquat Sci 45.

Nichols PD, Palmisano AC, Smith GA, White DC (1986) Lipids of the antarctic sea ice diatom Nitzschia cylindrus. Phytochemistry 25:1649–1653.

Parrish CC (2008) Essential Fatty Acids in Aquatic Food Webs. In: *Lipids in Aquatic Ecosystems*. Kainz M, Brett MT, Arts MT (eds) Springer, St. John's, Newfoundland, Canada, p 309–326

Paulsen M, Clemmesen C, Malzahn AM (2014a) Essential fatty acid (docosahexaenoic acid, DHA) availability affects growth of larval herring in the field. Mar Biol 161:239–244.

Paulsen M, Hammer C, Malzahn AM, Polte P, Dorrien C Von, Clemmesen C (2014b) Nutritional situation for larval Atlantic herring (Clupea harengus L.) in two nursery areas in the western Baltic Sea. ICES J Mar Sci 71:991–1000.

Pepin P, Dower JF, Benoît HP (2001) The role of measurement error on the interpretation of otolith increment width in the study of growth in larval fish. Can J Fish Aquat Sci 58:2204–2212.

Pepin P, Robert D, Bouchard C, Dower JF, Falardeau M, Fortier L, Jenkins GP, Leclerc V, Levesque K, Llopiz JK, Meekan MG, Murphy HM, Ringuette M, Sirois P, Sponaugle S (2015) Once upon a larva: revisiting the relationship between feeding success and growth in fish larvae. ICES J Mar Sci 72:359–373.

Perez KO, Fuiman LA (2015) Maternal diet and larval diet influence survival skills of larval red drum Sciaenops ocellatus. J Fish Biol 86:1286–1304.

Peters J, Renz J, Van Beusekom J, Boersma M, Hagen W (2006) Trophodynamics and seasonal cycle of the copepod Pseudocalanus acuspes in the Central Baltic Sea (Bornholm Basin): Evidence from lipid composition. Mar Biol 149:1417–1429.

Pyke GH (1984) OPTIMAL FORAGING THEORY : A CRITICAL REVIEW. Annu Rev Ecol Syst 15:523–575.

Raubenheimer D, Simpson SJ, Mayntz D (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct Ecol:4–16.

Richardson DE (2007) Physical and Biological Characteristics of Billfish Spawning Habitat in the Straits of Florida. University of Miami

Richardson DE, Cowen RK, Prince ED, Sponaugle S (2009a) Importance of the Straits of Florida spawning ground to Atlantic sailfish (Istiophorus platypterus) and blue marlin (Makaira nigricans). Fish Oceanogr 18:402–418.

Richardson DE, Llopiz JK, Leaman KD, Vertes PS, Muller-Karger FE, Cowen RK (2009b) Sailfish (Istiophorus platypterus) spawning and larval environment in a Florida Current frontal eddy. Prog Oceanogr 82:252–264.

Rilling GC, Houde ED (1999) Regional and temporal variability in distribution and abundance of bay anchovy (Anchoa mitchilli) eggs, larvae, and adult biomass in the Chesapeake Bay. Estuaries 22:1096–1109.

Robert D, Castonguay M, Fortier L (2008) Effects of intra- and inter-annual variability in prey field on the feeding selectivity of larval Atlantic mackerel (Scomber scombrus). J Plankton Res 30:673–688.

Robert D, Castonguay M, Fortier L (2009) Effects of preferred prey density and temperature on feeding success and recent growth in larval mackerel of the southern Gulf of St. Lawrence. Mar Ecol Prog Ser 377:227–237.

Robert D, Murphy HM, Jenkins GP, Fortier L (2014) Poor taxonomical knowledge of larval fish prey preference is impeding our ability to assess the existence of a 'critical period' driving year-class strength. ICES J Mar Sci 71:2042–2052.

Roman MR, Brandt SB, Houde ED, Pierson JJ (2019) Interactive effects of Hypoxia and temperature on coastal pelagic zooplankton and fish. Front Mar Sci 6:1–18.

Rooker JR, Simms JR, Wells RJD, Holt SA, Holt GJ, Graves JE, Furey NB (2012) Distribution and Habitat Associations of Billfish and Swordfish Larvae across Mesoscale Features in the Gulf of Mexico. PLoS One 7:e34180.

Sargent J, Mcevoy L, Estevez A, Bell G, Bell M, Henderson J, Tocher D (1999) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229.

Selander E, Heuschele JAN, Larsson ANNI (2017) Contribution to the Themed Section : Advances Using Copepods in Aquaculture Hydrodynamic properties and distribution of bait downstream of a zooplankton trap. 39:1020–1027.

Shiroza A, Malca E, Lamkin JT, Gerard T, Landry MR (2021) Active prey selection in developing larvae of Atlantic bluefin tuna (Thunnus thynnus) in spawning grounds of the Gulf of Mexico. J Plankton Res 00:1–19.

Simms JR (2009) Early life ecology of Sailfish, Istiophorus platypterus, in the northern Gulf of Mexico. Texas A&M University

Simms JR, Rooker JR, Holt SA, Holt GJ, Bangma J (2010) Distribution, growth, and

mortality of sailfish (istiophorus platypterus) larvae in the northern gulf of Mexico. Fish Bull 108:478–490.

Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D (2004) Optimal foraging when regulating intake of multiple nutrients. Anim Behav 68:1299–1311.

Sponaugle S, Denit KL, Luthy SA, Serafy JE, Cowen RK (2005) Growth variation in larval Makaira nigricans. J Fish Biol 66:822–835.

Sponaugle S, Llopiz JK, Havel LN, Rankin TL (2009) Spatial variation in larval growth and gut fullness in a coral reef fish. Mar Ecol Prog Ser 383:239–249.

Sponaugle S, Walter KD, Denit KL, Llopiz JK, Cowen RK (2010) Variation in pelagic larval growth of Atlantic billfishes: the role of prey composition and selective mortality. Mar Biol 157:839–849.

Suthers IM (1988) The use of condition indices in larval fish. Bur Rural Resour Proc 15:49–55.

de Sylva DP, Breder PR (1997) Reproduction, gonad histology, and spawning cycles of north atlantic billfishes (Istiophoridae). Bull Mar Sci 60:668–697.

Teodósio MA, Garrido S, Peters J, Leitão F, Ré P, Peliz A, Santos AMP (2017) Assessing the impact of environmental forcing on the condition of anchovy larvae in the Cadiz Gulf using nucleic acid and fatty acid-derived indices. Estuar Coast Shelf Sci 185:94–106.

Thompson PA, Harrison PJ (1992) Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. Mar Biol 113:645–654.

Tidwell (2008) The Feeding Ecology of Larval Billfishes (Family: Istiophoridae) in the

Northern Gulf of Mexico

Tidwell TM, Holt S, Rooker JR, Holt GJ (2008) The Distribution and Feeding Ecology of Larval Billfish in the Northern Gulf of Mexico. Proc 60th Gulf Caribb Fish Inst.

Tsadik GG, Kutty M. (1987) Influence of ambient oxygen on feeding and growth of the tilapia, *Oreochromis niloticus* (Linnaeus). Niomr ARAC/87/WP:pp: 1-13.

Uotani I, Ueyanagi S (1997) Feeding habits of Indo-Pacific blue marlin and shortbill spearfish larvae. J Sch Mar Sci Technol Tokai Univ 43:107–116.

Vaquer-Sunyer R, Duarte CM (2008) Thresholds of hypoxia for marine biodiversity. Proc Natl Acad Sci U S A 105:15452–15457.

Wexler JB, Chow S, Wakabayashi T, Nohara K, Margulies D (2007) Temporal variation in growth of yellowfin tuna (Thunnus albacares) larvae in the Panama Bight, 1990-97. Fish Bull 105:1–18.

Wong KC, Vivian CYL, Chan A (2008) Diel cycles of reproduction and vertical migration in the marine cladocerans Pseudevadne tergestina and Penilia avirostris. J Plankton Res 30:65–73.

Yasuda F, Kohno H, Yatsu A, Ida H (1978) Embryonic and early larval stages of the swordfish, Xiphias gladius, from the Mediterranean. J Tokyo Univ Fish 65:91–97.