UNIVERSITY OF CALIFORNIA
SANTA CRUZ

ADVANCING QUANTITATIVE FATTY ACID SIGNATURE ANALYSIS (QFASA) FOR CETACEAN DIET ESTIMATION: METHODOLOGIAL EVALUATION AND ECOLOGICAL APPLICATION

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

By

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August 2026

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Abstract

Advancing quantitative fatty acid signature analysis (QFASA) for cetacean diet estimation: Methodological evaluation and ecological application

by

Theresa-Anne Marie Tatom-Naecker

Quantifying cetacean diet is critical for understanding ecological and physiological processes but remains inherently challenging. Quantitative fatty acid signature analysis (QFASA) is a diet determination method with the potential to generate detailed, long-term diet estimates, but its use in cetaceans has been limited. My dissertation presents the first application of QFASA in bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphins"). It addresses key impediments to QFASA's broader application in cetaceans, evaluates its performance in professional-care and free-ranging dolphins, compares it to other diet determination methods, and explores the estimates' ecological implications. In Chapter 1, I used dolphins under professional care with known diets to derive calibration coefficients (CCs) and explore the temporal integration period of blubber fatty acids. I found that dolphin- and blubber layer-specific CCs produced lower-error estimates and identified major prey more accurately than non-dolphin CCs, and reflected prey consumption integrated over weeks to months. I also assessed how different model configurations impacted model performance and estimate accuracy, using professional-care dolphins in Chapter 1 and free-ranging dolphins in Chapter 2. While performance and estimates were sensitive to model configuration, the dietary suite of fatty acids and inner blubber

yielded better-performing, more consistent results in free-ranging dolphins. In Chapter 2, variable prey distinctiveness and bootstrap uncertainty resulted in multiple plausible estimates, but the variation typically involved redistribution among trophically-similar prey, suggesting that broader dietary patterns were robust for interpretation. In Chapter 3, I estimated free-ranging dolphin diet using QFASA and stable isotope analyses to compare the methods and evaluate contemporary diet estimates relative to historical studies. QFASA provided finer taxonomic resolution than stable isotopes but greater variability and uncertainty. However, both methods converged on broad dietary patterns, suggesting that dolphins selectively consumed a subset of available prey and exhibited modest individual- and demographic-level variation. Comparisons with previous studies suggested contemporary shifts in dominant prey that likely reflect both methodological differences and environmental changes. Together, these studies demonstrate that QFASA can successfully provide detailed, long-term diet estimates and valuable ecological insights for dolphins. However, careful model configuration selection and interpretation of uncertainty are essential, and QFASA is most powerful when applied alongside other diet determination methods.

Acknowledgements

I would like to start by thanking my advisors, Drs. Dan Costa and Randy Wells. My research and this dissertation would not have happened if not for you and your mentorship. I am incredibly grateful that you put your trust in me and provided the opportunity to complete a PhD. Your encouragement of my interests and my curiosity allowed me to pursue research that I was genuinely excited by and develop as an independent scientist, while your belief in me and support kept me motivated and gave me confidence even when I struggled. Your feedback improved my thinking process and my writing, and the generosity with which you included me in your research groups and connected me with your colleagues broadened my opportunities and grew my scientific community. Finally, thank you for your patience as I figured out the right approach, even when it took me down some rabbit holes! It has truly been an honor and a joy to work with you.

I am also incredibly grateful to Drs. Sue Budge, Steve Trumble, and Pete Raimondi, the mentors and committee members who helped make my dissertation what it is. Sue, I could not have completed a dissertation on QFASA if not for you, from your advice on sample processing protocols and data management to your feedback on my presentation and interpretation of the results. Thank you for patiently answering my endless questions and long emails, and for the valuable discussions about nitty gritty method mechanics. Steve, thank you for welcoming me into your lab to learn key methodologies, your thoughtful and honest feedback on my writing, and your nuanced advice on how best to approach and frame my research. Last but

certainly not least, Pete – I am forever grateful for the clear and patient way that you explain stats, your quick responses and generosity with your time, and your advice on my dissertation structure. You helped me quantify and statistically support my findings and challenged me to think more deeply about the statements I made and the approaches I took. Sue, Steve, and Pete, I am a stronger writer and scientist thanks to each of you.

This research would have been impossible without sample processing support. I would like to thank the Marine Lipids Lab at Dalhousie University, especially Carrie Greene for her careful and efficient work. I am also immensely grateful to high school and undergraduate students Vivian Chen, Jessica McCoppin, and KC McNamara and my fellow graduate student Jordann Young. Your dedication made it possible to analyze hundreds of samples, your curiosity helped me become a better teacher and communicator, and your positive attitudes made a sometimes-tedious process enjoyable and rewarding.

My PhD would have been equally impossible without my funding sources. First and foremost, I would like to thank the anonymous donor to Brookfield Zoo Chicago whose generosity supported multiple years of my PhD. I am so incredibly grateful for your unquestioning support of science, graduate students, and the Brookfield Zoo Chicago's Sarasota Dolphin Research Program. I was also privileged to have support from the National Science Foundation Graduate Research Fellowship Program, UC Santa Cruz's DoEE GANN Fellowship, the Sooy Foundation's Graduate Research Grant, the Lerner-Gray Fund's Marine Research grant, the PADI

Foundation, and UC Santa Cruz's EEB Department Research and Travel Grants. An immense thank you also to Dr. William Head, whose hours of guidance and feedback on how to craft effective fellowship and grant applications shaped the way I write about my research and achievements and without a doubt drove my success.

A big thank you to the EEB staff, especially Judy Straub, Stephanie Zakarian, and Nathalie Espinoza. I truly appreciate the work you do to keep this department running smoothly, and your help navigating graduate school administration and bureaucracy. Thank you for making our lives so much easier!

I would also like to recognize and thank the researchers at the National Marine Mammal Foundation, especially Celeste Parry, Dr. Forrest Gomez, and Dr. Cynthia Smith, and the U.S. Navy Marine Mammal Program. I truly appreciate your advice and support of my Chapter 1 research and your generous sharing of the biological samples that made that work possible.

I am so grateful to the academic communities I was part of during my PhD. To the multi-talented scientists at Brookfield Zoo Chicago's Sarasota Dolphin Research Program, thank you for welcoming me warmly into your midst, including me in your work and social activities, and sharing your knowledge and advice. Your dedication, broad range of skills, humor and ability to make work fun, and kindness are an inspiration to me. To the graduate students and post-docs of the Costa Lab, I am very lucky to have gone on this journey alongside you. Thank you for introducing me to elephant seal field work, sharing your knowledge and perspective during lab meetings and social gatherings, and providing examples of truly good people and

scientists. Finally, to the 2018 graduate cohort, thank you for your friendship, advice, and unwavering encouragement, and for creating a warm community of amazing scientists and humans.

I am equally grateful to the teachers and professors who shaped my academic experiences before graduate school. I have been privileged to learn from many incredible writers, thinkers, and scientists over the course of my many years of school – their names would take up multiple pages! However, I would like to specifically recognize several individuals. Dr. Scott Deken and Beth Waterman, your high school classes sparked my interest in biology and marine conservation and introduced me to the joys of field research, leading me to pursue biology in undergraduate and forever changing my path. Dr. Mark Smith, Mark Nicholas, and each of my fantastic high school English teachers, thank you for teaching me how to think critically, and write clearly and with impact. Dr. Mark Westneat, thank you for welcoming me into your lab as a motivated but largely untested undergraduate student and guiding me through my first independent research project and publication. You showed me how to conduct rigorous, meaningful research and present my findings, paving the way for my graduate career. Finally, Dr. Michael Labarbera, your classes, labs, and field opportunities truly cemented my interest in ecology, evolution, and pursuing an advanced degree. The joy, clarity, and engagement with which you taught, the opportunities you provided your students, and your kindness are an inspiration.

Finally, there are not enough words to express my gratitude to the family and friends who stood by my side throughout this journey. To my dad Paul, your

unwavering belief in me and unquestioning love and encouragement is without a doubt the reason I chose this path and completed this degree. To my late mother Jacqueline, you shaped the person I am today. You have supported me through the most difficult moments, the happiest ones, and everything in between. I am so proud and lucky to be the daughter of such incredible parents. To my fiancé Jacob, you are the light in my life that I was not sure I would ever find. Your belief in me and endless patience, encouragement, and love made it possible to persevere even when I was at my lowest without losing sight of my life outside of graduate school. To Sarah and Corina, my sisters in everything but blood, your patient listening, dedication, accomplishments, and love have inspired and supported me from day one. To the Naecker and Wiechmann families, especially Nancy and Jeannie, your guidance, support, generous acceptance, and love have been key sources of motivation and happiness. To Pat, thank you for welcoming me into your home and your family, sharing your advice and supporting my every endeavor, and for your unequivocal love. Finally, to the Zumba dazzle, your smiles, zest for life, exuberant dancing, and friendship brought me a necessary escape and immense joy.

To everyone listed here, thank you for your role in making this journey possible. I am so grateful to all of you!

Chapter 1 of this dissertation is a lightly edited reprint of the following published material, included with permission of the co-authors:

Tatom-Naecker, T.M., Budge, S.M., Parry, C.B., Trumble, S.J., Wells, R.S. and Costa, D.P. (2026). First application of quantitative fatty acid signature analysis in bottlenose dolphins (*Tursiops truncatus*) and its implications for diet estimation in free-ranging cetaceans. Front. Mar. Sci. 13:1804181. doi:10.3389/fmars.2026.1804181.

As the first author, I developed the central ideas, curated the data, acquired necessary funding, modified and supplemented existing lab methodologies, validated the data, conducted most lab analyses and all formal data analyses, created the figures, wrote the original draft, and edited the final product. However, this work would not have been possible without my co-authors and mentors Sue Budge, Steve Trumble, Celeste Parry, Randy Wells, and Dan Costa. They helped conceptualize the focus of the manuscript; develop methodologies; curate, validate, and analyze the data; and acquire key funding. They also provided critical suggestions and edits to the publication. As my advisors, Randy and Dan also directed and supervised the research that forms the basis of Chapter 1. Thank you, all, for your contributions, support, and guidance.

Introduction

Marine mammal predators play central roles in ecosystems, as consumers who drive top-down interactions but are also impacted by changes in prey composition, nutrient recyclers who feed and sometimes die at depth but release waste at the surface, and sentinel species whose high trophic levels, long lifespans, stored fat reserves, and large body sizes make them particularly vulnerable to changes in ocean conditions and thus indicators of ocean health and climate change impacts (e.g., Bowen, 1997; Wells et al., 2004; Bossart, 2011; Doughty et al., 2016; Roman et al., 2025). Concurrently, the prey consumed by an individual reflects morphological and physiological constraints but also behavioral decisions, social learning, prey availability, habitat use, and environmental conditions (e.g., Rosen et al., 2007; Krützen et al., 2014; Goundie et al., 2015; Drago et al., 2021; McHuron et al., 2025). As a result, quantifying diet allows the study of both ecological processes and factors that influence health, reproduction, behavior, and survival; these include nutrient acquisition, trophic role, habitat use, individual and demographic variation, social behavioral transmission, and response to natural and anthropogenic change (e.g., Johnson et al., 2009; Bowen and Iverson, 2013; Slifka et al., 2013; Krützen et al., 2014; Nielsen et al., 2018; Ning et al., 2020; Plint et al., 2023; García-Vernet et al., 2024). In turn, marine mammals face increasing disturbances – including harmful algal blooms, commercial and recreational fishing, and climate change, among others – that disrupt their access to prey and have impacts ranging from changes in prey choice and feeding behaviors to diminished body condition, decreased fitness, and

mortality impacts (e.g., Wells, 2010; Bentaleb et al., 2011; Rossman et al., 2013; Gavrilchuk et al., 2014; Booth, 2019; Senigaglia et al., 2019; Cloyed et al., 2021; Jory et al., 2021; Groß et al., 2020; D'Agostino et al., 2026). As diet influences which animals are most vulnerable and what actions mitigate negative impacts, understanding diet is more critical than ever.

However, most marine mammals are cryptic species whose behaviors, especially feeding, occur underwater. As a result, directly observing feeding is difficult and diet must often be estimated indirectly. Multiple diet determination methods exist for this purpose, including analyses of stomach contents, fecal samples, or regurgitates that identify prey using identifiable hard parts (e.g., whole prey, otoliths, squid beaks) or DNA metabarcoding (Dunshea et al., 2013; Luna et al., 2022; Stedt et al., 2025); stable isotopes (SIs) of carbon, nitrogen, sulfur, and/or oxygen extracted from tissue samples including bone, muscle, teeth, skin, or blood (Newsome et al., 2010; Teixeira et al., 2022); and fatty acids (FAs) that are transferred from consumed prey to predator milk, blood, and blubber (Budge et al., 2006; Nielsen et al., 2018). While these methods can be used to address critical research questions, each has inherent strengths and weaknesses and provides different degrees of taxonomic resolution, integrated diet periods, and associated uncertainties, as reviewed in Chapter 1. While deciding which to use depends on research objectives, sample availability, and processing capacity, studies are increasingly combining multiple complementary methods to account for their methodological

differences (Browning et al., 2014b; Guerrero et al., 2021; Facciola et al., 2022; Heße et al., 2025).

Of these methods, quantitative analyses of fatty acids, referred to as quantitative fatty acid signature analysis (QFASA), has been applied the least frequently in marine mammals. Its use has been especially limited in cetaceans, including only killer whales (*Orcinus orca*), belugas (*Delphinapterus leucas*), and Indo-Pacific humpback dolphins (*Sousa chinensis*) (Choy et al., 2019; Ning et al., 2020; Xie et al., 2022; Remili et al., 2022, 2023) at the time of this dissertation. This is despite QFASA's potential to generate diet estimates that identify the relative proportions of prey species consumed over an integrated period of weeks to months and requirement of only a small, minimally invasive tissue sample which can be collected remotely via dart biopsy, during catch-and-release operations, or from a freshly stranded animal (Budge et al., 2006). Together, these features allow for detailed, long-term pictures of diet, focal animal choice, and easier sampling of rare, endangered, or remote species. However, QFASA's broader application has largely been impeded by methodological limitations, including a lack of species-specific calibration coefficients (CCs) that account for structural changes when FAs are deposited from prey into predator tissues, multiple model inputs and parameters for which no "best-fit" options have been defined, and uncertainty surrounding the exact dietary time frame integrated by the estimates.

My dissertation addresses these limitations and provides an example of QFASA's potential for valuable ecological insights, expanding QFASA's application

in cetaceans. In Chapter 1, I use bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphins") under professional care with known diets to derive the first dolphin- and blubber-layer specific CCs and apply them alongside non-dolphin CCs and multiple model parameter options. This allowed me to explore CC and diet estimate variability, assess QFASA's ability to accurately estimate diet in a controlled system, and provide suggestions for evaluating model performance and choosing parameters in future QFASA applications in cetaceans. I built on these findings in Chapter 2, where I applied the CCs derived in Chapter 1 and the suggested model parameter options to free-ranging dolphins with unknown diets that were potentially more complex and variable than those of the dolphins under professional care. I again assessed how varying the model inputs and parameters impacted the performance of the model and interpretability of the resulting diet estimates, quantified by examining prey species distinctiveness, overlap between predator and prey fatty acid signatures, the role of different fatty acids, the bootstrap uncertainty of each estimate, and estimate variation with different model configurations. Finally, in Chapter 3, I compared the best-performing estimates from Chapter 2 to estimates generated for the same dolphins using SI analyses and historical diet studies in the same system. This allowed me to generate a more complete picture of diet, explore the methodological and ecological implications of the findings, and demonstrate QFASA's viability for application in free-ranging cetaceans.

Chapter 1: First application of quantitative fatty acid signature analysis in bottlenose dolphins (*Tursiops truncatus*) and its implications for diet estimation in free-ranging cetaceans

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Abstract

Quantitative fatty acid signature analysis (QFASA) can provide species level diet estimates integrated over weeks to months, which are valuable for assessing health, ecological roles, and disturbance vulnerability. However, the approach has seen limited use in cetaceans. Calibration coefficients (CCs) have mainly been derived from non-cetacean mammals, best-fit QFASA model parameters are undefined, and the temporal integration of blubber fatty acids (FAs) remains poorly resolved. We used bottlenose dolphins (*Tursiops truncatus*, n = 3, hereafter "dolphin") under professional care with known, varied diets to develop and evaluate species- and blubber layer-specific CCs and explore model performance under different parameter combinations. For each dolphin, we calculated CCs for the inner and outer blubber, compared these to published non-dolphin CCs, and evaluated QFASA-estimated diets across different FA sets, distance measures, CC sources (dolphin and non-dolphin), and FA integration periods. Model performance was assessed using prey distinctiveness, the percentage of predator FAs that fell outside prey ranges [predator-beyond-prey (PBP) values], and weighted error between estimates and the dolphins' known diets. Dolphin CCs differed between the inner and outer blubber and from non-dolphin CCs for many FAs. Dolphin-specific, layer-matched CCs produced lower-error estimates and identified key prey species more accurately than non-dolphin CCs. Inner and outer blubber estimates were consistent with prey consumption integrated over weeks to months, supporting QFASA's long-term nature. However, model performance was sensitive to FA set, distance measure,

CC source, and dolphin diet complexity. In some parameter combinations, the augmented FA contributed a large portion of the model signal, reducing interpretability. This highlights the need for cautious parameter selection. These results provide the first layer-specific CCs for bottlenose dolphins and illustrate the utility and limitations of QFASA for cetacean diet. We recommend that investigators use species- and layer-specific CCs where possible and consider prey distinctiveness, PBP values, and the augmented FA's contribution when selecting model parameters. We also caution against over-interpreting best-fit parameter sets and diet estimates derived from small calibration datasets. To yield the most complete understanding of free-ranging cetacean diet, QFASA is best applied as one of several complementary methods rather than as a standalone approach.

1 Introduction

A marine mammal's diet is fundamental to health, reproduction, and survival, and is shaped by morphological, physiological, social, behavioral, and environmental factors. Understanding diet is the basis for exploring many interconnected topics, including nutrient intake, foraging niche and diet specialization, response to ecological and environmental variability, and learning and cultural transmission of behaviors (Johnson et al., 2009; Bowen and Iverson, 2013; Slifka et al., 2013; Krützen et al., 2014; Nielsen et al., 2018; Ning et al., 2020; Plint et al., 2023; García-Vernet et al., 2024). Knowledge of a marine mammal's diet is especially critical as human actions disrupt the natural environment and directly and indirectly threaten

food sources. Diet influences the vulnerability of predators and informs the actions that may be taken to mitigate negative disturbance impacts (Wells, 2010; Bentaleb et al., 2011; Rossman et al., 2013; Gavrilchuk et al., 2014; Booth, 2019; Cloyed et al., 2021; Jory et al., 2021; Groß et al., 2020; McHuron et al., 2025).

Several approaches are available to estimate marine mammal diet (Pierce and Boyle, 1991; Bowen and Iverson, 2013; Trites and Spitz, 2017; Nielsen et al., 2018). As most marine mammals are cryptic underwater predators, directly observing feeding is difficult and rarely sufficient for complete diet estimation, especially over extended periods (Nielsen et al., 2018), although sea otters are an important exception (Estes et al., 2003; Tinker et al., 2012; Hale et al., 2019; LaRoche et al., 2023; Leach et al., 2024). Instead, analyses of stomach contents, fecal samples, or regurgitates that identify diet components using identifiable hard parts (e.g., whole prey, otoliths, and squid beaks) or DNA metabarcoding are frequently employed (Dunshea et al., 2013; Luna et al., 2022; Stedt et al., 2025; Coveney et al., 2025). These methods can identify prey to the species level and quantify their relative frequency in a predator's diet. However, they have certain limitations, including providing only snapshot views of recent meals in the hours to days before sampling, differential preservation of soft- and hard-bodied prey, challenges associated with obtaining adequate samples from live, healthy animals, the possibility that stomach contents of stranded marine mammals do not represent typical diets, and requiring hard part or molecular reference databases (Nielsen et al., 2018; Coveney et al., 2025).

Two alternative methods, stable isotope (SI) and fatty acid (FA) analyses, are also widely used to estimate diet and share some fundamental similarities. Both are based on the understanding that the chemical composition of a predator's tissues is influenced by the chemical signatures of its prey (and its environment, for SIs) (Iverson et al., 2004; Budge et al., 2006; Newsome et al., 2010; Rosen and Tollit, 2012; Teixeira et al., 2022). These methods can yield diet information integrated over weeks to months to years depending on the tissue used and require only small (<0.5 g) samples collected during catch-and-release operations, via dart biopsy, or from a freshly stranded marine mammal. However, the level of detail provided by either method depends on the number and type of trophic markers measured and the availability and distinctiveness of reference prey. SI analyses generally measure several isotopes (e.g., carbon, nitrogen, sulfur, and oxygen) via bulk or compound-specific analyses (Newsome et al., 2010; Teixeira et al., 2022). In comparison, FA analyses often quantify over 40 FAs, though not all are directly associated with prey consumption (Budge et al., 2006; Nielsen et al., 2018). If prey FA or SI values are not available or extensive, both analyses can provide diet-related information without estimating diet composition. For SI analyses, this can include foraging habitat, prey trophic levels, niche width, or trophic overlap between species (e.g., Burton and Koch, 1999; Troina et al., 2020; Feddern et al., 2022). Similarly, FAs may be used in so-called "qualitative" analyses to describe foraging patterns among predators, explore overlap between predator and prey FA profiles, or identify biomarkers indicative of certain prey species (Iverson et al., 1997b; Land-Miller et al., 2024;

Loseto et al., 2009; Pomerleau et al., 2016). In turn, if a reference "library" of potential prey SI or FA values is available, both methods can estimate the proportions of prey species or groups in a predator's diet (Iverson et al., 2004; Wilson et al., 2017; Goetsch et al., 2018; Lerner et al., 2018; Tessier-Larivière et al., 2026). However, the smaller number of SIs often restricts the number of unique diet items the method can identify (Phillips et al., 2014). In comparison, quantitative fatty acid signature analysis (QFASA) can estimate the percent contribution to diet of as many unique prey as the number of diet-associated FAs measured in the samples of interest (generally 15+) (Goetsch et al., 2018; Nielsen et al., 2018). Ultimately, each diet determination method can be used to answer critical research questions, but their different strengths and limitations mean some may be better suited to certain studies, systems, or available samples. As a result, studies are increasingly combining multiple methods (Browning et al., 2014b; Guerrero et al., 2021; Facciola et al., 2022; Heße et al., 2025).

However, unlike hard part, DNA metabarcoding, SI, or "qualitative" FA analyses, QFASA's application in marine mammals has been relatively limited, especially in cetaceans. QFASA has been applied in several pinniped species to date, although often only in a single study per species. These species include grey, harbor, harp, hooded, and northern elephant seals (*Halichoerus grypus, Phoca vitulina, Pagophilus groenlandicus, Cystophora cristata,* and *Mirounga angustirostris*, respectively), New Zealand and Steller sea lions (*Phocarctos hookeri* and *Eumetopias jubatus*, respectively), and Northern and Australian fur seals (*Callorhinus ursinus* and

Arctocephalus pusillus doriferus, respectively) (Iverson et al., 2004; Beck et al., 2007; Nordstrom et al., 2008; Tucker et al., 2009; Meynier et al., 2010; Rosen and Tollit, 2012; Meynier et al., 2014; Goetsch et al., 2018; Knox et al., 2019). It has also been applied in polar bears (*Ursus maritimus*) with some frequency (e.g., Thiemann et al., 2008, 2011; McKinney et al., 2013; Bourque et al., 2020). In comparison, application in cetaceans has been more limited, including only killer whales (*Orcinus orca*), belugas (*Delphinapterus leucas*), and Indo-Pacific humpback dolphins (*Sousa chinensis*) (Choy et al., 2019, 2020; Ning et al., 2020; Remili et al., 2022; Xie et al., 2022; Remili et al., 2023).

Multiple methodological factors have impeded QFASA's broader application. The most frequently cited is the lack of appropriate calibration coefficients (CCs) (e.g., Budge et al., 2006; Meynier et al., 2010; Knox et al., 2019; Choy et al., 2019; Remili et al., 2022; Xie et al., 2022). FAs, chains of carbon, hydrogen, and oxygen atoms, form energy storage building blocks in most animals. In marine mammals, FAs are concentrated in blubber, the primary fat storage tissue, and originate from several sources (Iverson et al., 2004; Samuel and Worthy, 2005; Budge et al., 2006). Some FAs are primarily biosynthesized by the predator, some are exclusively consumed via prey and incorporated into the blubber, and some originate from both dietary intake and biosynthesis. CCs are correction factors that account for small changes in prey FA proportions when they are digested and assimilated into predator tissues (Budge et al., 2006). Each FA in a FA signature (the mass percentage of FAs measured in a given sample) has a CC, calculated by comparing the signature of a

predator with a known diet to the signatures of its prey items (Budge et al., 2006). However, acquiring FA data from predators with known diets requires sampling mammals within research programs, aquariums, or zoos. As a result, CCs have to date been derived from only a limited number of mammals, including mink (*Mustela vison*), brown bears (*Ursus arctos*), six pinniped species, and killer whales (Iverson et al., 2004; Nordstrom et al., 2008; Thiemann et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012; Goetsch et al., 2018; Remili et al., 2022; Thiemann et al., 2022). The shortage means CCs have been applied across species and taxa when species-specific values are not available. QFASA studies in belugas and Indo-Pacific humpback dolphins with known diets applied CCs derived from mink that consumed herring and/or seal oil (Choy et al., 2019, 2020; Ning et al., 2020; Xie et al., 2022). Mink and brown bear CCs have also been used to estimate polar bear diet (e.g., Thiemann et al., 2008; Bourque et al., 2020; Thiemann et al., 2022). In pinnipeds, Steller sea lion CCs have been applied to Australian fur seals and New Zealand sea lions, while harp and grey seal CCs have been applied to hooded seals (Tucker et al., 2009; Meynier et al., 2010; Knox et al., 2019).

However, the interchangeability of CCs across species and taxa is uncertain. Studies in harbor seals, New Zealand sea lions, and Steller sea lions that applied cross-taxa and -species CCs found estimated prey contributions differed when different pinniped CCs were applied (Nordstrom et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012). Mink CCs applied in belugas and Indo-Pacific humpback dolphins estimated the identity and quantities of the most prevalent prey but

sometimes struggled to identify other prey (Choy et al., 2019; Xie et al., 2022). The only study with cetacean-specific CCs found inner and outer blubber killer whale CCs provided more accurate estimates than mink or pinniped CCs when applied to killer whales (Remili et al., 2022). Taxa- and species-specific physiological differences introduce further uncertainty. Digestive processes fundamentally differ among monogastric pinnipeds, mink, and brown bears (order Carnivora) and polygastric cetaceans (order Artiodactyla) (Horstmann, 2018). Blubber characteristics also vary significantly. In cetaceans, blubber is generally highly stratified (Koopman, 2007). The inner blubber contains a higher proportion of FAs derived mostly or exclusively from prey. It also experiences greater FA turnover, as proximity to the capillary and muscle layers leads to higher metabolic activity (Samuel and Worthy, 2005; Koopman, 2007; Ellisor et al., 2013). In contrast, the outer blubber is less vascularized (McClelland et al., 2012) and is more closely associated with thermoregulation (Koopman, 2007). Pinniped blubber is also stratified to varied extents (Best et al., 2003; Budge et al., 2006; Strandberg et al., 2008; Guerrero et al., 2016) but existing pinniped CCs are not layer specific (Iverson et al., 2004; Nordstrom et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012; Goetsch et al., 2018). Mink and brown bears, as non-marine mammals, lack blubber entirely. These differences in digestion and blubber structure suggest that despite their shared carnivorous diet, mink, brown bears, pinnipeds, and cetaceans differ in FA metabolism and thus CCs. Finally, the methods used to calculate CCs and diet estimates further affect CC interchangeability. Marine mammals under professional

care often consume simple diets (e.g., herring or herring and capelin only) with specific FA signatures. In turn, CCs derived from these predators may be pre-disposed to identify those prey or species with similar FA signatures (Budge et al., 2006; Rosen and Tollit, 2012; Happel et al., 2016). This may lead to estimates that over-estimate certain prey, mis-identify prey as important in diet due to FA similarities rather than actual consumption, or under-estimate prey not consumed by the species from which the CCs were derived (Nordstrom et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012). Together, studies questioning the cross-taxa interchangeability of CCs, taxa- and species-specific differences in digestive processes and blubber physiology, and the possibility for CC-related biases towards certain prey FA signatures suggest that the most accurate QFASA diet estimates are obtained using species and blubber layer-specific CCs, ideally derived from predators fed varied diets to diminish bias.

Uncertainty about the time frame of prey consumption (the "temporal window", Thiemann et al., 2022) and the accuracy of diet estimates recorded in the inner versus the outer blubber has further impacted QFASA's broader application. Inner blubber, with a higher FA turnover rate and larger proportion of prey-derived FAs, may more accurately reflect diet and/or capture a shorter temporal window (Budge et al., 2006; Remili et al., 2022). In comparison, the more structural outer layer may integrate a longer period of prey consumption, record diet from an earlier time, or be less reflective of a predator's diet overall. A study in belugas suggested that their inner blubber may represent diet from 2 to 5 weeks before sampling (Choy et al., 2019),

while studies in pinnipeds and polar bears indicated that their blubber, analyzed as a homogeneous layer, may reflect diet assimilated 1.5 to 3 months before sampling (Iverson et al., 2004; Budge et al., 2006; Nordstrom et al., 2008; Thiemann et al., 2022). Additionally, the only study comparing inner- and outer-blubber layer QFASA diet estimates, in killer whales, found that both layers accurately estimated the diet when layer-matched CCs (i.e., CCs derived from inner blubber applied to inner blubber samples and outer blubber CCs applied to outer blubber samples) were used (Remili et al., 2022). However, no studies have quantified FA turnover rate or temporal windows across both inner and outer cetacean blubber, or compared the estimates generated from different bottlenose dolphin blubber layers. Estimating cetacean diet using outer blubber only is sometimes necessary, as free-ranging cetacean blubber samples are frequently collected via dart biopsies, which may only extract the outer blubber (Remili et al., 2022). Accordingly, a clearer understanding of the temporal window integrated by the inner and outer cetacean blubber layers, and the relative accuracies of their estimates, is necessary to apply QFASA appropriately.

The lack of accepted "best-fit" model parameters to use when applying QFASA has further contributed to uncertainty. The QFASA statistical model estimates diet by determining which mixture of potential prey species yields a FA signature that most resembles the predator's signature (Budge et al., 2006). This involves two model parameter decisions: which subset of measured FAs to use in the analysis (the "FA suite") and the distance measure that quantifies the similarity between the prey mixtures and the predator. As blubber FAs originate both via biosynthesis and from

prey, two general FA suite categories exist. These include FAs derived entirely or primarily from dietary intake ("dietary" FAs) or the dietary FAs plus the FAs with relatively large contributions from both biosynthesis and diet ("extended dietary" FAs) (Iverson et al., 2004). Concurrently, three distance measures the Aitchison, Kullback–Leibler (KL), and chi-square distances can be used to determine the estimates that minimize the distance between predator and prey FA signatures. However, their analytical approaches differ and thus can yield different QFASA diet estimates. There is no consensus on which FA suite or distance measure is best or on the conditions under which one may be more appropriate than another (Bromaghin, 2015; Bromaghin et al., 2015; Bromaghin, 2017; Stewart et al., 2025). Existing QFASA studies in marine mammals have frequently used the KL distance with the extended dietary or dietary suites (e.g., Iverson et al., 2004; Beck et al., 2007; Tucker et al., 2009; Meynier et al., 2010; Remili et al., 2022, 2023), but the Aitchison distance with both suites have been employed as well (e.g., Goetsch et al., 2018; Choy et al., 2019; Ning et al., 2020; Xie et al., 2022). Assessing whether certain parameters consistently provide more accurate estimates would provide valuable guidance for future studies.

We used bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphin") under professional care as a controlled validation system to address these key methodological limitations impeding QFASA's broader application in cetaceans. These dolphins provided three advantages: archived inner and outer blubber, long-term records of prey consumption, and different diets that varied over time. Our

objectives were to determine whether (1) CCs differed among dolphin diets, blubber layers, species, and mammalian taxa, and identify drivers of variation; (2) CC differences translated into meaningful variation in diet estimates; (3) QFASA model parameters (FA suites and distance measures) affected model fit and accuracy, and a consistent "best-fit" parameter combination could be identified; (4) inner and outer blubber accurately reconstructed known dolphin diet; and (5) the two blubber layers integrated diet over different temporal windows. To address these objectives, we (i) derived the first bottlenose dolphin inner and outer blubber CCs from the individual professional care dolphins, (ii) compared these CCs with each other and with published non-dolphin CCs, (iii) evaluated how CC and model parameter choices influenced model fit and diet estimate accuracy, and (iv) assessed the temporal integration and accuracy of diet estimates from inner versus outer blubber. We hypothesized that CCs would vary most among taxa, followed by species, blubber layer, and diet, and that dolphin-specific, layer-matched CCs would outperform non-dolphin CCs. We further expected both inner and outer blubber to accurately estimate diet but integrated over different time scales. Finally, we expected that diet estimates would vary with different model parameters but no single optimal combination would be apparent. Through these objectives, our study addresses gaps limiting QFASA's application in cetaceans. We expand the available cetacean CC dataset, evaluate CC interchangeability and sources of variation, provide guidance on model parameter selection, and clarify the feasibility and temporal resolution of diet estimation from

inner and outer blubber. Together, our results expand our understanding of the capabilities and limitations of QFASA for bottlenose dolphins and other cetaceans.

2 Materials and methods

The QFASA model estimates diet by minimizing the statistical distance between the actual blubber FA signature of the marine mammal of interest (hereafter, "predator") and model FA signatures constructed from a "library" of prey FAs, corrected for effects of metabolism with CCs (Iverson et al., 2004; Budge et al., 2006). QFASA requires two inputs: FA signatures from (1) the predator and (2) a representative mixture of their potential prey. Three parameters influence the diet estimates: (1) the CCs applied to the predator or prey FAs, (2) the FAs used in the model, and (3) the statistical measure (Aitchison, KL, or chi-square) quantifying the distance between predator and prey FA signatures. Two model criteria must be considered: prey species distinctiveness and significant overlap in predator and potential prey FA signatures. Model inputs, parameters, criteria, and the diet estimates and their accuracy were addressed as follows. Analyses were conducted using Microsoft Excel and R (R Core Team, 2025).

2.1 Model inputs: dolphin and prey fish FA signatures

All blubber samples came from dolphins (n = 3, hereafter referred to as dolphins D1, D2, and D3; Supplementary Table 1) housed and cared for by the U.S. Navy Marine Mammal Program (MMP), Naval Information Warfare Center (NIWC)

Pacific, in San Diego Bay (CA, USA). Full-depth blubber biopsies were collected during post-mortem necropsies from the mid-lateral region below the dorsal fin, under the authority codified in U.S. Code, Title 10, Section 7524. Dolphins D1 and D2 died after brief illnesses (fungal vasculitis and fungal pneumonia), while D3 died of natural causes at an advanced age. Secretary of Navy Instruction 3900.41H directs that Navy marine mammals be provided the highest quality of care. NIWC Pacific is accredited by AAALAC International and adheres to the national standards of the U.S. Public Health Service policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. Dolphin prey fish specimens (n = 31 from 6 species; Supplementary Table 1) were also provided by the U.S. Navy MMP. Blubber and whole fish were stored at −80°C and −20°C, respectively, until processing. Each dolphin had a different diet: D1 consumed capelin (*Mallotus villosus*) and Pacific herring (*Clupea pallasii*, hereafter "herring"), D2 consumed capelin, herring, squid (*Loligo opalescens*), and mullet (*Mugil cephalus*), and D3 consumed capelin, herring, squid, mullet, Atlantic thread herring (*Opisthonema oglinum*), and croaker (*Micropogonias undulatus*). The consumed percentages of certain prey species varied during the months before each dolphin's blubber was sampled (Supplementary Table 2), addressed in Section 2.2.1.

Sample processing followed established protocols. Blubber was subsampled into three layers (inner, middle, and outer) based on color and texture, accounting for FA differences due to stratification (Samuel and Worthy, 2005; Koopman, 2007; Ellisor et al., 2013). Given the shallow blubber depth (<2 cm), the middle layer was treated

as a transition zone and excluded from further analyses. Since dolphins consume whole prey, fish were partially thawed, homogenized using a bullet blender and scalpel, and separated into 0.5 g subsamples. Blubber and fish lipids were extracted in 2:1 chloroform:methanol using a Soxtec apparatus (Anderson, 2004) and transformed into fatty acid methyl esters (FAMEs) using the Hilditch method, then each sample's FA signature was determined via gas chromatography with flame-ionization detection (GC-FID) as previously established (Budge et al., 2008). The relative contributions of 64 FAs, from 14:0 to 24:1n-9, were measured. FAs are named using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B denotes the number of double bonds, and X indicates the position of the double bond closest to the terminal methyl group. Lipid percentages in blubber and fish samples were determined using an internal standard to calculate mass percent FA, a proxy for percent lipid. Tissue sample processing and lipid extraction were performed at the University of California, Santa Cruz, USA, while FAME derivatization and GCFID were performed at Dalhousie University, Halifax, Canada.

Each dolphin's prey library consisted of their known prey species only. This study was designed as a controlled validation of QFASA methodology, and additional prey could confound our evaluation of model performance. QFASA is sensitive to prey species choice, and including unconsumed prey could lead to spurious misallocation of diet proportions (Bromaghin, 2015; Bromaghin et al., 2015, 2016b; Remili et al., 2022). This could obscure whether estimation error arises from the focus areas of our study: different CCs, model parameter combinations, blubber layers, and temporal

variation in diet. Previous validation studies in marine mammals under professional care have used the same approach (Iverson et al., 2004; Choy et al., 2019; Remili et al., 2022). Furthermore, even QFASA applications in free-ranging predators, where the exact diet is unknown, are encouraged to use ecologically likely prey only, owing to the same concerns about unnecessary misallocation (Budge et al., 2006).

2.2 Model parameters

2.2.1 Calibration coefficients

A FA's CC is determined by dividing the measured proportion of that FA in the predator by the expected proportion, calculated based on the FA proportions in the prey fish and their percentage in the predator's known diet (see Supplementary Methods for details). However, we did not have a uniform known diet for calculating dolphin CCs. Dolphins D1, D2, and D3 consumed different prey mixtures in compositions that varied over the months before sampling (Figure 1, Supplementary Table 2). No temporal windows or FA turnover rates exist for dolphin blubber that we could use to select a single known diet. Instead, we defined 13 integrated diets for each dolphin by calculating the average percentages of each prey species consumed over the entire period between 0 and x weeks before sampling, with x = 2, 4, 6, 8..., 26 (Supplementary Table 2). For example, D1 consumed an average of 51.8% capelin and 48.3% herring over the last 2 weeks before sampling (x = 2) and an average of 70.7% capelin and 29.3% herring over the entire 26 weeks (x = 26). The 26-week period was selected to expand on studies indirectly suggesting that blubber represents

the average diet consumed 2 to 5 weeks (in belugas, Choy et al., 2019) or 1.5 to 3 months before sampling (in pinnipeds and polar bears, Budge et al., 2006; Nordstrom et al., 2008; Thiemann et al., 2022). Then, for each of the 64 FAs measured and for the inner and outer blubber layers separately, we used each dolphin's 13 integrated diets, its FA signature, and the relevant prey items' FA signatures to derive one set of CCs from that dolphin. This yielded three sets of "one-dolphin" CCs, for D1, D2, and D3. We also calculated two sets of average CCs for each integrated diet period (x = 2, 4,..., 26 weeks before sampling), FA, and blubber layer: the average of the three dolphins' CCs ("three-dolphin" CCs) and the average CCs from each pair of dolphins (i.e., D1 and D2, D1 and D3, and D2 and D3; "two-dolphin CCs").

CCs from herring-fed mink, herring-fed harp, harbor, and grey seals, and the inner and outer blubber of killer whales came from the existing literature (Supplementary Table 3; Iverson et al., 2004; Nordstrom et al., 2008; Thiemann et al., 2008; Rosen and Tollit, 2012; Remili et al., 2022). We selected these CCs, hereafter referred to as "non-dolphin CCs", following their use in previous cetacean QFASA studies (Choy et al., 2019, 2020; Ning et al., 2020; Remili et al., 2022, 2023; Xie et al., 2022). However, we lacked non-dolphin CCs for certain FAs quantified in our dolphins, as some FAs were absent, not measured in the original study, or not part of the validated suite of published CCs. CCs were compared qualitatively and quantitatively. Boxplots were used to visualize how CCs varied among multiple factors: FA, blubber layer, dolphin identity, CC species, and integrated diet period.

We used R package VCA (Schuetzenmeister and Dufey, 2025) to conduct variance component analyses quantifying which factors contributed most to CC variation. To disentangle the factors' effects, we compared (1) all one-dolphin inner and outer blubber CCs together, (2) inner and outer blubber CCs separately, (3) CCs for each dolphin separately, and (4) all non-dolphin and one-dolphin CCs, separated by blubber layer. All factors were treated as nested random effects. CC comparisons allowed us to (1) explore how CCs derived from the same species were impacted by diet variation over time, diet complexity, and blubber layer, and (2) assess the interchangeability of CCs between species and taxa.

2.2.2 Distance measures and FA suites

As the majority of QFASA applications in marine mammals have used some combination of the Aitchison and KL distances and the extended dietary and dietary FA suites, we compared estimates generated using those four model parameter combinations (i.e., the Aitchison distance with both FA suites and the KL distance with both FA suites). The "dietary" suite included FAs derived entirely or primary from dietary intake (following established classifications, Iverson et al., 2004) that were greater than 0.1% of all dolphins' total FA signatures in each blubber layer (Supplementary Table 4). The "extended dietary" suite included the dietary FAs plus the FAs greater than 0.1% with relatively large contributions from both biosynthesis and diet (Supplementary Table 4). FA suites varied slightly between the inner and outer blubber, as FA proportions differed between blubber layers. We also excluded FAs lacking non-dolphin CCs when estimating diet with those CCs.

2.3 Calculating QFASA diet estimates

Inner and outer blubber estimates for D1, D2, and D3 were made using the two distance measures (Aitchison and KL), the two FA suites (extended dietary and dietary), and 97 CCs for each FA. CCs included the non-dolphin CCs and inner and outer blubber one-, two-, and three-dolphin CCs. Only one- and two-dolphin CCs from the other two dolphins were applied to each dolphin (i.e., one-dolphin CCs from D2 and D3 and their averages—the two-dolphin CCs—were applied to D1, etc.). After removing FAs not in the suites, the remaining FA proportions were augmented (Bromaghin et al., 2016b; Bromaghin, 2017, 2020). Compared to scaling remaining FAs to sum to 1, augmenting maintains the same FA proportions and accounts for removed FAs by combining their proportions into a single "augmented" FA with its own CC. Preserving the FA signatures' compositional features is important when unscaled FA proportions differ among prey species, as with our data (Supplementary Table 5; Bromaghin et al., 2016). Diet estimates were calculated in the predator space using the function "est_diet" in the R package qfasar (Bromaghin, 2020). Raw diet estimates (% lipid mass per prey species) were adjusted to account for each species' lipid content; thus, diet estimates indicated the % biomass per prey species. The diet estimates' means and standard errors (SEs) were obtained using bootstrap sampling (n = 100).

2.4 Assessing best-fit inputs and parameters using model criteria and estimate accuracy

We employed multiple metrics to explore which parameter combinations provided better-fitting models, acceptable estimate accuracy, and biologically interpretable diets. First, two criteria influence QFASA performance and indicate model fit: prey species distinctiveness and predator and prey FA signature overlap (Iverson et al., 2004; Bromaghin et al., 2015b; Bromaghin, 2017). We analyzed distinctiveness and overlap using, respectively, the "lopo" and "pred_beyond_prey" functions in the R package qfasar (Bromaghin, 2020). In LOPO (leave-one-prey-out) analyses, each prey sample is successively treated as an unknown species and grouped with other samples based on FA signature similarity. High self-attribution rates indicate that prey species have distinct FA signatures and are therefore more likely to be distinguished by the QFASA model and in predator diets. Predator-beyond-prey (PBP) values are the percentage of each predator's measured FAs whose proportions fall outside the observed range for that FA in the prey library after modification with CCs. High percentages suggest that predator signatures are poorly represented by the prey library or CC set and reduce confidence in the diet estimates for those parameter combinations.

Second, to quantify estimate accuracy, for each dolphin's 13 integrated diet options and estimates calculated using each combination of CCs, distance measure, and FA suite, we calculated species-specific percent error and a weighted error (WE) that summarized overall deviation between the estimated diet and the dolphin's

integrated diet (see Supplementary Methods for equations). Lower WEs indicate overall closer agreement with the integrated diet. WEs also account for the fact that prey constituting small portions of the diet will generate large errors when estimates differ from expected values by just a few digits, skewing apparent estimate accuracy. For estimates using dolphin CCs, we considered only WEs where the same integrated diet period was used to calculate both the CCs and WEs. For example, if D1's one-dolphin CCs were calculated using its diet averaged over the 2 weeks before sampling and then applied to estimate D3's diet, we only considered WEs calculated using D3's diet averaged over the 2 weeks before sampling.

To complement the WEs, we examined the average prey species percentages and SEs of individual estimates. As aggregate error measures, WEs are useful indicators of estimate accuracy but can conceal meaningful differences. Different prey percentage combinations can yield similar WEs when more than two prey species are considered. In addition, SEs are not included in WE calculations. For prey species that are small components (e.g., 0%10%) of a dolphin's diet, a SE of 1%–3% is significant when considering the overall estimate accuracy. Finally, QFASA diet estimates ideally identify all prey species and their percentages in the diet. In practice, however, some model parameters may provide more accurate percentages of major prey but struggle to identify minor diet components. In contrast, others may identify all species at the cost of less accurate percentages. Examining WEs alone can miss these nuances.

Finally, we quantified the percent contribution of each FA in the FA suite to each diet estimate using the PropDistCont measure in the R package QFASA (Stewart et al., 2024; see Supplementary Methods for details). While many FAs are included in each suite, not all contribute equally to the final diet estimate. Assessing FA contributions allowed us to explore how they varied with CC source, distance measure, and FA suite; to consider connections between predator and prey FAs; and to identify whether certain FAs dominated the estimates.

Overall, we considered "best-fit" parameter combination(s) to be those yielding reasonably distinctive prey, acceptable percentages of predator FAs falling beyond prey values, biologically realistic FA contributions, low WEs, and estimated diets with SEs that fell within range of the dolphins' integrated diet compositions.

Analyzing estimates calculated using multiple CC sets, FA suites, and distance measures served several purposes. First, estimating diet using dolphin CCs generated using 13 integrated diets made it possible to explore which of the diet periods most resembled the estimates, indirectly quantifying and comparing the temporal windows captured by inner and outer blubber. Second, applying both individual and average CCs allowed us to cross-validate QFASA's accuracy. Estimating each dolphin's diet using three-dolphin CCs—which include the dolphin's own CCs in the average value—alongside one- and two-dolphin CCs derived exclusively from the other dolphins addresses the tautological question of whether diet estimates are accurate simply because a dolphin's own CCs are part of the calculation. Third, by comparing estimates generated with CCs across multiple species, diets, and blubber layers, we

examined how these differences affected estimate accuracy and assess CC interchangeability. Finally, our comparison of estimates made using multiple model parameter combinations was an exploratory evaluation of parameter behavior rather than a formal model-selection exercise with cross-validation. Nonetheless, it adds to the existing literature and provides guidance for future studies.

3 Results

3.1 Dolphin and prey fish FA signatures

All three dolphins' FA signatures (Figure 2, Supplementary Table 6) shared general patterns. Of the 64 measured FAs, 18 comprised 88.1% and 88.2% of the total FAs in the outer and inner blubber, respectively. The total percentages of dietary FAs were almost twice as high in the inner as the outer blubber (38.1% ± 4.1% inner, 21.9% ± 0.7% outer, averaged across the three dolphins). Extended dietary FAs were overall more prevalent in the inner than in the outer blubber (89.9% ± 1.0% and 82.9% ± 1.9%, respectively). However, individual relationships varied by FA. For instance, the extended dietary FAs 18:1n-9 and 16:1n-7 were the most common FAs for both layers in all three dolphins but had higher percentages in the outer than in the inner blubber (24.7% ± 1.7% outer, 19.3% ± 1.5% inner; 21.9% ± 0.7% outer, 12.6% ± 1.9% inner). Other dietary-associated FAs had smaller percentages but were also among the most common FAs in all dolphins and had higher amounts in the inner than in the outer blubber. This included the dietary FAs 20:1n-9 (8.4% ± 1.7% inner, 4.3% ± 0.5% outer), 22:1n-11 (11.0% ± 3.5%, 2.6% ± 0.5%), and 22:6n-3 (5.3% ±

1.4%, 3.3% ± 0.5%) and the extended dietary FAs 14:0 (5.7% ± 0.3%, 4.8% ± 0.5%) and 16:0 (6% ± 1.1%, 3.9% ± 0.4%).

While the three dolphins shared the most common FAs, small differences in relative amounts were visible (Figure 2, Supplementary Table 6). For instance, dolphin D2's inner blubber had twice as much 17:0 as D1 or D3 (1.2% versus 0.6%) and more 22:6n-3 (6.8% versus 4.1% and 5.0%). D2 and D3's inner blubber also had twice as much 22:5n-3 as D1 (3.4% and 3.6% versus 1.8%), and slightly more 18:1n-9 in the outer blubber (25.6% versus 22.7%). In comparison, D1's inner blubber had slightly more 20:1n-9 (10.3% versus 7.4%) and 22:1n-11 (15.0% versus 9.0%).

Fish FA signatures (Figure 3, Supplementary Table 7) varied among species but shared common FAs with the dolphins, suggesting dietary links. For all six prey species, 12 of the 64 measured FAs accounted for 73.2%–92.7% of the total FAs. 16:0, 16:1n-7, 18:0, 18:1n-9, 20:1n-9, and 22:6n-3 were found at high levels in most species, consistent with the significant levels observed in the dolphins. In addition, individual species showed particularly high or low values for certain FAs, which also varied among dolphins. For instance, compared to other species, capelin had higher levels of 20:1n-9 (15.5% versus 0.4%–3.2% for the other species) and 22:1n-11 (19.1% versus 0.01%–3%). 20:1n-9 and 22:1n-11 were top dietary FAs in all three dolphins but were more prevalent in D1. This is consistent with capelin making up the largest portion of D1's diet and significant but lesser portions of D2 and D3's intake. Similarly, herring was another key part of all three dolphins' diets and had higher levels of 18:1n-9 (25.9% versus 2.3%9.3% for the other species), a common

extended dietary FA in dolphin blubber. Finally, squid, Atlantic thread herring, and croaker had higher levels of 22:6n-3 (36.6%, 21.4%, and 15.1% versus 4.7%7.8% for the other species). 22:6n-3 was more prevalent in D2, which ate the largest percentage of squid, and to a lesser extent D3, the only dolphin to consume Atlantic thread herring and croaker.

3.2 Calibration coefficients

The CCs for the FAs used for diet estimation varied to different extents by FA, between blubber layers, by dolphin and by species, and within dolphins due to the different integrated diets used to calculate the CCs (Figures 4, 5, Supplementary Tables 3, 8). In addition, dolphin D1's outer-layer CCs for 22:4n-6 and 22:5n-6 differed noticeably from all other CCs for those FAs, including D1's inner-layer CCs. While 21.1% of one-dolphin inner blubber CCs, 33.3% of pinniped CCs, 30% of killer whale inner blubber CCs, and 23.3% of mink CCs were between 0.8 and 1.2, just 7.7% of one-dolphin outer blubber and 3.3% of killer whale outer blubber CCs were within that range. All one-dolphin CCs were less than 3.0, except for CCs for 20:1n-11 (CC of 6.1–8.3), 22:5n-3 (2.3–4.0), and 22:4n-6 (1.5–5.7) for the inner blubber and 16:4n-3 (4.8–6.6), 20:1n-11 (3.3–4.1), 22:4n-6 (0.4–22.9), and 22:5n-6 (0.3–9.2) for the outer blubber. For all the inner blubber one-dolphin CCs, non-dolphin CCs varied by less than 30% for just 20 of the 150 CC–FA combinations, and just 18 of the combinations for the outer blubber dolphin CCs. In comparison, 30 and 54 of the combinations varied by more than 100% from the inner and outer blubber

one-dolphin CCs. One-dolphin inner and outer blubber CCs varied by less than 15% for just two FAs (18:3n-4 and 18:2n-6), by 15%–30% for 6 FAs, and by greater than 100% for 4 FAs (16:1n-7, 16:4n-3, 22:4n-6, and 22:5n-6).

VCA results quantify and support the CC variation visible in the boxplots. When 22:4n-6 and 22:5n-6 were excluded, patterns in CC variation were apparent. FA identity accounted for most of the CC variation (54.8%–96.1%) across all comparisons (Supplementary Tables 9A–D), indicating that the degree of modification and integration of FAs in dolphin blubber varied depending on the FA. The next largest contributor was blubber layer (36.2%–44.6%, Supplementary Tables 9A, C), indicating that CCs for each layer were more similar than the other layer's CCs for a given FA, even among dolphins. When applicable, CC species (i.e., mink, pinnipeds, killer whales, and dolphins) contributed the next 25% of the variation (Supplementary Table 9D), followed by dolphin identity (3.2%–6.3%, Supplementary Tables 9A, B). This reflects the greater physiological differences among species than among individuals of the same species. Visual differences among the pinniped CCs for certain FAs (e.g., 18:1n-9, 20:2n-6, and 22:1n11) and between the killer whale and bottlenose dolphin CCs even when separated by blubber layer (e.g., 16:3n-4, 18:3n-3, 20:3n-6, 21:5n-3, and 22:6n-3) further demonstrate species-specific differences (Figures 4, 5).

In comparison, including 22:4n-6 and 22:5n-6 changed FA contribution patterns, revealing the CCs' impacts. FA identity remained the largest contributor when considering inner blubber CCs alone (e.g., 88.6% and 71.9% with 22:4n-6 and 22:5n-

6, 92.8% and 74.2% without, Supplementary Tables 9B, D). However, dolphin identity and CC species became the largest contributors for any comparisons that included outer blubber CCs (e.g., 86.3% and 83.8% with 22:4n-6 and 22:5n-6, 3.2% and 24.6% without, Supplementary Tables 9B, D). The change in the outer but not the inner blubber comparisons and the greater contribution of dolphin identity when 22:4n-6 and 22:5n-6 are included suggest that D1's outer-layer CCs for 22:4n-6 and 22:5n-6 are outliers that could meaningfully impact diet estimates. The high CCs could have dietary or physiological explanations or result from sample processing errors. However, as significant outliers can bias estimates (Iverson et al., 2004; Rosen and Tollit, 2012; Remili et al., 2022), 22:4n-6 and 22:5n-6 were excluded from all further analyses.

Variation in CCs among dolphins due to diet or fundamental physiological differences (indicated by dolphin identity) was present but minor (3.2%–6.3%, Supplementary Tables 9A, B). Comparing each dolphin separately alongside the boxplot supported minor between-dolphin differences. Variation due to FA differed the most among dolphins (54.8% for D1, 61.5% for D2, and 62.0% for D3), while variation due to blubber layer decreased slightly from D1 to D3 (44.6%, 38.3%, and 36.6%) (Supplementary Table 9C). Residual errors within individuals (which include the effect of diet change over the 26 weeks before sampling) were overall very low, though increased slightly from D2 to D1 to D3 (0.2%, 0.6%, and 1.4%, respectively). Slightly greater variation among FAs and with diet variation for D1 and D3 compared to D2 is consistent with their integrated diets and visible in the CCs (Figures 1, 4, 5,

Supplementary Table 2). Overall, D2 had a more consistent diet over the 26 weeks and less spread in CC values, while D1 and D3 had greater variation in prey species percentages and visible spread in CC values for specific FAs (e.g., 16:3n-4, 17:0, 18:1n-9, 18:3n-4, 20:1n-7, 20:1n-9, 20:3n-6, 20:4n-6, and 22:5n-3). In turn, certain fish species had particularly small or large percentages of those FAs relative to the other species, explaining the CC variation (Figure 2, Supplementary Tables 6, 7). For instance, capelin had a low percentage of 17:0 and high percentages of 20:1n-9, 22:1n-9, and 22:1n-11 while herring had a high percentage of 18:1n-9, in line with variation in D1's CCs for those FAs. Similarly, mullet (and Atlantic thread herring and croaker, in some instances) had high percentages of 16:2n-4, 16:3n-4, 18:3n-4, 20:3n-6, 20:4n-6, and 22:5n-3, consistent with variation in D3's CCs for those FAs.

3.3 Model criteria and diet estimate accuracy

3.3.1 Prey distinctiveness

Prey species were overall distinct, but distinctiveness varied among species and with the FA suite and distance measure used (CCs do not impact LOPO results) (Supplementary Table 10). Squid had the highest self-attribution (>97.2%), followed by capelin (>91.2%), Atlantic thread herring (>91.0%), and mullet (>87.4%). Herring (74.6%–96.5%) had the most variable attribution: 81.2%–89.6% using the Aitchison distance and either FA suite, but lower values with the KL distance and the dietary suite (74%–83%) versus higher values with the extended dietary suite (86%–92%). Herring confounded most with mullet (up to 17.6% similarity) and Atlantic thread

herring (up to 7.1% similarity). Croaker had the lowest attribution (61.5%–68.9% with the Aitchison distance and 71.1%–76.4% with the KL distance, across both FA suites). It was most similar to Atlantic thread herring (up to 26.5%) and mullet (up to 11.7%).

3.3.2 Predator and prey overlap

The percentages of each dolphin's FAs with proportions outside the range of prey signatures (the "PBP percentage") were similar whether the Aitchison or KL distance was used and uniformly highest when applying non-layer matched and non-dolphin CCs (Supplementary Table 11). PBP percentages varied among dolphins and dolphin CCs; however, incorporating FA contributions added nuance. Results were straightforward for D3: all layer-matched dolphin CCs had <11.5% PBP (0% when using two- and three-dolphin CCs) no matter the FA suite or distance measure. In addition, none of the top contributing FAs (i.e., FAs together accounting for >80% of the FA contribution to the estimates) for the lowest error estimates had predator values falling beyond prey values (Supplementary Table 20). D2 had comparably low percentages (<13.6%) with layer-matched three- and two-dolphin CCs, no matter the other model parameters, but more variable percentages with one-dolphin CCs (0%–22.7% with D1's CCs and 0%–13.6% with D3's CCs) (Supplementary Table 11). In comparison, D1 had higher percentages with all CCs, though values were lower for three-dolphin CCs (11%–28%) than two- and one-dolphin CCs (22%–54%) (Supplementary Table 11). However, top contributing FAs had predator values

beyond prey values for D1 and D2's inner blubber estimates only when one- and two-dolphin CCs were used (Supplementary Tables 18, 19). By contrast, top contributing predator FAs were beyond prey values in D1's outer blubber whether one-, two-, or three-dolphin CCs were used (Supplementary Table 18).

3.3.3 Impacts of model parameters on diet estimate accuracy

For dolphin D1, multiple model parameter combinations yielded estimates with similar prey percentages and very low WEs. For the inner blubber, the Aitchison distance, the dietary suite, and layer-matched three- and two-dolphin CCs yielded estimates with the lowest errors (0.03% and 0.3% WE) and comparable prey percentages (68.8% ± 3.3% and 61.2% ± 3.6% capelin; 31.2% ± 3.3% and 38.8% ± 3.6% herring; mean ± SE) (Figure 6, Supplementary Table 12). However, several non-dolphin CCs and other layer-matched three-, two-, and one-dolphin CCs applied with the Aitchison distance and both FA suites also yielded estimates with similar prey percentages and WEs < 2% (Figure 6, Supplementary Tables 12, 13). In comparison, the KL distance estimated 10%–30% more capelin regardless of the CC set or FA suite, resulting in higher error estimates (Supplementary Table 13). For D1's outer blubber, the Aitchison distance (with either FA suite) and the KL distance (with the dietary suite) yielded diet estimates with prey percentages similar to those from the inner blubber. These estimates also had WEs <2% when both non-dolphin and layer-matched dolphin CCs were used (Figure 6, Supplementary Tables 12, 13). For both inner and outer blubber, the lowest-error estimates most closely matched

D1's actual diet averaged over 8 and 22 weeks before sampling. However, estimates with WEs <2% were similar to D2's diet averaged over at least 2 and up to 26 weeks before sampling.

For dolphin D2, multiple model parameter combinations again yielded low-error estimates, but WEs were higher (4.8%+), one- and non-dolphin CCs were less accurate, and estimated prey percentages varied more. In both blubber layers, layer-matched two- and three-dolphin CCs yielded the lowest-WE estimates (inner: 5.2%+ three-dolphin, 14.6%+ two-dolphin; outer: 10.9%+, 4.8%+), with multiple estimates differing by only 1%–2% error (Figure 7, Supplementary Tables 14, 15). Estimated prey percentages differed by only 2%–9% among these low-WE estimates when the same model parameter combination was used (e.g., the dietary suite with the Aitchison distance). However, best-fit model parameters differed: the lowest-WE estimates were generated by the Aitchison distance with the dietary and extended dietary suites in the inner blubber but the extended dietary suite with the Aitchison and KL distances in the outer blubber (Figure 7, Supplementary Table 14). Estimated percentages of certain species and the integrated diet period most resembling the low-WE estimates also differed across model parameter combinations. While percentages of capelin and squid were relatively similar across low-WE estimates in both layers (46.4% ± 3.6% to 57.6% ± 4.2% capelin; 6% ± 5.4% to 16.6% ± 3.5% squid), mullet was lower and herring was higher with the dietary than the extended dietary suite (0.7% ± 1.2% to 6.6% ± 3.1% versus 5.7% ± 0.9% to 14.4% ± 0.7% mullet; 33% ± 4.3% to 42.7% ± 4.1% versus 21.7% ± 2.3% to 29% ± 2.3% herring) (Figure 7,

Supplementary Tables 14, 15). As a result, estimates using the dietary suite and the Aitchison distance most closely resembled the actual diet integrated over longer periods (at least 22 and up to 26 weeks before sampling). In comparison, estimates with the extended dietary suite were most similar to intermediate diet periods (at least 10 and up to 16 weeks before sampling with the Aitchison distance, at least 4 and up to 18 weeks with KL). Finally, as for D1, harbor seal and mink CCs yielded estimates with comparable errors (16.7%27.7% WE) to some dolphin CCs, though all other layer-matched one-dolphin, non-layer matched, and non-dolphin CCs generated higher error estimates. Still, depending on the model parameters used, layer-matched one-dolphin CCs identified all prey species (Figure 7, Supplementary Table 15), though D1's CCs sometimes overestimated capelin and herring while D3's overestimated mullet and/or squid.

Dolphin D3 had the highest WEs (16.5%+), but layer-matched three- and two-dolphin CCs again generated the lowest-WE estimates (Figure 8, Supplementary Tables 16, 17). Unlike D1 and D2, however, only the KL distance and the dietary suite yielded the lowest error estimates in both blubber layers, and three-dolphin CCs yielded significantly lower WEs than two-dolphin CCs (inner: 23.5%+ three-dolphin, 36.4%+ two-dolphin; outer: 16.5%+, 38.5% +). While the estimates most resembled diet averaged over the 22 and 24 weeks before sampling, diets averaged over the 18 to 26 weeks before sampling had WEs only 1%–2% higher (Supplementary Table 16). Estimated prey percentages generated by three- and two-dolphin CCs were comparable, despite their different WEs, but estimates varied between layers. Three-

and two-dolphin CCs in the inner and outer blubber accurately estimated herring, mullet, and croaker, and underestimated Atlantic thread herring (Figure 8, Supplementary Table 16). However, while outer-layer CCs estimated capelin more accurately (29% ± 2.5% to 32.6% ± 2.8% for outer, 40.1% ± 3.3% to 49% ± 3.1% for inner, versus 30.6%–30.7% in the actual diet averaged over the 18 to 26 weeks before sampling), they overestimated squid (8% ± 4.9% to 18.4% ± 4.7% for outer, 0% ± 0.8% to 0% ± 2.1% squid for inner, versus 0.4% to 0.6% integrated). Distance measure also impacted estimated prey percentages. The Aitchison distance had higher WEs (46.6%+ inner blubber, 37.7%+ outer) and estimated more Atlantic thread herring (1.8%–4.5% with the Aitchison distance versus 0%–1.1% with the KL distance for inner; 7%–9.1% versus 3.5%–4% for outer) and croaker (16.5%–19.1% versus 4.9%–5.6% inner; 20.3%–25.3% versus 2.2%–4.8% outer) but less herring (19.5%–25.1% versus 31.5%–37.9% inner and outer) (Supplementary Tables 16, 17). Finally, as for D1 and D2, mink CCs yielded a low-error inner blubber estimate (35.4% WE; Supplementary Table 16), while all other dolphin and non-dolphin CCs had higher WEs (Supplementary Tables 16, 17). As for D1 and D2, one-dolphin CCs could identify all prey species (Figure 8, Supplementary Table 17), but sometimes overestimated capelin and herring (D1's CCs) or tended to overestimate croaker and/or mullet (D2's CCs).

3.3.4 Variation in FA contribution with different model parameters

One pattern was consistent among dolphins and model parameters: for each dolphin's inner and outer blubber, estimates generated using two- and three-dolphin CCs and the same FA suite and distance measure shared at least the top three contributing FAs, and most of the top 10 (Supplementary Tables 18 – 20).

For a given dolphin, however, the top FAs contributing to the lowest-WE estimates calculated using two- and three-dolphin CCs varied by distance measure and FA suite (Supplementary Tables 18 – 20). FAs present in large proportions generally had higher contributions when the KL distance was used, whereas lower-proportion FAs contributed more when the Aitchison distance was used. For example, for D1's inner-layer lowest-error estimates with the extended dietary suite (Supplementary Table 18), 16:3n-4, 16:4n-1, 18:4n-1, and 20:4n-6 were large contributors when the Aitchison distance was applied but were each <0.6% of D1's FA signature. Conversely, 16:0, 16:1n-7, and 18:0 were much larger contributors with the KL distance and were among the most prevalent FAs in D1's signature. Top contributing FAs necessarily differed between FA suites, as some FAs were in the extended but not dietary suites. However, the difference was greater for the KL than the Aitchison distance, as many smaller proportion FAs were of dietary origin. Accordingly, low proportion FAs were more likely to make large contributions when using the KL distance with the dietary suite than the extended dietary suite. For instance, 16:3n-4, 16:4n-1, 18:4n-1, and 20:4n-6 remained top contributors to D1's lowest-WE estimates calculated using the Aitchison distance and dietary suite, though their percent contribution increased. With the KL distance, however, 18:4n-1, 20:4n-

6, 22:1n-11, and 22:6n-3 became the top contributors, as the previous top contributing FAs were only in the extended dietary suite. While 22:1n-11 and 22:6n-3 were among the highest percentage dietary FAs measured in D1's inner blubber (15%, 4.1%), 18:4n-1 and 20:4n-6 were much smaller (0.1%, 0.4%).

Notably, the "augmented" FA also had a large percent contribution for some but not all low-error diet estimates, varying with blubber layer and model parameters (Supplementary Tables 18 – 20). For most dolphins and blubber layers, the augmented contribution was smallest (<4%) for the lowest-WE estimates calculated using the dietary suite, whether the KL or Aitchison distance measure was used. D2's outer blubber differed slightly: the augmented contribution was relatively low for estimates calculated using the dietary suite and the KL distance (<6%) but highest using the dietary suite and the Aitchison distance (<13.7%). Results were more variable when using the extended dietary suite. The augmented contribution remained low for some diet estimates no matter the distance measure, including D1's inner blubber (<3%), D2's outer blubber (<8%), and D3's inner blubber (<7%), and for D1's outer blubber with the Aitchison distance (<6%). However, the contribution increased for other estimates, including D1's outer blubber with the KL distance (33.3%–34.5%), D2's inner blubber with either distance measure (7.7%–21.5%), and D3's outer blubber with either distance measure (14.4%–23.3%). A large contribution by the augmented FA decreases the biological interpretability of the results and extreme cases were treated with caution, as discussed in Section 4.3.2.

4 Discussion

4.1 Dolphin and prey FA signatures

Dolphin and prey FA signatures followed patterns expected for marine mammals and temperate fish and squid, supporting their validity as QFASA model inputs. Dolphin FA signatures resembled previously reported values for free-ranging bottlenose dolphins (Samuel and Worthy, 2005), with 14:0, 16:0, 16:1n-7, 18:1n-9, and 22:6n-3 among the most common FAs. Inner and outer blubber differed in the percentages of dietary and extended dietary FAs, reflecting the known gradients in lipid metabolism and deposition in cetacean blubber (Koopman et al., 1996; Samuel and Worthy, 2005; Ellisor et al., 2013). Prey species also differed in their FA signatures, with fish and squid occupying distinct regions of FA space. Dietary and extended dietary FAs measured in higher percentages in the dolphins were also common in one or more of the prey species, and higher consumption of a prey species was associated with greater prevalence of their top FAs. This is expected if the dolphins are integrating available prey FAs. Finally, despite the outer blubber's lower percentage of dietary FAs, the presence of diet-associated FAs that varied with prey consumption maintains the possibility of using both inner and outer blubber for diet estimation.

4.2 CC variation with FA, species, blubber layer, and dolphin diet

Quantifying magnitude and variation among dolphin and non-dolphin CCs is essential to assessing their interchangeability and potential impacts on diet estimates.

CCs account for small structural changes as dietary and extended dietary FAs are integrated into a marine mammal's blubber and quantify the degree and form of modification occurring (Iverson et al., 2004; Budge et al., 2006; Rosen and Tollit, 2012). A CC of 1 (or 0 on the log scale used in our figures) indicates that a FA is deposited without modification. A CC greater than 1 indicates that the FA has been bio-transformed from other FAs and/or results from a combination of deposition from prey and biosynthesis, increasing its quantity in the predator relative to its prey. Conversely, a CC less than 1 indicates that the FA was not fully integrated from prey into the blubber and/or preferentially catabolized or bio-transformed within the predator. The variation in CCs among FAs and the fact that most dolphin and non-dolphin CCs differed noticeably from 1 underscores the need for CCs to account for FA metabolism. Simultaneously, large CC variation between species and between layers suggests layer- and species-specific CCs are necessary. Conversely, dolphin identity had a small effect on CC variation. This suggests that combining one-dolphin into two- or three-dolphin CCs does not conceal large dolphin- or diet-specific variations and supports the validity of those values. Diet change over time also minimally contributed to CC variation. This indicates that while changes in prey percentages over the 26 weeks caused CCs for certain FAs to vary, that variation was small relative to other differences and may not significantly impact diet estimates. We propose that this robustness to small changes makes sense. If prey amounts do not change significantly, the fundamental processes governing the degree of modification and integration of the FAs should not either. Together, our results suggest that CCs

are necessary and not interchangeable across taxa or blubber layers but less impacted by dietary differences between and within dolphins. CC variation likely reflects a combination of species-specific physiology, layer-specific metabolism, and diet composition, all of which can influence the extent to which individual FAs are absorbed, modified, and deposited in blubber. However, examining model performance and diet estimate accuracy is required to fully understand the impacts of CC type.

4.3 Model performance and best-fit model parameter suggestions

4.3.1 Model criteria

The first criterion for valid QFASA estimates is prey distinctiveness, as species with distinct FA signatures are more likely to be accurately estimated by the model. LOPO analyses confirmed that the model can distinguish among all prey species in our analyses, with most self-associations > 85%. However, the lower self-association of herring and croaker and their similarity with mullet and Atlantic thread herring may have impacted D2 and D3's diet estimates, as discussed below (see Section 4.4.1). Furthermore, herring, croaker, mullet, and Atlantic thread herring are known or potential free-ranging dolphin prey (Berens McCabe et al., 2010; Rossman et al., 2013; Wells et al., 2013; Browning et al., 2014b; Rossman et al., 2015a). Lower distinctiveness could thereby impact diet estimates if QFASA were applied in a free-ranging system, emphasizing the importance of conducting LOPO analyses before estimating diet.

The second criterion for valid estimates is that only a small percentage of FAs in a predator's signature fall outside the range observed in prey. Lower percentages indicate that the CCs and/or prey library are good fits to the predator FA data and are therefore expected to yield more accurate estimates (Bromaghin, 2017; Bromaghin, 2020). As each dolphin's prey library included only its known prey, higher percentages of FAs outside the prey range indicate poorer CC fit. No ideal “low” PBP percentage threshold is defined (Bromaghin, 2017, 2020). However, non-layer-matched and non-dolphin CCs had the highest PBP values for all dolphins. This indicates that they are the poorest fits for our data and further underscores the importance of species- and layer-specific CCs. In comparison, layer-matched dolphin CCs had lower percentages, though values were higher overall for dolphin D1 than D2 or D3 and when applying one-dolphin CCs. This suggests that predator and CC species diet affect model fit. We propose that D1’s simple diet of capelin and herring meant that CCs derived from dolphins consuming additional prey were poorer fits for D1 while its CCs were worse for D2 and D3. Conversely, D2 and D3's lower overall PBP percentages could be due to their greater dietary overlap with each other and D1. In turn, lower PBP percentages when applying two- and three-dolphin CCs and a good fit for the FAs most relevant to the diet estimates suggest that they tempered some of the one-dolphin biases and better accounted for more prey species. However, in some instances, two-dolphin CCs had predator values beyond prey values for top contributing FAs when three-dolphin CCs did not. This suggests that part of the better model fit of the three-dolphin CCs arises from the inclusion of that dolphin’s own

CCs in the average. While logical, this does not resolve the underlying tautological concern. Specifically, it remains unclear whether three-dolphin CCs are valid for estimating dolphin diet on their own merit and applicable to other individuals with no connection to the CCs, rather than appearing accurate simply because they include values derived from the same dolphin. Conversely, if two- and one dolphin CCs provide poorer fits to the predator data, it is unclear whether they can still yield accurate estimates. Thus, although layer-matched dolphin CCs better met the PBP criteria, evaluating diet estimates and their accuracy is necessary to address these tautological concerns and the effects of diet variation on estimate accuracy. Determining which diet estimates to consider—based on best-fit FA suites and distance measures—is required first, however.

4.3.2 Assessing best-fit FA suite and distance measure for estimate accuracy

The distance measure and FA suite used to estimate diet can impact the results, but no single recommended protocol exists and studies have used different parameter combinations (e.g., Iverson et al., 2004; Bromaghin et al., 2015a, b; Choy et al., 2019; Remili et al., 2022). Considering this inconsistency, we explored multiple parameter combinations. Unfortunately, suggesting best-fit options based on our results is complicated. Both FA suites and distance measures yielded low-WE estimates depending on which dolphins and blubber layers were analyzed, and parameters had interacting impacts on diet estimates. Thus, rather than declaring singular best-fit model parameters, we provide considerations for future QFASA applications.

The magnitude of the contribution of the "augmented" FA (i.e., the FA representing all FAs removed during FA suite selection) is the first consideration when selecting best-fit model parameters. A large contribution decreases explanatory power, because the augmented FA is a synthetic component that cannot be linked to an equivalent FA in the prey or to physiological or environmental data. Accordingly, we treat large contributions as a diagnostic red flag. A major contribution could indicate that FAs with explanatory importance were excluded. In our results, however, estimates from the dietary suite generally had smaller augmented contributions than those from the extended dietary suite, despite otherwise identical model parameters. The dietary suite removes the same and more FAs than the extended suite, suggesting that excluding explanatory FAs did not cause the higher contributions. The distance measures' different analytical approaches are another possible cause, and the augmented contribution was often highest when the KL distance and the extended dietary suite were used together. This does not appear to be the only driver, however, as the contribution was also elevated with the dietary FA suite and the Aitchison distance for D2's outer blubber. To elucidate what conditions cause increased augmented FA contribution, we suggest further studies with a larger sample size and greater diet consistency among dolphins to remove other sources of variation. We also recognize that the variation in and magnitude of the contribution could be unique to our data. However, it may be a previously unidentified consideration, as to our knowledge, this is the first publication combining the "augmenting" method of scaling a subsetted FA signature (available only in the R

package qfasar, Bromaghin, 2020) with quantifying each FA's contribution to the estimate (available only in the R package QFASA, Stewart et al., 2024). Accordingly, we suggest future applications examine FA contributions to guide model parameter selection and interpretation of results, and place greater weight on parameter combinations where biologically informative FAs have the highest contributions. Moving forward, our discussion will exclude the low-WE estimates where the augmented FA dominated the contribution. This includes D2's inner blubber estimates with the extended dietary suite and the Aitchison distance, and D2's outer blubber estimates with the dietary suite and the Aitchison distance.

Our second consideration for best-fit parameters concerns differences between the Aitchison and KL distance measures and how they interact with FA suite choice to impact diet estimates. The Aitchison and KL distances use different approaches to identify the prey percentages that minimize the statistical distance between predator and prey FA signatures. The Aitchison distance gives more importance to FAs present in smaller proportions, while the KL distance gives more to FAs in larger proportions (Bromaghin et al., 2015a). This is consistent with the patterns in our FA contributions. Similar to the analytical approaches, the Aitchison distance also overall gave greater weight to less common prey. This led to lower percentages of more prevalent prey and more evenly distributed prey percentages. In contrast, the KL distance tended to highlight the more prevalent prey. However, the FA suite also influenced these patterns. Extended dietary suite estimates tended to have a more

even prey distribution than dietary suite estimates even with the KL distance. This may reflect the larger number of FAs available for diet estimation.

Together, our results suggest that distance measure and FA suite choice depend on a study's goals, the expected complexity and variation in the predator's diet, and concerns about the augmented FA's contribution. The Aitchison distance may better identify the presence of rarer prey but risks overestimating some while underestimating more common prey. The KL distance may highlight the most common prey but struggle to identify lower percentage prey correctly. Thus, the Aitchison distance may be most useful if a predator is thought to have a varied diet but relatively consistent proportions of different species, or if identifying less common prey items has significance to a study (for instance, examining overlap with fisheries, investigating sources of contaminants, etc.). By contrast, if a predator has a more specialized diet or knowing the major prey is more significant, the KL distance may provide a simpler picture without overinflating the importance of minor prey. Using the extended dietary suite may yield more evenly distributed estimates with either distance measure, but the contribution of the augmented FA should be considered. Ultimately, we suggest future studies use both distance measures and FA suites and compare the results, further contributing to the literature surrounding best-fit model parameters for accurate QFASA analyses under different conditions.

4.3.3 Best-fit CCs for inner and outer blubber

Layer-matched three- and two-dolphin CCs overall provided the most accurate estimates for all three dolphins' inner and outer blubber. They identified similar prey percentages within range of the actual diets, demonstrated the same patterns in over- or underestimating prey, and identified the same FAs as top contributors to the estimates. In comparison, layer-opposite and non-dolphin CCs yielded estimates with overall higher PBP percentages, higher WEs, and larger over- or underestimations of prey, with some exceptions that will be discussed below (see Section 4.4.2). These results support the conclusion that differences in FA processing–arising from phylogenetic variation in FA digestion and incorporation, as well as differences in blubber structure among taxa and between layers–necessitate species- and layer-specific CCs for the most accurate diet estimates. Our findings are in line with the only other applications of cetacean-derived CCs to cetaceans of the same species (killer whales, Remili et al., 2022, 2023).

Accurate diet estimates in both inner and outer blubber indicate that QFASA can provide a valid estimate of dolphin diet when appropriate layer-matched CCs are applied. As methods to sample free-ranging cetaceans, such as dart biopsies, cannot always collect full-depth blubber samples, the ability to estimate diet using both inner and outer blubber has great scientific value. Still, the outer blubber's lower proportions of dietary FAs and differences from inner blubber estimates in some cases suggest that when possible, sampling both layers and comparing the results remain the best option for the most complete understanding of cetacean diet.

The higher accuracy and lower PBP percentages yielded by three-dolphin CCs compared to two- or one-dolphin values suggest, unsurprisingly, a benefit to including that dolphin's CCs when estimating diet. However, similar estimated prey species percentages and top contributing FAs when two- and three-dolphin CCs were used demonstrate that valid and accurate diet estimates can be generated even without that dolphin's CCs and despite sometimes poorer model fit. Simultaneously, it shows that the accuracy of three-dolphin CCs is not only due to including that dolphin's CCs, addressing our tautological concerns and supporting the validity of the three-dolphin CCs and their application beyond this study.

By contrast, one-dolphin CCs could identify all prey species but sometimes better identified certain prey consumed by the dolphin from which they were derived (e.g., capelin and herring with D1's CCs, mullet and squid with D2 and D3's CC) or prey with similar FA signatures (such as croaker instead of mullet or Atlantic thread herring). Previous studies in pinnipeds and belugas similarly suggested that prey species consumed by the predator from which CCs were derived could be overestimated when those CCs are applied in other systems (Nordstrom et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012; Choy et al., 2019). Thus, when applying CCs, even species-specific values, it is important to consider the diet of the predator(s) from which the CCs are derived and how it could influence estimates. This also suggests a benefit of our three-dolphin values: as averages of CCs derived from multiple dolphins with varied diets, they may temper some biases introduced by one-dolphin CCs. Combined with their higher accuracy and better model fit, this

leads us to suggest our three-dolphin CCs rather than one- or two-dolphin values for future QFASA applications.

4.3.4 Temporal feeding window and suggested three-dolphin CCs

Determining the temporal feeding window integrated in dolphin inner and outer blubber informs QFASA's future applications and which three-dolphin CCs to use. While diet change over the 26 weeks was a minor contribution to CC variation, diet estimate accuracy varied depending on which CCs were used. All dolphins had multiple lowest-error estimates generated by layer-matched three-dolphin CCs with similar prey percentages that varied by just 1%–2% WE and most resembled the diet averaged over intermediate to long temporal windows. This included at least 8 and up to 26 weeks before sampling for all dolphins and blubber layers except D1's outer blubber, which ranged over the 6 up to 12 weeks before sampling. We suggest that this range of low-WE estimates reflects the reduced variation in all three dolphins' diets during the 12–26 weeks before sampling. However, the simplicity of D1's diet and overall low-error estimates complicate defining a temporal window, while the greater complexity of D2 and D3's diets better resemble the varied diets of free-ranging dolphins. We thus propose that a temporal window of at least 8 and up to 26 weeks before sampling best reflects physiological reality and is most applicable for QFASA's future use.

Accordingly, three-dolphin CCs calculated using known diets integrated over that temporal window are our best-fit suggestion for future QFASA diet estimates. As diet

differences minimally impacted the CCs and many estimates within that temporal window were similar, we expect future estimates generated using any of the three-dolphin CCs from weeks 8 to 26 will also be similar. However, we recommend anyone applying these CCs in a new system select a subset (e.g., CCs from weeks 8, 16, and 24) and compare the resulting estimates to confirm.

Our results are overall consistent with existing research, with some deviations. This temporal window extends beyond those of some previous studies but remains within the ranges reported for belugas, pinnipeds, and polar bears (4–5 weeks and up to 3 months before sampling, respectively) (Iverson et al., 2004; Nordstrom et al., 2008; Choy et al., 2019; Thiemann et al., 2022). Additionally, inner blubber is thought to have a higher turnover rate than outer blubber and thus is proposed to reflect a more recent diet window (Koopman et al., 1996; Remili et al., 2022). The similar temporal windows for the inner and outer blubber in our study could indicate similar turnover rates in inner and outer blubber. However, small sample sizes and parameter uncertainty suggest that this finding is indicative rather than definitive. It is also possible that our sampling period was not long enough or that the diet differences over the 26 weeks were not large enough to register differences between blubber layers. A dedicated study of FA turnover rates in the inner and outer blubber of bottlenose dolphins is necessary to directly define the temporal feeding window. Regardless, our results support the assertation that QFASA can estimate long-term diet integrated over “weeks to months”.

4.4 Additional factors influence QFASA diet estimate accuracy

4.4.1 Predator and prey library complexity

While layer-matched dolphin CCs generated accurate diet estimates, WEs increased as dolphin diet complexity and prey library size increased. This suggests an important caveat: QFASA may yield less accurate estimates when applied to predators with more diverse diets, especially when estimating lower percentage prey. QFASA provides aggregate diet estimates, identifying prey consumed regularly throughout the feeding window, i.e., significant prey rather than species consumed in variable or occasional quantities (Budge et al., 2006). Prey species with small or variable percentages will be harder to estimate accurately. Greater diet complexity and prey library size also increase the likelihood of overlap in FA signatures among prey species, reducing the model's ability to distinguish among them and possibly leading to misidentification (Piché et al., 2010). Even with species- and layer-specific CCs, the most accurate diet estimates for dolphin D2 (four species with mixed distinctiveness, in large and medium percentages) and D3 (six prey species in a mix of percentages with some lower LOPO attributions) still contained errors. Underestimations of mullet, Atlantic thread herring, croaker, and squid could in part be because they were consumed in smaller amounts that varied over the 26 weeks before sampling. Croaker's lower distinctiveness and its similarity with Atlantic thread herring and mullet could also contribute to less accurate estimates by causing one species to be identified instead of another or underestimating them as a group while overestimating a distinct species like capelin. In comparison, the simplicity of

D1's diet (two distinct species consumed in large percentages) meant a variety of CCs, both dolphin and non-dolphin, could generate accurate estimates. Considering the impact of diet complexity on QFASA estimate accuracy is especially important when applying QFASA to free-ranging dolphins, whose diets are typically more diverse (Wells et al., 2013; Browning et al., 2014b; Rossman et al., 2015a, 2015b). QFASA can indicate the relative proportions of different prey species and identify the most prevalent prey. However, results for prey species that make up small percentages of the estimated diet or have lower self-associations should be interpreted with caution.

4.4.2 The role of non-dolphin CCs

High-WE estimates generated by killer whale CCs and low-WE estimates generated by mink and harbor seal CCs could dispute the conclusion that layer- and species-specific CCs are required for the most accurate estimates. Killer whale CCs were expected to generate relatively accurate dolphin diet estimates. Dolphins and killer whales are in the family Delphinidae, suggesting they should share physiological similarities that could remove some of the CC variation associated with lipid biochemistry. The killer whales from which the CCs were derived also consumed varied diets, which our results suggest could increase estimate accuracy. Conversely, harbor seals and mink are from physiologically different taxa than cetaceans, mink lack blubber and are not even marine mammals, and both species

consumed herring-only diets. Together, these factors led us to expect higher-error estimates with non-cetacean CCs.

Instead, killer whale CCs regularly yielded the highest-error estimates. However, CC limitations, possible FA biases, and physiological differences may explain these results. Fewer CCs were available for killer whales than for mink or pinnipeds, as CCs for several of the FAs measured at significant levels in the dolphins were not validated in the original study (Remili et al., 2022). This limited the FAs available to the QFASA model. Estimates could thus be biased towards prey with larger percentages of the limited FAs, even if those FAs and prey are not major components of dolphin diet. In addition, as observed with the one-dolphin CCs, similarities in the FA signatures of the dolphin prey and the prey consumed by the killer whales may have skewed estimates or made it harder to identify lower-prevalence prey. Finally, despite shared phylogeny, killer whales have a few key physiological differences from dolphins. Wax esters, a class of lipids primarily involved in insulation, are absent in dolphins but common in killer whale outer blubber, and found in the inner blubber to a much lesser extent (Krahn et al., 2004). FAs are derived from the wax esters but not necessarily related to diet (Budge et al., 2006). Killer whales are also significantly larger than bottlenose dolphins and often, though not always, found in colder habitats. FA composition can vary with thermal environment and with intrinsic lipid metabolism and tissue scaling in large-bodied marine mammals (Koopman, 2007; Guerrero and Rogers, 2017). Together, these physiological differences may influence killer whale metabolism and FA signatures, affecting CCs and making them

poorer fits to bottlenose dolphins. Ultimately, differences in CCs derived from species within the same taxa are also consistent with previous comparisons in pinnipeds (Rosen and Tollit, 2012).

Inconsistently accurate estimates when applying mink and harbor seal CCs underscores the value of species- and layer-specific CCs but does not rule out the use of alternative CCs. In our study, mink and harbor seal CCs sometimes yielded inner blubber estimates with WEs comparable to those from two- and three-dolphin CCs. This resembles results from QFASA applications in belugas and Indo-Pacific humpback dolphins, where estimates generated with the same mink CCs as our study identified the most prevalent prey (Choy et al., 2019, 2020; Xie et al., 2022). It also mirrors QFASA applications in free-ranging polar bears, where mink CCs have been widely used to accurately estimate diet (e.g., Thiemann et al., 2008, 2011; McKinney et al., 2013; Bourque et al., 2020). However, the accuracy of mink and harbor seal CCs in our results was neither complete nor consistent. The CCs were unable to identify squid in the low-WE estimates for D2 and D3's inner layers. As a mollusk with low lipid content, the FA signature of squid is more different from the other fish prey (Piché et al., 2010) and may have been harder to identify with CCs derived from non-dolphin predators that consumed only herring. Mink and harbor seal CCs also did not provide low-WE estimates for D2 or D3's outer blubber, unlike layer-matched dolphin CCs. The relative accuracy of these non-dolphin CCs in specific situations mirrors their previous applications and suggest they can provide viable, if less complete, diet estimates, even in cetaceans. This means cross-taxa CCs can be used if

more suitable CCs are not available. However, they were not uniformly better than layer-matched dolphin CCs, and we suggest species- and layer-specific values be applied when possible.

4.5 Study limitations and future work

Our results are subject to several limitations. The small number of calibration dolphins, single inner and outer blubber samples per dolphin, and single instance of each diet type limit our ability to quantify individual variability in CCs and to separate diet-driven effects from inherent physiological differences. Additionally, the dolphins' known diets, while more complex than single-prey trials, still represent a restricted subset of the prey diversity and environmental conditions encountered by free-ranging dolphins. FAs that are sensitive to factors like temperature, life stage, nutritional status, or prey type, or that occur at low percentages, may have CCs that differ in free-ranging populations. The model variables that we assessed may also respond differently when applied to larger prey libraries that include infrequently consumed prey with FA signatures similar to frequently consumed prey. This is likely in free-ranging applications where diet is unknown. While our restriction of each dolphin's prey library to known prey allowed us to investigate key methodological limitations without other confounding factors, it prevented us from directly addressing misidentifications of unconsumed prey species.

Thus, future studies have many possible focus areas. Larger calibration datasets incorporating more individuals per diet type, age class, and sex, along with more

complex diets and a broader range of environmental conditions, would help disentangle physiological differences from diet-driven effects while expanding the pool of cetacean CCs. Testing multiple potential prey libraries, including known, ecologically likely, and even ecologically unlikely prey, would address concerns about misidentification. This would bring us closer to testing QFASA in free-ranging conditions while retaining the control provided by studies with marine mammals under professional care, thereby improving interpretation when CCs are applied in free-ranging systems. Finally, full validation of QFASA in bottlenose dolphins requires applying dolphin CCs and multiple model parameter combinations in free-ranging systems and comparing the results with estimates from other diet determination methods.

5 Conclusions for the use of QFASA in cetaceans

This study extends QFASA to bottlenose dolphins under professional care and provides the first species- and blubber layer-specific CCs for this species. Using known diets, we show that dolphin-specific, layer-matched CCs improve model fit and diet estimate accuracy compared with non-dolphin CCs. Inner and outer blubber can both provide accurate diet estimates and capture long-term diet integrated over weeks to months, suggesting that QFASA can be applied with multiple sample types. Our results underscore the importance of species- and layer-specific CCs, validate our dolphin CCs, and suggest specific CCs for future use in cetaceans. However, model performance and estimate accuracy still depended on dolphin diet complexity,

blubber layer, prey distinctiveness, the diet consumed by the dolphins from which CCs were derived, and model parameter choice. Taken together, our findings support a cautious but constructive view of QFASA for cetaceans. We recommend that future studies prioritize species- and layer-specific CCs and consider prey distinctiveness, PBP values, and FA contributions when selecting model parameters. We also suggest using QFASA in conjunction with other diet determination tools, such as stomach contents, SIs, and molecular approaches. This may help inform model parameter selection and the interpretation of results and yield a more complete understanding of cetacean diet. When applied with these considerations, QFASA can provide valuable, temporally-integrated insights into cetacean diets that are otherwise difficult to obtain.

Ethics statement

The animal study was approved by the Naval Information Warfare Center (NIWC) Pacific and the U.S. Navy Bureau of Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing review & editing. SB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision,

Validation, Writing – review & editing. CP: Conceptualization, Data curation, Funding acquisition, Resources, Validation, Writing – review & editing. ST: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing. RW: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. DC: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Acknowledgments

We thank the researchers at the National Marine Mammal Foundation, especially Forrest Gomez and Cynthia Smith, for their advice and support of this effort, and the U.S. Navy Marine Mammal Program for providing the biological samples collected during routine Navy marine mammal care that made this science possible. We are grateful to Anaïs Remili and Gregory Thiemann for sharing their killer whale and mink calibration coefficients, which were fundamental to our analyses. We thank Peter Raimondi for his valuable insights into statistical analyses. This work would also have been impossible without sample processing by Vivian Chen, Jessica McCoppin, KC McNamara, and the Marine Lipids Lab at Dalhousie University, especially Carrie Greene.

Figures – Chapter 1

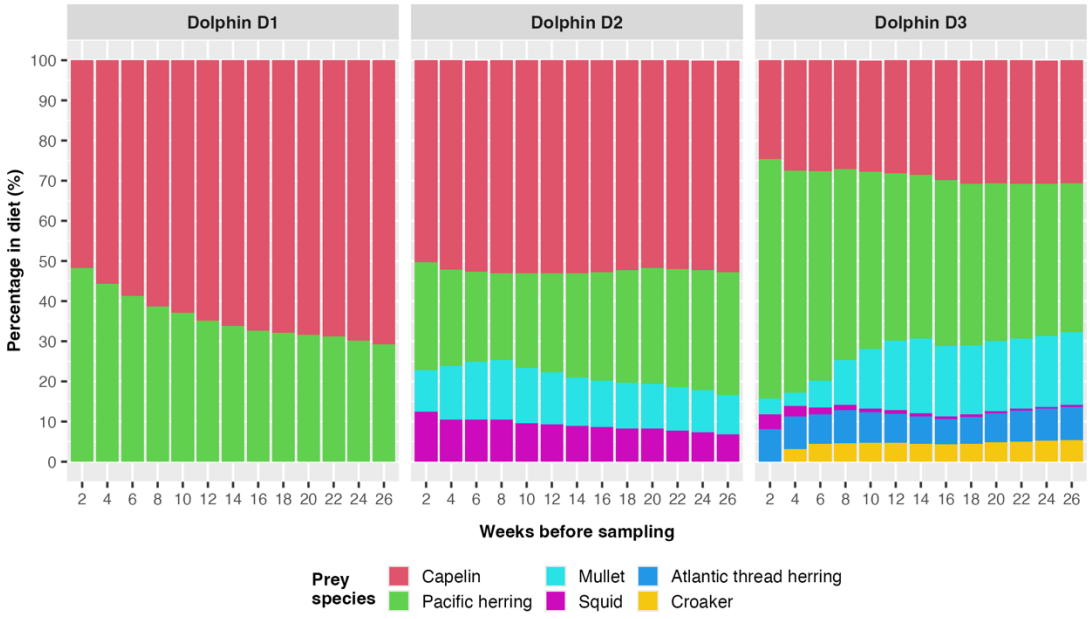

Figure 1: Diet composition of dolphins D1, D2, and D3, averaged over the 2, 4, 6, 8, ... to 26 weeks before blubber sampling occurred. Prey species included Canadian capelin (*Mallotus villosus*), Pacific herring (*Clupea pallasii*), mullet (*Mugil cephalus*), squid (*Loligo opalescens*), Atlantic thread herring (*Opisthonema oglinum*), and Atlantic croaker (*Micropogonias undulatus*).

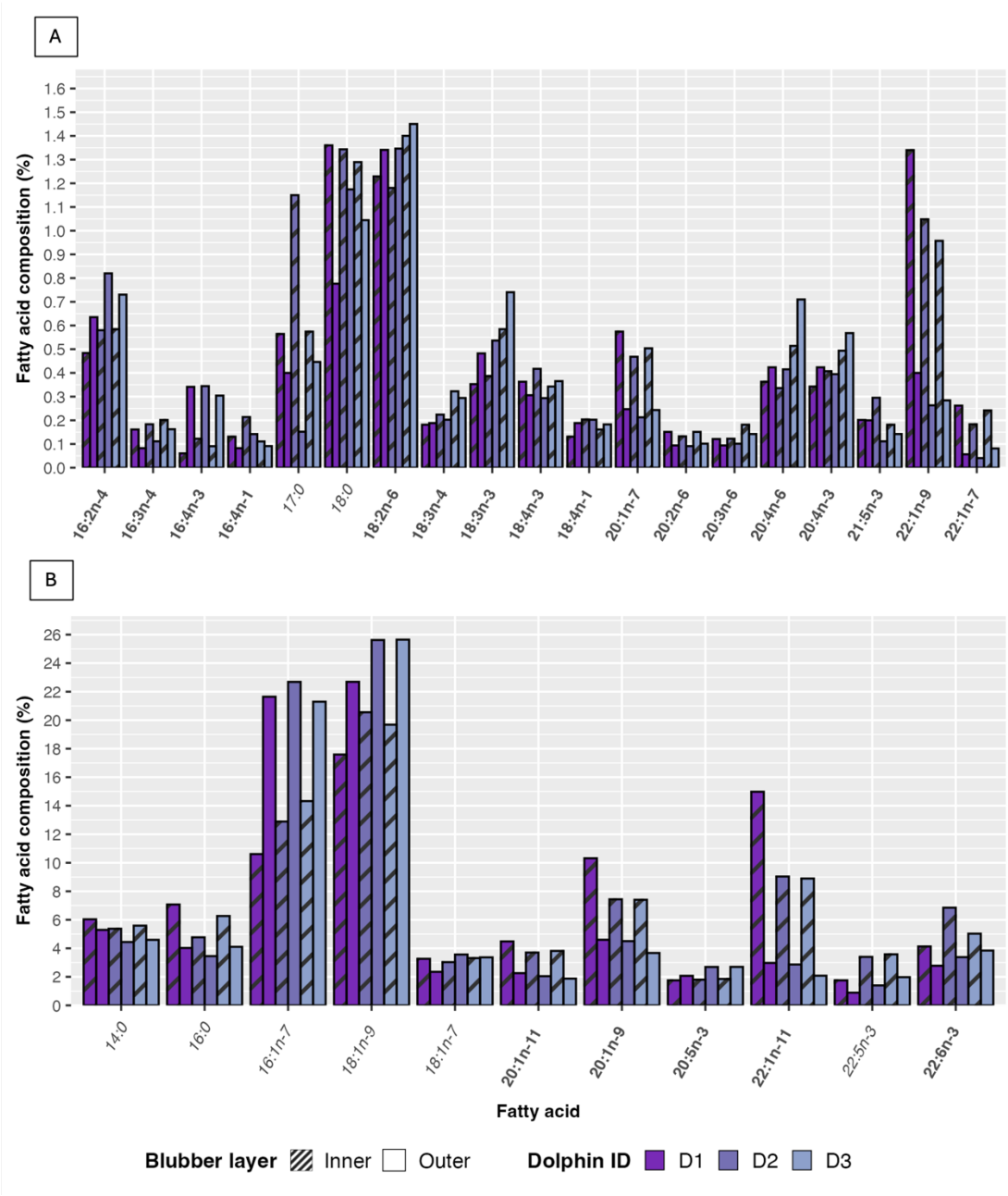

Figure 2: Fatty acid (FA) composition (%) of inner and outer blubber from dolphins D1, D2, and D3. Values represent the relative contribution of each FA to the total FA signature for each individual. Panels depict FAs with (A) smaller and (B) larger relative contributions and are shown on different x- and y-axis scales. Includes all dietary and extended dietary FAs used for diet estimation (see Methods).

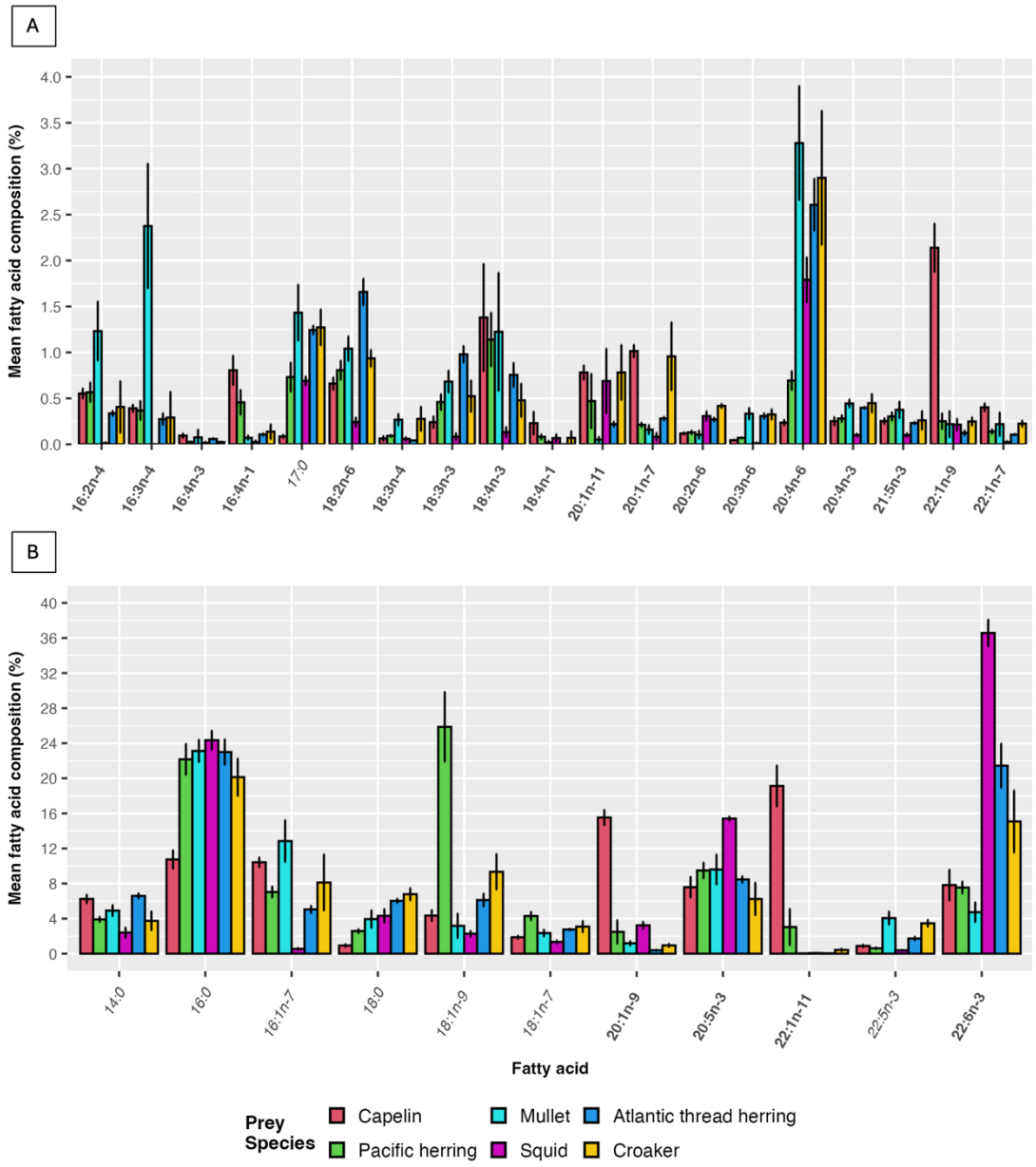

Figure 3: Mean fatty acid (FA) composition (%) of dolphin prey species. Values represent the average relative contribution of each FA to the total FA signature for each species, with error bars indicating ± standard deviation (SD) among samples. Panels depict FAs with (A) smaller and (B) larger relative contributions and are shown on different x- and y-axis scales. Includes all dietary and extended dietary FAs used for diet estimation (see Methods).

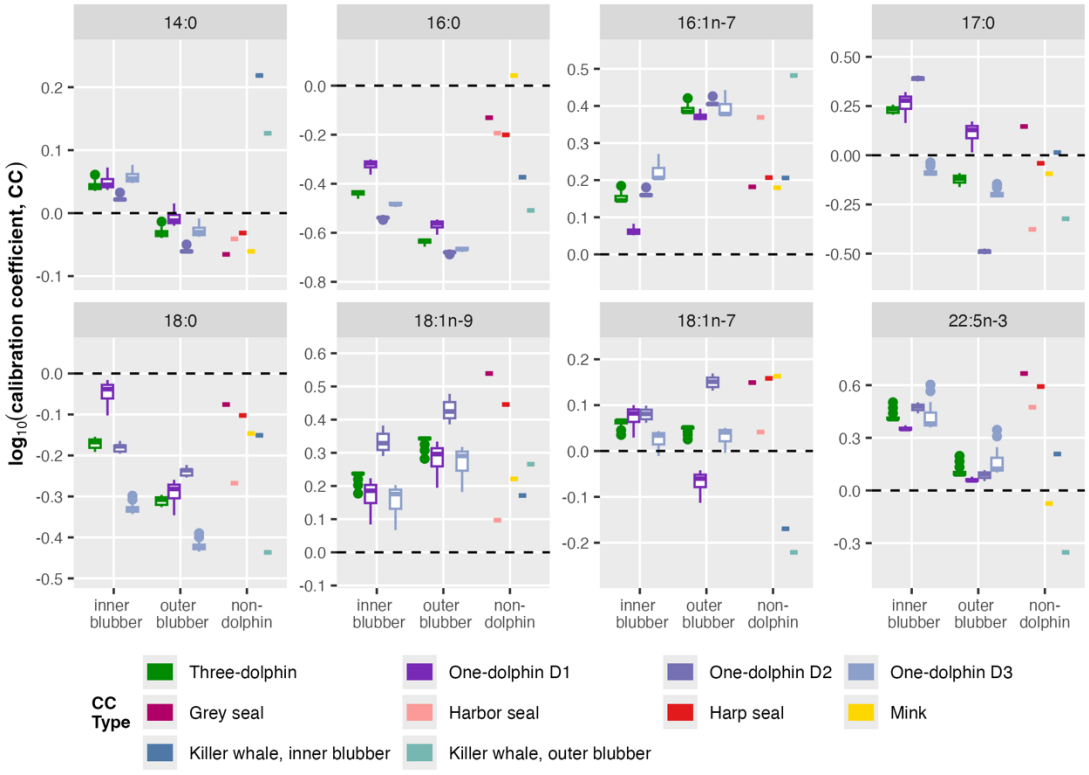

Figure 4: Individual ("one-dolphin") and average ("three-dolphin") bottlenose dolphin inner and outer blubber and non-dolphin calibration coefficients (CCs) for all FAs included only in the extended dietary FA suite used to estimate diet (see Methods). Includes dolphin CCs calculated for all integrated diet periods (x = 2, 4, 6, 8, ..., 26 weeks before sampling), leading to variation in each dolphin's CCs. CCs are shown on a $\log_{10}$ scale to preserve proportional differences across orders of magnitude and improve legibility. y-axis labels differ depending on the magnitude of the CCs and their relative variation. Dashed lines at y = 0 indicate the conditions when proportions of a FA are the same in the animal and its prey, indicating no structural change during deposition into blubber (Rosen and Tollit, 2012). Dolphin CCs were calculated from the three dolphins under professional care and their known prey species as part of this study. Killer whale, mink, and pinniped CCs come from existing literature (Remili et al., 2022; Thiemann et al., 2008; Iverson et al., 2004; Nordstrom et al., 2008; Rosen and Tollit, 2012).

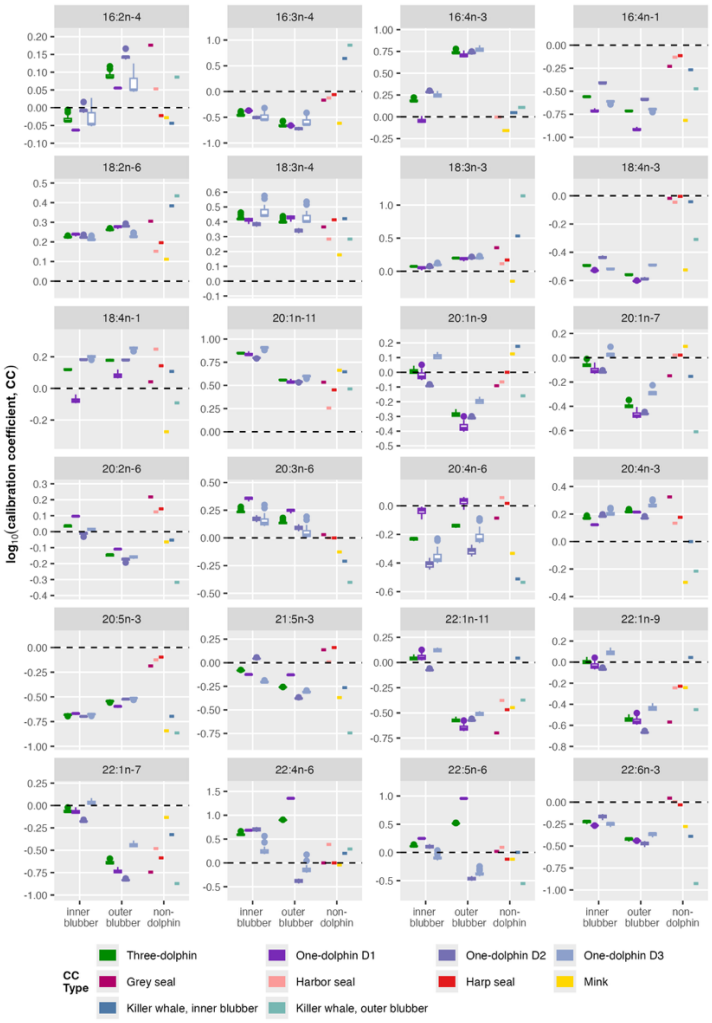

Figure 5: Individual ("one-dolphin") and average ("three-dolphin") bottlenose dolphin inner and outer blubber and non-dolphin calibration coefficients (CCs) for all FAs in the dietary FA suite used to estimate diet (see Methods). 22:4n-6 and 22:5n-6 are also included for reference, though the CCs were not used for diet estimation (see Results). Includes dolphin CCs calculated for each integrated diet period (x = 2, 4, 6, 8, ..., 26 weeks before sampling), leading to the variation in the CCs. CCs are shown on a log10 scale to preserve proportional differences across orders of magnitude and improve legibility. y-axis labels differ depending on the magnitude of the CCs and their relative variation. Dashed lines at y = 0 indicate the conditions when proportions of a FA are the same in the animal and its prey, indicating no structural change during deposition into blubber (Rosen and Tollit, 2012). Dolphin CCs were calculated from the three dolphins under professional care and their known prey species as part of this study. Killer whale, mink, and pinniped CCs come from existing literature (Remili et al., 2022; Thiemann et al., 2008; Iverson et al., 2004; Nordstrom et al., 2008; Rosen and Tollit, 2012).

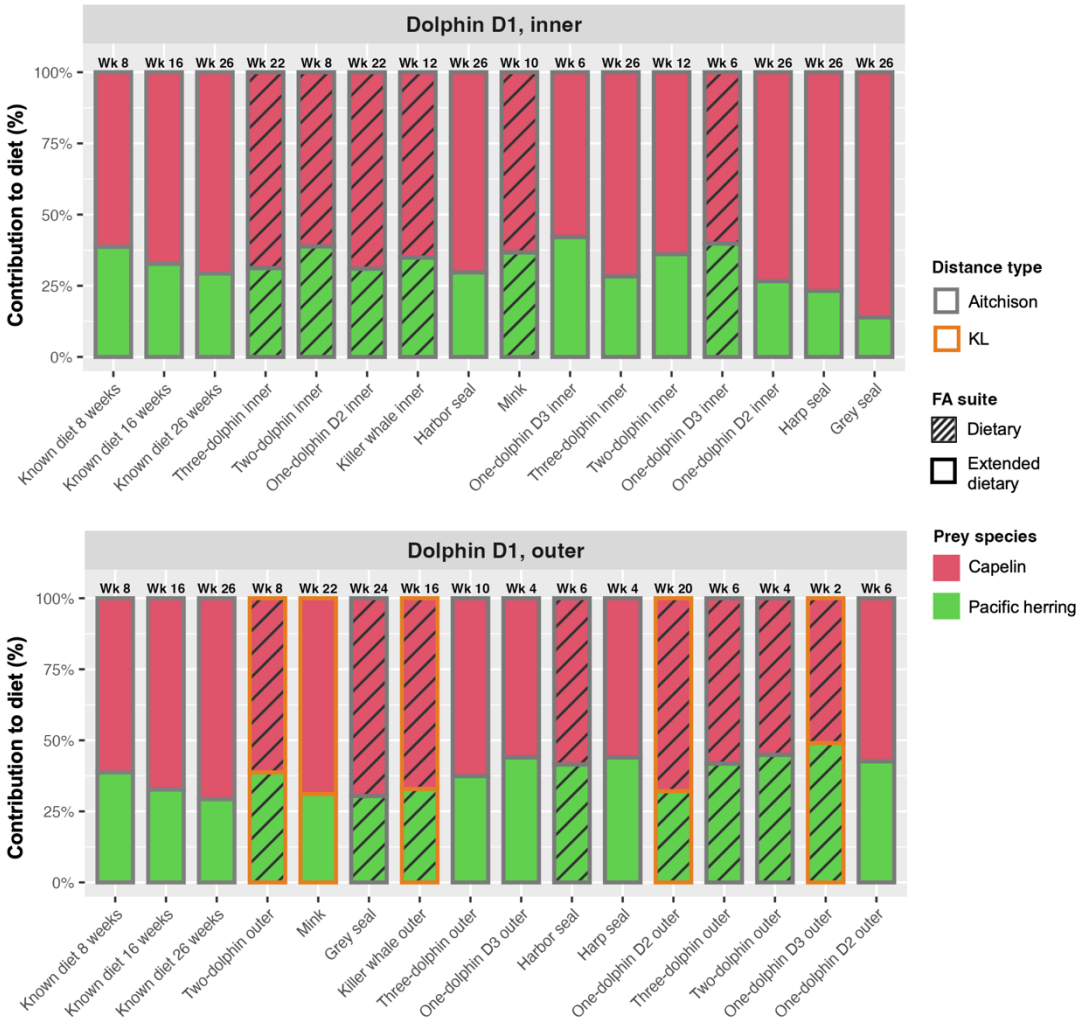

Figure 6: Prey species percentages for the lowest weighted error (WE) QFASA diet estimates for dolphin D1's inner (top) and outer (bottom) blubber layers. Includes the two lowest-WE estimates yielded by layer-matched three-, two-, and one-dolphin CCs using different model parameters and the lowest-WE estimates generated with non-dolphin CCs. Estimates are arranged from left to right by increasing WE and use the distance measure and FA suite that generated that estimate (Supplementary Tables 12, 13). Each dolphin's known diet averaged over the 8, 16, and 26 weeks before sampling is shown by the first three bars, as the lowest-WE estimates fell within that temporal window (see Results). The known diet that most closely matches the diet estimated using a given CC may differ from those weeks, however, and is indicated by the week number above each bar.

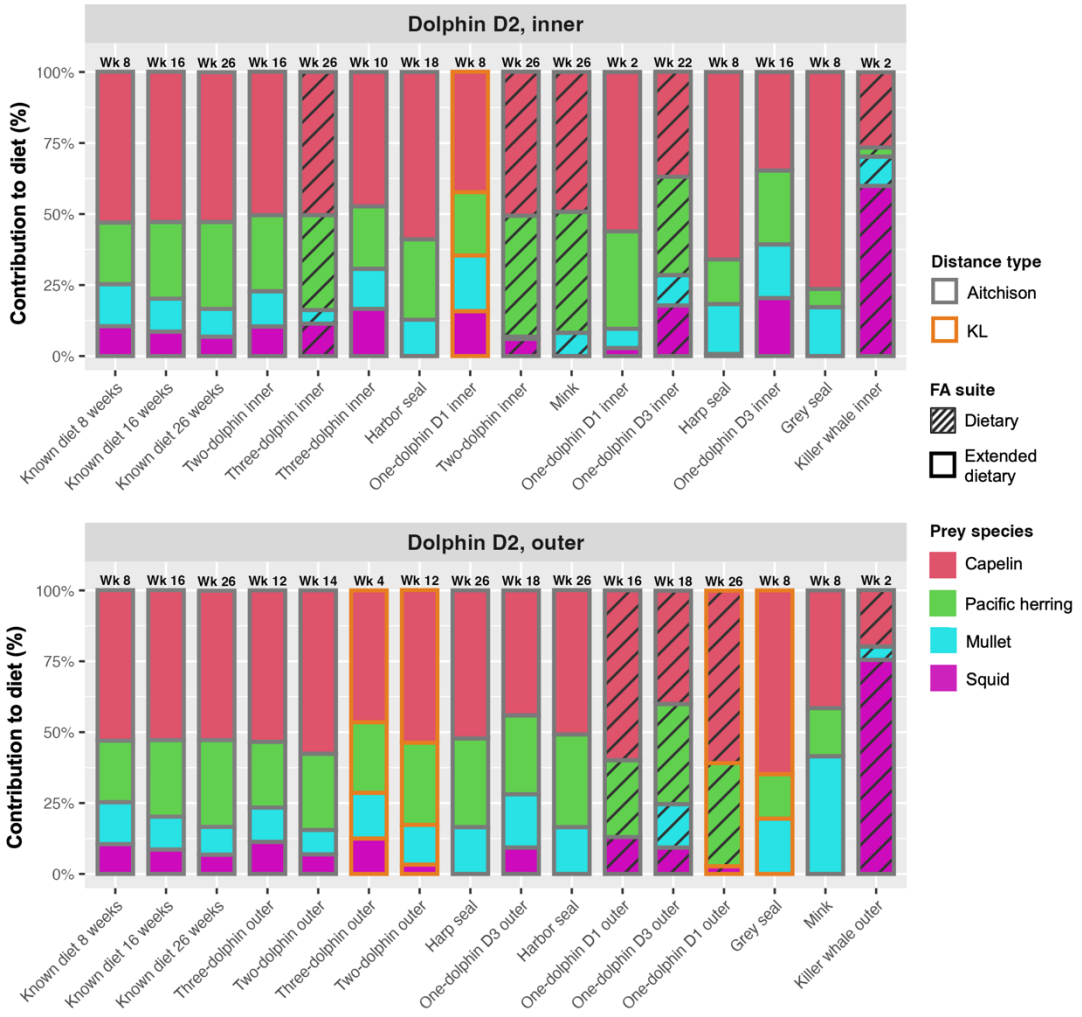

Figure 7: Prey species percentages for the lowest weighted error (WE) QFASA diet estimates for dolphin D2's inner (top) and outer (bottom) blubber layers. Includes the two lowest-WE estimates yielded by layer-matched three-, two-, and one-dolphin CCs using different model parameters and the lowest-WE estimates generated with non-dolphin CCs. Estimates are arranged from left to right by increasing WE and use the distance measure and FA suite that generated that estimate (Supplementary Tables 14, 15). Each dolphin's known diet averaged over the 8, 16, and 26 weeks before sampling is shown by the first three bars, as the lowest-WE estimates fell within that temporal window (see Results). The known diet that most closely matches the diet estimated using a given CC may differ from those weeks, however, and is indicated by the week number above each bar.

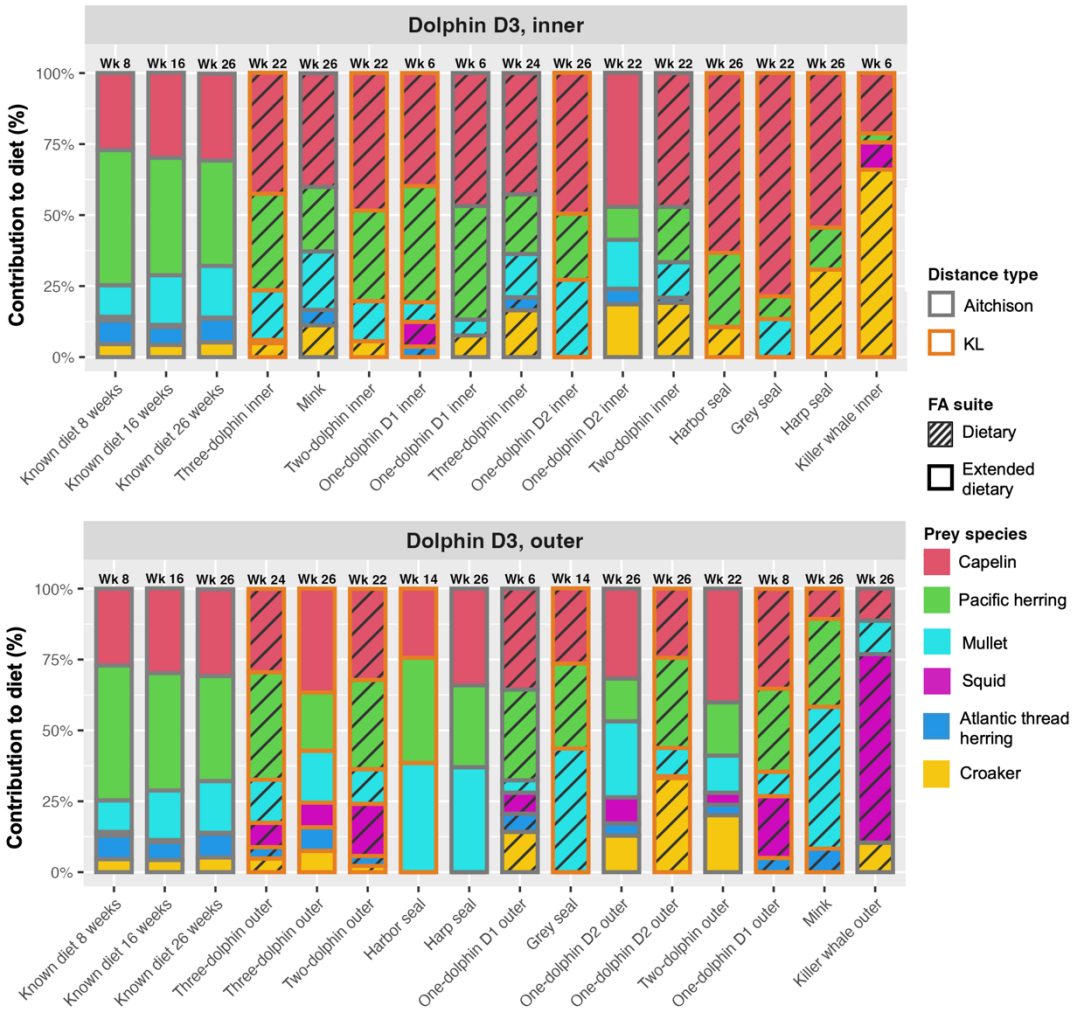

Figure 8: Prey species percentages for the lowest weighted error (WE) QFASA diet estimates for dolphin D3's inner (top) and outer (bottom) blubber layers. Includes the two lowest-WE estimates yielded by layer-matched three-, two-, and one-dolphin CCs using different model parameters and the lowest-WE estimates generated with non-dolphin CCs. Estimates are arranged from left to right by increasing WE and use the distance measure and FA suite that generated that estimate (Supplementary Tables 16, 17). Each dolphin's known diet averaged over the 8, 16, and 26 weeks before sampling is shown by the first three bars, as the lowest-WE estimates fell within that temporal window (see Results). The known diet that most closely matches the diet estimated using a given CC may differ from those weeks, however, and is indicated by the week number above each bar.

Chapter 2: Evaluating quantitative fatty acid signature analysis model performance and parameter sensitivity in free-ranging bottlenose dolphins (*Tursiops truncatus*)

Abstract

Quantitative fatty acid signature (QFASA) is a diet determination method with the potential to generate detailed estimates of diet integrated over weeks to months that is increasingly being applied to marine mammals. The mathematical model at the center of QFASA requires selecting specific inputs and parameters, of which there are multiple options. However, few studies have compared how different input and parameter combinations ("model configurations") impact model performance and the resulting estimates in free-ranging cetaceans. Here, I present the first application of QFASA in free-ranging bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphin", n = 15) to evaluate how alternative model configurations impact model performance and estimated prey contributions. Individual dolphin estimates were generated using multiple prey libraries, FA and calibration coefficient (CC) sets, distance measures, and blubber layers. Model performance and estimate interpretability were evaluated using prey distinctiveness, the percentage of predator FAs that fell outside prey ranges, the contribution of the "augmented" FA, and the uncertainty associated with each diet estimate, determined by assessing standard errors (SEs) and how estimates varied between configurations. I found that performance and estimated prey

contributions were sensitive to prey library composition, FA set, distance measure, and blubber layer. Generating single species-specific estimates for individual dolphins was complicated by variable prey species distinctiveness and SEs and the fact that multiple configurations provided estimates with similar performance metrics but different relative prey contributions. However, using the dietary FA suite and inner blubber yielded better performing and more consistent estimates. In addition, variation in individual dolphin estimates often involved redistribution among prey species with coherent trophic similarities. As a result, I suggest that broader diet patterns that are consistent across configurations with acceptable performance are robust for interpretation. I also propose that estimate uncertainty reflects real variation in prey and predator FAs, prey trophic relationships, and model parameter characteristics, and thus serves as a key indicator of reliability when analyzing diet estimates. Taken together, my results support the use of QFASA to estimate diet in free-ranging cetaceans, if performance metrics are used to inform model input and parameter selection and associated uncertainties are reported.

1 Introduction

Quantitative fatty acid signature analysis (QFASA) is a diet estimation method increasingly applied to marine mammals. Fatty acids (FAs) are chains of carbon, hydrogen, and oxygen atoms that serve as a primary form of energy storage in the blubber of marine mammals (Budge et al., 2006). Using the fact that FAs within consumed prey are deposited into blubber with few structural modifications and

remain in the blubber for weeks to months, QFASA has the potential to generate species-specific diet estimates that integrate diet consumption over that extended time frame (Budge et al., 2006). Such detailed, long-term estimates are ideal for addressing a range of research topics, from nutrient intake and diet specialization to disturbance vulnerability and social transmission of behavior (Johnson et al., 2009; Bowen and Iverson, 2013; Slifka et al., 2013; Krützen et al., 2014; Nielsen et al., 2018; Ning et al., 2020; Plint et al., 2023; García-Vernet et al., 2024). Despite its potential value, QFASA's application in cetaceans has to date been limited to four species: beluga whales (*Delphinapterus leucas)*, killer whales (*Orcinus orca*), and bottlenose dolphins (*Tursiops truncatus*) under professional care (Choy et al., 2019, 2020; Remili et al., 2022; Chapter 1 of this dissertation), and free-ranging Indo-Pacific humpback dolphins (*Sousa chinensis*) and killer whales (Ning et al., 2020; Xie et al., 2022; Remili et al., 2022, 2023).

Using the QFASA statistical model to estimate diet in marine mammals requires specific inputs and model parameters, as discussed in Chapter 1. QFASA generates estimates by determining the combination of prey species FA signatures (the mass percentage of FAs measured in a sample) that most closely matches the actual FA signature of the predator of interest (hereafter "predator"). This requires two inputs: FA signatures from 1) the predator's blubber, and 2) a "library" of potential prey species. Three model parameters influence the diet estimates. First, calibration coefficients (CCs) are correction factors used to account for small modifications that occur in prey FAs when they are digested and assimilated into

predator blubber. Second, only a subset of FAs measured in a sample, referred to as the "FA suite", are used for diet estimation. This is because blubber FAs originate both via biosynthesis and from dietary intake, and so not all measured FAs originate from the consumed prey and are informative for diet analyses. Finally, a statistical distance measure is used to quantify which mixture of potential prey has a FA signature that is most similar to the predator's signature.

Multiple decisions must be made when determining these inputs and model parameters. First, the appropriate CCs and distance measures must be selected from several possible options. To date, CCs have been derived from ten mammal species, including mink (*Mustela vison*), brown bears (*Ursus arctos*), six pinniped species, killer whales (*Orcinus orca*), and bottlenose dolphins (*Tursiops truncatus*) (Iverson et al., 2004; Nordstrom et al., 2008; Thiemann et al., 2008; Meynier et al., 2010; Rosen and Tollit, 2012; Goetsch et al., 2018; Remili et al., 2022; Thiemann et al., 2022; Chapter 1). Three distance measures can be used for diet estimation and have been applied in at least one study using QFASA to estimate diet in marine mammals: the Aitchison, Kullback-Leibler (KL), and chi-square measures (e.g., Iverson et al., 2004; Beck et al., 2007; Tucker et al., 2009; Meynier et al., 2010; Bromaghin et al., 2015a, b; Bromaghin et al., 2017; Goetsch et al., 2018; Choy et al., 2019; Ning et al., 2020; Xie et al., 2022; Remili et al., 2022, 2023; Stewart et al., 2025). Second, there are two general categories of FA suite: FAs derived entirely or primarily from dietary intake ("dietary" FAs) or the dietary FAs plus FAs with large contributions from both biosynthesis and diet ("extended dietary" FAs) (Iverson et al., 2004). Finally, prey

library composition must be considered, as the number and identity of included prey species can influence estimate accuracy and interpretability. Including too few species risks missing potentially important diet components, but the total number of species in the library cannot exceed the number of FAs used for diet estimation, as including more species than FAs yields non-unique diet estimates (Budge et al., 2006; Goetsch et al., 2018). Species FA signatures must also be distinct enough from each other for the QFASA model to accurately identify them (Budge et al., 2006; Bromaghin et al., 2017). Previous studies have formed prey libraries by pooling species with similar FA signatures (e.g., Piché et al., 2010; Iverson et al., 2011), iteratively removing species to test their effect on estimated diet and thus determine their importance (Goetsch et al., 2018; Ning et al., 2020), or using *a priori* knowledge from other diet determination methods (e.g., stomach contents, stable isotope analyses) to include or exclude certain species (Beck et al., 2007; Meynier et al., 2010; Xie et al., 2022; Remili et al., 2023).

Given the prey library considerations and variety of available model parameters, understanding how different inputs and parameter combinations impact the resulting diet estimates and which provide the most accurate results is critical. However, few studies have compared the accuracy of multiple parameter combinations, and the existing ones show that QFASA is sensitive to CC set, distance measure, FA suite, and prey library composition but lack consensus on "best-fit" options. For instance, the first paper to formally describe the QFASA method for marine mammal diet estimation evaluated multiple distance measures, FA suites, CC

sets, and prey library subsets in grey seals (*Halichoerus grypus*) (Iverson et al., 2004). The KL distance, the extended dietary suite, and CCs derived from grey seals provided the most accurate estimates, while accuracy decreased when using non-seal CCs and when simulated diets contained more prey species with similar FA signatures (i.e., less distinctive prey). Analyses of free-ranging polar bear (*Ursus maritimus*) diets showed that estimates differed depending on whether the Aitchison or KL distance was applied but suggested that which to use depended on multiple conditions (Bromaghin, 2015; Bromaghin et al., 2015). Validation studies in cetaceans have produced similarly variable results. In a single Indo-Pacific humpback dolphin (*Sousa chinensis*) under professional care, mink-derived CCs, the extended dietary suite, the Aitchison distance, and a prey library containing only known prey yielded the most accurate estimates (Xie et al., 2022). However, dolphin-specific CCs were not available for that study. Conversely, a study of killer whales (*Orcinus orca*) under professional care found that the KL distance, the extended dietary FA suite, and killer whale-specific CCs performed best, while the Aitchison distance and mink CCs increased error (Remili et al., 2022). Only known prey species were included in the library, however. Finally, most relevant to this chapter, our validation of QFASA in bottlenose dolphins (*Tursiops truncatus*) under professional care in Chapter 1 demonstrated that species-specific CCs and the dietary FA suite performed better than mink CCs or the extended dietary suite. However, both the Aitchison and KL distances were effective depending on dolphin diet complexity, and only known prey were included in the library. Together, these results support the value of species-

specific CCs when available but emphasize that different prey libraries can impact estimate accuracy and do not provide a unified recommendation regarding FA suite and distance measure selection. Furthermore, all previous evaluations in cetaceans have been conducted in animals under professional care with known diets; whether these conclusions hold for free-ranging cetaceans, where true diet is unknown and prey assemblages are generally more complex, remains unstudied.

Given QFASA's potential value but the lack of consensus surrounding critical best-fit model inputs and parameters, especially in free-ranging cetaceans, this chapter will evaluate how alternative model parameter combinations and prey library compositions impact the resulting diet estimates. My primary objectives are to: 1) determine the FA signatures of free-ranging bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphins") and a selection of their potential prey and compare them to existing values to assess their validity, 2) estimate dolphin diet use species-specific CCs derived in Chapter 1 and multiple FA suites, distance measures, and prey library compositions, 3) evaluate QFASA model performance and determine whether specific model configurations can be recommended for future applications, and 4) assess the interpretability and uncertainty of the resulting diet estimates. Together, these objectives will serve as the first evaluation of QFASA model performance and parameter- and prey library-sensitivity in free-ranging dolphins, providing insights into the strengths, limitations, and uncertainties associated with the diet estimates and establishing recommendations for future cetacean QFASA applications.

2 Materials and methods

2.1 Sample collection

All sample collection occurred in Sarasota Bay (SB), Florida (**Figure 1**). SB provides an ideal system to evaluate QFASA in free-ranging dolphins. The community of approximately 170 free-ranging dolphins has been nearly continuously studied by the Sarasota Dolphin Research Program (SDRP) since 1970 (Wells et al., 2025). As most dolphins reside in the area year-round and throughout most of their lives, this long-term research effort has yielded detailed information on individual dolphin identity, age, sex, maternal lineage, and social associations for more than 90% of the dolphins (Wells et al., 2025). Dolphin diet has also been estimated using hard part analyses (Barros and Wells, 1998; Berens McCabe et al., 2010; Barros et al., 2012; Wells et al., 2013), DNA metabarcoding (Dunshea et al., 2013), stable isotopes (Rossman et al., 2013, 2015a, 2015b), and direct observation (Ronje et al., 2017; SDRP unpublished data). Established dolphin catch-and-release and prey collection protocols further facilitate sample acquisition. Together, these features make the SB dolphins an ideal system for evaluating QFASA model performance and estimated prey contributions.

Blubber samples were collected in June 2019, during catch-and-release dolphin health assessments in SB. During these assessments, dolphins were encircled by a seine net and temporarily restrained to collect biological, physiological, and morphological data/samples (Wells et al., 2004; Barratclough et al., 2019). Blubber samples were taken from standard full-depth wedge biopsies collected under local

anesthesia by a veterinarian from the mid-lateral region below the dorsal fin. Samples were stored in liquid nitrogen in the field, then kept at –80 ℃ until analysis. An experienced veterinary team attended to the dolphins during examination and sampling, ensuring dolphin safety and welfare. Dolphin sex (male or female) was determined by visual examination of genital slit configuration and each individual was classified as maternally "independent" (> six years of age) or "dependent" (< six years of age). This division is based on the understanding that mother-calf associations in SB last three to six years, so dolphins greater than six can be considered independent from their mothers even if they are not yet sexually mature (Wells, 2014; Rossman et al., 2015a; Wells et al., 2025).

Potential prey fish were collected opportunistically via purse-seine net fishing during fish abundance surveys in SB during 2020 – 2022 (Berens McCabe et al., 2021, 2010). Whole fish were stored on ice in the field, then kept at –20 ℃ until analysis. Sampling targeted fish between 100 and 300 mm, the size range most often found in dolphin stomach contents (Barros and Wells, 1998). The diet of each species was estimated through a comparative study of published data (Smith, 1997; Ray et al., 1999; Gannon et al., 2009; Murdy et al., 2013). Based on their diet, fish were part of one of the following trophic groupings: demersal herbivore (browser, grazer), demersal invertivore, demersal piscivore, demersal omnivore (invertebrate, algae, and detritus feeder), benthic omnivore, pelagic filter feeder / planktivore, and pelagic piscivore (Gannon et al., 2009; Piché et al., 2010).

Dolphin sampling occurred under National Marine Fisheries Service/MMPA Scientific Research Permit No. 20455. Fish sampling was done under Florida Fish and Wildlife Conservation Commission Special Activity License No.19-0809-SR. Both projects were reviewed annually for IACUC approval through Mote Marine Laboratory.

2.2 Sample preparation and fatty acid analyses

Preparation of the dolphin blubber samples and the fish for FA analyses followed methods established in Chapter 1. Briefly, blubber was subsampled into layers (outer, middle, and inner or outer and middle, depending on sample depth) based on color and texture. This accounts for known FA differences due to the highly stratified nature of cetacean blubber. Generally, inner blubber contains a higher proportion of FAs derived mostly or exclusively from prey and is thought to experience greater FA turnover due to its proximity to capillary and muscle layers that leads to higher metabolic activity (Samuel and Worthy, 2005; Koopman, 2007; Ellisor et al., 2013). In contrast, the outer blubber is less vascularized (McClelland et al., 2012) and more closely associated with thermoregulation (Koopman, 2007). As a result of their structural differences, it has been suggested that inner blubber may capture a shorter integrated diet period than outer blubber (Budge et al., 2006; Remili et al., 2022) though our analyses in Chapter 1 comparing diet period between the layers suggested they reflect similar time frames. Despite these differences, however, both blubber layers have been shown to accurately estimate diet in cetaceans (Remili

et al., 2022; Chapter 1) and will be assessed in this chapter. Given the shallow blubber depth (<2 cm), the middle layer was treated as a transition zone and excluded from further analyses. Fish were partially thawed, homogenized using a bullet blender and scalpel, and separated into 0.5 g subsamples. Since dolphins generally consume whole prey, fish were homogenized whole, including their stomach contents. The only exception were hardhead catfish (*Ariopsis felis*), whose heads were removed before homogenization. The few observations of dolphins consuming catfish note that they generally remove the heads before consumption – which as indicated by their names are tough and equipped with sharp, venomous spines – by smacking the fish against the water or otherwise manipulating them (Ronje et al., 2017).

To measure dolphin and prey fish FA signatures, blubber and fish lipids were extracted in 2:1 chloroform:methanol using a Soxtec apparatus (Anderson, 2004) and transformed into FA methyl esters (FAMEs) using the Hilditch method, then each sample's FA signature was determined via gas chromatography with flame-ionization detection (GC-FID) as previously established (Budge et al., 2008). The relative contributions of 64 FAs, from 14:0 to 24:1n-9, were measured. FAs are named using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group. Lipid percentages in blubber and fish samples were determined using an internal standard to calculate mass percent FA, a proxy for percent lipid. Tissue sample processing and lipid extraction were performed at the

University of California, Santa Cruz, USA, while FAME derivatization and GC-FID were performed at Dalhousie University, Halifax, Canada.

2.3 QFASA model input and parameter selection

The QFASA model estimates diet by minimizing the statistical distance between the actual fatty acid (FA) signature of the dolphin of interest and model FA signatures constructed from a "library" of the prey fish FAs, corrected for effects of metabolism with calibration coefficients (CCs) (Iverson et al., 2004; Budge et al., 2006). The dolphins' inner and outer blubber FA signatures were used for diet estimation, along with two prey libraries. Prey libraries were constructed using *a priori* knowledge of the SB system, following the approach of previous QFASA studies in cetaceans and pinnipeds (Tucker et al., 2009; Meynier et al., 2010, 2014; Xie et al., 2022; Remili et al., 2022, 2023). The "full" library included all fish species and samples collected during 2020 – 2022 that met the following criteria: 1) more than two samples available, and 2) identified as potential SB dolphin prey based on previous stomach content, molecular prey detection, stable isotope (SI), and prey selection analyses, direct observation of feeding, and frequency of collection during prey sampling surveys (Barros and Odell, 1990; Barros and Wells, 1998; Berens McCabe et al., 2010; Dunshea et al., 2013; Wells et al., 2013; Rossman et al., 2015a; Ronje et al., 2017). The "SI" library consisted of the subset of the "full" library that matched species previously used to estimate SB dolphin diet using SIs and Bayesian mixing models (Rossman et al., 2015a). Using both prey libraries allowed me to

evaluate the sensitivity of diet estimates to prey library composition while also facilitating comparison with previous stable isotope analyses and existing information on SB dolphin diet.

I used CCs derived from dolphins with known diets in Chapter 1. CCs were selected based on the conclusions and recommendations of that chapter, where multiple sets of CCs were calculated to account for variation in the dolphins' diets in the months before sampling. There, we demonstrated that three-dolphin CCs (i.e., the average of each dolphin's individual CCs) calculated using their known diet averaged over at least 8 and up to 26 weeks before sampling differed minimally from each other and provided the most accurate reconstructions of the dolphins' known diets. Since a single CC set was not uniformly better, we suggested that future studies use multiple sets of three-dolphin CCs that reflected the 8-to-26-week period and assess whether the different CCs impacted the diet estimates when applied in a new system. Accordingly, for this chapter, I used three sets of three-dolphin CCs, calculated using the dolphins' known diets averaged over the 8, 16, and 24 weeks before sampling (**Table S1**).

Two additional model parameters – the FA suite and the distance measure – influence QFASA diet estimates, but multiple options exit and no single "best-fit" combination has consistently been applied or shown to provide the most accurate estimates (Iverson et al., 2004; Bromaghin et al., 2015; Bromaghin, 2015; Xie et al., 2022; Remili et al., 2022; Chapter 1). Thus, following previous cetacean QFASA studies, both the Aitchison and KL distances and the dietary and extended dietary FA

suites were used. Following established classifications (Iverson et al., 2004), the "dietary" suite included FAs derived entirely or primarily from dietary intake that were on average greater than 0.1% of the dolphins' total FA signatures in each blubber layer, while the "extended dietary" suite included the dietary FAs plus the FAs greater than 0.1% with relatively large contributions from both biosynthesis and diet (**Table S2**). FA suites varied slightly between the inner and outer blubber, as FA proportions differed between blubber layers.

2.4 Calculating and comparing QFASA diet estimates

Estimates for the SB dolphins were made using two blubber layers (inner, outer), two prey libraries (full and SI), two FA suites (extended dietary and dietary), two distances measures (Aitchison and KL) and three sets of layer-matched three-dolphin CCs (week 8, 16, and 24) for each FA. Hereafter, I will refer to these as the "model variables" and their combinations as the "model configurations". After removing FAs that were measured in the samples but not included in the FA suites, the remaining FA proportions were augmented (Bromaghin et al., 2016a; Bromaghin, 2017). Compared to scaling remaining FAs to sum to 1, augmenting maintains the same FA proportions and accounts for removed FAs by combining their proportions into a single "augmented" FA with its own CC. Diet estimates were calculated in the predator space using the function "est_diet" in R, using package qfasar (Bromaghin, 2020; R Core Team, 2025). Raw diet estimates (% lipid mass per prey species) were adjusted to account for each species' lipid content, which can be variable (see section

3.2.2), so diet estimates indicated the % biomass per prey species. The diet estimates' means and standard errors (SEs) were obtained using bootstrap sampling (n = 100).

I used a multi-factor permutational analysis of variance (PERMANOVA) with the different model variables (blubber layer, prey library, FA suite, distance measure, and CC set) as categorical fixed effects to quantify whether the diet estimates differed significantly between the different combinations. I accounted for repeated measurements of individuals by constraining the permutations within each dolphin. Prior to interpretation, I evaluated the key assumption of PERMANOVA models, the homogeneity of multivariate dispersion among groups. If dispersion differed significantly among groups, PERMANOVA results were interpreted cautiously, as significant dispersion suggests differences in the variability of the diet estimates with the different parameter options that can influence PERMANOVA outcomes.

I also employed a Mantel test to further assess diet estimate variability (or lack of variability) for different CCs sets. While PERMANOVA assesses whether diet estimates differ significantly, Mantel tests quantify the correlations between distance matrices generated by different CC sets. Higher positive correlations indicate greater agreement, or more similarity, in the overall patterns of diet estimates produced by the different sets.

All PERMANOVA and Mantel analyses were completed in R, with the package vegan and functions "betadisper", "permutest", "adonis2", and "mantel" (Oksanen et al., 2025; R Core Team, 2025).

2.5 Assessing model performance

Two criteria inform QFASA model performance, indicating how well the model fits the dolphin and prey FA data and the relative accuracy of the resulting diet estimates: 1) predator and prey FA signature overlap, and 2) prey species distinctiveness (Iverson et al., 2004; Bromaghin et al., 2015; Bromaghin, 2017). I analyzed overlap and distinctiveness using, respectively, the "pred_beyond_prey" and "lopo" functions in R package qfasar (Bromaghin, 2020). Predator-beyond-prey (PBP) values are the percentage of each predator's measured FAs whose proportions fall outside the observed range for that FA in the prey library after modification with CCs. High percentages suggest predator signatures are poorly represented by the prey library or CC set and reduce confidence in the diet estimates for those parameter combinations. In LOPO (leave-one-prey-out) analyses, each prey sample is successively treated as an unknown species and grouped with other samples based on FA signature similarity. High self-association rates indicate that prey species have distinct FA signatures and are therefore more likely to be distinguished by the QFASA model and in predator diets. Both LOPO and PBP values may vary depending on the prey library, FA suite, and distance measure used in the calculations, while CCs only impact PBP values. Accordingly, these analyses were repeated for all relevant model configurations.

As an additional test of model performance, I determined the percent contribution of the "augmented" FA to each diet estimate using the *PropDistCont* measure in R package QFASA (Stewart et al., 2024). If the augmented FA has a

particularly high contribution to the final estimate, it is a diagnostic red flag. First, it could suggest that the excluded FAs represented by the augmented proportion have explanatory importance. Second, it is an artificial FA that cannot be connected back to any biological or physiological considerations, diminishing its explanatory power. In Chapter 1, certain model parameter combinations, especially combining the extended dietary suite with either distance measure, yielded estimates where the augmented FA was one of the top 10 contributors to the diet estimate. Accordingly, I suggested that future studies check the contribution of the augmented FA and, if applicable, use the values to inform model parameter selection and result interpretation.

To quantify how PBP values and the augmented FA contribution varied with the different model variables (blubber layer, prey library, FA suite, distance measure, and CC set), I ran two separate linear mixed effects models using R package lme4 (Bates et al., 2025). Prior to modeling, I evaluated the distribution of the response variable and checked for potential skewness and outliers, to assess the model assumptions of linearity, homoscedasticity, and normality.

Finally, I used the standard errors (SEs) associated with each estimated prey contribution as an indicator of estimate uncertainty. QFASA's SEs are generated via a bootstrapping procedure. Prey FA profiles are resampled with replacement, averaged to create new prey means, and used to re-estimate diet percentages over 100 iterations (Bromaghin, 2017). Lower SEs indicate that a prey species' estimated contribution to dolphin diet is consistent across bootstrapped iterations, increasing certainty in the

estimate. By contrast, higher SEs indicate that multiple combinations of prey proportions produce similarly good fits to the predator FA signature, resulting in increased uncertainty when interpreting the estimates.

3 Results

3.1 Dolphin demographics and prey library variety

In total, 15 dolphins were sampled, including four independent females, six independent males, four dependent females, and one dependent male. Full-depth blubber samples were collected from 13 dolphins, while only outer blubber was collected from the remaining two (both independent males). See **Table S3** for complete dolphin sample and demographic details. Fish sampling for the "full" library collected 147 individuals from 20 species, with 3 – 12 individuals per species. The "SI" library included 13 of those species (n = 99 samples) which had been included in previous SI diet estimates (Rossman et al., 2015a). Prey fish came from a variety of trophic groups, including planktivores; benthic carnivores, omnivores, and detritivores; invertivores; and piscivores. See **Table S4** for complete fish sample details.

3.2 Dolphin and prey fish fatty acid signatures and lipid contents

3.2.1 Dolphin fatty acid signatures

All SB dolphin FA signatures shared general patterns, with certain dominant FAs and small differences between blubber layers (**Figure 2, Table S5**). Across both

blubber layers, 16:1n-7, 18:1n-9, 16:0, 22:6n-3, and 22:5n-3 accounted for most of the FA composition, and FA signatures were dominated by extended dietary FAs (61 – 67.9% of FA signatures across all groups and layers), compared to dietary (14.6 – 26.6%) and non-dietary (20.2 – 24.7%). Of the extended dietary FAs, 16:1n-7, 18:1n-9, 16:0, 14:0, 22:5n-3, 18:1n-7, and 18:0 were most prevalent, while of the dietary FAs, 22:6n-3, 20:5n-3, 20:4n-6, 18:2n-6, 18:3n-3, 16:2n-4, and 20:1n-9 had the highest percentages. Modest differences between blubber layers were also apparent. Across all samples, outer blubber had a slightly higher total percentage of monounsaturated FAs (MUFAs) (54.9 – 58.2% of outer blubber versus 43.1 – 54.7% of inner), especially 16:1n-7 and 18:1n-9. Similarly, inner blubber had a higher total percentage of polyunsaturated FAs (PUFAs) (18.1 – 30.8% of inner blubber for all groups versus 14.7 – 21.7% of outer blubber), especially 22:6n-3, and saturated FAs (SFAs) (25.8 – 30.7% versus 23.4 – 30.1%). Non-dietary FAs were slightly higher in inner than outer blubber for dependent dolphins (24.2 – 24.7% inner versus 21.1 – 21.9% outer), but the opposite was true for independent dolphins (20.2 – 21.9% versus 22.8 – 22.9%). In comparison, dietary FAs were higher in inner than outer blubber for all dolphins (16.4 – 26.6% inner versus 14.6 – 20.8% outer), while extended dietary FAs were slightly higher in outer or comparable between layers (61 – 67.4% inner versus 64.5 – 67.8% outer). However, most individual dietary and extended dietary FAs had higher percentages in inner than outer blubber, except for 16:1n-7, 18:1n-9, 16:2n-4, and 18:3n-3.

3.2.2 Fish fatty acid signatures and lipid composition

SB prey fish FA signatures shared a subset of dominant FAs, but the relative contributions differed (**Table 1, Figure 3, Table S6**). Across species, 16:0 (17.5 – 25.7%), 16:1n-7 (3 – 15%), 18:0 (5 – 10%), 18:1n-9 (5 – 20%), 20:4n-6 (2 – 10%), 20:5n-3 (2 – 10.5%), and 22:6n-3 (3.7 – 25%) were consistently among the top FAs. Some FAs (e.g., 14:0, 16:0, 18:0, 18:1n-7, 22:5n-3) were relatively consistent among species. A subset of FAs varied among species and drove differences, however. In particular, 22:6n-3 was highly variable across species. For instance, the highest values – in common snook, Atlantic thread herring, scaled sardine, and ladyfish – ranged from 21.5 – 25%, while the lowest values – in mullet, sheepshead, and pigfish – ranged from 3.7 – 4.3%. 18:1n-9 was also variable, with highest values in pigfish, hardhead catfish, silver perch, and white grunt (16.1 – 19.6%), and low values in mullet, menhaden, and Atlantic thread herring (5 – 5.4%).

Percent lipid varied among species and often within a given species (0.4 – 11.6%; mean ± SD: 3.7 ± 3.1%) (**Table S6**). It was highest in menhaden (11.6 ± 1.85), high but variable in mullet (9.2 ± 4.4%) and spot (8.6 ± 6.3%), and lowest in common snook (0.4 ± 0.04%), ladyfish (0.6 ± 0.1%), and gulf toadfish (0.7 ± 0.1%). However, most species had intermediate values around 2 – 6%.

3.3 QFASA model criteria

3.3.1 Predator and prey FA overlap

Qualitative comparisons (**Table S7**) and quantitative analyses via a linear mixed effect (LME) model (**Table 2**) revealed that predator-beyond-prey (PBP) values varied among dolphins, but that within a given dolphin, values were higher with the outer layer and the extended dietary FA suite, and the SI library to a lesser extent. Across dolphins, inner blubber PBP values ranged from 4 – 25% (full prey library) and 4 – 31% (SI prey library) and outer blubber values ranged from 12 – 42% (full) and 12 – 50% (SI) (**Table S7**). For a given dolphin, outer blubber PBPs were always higher than inner blubber values and extended dietary values were comparable to or higher than dietary values. The degree of difference varied among dolphins, however, ranging from 10 – 40% (inner versus outer, with most around 15 – 20%) and 3 – 20% (extended dietary versus dietary, with most around 5 – 10%). In comparison, estimates for a given dolphin generally varied by <5% between the distance measures, the CCs, and the prey libraries (**Table S7)**. The LME results (**Table 2**) support these qualitative differences: across dolphins, there was very strong evidence that PBP percentage differed between blubber layers ($p < 0.0001$), and weak evidence of differences between FA suites ($p = 0.08$). In comparison, there was no evidence of differences due to prey library, CC set, and distance measure ($p > 0.8$). LME model diagnostics indicated no major violations of the model assumptions. Together, qualitative and quantitative comparisons demonstrate that PBP values differed most depending on which blubber layer was used for diet estimation, with higher values for outer blubber than inner, differences due to FA suite and prey library were present but

not uniform across dolphins, and differences due to distance measure and CC set were minimal.

3.3.2 Augmented FA contributions to QFASA diet estimates

The contributions of the "augmented" FA were consistent across the different CC weeks but varied by different degrees depending on the model variables used to calculate the diet estimates. According to the LME model comparing the augmented FA contribution across the different dolphins and their diet estimates (**Table 3**), for inner blubber there was weak evidence of higher contribution with the extended dietary suite alone ($p = 0.07$) and the combined with the KL distance ($p = 0.05$) but strong evidence when the extended dietary suite, KL distance, and the SI library were used together ($p < 0.0001$). For the outer blubber, there was strong evidence of higher contribution when the extended dietary suite was used with the Aitchison distance ($p < 0.0001$) and when it was used with the KL distance and SI library ($p < 0.0001$). Model diagnostics indicated no major violations of assumptions for linear mixed-effects models. Examining the actual contributions for the different diet estimate subsets (**Table S7**) demonstrates this pattern qualitatively. Across all dolphins, augmented FAs never contributed > 6% when using the dietary suite and either distance measure, no matter the blubber layer, but using the extended dietary suite and the KL distance, in the inner layer, and the extended dietary suite and either distance measure, in the outer layer, yielded many more instances of large and very large contributions by the augmented FA (up to 36% in the inner layer and up to 56%

in the outer layer). Using the SI library only exacerbated the issue. Thus, while not all augmented FA contributions were meaningful when using the extended dietary suite, many were, especially in the outer blubber.

3.3.3 Prey species distinctiveness

Leave-one-prey-out (LOPO) analyses revealed variable species distinctiveness (**Figure 4**). Self-association values ranged from 5 – 97%, depending on species and model configuration. Associations varied noticeably with FA suite and distance measure but not prey library (CCs do not affect LOPO outcomes). Although certain model configurations yielded higher self-associations for some species, no consistent pattern emerged. For instance, using the dietary FA suite, self-associations were higher with the KL distance for pigfish, spotted seatrout, mojarra, lane snapper, and hardhead catfish, but higher with the Aitchison distance for mangrove snapper, spot, and white grunt. For other species, self-associations did not vary with the different combinations, but associations with other species did. For example, scaled sardine and grass porgy had higher associations with Atlantic thread herring and pigfish, respectively, when using the KL distance and with common snook and mojarra, respectively, when using the Aitchison distance.

Species present in both the full and SI libraries generally exhibited slightly higher self-associations with the SI library. For example, Atlantic thread herring and mojarra had higher self-associations with the SI library, largely reflecting the removal of menhaden and grass porgy, respectively. However, these differences did not

substantially alter overall association patterns. The only exception was spotted seatrout, who only self-associated with the full library and instead associated most strongly with ladyfish, common snook, and silver perch with the SI library.

LOPO values ranged widely among species. Five species were highly distinctive, with self-associations exceeding 70%: common snook (89 – 97%), gulf toadfish (71 – 88%), menhaden (87 – 93%), mullet (80 – 96%), and sheepshead (84 – 89%). Seven additional species had intermediate self-associations but clustered with specific subsets of species. Atlantic thread herring (49 – 66%) and scaled sardine (33 – 58%) were strongly associated with each other and menhaden. Both also had smaller associations with common snook and ladyfish. Ladyfish (32 – 51%) and spotted seatrout (7 – 44%) primarily associated with each other and common snook, along with silver perch and scaled sardines. Mojarra (50 – 75%), pigfish (30 – 76%), grass porgies (23 – 37%), and white grunt (20 – 64%) showed strong mutual associations. Hardhead catfish (14 – 51%) most frequently associated with pigfish, white grunt, and gulf toadfish, but also sometimes ladyfish. Five species – lane and mangrove snappers, silver perch, croaker, and spot – had intermediate to low self-associations (20 – 60%) and weak to intermediate associations (10 – 20%) with multiple species, depending on which model variables were combined. Finally, pinfish had the lowest self-association (< 19%) and low to intermediate associations (5 – 30%) with multiple species.

3.4 QFASA diet estimate variation with different model variables

There was strong evidence for diet estimate variation with all QFASA model variables (FA suite, distance measure, prey library, and blubber layer; $p < 0.001$) except CC set, where PERMANOVA results showed no evidence of variation with different CCs ($p = 0.97$) (**Table 4**). Furthermore, Mantel tests indicated extremely high concordance among diet estimates generated using the different CC sets ($r = 0.97 – 1.00$ across all pairwise comparisons and model configurations; $p < 0.001$), indicating that using different CCs led to minimal differences between the diet estimates (**Table S8**). Tests for homogeneity of dispersion, the core assumption of PERMANOVA, add some nuance to the results but do not change the major conclusions. Multivariate dispersion was homogeneous for CC set and prey library, indicating no evidence of dispersion differences. In comparison, FA suite, distance measure, and blubber layer had significantly non-homogeneous dispersions, suggesting that one FA suite, distance measure, and layer yielded more variable estimates than the other. This does not change the fundamental interpretation, however. Significant dispersion would make a significant PERMANOVA less rather than more likely; this suggests the diet estimates differences with FA suite, distance measure, and blubber layer are large enough to overcome any underlying variation. Detailed examination of how the diet estimates differ with the different model variables will occur below (see section 3.6).

3.5 Identifying retained model configurations

Following examinations of the QFASA model criteria and estimate variation with the different model variables, certain diet estimates were excluded from the remainder of the analyses.

As the different CC sets did not affect the LOPO results, PBP percentages, FA contributions, or estimated diet compositions, I focused only on the week 16 CC results. The week 16 CCs were selected as middle points between the week 8 and week 24 values, and for no other reason.

I also excluded all results generated using the extended dietary suite. First, many estimates generated using the extended dietary suite had high augmented FA contributions, especially with the KL distance and/or the SI prey library and in the outer blubber layer. This matches our application of QFASA in dolphins under professional care in Chapter 1, where the extended dietary suite tended to yield higher augmented FA contributions than the dietary suite. There, we suggested avoiding estimates with higher augmented contributions, as they diminish the estimates' explanatory powers and cast doubt on model accuracy. We also found that the dietary FA suite yielded the lowest-error diet estimates for the dolphins with the most complex diet. While that dolphin's diet consisted of six prey species, much smaller than the 20 species included in this chapter's prey library, it is the most similar in terms of species diversity and variation in LOPO associations. In further support of excluding the extended dietary suite, most dolphins' PBP values were consistently higher with the suite, suggesting a somewhat poorer model fit, though the difference was not strongly significant compared to the dietary suite. Finally, while previous

studies applying QFASA in cetaceans and other marine mammals have used the extended dietary suite (e.g., Iverson et al., 2004; Xie et al., 2022; Remili et al., 2022, 2023), only the dietary FAs are exclusively derived from prey consumption. Focusing on the dietary FAs removes interference in the model results that could come from considering FAs with some origin in biosynthesis. Taken together, these considerations support using and considering only estimates, PBP percentages, and LOPO values generated using the dietary FA suite moving forward. This still leaves multiple model variable combinations and multiple estimates per dolphin, however, generated using the full and SI prey libraries, Aitchison and KL distances, and inner and outer blubber.

3.6 Effect of model configuration on estimated diet composition

Across all model configurations, 12 prey species were identified in at least one dolphin's estimate. However, estimated diet compositions and the associated standard errors (SEs) varied substantially with blubber layer and distance measure, and to a lesser extent with prey library (**Figures 5, S1**). This aligns with the PERMANOVA results that showed significant variation between estimates with all model variables except for CC week.

Inner estimates were more consistent between model configurations and less diverse than outer blubber estimates but still varied between distance measures (**Figures 5, S1**). Estimates made using the Aitchison distance were the most straightforward, dominated by common snook (45 – 75% across dolphins), scaled

sardine (64 – 89%), or a combination both (41 – 65% snook, 24 – 38% sardine). Mullet (5 – 25%), gulf toadfish (12 – 35%), and Atlantic thread herring (8 – 19%) contributed smaller secondary proportions of the diet. The same prey species remained important for inner estimates with the KL distance, but their estimated percentages varied from the Aitchison results, and prey species (including silver perch, spotted seatrout, ladyfish, and mangrove snapper) not present in the Aitchison estimates were also identified, sometimes in large percentages (> 50%). With either distance measure, estimates generated using the SI prey library were broadly similar to those using the full library, although Gulf toadfish percentages tended to increase and common snook percentages tended to decrease. Across these estimates, SEs remained small for mullet (<9%), of intermediate size for common snook and scaled sardine (up to 25%), and variable but sometimes comparable to one-half or more of the estimated values for Atlantic thread herring, gulf toadfish, spotted seatrout, silver perch, ladyfish, and mangrove snapper. For some species, this was exacerbated by using the KL distance and the SI library together. For instance, although spotted seatrout was often absent or present at low proportions with small SEs, using the KL distance and SI prey library produced estimates of 40 – 80% with SEs up to 45% for some dolphins.

Relative to inner blubber estimates, outer blubber estimates calculated using both the Aitchison and KL distances included a greater diversity of prey species, different most prevalent prey, and comparable or higher SEs (**Figures 5, S1**). Common snook was overall reduced compared to inner blubber: whereas snook

frequently accounted for >50% of the diet in inner estimates, even with SEs, it was <30% of the diet for some dolphins and absent for the rest. Instead, estimates were mostly mixtures of scaled sardine, Atlantic thread herring, mullet, Gulf toadfish, croaker, mangrove snapper, and/or silver perch (KL distance and full library only) or spotted seatrout (KL distance and SI library only). Small amounts of lane snapper, spot, and ladyfish were also identified for some dolphins. Species that were larger contributors with the full library but not included in the SI library (croaker, mangrove snapper, and silver perch) were replaced by additional scaled sardine or other species, especially spotted seatrout and/or gulf toadfish. However, determining exact prey percentages was complicated, as SEs were high for most species except for mullet, tended to be either comparable to or larger than the inner blubber SEs, and were sometimes high even when the estimate was 0%. As with inner blubber estimates, this was especially exacerbated when using the SI library and the KL distance. For instance, mangrove snapper and scaled sardine comprised 7 – 45% and up to 95% of some estimates but had SEs of 14 – 25% and up to 70%.

Taken together, these results show several broad patterns. Inner blubber estimates were generally less diverse and more consistent across model configurations than outer blubber estimates, while estimates made using the KL distance tended to identify additional prey species and re-distribute prey proportions relative to the Aitchison estimates. Estimates were similar between the full and SI prey libraries, unless species absent from the SI library were important contributors in the full library estimates, which occurred more frequently for outer estimates than

inner estimates. Finally, SEs varied considerably among prey species and model configurations. While mullet consistently exhibited low uncertainty, other primary and secondary prey species frequently had SEs comparable to a substantial portion (one-half or more) of the estimated contribution. However, SEs were typically highest in outer blubber estimates and in estimates generated using the KL distance and SI library together.

4 Discussion

4.1 Sarasota Bay dolphin and prey fish sample size and prey library considerations

Dolphin sample size was relatively small, with 13 inner blubber and 15 outer blubber QFASA diet estimates. Consequently, the resulting diet estimates should be interpreted as representative of the sampled dolphins rather than the Sarasota Bay dolphin community as a whole. However, they were sufficient for evaluating QFASA model performance and parameter sensitivity across multiple model configurations.

Similarly, the "full" and "SI" prey libraries represent only a subset of the more than 100 fish species that occur in Sarasota Bay, although many of those species are uncommon or infrequently caught during fish sampling surveys . I created the library using *a priori* knowledge of the SB system following the precedent of previous QFASA analyses in cetaceans and pinnipeds (Tucker et al., 2009; Meynier et al., 2010, 2014; Xie et al., 2022; Remili et al., 2022, 2023). While this approach focuses analyses on the most likely prey species, it is necessarily restricted to well-studied

systems like SB and may exclude prey that are consumed but poorly represented in other diet determination methods (e.g., due to differential preservation of hard parts in stomach content analyses; Bowen and Iverson, 2013). Accordingly, the "full" and "SI" libraries should be viewed as two plausible subsets of a larger potential prey base rather than definitive representations of the complete dolphin prey community. However, they still allowed me to evaluate the sensitivity of diet estimates to prey library composition and generated estimates that are more directly comparable to previous SI and stomach content studies, both of which have contributed substantially to existing understanding of SB dolphin diet.

I also did not employ prey species pooling or drop core prey (DCP) analyses, two approaches that have been used to determine prey libraries in previous QFASA studies (Piché et al., 2010; Goetsch et al., 2018). Pooling prey species with similar FA signatures may result in mean FA profiles that do not represent biologically realistic prey signatures, reducing diet estimate accuracy (Goetsch et al., 2018). DCP analyses, where prey species are iteratively removed from the prey library and QFASA estimates are re-calculated to assess how the results change, provide an alternative option for library reduction (Goetsch et al., 2018; Ning et al., 2020). Although not computationally feasible within the scope of this dissertation, DCP analyses represent a promising quantitative approach for prey library refinement. Future applications of QFASA in this system could build on these libraries with additional prey sampling and targeted library reduction through DCP analyses to assess prey importance.

4.2 Dolphin and prey fish FA signatures

SB dolphin FA signatures followed patterns expected for marine mammals in general, and cetaceans and bottlenose dolphins in particular, supporting their validity. Dolphin FA signatures closely resembled previously reported values for free-ranging Gulf of Mexico (Texas and Louisiana) bottlenose dolphins (Samuel and Worthy, 2005). This includes similarly high percentages of 18:1n-9, 16:1n-7, 16:0, 14:0, 18:1n-7, 22:5n-3, and 22:6n-3 and increases in SFAs and PUFAs and decreases in MUFAs moving from outer to inner blubber. This variation across blubber layers, along with inner blubber having higher overall amounts of dietary FAs and outer blubber having more extended dietary FAs, is also consistent with known gradients in lipid metabolism and FA deposition in cetacean blubber. In cetaceans, the inner blubber contains a higher proportion of FAs derived mostly or exclusively from prey (i.e., dietary FAs). It is also thought to experience greater FA turnover, as proximity to the capillary and muscle layers leads to higher metabolic activity (Samuel and Worthy, 2005; Koopman, 2007; Ellisor et al., 2013). In contrast, the outer blubber is less vascularized (McClelland et al., 2012) and is more closely associated with thermoregulation (Koopman, 2007). These differences support evaluating QFASA estimates separately by blubber layer but also suggest that, because variation was subtle in magnitude, FA composition is largely conserved across layers and both inner and outer blubber can be used for QFASA diet estimation.

Prey fish FA signatures and lipid contents were also representative of subtropical fish and shared top contributing FAs with SB dolphins, supporting prey

fish FA integration into dolphin blubber. Few complete FA profiles of Gulf of Mexico prey fish exist in the published literature. However, FA and lipid values for Atlantic croaker, white grunt, ladyfish, mullet, spotted seatrout, sheepshead, and spot collected off the North Carolina coast (Gooch et al., 1987), pinfish, pigfish, mullet, and common snook from SB (Slifka et al., 2013; Hauville et al., 2015), Atlantic thread herring, sheepshead, mullet, and croaker from the northern Gulf of Mexico (Finne et al., 1980; Hale and Brown, 1983), and other Gulf of Mexico species not part of the prey library (Stickney and Torres, 1989) are available. These confirm 16:0, 16:1n-7, 18:1n-9, 20:4n-6, 20:5n-3, and 22:6n-3 as top contributing FAs across species. These FAs are also aligned with the most common FAs in dolphin FA signatures, which is expected if dolphins are integrating available prey FAs and supports the accuracy of this prey library for QFASA diet estimation. Those same studies also measured medium to low lipid contents (1 – 6% total lipids) in line with the range of values I measured. Furthermore, it is suggested that low productivity areas, like subtropical regions, are associated with lower lipid contents overall, as there is less need to accumulate large energy reserves (Stickney and Torres, 1989; Piche et al., 2010). Together, the alignment of prey FAs with dolphin FA patterns and existing literature support their validity for QFASA diet estimation.

4.3 QFASA model performance and estimate uncertainty

Understanding QFASA model performance and structural sources of uncertainty are necessary before the diet estimates themselves can be examined.

Overall, my results suggest that QFASA provides adequate data fit and intermediate estimate certainty but include some caveats that inform interpretation and serve as recommendations for future applications of this method.

4.3.1 Predator-beyond-prey (PBP) values

The degree of overlap between predator and prey FAs is a key QFASA model performance metric that indicates model fit and informs interpretation of uncertainty. For QFASA to perform optimally, dolphin FA signatures should largely fall within the range of prey FAs after CC correction; higher predator-beyond-prey (PBP) values indicate more FAs falling outside the range. Although no universal thresholds or "ideal" ranges are defined (Bromaghin, 2015, 2017), lower PBP values indicate closer predator-prey alignment and stronger model fit, while higher values suggest increasing mismatch and less certainty in model results. Using the dietary FA suite and both prey libraries and distance measures, observed PBP values were 4 – 25% for inner blubber estimates and 12 – 50% for outer blubber. These are comparable to or lower than those reported in other cetacean QFASA applications (Choy et al., 2019; Remili et al., 2022; Chapter 1). This suggests my results are in-line with previous peer-reviewed work.

Variation in PBP values among dolphins and model configurations could reflect multiple biological and analytical factors. First, imperfect CC correction may increase predator-prey mismatch (Bromaghin, 2015, 2017). The three-dolphin CCs applied here were derived from dolphins consuming controlled diets that differed in

composition and diversity from the SB dolphins. In Chapter 1, we showed that the variety and complexity of prey consumed by predators from which CCs are derived can influence the values, which could in turn lead to poorer model fit in some cases. Second, higher PBP values in outer blubber estimates, the only consistent and statistically significant variation, may reflect differential FA modification or increased variability in FA deposition. This finding is consistent with Chapter 1, where outer blubber PBPs were overall higher. Outer blubber contains fewer dietary FAs and may capture a different or less direct record of diet than inner blubber (Ellisor et al., 2013; Koopman, 2007; Samuel and Worthy, 2005). Accordingly, even layer-matched CCs, especially ones derived from dolphins with different diets, may not fully correct for prey FA modification. Finally, incomplete prey representation can elevate PBP values if predators consume prey not included in the reference library (Bromaghin, 2015; Bromaghin et al., 2015; Bromaghin, 2017). Estimates generated using the more limited SI prey library tended to have slightly higher values (though not statistically significant) for a given dolphin, which could reflect the smaller prey library. Dolphins with higher PBP values, even with the full library, may also have consumed additional prey missing from the library. While the library is large, sampling and modeling limitations mean that I could not include every possible prey species present in SB and available for the dolphins.

Importantly, however, low or high PBP values were not consistently associated with qualitative differences in diet composition. For certain dolphins, estimates with higher PBP values for the SI library produced similar prey proportions to the lower-

PBP full library estimates. Thus, even if the SI library was technically a poorer fit, it did not ultimately impact the resulting estimate. Conversely, some estimates with low PBP values for both prey libraries differed more substantially in prey allocation depending on the prey library used. This suggests different prey libraries can generate different estimates that are nonetheless similarly good fits for dolphin data. Finally, while outer blubber estimates had higher PBP values for a given dolphin, values were not uniformly high (and were often comparable to inner layer PBPs). Thus, while higher outer-layer PBPs may reflect layer-specific biochemical processes, they do not imply uniformly reduced reliability of outer blubber estimates. Together, these results suggest that while PBP values reflect overall predator-prey alignment, low values do not directly translate into more consistent and certain estimates for a given dolphin. In turn, I do not support excluding entire diet estimate categories (e.g., SI library estimates, outer blubber estimates) or specific dolphins based on PBP values alone.

Thus, my results support interpreting PBP values as relative indicators of model performance rather than strict determinants of estimate validity. Elevated PBP values, especially if consistently high, warrant consideration prey library completeness, layer-specific process, and applicability of the CCs. However, they do not entirely invalidate an estimate or imply reduced ecological interpretability. This aligns with Chapter 1, where estimates with higher PBP percentages still accurately estimated known prey composition in both blubber layers. Accordingly, I suggest PBP values should be considered as one component of a broader performance assessment.

Combining this consideration of PBP values and the fact that the values align with previous studies thus supports their validity for further interpretation.

4.3.2 Augmented FA contribution

The contribution of the "augmented" FA is another critical diagnostic metric of QFASA model performance. Elevated contributions suggest the model and its results are impacted by scaling adjustments rather than biologically representative predator-prey FA patterns. As using the extended dietary suite led to significantly larger augmented FA contributions, especially with the KL distance in inner blubber and both distance measures in outer blubber, I excluded estimates generated using that suite from further interpretation. Notably, the corresponding estimates produced using the dietary FA suite – which excludes the same and additional FAs as the extended suite – exhibited minimal augmented FA contributions. This suggests the issue is unlikely to result from the omission of specific fatty acids and may instead reflect interactions between the extended FA suite and the internal structure of the QFASA model. The recurrence of this pattern in both dolphins under professional care in Chapter 1 and free-ranging dolphins in this chapter suggest that it is not dataset-specific but may represent a broader methodological sensitivity when using the augmented scaling approach. While the mechanism underlying this pattern remains unclear, its repetition across studies means that I continue to recommend that future QFASA applications, if employing augmented scaling, evaluate augmented FA contribution as part of model performance. Elevated contributions should be

considered diagnostic red flags, and any estimates including them should be treated with caution.

4.3.3 Prey distinctiveness

LOPO analyses quantify the distinctiveness of prey FA signatures, and therefore the certainty with which a given prey species can be identified in QFASA diet estimates (Bromaghin, 2017). There is no defined LOPO cut-off; higher self-association indicates greater distinctiveness, while lower values indicate that a prey species may be difficult to distinguish from species with similar FA signatures. However, interpretation also depends on relative associations. For example, a species with 50% self-association may still be reliably identified if associations with other species are low (e.g., 5 – 10%) or may share a few clearly explainable strong associations with other species. Conversely, low self-association and diffuse low associations with other species increases uncertainty when interpreting the resulting diet estimates. In my results, distinctiveness varied between species but was overall ecologically coherent.

Some species formed clear groups consistent with a shared prey base and/or had high self-associations consistent with more specialized diets. Planktivorous clupeids – menhaden, Atlantic thread herring, and scaled sardine – formed clear trophic groupings, with strong or intermediate self-associations and associations with each other. Minor LOPO associations (8 – 17%) between Atlantic thread herring or scaled sardine and piscivorous common snook and ladyfish likely reflect trophic FA transfer,

as the piscivores integrate FAs derived from clupeid prey (Smith, 1997; Ray et al., 1999; Budge et al., 2006; Murdy et al., 2013). Distinctiveness among common snook, ladyfish, and spotted seatrout showed similar ecological and mechanistic dynamics as clupeids. Common snook was highly distinct in FA space (>89% self-association), whereas ladyfish and spotted seatrout had intermediate self-associations but strong associations with each other and/or common snook. As all are piscivorous species (Smith, 1997; Ray et al., 1999; Gannon et al., 2009; Murdy et al., 2013), this is consistent with shared prey bases producing partially convergent FA signatures. Finally, mullet, sheepshead, and gulf toadfish had similarly strong LOPO self-associations (>70%). This is consistent with the more specialized diets of mullet and gulf toadfish – primarily detritovores and carnivores, respectively – and could reflect the invertebrate-focus of sheepshead diet (Smith, 1997; Ray et al., 1999; Murdy et al., 2013).

Seagrass-associated omnivores and benthic invertivores shared greater overlap and more variable associations that nonetheless aligns with their trophic ecology. Pigfish and mojarra had intermediate self-associations but mutual associations with each other and white grunt and grass porgy, consistent with shared benthic invertivore and omnivore diets. Although pigfish and white grunt are also phylogenetically related (family Haemulidae, the “grunts”), their FA similarity with non-Haemulid species indicates that trophic overlap can be equally or more influential than taxonomy (Piché et al., 2010). Lane snapper had intermediate self-associations (32 – 54%) and small associations with planktivore, invertivore, and piscivore species,

consistent with their crustacean and fish diet. Several species with mixed invertivore, omnivore, or detritovore diets (e.g., mangrove snapper, silver perch, croaker, and hardhead catfish; Smith, 1997; Ray et al., 1999; Murdy 2013) exhibited low-to-intermediate self-associations (15 – 55%) and weak-to-moderate associations (10 – 20%) with multiple trophic groups, consistent with their varied diets. Finally, pinfish exhibited particularly low LOPO self-associations (< 10%) and diffuse associations (5 – 30%) across trophic groups. This low distinctiveness could reflect their well-documented ontogenetic transition from invertivory to increased omnivory and detritivory/herbivory with increasing size (Barbosa and Taylor, 2020). This transition aligns with the 100 – 300 mm size range targeted during prey fish sampling. Limited sample sizes precluded subdividing pinfish by size class, which could have accounted for increased within-species variability.

A final consideration remains to be addressed: could QFASA disproportionately identify prey with highly distinctive FA profiles, while under-identifying less distinctive prey? Diet estimate patterns do not support this concern. Species such as menhaden and sheepshead, with high LOPO self-associations, were not identified in significant proportions. For menhaden in particular, this is despite being more distinct than the other clupeids. In addition, species were not only or always estimated when they had higher self-associations. For instance, croaker had similar LOPO associations with both distance measures and blubber layers but was only substantially identified in outer blubber estimates using the Aitchison distance. Conversely, spotted seatrout was most frequently identified using the KL distance,

despite having its lowest self-association with that distance measure. In turn, these suggest that pinfish's particularly low self-association should not automatically prevent either it or species that are more distinct but have LOPO associations with pinfish (e.g., pigfish, mojarra, sheepshead) from being identified. These results suggest that bias towards distinct prey species is not the driving force of QFASA diet estimates. Thus, while LOPO results suggest that prey distinctiveness influences the certainty with which individual prey species can be interpreted, it does not appear to determine whether a species is identified in the resulting diet estimates.

Taken together, these results show that QFASA analyses identified coherent and trophically-structured prey groupings, with strong resolution at broad trophic levels but more variable certainty at the species level. High self-association values suggest QFASA estimates of those species can be interpreted with relatively high confidence; this applies to many of the top prey species in the diet estimates, including common snook, scaled sardines, Atlantic thread herring, mullet, and Gulf toadfish. Estimates involving species with lower self-association values should be interpreted more cautiously, however, because multiple species may possess similar FA signatures. More broadly, the variable distinctiveness across species and broader trophic associations emphasizes the importance of identifying prey trophic groupings and using LOPO analyses to understand prey library structure before proceeding with diet estimation.

4.3.4 Estimate uncertainty due to model parameter variation and standard errors

While PBP values, the contribution of the augmented FA, and prey distinctiveness inform understanding of diet estimate uncertainty by quantifying how well (or poorly) the model performs, uncertainty can also arise from fundamental variations in the resulting estimates. This is applicable to my results, where different model configurations yielded different diet estimates for the same dolphin, and where SEs were sometimes large enough that they changed the relative contribution of a given prey species. Previous cetacean QFASA studies have not reported SEs for individual diet estimates (Choy et al., 2020; Ning et al., 2020; Remili et al., 2023, 2022; Xie et al., 2022), instead presenting the standard deviations of estimates averaged across multiple individuals, demographic groups, or other categorizations. This limits direct comparison and makes it difficult to determine whether the magnitude and variation in SEs observed here are typical of QFASA applications or specific to this study or system. Expanded applications across systems and reporting of SEs will be necessary to distinguish system-specific effects from inherent model behavior. In the meantime, however, five interacting factors – prey FA similarity, prey library composition, distance measure, predator FA signatures, and blubber layer – influenced both estimated prey proportions and SE magnitude. Exploring these factors can help us understand the uncertainty of the resulting diet estimates and provide practical recommendations for future QFASA applications.

Convergence in prey FA signatures is a primary contributor to estimate variation and SE magnitude. Species with lower LOPO self-associations or strong associations with other species are more likely to be confounded during estimating and resampling (Iverson et al., 2004; Bromaghin et al., 2017; Bromaghin, 2017). This may cause a given species' re-sampled mean to vary and increases the possibility that a predator's FA signature resembles multiple species, yielding different diet estimates over iterations and thus increasing SEs. FA signature similarities between species also makes it more likely that when different model configurations are employed, species may be confounded with each other. Small sample sizes can exacerbate this effect: if within-species variability is high but sample size is small, bootstrap iterations and different configurations may more easily generate different prey means and thus higher SEs. Observed patterns in estimated prey contributions and their SEs are consistent with variability within species and FA similarities between species. For instance, mixed omnivore-invertivore-detritivore species (e.g., lane and mangrove snapper, croaker) had intermediate sample sizes but lower LOPO self-associations, making them susceptible to resampling-driven shifts. This could contribute to both their variable contributions across model configurations and their frequently large SEs. In comparison, the clupeids and piscivores had strong mutual LOPO associations within their trophic groups but also some overlap in signatures due to predator-prey relationships. In my results, this seemed to increase the likelihood that the estimated contributions "swapped" across estimates and had complementary SEs. Conversely, mullet – despite relatively small sample sizes – had high LOPO self-

associations, limited confounding in other species' LOPO results, and low SEs. As the only true detritovore in the library, its FA distinctiveness likely contributed to this stability.

Prey library composition can interact with prey FA similarity to further increase variability, highlighting a central tension in QFASA prey library selection. A larger library increases the likelihood of representing all true diet components but also increases the probability of FA similarity among species and potential misidentification, especially among trophically-similar prey. Including prey that are unlikely to be consumed could lead to further misidentifications, as the QFASA model will simply consider how that prey's FAs compare to the predator values (Bromaghin et al., 2015, 2016b; Remili et al., 2022). Conversely, smaller libraries may reduce confounding, but also risk omitting important prey and forcing the model to assign their signal to the next most similar species. Leaving remaining species to "occupy" a larger FA space might also increase the variability in their estimated proportions, leading to higher SEs. Thus, both larger and smaller prey libraries can influence estimate variation and SE magnitude, although the mechanisms may differ. This was apparent in my data. Some estimates were broadly similar between the full and SI prey libraries when other model variables were held constant, suggesting the reduced library captured dominant diet components. In other estimates, however, species missing from the SI library contributed substantially when using the full prey library. As a result, when the SI library was used, their contributions were redistributed among other species with whom they shared LOPO associations. For

instance, when croaker was estimated in full library outer blubber estimates, the relative percentage of scaled sardine generally increased in the accompanying SI library estimate; while these species are not obvious trophic pairs, scaled sardine was croaker's second largest LOPO association (after itself). The SI library estimates were also often accompanied by high SEs, however. This could suggest that the smaller library was insufficient, or that confounding occurred and caused diet estimate variation, and emphasizes the potential uncertainties introduced by library size and composition.

Distance measure selection further contributed to variation in both estimated prey contributions and SE magnitude. The Aitchison and KL measures use different equations to minimize the distance between predator and prey FAs, with Aitchison emphasizing lower-proportion FAs and KL emphasizing higher-proportion FAs (Bromaghin, 2015; Bromaghin et al., 2015, 2016b). Even when using the same dietary FA suite, this differential weighing means that if species differ primarily in FAs that are strongly weighted by one distance measure but not the other, diet estimates and SEs may vary accordingly. Aligned with the KL distance's emphasis, KL estimates were more likely to identify large percentages of prey that were less common in Aitchison estimates (e.g., silver perch, spotted seatrout, mangrove snapper), sometimes with small contributions from several other species. In Chapter 1, this made the KL distance better at estimating more complex diets, with large amounts of a few prey and small amounts of several others. While this may better detect prey with large amounts of certain FAs, it may also over-amplify their relative

diet importance. In contrast, the Aitchison distance's emphasis on smaller FAs may buffer against strong FA signals, leading to more consistent and intermediate proportions of multiple species as observed in the estimates. This was effective when professional care dolphin diets consisted of intermediate amounts of several prey species. However, it may under-emphasize the importance of some prey as a result. This emphasis not only leads to variation in estimated contribution between model configurations but also could explain why SEs for a given species differed between distance measures. Thus, while diet variation between distance measures was minimal and did not change the overall diet picture for some estimates, for others, certain species (e.g., spotted seatrout, croaker) appeared in diet estimates and with large SEs only with specific distance measures.

Predator FA characteristics also influence estimated diet contributions and SEs. If a dolphin's FA signature falls between the mean signature of multiple prey species, multiple prey combinations may provide similarly good fits, thus increasing bootstrap variability (and SEs) and the likelihood that different model configurations will lead to different estimates. Small shifts in re-sampled prey means can also redistribute proportions among species that provide similarly good predator-prey matches, increasing SEs. Dolphins with temporal diet variation or those consuming many prey species in relatively small percentages (i.e., a more generalist diet) may also produce integrated blubber signatures that lack a dominant FA signal, increasing uncertainty. In this context, even prey species that are highly distinct may exhibit elevated SEs. For instance, common snook and gulf toadfish had high LOPO self-associations,

indicating strong distinctiveness from other prey, but for certain dolphins their estimated contributions included large SEs. This suggests these species were not uniquely required to explain specific dolphin FA profiles. These same mechanisms could explain why SE magnitudes varied among dolphins and why a given prey species could exhibit high SEs in one dolphin but low SEs in another.

Physiological differences between inner and outer blubber are also inherent to the dolphin predator but may influence prey contribution and SE variability and magnitude. As described earlier and seen in dolphin FA signatures, inner blubber contains a higher percentage of dietary FAs and has been proposed to experience greater FA turnover than outer blubber (Samuel and Worthy, 2005; Koopman, 2007; Ellisor et al., 2013). In turn, this difference has led it to be suggested that outer blubber may either less accurately estimate diet or represent a more extended integrated diet period ("temporal window") (Remili et al., 2022). The differences between inner and outer blubber estimates, greater variability between model configurations for outer blubber estimates, and higher outer blubber SEs that I observed could be consistent with this physiological consideration. While layer-specific CCs account for differences in deposition and mobilization between layers, they do not cause the FA profiles of the layers to become more similar to each other; thus, different FA proportions would be expected to lead to different diet proportions. Furthermore, if outer blubber provides a less direct or more integrated dietary record, small changes in prey means during bootstrapping could produce proportionally larger shifts in estimated prey contributions, leading to higher SEs. In Chapter 1,

inner and outer blubber estimates differed slightly, especially for the dolphin with the most complex and variable diet, consistent with these results. There, however, differences were small enough that both layer estimates had similar weighted errors relative to known diet and captured similar temporal diet window ranges. However, that was in a small sample (n = 3) of dolphins under professional care with more limited diets, smaller prey libraries, and potentially less diet variation over time compared to the free-ranging dolphins in this chapter, which could have obscured greater differences between inner and outer blubber. The only other applications of QFASA in cetaceans where blubber stratification was taken into consideration either used inner blubber only (Choy et al., 2019; Ning et al., 2020; Xie et al., 2022) or used both inner and outer blubber but did not provide any direct comparisons or details (Remili et al., 2022), complicating comparisons. Thus, larger validation studies are needed to properly explore the relative accuracy of inner and outer blubber. Larger applications of QFASA in free-ranging dolphins, to see if differences in estimates and certainty between inner and outer blubber are a shared feature, would also provide further clarification.

Taken together, my results suggest that QFASA estimates of SB dolphin diet are subject to a meaningful degree of uncertainty at the species- and dolphin-specific level, due to interactions among prey distinctiveness, prey library composition, distance measure variation, predator FA characteristics, and blubber layer physiology. However, I suggest that this does not represent a fundamental flaw in the method. Rather, this uncertainty quantifies the sensitivity of diet estimates to realistic variation

in prey and predator FA values, prey species FA signature convergences and trophic relationships, and different model configurations. Some diet estimates were relatively stable across bootstrap iterations and parameter choices, while others were more variable. In many cases, however, species with strong trophic or LOPO-based similarities exhibited complementary SE patterns and/or appeared to "swap" across different model configurations for the same dolphin, suggesting the model viewed them as functionally similar FA contributors. This was especially clear for the clupeids, the piscivores, and some mixed seagrass-associated species (e.g., gulf toadfish, croaker, snappers, silver perch). This suggests different model configurations and higher SEs are not producing fundamentally wrong or contradictory diet estimates. Instead, they appear to emphasize different components of the dolphins' FA signatures, shifting proportional contributions among prey that are trophically related or share other FA similarities. While this does not resolve species-level uncertainty, it reinforces the biological interpretation that these taxa occupy similar trophic roles within the model framework and in dolphin diet. Accordingly, I propose that variation should be interpreted not as evidence of inaccuracy but rather as an emphasis on the complexity of the underlying FA data and the sensitivity of proportional diet estimates to how that FA information is grouped and incorporated by the QFASA model framework.

Critically, despite these uncertainties, QFASA estimates also provided broadly coherent trophic-level ecological patterns. Where variation occurred among QFASA model parameters or individual dolphins, it often involved redistribution among

trophically-related prey, so that shared prey types and trophic groupings repeatedly emerged. Thus, while exact proportions may be sensitive to methodological decisions, I propose that higher-level inferences – such as dominant prey categories across model parameter combinations, trophic positioning, relative demographic differences, and comparisons between diet determination methods – are robust for interpretation.

My examination of this uncertainty, combined with the assessment of model performance under different model configurations, also provides several practical considerations for future applications of QFASA in free-ranging cetaceans.

4.4 Considerations for future QFASA applications

While no single model configuration provided a single "best-fit" option, patterns in model performance and uncertainty suggest several considerations for future applications that inform and may sometimes improve the reliability and interpretability of the resulting diet estimates.

My comparisons of multiple CC sets and FA suites provide the clearest recommendations. First, bottlenose dolphin-specific CCs derived in Chapter 1, calculated using the dolphins' known diets averaged over the 8, 16, and 24 weeks before sampling, yielded estimates that were statistically highly similar. This suggests that any single set of CCs within that time frame are viable for future use; however, I suggest future studies use week 16 CCs for consistency. Second, the extended dietary FA consistently generated estimates where the augmented FA was a significant

contributor, a diagnostic red flag. While the extended suite may capture additional information in some systems, I strongly suggest studies assess this indicator of model performance. Furthermore, the lower augmented FA contribution of the dietary FA suite suggests it may provide the stronger starting point for cetacean QFASA applications. In particular, future applications in the SB system should prioritize the dietary FA suite.

Similarly, although both blubber layers generated estimates with sometimes comparable model performance criteria (PBP values, augmented FA contributions, prey species distinctiveness), inner blubber overall had lower PBP values, estimates were more consistent among model configurations, and SEs were frequently thought not consistently lower. While outer blubber remains a possible source of diet estimates and may represent a longer integrated diet period than inner blubber that provides valuable ecological insights, I would not suggest using it exclusively until larger validation studies can be accomplished. If possible, it should be considered alongside inner blubber for comparison purposes, and inner blubber should be considered the priority when available. If only outer blubber is available for analysis (e.g., a dart biopsy frequently does not capture full-depth blubber samples), it is critical to assess model performance and address any associated uncertainties.

In comparison, both the Aitchison and KL distance measures produced estimates with comparable model performance when the full prey library was used, though the KL distance was more associated with variability and higher errors when paired with the more restricted SI library. Thus, it is important to be aware that the

KL distance may be more susceptible to incomplete prey libraries. However, estimates generated using these different measures were not consistently similar, suggesting they emphasize different aspects of the FA data. Rather than selecting one method, I propose that future research apply multiple distance measures, and, if their model performance remains comparable, assess the consistency of the resulting diet estimates.

Prey library composition and prey distinctiveness were major interconnected sources of uncertainty and remain a fundamental tension point when applying QFASA. Overly restricted libraries may exclude important prey, yielding diet estimates that misclassify or over-estimate prey that share FA similarities to excluded prey. Conversely, including all or even most prey in a system is not possible. Along with being logistically complex, overly broad libraries increase the risk of confounding between prey with similar FA signatures. Furthermore, QFASA requires that there are more FA in the FA suite than prey species in the library to generate unique diet estimates (Goetsch et al., 2018). Depending on FA suite parameters, this may restrict the prey library size. Thus, ideally, prey libraries should be constructed using all ecologically plausible prey when possible, combining independent *a priori* knowledge of prey availability, predator habitat use, and potential diet patterns as estimated via other diet determination methods (e.g., stomach contents, molecular barcoding, stable isotopes). Drop core prey analyses (Goetsch et al., 2018) may be used to further reduce the library. However, it is important to remember that while this technique can identify prey species that never contribute to the diet estimates, it

does not guarantee whether prey species that are identified are true diet items or simply share FA signature similarities with true prey. For this reason, LOPO analyses are critical for understanding prey distinctiveness and patterns of association between species, and for interpreting the resulting estimates. Estimates involving prey with low self-associations and diffuse associations with other species should be interpreted cautiously, especially if they contribute relatively small portions of the estimated diet, whereas distinct species can be interpreted with more confidence. Conversely, species with larger estimated contributions but lower self-associations and consistent associations with other species sharing similar trophic categories may need to be considered as part of a wider trophic group. When appropriate, this may generate higher-confidence estimates.

In general, SEs provide valuable insights into the stability of estimated prey contributions and serve as a broad measure of uncertainty and should be reported alongside individual predator diet estimates when possible. Large SEs may be inherent elements of QFASA estimates, whether due to fundamental model limitations, variability in predator diet, blubber physiology, or the complexities of prey library composition, though are likely particularly prevalent in systems with greater similarity in prey FA signatures. However, they also serve as important indicators of these factors that directly inform interpretation of the resulting diet estimates.

Finally, considering the multiple sources of uncertainty surrounding QFASA, greater confidence should be placed on estimated prey contributions that re-occur

across multiple supported model configurations and broader patterns shared across individual predators, rather than exact estimates for the contribution of an individual prey species or for an individual predator's diet.

4.5 Study limitations and future research

Several limitations in model inputs and parameter choices are present in this study. Only 15 free-ranging dolphins were sampled across multiple demographic groups. Differences across sexes and age classes due to dietary, physiological, behavioral, or environmental differences likely impact their respective FA signatures and add natural variation to the resulting estimates and associated assessments of model performance. The maximum size of the prey library also could not encompass all theoretically "possible" prey in the SB system. While my exclusion of certain prey species had logistical and ecological justifications, it is possible that the results of other diet determination methods used to reduce the prey library fundamentally missed certain prey which could be important if unexpected contributors to dolphin diet. Sampling and processing limitations meant that sample sizes were small for some species, and samples were collected over the course of multiple months, which could encompass natural seasonal variation in prey FAs or lipid contents that were not taken into consideration in my analyses. Finally, CCs were derived from the same species, but from dolphins under professional care with less complex diets and potential physiological differences due to habitat, diet, and environmental conditions. This could yield CCs that are perhaps less than a complete fit for the free-ranging

dolphins, potentially impacting the resulting model performance criteria and estimates.

Nonetheless, this represents the first application of species-specific CCs and QFASA to free-ranging bottlenose dolphins and provides a valuable framework and key considerations for future research in this system and beyond. Future studies that apply this approach to diet determination for a free-ranging population should expand the numbers of sampled dolphins and extend sampling across all seasons. Improving the prey library by increasing prey species sample sizes, especially across seasons, size classes, and habitats, could allow us to better account for within-species variability. With larger prey datasets, it may be possible to subdivide species into ecologically meaningful groups (e.g., by size class, collection season, or habitat), as has been done in previous QFASA studies (Budge et al., 2002; Iverson et al., 2002). Together, these improvements could reduce misallocation among species and increase prey distinctiveness, potentially lower estimate uncertainty. This would necessarily increase prey library size, with its own implications as discussed. However, drop core prey analyses could be applied to reduce the prey library size if needed. Finally, further validation of QFASA in cetaceans is a key priority. Increased application of QFASA in free-ranging cetaceans, both in SB and beyond, would allow for continued exploration of model parameter choice implications and the methodological uncertainties (i.e., multiple viable diet estimates, higher SEs, etc.) associated with QFASA diet estimates.

5 Conclusion

This chapter represents the first application of QFASA and bottlenose dolphin-specific CCs to free-ranging bottlenose dolphins. I demonstrate that QFASA is sensitive to prey FA similarity and model parameter variation. I show that outer blubber is not as useful as inner blubber for interpreting diet estimation because higher SEs and poorer model fit introduce more uncertainty to diet estimate interpretation. However, I also show that QFASA can yield viable estimates of free-ranging dolphin diet, especially using inner blubber, and that variation in estimates often involved redistribution among prey species with coherent trophic similarities. As a result, broader diet patterns that are consistent across methods and model configurations are available for ecological interpretation. Furthermore, I propose that the uncertainty associated with the diet estimates reflect real variation in prey and predator FA values, trophic relationships between prey species, and parameter configurations, and thus provides a valuable indicator of reliability when interpreting the resulting diet estimates. It also emphasizes the complexity of the underlying data and the importance of considered model input and parameter choice. Taken together, my results support the use of QFASA diet estimation in cetaceans moving forward, if model variable choice and potential uncertainty of the resulting estimates are carefully taken into consideration, but suggest it should be used alongside, rather than instead of, other diet determination methods.

Tables – Chapter 2

Species	Top contributing FAs	FA contribution (%)	Cumulative contribution (%)
Atlantic thread herring	16:0, 22:6n-3, 20:5n-3, 18:0, 14:0, 18:1n-9, 16:1n-7, 20:4n-6	22.8 ± 1.6, 22.2 ± 7.5, 8.7 ± 2.1, 7.0 ± 1.4, 5.5 ± 3.4, 5.3 ± 1.5, 5.3 ± 3.0, 3.0 ± 1.2	79.9
common snook	22:6n-3, 16:0, 18:0, 20:4n-6, 18:1n-9, 22:5n-6, 22:5n-3	25.0 ± 1.1, 20.9 ± 0.4, 9.9 ± 0.5, 9.4 ± 0.3, 9.0 ± 0.3, 3.2 ± 0.2, 2.4 ± 0.2	79.8
croaker	16:0, 22:6n-3, 18:1n-9, 18:0, 16:1n-7, 20:5n-3, 22:5n-3, 20:4n-6, 14:0, 18:1n-7	19.9 ± 1.9, 17.0 ± 5.3, 9.6 ± 1.8, 7.2 ± 1.0, 7.0 ± 3.7, 5.9 ± 1.8, 3.3 ± 0.5, 3.3 ± 1.1, 3.3 ± 1.4, 2.9 ± 0.7	79.4
grass porgy	16:0, 18:1n-9, 18:0, 22:6n-3, 20:5n-3, 16:1n-7, 20:4n-6, 18:1n-7, 22:5n-3, 14:0	23.4 ± 1.2, 12.4 ± 3.7, 9.5 ± 0.4, 6.3 ± 0.9, 6.1 ± 1.2, 5.9 ± 1.7, 4.3 ± 0.5, 3.8 ± 0.9, 3.4 ± 0.5, 3.2 ± 0.3	78.3
Gulf toadfish	16:0, 18:1n-9, 22:6n-3, 18:0, 20:4n-6, 22:5n-3, 18:1n-7, 20:5n-3, 16:1n-7, 22:4n-6	17.5 ± 1.5, 10.9 ± 1.0, 9.5 ± 2.0, 8.4 ± 1.4, 8.0 ± 1.2, 6.3 ± 1.1, 5.8 ± 0.7, 5.0 ± 1.3, 4.1 ± 0.5, 3.5 ± 0.6	79.0
hardhead catfish	16:0, 18:1n-9, 18:0, 22:6n-3, 16:1n-7, 20:4n-6, 20:5n-3, 18:1n-7	24.4 ± 4.8, 19.1 ± 3.4, 9.7 ± 2.3, 6.4 ± 2.6, 5.8 ± 2.2, 5.3 ± 3.5, 4.0 ± 0.9, 3.4 ± 0.5	78.1
ladyfish	22:6n-3, 16:0, 18:1n-9, 18:0, 20:4n-6, 20:5n-3, 18:1n-7	21.5 ± 4.2, 19.8 ± 2.5, 11.2 ± 2.5, 10.7 ± 0.9, 6.0 ± 1.5, 4.2 ± 0.3, 3.1 ± 0.4, 3.1 ± 1.3	79.6
lane snapper	16:0, 22:6n-3, 18:1n-9, 18:0, 20:5n-3, 20:4n-6, 16:1n-7, 18:1n-7	22.4 ± 3.2, 14.4 ± 4.6, 13.0 ± 4.9, 9.1 ± 0.6, 6.4 ± 1.3, 4.7 ± 1.0, 4.1 ± 0.7, 3.6 ± 0.6	77.5
mangrove snapper	16:0, 18:1n-9, 22:6n-3, 18:0, 20:4n-6, 20:5n-3, 16:1n-7, 18:1n-7, 22:5n-3	23.8 ± 2.5, 12.4 ± 3.0, 9.9 ± 2.7, 9.6 ± 1.2, 5.7 ± 1.6, 5.4 ± 1.2, 5.3 ± 1.2, 4.6 ± 0.5, 3.4 ± 0.3	80.0
menhaden	16:0, 16:1n-7, 14:0, 20:5n-3, 22:6n-3, 18:1n-9, 18:0, 18:1n-7	23.4 ± 1.0, 12.3 ± 0.4, 11.1 ± 0.8, 10.5 ± 0.1, 7.6 ± 1.2, 5.2 ± 0.9, 5.2 ± 0.1, 3.4 ± 0.4	78.7

mojarra	16:0, 18:1n-9, 18:0, 22:6n-3, 16:1n-7, 22:5n-3, 20:5n-3, 18:1n-7, 20:4n-6, 14:0,	25.7 ± 3.8, 13.9 ± 2.9, 7.0 ± 0.9, 6.1 ± 2.0, 5.7 ± 0.9, 4.0 ± 0.7, 3.9 ± 1.3, 3.6 ± 0.2, 3.5 ± 0.9, 3.3 ± 0.6	76.7
mullet	16:0, 16:1n-7, 15:0, 20:5n-3, 14:0, 18:1n-9, 22:5n-3, 22:6n-3, 17:1, 18:1n-7, 20:4n-6	22.9 ± 2.6, 15.0 ± 2.8, 7.6 ± 5.7, 7.0 ± 1.8, 6.4 ± 2.2, 5.0 ± 2.0, 3.8 ± 0.9, 3.7 ± 1.3, 3.3 ± 2.3, 2.6 ± 0.9, 2.6 ± 0.7	79.9
pigfish	16:0, 18:1n-9, 18:0, 20:5n-3, 22:6n-3, 20:4n-6, 18:1n-7, 16:1n-7, 14:0, 22:5n-3, 17:0	21.4 ± 2.1, 19.6 ± 7.0, 8.4 ± 1.0, 6.2 ± 1.6, 4.3 ± 1.5, 3.9 ± 1.5, 3.8 ± 1.2, 3.8 ± 0.8, 2.9 ± 0.5, 2.8 ± 0.6, 2.2 ± 0.7	79.4
pinfish	16:0, 18:1n-9, 22:6n-3, 18:0, 20:5n-3, 16:1n-7, 20:4n-6, 14:0, 18:1n-7, 22:5n-3	22.7 ± 3.7, 10.8 ± 4.8, 7.4 ± 4.1, 7.2 ± 1.5, 6.9 ± 2.8, 6.0 ± 1.9, 4.9 ± 2.4, 3.6 ± 1.6, 3.6 ± 1.0, 3.6 ± 0.9	76.8
scaled sardine	22:6n-3, 16:0, 18:0, 20:5n-3, 18:1n-9, 20:4n-6, 16:1n-7	22.7 ± 4.4, 22.1 ± 1.5, 9.4 ± 1.6, 7.2 ± 2.3, 6.4 ± 1.0, 4.1 ± 1.4, 3.3 ± 3.1, 3.2 ± 1.2	78.4
sheepshead	16:0, 18:1n-9, 16:1n-7, 20:5n-3, 18:0, 18:1n-7, 20:4n-6, 22:6n-3, 22:5n-3, 18:3n-3, 18:2n-6, 14:0	22.3 ± 1.2, 13.6 ± 1.8, 7.6 ± 0.8, 5.9 ± 0.7, 5.0 ± 0.1, 4.7 ± 0.3, 4.0 ± 0.4, 3.9 ± 0.4, 3.8 ± 0.3, 3.8 ± 0.8, 2.7 ± 0.4, 2.5 ± 0.2	79.7
silver perch	16:0, 18:1n-9, 22:6n-3, 20:5n-3, 18:0, 16:1n-7, 20:4n-6	23.6 ± 1.3, 16.6 ± 4.9, 11.7 ± 2.7, 7.4 ± 1.6, 7.3 ± 1.4, 6.3 ± 1.0, 3.8 ± 1.5	76.9
spot	16:0, 22:6n-3, 18:1n-9, 20:5n-3, 16:1n-7, 18:0, 20:4n-6, 18:1n-7, 22:5n-3, 14:0	21.6 ± 4.3, 11.0 ± 6.0, 10.5 ± 3.6, 8.1 ± 1.1, 8.0 ± 2.7, 7.2 ± 1.4, 4.1 ± 1.7, 3.9 ± 0.5, 2.8 ± 0.3, 2.3 ± 0.7	79.4
spotted seatrout	16:0, 22:6n-3, 18:1n-9, 18:0, 16:1n-7, 20:4n-6, 20:5n-3, 18:1n-7	22.5 ± 2.9, 16.3 ± 4.4, 10.5 ± 1.8, 8.3 ± 1.9, 8.1 ± 4.0, 5.7 ± 2.7, 5.1 ± 1.1, 3.1 ± 0.5	79.5
white grunt	16:0, 18:1n-9, 18:0, 20:5n-3, 20:4n-6, 22:6n-3, 16:1n-7, 18:1n-7, 17:0	23.2 ± 4.4, 16.1 ± 4.0, 8.7 ± 0.2, 6.5 ± 1.6, 5.7 ± 2.2, 5.1 ± 5.6, 4.4 ± 0.9, 4.0 ± 0.6, 2.2 ± 0.5	76.4

Table 1: Top fatty acids (FAs) for each SB prey species, with mean ± standard deviation (SD) % of each FA's relative contribution to the FA signature. Includes all FAs together contributing between 70 and 80% of the total FA signature (indicated by the cumulative contribution column).

Fixed Effects

Predictor	Estimate	SE	df	t-value	p-value
Intercept	12.38	1.8	140	6.90	**<0.0001**
Distance measure (KL)	1.26×10^{-12}	2.15	610	0.00	0.99
FA suite (extended dietary)	3.77	2.15	610	1.76	0.08
Blubber layer (outer)	10.39	2.08	610	5.01	**<0.0001**
CC set (week 24)	1.47×10^{-12}	2.5	610	0.00	0.99
CC set (week 8)	-0.32	2.15	610	-0.15	0.88
Prey library (SI)	0.64	2.15	610	0.30	0.77

Random Effects

Effect	Variance	SD
Dolphin ID	13.66	3.70
Residual	29.91	5.47

Table 2: Results of linear mixed-effect (LME) model quantifying the effects of distance measure (Aitchison or KL), FA suite (extended dietary or dietary), blubber layer (inner or outer), CC set (week 8, 16, or 24), and prey library (full or SI) on QFASA estimate predator-beyond-prey (PBP) values. Significant effects ($p < 0.05$) are shown in bold. All interactions were calculated, but none were significant; thus, none are included here. Dolphin identity was included as a random intercept to account for repeated measurements of individuals. Fixed effects are reported as estimate +/- standard error (SE), with associated degrees of freedom (df), t-values, and p-values. Reference levels were the Aitchison distance, the dietary FA suite, inner blubber, week 16 CCs, and the full prey library. The random effects table reports the variance and standard deviation (SD) associated with the random effect (i.e., dolphin identity) and the residual error.

Fixed Effects

Predictor	Estimate	SE	df	t-value	p-value
Intercept	0.81	1.13	308	0.72	0.47
Distance measure (KL)	0.97	1.46	610	0.67	0.51
FA suite (extended dietary)	2.64	1.46	610	1.81	0.07
Blubber layer (outer)	-0.31	1.41	610	-0.22	0.83
CC set (week 24)	-0.014	1.46	610	0.01	0.99
CC set (week 8)	0.024	1.46	610	-0.02	0.99
Prey library (SI)	0.017	1.46	610	0.01	0.99
Distance measure x FA suite	4.0	2.07	610	1.93	0.054
FA suite x Blubber layer	6.77	2.0	610	3.39	**<0.0001**
Distance measure x FA suite x Prey library type	15.73	2.92	610	5.38	**<0.0001**
Distance measure x FA suite x Blubber layer x Prey library type	13.54	3.99	610	3.39	**<0.0001**

Random Effects

Effect	Variance	SD
Dolphin ID	2.95	1.72
Residual	13.88	3.73

Table 3: Results of linear mixed-effect (LME) model quantifying the effects of distance measure (Aitchison or KL), FA suite (extended dietary or dietary), blubber layer (inner or outer), CC set (week 8, 16, or 24), and prey library (full or SI) on the percent contribution of the augmented FA to all QFASA diet estimates. Significant effects ($p < 0.05$) are shown in bold. All interactions were calculated, but only significant ones are shown. Dolphin identity was included as a random intercept to account for repeated measurements of individuals. Fixed effects are reported as estimate +/- standard error (SE), with associated degrees of freedom (df), t-values, and p-values. Reference levels were the Aitchison distance, the dietary FA suite, inner blubber, week 16 CCs, and the full prey library. The random effects table reports the variance and standard deviation (SD) associated with the random effect and the residual error.

Factor	df	Pseudo-F	R^2	p-value
Distance measure	1	179.44	0.17	**<0.001**
FA suite	[1]	65.14	0.06	**<0.001**
Blubber layer	1	84.66	0.08	**<0.001**
CC set	2	0.26	0.0005	0.97
Prey library	1	83.24	0.08	**<0.001**
Residual	665	-	0.62	-
Total	671	-	1	-

Table 4: Results of permutational multivariate analysis of variance (PERMANOVA) testing the effects of distance measure (Aitchison or KL), FA suite (extended dietary or dietary), blubber layer (inner or outer), CC set (week 8, 16, or 24), and prey library (full or SI) on QFASA diet estimate variation. Analyses were conducted on a distance matrix of diet estimates using Bray-Curtis distances with 999 permutations. Permutations were restricted within dolphin identity to account for repeated measures of individuals. Pseudo-F values represent the ratio of among-group to within-group variation, and R^2 values indicate the proportion of variance explained by each factor. Significant effects ($p < 0.05$) are shown in **bold**.

Figures – Chapter 2

Figure 1: Map of Sarasota Bay, Florida, USA, where all sample collection occurred.

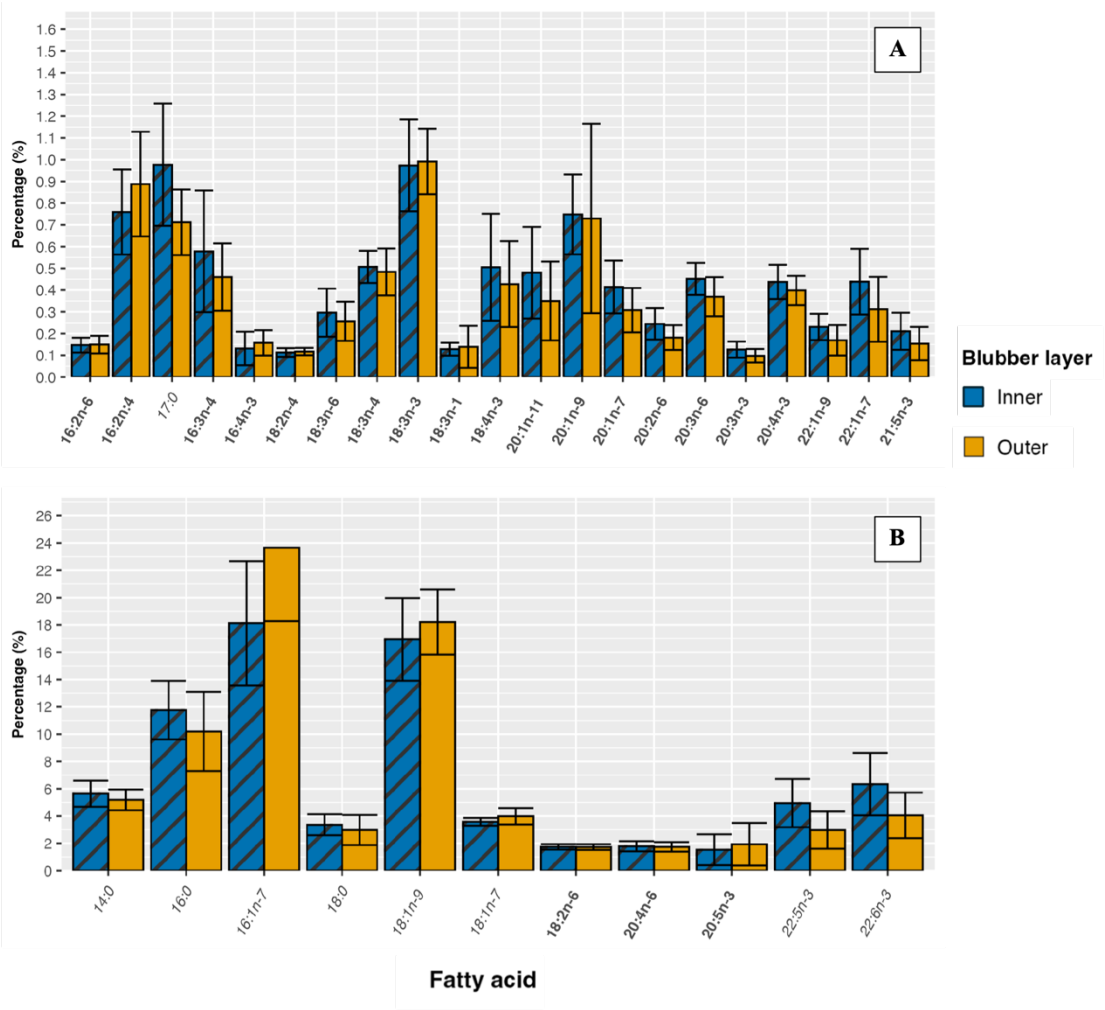

Figure 2: Fatty acid (FA) composition of inner and outer blubber from all SB dolphins. Values represent the average relative contribution of each FA ± standard deviation (SD) to the total FA signature of all dolphins. Panels depict FAs with **(A)** smaller and **(B)** larger relative contributions. Note that the x- and y-axes differ between panels. Includes all ***dietary*** and *extended dietary* FAs used for diet estimation (see Methods).

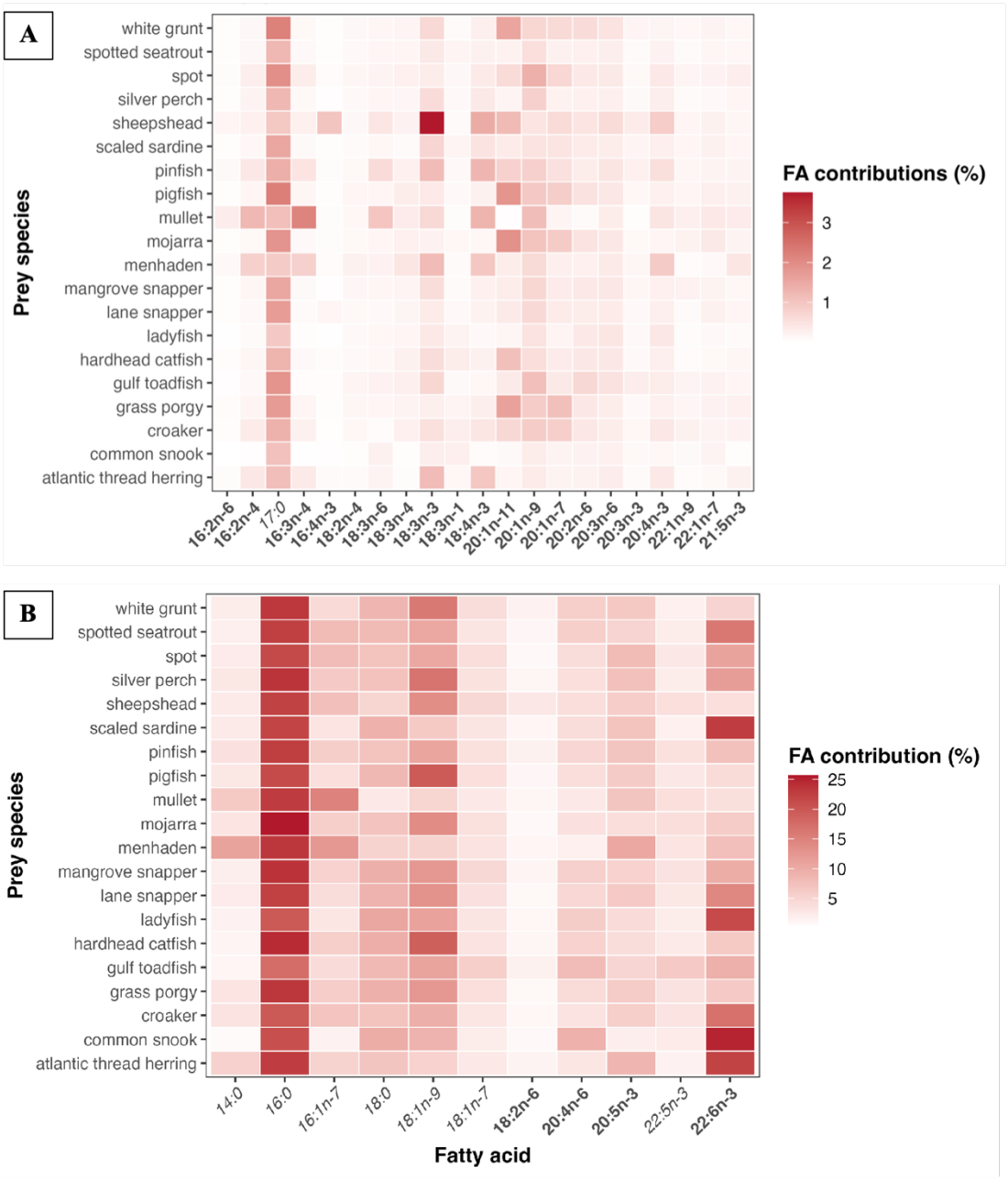

Figure 3: Mean fatty acid (FA) composition of all SB prey species. Values represent the average relative contribution of each FA to the total FA signature of each species, with darker colors indicating higher FA contribution. Panels depict FAs with **(A)** smaller and **(B)** larger relative contributions. Note that x-axes and contribution strengths differ between panels. Includes all ***dietary*** and *extended dietary* FAs used for diet estimation (see Methods). Some FAs (e.g., 14:0, 16:0, 18:0, 18:1n-7, 22:5n-3) were consistently high or low among most species. A subset of FAs (e.g., 18:1n-9, 22:6n-3) varied among species and drove differences, however.

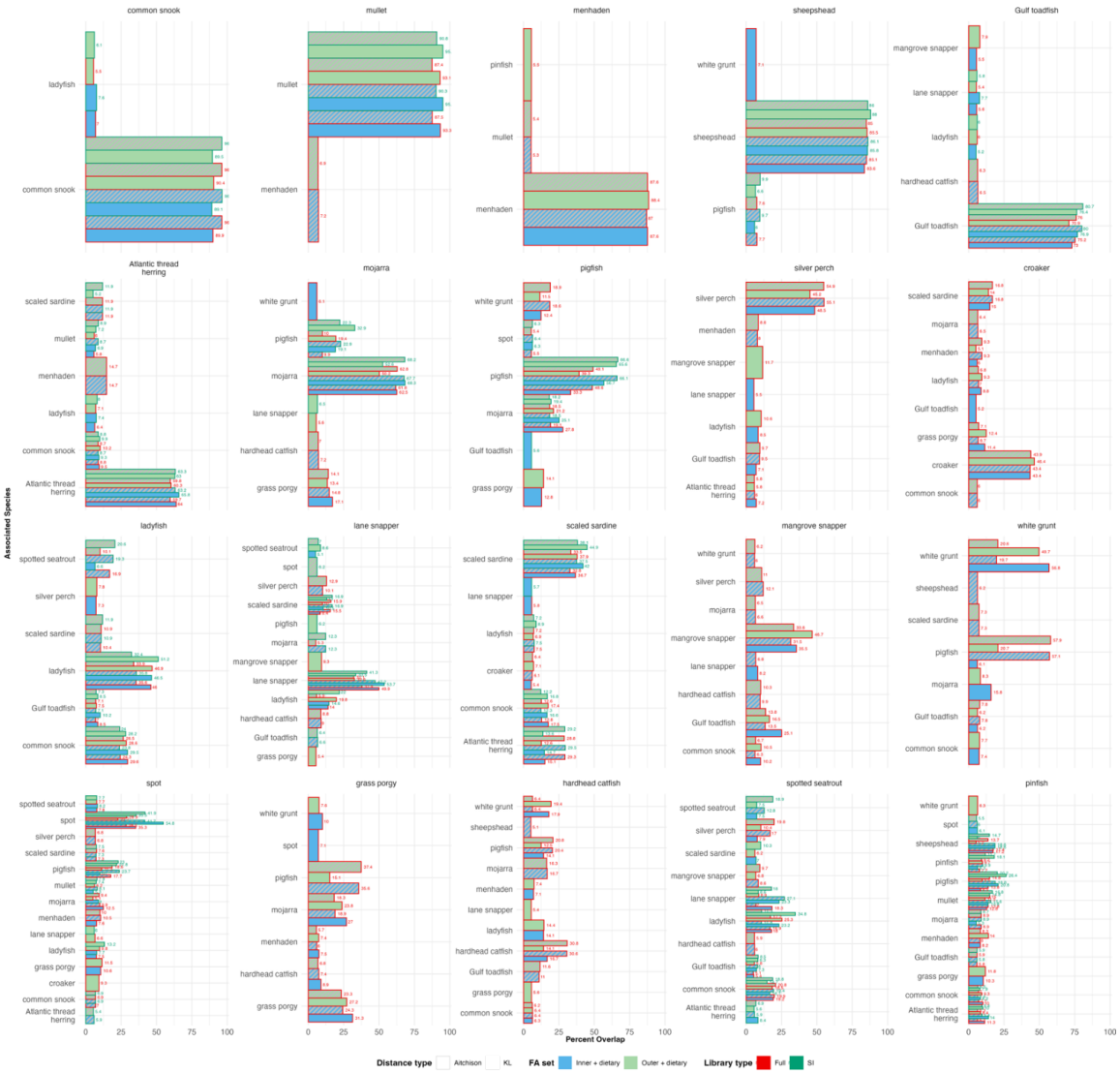

Figure 4: Leave-one-prey-out (LOPO) results for Sarasota Bay prey fish. Values indicate species self-associations and associations with other species that were >5% and are arranged by species from highest to lowest self-association. Higher self-association values indicate greater FA signature distinctiveness for that species, and therefore greater confidence in species-level QFASA estimates. Results are shown for the dietary FA suite using the Aitchison and KL distance measures, inner and outer blubber, and the full and SI prey libraries. Results for the extended dietary suite, which was excluded from diet estimation (see Results), are available upon request. Some species were absent from the SI library, leading to fewer LOPO values.

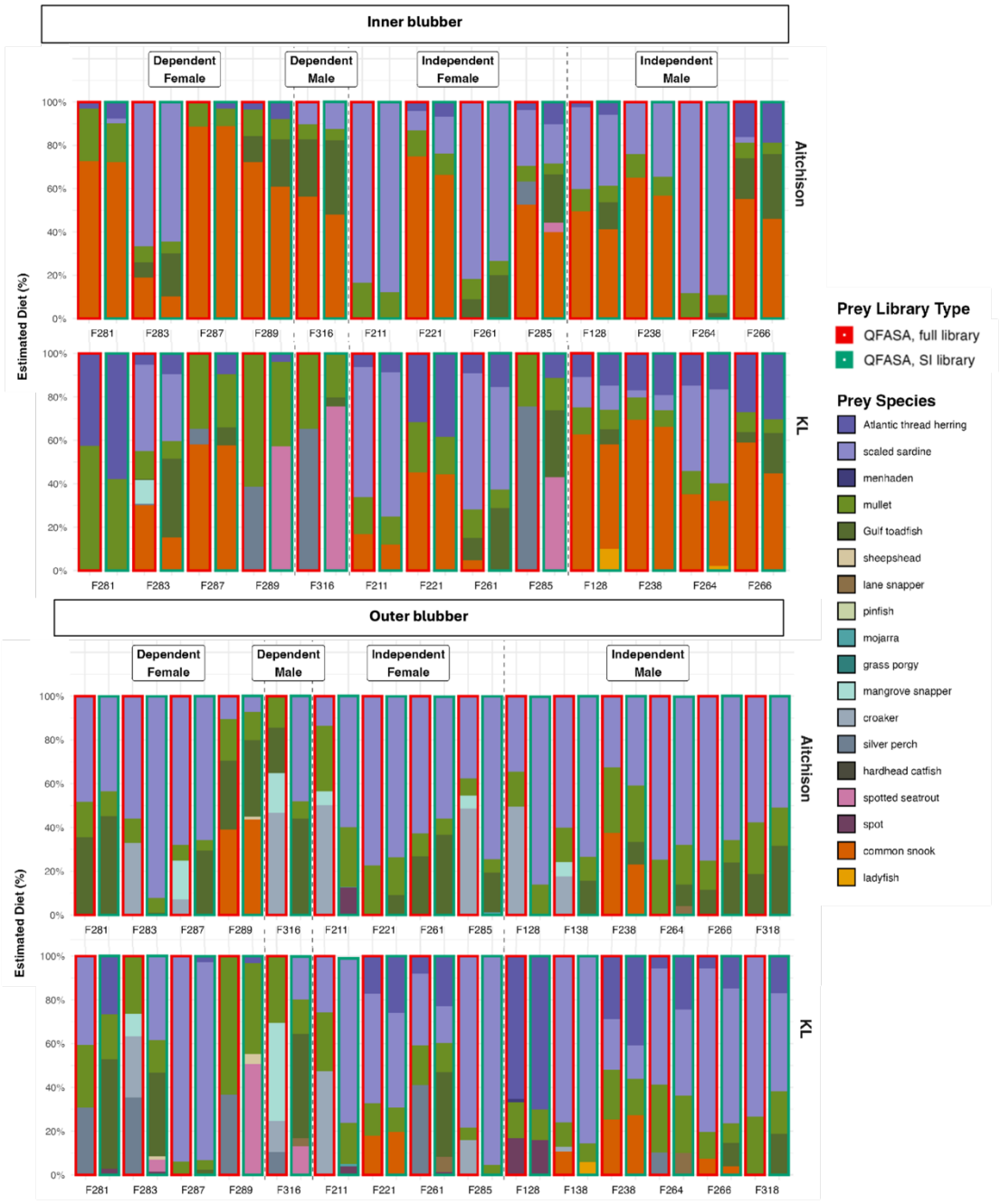

Figure 5: QFASA diet estimates for all dolphins and model configurations, separated by blubber layer. Dolphin IDs are indicated on the x-axis, the estimated diet contribution (%) is shown on the y-axis, Aitchison and KL estimates are shown in the top and bottom boxes, respectively, and the age class and sex of the dolphins are indicated by the labels and dashed line separators.

Chapter 3 – Integrating fatty acid and stable isotope approaches to reconstruct contemporary bottlenose dolphin (*Tursiops truncatus*) diet in Sarasota Bay: methodological comparison and ecological implications

Abstract

While multiple diet determination methods exist to quantify the diet of cryptic marine mammal predators, each integrate prey consumption over different temporal windows, provide different degrees of prey species resolution, and are subject to different sources of uncertainty. Combining multiple methods may provide a more complete picture of diet, but understanding and separating the methodological and ecological drivers of the resulting diet patterns is critical. This study compares the performance, estimated prey contributions, and associated uncertainties of Bayesian stable isotope and quantitative fatty acid signature analyses (QFASA) applied to free-ranging bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphins", n = 15) in Sarasota Bay, Florida. I show that while QFASA can yield high-resolution diet estimates, it is accompanied by meaningful uncertainty due to sensitivity to similarity in prey fatty acid signatures, estimate variation and unequal model performance with different model inputs and parameters, and variable standard errors. In comparison, SI analyses offered trophic-level insights and lower relative uncertainty than QFASA but

lacked its prey resolution. Despite these differences, however, both methods produced estimates with broad dietary patterns that were consistent within and between methods. During the limited period represented by this study, dolphins predominantly consumed piscivores and clupeids, with secondary contributions from species such as mullet and Gulf toadfish. When considered alongside previous studies in this system, my findings suggest dolphins select from similar subsets of available prey, including soniferous species, and exhibit some degree of variation among individuals and demographic groups. However, I also identified possible shifts in prey species use over time, which may be the result of both methodological differences and true ecological change. This study emphasizes the complexity of studying diet, even in an extremely well-studied system, and the value of incorporating multiple diet determination methods. Through this method comparison, I suggest that dolphin diet is focused on prey that share ecological features but is also flexible and responsive to current conditions, characteristics with key implications for future dolphin health.

1 Introduction

Understanding marine mammal diets is fundamental to exploring a wide variety of interconnected ecological and physiological processes, yet reconstructing the diets of cryptic predators remains inherently challenging. Multiple diet determination methods are available; however, each has distinct strengths and limitations, and integrate diet over different temporal and taxonomic scales (Bowen and Iverson, 2013; Nielsen et al., 2018; Texeira et al., 2022; Chapter 1; Chapter 2). Accordingly,

studies increasingly incorporate multiple approaches. Comparisons can provide insights into both diet determination methodology and predator ecology, identifying consistent trophic patterns, revealing areas of uncertainty and inconsistency, and improving interpretation of dietary variation among individuals and between demographic groups.

As top predators, the free-ranging bottlenose dolphins (*Tursiops truncatus*, hereafter "dolphins") residing in Sarasota Bay, Florida are considered sentinels of coastal ecosystem health (Wells et al., 2004). Understanding their diet is central to understanding trophic interactions and their potential impact on prey communities, but also dolphin health, reproductive success, contaminant exposure, and vulnerability to disturbances that impact their prey, such as harmful algal blooms, interactions with recreational and commercial fisheries, and changes in climate and ocean conditions (Nowacek et al., 2001; McHugh et al., 2011; Powell and Wells, 2011; Berens McCabe et al., 2021; Longden et al., 2022; Hart et al., 2022; Conger et al., 2024; Bankhead et al., 2025; Estella Martin et al., 2026). As described in Chapter 2, the approximately 170 dolphins in this community reside in the area year-round and throughout most of their lives and have been nearly continuously studied by the Sarasota Dolphin Research Program (SDRP) since 1970 (Wells et al., 2025). As a result, detailed information on individual dolphin identity, age, sex, maternal lineage, and social associations is available for more than 90% of the dolphins (Wells et al., 2025). Furthermore, sample acquisition is facilitated by established dolphin catch-and-release and prey collection protocols. Together, these features have made

Sarasota Bay one of the best-characterized dolphin communities, with extensive knowledge of diet, behavior, physiology, and ecology. Accordingly, the SB dolphins present an ideal system in which to both compare different diet determination methods and explore the ecological implications of dietary findings.

This chapter involves two primary objectives. First, I will compare QFASA and SI diet determination methods applied to the same dolphins, using the set of viable QFASA estimates identified in Chapter 2. I will examine both the model performance and associated uncertainties of the methods and the actual diet estimates. Second, I will place these results in the context of previous SB dolphin diet studies based on hard part analyses, DNA metabarcoding, and stable isotopes to (a) identify dietary patterns across methods, and (b) evaluate potential methodological, ecological, and nutritional drivers of diet variation. Through this integrated approach, I aim to identify dietary patterns, evaluate how methodological uncertainty influences estimated diet and ecological interpretations, and address the complementary strengths and limitations of each method.

2 Materials and methods

2.1 Sample collection

All sample collection occurred in Sarasota Bay (SB), Florida (**Figure 1**). Using the methods described in Chapter 2, skin and blubber samples were collected during catch-and-release dolphin health assessments in June 2019 and dolphin sex and age class were determined. Prey fish collection and trophic group identification also followed

the same protocols described in Chapter 2, except that prey fish used in this chapter were collected during both 2009 – 2012 and 2020 – 2022.

Dolphin sampling occurred under National Marine Fisheries Service/MMPA Scientific Research Permit No. 20455. Fish sampling occurred under Florida Fish and Wildlife Conservation Commission Special Activity License No. 19-0809-SR. Both projects were reviewed annually for IACUC approval through Mote Marine Laboratory.

2.2 Fatty acid analyses

Sample preparation, data analyses, assessment of QFASA model performance, and diet estimation using the dolphin blubber samples and fish collected during 2020 – 2022 were the same as described in Chapter 2.

2.3 Stable isotope analyses

2.3.1 Sample preparation

Sample preparation for SI analyses of the skin samples and fish collected only during 2009 – 2012 followed established methods (Rossman et al., 2015a). Dolphin skin and fish white muscle tissue were freeze-dried, lipid-extracted, and homogenized to a fine powder in a high-energy ball mill (SPEX SamplePrep) and a 1 mg aliquot of homogenate was transferred to tin capsules for stable carbon (^{13}C) and nitrogen (^{15}N) isotope analysis. Isotope values were determined using an elemental analyzer (Eurovector) interfaced to an Isoprime mass spectrometer (Elementar) and reported as

δ13C and δ15N. In-house standards were calibrated with respect to international scales V-PDB and air respectively. In-house precision was ±0.2‰ for δ13C and δ15N values. All SI sample processing was conducted by Sam Rossman and Peggy Ostrom at Michigan State University.

2.3.2 Data analyses, SI model performance, and diet estimation

I used R and the package simmr (Govan and Parnell, 2024; R Core Team, 2025) to generate diet estimates for each dolphin. simmr requires isotope values for consumers and prey species and a trophic enrichment factor (TEF) that accounts for the isotopic difference caused by the consumer's digestion and assimilation of prey SIs. However, having just two SIs for analysis (^{13}C and ^{15}N) means that simmr cannot differentiate between prey species with similar SI values; prey species must instead be grouped into clusters. I evaluated cluster solutions across a range of cluster numbers (*k*s) using both the elbow method and silhouette analysis. The elbow method identifies values of *k* beyond which additional clusters do not yield meaningful reductions in within-cluster variance, while silhouette analysis indicates cluster cohesion, with higher silhouette widths suggesting better-defined clusters. To visualize the uncertainty and variability of each cluster, I calculated the centroids of each cluster, used re-sampling to determine the 95% confidence interval (CI) around those means, and plotted both 40% and 95% prediction intervals (PIs) around the means. The 95% CI quantifies uncertainty in estimating the mean isotopic composition of each cluster and is expected to be much smaller than the PIs. PIs

represent the expected spread of individual observations within each cluster, reflecting core (40% PI) and broader (95% PI) within-cluster biological variability rather than centroid uncertainty. To determine the appropriate number of clusters, I used the diagnostic tests (silhouette and elbow plots), centroid separation, visual depictions of within-cluster dispersion and cluster overlap, and previous analyses of these fish SIs (Rossman et al., 2015a). Finally, I applied TEF values previously derived from SB dolphin muscle samples (Rossman et al., 2015a): 0.0‰ for $\delta^{13}C$ and 2.0‰ for $\delta^{15}N$. For each dolphin, Bayesian stable isotope mixing models were fit using Markov chain Monte Carlo sampling and used to estimate the proportional diet contributions for each prey source. Estimate uncertainty was quantified as standard errors (SEs) for each estimate, calculated by dividing the standard deviation of each estimate by the square root of the number of posterior draws for each prey proportion.

Thus, SI model performance and uncertainty were quantified through two main avenues. First, the prey clusters: the level of prey species detail captured by the clusters and the variability associated with them are comparable to the prey distinctiveness metric used to evaluate QFASA estimates. Clusters that contain fewer species, are more trophically coherent, and/or have lower variability provide more distinctive prey types for diet estimation. Conversely, fewer large clusters containing multiple species, especially if they are not trophically coherent and/or have large variability and thus uncertainty associated with them, mean fewer distinctive prey types are available for diet estimation. The SEs associated with each dolphin's SI diet

estimate is the second measure of estimate uncertainty: large SEs suggest estimates are less certain.

2.4 Previous diet estimates of Sarasota Bay dolphins

Given the long-term research of the SB dolphins, previous studies have described their diet using a variety of methods. These include stomach content and prey selection analyses of stranded dolphins sampled from 1984 through 2006 (Barros and Wells, 1998; Berens McCabe et al., 2010; Barros et al., 2012; Wells et al., 2013), DNA metabarcoding (molecular prey detection) using fecal and gastric samples collected from live dolphins sampled during health assessments in 2005 and 2006 (Dunshea et al., 2013), stable isotope analyses of dolphins sampled before 2015 (Rossman et al., 2013, 2015a, 2015b), and direct observation of dolphin feeding in SB (Ronje et al., 2017; SDRP unpublished data). However, no diet analyses of SB dolphins have been carried out since 2015; while collection of fecal swabs for DNA metabarcoding began in 2022, results are not yet available (Krystan Wilkinson, personal communication). As a result, neither observations of feeding behavior nor individual stomach content or fecal sample analyses were available for the 15 dolphins sampled in June 2019 or any dolphins sampled more recently. Accordingly, I compared the QFASA and SI diet estimates generated in this study to the previous results to qualitatively explore differences between diet determination methods and historical and contemporary diet estimates.

3 Results

3.1 Dolphin demographics and prey library variety

In total, 15 dolphins were sampled, including four independent females, six independent males, four dependent females, and one dependent male. This included three mother-daughter pairs: F261 (mother) and F281, F211 and F289, and F221 and F287. Skin and full-depth blubber samples were collected from 13 dolphins, while skin and outer blubber only were collected from the remaining two (both independent males). See **Table S1** for complete sample and demographic details.

Fish were caught separately for SI and FA analyses. 177 fish from 13 species, with 3 – 27 individuals per species, were collected during 2009 – 2012 and processed for SIs before the FA portion of this study was completed. 147 fish from 20 species, with 3 – 12 individuals per species, were collected during 2020 – 2022 for FA analyses. Accordingly, for QFASA analyses, the "full" library included 20 species and 147 samples, while the "SI" library included the 13 species (99 samples) for which I also had SI data. SI analyses were conducted using the 177 samples from 13 species for which SI data was available. Prey fish came from a variety of trophic groups, including planktivores; benthic carnivores, omnivores, and detritivores; invertivores; and piscivores. See **Table S2** for complete sample details.

3.2 Diet estimation using QFASA

3.2.1 Dolphin and prey fish fatty acid signatures and lipid composition

General patterns in SB dolphin FA signatures and prey fish FA signatures and lipid composition are discussed in Chapter 2. However, modest demographic differences, involving both broader FA categories and specific FAs, were also apparent (**Table S3**). Independent dolphins, and males more than females, had higher overall polyunsaturated FA (PUFA) percentages, especially in inner blubber (inner: 25.7 – 30.8% independent versus 18.1 – 21.9% dependent; outer: 21.5 – 21.7% versus 14.7 – 17.8%). Independent dolphins also had higher percentages of individuals PUFAs such as 22:6n-3 (inner: 6.7 ± 1.4% and 8.7 ± 1.0% independent females and males versus 4.3 ± 1.8% and 3.8% dependent females and male; outer: 4.7 ± 1.7% and 4.49 ± 1.07% versus 3.3 ± 2.25% and 2.1%) and 22:5n-3 (inner: 5.2 ± 1.5% and 6.5 ± 0.8% versus 3.5 ± 1.6% and 3.2%; outer: 3.7 ± 1.5% and 3.0 ± 1.3% versus 2.5 ± 1.4 and 1.7%). In contrast, dependent females exhibited somewhat greater variability in both overall FA patterns and several dominant FAs, particularly in inner blubber, while independent males tended to have smaller standard deviations (SDs). For instance, for the total inner monounsaturated FAs (MUFAs) and PUFAs, SDs were 14.2 and 9.9 for dependent females, 9.4 and 5.8 for independent females, and 5.2 and 4.9 for independent males. Similarly, for inner 16:1n-7, SDs were 6.8 in dependent females versus 3.9 and 2.4 in independent females and males, respectively. This pattern was not as consistent in outer blubber, however, where SDs were similar or higher for independents compared to dependents. For instance, for overall outer blubber MUFAs, the SD for dependent females was 8.7, compared to SDs of 12.9 and 14.8 for independents females and males, respectively. Finally, overall differences

between inner and outer blubber were slightly greater in independent dolphins than dependents. For instance, both 22:6n-3 and 22:5n-3 differed more between layers in independents than dependents.

3.2.2 QFASA model performance and retained QFASA estimates

In Chapter 2, I evaluated QFASA model performance and the impact of alternative model configurations (different combinations of calibration coefficient set, FA suite, distance measure, prey library, and blubber layer) to assess estimate certainty and whether "best-fit" configurations could be recommended for future use. Different calibration coefficient (CC) sets yielded comparable estimates and did not impact model performance and so estimates generated using three-dolphin CCs derived in Chapter 1 from known professional-care dolphin diet integrated over the 16 weeks before sampling are presented here. In comparison, FA suite, distance measure, prey library composition, and blubber layer all influenced diet estimates and model performance to varying degrees. Based on model performance metrics, including predator-beyond-prey (PBP) values and the contribution of the augmented FA, only estimates generated using the dietary FA suite were recommended for further interpretation. PBP values and assessment of the SEs of the estimates also led me to suggest that while both inner and outer blubber generated viable estimates, outer blubber estimates should be interpreted cautiously and only alongside inner blubber estimates, due to poorer model performance and higher SEs contributing to higher uncertainty. However, because neither the Aitchison nor KL distance

consistently outperformed the other, and as both prey libraries produced estimates with overall comparable model performance, estimates from both distance measures and libraries are presented here. In summary, this chapter will be based on estimates generated using the dietary FA suite and dolphin-specific CCs but incorporating the range of valid estimates produced by the alternative distance measures, prey libraries, and blubber layers presented in Chapter 2.

3.2.3 QFASA prey distinctiveness

Interpretation of the QFASA estimates should be considered in the context of the prey distinctiveness analyses presented in Chapter 2. Leave-one-prey-out (LOPO) analyses found that prey species varied in FA distinctiveness. Some species – common snook, gulf toadfish, menhaden, mullet, and sheepshead – were highly distinctive, while others – Atlantic thread herring, scaled sardine, ladyfish, spotted seatrout, mojarra, pigfish, grass porgy, and white grunt – were less distinctive individually but consistently associated with other species in trophically coherent groups. In comparison, hardhead catfish, lane and mangrove snappers, silver perch, croaker, spot, and pinfish were less distinctive and had weak to intermediate associations with multiple species.

3.2.4 Major prey identified across retained QFASA estimates and associated estimate uncertainty

As evaluated in Chapter 2, estimated prey proportions varied depending on the model variable configurations and bootstrap-derived SEs added uncertainty to some estimates (**Figures 2, S1**). Estimates differed most between distance measures and blubber layers, whereas differences between prey libraries were generally smaller unless a prey species that was absent from the "SI" library was a large contributor when the "full" library was used. SEs differed among prey species and model configurations but were sometimes large relative to the estimated prey contributions. Nonetheless, broad patterns in identified prey species and estimated contributions across individuals and demographic groups could be identified.

Using the Aitchison distance and the inner blubber yielded the most consistent and structured estimates across dolphins, while KL estimates with the inner blubber shared similarities but were slightly more diverse and variable (**Figures 2, S1**). With both distance measures, common snook, scaled sardine, or both together were top diet contributors, with intermediate SEs (7 – 27%) that changed their relative proportions but not their status as top prey. Mullet was of secondary importance, with consistently low SEs (< 9%). Of the three mother-daughter pairs, one (mother F221 and daughter F287) had similar estimated percentages of common snook and mullet, but neither of the other pairs had noticeable similarities. However, whereas Aitchison estimates identified gulf toadfish and Atlantic thread herring as contributors of secondary importance, estimated diet diversity increased with the KL distance, as spotted seatrout, silver perch, mangrove snapper, and ladyfish were also identified, sometimes in large percentages (>50%). For both distance measures, however, these

estimates of secondary contributors had variable SEs that were sometimes equivalent to one-half or more of the estimated values, adding uncertainty to their relative contribution. In addition, while with the Aitchison distance both age classes and sexes shared similar diet patterns, with the KL distance, dependent dolphins of both sexes and independent females tended to have more varied diets than independent males.

In comparison, outer blubber estimates calculated using both distance measures tended to identify a greater diversity of species, differed in the most prevalent prey, and had comparable or higher SEs (**Figures 2, S1**). Common snook was less frequently identified (<30% of any estimated diet, compared to >50% in many inner blubber estimates). Instead, estimates mostly included mixtures of scaled sardine, Atlantic thread herring, mullet, gulf toadfish, croaker, mangrove snapper, silver perch, and spotted seatrout, along with small amounts of lane snapper, spot, or ladyfish. Thus, compared to inner blubber, all demographic groups had more varied diets and greater differences between model configurations (e.g., all demographic groups had croaker, spot, and lane snapper). However, dependent dolphins of both sexes and independent females still consumed an overall greater diversity of species – including spotted seatrout (dependents only) and silver perch (both) – and were more likely than independent males to have significant diet changes between the distance measures. In addition, two mother-daughter pairs (F261 and F281; F221 and F287) shared some diet overlaps. F261 and F281 were more alike, with similar relative amounts of Atlantic thread herring, mullet, gulf toadfish, and/or silver perch, depending on the model configuration. F221 and F287 shared scaled-sardine

dominated diets but had differing amounts and types of secondary prey. Overall, however, quantifying exact prey contributions was difficult for all estimates, as SEs tended to be comparable to or higher than inner blubber SEs (except for mullet, for which they remained low) and were sometimes large even when the estimated contribution of a species was 0%.

Thus, across all estimates, of the 20 prey species in the full library and the 13 in the SI library, nine species from both libraries – scaled sardine, Atlantic thread herring, common snook, spotted seatrout, ladyfish, gulf toadfish, mullet, spot, and lane snapper – and three only in the full library – silver perch, mangrove snapper, and croaker – were estimated in at least one dolphin and model configuration. Of these, common snook, scaled sardine, Atlantic thread herring, mullet, and gulf toadfish were the species most consistently identified across dolphins. Dependent dolphins of both sexes and independent females were more likely to also have small amounts of additional prey species, leading to more diverse and/or variable diets than independent males. Inner blubber estimates also tended to have higher proportions of common snook relative to outer blubber estimates, where estimated proportions of scaled sardine and/or Atlantic thread herring were higher in outer blubber. Finally, while some mother-daughter pairs shared diet similarities, especially in the outer blubber, overlaps were neither complete nor consistent between model configurations. However, relative prey proportions varied with distance measure, and many prey species estimates had large SEs. This added uncertainty to the results, as multiple possible diet estimates existed for each dolphin, even within a blubber layer.

3.3 Diet estimation using stable isotopes

3.3.1 Prey species SI and distinctiveness

Average δ13C values ranged from –17.6‰ and –17.5‰ (scaled sardine and ladyfish) to – 12.4‰ (mullet), while average δ15N ranged from 7.6‰ (mojarra) to 11.7‰ (ladyfish) (**Table 1**).

Clustering diagnostics were used to evaluate the number and structure of prey clusters. Silhouette analyses showed a local maximum in mean silhouette width at k = 2 and the elbow plot exhibited an inflection point at k = 2, indicating strong support for at least two trophic clusters (**Figure S2**). However, silhouette width remained relatively high for k = 3 and 4, and the elbow plot suggested k = 3 – 5 further reduced cluster variance, although with diminishing returns. Cluster centroids and their 95% CIs were distinct across all evaluated k values (2 – 5) (**Figure 3**). In contrast, both 40% and 95% PIs indicated substantial within-cluster variability and partial overlap among clusters, particularly at higher k values (**Figure 3**). Overlap was most pronounced for the 40% PIs at k = 5 and for the 95% PIs at k = 3 – 5. These patterns indicate that although clusters differed consistently in their mean isotope composition, the underlying data exhibited considerable variability and gradual transitions among clusters rather than discrete boundaries. Previous analyses of these same prey fish SI data (Rossman et al. 2015a) identified four clusters mostly consistent with expected ecological groupings and closely matching the k = 4 solution. Based on clustering diagnostics, centroid separation, uncertainty visualization, and prior analyses, k = 4 was selected for subsequent diet modeling.

Under this solution, Cluster 1 included Atlantic thread herring and scaled sardine, Cluster 2 included pigfish, pinfish, lane snapper, mojarra, mullet, gulf toadfish, and sheepshead, Cluster 3 included only spotted seatrout, and Cluster 4 included common snook, ladyfish, and spot (**Table 1)**.

3.3.2 Dolphin SI values and diet estimates

Dolphin skin SI values ranged from –18.6‰ to –13.3‰ (δ13C) and 8.7‰ to 12.8‰ (δ15N) (**Table S4**). The δ13C values (reported as means ± SDs) of the dependent females and independent females and males (–15.7 ± 1.3‰, –15.6 ± 0.6‰, –16.1 ± 1.0‰, respectively) differed from the single dependent male (–18.6‰). In comparison, the mean δ15N values were similar across the four groups: (11.3 ± 0.9‰, 11.4 ± 0.8‰, 11.1 ± 1.6‰, 11.4‰). The mother-daughter pairs (F261 – F281, F211 – F289, and F221 – F287) also had very similar δ13C and δ15N values within each pair (**Figure 4; Table S4**).

SI estimates had low uncertainties, with SEs < 1%, and diet estimates were relatively similar across all dolphins, age classes, and sexes, with 20 – 30% Cluster 1 (Atlantic thread herring and scaled sardine), 20 – 30% Cluster 4 (common snook, ladyfish, and spot), 10 – 20% Cluster 3 (spotted seatrout), and the remaining 15 – 20% Cluster 2 (pigfish, pinfish, lane snapper, mojarra, mullet, gulf toadfish, and sheepshead) (**Figure 5; Table S5**). Four dolphins had estimates that differed from this general pattern: dependent female F283 consumed mostly Cluster 2, independent females F283 and F285 had >50% Cluster 2, and dependent male F316 had around

45% Cluster 1. Taken together, dependent dolphins had slightly more variable diets than independents, but there were no large differences across the demographic groups. All mother-daughter pairs had very similar percentages of each cluster, although they shared the same broad divisions among clusters as other dolphins and thus were not notably more similar to each other than to the other dolphins.

3.4 Comparing QFASA and SI estimates within individual dolphins

To facilitate comparison between QFASA and SI estimates (**Figure S3**), QFASA prey species were considered relative to the SI clusters: Atlantic thread herring and scaled sardine versus Cluster 1, spotted seatrout versus Cluster 3, common snook, ladyfish, and spot versus Cluster 4, and all other QFASA prey species (e.g., gulf toadfish, mullet, croaker, silver perch) versus Cluster 2.

QFASA and SI estimates for individual dolphins shared some proportional contribution similarities in limited cases with specific model configurations. These included F128's inner blubber estimates with both distance measures, F211's inner blubber estimates with the KL distance, F238's outer blubber estimates with the Aitchison distance, and F283 and F285's outer blubber estimates with the KL distance (**Figure S3**). For instance, F221's inner blubber estimate with the KL distance and SI estimates had, respectively, ~25% scaled sardine and Cluster 1, ~25% mullet and Cluster 2, and the remainder common snook and Clusters 3 and 4. SI estimates for F283 and F285 had 60 – 70% Cluster 2, different from the other dolphins, and some of the QFASA estimates for those dolphins had similarly unusual

prey species that were mixed invertivores that could fall into that cluster. This included F283's outer blubber estimates with the KL distance, where ~75% of the diet was composed of a mix of mangrove snapper, croaker, and silver perch, and F285's inner blubber estimates with the KL distance and outer blubber estimates with the Aitchison distance, which had ~75% silver perch and 50% croaker, respectively. Even in these limited cases, however, similarities did not consistently occur with certain blubber layers, distance types, or prey libraries.

Beyond these cases, individual dolphin QFASA and SI estimates differed (**Figure S3**). For instance, all QFASA estimates for F261 included 40 – 70% scaled sardine and the remainder divided between mullet, gulf toadfish, and silver perch. In comparison, the SI estimates included ~25% Cluster 1 (which would include scaled sardine), 25% Cluster 2 (which would encompass mullet, toadfish, and perch), and the rest of the estimate divided between Clusters 3 and 4. Similarly, F266's inner blubber estimates included ~50% common snook, the outer blubber estimates had ~75% scaled sardine and Atlantic thread herring, but Cluster 2 made up 50% of the SI estimates. Even for dolphins with high variability among their QFASA diet estimates (e.g., F289, F316), no single QFASA estimate was consistently aligned with the SI estimates. For instance, F289 had ~50 – 70% snook in the inner and outer blubber with the Aitchison distance and ~50 – 60% mullet with the KL distance, with the remainder silver perch (full library) or spotted seatrout (SI library), whereas SI estimates included ~30% Cluster 1 and Cluster 4, ~15% Cluster 3, and ~25% Cluster

2, each much less than the amounts of common snook, spotted seatrout, mullet, or silver perch in the QFASA estimates.

4 Discussion

4.1 Dolphin and prey fish FA and SI sampling

My sample size is relatively small, with 15 SI diet estimates, 13 inner blubber QFASA diet estimates, and 15 outer blubber QFASA diet estimates. This is especially true once the samples are divided into demographic groups, yielding four dependent females, one dependent male, four independent females, and six independent males. Furthermore, the 20 and 13 prey species included in the QFASA and SI estimates represent only a subset of the 100+ species that can be found in SB and could theoretically be considered potential dolphin prey fish. As discussed in Chapter 2, the choice of species included in the "full" prey library were informed by *a priori* knowledge and sample availability, while the species included in the "SI" library were further reduced to match the species for which I also had SI data. As a result, these prey libraries are not definitive representations of the complete SB dolphin prey base. However, they do represent justified and viable subsets appropriate for addressing this chapter's objectives: comparing different diet determination methods and exploring the ecological implications of the resulting estimates.

4.2 Dolphin and prey fish FA signatures and lipid contents

Overall patterns in SB dolphin and prey fish FA signatures and lipid contents were discussed in Chapter 2 and shown to follow patterns expected for bottlenose dolphins and subtropical estuarine fish, supporting their validity for use in this study. Similarities between dolphin and prey fish FAs were also consistent with dolphins consuming those fish. However, demographic differences were not addressed in Chapter 2. Few studies have examined demographic patterns in FA signatures in cetaceans, though studies in pinnipeds are more common. However, previous analyses suggest that FA stratification in cetaceans increases with age (Koopman, 2007; Koopman et al., 1996) and that while dependent seals have higher total percentages of saturated FAs (SFAs) and MUFAs and independent seals have higher amounts of PUFAs, differences between sexes were minimal (Kirkpatrick et al., 2022; Neises et al., 2021). My results are consistent with these findings, with slightly greater stratification in independent dolphins, especially males, than dependents, and higher percentages of PUFAs in independents and SFAs in dependents. I also found slightly greater variability (measured via the SDs of the mean values for different FAs) in dependent female inner blubber than independent dolphin inner blubber, with independent males having particularly low inner blubber SDs for many FAs. This suggests males may have more consistent diets overall compared to dependent dolphins of both sexes, though examination of the QFASA estimates is necessary to further support this. Taken together, these results demonstrate that SB dolphin FA signatures share the same patterns as previous analyses, confirming their accuracy

and use for QFASA diet estimation, and suggest dietary differences may exist between demographic groups.

4.3 Dolphin and prey fish SI values

Prey fish SI values were automatically consistent with the previous analysis of SB fish SIs (Rossman et al., 2015a) as they were the same values, but SB dolphin SIs were also broadly in line with those results, though with some variations. In the previous study, δ13C values for dependent and independent dolphins were between –14.9 ± 0.9 ‰ and –14.2 ± 0.4 ‰, slightly higher than my results (–15.6 ± 0.6‰ to –16.1 ± 1.0‰ for all groups except the dependent male, which had –18.6‰). While these values are broadly within the same range, lower δ13C could suggest increased consumption of prey species that use environments with lower δ13C values – open bay and mangroves – and/or increased use of those environments by the dolphins (Peterson and Fry, 1987; Wada, 2009). On the other hand, δ15N values were consistent between studies, around 11‰ for all demographic groups in this study and 10.6 ± 0.6 ‰ to 11.2 ± 0.9 ‰ in the previous analyses. These are expected for a higher-trophic level predator like dolphins (Peterson and Fry, 1987; Wada, 2009), and suggest all demographic groups were feeding across broadly similar trophic levels in this study.

4.4 QFASA and SI model performance and estimate uncertainty

Before interpreting diet estimates, both model performance and structural sources of uncertainty in the diet estimates, and how they compare between methods, were evaluated. QFASA and SI approaches have both similarities and differences in where uncertainty arises, how it is quantified, and how it affects ecological inference. Prey distinctiveness is a key component of both QFASA and SI model performance that indicates how definitive and informative a given prey species or prey cluster estimate is, and how that resolution compares between the methods. QFASA estimates also had two additional metrics of model performance: predator-beyond-prey (PBP) values and the contribution of the augmented FA. Finally, both QFASA and SI estimates had standard errors (SEs) that quantify uncertainty of the actual estimates, while QFASA had the additional complexity of multiple model configurations that yielded multiple potential diet estimates for each dolphin.

4.4.1 Comparison of prey resolution between QFASA and SI analyses

Prey distinctiveness is a component of model performance relevant to both FA and SI analyses which is critical for interpreting each method's estimates but also informs their comparison. For QFASA estimates, leave-one-prey-out (LOPO) values quantify the distinctiveness of a species' FA signature. There is no defined LOPO cut-off. Higher self-association indicates greater distinctiveness and thus greater certainty in the identification of that species; conversely, lower self-association and distinctiveness increase the chance of confounding between species, where the identification of one species could represent the presence of a difference species with

a similar FA signature (Bromaghin, 2017). LOPO values for these QFASA estimates were covered extensively in Chapter 2, where I showed that distinctiveness varied among species. However, the ecological implications of that variation and how it relates to SI species resolution will be discussed here. For SI estimates, prey resolution is considered at the cluster level, using the number and sizes of clusters, trophic coherence, and the quantitative metrics of centroid confidence intervals (CIs), prediction intervals (PIs), and their overlap. No formal cut-offs exist for these metrics, but less overlap indicates stronger separation. In this study, all clusters had distinct centroid CIs and 40% core PIs, but substantial overlap occurred among 95% PIs, indicating within-cluster variability and adding uncertainty in SI prey resolution.

Both QFASA and SI analyses found that clupeids – including menhaden (FA "full" library only), Atlantic thread herring, and scaled sardine – formed coherent trophic groupings, despite differences in species-level resolution. SI analyses grouped Atlantic thread herring and scaled sardine into Cluster 1, characterized by lower $\delta^{15}N$ and $\delta^{13}C$ values consistent with their low-trophic-level planktivorous diet and more open-bay habitat (Smith, 1997; Ray et al., 1999; Murdy et al., 2013; Rossman et al., 2015a). As demonstrated in Chapter 2, planktivorous menhaden was distinct in FA space, while Atlantic thread herring and scaled sardine associated with each other and menhaden. Minor FA associations also occurred between Atlantic thread herring or scaled sardine and piscivorous common snook and ladyfish. This association likely reflects trophic FA transfer, as the piscivores integrate FAs derived from clupeid prey (Smith, 1997; Ray et al., 1999; Budge et al., 2006; Murdy et al., 2013). While SI

analyses also showed modest overlap between Clusters 1 and 4 (the latter of which contains common snook and ladyfish) at the 95% PI level, their centroids and 40% core PIs remained distinct. These patterns illustrate mechanistic differences between the methods. QFASA leverages numerous FA markers, which can allow finer within-trophic-level resolution and thus distinguish one clupeid species from the others to some extent, but FA transfer between predators and prey can also increase signature similarity (Budge et al., 2006). SI analyses, in comparison, better separated predators and prey via $\delta^{15}N$ values, separating Clusters 1 and 4, but often offer less species-level resolution within trophic groups unless they live in different habitats and thus have different $\delta^{13}C$ values, resulting in a single planktivorous Cluster 1 dolphins (Peterson and Fry, 1987; Wada, 2009). Taken together, while the methods differed in species-level resolution, both methods resolved the same underlying planktivorous grouping with minor piscivorous overlap.

Similarly, both QFASA and SI analyses identified common snook, ladyfish, and spotted seatrout as piscivorous prey with FA and SI similarities, although they again differed in the degree of species-level resolution. Spot, in contrast, highlighted an important difference between the two methods. Common snook was highly distinct in FA space, while ladyfish and spotted seatrout were of intermediate distinctiveness but associated with each other and/or common snook. Partially convergent FA signatures are consistent with their shared prey bases as piscivores (Smith, 1997; Ray et al., 1999; Gannon et al., 2009; Murdy et al., 2013). In turn, SI analyses grouped ladyfish and common snook (Cluster 4) and separated spotted seatrout into Cluster 3. Similar

$\delta^{15}N$ values reflect their shared higher-trophic-level piscivorous diet, but higher $\delta^{13}C$ values distinguished the more seagrass-associated spotted seatrout from ladyfish and common snook, which may use non-seagrass habitats like mangroves and estuaries (Smith, 1997; Ray et al., 1999; Murdy et al., 2013; Rossman et al., 2015a). The primary disagreement between methods involved spot. SI analyses grouped spot with common snook and ladyfish in Cluster 4, despite its benthic invertivore diet. Although its mean $\delta^{15}N$ was lower than the piscivores, it remained distinct from Cluster 2 (mixed benthic carnivores, omnivores, and detritivores) where it would be expected to cluster based on trophic ecology. In contrast, QFASA associated spot with other benthic invertivores, better reflecting its feeding ecology. Together, these results demonstrate that distinctiveness can depend on the method and related dietary marker (i.e., SIs or FAs). In this case, while SIs identified differences associated with both trophic position and habitat use, they did not have enough resolution to group spot in line with its trophic ecology. In comparison, the greater number of FA markers allowed QFASA to distinguish trophically similar species and maintain trophically coherent prey groupings.

Seagrass-associated benthic carnivores, omnivores, and detritivores showed the greatest overlap and most variable associations in both QFASA and SI analyses, though the results remained trophically coherent. SI analyses grouped pigfish, pinfish, lane snapper, mojarra, mullet, sheepshead, and gulf toadfish into Cluster 2, characterized by higher $\delta^{13}C$ consistent with their seagrass and muddy bottom habitats and lower $\delta^{15}N$ reflecting their low-intermediate trophic positions (Peterson and Fry,

1987; Rossman et al., 2015a; Wada, 2009). While species means revealed subtle trophic differences – slightly higher $\delta^{15}N$ values for sheepshead, pigfish, gulf toadfish, and lane snapper and slightly lower values for pinfish, mojarra, and mullet were consistent with diets that were more carnivorous versus more omnivorous and detritivorous – these did not lead to additional species-level resolution (Smith, 1997; Ray et al., 1999; Murdy et al., 2013). In comparison, QFASA provided greater resolution for some species but could not fully overcome their shared ecology. As discussed in Chapter 2, species like mullet, sheepshead, and gulf toadfish with more specialized diets (primarily detritivorous, invertebrate-focused, and carnivorous, respectively; Smith, 1997; Ray et al., 1999; Murdy et al., 2013) were highly distinct. However, pigfish, mojarra, white grunt, and grass porgy were individually less distinct but associated in a coherent benthic invertivore/omnivore group, while lane snapper had diffuse associations consistent with its mixed crustacean and fish diet, and pinfish had the lowest FA distinctiveness, likely reflecting its well-documented ontogenetic transition from invertivory towards omnivory and herbivory (Smith, 1997; Ray et al., 1999; Murdy et al., 2013; Barbosa and Taylor, 2020). Finally, mangrove snapper, silver perch, croaker, and hardhead catfish were present only in the FA prey library but exhibited the same intermediate-to-low distinctiveness and diffuse mixed associations. This is consistent with the flexible invertebrate-fish diets of mangrove snapper and silver perch, and the focus on benthic invertebrates and detritus by croaker and hardhead catfish (Smith, 1997; Ray et al., 1999; Murdy et al., 2013). Thus, QFASA overall provided greater species-level resolution than SI

analyses, but both methods reflected the same underlying ecological relationships that could decrease distinctiveness. Shared habitats, benthic food resources, and potential ontogenetic transitions limited separation between species, indicating that estimates involving these species may need to be interpreted with greater caution than those involving more distinct species.

Interacting methodological and ecological considerations likely contributed to some of the observed prey resolution patterns in both QFASA and SI analyses. Prey fish were primarily collected from shallow seagrass and muddy bottom habitats where SB dolphins commonly forage, and sampling targeted fish 100 – 300 mm in length, consistent with the size range in dolphin stomach contents (Barros and Wells, 1998). Sampling from similar habitats likely further constrained $\delta^{13}C$ variation and may have increased dietary (and thus SI and FA) similarity among species occupying similar areas. This could have contributed to the broadness of SI Cluster 2 and the FA similarities between many of the benthic carnivore-omnivore-detritovore species, along with the unexpected placement of spot with piscivores in SI analyses. Sampling within this size range may also have captured individuals during ontogenetic diet transitions, potentially compressing trophic distinctions. This is relevant for pinfish but also piscivores and benthic carnivores that may transition from invertebrate- to fish-based diets with increasing size (Smith, 1997; Ray et al., 1999; Murdy et al., 2013). QFASA prey fish were also homogenized whole, including stomach contents, as dolphins typically consume whole prey (Barros and Wells, 1998; Wells and Scott, 2017). However, this may have increased apparent FA similarity between predators

and prey, if predator stomachs contained prey. Some species also had small sample sizes (as few as n = 3) and were collected across seasons, which may limit representation of seasonal, ontogenetic, and sex-based variation in FA and SI profiles related to nutritional status, reproductive cycles, or environmental conditions (Iverson et al., 2002; Budge et al., 2002; Sweeting et al., 2007; Lane et al., 2011; Leaf et al., 2018). Finally, because different individuals were used in the FA and SI libraries due to sample availability, unavoidable differences in the size, diet, or physiological state of sampled fish may have contributed to some of the discrepancies between methods.

Overall, the QFASA and SI prey libraries shared broad trophic categories and exhibited structurally similar patterns of prey overlap, but patterns in prey distinctiveness also reflected methodological differences. Both methods identified planktivore, piscivore, and mixed seagrass-associated carnivore, omnivore, and detritovore prey groups, and species clustering in SI space often corresponded with associations in FA space. Lower distinctiveness in some prey species was also largely consistent with trophic similarity, predator-prey overlaps, habitat similarity, and sampling limitations. Despite this, QFASA overall provided finer species-level resolution than SI analyses when FA divergence was sufficient. This was particularly apparent within groups merged by SI analyses such as Clusters 2 and 4. Conversely, SI analyses provided more robust separation across trophic levels, better distinguishing predator and prey species in some cases were FA overlap occurred due to likely trophic transfer. This difference is reflective of the number and type of dietary tracers (i.e., different FAs or SIs) underlying each method (Nielsen et al.,

2018). My QFASA analyses involved 23 FAs, allowing up to 22 separate prey species in the library, but requires that their FA profiles be distinct enough to do so. In, comparison, SI analyses involved just two SIs (^{13}C and ^{15}N), automatically requiring prey to be clustered. However, the nitrogen stable isotope allowed us to better distinguish between prey feeding at different trophic levels, avoiding the predator-prey confounding observed in the FA analyses. Thus, taken together, species with lower FA distinctiveness warrant careful interpretation when estimates are considered at the species-level, and SI cluster assignments inherently represent broader functional groups, lending some uncertainty to the resulting diet estimates. Nevertheless, convergence between QFASA and SI prey patterns supports inference of dolphin diet at trophic and functional group levels within the SB prey base.

4.4.2 QFASA model performance: PBP values and augmented FA contribution

I also considered two additional QFASA model performance metrics which were not applicable to the SI analyses: predator-beyond-prey (PBP) values and the contribution of the augmented FA. This was addressed extensively in Chapter 2. Briefly, augmented FA contributions demonstrated that the dietary FA suite yielded more biologically viable estimates than the extended dietary suite. After excluding estimates generated using the extended dietary suite, however, no single configuration of the remaining model variables – distance measure, prey library, or blubber layer – consistently outperformed the others. While the results did suggest inner blubber overall provided better overlap between predator and prey signatures than outer

blubber, the difference was not enough to entirely exclude outer blubber estimates from consideration. Instead, I recommended that inner blubber estimates be prioritized, and that outer blubber only be used alongside inner estimates. As a result, this left multiple viable estimates for each dolphin, adding a meaningful amount of uncertainty to the QFASA results which was not present for the SI analyses.

4.4.3 Estimate uncertainty due to model parameter variation and standard errors

While model performance – captured by prey species resolution for both QFASA and SI analyses, and PBP values and augmented FA contribution for QFASA only – represents one metric of uncertainty assessed before interpreting the resulting diet estimates, uncertainty can also arise from variability within the estimates themselves.

The SI estimates were less subject to this form of uncertainty than the QFASA estimates. SI estimates had small SEs (<1%) and, at least in these analyses, were not subject to variation in model variables due to uncertainty in "best-fit" parameters. In comparison, as shown above (section **3.2.4)** and discussed in Chapter 2, QFASA-estimated prey contributions and their SEs varied with the different dolphins, prey species, and model variables, and SEs were sometimes large enough (up to 70%) that they changed the relative contribution of a given prey species to a dolphin's diet. Thus, I found that QFASA estimates of SB dolphin diet were subject to a meaningful degree of uncertainty at the prey species- and dolphin-specific level. However, I also

suggested that this uncertainty reflected the sensitivity of the diet estimates to realistic variation in prey and predator FA values, the analytical differences of the model variables, and similarities in prey FA signatures due to trophic overlap. In that way, different model configurations and higher SEs emphasize different components of the dolphins' FA signatures and the complexity of the underlying data. Furthermore, while prey species- and dolphin-level resolution was lower and associated with meaningful uncertainty, QFASA still provided broadly coherent trophic-level ecological patterns. Considering the distinctiveness of the different prey species and their trophic relationships allowed me to recognize that where variation occurred among QFASA model parameters or individual dolphins, it often involved redistribution among trophically-related prey. This meant that shared prey types and trophic groupings repeatedly emerged. Accordingly, I proposed that while exact species proportions are sensitive to methodological decisions, dominant prey species and categories that re-appear across model parameter combinations, especially when combined with higher prey distinctiveness, and relative demographic differences are robust for interpretation.

4.4.4 Overall conclusions on QFASA and SI diet estimate uncertainty

The preceding sections illustrate that both QFASA and SI estimates have associated uncertainty, though its degree and structure differ between approaches. Importantly, however, the uncertainties of each method do not make them fundamentally unreliable. Rather, I propose that each method captures different

dimensions of diet that can be seen as complementary trade-offs rather than competing weaknesses. QFASA can resolve prey at finer taxonomic scales when FA signatures are distinct but is sensitive to FA similarity and model structure. However, different model configurations emphasize different components of the dolphins' FA signatures and elucidate how realistic variation in prey FA means and predator-prey overlap impact the diet estimates. In comparison, uncertainty around SI analyses arose from decreased prey distinctiveness at the species level due to clustering, itself due to the limited number of dietary tracers available, rather than variation in parameter choice or large SEs. Thus, SI estimates are lower resolution than QFASA estimates but more stable.

Critically, each method also identified ecological patterns that were trophically coherent and consistent within and between methods. Both QFASA and SI analyses classified prey into the same broad groupings, including clupeids, piscivores, and mixed carnivore-omnivore-detritovore groups. Furthermore, where variation occurred among QFASA model parameters or individual dolphins, it often involved redistribution among trophically-related prey, so that shared prey types and trophic groupings repeatedly emerged. Thus, both methods provide higher-level information about key prey species and/or trophic categories that are valid for interpretation and comparison between diet determination methods.

Taken together, these analyses suggest that QFASA and SI estimates should be considered complementary sources of information and used together when possible, providing a more complete understanding of diet. In this way, uncertainty is not

concealed as a limitation but a guide for rigorous study design and more nuanced, if also more complicated, interpretation.

4.5 Comparing QFASA and SI diet estimates between dolphins and demographic groups

On an individual dolphin level, QFASA and SI estimates generally differed. This was true even when considering the QFASA prey species in broad trophic categories resembling the SI clusters and when using the "SI" library for QFASA estimates, demonstrating that the differences are not simply due to lower SI prey distinctiveness or different prey bases. It was also true no matter the blubber layer or distance measure used, meaning that I cannot use SI estimates to determine whether one of the different QFASA estimates for a given dolphin is more "accurate" than the others, at least in this system. A fundamental difference in the temporal windows integrated by the different methods could account for some of this variation. Stable isotope estimates of dolphin skin record a diet period of about two to four weeks before sampling, providing a view of average prey consumption over an intermediate temporal window (Browning et al., 2014). In comparison, QFASA estimates in dolphin blubber capture diet integrated over at least eight and up to 26 weeks before sampling according to the analyses in Chapter 1. Thus, the dolphins could have consumed diets that contained different relative proportions of prey in the medium term, as represented by SI, than how they appear integrated over the longer term, as represented by the QFASA estimates. Simultaneously, this difference could suggest

the dolphins are consuming different diets in the intermediate versus the longer term, whether due to seasonal differences or other environmental factors that impact prey availability. These implications will be discussed further below (section **4.7.2**).

Despite limited agreement on the individual dolphin level, however, both QFASA and SI estimates converged on several broader ecological patterns. SI estimates suggested most dolphin diets were dominated by the piscivore-associated Clusters 3 and 4 and clupeid-associated Cluster 1, while Cluster 2 – the mixed benthic carnivore-omnivore-detritivore group – made up about a quarter of estimated diet. Similarly, piscivorous common snook and the clupeid scaled sardine were top contributors in the inner blubber QFASA diet estimates, with mullet and gulf toadfish, part of Cluster 2, serving as secondary prey. Lower similarity between outer blubber QFASA estimates, where diet diversity and uncertainty were higher but clupeid contribution appeared to increase relative to other species, and the SI estimates could again be a result of temporal differences in integration period. As a result of structural differences between inner and outer blubber, it has been suggested that inner blubber may capture a shorter integrated diet period than outer blubber (Budge et al., 2006; Remili et al., 2022), though Chapter 1 analyses comparing temporal windows between dolphin blubber layers suggest they may reflect similar time frames. If these temporal differences do exist, however, inner blubber would capture estimates closer in temporal window to SI estimates, increasing the chance of estimate similarity. Demographic patterns were also subtle in both methods. SI estimates were overall comparable between demographic groups, though dependent

dolphins qualitatively had slightly greater variability. Inner blubber QFASA estimates using the Aitchison distance were also similar across all demographic groups, while inner blubber KL estimates and outer blubber estimates suggested dependent dolphins of both sexes and independent females may have more diverse diets. Finally, neither SI nor QFASA estimates provided conclusive evidence of greater similarity in mother-daughter diet relative to other unrelated dolphins. Together, these broad scale similarities support the idea that both methods are capturing true patterns in dolphin diet, but at different time scales and levels of prey resolution due to methodological differences.

4.6 Comparison to previous Sarasota Bay diet estimates and ecological implications of prey contribution patterns

4.6.1 Estimate consistencies across diet determination methods

Across the different methods, SB dolphins consistently exploited only a subset of available prey despite access to a diverse community of over 100 fish species under non-disturbed conditions. Of the 13 and 20 species in the SI and full libraries, QFASA estimates consistently identified just five species – common snook, scaled sardine, Atlantic thread herring, gulf toadfish, and mullet – in large or intermediate percentages across multiple dolphins and model configurations, while another seven – silver perch, spotted seatrout, ladyfish, croaker, spot, and mangrove and lane snapper – appeared only in some dolphins or model configurations. Furthermore, individual dolphins consumed only four to nine of these species. Identifying a comparable

pattern in the SI results is difficult, as cluster resolution does not allow me to distinguish whether certain species drove the otherwise relatively equal contribution of each cluster. However, previous prey selection, stomach content, DNA metabarcoding, and SI analyses showed similar patterns. These methods repeatedly identified that fish in the families Sparidae (including pinfish, sheepshead, and grass porgy) and Scianidae (including croaker, silver perch, spot, and spotted seatrout), piscivorous species (including common snook, spotted seatrout, and ladyfish), mullet, and soniferous (sound-producing) species – especially Gulf toadfish, croaker, and pigfish – made up the majority of dolphin diet (Barros and Wells, 1998; Berens McCabe et al., 2010; Barros et al., 2012; Wells et al. 2013; Dunshea et al., 2013; Rossman et al., 2015a). Together, these findings support the suggestion that SB dolphins are not "opportunistic generalists" feeding on any available prey but instead select certain species (Berens McCabe et al., 2010). Concurrently, previous SI analyses have suggested that dependent dolphins of both sexes and independent females may exploit broader prey bases as demographic groups but exhibit greater individual specialization, whereas males share more similar diet patterns (Rossman et al., 2015a, 2015b). This is consistent with my QFASA findings using inner blubber KL estimates and outer blubber, which suggested independent males may consume less diverse diets than other demographic groups. Thus, despite the different methodologies, all results suggest that dolphins exploit a more limited prey subset than is broadly available, with the possibility of further demographic specialization,

and focus on fish in the families Scianidae and Sparidae, certain piscivorous species, gulf toadfish, and mullet.

In addition, many of the species that were consistent diet components across methods could be considered soniferous, providing further support for the hypothesis that dolphins use passive listening as one technique for finding prey. Many SB fish are known to produce sounds by grinding pharyngeal teeth and/or vibrating their swim bladders, often increasing the frequency and intensity of calls during spawning periods (Barros and Wells, 1998; Berens McCabe et al., 2010). These include gulf toadfish, spotted seatrout, spot, pigfish, croaker, and silver perch. In addition, while neither common snook nor mullet are known to produce comparable internal sounds, both are known to produce distinctive external noises. This includes the "pop" made by common snook feeding at the surface and the distinctive splash of mullet which leap frequently from the water (Hoese, 1985; Zoffinger, 2015; Bruun, 2022). This would suggest large portions of many dolphin diets, based on stomach content, prey selection, and QFASA analyses, include prey that make regular and recognizable noises. In turn, large diet contributions by sound-producing fish support the hypothesis that SB dolphins use passive listening while foraging (i.e., finding prey using the sounds they naturally produce) (Barros and Odell, 1990; Barros and Wells, 1998; Gannon et al., 2005). This hypothesis arose in part because although dolphins have a sophisticated echolocation system, foraging click rates in SB are relatively low (Gannon et al., 2005; Jones and Sayigh, 2002). Echolocation incurs a cost due to a metabolic expense associated with click production or the risk of fish prey or shark

predators detecting the signals (Gannon et al., 2005). Visible prey detection may also be used, although the turbid waters of SB make this less likely, at least while actively searching for prey from a distance (Wells, 2019). Still, it is important to note that other frequently identified prey – notably pinfish and clupeids – are not known noise producers. This indicates that passive listening is just one of several prey detection methods; for instance, species like clupeids cluster in large schools, facilitating their detection, while others, like pinfish, are widely available throughout dolphin habitat.

Together, these consistencies suggest that SB dolphins preferentially exploit a subset of all available prey with shared ecological characteristics, even if the relative importance of individual prey species varies among methods.

4.6.2 Diet estimate divergence across methods

Along with the broad consistencies, however, several key divergences emerge when comparing the QFASA and SI results in this study to previous SB dolphin diet estimates.

First, this study's QFASA and SI estimates suggest greater reliance on clupeids and piscivores and reduced consumption of fish in the family Sparidae ("sparids") relative to previous estimates. The previous stomach content and DNA metabarcoding analyses discussed above, supported by field observations (Barros and Odell, 1990; Barros and Wells, 1998; Barros et al., 2012; Wells et al., 2013; Dunshea et al., 2013; SDRP, unpublished data) showed that SB dolphins across demographic groups primarily consumed sparids (especially pinfish, sheepshead, and grass porgy)

and gulf toadfish, while clupeids were infrequently present in diet. Consumption of piscivores and mullet was more variable, found in as few as 1.3% (Wells et al., 2013) and as high as 28% (Barros et al., 2012) of stomachs depending on the subset of dolphins considered. Previous SI estimates (Rossman et al., 2015a) similarly identified a cluster containing benthic carnivore, omnivore, and detritovore prey (including sparids, gulf toadfish, and mullet) as dominant diet contributors, while piscivores were minor contributors. The cluster containing clupeids did represent the second largest prey group in those analyses. However, this interpretation is complicated by the inclusion of non-seagrass pinfish within that cluster, making it difficult to disentangle whether the results indicate greater consumption of clupeids or a different kind of pinfish. While prey selection analyses on the stomach content data indicated selection against pinfish, it was still frequently found in stomach contents but was less common than expected based on its high abundance in the environment (Berens McCabe et al., 2010). Those analyses also found selection against clupeids, which were abundant in the environment but infrequently found in stomachs. Thus, previous SB diet estimates suggest reliance on sparids and gulf toadfish, while consumption of piscivores, mullet, and clupeids occurs but is generally an intermediate or minor portion of the diet. This differs from my SI and QFASA estimates. Most SI estimates included at least 50% piscivore- and clupeid-associated clusters, while QFASA estimates similarly identified common snook, scaled sardine, and Atlantic thread herring as major prey. In addition, none of the QFASA estimates showed any sparid species acting as significant or even minor diet contributors. My

results did not completely diverge from previous estimates: SI estimates for three of the 15 dolphins did have diets dominated by the mixed carnivore-omnivore-detritivore cluster that includes sparids and gulf toadfish, while that same cluster made up about a quarter of the rest of the dolphins' diets. Similarly, gulf toadfish was of secondary importance in many QFASA estimates, and sciaenids like silver perch, croaker, and spotted seatrout did appear in some diet estimates. Thus, although gulf toadfish, mullet, and some Sciaenid species remain important diet components, the results overall suggest a shift away from sparid-dominated diets and increased reliance on piscivorous and clupeid prey, at least during the first few months of 2019 represented by the blubber samples analyzed for this study.

Demographic patterns in my results were also weaker and less consistent than previously reported. Previous SI analyses (Rossman et al., 2015a, 2015b) found separation among demographic groups, with dependent dolphins feeding at lower trophic levels than independents and consuming somewhat larger amounts of the cluster containing clupeids and non-seagrass associated pinfish. Males were also found to consume larger quantities of the cluster containing mixed carnivore-omnivore-detritivore species, while females were suggested to have more varied diets as a group. As discussed, certain model configurations of my QFASA results also suggested greater diversity in female and independent diet. However, both the QFASA and SI estimates identified similar consumption of piscivores and clupeids in all groups, differing from previous findings of trophic separation. Inner blubber Aitchison estimates and SI estimates also suggested overall similar relative prey

proportions across groups, suggesting minimal demographic variation. Thus, while some demographic structure may exist in these new estimates, it was less definitive than in previous studies.

4.7 Drivers of diet estimate differences between methods

4.7.1 Methodological drivers

Methodological differences likely contribute to some of the differences between my findings and previous studies. First, the methods capture different diet consumption time frames. While hard part and DNA metabarcoding capture recent feeding in the hours to days before sampling and provide a single "snapshot" of diet, skin stable isotopes integrate diet over two to four weeks before sampling and QFASA reflects diet integrated over weeks to months (eight to 26 weeks before sampling) (Pierce and Boyle, 1991; Budge et al., 2006; Dunshea, 2009; Dunshea et al., 2013; Browning et al., 2014a; Chapter 1). Consequently, differences among methods could arise because dolphins consume different prey over time or because a single meal does not represent average consumption. Each method also has distinct biases in prey identification and resolution. As discussed, SI and QFASA estimates are particularly limited by prey distinctiveness – for SIs, isotopic overlap among prey always requires a degree of clustering, while for FAs, similarity in FA signatures can reduce resolution. In comparison, hard part and DNA metabarcoding analyses require sufficient samples (whether hard parts like bones or genome sequences) and appropriate reference material to identify the samples (Nielsen et al. 2018). There is

also some uncertainty as to whether samples are truly representative of diet or biased by differential digestion of hard parts or sampling of stranded animals who, if death was not acute from sources such as severe injury or asphyxiation, may have been ill or disabled and thus not representative of a healthy animal's regular diet (Bowen, 2000; Nielsen et al., 2018; Pierce and Boyle, 1991; Tollit et al., 2007). Thus, while hard part and DNA metabarcoding may identify prey at a finer resolution than FA or SI analyses, biases in the presence and identification of those species introduce new sources of uncertainty. Finally, previous SI estimates were made with a prey base that is broadly comparable but not identical to the one in this study – namely, the previous study included non-seagrass associated pinfish, crevalle jack (*Caranx hippos*), and red drum (*Sciaenops ocellatus*), which occurred in the clusters equivalent with Clusters 1 (clupeids), 3 (spotted seatrout), and 2 (mixed carnivores, omnivores, and detritivores), respectively. These three species were not available for the QFASA library, meaning my comparisons to previous SI estimates are not directly comparable. Taken together, these methodological differences mean some disagreement among methods is likely, even if they all accurately represent dolphin diet.

Sampling biases and differences in data presentation introduce additional sources of estimate variation. Small sample sizes, lack of re-sampling of the same dolphins, and data presentation methods mean that all comparisons of old and new results are qualitative. Previous studies and this study's analyses included 13 – 18 dolphins per study, a sample size that is representative but not fully inclusive of the

approximately 170 individual dolphins in the SB community, in which different dolphins may emphasize different habitats, with different prey availability, in their core area usage (Wells and McHugh, 2025). All hard part analyses were conducted using stranded dolphins, removing the possibility of direct comparison to the live animals sampled for DNA metabarcoding and this study. While live dolphins were used for metabarcoding and SI analyses, only one dolphin (F238) was identified in both the metabarcoding analyses (Dunshea et al., 2013) and this study. As dolphin-specific results were not presented in that study, however, we could not directly compare the estimates. Similarly, many stomach content and metabarcoding analyses report the overall frequency of consumption of a given species across the sampled population, rather than the proportional frequency of prey found in each sample (stomach, fecal, etc.). While often methodologically necessary, this means that prey that are consumed widely but in smaller relative amounts could theoretically be given more weight than prey that are consumed in high quantities but less frequently. Finally, sample timing presents another potential source of variation. While my estimates were made using dolphins sampled in June 2019 only, metabarcoding analyses were based on dolphins sampled in June 2005 and 2006 and stomach content and SI analyses involved dolphins sampled across multiple seasons and over the course of several decades. It is likely for diet to vary by season and over the course of time due to both natural variation, disturbances, and environmental changes, as will be discussed below (see section **4.7.2**). Thus, as before, these sampling differences

mean that accurate results from different methods could nonetheless differ in their estimated prey contributions.

Despite these methodological differences, however, historical studies have converged on a relatively consistent picture of SB diet and suggest some of these sources of variation are more impactful than others. The sparid- and gulf toadfish-dominated diet identified by the stomach content and DNA metabarcoding studies is consistent, even though those samples were taken over the course of about 22 years, in multiple seasons, and from both stranded and live animals (Dunshea et al., 2013). The diet pattern is also consistent with previous SI analyses (Rossman et al., 2015a), which included skin samples from 42 live dolphins which were also collected over multiple years (the exact time frame is not given in the paper). Furthermore, as both the metabarcoding samples and the blubber and skin for this study's new analyses were collected in June of a single year, this also diminishes the likelihood that sampling during different seasons would lead to differences – if the sparid-dominated diet pattern is consistent across seasons, I would expect to find it in my new analyses as well. Together, these previous results suggest that the identified sparid-dominated diet pattern remains consistent across short and intermediate temporal windows, live and stranded animals, multiple seasons and decades, and multiple diet determination methods with their specific biases.

This relative consistency in previous estimates, despite the methodological differences, suggest those differences alone are unlikely to fully explain the shift observed in this study's QFASA and SI estimates relative to these historical studies.

Concurrently, the modest sample size of this study could contribute to diet estimate differences. However, it is comparable to those of the earlier studies that nonetheless produced estimates shown to be representative of the wider community. Thus, while methodological and sampling effects undoubtedly contribute to some of the observed differences in prey composition between previous studies and this one, they are unlikely to fully account for them. This suggests that other environmental or ecological factors could be driving the differences.

4.7.2 Ecological and environmental drivers

4.7.2.1 Demographic differences

Multiple interacting factors could explain the greater diet similarity between dependent and independent dolphins observed in this study.

Limitations in dependent dolphin gape size and foraging skills are ecological explanations for previous SI analyses suggesting dependents feed on lower trophic level prey and clupeids (Rossman et al., 2015a, 2015b). However, dependents may simply consume smaller individuals of the same prey species as independents, producing similar FA profiles. This interpretation is consistent with prey sampling in this study, which focused on fish in the 100 – 300 mm size range. As a result, FA signatures for species like common snook, that can grow quite large, may represent smaller size classes accessible to dependents and independents alike.

Continued milk consumption and/or shared dietary patterns transmitted through social learning could also theoretically cause dependent dolphin profiles to more

closely resemble independents. The dependent dolphins in this study were 2 – 4 years old and were likely weaned or approaching nutritional weaning and catching their own prey fish. Nonetheless, SB dolphins remain with their mothers for three to six years, and while calves transition from milk to fish during their first one to two years of life, low levels of nursing may continue until calves permanently separate from their mother (Wells, 2014, 2003; Wells et al., 2025). This could explain SI estimate similarity, as previous SI analyses found elevated δ15N values in early life consistent with trophic enrichment from milk, followed by gradual declines during weaning until two years old (Rossman et al., 2015b). However, whether dolphin milk directly reflects the mother's dietary FA profile is unclear. While studies in pinnipeds have shown correspondence between pup blubber, maternal diet, and milk FA composition (Iverson, 1993; Iverson et al., 1995, 1997a; Baylis et al., 2009), studies in beluga whales did not find direct resemblances between calf blubber and milk composition (Birkeland et al., 2005). The only analysis of dolphin milk found dominant FAs corresponded with prey (Martini et al., 2022), but how directly this signal is incorporated into offspring blubber has not been studied. The extended period of mother-offspring association may also allow for social transmission / learning of foraging strategies (Weiss, 2006), causing dependent dolphins to consume similar diets to their mothers. However, while mother-daughter pairs had similar SI values and shared some QFASA-estimated prey contributions these similarities were not stronger or more consistent than those observed among unrelated individuals. Taken together, my findings suggest that while prey size differences, milk consumption, and

social learning may contribute to some portion of the observed similarities between age classes, none are dominant drivers.

4.7.2.2 Harmful algal bloom disturbances may cause dietary differences

Large-scale differences in dolphin diet estimates across methods and blubber layers may be partially explained by changes in prey availability associated with harmful algal blooms (HABs). An extended HAB occurred in SB between June 2018 and February 2019, ending approximately four months before the collection of blubber and skin samples used in this study (Berens McCabe et al., 2021). Prey diversity and catch rates for several potential prey species – especially pigfish and pinfish but also mullet, spotted seatrout, and gulf toadfish to lesser extents – decreased, while clupeid abundances increased (Berens McCabe et al., 2021). Passive acoustic monitoring also recorded decreased underwater sound during that period, aligning with reductions in soniferous fish abundances (Rycyk et al., 2020). Previous severe HABs in 2005 and 2006 were accompanied by similar changes in prey abundance and by dolphin behavioral changes – including increased foraging effort, grouping feeding, and use of open-water habitats – consistent with decreases in traditional prey and likely increased clupeid consumption (Gannon et al., 2009; Flaherty and Landsberg, 2011; McHugh et al., 2011; Berens McCabe et al., 2021). HAB impacts on prey fish numbers may last up to a year after the end of the event, depending on the timing and magnitude of juvenile fish recruitment, and differences in recruitment timing means species recovery rates may differ, further impacting prey

availability (Berens McCabe et al., 2021; Gannon et al., 2009). Furthermore, HAB impacts on dolphins may last longer than suggested by pure abundance numbers, as it takes time for recruits to reach the sizes typically consumed by dolphins. Despite these significant effects on prey fish, however, it is important to note that HAB impacts were not uniform across species. For instance, Berens McCabe et al. (2021) did not measure whether common snook, silver perch, or croaker were impacted, Gannon et al. (2009) did not record decreases in the trophic guild containing mullet, and a study in Tampa Bay, adjacent to SB, during the 2005/2006 HABs did not find significant changes in adult or juvenile common snook (Flaherty and Landsberg, 2011).

Many of the patterns observed in this study's estimates align with these HAB impacts on prey availability, especially when considering the different diet integration periods captured by QFASA and SI estimates. Integration periods of eight to 26 weeks and two to four weeks before sampling mean both methods would overlap with HAB impacts that continue through the end of the HAB and/or into the year following the end of the HAB in February 2019 but may capture different prey recovery patterns. Decreases in the contributions of pinfish, other sparids, and Cluster 2 in the QFASA and SI estimates relative to previous studies could reflect the significant decreases in their abundance and/or availability of appropriate size classes in SB. In turn, higher diet contributions by clupeids and piscivores and lower δ13C in the dolphins are consistent with greater use of non-seagrass habitats, including open bays, where clupeids are frequently found, and mangroves, where adult common

snook may live (Smith, 1997; Ray et al., 1999; Murdy et al., 2013; Rossman et al., 2015a). Higher contributions may also reflect continued or increased availability of these species during the HAB or shortly after. For instance, it has been suggested that common snook may seek refuge in lower-salinity estuarine, creek, and mangrove habitats areas during HABs, while juvenile snook typically already live in these habitats; this could allow common snook to recover more quickly than other species (Flaherty and Landsberg, 2011; Lowerre-Barbieri et al., 2014). Concurrently, continued consumption of some HAB-impacted species (e.g., croaker, silver perch, spotted seatrout, and gulf toadfish) could reflect increased foraging effort, exploitation of remaining available patches of those species, and/or further use of alternative feeding habitats. The higher SEs of QFASA estimates, particularly in outer blubber, could also be linked to increased diet variability over this time frame, as dolphins respond to prey abundance changes.

Considering the different diet integration periods captured by the sample types adds further support for the connection between diet estimate differences and HAB impacts. Analyses in Chapter 1 suggested inner and outer blubber may capture similar temporal windows in dolphins under professional care. However, differences in the diet estimates in this study, along with the known stratification of cetacean blubber, suggest they could integrate different diet time periods in this free-ranging dolphin application. If so, the observed pattern of higher clupeid contributions in outer blubber estimates relative to inner blubber would be consistent with the strongest HAB effects occurring in the diet estimates that capture time furthest from sampling,

i.e., nearest to the end of the HAB. Similarly, lower relative clupeid contributions and increased contributions of Cluster 2 in my SI estimates would be consistent with that method capturing the most recent time period, i.e., furthest from the end of the HAB, as prey species returned to SB or recovery was beginning to occur. Together, dietary differences across the varied time frames captured by the blubber layers and skin could suggest the beginning of a return to "normal" diet patterns as represented by previous studies.

Finally, it is important to note that the previous DNA metabarcoding analyses used samples collected within one year of HAB conditions but showed neither clupeid dominance nor diminished sparid contribution (Dunshea et al., 2013). However, these samples were collected more than six months after the end of a severe HAB or following a winter HAB. Together, a combination of prey recovery, the fact that winter HABs are thought to have less significant prey impacts than summer blooms (Berens McCabe et al., 2021), and the short diet period captured by metabarcoding analyses could explain why these analyses did not show diet shifts. Thus, overall, many of the patterns in prey contributions, SEs, and SI values across my QFASA and SI analyses could be the result of HAB-induced prey availability disturbances.

4.7.2.3 Dietary differences may also reflect long-term ecosystem changes

While differences between this study's SI and QFASA estimates and previous studies may largely be explained by HAB impacts, longer-term ecosystem changes in

SB could also contribute to the patterns. Previous stomach content analyses (Barros et al., 2012) have identified that following a ban on large-scale gill-net fishing in SB instituted in 1995 – 1996, dolphin diets became more diverse and included greater consumption of gulf toadfish, spotted seatrout, and sheepshead – all major bycatch species of the gill-nets – along with common snook, while pinfish remained important but decreased in frequency relative to the pre-ban period. Concurrent SI analyses and behavioral observations also suggested increased use of non-seagrass and open-bay habitats (Barros et al., 2012; Rossman et al., 2013; Wells, 2003; Wells et al., 2013). These changes have been attributed to reduced competition with fisheries, increased availability of previously harvested species, and changes in seagrass coverage and recreational fishing and boating patterns following the ban. My QFASA and SI results are consistent with this pattern of change. Increased contributions of species such as silver perch, spotted seatrout, common snook, and gulf toadfish, and reduced importance of seagrass-associated prey like pinfish and pigfish, suggest continued diversification of diet. Simultaneously, increased clupeid and piscivore consumption and lower δ13C in the dolphins are again consistent with greater use of open bay and non-seagrass habitats. Finally, the possibility that dolphins are selecting against pinfish (Berens McCabe et al., 2010) also align with longer-term dietary change. Taken together, the similarities between this study's QFASA and SI estimates and the recorded post-net-ban changes suggest that some of the differences between new and previous diet estimates could represent a natural continuation of the changes first reported several decades ago.

5 Study limitations and future research

Several limitations must be considered alongside my results and suggest natural directions for future research. First and foremost, this study includes fifteen dolphins from a community of more than 170 individuals, sampled over two weeks following a severe HAB that changed the prey community, using two diet determination methods with inherent associated uncertainties. This necessarily introduces the possibility that my results, regarding both QFASA and SI model considerations and the observed dietary patterns, are not representative of the broader community feeding under "normal" conditions. Thus, increasing the number of sampled dolphins and collecting over a longer period and across seasons, during both HAB-impacted and non-disturbance conditions, would allow for greater insight into the timing, consistency, and drivers of the apparent diet change observed in this study's results. Re-sampling individuals would especially help disentangle short-term dietary variation from long-term ecological shifts. Adding additional diet determination methods would also strengthen these efforts by allowing for diet evaluation across different integration periods while diminishing the SI and QFASA uncertainties surrounding prey distinctiveness and multiple model configurations.

Improvements to both the QFASA and SI prey libraries could also increase prey distinctiveness, addressing one of the key uncertainties of both methods and providing higher-resolution diet estimates. Increasing species sample sizes, including greater collection across seasons, size classes, and habitats, could allow better accounting of within-species variability and improve the representation of FA and

isotopic space. With larger prey datasets, it may be possible to subdivide species into ecologically meaningful groups such as size class, collection season, or habitat, as in previous QFASA studies (e.g., Iverson et al., 2002; Budge et al., 2002). These improvements could create more distinctive species subsets, reducing misallocation and trophic overlap.

The imperfect comparison between the SI and QFASA prey libraries – due to different prey fish collection seasons and fewer available SI prey data – and the overall lower resolution of SI estimates are also key limitations of this study. First and foremost, measuring δ13C and δ15N values in the prey fish samples collected during 2020 – 2022 and using those for SI diet estimation would remove the question of whether underlying differences in the QFASA and SI prey libraries are contributing to differences in the resulting diet estimates and yield cleaner method comparisons. Incorporating additional isotopes, such as sulfur (δ34S), could also improve discrimination among prey species or predators, somewhat improving estimate resolution (Pinzone et al., 2019; Rossman et al., 2013; Teixeira et al., 2022). In addition, analyzing additional tissue types (e.g., blood, blubber) would provide access to the different diet integration periods that they capture (Newsome et al., 2010; Teixeira et al., 2022). This would allow for more direct comparisons with QFASA, DNA metabarcoding, and hard part analyses, while adding additional information about diet patterns over different time frames.

Finally, while validation of QFASA in cetaceans was not the focus of this chapter, expanding it remains a key priority. Additional studies with dolphins under

professional care could refine or expand our understanding of different CCs and prey library configurations and allow further evaluation of blubber layer dynamics. Increased application of QFASA in free-ranging cetaceans, both in the SB system and beyond, would also allow for continued exploration of methodological uncertainties and model parameter implications outside of controlled conditions.

6 Conclusion

This chapter represents the first direct comparison of different diet methods in the SB system, and the first new SB dolphin diet estimates in a decade. Through it, I highlight the complexity of studying diet, even in an extremely well-studied system, and the value of incorporating multiple diet determination methods. I showed that QFASA can yield high-resolution diet estimates but is sensitive to prey FA similarity and model parameter variation, while higher SEs and the poorer fit of outer blubber introduce some uncertainty to estimate interpretation. Concurrently, SI analyses offered robust trophic-level insights and lower relative uncertainty than QFASA. However, the necessary aggregation of prey into clusters yielded lower-resolution estimates and made it harder to explore variation and specialization at the prey species level. Given their differences and associated uncertainties, I propose that QFASA is best used alongside other diet determination methods, and that future studies should prioritize the use of multiple methods for a more complete, if potentially more complex, picture of cetacean diet.

Importantly, however, both methods produced estimates that were trophically consistent both within and between methods, allowing for broader ecological interpretation. Variation in QFASA estimates often involved redistribution among trophically-similar species, SI clusters were trophically coherent, and both FA and SI analyses recovered largely the same trophic relationships between species. As a result, while exact species-level proportions could not be resolved with certainty, broad patterns were consistent across methods and model configurations. During the limited and atypical period represented by the dolphin samples, dolphins predominantly consumed piscivores and clupeids, with recurring secondary contributions from species such as mullet and Gulf toadfish, and I found evidence for both shared and individual-level variation in diet. When interpreted alongside previous diet studies, my findings suggest retained diet patterns: dolphins selected a similar subset of available prey, including soniferous species, even if their relative proportions varied across methods, and some degree of variation occurred among individuals and demographic groups. However, my findings also suggested possible shifts in prey species use over time, potentially reflecting a combination of methodological and sampling differences and true ecological changes in prey availability and habitat use. This presents a view of dolphin foraging that is focused on prey that share ecological characteristics but also shows that they are flexible and responsive to environmental conditions. Facing changing climate conditions and increasing anthropogenic disturbances, dolphins' apparent ability to respond to change will be critically important.

Tables – Chapter 3

Common name	Species	n	$\delta^{13}C$	$\delta^{15}N$	Prey cluster
Atlantic thread herring	*Opisthonema oglinum*	11	-15.6 ± 2.2	9.4 ± 1.4	1
Common snook	*Centropomus undecimalis*	3	-16.1 ± 0.7	11.1 ± 1	4
Gulf toadfish	*Opsanus beta*	14	-13.8 ± 11.6	8.7 ± 2.2	2
Ladyfish	*Elops saurus*	11	-17.5 ± 1.6	11.7 ± 1.1	4
Lane snapper	*Lutjanus synagris*	6	-14.2 ± 1.3	8.3 ± 0.9	2
Mojarra	*Gerreid* sp.	14	-13.6 ± 0.9	7.6 ± 0.6	2
Pigfish	*Orthropristis chrysoptera*	15	-14.2 ± 0.7	9.3 ± 1	2
Pinfish	*Lagodon rhomboides*	27	-13.7 ± 1.2	8 ± 1.3	2
Scaled sardine	*Harengula jaguana*	7	-17.5 ± 1.7	9.2 ± 0.4	1
Sheepshead	*Archosargus probatocephalus*	11	-14.7 ± 1.8	8.9 ± 0.9	2
Spot	*Leiostomus xanthurus*	20	-16.8 ± 2.2	10.4 ± 1.5	4
Spotted seatrout	*Cynoscion nebulosus*	26	-14.3 ± 1.8	11.5 ± 1.7	3
Mullet	*Mugil cephalus*	12	-12.4 ± 12.1	10.5 ± 4.3	2

Table 1: Stable isotope values for prey species included in the SI dataset (mean ± standard deviation, SD), sample size, and cluster assignment in the $k = 4$ solution (see Results).

Figures – Chapter 3

Figure 1: Map of Sarasota Bay, Florida, USA, where sample collection occurred.

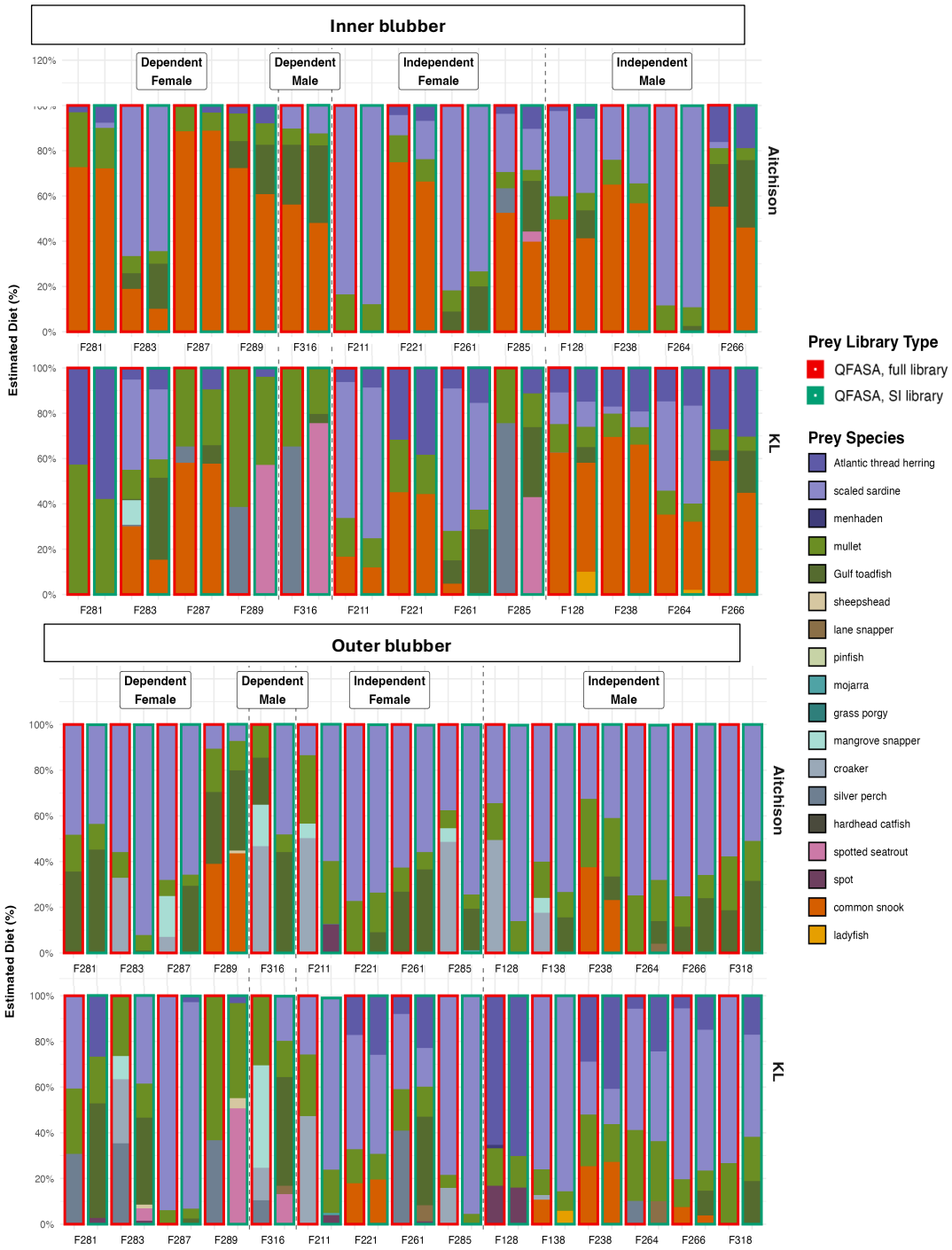

Figure 2: QFASA diet estimates for all dolphins and model configurations, separated by blubber layer. Dolphin IDs are indicated on the x-axis, the estimated diet contribution (%) is shown on the y-axis, Aitchison and KL estimates are shown in the top and bottom boxes, respectively, and the age class and sex of the dolphins are indicated by the labels and dashed line separators.

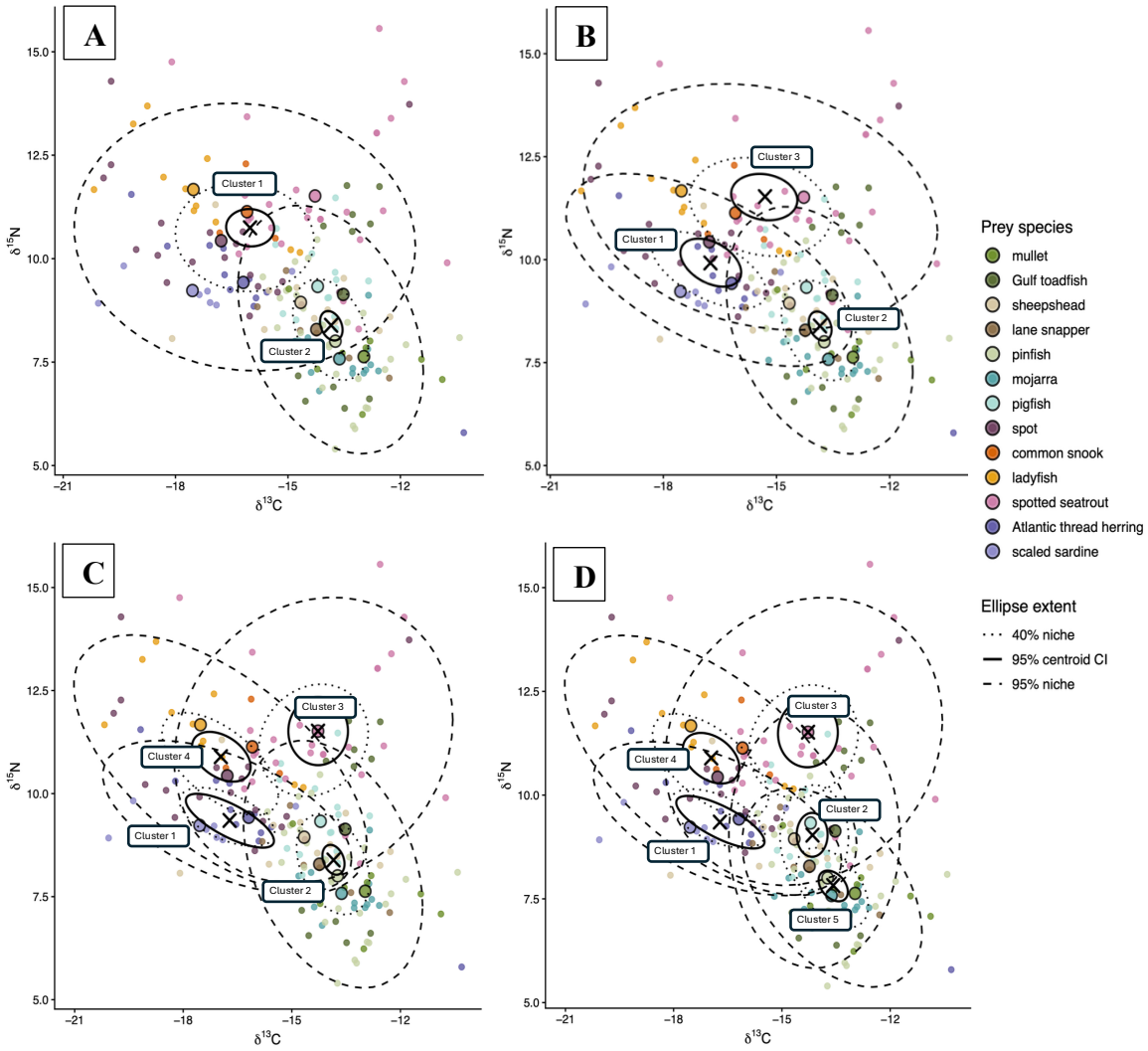

Figure 3: Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values for prey species included in the SI dataset and their cluster assignments, for all evaluated *k* values (2 – 5, A – D). Individual samples are shown as colored points without outlines, and species means are indicated by points with black outlines. Cluster centroids and their 95% confidence intervals (CIs), calculated using re-sampling, are indicated by the Xs and the solid ellipses, respectively, and represent uncertainty in centroid location. Dotted and dashed ellipses denote 40% and 95% prediction intervals (PIs), respectively, indicating the spread of individual observations within each cluster.

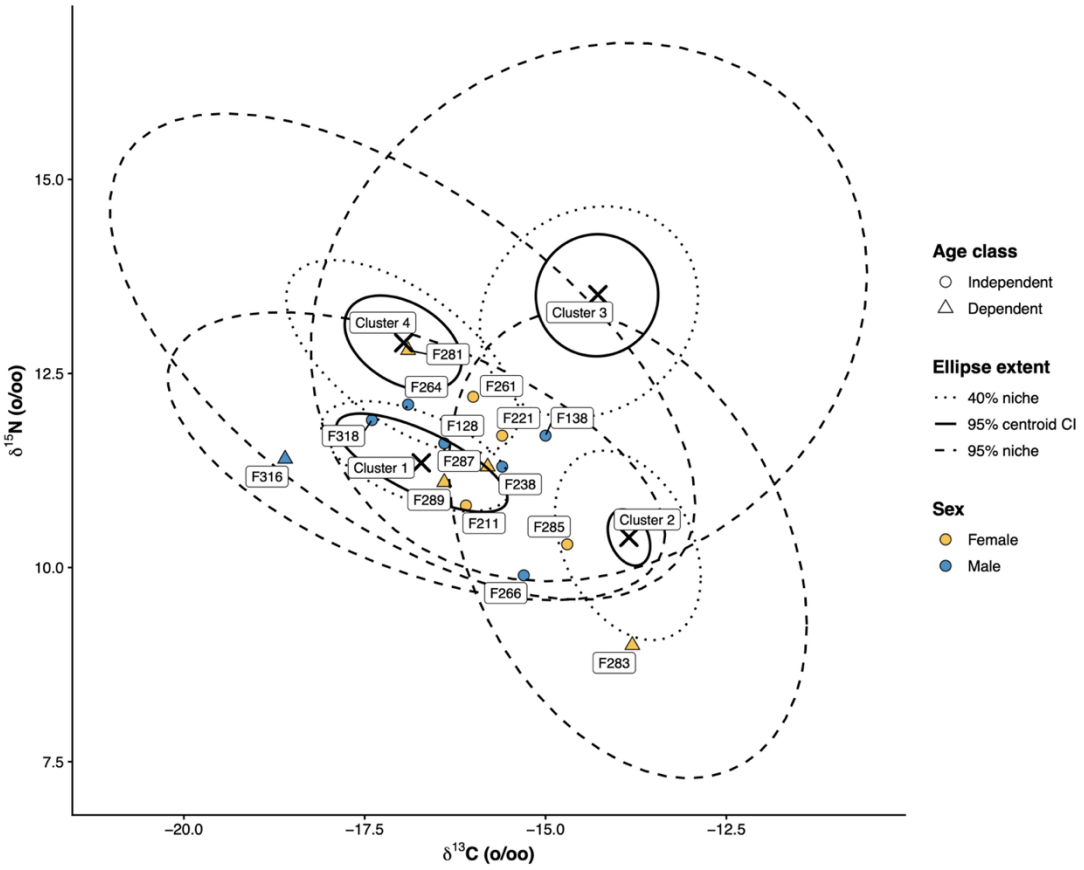

Figure 4: Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of prey clusters and dolphin skin from all individual dolphins (n = 15), including adult males (>6 years old), adult females (>6 years old), and male and female juveniles (2 – 6 years old). Prey clusters include the centroid and 95% CIs, along with the 40% and 90% PIs, to visualize cluster uncertainty and within-group variability. Prey clusters were adjusted for trophic enrichment (cluster average + trophic enrichment factor, TEF), using the TEF estimates derived in Rossman et al., 2015a and applied for SI diet estimation in this study.

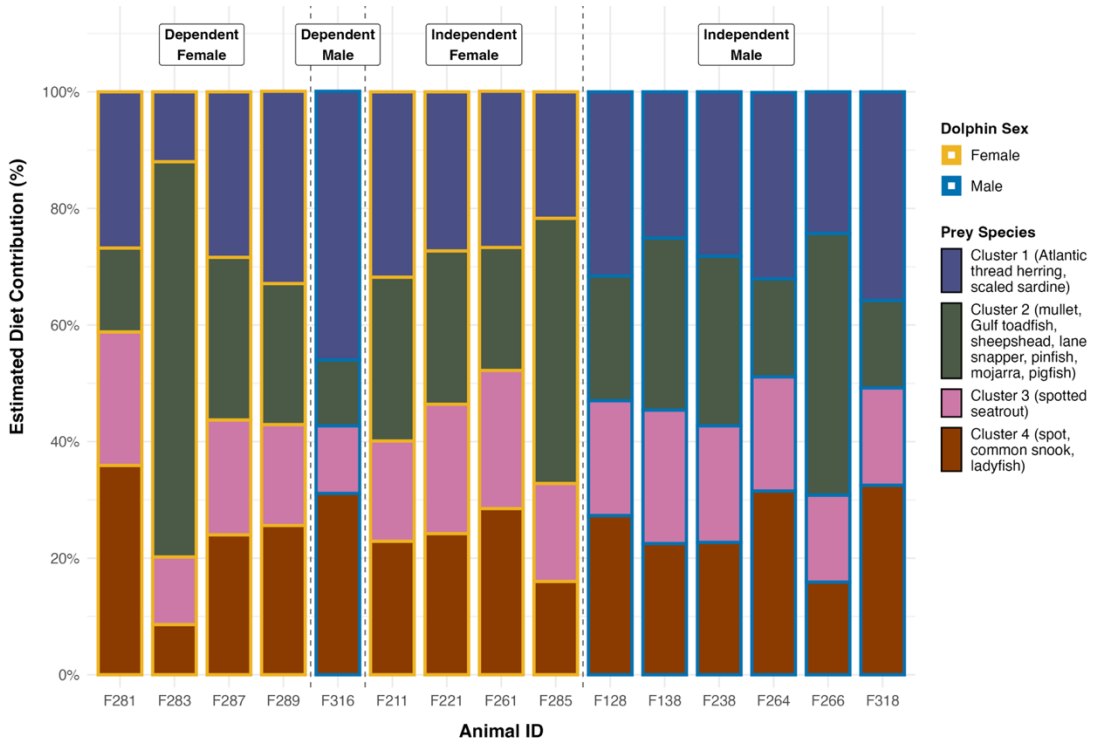

Figure 5: Stable isotope diet estimates for all SB dolphins, separated by dolphin ID. Dolphin IDs are indicated on the x-axis, the estimated diet contribution (%) is shown on the y-axis and the age class and sex of the dolphins are indicated by the labels and dashed line separators.

Synthesis

Quantifying marine mammal diet serves as a critical lens through which to study ecological, physiological, and conservation processes (Johnson et al., 2009; Bowen and Iverson, 2013; Slifka et al., 2013; Krützen et al., 2014; Nielsen et al., 2018; Ning et al., 2020; Plint et al., 2023; García-Vernet et al., 2024), driving continual refinement of existing diet determination methods and development of new approaches. QFASA is an approach with considerable potential, but methodological limitations have impeded its broader use, particularly in cetaceans. By combining applications professional-care and free-ranging dolphins, my dissertation advances the application of QFASA to cetaceans while also clarifying the method's limitations, uncertainties, and appropriate interpretation.

I started by applying QFASA in a controlled system in Chapter 1, using dolphins under professional care with known diets to explore whether QFASA was valid for use in dolphins. I derived the first set of dolphin-specific CCs, showed that the method accurately estimated diet, and provided recommendations on testing model performance and choosing model configurations for future applications. This study established the starting point for Chapters 2 and 3 and for others looking to apply QFASA in their own cetacean study systems.

In Chapter 2, I moved to free-ranging dolphins with more complex, unknown diets and a broader potential prey base to ask how well QFASA performed when applied in real conditions and whether different model configurations meaningfully impacted the resulting diet estimates. In this first-ever application of QFASA in free-

ranging dolphins, I demonstrated that QFASA estimates were sensitive to model configuration and prey species distinctiveness, introducing meaningful uncertainty into species-level diet estimates. However, despite variability at the prey species level, estimates were more consistent when considering broader trophic groups and QFASA model performance was acceptable, yielding a set of estimates that could be used for ecological interpretation. Through this study, I show that QFASA can be applied to estimate diet in free-ranging cetaceans if model performance is properly used to inform model configuration choices. I also argue that estimate uncertainty should be reported rather than minimized or concealed, as it reflects genuine variation in the data and provides important information about estimate reliability.

Finally, in Chapter 3, I compared the QFASA estimates generated in Chapter 2 to stable isotope (SI) analyses applied in the same dolphins and to previous diet estimates made in the same system using other methods, to compare the performance and results of multiple methods and explore the ability of QFASA estimates to address ecological research questions. I found that QFASA estimated diet at a finer prey species resolution than SIs but was associated with greater variability and uncertainty. However, both methods identified shared broad-scale dietary patterns which differed from historical studies, suggesting a combination of methodological and ecological drivers. Together, these comparisons demonstrated that QFASA is well-suited to ecological applications, but that integration with complementary diet determination methods provides a more complete understanding of cetacean diet.

While my dissertation removed key impediments to QFASA's broader use

and provided recommendations for future studies, it also highlighted areas where more research is needed. Additional validation studies in controlled systems, CCs derived from dolphins with even more complex known diets, and prey libraries with larger sample sizes for free-ranging applications would deepen our understanding of CC variation and how it impacts estimate accuracy and could improve prey distinctiveness, improving the method overall. Larger dolphin sample sizes to increase demographic and temporal diversity and applications in other study systems would expand the ecological applications of this method while exploring whether the sources of uncertainty identified in my dissertation are unique to the Sarasota Bay dolphins or a general QFASA characteristic.

Together, my dissertation chapters demonstrate that QFASA is a valuable tool for reconstructing cetacean diet, but its successful application depends on careful evaluation of model performance, informed model configuration selection, and transparent reporting of estimate uncertainty. At present, I recommend that QFASA be used alongside complementary diet determination methods rather than as a replacement for them. As additional validation studies and applications become available, QFASA can become an increasingly powerful approach to understanding cetacean foraging ecology and responses to environmental change. As natural and anthropogenic disturbances continue to impact marine food webs worldwide, improving our ability to quantify cetacean diets will be increasingly important for research and conservation of these critical top-predators.

List of supplemental files

TatomNaecker_dissertation_supplementary material.pdf

- PDF with all supplemental methods, tables, and figures for the entire dissertation

TatomNaecker_Chapter 1_coauthor permissions.pdf

- PDF with copies of emails from co-authors to Chapter 1 giving their permission for its inclusion in this dissertation

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