A library of fish fatty acid signatures for use in QFASA in bottlenose dolphins (*Tursiops truncatus*) in Sarasota, FL

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Introduction

- Detailed, long-term diet estimates are critical for evaluating cetacean vulnerability to disturbances that impact access to prey, such as harmful algal blooms, commercial and recreational fishing, and climate change
- Quantitative fatty acid signature analysis (QFASA) provides a months-long record of prey species proportions in predator diet by comparing predator fatty acid signatures (FASs) to a "library" of potential prey FASs[1], relying on the fact that FAs from prey are deposited in predators with minimal structural change
- If within-species prey FAS variation is less than between-species variation, individual prey species can be identified in predator diet
- QFASA has rarely been used in cetaceans, in part due to the challenges (e.g., time and cost of sample processing, uncertainty about prey species FAS distinctiveness) of developing a prey library

(1) Prey fish collection via purse seine net
(2+3) Prey fish homogenization
(4) Soxtec apparatus for lipid extraction

Methods

- Collected 2-5 individuals of each fish species caught via purse seine net fishing in Sarasota Bay, Florida during January through March and June through September 2020
- Homogenized 125 fish (28 species), extracted lipids from tissue aliquots with a Soxtec apparatus (Foss 2018), derivatized FA methyl esters, and quantified FASs (72 FAs) using gas chromatography with flame-ionization detection[1]
- Determined species diet type and habitat from literature[2,3]
- Used hierarchical clustering, PERMANOVA with post-hoc pairwise tests, and leave-one-prey-out analysis (LOPO, in R package qfasar[4]) to visualize and quantify within- and between-species FAS variation

Some but not all fish species in Sarasota Bay, Florida have **distinct fatty acid signatures** appropriate for determining bottlenose dolphin diet via quantitative fatty acid signature analysis (QFASA)

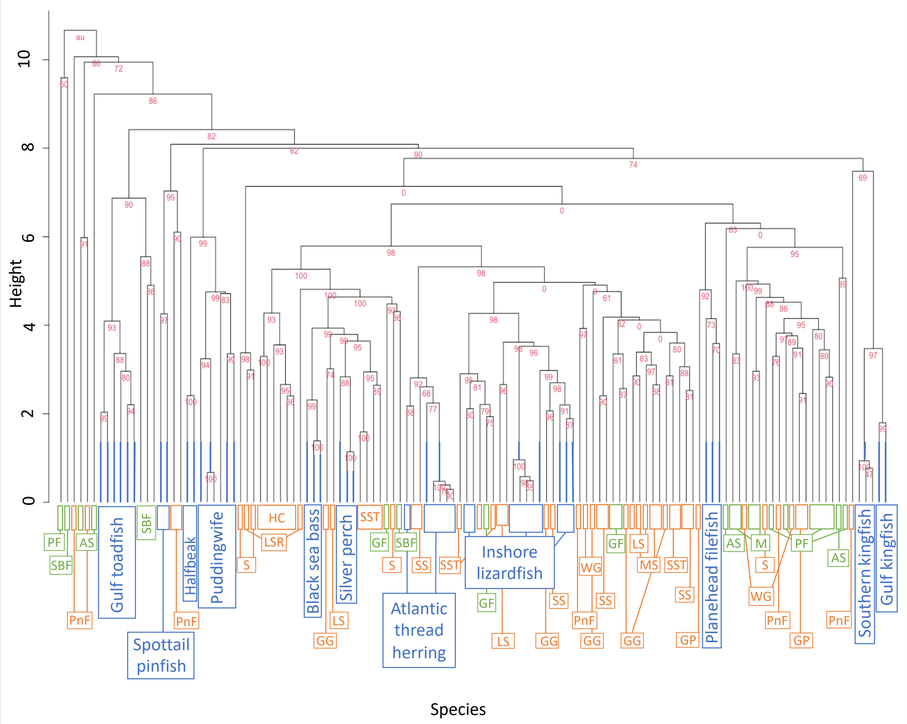

Figure 1. Hierarchical clustering of prey species, using "average" linkage method. Text colors indicate the FAS "similarity group" (see **Results**) of each species; species in **group A** had a high degree of clustering, low confounding, and significantly different FASs, while species in **group B** and **group C** had progressively less clustering, more confounding, and more similar FASs. p-values calculated by multiscale bootstrap resampling indicate likelihood of correct cluster relationship. Species abbreviations: AS (Atlantic spadefish), GF (Gulf flounder), GG (gag grouper), GP (grass porgy), HC (hardhead catfish), LS (lane snapper), LSR (leopard sea robin), M (mojarra), MS (mangrove snapper), PF (pigfish), PnF (pinfish), S (spot), SBF (striped burrfish), SS (scaled sardine), SST (spotted sea trout), and WG (white grunt).

Results and Implications

- 11 species showed distinct clusters and had a low degree of prey confounding (>70% of samples attributed to the correct species) and FASs that were largely significantly different ($p<0.05$) from other species (Fig. 1, **group A**)
- 6 species had multiple clusters, intermediate confounding (30 - 60% correct attribution), and significantly similar FASs to a small number of other species (Fig. 1, **group B**)
- 11 species lacked clear clustering, and had high confounding (<20% correct attribution) and significantly similar FASs to many other species (Fig. 1, **group C**)
- Shared diet types may explain FAS similarities between species, as fish FASs are influenced by prey consumption
  - The least distinct species (**group C**) were either invertebrate-specialists or generalists that consume many varieties of invertebrates and fish
  - Species in groups **A** and **B** with similar FASs also tended to have shared diet items
- Since fish were caught across several months, seasonal FAS variation—recorded in other fish species[5,6]—may contribute to within-species differences, decreasing species FAS distinctiveness

Next Steps

- By combining this prey FAS library with calibration coefficients calculated for managed bottlenose dolphins (in-hand), we will use QFASA to obtain a detailed, long-term view of diet variation and response to harmful algal bloom disturbances for free-ranging bottlenose dolphin demographic groups in Sarasota Bay, Florida and for those in the Gulf of Mexico who share similar prey items
- However, before the prey library can be used for diet estimates, species sample sizes will be increased (currently in progress), allowing for improved and additional analyses (e.g., discriminant function and CART analyses), and some prey species may require grouping by diet type or other factors if confounding remains high

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