RNA:DNA and total lipid measurements for laboratory-based experimental animals collected from the Gulf of Mexico Estuary near Port Aransas, Texas from 2020 to 2022

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Data Type: experimental
Version: 1
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Project
» Counter-gradient Flow of Fatty Acids in Marine Food Webs Through Egg Boons (Egg Boon Food Webs)

Contributors	Affiliation	Role
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Abstract
RNA:DNA and total lipid measurements for laboratory-based experimental animals collected from the Gulf of Mexico Estuary near Port Aransas, Texas from 2020 to 2022. Laboratory experiments took place at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute from July 2021 to November 2022.

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Coverage

Spatial Extent: **N**:27.8611 **E**:-97.0726 **S**:27.8035 **W**:-97.0898
Temporal Extent: 2020-07-01 - 2022-11-01

Dataset Description

These data were published in Nair et al. (2023).

Methods & Sampling

Mnemiopsis leidyi and juvenile Callinectes similis were collected in March – April 2022, and Beroe ovata was collected in November 2022, using a plankton net (50 cm diameter, 500 µm mesh) from Aransas Pass inlet at Port Aransas (27.8396° N, 97.0726° W). Palaemon pugio was collected using a plankton net from Corpus Christi Bay (27.8035° N, 97.0898° W) in Port Aransas in July 2021. Opisthonema oglinum and Lagodon rhomboides were collected using a seine (6.4 m wide by 1.2 m high with 5 mm square mesh) from Aransas

Pass inlet at Port Aransas (27.8396° N, 97.0726° W) in August 2021 and Redfish Bay at Aransas Pass (27.8611° N, 97.07632° W) in May 2022, respectively.

The live animals of each species were divided into two treatments (control and experimental). Both treatments were fed a common diet of either live Artemia sp. nauplii (enriched with Alga-Mac 3050; Aquafauna Bio-Marine, Inc.) or commercial fish food, Otohime (EP1, Reed Mariculture, Inc.) during the acclimation period of 10 – 45 days. After acclimation (Day 0), both treatments received a common diet of Artemia or Otohime, and the diet of experimental treatments was supplemented with red drum eggs for a period of 10 – 94 days. Controls did not receive eggs. Three to eight tanks of study species were sampled at the end of acclimation (day 0). Three to eight replicate tanks were sampled from each treatment 24 h and 2 – 10 days after the experimental treatment received eggs.

Mnemiopsis leidyi, B. ovata, and C. similis were held in rectangular tanks (26.7 cm long x 16.5 cm high x 16.5 cm wide), and P. pugio and fishes were held in circular tanks (12 cm in diameter, 6.4 cm deep) with recirculating filtered water. Within each rectangular tank, individuals of C. similis were held separately in round plastic containers (106.7 cm in diameter, 43.2 cm deep) with perforated lids to prevent aggressive contact. For the same reason, individuals of L. rhomboides were kept in separate perforated cylindrical enclosures (30 cm in diameter, 45 cm high) within each circular tank. Excess food and solid waste were siphoned daily from all tanks, and complete water changes were performed in rectangular tanks every 2 – 4 days. Environmental conditions were measured daily and were constant throughout the experiment (temperature: 21 – 24°C, salinity: 28 – 35 ppt, and photoperiod: 12-h light and 12-h dark).

Invertebrates removed from both treatments on sampling days were kept in clean sea water overnight to evacuate their guts and were sacrificed the following morning. For taxa with low dry weight, i.e., ctenophores, 3 – 4 individuals from each tank were pooled together to make a replicate. A single individual per tank of C. similis, and three individuals of P. pugio (subsamples, n=3) per tank were removed at each sampling day. Invertebrates were analyzed whole, except for C. similis, for which the exoskeleton was excluded. On each sampling day, one fish per tank was removed and immediately euthanized with tricaine methanesulfonate (MS-222). Euthanized fish were placed on ice where a fillet of dorsal white muscle tissue, liver and muscle plug were collected. All samples were rinsed twice in distilled water and frozen at -80°C until analysis.

For total lipids, each sample was lyophilized, homogenized, and weighed. Total lipid was measured by the phosphosulphovanillin method (Barnes and Blackstock, 1973). Briefly, lipids were cold extracted from lyophilized and homogenized samples with 2:1 chloroform: methanol (v/v). A calibration curve was prepared by performing 1:2 serial dilutions on a cholesterol standard (Millipore-Sigma, Burlington, MA, USA) dissolved in 2:1 chloroform:methanol (v/v). Blank, standards, and extracted lipid samples were reacted with concentrated sulphuric acid and vanillin (vanillin in 4:1 85% phosphoric acid: water v/v) and were run in duplicate. Absorbance was measured using a Spectramax 190 Microplate Reader (Molecular Devices, San Jose, CA, USA) at a wavelength of 520 nm. Total lipids were expressed as mg g−1 dry weight.

Measurements of RNA:DNA were made on individuals. DNA and RNA were measured using the ethidium bromide (EB) fluorometric technique (Westerman and Holt 1988) based on aliquots (10 µL) of homogenates. Calculations were based upon comparisons with DNA-EB and RNA-EB calibration curves from calf thymus DNA and yeast RNA (Type 111) standards. RNA:DNA ratios were normalized using a standardization factor based on the common RNA:DNA slope ratio procedure described by Caldarone et al. (2006). Results of the analysis were reported as the ratio of RNA content to DNA content. Measurements of RNA:DNA were unsuccessfully attempted for invertebrates.

Laboratory experiments took place at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute from July 2021 to November 2022.

Data Processing Description

The measured absorbance was subtracted from the blank (2:1 chloroform:methanol), and the calibration curve was used to calculate total lipids in mg g−1dry weight.

The microplate fluorometer is operated using SoftMax Pro version 5.4 by Molecular Devices. Raw data were exported to Microsoft Excel for further processing.

Quality control procedure:

Individual samples for which the means were greater than 3 standard deviations from the taxon mean were removed from the data set.

A primary check value was assigned as follows:
1 = Perfectly fine

BCO-DMO Processing Description

BCO-DMO Data Manager Processing Notes:
* Sheet 1 of file "Total lipids and RNA-DNA experimentals.xlsx" (submitted in our online submission system 2023-06-23) was imported into the BCO-DMO data system.
** Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.
* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]
* Taxon name and associated LSID for names in this dataset as of 2023-06-23 (source: World Register of Marine Species). Added this list to Methods and Sampling section.

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Data Files

File	
908155_v1_dna-rna_total-lipids.csv	(Comma Separated Values (.csv), 22.89 KB) MD5:01033304cdb4090c080923d2c65b9ae8
Primary data table for dataset 908155 version 1.	

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Supplemental Files

File	
Sampling information filename: sampling_info.csv	(Comma Separated Values (.csv), 814 bytes) MD5:bffa03b3eb5663faa995ed831f850446
Sampling information table with columns: Collected_organisms,collection_date,sampling_method,location,lat,lon	
Taxon identifiers filename: taxon_identifiers.csv	(Comma Separated Values (.csv), 379 bytes) MD5:531b8df1e794dcfc718d4dbc11b862dc
Taxon name and associated LSID for names in datasets 878635, 908155, and 908200 version 1 as of 2023-06-23 (source: World Register of Marine Species).	

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Related Publications

Barnes, H., & Blackstock, J. (1973). Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanilun method for ‘total’ lipids. Journal of Experimental Marine Biology and Ecology, 12(1), 103–118. https://doi.org/10.1016/0022-0981(73)90040-3
Methods

Caldarone, E. M., Clemmesen, C. M., Berdalet, E., Miller, T. J., Folkvord, A., Holt, G. J., Olivar, M. P., & Suthers, I. M. (2006). Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. Limnology and Oceanography: Methods, 4(5), 153–163. Portico. https://doi.org/10.4319/lom.2006.4.153
Methods

Molecular Devices, LLC (2012). SoftMax Pro version 5.4.

https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software
Software

Nair, P., Miller, C. M., & Fuiman, L. A. (2023). Tracing exploitation of egg boons: an experimental study using fatty acids and stable isotopes. Journal of Experimental Biology, 226(22). https://doi.org/10.1242/jeb.246247
Results

Westerman, M., & Holt, G. J. (1994). RNA:DNA ratio during the critical period and early larval growth of the red drum Sciaenops ocellatus. Marine Biology, 121(1), 1–9. https://doi.org/10.1007/bf00349468 https://doi.org/10.1007/BF00349468
Methods

Zöllner, N., & Kirsch, K. (1962). Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Zeitschrift Für Die Gesamte Experimentelle Medizin, 135(6), 545–561. https://doi.org/10.1007/bf02045455 https://doi.org/10.1007/BF02045455
Methods

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Parameters

Parameter	Description	Units
Taxon	Taxonomic grouping of sample	unitless
Tissue_sampled	Animal tissue sampled	unitless
Length	Total length of fish carapace length of crabs in centimeters	centimeters (cm)
Tank_number	Tank that animal was assigned to	unitless
Acclimation_days	Acclimation days. Acclimation period began soon after animals were collected from the wild. During acclimation animals were fed Artemia or Otohime	days
Days_after_acclimation	Days in control or experimental treatment after acclimation. End of Acclimation marked by Day 0	days
Treatment	Control or Experimental	unitless
Diet_fed	Diets provided to control and experimental treatment. Controls were fed Artemia/Otohime only. Diet of experimentals supplemented with red drum eggs	unitless
Notes	notes about sample	unitless
Primary_check	Primary QC check	unitless
Total_lipids	Total lipids	milligrams per gram of dry weight (mg g-1 dw)
RNA_DNA	Ratio of RNA to DNA in sample	unitless

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Instruments

Dataset-specific Instrument Name	Microplate fluorometer (Molecular Devices, model Gemini XPS)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Spectramax 190 Microplate Reader
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Project Information

Counter-gradient Flow of Fatty Acids in Marine Food Webs Through Egg Boons (Egg Boon Food Webs)

Coverage: Gulf of Mexico estuary at Port Aransas, Texas

NSF Award Abstract:

Marine animals release extremely large numbers of eggs when they spawn. Most of these eggs are eaten by animals ranging from microscopic plankton to fish. Many egg consumers are smaller than the animals that released the eggs, representing a reversal of the usual food web. The consumption of eggs provides animals with highly nutritious molecules called essential fatty acids which are very concentrated in eggs. These essential fatty acids are important for the health of animals and the health of the whole ecosystem. When marine fishes form spawning aggregations to coordinate the timing and location of spawning, they release trillions of eggs. This results in an "egg boon" an immense but temporary concentration of highly nutritious fatty acids. This project combines field-based sampling with laboratory experiments to assess how fatty acids in the egg boons affect food webs. The project is determining whether consumption of eggs is beneficial to the condition of the egg consumers. New findings from this project are advancing the understanding of aquatic food webs and contributing to improved management of marine resources. For example, commercial harvest of fish can remove tons of fatty acids from an ecosystem by reducing egg boons and leading to cascading and unforeseen effects on those biological communities. The project is fostering the participation of women in science by substantially advancing the professional training of a female

postdoctoral fellow. The project is supporting K-12 STEM education through inquiry-based and place-based programs for teachers and youth. Findings are being communicated to the public locally and nationally through participation in public lectures and contributions to the Science and the SeaTM radio program, podcast, and website.

Super-abundances of eggs released in temporally and spatially discrete patches create pulsed nutritional resources for egg consumers, called "egg boons", which are potentially important components of marine food webs. While various marine animals have been shown to consume eggs, the role of egg boons in energy transfer through food webs has received little attention. Three hypotheses are being tested: 1) egg boons provide a pathway through which essential fatty acids (EFAs) are redistributed counter to the main direction of trophic flow; 2) stores of EFAs in egg consumers increase during egg boons and remain elevated after the spawning season; and 3) egg boons are beneficial to the condition of egg consumers. The proposed research takes advantage of an annual egg boon produced by a spawning aggregation of the marine fish, red drum (Sciaenops ocellatus) near Port Aransas, Texas. In a combination of field sampling and laboratory experiments, fatty acid profiles, lipid content, and bulk stable isotope ratios are measures used to define trophic links between the egg boon and a selection of lower-trophic-level taxa. Egg boons are simulated in laboratory feeding experiments that are designed to enhance interpretation of data collected from field based sampling by comparing taxa that consume fish eggs with those that do not. A nucleic acid biomarker (RNA/DNA ratios) is being used to assess changes in condition that can be attributed to egg consumption in target taxa. In the environment, the importance and persistence of counter-gradient flow of fatty acids in the food web is being gauged through comparisons of samples taken inside and outside the spatial and temporal extent of the egg boon. The effects of egg consumption on consumers is being quantified in controlled experiments to identify dietary biomarkers of egg consumption in consumer tissues that can be applied to field samples. The proposed research examines how egg consumption redistributes EFAs within food webs and provides a context for considering potential controls and trophic bottlenecks that cannot be explained from the traditional element-limitation models. The integration of fatty acid and stable isotope approaches is expected to provide greater resolution for tracking organic matter through food webs and to advance the application of multi-tracer techniques in trophic investigations. Further, if egg boons are indeed an important nutritional subsidy to select groups of consumers, then subsequent studies investigating the energetic contribution of egg boons to secondary production in marine food webs are warranted. An analysis of how reduction or removal of egg resources through the harvest of fishes in spawning aggregations changes nutrient flow in food webs could have implications for ecosystem-based fisheries management.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2023618

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