Compound-specific isotope analysis of amino acids (CSIA-AA) from a subset of siphophore samples collected during four research cruises on the R/V Wester Flyer in the California Current Ecosystem between 2019 and 2021

Website: https://www.bco-dmo.org/dataset/917239
Data Type: Cruise Results
Version: 1
Version Date: 2023-12-19

Project
» Collaborative research: The effects of predator traits on the structure of oceanic food webs (SiphWeb)

Contributors	Affiliation	Role
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Abstract
Samples of siphonophores (Cnidaria, Hydrozoa) were collected using blue-water diving, midwater trawls, and remotely operated vehicles in the California Current Ecosystem, from 0 to 3,000 meters depth. Siphonophore samples were collected on four research cruises on the R/V Wester Flyer between 2019-2021. To remove potential biases associated with tissue-specific variability in stable isotope values, the gelatinous swimming bells (nectophores) of siphonophores were sampled. This approach was possible for most specimens, except for physonect species that are extremely fragile or have nectosomes that are a small fraction of the colony length and are often not collected. For these species (e.g., Apolemia spp.), the gelatinous bracts and pieces of the siphosome, excluding gastrozooids, were used. For small individuals (Diphyes dispar, Nanomia bijuga, and Sphaeronectes koellikeri), nectophores from several colonies that were captured at the same time and sampling location were pooled to obtain an adequate mass for isotope analyses. A subset of samples was selected for compound-specific isotope analysis of amino acids. These specific taxa were selected as representatives of different depth habitats, suborders, and hypothesized diets. Bulk and compound-specific isotope analyses were performed at the University of Hawaii's Biogeochemistry Stable Isotope Facility. This dataset includes the compound-specific isotope analysis data.

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Coverage

Spatial Extent: **N**:37.1967 **E**:-117.717 **S**:32.72 **W**:-125.038
Temporal Extent: 2019-03 - 2020-01

Methods & Sampling

Samples were collected in the central and southern California Current. Most samples were collected in the Monterey Bay region, but a subset of samples were collected in southern California in 2020 and 2021. Samples were collected from between 0 to 3,000 meters depth. Samples were collected on four cruises across three years. All cruises were on the R/V Western Flyer. Dr. Steven Haddock (haddock@mbari.org) was the Chief Scientist on all cruises. Cruises occurred in March 2019 (Cruise ID: WF0319), January 2020 (Cruise ID: WF0120), July 2020 (Cruise ID: WF0720), and July 2021 (Cruise ID: WF0721). Sample locations and dates are provided as columns in the data file.

Siphonophores were collected using three methods: (1) a remotely operated vehicle, (2) blue water diving, and (3) a midwater trawl.

(1) We used the Remotely Operated Vehicle (ROV) Doc Ricketts (https://www.mbari.org/technology/rov-doc-ricketts/) to collect siphonophores, which is an electro-hydraulic vehicle that operates between 200 and 4000 meters. The vehicle was fitted with high-definition video cameras, environmental data instrumentation (e.g. depth, temperature, salinity, and oxygen sensors), and suction and detritus samples to collect in-tact siphonophore specimens. ROV collections occurred during daylight hours.

(2) Siphonophores were collected by blue water diving between 0 and 20 meters during daylight hours. Blue water diving techniques followed the guidelines in the following publication: Haddock, Steven HD, and John N. Heine. "Scientific blue-water diving." (2005). From Haddock and Heine (2005): "In a typical blue-water dive, working divers are connected to a surface platform (and indirectly to each other) by tethers attached to a central hub, which is tended by a safety diver. This hub is connected to a down-line, providing a vertical point of reference. A surface float allows the divers to drift freely through the upper water-column, focusing on their work while the safety diver acts as a buddy for everyone."

(3) A Tucker Trawl with a frame area: 2 square meters (m^2), mesh size: 500 micrometers (µm) was towed obliquely for ~2 hours between 900 meters and the surface at night.

Upon collection, siphonophores were identified to the finest taxonomic level, which was either genus or species. For some genera, there are likely undescribed and/or cryptic species (e.g., *Apolemia*) and for these taxa, genera-level identifications were used. All siphonophores were rinsed with DI water and frozen at -80°C until further processing. Siphonophore tissues were weighed, lyophilized, packaged into tin capsules for bulk isotope analysis, and analyzed at the University of Hawaii's Isotope Geochemistry Facility.

For bulk stable isotope analysis, siphonophore samples were analyzed using a Costech (Valencia, CA, USA) elemental combustion system coupled to a Thermo-Finnigan Delta XP isotope ratio mass spectrometer with N2 standard for nitrogen and Vienna Pee Dee Belemnite for carbon. The bulk stable isotope data are available in a separate BCO-DMO dataset (see 'Related Datasets').

A subset of samples was selected for compound-specific isotope analysis of amino acids (CSIA-AA). CSIA-AA was also conducted at the University of Hawaii's Isotope Geochemistry Facility using acid hydrolysis followed by derivatization (see Popp et al. (2007) and Hannides et al. (2013) for details). Derivatives were analyzed using a Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS), interfaced with a Thermo Trace GC gas chromatograph via GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap. Samples were injected (split/splitless injector, splitless mode) with a 180°C injector temperature and a constant helium flow rate of 1.4 milliliters per minute (mL min-1). For quality control, we analyzed an amino acid suite, with known δ15N values of 14 amino acids, every 3-4 sample injections. Internal reference compounds, L-2-Aminoadipic acid and L-(+)-Norleucine of known nitrogen isotopic composition, were co-injected with samples and suites and used as a measure of accuracy and instrument precision. Samples for CSIA-AA are typically analyzed in triplicate runs. Our samples, however, required six runs to obtain peaks for all amino acids (AAs) due to the inordinate relative abundance of glycine compared to all other amino acids. It is unclear why glycine peaks were large, although we note that the relative abundances of the different AAs can vary depending on the taxa and tissue type observed. It is unknown whether this is common for siphonophores since there are no other published siphonophore CSIA studies. Glycine peaks were so large that the chromatography surrounding glycine was deleteriously affected when injecting volumes large enough to detect all AAs of interest. To overcome this, we analyzed samples in triplicate at injection volumes that allowed for good chromatography around glycine, and then again in triplicate at a larger injection volume to allow smaller AAs to be detected while back flushing the large glycine peak out of the chromatogram. We obtained well-defined peaks for 14 amino acids, which were grouped into standard 'trophic' and 'source' categories based on previous studies.

Related Resources:

Some of the siphonophores collected in this dataset were also used for metabarcoding. Those data are published Damian-Serrano, et al. (2022) (doi: 10.1371/journal.pone.0267761)

Illumina sequencing data files can be found in the NCBI BioProject PRJNA733192 (https://www.ncbi.nlm.nih.gov/bioproject/733192)

Prey 18S reference database enhancement sequences are available in NCBI (accession numbers between MZ333540 - MZ333629)

Other data, intermediary files, and all code can be found in the GitHub repository: https://github.com/dunnlab/siphweb_metabarcoding (see DOI: doi.org/10.1371/journal.pone.0267761)

BCO-DMO Processing Description

- Imported original file "Siphonophore CSIA-AA data (NSF Project Number 1829812).csv" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Added separate columns for Year and Month based on the Collection_Date column.
- Saved the final file as "917239_v1_siphonophore_csia-aa.csv".

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Data Files

File
917239_v1_siphonophore_csia-aa.csv (Comma Separated Values (.csv), 4.57 KB) MD5:5b40410153edd6c885b23ff2bdd9a1d4
Primary data file for dataset ID 917239, version 1

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Related Publications

Damian-Serrano, A., Hetherington, E. D., Choy, C. A., Haddock, S. H. D., Lapides, A., & Dunn, C. W. (2022). Characterizing the secret diets of siphonophores (Cnidaria: Hydrozoa) using DNA metabarcoding. PLOS ONE, 17(5), e0267761. https://doi.org/10.1371/journal.pone.0267761
Results

Haddock, S. H. D., Heine, J. N., United States. National Oceanic and Atmospheric Administration, California Sea Grant College Program, & National Sea Grant College Program (U.S.). (2005). Scientific blue-water diving. California Sea Grant College Program. https://isbnsearch.org/isbn/978-1-888691-13-9
Methods

Hannides, C. C. S., Popp, B. N., Choy, C. A., & Drazen, J. C. (2013). Midwater zooplankton and suspended particle dynamics in the North Pacific Subtropical Gyre: A stable isotope perspective. Limnology and Oceanography, 58(6), 1931–1946. doi:10.4319/lo.2013.58.6.1931
Methods

Hetherington, ED, Close, H, Haddock, SHD, Damian-Serrano, A, Dunn, CW, Wallsgrove, N, Doherty, S, and Choy, CA. Vertical trophic structure and niche partitioning of gelatinous predators in a pelagic food web: insights from stable isotopes of siphonophores. Under Review at Limnology and Oceanography.
Results

Popp, B. N., Graham, B. S., Olson, R. J., Hannides, C. C. S., Lott, M. J., López-Ibarra, G. A., … Fry, B. (2007). Insight into the Trophic Ecology of Yellowfin Tuna, Thunnus albacares, from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids. Terrestrial Ecology, 173–190. doi:10.1016/s1936-7961(07)01012-3
Methods

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Related Datasets

IsRelatedTo

Hetherington, E. D., Choy, C. A. (2024) **Bulk stable isotopes from siphonophores collected during four research cruises on the R/V Wester Flyer in the California Current Ecosystem between 2019 and 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-01-11 doi:10.26008/1912/bco-dmo.916958.1 [view at BCO-DMO]

Yale University. DNA Metabarcoding of Siphonophore Gut Contents. 2021/05. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA733192. NCBI:BioProject: PRJNA733192.

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Parameters

Parameter	Description	Units
Collection_Date	Month and year of the specimen collection	unitless
Year	Year of sample collection	unitless
Month	Month of sample collection	unitless
Genus	Taxonomic genus of specimen	unitless
Best_Taxonomic_ID	Best taxonomic identification of specimen, usually at the genus or species level	unitless
Alanine	mean d15N value (in parts per thousand) from multiple sample injections for alanine	parts per thousand
Glycine	mean d15N value (in parts per thousand) from multiple sample injections for glycine	parts per thousand
Threonine	mean d15N value (in parts per thousand) from multiple sample injections for threonine	parts per thousand
Serine	mean d15N value (in parts per thousand) from multiple sample injections for serine	parts per thousand
Valine	mean d15N value (in parts per thousand) from multiple sample injections for valine	parts per thousand

Leucine	mean d15N value (in parts per thousand) from multiple sample injections for leucine	parts per thousand
Isoleucine	mean d15N value (in parts per thousand) from multiple sample injections for isoleucine	parts per thousand
Proline	mean d15N value (in parts per thousand) from multiple sample injections for proline	parts per thousand
Aspartic_acid	mean d15N value (in parts per thousand) from multiple sample injections for aspartic acid	parts per thousand
Methionine	mean d15N value (in parts per thousand) from multiple sample injections for methionine	parts per thousand
Glutamic_acid	mean d15N value (in parts per thousand) from multiple sample injections for glutamic acid	parts per thousand
Phenylalanine	mean d15N value (in parts per thousand) from multiple sample injections for phenylalanine	parts per thousand
Tyrosine	mean d15N value (in parts per thousand) from multiple sample injections for tyrosine	parts per thousand
Lysine	mean d15N value (in parts per thousand) from multiple sample injections for lysine	parts per thousand
TP	Trophic position estimate, using the equation from Chikaraishi et al. 2009: (Glutamic acid-Phenylalanine-3.4)/7.6 + 1	unitless
Alanine_SD	standard deviation of d15N value (in parts per thousand) for alanine	parts per thousand
Glycine_SD	standard deviation of d15N value (in parts per thousand) for glycine	parts per thousand
Threonine_SD	standard deviation of d15N value (in parts per thousand) for threonine	parts per thousand
Serine_SD	standard deviation of d15N value (in parts per thousand) for serine	parts per thousand
Valine_SD	standard deviation of d15N value (in parts per thousand) for valine	parts per thousand
Leucine_SD	standard deviation of d15N value (in parts per thousand) for leucine	parts per thousand

Isoleucine_SD	standard deviation of d15N value (in parts per thousand) for isoleucine	parts per thousand
Proline_SD	standard deviation of d15N value (in parts per thousand) for proline	parts per thousand
Asparticacid_SD	standard deviation of d15N value (in parts per thousand) for aspartic acid	parts per thousand
Methionine_SD	standard deviation of d15N value (in parts per thousand) for methionine	parts per thousand
Glutamic_acid_SD	standard deviation of d15N value (in parts per thousand) for glutamic acid	parts per thousand
Phenylalanine_SD	standard deviation of d15N value (in parts per thousand) for phenylalanine	parts per thousand
Tyrosine_SD	standard deviation of d15N value (in parts per thousand) for tyrosine	parts per thousand
Lysine_SD	standard deviation of d15N value (in parts per thousand) for lysine	parts per thousand

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Instruments

Dataset-specific Instrument Name	combustion furnace
Generic Instrument Name	furnace
Dataset-specific Description	Derivatives were analyzed using a Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS), interfaced with a Thermo Trace GC gas chromatograph via GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap.
Generic Instrument Description	An enclosed chamber designed to produce heat.

Dataset-specific Instrument Name	reduction furnace
Generic Instrument Name	furnace
Dataset-specific Description	Derivatives were analyzed using a Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS), interfaced with a Thermo Trace GC gas chromatograph via GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap.
Generic Instrument Description	An enclosed chamber designed to produce heat.

Dataset-specific Instrument Name	Thermo Trace GC gas chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Derivatives were analyzed using a Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS), interfaced with a Thermo Trace GC gas chromatograph via GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	CSIA-AA was also conducted at the University of Hawaii's Isotope Geochemistry Facility using acid hydrolysis followed by derivatization (see Popp et al. [2007] and Hannides et al. [2013] for details). Derivatives were analyzed using a Thermo-Finnigan Delta V Plus isotope ratio mass spectrometer (IRMS), interfaced with a Thermo Trace GC gas chromatograph via GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	blue water diving
Generic Instrument Name	Manual Biota Sampler
Dataset-specific Description	Siphonophores were collected by blue water diving between 0-20 meters during daylight hours. Blue water diving techniques following the guidelines in the following publication: Haddock, Steven HD, and John N. Heine. "Scientific blue-water diving." (2005).
Generic Instrument Description	"Manual Biota Sampler" indicates that a sample was collected in situ by a person, possibly using a hand-held collection device such as a jar, a net, or their hands. This term could also refer to a simple tool like a hammer, saw, or other hand-held tool.

Dataset-specific Instrument Name	Remotely Operated Vehicle Doc Ricketts
Generic Instrument Name	ROV Doc Ricketts
Dataset-specific Description	We used the Remotely Operated Vehicle Doc Ricketts (https://www.mbari.org/technology/rov-doc-ricketts/) to collect siphonophores, which is an electro-hydraulic vehicle that operates between 200 and 4000 meters. The vehicle was fitted with high-definition video cameras, environmental data instrumentation (e.g. depth, temperature, salinity, and oxygen sensors), and suction and detritus samples to collect in-tact siphonophore specimens. ROV collections occurred during daylight hours.
Generic Instrument Description	The remotely operated vehicle (ROV) Doc Ricketts is operated by the Monterey Bay Aquarium Research Institute (MBARI). ROV Doc Ricketts is capable of diving to 4000 meters (about 2.5 miles). The R/V Western Flyer is the support vessel for Doc Ricketts and was designed with a center well whose floor can be opened to allow Doc Ricketts to be launched from within the ship into the water below. For a complete description, see: https://www.mbari.org/at-sea/vehicles/remotely-operated-vehicles/rov-doc...

Dataset-specific Instrument Name	Tucker Trawl
Generic Instrument Name	Tucker Trawl
Dataset-specific Description	A Tucker Trawl with a frame area of 2 square meters and a mesh size of 500 micrometers was towed obliquely for ~2h hours between 900 meters and the surface at night.
Generic Instrument Description	The original Tucker Trawl, a net with a rectangular mouth opening first built in 1951 by G.H. Tucker, was not an opening/closing system, but shortly thereafter it was modified so that it could be opened and closed. The original had a 183 cm by 183 cm flexible rectangular mouth opening 914 cm long net with 1.8 cm stretched mesh for the first 457 cm and 1.3 cm mesh for last 457 cm. 152 cm of coarse plankton or muslin netting lined the end of the net. Tucker designed the net to collect animals associated with the deep scattering layers, principally euphausiids, siphonophores, and midwater fish. (from Wiebe and Benfield, 2003). Currently used Tucker Trawls usually have 1-m2 openings and can have a single net or multiple nets on the frame.

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Deployments

WF0319

Website	https://www.bco-dmo.org/deployment/917024
Platform	R/V Western Flyer
Description	Cruise occurred in March 2019

WF0120

Website	https://www.bco-dmo.org/deployment/917025
Platform	R/V Western Flyer
Description	Cruise occurred in January 2020

WF0720

Website	https://www.bco-dmo.org/deployment/917030
Platform	R/V Western Flyer
Description	Cruise occurred in July 2020

WF0721

Website	https://www.bco-dmo.org/deployment/917032
Platform	R/V Western Flyer
Description	Cruise occurred in July 2021

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Project Information

Collaborative research: The effects of predator traits on the structure of oceanic food webs (SiphWeb)

Coverage: North Pacific

Food webs describe who eats whom, tracing the flow of energy from plants up to large animals. While many connections in food webs on land are quite familiar (lions eat antelope and antelope eat grass, for example), there are large gaps in our understanding of ocean food webs. Closing these gaps is critical to understanding how nutrients and energy move through ocean ecosystems, how organisms interact in the ocean, and how best to manage ocean resources. This project will study ocean food web structure with a focus on siphonophores, an abundant group of predators in the open ocean that range in length from less than an inch to more than one hundred feet. Siphonophores are closely related to corals and many jellyfish. They are known to be important predators within ocean food webs, but they are difficult to study because they live across great ocean depths and are gelatinous and fragile. The details of what they eat, as well as many other features of their biology, remain poorly known. This project will combine direct observations of feeding, genetic analysis of siphonophore gut contents, and stable isotope analyses to identify what different species of siphonophores eat. The team will also examine why they eat what they do. This will provide a new understanding of how the structure of food webs arise, aiding in our ability to predict future changes to food webs as the global climate shifts. Siphonophores feed in a very unique manner--they have highly specialized tentacles that are used solely for capturing prey--thus, the prey captured is determined largely by the anatomy and function of these tentacles. The project will describe these tentacles, reconstruct their evolutionary history, and investigate how evolutionary shifts in tentacle structure have led to changes in diet. This project will train one PhD student, one Master's student, a postdoc, and undergraduate students, including individuals of underrepresented groups. This project will support the production of scientifically rigorous yet engaging videos, foster the expansion of a citizen-science program, and create K-12 teaching modules.

This project will advance three scientific aims: First, it will identify the diet of a diverse range of siphonophores using DNA metabarcoding of gut contents and prey field, remotely operated vehicle (ROV) video of prey encounters, and stable isotope analysis. These approaches are highly complementary and allow for extensive cross validation. Second, the project will characterize the selectivity of siphonophore diets by comparing them to the relative prey abundances in the habitats of each of these species. Third, the project will characterize the structure of the siphonophore prey capture apparatus across species through detailed morphological analysis of their tentacles and nematocysts. These data will be integrated in an ecological and evolutionary framework to identify predator features associated with prey specialization. In a larger context, addressing these questions will advance our understanding of oceanic predation by revealing how evolutionary changes in predator selectivity correspond to evolutionary changes in habitat and feeding apparatus and how these changes shape current food web structure in the open ocean. We will test and refine an integrated approach to describing the structure and origin of food web topology, and evaluate the potential for phylogenetic

relationships to explain prey selectivity.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829812

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