The California State University
COUNCIL ON OCEAN AFFAIRS, SCIENCE & TECHNOLOGY (COAST)

Dr. Kenneth H. Coale Graduate Scholar Awards
AY 2023-2024 Application Form
Application Deadline: Wednesday, January 24, 2024, 5:00 p.m. PST

Please read the information on Dr. Kenneth H. Coale Graduate Scholar Awards on the COAST Webpage Announcement for full details and instructions.

Submit this form (which includes the Advisor Sign-Off Form) as both a Word document and a PDF file named as follows: *LastName_FirstName_App.docx* and *LastName_FirstName_App.pdf*. Submit both files as attachments, along with your **Department Commitment Form** (if needed) in **ONE** email to graduate@share.calstate.edu. **Please note**: A signature is required from your advisor on the **Advisor Sign-Off Form** only in the PDF version of your application that you submit. Your Advisor must submit your LOR to gradletter@share.calstate.edu separately.

Student Applicant Information

First Name:	Anthony	Last Name:	Donahue
CSU Campus:	SFSU	Student ID#:	
Email:		Phone:	
Degree Program:		Degree Sought (e.g., MS, PhD):	MS
Matriculation Date (mm/yy):		Anticipated graduation date (mm/yy):	
GPA in Major Courses:		Thesis-based? (Y/N):	Y

Advisor Information

First Name:	Michelle	Last Name:	Jungbluth
CSU Campus:	SFSU	Department:	Estuary & Ocean Science Center
Email:		Phone:	

Research Project Title:	Larval Fish Diets as Indicators of Food Web Dynamics in Tidal Wetland Restoration
Project Keywords (5-7 keywords related to your project):	SF Estuary Wetlands, Larval Fishes, Zooplankton, Food web Dynamics, High-throughput DNA Sequencing

Budget Summary (must add up to $4,000)

Award amount directly to awardee (through financial aid):	4,000

Award amount to Department (DCF required for department funding):	0

The information on this page is for COAST use only and will not be shared with potential reviewers.

Have you previously received a COAST Graduate Student Research Award? (Y/N)	Y
If yes, please provide year(s) of award(s):	2023

Committee Members (Required)

Name	Department	Campus

CSU Suggested Reviewers (Required): Suggested reviewers must be from the CSU. Do not suggest any reviewers from your campus or reviewers with a potential conflict of interest.

Name:		
CSU Campus:		
Department:		
Email:		

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Please refer to the **Award Announcement** for detailed instructions on the information required for each of the following sections. All the boxes below will expand as you type.

Project Description (65 points total): 1,500-word maximum; any text over this limit will be redacted

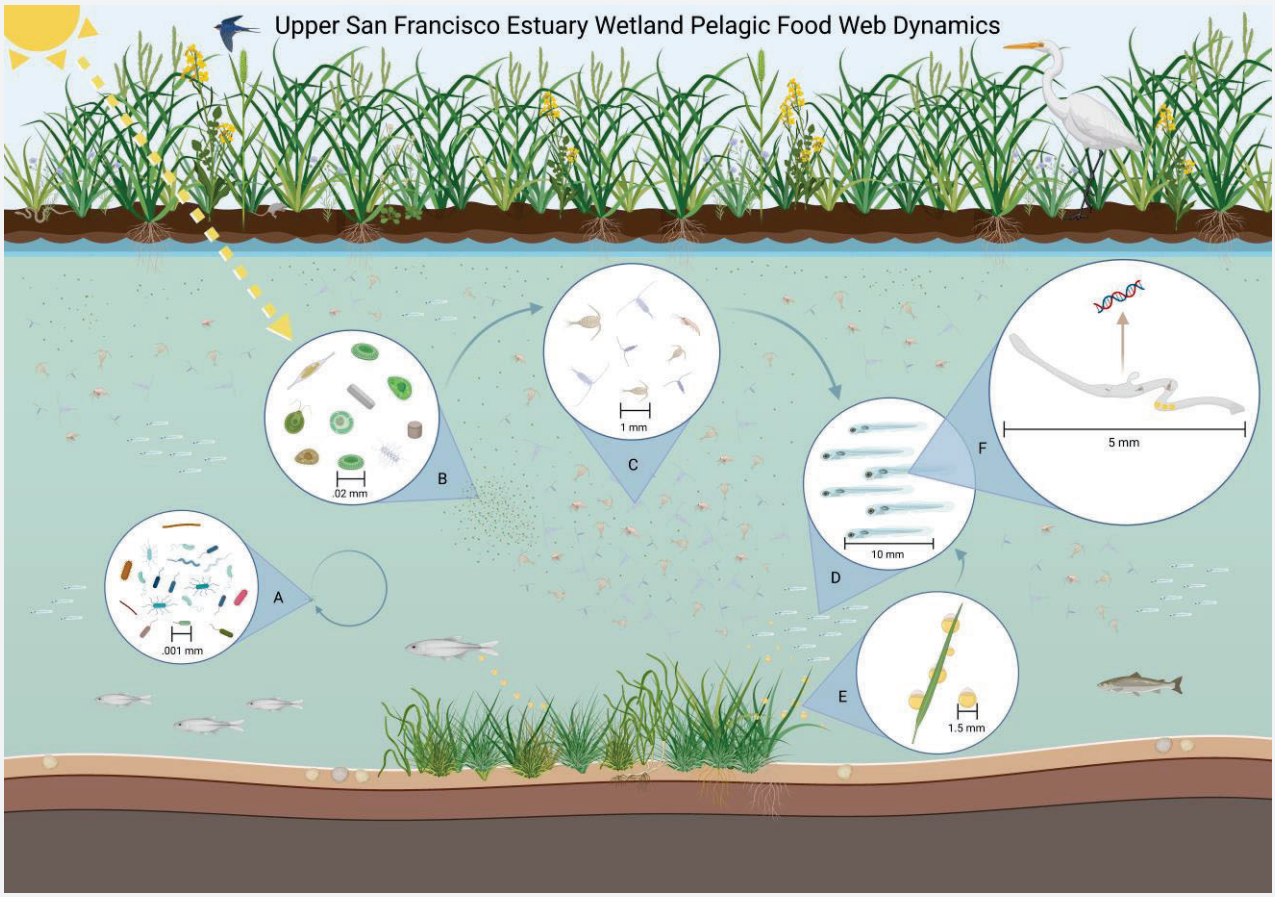
Background and Significance

Fish populations in the San Francisco Estuary (SFE) have been declining for decades and a decrease in zooplankton food web resources are one likely cause (Feyrer et al., 2007). The decrease in zooplankton is likely due to the reduction in tidal wetlands in the SFE by ~85%. Currently, wetlands in the SFE are being restored in an effort to increase fish populations with the assumption that the wetlands provide beneficial habitat (shelter, lower salinity zone) and food web resources for fishes utilizing the habitat. While the restoration of wetlands in the SFE will most likely improve food web dynamics and be beneficial for fishes that utilize them as nursery grounds (Herbold et al., 2014) the restoration of wetlands does not necessarily recreate a native/ideal ecosystem (Lockwood & Pimm, 1999) (Moy & Levin, 1991) (Simenstad & Thom, 1996). The focus of wetland restoration monitoring efforts is typically on the vegetation and benthic macrofauna (Zedler & Callaway, 1999) (Weilhoefer, 2011). While benthic macrofauna are consumed by some fishes (Grimaldo et al., 2009), they are not vital prey for the majority of larval fishes utilizing wetlands as nursery grounds. In addition, it may take decades for benthic infauna in early stage restoration projects to become similar in composition to those in mature wetlands (Craft & Sacco, 2003)(Moseman et al., 2004). Zooplankton species abundance may be a more immediate indicator of which restoration techniques achieve wetland maturity in the water column.

Longfin smelt (LFS; *Spirinchus thaleichthys*) is one of the main declining fish species of concern in the SFE (Rosenfield & Baxter, 2007), as it has been considered threatened under the California Endangered Species Act since 2009. LFS are semi-anadromous fish that spawn in the low salinity waters of the Suisun Bay and San Pablo Bay (Hobbs et al., 2010) (Grimaldo et al., 2020) and utilize the surrounding wetlands as nursery grounds. The food web dynamics of these wetlands directly affects LFS abundance (MacNally et al., 2010), as larval LFS (Fig. 1 - D) primarily feed on calanoid and cyclopoid copepod zooplankton (*Eurytemora carolleeae, Acanthocyclops americanus*, *Pseudodiaptomus forbesi*) (Fig. 1 - C) (Hobbs et al, 2006) (Jungbluth et al., 2021).Therefore, where and when these copepods are in wetlands will likely affect larval LFS feeding and their survival.

This study will utilize high-throughput sequencing (HTS) to discover indicator species in the water column within close proximity to wetlands at different phases of restoration (early, intermediate, mature), and identify the diversity of prey and indicator species in diets of larval fishes, specifically LFS, Pacific herring (*Clupea pallasii*), and Prickly sculpin (*Cottus asper*), which utilize the wetlands as nursery grounds. Studying the diets of larval fishes with HTS will provide a better understanding of the influences on declining fish populations and the extent to which wetland restoration efforts are directly supporting larval fish habitats. HTS is advantageous for DNA diet analysis as it can identify diverse zooplankton in the diets of larval LFS, which were previously unknown species in the food web that microscopy was not able to achieve (Jungbluth et al., 2021).

Figure 1



A: Microbes remineralize nutrients from dead organic matter. B: Phytoplankton photosynthesize and are prey for zooplankton. C: Copepods (*Eurytemora carolleeae*, *Acanthocyclops americanus*, *Pseudodiaptomus forbesi*) consume phytoplankton and are prey for larval fishes. D: Larval Longfin smelt (*Spirinchus thaleichthys*) consume copepods and E: fish eggs settled on seagrasses as well as in the water column. F: Larval Longfin smelt gut is dissected, then DNA sequencing analysis specifies consumed prey.

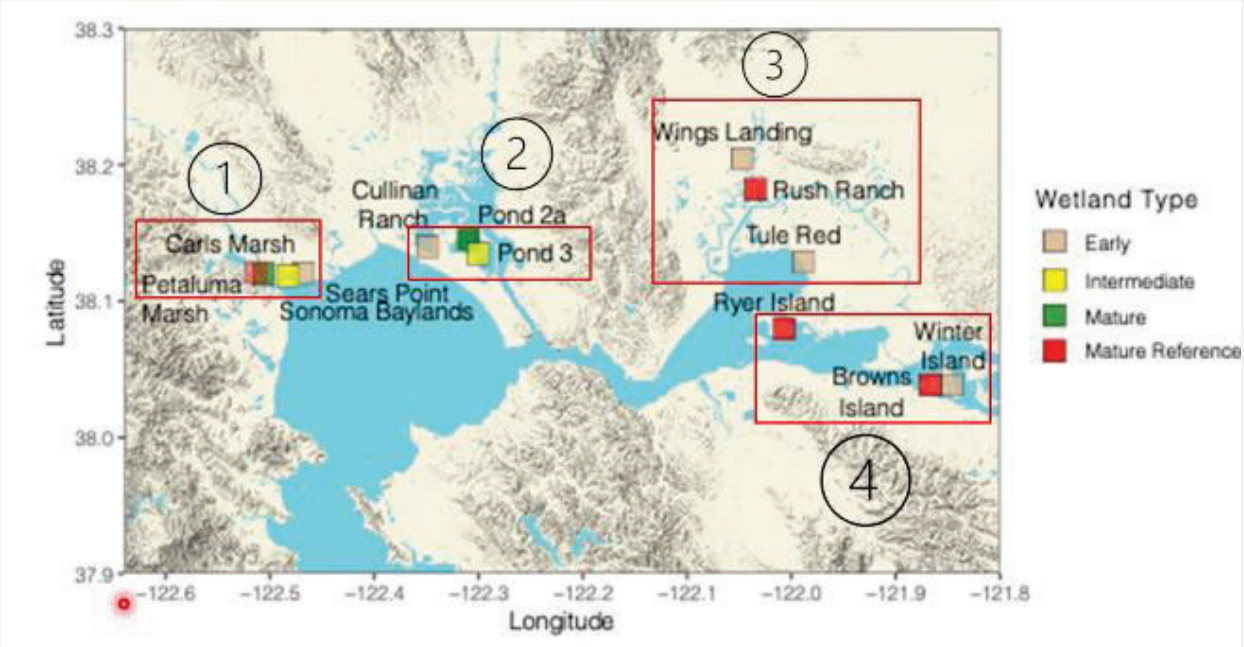
Methods

Wetland Collection Sites

San Pablo Bay: Black John, Carl's Marsh, Cullinan Ranch, Petaluma Marsh, Sears Point, and Sonoma Baylands (Fig. 2).

Suisun Bay: Brown Island, Rush Ranch, Ryer Island, Tule Red, Wings Landing, and Winter Island (Fig. 2).

Figure 2



Wetland collection sites in San Pablo Bay (Region 1: Carls Marsh, Sonoma Baylands, Sears Point; Region 2: Cullinan Ranch, Pond 2a, Pond 3) and Suisun Bay and Marsh (Region 3: Wings Landing, Rush Ranch, Tule Red; Region 4: Ryer Island, Browns Island, Winter Island).

Larval Fish Collection and Processing

Larval fishes will be collected from the 12 wetland sites with a 500 µm mesh net in open water areas or central marsh channels during the ebb tide. The net contents will be washed into the cod end, concentrated on a clean sieve, and then preserved in 95% ethyl alcohol (EtOH). Samples will be put on ice, transported to the Estuary & Ocean Science Center (EOS) laboratory, and stored in a freezer. Larval fishes in the samples will then be identified, counted, and organized into appropriate vials by a taxonomist at ICF Inc. Larval fish length (mm) will be measured and the guts will be dissected with sterile tools under microscopy, placed in a sterile tube with 95% EtOH, stored in a -20 °C freezer, then HTS will be conducted on the gut for diet analysis (Jungbluth et al., 2021).

Molecular Analysis

The guts (esophagus, stomach) (Fig. 1 - F) of the larval fish samples will be dissected under microscopy. DNA from the gut samples will be extracted with the DNeasy Blood and Tissue kit at EOS. Bead-beating will be conducted to rupture the prey DNA in the gut (Jungbluth et al., 2013). Zooplankton sample community eDNA will also be extracted to characterize the prey assemblage available at each wetland site (Figure 1 - C). Zooplankton subsamples DNA will be extracted with the OMEGA EZNA soil DNA kit following the protocol for 250–1000 mg samples (Jungbluth et al., 2021). The Illumina MiSeq, located on the SFSU main campus, will be used to sequence the species-level marker genes (mtCOI, 18S). Both marker genes are present in eukaryotes, therefore, blocking primers will be used to reduce amplification of the predator DNA in diet analyses (Jungbluth et al., 2021). HTS will be conducted and the amplified sequences from the gut and zooplankton samples will be used to identify food web resources and indicator species in the prey assemblage. The custom reference database will be created by identifying mtCOI and 18S for each species, then combining the data in the SILVA database, Protist Ribosomal Reference database, and NCBI Genbank database.

Data Analysis

The ANACAPA toolkit (Curd et al., 2019) will be used to clean the sequence data and assign them into Amplicon sequence variants (ASV) with DADA2 (Callahan et al., 2016), create a custom reference database for each marker gene, and classify the sequences. The custom reference database will combine the SILVA database (18S) (Quast et al., 2012), Protist Ribosomal Reference database (PR2; 18S) (Guillou et al., 2012), and NCBI Genbank database (mtCOI, 18S) (Clark et al., 2016) to include the range of taxa expected in the study. Analysis of Similarity will be applied to Bray-Curtis distances to determine the assemblage difference between larval fishes from different wetlands. Percent similarity analysis will be used to determine which prey are responsible for differences between larval fishes samples, and to determine whether water samples or zooplankton are the more significant link to the different stages of wetland restoration. The ASV will then be identified to the species level using a sequence alignment-based method with Bowtie2 and the Bayesian Lowest Common Ancestor Algorithm. Species that are identified in each type of sample (zooplankton, larval fishes) from each wetland will be identified via indicator species analysis with R Studio and TITAN2.

Expected Outcomes

HTS will identify the majority of the DNA present from prey (primarily copepods) and other food sources (copepod eggs, fish eggs) (Fig. 1 - E) consumed by the larval fishes, which will determine the food web resources and indicator species at each wetland restoration site. Therefore, this study will provide molecular methods for the SFE regional wetland monitoring community to more accurately quantify the progress of wetland restoration and if the ecology of the SFE is enhancing food web resources for larval fishes in their nursery grounds. This study will yield information that can be utilized by wetland restoration managers to more accurately assess the benefits provided by wetlands in the SFE and determine the prey availability of specific species of zooplankton for larval fishes as restoration projects progress. The restoration of wetlands in the SFE will ultimately help restore multiple declining aquatic species.

References (0 points): no limit

References

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Timeline (10 points total): 250-word maximum; any text over this limit will be redacted.
Please note: If you reference activities occurring prior to May 15, 2024, for context, be sure to clearly identify the activities an award would fund. **Requests for funds for expenses or work done prior to start date will result in your application being returned without review.**

Activities Prior to 2024 Award Funding

February – April 2023: Field Collection of larval fish, zooplankton, and subsequent morphological classification.

May – August 2023: Continue larval fish gut dissections on 2022 samples, begin DNA extraction of larval fish gut 2022 samples, begin larval fish gut dissections of 2023 samples.

August 2023 – December 2023: Finished larval fish gut dissections of 2022 samples, finished DNA extraction of larval fish gut 2022 samples, began larval fish gut dissections of 2023 samples, began writing thesis, conducted data analysis on population numbers of larval fishes at each wetland site.

January – May 2024: Continue larval fish gut dissections of 2023 samples, continue writing thesis, begin DNA extraction of zooplankton 2022 & 2023 samples, begin DNA extractions of larval fish gut 2023 samples, first committee meeting.

Activities After 2024 Award Funding

May 2024: Receive Dr. Kenneth H. Coale Graduate Scholar Award.

May 2024 – July 2024: Finish DNA extraction of zooplankton 2022 & 2023 samples and larval fish gut 2023 samples, create PCR Library of 2022 & 2023 larval fish gut samples and zooplankton samples, conduct HTS analysis of 2022 & 2023 larval fish gut and zooplankton samples.

August – December 2024: Finish HTS analysis of 2022 & 2023 larval fish gut and zooplankton samples, continue writing thesis, second committee meeting, conduct analysis on HTS data.

January – May 2025: Finish data analysis and writing thesis, final committee meeting.

May 2025: Defend thesis, graduate from SFSU | IMES Program, then submit thesis for publication.

Need for Research (7 points total): 250-word maximum; any text over this limit will be redacted

Previous research in the SFE to determine the food web dynamics in and around wetlands undergoing restoration have predominately focused on dissecting the diets of adult fishes, which have indicated that crustaceans are the main component of the diet of pelagic fishes in the SFE (Canuel et al., 1995). Minimal work has focused on the diets of larval fishes due to the challenges in identification of partly digested and microscopic prey. A previous study analyzed the diets of larval fishes in the estuary using molecular methods and found evidence of spatial differences in fish diets, unfortunately the study did not have a large enough sample size to be statistically significant (Jungbluth et al., 2021). Furthermore, previous research has exhibited that restoration efforts do not necessarily yield an ideal ecosystem structure (Lockwood & Pimm, 1999) or function (Kentula, 1996)(Simenstad & Thom, 1996) and disparities in prey availability for fishes can last for years following restoration of wetlands in the SFE. In addition, current techniques for monitoring wetlands do not accurately identify zooplankton that are critical prey during the sensitive larval stages of fish. For example, calanoid and cyclopoid copepods are difficult to visually identify to the species level in diet studies. In order to successfully restore wetlands in the SFE while conserving and supplementing declining fish populations, it is necessary to know what prey or food sources the restored wetlands are providing larval fishes, and if such prey or food sources vary in wetlands at different stages of restoration.

Relevance to state of California (3 points total): 100-word maximum; any text over this limit will be redacted

Tidal wetlands have been reduced by ~85% in the SFE. A substantial amount of wetland habitat restoration efforts are currently in operation to restore multiple declining aquatic species, such as LFS, and the critically endangered Delta smelt (*Hypomesus transpacificus*). However, despite such efforts, it is unknown as to which species restoration operations are helping, how to quantify benefits over specific periods of time, or determine how efforts benefit higher levels of the foodweb. HTS will remedy these limitations by providing molecular methods for the SFE regional wetland monitoring community to more accurately assess the benefits of wetland restoration projects.

Budget and Justification (15 points total)

Example Budget (to use this format, erase the content below and add additional rows as necessary; alternatively, you are welcome to create your own table):
Please note: Funds can only be requested for costs incurred ON or AFTER the project start date (May 15, 2024). Award funds may not be used for activities that occur prior to this date. **Requests for funds for expenses or work done prior to start date will result in your application being returned without review.**

Item/Description	Unit Price	Quantity	Amount to Awardee (via Financial Aid)	Amount to Department
Gasoline for Travel (1 month)	$230	1	$230	-
Living Expenses (1 month)	$1694 (rent) + $200 (food) + $50 (internet) + $40 (utilities)	1	$1984	-
Tuition	$4,481- $3,588 (Grant) = $893	2	$1786	-
		Subtotals:	*$4,000*	-
		Grand Total	**$4,000.00**	

Justification (250-word maximum; any text over this limit will be redacted):

The current grant funding for the Microbes to Zooplankton project my research is part of will cover the costs of lab supplies for DNA extractions, PCR libraries, and HTS analysis. However, the grant funds I've received monthly (~$1,700 net) for the Summer and Fall of 2023, and Spring of 2024, will be depleted at the end of May 2024. Therefore, I will need to find either part-time or full-time work, or other sources of funding to complete the final year or so of my research and classes, while financially supporting my 10-year-old daughter. We are currently receiving Medi-Cal benefits, but I cannot afford to live in areas closer to SFSU and EOS. Therefore, I drive ~85-105 miles round-trip to and from my apartment in Vallejo, plus pay one - two $8 - 9 tolls per day. Based on my financial needs, all of the Dr. Kenneth H. Coale Graduate Scholar Awards' funding will go to myself, since the cost of living in the Bay Area is expensive, as the majority of my income is used to cover living expenses, which is evident in only one month of living and travel expenses combined with two semesters of tuition (minus CSU tuition grant) is approximately equal to the grand total of the award. Otherwise, completing my degree and research will be delayed and negatively impact the quality and viability of the Microbes to Zooplankton project.

Application Deadline: Wednesday, January 24, 2024, 5:00 p.m. PST
Save as both a Word document and a PDF file named as follows:
***LastName_FirstName_App.docx* and *LastName_FirstName_App.pdf*.**
Submit both files as email attachments in ONE email (with other required forms) to graduate@share.calstate.edu.

Within 24 hours of application submission, you will receive a confirmation email from COAST. Please save this confirmation email for future reference. If you do not receive a confirmation email, please contact Kimberly Jassowski (kjassowski@csumb.edu) to ensure your application was received.

CSU The California State University
COUNCIL ON OCEAN AFFAIRS, SCIENCE & TECHNOLOGY (COAST)

Dr. Kenneth H. Coale Graduate Scholar Awards
AY 2023-2024 Advisor Sign-Off Form

To encourage you to engage with your CSU Advisor as you develop your application, **we require this form for all applications submitted to the Dr. Kenneth H. Coale Graduate Scholar Awards Program**. By signing this form, your advisor indicates that they have reviewed your application, provided guidance and input, and approved it for submission. All information except signatures must be typed. Electronic signatures are acceptable. **Please note:** A signature is required from your advisor on this **Advisor Sign-Off Form** in the **PDF version** of your application that you submit (the word document does NOT need to be submitted with a signature)

Please note: this form is NOT a substitute for a letter of recommendation (LOR). Your Advisor must submit your LOR to gradletter@share.calstate.edu separately.

Applicant Name:

Anthony Donahue

CSU Advisor Information:

Name:	Michelle Jungbluth	Phone:	
Department:	SFSU | Estuary & Ocean Science Center	Email:	

I have reviewed my student's application and provided guidance and input. My signature below indicates my approval of the application.

CSU Advisor Signature: DocuSigned by: Michelle Jungbluth 1BE73D89350A4AD... Date: 01/30/2024 | 9:53 PM PST