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

Graduate Student Research Award Program
AY 2020-2021 Application Form
Application Deadline: Monday, February 1, 2021, 5:00 p.m. PST

Please see information on Graduate Student Research Awards on the COAST **website** and read the **Announcement** for full details and instructions.

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 attachments (along with your Department Commitment Form if needed) in **ONE** email to **csucoast@csumb.edu**. Your Advisor must submit your LOR to **csucoast@csumb.edu** separately.

Student Applicant Information

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

Advisor Information

First Name:	Wim	Last Name:	Kimmerer
CSU Campus:	San Francisco	Department:	Biology
Email:		Phone:	

Research Project Title:	Using high-throughput sequencing to determine the prey composition within the diets of the copepods Eurytemora carolleeae and Psudiodiaptomus forbesi in the San Francisco Estuary
Project Keywords (5-7 keywords related to your project):	San Francisco Estuary, zooplankton, copepods, high-throughput sequencing

Budget Summary (must add up to $3,000)

Award amount directly to awardee:	3000

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

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

Committee Members (Required)

Name	Department	Campus

CSU Suggested Reviewers (Required): Suggested reviewers must be from the CSU. Use the COAST member database to help identify potential reviewers. 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:

The San Francisco Estuary (SFE) has gone through many complex changes due to human development; it has become a high-traffic area for shipping, most of the wetlands have been diked and filled, and more than half of its freshwater inflow has been diverted towards the rest of the state of California for industrial, agricultural, and residential use (Nichols et al., 1986). Due to these extensive anthropogenic influences, we have seen an intense shift in the species composition of the SFE in recent decades. This shift includes rise of introduced planktonic and benthic species since the 1980's, that likely arrived to the SFE through the ballast waters of ships coming from Asia (Carlton et al., 1990, Orsi and Ohtsuka, 1999, Choi et al., 2005). The shift also includes the fall of pelagic organisms, including notable fishes such as the delta smelt, longfin smelt, and striped bass, termed the Pelagic Organism Decline (POD) (Baxter, 1999; Moyle, 2002; Sommer et al., 2007; Thomson et al., 2010). The rapid decline and near extinction of native species such as the delta and longfin smelt have steered researchers studying the SFE to determine the mechanisms behind the POD. Currently known causes include fish entrainment from water diversions (Kimmerer, 2008; Kimmerer 2011), changes in the position of salinity range, increasing water clarity, and increasing summer water temperatures (Mac Nally et al., 2010). While multiple factors may contribute to the fish's decline, I aim to research an essential factor for larval fish survival: the availability of zooplankton as food resources (Platt, 2003).

Delta smelt and longfin smelt residing in the low salinity zone (LSZ) of the SFE primarily feed on two calanoid copepod zooplankton species, *Eurytemora carolleeae* (formerly *Eurytemora affinis*) and *Psuedodiaptomus forbesi* (Slater and Baxter, 2014). Before 1986, *E. carolleeae* was a dominant copepod species in the SFE (Winder and Jassby, 2011) with a year-round abundance (Ambler et al., 1985). However, the introduction of the clam *Potamocorbula amurensis* in 1986 decimated *E. carolleeae* through competition as well as predation (Kimmerer et al., 1994). The invasive clam established itself in the SFE during the summer to fall (Carlton et al., 1990), when *E. carolleeae* had its peak season (Merz et al., 2016). *E. carolleeae*, in every year since 1987, was thereby limited to a fall to spring season, declining every April/May and only appearing again in November (Merz et al., 2016; Kimmerer et al., 2014).

While this phenomenon would otherwise have left a vast gap in larval fish diet, *P. forbesi* was introduced to the SFE in 1988 and filled in this summer absence of *E. carolleeae* (Winder and Jassby, 2011). *P. forbesi* now maintains a seasonal abundance from summer to fall due to source populations outside of the clam's range that travel up stream and provide a moderate population in the LSZ (Kimmerer et al., 2018). However, even with combining the two species' seasonal abundances, a temporal gap remains. *E. carolleeae*'s population drops off in April/May, while *P. forbesi* doesn't reach its peak abundance until July (Merz et al., 2016). Peak spawning for delta smelt is in March/April (Sommer et al., 2011), and the larval fish require food during this critical springtime period to progress to the juvenile stage.

Objectives:

My two research objectives are to:

1) Measure the *in-situ* diets of *P. forbesi* and *E. carolleeae* during their current peak seasons and determine what limits each species during their respective declining periods: spring decline in *E. carolleeae* abundance and late fall for *P. forbesi*.

There may be a combined effect of declining food resources appropriate for each species during these time periods, in addition to the detrimental effects of *P. amurensis,* which have been well studied.

2) Compare the diet of both species when offered the same food during the summer period when *E. carolleeae* is not present and *P. forbesi* populations are increasing.

I hypothesize that *P. forbesi* may be better than *E. carolleeae* at utilizing the summer prey assemblage, preventing *E. carolleeae* population growth on the summer prey and allowing *P. forbesi* populations to grow. It has been suggested that *P. forbesi* serve as a good food source for larval fish because they selectively feed on more nutritious prey items (Winder and Jassby, 2011; unpublished results from A. Müller-Solger, Department of Water Resources, Sacramento). However, recent research using high throughput sequencing (HTS) targeting the 16S rRNA gene on *P. forbesi* gut contents (Holmes, 2018) has revealed a surprising diet consisting of low nutritional value prey items. In my proposed experiment, I aim to use HTS to target the 18S rRNA and 16S rRNA genes, to provide support to prior research and a more accurate representation of the range of prokaryotes and eukaryotes in the diets of these two important zooplankton species. This information is essential for further management of the estuary to improve foodweb productivity and mitigate the decline of native fishes such as the delta and longfin smelt.

Methods:

Field Sampling

Both species will be collected from various sites in the low-salinity zone of the SFE, within Suisun Bay or the western Delta. I will collect live zooplankton through subsurface tows with a 150 µm mesh net. Experimental *E. carolleeae* will be collected from the field between March and June 2021 and kept alive in the laboratory until the summer. Experimental *P. forbesi* will be collected from the field between June and October 2021.

For *in-situ* diet composition of each species (Objective 1), a subsample of field-collected zooplankton will be immediately preserved in 95% molecular ethanol and transported back to the lab to be stored in a -20 ºC freezer for molecular analysis.

During both periods of sampling whole water samples will be taken from the surface with sterile buckets and brought back to the laboratory to determine the *in-situ* prey assemblages available in each season. The summer whole water samples will also be used as *in situ* food to be tested with both copepod species in incubation experiments.

Laboratory Work

During both sampling periods, three replicates of 1-2 L of field-collected water will be immediately filtered onto three 0.2 µm Sterivex filter cartridges which will be stored in a -20 ºC freezer for later molecular analysis.

Bottle Incubation Experiments

To determine the prey composition for each species when offered the same prey assemblage from the summer (Objective 2), I will incubate both species, live, in the whole water samples collected from the summer.

I will size fractionate the live *E. carolleeae* using reverse filtration to isolate individuals in the late copepodite stages. I will then add 20-30 individuals each into 10 sterile 1.0 L incubation containers containing the collected summer water (35 µm pre-filtered). To serve as a control and initial gut content measurement, I will do the same for 5 identical containers containing only filtered water. After 24 hours of incubation, I will filter the contents of each incubation container through a 100 µm-sieve, preserve the zooplankton in 95% ethanol, and store in a -20 ºC freezer for later molecular analysis. The live *P. forbesi* will be incubated using the same method.

Molecular Analyses

I will extract the DNA from the filtered water samples using the PowerWater Kit (Qiagen), and from sets of five individual copepods using the DNeasy Tissue Extraction kit (Qiagen). All copepods from field subsamples and bottle incubation experiments will be carefully cleaned to prevent contamination by any attached organisms.

To determine the prey items from the water and copepod samples, I will use high-throughput DNA sequencing (HTS) targeting the 18S rRNA gene (eukaryotic phytoplankton and microzooplankton) and 16S rRNA gene (prokaryotic phytoplankton and bacteria) following protocols similar to those of Yeh et al. (2020). All samples will be sequenced using an Illumina MiSeq sequencer (V2 x 250 kit).

Data Analysis

Analyses will include primer trimming with the program *cutadapt*, read pairing with the program *pear* (Zhang et al., 2014), and data quality control using *DADA2* (Callahan et al., 2016). I will download reference databases from the Protist Ribosomal Database (PR2) (Guillou et al., 2013) for eukaryote prey and SILVA reference database for prokaryotes (Quast et al., 2013). Using these databases, I will use BLASTn (Altschul et al., 1990) implemented in Qiime (Caporaso et al., 2010) to classify sequences to identify prey taxa.

Subsequent analyses will be performed in R (R Core Team, 2014). All sequence data will be normalized by total sequence abundance per sample. Bray-Curtis distances will be calculated to assess the similarity of prey assemblages recovered from each sample. Analysis of Similarity (ANOSIM) analysis (*vegan)* will be used to determine differences in prey assemblages found between species and stations, and SIMPER analysis (*vegan*) will be used to determine which prey items drive any observed differences between species (Oksanen et al, 2012).

References (0 points): no limit

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Timeline (10 points total): 250-word maximum

May 15th, 2021- continue field work season

June 2021 – begin bottle incubation experiment

Summer 2021 – begin molecular analysis

Fall 2021 – collect field samples of *P. forbesi* and perform molecular analyses. Begin bioinformatics analysis of summer experiment data and begin writing thesis

Spring 2022 – continue writing thesis and prepare manuscript for submission to peer-reviewed journals, defend and graduate from SFSU

Need for Research (NEW SECTION, 7 points total): 250-word maximum

By understanding zooplankton diets, we can better inform bottom-up methods of restoring the SFE's lower food web to mitigate the effects of POD. Although researchers have been studying *P. forbesi and E. carolleeae* for some time because of their importance in the diets of larval longfin and delta smelt, we need further exploration on their diets.

So far, studies on zooplankton diets have been largely through bottle incubation observations by measuring prey removal, which may not serve as a true representation of prey composition. These tiny prey items are hard to identify in general, and even more so if half-digested. High throughput sequencing is a way to more accurately determine zooplankton diet. There is a lack of clarity on what exactly zooplankton eat in their natural environment, and that was made clear when a master's student in the Kimmerer lab used HTS on *P. forbesi* and revealed an unexpected diet very different than what has been assumed so far in the literature. With further research by targeting the 18s rRNA gene as well, I will help determine a more accurate prey composition for these two important species of zooplankton. This may help us increase population levels of these critical prey items for the endangered larval fish and understand why there is a temporal gap of prey items during the late spring.

Relevance to state of California (NEW SECTION, 3 points total): 100-word maximum

This research is relevant to the state of California because the San Francisco Estuary is the largest estuary in California and the management of its waters has impact on the entire state. Many decisions about California's water resources are already hinged on their effects on endangered native fish such as delta and longfin smelt. For example, there are extensive regulations on diversions of water to avoid entrainment and fish mortality. By further illuminating the mechanisms behind the POD, specifically the availability of food resources for larval fish, we can better inform management of the water distributed throughout California.

Budget and Justification (15 points total)

Example Budget (feel free to erase the content and use this format, adding additional rows as necessary, or create your own):

Item/Description	Unit Price	Quantity	Amount to Awardee (via Financial Aid)	Amount to Department
1.5 mL microcentrifuge tubes (pack of 500)	$8.00	2	$16.00	$0.00
100mL plastic jars	$1.00	12	$12.00	
Power Water Kit	$546.00	1	$546.00	
DNEasy kit	$690.00	1	$690.00	
High fidelity polymerase: Kapa Hifi, 25 ul reactions	$260.00	2	$520.00	
MiSeq kit: V2x500	$1216.00	1	$1216.00	
		Subtotals:	$3,000.00	*$0.00*
		Grand Total:	**$3,000.00**	

Justification (250-word maximum):

While I am generously being supported by a research assistantship in the Kimmerer lab and the Grad State University Grant, I still need funds to acquire laboratory materials. This project requires DNA extraction and sequencing to determine the prey items within the zooplankton. The materials needed for molecular analyses can be quite expensive, however for completion of project, I will need to run many samples for a robust analysis. This award would allow me to cover some of the materials needed for the bottle incubation experiment and all subsequent molecular analyses for the water and zooplankton samples.

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***LastName_FirstName_App.docx* and *LastName_FirstName_App.pdf*.**
Submit both files as email attachments in ONE email to csucoast@csumb.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.