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:	Isabel	Last Name:	Villafuerte
CSU Campus:	California Polytechnic State University, San Luis Obispo	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:	Sean	Last Name:	Lema
CSU Campus:	California Polytechnic State University, San Luis Obispo	Department:	Biological Sciences
Email:		Phone:	

Research Project Title:	Effects of marine heatwaves on fish reproduction: assessing the potential for reproductive impairment via hormone changes at elevated temperature
Project Keywords (5-7 keywords related to your project):	marine heatwaves, climate change, fish, reproduction, hormone

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

Award amount directly to awardee (through financial aid):	2600
Award amount to Department (DCF required for department funding):	1400

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)	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. 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

Marine systems are increasingly experiencing higher than average temperatures[1] and more frequent shorter-term anomalous marine 'heat wave' events[2-5] due to climate change. As overall ocean temperatures continue to rise, marine heat waves are predicted to become increasingly severe and frequent[6]. These elevated ocean temperatures and anomalous heat wave events are expected to have varied impacts on marine life[7].

In marine fishes, reproduction has been shown to be especially susceptible to elevated temperatures[8-11]. Fishes reproduce within a narrower thermal range than what is required for their survival[8,12], and exposure of fish to elevated temperatures beyond what their species would normally experience can impair gametogenesis and spawning[8,10,11,13,14]. As successful reproduction is necessary for the persistence of populations, understanding how elevated temperatures impact reproductive performance, and physiological mechanisms, in marine fishes is crucial for predicting how warming oceans will impact the reproductive output and recruitment dynamics of fish populations.

In fishes, reproduction is regulated by several hormonal pathways, with hormones of the hypothalamic-pituitary-gonadal (HPG) endocrine axis playing critical roles in regulating reproductive processes. However, several aspects of HPG axis signaling in fishes have been shown to be thermally sensitive and thus can be impaired by environmental temperature conditions[11]. Specifically, it has been shown that high temperatures can impact the hormonal pathways regulating reproductive processes at three critical developmental periods necessary for reproduction: sex determination and differentiation (early development), steroidogenesis and gametogenesis, and spawning events[11]. At each of those developmental periods, the impacts of elevated temperature are mediated in part by changes in hormone signaling. For instance, blood concentrations of the sex steroid hormone 17β-estradiol (E_2) – a key steroid regulating oogenesis – have been shown to decline in females of several fish species under elevated temperatures[15-19]. Such declines in E_2 hormone production can lead to impaired egg production. Across several fish species, females exposed to elevated temperatures experience decreases in ovary mass and ovarian gonadosomatic index (GSI) values, resulting in smaller and less viable eggs (for review, see Alix et al. 2020). Males

of some fishes have also shown impaired gamete production under elevated temperatures[20,21], although effects in males appear to be more variable across fish taxa than for females.

While several studies have examined how higher average temperatures can impair fish reproductive endocrinology and performance (Alix et al. 2020), it remains unclear how exposure to shorter-term 'heat wave' events might affect fish reproduction, or whether fishes exposed to such heat waves during critical reproductive periods (e.g., gametogenesis) might lead to long-term reproductive impacts. Additionally, few studies have examined how elevated temperatures from heat wave events impact fish reproduction in estuarine marine fishes, despite these habitats having a higher likelihood of experiencing severe heat wave conditions.

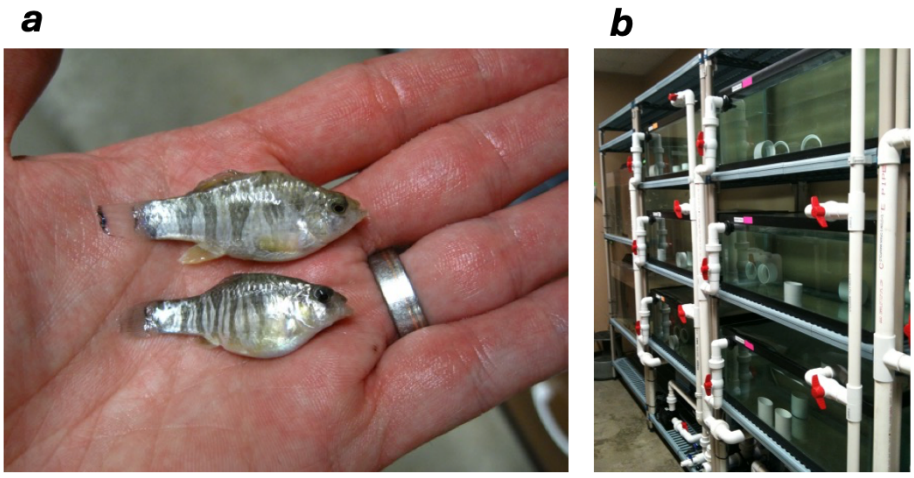

Fig. 1. a) Adult Sheepshead Minnow (*Cyprinodon variegatus*). **b)** Experimental tank set-up.

My research aims to address how the HPG axis is affected during and after marine heat wave conditions in the eurythermal Sheepshead Minnow (*Cyprinodon variegatus*)(**Fig. 1a**). The Sheepshead Minnow is an estuarine fish that has been used as a model organism for studying the effects of endocrine disrupting chemicals and other pollutants[22-24]. This eurythermal fish lives in shallow water estuarine and salt marsh habitats along the coast of Eastern North America[25], and can survive temperatures from -1.9°C to 45.4°C[26]. Despite having a broad thermal tolerance range, reproduction is susceptible to temperature fluctuations[17,25]. A recent study by Bock et al.[17] found that adult female Sheepshead Minnow exposed to a higher stable temperature regime (37°C) experienced extensive alterations to HPG axis signaling and an inhibition of oogenesis, as compared to females held at a lower stable temperature (27°C). That study, however, used stable thermal

regimes, which are not representative of the dynamic thermal conditions that this species typically experiences in its natural habitats.

With this proposed research, the following hypotheses will be tested:

Short-term exposure to elevated, ecologically-relevant fluctuating temperature conditions representative of a marine heat wave will negatively impair:

1) **HPG axis signaling in sexually-mature Sheepshead Minnow.**
2) **Indicators of Sheepshead Minnow reproductive performance (i.e., gonadal GSI values, proportion of spawning-ready oocytes, etc.).**

Methods

Adult Sheepshead Minnows (*C. variegatus*) will be obtained from Aquatic Biosystems (Fort Collins, CO, USA) where fish are bred and raised at stable 25°C. Fish will be maintained in 208 L tanks under stable temperatures (25°C) and 15L:9D photoperiods during a 14 d acclimation period, after which fish will be assigned systematically to experimental 114 L tanks (**Fig. 1b**) in mixed-sexed groups of 20 males and 20 females per tank, with the body size distribution for each sex balanced across tanks. Fish in these tanks will be transitioned to a fluctuating temperature regime with daily oscillations corresponding to the 14L:10D photoperiod from 22°C at the start of daily light (L) photoperiod in the morning to a high of 32°C, just before the switch to dark (D) photoperiod. In three replicate 114 L tanks, one group of fish (*control*) will be maintained in 22-32°C fluctuating conditions for the duration the experiment. A second set of three replicate tanks will experience similar fluctuating conditions for 15 d, then temperatures will increase to a regime of 27-37°C for 5 d (simulated 'heat wave') (**Fig. 2**). Temperatures within all tanks will be recorded by HOBO data loggers every 5 minutes. Fish will be fed spirulina and brine shrimp flake feeds *ad libitum* throughout the experiment.

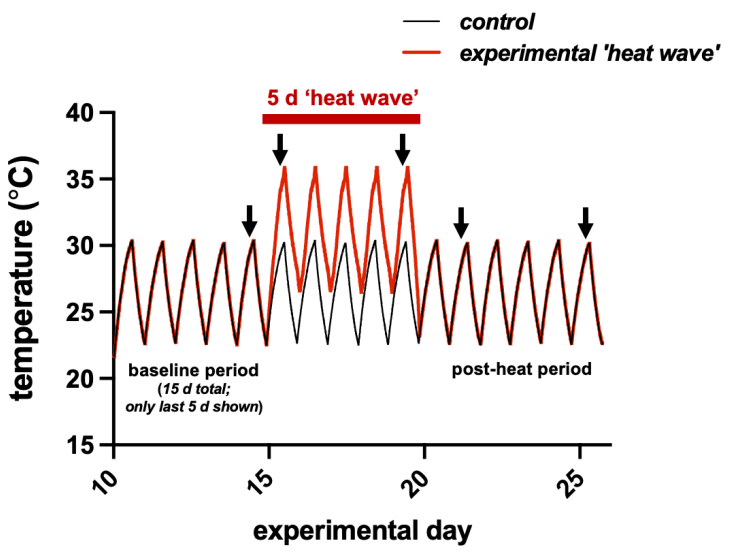

Fig. 2. Planned experimental *'heat wave'* and *control* temperature treatments. Both treatments will be maintained under fluctuating 'baseline' temperatures (daily fluctuation from 22-32°C) for 15 d, then the experimental group will experience 'heat wave' temperatures of > 5°C for 5 d, and returned to 'baseline' conditions for a 'post-heat wave' 6 d period. Arrows indicate experimental sampling days.

Sampling of both treatment groups will occur on several days (**Fig. 2**): day 15 (1 d immediately prior to 'heat wave'), day 16 (+1 d of heat wave), day 20 (+5 d of heat wave), and at two points following the heat wave (day 22 and day 26: respectively 2 and 6 d after end of heat wave). A total of 12 fish of each sex per treatment group (4 per tank replicate) will be sampled each day. Capillary tubes will be used to collect blood; blood samples will be centrifuged (3000 x g for 10 min at 4°C), and resulting plasma stored (-80°C). The sex steroids 17β-estradiol (E_2) and testosterone (T) in female minnows, and 11-ketotestosterone (11-KT) and T in males will be measured in plasma using ELISAs (Cayman Chemical Co., Ann Arbor, MI). The following tissues will be dissected from each fish for analysis of HPG axis-associated gene expression: hypothalamus, pituitary gland, gonads, and liver (*females only*). Gonads will be weighed to calculate the GSI, and then subdivided for gametogenic staging using histology (fixed in Bouin's solution and embedded in paraffin) and for gene expression analyses (flash-frozen in liquid N_2) using quantitative real-time PCR (qPCR). qPCR will be used to quantify relative mRNA levels of genes related to HPG axis signaling and regulation of steroidogenesis and gametogenesis **(Fig. 3)**. That list of genes will include key steroidogenesis enzymes (i.e., ovarian aromatase *cyp19a1a*), hormone-encoding genes (i.e., follicle-stimulating hormone [Fsh] β-subunit, *fshb*) or hormone receptors (Fsh receptor, *fshr*), or oogeneic proteins. The full list of mRNAs to be quantified will correspond to those measured in Bock et al.[17].

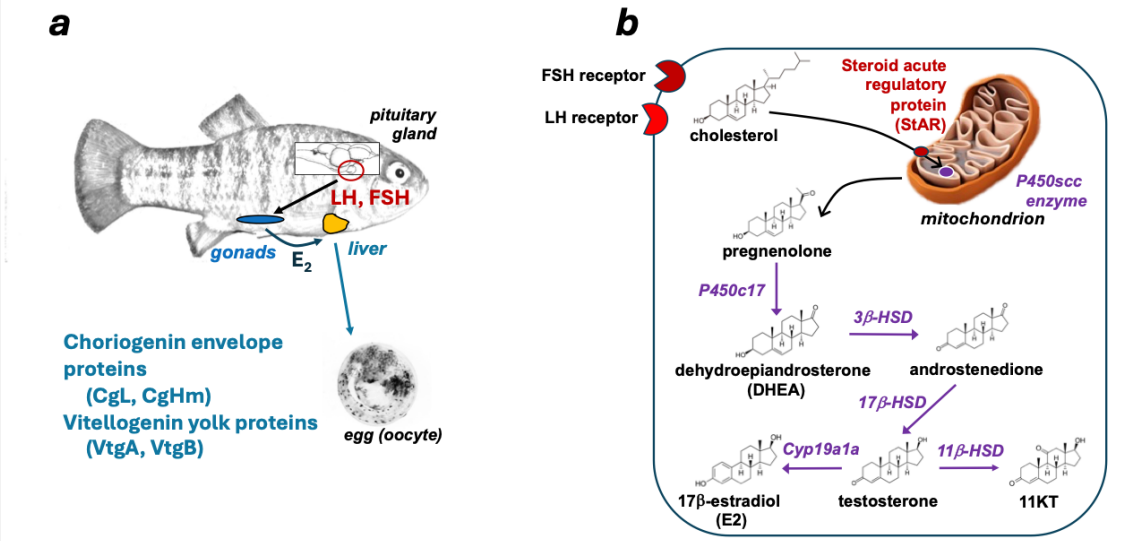

Fig. 3. a) HPG axis pathways and liver oogeneic proteins (choriogenins, vitellogenin) to be measured using ELISAs or qPCR. **b)** Schematic of steroidogenesis in gonadal cell. Relative mRNA levels of shown hormone receptors and enzymes (purple) will be measured using qPCR.

Data Analyses

Plasma hormone concentrations of E_2, T, and 11-KT will be analyzed for each sex separately using 2-factor ANOVA models with temperature treatment, sampling time/day, and the interaction between temperature and sampling day. Gonad GSI values and measures recorded from histological staging of the gonads will similarly be compared using a 2-factor ANOVA model for each sex separately. Quantified gene transcripts obtained from the dissected tissues will be compared through an ANOVA model with temperature, sex, and temperature-sex interactions. For all ANOVA models, *post-hoc* multiple comparison tests will be used to make pairwise comparisons between temperature treatments on the sampling day, or across sampling days within a temperature treatment.

Broader Implications

Understanding how heat wave events affect fish reproductive physiology and performance is critical for predicting impacts of these events on marine and estuarine fish populations. At present, major gaps remain in our understanding of how short-term extreme temperature events affect fish reproductive processes, even though many of the hormone pathways that regulate reproductive processes in fish are known to be thermally sensitive and potentially impaired by atypically high

temperatures[11]. This proposed research will help provide crucial insights into how fishes respond to short-term increases in temperature under a thermal regime of fluctuating temperatures, which is ecologically relevant for coastal habitats like estuaries and tidepools. With average ocean temperatures rising and marine and atmospheric heat wave events becoming more common and severe, it is crucial to establish a solid foundation for how fishes will respond to warming waters.

References (0 points): no limit

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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.**

Summer of 2024

- Begin experiment: acclimation period for sheepshead minnow (June 2024); assignment of fish to experimental tanks (late-June to early July 2024); run and complete experimental 'heat wave' treatments (early-July to early-Aug 2024)
- ELISA measurement of E_2, T and 11-KT hormones from plasma (early- to mid-Aug 2024)
- Analyses of plasma hormone data (late-Aug 2024)

Fall 2024

- Collection data from tissues using real-time quantitative PCR: RNA extraction, reverse transcription, and qPCR measurement of cDNAs of interest (Aug 2024 – Feb 2025)
- Draft and submit abstract for presentation of initial data results for conference presentation in Winter/Spring (Society for Integrative and Comparative Biology meeting; region CalNeva American Fisheries Society (AFS) meeting)
- Write Methods sections for manuscript(s)/thesis

Winter 2025

- Continued collection and analyses of qPCR data from gonad, hypothalamus, pituitary gland and liver tissues
- Sectioning and histological staining of fixed gonadal tissues for gametogenic staging Write methods sections for manuscript(s)/thesis
- Write Results sections for manuscript(s)/thesis

Spring 2025

- Complete manuscript/thesis writing and M.S. thesis defense

Throughout the duration of this project, I will also be working alongside the Cal Poly Santa Rosa Creek Foundation (SRCF) to increase marine science communication to underserved high school students. These outreach events occur twice per academic quarter at Cal Poly and consist of both open house events and in-person school visits in the Central Valley of California. In each visit, we present engaging presentations about our own personal M.S. degree research as well as marine-related research and broader topics.

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

With the rising threat of climate change, the ocean is becoming a more variable environment. Such drastic changes will impact populations of marine organisms; however, the underlying physiological mechanisms are yet to be explored under conditions of high fluctuating temperatures, or more specifically, anomalous marine heatwave events. Physiological mechanisms associated with reproduction are thermally sensitive, and for this reason, there is a dire need to further research the impact of marine heatwaves on fish reproduction. Most fish reproductive studies often use stagnant thermal regimes to understand signaling inhibition at high temperatures. Such methods are not ecologically relevant to the thermal conditions occurring within the coastal ocean. The novel thermal regime within this project will simulate the thermal fluctuations exhibited by marine heatwaves. Very few studies, and none in the Sheepshead Minnow, have examined HPG axis signaling under such temperature fluctuations. This research will play a critical role in expanding our understanding of fish reproduction during periods of environmental uncertainty, and the consequences these conditions pose to fish populations both in the wild and in aquaculture. The fluctuating thermal regime will serve as a source of reference for future experiments that aim to adequately address how other thermally sensitive physiological processes are impacted by the high temperature fluctuations brought on by climate change.

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

The Sheepshead minnow is not native to California, but it is a model organism that can withstand the thermal stress of this experiment. This project will provide information on how fish reproduction is affected by marine heatwaves, which in California are gradually increasing in duration and frequency (Oliver et al. 2018). Recently, California's coastal ecosystem has been recorded to have fluctuating environmental variables such as rising sea levels, temperatures, and acidity (CEC 2018). California's coastline is an abundantly diverse ecosystem that supports many marine organisms, so understanding the lasting effects of marine heatwaves, especially on fish reproduction, is crucial.

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
Electric Tank Chiller (Active Aqua)	$450	1	-	$450
qPCR assay reagents (PowerUp SYBR Green qPCR master mix, 2x 5ml)	$950	1	-	$950
Living Expenses (2 months)	-	-	$2,600.00	
		Subtotals:	*$2,600.00*	*$1,400.00*
		Grand Total	**$4,000.00**	

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

Graduate Student Salary

I will be allocating $2,600 of the award amount towards personal expenses such as rent, utility bills, and gas. This will enable me to work roughly 125 hours during the summer and dedicate the necessary amount of time to accomplishing the experimental procedures of my project (assessing the impact of marine heatwaves on fish reproduction). This is the most time-sensitive and labor-intensive portion of the proposal, so the funds incurred during that period will buffer my financial security and increase my work efficiency.

Equipment and Reagents

The Cal Poly SLO William and Linda Frost Center for Research and Innovation is a state-of-the-art interdisciplinary lab space that is home to my PI's (Dr. Lema) research lab. This lab space is equipped with plate readers for ELISA assays and two real-time qPCR systems, so funds are requested here for select additional equipment (tank water chiller) and reagents (qPCR master mix) to support the experimental design and data collection for this project. Electrical thermostat chillers and heaters will be used to simulate marine heatwaves and temperature loggers will record the changes in temperature throughout the experiment. Funds will be requested for an additional tank chiller need to generate the fluctuating temperature regimes and for some quantitative PCR (qPCR) assay reagents (Applied Biosystems PowerUp SYBR Green master mix).

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.

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:

Isabel Villafuerte

CSU Advisor Information:

Name:	Dr. Sean Lema	Phone:	
Department:	Biological Sciences	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:  Date: **1/31/2024**