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

Graduate Student Research Award Program
AY 2019-2020 Application Form

Application Deadline: Thursday, January 30, 2020, 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 to graduate@share.calstate.edu.

Student Applicant Information

First Name:	Peter	Last Name:	Nilsson
CSU Campus:	Long Beach	Student ID#:	
Email:		Phone:	
Degree Program:	Biology	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:	Bruno	Last Name:	Pernet
CSU Campus:	Long Beach	Department:	Biological Sciences
Email:		Phone:	

Research Project Title:	Effects of naturally occurring inedible particles on the feeding and time to metamorphic competence of echinoderm larvae
Project Keywords (5-7 keywords related to your project):	Larval ecology, planktonic larval duration, Echinodermata

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

Award amount directly to awardee:	$749.00
Award amount to Department:	$2,251.00

Please refer to the Award Announcement for detailed instructions on the information required for each of the following sections.

Project Description (60 points total)-1,500 word maximum; any text over this limit will be redacted

Background

Many marine invertebrates spend their larval period feeding on particles in the plankton, developing until they are ready to metamorphose into juveniles. The time it takes larvae to consume enough particles to develop to the point that they can metamorphose is known as the minimum planktonic larval duration (mPLD.)

The length of the mPLD is important because it affects larval mortality and possibly dispersal. Field surveys show echinoderm larvae have an average instantaneous mortality rate of 0.15 per day.[1] Any factor that lengthens mPLD thus increases the cumulative chance of mortality and therefore reduces the number of larvae that survive to the juvenile stage. Larval dispersal distance may also increase with the mPLD.[2] Dispersal distance affects recruitment[3] and genetic connectivity,[4] and is especially important to the population dynamics of benthic invertebrates, many of which are sedentary or sessile as adults.

Numerous studies have investigated factors that affect mPLD. Higher temperatures generally increase the rate of development[5, 6] while ocean acidification may increase or decrease it depending on temperature[7]. Low food concentrations, which larvae in nature often experience,[8,9,10] lengthen mPLD. While previous studies have examined food limitation as a function of food concentration, the concentration of inedible particles may also impact food availibility. Larvae are limited in the size of particle that they can ingest, and particles that are too large to be ingested might interfere with feeding. This interference might reduce clearance rates enough to extend mPLD and thus affect larval mortality and dispersal.

Such effects are suggested by the three studies that have examined larval feeding in the presence of large particles. In one study, plutei consumed less of a small alga when a large rod-shaped diatom was present.[11] In a second study, large latex beads reduced clearance rates of veligers and copepodites.[12] In the third study, the larvae of five out of six tested echinoderm species exhibited lower clearance rates in the presence of low concentrations of inedible particles.[13]

These studies have three key limitations. First, they have not identified the mechanism responsible for feeding interference. Second, they do not approximate natural conditions, using only a single type of inedible particle in contrast to the heterogenous particle populations that occur in the plankton. Finally, they only examine the immediate effects of inedible particles on food clearance, leaving the long-term effects on larval development unknown.

I will address these questions in my master's thesis research. I will use an echinoid (*Dendraster excentricus*) and an asteroid (*Astropecten armatus*) as models. Both species have long reproductive seasons and have been used for prior research in this lab.[13,14]

Research objectives

The goal of this research is to expand our understanding of how inedible particles affect the mPLD of planktonic echinoderm larvae. I will investigate three research questions:

Question 1: By what mechanism do inedible particles reduce clearance rates of echinoderm larvae?

Hypothesis 1: Inedible particles small enough to enter the mouth but too large to be swallowed decrease the feeding performance of echinoderm larvae by blocking the ingestion of food particles.

Question 2: Do inedible particles found naturally in the plankton reduce clearance rates of echinoderm larvae?
Hypothesis 2: Natural assemblages of inedible particles reduce the clearance rates of echinoderm larvae.

Question 3: Do inedible particles have long term effects on the development of echinoderm larvae?
Hypothesis 3: The presence of inedible particles increases the minimum planktonic larval duration of echinoderm larvae.

Experimental design and methods
Echinoderm spawning and larval culture

Adult *D. excentricus* and *A. armatus* will be collected and housed in a seawater system at CSULB. I will inject individuals with KCl (*D. excentricus*)[15] or 1-methyladenine (*A. armatus*)[14] to induce spawning. Larvae will be reared at concentrations of 0.25 embryos · mL^{-1} at 16 °C while stirred by an automated paddle system.[15] They will be fed laboratory cultured *Rhodomonas lens* at a concentration of 5000 cells · mL^{-1}. I will also culture *Coscinodiscus radiatus* to serve as inedible particles in the long-term exposure experiment.

By what mechanism do inedible particles reduce clearance rates of echinoderm larvae?

Small inedible particles (SIPs) that can enter a larva's mouth but not the esophagus might slow a larva's feeding by blocking food particles from being swallowed. To determine whether this is a source of feeding interference I will compare clearance rates of larvae feeding in the presence of SIPs to those feeding in the presence of larger particles (which would not block food particles). I predict that the clearance rates of larvae feeding among SIPs will be lower than that of larvae feeding among larger particles.

I will prepare three treatments: filtered seawater (FSW) with SIP-sized beads, FSW with larger beads, and FSW without inedible particles (control). Inedible particles will be at concentrations of 500 beads · mL^{-1} (which had a strong effect in previous experiments[13]). Each treatment will also contain edible (6 µm) fluorescent beads at a concentration of 650 beads · mL^{-1}. I will prepare six 20 mL scintillation vials for each treatment (for a total of 18). I will starve larvae for three hours, then transfer 10 into each vial and place each vial on a plankton wheel. I will allow the larvae to feed for 10 minutes, then terminate feeding with formalin. I will examine the gut contents of each larva and count ingested fluorescent beads. Using R v3.6.1[16], I will determine whether the data meet assumptions of normality (Shapiro-Wilk test) and equal variance (Levene's test) then perform a one-way ANOVA (on transformed data if needed) to assess whether clearance rates differed between treatments. If SIPs yield the lowest food clearance rates, this will support the hypothesis that inedible particles interfere with feeding by blocking the ingestion of food particles.

Do inedible particles found naturally in the plankton reduce clearance rates of echinoderm larvae?

To determine whether large particles found in natural plankton assemblages interfere with larval feeding, I will compare clearance rates of larvae feeding in plankton with and without the

large particles filtered out. I will collect seawater from the mouth of Alamitos Bay and prepare three suspensions: one of seawater filtered to remove all particles too large to be consumed ("Inedible Absent"), one of seawater filtered through a larger (250 µm) mesh to remove only the largest items such as potential predators ("Inedible Present"), and one of 250 µm-filtered seawater with an additional 500 cells · mL^{-1} of *C. radiatus* ("Inedible Enhanced"). Each treatment will also contain 650 beads · mL^{-1} of 6 µm fluorescent beads. The treatments will be added to six scintillation vials each (total of 18), and 10 larvae will be added to each vial and allowed to feed for 10 minutes as in the previous experiment. I will use a FlowCAM (an imaging cell counter) to characterize the particle size distributions of all three suspensions while also capturing particle images for identification, which will allow me to generate hypotheses about whether specific types of inedible particles are particularly problematic for larval feeding. I will also use the FlowCAM data to estimate the concentration of inedible particles.

I will perform one-way ANOVAs on clearance rates for both species in order to determine whether natural inedible particles significantly interfered with feeding. I will repeat this experiment four times over the course of a year to obtain particle assemblages from each season. I predict that in each case clearance rate will be highest when large particles are filtered out. I will create a generalized linear model in R relating clearance rate in the "inedible present" treatments of each experimental run to the concentration of inedible particles. I predict that clearance rates in the "Inedible Present" and "Inedible Enhanced" treatments will be negatively correlated with the concentration of inedible particles.

Do inedible particles have long term effects on the development of echinoderm larvae?

In order to establish whether the immediate effects on feeding translate into long term effects on development, I will compare the growth of larvae reared with the same concentration of food (*Rhodomonas lens* at growth-limiting concentrations) in the presence of different concentrations of inedible particles. I will rear larvae in three treatments with different concentrations of *C. radiatus* (0, 100, and 500 cells · mL^{-1}), plus a fourth control with the same quantity of *C. radiatus* as the 500 cells · mL^{-1} treatment, but with the cells in a plastic container covered in a 20 µm mesh as a control to ensure that effects of toxic exudate – if any – are not mistaken for effects of particle interference. I will measure the protein mass of larvae at 7 days post fertilization and (for *D. excentricus* only) test for metamorphic competence daily beginning at day 10. I will perform ANOVAs for protein mass and time to 50% metamorphic competence to determine whether they differ between treatments. In both species, I predict larval protein mass will be inversely related to the treatment's inedible particle concentration. I also predict *D. excentricus* larvae reared in higher concentrations of inedible particles will take longer to reach metamorphic competence.

References (0 points)-no limit

1. Rumrill, S. S. (1990). Natural mortality of marine invertebrate larvae. *Ophelia*, *32*(1–2), 163–198.
2. Shanks, A. L. (2009). Pelagic larval duration and dispersal distance revisited. *Biological Bulletin*, *216*(3), 373–385.
3. Gaines, S. D., & Lafferty, K. D. (1995). Modeling the dynamics of marine species: The importance of incorporating larval dispersal. In L. R. McEdward (Ed.), *Ecology of marine invertebrate larvae* (pp. 389–412). Boca Raton: CRC Press.
4. Padrón, M., Costantini, F., Baksay, S., Bramanti, L., & Guizien, K. (2018). Passive larval transport explains recent gene flow in a Mediterranean gorgonian. *Coral Reefs*, *37*(2), 495–506.
5. Whalan, S., Ettinger-Epstein, P., & De Nys, R. (2008). The effect of temperature on larval pre-settlement duration and metamorphosis for the sponge, *Rhopaloeides odorabile*. *Coral Reefs*, *27*(4), 783–786.
6. Wangensteen, O. S., Dupont, S., Casties, I., Turon, X., & Palacín, C. (2013). Some like it hot: Temperature and pH modulate larval development and settlement of the sea urchin *Arbacia lixula*. *Journal of Experimental Marine Biology and Ecology*, *449*, 304–311.
7. Gianguzza, P., Visconti, G., Gianguzza, F., Vizzini, S., Sarà, G., & Dupont, S. (2014). Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula* early life stages to CO2-driven acidification. *Marine Environmental Research*, *93*, 70–77.
8. Paulay, G., Boring, L., & Strathmann, R. R. (1985). Food limited growth and development of larvae: Experiments with natural sea water. *Journal of Experimental Marine Biology and Ecology*, *93*, 1–10.
9. Fenaux, L., Strathmann, M. F., & Strathmann, R. A. (1994). Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. *Limnology and Oceanography*, *39*(1), 84–98.
10. Reitzel, A. M., Webb, J., & Arellano, S. (2004). Growth, development and condition of *Dendraster excentricus* (Eschscholtz) larvae reared on natural and laboratory diets. *Journal of Plankton Research*, *26*(8), 901–908.
11. Strathmann, R. R. (1971). The feeding behavior of planktotrophic echinoderm larvae: Mechanisms, regulation, and rates of suspension feeding. *Journal of Experimental Marine Biology and Ecology*, *6*, 109–160.
12. Hansen, B., Hansen, P. J., & Nielsen, T. G. (1991). Effects of large nongrazable particles on clearance and swimming behaviour of zooplankton. *Journal of Experimental Marine Biology and Ecology*, *152*(2), 257–269.
13. Lizárraga, D., Danihel, A., & Pernet, B. (2017). Low concentrations of large inedible particles reduce feeding rates of echinoderm larvae. *Marine Biology*, *164*(5), 1–12.
14. Pernet, B., Livingston, B. T., Sojka, C., & Lizárraga, D. (2017). Embryogenesis and larval development of the seastar *Astropecten armatus*. *Invertebrate Biology*, *136*(2), 121–133.
15. Strathmann, M. F. (1987). *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press.
16. R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Timeline (10 points total)-250 word maximum

Spring 2020 (prior to COAST funding)	Preliminary experiments Trial runs with FlowCAM
Summer 2020	Experiment #1 First run of Experiment #2
Fall 2020	Experiment #3 on *A. armatus* Second run of Experiment #2
Winter 2020	Experiment #3 on *D. excentricus* Third run of Experiment #2
Spring 2021	Fourth run of Experiment #2
Summer 2021	Write thesis
Spring 2022	Defend thesis

Relation to COAST Goals (15 points total)-300 word maximum

This research supports the COAST goal of advancing knowledge of coastal and marine resources and the processes that affect them. Specifically, this study will elucidate the impact of inedible particles on the feeding performance and time to metamorphic competence of planktotrophic larvae of marine invertebrates. This poorly studied aspect of the feeding environment has consequences for the minimum planktonic larval duration and population dynamics of echinoderms. This study will improve our understanding of how the distribution of algae impacts larvae. Likewise, this study may improve our picture of how particulate matter from pollution and runoff–including microplastics–affects larvae. As invertebrates play a vital role in the food webs of coastal and marine ecosystems, this study will inform our view of threats to these ecosystems incurred at a very vulnerable stage of invertebrate life cycles.

Budget and Justification (15 points total)

Item/Description	Unit Price	Quantity	Amount to Awardee (via Financial Aid)	Amount to Department
Algal starter cultures	$175.00	2	-	$350.00
Algal f/2 growth medium	$75.00	1	-	$75.00
Animal collection (per animal)	$5.00	72	-	$360.00
6 µm fluorescent beads (2 ml bottle)	$356.00	1	-	$356.00
30 µm beads (15 ml bottle)	$348.00	1	-	$348.00
45 µm beads (5 ml bottle)	$194.00	1	-	$194.00
60 µm beads (15 ml bottle)	$348.00	1	-	$348.00
BD Accuri C6 flow cytometer fluid kit	$220.00	1	-	$220.00
Rent	$749.00	1	$749.00	-
		Subtotals:	*$749.00*	*$2,251.00*
		Grand Total:	**$3,000.00**	

Justification (250-word maximum):

I will use the algal starter cultures to create lab cultures of *Rhodomonas lens* (for food) and *Coscinodiscus radiatus* (to serve as inedible particles), which I will maintain using the algal growth medium. These will be supplied to the larvae spawned from the adults gathered during animal collection by a commercial collection company.

I will use 6 µm fluorescent beads in food clearance rate trials as they are easy to identify and count in larval stomachs. I will use the 30, 45, and 60 µm beads as inedible particles.

I will use the BD Accuri C6 flow cytometer fluid kit to replace cleaning and sheath fluid consumed while I use the flow cytometer to determine the concentrations of algal cultures, as well as the concentration of 6 µm beads.

I will use $749 to pay for rent, which will reduce the number of hours I need to work and thus leave me more time to work on research.

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