

**APPLICATION FOR
FEDERAL ASSISTANCE**

99E-112
OMB Approval No. 33-12-00

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|--|-------------------------------------|------------------------------|
| 1. TYPE OF SUBMISSION: Application <input type="checkbox"/> Construction <input checked="" type="checkbox"/> Non-Construction Preapplication <input type="checkbox"/> Construction <input type="checkbox"/> Non-Construction | 2. DATE SUBMITTED April 16, 1999 | Applicant Identifier |
| | 3. DATE RECEIVED BY STATE | State Application Identifier |
| 4. DATE RECEIVED BY FEDERAL AGENCY | | Federal Identifier |

5. APPLICANT INFORMATION

| | |
|---|--|
| Legal Name: <u>Tsukimura, Brian</u> | Organizational Unit: |
| Address (give city, county, State, and zip code): Department of Biology 2555 E. San Ramon Ave. California State University, Fresno, CA 93740 | Name and telephone number of person to be contacted on matters involving this application (give area code) Dr. Thomas McClanahan (559) 278-0840 |

6. EMPLOYER IDENTIFICATION NUMBER (EIN):

9 4 - 6 0 0 3 2 7 2

8. TYPE OF APPLICATION:

New Continuation Revision

If Revision, enter appropriate letter(s) in box(es)

A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other(specify):

7. TYPE OF APPLICANT: (enter appropriate letter in box)

A. State H. Independent School Dist. A
B. County I. State Controlled Institution of Higher Learning
C. Municipal J. Private University
D. Township K. Indian Tribe
E. Interstate L. Individual
F. Intermunicipal M. Profit Organization
G. Special District N. Other (Specify) _____

9. NAME OF FEDERAL AGENCY:

Multiple-CalFed Agencies

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

-

TITLE:

12. AREAS AFFECTED BY PROJECT (Cities, Counties, States, etc.):

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Reproductive Life History of Chinese Mitten Crab, *Eriocheir sinensis*: Identification of possible reproductive disrupters to reduce ecological impact on endangered delta species.

13. PROPOSED PROJECT

Start Date Ending Date

14. CONGRESSIONAL DISTRICTS OF:

a. Applicant
19th

b. Project

Delta Districts

15. ESTIMATED FUNDING:

| | | | |
|-------------------|----|-----------|----|
| a. Federal | \$ | 1,095,708 | 00 |
| b. Applicant | \$ | 250,000 | 00 |
| c. State | \$ | | 00 |
| d. Local | \$ | | 00 |
| e. Other | \$ | | 00 |
| f. Program Income | \$ | | 00 |
| g. TOTAL | \$ | 1,345,708 | 00 |

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE 4/16/99

b. No. PROGRAM IS NOT COVERED BY E. O. 12372
 OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?

Yes If "Yes," attach an explanation. No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT, THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

| | | |
|--|--------------------------------------|---------------------------------------|
| a. Type Name of Authorized Representative <u>Thomas McClanahan, Ph.D.</u> | b. Title Associate Vice President | c. Telephone Number (559) 278-0840 |
| d. Signature of Authorized Representative <i>Thomas McClanahan</i> | e. Date Signed April 16, 1999 | |

Previous Edition Usable
Authorized for Local Reproduction

Standard Form 424 (Rev. 7-97)
Prescribed by OMB Circular A-102

CALFED Bay-Delta Program: February 1999 PSP

Title of Project: Reproductive Life History of Chinese Mitten Crab, *Eriocheir sinensis*:
Identification of possible reproductive disrupters to reduce ecological impact on
endangered delta species.

Principal Investigator: Brian Tsukimura

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Graduate students (to be named)
Undergraduate students (to be named)

Type of Organization: CSU Foundation - Non-Profit

CSU Foundation Employer ID # 94-6003272-A2

99E-112

EXECUTIVE SUMMARY

1. Title: Reproductive Life History of Chinese Mitten Crab, *Eriocheir sinensis*: Identification of possible reproductive disrupters to reduce ecological impact on endangered delta species.

Applicant Names: Brian Tsukimura, Department of Biology, California State University, Fresno.

2. Project Description and primary Biological Objectives: This project will determine the reproductive life history of the invasive mitten crab, *Eriocheir sinensis*, and identify possible biological and environmental cues that stimulate the onset of gonadal maturation and downstream migration. Presently, scientifically sound programs cannot be initiated because the basic life history of these invasive crabs is unknown in California. Data from this project will allow the development of proactive programs to control and possibly eradicate these invasive species. To this end, an ELISA (Enzyme-linked Immunosorbant assay) will be developed to quickly assess the reproductive status of females, both prior to and during their downstream migrations. These data are necessary because the reproductive life histories of the mitten crab in Europe are dissimilar to that of their native China. Gonadal maturation data will be collected from both juvenile and adult mitten crabs throughout the year. In addition, environmental factors, such as temperature and light cycle, will be studied to determine their effects on reproduction and downstream migration. These data will identify targets for chemical mediation of reproduction or behavior. These targets will then be investigated using known hormones that function in other crustaceans. Known chemical mediators will be administered to both juvenile and maturing females to determine the effectiveness of these agents in retarding the maturation of gonadal development. Any environmental treatment (with chemicals) might also effect the local crayfish. Thus, similar experiments will also be conducted upon the local species of crayfish to ensure that any proposed treatments will not negatively impact the crayfish industry in the Sacramento River/San Joaquin Delta system. These data will provide insights into the timing and possible mechanisms that stimulate gonadal maturation and the downstream migration. Interference of the stimuli for reproductive maturation would prevent either gonadal maturation or the downstream migration and thus decrease the damage to the endangered and threatened fish caught in the BOR and DWR fish screens. Data from this proposal will greatly enhance the ability of the IEP and Mitten Crab PWT in making critical decisions concerning the regulation, control, and management of the this invasive crab. In addition, the IEP will be able to avoid facing rash measures to control the mitten crab in the event that the exponential increase crab population continues and potentially forces the cessation of water pumping to avoid killing endangered fish species that inhabit the delta system.

3. Approach/Tasks/Schedule: This 3 year project will develop assays to quickly determine the reproductive state of mitten crabs and determine what environmental stimuli are required to initiate gonadal development and downstream migration. Without these data, it will be difficult for any regulatory agency to make scientifically sound decisions on the regulation and control of the invasive mitten crab. The characterization of the vitellin, development of an antibody, and development of the ELISA and Western Blot assays will occur during year 1. Samples from wild crabs and those grown in environmental chambers can be stored until the ELISA is

developed. Sample collection can thus be carried out before the assay is completed operational. Samples will be collected to determine the normal reproductive cycle of the mitten crab in California. In particular, the status of gonadal development during their downstream migration will be examined. The 2nd and 3rd years will involve growth of juvenile crabs in environmental chambers to determine what environmental stimuli, if any, are required to initiate gonadal development and the downstream migration.

Justification for project: CALFED is investing significant effort into restoring habitats and ensuring a proper water supply and quality for endangered species. The invasive Mitten Crab population has increased exponentially over the last 4 years. Their immediate impact on the ecosystem has yet to be determined. It appears that this omnivorous crab will greatly impact its environment. As plans to control these crabs have been developed, the absence of information on this crabs basic life history, particularly its reproductive life history, makes difficult any logical decisions. Determination of the reproductive life history was cited as one of the most important pieces of information required by the Mitten Crab PWT of the IEP.

Budget Cost and Third Party Impacts. This project requests \$1,095,708 for 3 years. It covers personnel, expenses and indirect costs. Matching funds are provided by the purchase of 2 new environmental chambers for the Department of Biology over the next 2 years. The School/Department will release my time 0.25/semester/year. Over the three year period, \$12,300 will be made available in disposable supply finds and \$1,500 in travel. Because most of this work will be completed on the CSU Fresno campus, there are no anticipated third party impacts.

Applicant Qualifications: The PI has been working in crustacean reproduction through his Master's, Ph.D., Post-doc, and now as an assistant professor, for a total of 17 years. The PI has 8 publications (and 2 in press) relating to crustacean reproduction. In addition, the PI has 9 presentation in the last 4 years on crustacean reproduction, all of which have involved graduate students. The PI has been able to obtain internal funding every year at Fresno State, including release time to conduct research, and external funding from California Sea Grant to study shrimp reproduction. The PI is a member of 6 national

Monitoring and Data Evaluation: The description of vitellin will be published in peer reviewed journals. The data collected in this study will be analyzed using standard biometric techniques including repeated measures ANOVA, linear regression and ANCOVA. Data will be presented at national (SICB) and regional meetings, and published in peered reviewed journals.

Compatibility with CALFED Objectives: This project directly addresses the concerns of invasive species, in particular, the Chinese mitten crab (p.419, 467). The exponential population growth of this crab has made difficult any assessment of its impact on native species, particularly, the endangered, threatened and recovering species. The massive downstream migrations each Fall must have some effect on the local habitats and their ability to provide sufficient food for all the species. In addition, the ERP sites the potential for levee damage and thus the control of this species has become a priority. These crabs have become a significant problem at the DWR and BOR, clogging the fish screens and compressing recovered fish. This indicates the urgency to acquire basic biological information, particularly reproductive life histories, so that each agency can develop scientifically sound plans to control this invasive crab.

PROJECT DESCRIPTION

This project plans to provide the basic reproductive life history of this invasive species so that the appropriate agencies will be able to develop proactive scientifically sound management plans to control, and possibly eradicate, this potentially devastating crab. To this end, this project plans to develop an assay that will allow the rapid examination of the reproductive status of females. With this assay, data can be collected that will describe the reproductive maturation of female gonads during their downstream migration to the San Pablo Bay. These data will provide information on the timing of gonadal development and suggest mechanisms that regulate gonadal development and the downstream migration.

Project Approach

Animals: Females on their downstream migration will be collected at the BOR facility in Tracy and transported back to the Animal facility in the Department of Biology at California State University, Fresno. Juvenile mitten crabs, *Eriocheir sinensis*, will be collected in South San Francisco Bay along tributary streams. Permits to collect (#801162-05), transport and maintain (#2653) crabs have already been obtained from DFG.

Vitellin Isolation: Vitellin (Vn), the most common yolk protein in oocytes, will be isolated from the excised ovarian tissue using a common technique (Riley and Tsukimura, 1998; Tsukimura et al., in prep). The ovaries will be homogenized in 10 ml/g (wet tissue weight) of Homogenization Buffer (0.1 M NaCl, 0.05 M Tris, 1 mM EDTA, and 0.1% Tween-20; 10 µg/ml each of leupeptin and PSMF, pH 7.8). The homogenate will be centrifuged at 4,000 x g for 5 min. The supernatant will be collected and centrifuged at 20,000 x g for 20 min. This successive supernatant will be combined with saturated Ammonium Sulfate (SAS) to produce a 25% SAS solution, incubated for 1 hr., and centrifuged at 20,000 x g for 10 min. The supernatant will be collected and sequentially combined with SAS to produce 40%, 50% and 60% solutions. Pellets from the 60% solution will be resuspended in homogenization buffer and dialyzed at 4°C for 12 hours with three changes of homogenization buffer. Purified Vn will be stored at -20°C until needed.

Antisera Preparation: New Zealand white rabbits will be injected with 500 µg of purified Vn in Freund's Complete Adjuvant. Three boosters of 100 µg Vn in Freund's Incomplete Adjuvant will be administered over the following eight weeks. The final antiserum titer will be 1:12,000 or higher as determined by enzyme-linked immunosorbent assay (ELISA) as performed below. Antisera will be diluted with phosphate buffer solution (PBS: 150 mM NaCl; 1.6 mM KH₂PO₄; 8 mM Na₂HPO₄; 2.7 mM KCl; pH 7.4).

Electrophoresis: To determine the purity of the Vn preparations, both from ovaries and eggs, non-reducing electrophoresis will be performed. To determine molecular composition, Vn preparations were analyzed by SDS-PAGE (Laemmli, 1970) with two minor modifications: (1) not boiling the yolk preparations prior to electrophoresis and; (2) adjusting the pH of buffers above 7.0 to reduce precipitation of Vn. Proteins were fixed and stained with Coomassie stain (50% methanol; 10% acetic acid; and 0.2% Coomassie Brilliant Blue R-250).

Western Blot Analysis: SDS-PAGE gels will be rinsed in transfer buffer (48mM Tris; 39 mM glycine; 20% methanol, pH at 9.2) for 15 min. Proteins will be electroblotted to a transfer membrane (Immobulon-P PVDF, Millipore) for 1 hour. The membrane will be blocked with 5% nonfat dry milk in PBS-T (0.7% Tween-20 in PBS; pH 7.4). Visualization will be attained by

incubation of the blotted membrane with anti-Vn antisera (1:16,000, preabsorbed with male hemolymph), followed by incubation with the secondary antibody (Goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (Gt α Rb IgG -HRPO, Sigma #A9169)) at a concentration of 1:1,000. The blot will be developed with (2% CoCl₂; 0.08% 3'3'diamino-benzidene, 0.03% H₂O₂) in PBS.

Chromatography: To quantitate the molecular weight of the yolk, Vn will be passed through a gel filtration column (Superose-12, Pharmacia) using Homogenization buffer as a solvent, with a flow rate of 0.25 ml/min (ESA) and elution time will be detected with a Beckman 166 detector. Column elution times were equilibrated with standard molecular weight markers.

ELISA: An ELISA will be developed to determine the hemolymph levels of Vn. The 96-well EIA plate will be coated with 5 μ g/ml Vn in carbonate buffer (60 mM NaHCO₃, pH 9.6). The wells were blocked with 0.05% gelatin. Competition reactions of anti-Vn antibody (1:12,000) and antigen will be completed at room temperature for 1 hour or overnight at 4°C in separate culture tubes (12 x 75 mm). The antigen sample consisted of hemolymph diluted 1:50 or 1:100, and the standard curve samples will be of serially diluted amounts of Vn (standards ranged from 0.1 ng to 1,000 ng in half log units). Competition reaction mixture (100 μ l) will be added to wells of the ELISA plate and allowed to come to equilibrium for 1 hr. A secondary antibody (Gt α Rb IgG -HRPO) at 1:1000 dilution will be incubated for 1 hr. The colorometric analysis was accomplished by adding development solution (50 mM citrate acid at pH 4.0; 0.48 mM 2'-2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) diammonium salt; and 2.4 mM H₂O₂) and detected on an ELISA plate reader (BioRad 2550 EIA Reader) at 405 nm. To determine the linearity of measurement, hemolymph from males and females will be serially diluted from 1:50 dilution to 1:3,200. In addition, to determine whether diluting hemolymph affects the linearity of measurement, hemolymph from a reproductive female will be serially diluted to decrease the antigen concentrations, and a constant level of hemolymph will be maintained by adding male hemolymph.

Reproductive Cycle: Hemolymph will be drawn from the posterior leg sutures in the ventral sinus of the female crabs. The samples will be diluted in hemolymph buffer and stored at -20°C until examined by ELISA. These data will initially be collected from 10 individuals biweekly from two-year old females starting in June. In addition, crabs collected at the BOR Tracy facility will be bled and dissected to determine their reproductive state through out the downstream migration season, which is from August through February. These data will be used to create a table against which vitellin levels from females can be compared to estimate their reproductive progress.

Environmental Factors: Two- and three- year old female crabs (n=10) will be housed in environmental chambers (temperature and light controlled) where the daylight cycles will be slowly reduced to emulate the onset of Fall and Winter. Biweekly samples of hemolymph will be collected and analyzed by ELISA. In addition, a separate group of females will be housed in the environmental chambers, where the temperature will be slowly decreased to emulate the declining water temperatures associated with the onset of Fall and Winter. Lastly, both the temperature and daylight will be reduced to ascertain whether both stimuli are required to commence downstream migrations or gonadal maturation. To determine if male behavior is stimulated by the same environmental factors as females, male crabs of the same ages will be treated with decreasing light, temperature or both. If the downstream migration behavior is sexually dimorphic then it might be possible to eventually test whether the males and females can be separated from each other during this downstream migration and reduce their fecundity.

This might provide an eventual avenue for control, by controlling major river temperatures through water releases from dams to separate males from females during the mating periods. **Hormonal Factors:** In an attempt to inhibit the downstream migration behavior, injections of methyl farnesoate will be made into males and females. This hormone is thought to act like insect juvenile hormone and promote juvenile characters by inhibiting adult characters such as maturation of the gonads. Recent data from our lab indicates that very small doses can inhibit the gonad development in tadpole shrimp, *Triops longicaudatus*.

Proposed Scope of Work

Project Implementation: Task 1. Purification, Isolation, and Characterization of Vitellin (yolk protein). This process should require less than 4 months. This step will identify a protein that can be easily monitored to assess the reproductive state of female crabs. The characterization of vitellin will be achieved using FPLC and electrophoresis.

Task 2. Develop antibody. The purified vitellin will be used to develop an anti-vitellin antibody, which will take about 6 months. The antibody should have a titer over 10,000 such that it will be usable to develop both ELISA and Western Blot assays. The characterization of vitellin will be achieved using FPLC and electrophoresis. Development of both assays requires about 2-3 additional months.

Task 3. Collect downstream migrating females and monitor the reproductive state of females through time. Hemolymph samples will be collected from females and yolk concentrations determined by ELISA. These data will be correlated to the state of oocyte development to create a table that can be used by researchers to determine the reproductive state of crabs without having to sacrifice each animal. These samples will be taken during each downstream migration. As the ELISA is developed, the vitellin in each sample will be quantitated.

Task 4. Determine the Normal Reproductive Cycle. Young females will be collected and raised in environmental chambers under conditions that emulate the ambient environment. These individuals will be bled and the samples stored until the ELISA is operational. Data from these studies will provide a baseline for future treatments.

Task 5. Environmental Stimulation of Gonad Maturation and Downstream Migration. Using environmental chambers, day length, temperature or both will be slowly decreased to determine whether these stimuli are used as environmental cues to bring about the onset of gonad maturation and downstream migration. To ascertain the effectiveness of methyl farnesoate injections on inhibiting the downstream migration or gonadal maturation, daily injections will be administered.

Task Separation: Tasks 1 & 2 are required for the completion of tasks 3, 4, and 5. Each of these latter tasks do not require each other, however, the strength of the data collected in each task is greatly enhanced by each other. In other words, the whole is greater than the sum of its parts.

Location and Geographic Boundaries: Other than collecting juvenile crabs in South San Francisco Bay tributaries, and downstream crabs from the BOR Tracy facility, most of this project will be conducted on the campus of CSU Fresno (Fresno County), in the Biology Department and its environmental chambers.

Ecological/Biological Benefits

The Chinese mitten crab, *Eriocheir sinensis* (Decapoda: Grapsidae), was first found in California in South San Francisco Bay in 1992. Since that time, their population has exploded into the Sacramento-San Joaquin Delta system and its associated tributaries. These crabs spend most of their lives as juveniles living in freshwater habitats. In California, these now range from as far North as Colusa and East as far as Marysville, and South down to San Luis National Wildlife Refuge in Merced County (Veldhuizen, 1997; K. Heib, pers. comm.). A few crabs have been reported to the California Department of Fish and Game (DFG) in Kettleman City walking downstream.

The threat to California is that these crabs are omnivores and supposedly, young juveniles eat mostly vegetation. Farmers have become concerned because the extensive canal system throughout our State, is a perfect vehicle for a wide distribution of these crabs. Row crops (e.g. cotton, rice and vegetables) are particularly susceptible to destruction by the young crabs, which are primarily herbivorous. The adult crabs are also becoming a problem to agriculture, by clogging the fish screens of the intakes of the Delta-Mendota Canal and California Aquaduct during the downstream migration to breed in the saline waters of the San Francisco Bay. By clogging and damaging with the fish screens that recover endangered fish species, the crabs may prohibit the pumping of water during their downstream migration to protect the endangered and threatened fish. As this is unacceptable to agricultural interests, a great conflict is about to unfold.

Primary Stressor: Invasive aquatic organisms (V1 - p. 460) are a threat to the Bay-Delta system because they have the potential to displace or replace native organisms. The mitten crab, is believed to threaten delta levees, though more recent discussions have focused on the effects on crayfish and potentially the eggs, fry and parr of fish that breed or develop in the Delta region and its immediate tributaries. Damage to these populations could occur both on the upstream and downstream migration, as well as during the crab juvenile inhabitation of the delta region. As mentioned, the impact on recovery of endangered fish in fish screens will also be of major concern.

Identification and of expected benefits: This project proactively addresses the 7th strategic objective in Invasive Aquatic Organisms, which is to eliminate or control to a level of little significance all undesirable non-native species. Data generated from this study will outline the reproductive life history of the Mitten crab, identify peculiarities in this life history to California, identify the stimuli for gonad maturation and downstream migration. These data will then allow the appropriate regulatory agencies to make scientifically sound decisions concerning the regulation and control of this invasive crab species. A probable cause for the downstream migration is a change in the water temperature. This adaptation may be sexually dimorphic, where males and females respond differentially to the changes in water temperature. This might provide an eventual avenue for control, by controlling major river temperatures through water releases from dams to separate males from females during the mating periods. **Secondary Benefits:** In addition, several assays that will rapidly identify the reproductive state of the mitten crabs will be developed. Hormonal inhibition of reproductive maturation and downstream migration will be tested with methyl farnesoate injections. Stimuli of reproductive activities will provide insights into possible sites of regulation that might be retarded or blocked. Determination of the stimuli for downstream migration will provide a target against which agencies can focus to reduce the adverse effects of the crabs on native habitats and foodwebs.

Certainly, the movement of hundreds millions of crabs will negatively impact both. Additionally, the destruction to levees by the mitten crabs has yet to be fully determined. But with 30 burrows, 18" in depth or more, per square meter, the likelihood of severe levee damage is high. This places an increased importance on the regulation and control of mitten crabs. **Benefits to Third Parties, Other Restoration Programs, and CALFED Non-ecosystem Objectives.** By determining environmental targets for mitten crab reproductive control, alternative measures to shutting down the Delta-Mendota Canal and California Aquaduct of removing the fish screens while the crabs are in their downstream migration will be provided. Removing the fish screens will incite lawsuits under both CESA and ESA. Thus, third parties that benefit will thus be BOR, DWR, DFG, FWS, farmers of the San Joaquin Valley and Southern California water users. The potential damage to levees by the burrowing behavior of these crabs is also of great interest to ACOE.

Linkages: This project directly addresses the concerns of the invasive aquatic organisms (p.461), Chinese Mitten crab. In particular, this project will provide data with which strategic plans to control eliminate the mitten crab can be derived (V1 - pp.419, 467). The primary potential damage outlined the ERP is damage to levees (V1 - p. 461). The mitten crab will interfere with restoration plans through their burrowing behavior (V1 - p. 438). Because the Chinese mitten crab is still in exponential growth, it is unclear at what level the population will start to level off and what there final impact on the native habitat and ecosystems will be. Current studies by other researchers (at UC Berkeley) are investigating the role of the mitten crab on the signal crayfish (V1 - p. 415). If the mitten crab does have a negative impact on the crayfish, a method of control will be necessary for the maintenance of this species that then requires a plan to eliminate or reduce the mitten crab, which is interfering with the establishing and enhancing the sustainable commercial species (V2 - p 18). The impact of the mitten crab on grass shrimp is being studied by DFG (V1 - p. 417). Again the mitten crab will likely threaten this recovery effort if the crabs are not controlled. The impact of the mitten crab on native fish species is still unknown, but is believed these omnivorous crabs will devour any small fry and pair unable to escape their claws. This has potential impacts on endangered fish species, particularly the anadromous species. With the crabs increasing at exponential rates, it appears that predation upon the endangered fish (delta smelt (V1 - p. 194), longfin smelt (V1 - p.201), green sturgeon (V1 - p. 205), splittail (V1 - p.209), and salmonids (V1 - p. 219, 229) will soon become a significant problem. In addition, there is no definitive data on the impacts by crabs on the fry and eggs of the white sturgeon, striped bass and warm-water game fish which breed in the delta region.

Non-ecosystem Objectives: If indeed water temperature is a sexually dimorphic trait that can be used to separate males from females, this would link to Central Valley Stream flows and greater protection for native anadromous fish species, particularly with the Central Valley water temperatures (V1 - p. 57). In addition, periodic purges of cold water could be used to reduce fine sediment build-up (V1 - p. 66).

Technical Feasibility and Timing

Currently, there is little data concerning the activity of the mitten crab in the Bay-Delta region. Most, if not all, of the data on the mitten crab in California is anecdotal. Thus, without experimental data, no agency will be able to develop scientifically sound plans to control and eradicate this invasive crab. Thus the data derived from this project is essential to achieve any regulation of the crab population explosion.

The equipment to conduct this project exists within the Department of Biology, CSU Fresno. We are acquiring two new environmental chambers in which the environmental experiments can be conducted.

Permits to collect (#801162-05), transport, and maintain (#2653) crabs have already been obtained from DFG. The Department of Biology possess a USDA licensed animal facility in which some of the crabs will be maintained.

The only constraint that might arise is our inability to collect juvenile crabs. With the current population explosion, we do not believe that this will be a problem.

Monitoring and Data Collection

See Table 1

The complete absence of scientifically derived data concerning the Chinese mitten crab makes difficult any attempt to derive a strategic plan to control and regulate the expansion of this invasive crab. The data collected here will be used to establish baselines from which strategic plans and experiments can be drawn. The project will determine the normal reproductive cycle in California (since the European crabs differ so greatly from China). Also, the stimuli for gonad maturation and downstream migration will be determined. It is probable that the downstream migration environmental signal is water temperature or daylight. Both will be examined in this project. In addition, the effect of methyl farnesoate will be examined in lab. This terpenoid compound has been found in our lab to inhibit the formation of ovarian tissue in the invasive tadpole shrimp, *Triops longicaudatus*. We will feed and inject crabs with this material to determine if this same effect occurs in crabs. Crayfish also will be tested to determine if there is a possible detrimental effect.

The critical data collected from this project require the development of the ELISA, if we are to avoid having to sacrifice every animal examined. To properly conduct these experiments without the ELISA would require the storage of copious amounts of crabs. This would entail a significantly higher budget for feeding and care of all these organisms. In addition, the sampling error within each experiment would greatly increase.

The proposed work will be conducted under laboratory conditions, and thus the timing of the research is independent of weather and seasonal conditions. Protocols that will be used are published in Riley and Tsukimura, 1998 (see PI CV). Data from this project will be disseminated through refereed journals and through presentations at CALFED, national and regional meetings.

Local Involvement

Whereas this project is primarily research, the amount of local involvement will be limited. The BOR has offered to supply crabs that are caught in their fish screens when available. In years 2 and 3, the PI will involve the San Joaquin River Parkway in monitoring for the juvenile and adult mitten crabs on their property below the Friant Dam on the San Joaquin River. At this time this agreement has yet to be arranged, but the PI sees no future problems.

Cost - Budget Justification
Budget Cost: See attached tables

The proposed project develops assay to quickly ascertain the reproductive state of female crabs and provides data on possible stimuli for downstream migration and gonadal development. Tasks 1 and 2 are required for the completion of tasks 3-5. The PI acknowledges that Tasks 3-5 are not interconnected but believes that each portion of data will strengthen the information derived from each of the other tasks.

Schedule Milestones:

| Year 1 | Year 2 | Year 3 |
|--------|--------|--------|
|--------|--------|--------|

-----Task 1----- (Isolation & characterization of vitellin)

-----Task 2----- (Develop antibody)

-----Task 3-----
(Monitor downstream migration and reproductive state)

-----Task 4-----
(Determine normal reproductive cycle)

-----Task 5-----
(Environmental stimulation of reproduction)

The requested funds are primarily for personnel (PI, graduate assistants and undergraduate assistants to maintain the animals and lab). Funds are requested for disposable supplies, including chemicals. Funds for travel are to the collecting locations for the PI and research assistants. In addition, travel funds to national meetings are also requested for the PI and 1 graduate student per year. An HPLC is requested to greatly expedite the characterization of vitellin. Chromatographic runs on this HPLC system average 5 minutes including recharge time, whereas, traditional systems average over 1 hour with an addition 45 minutes to recharge the column. To offset the cost of publication, yearly publication costs are requested.

Cost Sharing

The Department of Biology, School of Natural Sciences will be purchasing 2 \$32,000 environmental chambers over the next two years to research purposes. These chambers can be used to determine the temperature and daylight requirements for stimulating the downstream migration and gonad development. In addition, the School/Department will release my time 0.25/semester/year, which equates to \$14,614/year (including fringes). Over the three year period, \$12,300 will be made available in disposable supply funds and \$1,500 in travel. These funds will help offset the required funding to conduct this project.

Applicants Qualifications

Brian Tsukimura

Assistant Professor

| | | | |
|-------------------------------------|-------|-------------|---------|
| University of California, Berkeley. | A.B. | 1977 - 1981 | Zoology |
| University of Hawaii | M.S. | 1981 - 1984 | Zoology |
| University of Hawaii. | Ph.D. | 1985 - 1988 | Zoology |

POSITIONS

| | |
|------------------|--|
| 1988 to 1993: | Post-Doctoral Fellow, Illinois State University. |
| 1990: | Lecturer, Illinois State University. |
| 1993 to 1994: | Acting Assistant Professor, Illinois State University. |
| 1994 to present: | Assistant Professor of Physiology, CSU Fresno. |

AWARDS (last 4 years):

1998 - CSU Fresno - In House - \$4,900 + 6 WTU Release time

1997 - CSU Fresno - In House - \$4,000 + 6 WTU Release time

2 Graduate Student Stipends

University Performance Salary Step Increase Award '96 - '97.

California Sea Grant: Rapid Response Grant - Funded

For: "Regulation and Gonadal Maturation in Penaeid Shrimp."

1996 - CSU Fresno - In House - \$5,165 + 9 WTU

1 Graduate Student Stipend

1995 - CSU Fresno - In House - \$5,450 + 3 WTU

AFFILIATIONS:

Society for Integrative and Comparative Biology: 1984 to present.

Sigma Xi: 1985 to present.

Crustacean Society: 1983-1984, 1995 to present

American Microscopical Society: 1995 to present

AAAS: 1989 to present.

Interagency Ecology Project, CA - Mitten Crab Project Workteam: 1/99 to present

Sierra Foothill Conservancy: 1996 to present

Current Collaborations:

Drs. E.S. Chang, Fred Griffin, and M. Snyder - shrimp collection in Santa Barbara

Drs. D.W. Borst, E.S. Chang and H. Rees - methyl farnesoate inhibiting peptide

Drs. D.W. Borst, S.L. Waddy, and C.J. Linder - vitellogenin in American lobster, *Homarus americanus*

RECENT PRESENTATIONS:

Murphy, K.M., B. Tsukimura and R.H. Abhold. 1998. Temperature effects the induction of diapause in tadpole shrimp, *Triops longicaudatus*, embryos. At the annual meeting of SICB, Denver 1999. Am. Zool. 38:69A

- Pooyan, R. and B. Tsukimura. 1998. Determination of the site of vitellogenin synthesis in the ridgeback shrimp, *Sicyonia ingentis*. At the Annual meeting of SICB, Denver 1999. Am. Zool. 38:187A.
- Stukovsky, S.S. and B. Tsukimura. 1997. A possible novel yolk protein transported in the ridgeback shrimp, *Sicyonia ingentis*. Am. Zool. 37:154A. At the Annual meeting of SICB, Boston 1998.
- Riley, L.G., Jr. and B. Tsukimura. 1996. Isolation and characterization of vitellin in the tadpole shrimp, *Triops longicaudatus*. Proceedings of the Third International Large Branchiopod Symposium/ Crustacean Society Meeting, University of San Diego, CA. pp. 31.
- July '96 - Riley, L.G., Jr. and B. Tsukimura. Isolation and characterization of vitellin in the tadpole shrimp, *Triops longicaudatus*. Presentation at the Third International Large Branchiopod Symposium/Crustacean Society Meeting, University of San Diego, CA.
- March '96 - Bender, J.S. and B. Tsukimura. Vitellin ELISA used to determine the effects of steroids on vitellogenesis in the Ridgeback Shrimp, *Sicyonia ingentis*. Presentation at Western Regional Conference on Comparative Endocrinology, University of California, Berkeley.
- December '95 - Bender, J.S. and B. Tsukimura. Development of a vitellin ELISA for the ridgeback shrimp, *Sicyonia ingentis*. Presentation at American Society of Zoologists Annual Meeting, Washington, D.C. Am. Zool. 35:77A.
- August, '95 - Tsukimura, B., S.L. Waddy and D.W. Borst. Steroid hormone treatment had no effect on lobster vitellogenesis. Presented at the 7th International Congress on Invertebrate Reproduction at University of California at Santa Cruz.

PUBLICATIONS:

- Riley, L.G. and B. Tsukimura. 1998. Yolk protein synthesis in the rice land tadpole shrimp, *Triops longicaudatus*, measured by in vitro incorporation of ³H-leucine. J. Exp. Zool. 281:238-247.
- Borst, D.W., B. Tsukimura and M. Frinsko. 1995. Methyl farnesoate levels in the crayfish, *Orconectes virilis*. pp. 462-474. In: Freshwater Crayfish VIII. Ed: R.P. Romaine. Louisiana State Printing Office, New Orleans.
- Borst, D.W., B. Tsukimura, Couch, E.F., J.K. Butler and H. Laufer. 1994. Regional differences in methyl farnesoate production by the mandibular organ of the lobster, *Homarus americanus*. Biol. Bull. 186:9-16
- Tsukimura, B., F.I. Kamemoto and D.W. Borst. 1993. Cyclic nucleotide regulation of methyl farnesoate synthesis by the mandibular organ of the lobster, *Homarus americanus*. J. Exp. Zool. 265:427-431.
- Tsukimura, B. and D.W. Borst. 1992. Regulation of methyl farnesoate in the hemolymph and mandibular organ of the lobster, *Homarus americanus*. Gen. Comp. Endocrinol. 86:297-303.
- Borst, D.W. and B. Tsukimura. 1992. Methyl farnesoate levels in crustaceans. pp. 27-35. In: Insect Juvenile Hormone Research. B. Mauchamp, F. Couillaud and J.C. Bachr, Eds. Institut National de la Recherche Agronomique, Paris, France.
- Tsukimura, B. and F.I. Kamemoto. 1991. *In vitro* stimulation of oocytes by presumptive mandibular organ secretions in the shrimp, *Penaeus vannamei*. Aquaculture 91:59-66.
- Borst, D.W. and B. Tsukimura. 1991. Liquid chromatography method for measuring levels of methyl farnesoate. J. Chromatog. 545:71-78.

Table 1. Biological/Ecological Objectives

| Hypothesis to be evaluated | Data Collection Approach | Data Evaluation Approach | Comments Data Priority |
|--------------------------------------|--|----------------------------------|--|
| Characterization of Vitellin | Vitellin isolation, Electrophoresis Western Blot | Publication in refereed Journal. | Required for Tasks #2-5 |
| Develop antibody | Antibody prep | ELISA Western Blot | Required for tasks #3-5 |
| Female Reproductive states | Downstream migration | ELISA | Date for baseline activity of crab |
| Determine normal reproductive cycle | Environmental chambers | ELISA | Data used to establish baseline |
| Stimulation of reproductive activity | Environmental chambers and hormones (MF) | ELISA Western Blot | Data used to identify control targets. |

The critical data collected from this project require the development of the ELISA, if we are to avoid having to sacrifice every animal examined. To properly conduct these experiments without the ELISA would require the storage of copious amounts of crabs. This would entail a significantly higher budget for feeding and care of all these organisms. In addition, the sampling error within each experiment would greatly increase.



CALIFORNIA
STATE
UNIVERSITY,
FRESNO

April 12, 1999

Fresno County Board of Supervisors
2220 Tulare Street
Fresno, CA 93721

Dear Sirs,

I am writing to inform you that I am submitting a proposal to CALFED to study the reproductive life histories of the Chinese Mitten Crab. This project will involve collecting data on the stimulation of gametogenesis and downstream migration. In addition, the yolk proteins that compose a significant volume of their eggs will be characterized, purified and isolated for use in making antibodies. The crabs have not yet arrived in Fresno County but may pose a serious threat to our row crops, as the juvenile crabs are walking through irrigation canals and are omnivorous. If you have any questions please feel free to call.

Sincerely,

A handwritten signature in cursive script, appearing to read "Brian Tsukimura", with a long horizontal flourish extending to the right.

Brian Tsukimura
Assistant Professor of Physiology

School of Natural Sciences
Department of Biology
2555 East Sun Raemon Ave. M/S 73
Fresno, CA 93740-8034
Tel. 278-2001
Fax 209. 278-3963

THE CALIFORNIA STATE UNIVERSITY

1 - 0 2 0 0 1 2

I-020012



CALIFORNIA
STATE
UNIVERSITY,
FRESNO

April 12, 1999

Fresno County Planning Department
2281 Tulare Street
Fresno, CA 93721

Dear Sirs,

I am writing to inform you that I am submitting a proposal to CALFED to study the reproductive life histories of the Chinese Mitten Crab. This project will involve collecting data on the stimulation of gametogenesis and downstream migration. In addition, the yolk proteins that compose a significant volume of their eggs will be characterized, purified and isolated for use in making antibodies. The crabs have not yet arrived in Fresno County but may pose a serious threat to our row crops, as the juvenile crabs are walking through irrigation canals and are omnivorous. If you have any questions please feel free to call.

Sincerely,

A handwritten signature in cursive script, appearing to read "Brian Tsukimura".

Brian Tsukimura
Assistant Professor of Physiology

School of Natural Sciences
Department of Biology
1999 East San Ramon Ave. MS 73
Fresno, CA 93740-8034
Ph. 278-2001
Fax 209. 278-3963

THE CALIFORNIA STATE UNIVERSITY

I - 0 2 0 0 1 3

I-020013



CALIFORNIA
STATE
UNIVERSITY.
FRESNO

April 12, 1999

Delta Protection Commission
14215 River Road
P.O. Box 530
Walnut Grove, CA 95690

Dear Sirs,

I am writing to inform you that I am submitting a proposal to CALFED to study the reproductive life histories of the Chinese Mitten Crab. This project will involve collecting data on the stimulation of gametogenesis and downstream migration. In addition, the yolk proteins that compose a significant volume of their eggs will be characterized, purified and isolated for use in making antibodies. The crabs have not yet arrived in Fresno County but may pose a serious threat to our row crops, as the juvenile crabs are walking through irrigation canals and are omnivorous. If you have any questions please feel free to call.

Sincerely,

A handwritten signature in cursive script, appearing to read "Brian Tsukimura".

Brian Tsukimura
Assistant Professor of Physiology

School of Natural Sciences
Department of Biology
3555 East San Ramon Ave. M/S 73
Fresno, CA 93740-8034
209. 278-2001
Ext 209. 278-3963

THE CALIFORNIA STATE UNIVERSITY

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I-020014

FAX COVER SHEET



U.S. BUREAU OF RECLAMATION

Tracy Office
RR1 Box 35
Byron CA 94514-9614

FAX NO. (209) 836-6264

Number of pages including cover sheet: 1

To: 559-278-3963

Attention: Dr. Brian Tsukimura

From: Brent Bridges

Date: April 15, 1999

Remarks: 1 Brent Bridges, a fisheries biologist at the Tracy Fish Collection Facility, will provide Dr. Brian Tsukimura a limited number of Chinese mitten crabs during any time of the year that they are available in 1999 and 2000. These crabs are to be used for research purposes only and Dr. Brian Tsukimura must obtain a permit from California Department of Fish and Game to transport the mitten crabs if such a permit applies.

IF YOU SHOULD HAVE ANY PROBLEMS RECEIVING

PLEASE CALL -- Brent Bridges at (209)833-0340

| BUDGET INFORMATION - Non-Construction Programs | | | | | | |
|--|---|------------------------------------|-----------------|-----------------------|-----------------|------------|
| Grant Program Function or Activity (a) | Catalog of Federal Domestic Assistance Number (b) | Estimated Unobligated Funds | | New or Revised Budget | | |
| | | Federal (c) | Non-Federal (d) | Federal (e) | Non-Federal (f) | Total (g) |
| 1. Tasks 1 and 2 | | \$ | \$ | \$ 160,948 | \$ 56,870 | \$ 217,817 |
| 2. Task 3 | | | | 24,846 | 8,445 | 33,291 |
| 3. Task 4 | | | | 42,354 | 8,945 | 52,883 |
| 4. Task 5 | | | | 82,948 | 20,770 | 103,217 |
| 5. Totals | | \$ | \$ | \$ | \$ | \$ 407,208 |
| SECTION 101(a)(2)(B) | | | | | | |
| 6. Object Class Categories (Year I) | | GRANT PROGRAM FUNCTION OR ACTIVITY | | | | Total (5) |
| | | (1) tasks 1 & 2 | (2) task 3 | (3) task 4 | (4) task 5 | |
| a. Personnel | | \$ 55,130 | \$ 16,822 | \$ 27,210 | \$ 55,130 | \$ 154,292 |
| b. Fringe Benefits | | 9,482 | 3,215 | 4,357 | 9,482 | 26,536 |
| c. Travel | | 3,500 | 500 | 3,500 | 2,500 | 10,000 |
| d. Equipment | | 110,000 | | | | 110,000 |
| e. Supplies | | 14,000 | 5,000 | 5,000 | 10,000 | 34,000 |
| f. Contractual | | | | | | |
| g. Construction | | | | | | |
| h. Other | | 600 | | 600 | 600 | 1,800 |
| i. Total Direct Charges (sum of 6a-6h) | | 192,812 | 25,537 | 40,667 | 78,212 | 337,228 |
| j. Indirect Charges | | 25,005 | 7,754 | 12,216 | 25,005 | 69,980 |
| k. TOTALS (sum of 6i and 6j) | | \$ 217,817 | \$ 33,291 | \$ 52,883 | \$ 103,217 | \$ 407,208 |
| 7. Program Income | | \$ | \$ | \$ | \$ | \$ |

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Prescribed by OMB Circular A-102

1-020016

1-020016

1-020017

| (a) Grant Program | | Year I | (b) Applicant | (c) State | (d) Other Sources | (e) TOTALS |
|---|--------------------------------|--------------------------------|---------------------------------|-------------|-------------------|--------------|
| 8. | Tasks 1 & 2 | | \$ 56,870 | \$ | \$ | \$ 56,870 |
| 9. | Task 3 | | 8,445 | | | 8,445 |
| 10. | Task 4 | | 8,945 | | | 8,945 |
| 11. | Task 5 | | 20,770 | | | 20,770 |
| 12. | TOTAL (sum of lines 8 - 11) | | \$ 950,300 | \$ | \$ | \$ 950,300 |
| SECTION 7 - BUDGET ESTIMATES OF FEDERAL FUND NEEDS - BALANCE OF THE PROGRAM | | | | | | |
| | | Total for 1st Year | 1st Quarter | 2nd Quarter | 3rd Quarter | 4th Quarter |
| 13. | Federal | \$ 311,096 | \$ 100,000 | \$ 100,000 | \$ 75,000 | \$ 36,096 |
| 14. | NonFederal | 95,030 | 23,758 | 23,758 | 23,757 | 23,757 |
| 15. | TOTAL (sum of lines 13 and 14) | | 406,126 | 123,758 | 123,758 | 99,853 |
| SECTION 8 - BUDGET ESTIMATES OF FEDERAL FUND NEEDS - BALANCE OF THE PROGRAM | | | | | | |
| (a) Grant Program | | FUTURE FUNDING PERIODS (Years) | | | | |
| | | (b) First | (c) Second | (d) Third | (e) Fourth | |
| 16. | | \$ 311,096 | \$ 383,201 | \$ 400,911 | \$ 1,095,208 | |
| 17. | | | | | | |
| 18. | | | | | | |
| 19. | | | | | | |
| 20. | TOTAL (sum of lines 16-19) | | \$ 311,096 | \$ 385,201 | \$ 400,911 | \$ 1,095,208 |
| SECTION 9 - BUDGET ESTIMATES OF FEDERAL FUND NEEDS - BALANCE OF THE PROGRAM | | | | | | |
| 21. Direct Charges: \$931,084 | | | 22. Indirect Charges: \$164,624 | | | |
| 23. Remarks: There was insufficient space to type in names of tasks; therefore, we used numbers. We also conflated Tasks 1 & 2 into one line to fit the 5 tasks into 4 lines. | | | | | | |

1-020017

YEAR ONE

| Tasks 1&2: Isolation, Purification and Characterization of vitellin, the development of the antibody against vitellin and the development the ELISA and Western Blot assays | | | | |
|--|---------------------------------|------------------|-----------------|------------------|
| | | Request | Match | Total |
| Salaries | | | | |
| Brian Tsukimura | 25% time x 1 Acad. Year | \$11,691 | | \$11,691 |
| | Match: 1/4 time x 1 Acad. Year | | \$11,691 | \$11,691 |
| | 2 months summer salary | \$10,388 | | \$10,388 |
| Graduate Assistant | \$1,000 x 12 mos. X 20 hrs/wk | \$12,000 | | \$12,000 |
| Student Assistant | \$9.00/hr. x 20 hrs. x 52 weeks | \$9,360 | | \$9,360 |
| Fringe Benefits | | | | |
| Brian Tsukimura | Academic year release rate=25 | \$2,923 | | \$2,923 |
| | Academic year release rate=25% | | \$2,923 | \$2,923 |
| | Summer/overload rate=11% | \$1,286 | | \$1,286 |
| Graduate Assistant | Adjunct faculty rate=11% | \$1,320 | | \$1,320 |
| Student Assistant | Student benefit rate=11% | \$1,030 | | \$1,030 |
| Subtotal, Salaries, Wages & Benefits | | \$49,998 | \$14,614 | \$64,612 |
| Other Direct Costs (Tasks 1&2) | | | | |
| Supplies and disposables | | \$10,000 | \$4,100 | \$14,100 |
| Equipment -HPLC | | \$78,000 | \$32,000 | \$110,000 |
| Travel to meetings | | \$2,500 | \$500 | \$3,000 |
| Travel to collecting sites | | \$500 | \$0 | \$500 |
| Publication and printing | | \$600 | \$0 | \$600 |
| Subtotal, Other Direct Costs | | \$91,600 | \$36,600 | \$128,200 |
| Total Direct Costs, Tasks 1&2 | | \$141,598 | \$51,214 | \$192,812 |
| Indirect Costs (38.8% of total direct costs) | | \$19,350 | \$5,656 | \$25,005 |
| CSUF Federal rate is 38.7% of salaries and benefits | | | | |
| Total Tasks 1&2 | | Request | Match | Total |
| | | \$160,948 | \$56,870 | \$217,817 |

YEAR ONE

| Task 3:Determination of female reproductive states | | | | | |
|---|--------------------------------|--|-----------------|----------------|-----------------|
| Salaries | | | Request | Match | Total |
| Brian Tsukimura | 25% Release time-1 semester | | \$4,871 | | \$4,871 |
| | Match: 1/4 time x semester | | | \$4,871 | \$4,871 |
| Graduate Assistant | \$1,000 x 6mos. X 20 hrs. | | \$8,000 | | \$6,000 |
| Student Assistant | \$9.00/hr. x 6 mos. X 20 hrs. | | \$1,080 | | \$1,080 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$1,218 | | \$1,218 |
| | Academic year release rate=25% | | | \$1,218 | \$1,218 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$660 | | \$680 |
| Student Assistant | Student benefit rate=11% | | \$119 | | \$119 |
| Subtotal, Salaries, Wages & Benefits | | | \$13,948 | \$6,089 | \$20,037 |
| Other Direct Costs(Task3) | | | | | |
| Supplies and disposables | | | \$5,000 | \$0 | \$5,000 |
| Travel to collecting sites | | | \$500 | \$0 | \$500 |
| Subtotal, Other Direct Costs | | | \$5,500 | \$0 | \$5,500 |
| Total Direct Costs, Task 3 | | | \$19,448 | \$6,089 | \$25,537 |
| Indirect Costs (38.7% of total direct costs) | | | \$5,398 | \$2,356 | \$7,754 |
| CSUF Federal rate is 38.7% of salaries and benefits | | | | | |
| | | | Request | Match | Total |
| Total Task 3 | | | \$24,846 | \$8,445 | \$33,291 |

YEAR ONE

| Task 4: Determine normal reproductive cycle | | | | | |
|---|--|--|-----------------|----------------|-----------------|
| | | | Request | Match | Total |
| Salaries | | | | | |
| Brian Tsukimura | 25% Release time-1 semester and summer salary | | \$4,871 | | \$4,871 |
| | | | \$10,388 | | \$10,388 |
| | Match 25% time x 1 semester | | | \$4,871 | \$4,871 |
| Graduate Assistant | \$1,000 x 6mos. X 20 hrs. | | \$6,000 | | \$6,000 |
| Student Assistant | \$9.00/hr. x 6 mos. X 20 hrs. | | \$1,080 | | \$1,080 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$1,218 | | \$1,218 |
| | Summer release rate=11% | | \$1,142 | | \$1,142 |
| | Academic year release rate=25% | | | \$1,218 | \$1,218 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$660 | | \$660 |
| Student Assistant | Student benefit rate=11% | | \$119 | | \$119 |
| Subtotal, Salaries, Wages & Benefits | | | \$24,336 | \$6,089 | \$31,687 |
| Other Direct Costs (Task4) | | | | | |
| Supplies and disposables | | | \$5,000 | \$0 | \$5,000 |
| Travel to collecting sites | | | \$500 | \$0 | \$500 |
| Travel to meetings | | | \$2,500 | \$500 | \$3,000 |
| Publication costs | | | \$600 | \$0 | \$600 |
| Subtotal, Other Direct Costs | | | \$8,600 | \$500 | \$9,100 |
| Total Direct Costs, Task 4 | | | \$32,936 | \$6,589 | \$40,667 |
| Indirect Costs (38.7% of total direct costs) | | | \$9,418 | \$2,356 | \$12,216 |
| CSUF Federal rate is 38.7% of salaries and benefits | | | | | |
| | | | Request | Match | Total |
| Total Task 4 | | | \$42,354 | \$8,945 | \$52,883 |

YEAR ONE

| Task 5: Environmental Stimulation of gonad maturation | | | | | | |
|--|---------------------------------|--|--|-----------------|-----------------|------------------|
| | | | | Request | Match | Total |
| Salaries | | | | | | |
| Brian Tsukimura | 25% release time-1 year + summe | | | \$11,691 | | \$11,691 |
| | Match: 1/4 time x Academic year | | | | \$11,691 | \$11,691 |
| | 2 months summer salary | | | \$10,388 | | \$10,388 |
| Graduate Assistant | \$1,000 x 12 mos. X 20 hrs. | | | \$12,000 | | \$12,000 |
| Student Assistant | \$9.00/hr. x 12 mos. X 20 hrs. | | | \$9,360 | | \$9,360 |
| Fringe Benefits | | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | | \$2,923 | | \$2,923 |
| | Academic year release rate=25% | | | | \$2,923 | \$2,923 |
| | Summer/overload rate=11% | | | \$1,286 | | \$1,286 |
| Graduate Assistant | Adjunct faculty rate=11% | | | \$1,320 | | \$1,320 |
| Student Assistant | Student benefit rate=11% | | | \$1,030 | | \$1,030 |
| Subtotal, Salaries, Wages & Benefits | | | | \$49,998 | \$14,614 | \$64,612 |
| Other Direct Costs(Task5) | | | | | | |
| Supplies and disposables | | | | \$10,000 | \$0 | \$10,000 |
| Travel to collecting sites | | | | \$500 | \$0 | \$500 |
| Travel to meetings | | | | \$2,500 | \$500 | \$2,500 |
| Publication costs | | | | \$600 | | \$600 |
| Subtotal, Other Direct Costs | | | | \$13,600 | \$500 | \$13,600 |
| Total Direct Costs, Task 5 | | | | \$63,598 | \$15,114 | \$78,212 |
| Indirect Costs (38.7% of total direct costs) | | | | \$19,350 | \$5,666 | \$25,006 |
| CSUF Federal rate is 38.7% of salaries and benefits | | | | | | |
| | | | | Request | Match | Total |
| Total Task 5 | | | | \$82,948 | \$20,770 | \$103,217 |

YEAR TWO

| Tasks 1&2: Isolation, Purification and Characterization of vitellin, the development of the antibody against vitellin and the development the ELISA and Western Blot assays | | | | | |
|--|-------------------------------------|--|------------------|-----------------|------------------|
| | | | Request | Match | Total |
| Salaries | | | | | |
| Brian Tsukimura | 25% time x 1 Academic Year | | \$12,041 | | \$12,041 |
| | Match: 1/4 time x 1 Acad. Year | | | \$12,041 | \$12,041 |
| | 2 months summer salary | | \$10,700 | | \$10,700 |
| Graduate Assistant | \$1,000 x 12 mos. X 20 hrs @ 1.03 | | \$12,360 | | \$12,360 |
| Student Assistant | \$9.00/hr. x 12 mos. X 20 hrs @1.03 | | \$9,641 | | \$9,641 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$3,010 | | \$3,010 |
| | Academic year release rate=25% | | | \$3,010 | \$3,010 |
| | Summer/overload rate=11% | | \$1,325 | | \$1,325 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$1,360 | | \$1,360 |
| Student Assistant | Student benefit rate=11% | | \$1,061 | | \$1,061 |
| Subtotal, Salaries, Wages & Benefits | | | \$50,173 | \$15,051 | \$63,539 |
| Other Direct Costs (Tasks 1&2) | | | | | |
| Supplies and disposables | | | \$10,000 | \$4,100 | \$14,100 |
| Equipment-HPLC | | | \$0 | \$32,000 | \$32,000 |
| Travel to meetings | | | \$2,500 | \$500 | \$3,000 |
| Travel to collecting sites | | | \$500 | \$500 | \$1,000 |
| Publication and printing | | | \$600 | \$600 | \$1,200 |
| Subtotal, Other Direct Costs | | | \$13,600 | \$37,700 | \$51,300 |
| Total Direct Costs, Tasks 1&2 | | | \$63,773 | \$52,751 | \$114,839 |
| Indirect Costs (38.7% of total direct costs) | | | \$19,417 | \$6,825 | \$24,590 |
| CSUF Federal rate is 38.7% of Salaries and benefits | | | | | |
| Total Tasks 1&2 | | | Request | Match | Total |
| | | | \$133,363 | \$73,627 | \$202,968 |

YEAR TWO

| Task 4: Determine normal reproductive cycle | | | | | |
|---|--------------------------------|------------------|-----------------|-----------------|-----------------|
| | | | | | |
| | | | Request | Match | Total |
| Salaries | | | | | |
| Brian Tsukimura | 25% time | 1 semeste and | \$5,017 | | \$5,017 |
| | Summer salary | | \$10,700 | | \$10,700 |
| | Match 25% time x 1 semester | | | \$5,017 | \$5,017 |
| Graduate Assistant | \$1,000 x 6 mos. | X 20 hrs @ 1.03% | \$6,180 | | \$6,180 |
| Student Assistant | \$9.00/hr. x 12 mos. | X 20 hrs @1.03 | \$1,112 | | \$1,112 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$1,254 | | \$1,254 |
| | Academic year release rate=25% | | | \$1,254 | |
| | Summer/overload rate=11% | | \$1,177 | | \$1,177 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$680 | | \$680 |
| Student Assistant | Student benefit rate=11% | | \$122 | | \$122 |
| Subtotal, Salaries, Wages & Benefits | | | \$25,130 | \$6,271 | \$31,269 |
| Other Direct Costs (Task 4) | | | | | |
| Supplies and disposables | | | \$5,000 | \$4,100 | \$9,100 |
| Travel to meetings | | | \$2,500 | \$500 | \$3,000 |
| Travel to collecting sites | | | \$500 | \$500 | \$1,000 |
| Publication and printing | | | \$600 | \$600 | \$1,200 |
| Subtotal, Other Direct Costs | | | \$8,600 | \$5,700 | \$14,300 |
| Total Direct Costs, Task 4 | | | \$33,730 | \$11,971 | \$45,559 |
| Indirect Costs (38.7% of total direct costs) | | | \$9,725 | \$2,427 | \$12,097 |
| CSUF Federal rate is 38.7% of Salaries and benefits | | | | | |
| Total Task 4 | | | Request | Match | Total |
| | | | \$68,585 | \$20,669 | \$88,915 |

YEAR TWO

| Task 5 : Environmental Stimulation of Gonad maturation | | | | | |
|---|------------------------------------|--|------------------|-----------------|------------------|
| | | | | | |
| Salaries | | | Request | Match | Total |
| Brian Tsukimura | 25% time x 1 Academic Year | | \$12,041 | | \$12,041 |
| | Match: 1/4 time x 1 Acad. Year | | | \$12,041 | \$12,041 |
| | 2 months summer salary | | \$10,700 | | \$10,700 |
| Graduate Assistant | \$1,000 x 12 mos. X 20 hrs @ 1.03 | | \$12,360 | | \$12,360 |
| Student Assistant | \$9.00/hr. x 12 mos. X 20 hrs @1.0 | | \$9,641 | | \$9,641 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$3,010 | | \$3,010 |
| | Academic year release rate=25% | | | \$3,010 | |
| | Summer/overload rate=11% | | \$1,325 | | \$1,325 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$1,360 | | \$1,360 |
| Student Assistant | Student benefit rate=11% | | \$1,061 | | \$1,061 |
| Subtotal, Salaries, Wages & Benefits | | | \$50,173 | \$16,061 | \$63,539 |
| Other Direct Costs Task 5 | | | | | |
| Supplies and disposables | | | \$10,000 | \$4,100 | \$14,100 |
| Travel to meetings | | | \$2,500 | \$500 | \$3,000 |
| Travel to collecting sites | | | \$500 | \$500 | \$1,000 |
| Publication and printing | | | \$600 | \$600 | \$1,200 |
| Subtotal, Other Direct Costs | | | \$13,600 | \$5,700 | \$18,300 |
| Total Direct Costs, Task 5 | | | \$63,773 | \$20,761 | \$82,839 |
| Indirect Costs (38.7% of total direct costs) | | | \$19,417 | \$5,825 | \$24,590 |
| CSUF Federal rate is 38.7% of Salaries and benefits | | | | | |
| Total Task 5 | | | Request | Match | Total |
| | | | \$133,363 | \$41,627 | \$170,968 |

YEAR THREE

| Task 1&2: Determination of female reproductive states the development of the antibody against vitellin and the development the ELISA and Western Blot assays | | | | |
|---|---|-----------------|-----------------|-----------------|
| | | Request | Match | Total |
| Salaries | | | | |
| Brian Tsukimura | 25% time x 1 Academic Year | \$12,402 | | \$12,402 |
| | Match: 1/4 time x 1 Acad. Year | | \$12,402 | \$12,402 |
| | 2 months summer salary | \$11,021 | | \$11,021 |
| Graduate Assistant | \$1,000 x 12 mos. X 20 hrs. @ 1.03 | \$12,730 | | \$12,730 |
| Student Assistant | \$9.00/hr. x 12 mos. X 20 hrs @ 1.03 | \$9,930 | | \$9,930 |
| Fringe Benefits | | | | |
| Brian Tsukimura | Academic year release rate=25% | \$3,101 | | \$3,101 |
| | Academic year release rate=25% | | \$3,101 | |
| | Summer/overload rate=11% | \$1,364 | | \$1,364 |
| Graduate Assistant | Adjunct faculty rate=11% | \$1,400 | | \$1,400 |
| Student Assistant | Student benefit rate=11% | \$1,092 | | \$1,092 |
| Subtotal, Salaries, Wages & Benefits | | \$53,040 | \$15,503 | \$64,542 |
| Other Direct Costs (Tasks 1&2) | | | | |
| | Supplies and disposables | \$10,000 | \$4,100 | \$14,100 |
| | Travel to meetings | \$2,500 | \$1,000 | \$3,500 |
| | Travel to collecting sites | \$500 | \$500 | \$1,000 |
| | Publication and printing | \$600 | \$600 | \$600 |
| Subtotal, Other Direct Costs | | \$13,600 | \$6,200 | \$19,200 |
| Total Direct Costs, Tasks 1&2 | | \$66,640 | \$21,703 | \$83,742 |
| Indirect Costs | | | | |
| | CSUF Federal rate is 38.7% of Salaries and benefits | \$20,526 | \$6,000 | \$24,978 |
| Total Tasks 1&2 | | Request | Match | Total |
| | | \$140,206 | \$43,206 | \$173,262 |

YEAR THREE

| Task 3: Determination of female reproductive states | | | | | | |
|--|------------------------------------|--|-----------------|-----------------|-----------------|--|
| | | | | | | |
| | | | | | | |
| Salaries | | | Request | Match | Total | |
| Brian Tsukimura | 25% release time x 1 semester | | \$5,168 | | \$5,168 | |
| | Match: 1/4 time x 1 Acad. Year | | | \$5,168 | \$5,168 | |
| | | | | | | |
| Graduate Assistant | \$1,000 x 6 mos. X 20 hrs. @ 1.03% | | \$6,365 | | \$6,365 | |
| Student Assistant | \$9.00/hr. x6 mos. X 20 hrs @ 1.03 | | \$1,145 | | \$1,145 | |
| | | | | | | |
| Fringe Benefits | | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$1,292 | | \$1,292 | |
| | Academic year release rate=25% | | | \$1,292 | | |
| | | | | | | |
| Graduate Assistant | Adjunct faculty rate=11% | | \$700 | | \$700 | |
| Student Assistant | Student benefit rate=11% | | \$126 | | \$126 | |
| | | | | | | |
| Subtotal, Salaries, Wages & Benefits | | | \$14,796 | \$6,460 | \$19,964 | |
| Other Direct Costs Task 3 | | | | | | |
| Supplies and disposables | | | \$10,000 | \$4,100 | \$14,100 | |
| Travel to meetings | | | \$2,500 | \$1,000 | \$3,500 | |
| Travel to collecting sites | | | \$500 | \$500 | \$1,000 | |
| Publication and printing | | | \$600 | \$600 | \$600 | |
| | | | | | | |
| Subtotal, Other Direct Costs | | | \$13,600 | \$6,200 | \$19,200 | |
| Total Direct Costs Task 3 | | | \$28,396 | \$12,660 | \$39,164 | |
| Indirect Costs | | | | | | |
| CSUF Federal rate is 38.7% of Salaries and benefits | | | \$5,726 | \$2,500 | \$7,726 | |
| | | | | | | |
| Total Task 3 | | | Request | Match | Total | |
| | | | \$48,918 | \$21,620 | \$66,854 | |

YEAR THREE

| Task 4: Determine normal reproductive cycle | | | | | |
|---|--------------------------------|-------------------|-----------------|-----------------|-----------------|
| | | | | | |
| | | | | | |
| Salaries | | | Request | Match | Total |
| Brian Tsukimura | 25% time | 1 semester and | \$5,168 | | \$5,168 |
| | Summer salary | | \$11,021 | | \$11,021 |
| | Match 25% time x 1 semester | | | \$5,168 | \$5,168 |
| Graduate Assistant | \$1,000 x 6 mos. | X 20 hrs. @ 1.03% | \$6,365 | | \$6,365 |
| Student Assistant | \$9.00/hr. x 6 mos. | X 20 hrs @ 1.03 | \$1,145 | | \$1,145 |
| Fringe Benefits | | | | | |
| Brian Tsukimura | Academic year release rate=25% | | \$1,292 | | \$1,292 |
| | Academic year release rate=25% | | | \$1,292 | |
| | Summer/overload rate=11% | | \$568 | | \$568 |
| Graduate Assistant | Adjunct faculty rate=11% | | \$700 | | \$700 |
| Student Assistant | Student benefit rate=11% | | \$126 | | \$126 |
| Subtotal, Salaries, Wages & Benefits | | | \$26,385 | \$8,460 | \$31,553 |
| Other Direct Costs Task 4 | | | | | |
| Supplies and disposables | | | \$5,000 | \$4,100 | \$9,100 |
| Travel to meetings | | | \$2,500 | \$1,000 | \$3,500 |
| Travel to collecting sites | | | \$500 | \$500 | \$1,000 |
| Publication and printing | | | \$600 | \$600 | \$1,200 |
| Subtotal, Other Direct Costs | | | \$8,600 | \$6,200 | \$14,800 |
| Total Direct Costs Task 4 | | | \$34,985 | \$12,660 | \$46,353 |
| Indirect Costs | | | | | |
| CSUF Federal rate is 38.7% of Salaries and benefits | | | \$10,211 | \$2,500 | \$12,211 |
| Total Task 4 | | | Request | Match | Total |
| | | | \$71,581 | \$21,620 | \$90,117 |

