

Quantitative Indicators And Life History Implications Of Environmental Stress On Sturgeon

submitted to Science Program 2006

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lead investigators:

Kueltz, Dietmar

Doroshov, Serge

Hung, Silas

Cech, Joseph

Gingras, Marty

Project Information And Executive Summary

Quantitative Indicators And Life History Implications Of Environmental Stress On Sturgeon

This is proposal #0025 for the Science Program 2006 solicitation.

Frequently asked questions and answers for this PSP are now available.

The submission deadline for this proposal has passed. Proposals may not be changed.

Instructions

Please complete the Project Information and Executive Summary Form prior to proceeding to the other forms contained on this website and required to be completed as part of your PSP application submittal. Information provided on this form will automatically support subsequent forms to be completed as part of the Science PSP submission process. Information provided on this form will appear in the Contacts and Project Staff, Task and Budget Summary, and Conflict of Interest forms.

Proposal Title: Quantitative indicators and life history implications of environmental stress on sturgeon
This field is limited to 255 characters. All proposal titles must be entered in title case. No abbreviations or acronyms will be accepted.

Applicant Information

Applicant Organization Name: Davis, California University of

Please provide the name of the organization submitting the application as follows: Davis, California University of; Fish and Game, California Department of; California Waterfowl Association, etc.

Applicant Organization Type:

public institution of higher education
eligibility

Below, please provide contact information for the representative of the applicant organization who is authorized to enter into a contractual agreement with the State of California and who has overall responsibility for the operation, management, and reporting requirements of the applicant organization. (This should be the same individual who signs the signature page.)

Salutation: **Ms.**

First Name: **Kimberly**

Last Name: **Lamar**

Street Address: **UC Davis Office of Research - Sponsored Programs - 1850 Research Park, Suite 300**

City: **Davis**

State or Province: **CA**

Zip Code or Mailing Code: **95618**

Telephone: **(530) 747-3924**

E-mail Address: **kdlamar@ucdavis.edu**

Below, please provide contact information for the primary point of contact for the implementation of the proposal. This person should be the same individual who is serving as the project Lead Investigator/Project Director.

Salutation: **Prof.**

First Name: **Dietmar**

Last Name: **Kueltz**

Telephone: **530-752-2991**

E-mail Address: **dkueltz@ucdavis.edu**

Proposal Information

Total Amount Requested: \$1,008,881

The figure represented above is provided by the total amount requested on your completed Task and Budget Summary Form. The applicant must ensure the amount indicated above is correct and equal to the total amount requested in the budget document uploaded via the Budget and Justification Form for this project.

Select one primary and up to three secondary topic areas that best apply to this proposal:

Habitat Availability and Response to Change (Primary)

Environmental Water

Trends and Patterns of Populations and System Response to a Changing Environment

Select up to five keywords to describe this project.

- *agriculture*
- *agricultural economics*
- *agricultural engineering*
- *agronomy*
- *agro-ecology*
- *benthic invertebrates*
- *benthos*
- *biochemistry*
- X *biological indicators*
- *birds*
- *channels and sloughs*
- *climate change*
- *conservation or agricultural easements*
- *conservation program management*
- *database management*
- *ecotoxicology*
- *economics*
- *engineering*
- *erosion control*
- *environmental education*
- *evapotranspiration*
- X *fish biology*
- *delta smelt*
- *salmon and steelhead*
- *other species*
- *otoliths*
- *tagging*
- *fish management and facilities*
- *flooded islands*
- *floodplains and bypasses*
- *forestry*
- *genetics*
- *geochemistry*
- *geographic information systems (GIS)*
- *geology*
- *geomorphology*
- *groundwater*
- *human health*
- *hydrodynamics*
- *hydrology*
- *insects*
- *integrated pest management*
- *integrated resource planning*
- *invasive species / non-native species / exotic species*
- *irrigation systems*
- *land use laws and regulations*
- *land use management*
- *land use planning and policy*
- *levees*
- *mammals*
- *microbiology / bacteriology*
- *conceptual*
- *quantitative*
- *oceanography*
- *performance measures*
- *phytoplankton*

- *plants*
- terrestrial
- aquatic
- wetland
- *remote sensing / imaging*
- *reptiles*
- *reservoirs and lakes*
- *restoration*
- *riparian zone*
- *rivers and streams*
- *sediment*
- *soil science*
- *statistics*
- *subsidence*
- *sustainable agriculture*
- *trophic dynamics and food webs*
- *water operations (diversions, pumps, intakes, exports, barriers, gates, etc.)*
- *water quality*
- other
- X temperature
- X contaminants
 - nutrients, organic carbon, and oxygen depleting substances
- X salinity
 - sediment and turbidity
 - *water supply*
 - *watershed assessment*
 - *watershed management*
 - *wetlands*
 - *zooplankton*

Provide the geographic coordinates that best describe the center point of your project. (Note: If your project has more than one site, provide a center point that best captures the central location.)

Example: Latitude: 38.575; must be between 30 and 45
 Longitude: -121.488; must be between -120 and
 -130

Help for finding a geographic location.

Latitude: **38.539**
 Longitude: **-121.753**

Provide the number miles radius from the center point provided above, to demonstrate the radius of the entire project.

62

Provide a description of the physical location of your project. Describe the area using information such as water bodies, river miles and road intersections.

This project is mainly located at the UC Davis campus and the Center for Aquaculture and Aquatic Biology (CABA) in Davis. Task 5 of this project is coordinated at the CDFG in Stockton. Biopsies from wild sturgeon will be obtained in the field at the following locations: San Pablo Bay and nearby areas (e.g., Suisun Bay and Montezuma Slough), Skinner Fish Protective Facility near Tracy, and Yolo Bypass.

Successful applicants are responsible for complying with all applicable laws and regulations for their projects, including the National Environmental Policy Action (NEPA) and the California Environmental Quality Act (CEQA). Projects funded through this PSP that tier off the CALFED Programmatic EIS/EIR must incorporate applicable mitigation strategies described in the CALFED Programmatic Record of Decision to avoid or minimize the project's adverse environmental impacts. Applicants are encouraged to review the Programmatic EIS/EIR and incorporate the applicable mitigation strategies from Appendix A of these documents for their projects.

If you anticipate your project will require compliance of this nature (ie applications for permits, other environmental documentation), provide below a list of these items, as well as the status of those applications or processes, if applicable. If you believe your project will not require these regulatory actions, please provide one or two lines of text outlining why your proposed project will not be subject to these processes. Further guidance is available in The Guide to Regulatory Compliance for Implementing CALFED Activities.

All collections will be made under the terms of any necessary agreements and permits issued to the Interagency Ecological Program and/or the Department of Fish and Game.

Is this proposal an application for next phase funding of an ongoing project funded by CALFED Science Program?

No. Yes.

If yes, identify the ongoing project:

Project Title:

CALFED Contract Management Organization:

Amount Funded:

Date Awarded:

Lead Organization:

Project Number:

Have primary staff and/or subcontractors of the project team (those persons listed on the Contacts and Project Staff form) received funding from CALFED for a project *not* listed above?

- No. Yes.

If yes, list the projects below: (only list up to the five most recent projects)

Project Title: **Selenium effects on health and reproduction of white sturgeon in the Sacramento-San Joaquin Estuary**

CALFED Contract Management Organization: **GCAP Services, Inc.**

Amount Funded: **\$150,047 (16 months)**

Date Awarded: **July 1, 2003**

Lead Organization: **University of California, Davis (Dr. Doroshov, PI)**

Project Number: **ERP-02-P35**

Project Title: **Biological assessment of green sturgeon in the Sacramento-San Joaquin watershed**

CALFED Contract Management Organization: **GCAP Services, Inc.**

Amount Funded: **\$1,271,272**

Date Awarded: **October 1, 2003 (3 yrs)**

Lead Organization: **University of California, Davis (PI was Dr. Klimley, WFCB; Drs. Doroshova and Cech were Co-PIs)**

Project Number: **ERP-02D-P57**

Project Title: **Delta smelt culture and research program**

CALFED Contract Management Organization: **GCAP Services, Inc.**

Amount Funded: **\$400,000 (2 yrs)**

Date Awarded: **November 1, 2003**

Lead Organization: **University of California, Davis (Dr. Doroshov, PI)**

Project Number: **ERP-02-P31**

Project Title: **Chronic toxicity of environmental contaminants in Sacramento**

CALFED Contract Management Organization: **CALFED Ecosystem Restoration**

Amount Funded: **\$924,276.20**

Date Awarded: **1/1/2000**

Lead Organization: **University of California, Davis (Dr. Hung, PI)**

Project Number: **ERP 99-N07**

Project Title: **Restoration of Sacramento Perch to San Francisco Estuary**

CALFED Contract Management Organization: **GCAP Services**

Amount Funded: **\$507,432**

Date Awarded: **8-1-03**

Lead Organization: **UC Davis - John Muir Institute of the Environment (Dr. Klimley, PI; Dr. Cech, Co-PI)**

Project Number: **ERP-02-P34**

Has the Lead Investigator, the applicant organization, or other primary staff or subcontractors of your project team ever submitted a proposal for this effort or a similar effort to any CALFED PSP?

- No. Yes.

If yes, list the submission below: (only list up to the five most recent projects)

Project Title: **Quantitative indicators and life history implications of environmental stress on sturgeon**

CALFED Program: **Science PSP**
Date of PSP: **2004**

Project Title:
CALFED Program:
Date of PSP:

Note: Additional information on this or prior applications submitted -- or proposals funded -- may be required of applicants.

List people you feel are qualified to serve as scientific and/or technical reviewers for this proposal and are not associated with your organization or CALFED.

Full Name	Organization	Telephone	E-Mail	Expertise
George Somero	Stanford University	(831) 6556243	somero@stanford.edu	
Jason Podrabsky	Portland State University	(503) 7255772	podrabsj@pdx.edu	
Lars Tomanek	Cal Poly State Univ. - San Luis Obispo	(805) 756-2437	ltomanek@calpoly.edu	
Jonathon Stillman	Romberg Tiburon Lab, SFSU	(415) 435-7144	stillmaj@sfsu.edu	

Provide additional comments, information, etc. here:

This proposal was developed in response to the CALFED 2006 Science Program PSP. It is based on a previous proposal that received a technical rating of "superior" in the 2004 CALFED Science PSP. Despite initial selection for funding and superior ratings the previous proposal was not funded because of a cut-back in 2004 CALFED Science PSP funds. We have modified our current proposal somewhat to 1) optimally align it with the more focused priorities of the 2006 CALFED Science PSP, 2) address any suggestions for improvements made by the previous reviewers, and 3) incorporate new, relevant material that was produced by the research groups participating in this proposal since the submission of the previous proposal.

Executive Summary

Provide a brief but complete summary description of the proposed project; its geographic location; project objective; project type, approach to implement the proposal; expected outcomes; and adaptive management approach and relationship to the Science Program goals. The Executive Summary should be a concise, informative, stand-alone description of the proposed project and be no longer than one page in length. Please note, this information will be made public on our website shortly after the closing date of this PSP.

This proposal seeks to analyze the effects of the pollutant stressor selenomethionine (SeMet), singly and in combination with methyl mercury (MeHg), temperature, and salinity stresses on the biochemistry, physiology, and overall fitness of different life stages of sturgeon. Because white sturgeon (*Acipenser transmontanus*) and green sturgeon (*Acipenser medirostris*) both occur in the San Francisco (SF) BayDelta this study will focus on both species. Our proposal aims at better understanding the mechanistic basis for bioaccumulation of SeMet and MeHg, and unraveling key elements of the molecular response to such pollutant stresses in sturgeon. This proposal represents a collaborative effort by four experienced UC Davis-based laboratories and CDFG in Stockton. The research team has excellent prerequisites for addressing key areas that are of primary concern to CALFED using an innovative and modern approach, and utilizing the power of combining state-of-the-art expertise in multiple areas of science. This collaborative effort is expected to yield more useful and comprehensive information than would have been possible by five individual proposals. Furthermore, the proposed collaboration streamlines procedures by allowing efficient sharing of expertise, personnel, equipment, and supplies. Our proposed

research will significantly enhance the depth of knowledge about the stress biology of sturgeon and allow further development of life history models by performing a series of integrated tasks. These tasks will test the overall hypothesis that specific stress proteins in combination with biochemical, cellular, and physiological parameters a) indicate exposure of sturgeon to specific types and defined combinations of stresses and b) allow us to deduce molecular mechanisms and biological processes of stress adaptation in sturgeon. We will breed sturgeon and obtain samples from laboratory acclimation experiments and obtain biopsies from wild sturgeon in the field. Using these samples we will quantify the toxicokinetics, bioaccumulation, and chronic toxicity of selenium when present alone or in combination with MeHg in the diet. Moreover, we will quantify effects of chronic exposure to dietary SeMet stress, singly and in combination with MeHg, salinity, and temperature stresses on swimming performance, resting metabolism, and hematology. Furthermore, we will identify novel bioindicators and entire proteome signatures and biochemical pathways affected by the stressors outlined above. These 'stress-signatures' will be compared to the proteome of sturgeon biopsies obtained in the field to deduce exposure history in field specimen. Finally, we will construct tissue microarrays (TMAs) from sturgeon tissues to enable robust, stressor-specific, and high-throughput bio-monitoring field assays in the future. This was a significant weakness in existing programs, which will be addressed directly by our proposed approach. By knowing the proteins and biochemical pathways associated with stressful levels of selenium, mercury, salinity, and temperature we can identify physiological functions that are affected by such stresses in endangered fish species such as sturgeon. Knowledge of physiological functions, in turn, is essential for modeling the impact of future environmental changes on the SF BayDelta ecosystem as a whole.

Contacts And Project Staff

This is proposal #0025 for the Science Program 2006 solicitation.

Frequently asked questions and answers for this PSP are now available.

The submission deadline for this proposal has passed. Proposals may not be changed.

INSTRUCTIONS

Use this form to provide titles, affiliations, qualifications, and descriptions of roles of the primary and secondary project staff. Include any consultants, subcontractors and/or vendors. The Lead Investigator or Project Director, as identified in the Project Information and Executive Summary Form, is required to upload a PDF version of their resume. To complete the qualification field of this form, please provide a bulleted list of relevant project/field experience and any publications/reports that support your participation in the proposed project.

Information provided on this form will automatically support subsequent forms to be completed as part of the Science Program PSP submission process. Please note that information you enter in this form will appear in the Task and Budget Summary and Conflict of Interest forms.

Information on subcontractor services must be provided even if the specific service provider has not yet been selected. If the specific subcontractor has not been identified or selected, please list TBD (to be determined) in the last name field and the anticipated service type in the title field (example: Fish Biologist).

Please provide this information before continuing to the Tasks and Deliverables Form.

Applicant

Davis, California University of
Ms. Kimberly Lamar
UC Davis Office of Research – Sponsored Programs – 1850 Research Park, Suite 300
Davis CA 95618
(530) 747-3924
kdlamar@ucdavis.edu

Lead Investigator/Project Director

Salutation: **Prof.**
Last Name: **Kueltz**
First Name: **Dietmar**
Title: **Associate Professor**
Organization: **UC Davis – Department of Animal Science**
Responsibilities: **PI, tasks 1, 6, 7**
Resume:

You have already uploaded a PDF file for this question. Review the file to verify that appears correctly.

Mailing Address: **Department of Animal Science, Meyer Hall, UC Davis**
City: **Davis**
State: **CA**
Zip: **95616**
Telephone: **530-752-2991**
E-Mail: **dkueltz@ucdavis.edu**

All Other Personnel

Salutation: **Prof.**
Last Name: **Doroshov**
First Name: **Serge**
Title: **Professor**
Organization: **UC Davis – Department of Animal Science**
Position:

Co-PI

Responsibilities: tasks 2, 7

Qualifications:

EDUCATION B.S. &M.S. (Zoology), 1959. University of Moscow, Russia. Ph.D. (Biology), 1967. Institute of Oceanology, Russian Academy of Science.

POSITIONS Researcher, Institute of Freshwater Reservoirs, Academy of Sciences, Russia: 1959 - 1960; Research Scientist, Institute of Marine Fisheries and Oceanography, Moscow, Russia: 1961 - 1967; Head, Department of Marine Aquaculture, Institute of Marine Fisheries and Oceanography, Moscow: 1969 - 1975; Aquaculture Expert, Food and Agriculture Organization, United Nations: 1975 - 1977; Associate Professor, Department of Animal Science, University of California, Davis: 1978 - 1983; Professor, Department of Animal Science, University of California, Davis: 1984 - present.

AWARDS Distinguished Service Award, California Aquaculture Association (1988) Honorary Life Membership, World Aquaculture Society (2000) Outstanding Contribution to Development of Aquaculture, USDA (2004)

SELECTED PUBLICATIONS Doroshov, S.I., W.H. Clark, Jr., P.B. Lutes, R.L. Swallow, K.E. Beer, A.B. McGuire and M.D. Cochran. 1983. Artificial propagation of the white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture* 32: 93 104.

Binkowski, F.P. and S.I. Doroshov (ed). 1985. North American sturgeons: biology and aquaculture potential. Dr. W. Junk Publishers, Dordrecht.

Doroshov, S.I. 1985. Biology and culture of sturgeon, *Acipenseriformes*. Pp. 251 274 in: J.F. Muir and R.L. Roberts (ed). *Recent Advances in Aquaculture*. Croom Helm, London.

Wang, Y.L., F.P. Binkowski and S.I. Doroshov. 1985. Effect of temperature on early development of white and lake sturgeon, *Acipenser transmontanus* and *A. fulvescens*. *Env. Biol. Fish.* 14:43 50.

Buddington, R.K. and S.I. Doroshov. 1986. Structural and functional relations of the white sturgeon alimentary canal (*Acipenser transmontanus*). *J. Morphology* 190: 201 213.

Wang, Y.L., R.K. Buddington and S.I. Doroshov. 1987. Influence of temperature on yolk utilization by the white sturgeon, *Acipenser transmontanus*. *J. Fish Biol.* 30: 263- 271.

Lutes, P.B., S.I. Doroshov, F.A. Chapman, J. Harrah, R. Fitzgerald and M. Fitzpatrick. 1987. Morpho-physiological predictors of ovulatory success in white sturgeon, *Acipenser transmontanus*. *Aquaculture* 66:43-52.

Conte, F.S., S.I. Doroshov, P.B. Lutes and E. M. Strange. 1988. Hatchery manual for the white sturgeon, *Acipenser transmontanus*, with application to other North American *Acipenseridae*. Division of Agriculture and Natural Resources, University of California. Publ. 3322.

Brown, C.L., S.I. Doroshov, J.M. Nunez, C. Hadley, J.P. Van Eenennaam, R.S. Nishioka and H.A. Bern. 1988. Maternal triiodothyronine injections cause increases in swimbladder inflation and survival rates in larval striped bass, *Morone saxatilis*. *J. Exp. Zool.* 248: 168-176.

Kroll, K.J. and S.I. Doroshov. 1991. Vitellogenin: potential vehicle for selenium bioaccumulation in the oocytes of the white sturgeon. Pp. 99 106 in: P.Williot (ed). *ACIPENSER*. Cemagref, Bordeaux.

Bidwell, C.A., K.J. Kroll, E. Severud, S.I. Doroshov, and D.M. Carlson. 1991. Identification and preliminary characterization of white sturgeon (*Acipenser transmontanus*) vitellogenin mRNA. *Gen. Comp. Endocrinol.* 83: 415 424.

Sherwood, N.M., S. Doroshov, and V. Lance. 1991. Gonadotropin Releasing hormone (GnRH) in bony fish that are phylogenetically ancient: reedfish (*Calamoichthys calabaricus*), sturgeon (*Acipenser transmontanus*), and alligator gar (*Lepisosteus spatula*). *Gen. Comp. Endocrinol.* 84: 44 57.

Buddington, R.K., J.R. Hazel, S.I. Doroshov, and J.P. Eenennaam. 1993. Ontogeny of the capacity for homeoviscous adaptation in white sturgeon (*Acipenser transmontanus*). *J. Exp. Zool.* 265: 18-28.

Doroshov, S.I., J.P. Van Eenennaam, and G.P. Moberg. 1994. Reproductive management of cultured white sturgeon. Pp. 156-161 in: D. MacKinlay (ed). *High Performance Fish*. Fish Physiology Association, Vancouver BC.

- Moberg, G.P., J.G. Watson, S. Doroshov, H. Papkoff, and R.J. Pavlick, Jr. 1995. Physiological evidence for two sturgeon gonadotropins in *Acipenser transmontanus*. *Aquaculture* 135: 27-39.
- Moberg, G.P. and S.I. Doroshov. 1996. Probing the endocrine control of sturgeon reproduction. Pp.105-111 in: S. Doroshov, F. Binkowski, T. Thuemler and D. MacKinlay (ed). *Culture and Management of Sturgeon and Paddlefish*. American Fisheries Society, San Francisco.
- Van Eenennaam, A., J.P. Van Eenennaam, J.F. Medrano, and S.I. Doroshov. 1996. Rapid identification of meiotic gynogenesis and polyploidy in white sturgeon (*Acipenser transmontanus* Richardson). *Aquaculture* 147: 177-189.
- Chapman, F.A., J.P. Van Eenennaam, and S.I. Doroshov. 1996. The reproductive condition of white sturgeon, *Acipenser transmontanus*, in San Francisco Bay, California. *Fish. Bull.* 94: 628-634.
- Doroshov, S.I., G.P. Moberg, and J.P. Van Eenennaam. 1997. Observations on the reproductive cycle of cultured sturgeon (*Acipenser transmontanus*). *Env. Biol. Fish* 48: 265-278.
- Van Eenennaam, J.P. and S.I. Doroshov. 1998. Effects of age and body size on gonadal development of Atlantic sturgeon. *J. Fish Biol.* 53: 624-637.
- Van Eenennaam, A.L., J.P. Van Eenennaam, J.F. Medrano, S.I. Doroshov. 1999. Evidence of female heterogametic sex determination in white sturgeon. *J. Heredity* 90:231-233.
- Webb, M.A.H., J.P. Van Eenennaam, S.I. Doroshov, G.P. Moberg. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture* 176:315-329.
- Doroshov, S.I., J.P. Van Eenennaam and G.P. Moberg. 1999. Development of white sturgeon broodstock. *J. Appl. Ichthyol.* 15: 326-327.
- Webb, M.A.H., J.P. Van Eenennaam, S.I. Doroshov. 2000. Effects of steroid hormones on in vitro oocyte maturation in white sturgeon (*Acipenser transmontanus*). *Fish Physiol. Biochem.* 23: 317-325.
- Van Eenennaam, J.P. , M.A.H. Webb, Xin Deng, S.I. Doroshov, R. Mayfield, JJ. Cech, Jr., D.C. Hillemeier, and T.E. Willson. 2001. Artificial spawning and larval rearing of Klamath River green sturgeon. *Trans. Am. Fish. Soc.* 130:159-165.
- Gisbert, E., J.J. Cech Jr., and S.I. Doroshov. 2001. Routine metabolism of larval green sturgeon (*Acipenser medirostris* Ayres). *Fish Physiol. Biochem.* 25:195-200.
- Webb, M.A.H., J.P. Van Eenennaam, G.W. Feist, J. Linares-Casenave, M.S. Fitzpatrick, C.D. Schreck, and S.I. Doroshov. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon. *Aquaculture* 201:137-151.
- Belanger, J.M., J.H. Son, K.D. Laugero, S. Lankford, G.P. Moberg, S.I. Doroshov, and JJ. Cech, Jr. 2001. Effects of short-term management stress and ACTH injections on plasma cortisol levels in cultured white sturgeon. *Aquaculture* 203:165-176.
- Deng, X., J.P. Van Eenennaam, and S.I. Doroshov. 2002. Comparison of early life stages and growth of green and white sturgeon. *Am. Fish. Soc. Symp.* 28:237-248. Webb, M.A.H., G.W. Feist, J.M. Trant, J.P. Van Eenennaam, M.S. Fitzpatrick, C.B. Schreck, and S.I. Doroshov. 2002. Ovarian steroidogenesis in white sturgeon (*Acipenser transmontanus*) during oocyte maturation and induced ovulation. *Gen. Comp. Endocrinol.* 129:27-38.
- Rosenthal, H., R.M. Bruch, F.P. Binkowski, S.I. Doroshov (eds.). 2002. Proceedings of the 4th International Symposium on Sturgeon. *J. Appl. Ichthyol.* 18 (Special Issue)
- Linares-Casenave, J., K.J. Kroll, J.P. Van Eenennaam, S.I. Doroshov. 2003. Effect of ovarian stage on plasma vitellogenin and calcium in cultured white sturgeon. *Aquaculture* 221:645-656.
- Gisbert, E. and S.I. Doroshov. 2003. Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*). *Aquat. Liv. Res.* 16:77-89.
- Feist, G., J.P. Van Eenennaam, S.I. Doroshov, C.B. Schreck, R.P. Schneider, M.S. Fitzpatrick. 2004. Early identification of sex in culture white sturgeon, using plasma steroid levels. *Aquaculture* 232:

581-590.

Cech, J.J., Jr. and S.I. Doroshov. 2004. Environmental requirements, preferences, and tolerance limits of North American sturgeons. Pp. 73-86 in: G.T.O. LeBreton, F.W.H. Beamish and R.S. McKinley (eds.) Sturgeons and paddlefish of North America. Kluwer Academic Publishers, Dordrecht.

Van Eenennaam, J.P., J. Linares-Casenave, X. Deng, and S.I. Doroshov. 2005. Effect of incubation temperature on green sturgeon embryos, *Acipenser medirostris*. *Env. Biol. Fish.* 72: 145-154.

Van Eenennaam, J.P., J. Linares-Casenave, S.I. Doroshov, D.C. Hillemeier, T.E. Willson, A.A. Nova. 2006. Reproductive conditions of the Klamath River green sturgeon. *Trans. Am. Fish. Soc.* 135:151-163.

Werner, I., J. Linares-Casenave, J.P. Van Eenennaam, and S.I. Doroshov. The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (*Acipenser medirostris*). *Env. Biol. Fish.* (in press).

Gessner, J., J.P. Van Eenennaam, S.I. Doroshov. North American green and European Atlantic sturgeon: comparisons of life histories and human impacts. *Env. Biol. Fish.* (in press).

List relevant project/field experience and publications/reports.

Salutation: Prof.

Last Name: Hung

First Name: Silas

Title: Professor

Organization: UC Davis - Department of Animal Science

Position:

Co-PI

Responsibilities: tasks 3, 7

Qualifications:

Education CEGEP (Gen.Sci.) MacDonald College, McGill University, 1970-72 B.S. (Fd.Biochem.) MacDonald College, McGill University, 1972-75 M.S. (Nutrition) University of Guelph, 1975-77 Ph.D. (Nutrition) University of Guelph, 1977-80

Positions Professor (Animal Science), UCD, 1995-present Associate Professor (Animal Science), UCD, 1989-1995 Assistant Professor (Animal Science), UCD, 1983-1989 Postdoctoral Fellow (Biology), University of Ottawa, 1981-82 Postdoctoral Fellow (Nutrition) University of Guelph, 1980-81

Professional Society Memberships American Fisheries Society American Institute of Nutrition Asian Fisheries Society Comparative Nutrition Society

Adjunct Professorships Adjunct Professor, Sciences et technologie des aliments, Université Laval, Quebec, Canada, 1992-1995.

Visiting Professor, Department of Marine Food Sciences, National Taiwan Ocean University, Keelung, Taiwan, 1997-1998.

Adjunct Professor, College of Life Sciences, Zhongshan University, Guangzhou, China, 1995-1998 & 2002-2005.

Honorary Research Professor, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, China, 1995-present.

Chaired Meetings and Symposiums Chair and Principle Organizer, 21st Fish Feed and Nutrition Workshop, Ramada Inn, Davis, 7-9 October 1992.

Chair and Principle Organizer, 29th Fish Feed and Nutrition Workshop, University of California, Davis, June 22nd to 25th, 2003.

Chair of Scientific and Local Organizing Committee and Symposium editor-in-chief, on The Fifty Symposium of World's Chinese Scientists On Nutrition and Feeding of Finfish and Shellfish, 5-9th September, Zhuhai, Guangdong, China.

Associate Editor Associate Editor, the Progressive Fish-Culturist, 1993-1995. Associate Editor, Aquaculture Nutrition, 1994-2004. Associate Editor, Journal of the Chinese Nutrition Society, Chinese Nutrition Society/Taipei, 1994-present. Member, Editorial Advisory Board, Aquaculture, 2005-Present.

Scientific Publications Since 2000 (Out of total 86 since 1977) Hemre, G.I., Shiau, S.Y., Deng, D.F., Storebakken, T. & S.S.O. Hung. 2000. Utilization of hydrolysed potato starch by juvenile Atlantic salmon (*Salmo salar* L.), when using a restricted feeding regime. *Aquaculture Research*. 31:207-212.

Deng, D.F., Refstie, S., Hemre, G.I., Crocker, C.E., Chen, H.Y., Cech, J.J., Jr. & Hung, S.S.O. 2000. A new technique of feeding, repeated sampling of blood and continuous collection of urine in white sturgeon. *Fish Physiology and Biochemistry*. 22:191-197.

Fu, C., Cui, Y., Hung, S.S.O. & Zhu, Z. 2000. Whole-body amino acid pattern of F4 human growth hormone gene-transgenic red common (*Cyprinus carpio*) fed diets with different protein levels. *Aquaculture* 189:287-292.

Deng, D.F., Refstie, S. & Hung, S.S.O. 2001. Glycemic and glycosuric responses in white sturgeon after oral administration of simple and complex carbohydrates. *Aquaculture*. *Aquaculture* 199:107-117.

Chou, B.S., Shiau, S.Y. & Hung, S.S.O. 2001. Effect of dietary cod liver oil on growth and tissue fatty acids of juvenile hybrid tilapia. *North American Journal of Aquaculture*. 63:277-284.

Hung, S.S.O. & Deng, D.F. 2002. Sturgeon, *Acipenser* spp. In: Webster, C.D., Lim, C. (Eds.), *Nutrient Requirements and Feeding of Finfish for Aquaculture*, CABI Publishing, Wallingford, UK., pp. 344-357.

Teh, S.J., Deng, X., Teh, F-C. & Hung, S.S.O. 2002. Selenium-induced teratogenicity in Sacramento splittail (*Pogonichthys macrolepidotus*). *Journal of Marine Environmental Research*. 54:605-608.

Deng, D.F., Teh, S.J., Teh, F-C. & Hung, S.S.O. 2002. Effect of Diets and Water Temperatures on the Growth of Sacramento Splittail (*Pogonichthys macrolepidotus*) Larvae. *North American Journal of Aquaculture*. 64:242-247.

Gawlicka, A., Herold, M.A., Barrows, F.T., de la Noüe, J. & Hung, S.S.O. 2002. Effects of dietary lipids on growth, fatty acid composition, intestinal absorption and hepatic storage in white sturgeon (*Acipenser transmontanus* R.) larvae. *Journal of Applied Ichthyology*. 18:673-681.

Deng, D.F., Koshio, S., Yokoyama, S. Bai, S.C., Shao, Q.J., Cui, Y. & Hung, S.S.O. 2003. Effects of Feeding Rates on Growth Performances of White Sturgeon Larvae. *Aquaculture*. 217:589-598.

Gisbert, E., Sainz, R.D. & Hung S.S.O. 2003. Glycemic responses in white sturgeon after oral administration of graded doses of glucose. *Aquaculture* 217:589-598.

Tian, L.-X., Liu, Y.-J. & Hung, S.S.O. 2004. Utilization of glucose and cornstarch by juvenile grass carp. *North American Journal of Aquaculture* 66:141-145.

Deng, D.F., Teh, S.J., Min, T.S., & Hung, S.S.O. 2004. Effect of dietary lipid level on growth performance of splittail at 18C. *North American Journal of Aquaculture*. 66:299-304.

Teh, S.J., Deng, X., Deng, D.F., Teh, C-F., Hung, S.S.O., Fan, T.W.F., Liu, J. & Higashi, R. M. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (*Pogonichthys macrolepidotus*). *Environmental Science and Technology*. 38:6085-6093.

Teh. S.J., Deng, D.F., Werner, I., Teh, F.C., & Hung, S.S.O. 2005. Sublethal toxicity of orchard stormwater runoff contaminated with esfenvalerate and diazinon in Sacramento splittail (*Pogonichthys macrolepidotus*). *Environmental Science and Technology*. 59:203-216.

Park, G.J., Bai, S.C., Ok, I.H., Han, K.M., Hung, S.S.O., Rogers, Q.R., & Min, T.S. 2005. Post prandial plasma free arginine concentrations increase in rainbow trout fed arginine deficiency diets. *Asian-Australian J. Animal Science*. 18:395-402.

Deng, D.F., Hemre, G-I., Storebakken, T., Shiau, S.Y., & Hung S.S.O. 2005. Utilization of diets with hydrolyzed potato starch, or glucose by juvenile white sturgeon as affected by Maillard reaction during processing. *Aquaculture* 248:103-109.

Tashjian, D.H. & Hung, S.S.O. 2006. Selenium Absorption, Distribution, and Excretion in White Sturgeon

Orally Dosed With Graded Levels of L-Selenomethionine. *Environmental Toxicology and Chemistry*. (in press).

Tashjian, D.H., Teh, S.J., Sogomonyan, A. & Hung, S.S.O. 2006. Bioaccumulation and Chronic Toxicity of Dietary L-Selenomethionine in Juvenile White Sturgeon (*Acipenser transmontanus*). *Aquatic Toxicology*. (in press)

List relevant project/field experience and publications/reports.

Salutation: **Prof.**

Last Name: **Cech**

First Name: **Joseph**

Title: **Professor**

Organization: **UC Davis - Department of Wildlife, Fisheries, and Conservation Biology**

Position:

Co-PI

Responsibilities: **tasks 4, 7**

Qualifications:

EDUCATION B.S. University of Wisconsin, Madison, 1966 (Zoology) M.A., Ph.D. University of Texas, Austin, 1970, 1973 (Zoology)

POSITIONS Resident Zoologist, Sea Search I, R/V Dante Deo, Caribbean Sea and S. Pacific Ocean, 1965-66; Research Asst., Univ. Texas Marine Sci. Inst., 1966, 1968-72; Teaching Asst., Univ. Texas, 1967; Research Assoc. Univ. Texas Marine Sci. Inst., 1973; Research Assoc., The Research Institute of the Gulf of Maine, 1973-1975; Lecturer, Univ. Maine at Portland-Gorham, 1975; Asst. Professor 1975-1981, Assoc. Professor 1981-1987, Professor of Fisheries Biology, Univ. California, Davis, 1987-present; Associate Editor, *Transactions of the American Fisheries Society*, 1991-1993; Chair, UC Davis Dept. Wildlife, Fish, and Conservation Biology, 1992-1997; Member, Copeia Editorial Board, 1997-1998, 2000-2002; Director, UC Davis Center for Aquatic Biology and Aquaculture, 2002-present.

AWARDS AND HONORS Member: Phi Sigma, Phi Kappa Phi, Sigma Xi; NIH Predoctoral Fellow 1970-73; Invited participant: NATO Advanced Study Institute on Environ. Physiol. Fishes, 1979, Lennoxville, Quebec; NATO Advanced Research Workshop on Evol. Biol. Primitive Fishes, 1985, Bamfield, B.C. Canada; IUPS Discussion Panel on Controversies: Circulation and Respiration, 1986, Vancouver, B.C.; Organizer: 2nd Biennial International Symposium on "Fish Physiology, Toxicology, and Water Quality Management", 1990, Sacramento, Calif.; Invited speaker: 3rd Biennial International Symposium on "Fish Physiology, Toxicology, and Water Quality Management, 1992, Nanjing, PRC; Fellow: American Institute of Fishery Research Biologists, 1992; Honorable Mention, Most Significant Paper in *Transactions of the American Fisheries Society*, Vol.121, 1992; Outstanding Faculty Adviser Award, College Agric..Environ. Sci.: 1992-93; Plenary speaker, "High Performance Fish" First International Fish Physiology Symposium, Vancouver, B.C.: 1994; Excellence in Fisheries Education Award (with P.B. Moyle), American Fisheries Society, 1995; Fellow: American Association for the Advancement of Science, 1996; Outstanding Mentor Award: UC Davis ProFemina Research Consortium 1997; Mentoring for Professional Diversity Award: Equal Opportunities Section, American Fisheries Society, 1999. Award of Excellence, California-Nevada Chapter, American Fisheries Society, 2000; Congressional Legion of Honor, Physiology Section, American Fisheries Society, 2000; UC Davis Prize for Teaching and Scholarly Achievement, 2001; USDA Excellence in Teaching Award, Western Region, 2003.

SELECTED (last 10 years) PUBLICATIONS (from >130 peer-reviewed articles and books) Cech, J.J., Jr., S.D. Bartholow, P.S. Young, and T.E. Hopkins. 1996. Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. *Trans. Am. Fish. Soc.* 125:308-320. Young, P.S. and J.J. Cech, Jr. 1996. Environmental tolerances and requirements of splittail. *Trans. Am. Fish. Soc.* 125:664-678. Crocker, C.E. and J.J. Cech, Jr. 1996. The effects of hypercapnia on the growth of juvenile white sturgeon, *Acipenser transmontanus*. *Aquacult.* 147:293-299. Heath, A.G., J.J. Cech, Jr., L. Brink, P. Moberg, and J.G. Zinkl. 1997. Physiological responses of fathead minnow larvae to rice pesticides. *Ecotox. Env. Saf.* 37:280-288. Crocker, C.E. and J.J. Cech, Jr. 1997. Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile white sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. *Env. Biol. Fish.* 50:383-389. Choi, M.H., J.J. Cech, Jr., and M.C. Lagunas-Solar. 1998. Bioavailability of methylmercury to Sacramento blackfish (*Orthodon microlepidotus*): dissolved organic carbon (DOC) effects. *Env. Tox. Chem.* 17:695-701. Cech, J.J., Jr., B.W. Wilson, and D.G. Crosby. 1998. Multiple stresses in ecosystems. *Lewis/CRC Publ.*, Boca Raton. Webber, J.D. and J.J. Cech, Jr. 1998. Nondestructive diet analysis of the

leopard shark from two sites in Tomales Bay, California. *Calif. Fish and Game* 84:18-24. Sanderson, S.L., M.E. Mort, and J.J. Cech, Jr. 1998. Particle retention by non-suspension-feeding cyprinid fishes. *Can. J. Fish. Aquat. Sci.* 55:861-868. Swanson, C., P.S. Young, and J.J. Cech, Jr. 1998. Swimming performance of delta smelt: maximum performance, and behavioral and kinematic limitations on swimming at submaximal velocities. *J. Exp. Biol.* 201:333-345. Crocker, C.E. and J.J. Cech, Jr. 1998. Effects of hypercapnia on blood-gas and acid-base status in the white sturgeon, *Acipenser transmontanus*. *J. Comp. Physiol.* B168:50-60. Magee, A., C.A. Myrick, and J.J. Cech, Jr. 1999. Thermal preference of female threespine sticklebacks under fed and food-deprived conditions. *Calif. Fish Game* 85 :102-112. Matern, S.A., J.J. Cech, Jr., and T.E. Hopkins. 2000. Diel movements of bat rays, *Myliobatis californica*, in Tomales Bay, California: evidence for behavioral thermoregulation? *Env. Biol. Fish.* 58:173-182. Deng, D.F., S. Refstie, G.-I. Hemre, C.E. Crocker, H.Y. Chen, J.J. Cech, Jr., and S.S.O. Hung. 2000. A new technique of feeding, repeated sampling of blood and continuous collection of urine in white sturgeon. *Fish Physiol. Biochem.* 22:191-197. Ackerman, J.T., M.C. Kondratieff, S.A. Matern, and J.J. Cech, Jr. 2000. Tidal influence on spatial dynamics of leopard sharks, *Triakis semifasciata*, in Tomales Bay, California. *Env. Biol. Fish.* 58:33-43. Myrick, C.A. and J.J. Cech, Jr. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiol. Biochem.* 22:245-254. Myrick, C.A. and J.J. Cech, Jr. 2000. Swimming performances of four California stream fishes: temperature effects. *Env. Biol. Fish.* 58:289-295. Swanson, C., T. Reid, P.S. Young, and J.J. Cech, Jr. 2000. Comparative environmental tolerances of threatened delta smelt (*Hypomesus transpacificus*) and introduced wakasagi (*H. nipponensis*) in an altered California estuary. *Oecologia* 123:384-390. Crocker, C.E., A.P. Farrell, A.K. Gamperl, and J.J. Cech, Jr. 2000. Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Amer. J. Physiol.: Regul., Int., and Compar. Physiol.* 279:R617-R628. Katzman, S.M. and J.J. Cech, Jr. 2001. Juvenile coho salmon locomotion and mosaic muscle are modified by 3', 3', 5' Tri-iodo-L-thyronine (T3). *J. Exp. Biol.* 204:1711-1717. Meloni, C.J., J.J. Cech, Jr., and S.M. Katzman. 2002. Effect of brackish salinities on oxygen consumption of bat rays (*Myliobatis californica*). *Copeia* 2002(2):462-465. Cech, J.J., Jr. and C.E. Crocker 2002. Physiology of sturgeon: effects of hypoxia and hypercapnia. *J. Appl. Ichthyol.* 18:320-324. Danley, M.L., S.D. Mayr, P.S. Young, J.J. Cech, Jr. 2002: Swimming performance and physiological stress responses of splittail exposed to a fish screen. *N. Amer. J. Fish. Manage.* 22:1241-1249. Myrick, C.A. and J.J. Cech, Jr. 2002. Growth of American River fall-run Chinook salmon in California's central valley: temperature and ration effects. *Calif. Fish Game* 88:35-44. Brick, M.E. and J.J. Cech, Jr. 2002. Metabolic responses of juvenile striped bass to exercise and handling stress with various recovery environments. *Trans. Am. Fish. Soc.* 131:855-864. Swanson, C., D.V. Baxa, P.S. Young, J.J. Cech, Jr., and R.P. Hedrick 2002. Reduced swimming performance in delta smelt infected with *Mycobacterium* spp. *J. Fish Biol.* 61:1012-1020. Myrick, C.A. and J.J. Cech, Jr. 2003. The physiological performance of golden trout at water temperatures of 10-19°C. *Calif. Fish Game* 89:20-29. Lankford, S.E., T.E. Adams, and J.J. Cech, Jr. 2003. Time of day and water temperature modify the physiological stress response in green sturgeon, *Acipenser medirostris*. *Comp. Biochem Physiol.* 135A:291-302. Swanson, C., P.S. Young, and J.J. Cech, Jr. 2004. Swimming in two-vector flows: performance and behavior of juvenile Chinook salmon near a simulated screened water diversion. *Trans. Am. Fish. Soc.* 133:265-278. Moyle, P.B. and J.J. Cech, Jr. 2004. *Fishes: introduction to ichthyology*. 5th ed., Prentice Hall. Myrick, C.A., D.K. Folgner, and J.J. Cech, Jr. 2004. An annular chamber for aquatic animal preference studies. *Trans. Am. Fish. Soc.* 133:426-432. Marine, K.R. and J.J. Cech, Jr. 2004. Effects of high water temperature on growth, smoltification, and predator avoidance in juvenile Sacramento River Chinook salmon. *N. Amer. J. Fish. Manage.* 24:198-210. Cech, J.J., M. McEnroe, and D.J. Randall. 2004. Coho salmon haematological, metabolic, and acid-base changes during exercise and recovery in sea water. *J. Fish Biol.* 65:1223-1232. Roessig, J.M., C.M. Woodley, J.J. Cech, Jr., and L.J. Hansen. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. *Rev. Fish Biol. Fisheries* 14:251-275. Myrick, C.A. and J.J. Cech, Jr. 2004. Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? *Rev. Fish Biol. Fisheries* 14:113-123. Lankford, S.E., T.E. Adams, R.A. Miller, and J.J. Cech, Jr. 2005. The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiol. Biochem. Zool.* 78:599-609. Swanson, C., P.S. Young, and J.J. Cech, Jr. 2005. Close encounters with a fish screen: integrating physiological and behavioral results to protect endangered species in exploited ecosystems. *Trans. Amer. Fish. Soc.* 134:1111-1123. Myrick, C.A. and J.J. Cech, Jr. 2005. Effects of temperature on the growth, food consumption, and thermal tolerance of age-0 Nimbus-strain steelhead. *N. Amer. J. Aquacult.* 67:324-330. Solomon, C.T., P.K. Weber, J.J. Cech, Jr., B.L. Ingram, M.E. Conrad, M.V. Machavaram, A.R. Pogodina, and R.L. Franklin. 2006. Experimental determination of the sources of otolith carbon and associated isotopic fractionation. *Can. J. Fish. Aquat. Sci.* 63:79-89. Allen, P.J., M. Nicholl, S. Cole, A. Vlazny, and J.J. Cech, Jr. 2006. Growth of juvenile green sturgeon in elevated temperature regimes. *Trans. Amer. Fish. Soc.* 135:89-96. Lankford, S.E., B.M. Adams, T.E. Adams, and J.J. Cech, Jr. 2006. Using specific antisera to neutralize ACTH in sturgeon: A method for manipulating the interrenal response during stress. *Gen. Comp. Endocr.* 147:384-390. Allen, P.J., B. Hodge, I. Werner, and J.J. Cech, Jr. 2006. Effects of ontogeny, season, and temperature on the swimming performance of juvenile green sturgeon (*Acipenser medirostris*). *Can. J. Fish. Aquat. Sci.* 63:1360-1369.

List relevant project/field experience and publications/reports.

Salutation: Mr.

Last Name: Gingras

First Name: Marty

Title: Supervising Biologist (Fisheries)

Organization: California Department of Fish and Game, Central Valley Bay Delta Branch, Stockton

Position:

Co-PI

Responsibilities: task 5

Qualifications:

Marty Gingras is a Supervising Biologist managing the Department of Fish and Game's Central Valley Bay Delta Branch Fisheries Research and Monitoring Program (Program). This program includes units lead by Senior Biologist Supervisors working on sport fish populations (Mr. Kyle Murphy), fish facility operations (Mr. Robert Fujimura), native fishes investigations (Mr. Kevin Fleming), and long-term monitoring of fishes and zooplankton (Mr. Randy Baxter). Day-to-day coordination of the proposed activities will be made by one or more of the Program's Senior Biologist Supervisors. This Program has a long history of collaboration with interested parties, including previous and on-going collections for investigators at UC Davis of delta smelt, live striped bass and striped bass tissues, live sturgeon and sturgeon tissues.

Fisheries Research and Monitoring Program publications regarding sturgeon include:

California Department of Fish and Game. 1992. Sturgeon in relation to water development in the Sacramento-San Joaquin Estuary. Entered by California Department of Fish and Game for the State Water Resources Control Board 1992 Water Rights Phase of the Bay-Delta Estuary Proceedings.

California Department of Fish and Game. 2002. California Department of Fish and Game Comments to NMFS Regarding Green Sturgeon Listing. 129 p

Kohlhorst, D. W. 1976. Sturgeon spawning in the Sacramento River in 1973, as determined by distribution of larvae. Calif. Fish Game 62(1):32-40.

Kohlhorst, D. W. 1980. Recent trends in the white sturgeon population in California's Sacramento-San Joaquin Estuary. California Fish and Game 66:210-219.

Kohlhorst, D. W., L. W. Miller, and J. J. Orsi. 1980. Age and growth of white sturgeon collected in the Sacramento-San Joaquin Estuary, California: 1965-1970 and 1973-1976. California Fish and Game 66:83-95.

Kohlhorst et al. 1991. Aspects of the structure and dynamics of an exploited central California population of white sturgeon (*Acipenser tranmontanus*). Pages 227-293 in P. Williot, Editor. *Acipenser*. Cemagref Publications, California.

Miller, L. W. 1972a. White sturgeon population characteristics of the Sacramento- San Joaquin Estuary as measured by tagging. California Fish and Game 58:94-101.

Miller, L. W. 1972b. Migrations of sturgeon tagged in the Sacramento-San Joaquin Estuary. California Fish and Game 58:102-106.

Schaffter, R. G. 1997. White sturgeon spawning migrations and location of spawning habitat in the Sacramento River, California. California Fish and Game 83:1-20.

Schaffter, R. G., and D. W. Kohlhorst. 1999. Status of the white sturgeon in the Sacramento-San Joaquin Estuary. California Fish and Game 85:37-41.

Stevens, D. E. and L. W. Miller. 1970. Distribution of sturgeon larvae in the Sacramento-San Joaquin River system. Calif. Fish and Game 56(2):80-86.

List relevant project/field experience and publications/reports.

Salutation: Dr.

Last Name: Kaufman

First Name: Robert

Title: Ph.D.

Organization: UC Davis - Department of Wildlife, Fisheries, and Conservation Biology

Position:

primary staff

Responsibilities: tasks 3, 7

Qualifications:

Education: BS in Environmental Toxicology from UC Davis (1995) (GPA: 3.858) Awarded Department Citation for Excellence High Honors 1995-present: Pharmacology and Toxicology Graduate Group as Ph.D. candidate (GPA: 3.939)

Scholarships: Teresa Barbera S.C. Trustees-Natural Science Shasta-Trinity Medical Health Henry A. Jastro (2 years undergraduate) Henry A. Jastro (96-97 &98-99 as graduate) Superfund Trainee (1 year graduate) Ecotoxicology (TSR Lead Campus Program) Funding Years: 1996-1997 &1997-1998 John Muir Institute of Ecology Graduate Student Fellowship (2004-2005)

Work Experience and Skills: 20 years as commercial, residential and custom carpentry. Cabinet maker. Part time work in a toxicology lab: primarily in design, construction and installation of bioassay laboratories. Experience in plant and animal husbandry 1982-1993 EMT I first responder and volunteer fireman 1988-1993 Trinity County Search and Rescue and Dive Team member

Professional Societies: American Fisheries Society NorCal Society of Environmental Toxicology and Chemistry National Society of Environmental Toxicology and Chemistry

Research Publications:

2006- Kaufman, R., A. Houck, and J.J. Cech, Jr. "Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria." Environmental Biology of Fishes (online first 2006)
[http://www.springerlink.com/\(ucxoyujtf43pbk2lmucq55\)/app/home/contribution.asp?referrer=parent=issue,59,1](http://www.springerlink.com/(ucxoyujtf43pbk2lmucq55)/app/home/contribution.asp?referrer=parent=issue,59,1)

1995-Second author of a project report with Dr. Jim Peterson (Acting Director of the National Biological Service Columbia River Research Laboratory, Cook, Washington) "Smallmouth Bass in the Horseshoe Bend Reach of the San Joaquin River: Limiting Factors and Bioenergetics Modeling"

1995- Second author of a report with Dr. Peter Moyle (professor, UCD) presented to BLM "Distribution of and Status of the Fishes of Willow Creek, Lassen County, California"

Professional Poster and Oral Presentations: 1996-National Society of Environmental Toxicology and Chemistry "Biochemical Indicators in Benthic Aquatic Organisms" Principle Author 1997-National Society of Environmental Toxicology and Chemistry "Biochemical Profiles as Biomarkers in Benthic Invertebrates" Principle Author 1998- Society of Environmental Toxicology and Chemistry-Europe "Biochemical Profiles as Biomarkers in Benthic Invertebrates" Principle Author 1999- National Society of Environmental Toxicology and Chemistry "Spatial and Temporal Profiles of Stress Protein (HSP70) and Metallothionein in Asian Clam ("Potamocorbula amurensis") in Northern San Francisco Bay." Second Author

2004- California Bay-Delta Authority Annual Science Meeting. "Effects of methylmercury on green sturgeon, *Acipenser medirostris*, bioenergetics at various lifestages." Robert C. Kaufman and Joseph J. Cech, Jr.

2005- Interagency Ecological Program for the San Francisco Bay Estuary "Green sturgeon blood-oxygen equilibria: In vitro effects of temperature and methylmercury." Robert C. Kaufman and Joseph J. Cech, Jr.

2005- American Fisheries Society Cal-Neva Chapter, Sacramento, California. "Green sturgeon blood-oxygen equilibria: Effects of temperature and carbon dioxide." Robert C. Kaufman and Joseph J. Cech, Jr.

2005- American Fisheries Society Meeting, Anchorage, Alaska. "Effects of Temperature and Carbon Dioxide on Green Sturgeon Blood-Oxygen Equilibria" Robert C. Kaufman, Ann G. Houck, and Joseph J. Cech, Jr.

2006-American Fisheries Society Cal-Neva Meeting San Luis Obispo, California. "Dietary Effects of Methylmercury on Green Sturgeon Bioenergetics" Bob Kaufman, Ann G. Houck and Joseph J. Cech, Jr

2006- American Fisheries Society International Meeting, Newfoundland, Canada. "Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria" Robert C. Kaufman, Ann G. Houck, and Joseph J. Cech, Jr.

Additional Projects and Information:

1999- Toxicity and effects of organophosphate insecticides mobilized by stormwater runoff events in a urban stream: Arcade Creek, Sacramento California. Conducted in collaboration with Aqua-Science, an environmental consulting firm.

November 2003 Contract with East Bay Municipal Utility Districts (EBMUD) to develop a salmon fecundity model specific to the Mokelumne River to be used in their management decisions on future Mokelumne River hydrographs and ecological restoration projects.

List relevant project/field experience and publications/reports.

Salutation: Dr.

Last Name: **Fiol**

First Name: **Diego**

Title: **Ph.D.**

Organization: **UC Davis - Department of Animal Science**

Position:

primary staff

Responsibilities: **tasks 6, 7**

Qualifications:

Education:

PhD in Sciences, Biological Area Facultad de Ciencias Exactas y Naturales -Universidad Nacional de Mar del Plata (FCEyN, UNMDP) - Argentina.: "Biochemical and physiological studies on trehalose-phosphate synthase in *Euglena gracilis*" Director: Dr. Graciela L. Salerno Thesis Presented in December 2002

Bachelor in Biological Sciences (Program equivalent to a master degree, a thesis is presented and defended to obtain the degree) Facultad de Ciencias Exactas y Naturales -Universidad Nacional de Mar del Plata (FCEyN, UNMDP) - Argentina "Functional analysis of the chaperonin cpn60 of *A. thaliana* expressed in *E. coli*." Director: Dr. Graciela L. Salerno Co-Director: Dr. Néstor R. Cortez Thesis Presented in October 1995

Research Experience:

Post-doctoral research

At Dr. Dietmar Kueltz Laboratory. Department of Animal Science, University of California at Davis. "Mechanisms of cellular osmoregulation and intracellular stress signaling in gill cells of euryhaline tilapia (*Oreochromis mossambicus*)" From January 2003

Post-graduate research

At Dr. Graciela L. Salerno Laboratory. Centre for Applied Biological Research (CIB-FIBA) Argentina. "Biochemical and physiological studies on trehalose-phosphate synthase in *Euglena gracilis*" (PhD Thesis) From August 1996 to December 2002

At Dr Luis Herrera-Estrella Laboratory. CINVESTAV - Irapuato- Mexico. "Cloning of the sucrose phosphate synthase from *Synechocystis*" (UNESCO short-term fellowship) From October 1995 to December 1995
Undergrad research

At Dr. Graciela L. Salerno Laboratory. Centre of Biological Research (CIB-FIBA) Argentina. "Functional analysis of the chaperonin cpn60" (Bachelor Thesis) From March 1994 to October 1995

Publications:

Fiol, Diego F. Mak, Sally and Kultz, Dietmar. TSC22 transcripts are hypertonically induced and alternatively spliced to protect mouse kidney cells during osmotic stress. Submitted to FEBS J.

Fiol, Diego F. Chan S.Y. and Kültz, Dietmar. Identification and pathway analysis of immediate hyperosmotic stress responsive molecular mechanisms in tilapia (*Oreochromis mossambicus*) gill. *Comp. Biochem. Physiol.*, part D: Genomics and Proteomics In press

Fiol, Diego F. Chan S.Y. and Kültz, Dietmar. Regulation of osmotic stress transcription factor 1 (*Ostf1*) in tilapia (*Oreochromis mossambicus*) gill epithelium during salinity stress. *J. Exp. Biol* (2006) 209:3257-3265

Fiol, Diego F. and Kültz, Dietmar. Use of suppressive subtractive hybridization to identify osmotic stress transcription factors in tilapia (*Oreochromis mossambicus*). *ClonTechniques* (2006) April issue.

Fiol, Diego F. and Kültz, Dietmar. Rapid hyperosmotic co-induction of two novel tilapia (*Oreochromis mossambicus*) transcription factors in gill cells. *Proc. Natl. Acad. Sci. USA* (2005) 102:927-932

Fiol, Diego F. and Salerno, Graciela Trehalose synthesis in *Euglena gracilis* (euglenophyceae) occurs through an enzyme complex" *J Phycology* (2005) 41:812-818

Pagnussat, Gabriela C., Fiol, Diego F. and Salerno, Graciela L. "A CDPK type protein kinase is involved in rice SPS light modulation" *Physiologia Plantarum* (2002) 115:183-189

Porchia, Andrea C., Fiol, Diego F. and Salerno, Graciela L. "Sucrose and trehalose synthesis in *Euglena gracilis*. *Plant Science* (1999) 149:43-49

Grants/Fellowships:

Period: 12/98 to 12/02. Type: Posgraduate Formation fellowship from Argentine national Council of Scientific Research, CONICET Period: 4/97 to 12/98 Type: Posgraduate Formation fellowship from Comisión de Investigaciones Científicas (CIC), Argentina

Period: 4/96 to 4/97 Type: Fellowship for Scientific Training from Fundacion para Investigaciones Cientificas Aplicadas Period: 10/95 to 12/95 Type: UNESCO short-term fellowship Presentations:

Journal of Experimental Biology Meeting, September 2006, Banff, Alberta, Canada. Title: "Functional genomics of the cellular osmotic stress response in 'non-model' organisms". Authors: Kültz D, FIOLE DF, Valkova N, Gomez-Jimenez S, Chan SY, and Lee JY.

XXXV International Congress of Physiological Science, April 2005, San Diego, CA. Title: "Identification of novel osmotic stress transcription factor 1 in gills of euryhaline tilapia". Authors: FIOLE DF and Kültz D. *The FASEB Journal*. (2005) 19, A1583

43th Annual Meeting of the American Society of Cell Biology, December 2003 San Francisco, CA. Title: "Osmotic induction of novel immediate early genes in fish gill epithelial cells". Authors: FIOLE DF and Kültz D

XXXVII Congress of the Argentine Society of Biochemical and Molecular Biology Research. October 2001. Córdoba, Argentina.. Title: "Early response of trehalose metabolism in stressed euglena cells". Authors: FIOLE DF and Salerno GL

XXIII Meeting of the Argentine Society of Plant Physiologists. November 2000. Rio IV, Argentine Title: "Evolutive analysis of trehalose and sucrose synthesis in photosynthetic organisms". Authors: FIOLE DF, Ghiringhelli PD, Salerno GL and Curatti L

XXXVI Congress of the Argentine Society of Biochemical and Molecular Biology Research. October 2000. Viña del Mar, Chile. Title: "Trehalose synthesis in *Euglena gracilis*. Properties of trehalose phosphate phosphatase". Authors: FIOLE DF and Salerno GL

XXII Meeting of the Argentine Society of Plant Physiologists. September 1998, Mar del Plata, Argentina. Title: "Role of trehalose metabolism in *Euglena gracilis* in response to abiotic stresses". Authors: FIOLE DF and Salerno GL

XXXIII Congress of the Argentine Society of Biochemical and Molecular Biology Research. November 1997. Villa Giardino, Córdoba. Argentina. Title: "Partial purification and characterization of trehalose synthase of *Euglena gracilis*". Authors: FIOLE DF and Salerno GL

XXXI Congress of the Argentine Society of Biochemical and Molecular Biology Research, October 1995,

Villa Giardino, Córdoba, Argentina. Title: "Study of the chaperonine Cpn60- of Arabidopsis expressed in E. coli". Authors: Cortez N, FIOLE DF, Zabaleta E and Salerno G

First International Symposium on Sucrose Metabolism. May 1995. Mar del Plata, Argentina. Title: "Separation of two sucrose-phosphate synthase from etiolated rice". Authors: Pagnussat GC, Cortez N, FIOLE, DF and Salerno GL

List relevant project/field experience and publications/reports.

Salutation: Mr.

Last Name: Van Eenennaam

First Name: Joel

Title: M.S.

Organization: UC Davis - Department of Animal Science

Position:

primary staff

Responsibilities: tasks 2, 7

Qualifications:

EDUCATION B.S. (Fisheries & Wildlife), Michigan State University, East Lansing, 1977. M.S. (International Agricultural Development, Reproductive Biology of Fish). University of California, Davis, 1985.

POSITIONS 1985-present, Staff Research Associate, Department of Animal Science, University of California, Davis; 1983-1985, Research Assistant, Department of Animal Science, University of California, Davis; 1982, Aquaculture Technician, Fish Breeders of California, Niland, California; 1977-1981, Fisheries Extension Agent, Accelerated Rural Development Program, Khon Kaen, Thailand.

AWARDS 1999-00 Department of Animal Science Staff Performance Award 1998-99 Department of Animal Science Staff Recognition Award 1995-96 Department of Animal Science Staff Recognition Award 1990 Outstanding Performance Award, Administrative and Professional Staff Program

MEMBERSHIPS AND AFFILIATIONS World Aquaculture Society National Aquaculture Association American Fisheries Society National Society for Histotechnology World Sturgeon Conservation Society

PUBLICATIONS (last ten years) Van Eenennaam, A.L., J.P. Van Eenennaam, J.F. Medrano, and S.I. Doroshov. 1996. Rapid verification of meiotic gynogenesis and polyploidy in white sturgeon (*Acipenser transmontanus* Richardson). *Aquaculture* 147:177-189.

Van Eenennaam, J.P., S.I. Doroshov, G.P. Moberg, J.G. Watson, D.S. Moore, and J. Linares. 1996. Reproductive conditions of the Atlantic sturgeon (*Acipenser oxyrinchus*) in the Hudson River. *Estuaries* 19:769-777.

Chapman, F.A., J.P. Van Eenennaam, and S.I. Doroshov. 1996. The reproductive condition of white sturgeon, *Acipenser transmontanus*, in San Francisco Bay, California. *Fishery Bulletin*, 94: 628-634.

Doroshov, S.I., G.P. Moberg, and J.P. Van Eenennaam. 1997. Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. *Environmental Biology of Fishes* 48: 265-278.

Van Eenennaam, J.P., and S.I. Doroshov. 1998. Effects of age and body size on gonadal development in Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill). *Journal of Fish Biology* 53: 624-637.

Van Eenennaam, A.L., J.P. Van Eenennaam, J.F. Medrano, and S.I. Doroshov. 1999. Evidence of female heterogametic sex determination in white sturgeon. *Journal of Heredity* 90(1): 231-233.

Webb, M.A.H., J.P. Van Eenennaam, S.I. Doroshov, and G.P. Moberg. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture* 176: 315-329.

Webb, M.A.H., J.P. Van Eenennaam and S.I. Doroshov. 2000. Effects of steroid hormones on in vitro oocyte maturation in white sturgeon. *Fish Physiology and Biochemistry* 23: 317-325.

Czesny, S., K. Dabrowski, J.E. Christensen, J. Van Eenennaam and S. Doroshov. 2000. Discrimination of

wild and domestic origin of sturgeon ova based on lipids and fatty acid analysis. *Aquaculture* 189: 145-153.

Van Eenennaam, J.P., M.A.H. Webb, X. Deng, S. I. Doroshov, R. Mayfield, J.J. Cech, Jr., D. Hillemeier, and T. Willson. 2001. Artificial spawning and larval rearing of Klamath River green sturgeon. *Transactions of the American Fisheries Society* 130: 159-165.

Webb, M.A.H., J.P. Van Eenennaam, G. Feist, J. Linares-Casenave, M. Fitzpatrick, C.B. Schreck, and S.I. Doroshov. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture* 201 (1-2): 137-151.

Deng, X., J.P. Van Eenennaam, and S.I. Doroshov. 2002. Comparison of early life stages and growth of green and white sturgeon. Pages 237-248 in W. Van Winkle, P.J. Anders, D.H. Secor, and D.A. Dixon (editors). *Biology, management and protection of North American sturgeon*. American Fisheries Society, Symposium 28, Bethesda, Maryland.

Yesaki, T.Y., R. Ek, J. Siple, J.P. Van Eenennaam, and S.I. Doroshov. 2002. The effects of iodophor disinfection and transportation on the survival to hatch of fertilized white sturgeon (*Acipenser transmontanus*) eggs. *Journal of Applied Ichthyology* 18: 639-641.

Linares-Casenave, J., J.P. Van Eenennaam, and S.I. Doroshov. 2002. Ultrastructural and histological observations on temperature-induced follicular ovarian atresia in the white sturgeon. *Journal of Applied Ichthyology* 18: 382-390.

Linares-Casenave, J., K.J. Kroll, J.P. Van Eenennaam, and S.I. Doroshov. 2003. Effects of ovarian stage on plasma vitellogenin and calcium in cultured white sturgeon. *Aquaculture* 221:645-656.

Feist, G., J.P. Van Eenennaam, S.I. Doroshov, C.B. Schreck, R.P. Schneider, and M.S. Fitzpatrick. 2004. Early identification of sex in cultured white sturgeon, *Acipenser transmontanus*, using plasma steroid levels. *Aquaculture* 232: 581-590.

Van Eenennaam, J.P., F.A. Chapman, and P.L. Jarvis. 2004. *Aquaculture*. Pp. 277-311 in G.T.O. LeBreton, F.W.H. Beamish and R.S. McKinley (ed). *Sturgeons and Paddlefish of North America*. Fish and Fisheries Series Vol. 27. Kluwer Academic Publishers, Dordrecht.

Mager, R.C., S. I. Doroshov, J.P. Van Eenennaam, and R.L. Brown. 2004. Early life stages of delta smelt. *American Fisheries Society Symposium* 39: 169-180.

Van Eenennaam J.P., J. Linares-Casenave, X. Deng, and S.I. Doroshov. 2005. Effect of incubation temperature on green sturgeon embryos, *Acipenser medirostris*. *Environmental Biology of Fishes* 72:145-154.

Van Eenennaam J.P., J. Linares-Casenave, S.I. Doroshov, D. C. Hillemeier, T. E. Willson, and A. A. Nova. 2006. Reproductive conditions of the Klamath river green sturgeon (*Acipenser medirostris*). *Transactions of the American Fisheries Society* 135: 151-163.

Werner, I., J. Linares-Casenave, J.P. Van Eenennaam, and S.I. Doroshov. In Press. The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (*Acipenser medirostris*). *Environmental Biology of Fishes*.

Gessner, J., J.P. Van Eenennaam, and S.I. Doroshov. In Press. North American green and European Atlantic sturgeon: comparisons of life histories and human impacts. *Environmental Biology of Fishes*.

List relevant project/field experience and publications/reports.

Salutation: Ms.

Last Name: Houck

First Name: Ann

Title: M.S.

Organization: UC Davis - Department of Wildlife, Fisheries, and Conservation Biology

Position:

primary staff

Responsibilities: tasks 4, 7

Qualifications:

SCHOLASTIC ACHIEVEMENTS

Graduated UC Davis (Dept. Wildlife, Fisheries, and Conservation Biology) with High Honors (B.S., major: Fisheries Biology, minor: Environmental Toxicology)

Awarded Departmental Citation for Academic Excellence

Cumulative Undergraduate UC Davis GPA: 3.75

Graduated Ecology Graduate Group with an M.S. (area of emphasis Aquatic Toxicology) GPA:

Fellowships: Two year Ecotoxicology Traineeship from the EPA with matching funds for research from the Center for Ecological Health Research

RESEARCH SKILLS and RELATED WORK EXPERIENCE Three years raising Sacramento blackfish from larvae to juveniles Three years raising green sturgeon from egg to sub-adults 6 years experience in the investigation and assessment of the effects of MeHg on fish bioenergetics and performance Developing blood oxygen equilibrium curves (standard tonometry) and the effects of MeHg (both in vitro and in vivo Mercury analytical techniques (both AA and CVAA) Extraction procedures for biochemical profiling tissue samples (amino acids, glycolysis and Krebs cycle intermediates, etc.) SDS:PAGE and modified Western blot protein separation Aquatic lab design and construction

PROFESSIONAL SOCIETIES American Fisheries Society (also Cal-Neva chapter) NorCal Society of Environmental Toxicology and Chemistry National Society of Environmental Toxicology and Chemistry

COMPLETED RESEARCH AND PUBLICATIONS

2006- Kaufman, R., A. Houck, and J.J. Cech, Jr. "Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria." Environmental Biology of Fishes (online first)
[http://www.springerlink.com/\(ucxoyujtf43pbk2lmucqe55\)/app/home/contribution.asp?referrer=parent=issue,59,1](http://www.springerlink.com/(ucxoyujtf43pbk2lmucqe55)/app/home/contribution.asp?referrer=parent=issue,59,1)

Houck, Ann, R. Kaufman, and J. Petersen (Acting Director of the National Biological Service, Columbia River Research Laboratory, Cook, Washington) "Smallmouth Bass in the Horseshoe Bend Reach of the San Joaquin River: Factors and Bioenergetics Modeling"

Houck, A., Kaufman, R. and P. B. Moyle "Distribution of and Status of the Fishes of Willow Creek, Lassen County, California" furnished for

J.J. Cech Jr., M. Choi, and A. Houck. Trans-gill and Dietary Uptake of Methyl Mercury by the Sacramento Blackfish, a Planktivorous Freshwater Fish. Pg1273-1283 in Managing for Healthy Ecosystems. Ed Rapport, D. J. et al, Lewis Pub. CRC, Boca Raton

Houck, Ann and J.J.Cech. 2004. Effects of dietary methylmercury on juvenile Sacramento blackfish bioenergetics. Aquatic Toxicology 69 (2004) 107-123

2005- American Fisheries Society Meeting, Anchorage, Alaska. "Effects of Temperature and Carbon Dioxide on Green Sturgeon Blood-Oxygen Equilibria" Robert C. Kaufman, Ann G. Houck, and Joseph J. Cech, Jr.

2006-American Fisheries Society Cal-Neva Meeting San Luis Obispo., California. "Dietary Effects of Methylmercury on Green Sturgeon Bioenergetics" Bob Kaufman, Ann G. Houck and Joseph J. Cech, Jr

2006- American Fisheries Society International Meeting, Newfoundland, Canada. "Effects of temperature and carbon dioxide on green sturgeon blood-oxygen equilibria" Robert C. Kaufman, Ann G. Houck, and Joseph J. Cech, Jr.

Additional Projects and Information:

1999- Toxicity and effects of organophosphate insecticides mobilized by stormwater runoff events in a urban stream: Arcade Creek, Sacramento California. Conducted in collaboration with Aqua-Science, an environmental consulting firm.

November 2003 Contract with East Bay Municipal Utility Districts (EBMUD) to develop a salmon fecundity model specific to the Mokelumne River to be used in their management decisions on future Mokelumne

River hydrographs and ecological restoration projects.

WORK IN PROGRESS

Finish out remaining work of the green sturgeon project (funded by Cal-Fed): the Hg analysis and histological samples from the MeHg growth study on green sturgeon Completing the second chapter of my thesis for publication: "The effects of MeHg on the swimming performance and optomotor response of Sacramento blackfish"

List relevant project/field experience and publications/reports.

Salutation: **Mr.**

Last Name: **Lee**

First Name: **Jang-Won**

Title: **PhD student**

Organization: **UC Davis - Department of Animal Science**

Position:

primary staff

Responsibilities: **tasks 3, 7**

Qualifications:

Education

Master of Science (Candidate) in Fish Physiology, Humboldt State University, Arcata, CA

Dissertation Title: The Effects of Suspended Particles on Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Energetics; Individual-based Bioenergetics Approach.

Master of Science in Animal Physiology, Department of Marine Biology, Pukyong National University, Busan, Korea, 2000.

Dissertation Title: The Sub-lethal Effects of Trace Metals (Cd, Cu and Hg) on Bioenergetics (Scope for Growth) of Barnacle, *Megabalanus rosa* for Marine Pollution Assessment.

Bachelor of Science in General Biology, Dong-A University at Busan, Korea, 1998.

Language Experience

Student in English Language School of International Department, University of Chichester, West Sussex, England, January-June, 1996.

Honors and Fellowships

First Place in the 2000 Graduation Examination in English, Department of Marine Biology, Pukyong National University at Busan, Korea 2000.

Graduate Summer Research Fellowship, Department of Marine Biology, Pukyong National University at Busan, Korea, summer of 1998.

Research Employment and Experience

Graduate Research Assistant, California Cooperative Fisheries Research Unit, Department of Fisheries Biology, Humboldt State University, Arcata, January 2004-Present.

Research Technician, California Cooperative Fisheries Research Unit, Department of Fisheries Biology, Humboldt State University, Arcata, January 2003-December 2003.

Collection Manager, Fish Collection, California Cooperative Fisheries Research Unit, Department of Fisheries Biology, Humboldt State University, Arcata, August 2003-May 2004.

Research Scientist, Pukyong Fisheries Aquaculture Corporation, Korea, President Min-suk Kim, 082-(055)-632-4012, March 2000-May 2002.

Graduate Research Assistant, Department of Marine Biology, Pukyong National University at Busan, January 1998-February 2000.

Research Technician, Fish Physiology Laboratory, National Fisheries Research and Development Institute (NFRDI), July-August 1997.

Teaching Employment and Experience

Teaching Assistant in Animal Physiology Laboratory under Professor Pyung Chin, Department of Marine Biology, Pukyong National University at Busan, Korea, Spring Semester 1999.

Science Teacher (part-time) in science class for middle school student and Biology I for high school students, Jongro Middle School, Sagik-dong, Busan, Korea.

Areas of Interest

Ecological physiology of aquatic animals, nutrition of fish and aquaculture, sustainable aquaculture, environmental adaptation of fish and its molecular mechanisms, applied physiology of fish, genomics.

Publications

Lee, Jang-Won and Pyoung Chin. Development of physiological and biochemical bioindicators of barnacle, *Megabalanus rosa*, for Marine Pollution Assessment. *Journal of Korean Fisheries Society*. 36(3):276-282.

Technical Report

Pyoung Chin, Jeong-A Lee, Jang-Won Lee. The assessment of adverse effect on the aquatic organism as a result of marine construction. Korea Science and Engineering Foundation, Korea Government Grant, 98, 2000.

Presentations

Poster Presentation on "Monitoring of Marine Pollution Using Common Mussels (*Mytilus edulis*) in Yon-Ho Bay", Korean Fisheries Society Annual Conference, Gyeongsang National University, Tongyeong, Korea, April 1999.

Poster Presentation on "The adverse effects of silt and clay on Common Mussels (*Mytilus edulis*) and founder (*Pleuronichthys cornutus*), Korean Fisheries Society Annual Conference, Yosu National University, Kosu, Korea, April 1998.

List relevant project/field experience and publications/reports.

Salutation: Ms.

Last Name: Kammerer

First Name: Brittany

Title: PhD student

Organization: UC Davis - Department of Animal Science

Position:

primary staff

Responsibilities: tasks 6, 7

Qualifications:

EDUCATION Ph.D., Molecular Cellular and Integrative Phys., University of California, Davis. Expected Fall 2009.

Bachelor of Science, Cum Laude, Biology and Environmental Studies, Tufts University. 2002.

HONORS Hart, Cole, and Goss Research Fellowship. University of California, Davis. 2005 & 2006. Graduate Scholar Fellowship, University of California, Davis. 2004-2005. Molecular, Cellular, and Integrative Physiology Fellowship. 2004-2005. Golden Key National Honor Society, Tufts University, 2000-2002. Americorps National Service Education Award Scholarship, 2001 Tufts University Environmental Studies Scholarship, 2000.

RESEARCH EXPERIENCE

Dissertation Research, University of California, Davis. Short-term mechanisms of seawater adaptation in teleost gill cells, 2005-present.

Research Intern. University of California, Davis. Is a "Sturgeon-Friendly" Fish Ladder Possible? Hydraulics and Passage Efficacy. Winter 2004-2005.

Research Technician. University of Washington, Department of Medicine. 2004. Effects of Transforming Growth Factor-beta on Vascular Disease.

Research Intern, College of the Atlantic, Mt. Desert Rock Field Station. 2003. Population biology of Fin Whales (*B. physalus*) using genetic techniques. Research Intern, New England Aquarium North Atlantic Right Whale Research Project. 2002-2003. Population dynamics and monitoring of the North Atlantic Right Whale.

Biotech Intern. Mt. Rainier National Park, Student Conservation Association. 2001. The Influence of Marine-Derived Nutrients on Water Quality and Biological Productivity; Genetics and Demographics of Amphibians. **TEACHING EXPERIENCE**

Teaching Assistant Neurobiology, Physiology, and Behavior 101L: Systemic Physiology Laboratory. University of California, Davis. Summer, Fall 2005.

Program Educator New England Aquarium Boston, MA. July-Nov. 2002.

Undergraduate Teaching Assistant Biology 13: Cells and Organisms Laboratory. Tufts University, Fall 2000.

SERVICE American Fisheries Society Secretary. CAL-NEVA Chapter, Davis Subunit. Davis, CA. 5/2006-present. Molecular, Cellular, and Integrative Physiology Student Steering Committee. 2006. Solar Community Housing Association Board of Director & Secretary, Davis, CA. 2005-present.

List relevant project/field experience and publications/reports.

Salutation: **Mr.**

Last Name: **Sardella**

First Name: **Brian**

Title: **Graduate Student (will have acquired PhD by 12/31/06)**

Organization: **UC Davis - Department of Animal Science (starting with this project)**

Position:

primary staff

Responsibilities: **tasks 3, 6, 7**

Qualifications:

Funded by the U.S. Bureau of Reclamation, and The Salton Sea Authority, I have spent the last four years conducting physiological research within the ecological framework of the Salton Sea, in southeastern CA, which poses multiple environmental challenges to its inhabitants. This research has strong management implications, as there are massive efforts at both the state and federal level to mitigate damage to the Salton Sea fishery due to rising salinity, eutrophism, hypoxia/anoxia, high ammonia levels, and large season fluctuations in temperature. My focuses have been in three main areas; investigating the physiological basis of temperature/salinity interactions and their possible role in Salton Sea fish kills, identifying the most sensitive indicators of osmoregulatory stress in order to develop a bioindicator for wild Salton Sea fishes, and investigating the mechanisms behind the incredible salinity tolerance of the Mozambique tilapia (as high as 120 g/l). Our results have shown that wide-scale fish kills of tilapia in the Salton Sea are most likely due to their poor osmoregulatory performance in low ambient temperatures, and over short term transfer protocols, the rate of chloride cell apoptosis has been shown to be the most sensitive indicator of osmoregulatory stress, with increases up to eight-fold in our highest salinity treatments. With regard to the final objective, research is still ongoing; however, I have shown that this species reduces its metabolic rate, as well as the activities of key energy-demanding enzymes, in response to elevated external salinity. This has never been previously observed and may be a key in understanding the impressive salinity tolerance of this species. In addition, I have participated in and/or conducted other lines of research outside the Salton Sea project, including the effects of hypoxic exposures on the common

killifish, the effects of acid-base and salinity disturbances on the Pacific hagfish, and the effects of temperature/salinity interaction on the osmoregulatory ability of the tide pool sculpin. My work on the Salton Sea tilapia hybrid has been published, submitted, or is in preparatory phases for publication, and has been presented at professional scientific meetings worldwide, as well as to our funding agencies by both me and my current supervisor.

Selected Relevant Publications Sardella, B. A., and C.J. Brauner, 2006. Temperature shifts result in osmotic imbalance in the California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*); a physiological basis for wide-scale mortality in the Salton Sea. Journal of the California Department of Fish and Game, Submitted August 2006.

Sardella, B. A., and C.J. Brauner, 2006. Coping with multiple stressors: physiological mechanisms and strategies of fishes in the Salton Sea. *Hydrobiologia*, In Press.

Sardella, B. A., and C.J. Brauner, 2006. The effect of physiological state and the environment on the osmoregulatory compromise in fish. In: *Fish Respiration and Environment*, Eds. M.N. Fernandez, F.T. Rantin, M.L. Glass, and B.G. Kapoor. Science Publisher, Enfield, NH, USA (In Press)

Sardella, B. A., J. Cooper, V. Matey, R. Gonzalez, and C.J. Brauner. 2004. Physiological, biochemical, and morphological indicators of osmoregulatory stress in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to hypersaline water. *Journal of Experimental Biology*, 207: 1399-1414.

Sardella, B. A., J. Cooper, R. Gonzalez, and C.J. Brauner. 2004. The effects of temperature on the salinity tolerance of juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*). *Comparative Biochemistry and Physiology A*, 137: 621-629.

Invited Seminar Speaker

Wednesday Evening Comparative Physiology Seminar, Department of Zoology, University of British Columbia, October 2003 Salton Sea Science Committee, January 2003 Salton Sea Technical Advisory Committee, November 2002 Salton Sea Science Committee, November 2002

Selected Presentations at Professional Meetings (Published Abstracts)

Sardella, B. A., V. Matey, and C.J. Brauner. Apoptosis in gill mitochondrial-rich cells as a bioindicator of salinity stress in a Mozambique tilapia hybrid (*Oreochromis mossambicus* x *O. urolepis hornorum*). *International Congress on the Biology of Fishes*, St. John's Newfoundland, Canada, July 2006.

Sardella, B. A., and C.J. Brauner. A biochemical basis for reductions in metabolic rate following hypersaline exposures in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*). *International Congress on the Biology of Fishes*, St. John's Newfoundland, Canada, July 2006.

Sardella, B. A., J. Cech Jr., D. Kültz, and C.J. Brauner. The effects of temperature on chloride cell characteristics of Mozambique tilapia assessed using tissue microarrays and laser scanning cytometry. *Annual Meeting of the Canadian Society of Zoologists*, Kingston, Ontario, Canada, May 2005.

Sardella, B. A., and C.J. Brauner. The effect of temperature/salinity interaction on 24 hour saline challenges in juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*). *Annual Meeting of the Canadian Society of Zoologists*, Wolfville Nova Scotia, Canada, May 2004.

Sardella, B. A., J. Cooper, V. Matey, R. Gonzalez, and C.J. Brauner. Salinity tolerance of juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*). *International Congress on the Biology of Fishes*, Manaus, Amazonas, Brazil, August 2004.

Davis, P. and B.A. Sardella. Saline tolerance of the red belly tilapia (*Tilapia zilli*) from the Salton Sea drainage. *Annual Meeting of the Western Division of the American Fisheries Society*, San Diego, CA, USA, April 2003

List relevant project/field experience and publications/reports.

Conflict Of Interest

This is proposal #0025 for the Science Program 2006 solicitation.

Frequently asked questions and answers for this PSP are now available.

The submission deadline for this proposal has passed. Proposals may not be changed.

Instructions

To assist Science Program staff in managing potential conflicts of interest as part of the review and selection process, we are requesting applicants to provide information on who will directly benefit if your proposal is funded. Please provide the names of individuals who fall in the following categories and are not listed in the Personnel Form:

- Persons listed in the proposal, who wrote the proposal, will be performing the tasks listed in the proposal, or who will benefit financially if the proposal is funded; and/or
- Subcontractors listed in the proposal, who will perform tasks listed in the proposal, or will benefit financially if the proposal is funded.

Applicant
Submittor
Lead Investigator/Project Director
Primary Staff
Secondary Staff
Subcontractor

Provide the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

Last Name First Name Organization Role

Task And Budget Summary

This is proposal #0025 for the Science Program 2006 solicitation.

Frequently asked questions and answers for this PSP are now available.

The submission deadline for this proposal has passed. Proposals may not be changed.

Instructions

Use the table below to delineate the tasks needed to carry out your proposal. Tasks in this form should support the narrative description of your project in your proposal document and the information provided in your detailed budget spreadsheet. Each task and subtask must have a number, title, timeline, list of personnel or subcontractors providing services, and associated budget figure.

When creating subtasks, ensure that each activity is counted only once. Please note, the initial task of your table (Task 1) must present all project management/administrative activities supporting your overall proposal.

For proposals involving multiple agencies or organizations (including subcontractors), the table must clearly state the tasks and subtasks performed by each entity.

Task #	Task Title	Start Month	End Month	Personnel Involved	Description	Task Budget
1	General coordination and management	1	36	Kueltz, Dietmar	Prepare and facilitate meetings; Coordinate monthly progress; Integrate results; Progress reports will be generated.	8,625
2	Sturgeon culture and larval exposure	1	24	Doroshov, Serge Van Eenenaam, Joel	Sturgeon breeding and histological analysis of early life stages exposed to SeMet and thermal stress; White and green sturgeon will be raised for this project for tasks 3,4, 6; Information on developmental defects during SeMet and temperature stress will be generated.	123,576
3	Toxicological effects of SeMet with and without additional stresses	1	24	Hung, Silas Lee, Jang-Won Sardella, Brian	Quantifying the toxicokinetics, bioaccumulation, and chronic toxicity of selenium when present alone or in combination with MeHg in the diet; Information about critical SeMet and MeHg concentrations that are toxic to sturgeon and lead to 1) mortality, 2) growth depression will be generated; Information about rates of bioaccumulation of SeMet and MeHg in sturgeon tissues will be generated; Information about potential additive effects of SeMet and MeHg on sturgeon health will be generated; Tissues will be provided for analysis in task 6; acclimated animals will be provided for analysis in task 4.	154,724
4	Physiological effects of SeMet with and without additional stresses	1	24	Cech, Joseph Kaufman, Robert Houck, Ann	Quantifying effects of chronic exposure to dietary SeMet stress alone and in combination with MeHg, salinity, and temperature stresses on swimming performance, resting metabolism, and hematology; Information about critical SeMet and MeHg concentrations that impair sturgeon physiology will be generated; Information about additive effects of temperature and salinity stresses on sturgeon that are exposed to SeMet and MeHg will be generated; Tissues will be provided for analysis in task 6.	159,131
5	Collection of biopsies and data from wild sturgeon	1	24	Gingras, Marty Kammerer, Brittany	Wild sturgeon will be monitored through existing fish salvaging and monitoring programs by CDFG; Biopsies from gill and muscle tissues will be obtained and delivered to task 6; Data on locations and dates of sturgeon catches will be collected and correlated with environmental water quality data.	0

6	Stress proteome identification and tissue microarray construction	1	36	Kueltz, Dietmar Fiol, Diego Kammerer, Brittany Sardella, Brian	Identification of proteome changes occurring in gill, liver, and kidney during SeMet stress, singly and in combination with MeHg, salinity, and temperature stresses; Construction of tissue microarrays (TMAs) to reveal effects of these stressors and provide a platform for future high-throughput bio-indicator assays; Protein sequences for antibody generation and development of ELISAs will be generated; More sensitive molecular bio-indicators that can detect responses to lower levels of SeMet and MeHg than can be detected at the whole animal level (e.g. compared to growth or mortality) will be generated; Tissue microarrays (TMAs) that can be used as a reference for comparison with future field samples will be generated.	554,575
7	Data management, analysis, and submission to public databases	1	36	Kueltz, Dietmar Doroshov, Serge Hung, Silas Cech, Joseph Gingras, Marty Kaufman, Robert Fiol, Diego Van Eenenaam, Joel Houck, Ann Lee, Jang-Won Kammerer, Brittany Sardella, Brian	Management and analysis of data allowing crosscomparison of results obtained in all tasks; Preparation and submission of proteomics data into a public database that is accessible via internet; Coordination of collaborative publications resulting from this project.	8,250

total budget=\$1,008,881

Detailed Budget Upload And Justification

This is proposal #0025 for the [Science Program 2006 solicitation](#).

[Frequently asked questions and answers for this PSP are now available.](#)

The submission deadline for this proposal has passed. Proposals may not be changed.

Using the [budget provided via this link as a guide](#), please complete a budget for your proposal in the software of your choice (e.g. Excel). This document must be in a format and software that can be converted to PDF prior to uploading on the web system.

It is incumbent upon the applicant to fully explain/justify the significant costs represented in the attached budget. This information can be provided either in a text document and uploaded below, or included in your proposal text in a clearly defined budget justification section. If it is not abundantly clear to reviewers what project costs are commensurate with which efforts and benefits, the proposal may receive a poor review and denied funding.

Costs for each task described in the Task and Budget Summary Form and each staff or subcontractor described on the Contacts and Project Staff Form, must be included in your budget. The budget for Task One should represent project management activities, including but not limited to cost verification, environmental compliance, data handling, report preparation, project oversight, and public outreach. The total amount of your budget must equal the total amount represented on your Task and Budget Summary Form and the total budget amount represented on your Project Information and Executive Summary Form.

In a separate text document to be uploaded below, identify any cost share and other matching funds available to support your proposed project. If you identify cost share or matching funds, you must also describe them in the text of your proposal (see explanation of "cost share and other matching funds" in Section Two of the solicitation document).

CBDA may request additional information pertaining to the items, rates and justification of the information presented in your budget. Applications without completed budgets will not be considered for funding.

Uploading The Completed Budget Template

First, convert your completed Budget to a PDF file. Then, use the browse function to locate the PDF version of your document, select the document and click on the upload prompt below.

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Uploading The Description Of Cost Share/Matching Funds

First, convert your completed Description of Cost Share/Matching Funds text file to a PDF file. Then, use the browse function to locate the PDF version of your document, select the document and click on the upload prompt below.

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Schedule Of Deliverables

This is proposal #0025 for the Science Program 2006 solicitation.

Frequently asked questions and answers for this PSP are now available.

The submission deadline for this proposal has passed. Proposals may not be changed.

Use the table below to delineate the key deliverables and the time necessary to complete them (in months from the date the project's grant agreement is executed). Each Science Program 2006 PSP grant recipient must provide the required minimum deliverables for each project. The required minimum deliverables for each funded proposal are as follows:

- Semi-annual report(s)
- Final Report
- One page project summary for public audience at beginning of project
- One page project summary for public audience upon project completion
- Project closure summary report or copy of draft manuscript
- Presentation at CALFED Science Conference
- Presentations at other events at request of CALFED Science Program staff
- Copy of all published material resulting from the grant

Deliverable	Description	Delivered By: # (In Months From Project Start Date)
Public project start-up summary	One page project summary for public audience at beginning of project	1
1. Semi-annual report	Summary of progress, data gathered, conclusions, and directions	6
2. Semi-annual report	Summary of progress, data gathered, and conclusions, and directions	12
3. Semi-annual report	Summary of progress, data gathered, and conclusions, and directions	18
Conference presentation	Presentation of data at CALFED Science Conference	20
Presentation at other events	Presentations at other events at request of CALFED Science Program staff	24
4. Semi-annual report	Summary of progress, data gathered, and conclusions, and directions	24
5. Semi-annual report	Summary of progress, data gathered, and conclusions, and directions	30
Final Report	Summary of progress, data gathered, and conclusions, and directions	36
Public project completion summary	One page project summary for public audience upon project completion	36
Project closure report	Project closure summary report or copy of draft manuscript	36
Conference presentation	Presentation of data at CALFED Science Conference	36
Presentation at other events	Presentations at other events at request of CALFED Science Program staff	36
Presentation at Society meetings	Presentation of Data and Conclusions at National and International Scientific Society meetings	36
Published material	Copy of all published material resulting from the grant	36

If you are unable to provide a Schedule of Deliverables as outlined above, please provide your justification of non-compliance in the text box provided below. The Science Program reserves the right to determine a proposal non-eligible based on an applicants inability to provide the materials requested above.

Quantitative indicators and life history implications of environmental stress on sturgeon

1. Introduction and Project Purpose

This proposal was developed in response to the CALFED 2006 Science PSP. It is based on a previous proposal that received a technical rating of "superior" in the 2004 CALFED Science PSP but despite superior ratings was not funded because of a cut-back in 2004 CALFED funds. The proposal was modified to 1) focus on the priorities of the 2006 CALFED Science PSP, 2) address suggestions for improvements made by the previous reviewers, and 3) incorporate new, relevant material produced by the participating researchers since the submission of the previous proposal.

Overall, the reviewer's comments on the previous version of this proposal were highly positive and enthusiastic and only a small criticism was pointed out. In response to a reviewer's comment we excluded the HSP70 analysis from the project because it did not have the same degree of novelty and was not as essential as the other parts of the project. In addition, we limited the project description to 20 pages (excluding figures and tables). Moreover, we made it clearer that our proposed approach does not only represent an innovative new way of identifying novel biomarkers in a key species but that we will also identify entire biochemical networks and biological processes modulated by stresses that are of critical relevance for the San Francisco Bay-Delta (SFBD) water system. The proposed approach will enable high-resolution modeling of biochemical networks and biological processes and, once established, can be used for any organism of interest in the SFBD area. One concern about our previous proposal was that it did not directly extend knowledge from the molecular and organismal levels to population level responses although the reviewers acknowledged that this remains a general challenge in the field of ecotoxicology. We agree that it would be highly desirable to link molecular and organismal responses to population level responses and our proposal is specifically designed to provide the basis for such undertaking. However, our current state of knowledge on any SFBD aquatic species simply does not permit wide-ranging conclusions about population-level effects based on biochemical indicators sampled from fish or invertebrates in the field. This will only become possible if more sensitive biomarkers are identified and if the biochemical pathways and biological processes affected by relevant stresses in key species (e.g. molecular pathways and functions controlling growth, development, reproduction, swimming performance, food consumption, detoxification, immunity, aging/longevity etc.) can be modeled at the organismal level. Such modeling will allow more accurate predictions of population-level effects in key species, and will ultimately improve our ability to predict consequences of water management-induced stress on the ecosystem.

In our opinion, tangible water management solutions have to be based on knowledge about a) how changes in water regimes affect important environmental variables such as salinity, temperature and toxicant levels, but also b) how these environmental changes (stresses) impact the key species that are critical elements of the ecosystem. Our proposal comprehensively addresses the second question by focusing on sturgeon as one of those key species to help define limits of water management operations that are compatible with ecosystem health. Because we will use an innovative, multi-disciplinary, state-of-the-art approach, we anticipate that results of this project will find broad application in many aquatic systems and will be published in high-impact scientific journals.

This proposal directly addresses multiple areas that are critical for improving ecosystem quality to support sustainable populations of key aquatic species. In particular, we seek to find out which of the many stressful conditions in the SFBD are most detrimental to population-sustaining biological processes in sturgeon (reproductive performance, immunity, growth, swimming performance, etc.). Such knowledge will support the long-term performance of CALFED actions by identifying the critical stressors or combinations of stressors that are most detrimental to sturgeons. We will model biological processes affected by the **pollutant stress selenomethionine** (SeMet), singly and in combination with **methyl mercury** (MeHg), **temperature**, and **salinity** in sturgeon, including

biochemical pathways, physiological functions, and overall fitness. In addition, we propose to investigate the acclimation and adaptation potential of early life stages of sturgeon to these stresses. Because **white sturgeon** (*Acipenser transmontanus*) and **green sturgeon** (*Acipenser medirostris*) both occur in the SFBD this study will focus on both species. These two endemic species represent the ancient lineage of modern ray-finned fishes and have an exceptional biological value (Gardiner, 1984). White sturgeon have commercial (sport fishing and aquaculture) and ecological (control populations of invasive clams) values in the SFBD.

Green sturgeon is a California Species of Special Concern and listed as a federally threatened species (CNDDDB, 2006). Therefore, better knowledge about how hydrological and other environmental factors affect this species in the SFBD is urgently needed. However, sampling programs are much more likely to yield data on white sturgeon than on green sturgeon and, therefore, it is critical to evaluate stress responses of both species to know whether field data gathered on white sturgeon can be used in models predicting green sturgeon population dynamics.

The purpose of this project is to address the significance and mechanistic basis of responses of threatened sturgeon to pertinent stresses in the SFBD. The research will directly support CALFED's needs for data that allow modeling the impact of water operations, climate change, and pollution on fragile key species of fish in the SFBD. Four types of stress are particularly relevant for the SFBD: two environmental stresses, temperature and salinity, and two pollutant stresses SeMet and MeHg. Effects of these four major types of stress on the molecular biology, physiology, and development of sturgeon can be studied in laboratory acclimation experiments. However, these stresses do not occur isolated but in combination in the field. For instance, global and periodic climate change influences snow pack, tidal cycle, and sea level affecting salinity, the surface area covered by water, water depth, and water temperature in the SFBD. These factors, in turn, are critical for the influx, bioavailability, and concentration of SeMet and MeHg in the SFBD.

2. Background and conceptual model

Because of complex interactions of multiple stressors, it is imperative to study not only the effect of one particular type of stress but to also study the interactions between major stresses affecting the well-being of species in the SFBD at the molecular, cellular, tissue, organism, and population levels (Figure 1). Such a study represents a formidable problem requiring considerable breadth and depth in the approach. However, we have to tackle this complex issue to better understand key relationships between hydrologic changes and environmental factors that are critical to the biology of key species and ecosystem management. Our proposal embodies a comprehensive approach to this problem that is feasible because it integrates the expertise of researchers in the fields of developmental, biochemical, physiological, ecological, behavioral, and molecular biology of stress in fishes. Critical questions that are directly addressed in our proposal include: How do sturgeons respond to SeMet stress, singly and in combination with other relevant stresses? Which combinations of stresses are most detrimental and at what levels? Do combinations of different types of stress potentiate negative biological effects? How can exposure of fish to a particular type of stress or combinations of multiple stresses be recognized in the field before irreversible consequences at the population level take effect? The answers to these critical questions are needed to assess and mitigate the impact of hydrologic changes, urbanization, water operations, and global warming on the SFBD ecosystem.

An important general problem of interest for CALFED actions concerns effects of anthropogenic influences on the life history of endangered fishes in the SFBD. We will focus on investigating effects of the pollutant stresses **selenomethionine (SeMet)** and **methyl mercury (MeHg)**, as well as **temperature and salinity** stresses. SeMet stress is of great concern for SFBD fish populations, impacting them in the field often in combination with MeHg, temperature, and salinity stresses. Selenium (Se), mainly in its organic form through food chain transfer, represents a major

environmental problem in the SFBD because this region is heavily used for agriculture and oil refineries (Zawislanski et al., 2001; Zawislanski et al., 2003; Lemly, 2004). Although legislative action has led to the reduction of Se concentrations in the SFBD in recent decades, there is legitimate concern that significant disposal of Se-rich agricultural drainage water into the SFBD could occur again (Luoma and Presser, 2000; Hug et al., 2000; Wu, 2004; Chow et al., 2004). The US Geological Survey forecasts that in the event of such disposal SeMet bioaccumulation in white sturgeon would exceed levels that are toxic to other fishes, including fathead minnow (Ogle, 1998), bluegill (Hermanutz et al., 1992), and Chinook salmon (Hamilton et al., 1990). The high variability of bioaccumulation of Se in different species is well documented but its mechanistic basis is not understood (Stewart et al., 2004).

Selenium stress often occurs in combination with MeHg stress. Mercury leaches from abandoned gold and mercury mines abundant in California, and accumulates in agricultural land (Ganguli et al., 2000; Rytuba, 2000). When such land is flooded, water-borne bacteria convert mercury to the highly toxic organic derivative MeHg (Marvin-DiPasquale et al., 2003), accumulating to a high degree in fish (Houck and Cech, Jr., 2004) and creating significant health concerns for humans (Harnly et al., 1997; Hightower and Moore, 2003). This represents a major problem for the SFBD, not only with regard to flooding due to severe weather or long-term climate change, but also in the context of efforts to restore agricultural land back to tidal marshland. Our proposal focuses on better understanding the mechanistic basis for bioaccumulation of SeMet and MeHg, and modeling biochemical pathways and biological processes affected by such pollutant stresses in sturgeon. Because pollutant and environmental stresses often act synergistically, our proposal will also **investigate how SeMet or SeMet + MeHg stresses interact with salinity and temperature stresses.** Interaction of salinity and SeMet toxicity was shown to be of great significance in other species of fish. For example, exposure of rainbow trout to increased salinity diminishes the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) when these fish are fed with SeMet-rich diet (Schlenk et al., 2003). By comparing critical combinations of environmental and pollutant stresses, our project will gain knowledge about alternative threats to sturgeon populations in the SFBD and the relative importance of such threats compared to other threats such as water-export pumping.

Another critical question relevant to long-term CALFED actions is the accuracy of predictions about consequences of future changes in hydrological and meteorological conditions that have not yet been experienced. This question can be addressed in laboratory experiments that simulate possible future changes in key environmental factors such as **salinity** and **temperature** on key fish species. Salinity and temperature are important determinants of water quality and of population dynamics, spawning success, development, growth, and survival of many fish species in the SFBD, which represents California's primary hydrologic system. This system is vulnerable to the regional hydrologic consequences of projected global climate change, in particular alterations in temperature and salinity (Knowles and Cayan, 2002; Knowles and Cayan, 2004).

By knowing the biochemical pathways, molecular functions, and physiological variables associated with stressful changes in salinity and temperature we can identify biological processes that are affected by such stresses in endangered fish species such as sturgeon. Knowledge of biological processes, in turn, is essential for modeling the impact of future climate change on the SFBD ecosystem as a whole. Knowledge of the proteome underlying such biological processes will allow us to develop powerful and specific bio-indicator assays for monitoring exposure of fish to stresses in the field. Moreover, identification of stressor-specific indicator proteins will allow conclusions about the predominant type of stress in the field. This was a significant weakness in the existing CALFED programs, which will be addressed directly by our proposed approach. Although we do not propose to analyze all potential combinations of different stresses to maintain feasibility of this project, we will focus on those that are known to be of great significance in the SFBD. This approach can easily be

extended in the future by applying the procedures proposed in task 6 of this project to additional types of stress and other species, e.g. Chinook salmon, splittail, and Delta smelt.

This proposal represents a **collaborative effort** by four experienced UC Davis-based laboratories and CDFG. The research team has excellent prerequisites for addressing key areas that are of primary concern to CALFED using an innovative and modern approach, and utilizing the power of combining state-of-the-art expertise in multiple areas of science. This collaborative effort is expected to yield more useful and comprehensive information than would have been possible by five individual proposals. Furthermore, the proposed collaboration streamlines procedures by allowing efficient sharing of expertise, personnel, equipment, and supplies.

By focusing on **sturgeon** our project will directly address information needs on species that are of special concern and have been identified as being of particular interest to CALFED objectives. The project represents a collaboration between long-standing experts in sturgeon biology and experts in the molecular biology and proteomic basis of the cellular stress response. The legal status of SFBD sturgeon is based on the magnitude and reversibility of threats to these endemic species of high biological and commercial value. Little is known about the molecular biology and biochemical basis of the stress response in these fish. Such knowledge is, however, critical for understanding the major threats to the survival, fecundity, and development of SFBD sturgeon populations. Such knowledge would also aid in understanding seasonal and annual variations in population abundance and distribution. Therefore, the proposed research directly supports the long-term CALFED goal of developing quantitative models of overall population changes in response to multiple stresses by performing 7 specific tasks (**Figure 2**). These tasks will test the overall **hypothesis that specific stress proteins in combination with biochemical, cellular, and physiological parameters a) indicate exposure of sturgeon to specific types and defined combinations of stresses and b) allow us to deduce biochemical pathways, molecular functions, and biological processes that are most affected by environmental stress in sturgeon.**

3. Approach and scope of work

To test the hypothesis stated in the preceding paragraph we have designed a series of tightly integrated tasks to understand the effects of environmental stresses on sturgeon biology:

Task 1: General management of this project (Leader: Dietmar Kültz)

Task 1 will ascertain that the overall approach to these questions is highly integrative and interactive, and will coordinate the communication of progress, optimize sharing of resources, expertise, and personnel during the project. Quarterly meetings will be organized to coordinate progress in each of the tasks and interactions between different tasks. In addition, progress reports will be discussed at these meetings and later prepared in writing. Each laboratory will present a short overview of activities within each quarter period and the most effective ways of disseminating the results presented will be discussed. The preparation of scientific publications resulting from this project will be coordinated at these quarterly meetings (see previous paragraph).

Task 2: Experimental animals and larval stressors (Leader: Serge Doroshov)

Task 2 will provide larval and juvenile sturgeon for the project and will include the study of temperature and selenium stresses in larval sturgeon linked to the proteome analysis in Task 6.

Part I. Procurement of experimental animals.

Fish for laboratory experiments will be obtained by induced spawning of captive stocks at sturgeon farms and the UC Davis Center for Aquatic Biology and Aquaculture (CABA). The laboratory in charge has a long experience in sturgeon reproduction and collaborates with the aquaculture industry in breeding white sturgeon (Doroshov et al., 1997; Van Eenennaam et al., 2004). Proper rearing,

handling, spawning, and the quality assessment of sturgeon gametes and early life stages will ensure the use of suitable quality progenies obtained from known parents. Metrics and seasonal availability of sturgeon life stages are listed in Table 1. The normal stages of early development were characterized by Dettlaff et al. (1993) in Eurasian sturgeons, and by Beer (1981), Deng (2000), Deng et al. (2002), and Bolker (2004) in white and green sturgeons.

Captive breeding is fully established for white sturgeon (Conte et al., 1988; Doroshov et al., 1997; Van Eenennaam et al., 2004). The readiness of a female to spawn is determined by the stage of germinal vesicle migration and *in vitro* egg maturation assay. Ovulation and spermiation are induced by mammalian GnRH agonist. The fertilized eggs are incubated in jars with upwelling flow, and the larvae and juveniles are reared in circular flow-through tanks, using artificial diets (Van Eenennaam et al., 2004). Larval and juvenile white sturgeon will be obtained from Stolt Sea Farm located close to campus. We will collect information on brood fish (size and breeding history) and evaluate the quality of the offspring (fertilization, hatching and abnormality rates, size and condition of larvae and juveniles). Task 2 will use newly hatched larvae, tasks 3 and 4 will use grown juveniles. Hatchery spawning and distribution of white sturgeon will be coordinated by Joel Van Eenennaam.

We currently rear at CABA two year-classes (born in 1999 and 2000) of green sturgeon originated from the Klamath River brood fish. The males produce viable sperm and some females are expected to mature and spawn in the springs of 2007-2009. We previously successfully bred wild-caught green sturgeon (Van Eenennaam et al., 2001, 2005), using techniques developed for white sturgeon.

Part II. Temperature stress and notochord deformity in larval sturgeon.

The notochord is a transient axial skeleton of the embryos and larvae, functioning in adult sturgeon (Long, 1995; Schmitz, 1998a; Schmitz, 1998b). The notochord is made up of large cells with water-filled vacuoles that generate turgor pressure on a thick collagen sheath constraining the notochord, resulting in stiffness and resistance to bending. The function of the notochord is affected by environmental factors, including temperature and toxic stresses. The exposure of green sturgeon embryos and yolk sac larvae to 22-26°C induced notochord deformities, impaired swimming, and compromised larval survival (Van Eenennaam et al., 2005; Linares-Casenave et al., 2005). Larvae with a dysfunctional notochord were temporarily or permanently bent and were unable to swim normally (Figure 3). This notochord deformity was accompanied by the high expression of heat-shock proteins hsp72/hsp79 and was reversible in suboptimal temperatures (Werner et al., 2006). Notochord deformities may be caused by regulation of river flow and climate changes, and they may significantly increase larval mortality due to predation and starvation at the onset of feeding. While there were several studies with green sturgeon, there is no information on the effect of temperature stress on larval white sturgeon. The impact on white sturgeon may be more severe since, in contrast to green sturgeon larvae (Deng et al., 2002), sturgeon larvae become pelagic immediately after hatching and must be able to swim normally to drift downstream. The following experiment with yolk sac larvae (stages 36-45, Figure 4) will determine the effect of temperature stress on the incidence and severity of notochord deformities in white sturgeon and will identify the proteins altered in response to temperature stress (task 6).

The groups (n=100) of newly hatched stage 36 larvae will be held in optimal 18°C (control) and stressful 23, 25, 27, 29°C temperatures after brief acclimation 2°C /h to determine the temperature at which most notochord deformations are induced. The exposure duration will include: 1) the entire period of yolk absorption (stage 36-45), to evaluate adaptability of larvae to high temperatures and the impact on swimming and survival; 2) the short exposures for 2 and 4 h at stage 43, with a subsequent holding in control temperature, to establish best temperature treatment for the experiment with combined stressors (Part III). In the continuous exposure, the notochord abnormalities will be monitored daily using digital photography and the images will be analyzed to estimate the percent of

abnormal larvae, severity of notochord deformities, and the rate of recovery (straightening of larval body and acquisition of normal swimming), if it occurs. Mortalities will be recorded daily, and the larvae that reach stage 45 will be euthanized (overdose of MS 222) and fixed in 10% buffered formalin for histology. The experiment will include tank replications. In the short exposure treatments, “bent” and “straight” larvae at stage 45 (devoid of yolk) will be counted and snap-frozen separately for proteome analysis (task 6). Conventional histological techniques will be used for the tissue processing and paraffin embedding. Sagittal and transverse serial sections will be stained by hematoxylin and eosin, Gomori trichrome, and PAS stains. Slides will be examined and photographed using high-resolution light microscopy and a digital camera with computer-assisted imaging. We will investigate the impacts of temperature stress on the integrity of the notochord core cells and collagen sheath; larval mortality and notochord deformities, and the recovery rates. The statistical tests will employ analysis of variance and appropriate mean comparison tests. This experiment and the one described in Part III will be conducted in an environmental room at CABA, equipped with 6 closed recirculation systems, 80 small tanks, temperature control, biofilters, chiller and heaters, and aeration.

Part III. Selenium (SeMet) and temperature stresses in larval sturgeon.

Sturgeons are exposed to organic selenium through their benthic food, particularly bivalves (Linville et al., 2002). Most white sturgeon remain year-round in the estuary and tributary rivers, based on the 1974-1988 tagging studies of the CDFG (Kohlhorst et al. 1991). High Se levels, ranging 7-30 µg/g dw, were repeatedly found in white sturgeon muscle, liver, kidney, and eggs (White et al., 1988; Kroll and Doroshov, 1991; Urquhart and Regalado, 1991; Stewart et al., 2004). In a recent field study (Linares-Casenave et al. 2006), high Se levels were found in the ovaries and livers of maturing, vitellogenic white sturgeon (20.8 ± 4.1 and 21.8 ± 2.1 µg/g, respectively, n=8), compared to immature females (5.2 ± 2.5 and 8 ± 1 µg/g, n=18). A Se concentration of 12.4 ± 3.6 (range 3.6-29.3 µg/g, n=6) was reported for ovulated eggs of six females captured in the lower Sacramento River for captive breeding (Kroll and Doroshov, 1991). Mechanism of maternal Se transfer to sturgeon eggs with hepatic vitellogenin and the yolk proteins lipovitelline and phosvitin was proposed by Kroll and Doroshov (1991) and verified by exposing vitellogenic females to Se-enriched artificial diet (Linville et al., 2004). During the early development, particularly in yolk sac larvae, the organic Se is metabolized but a significant amount of Se is retained in larval tissue, apparently in the assimilated organic form. Se levels of 10-15 µg/g dw caused edema and lordosis in the white sturgeon yolk sac larvae (Linville et al., 2004). The field and laboratory observations on white sturgeon indicate that the egg Se levels approach, and in some fish exceed, the reproductive toxicity threshold of 10 µg/g proposed by Lemly (2002). This threshold can be modified by other stressors, e.g. temperature affecting development and metabolism. We will determine effect of organic Se (SeMet) and temperature stress (alone and in combination) on larval development, survival, and stress-induced changes in proteome. Se bioaccumulation will be simulated by microinjection of SeMet into the larval yolk sac, since the larval response to maternal or microinjected organic Se has been similar in our previous study (Linares et al. 2004).

The experiment will include three groups of sturgeon larvae (n=150), replicated in three different progenies: noninjected (progeny control), injected with L-Met (treatment control), and injected with Se-L-Met (treatment), at below lethal dose of 7-8 µg/g Se, dw. Newly hatched larvae (stage 36) will be microinjected and held at optimal 18°C to the stage 43, when a half of each group will be exposed to a 2 or 4h temperature stress (from Part II experiment) and then held for 24 h in 18°C. All groups, heat-stressed and (Se+heat)-stressed, with respective controls, will be sampled at stage 45 (fully absorbed yolk) for the type and percent abnormality, tissue selenium concentration (40 larvae pooled, dw~280 mg), and for proteome analysis (frozen larvae). The Se concentrations will be determined by micro-digestion, as described in Task 3 (Tashjian et al. 2006). The selected samples with two types of abnormalities (Se-specific edema of anterior yolk sac, and bent larval body observed in both Se and

temperature stresses) will be fixed in formalin and characterized morphologically and histologically. The statistical analysis will employ analysis of variance and appropriate mean comparison tests. The experiment will compare two stressors applied separately or in combination.

Microinjection procedure. We previously established a microinjection technique suitable for sturgeon larvae (Linares et al. 2004). In microinjected controls (> 200 of stage 36 larvae) we observed normal development and survival to stage 45. In larvae microinjected with SeMet we observed dose-dependent mortalities and abnormalities (edema and lordosis), as well as high Se concentrations, in microinjected stage-45-larvae (Linares et al., 2004). Yolk sac larvae are injected under a stereoscope, using a programmable pressure picoinjector IM 300, micromanipulator MN-151 (Narishige, Japan), and aluminosilicate needles (Sutter Instruments Co., Novato, CA) pulled to a 10 μm OD. The coated (sigmacote, Sigma) and beveled (EG-44 Microgrinder, Narishige) needles are filled with the injection solution and oil plug, and calibrated under a stereoscope. High purity (>98%) Se-L-Methionine (Sigma) is dissolved in doubly distilled sterile water. The amount of Se-L-Met is calculated per dry weight of sturgeon larva (7.03 ± 0.09 mg at stage 36, Wang, 1984). Larvae are anesthetized in buffered MS 222 and transferred onto filter paper for microinjections of less than 25 nl into the region of the yolk sac between Cuvier's duct and yolk sac veins. After injection, larvae are held for recovery in a dish with aerated water from the culture system, and then returned to their respective tanks.

Task 3: The toxicokinetics, bioaccumulation, and chronic toxicity of SeMet when present alone or in combination with MeHg in the diet (Leader: Silas Hung).

The purpose of task 3 is to quantify the toxicokinetics, bioaccumulation, and chronic toxicity of SeMet when present alone or in combination with MeHg in the diet. The effects of chronic SeMet, and SeMet + MeHg stresses on growth and mortality of sturgeon will be measured. We will investigate effects of a range of SeMet and MeHg concentrations in the diet to determine at what concentration of SeMet and MeHg in the food supply of sturgeon significant effects on tissue bioaccumulation, growth, and mortality can be observed. Analysis of such effects at the whole animal level (task 3) will be complemented with analysis of effects at the molecular level by proteomics (task 6) because effects of stress are generally apparent at lower doses at the molecular level than at higher levels of organization (Fig. 1). These studies are critical because sturgeon are bottom feeders and bioaccumulation of Se through the benthic food chain in the SFBD represents a significant problem (Stewart et al., 2004). Our data will provide a better basis for construction of bioaccumulation models and enable more accurate predictions about physiological effects of metal stress. The experiments, in which sturgeon will be exposed chronically to SeMet, singly and in combination with MeHg, will serve also to supply long-term acclimated fish to task 4 and tissue samples to tasks 5 and 6. The data obtained in task 3 will provide crucial information needed for guiding management decisions on amounts of selenium that can be disposed of without harming green or white sturgeon populations in the SFBD.

Objective a) Determination of Se tissue distribution, speciation, and depuration of SeMet when present alone or in combination with MeHg in the diet.

Part I: Selenium tissue distribution, speciation, and depuration will be studied using the gelatin capsule oral dosing technique (Hung, 1991a). Twenty sturgeon subyearlings (200-300 g) will be anesthetized, weighed, and tagged with chicken wing bands at the pectoral fin (Hung, 1991a), and kept in a large tank. The sturgeon will not be fed on the day of tagging and weighing. They will be force-fed $400 \text{ mg Se kg}^{-1}$ body weight in the form of L-(+)-selenomethionine (SeMet) in gelatin capsules together with low-Se sturgeon purified diet (Hung, 1991b) as a filler. We selected this dosage based on a previous study that we recently conducted (Tashijan and Hung, 2006). The fish will be deprived of feed for 24 hrs after force-feeding the capsules and fed the purified diet until samplings. Five fish each will be euthanized with tricaine methanesulfonate (MS222, 2 g l^{-1}) at 0, 1, 2, 4, and 8

days after dosing. Blood plasma will be collected; gill, liver, kidney, gastrointestinal tract, white muscle, and gonads will be dissected. Blood plasma will be stored in three mini-vials, one for total Se determination (Tashjian and Hung, 2006), one for separating the protein and protein free water-soluble fractions, and one for protein speciation. Tissues will be divided into five parts, one preserved in 10% phosphate-buffered formalin for histopathological examination and TMA construction (task 6), and the other four wrapped in aluminum foil, quickly frozen in liquid nitrogen, and stored at -80°C. The first set of frozen samples will be used for HSP determination (task 5). The second set of frozen samples will be lyophilized with holes poked through the aluminum foil; the lyophilized samples will be grinded in liquid nitrogen, and total Se in dry tissues will be determined (Tashjian et al., 2006).

Determination of Se concentration in diet, culture water, and tissue: Diet Se concentration will be determined as previously described (Tashjian et al., 2006) and Se concentration in culture water will be determined by inductively coupled plasma mass spectrometry (ICPMS) (Sugihara et al., 2004). For Se tissue fractionation and speciation, the third set of frozen samples will be lyophilized and extracted for protein-free, water-soluble metabolites using 10% trichloroacetic acid (TCA) and for proteins using Tris-SDS buffer (BioRad), followed by TCA precipitation. The TCA extracts will be analyzed for Se (Tashjian et al., 2006). Selenium will also be determined in the TCA precipitates and if they contain high levels, selenoprotein speciation will be conducted.

For protein speciation, the fourth package of frozen samples will be homogenized with buffered KCl solution and protein separated by size using either dialysis with different pore sizes and/or size-exclusion chromatography with different columns to separate the proteins into three sizes: small, medium, and large. The corresponding size fractions will be analyzed for Se content to estimate the approximate sizes of the selenoprotein where Se has been incorporated. Other protein separation techniques such as 1-D (SDS-PAGE) or 2-D (IEF + SDS-PAGE) will be explored to determine the precise molecular weight of the seleno-proteins. We will not attempt to fractionate tissues into lipid and non-lipid fractions because preliminary studies in our laboratory showed that splittail fed high Se yeast diet for 5 months retained very little Se in the lipid fraction.

Part II: The same experimental procedures as described in Part I will be used to determine the uptake, distribution, speciation, and depuration of Se and Hg in sturgeon force-fed with a combination of SeMet and MeHg (400 mg Se and 40 mg Hg kg⁻¹ body weight, respectively). Selenium analyses will be conducted as described in part I above. Tissue samples will also be prepared as described in part I for use in tasks 5 and 6.

Determination of Hg concentration in diet, culture water, tissue: Total Hg concentration in the culture water will be determined by the IC-PMS. Total Hg concentration in the diet and fish tissue will be determined using cold vapor atomic absorption spectrometry (Slotton et al., 1995). Previous work has shown that >95% of the Hg in fish is in the form of MeHg (Bloom, 1992) and due to the high MeHg analytical costs, EPA recommends that total Hg (rather than MeHg) be determined and the conservative assumption be made that all Hg present is in the form of MeHg (EPA Document 823-R-93-002 1993).

Objective b) Chronic toxicity of SeMet alone or combined with MeHg in the diet.

Part I: The chronic toxicity (16 weeks) of dietary SeMet will be studied using the traditional dose response growth method (Hung and Lutes, 1987). One thousand juvenile sturgeon (3-5 g) will be obtained from task 2 and kept in large stock tanks for one week. Seven hundred and twenty sturgeon of similar size will be selected and randomly distributed into 24 small circular fiberglass tanks (Hung and Lutes, 1987) with 30 fish per tank. The fish will be acclimated to these tanks and weaned to sturgeon purified diet (Hung, 1991b) for two weeks. Four sturgeon purified diets containing 0, 10, 20, and 40 mg of L-(+)-selenomethionine kg⁻¹ diet will be prepared as described previously (Hung and

Lutes, 1987) and assigned randomly to six replicate tanks of fish. This large number of animals is necessary to provide enough tissue samples for all subsequent tasks and to account for possible mortality. We select this dietary Se concentration based on an 8-wk growth trial of white sturgeon fed SeMet diets containing 0-160 mg Se kg⁻¹ diet (Tashjian et al., 2006). Fish will be weighed once every 2 weeks and the rations will be adjusted according to body weight using the optimum feeding rate model (Cui and Hung, 1995). At the beginning of the trial, five control fish from one tank of each group will be euthanized, dissected, a small piece of all major tissues including gill, liver, and kidney preserved in 10% phosphate-buffered formalin, and the remaining tissue pieces frozen at -80°C for determination of Se bioaccumulation and use in tasks 5 and 6. Three out of the six tanks of fish on each diet will be transferred to Prof. Cech's laboratory for use in task 4. At 8 and 16 weeks, five fish from each of the remaining three tanks will be euthanized and tissues prepared as described above for the control fish for determination of Se bioaccumulation and use in tasks 5 and 6.

In addition, we will determine survival, growth, food conversion, retention, hepatosomatic index, gonadosomatic index, and bioaccumulation of SeMet within the 16 week acclimation period. Growth performances will be determined as described previously (Stuart and Hung, 1989) and tissue Se burden, speciation, and metabolites will be determined as described in objective 3a (see above).

Part II: The chronic toxicity (16 weeks) of SeMet in combination with MeHg in the diet will be studied using the dose response growth method (Hung and Lutes, 1987) in a 4 by 4 factorial design with three replicate tanks per treatment. Two thousand five hundred juvenile sturgeon (3-5 g) will be obtained and kept in large stock tanks for one week. Nineteen hundred and twenty sturgeon of similar size will be selected and randomly distributed into 48 small circular fiberglass tanks (Hung and Lutes, 1987) with 40 fish per tank. Sixteen purified diets (Hung and Lutes 1987) will be prepared according to a factorial design consisting of 4 levels of SeMet (0, 10, 20, and 40 mg Se kg⁻¹ diet) and 4 levels of MeHg (0, 0.1, 1 and 10 mg Me kg⁻¹ diet) and assigned randomly to three replicate tanks of fish. Care and maintenance of fish will be similar to those describe in Part I. At the end of the growth trial, half of the fish in each tank will be sampled for analysis of tissues in tasks 5 and 6 as described for the growth trial in objective b, part I. The remaining half of the fish will be transferred to Prof. Cech's laboratory for use in task 4. In addition to determining Se concentrations in tissues as described for objective b, part I, we will also determine rates of bioaccumulation and distribution of Hg in tissues.

Data Management/Statistical Analyses: Statistical analyses of endpoints in treatment groups will be conducted with JMP 4.0 statistical software (JMP IN, SAS Institute, Cary, NC) utilizing the appropriate statistical models (ANOVA, Kruskal-Wallis, post-hoc tests).

Task 4: Physiological effects of chronic dietary SeMet and SeMet + MeHg exposure (Leader: Joseph Cech)

Task 4 will provide us with an overall understanding of how chronic dietary ingestion of SeMet, singly and in combination with MeHg, salinity, and temperature stresses affects critical performance and physiological parameters of sturgeon. This task will generate key data for integrative analysis in the overall context of data gathered in the other tasks of this project. For example, the cellular function of stress proteins identified in task 6 may directly relate to a physiological function at the whole animal level identified in task 4. In addition, data generated in task 4 will nicely complement efforts described in another project that focuses on analysis of effects of single MeHg stress on physiological and performance parameters of green sturgeon. Sturgeon that were chronically exposed to SeMet, singly and in combination with MeHg in task 3 will be used directly in task 4 for tests of swimming performance, resting metabolism, and hematological analysis of key physiological parameters.

Some of these sturgeon will be further exposed to salinity and temperature stress to determine whether their tolerance, performance, and physiology to these critical types of abiotic environmental

stress is affected by prior chronic exposure to SeMet and SeMet + MeHg stress. The purpose of task 4 of our project is to gain knowledge about physiological effects of combinations of multiple types of stress that are most relevant for the SFBD. The physiological parameters measured in task 4 are good indicators of the overall physiological state of sturgeon, which is critical for reproductive success. Thus, the data acquired in task 4 will aid in the development of better models for predicting effects of stress on sturgeon populations. Tissue samples collected in task 4 will also be utilized for tasks 5 and 6. We will test critical performance parameters in fish exposed for 16 weeks to dietary levels (see task 3) of SeMet or SeMet + MeHg and then challenged with salinity and temperature extremes found in the SFBD. Performance experiments will include fish exposed to baseline conditions, as well as fish exposed to salinity and temperature extremes found in the SFBD.

Part I: Juvenile sturgeon acclimated to SeMet, singly and in combination with MeHg in objective b of task 3 will be used for measurements of metabolism and swimming performance following an overnight acclimation to 1) an increase in temperature (from 18°C to 25°C); 2) an increase in salinity (zero to 30 ppt), simulating a tidal cycle in the SFBD; or 3) no additional stress to serve as a control (n=8 for each treatment). In the temperature-stress experiment, fish will be situated in a water bath and raised from 18 to 25°C over 6 hours and held at 25°C for an additional 8-hour acclimation, and through the subsequent performance experiments. In the salinity stress challenge, fish will be held as described above with salinities increasing from zero to 30 ppt.

Resting routine metabolic rates (RMR) and active metabolic rates (AMR) will be determined using oxygen consumption techniques (Cech, Jr., 1990) with Blazka-type or Brett-type swimming (or sham) respirometers on fish from each experimental treatment. The use of sham respirometers assures that confinement stress is identical for all fish during acclimation to environmental stresses, e.g., temperature and salinity. Scope for activity (SFA) will be calculated as described previously (Heath, 1990):

$$\text{SFA} = \text{AMR} - \text{RMR}$$

The value of these metabolic performance measures, regarding stress exposure, has been demonstrated in another California native fish (Houck and Cech, Jr., 2004). After 14-h acclimation, fish in the sham respirometers will be sacrificed and plasma and liver samples collected for baseline determinations. The remaining four fish (in Blazka-type respirometers) will be used for resting routine and active metabolism measurements, critical swimming velocity (U_{crit}) estimates (Drucker, 1996), and burst swimming performance determinations (Peake and Farrell, 2004). Recent research results covering several fish species show a tight link between U_{crit} and the onset of the burst and glide swimming gait (Drucker, 1996; Peake and Farrell, 2004; Young et al., 2004). Burst swimming ability will be determined as previously described (Odell et al., 2003). Fish will be rested for a 15-min period in aerated water following scope for activity experiments. After resting, water velocities will be immediately increased to 80% of estimated critical swimming velocity for a 20 s burst-swimming bout, after which velocity will be returned to zero. This protocol will be repeated following 30-s rest intervals, for a total of ten bouts per fish. Repeat burst-swimming performance will be determined by the number of successful bouts, before fatigue (inability to swim at 80% of U_{crit} for 20 s).

Part II: For testing optomotor response additional juvenile sturgeon acclimated to SeMet, singly and in combination with MeHg in objective b of task 3, will be used. These sturgeon again will be acclimated to 1) temperature stress; 2) salinity stress; and 3) no stress (control) as described in part I (n=8 for each treatment). Fish will be placed in a cylindrical optomotor chamber (Dutta et al., 1992). They will be acclimated for 15 min with no movement of the visual field (consisting of alternating dark and light stripes). Experiments will use a series of reversals in the moving visual field to evaluate the fish's response to this visual stimulus, including the latency (time lag) response when the direction of movement of the visual field is reversed.

Part III: Another set of sturgeon obtained from treatments performed in objective b of task 3 will be further acclimated to 1) temperature stress; 2) salinity stress; and 3) no stress (control) as described in part I (n=8 for each treatment). Blood and tissue samples will be collected to determine effects of long-term exposure to SeMet, singly or in combination with long-term MeHg exposure, short-term temperature stress, and short-term salinity stress on additional physiological indicators. Hematocrit (packed red cell volume) and plasma osmolality, lactate, and glucose will be measured using standard methods (Wedemayer et al., 1990). A piece of each liver sample will be analyzed for glycogen content via an amyloglucosidase method (Murat and Serfaty, 1974; Hung et al., 1989) and hepatosomatic index (HIS = liver mass/ body mass) to assess the effects of chemical and environmental stresses on sturgeon nutritional status and energy reserves. Treatment means will be compared using ANOVA-type models. The left 4 gill arches, a small piece of liver, and a small piece of kidney will be fixed in 10% phosphate-buffered formalin for TMA construction (task 6). The 4 right gill arches and remaining pieces of liver and kidney will be frozen at -80°C for proteome analysis (task 6).

Task 5 Gill and muscle biopsies from field sturgeon (Leader: Marty Gingras)

Task 5 will integrate our laboratory experiments on sturgeon with already ongoing field work. Specifically, we will collect small biopsies from sturgeon obtained from ongoing sampling programs of the California Department of Fish and Game (CDFG) and analyze those biopsies for tissue selenium burden and expression of stress protein biomarkers identified during our laboratory acclimation studies. Tissue selenium burden will be assessed on muscle biopsies and stress protein expression will be analyzed by proteomic analysis of gill biopsies. Gill biopsies will be collected as previously described (Cooke et al., 2006) and muscle biopsies will be obtained by the dermal punch technique (Baker et al., 2004).

Collection of sturgeon tissue samples in 2007 and 2008 will be attempted from several surveys. The preponderance of samples will be from sturgeon collected during routine adult sturgeon population monitoring Aug. - Oct. 2007, when fish will be captured in trammel nets being set in San Pablo Bay and perhaps at areas adjacent to San Pablo Bay (e.g., Suisun Bay and Montezuma Slough). It has not yet been determined whether CDFG will attempt to capture sturgeon in trammel nets set in and near San Pablo Bay in 2008. However, in 2007 and 2008 samples will also be collected opportunistically from CDFG efforts including (1) a routine year-round creel survey and (2) the March-July '20-mm survey', and from routine year-round fish-salvage operations at the Skinner Fish Protective Facility near Tracy. Additional samples may be collected during opportunistic rescue of sturgeon stranded in the Yolo Bypass from approximately Dec. through May.

Muscle and gill biopsies will be shock-frozen on dry ice, transported to UC Davis, and stored at -80°C until being analyzed for tissue selenium burden (task 3) and stress protein expression (task 6). These studies are important because they will provide critical information about the state of sturgeon exposure to stress in the SFBD. For instance, tissue selenium burden and stress protein profiles obtained can be compared with those obtained during exposures to specific types and combinations of stress in the laboratory and conclusions as to possible exposure histories in the field can be made. In addition, these data will also be correlated with temperature, salinity, and water quality data from monitoring stations near sampling sites. Because green sturgeons are less abundant than white sturgeons we expect to obtain more field biopsies from white sturgeons. Therefore, it will be particularly important to determine whether green and white sturgeons show similar responses in their proteomes to the stresses studied in this proposal that are pertinent for the SFBD. If yes, then white sturgeon can be used as a surrogate for green sturgeon during stress-protein-based biomarker monitoring. If no, then the differences will provide clues to biochemical pathways and biological processes that are more or less vulnerable to environmental stress in green sturgeon.

Task 6: Proteomic analysis of sturgeon stress response (Leader: Dietmar Kültz)

Task 6 represents the center piece of this proposal. It will utilize samples collected in tasks 2 – 5, generate comprehensive proteome maps from those samples, and identify the most informative stress proteins associated with exposure of sturgeon to SeMet stress, singly and in combination with MeHg, temperature, and salinity stresses. Information about such proteins is critical in two major regards: *First*, it will provide us with insight into which biochemical pathways and biological processes that are most affected by SeMet stress, singly or in combination with other relevant types of stresses. *Second*, it will enable us to develop more robust and reliable bioindicator assays in the future. Existing monitoring programs can be greatly improved by developing more stressor-specific biomarkers that provide reliable quantitative readout rather than just qualitative information. Furthermore, state-of-the-art proteomics in conjunction with bioinformatics approaches now allow modeling of entire biochemical pathways and biological processes that are altered by different types of stress.

The novel approach proposed in task 6 can be easily adapted to species other than sturgeon in future projects (e.g. Chinook salmon, splittail, Delta smelt, etc.). Because protein function determines organismal physiology and ultimately fitness of entire populations (Figure 1) we will correlate data obtained in task 6 with the data obtained in tasks 2 – 5. This is particularly important because stress responses are first evident at the molecular level as posttranslational modifications of proteins and changes in protein localization and abundance (Kültz, 2004a). In contrast to changes in mRNA abundance, which may or may not translate into functional consequences, changes in proteins always have functional consequences and are, therefore, much more reliable bioindicators. Thus, catastrophic and irreversible consequences at higher levels of organization (organisms, populations, Figure 2) may be correctly modeled and predicted and, perhaps, prevented by monitoring programs based on reliable and informative bioindicators. The approach developed in this proposal is very powerful because it does not depend on already known bioindicators, which are often not very specific, e.g. Na⁺/K⁺-ATPase (Kültz and Jürss, 1991; Kültz et al., 1992; Kültz and Somero, 1995). Instead, we aim to identify the MOST PREDICTIVE and INFORMATIVE stress proteins for further development of reliable and specific bioindicators using a completely unbiased scientific approach. Thus, this proposal will yield scientific information that provides a firm basis for a variety of follow-up projects.

Novel methods and technology development in analytical chemistry and genome-wide biology have enabled large scale analysis of genes and proteins that are targets during biological processes, e.g. during stress adaptation. Thus, we are not limited anymore to assessing stress in fish based on long-standing bioindicators (e.g. cortisol, T3, T4, Na⁺/K⁺-ATPase, heat shock proteins, etc) but it is now possible to obtain more comprehensive insight into changes of the proteome (= entire protein complement) and identify more reliable, more consistent, and more responsive proteins and entire biochemical pathways and biological processes during stress. This can be done by looking at more than a thousand proteins in each tissue at the same time after exposure of fish to a particular stresses.

Task 6 will identify proteins, biochemical pathways, and biological processes altered by SeMet stress, singly and in combination with MeHg, salinity, and temperature stresses in sturgeon larvae (samples provided by task 2), gill, liver, and kidney of sturgeon acclimated in the laboratory (samples provided by tasks 3 and 4), and gill biopsies obtained in the field (samples provided by task 5). In addition, we will compare normal and malformed, thermally stressed sturgeon larvae at yolk sac resorption (stage 45, samples provided by task 2) to identify the proteomic basis of thermally induced abnormal development. TMAs of the samples will also be constructed to allow for future development of specific biomarker assays that are based on stressor-specific proteins identified in this project.

Objective a) Identification of proteins associated with specific stresses in sturgeon

A proteomics approach based on two-dimensional protein mapping (Figure 5) and mass spectrometry (Figure 6) will be used on tissue samples collected in tasks 2 - 5. N- and C-terminal amino acid sequences of such proteins will be determined by T3 sequencing. In a follow-up project, these peptide sequences can then be utilized for generation of antibodies based on immunization of rabbits with synthetic peptides representing N- and C-termini of such proteins. In addition, degenerate primers can be designed based on these N- and C-terminal peptide sequences and the full-length cDNAs for novel sturgeon stress genes can be cloned. Antibodies against novel stress proteins are of particular interest because they enable robust screening of tissue samples collected from sturgeon (and perhaps other fish species) in the field. This can be done using high-throughput enzyme-linked immuno-absorbent assays (ELISA) or tissue microarrays (TMAs, see objective b).

Part I: Construction of reference proteome maps of sturgeon larvae, gill, liver, and kidney. Proteome maps will be constructed from green and white sturgeon larvae, gill, liver, and kidney. We will dissect these tissues from juvenile sturgeon sub-yearlings that originate from the same offspring as those used for acclimation experiments in tasks 3 and 4. Construction of these proteome maps will 1) ensure that all our proteomics procedures (extraction buffers, etc.) are optimized for these sturgeon tissues before we analyze experimental samples from tasks 2 - 5; 2) serve as a basis for comparing proteome maps generated from task 2 - 5 samples; and 3) provide material for optimization of T3 sequencing of sturgeon proteins during the first year of this project.

Proteins will be extracted from tissues using 3 different extraction buffers (urea-based buffer; hypotonic buffer, and RIPA buffer). All buffers will contain 1 – 4% of zwitterionic detergents (CHAPS, C7) and the buffer that extracts most protein will be selected for each tissue. Proteins will be precipitated using 10% TCA in acetone and the pellet dissolved in 7M urea, 2M thiourea, and 2% CHAPS. They will be reduced and alkylated with tributylphosphine and acrylamide monomer and loaded on immobilized pH gradient (IPG) gels by rehydration loading. The optimal pH range for each tissue will be determined by comparison of pH 3-10, 4-7, and 5-8 IPG strips. IPG gels will be focused using an IsoElectrIQ2 unit (Proteome Systems) at 100,000 Vh. IPG strips will be stored at -80°C until the second dimension separation by SDS-PAGE is performed. Before SDS-PAGE, IPG strips will be thawed and equilibrated in SDS-PAGE running buffer for 2 x 10 min. They will then be positioned on top of an 11% SDS-PAGE slab gel and electrophoresed at 30W constant power and 10°C in a Criterion Dodeca Cell (Biorad). Twelve samples (1 per gel) will be processed simultaneously during each 1st and 2nd dimension separation. Gels will be briefly rinsed in water and stained with colloidal Coomassie Blue. They will be imaged with an Epson 1680 densitometer and gel images will be analyzed with Delta 2D software (Decodon). Some protein spots will be picked and proteins extracted from the gel using a gel nebulizer (Millipore) and electroelution. These samples will be purified over a Zip-plate (Millipore), mixed with MALDI matrix (dihydrobenzoic acid) and applied to an anchor chip target for the Ultraflex II mass spectrometer. Spectra will be acquired using T3 sequencing mode, evaluated using Compass software (Bruker), and verified by manual analysis. Larval, liver, gill, and kidney samples from 4 green sturgeon and 4 white sturgeon will be processed for this objective. Thus, a total of thirty-two 2D gels will be generated in this part of the project.

Part II: Stress proteome analysis in sturgeon gill, liver, and kidney. We will focus on proteome analysis of gill, liver, and kidney because they are functionally directly involved in stress resistance and are, therefore, most informative. Other tissues will not be considered in this proposal to maintain feasibility within the proposed budget and timeline but they will be stored and can be analyzed in a follow-up project (e.g. muscle, intestine, heart, spleen, pyloric caecae, etc.). The following samples will be analyzed using the proteomics approach outlined in objective 6a, part I:

Frozen gill, kidney, and liver samples collected in task 3 will be analyzed. To maintain feasibility we will not analyze all samples but only those shown in **Figure 7**. We chose these particular samples because they represent a good selection from sturgeon exposed to no stress (control), low and high SeMet stress alone, and low and high SeMet stress in combination with low and high MeHg stress. Samples from additional treatments will be utilized if low abundance stress proteins are identified on 2D gels that require extensive pooling from multiple gels for T3 sequencing. Based on the outline provided in **Figure 7** seven different treatment groups will be analyzed and we will run 2D gels for 3 sturgeon for each treatment (n=3). This low number of replicates is commonly used for large-scale 2D electrophoresis experiments to maintain feasibility (only 1 sample is represented on a 2D gel). Because we will analyze 7 treatments, 3 replicates, and 3 different tissues from 2 species of sturgeon the total number of gels prepared in objective a, part II will be $7 \times 3 \times 3 \times 2 = 126$ gels. We will run 36 gels simultaneously in the first dimension (IPG) on a Proteome Systems IsoElectrIQ2 instrument and 12 gels simultaneously in the second dimension in a Criterion Dodeca Cell (Biorad). Gels will be stained, analyzed, and spots prepared for MS as described above under objective a, part I.

Frozen gill, kidney, and liver samples collected in task 4 from green and white sturgeon exposed to short-term temperature stress (see task 4), and short-term salinity stress (task 4) will be analyzed. These samples will complement samples collected in task 3 (**Figure 7**). Because we will analyze 14 treatments, 3 replicates, 3 different tissues from 2 species of sturgeon the total number of gels prepared in objective b, part I will be $14 \times 3 \times 3 \times 2 = 252$ gels. We will run 36 gels simultaneously in the first dimension (IPG) on a Proteome Systems IsoElectrIQ2 instrument and 12 gels simultaneously in the second dimension in a Criterion Dodeca Cell (Biorad). Gels will be stained, analyzed, and spots prepared for MS as described above under objective a, part I.

Frozen gill biopsies from green and white sturgeon obtained in the field will also be analyzed using the proteomics approach outlined above. The number of samples to be processed depends on the number of specimens obtained in the field (task 5). Gill proteomes from field sturgeon will be compared with those acclimated in the laboratory to detect proteome signatures that are indicative of exposure to SeMet, MeHg, temperature, or salinity stresses in the field. The anticipated outcome of this approach is the identification of proteins that are altered in abundance and/ or posttranslational modification (PTM) state in response to the types of stress that sturgeon will be exposed to in tasks 3 - 5 (**Figure 7**). An advantage of this approach is that stress proteins can be identified based on PTM in addition to changes in abundance. This will allow development of PTM-specific antibodies, such as phospho-specific antibodies that will be useful for ELISA and TMA assays.

Part III: Stress proteome analysis in sturgeon larvae. Green and white sturgeon larvae will be subjected to control temperature (18°C) and transient sublethal heat stress (optimal temperature determined in task 2), allowed to recover for 24 h at 18°C, and collected at developmental stage 45 in task 2. Samples will be analyzed to determine which proteins and corresponding pathways are associated with notochord malformation caused by temperature stress. The group subjected to heat stress will be divided into sturgeon showing notochord malformation (flexure) and those that do not show any visible malformation (see task 2). In addition, larvae exposed to SeMet alone or in combination with heat stress will be analyzed (see task 2). Thus, 5 groups of larvae will be analyzed: 1) larvae that will not be exposed to any stress (control); 2) larvae that will be exposed to heat stress and show notochord flexure; 3) larvae that will be exposed to heat stress but show no notochord flexure; 4) larvae that will be exposed to SeMet stress alone; and 5) larvae that will be exposed to Se Met stress in combination with heat stress (**Figure 6**). We will perform sixty 2D gel separations of whole larval proteins (5 conditions x 2 sturgeon species x 6 replicates = 60 samples). Several larvae will be pooled per sample within each group to extract 600 µg of total protein. This amount is needed for loading onto IPG strips to yield at least 1000 proteins after Coomassie blue staining for

quantitative analysis. Thirty-six gels will be run simultaneously in the first dimension (IPG) on a Proteome Systems IsoElectrIQ2 instrument and 12 gels will be run simultaneously in the second dimension in a Criterion Dodeca Cell (Biorad). Gels will be stained, analyzed, and spots prepared for MS as described above under objective a. The total number of 2D gels proposed ($24 + 126 + 252 + 60 = 462$) is feasible within the scope of this project based on prior experience of running such gels in Prof. Kültz's laboratory, which is set up well for high-throughput proteomics.

Objective b) Modeling of biochemical pathways and biological processes altered by stress in sturgeon larvae, gill, liver, and kidney.

Gene ontology analysis will be carried out using the sets of stress-responsive proteins identified in objective a/ task 6. Recently, algorithms that are sufficiently powerful for such analysis became publicly available and they will be used for this project. In particular, the PANTHER (Protein ANalysis THrough Evolutionary Relationships) bioinformatics tool will be utilized for this analysis. PANTHER allows identification of molecular functions, biochemical pathways, and entire biological processes that are associated with subsets of a particular proteome. All identified sturgeon stress proteins will be scored against the PANTHER database of hidden Markov models (HMMs) to determine the corresponding/ homologous PANTHER record for each protein (Mi et al., 2005). Biological processes and molecular functions associated with stressor-specific protein lists will then be identified using PANTHER. Statistical significance of assigning a particular pathway and biological process to stressor-specific protein sets will be verified using the binomial test method (Cho et al., 2000). The Bonferroni correction method will be used because many statistical tests (one for each pathway) will be automatically performed at the same time. This conservative correction method multiplies the single-test P-value by the number of independent tests to obtain an expected error rate and account for the possibility that some proteins participate in multiple pathways. Biochemical pathways will be visualized with PANTHER and customized with CellDesigner software (Kitano et al., 2005). The association of identified biological processes with population level events will be evaluated using Gene ontology PubMed (GO PubMed) software (Doms and Schroeder, 2005). Prof. Kültz's laboratory has recently successfully applied these approaches to proteome sets generated from human and shark tissues (Perroud et al., 2006; Lee et al., 2006).

Objective c) Construction of TMAs from tissues of sturgeon exposed to stress.

Task 6 will generate tissue microarrays (TMAs) yielding hundreds of microscopic slides, each containing an array of 50 – 200 tissue samples. These slides can be used for histological analysis and immunohistochemical quantification of stress proteins in many different samples under identical staining conditions (Lima and Kültz, 2004; Kültz, 2004b). They will be distributed to field stations and research units supporting CALFED's scientific objectives to serve as a reference for evaluating the physiological status, and stress exposure history of fish in the field based on comparison of histology and stress protein abundance and cellular/ subcellular compartmentation. TMA analysis has an advantage over ELISA tests because a) it can be more readily adapted to field surveys and b) it generates more information. This method can readily be adapted to the field because all it requires is simple fixation of tissues in 10% formalin. Thus, no cooling or freezing of field samples is necessary and all that is needed in the field is a sample vial filled with 10% formalin. Fixed samples can be stored for several months before embedding in paraffin and sectioning for microscopic analysis. TMAs provide quantitative information about the abundance of stress proteins similar to that obtained with ELISAs. However, in contrast to ELISAs, TMAs provide additional information on stress protein characteristics, including their cellular and subcellular localization (Kültz, 2004a). All tissues fixed in 10% phosphate-buffered formalin in tasks 3-5 will be dehydrated in an ethanol series, embedded in paraffin, and used for construction of tissue microarrays (TMAs) as previously described (Lima and Kültz, 2004). Briefly, TMA blocks will be constructed using an MT-1 tissue microarrayer (Beecher

Instruments) and sectioned using an ultramicrotome (LKB) present in the histology core facility of the Department of Animal Science to a thickness of 4 – 5 μm . These sections will then be floated onto poly-L-lysine-coated glass slides and the slides containing the TMAs stored at 4°C for later development with antibodies, histological stains, or distribution to other CALFED investigators. Mr. Peter Allen, who is a graduate student of Prof. Cech has already constructed TMAs of gill, intestine, kidney, and pyloric caecae of green sturgeon as part of his thesis project in Prof. Kültz's laboratory during the past 2 years demonstrating that this approach is straightforward (Figure 8). Because all samples in a TMA are treated identically during antibody staining they can be quantified and compared directly. In addition, stress-induced changes in cellular and subcellular localization of proteins can be detected. TMA construction will be done in years 2 and 3 of this project because it depends on a sufficient number of samples provided by tasks 3 - 5. In addition, this part of task 6 is not as work-intensive as the proteomics approach and, therefore, can be completed in less time.

Task 7: Coordination - data analysis and dissemination (Leader: Dietmar Kültz)

Task 7 will coordinate data management, analysis, and dissemination of the results produced in this study. Quarterly meetings of all participants will be organized. A major strength of this project is its collaborative, interdisciplinary, and integrative nature and we anticipate contributing major new findings to the existing scientific literature in the field of sturgeon biology and life history. Thus, our results will be disseminated in the form of publications in scientific journals. Moreover, we will participate in regional and other scientific meetings to present results from this project. Scientists involved in this project regularly participate in such meetings. For instance, Prof. Kültz gave an overview of emerging TMA technology and its use for fish biology at the American Fisheries Society's California-Nevada regional meeting in Redding, CA in Spring 2004 (Kültz, 2004b).

In addition, all proteomics data resulting from this project, including 2D gels, mass spectrometry data, peptide sequences derived from the MS data, etc. will be prepared for and deposited in a publicly accessible database that was developed in Prof. Kültz's laboratory. This Stress Proteome and Environmental Adaptation Database (SPEAD) can be accessed at: <http://kueltzlab.ucdavis.edu/SPEAD.html>. The Delta 2D software is installed on a 2.8 Ghz Pentium 4 computer with AGP 8x 3D graphics card, 2 Gb RAM and 150 GB storage. All initial graphics files, the annotated gel files and all intensity information files are stored directly on this computer. A Coldfusion Server (Macromedia) with 36 GB of storage space is available for deposition of proteomics data and long-term storage of all proteomics data generated in this and other projects in the SPEAD database. This server has 4 swappable hard-disks making it easy to extend storage space as the need arises in the future. The SPEAD database is based on an interactive relational database design that allows complex multi-parameter queries over the internet. In the future we also intend to develop a TMA database that works similarly to the SPEAD proteomics database and will also be available for dissemination of TMA data generated in this project. TMA slides generated in this project will be distributed to interested CALFED agencies working on sturgeon biology.

4. Project Feasibility and Justification

Feasibility and justification of the proposed MS-based proteomics approach

Prof. Kültz is an expert in proteomics and protein mapping by 2D gel electrophoresis who has been using and refining these techniques for more than 10 years (Kültz, 1996; Kültz and Somero, 1996; Valkova et al., 2005; Valkova and Kültz, 2006; Lee et al., 2006; Perroud et al., 2006). An example depicting a 2D gel of shark intestine that was generated in Prof. Kültz's laboratory is shown in Figure 4. All necessary equipment for proteomics (except for the mass spectrometer) is present in Prof. Kültz's laboratory, including three high-capacity isoelectric focusing units (Biorad IEF-Cell, Amersham IPGphor, Proteome Systems IsoElectrIO2), two multicompartment electrolyzer units

(Biorad Rotofor, Proteome Systems IsoElectrIQ2), three multi-gel SDS-PAGE units (Biorad 2D cells for 12 six gels, Biorad Criterion Dodeca Cell for 12 gels, Amersham EttanDaltSix for 6 gels), an automatic gel staining unit, a sterile spot picking hood and spot pickers, shakers, centrifuges, densitometers, a SpeedVac (Savant), etc. Two licenses for Delta2D software (Decodon, Inc.) that is used to perform complex analysis of 2D gels are installed in Prof. Kültz's laboratory.

Sensitive (mass spectrometry-based) protein identification can only be achieved with a reasonable success rate if the whole genome of the organism of interest has been sequenced because it relies on database comparison of peptide mass fingerprints and random sequence fragments of peptides. Unfortunately, a sturgeon genome has not been sequenced and very little sequence information is available. Even though limited internal peptide sequence can be generated with mass spectrometers commonly used for model organisms (i.e. those available at UC Davis and other campuses or pharmaceutical companies) it is almost always insufficient for unambiguous protein identification in non-model species on its own. Thus, proteomics for comparative biology requires a unique approach that is not widely practiced because most medical schools and biology departments focus on the analysis of model organisms instead of organisms with little sequence information. Our proposal seeks to implement novel proteomics approaches and employ them to achieve major objectives identified in the CALFED Science program solicitation. Unfortunately, none of the key species in the SFBD that is of CALFED interest can be analyzed effectively in this way because very little sequence information is available for those species.

However, a novel technique called T3 sequencing enables de novo sequencing of N- and C-terminal ends of whole proteins by a pseudo MS³ top-down approach that does not rely on protein digestion (Suckau and Resemann, 2003). N- and C-terminal sequences can be used for antibody generation or for full-length cDNA sequencing. In addition, they can be used successfully for homology searches in protein databases because search parameters can be defined with regard to the position of the peptide in the protein sequence and the direction of the protein sequence (N->C), both of which are not known for internal fragments generated by PMF/MS-MS. T3 sequencing is ideal for species such as sturgeon and many others that are of interest to CALFED and whose genomes have not been sequenced and will not be sequenced in the foreseeable future. This approach requires a novel type of mass spectrometer, the Ultraflex II T3 sequencing mass spectrometer, which is only available from Bruker Daltonics. Availability of this instrument makes it possible to propose the interdisciplinary studies outlined here and to utilize proteomics approaches effectively for studies on sturgeon and other non-model species. Thus, this proposal was designed and written based on using an Ultraflex II MS as a core piece of equipment. This mass spectrometer is absolutely essential for the proposed project because identification of sturgeon proteins associated with stress cannot be achieved without this mass spectrometer. The funds requested from CALFED for this mass spectrometer (\$299,989) will be matched by \$100,000 from the University of California, Davis (see letter from UC Davis Vice Chancellor for Research Barry Klein in supplementary material). Together with a \$179,260.78 special academic discount given by Bruker these funds will allow acquisition of this instrument (see quotation from Bruker Daltonics in description of matching funds and cost sharing).

To test the feasibility of using the Ultraflex mass spectrometer for T3 protein sequencing we have analyzed a sample of horse myoglobin that was prepared in our laboratory. For the purpose of performing this test we have used an Ultraflex II mass spectrometer at a Bruker Daltonics factory facility in Massachusetts. The spectrum we have obtained with this instrument from a small amount of horse myoglobin that is representative of an average Coomassie-stained 2D gel spot is very impressive. It shows a complete c ion series for 56 N-terminal amino acids and partial y ion series for the same amino acids (Figure 9).

We would like to emphasize that this approach can be extended to any species in the SFBD. Other projects that meet CALFED objectives will also be able to profit from this equipment as we would be happy to extend training to other researchers that work on problems that are relevant to CALFED interests.

Justification and feasibility of focus on green and white sturgeon

Since the early 1900's, sturgeon populations in California have been under extreme pressure as a result of over-harvesting, loss of habitat, pollution, and the establishment of dominant invasive species (Kohlhorst, 1980; Norton, 2001; Linville et al., 2002). Sturgeons are bottom feeders that feed on shrimp, amphipods, mollusks, and fish (Billard and Lecointre, 2001). The main food source of sturgeon has shifted to the Asian clam (*Potamocorbula amurensis*) (McKechnie and Fenner, 1971; SWRCB, 1991), which is an invasive species that dominates the benthic macro-invertebrate community in the SFBD since 1986 (Carlton et al., 1990; Nichols et al., 1990). This clam accumulates Se to unusually high levels (Johns and Luoma, 1988; Brown and Luoma, 1995), presumably due to its efficient filtration capacity. It is well-established that Se biotransformation yielding seleno-amino acid derivatives and the accumulation of such derivatives in aquatic top predators via their diet hold the key to the ecotoxicology of Se (Saiki et al., 1993; Adams et al., 1997). Therefore for sturgeon, data on dietary assimilation and metabolism of seleno-amino acid derivatives, the most common of which is SeMet, are needed. Our proposal directly addresses the current lack of such data.

CALFED directives outlined in the - *Mercury Strategy for the Bay-Delta Ecosystem: A Unifying Framework for Science, Adaptive Management, and Ecological Restoration, 2003 Final report to the California Bay Delta Authority* - indicate that the focus of scientific study should be less on simple description and more on mechanistic processes addressing questions such as: How does intoxication manifest itself? Which life-stages are most vulnerable? What are the effects when an organism is exposed to more than one type of stress? Our proposal directly addresses these questions for green and white sturgeon. We anticipate gaining new insight into fundamental mechanisms of Se toxicity, singly and in combination with other types of stress, that are most relevant for the SFBD by identifying proteins, pathways, and entire biological processes that are regulated during such stresses.

Two of the stated CALFED ecosystem restoration goals are to assist and recover at-risk native species (e.g., green sturgeon) and to maintain or enhance species that are harvested by the public for food or recreation (e.g., white sturgeon). In this regard, green sturgeon is listed as a species of special concern. To assist in the recovery of green sturgeon in the Sacramento-San Joaquin Watershed, a better understanding of the effects of relevant contaminants and environmental stresses such as SeMet, MeHg, temperature, and salinity on its performance is essential.

Our proposal focuses on both, green and white sturgeon because it will be important to know whether these species respond similarly or differently to the types of stresses affecting the SFBD. This is particularly important to know because field sampling programs rely essentially on white sturgeon and green sturgeon samples are difficult to collect on a regular basis in the field. In addition, the usefulness of using white sturgeon as a model for future toxicity studies that focus on stress response mechanisms that would also be applicable to green sturgeon will be evaluated.

This proposal is build on previously conducted stress acclimation studies on white sturgeon (Prof. Hung's laboratory) and green sturgeon (Prof. Cech's laboratory) that establish the feasibility of performing the proposed experiments. Preliminary experiments have shown that white and green sturgeon sub-yearlings tolerate the proposed doses of SeMet, MeHg, temperature, and salinity stresses reasonably well such that mortality, although some is expected at the higher concentrations of toxicants, should be within manageable limits and not prevent subsequent experiments planned in tasks 4, 5, and 6. Should, contrary to our expectations, mortality be a significant problem then we will

adjust down the concentrations of SeMet and MeHg used in task 3. Besides demonstrating the feasibility to conduct growth studies at the UC Davis campus, preliminary experiments conducted on white sturgeon have demonstrated the potency of SeMet as a toxic agent in sturgeon. Furthermore, additional preliminary experiments have shown additive effects of multiple types of stress. For instance, white sturgeon survival is affected at much lower doses of SeMet and salinity stresses when these stresses are encountered in combination. Because sturgeon are exposed to Se and salinity stresses and even additional Hg and temperature stresses in combination in the SFBD, the studies outlined in this proposal will obtain a more complete, ecologically more relevant picture of how multiple stresses affect both white and green sturgeon.

Justification of focus on combined Se, Hg, salinity, and temperature stresses

Recent evidence suggests that Se contamination from Sacramento River inflows, effluents from refineries in the San Francisco Bay, and irrigation drainage removing salts from agricultural lands of the San Joaquin Valley may contribute to the decline in the abundance and distribution of sturgeon in the SFBD (Luoma and Presser, 2000). Field studies on sturgeon in the SFBD over the past 25 years have revealed that liver Se can be as high as 30 μg per g dry weight and muscle Se can reach up to 15 μg per g dry weight, which is well above toxic levels for other fishes (Urquhart and Regalado, 1991; Linville et al., 2002). The high Se levels found in sturgeon tissue are due to a combination of environmental Se dynamics, feeding habits, metabolism, and life history traits of sturgeon.

Selenium-rich soils, especially along the western slope of the San Joaquin Valley, as well as the refining of selenium-enriched crude oil contribute to selenium loading of Sacramento-San Joaquin Watershed and SFBD aquatic habitats. Both the historic mining of mercury-rich cinnabar ores and the extensive use of mercury (Hg) in gold mining in the Sacramento River watershed have contributed to current levels of Hg in the SFBD. Furthermore, the continued transport of this historic Hg contamination may increase environmental levels of methylmercury (MeHg) via mobilization and methylation. This Hg methylation rate could actually increase through efforts to mitigate Delta habitat losses by re-flooding shallow islands (wetland restoration) or “feathering” of riverbanks (channel reconstruction). It is well documented that biomagnification of Se and MeHg occurs in aquatic food webs (e.g. Luoma and Presser, 2000). Dietary exposure and concentrations in fish flesh increase with trophic position, exposure concentrations, and duration of exposure. Dietary intake is recognized as the primary source of exposure to Se and MeHg in aquatic food webs. Increased Se and MeHg levels translate directly into wildlife exposure concerns, including several species of fish already considered to be at risk (e.g., green sturgeon). Sturgeons (both white and green) are known to feed on benthic invertebrates, which are directly in contact with Se-and Hg-contaminated sediments (the apparent source of Se and MeHg in the SFBD).

While the effects of Se and Hg are well documented in mammals and birds, relatively few studies have focused on the specific physiological effects on fishes, especially when combined with other potential stresses, e.g. other contaminants and seasonal/ tidal extremes of salinity, and temperature that occur in the SFBD. Little is known about effects of these multiple stresses in combination on the fishes’ ability to successfully forage and escape predation. According to a previously described model (Wainwright, 1994) as recently modified (Cech, Jr. and Crocker, 2002), organismal performance (e.g., including growth, swimming, neuromuscular integration) critically links the fishes’ phenotypic design with their patterns of resource use and survival, which in turn determines population viability and evolutionary fitness.

Aquatic species, in particular highly evolved vertebrate species (fishes) rely on regional hydrology and environmental conditions for their survival. Regional climate-driven hydrologic changes (such as salinity or temperature changes) and consequences of urban activity (such as toxicant influx) can have dramatic effects on the structure of local ecosystems. Therefore, we need to know how salinity,

temperature, and toxicants affect the biology of fishes, what stress proteins and pathways are activated by changes in these environmental factors, and how such proteins alter the biology, fecundity, and survival prospects of endangered species. A critical question associated with climate change concerns the implications of likely shifts in salinity (due to either increased rainfall or elevated sea levels) and temperature (due to droughts, greenhouse gases, and recession of freshwater water levels) on hydrological conditions, key fish species, and ecosystem structure and function in the SFBD.

5. Relevance to the CALFED Science Program

This proposal addresses four of the three topics of the 2006 CALFED Science PSP priority research topic list. First, Environmental water quality is a critical determinant for protection and recovery of fish species that are at risk. In this regard, we are addressing the question of the relative importance of key environmental stressors and combinations of such stressors (selenium, methyl mercury, temperature, salinity) for life- and population-sustaining biological processes in sturgeons. Because temperature, salinity, and toxicant levels in many parts of the SFBD depend directly on the amount of water exports it is important to know to what degree these variables can be altered before measurable effects on biological processes in sturgeons are detectable. Our biological data can then be taken into consideration in conjunction with data from the existing water quality monitoring network for optimizing water management operations to ensure most effective use of environmental water and provide the largest benefits to fish populations that are at risk.

Second, trends and patterns of sturgeon populations are influenced by a changing environment. Our proposal directly analyzes how changes in key environmental variables (2 toxicants, temperature, salinity) alter biological processes in sturgeons that are critical for sustaining healthy populations. Changes in the environmental parameters under study are highly relevant for the SFBD. For instance, global climate is expected to change the hydrology of watershed rivers and raise ocean levels, which may significantly alter the salinity and temperature in large parts of the SFBD. In addition, urbanization and agricultural activities may significantly increase levels of toxicants such as selenium in the SFBD. Water management operations could significantly potentiate adverse effects of global environmental change but also serve to alleviate such effects. To maximize benefits to water consumers as well as the ecosystem it is imperative to be able to predict to what extent key environmental parameters can be altered before irreversible damage occurs. This requires accurate modeling of such effects before global changes actually occur, which, in turn, has to be based on knowledge of how changes in key environmental variables affect key species in the ecosystem. Currently, our knowledge on the impact of temperature, salinity, and toxicant stress on biological processes in sturgeon and other aquatic species is very superficial and insufficient to support accurate models at higher levels of organization (populations, ecosystem). Our proposal aims to narrow this knowledge gap for two of the key species, green and white sturgeon.

Third, anticipated changes in climate are expected to alter salinity and temperature in the SFBD. Therefore, it is critical to understand how key aquatic species such as sturgeon respond to changes in these abiotic environmental factors. Moreover, combinations of multiple stressors beyond just temperature and salinity (e.g. toxicants) have to be evaluated to know whether such combinations act synergistically. The potential for remedial action can then be assessed based on what biological processes are affected by these stresses and combinations of stresses.

The relevance of this proposal to broader CALFED issues, larger CALFED goals and efforts, and past CALFED activities outside this PSP has been incorporated throughout the proposal and will not be repeated here in the interest of brevity.

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Tables

Table 1: Life stages of cultured White and Green sturgeon: D/L – diameter or length, W- live weight. Source of data: (Deng et al., 2002) and Van Eenennaam, J.P. (unpublished).

Life Stages	White sturgeon		Green sturgeon	
	D/L, W	Season	D/L, W	Season
Fertilized egg	3.6 mm, 22 mg	May-Jun	4.6 mm, 42 mg	Apr-May
Larva at hatch	10.6 mm, 16 mg	May - Jun	13.7 mm, 36 mg	May
Onset of feeding	18 mm, 42 mg	May-Jun	27 mm, 110 mg	May-Jun
Metamorphosis	6.1 cm, 1.5 g	Jun -July	7.4 cm, 2.5 g	Jun-July
Subadult	70 cm, 2.2 kg	Mar (2nd yr)	59 cm, 1.0 kg	Mar (1st yr)

Figures:

Figure 1: Conceptual model illustrating effects of urban and environmental influences on the quality of the aquatic environment in the SFBD. Note that stress responses of fish are first and rapidly apparent at the molecular level. Therefore, molecular indicators of stress are most powerful and specific. Abiotic Env. Setpoints = Setpoints for abiotic environmental factors, including SeMet, MeHg, salinity, and temperature; PTM = Posttranslational Modifications.

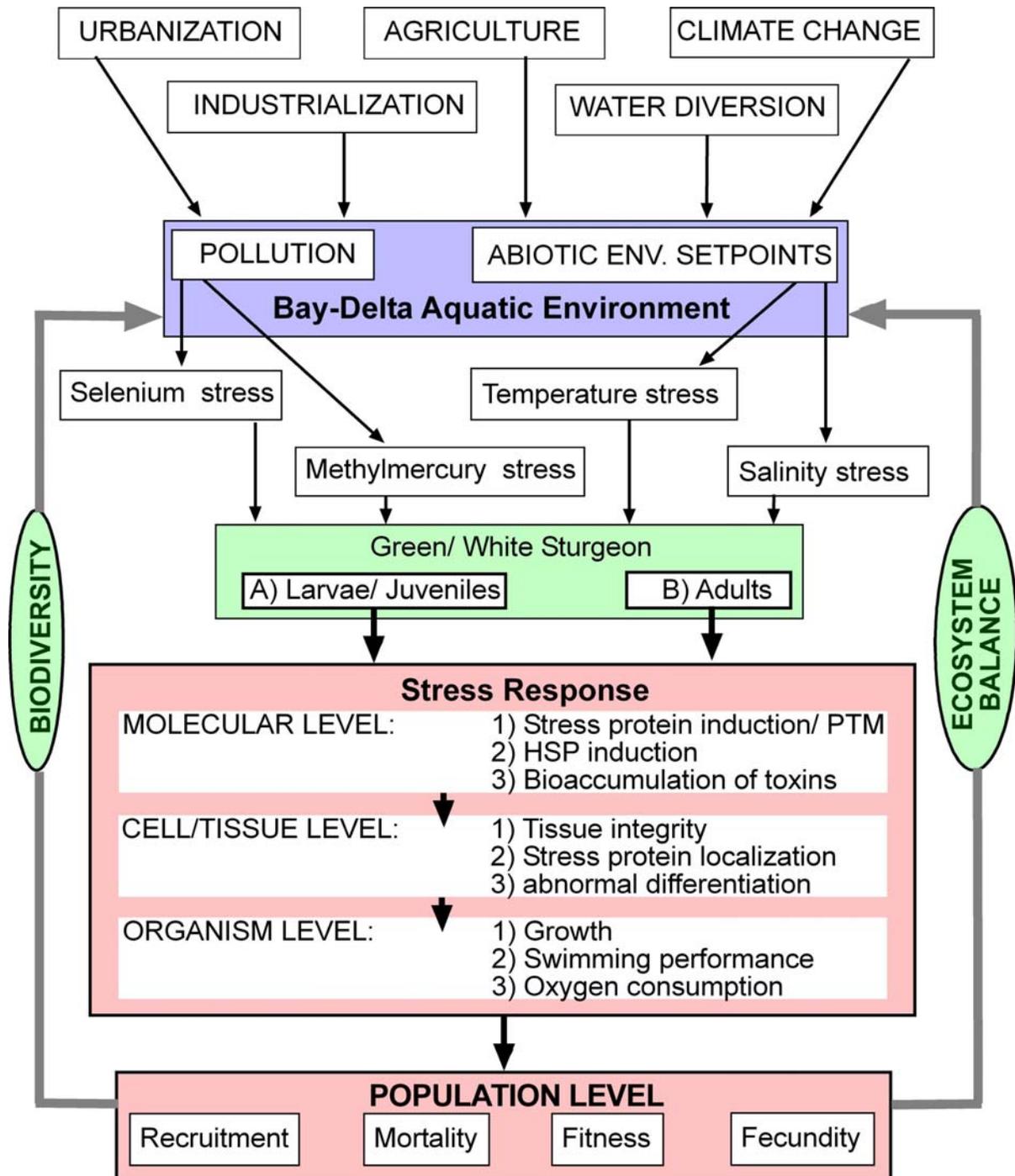


Figure 2: Flow chart illustrating the general work flow and integration of the proposed tasks. The roles of PI's within the overall project and the corresponding task numbers are indicated by boxes and arrows. The tasks depend on each other and are arranged in a logical sequence and slightly staggered with regard to their timing. For example, tasks 3 – 5 depend on task 2 and all four of these tasks will be processed in years 1 – 2 of this project. Tasks 6 – 7 depend on tasks 2 –5 and will require an additional third year of processing time. Larvae acclimations in tasks 2 and 5 will be performed together.

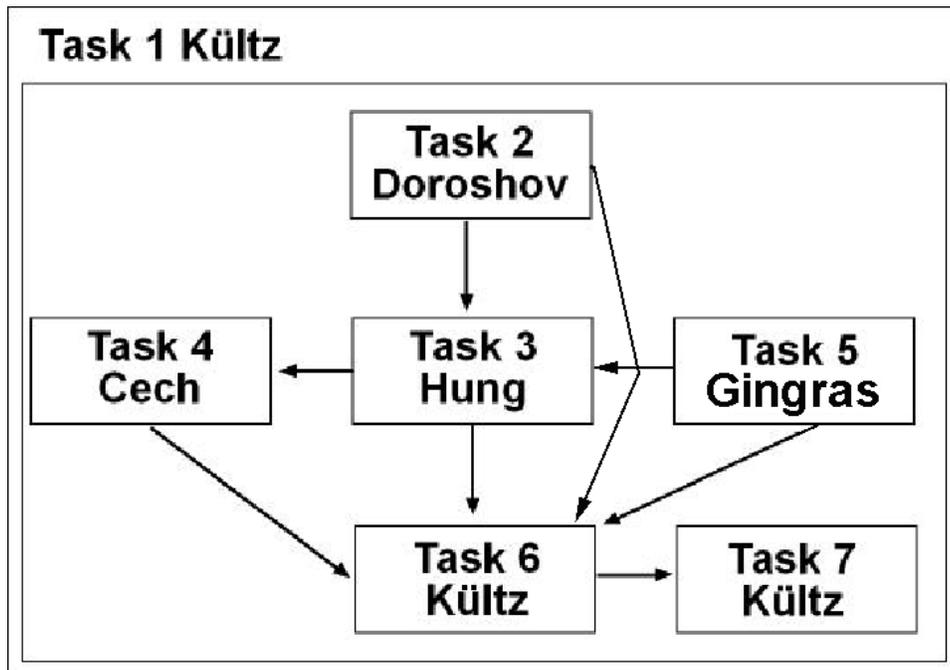


Figure 3: Notochord flexure induced by thermal stress in green sturgeon larvae hatched at incubation temperature 16°C (stage 36) and transferred to the range of temperatures (18-28°C) after hatching. 1) normal clumping larvae at 18°C; 2) bent larvae after exposure to 28°C; 3, 4) sagittal sections of normal (3) and bent (4) larvae: notochord is constricted and collagen sheath (green) involuted in the region of flexure (Gomori one-step trichrome stain, NT - notochord cells, arrows – collagen sheath); 5) Notochord deformities at stage 45 after exposure to 26°C (upper left = normal larva). Linares et al. (unpublished).

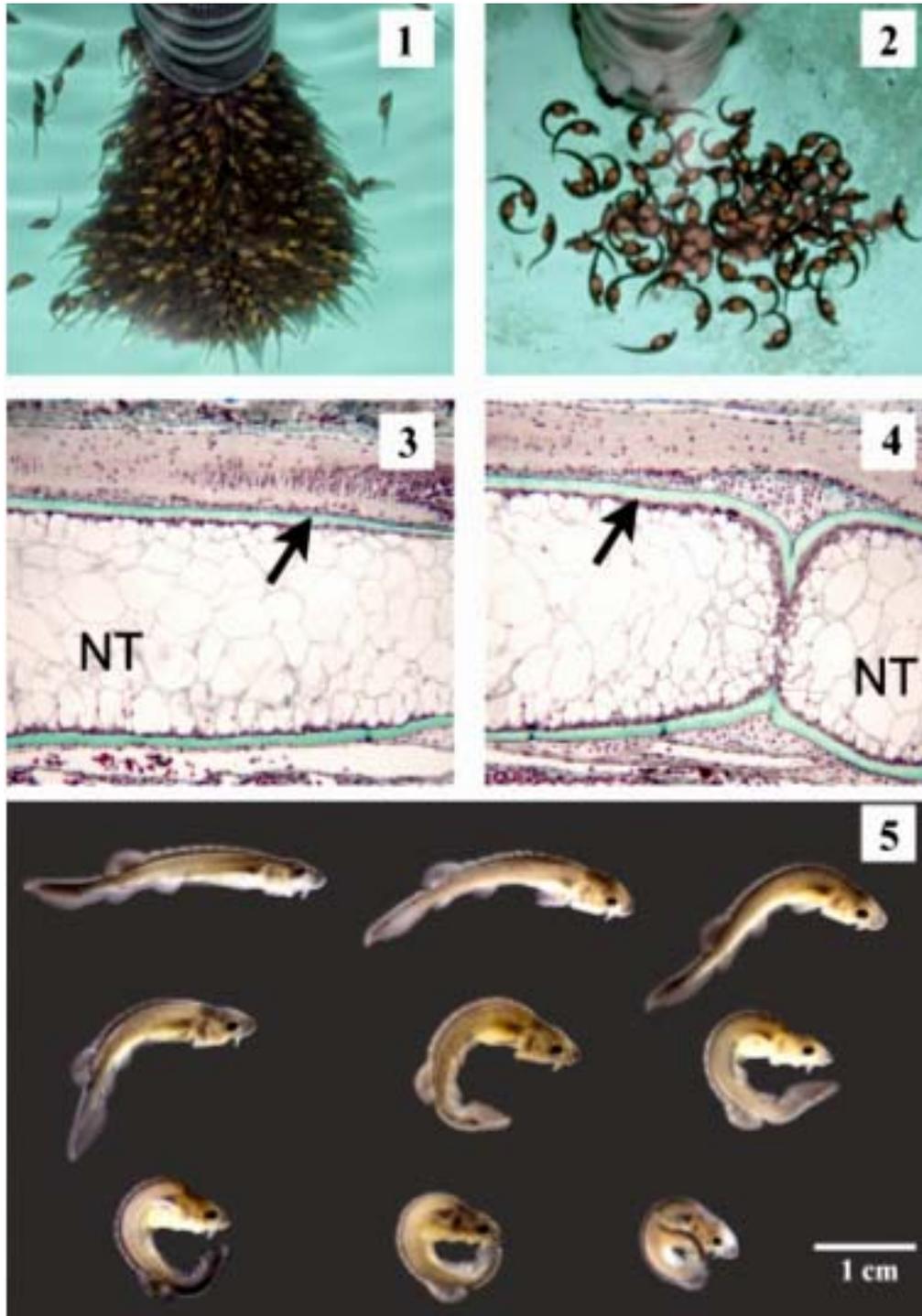


Figure 4: Normal stages of yolk sac larvae in sturgeon. Stage 36: newly hatched; stage 45: complete absorption of yolk, approximately 9 d post-hatch at 18°C (Dettlaff et al., 1993). Note development of the gut and visceral organs from the endodermal yolk sac.

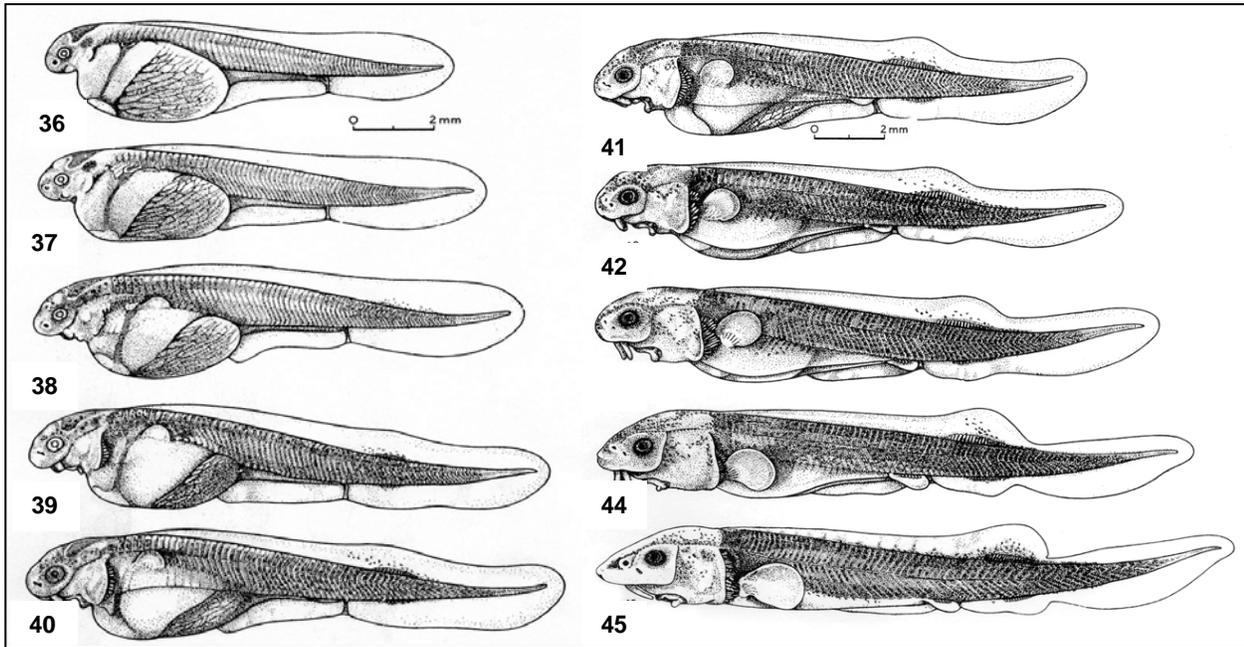


Figure 5: 2D gel from dogfish shark (*Squalus acanthias*) intestine separated by IPG electrophoresis in the first dimension (horizontal) and by SDS-PAGE in the second dimension (vertical). pH is indicated on the top of the gel and apparent molecular mass is indicated on the right of the gel.

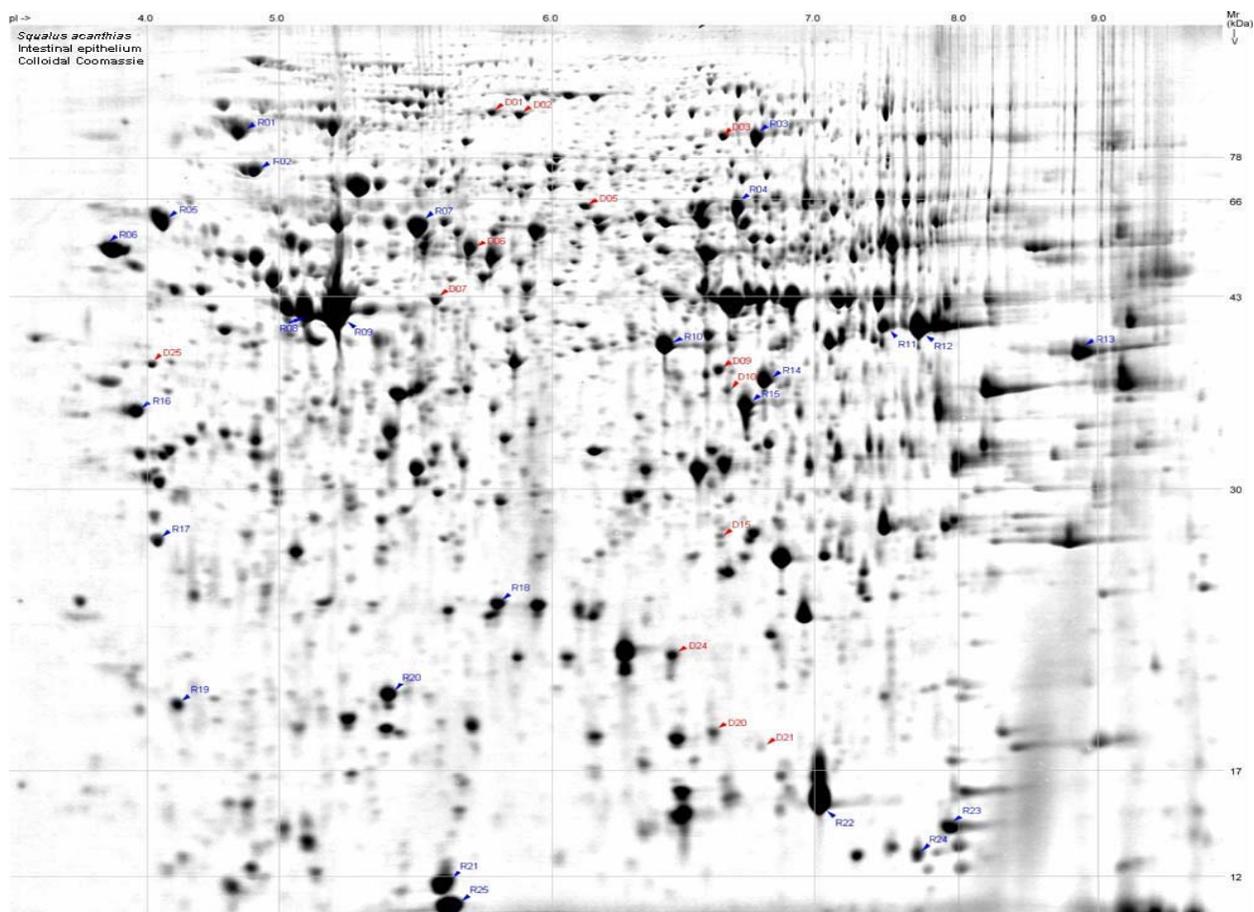


Figure 6: MALDI-TOF MS (A) and MALDI-TOF-TOF MS-MS (B) spectra of a spot from a 2D gel of mIMCD3 mouse kidney cells. An LCQ Deca Plus MS from ThermoFinnigan was used for this analysis. **A)** The spot was identified as the small heat shock protein HSP27 by peptide mass fingerprinting after partial in-gel digestion with trypsin. Peptides matched by the search engine MASCOT are shown in bold. **B)** MS-MS spectrum of the peptide peak m/z 1799. The theoretical sequence VSLDVNHFAPEELTVK of the entire peptide can be confirmed from the y- and b-ion series indicating that the corresponding spot is HSP27.

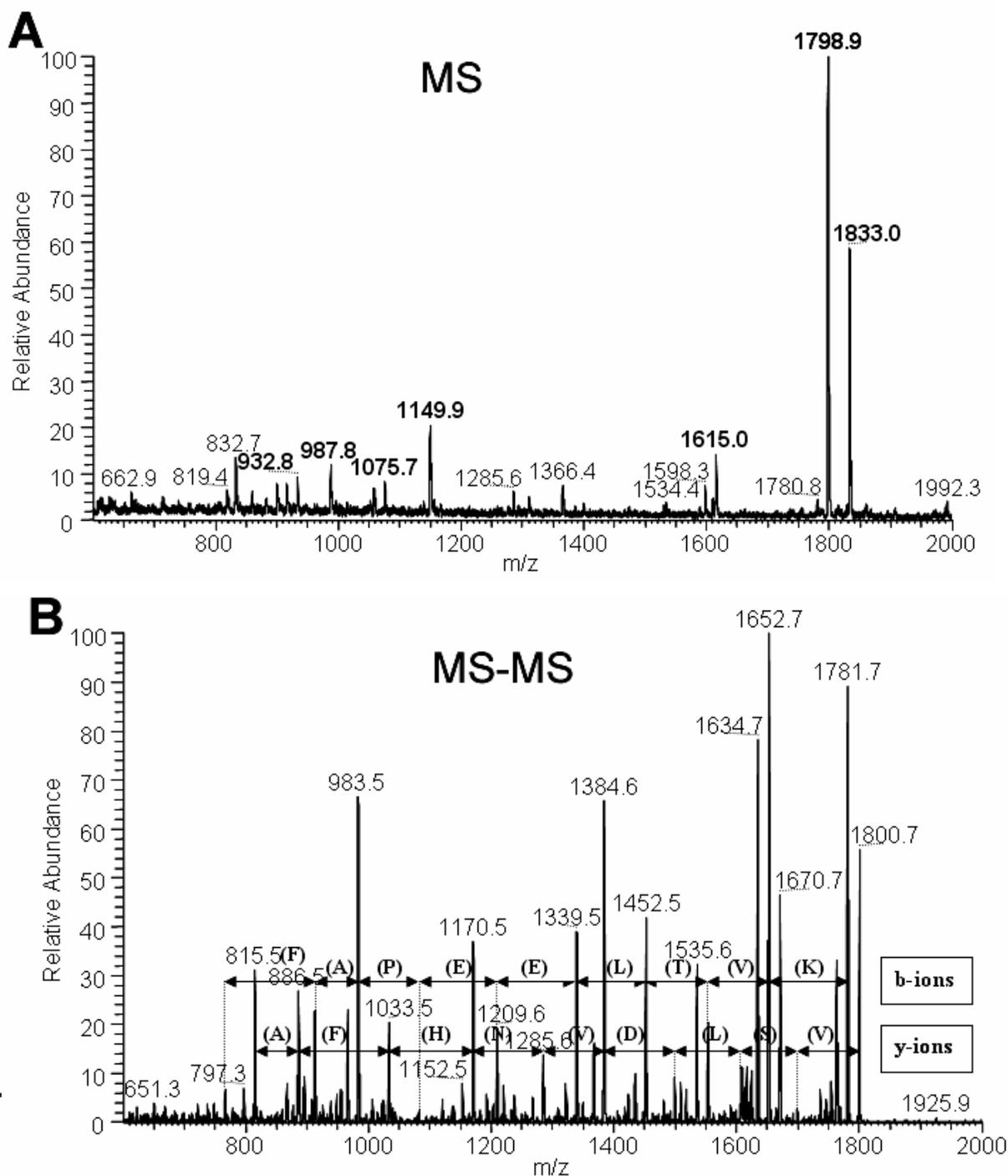


Figure 7: Overview of samples used in task 6. Gill, liver, and kidney samples from both green and white sturgeon will be used for juvenile sub-yearlings. Larvae from white sturgeon and, if captive spawning from Sacramento river broodstock succeeds (task 2), green sturgeon will be used.

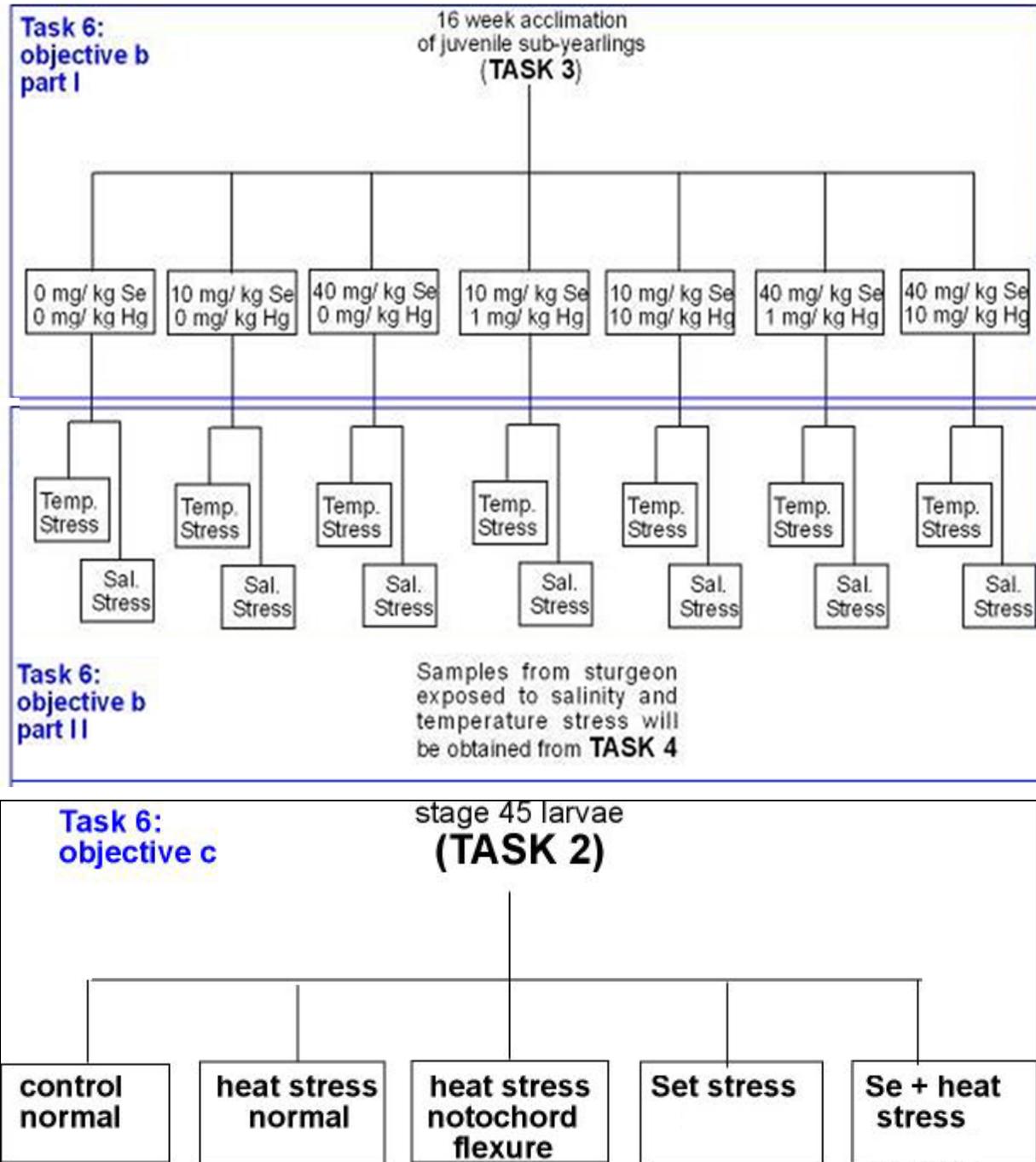


Figure 8: Tissue microarray (TMA) of green sturgeon kidney stained with an antibody against the Na^+/K^+ -ATPase β subunit (blue) and propidium iodide, which stains nuclear DNA (red). **Left panel)** The TMA was scanned with a laser scanning cytometer to quantify Na^+/K^+ -ATPase fluorescence in kidney sections from 96 different sturgeon acclimated to various salinities. Areas in which Na^+/K^+ -ATPase is present and that emit blue light after laser excitation give a signal and are depicted as black pixels on the XY scattergram. LSC scanning resolution was $0.5 \mu\text{m}$. **Center Panel)** LSC image of a small area of one of the 1 mm tissue cores shown in panel A from a freshwater acclimated sturgeon. Note, that there are two types of renal tubules – one that has large lumen and Na^+/K^+ -ATPase only localized in the basolateral membrane and a second type located in the lower part of the image with a smaller lumen and stronger, more uniform Na^+/K^+ -ATPase staining. **Right panel)** LSC image of a kidney from seawater acclimated sturgeon. The cells with small lumen (upper right part of image) are unaffected by salinity but the cells with large lumen become bigger and have more Na^+/K^+ -ATPase throughout, indicating higher rates of synthesis, turnover, and presence in intracellular ER and Golgi membranes during maturation of this enzyme in kidney of seawater fish.

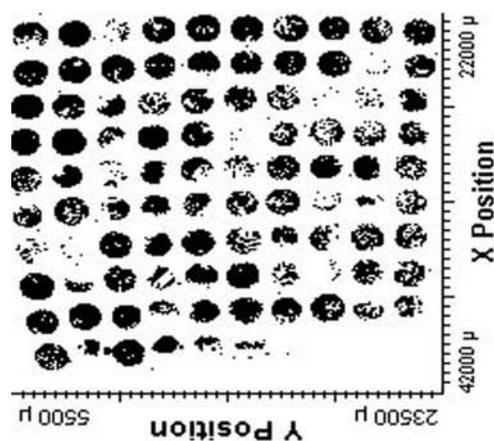
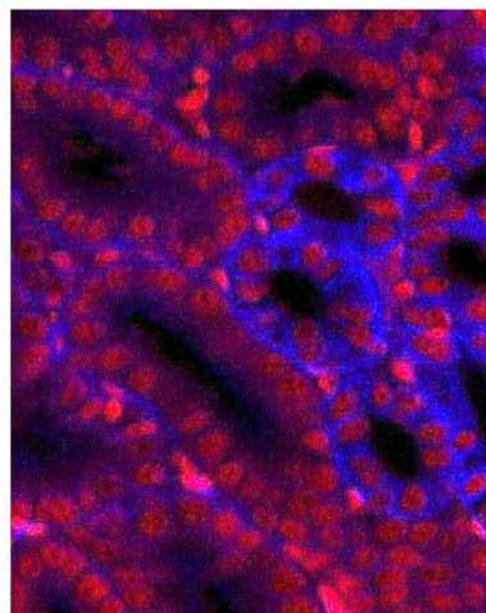
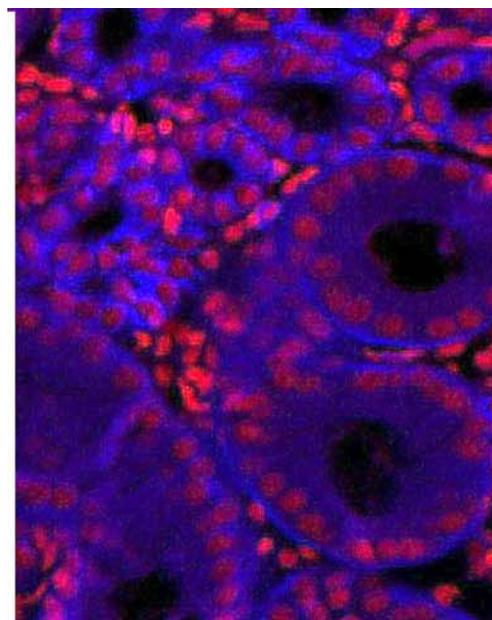
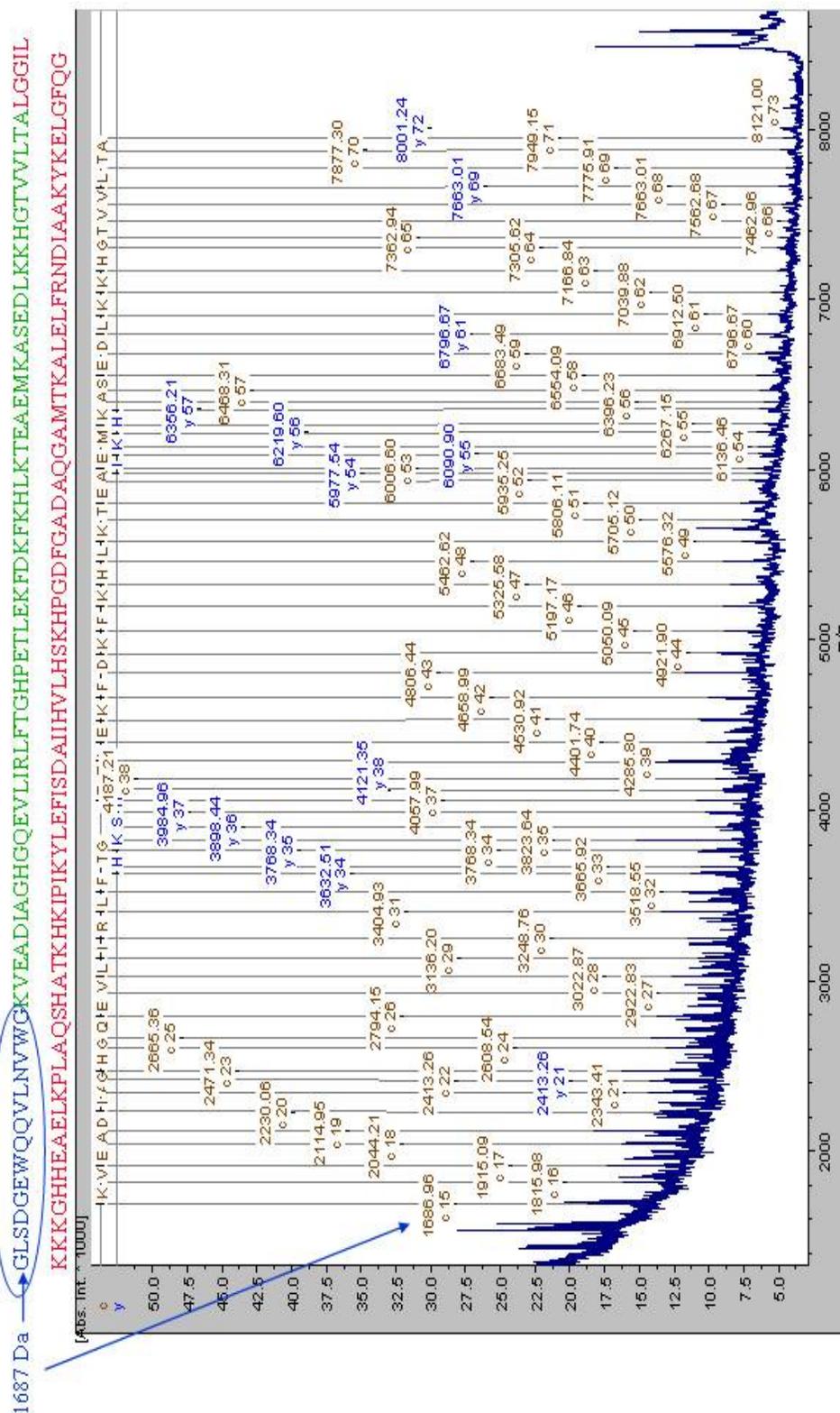


Figure 9: In source decay (ISD) of horse myoglobin. The spectrum was generated by an Ultraflex MS, annotated automatically by Compass software, and confirmed manually. Three pg of undigested intact protein, an amount that is representative of a typical colloidal Coomassie-stained spot on a 2D gel, were used as starting material. ISD capability is unique to the Ultraflex and a prerequisite for T3 sequencing. The c-ion series resulting from the ISD spectrum is shown in brown, the y-ion series is shown in blue. Amino acids identified by ISD are shown in green and the N-terminal 1687 Da peptide (singly charged in the ISD spectrum) is shown in light-blue. The corresponding peak of m/z 1687 in the spectrum was selected for TOF/TOF MS-MS T3 sequencing. Note that the first amino acid of horse myoglobin is glycine and not methionine (Genbank accession # P68083).



DIETMAR KÜLTZ

EDUCATION

M.S. University of Rostock, Germany, 1989 (Animal Physiology)

Ph.D. University of Rostock, Germany, 1992 (Zoology)

POSITIONS

- 1987-1989 Research Assistant: Experimental phylogeny of limnic and marine stickleback populations, Department of Animal Physiology, University of Rostock, Germany (Dr. Karl Jürss)
- 1989-1992 Research Student: The structure and function of mitochondria-rich cells in teleost gills, Department of Animal Physiology, University of Rostock, Germany (Dr. Karl Jürss)
- 1990 Guest Researcher: The significance of carbonic anhydrase for ion transport in fish and crustaceans, Biological Station Helgoland, Headquarters Hamburg, Germany (Dr. Dietrich Siebers)
- 1991 Guest Researcher: Active ion transport across teleost opercular epithelium, Department of Animal Physiology, Free University of Berlin, Germany (Dr. Kai Graszynski)
- 1993-1995 Postdoctoral Research Associate: Adaptive energy and protein metabolism of marine fishes and invertebrates, Department of Zoology, Oregon State University, Corvallis, USA (Dr. George N. Somero)
- 1995-1998 Fogarty Visiting Fellow: Osmosensing signal transduction in ion-transporting epithelial cells, Laboratory of Kidney & Electrolyte Metabolism, NIH, Bethesda, USA (Dr. Maurice B. Burg)
- 1998-2002 Assistant Professor of Physiology and Functional Genomics: The Whitney Laboratory & Department of Physiology and Functional Genomics, University of Florida, St. Augustine & Gainesville, USA
- 1999-2003 Principal Investigator: Mount Desert Island Biological Laboratory, Salisbury Cove, Maine, USA
- 2002-2004 Assistant Professor of Physiological Genomics: Department of Animal Science, University of California, Davis, Davis, USA
- 2004-pres. Associate Professor of Physiological Genomics: Department of Animal Science, University of California, Davis, Davis, USA
- 2002-pres. Member, Center for Aquaculture and Aquatic Biology, University of California, Davis, Davis, USA
- 2005-pres. Member, Genome Center, University of California, Davis, Davis, USA
- 2005-pres. Member, Center for Environmental Health Science, University of California, Davis, Davis, USA

AWARDS AND HONORS

- 1980-1982 The Student Society of Natural Sciences at Humboldt-University (Berlin, Germany)
- 1987-1989 Outstanding Student Promotion (University of Rostock, Germany)
- 1989-1992 Research Fellowship (University of Rostock, Germany)

- 1992 Doctor rerum naturalium with "summa cum laude" (University of Rostock, Germany)
- 1992 International Travel Award (German Academic Exchange Service)
- 1993-1995 International Postdoctoral Fellowship (German Academic Exchange Service)
- 1995-1998 Fogarty Visiting Fellowship (National Institutes of Health, USA)
- 1997 Fellows Award of Research Excellence (National Institutes of Health)
- 1997 Habilitation Fellowship (German Science Foundation)
- 1998 Hoechst Marion Roussel Award for Excellence in Renal Research (American Physiological Society)
- 1999-2001 New Investigator Award (Mount Desert Island Biological Laboratory)
- 2000 A. Clifford Barger Memorial Symposium Award (sponsored by the William Townsend Porter Foundation)
- 2001 Travel Award, XXXIV International Congress of Physiological Sciences in Christchurch, New Zealand (American Physiological Society)
- 2004-pres. Associate Editor, Journal of Experimental Zoology A: Molecular and Comparative Physiology
- 2005-pres. Editorial Board, American Journal of Physiology - Renal Physiology
- 2005-pres. Associate Editor, Comparative Biochemistry and Physiology D: Genomics and Proteomics
- 2006-pres. Board of Directors, NORCAL-SETAC

RECENT PUBLICATIONS:

- Fiol, D.F., Chan, S.Y. & Kültz, D. (2006) Identification and pathway analysis of immediate hyperosmotic stress responsiveness molecular mechanisms in tilapia (*Oreochromis mossambicus*) gill. CBP-D: Genomics and Proteomics, in press.
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- Kültz, D. & Avila, K. (2001) Mitogen-activated protein kinases are in vivo transducers of osmosensory signals in fish gill cells. *CBP-B: Biochem. Mol. Biol.* 129, p. 821-829.
- Kültz, D. (2001) Evolution of osmosensory MAP kinase signaling pathways. *Amer. Zool.* 41, p. 743-757.

For more publications visit:

<http://kueltzlab.ucdavis.edu/LPub.html>

Note: This budget summary **automatically links** to the costs and totals on the "**Budget Detail**" worksheet.
DO NOT CHANGE FORMULAS OR ENTER NUMBERS INTO ANY CELLS EXCEPT THE SHADED CELLS for "Cost Share" and "Other Matching Funds"

BUDGET SUMMARY	Total Amount for Year 1	Total Amount for Year 2	Total Amount for Year 3	Total Amount for All Years
Total Costs for Task One	\$ 2,875.00	\$ 2,875.00	\$ 2,875.00	\$ 8,625.00
Total Costs for Task Two	\$ 61,788.00	\$ 61,788.00	\$ -	\$ 123,576.00
Total Costs for Task Three	\$ 77,362.00	\$ 77,362.00	\$ -	\$ 154,724.00
Total Costs for Task Four	\$ 79,565.75	\$ 79,565.75	\$ -	\$ 159,131.50
Total Costs for Task Five	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Six	\$ 384,851.00	\$ 84,862.00	\$ 84,862.00	\$ 554,575.00
Total Costs for Task Seven	\$ 2,750.00	\$ 2,750.00	\$ 2,750.00	\$ 8,250.00
Total Costs for Task Eight	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Nine	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Ten	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Eleven	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Twelve	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Thirteen	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Fourteen	\$ -	\$ -	\$ -	\$ -
Total Costs for Task Fifteen	\$ -	\$ -	\$ -	\$ -
Total Costs for Project Tasks	\$ 609,191.75	\$ 309,202.75	\$ 90,487.00	\$ 1,008,881.50
1/Cost Share	\$ 50,434.00	\$ 50,434.00	\$ 9,150.00	\$ 110,018.00
2/ Other Matching Funds	\$ 279,250.00	\$ -	\$ -	\$ 279,250.00

1/ *Cost share funds* are specifically dedicated to your project and can include private and other State and Federal grants. Any funds listed in this line must be further described in the text of your proposal (see Chapter 3, Section D, of the PSP document)

2/ *Other matching funds* include other funds invested consistent with your project in your project area for which the ERP grant applicant is not eligible. Any funds listed in this line must be further described in the text of your proposal (see Chapter 3, Section D, of the PSP document)

BUDGET FOR TASK ONE (Administrative)	TOTAL AMOUNT TASK 1 All Years	Year 1			Year 2			Year 3		
		Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
Dietmar Kueltz (Assoc. Prof., 10% = \$27,450 cost sharing salary + be	\$ -	\$ -	0	\$ -	\$ -	0	\$ -	\$ -	0	\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
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	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Personnel Subtotal	\$ -			\$ -			\$ -			\$ -
^{1/} Benefits as percent of salary	0%			\$0.00			\$0.00			\$0.00
Personnel Total (salary + benefits)	\$0.00			\$0.00			\$0.00			\$0.00
Other Costs										
	Total All Years			Total Year 1			Total Year 2			Total Year 3
Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ 3,000.00			\$ 1,000.00			\$ 1,000.00			\$ 1,000.00
2/ Travel and Per Diem	\$ 3,900.00			\$ 1,300.00			\$ 1,300.00			\$ 1,300.00
3/ Equipment	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
Other Costs Subtotal	\$ 6,900.00			\$ 2,300.00			\$ 2,300.00			\$ 2,300.00
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)	25%			\$ 575.00			\$ 575.00			\$ 575.00
Total Costs for Task One	\$ 8,625.00			\$ 2,875.00			\$ 2,875.00			\$ 2,875.00

1/ Indicate your rate, and change formula in column immediately to the right of this cell

2/ Travel expenses and per diem must be at rates specified by the Department of Personnel Administration. The contractor is required to maintain travel receipts and records for auditing purposes. No travel out of the state of California shall be reimbursed unless prior written authorization is obtained from the State.

3/ Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

4/ Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

5/ Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

BUDGET FOR TASK TWO	TOTAL AMOUNT TASK 2 All Years	Year 1			Year 2			Year 3		
		Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
Serge Doroshov (Prof., 10% = \$29,670 cost sharing salary + benefits)	\$ -	\$ -	0	\$ -	\$ -	0	\$ -	\$ -	0	\$ -

Proposal Number
Proposal Name

Detailed Budget Breakdown by Task and by Fiscal Year

Applicant Name

Joel Van Eenenaam	\$ 24,804.00	\$ 29.25	424	\$ 12,402.00	\$ 29.25	424	\$ 12,402.00	\$ -	\$ -
TBN (Postdoctoral Fellow)	\$ 27,580.00	\$ 19.70	700	\$ 13,790.00	\$ 19.70	700	\$ 13,790.00	\$ -	\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -	\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -	\$ -
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	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -	\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -	\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -	\$ -
Personnel Subtotal	\$ 52,384.00			\$ 26,192.00			\$ 26,192.00	\$ -	\$ -
^{1/} Benefits as percent of salary	20%			\$5,238.40			\$5,238.40	\$0.00	\$0.00
Personnel Total (salary + benefits)	\$62,860.80			\$31,430.40			\$31,430.40	\$0.00	\$0.00
Other Costs	Total All Years			Total Year 1			Total Year 2	Total Year 3	
Operating Expenses: (ex: fish, recirculation system supplies, micro injection supplies, water treatment, selenium analyses, waste disposal, fish tank recharges, general lab and office supplies, etc)	\$ 34,000.00			\$ 17,000.00			\$ 17,000.00		
^{2/} Travel and Per Diem	\$ 2,000.00			\$ 1,000.00			\$ 1,000.00	\$ -	\$ -
^{3/} Equipment	\$ -			\$ -			\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -			\$ -			\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -			\$ -			\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -			\$ -			\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -			\$ -			\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -			\$ -			\$ -	\$ -	\$ -
Other Costs Subtotal	\$ 36,000.00			\$ 18,000.00			\$ 18,000.00	\$ -	\$ -
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)	25%			\$ 12,357.60			\$ 12,357.60	\$ -	\$ -
Total Costs for Task Two	\$ 123,576.00			\$ 61,788.00			\$ 61,788.00	\$ -	\$ -

1/ Indicate your rate, and change formula in column immediately to the right of this cell

2/ Travel expenses and per diem must be at rates specified by the Department of Personnel Administration. The contractor is required to maintain travel receipts and records for auditing purposes. No travel out of the state of California shall be reimbursed unless prior written authorization is obtained from the State.

3/ Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

4/ Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

5/ Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

BUDGET FOR TASK THREE	Year 1			Year 2			Year 3			
	TOTAL AMOUNT TASK 3 All Years	Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
Silas Hung (Prof., 10% = \$24,644 cost sharing for salary + benefits for	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Jang-Won Lee (Graduate Student)	\$ 33,072.00	\$ 10.40	1590	\$ 16,536.00	\$ 10.40	1590	\$ 16,536.00	\$ -		\$ -
Brian Sardella (Postdoctoral Fellow)	\$ 41,764.00	\$ 19.70	1060	\$ 20,882.00	\$ 19.70	1060	\$ 20,882.00	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -

Proposal Number
Proposal Name

Detailed Budget Breakdown by Task and by Fiscal Year

Applicant Name

	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
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	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
Personnel Subtotal	\$ 74,836.00	\$ 37,418.00	\$ -				
^{1/} Benefits as percent of salary	20%	\$ 7,483.60	\$ 7,483.60	\$ 7,483.60	\$ 7,483.60	\$ 7,483.60	\$ 0.00
Personnel Total (salary + benefits)	\$89,803.20	\$44,901.60	\$44,901.60	\$44,901.60	\$44,901.60	\$44,901.60	\$0.00
Other Costs	Total All Years	Total Year 1	Total Year 1	Total Year 2	Total Year 2	Total Year 3	Total Year 3
Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ 21,000.00	\$ 10,500.00	\$ 10,500.00	\$ 10,500.00	\$ 10,500.00	\$ 10,500.00	\$ -
^{2/} Travel and Per Diem	\$ 2,000.00	\$ 1,000.00	\$ 1,000.00	\$ 1,000.00	\$ 1,000.00	\$ 1,000.00	\$ -
^{3/} Equipment	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
Grad Student Fee Remission	\$ 13,720.00	\$ 6,860.00	\$ 6,860.00	\$ 6,860.00	\$ 6,860.00	\$ 6,860.00	\$ -
^{4/} Sub-Contractor	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
^{4/} Sub-Contractor	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -
Other Costs Subtotal	\$ 36,720.00	\$ 18,360.00	\$ -				
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)	25%	\$ 14,100.40	\$ 14,100.40	\$ 14,100.40	\$ 14,100.40	\$ 14,100.40	\$ -
Total Costs for Task Three	\$ 154,724.00	\$ 77,362.00	\$ -				

^{1/} Indicate your rate, and change formula in column immediately to the right of this cell

^{2/} Travel expenses and per diem must be at rates specified by the Department of Personnel Administration. The contractor is required to maintain travel receipts and records for auditing purposes. No travel out of the state of California shall be reimbursed unless prior written authorization is obtained from the State.

^{3/} Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

^{4/} Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

^{5/} Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

BUDGET FOR TASK FOUR	Year 1			Year 2			Year 3			
	TOTAL AMOUNT TASK 4 All Years	Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
Joseph Cech (Prof., 10% = \$28,254 cost sharing salary + benefits for	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Robert Kaufman (Postdoctoral Fellow)	\$ 41,764.00	\$ 19.70	1060	\$ 20,882.00	\$ 19.70	1060	\$ 20,882.00	\$ -		\$ -
Ann Houck (Graduate Student)	\$ 33,072.00	\$ 10.40	1590	\$ 16,536.00	\$ 10.40	1590	\$ 16,536.00	\$ -		\$ -
To Be Named (Undergraduate Student)	\$ 6,450.00	\$ 7.50	430	\$ 3,225.00	\$ 7.50	430	\$ 3,225.00	\$ -		\$ -
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	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Personnel Subtotal	\$ 81,286.00			\$ 40,643.00			\$ 40,643.00			\$ -

Proposal Number
Proposal Name

Detailed Budget Breakdown by Task and by Fiscal Year

Applicant Name

^{1/} Benefits as percent of salary	20%	\$8,128.60	\$8,128.60	\$0.00
Personnel Total (salary + benefits)	\$97,543.20	\$48,771.60	\$48,771.60	\$0.00
Other Costs	Total All Years	Total Year 1	Total Year 2	Total Year 3
Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ 17,250.00	\$ 8,625.00	\$ 8,625.00	\$ -
2/ Travel and Per Diem	\$ 1,536.00	\$ 768.00	\$ 768.00	\$ -
3/ Equipment	\$ -	\$ -	\$ -	\$ -
Graduate Student Fees	\$ 13,720.00	\$ 6,860.00	\$ 6,860.00	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
Other Costs Subtotal	\$ 32,506.00	\$ 16,253.00	\$ 16,253.00	\$ -
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)	25%	\$ 14,541.15	\$ 14,541.15	\$ -
Total Costs for Task Four	\$ 159,131.50	\$ 79,565.75	\$ 79,565.75	\$ -

1/ Indicate your rate, and change formula in column immediately to the right of this cell

2/ Travel expenses and per diem must be at rates specified by the Department of Personnel Administration. The contractor is required to maintain travel receipts and records for auditing purposes. No travel out of the state of California shall be reimbursed unless prior written authorization is obtained from the State.

3/ Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

4/ Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

5/ Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

	Year 1		Year 2		Year 3					
BUDGET FOR TASK FIVE	TOTAL AMOUNT TASK 5 All Years	Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
Marty Gingras	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
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	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Personnel Subtotal	\$ -			\$ -			\$ -			\$ -
^{1/} Benefits as percent of salary				\$0.00			\$0.00			\$0.00
Personnel Total (salary + benefits)	\$0.00			\$0.00			\$0.00			\$0.00
Other Costs	Total All Years			Total Year 1			Total Year 2			Total Year 3

Proposal Number
Proposal Name

Detailed Budget Breakdown by Task and by Fiscal Year

Applicant Name

Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ -	\$ -	\$ -	\$ -
2/ Travel and Per Diem	\$ -	\$ -	\$ -	\$ -
3/ Equipment	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
4/ Sub-Contractor	\$ -	\$ -	\$ -	\$ -
Other Costs Subtotal	\$ -	\$ -	\$ -	\$ -
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)		\$ -	\$ -	\$ -
Total Costs for Task Five	\$ -	\$ -	\$ -	\$ -

1/ Indicate your rate, and change formula in column immediately to the right of this cell

2/ Travel expenses and per diem must be at rates specified by the Department of Personnel Administration. The contractor is required to maintain travel receipts and records for auditing purposes. No travel out of the state of California shall be reimbursed unless prior written authorization is obtained from the State.

3/ Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

4/ Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

5/ Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

	Year 1			Year 2			Year 3			
	TOTAL AMOUNT TASK 6 All Years	Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
BUDGET FOR TASK SIX										
<i>Personnel</i>										
Dietmar Kueltz (Assoc. Prof., cost sharing 3 years - see task 1)	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Diego Fiol (Postdoctoral Fellow)	\$ 62,646.00	\$ 19.70	1060	\$ 20,882.00	\$ 19.70	1060	\$ 20,882.00	\$ 19.70	1060	\$ 20,882.00
Brittany Kammerer (Graduate Student)	\$ 49,608.00	\$ 10.40	1590	\$ 16,536.00	\$ 10.40	1590	\$ 16,536.00	\$ 10.40	1590	\$ 16,536.00
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
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Personnel Subtotal	\$ 112,254.00			\$ 37,418.00			\$ 37,418.00			\$ 37,418.00
^{1/} Benefits as percent of salary	20%			\$7,483.60			\$7,483.60			\$7,483.60
Personnel Total (salary + benefits)	\$134,704.80			\$44,901.60			\$44,901.60			\$44,901.60
<i>Other Costs</i>										
	Total All Years			Total Year 1			Total Year 2			Total Year 3
Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ 48,000.00			\$ 16,000.00			\$ 16,000.00			\$ 16,000.00
2/ Travel and Per Diem	\$ 4,500.00			\$ 1,500.00			\$ 1,500.00			\$ 1,500.00
3/ Equipment	\$ 299,989.00			\$ 299,989.00			\$ -			\$ -

Proposal Number
Proposal Name

Detailed Budget Breakdown by Task and by Fiscal Year

Applicant Name

Other Costs Subtotal	\$ 6,600.00	\$ 2,200.00	\$ 2,200.00	\$ 2,200.00
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)	25%	\$ 550.00	\$ 550.00	\$ 550.00
Total Costs for Task Seven	\$ 8,250.00	\$ 2,750.00	\$ 2,750.00	\$ 2,750.00

1/ Indicate your rate, and change formula in column immediately to the right of this cell

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3/ Please provide a list and cost of major equipment (\$5,000 or more) to be purchased, and complete "Equipment Detail" Worksheet

4/ Please list each subcontractor and amounts (if subcontractor not selected yet, use function like "ditch construction subcontractor")

5/ Indicate rate in column immediately to the right of this cell; and provide a description of what expenses are covered by overhead. If overhead is > 15% must provide justification

	Year 1		Year 2		Year 3					
BUDGET FOR TASK EIGHT	TOTAL AMOUNT TASK 8 All Years	Amount per hour	Number of Hours	Total Amount for Year 1	Amount per hour	Number of Hours	Total Amount for Year 2	Amount per hour	Number of Hours	Total Amount for Year 3
Personnel										
	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
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	\$ -	\$ -		\$ -	\$ -		\$ -	\$ -		\$ -
Personnel Subtotal	\$ -			\$ -			\$ -			\$ -
^{1/} Benefits as percent of salary				\$0.00			\$0.00			\$0.00
Personnel Total (salary + benefits)	\$0.00			\$0.00			\$0.00			\$0.00
Other Costs	Total All Years			Total Year 1			Total Year 2			Total Year 3
Operating Expenses: (ex: seed, plant materials, irrigation supplies, software, office supplies, etc)	\$ -			\$ -			\$ -			\$ -
2/ Travel and Per Diem	\$ -			\$ -			\$ -			\$ -
3/ Equipment	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
4/ Sub-Contractor	\$ -			\$ -			\$ -			\$ -
Other Costs Subtotal	\$ -			\$ -			\$ -			\$ -
^{5/} Overhead Percentage (Applied to Personnel & Other Costs)				\$ -			\$ -			\$ -
Total Costs for Task Eight	\$ -			\$ -			\$ -			\$ -

EQUIPMENT DETAIL

Use this worksheet as a sample of how to present project equipment costing more than \$5,000. Applicants must complete a spreadsheet as shown below to present project equipment costing more than \$5,000.

Task No	List of Equipment	Unit Cost	Task Total
6	Bruker Ultraflex II T3 Sequencing Mass Spectrometer	\$ 299,989.00	
	Cost sharing: \$ 100,000 match by UC Davis		
	Cost sharing: \$ 1,000 match for UC Davis		
	Cost sharing: \$ 179,260.78 special discount for UC Davis		\$ 299,989.00
	Total equipment cost: \$ 579,250		\$ -
		TOTAL	\$ 299,989.00

Equipment purchased for a project shall be purchased by (*Name of Contractor*) and shall adhere to State of California Contracting rules and regulations as stated in State Contracting Manual (SCM) 7.29 Equipment Purchases.

For further information please go to: <http://www.ols.dgs.ca.gov/Contract+Manual/default.htm>

The Contractor shall maintain an inventory record for each piece of non-expendable equipment purchased with the funds provided under the terms of this agreement. The inventory record for each piece of such equipment should include the date acquired, total cost, serial number, model identification, and any other information or description necessary to identify said equipment. Non-expendable equipment are those **items** of equipment that have a normal life expectancy of one year or more and an approximate cost of \$5,000 or more.

Contractor shall provide DFG with a copy of the inventory record at the time an invoice is presented for reimbursement for such equipment purchase.

NOTE: Ownership and reporting requirements for equipment purchased depends upon the Contractor's type of organization (state agency, local entity, private, etc.). Specific provisions for equipment purchases shall be provided at the time contract documents are prepared.

Budget justification (CALFED 2006 Science PSP - proposal #0025)

Task 1:

Operating Expenses: Funds for preparing meeting outlines for all participants as well as for generation of reports, presentations, and meeting agendas are needed.

Travel: Lodging and mileage to cover participation of the PI in regional and national meetings each year are requested.

Task 2:

Operating Expenses: Funds for fish tank recharges, micro-injection supplies, recirculation temperature system supplies, selenium water treatment supplies, water and tissue selenium analyses recharges, waste disposal recharges, fish transportation supplies, miscellaneous laboratory and office supplies.

Travel: Funds to cover trips to sturgeon farms and participation of Prof. Serge Doroshov and Joel Van Eenennaam in regional conferences are requested.

Salary: Twenty % SRA salary for 2 years is requested for Joel Van Eenennaam (MS), who will be instrumental for sturgeon acquisition for all tasks and organizing and participating in the micro-injection and water temperature experiments with sturgeon larvae, and conduct all histological processing of samples. A Postdoctoral Fellow (TBN) will be hired to work 50% time for eight months, each year, to prepare culture systems and the micro injection systems, perform temperature exposure and microinjection experiments, data analyses, and assist with histological analyses, and preparation of reports.

Benefits: Funds to cover benefits for Joel Van Eenennaam and the Postdoctoral Fellow at a rate of 20% of the salary requested.

Task 3:

Operating Expenses: Funds for Publication costs, Nutrition lab user fees, Cost of Hg and Se analysis, and Laboratory supplies for experiments are needed.

Travel: Funds to cover Lodging and mileage for participation of Prof. Hung and Mr. Lee in a regional meeting each year are requested.

Salary: Salary for 2 years is requested for Jang-Won Lee (PhD student). Mr Lee will perform acclimation and biochemical experiments and Se and Hg tissue burden analysis.

Benefits: Funds to cover tuition/ student fees for Jang-Won Lee at a rate of 20% of his salary are requested.

Task 4:

Operating Expenses: Funds for Publication costs, laboratory supplies, and office supplies and expendables are needed.

Travel: Funds to cover Lodging and mileage for participation of Prof. Cech and Dr. Kaufman in several regional and national meetings each year are requested. (6 trips to CALFED informational meetings, 3–day trips to scientific meetings)

Graduate Student Fees: 75% of Student Tuition fees for Ms. Houck are requested at \$6,860 per year. The remaining 25% of the required fees will be covered by the campus as part of the Graduate Student Researcher buy-down program.

Salary: 50% Salary for 2 years is requested for Robert Kaufman (Postdoctoral Fellow) and 75% of salary for 2 years is requested for Ann Houck (graduate student). Dr. Kaufman and Ms. Houck will perform further acclimation, hematological, swimming performance, and physiological test analysis of sturgeon.

Benefits: Funds to cover benefits for Dr. Kaufman at a rate of 20% of his salaries is requested.

Task 5:

No budget is requested from CALFED for this task because the necessary sturgeon biopsies will be obtained through existing fish monitoring and salvaging programs that are already funded by CDFG.

Task 6:

Operating Expenses: Funds for Publication costs, supplies for proteomics, including IPG gel strips, acrylamide, buffers, CHAPS, stains, MS anchorchip targets, zip tips and zip plates, and other reagents are needed. In addition, funds for supplies and reagents for construction of TMAs (tissue microarrays) are required.

Travel: Funds to cover Lodging and mileage for participation of Dr. Fiol and Ms. Kammerer in several regional and national meetings each year are requested. (6 trips to CALFED informational meetings, 3–day trips to scientific meetings)

Salary: 50% Salary for 3 years is requested for Diego Fiol (Postdoctoral Fellow) and 50% of salary for 2 years is requested for Brittany Kammerer (graduate student). Dr. Fiol and Ms. Kammerer will perform proteomic

and bioinformatic analysis of sturgeon samples and also construct tissue microarrays from those samples.

Benefits: Funds to cover benefits/ for Dr. Fiol at a rate of 20% of salaries is requested.

Equipment: The proposal is built on a new approach that is only possible with this new instrument. Thus, funds to cover a portion of the initial cost are requested. Matching funds are provided by UC Davis and a special UC Davis academic discount for this equipment is being applied. Please refer to detailed justification in the main text of the proposal.

Graduate Student Fees: 75% of Student Tuition fees for Ms. Kammerer are requested at \$6,860 per year. The remaining 25% of the required fees will be covered by the campus as part of the Graduate Student Researcher buy-down program.

Task 7:

Operating Expenses: Funds for ColdFusion server software updates for proteomics database publication on the internet, Office/Presentation Supplies, Supplies for data management and analysis, Reproduction, Copier charges for distribution of data analysis results to all PIs in this project are requested.

All Tasks:

Overhead Percentage: Please note that the University of California, Davis Federally Negotiated Indirect Cost Rate Agreement is currently 51.5% of Modified Total Direct Costs (MTDC). However, the University has an approved rate with State Agencies for 25% MTDC. The MTDC base excludes equipment capital expenditures in excess of \$5,000, patient care costs, tuition remission, rental costs, scholarships and fellowships, as well as the portion of each subcontract in excess of \$25,000. When applicable, these items have been excluded when calculating the indirect costs.

Description of matching funds and cost sharing (CALFED 2006 Science PSP - proposal #0025)

Matching Funds

Matching funds are provided by the UC Davis office of Research for the equipment (mass spectrometer) needed for this project. In addition to these \$100,000 matching funds a special academic discount of \$179,260.78 is provided by Bruker to UC Davis. A letter by UC Davis Vice Chancellor for Research Prof. Barry Klein and a signed quotation specifying the academic discount for the Ultraflex II mass spectrometer are attached.

Cost sharing

Cost sharing is provided in the form of salary and benefits for Profs. Kültz, Doroshov, Hung, and Cech from UC Davis for 10% effort devoted exclusively to this project.

August 29, 2006

ASSOCIATE PROFESSOR DIETMAR KUELTZ

Department of Animal Science

**RE: "Quantitative Indicators and Life History Implications of Environmental Stress on Sturgeon"
CALFED
UC Davis Capital Equipment Matching Funds Program**

Dear Associate Professor Kultz:

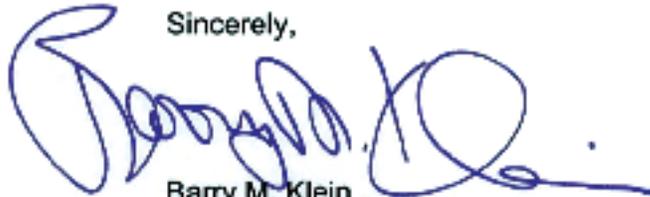
I strongly support your proposal "Quantitative Indicators and Life History Implications of Environmental Stress on Sturgeon" which you are submitting to the Extramural Funding Agency CALFED.

The Office of Research is prepared to commit up to \$50,000 in Capital Equipment Matching Funds. These funds will be used to support the purchase of a high mass range MS with MALDI ionization and T3 sequencing capability. With the College of Agricultural and Environmental Sciences contribution of \$50,000, this brings the total campus commitment to \$100,000. UC Davis' commitment level will be adjusted proportionately if the proposal is partially funded. When you require details on transferring funds to your account, please contact Financial Officer Jessie Catacutan at 747-3833 or jfcatacutan@ucdavis.edu. The Program requires that any funds remaining from your Capital Equipment Matching Funds Program commitment be returned to our office for reallocation to another project.

The campus administration is cognizant of the importance of your research. UC Davis contributes significantly in terms of cost sharing on academic year salaries in support of faculty research. The addition of Capital Equipment Matching Funds to this campus commitment is another demonstration of the value placed on the comprehensive program you are developing.

You have my best wishes for success in your project.

Sincerely,



Barry M. Klein
Vice Chancellor for Research

/kmo

c: Dean Neal Van Alfen
Associate Dean Michael Parrella
MSO Gary Crawford
Financial Officer Catacutan
Contracts & Grants Analyst David Ricci

BRUKER DALTONICS[®]

2859 BAYVIEW DRIVE
FREMONT, CA 94538 USA
TEL. (510) 683-4300
FAX. (510) 490-6586

Quote Number: MS-MLS073106-02

Date: 7/31/2006

Quote Expires on: 9/29/2006

TO: Dr. Dietmar Kueltz
UC Davis
Animal Science, Meyer Hall 2207
One Shields Drive
Davis, CA 95616
Phone: 530-752-2991

Shipment:

F.O.B.: Billerica

SHIP VIA: Best Way

PREPARED BY: Maryann Shen

SALES REPRESENTATIVE: Maryann Shen

Part #	Description	Retail	Qty	Extend
246420	ultraflex [™] II TOF/TOF200 MALDI-TOF System: MALDI-Time-of-flight Mass Spectrometer for accurate mass determination and structure identification of bio-molecules. Includes scoutMTP [™] ion source with extended pulsed ion extraction PAN [™] ; modified 200 Hz all-solid-state laser with smartbeam [™] technology for versatile applications with different sample preparation techniques; TOF analyzer for linear and reflectron measurements; TOF/TOF capability for LIFT [™] measurements; high-energy CID (Collision-Induced Dissociation) accessory; Data System with digitizer, WIN-XP Operating System, =20" LCD-Display, Laser printer and Compass [™] for Flex software package for MS control, data acquisition and processing; Installation; Familiarization upon installation; 1-year warranty; Voucher for factory training course.	\$575,000.00	1	\$575,000.00
223664	Biotoools [™] 3.0 software package : Supports processed spectra and/or peak picking results from FLEX [™] series of MALDI-TOF and -TOF/TOF mass spectrometers, microTOF and BioTOF [™] series of orthogonal ESI-TOF and ESI-Q-q-TOF systems, ESQUIRE [™] series of API ion trap systems, and APEX [™] series of FTMS systems. Communication and File Import/Export of XMASS and Flex Analysis [™] spectra, FAST [™] and LIFT [™] (post-source decay) spectra, LC ESI-MS/MS deconvoluted and non deconvoluted profile spectra from Bruker systems, GPMAS Sequence Files (unmodified amino acids only), and clipboard support for spectra and data. Annotation of spectra and sequence data to visualize and score the match between MS/MS spectra and the sequence. Automatic generation of sequence tags for database searches. Internet library searches are fully integrated in the software using EMBL PeptideSearch and MASCOT (peptide fingerprint and MS/MS). Local MASCOT installations are only supported under windows with IIS. WARP [™] support for TOF and TOF/TOF. Combined MS + multi MS/MS search and	\$100.00	1	\$100.00

BRUKER DALTONICS®

2859 BAYVIEW DRIVE
FREMONT, CA 94538 USA
TEL. (510) 683-4300
FAX. (510) 490-6586

Quote Number: MS-MLS073106-02

Date: 7/31/2006

Quote Expires on: 9/29/2006

TO: Dr. Dietmar Kueltz
UC Davis
Animal Science, Meyer Hall 2207
One Shields Drive
Davis, CA 95616
Phone: 530-752-2991

Shipment:

F.O.B.: Billerica

SHIP VIA: Best Way

PREPARED BY: Maryann Shen

SALES REPRESENTATIVE: Maryann Shen

Part #	Description	Retail	Qty	Extend
223662	reporting; CAF support. Includes CD and manual; requires separate licence key. Licence for biotools™ 3.0: Licence key for biotools™ 3.0 Special Package Discount for UC Davis	\$4,150.00	1	\$4,150.00 ((\$179,260.78))

Total \$399,989.22

Quote valid only with accompanying **TERMS & CONDITIONS** and **WARRANTY** pages

Total price does not include sales tax, sales tax will be added to invoice

QUOTATION ONLY VALID WHEN SIGNED BY A CORPORATE OFFICER

BY:


AUTHORIZED SIGNATURE

STANDARD TERMS AND CONDITIONS

1. GENERAL CONDITIONS

a. The terms and conditions contained in this quotation, unless otherwise agreed to by the parties in writing, constitute the sole terms and conditions governing the purchase by the customer described on page 1 hereof ("Customer") from Bruker Daltonics Inc. ("BDAL") of all products and services. Any terms and conditions different from or in addition to those contained herein, including any contained in Customer's purchase order or in any other document furnished by Customer, shall be of no force or effect in connection with the sale of any products and services by BDAL to Customer, and BDAL hereby objects to and rejects in their entirety all such terms and conditions, as BDAL's agreement to sell such products and services is expressly made conditional upon the use of these terms and conditions.

b. In the absence of written acceptance of these terms and conditions, an acceptance of any goods or services shipped or provided by BDAL based on an order received from Customer shall constitute an acceptance of these terms and conditions. The terms and conditions herein shall prevail against the terms and conditions of any order.

c. The rights and obligations of the parties shall be governed in all respects by the laws of the Commonwealth of Massachusetts, and the parties shall submit themselves to the jurisdiction thereof.

d. BDAL shall retain copyright, trademark, patent and proprietary rights in all drawings, technical information and know-how. Customer shall not disclose to third parties any proprietary information gained from BDAL. In the event Customer breaches any of the conditions set forth herein, in addition to any other remedy, BDAL may discontinue all service to Customer and all guarantees and warranties shall be terminated without notice.

e. Documentation such as software listings, detailed drawings and other confidential and proprietary documentation normally not distributed may only be provided by BDAL on the condition that the recipient of such documentation signs a Confidentiality Agreement.

f. Clerical errors and mistakes of fact in the quotation are subject to correction by BDAL at any time.

g. No action, regardless of form, arising out of, or in any way connected with, the products or services furnished or to be furnished by BDAL may be brought by Customer more than one (1) year after the cause of action has accrued.

h. Customer shall not have the right to assign any of its rights or obligations hereunder, whether by operation of law or otherwise.

i. These terms and conditions, together with any documents incorporated by reference herein, are the sole and exclusive statement of any agreement between the parties which may result from this quotation and supersede any prior or contemporaneous agreements, demonstrations, samples, purchase orders or understandings in connection therewith.

2. PRICE AND PAYMENT

- a. All quotations are firm for a period of 60 days from the date of issuance unless otherwise specified.
- b. All prices are F.O.B. shipping point unless otherwise stated. Responsibility for risk of loss of or damage to items and title pass to Customer upon delivery to the F.O.B. point.
- c. Prices quoted do not include city, state or federal sales or similar taxes. Customer shall provide evidence of exemption from such taxes, or the applicable sales/use taxes will be added to each invoice. Prices exclude any applicable import duty.
- d. Payment for system orders shall occur as follows unless otherwise specified:
 - 60% upon order placement,
 - 30% upon on delivery, and
 - 10% upon signed Customer acceptance that the system complies with its specifications.If delivery/installation of the system is delayed by request of Customer, then the amount due upon delivery/installation will be considered payable on the quoted delivery date. Invoices for other than system orders are due upon receipt.
- e. Invoices are due without deduction upon presentation. Orders shipped and invoiced in separate parts shall be due upon receipt of such parts. Failure to make payment when due on any one part shall relieve BDAL of delivery of any remaining parts.
- f. If a bank guarantee or bid bond is required by Customer, the prices set forth herein shall be increased by the cost of such guarantee or bond.
- g. Upon demonstration by BDAL that the product complies with its specifications, any bank guarantee or bid bond will be returned by Customer, but in any event no later than ninety (90) days after delivery.

3. ORDERS AND ORDER SPECIFICATIONS

- a. Customer orders are subject to acceptance by BDAL.
- b. In a case where a new development is included in an order or the execution of any order depends upon successful completion of a new development, BDAL reserves the right to cancel such order, without incurring any obligation to Customer, if such development cannot be completed successfully in BDAL's discretion.

4. DELIVERY AND SHIPMENT

- a. Delivery time is computed from the date of acknowledgement by BDAL of Customer's order.
- b. BDAL shall not be liable for delivery delays due to circumstances beyond its control, including, but not limited to, fire, flood, war, acts of terrorism, labor disputes, accidents or delay of carriers, subcontractors or suppliers.
- c. Changes to an order by Customer after the original order is accepted by BDAL shall be subject to reacceptance by BDAL, in which case the originally quoted delivery time may no longer be applicable. Should BDAL decline acceptance of a change order, the original order remains binding.
- d. Cancellation of an order due to reasonable delay of delivery is excluded. Any rights of Customer for claims or compensation for damage or loss of any kind whatsoever due to delay of delivery are excluded.
- e. An order covering several independent functional units may be delivered and invoiced in part based upon functional units and payment thereof shall be due for such unit upon presentation of an invoice.

5. INSTALLATION

Complete Systems/Instruments:

Installation is included with a system familiarization on-site by the installation engineer. Additional factory training is available as an option. Cryogenics for FTMS systems and any required internal rigging are the responsibility of Customer and are not covered in this quotation.

Accessories & Consumables:

When purchased standalone, accessories and consumables, unless otherwise provided in this quotation, are shipped

6. WARRANTY

a. Systems

All systems sold by BDAL include a spectrometer and carry the limited warranty provided herein. Systems are warranted for one (1) year to be free of defects in material and workmanship. BDAL's warranty obligation is limited in accordance with the periods of time and all other conditions contained herein. The system warranty period shall begin upon demonstration by BDAL that the system complies with its specifications. Short shipment of individual items does not delay commencement of the warranty period. In no event shall any warranty period extend more than fifteen (15) months from the date of shipment.

b. Coverage

This warranty is subject to all the following limitations:

1. This warranty applies only to defects in material and workmanship and is not to be interpreted as providing full service coverage for such items as routine maintenance, adjustments, or recalibration as defined by the instruction manual.
2. This warranty covers only parts and labor furnished by BDAL. This warranty does not cover products or services provided by an outside manufacturer, which may be repaired or replaced according to the original manufacturer's warranty terms, if any. BDAL accepts no responsibility for failure of the original manufacturer to perform under its own warranty obligations.
3. The following are expressly not covered under this warranty:
 - a) Any loss, damage or instrument malfunction relating in any way to:
 - Shipping or storage;
 - Accident, abuse, alteration, misuse, or neglect;
 - Breakage or abuse of parts;
 - Operation other than in accordance with correct operating procedures;
 - Tampering with the system;
 - Lack of routine care and maintenance, such as lubrication and cleaning, as indicated in the instruction manual;
 - Change of location of the system from its installation site unless otherwise approved in writing by BDAL;
 - Inadequate utility service, failure of electrical or other energy supplies, incorrect physical environment, or other inadequate facilities or utilities as indicated in the instruction manuals and/or pre-installation instructions;
 - Chemical action or contamination; or
 - Failure to maintain proper helium level in superconducting magnets.
 - b) Items, parts, accessories, subassemblies, or components which are expendable in normal use or operation, or those of limited life, such as, but not limited to, filters, glassware, glass accessories, fuses, probe inserts, variable temperature dewars, and transfer lines.
4. The sole and exclusive remedy under this warranty shall be repair of malfunctions which, in the sole opinion of BDAL are due or traceable to defects in original materials or workmanship or, at BDAL's option, replacement of defective parts.
5. In-warranty repaired or replacement items are covered by this warranty only for the remaining unexpired portion of the original warranty period applicable to the repaired or replaced items. Repair or replacement of items under warranty does not extend the original warranty period. Any defective item which is replaced by BDAL must be returned to BDAL or Customer will pay the price therefor.
6. After expiration of the applicable warranty period, BDAL will be available to provide service for which Customer shall be charged at BDAL's then current prices for parts, labor, and transportation.

(Continued on next page)

6. WARRANTY (Continued from previous page)

c. Replacement and Adjustment

All claims under this warranty must be made promptly after occurrence of the circumstances giving rise thereto and must be received within the applicable warranty period by BDAL. Such claims shall include the system type and serial numbers, and a full description of the circumstances giving rise to the claim. BDAL reserves the right in its sole discretion to determine whether repair under valid warranty claims shall be made by (a) sending a field service engineer to the site, (b) having Customer send the defective part, assembly, or instrument to a service shop or facility as authorized by BDAL, or (c) authorizing Customer to return the same to BDAL. Before any systems, parts or assemblies are sent to a service shop or facility or are returned to BDAL for repair and/or adjustment, authorization from BDAL or its authorized representative for the return and instructions how and where the same should be shipped must be obtained. Any system, part, or assembly sent to an authorized service shop or facility or returned to BDAL for examination shall be sent prepaid via the means of transportation indicated as acceptable by BDAL with all transportation at the expense of Customer. BDAL reserves the right to reject any warranty claim not promptly reported and any warranty claim on any item that has been altered or has been shipped by a non-acceptable means of transportation. When any system, part, or assembly is sent to a service shop or facility or is returned to BDAL for examination and inspection or for any other reason, Customer retains all risks of ownership including, without limitation, responsibility for all damage resulting from improper packing or handling, and for loss in transit, notwithstanding any defect or non-conformity in the system, part, or assembly. In all cases, BDAL has sole responsibility for determining the cause and nature of failure, and BDAL's determination with regard thereto shall be final.

Reasonable care must be used to avoid hazards. BDAL expressly disclaims responsibility for loss or damage caused by use of its systems other than in accordance with proper operating procedures. All obligations of BDAL under this warranty shall cease in the event its systems or accessories have been subject to accident, abuse, alteration, misuse or neglect, or have not been operated and maintained in accordance with proper operating procedures. All products and services provided within the scope of their warranty must be provided through, or with the knowledge and approval of, BDAL. BDAL makes no warranty concerning services or components supplied through unapproved sources. What constitutes an approved source shall be determined by BDAL. THIS WARRANTY IS EXPRESSLY IN LIEU OF AND EXCLUDES ALL OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF MERCHANTABILITY, INFRINGEMENT AND FITNESS FOR A PARTICULAR PURPOSE, USE, OR APPLICATION, AND ALL OTHER OBLIGATIONS OR LIABILITIES ON THE PART OF BDAL. Statements made by any person, including representatives of BDAL, which are inconsistent or in conflict with the terms of this warranty, shall not be binding upon BDAL unless reduced to writing and approved by an officer of BDAL.

d. Accessories

In lieu of the one (1) year period applicable to systems, accessories (provided separately from systems) or service furnished by BDAL are warranted to be free of defects in material and workmanship for a period of ninety (90) days from the date of shipment, or if BDAL specifically agrees in writing to provide installation, ninety (90) days from the date of installation. All such accessory/service warranties are limited in accordance with all the terms, conditions, and other provisions stated in this warranty. However, the warranty for accessories shall commence upon shipment from BDAL.

7. LIMITATION OF LIABILITY

IN NO EVENT SHALL BDAL BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL, CONSEQUENTIAL OR RESULTING LOSS OR DAMAGE OF ANY KIND, HOWEVER CAUSED, AND BDAL'S LIABILITY FOR DAMAGES SHALL NOT EXCEED THE PAYMENT, IF ANY RECEIVED BY BDAL FOR THE PRODUCT OR SERVICE FURNISHED OR TO BE FURNISHED, AS THE CASE MAY BE, WHICH IS THE SUBJECT OF THE CLAIM OR DISPUTE.

Signature

<https://solicitation.calwater.ca.gov/solicitations/2006.01/proposals/0...>

California Home



Signature

The applicant for this proposal must submit this form by printing it, signing below, and faxing it to +1 877-408-9310. Send exactly one form per transmission.

Failure to sign and submit this form will result in the application not being considered for funding.

The Individual submitting this proposal will receive e-mail confirmation as soon as this signature page has been processed.

The Individual signing below declares that:

- all representations in this proposal are truthful;
- the individual signing the form is authorized to submit the application on behalf of the applicant (If applicant is an entity or organization);
- the applicant has read and understood the conflict of Interest and confidentiality discussion under the Confidentiality and Conflict of Interest Section in the main body of the PSP and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent provided in this PSP; and
- the applicant has read and understood all attachments of this PSP.

Proposal Title: Quantitative indicators and life history implications of environmental stress on sturgeon

Proposal Number: 2006.01-0025

Applicant Organization: Davis, California University of

Applicant Contact: Ms. Kimberly Lamar

Kimberly Lamar

8/29/06

Applicant Signature

Kimberly Lamar
Contracts and Grants Analyst

Date

Help is available: help@solicitation.calwater.ca.gov, +1 877 408-9310

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time: 2006-08-25 10:04:20 PST

user ID: dkueltz

client IP: 169.237.28.21

UNIVERSITY OF CALIFORNIA, DAVIS

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SANTA BARBARA • SANTA CRUZ

Kimberly Lamar, Contracts and Grants Analyst
Office of Research, Sponsored Programs
1850 Research Park Drive, Suite 300
Davis, California 95618

Sponsored Programs, 118 Everson Hall
Telephone: (530) 747-3924
Fax: (530) 747-3929
e-mail: kdlamar@ucdavis.edu

August 29, 2006

California Bay-Delta Authority
650 Capitol Mall, 5th Floor
Sacramento CA 95814

To Whom It May Concern:

Letter in Support of Project Entitled
"Quantitative Indicators and Life History Implications of Environmental Stress on Sturgeon"
Principal Investigator- Dr. Dietmar Kueltz, UCD

It is our pleasure to forward institutional support and approval of the collaboration by UCD's Dr. Kueltz on the referenced research project to the California Bay-Delta Authority Science Program.

Please note as outlined in Attachments 1 and 2 of the CALFED Science Program Solicitation UCD takes exception to the following proposed standard clauses:

- Exhibit C – General Terms and Conditions for Science Program Grants (specifically Indemnification and Termination clauses)
- Exhibit D – Special Terms and Conditions for Science Program Grants

Should CALFED make an award to the University, we would anticipate negotiating terms that comply with University guidelines as they pertain to the higher learning institutions and retention of intellectual property rights.

Please contact the principal investigator for scientific information. Administrative questions may be directed to me by telephone, facsimile or electronic mail at the numbers cited above.

Sincerely,

A handwritten signature in cursive script that reads 'Kimberly Lamar'.

Kimberly Lamar, CRA
Contracts & Grants Analyst

cc: Dr. Kueltz



August 24, 2006

To: CALFED 2006 Science Program PSP

Re: Proposal "Quantitative Indicators and Life History Implications of Environmental Stress on Sturgeon" (Project Leader: Dietmar Kueltz)

Sterling Caviar LLC supports the proposed project aimed to determine stress-specific bioindicators or environmental and toxic stressors in sturgeon. This project would greatly benefit the existing and future wild populations of sturgeon and would also provide us with information that could lead to improved culture practices for the sturgeon farming industry. We have had a collaborative Sturgeon Broodstock Development Program with UC Davis for 18 years which has been mutually beneficial for our California sturgeon aquaculture industry and various research projects at UC Davis. The availability of cultured fish in a sustainable production system greatly expanded our knowledge of sturgeon biology and physiology during the recent years. Our company will be willing to supply the researchers in this project with the white sturgeon larvae required for their specific tasks.

Sincerely,

A handwritten signature in dark ink, appearing to read "Peter Struffenegger". The signature is fluid and cursive, with a long horizontal stroke extending from the end of the name.

Peter Struffenegger
Manager



DEPARTMENT OF ENVIRONMENTAL SCIENCES - 084
2217 GEOLOGY BUILDING
RIVERSIDE, CALIFORNIA 92521-0424

<http://envisci.ucr.edu>
FAX: (909) 787-3993

August 30, 2006

Dietmar Kueltz, PhD
Department of Animal Science
I Shields Avenue
2207 Meyer Hall
University of California, Davis
Davis, CA 95616-0340

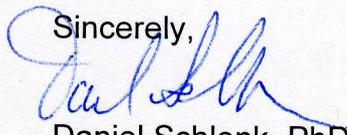
Dear Dietmar:

This is a letter to support your CALFED grant proposal entitled "**Quantitative indicators and life history implications of environmental stress on sturgeon**". I feel that is imperative to understand the interactions of mercury and selenium within sensitive aquatic species such as sturgeon and I applaud your efforts. I also think that is important to link the biochemical and histological effects of these compounds to life history in order to better understand the sublethal impacts of these compounds to the population of these organisms.

I look forward to potential collaboration with your group particularly in evaluating the effects of these compounds on oxidative pathways within the cell. Our laboratory would be more than willing to help you in evaluating oxidative stress within selected tissues.

I wish you the best of luck with your proposal and if you need any further information, please do not hesitate to contact me.

Sincerely,



Daniel Schlenk, PhD
Professor