Addendum to Proposal Entitled:

Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

Lead PI: Nann A. Fangue
Co-PI's: Serge Doroshov, Silas Hung, Ermias Kebreab
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This proposal was originally submitted to the Delta Science Program for consideration in their 2010 Focused Proposal Solicitation Package, and was forwarded to the California Dept. of Fish and Game: Ecosystem Restoration Program for funding consideration. The proposal underwent an extensive review process with the Delta Science Program and the reviews accompany our proposal. We would like to take the opportunity to address the thoughtful comments put forward by the reviewers to improve our proposal, and we have outlined and responded to the comments below.

Response to prior reviews:

1) One of the unique aspects of this proposal, pointed out by external reviewer #2, is our experimental focus on a graded series of young sturgeon life stages. Most of the relevant experimental work on sturgeon to date has ignored young sturgeon, and this data gap is important to fill, particularly since it is very likely that there will be differential sensitivity to stress between stages. However, the summary project review, based on final review panelist #1, found that our laboratory approaches could be improved by including an assessment of the general condition and nutritional status of wild fish using condition indices or bioimpedance analysis. Although this is a task that we considered during preparation of this proposal to compliment the laboratory experiments, it was determined to be not feasible at this time. To the best of our knowledge there is no documented information on the habitat use of sturgeon at the life stages we propose to study (first feeding larvae to 12 month old juveniles) making assessment of wild fish of these stages nearly impossible. In addition, the southern distinct population segment of green sturgeon has been listed as "threatened" under the ESA, and so the capture and sampling of any of these fish is not allowed, without special state and federal permits. Lastly, although authorization for capture and sampling of white sturgeon is a possibility, at this time no agency has identified where to find or how to capture juvenile white sturgeon, at the size range used in our experiments. Current monitoring studies capture much larger fish in the 1.0 to 1.7 m subadult to adult size range (M. Gingras, California Department of Fish and Game, pers. comm.). While beyond the scope of this project, a comprehensive study to determine the habitat of juvenile sturgeon within the river, delta and bay is warranted as very little is currently known about the distribution of sturgeon at these very critical young life stages.

2) Final review panelist #1, pointed out that the magnitude of the proposed thermal and salinity challenges (Task #4) do not accurately mimic conditions of stress likely experienced by sturgeon in the wild. This is a common critique of the use of standard, quantitative physiological tools to address ecosystem-level questions. The reviewer suggested that the inclusion of measures of physiological tolerance taken from individuals captured from the field could shed light on this matter, but as pointed out above these small size classes are simply unavailable for sampling. Currently we know very little about where small sturgeon spend the first year of their lives, and therefore what ecological gradients in environmental salinity and temperature are encountered historically or with the onset of climate change are unknown. Our experimental approach is therefore designed to utilize standard methodologies as comparative indices across fish species and among species of different lifestages, which the reviewer acknowledged as typical practice in physiological ecology. The value of gathering data from well-controlled laboratory experiments is that they reveal the mechanistic underpinnings that determine an animal's sensitivity to environmental change and lead to an explanation of the trade-offs and constraints that govern physiological performance. As habitat use of young sturgeon becomes better described and as ongoing climate change modeling efforts for Bay-Delta salinity and temperature projections become available, we look forward to future experiments that can be parameterized using these projections. Lastly, it is important to point out that even when more ecologically relevant exposure scenarios become available, completely capturing all possible scenarios experimentally quickly becomes overwhelming. For example, the two abiotic factors of interest in this proposal, temperature and salinity, vary over time (daily, seasonally, with the onset of climate change), over spatial scales, and with differing magnitudes. Moreover, we know that multiple environmental stressors often act synergistically to influence an animal's physiological performance. We cannot conduct all of these exposure scenarios experimentally, but we propose to use these data to develop an ecological model based on a structural equation model. Since the model is based on the principle of cause and effect, it can be applied to study effects of a wide range of environmental stressors on an animal's performance in the field.

3) Final review panelist #2 indicated that the details of how the data from Task 3 and 4 would be handled statistically were missing. Our proposal included these details, but admittedly they were a bit hidden. In Task 5, Dr. Kebreab and his team will not only build the statistical model, they will also perform the basics statistics for Task 3 and 4 data. See first paragraph, Task 5, 3rd line from the end. But, for clarity we have included a more detailed description here.

<u>Statistical analysis:</u> Nutritional status including mortality, specific growth rate, feed efficiency, hepatosomatic index, viscerosomatic index, plasma glucose, protein, and triacylglycerol concentrations, whole body RNA/DNA, moisture, protein, lipid, energy contents, and liver glycogen and lipid concentrations will be analyzed by one way analysis of variance (ANOVA) and optimum feeding rates will be determined by broken line model (Robbins, K.R., Norton, H.W. & Baker, D.H. (1979) Estimation of nutrient requirements from growth data. *J. Nutr.,* **109**, 1710–

1714). Similarly, the effect of nutritional status on physiological performance and tolerance variables will be determined using ANOVA methods. All statistical analyses of nutritional status will be conducted either with R or SAS statistical software (SAS 9.2, SAS Institute, Cary, NC).

4) One of the areas of the proposal we hope to clarify in this addendum is our linkage of laboratory data to the development of a management tool for wild sturgeon. Because working with field-acclimatized sturgeon of relevant size classes is not possible for many reasons, we propose to bridge this gap using a structural equation model. This model will bring together the data collected in Tasks 3 & 4 into a decision-support tool that can be used by resource managers. The structural equation model is essentially a multi-dimensional, multiple linear regression because it is a multivariate problem. The responses are a result of multiple effects from various stressors. Parameter estimation is done by comparing the actual covariance matrices representing the relationships between variables and the estimated covariance matrices of the best fitting model. The model will be able to predict growth and mortality based on inputs such as changes in salinity, temperature and other environmental stressors. The user will then be able to make management decisions based on the predictions from the model.

Ecological Performance Of Fishes In An Ever-Changing Estuary: The Effects Of Nutritional Status On Environmental Stress Tolerance In Sturgeon

submitted to Science Program 2010 Solicitation

compiled 2010-06-30 10:40:18 PST

Primary Investigator: Nann Fangue

Project Information and Executive Summary

Proposal Title	Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon
Primary Contact Organization Name	Davis, California University of
Primary Contact Organization Type	public institution of higher education
Salutation of Primary Contact	Dr.
First Name	Nann
Last Name	Fangue
Street Address	1088 Academic Surge
City	Davis
State or Province	CA
Mailing Code	95616
Telephone	530-752-0388
E-mail Address	nafangue@ucdavis.edu
Total Amount Requested	$446,690
Primary Topic Area	Native Fish Biology and Ecology
Secondary Topic Area(s)	Food Webs of Key Delta Species and their Relationship to Water Quality and other Drivers
Descriptive Keywords	**biological indicators**; **climate change**; **contaminants / toxicants / pollutants**: salinity; **fish biology**: sturgeon; **water quality**: salinity, temperature
Compliance statement	N/A. Our project is not subject to the above requirements.
Staff and/or subcontractors received funding for at least one project *not* listed above:	*Project Title:* Selenium effects on health and reproduction of white sturgeo *Amount Funded:* $150,047 *Date Awarded:* July 1, 2003 *Lead Organization:* *Project Number:* ERP-02-P35 *Project Title:* Biological assessment of green sturgeon in the Sacramento-Sa *Amount Funded:* $1,271,272 *Date Awarded:* October 1, 2003 *Lead Organization:* *Project Number:* ERP-02D-P57 *Project Title:* Delta smelt culture and research program *Amount Funded:* $400,000 *Date Awarded:* November 1, 2003 *Lead*

Organization:	
Project Number:	ERP-02-P31
Project Title:	Chronic toxicity of environmental contaminants in Sacramento
Amount Funded:	$924,276.20
Date Awarded:	January 1, 2000
Lead Organization:	
Project Number:	ERP-99-N07
Project Title:	Quantitativ Indicaors and Life history implications of Envir
Amount Funded:	$700,000
Date Awarded:	January 1, 2007
Lead Organization:	
Project Number:	#SP 2006-1035

Recommend Reviewers

Full Name	Organization	Telephone	E-Mail	Expertise
Dr. Colin Brauner	University of British Columbia	604-822-3372	brauner@zoology.ubc.ca	fish biology, sturgeon
Dr. Carlos Crocker	Western University of Health Sciences	(909) 623-6116	ccrocker@westernu.edu	fish biology, sturgeon
Dr. Brian Sardella			brian.sardella@gmail.com	fish biology, sturgeon
Dr. Kofi Fynn-Aikins	Chief, Lower Great Lakes Fishery Resources Office	716-691-5456	Kofi_FynnAikins@fws.gov	fish biology

Executive Summary

This proposal addresses Delta Science PSP 2010 Topic 1: Native Fish Biology and Ecology: feeding and diets on physiological tolerances of sturgeon to key environmental stressors, and Topic 2: Food web effects on key Delta species. Not only are food webs in the Delta changing, potentially affecting the food source for sturgeon, but climate change effects (e.g. temperature and salinity) stand to impact sturgeon physiological stress tolerance. We will manipulate nutritional status in green and white sturgeon and assess the effects on physiological tolerance to environmental stressors. Feed restriction will be used to manipulate the nutritional status in sturgeon larvae, fingerlings, and juveniles. Measurements include mortality, growth rate, feed efficiency, morphology, body composition, muscle RNA/DNA ratios, and liver glycogen and lipid levels. Critical swimming velocities and metabolic rates will be measured, and fish will also be challenged with increasing temperature and salinity, singly and in combination. Stress indicators such as mortality, hematocrit, cortisol, plasma ions, glucose, and tissue heat shock protein levels will be measured, and levels of key metabolites such as protein, triacylglycerol, free fatty acid, glycogen and lipid will be determined. Understanding the physiological responses of sturgeon exposed to climate-change-relevant, environmental stressors is critical in our ability to compatibly link ecosystem health with management decisions.

Contacts and Project Staff

	Primary Contact
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Organization	Davis, California University of
Work Telephone	530-752-0388
	Primary Investigator
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Last Name	Nann
First Name	Fangue
Organization	UC Davis
Work Telephone	530-752-0388
Qualifications	See Appendix for complete CV of this Participant.

	Participant #2
Salutation	Dr
Last Name	Doroshov
First Name	Serge
Title	Professor, Dept of Animal Science
Organization	UC Davis
Position	Co-PI
Responsibilities	Performance of Task 2
E-mail	sidoroshov@ucdavis.edu
Qualifications	See Appendix for complete CV of this Participant.

	Participant #3
Salutation	Dr
Last Name	Hung
First Name	Silas
Title	Professor, Animal Science
Organization	UC Davis
Position	Co-PI
Responsibilities	Task 3
E-mail	sshung@ucdavis.edu
Qualifications	See Appendix for complete CV of this Participant.

	Participant #4
Salutation	Dr
Last Name	Kebreab
First Name	Ermias
Title	Professor, Animal Science

Organization	UC Davis
Position	Co-PI
Responsibilities	Task 5
E-mail	ekebreab@ucdavis.edu
Qualifications	See Appendix for complete CV of this Participant.

Conflict of Interest

Primary Investigator Nann Fangue

To assist Science Program staff in managing potential conflicts of interest as part of the review and selection process, we requested applicants provide information on who will directly benefit if their proposal is funded, that were not listed on the Contacts and Project Staff Form.

Co-PI(s)

Subcontractor

Individuals who helped with proposal development

Last Name	First Name	Organization	Role

Task and Budget Summary

Task #	Task Title	Start Month	End Month	Personnel Involved	Description	Task Budget
1	Management of Tasks and Publication of Results	1	36	Nann Fangue	Coordinate and facilitate quarterly meetings; write and submit semi-annual reports; prepare scientific manuscripts for publication	$0
2	Procurement of Experimental Animals for Tasks 3 & 4	1	24	Dr Serge Doroshov	Breeding of white and green sturgeon for experimentation in tasks 3 and 4. Joel Van Eenennaam is a participant on this task.	$43,069
3	Feed Restriction and Nutritional status	1	36	Dr Silas Hung	Rearing of green and white sturgeon to 3 age classes on differing dietary regimes; measurements of the nutritional status will be completed on all groups; fish of known nutritional status will be passed to Task 4 for physiological stress testing. Mr. Seung-hyung Lee is the graduate student participant on this task.	$157,941
4	Physiological Tolerance to Key Environmental Stressors	1	36	Nann Fangue	Quantify the effects of temperature/salinity stress on physiological performance in sturgeon of all age classes; Mr. Robert Coalter is the graduate student participant on this task.	$173,456
5	Data Management/ Statistical Analysis/ Modeling	1	36	Dr Ermias Kebreab	Provide statistically-sound advice for experimental design; statistical analysis of physiological data; develop a Bayesian structural equation model in green and white sturgeon. Dr. Anders Strathe is the post-doctoral scholar on this task.	$72,224
					Budget total	$446,690

Schedule of Deliverables

Each Science Program 2010 Solicitation grant recipient must provide the required minimum deliverables (listed below) for each project.

Required minimum deliverables

- Semi-annual Progress Reports (due July 15 and January 15)
- Final Progress Report (Due at end of project)
- One page project summary for public audience at beginning of project
- One page project summary for public audience upon project completion
- Management implications of project findings
- Project closure summary report or copy of draft manuscript
- Presentation at Bay-Delta Science Conference
- Presentations at other events at request of Delta Science Program staff
- Copy of all published material resulting from the grant

Additional deliverables	Description	Start Month	End Month
Presentation at Society meetings	Presentations at Natiional/International Scientific Meeting	12	36

TOTAL PROJECT BUDGET SUMMARY BY TASK AND BY FISCAL YEAR

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	-	-	-	
Total Costs for Task Two	$ 11,231.00	$ 31,838.00	-	$ 43,069.00
Total Costs for Task Three	$ 65,223.00	$ 45,796.00	$ 46,922.00	$ 157,941.00
Total Costs for Task Four	$ 71,726.00	$ 50,302.00	$ 51,428.00	$ 173,456.00
Total Costs for Task Five	$ 6,311.00	$ 6,311.00	$ 59,602.00	$ 72,224.00
Total Costs for Project Tasks	$ 154,491.00	$ 134,247.00	$ 157,952.00	$ 446,690.00
2/ Other Matching Funds	$ 40,726.00	$ 40,726.00	$ 40,726.00	$ 122,178.00

BUDGET FOR TASK 1
(Administrative)

			MATCHING FUNDS		
			SALARY	BENEFITS	TOTAL
NANN FANGUE - 2%	PI	3 yrs.	$ 4,140.00	$ 1,403.00	$ 5,543.00
TOTAL COST FOR TASK ONE					

BUDGET FOR TASK 2

			MATCHING FUNDS		
			SALARY	BENEFITS	TOTAL
SERGE DOROSHOV - 5%		3 yrs.	$ 22,881.00	$ 3,660.00	$ 26,541.00

			YEAR 1				YEAR 2				YEAR 3	
PERSONNEL			SALARY PER MONTH	MONTHS ON PROJECT	TOTAL AMOUNT	BENEFITS	SALARY PER MONTH	MONTHS ON PROJECT	TOTAL AMOUNT	BENEFITS		TOTAL TASK 2 ALL YEAR
Joel Van Eenennaam	SRA*		$ 299.00	12	$ 3,588.00	$ 897.00	$ 598.00	12	$ 7,176.00	$ 1,794.00		$ 13,455.00
- Benefits Rate		0.25										
SUPPLIES					$ 4,500.00				$ 16,500.00			$ 21,000.00
TASK 2 SUB-TOTAL					$ 8,088.00	$ 897.00			$ 23,676.00	$ 1,794.00		
Overhead Percentage		0.25			$ 2,022.00	$ 224.00			$ 5,919.00	$ 449.00		$ 8,614.00
TOTAL COSTS FOR TASK TWO					$ 10,110.00	$ 1,121.00			$ 29,595.00	$ 2,243.00		**$ 43,069.00**

BUDGET FOR TASK 3

			MATCHING FUNDS		
			SALARY	BENEFITS	TOTAL
SILAS HUNG - 10%	Co-PI	3 yrs.	$ 35,940.00	$ 7,547.00	$ 43,487.00

			YEAR 1				YEAR 2				YEAR 3				
PERSONNEL			SALARY PER MONTH	MONTHS ON PROJECT	TOTAL AMOUNT	BENEFITS	SALARY PER MONTH	MONTHS ON PROJECT	TOTAL AMOUNT	BENEFITS	SALARY PER MONTH	MONTHS ON PROJECT	TOTAL AMOUNT	BENEFITS	TOTAL TASK 3 ALL YEAR
Seung Hyung Lee	GSRII**		$ 1,456.00	9	$ 13,104.00	$ 524.00	$ 1,514.00	9	$ 13,626.00	$ 545.00	$ 1,575.00	9	$ 14,175.00	$ 567.00	$ 42,541.00
-Benefits Rate		0.04													
SUPPLIES					$ 13,193.00				$ 13,193.00				$ 13,193.00		$ 39,579.00
TRAVEL					$ 1,000.00				$ 1,000.00				$ 1,000.00		$ 3,000.00
TASK 3 SUB-TOTAL					$ 27,297.00	$ 524.00			$ 27,819.00	$ 545.00			$ 28,368.00	$ 567.00	
- Overhead Percentage		0.25			$ 6,824.25	$ 131.00			$ 6,954.75	$ 136.25			$ 7,092.00	141.75	$ 21,280.00
EQUIPMENT															
- Sanyo Uthalow Freezer, MDF-73VC					$ 8,969.00										$ 8,969.00
- Fluorospectrometer					$ 11,535.00										$ 11,535.00
STUDENT FEES															
GSR Tuition Fees					$ 9,943.00				$ 10,341.00				$ 10,753.00		$ 31,037.00
TOTAL COSTS FOR TASK THREE					$ 64,568.25	$ 655.00			$ 45,114.75	$ 681.25			$ 46,213.00	$ 708.75	**$ 157,941.00**

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BUDGET FOR TASK 4

NANN FANGUE - 10% PI

				MATCHING FUNDS		
				SALARY	BENEFITS	TOTAL
NANN FANGUE - 10%	PI		3 yrs.	$ 20,700.00	$ 7,014.00	$ 27,714.00

PERSONNEL			YEAR 1 SALARY PER MONTH	YEAR 1 MONTHS ON PROJECT	YEAR 1 TOTAL AMOUNT	YEAR 1 BENEFITS	YEAR 2 SALARY PER MONTH	YEAR 2 MONTHS ON PROJECT	YEAR 2 TOTAL AMOUNT	YEAR 2 BENEFITS	YEAR 3 SALARY PER MONTH	YEAR 3 MONTHS ON PROJECT	YEAR 3 TOTAL AMOUNT	YEAR 3 BENEFITS	TOTAL TASK 4 ALL YEAR
Robert Coalter	GSR II		$ 1,456.00	9	$ 13,104.00	$ 524.00	$ 1,514.00	9	$ 13,626.00	$ 545.00	$ 1,575.00	9	$ 14,175.00	$ 567.00	$ 42,541.00
- Benefits Rate		0.04													
SUPPLIES					$ 16,798.00				$ 16,798.00				$ 16,798.00		$ 50,394.00
TRAVEL					$ 1,000.00				$ 1,000.00				$ 1,000.00		$ 3,000.00
TASK 4 SUBTOTAL					$ 30,902.00	$ 524.00			$ 31,424.00	$ 545.00			$ 31,973.00	$ 567.00	
- Overhead Percentage		0.25			$ 7,726.00	131			$ 7,856.00	$ 136.00			$ 7,993.00	$ 142.00	$ 23,984.00
EQUIPMENT															
-Swimming Respirometer					$ 22,500.00										$ 22,500.00
STUDENT FEES															
GSR Tuition Fees					$ 9,943.00				$ 10,341.00				$ 10,753.00		$ 31,037.00
TOTAL COSTS FOR TASK FOUR					$ 71,071.00	$ 655.00			$ 49,621.00	$ 681.00			$ 50,719.00	$ 709.00	**$ 173,456.00**

BUDGET FOR TASK 5

ERMIAS KEBREAB - 5%

				MATCHING FUNDS		
				SALARY	BENEFITS	TOTAL
ERMIAS KEBREAB - 5%			3 yrs.	$ 14,760.00	$ 4,133.00	$ 18,893.00

PERSONNEL			YEAR 1 SALARY PER MONTH	YEAR 1 MONTHS ON PROJECT	YEAR 1 TOTAL AMOUNT	YEAR 1 BENEFITS	YEAR 2 SALARY PER MONTH	YEAR 2 MONTHS ON PROJECT	YEAR 2 TOTAL AMOUNT	YEAR 2 BENEFITS	YEAR 3 SALARY PER MONTH	YEAR 3 MONTHS ON PROJECT	YEAR 3 TOTAL AMOUNT	YEAR 3 BENEFITS	TOTAL TASK 5 ALL YEAR
Anders Strathe	Post-Doc***		$ 311.65	12	$ 3,740.00	$ 1,309.00	$ 311.65	$ 12.00	$ 3,740.00	$ 1,309.00	$ 2,943.23	12	$ 35,319.00	$ 12,362.00	$ 57,779.00
-Benefits Rate		0.35													
INDIRECTS		0.25			$ 935.00	$ 327.00			$ 935.00	$ 327.00			$ 8,830.00	$ 3,091.00	$ 14,445.00
GRAND TOTAL					$ 4,675.00	$ 1,636.00			$ 4,675.00	$ 1,636.00			$ 44,149.00	$ 15,453.00	**$ 72,224.00**

*STAFF RESEARCH ASSOCIATE, 5% YEAR 1; 10% Yr. 2
**GRADUATE STUDENT RESEARCHER
Graduate Student Researchers receive 4% salary increase per year per compensation plan
4% increase in Graduate Student Researcher Tuition Fees per year
***Postdoctoral Scholar, 10% Year 1, 10% Year 2; 80% Year 3

TOTAL PERSONNEL	$ 135,373.00
TOTAL BENEFITS	$ 20,943.00
TOTAL SUPPLIES	$ 110,973.00
TOTAL TRAVEL	$ 6,000.00
TOTAL EQUIPMENT	$ 43,004.00
TOTAL INDIRECTS	$ 68,323.00
TOTAL STUDENT FEES	$ 62,074.00
GRAND TOTAL FOR THREE YEARS	$ 446,690.00

EQUIPMENT DETAIL

Task No	List of Equipment	Unit Cost	Task Total
3	Sanyo Uthalow Freezer, MDF-73VC	$ 8,969.00	
	Fluorospectrometer	$ 11,535.00	$ 20,504.00
4	Swimming Respirometer	$ 22,500.00	$ 22,500.00
		TOTAL	**$ 43,004.00**

Equipment purchased for a project shall be purchased by the University of California, Davis 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 (Delta Science PSP - proposal # 0090)

Task 1:
No budget is requested for this task.

Task 2:
Supplies and Expenses: Funds for fish tank recharges, feed and irrigation water recharges, recirculation incubation system supplies, and sampling and spawning supplies. Funds for tanks, water and feed recharges are mostly covered the first year by current funding.

Salary and Benefits: Five and ten percent research associate salary for years one and two, respectively, is requested for Joel Van Eenennaam who will be instrumental for sturgeon acquisition for all tasks. Funds to cover benefits at a rate of 25% of the salary are also requested.

Task 3:
Supplies and Expenses: Funds for fish tank recharges, water recharges, feed, purified diet ingredients, Nutrition Lab user fees, and laboratory supplies for histopathological, chemical, and biochemical analyses for the six feed restriction growth trials are requested.

Travel: Funds to cover lodging and mileage for participation of Prof. Hung and Mr. Lee Seung-hyung in a regional meeting each year is requested.

Salary: GSR II for 3 years is requested for Mr. Seung-hyung Lee (incoming PhD student). Mr. Lee will perform the six growth trials on feed restriction, collect the biological samples, and conduct the appropriate histopathological, chemical, and biochemical analyses.

Benefits: Funds to cover tuition/student fees for Mr. Lee are requested.

Equipment: Funds to purchase an ultralow (-80°C) freezer are requested because a large amount of samples will be collected from the six growth trials. The samples will be stored at -80°C until they are ready to be analyzed biologically and biochemically. Funds are also requested to purchase a fluorospectrometer (Nanodrop 3300) because the equipment is needed to determine RNA/DNA ratio using a very small amount of tissue samples.

Task 4:
Supplies and Expenses: Funds for publication costs and general lab supplies (plumbing supplies and fittings, water quality monitoring and control, and fish food are requested). Funds for supplies and reagents for the construction of tissue microarrays (TMA's), heat shock protein analyses, and the analyses of tissue/plasma metabolites and stress variables are also requested.

Travel: Funds to cover Lodging and mileage for participation of Prof. Fangue and Mr. Coalter in a regional meeting each year is requested.

Salary: GSR II for 3 years is requested for Mr. Robert Coalter (incoming PhD student). Mr. Coalter will perform the heat stress and salinity challenge experiments, collected the biological samples, and conduct the appropriate chemical, biochemical, and molecular analyses.
Benefits: Funds to cover tuition/ student fees for Mr. Coalter are requested.

Equipment: Funds to purchase a 30-L swimming respirometer (Loligo Systems) are requested to conduct metabolic swim trials described in Task 4 on 30 and 100g sturgeon. In the Fangue laboratory we already own a 5-L tunnel, which is the appropriate size for our smallest sturgeon.

Task 5:
Salary: Ten percent each in years 1 and 2 and 80 percent in year 3 post-doctoral fellow salary is requested for Dr Anders Strathe who will be working on design of the experiments in year 1, building up database in year 2 and development of the structural ecological equation and other statistical analysis in year 3.

Benefits: Funds to cover benefits at a rate of 35% of the salary are also requested.

Description of matching funds and cost sharing (Delta Science PSP – proposal # 0090)

Our proposal will take all the advantage of cost savings by heavily relying on existing facilities in the Center of Aquatic Biology and Aquaculture, the Department of Animal Science, and the Fish and Wildlife Conservation Biology Department, at the University of California, Davis. Considered together, these savings have reduced the cost of our proposal by more than 30% over the three-year study period.

Cost savings of $122,178 will be obtained through the University of California, Davis paying for the salaries and benefits of PI Nann Fangue (2+10% for 3 years), Co-PI Serge Doroshov (5% for 3 years), Co-PI Silas Hung (10% for 3 years), and Co-PI Ermias Kebreab (5% for 3 years).

Cost savings of over $50,000 will be obtained through the use of existing facilities for rearing and spawning green sturgeon, and conducting growth trials of green and white sturgeon at the Center of Aquatic Biology and Aquaculture. White sturgeon larvae will be provided at no cost by Sterling Caviar, a savings of $7,500.

Cost savings of over $50,000 will be obtained through the use of existing facilities and equipment at the Fish Nutrition and Toxicology Laboratory, Department of Animal Science for determining the nutritional status such as body moisture, protein, lipid, muscle RNA/DNA, liver glycogen and lipid, etc. Otherwise equipment for these analyses has to be purchased or the samples collected to be contracted out to certified commercial laboratory.

Cost savings of $110,000 will be obtained through the use of existing facilities and equipment at the Fish Conservation Physiology Laboratory, Wildlife Conservation Biology Department for the physiological performance component, which would otherwise have to be purchased and fabricated.

Cost savings of over $5,000 will be obtained through the use of existing computing hardware and software in the UCD Sustainable Agriculture Modeling Laboratory at the Department of Animal Science to conduct the modeling analysis.

Ecological Performance of Fishes in an Ever-changing Estuary: Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

1. Project Purpose -

Our proposal addresses uncertainties in the ecological performance of sturgeon in an ever-changing estuary. The San Francisco Bay Delta (SFBD) system is among the most impacted estuary systems in the world, with a multitude of well-documented abiotic and biotic changes occurring over a variety of time scales. In this proposal, we directly address Delta Science Program PSP 2010. **Topic 1: Native Fish Biology and Ecology-**The effect of **feeding and diets** on **physiological tolerances** of green and white sturgeon **to key environmental stressors** in a changing estuary. We interpret **'feeding and diets'** as the quantity and quality of the natural food of the green and white sturgeon and have chosen temperature and salinity as key **'environmental stressors'** of concern in the SFBD. Nutritional status is an important factor for determining survival and fitness in fishes and is closely associated with quantity of food supply, food quality and feeding success. Water temperature and salinity are projected to change in magnitude, timing, and space in the SFBD and watershed as a result of anthropogenic climate change (Knowles & Cayan 2002; 2004; Meehl et al. 2007; Cayan et al. 2008a; 2008b). Both diet and environmental stressors have the potential to negatively impact the **'physiological tolerance'**, performance and overall health of sturgeon populations. These data are also relevant to **Topic 2: Food web effects on Key Delta Species.** Food web dynamics have been changing in the SFBD over the past few decades, and these changes have at least partially been attributed to anthropogenically-driven climate change. While the underlying mechanisms responsible for these changes are not fully understood, it is clear that dramatic changes in productivity have the potential to negatively impact native fishes. Since ocean productivity and productivity in SFBD are closely linked (Cloern et al. 2007) and unprecedented variability in oceanic conditions have been recently detected (Brander et al. 2010), food-webs in the SFBD are likely to continue to change in the coming decades. Green and white sturgeon are semi-anadromous, spending part of their life cycle in the SFBD (Moyle 1976), and thus are likely to experience shifts in prey abundance and/or prey quality, as well as increasing temperatures and salinities associated with global climate change. To date, however, there has not been a systematic study designed to establish the relationship between nutritional status, an indicator of dietary quality and quantity, and physiological performance of green and white sturgeon when faced with key environmental stressors. In our proposed studies, we will manipulate the nutritional status of sturgeon using feed restriction and examine how these manipulations impact sturgeon physiology and resilience to environmental stressors. ***Understanding the physiological responses of sturgeon exposed to ecologically relevant, and potentially novel, environmental stressors will be critical in our ability to compatibly link ecosystem health with management decisions in the rapidly changing SFBD system***.

Three general hypotheses will be tested in this proposal:

1) Higher nutritional status is positively correlated with tolerance to thermal and salinity stressors and to these stressors in combination.

2) Sensitivity to environmental stressors is age dependent with younger stages of sturgeon showing increased sensitivity.

3) Green and white sturgeon species, with the same nutritional status, will respond differently to environmental stressors.

Foundational studies have been conducted that provide reliable methodologies for rearing green and white sturgeon, for manipulation of nutritional status (Hung and Lutes 1987; Hung et al. 1989; Hung et al. 1993; Hung et al. 1995a; Deng et al. 2003) as well as for measurement of physiological tolerance to temperature and salinity challenge of some life stages (Allen and Cech 2007; Sardella et al. 2008). In the proposed studies, we will manipulate nutritional status in a series of laboratory growth experiments by feeding green and white sturgeon larvae (50 mg wet body weight), fingerlings (30 g) and juveniles (100 g) at 25, 50, 75, and 100% of optimum ration for 1, 2 and 4 weeks, respectively. A subset of fish from each ration/size class will be sampled to determine their nutritional status by measuring mortality, growth rate, feed efficiency, condition factor, hepatosomatic index, viscerosomatic index, body composition (moisture, protein, lipid, ash, and energy), muscle RNA/DNA ratios, and liver glycogen and lipid levels. To evaluate tolerance to the environmental stressors, the remaining fish will be challenged with exercise as well as exposed to environmental thermal stress and salinity challenge, singly and in combinations. Sublethal stress will be assessed by measurements of the generalized stress response (e.g. plasma cortisol, glucose, and hematocrit), ionoregulatory changes (e.g. plasma osmolarity and ion concentrations), muscle water content, and tissue energy mobilization (e.g. glycogen and triacylglycerol concentrations) during exercise challenge. Additionally, the cellular stress response will be assessed by examining heat shock protein levels (Hsp 90, 70, 30 levels) and indices of apoptosis (liver caspase activity and levels of DNA fragmentation). Our proposal addresses the Delta Science Program's goal of advancing our understanding of the complex biology and ecology of green and white sturgeon with an eye towards the conservation and management of these ecological, economical, and recreationally important species. By quantifying and modeling the nutritional status and physiological performance of sturgeon, we provide important management tools for the determination of overall sturgeon population health.

2. Background and Conceptual Models –

Global Climate Change in the SFBD system

Global climate change has profound implications for aquatic ecosystems, and current predictions include increases in average temperature and sea level (Meehl et al. 2007). Initial predictions are that, as temperatures rise, the distribution and abundance of organisms will shift according to their thermal tolerance limits and their ability to adjust to new environmental temperatures (Fields et. al. 1993; Lubchenco et al. 1993). In fact, recent evidence from polar, temperate, and tropical ecosystems has shown that climate change has already altered the distribution and local abundance of many organisms (reviewed in Parmesan, 2006). While temperature is often the key driver of ongoing climate change (Pörtner and Knust 2007), these thermal changes co-occur with many other anthropogenically-derived changes. Anthropogenic climate change stressors particularly relevant to the SFBD include increasing water temperatures as well as increasing salinity as a result of sea level rise and seawater intrusion into the SFBD, changes in precipitation patterns, smaller snowpack contributing to a lower spring freshwater runoff (Knowles & Cayan 2002; 2004; Cayan et al. 2008), and impacts in both salinity and temperature regimes associated with SFBD water withdrawal projects. These climate change impacts may affect sturgeon through direct physiological effects, but also indirectly through impacts on the prey organisms of sturgeon quantitatively and qualitatively. In fact, recent evidence suggests that the food webs in the SFBD system are changing and that sturgeon diets can shift to reflect availability and abundance of prey items (Linville et al. 2002). The recent shift to Asian clams of their major prey species in white sturgeon no doubt will affect the qualitative and quantitative nature of their diets. Thus, to fully appreciate the impacts of climate change, consideration of multiple, emerging stressors is a significant area of emphasis for the conservation of the aquatic organisms in the SFBD and its upstream watershed.

The **goal of this proposal** is to address the question of whether early life stages of green and white sturgeon possess the physiological capacity to survive and thrive when faced with the potentially novel environmental challenges associated with anthropogenic climate change (Figure 1). Physiologists worldwide are trying rapidly to answer this question for many sensitive species across ecosystems from pole to pole, and several recent reviews have highlighted the important and necessary contributions of animal physiology in the study of global climate change (Pörtner and Farrell 2008; Widdicombe and Spicer 2008; Hofmann and Todgham 2010; Somero 2010). Contemporary organisms have 3 general options when faced with climate change stressors: 1) disperse to a more suitable habitat, 2) acclimatize to new conditions if their current suite of physiological mechanisms are sufficiently plastic, or 3) adapt to these new conditions via genetic change. Finding suitable habitat is not a viable option for many organisms, particularly those with very specialized requirements to complete their life cycles. For other organisms, particularly those with long generation times, adaptation may not be a viable option given the current, unprecedented rate of environmental climate change which is very likely to outpace the ability of many organisms to adapt (Bell and Collins 2008, Gienapp et al. 2008, Visser 2008, Somero 2010). Most contemporary organisms will thus have to rely on their current suite of physiological mechanisms to 'cope' with rapid environmental change (Hofmann and Todgham 2010). It is therefore critical that we: 1) determine which physiological mechanisms underlie the capacity of animals to tolerate these changes in environmental conditions, 2) determine if these mechanisms are currently in place for an organism to compensate for and offset any detrimental effects of climate change on organismal performance, and 3) carefully consider any important physiological tradeoffs that may result from compensation (i.e., does compensation for one environmental stressor impact an animal's ability to respond to another stressor?).

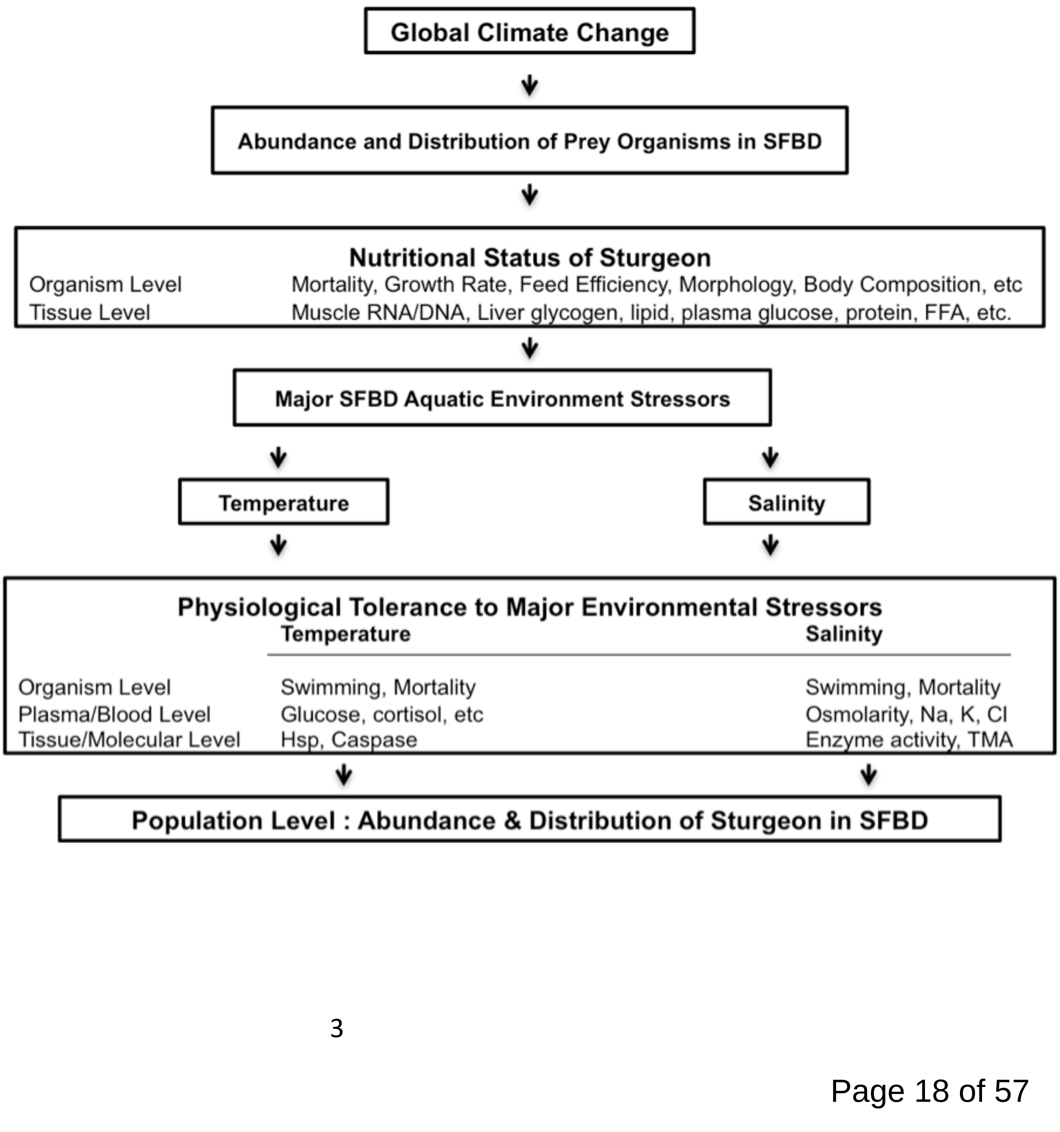

Figure 1: Conceptual Model. Global climate change will alter major environmental stressors (such as increasing water temperatures and salinities) in the SFBD. These changes may affect the abundance and distribution of food organisms for green and white sturgeon and result in differential nutrition status in these fishes. A consequence of poor nutritional status may be compromised physiological tolerance to environmental stressors, and associated changes in the distribution, survival and well being of the green and white sturgeon living the SFBD and its river systems.

Experimental Approach

To understand whether sturgeon in the SFBD system have the necessary physiological resilience to respond to climate change stressors, one important approach will be to focus on assessing physiological tolerance thresholds to environmental stressors, thus providing ranges of organismal tolerance. Only recently have clear links been established between changes seen at the ecosystem level and physiological limitations detected through well-controlled laboratory experiments (reviewed in Pörtner 2010). For example, detailed studies of the physiological performance of Fraser river salmonids have revealed that these fish are migrating in waters very near their upper thermal limits and that a subtle increase in water temperatures during migration (already detectable in this system due to climate change) is enough to prevent spawners from reaching their spawning grounds (Farrell et al. 2008). Another particularly fruitful avenue of study has been to unite whole-organism measures of physiological performance with biochemical and molecular measures of sublethal stress as a way forward to developing an understanding of physiological thresholds and tipping points. For example, it has been recently shown that exposure of a variety of aquatic species to climate-change-relevant stressors results in subtle changes is growth patterns but also dramatic changes at the biochemical level, many which were not initially predictable from whole organism studies (reviewed in Hofmann et al. 2010; O'Donnell et al. 2009; Todgham and Hofmann 2009). Not only do sublethal indicators of physiological stress reveal sensitivity of physiological pathways to climate change stressors, they have also shown us that there are species-specific patterns and thresholds of response that must be considered to fully understand the impacts of climate change.

Global climate change will likely present organisms with changes in multiple stressors simultaneously (Pörtner 2008; Hofmann and Todgham 2010). At present there is a need for climate change physiologists to move beyond single-stressor experiments to consider multiple stressors simultaneously, which are aligned with climate change predictions for the coming decades (Meehl et al. 2007). Consideration of interactive and possibly synergistic stressors is of critical importance as global change biologists move forward with forecasts of the impacts of global climate change on contemporary species. The global climate change community has recognized also the importance of studying all organismal life-stages, because identifying a potentially weak link in the developmental chain will be key to forecasting climate-change effects up to the level of populations (Hofmann et al. 2010). Recent emphasis has been placed on the investigation of early life-stages, which are often thought to be more sensitive to environmental stressors than adults (Hamdoun and Epel, 2007). This is particularly relevant in the case of sturgeon where not only are young stages relatively poorly understood, but the success of young fish as they move through the SFBD is at least partly dependent on the proper timing and pattern of the development of physiological mechanisms well matched to their migration habitats, (e.g. the development of seawater tolerance as these animals migrate out of freshwater habitats).

Sturgeon Biology, Ecology, and Population Dynamics

Green and white sturgeon are species of high conservational, recreational, and economic interest in California. Their unusual biological characteristics of longevity, late sexual maturity, and long intervals between spawning make them especially susceptible to anthropologic activities such as overfishing, destruction of spawning grounds, contamination of habitat, entrainment due to water diversions, and physical drivers of climate change.

White Sturgeon

California Fish and Game estimates the population size of white sturgeon using tag and recapture methods, creel census, and monitoring fish salvage at the state and federal pumping stations. These studies have been conducted 17 times, beginning in 1954, with the most recent tagging done in 1998, 2001, 2002, 2005, and 2008. Data from tagging studies is then used to calculate 'absolute' and 'relative' abundance, length- and age-frequency, harvest rate, and survival rate (Schaffter and Kohlhorst 1999). The current white sturgeon population is about 10% of the estimated 1998 population of 144,000.

The semi-anadromous white sturgeon (*Acipenser transmontanus*) is one of two sturgeons found along the Pacific Northwest. Although white sturgeon have been found in areas ranging from Mexico to Alaska, spawning white sturgeon have only been found in and north of the Sacramento-San Joaquin river systems (Moyle 2002). White sturgeon spawn in freshwater rivers, then spend most of their lives in the estuaries of large rivers such as the Sacramento and Columbia Rivers. Adult white sturgeon move back into the rivers during March-June to spawn, preferring cobble and boulders as substrate in cool (10-16°C), fast-flowing river sections (3-23 m depth, and 0.8 m/s velocity) (Billard and Lecointre 2001). White sturgeon have been found to reach a large size (6.1 m in length and 816 kg in weight), and require a long time to reach sexual maturity in the wild (12 y for males, 16-35 y for females) (Billard and Lecointre 2001).

The white sturgeon has a State S2 status (low abundance, restricted range and potentially endangered species) from the California Department of Fish and Game, California Natural Diversity Database (CNDDB 2009) and is considered an endangered species by the American Fisheries Society (Jelks et al. 2008). Adult (> 102 cm TL) white sturgeon abundance has varied greatly between 1967 and 1998 (Schaffter and Kohlhorst 1999). Since peaking at about 144,000 in 1998, the estimated abundance of California's legal-sized white sturgeon has declined, likely due to factors including, but not limited to, poor spawning success, variation in streamflow, passage impediments, entrainment, and legal and illegal harvest. Information developed in 2005 suggests that the abundance of legal-sized white sturgeon in now at a 50-year low and abundance is not likely to increase substantially during the next 10 years (California Fish & Game Commission 2006).

Green Sturgeon

The anadromous green sturgeon, *Acipenser medirostris*, inhabits the North American shorelines of the Pacific Ocean, from the Bering Sea to Mexico (Moyle 2002), and are considered rare or vulnerable in the United States and Canada (Birstein 1993; Campbell 1997; Musick et al. 2000). The average annual harvest has decreased from 6,494 fish in 1985-1989 to 1,072 fish in 2000-2003, with the Klamath and Columbia River Tribal fisheries accounting for 65% of the total catch (Adams et al. 2007). In 2001, green sturgeon was petitioned to be listed under the Endangered Species Act, and after the Biological Review Team's updated status report (BRT 2005), the National Marine Fisheries Service listed the southern distinct population segment (DPS) as threatened (NMFS 2006). The southern DPS encompasses the region south of the Eel River, California, including the coastline, San Francisco Bay and Sacramento River (Israel et al. 2004).

Past green sturgeon population estimates were obtained in San Pablo Bay while monitoring the size of the white sturgeon population. Due to the generally lower numbers of this species it was historically never directly targeted for population studies using tag and recapture methods. The size of the green sturgeon population is estimated indirectly by multiplying the ratio of legal size green sturgeon to legal-size white sturgeon caught in the tagging program, by the legal-size white sturgeon population estimate. This indirect method is used because only 233 green sturgeon have been tagged with no recaptures. The estimates of green sturgeon abundance ranged from 175 during 1993 to 8,421 during 2001 (Adams et. al. 2002). However, these estimates may not reflect the true population size of the species because the individuals within the San Pablo Bay may be only temporary summer residents with a larger fraction of the population still within the marine environment. In addition, these estimates assume equal vulnerability of both species to the collection gear, and the same habitat use by both species. Of all the sturgeon caught from 1954-2001 that were over 102 cm, only 1.6% were green sturgeon. However, green sturgeon made up 23.7% of the sturgeon smaller than 102 cm (data from CDFG, 2002 cited in Adams et. al. 2002). The most recent tagging study was in Suisun Bay, during August-October, 2008, when 615 white sturgeon and 14 green sturgeon were caught (DFG News

Release, 2008). Although absolute abundance is not known, it is fair to say that the numbers of green sturgeon are lower relative to those of white sturgeon in the SFBD.

The green sturgeon is a highly migratory species, which is able to enter seawater within its first or second year (Allen and Cech 2007) and to migrate long distances along the Pacific coast (Moser and Lindley 2007). In contrast to white sturgeon, green sturgeon have very large eggs and low fecundity (Van Eenennaam et al. 2006), and newly emerged larvae are demersal and are highly sensitive to light (Deng et al. 2002). The only known breeding grounds of green sturgeon are the Klamath and Sacramento Rivers in California (Moyle 2002; Van Eenennaam et al. 2006; Brown 2007) and the Rogue River in Oregon (Moyle 2002; Erickson and Webb 2007; Webb and Erickson 2007). There is also some evidence for spawning in the Umpqua River in Oregon, and the Trinity and Eel Rivers in California (Adams et al. 2007). It is believed that green sturgeon spawn in fast-flowing rivers; however, there is no published information on spawning and larval nursery habitat. Although natural reproduction of green sturgeon is likely to be compromised by altered river flows, reduced spawning habitat, and climate change (Van Eenennaam et al. 2005; Bartholow 2005), the recently established techniques for artificial breeding and culture (Van Eenennaam et al. 2001; 2008) are important tools for allowing researchers to study critical aspects of this species biology and physiology.

Nutritional Status of Sturgeon

Nutritional status of young fishes is an important factor determining their survival. Individuals in poor nutritional status are not only more vulnerable to predation and disease but also to unfavorable environmental conditions. In larvae, adequate nutritional status is critical for survival to metamorphosis - as it is a period of rapid growth and development (Watanabe and Kiron 1994). Although tolerance to unfavorable feeding conditions improves with age and size of animals, nutrient inadequacy in young fish can lead to abnormal development, smaller final weight and reduced tolerance to stress, which has been observed in white sturgeon larvae (Deng et al. 2003; 2009). Nutritional status is closely associated with food supply and quality and thus, is indicative of variation in the trophic environment (Islam and Tanaka 2005). Therefore, careful monitoring of the nutritional status of populations in their early life stages is a critical management consideration (Richard et al. 1992).

Larval fish show high mass specific metabolic rates during early exponential growth and development phases. In support of these high physiological demands, fish larvae require a more diverse suite of nutrients than older life stages (Heming and Buddington 1988). They also require high amounts of dietary proteins for tissue growth and lipids for structural components of the cell membrane. The high nutrient and metabolic demand renders fish larvae very susceptible to sub-optimal feeding. Essential nutrients such as amino acids and fatty acids must be obtained in adequate amounts as de novo synthesis in larvae has been shown to be insufficient to meet the developmental requirements (Kanazawa 1985; Dabrowski 1991). In Siberian sturgeon larvae, onset of feeding and food availability affected survival, body size and specific growth rate (Gisbert and Williot 1997). In white sturgeon larvae, sub-optimal feeding rates increased mortality and decreased growth performance, nutrient utilization, and energy retention (Deng et al. 2003). Body composition, especially moisture and lipid content, were also found to be significantly affected by feeding rate. Furthermore, RNA/DNA ratios have been shown to decrease in fish larvae during food restriction or deprivation, reflecting reduced or stunted growth (Raae et al. 1988; Deng et al. 2009). Lutes and colleagues (1990) noted that white sturgeon larvae fed diets with high protein and lipid content (53-56% and 18-25% respectively) had better survival, larger final body weights and lower body moisture content.

Aside from poor growth performance, larvae in poor nutritional status are also more vulnerable to changes in their abiotic environment than older stages. For example, striped base larvae showed increased rate of weight loss with increasing temperature (Roger and Westin 1981), and Yin and Blaxter (1987) found that temperature tolerance is reduced in marine fish larvae during starvation. The ability of individuals to withstand food restriction or starvation is strongly dependent on body size (Post and

Parkinson 2001; Bystrom et al. 2006) with survival improving in post-larval stages. However, the physiology and other constituents of the animals can still be affected. In juvenile catfish, the aerobic and biosynthetic capacity was substantially impaired by starvation (Tripathi and Verma 2003). Other biochemical and haematological changes were noted in fish during periods of fasting or feed restriction (Reviewed in Tripathi and Verma 2003). These cellular changes under sub-optimal feeding conditions suggest physiological impairment. Thus, nutritional status is an important factor of consideration in all stugeon life stages.

Effects of Environmental Stress on the Physiological Response of Sturgeon

Animals have a limited amount of energy to allocate to biological processes such as growth, reproduction, activity, and stress tolerance. We predict that if juvenile sturgeon, during the critical phases of early development, are sensitive to feed restriction, these fish may have a reduced ability to tolerate abiotic stress. In this proposal, we will measure a suite of physiological and biochemical responses to salinity and temperature in sturgeon with differing energetic potentials. There are a number of physiological and biochemical approaches to understanding the relationships between nutritional/energetic status, thermal performance, and salinity tolerance. A brief introduction to each approach is described here.

Swimming performance and energetics

Migration by anadromous fishes like sturgeon is energetically expensive, particularly when these fish are transitioning between seawater and freshwater phenotypes or when the environmental conditions encountered are adverse. Critical swimming speed (U_{crit}) is a method to determine the theoretical maximum prolonged swimming speed in fish (Brett, 1964) and is thought to be an ecologically relevant measure of a fish's ability to survive ecological challenges (reviewed in Plaut 2001). When swimming performance measurements are combined with metabolic rate (MO_2) measurements at rest, while swimming, and during recovery, several metrics including scope for activity, and oxygen cost of transport can be calculated and used to assess swimming energetics in fishes (Lee et al. 2003a; 2003b). Prolonged swimming is energetically demanding and largely supported by muscle glycogen stores (Keiffer, 2000). Measurements of biochemical fuels (i.e., glycogen/glucose, lactate, lipids) in resting and exercising fish are important to understanding patterns of metabolic fuel use. For example, low U_{crit} values have been attributed to insufficient biochemical fuel reserves in both laboratory studies (Fangue et al. 2008; McFarlane and McDonald 2002) as well as in studies on wild fish during spawning migrations (e.g., Standen et al. 2002). It has also been recently shown that migrating salmonids with poor energy reserves demonstrate substantial differences in their stress response variables indicating that physiological condition may interact negatively with these animals' ability to deal with stressors during migration (Nadeau et al. 2010).

Critical swimming speed in fish is influenced by a variety of environmental factors such as perturbations in temperature, oxygen, pH, and salinity (reviewed in Keiffer 2000) in addition to physiological condition. In particular, swimming performance is sensitive to temperature and salinity such that declines in U_{crit} values from the fish's optima may be observed as fish are challenged with abiotic stressors. Taken together, these data suggest that sturgeon exposed to suboptimal feeding and diets in the SFBD may show a reduction in swimming performance, and this reduction may be further exacerbated by temperature and salinity stress. Because swimming performance is considered a main characteristic determining survival in many species of fish, and maximal swimming performance may strongly influence fitness (Jones et al. 1974; Rome et al. 1992; Young and Cech 1993; Swanson 1998), reduced U_{crit} may have significant ecological consequences for sturgeon. In juvenile, anadromous sturgeon, there have been several studies of swimming performance (McKenzie et al. 2001; Mayfield and Cech 2004; Peake 2004), but each of these studies has investigated sizes/ages that are significantly more mature than those proposed as the focus of these experiments, (although see Allen et al. 2006). In addition, only a single study of swimming in green sturgeon manipulated food consumption, and these experiments were conducted on one-year-old individuals (Mayfield and Cech 2004). Finally, Allen et al. (2006) showed an ontogenetic shift in green sturgeon swimming performance such that their U_{crit} values declined when these fish were transitioning to a seawater-ready phenotype (~100-150 dph). These data highlight the tradeoffs that can result when energy resources are allocated to a particular process (e.g., a physiological transition from hyposmotic to hyperosmotic environments) leading to declines in another energetically costly process (e.g., swimming performance).

Quantifying Thermal Performance in Sturgeon

Temperature has been coined as the 'ecological master factor' for fishes (Brett, 1971). Fish often show species-specific temperature optima in a variety of important physiological functions at or near common habitat temperatures. Fishes show adaptive patterns in thermal tolerance limits such that thermal tolerance thresholds are correlated with habitat temperature. There is also a positive correlation between thermal tolerance limits and thermal acclimation/acclimatization temperatures. Measurements of thermal tolerance in fishes can be achieved using Critical thermal methodology (CTM). Simply stated, a CTMax is determined for fish of known thermal history by gradually increasing the experimental water temperature by 0.3°C/min until a predetermined, non-lethal endpoint is reached (Beitinger et al. 2000; Fangue and Bennett, 2003). Our information on CTMax in green sturgeon is limited to a single study (Sardella et al. 2008) where nutritional status was not considered.

One of the most well-studied biochemical mechanisms underlying an organism's ability to tolerate thermal stress is the role played by heat shock proteins (Hsp). The importance of Hsps hinges upon two major functions, both designed to maintain the integrity of the cellular protein pool: minimizing non-native protein aggregations and targeting these aggregations for either stabilization and repair or degradation and removal from the cell (Lindquist 1986; Hightower 1991; Morimoto 1998; Feder and Hofmann 1999). Hsps are members of a group of highly conserved cellular proteins synthesized in response to a variety of stressors (Senders 1993). Hsp are encoded by multiple genes that are assigned to families based on sequence similarity and molecular mass: Hsp90 (85-90 kDa), Hsp70 (68-73 kDa) and low molecular weight Hsps (16-47 kDa) (Gething 1997; Basu et al. 2002). Hsp's differ in thermal inducibility, intracellular location, and function both within and between gene families (Gething 1997). In unstressed cells, Hsps are expressed constitutively, assisting in the folding, assembly, and translocation of newly synthesized proteins, and the degradation of misfolded proteins (Gething and Sambrook 1992; Hartl 1996; Hartl and Hayer-Hartl 2002).

Heat stress is known to compromise protein function at physiologically relevant temperatures, and a wealth of literature has shown a correlation between an increase in Hsp levels with increasing thermal stress (reviewed in Feder and Hofmann 1999). The functional significance of Hsps has been established by direct evidence showing that Hsp expression is an essential component of inducible thermal tolerance in a variety of organisms (Feder and Krebs 1998; Sanchez and Linquist 1990; Parsell and Lindquist 1993) and has been shown to enhance the survival of fish exposed to stress (Iwama et al. 1998; Basu et al. 2002). The induction of Hsps has been shown to occur in response to many stressors in addition to temperature, including pathogens (Ackerman and Iwama 2001), heavy metals (Heikkila et al. 1982; Duffy et al. 1999), hypoxia (Airaksinen et al. 1998; Sørensen et al. 2003), osmotic shock (Smith et al. 1999), parasitism (Merino et al. 1998; Rinehart et al. 2002) and the presence of predators (Pauwels et al. 2005). In addition, the nutritional status of fishes has also been shown to influence a fish's ability to express Hsps. For example, a previous study by Cara et al. (2005) reported that a 7-day period of feed deprivation induced elevated Hsp70 protein levels in rainbow trout larvae, and Yengkokpam et al. (2008) showed that in the Indian carp, the length of starvation was positively correlated with tissue Hsp70 levels. In larval white sturgeon (230 mg wet body weight) placed on feed restriction for 2 weeks and exposed to a 4-hour thermal challenge of 26°C, the heat shock response differed such that Hsp60 and Hsp70 protein levels were depressed in the feed restricted group (Deng et al. 2009). While investigations linking nutritional status and Hsp expression are limited to a handful of studies, the findings to date indicate that the nutritional status of fish may have a significant effect on their heat shock response and may therefore influence their ability to respond to thermal stress in their environment.

Quantifying Salinity Stress in Sturgeon

Osmoregulatory ability that is well-matched with environmental salinity variation is a key life-history factor in anadromous fish species like sturgeon. Salinity fluctuations pose osmotic challenges to these organisms and can be particularly challenging to early life-history stages both in terms of the physiological readiness in developing osmoregulatory organs (i.e., gills) as well as the energy demanded by the physiological mechanisms involved in osmoregulation. Therefore, it is critical to evaluate these thresholds in the context of a changing energetic and salinity landscape in the SFBD.

White sturgeon are known to spawn in the Sacramento River as adults and move downstream into the San Francisco Bay-Delta as juveniles (Billard and Lecointre 2001, Doroshov 1985). Although the precise age or size of white sturgeon juveniles during seaward migration is unknown, McEnroe and Cech (1985) demonstrated that juvenile (18-56 g wet body weight) white sturgeon were able to tolerate abrupt transfer into 15 $g.L^{-1}$ brackish water, while Amiri et al. (2009) showed that 30 g sturgeon suffered 30% mortality rates upon transfer to 15 $g.L^{-1}$. These results suggests that sturgeon are capable of moving into brackish water as juveniles weighing <100 g, but that early developmental stages of sturgeon are differentially sensitive to salinity increases. Salinity tolerance in green sturgeon differs from that of white sturgeon with 30 g or larger green sturgeon juveniles being able to tolerate fresh and brackish waters of 10 $g.L^{-1}$, but unable to tolerate full-strength seawater until reaching approximately 140 g (Allen and Cech, 2007).

Sturgeon survival may be impacted during unexpected salinity shifts by the direct toxicity of elevated Na^+ and Cl^- concentrations on the neuromuscular system (Brauner et al. 1992). Survival may also be compromised through indirect effects of the inability to maintain ionic homeostasis. Such indirect effects include the depletion of body energy resources, changes in foraging activity, and increased susceptibility to predation. Salinity tolerance can be assessed by monitoring mortality during seawater acclimation. In addition, maintenance of osmotic/iono- homeostasis during seawater challenge can be monitored by measuring sub-lethal indices such as plasma ion concentrations, plasma osmolarity, hematology, muscle water content, Na^+/K^+ ATPase enzyme activity, and blood stress indices such as cortisol, glucose, and lactate concentrations.

3. Approach and Scope of Work

Task 1: Management of Tasks and Publication of Results (Project Leader: Fangue)

Each of the proposed tasks are interdependent and therefore coordination of project management decisions will be achieved through close coordination among the principal and co-principal investigators (see flowchart, Figure 2). The project leader will ensure that all tasks are highly integrated and will coordinate manpower among different laboratories to run each task most efficiently. The project leader will hold quarterly meetings, and write and submit semi-annual progress reports. Scientific manuscripts resulting from this project will be prepared as soon

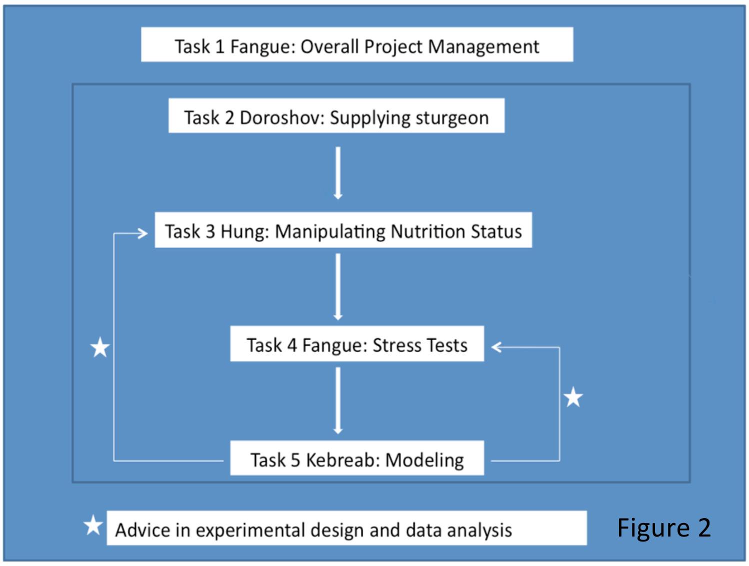

Figure 2

as sufficient data are gathered. An annual meeting with all personnel involved will be held at the end of each year, and participants will present an overview of achievements accomplished during the current year and propose a detailed work plan for the subsequent year.

Task 2: Procurement of Experimental Animals for Tasks 3 & 4 (Leader: Doroshov)

Sturgeon for laboratory experiments will be obtained by induced spawning of captive white and green sturgeon stocks, at sturgeon farms and the UC Davis Center for Aquatic Biology and Aquaculture (CABA), respectively. The laboratory in charge has a long history 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 viability assessment of sturgeon gametes and early life stages will ensure the use of high-quality progenies obtained from known parents.

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 an *in vitro* egg maturation assay. Ovulation and spermiation are induced by injecting a mammalian gonadotropin releasing hormone (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 white sturgeon will be obtained from Sterling Caviar LLC 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). Hatchery spawning and distribution of white sturgeon will be coordinated by Joel Van Eenennaam.

At CABA, we currently rear two year-classes (born in 1999 and 2000) of green sturgeon which originated from the northern distinct population segment (DPS) Klamath River brood fish. The Klamath River fish are from the 1999 year spawn (n=9, averaging 31 kg wet body weight) and the 2000 year spawn (n=16, averaging 24 kg). These stocks are maintained in two, 7 m diameter outdoor tanks. Fish are individually tagged with a passive integrated transponder (PIT tag) and are sampled annually (Oct-Nov) *in vivo* to determine histological stage of gonad development and changes in length and weight.

Techniques for spawning wild-caught green sturgeon (Van Eenennaam et al. 2001; 2008) have been used on these captive-reared year classes, beginning in 2007, and several males have been successfully induced to produce viable sperm each spring. Thirteen females (four in 2007, two in 2008, three in 2009, and four in 2010) have reached final maturity, and eight were successfully induced to ovulate, resulting in a 2007, 2009 and 2010 year-class progenies available for research. We anticipate that 2-4 females each year will be potential candidates for spawning induction.

Task 3: Feed Restriction and Nutritional status (Leader, Hung)

We propose to conduct six feed restriction experiments. In the feed restriction experiments, we will rear three different starting sizes (50 mg, 30 g, and 100 g wet body weight) of both green and white sturgeon, and feed them at 25, 50, 75, and 100% of optimum feeding rates at 18°C ± 0.5°C. We select these sizes because they are the critical stages influencing the long-term survival and well being of the sturgeon in the SFBD and its upstream watershed: initiation of external feeding (50 mg 12 days post hatch, DPH) and two stages (30 g, 3-6 months old; and 100 g, about 1 year old) that encompass the ages where these fish are entering brackish water as they move through the SFBD system to sea. Remaining sturgeon after the feed restriction experiments will be passed to leader of Task 4 (Fangue) for physiological stress testing. The feed restriction and nutritional status experiments will be conducted in the Fish Nutrition and Toxicology Laboratory at UC Davis.

Part I: Sturgeon larvae (50 mg, 12 DPH) feed restriction and nutrition status trials

Two, 1-week growth trials will be conducted to manipulate nutritional status of green and white sturgeon larvae by four different feeding rates (25, 50, 75, and 100% of optimum feeding rates). The four different feeding rates will be 7, 14, 21, 28% body weight per day ($BW.d^{-1}$) because the optimum feeding rate of 50 mg size white sturgeon (12 days post hatch, DPH) was determined in our lab (Deng et al. 2003) to be around 28% $BW.d^{-1}$ at 20°C based on a commercial salmonid starter feed. Twelve replicate tanks with 300 sturgeon larvae per tank will be randomly assigned to one of the four feeding rates with three replicate tanks per feeding rate. Details of the feed restriction experiments and nutritional status including mortality, specific growth rate (SGR), and feed efficiency (FE) will be determined according to Deng et al. (2003). Three groups of 140 larvae will be euthanized with an overdose of tricaine methanesulfonate (MS-222, 500 $mg.L^{-1}$, Argent Chemical Laboratories, Redmount, WA) to obtain enough samples for subsequent analyses. Larvae will be frozen in liquid nitrogen and kept at -80°C for later determinations of whole body RNA/DNA ratios by an ethidium bromide fluorometric technique (Wagner et al. 1998), and proximate composition and energy content by the AOAC method (Jones, 1984) and bomb calorimetry methods, respectively. Three groups of five larvae each will be euthanized with MS-222, their body weight and length measured to determined condition factor (CF), and the whole larvae will be preserved in neutral buffered formaldehyde and their liver glycogen and lipid will be examined histologically after Periodic Acid Schiff and Oil Red O staining.

Part II: Sturgeon fingerling (30 g, 3-6 months old) feed restriction and nutrition status experiments

Two 2-week growth experiments will be conducted to manipulate nutritional status of green and white sturgeon by different feed restriction rates (25, 50, 75, and 100% of optimum feeding rates). Twelve replicate tanks with 100 sturgeon fingerlings per tank will be randomly assigned to one of four feeding rates (0.5, 1.0, 1.5, and 2.0% $BW.d^{-1}$) because the optimum feeding rate of 30 g size white sturgeon was determined to be 2% $BW.d^{-1}$ previously in our laboratory (Hung and Lutes 1987). The sturgeon will be fed these rates using our sturgeon purified diet because the optimum feeding rate was determined based on the same diet (Hung and Lutes 1987). Details of the feed restriction trials and nutritional status including mortality, SGR, and FE will be determined similar to those described by Hung and Lutes (1987). At the end of the growth experiments, five fingerlings from each tank will be euthanized with an overdose of MS-222 and their weight and length measured to determine their CF, their liver and viscera dissected and weighed to determine their hepatosomatic index (HSI) and viscerosomatic index (VSI), respectively. These fingerlings will be pooled and kept frozen at -20°C for later determinations of moisture, protein, and lipid by the AOAC method (Jones 1984) and their energy content determined by bomb calorimetry. Another three fingerlings from each tank will be anesthetized with MS-222 solution and their blood sampled individually from caudal vein with a 22 gauge needle (Monoject, Division of Sherwood Medical, St. Louis, MO) and a vacutainer (Becton Dickinson, Franklin, NJ). The blood samples collected from individual fingerlings will be centrifuged at 4,500 *g* for 5 minutes at room temperature to obtain plasma which will be separated into several aliquots, frozen in liquid nitrogen, and kept at -80°C for later determination of glucose, protein, and triacylglycerol concentrations. Plasma glucose, protein, and triacylglycerol concentrations will be determined by the colorimetric end point methods using a Glucose Assay kit, Micro Lowry Total Protein Kit, and Serum Triglyceride Determination Kit (Sigma, Saint Louis, MO), respectively. After the blood sample, the fingerlings will be euthanized by cervical dislocation, their liver and muscle dissected, pooled, and kept frozen for later determination of RNA/DNA ratios as described earlier. Liver glycogen and lipid will be determined according Murat and Serfaty (1974) and Folch et al. (1957) respectively.

Part III: Sturgeon juvenile (100 g, 9-12 month old) feed restriction and nutrition status trials

Two 4-week growth trials will be conducted to manipulate nutritional status of green and white sturgeon juveniles by different feeding rates (25, 50, 75, and 100% of optimum feeding rates). The optimum feeding rate of 100 g white sturgeon was determined to be 1% $BW.d^{-1}$ based on our sturgeon purified diet in a previous growth trial in our laboratory (Hung, unpublished data). Therefore, 12 tanks of sturgeon juvenile with 25 fish per tank will be randomly assigned to one of four feeding rates (0.25, 0.50, 0.75, and 1.00% BW.d^{-1}). Details of the feed restriction trials and tissue samples after the trials will be similar to those described by Hung and Lutes (1997) except only two juveniles from each tank will be used because they are large enough to be sampled individually, obviating sample pooling. Nutritional status including mortality, SGR, FE, CF, HIS, VSI, whole-body moisture, protein, lipid, and energy content, and plasma glucose, protein, and triacylglycerol concentrations will be determined as described earlier.

Task 4: Physiological Tolerance to Key Environmental Stressors (Leader: Fangue)

The goal of Task 4 is to understand how the differential nutritional status of sturgeon affects their ability to maintain critical physiological performance and tolerance levels when challenged with climate change relevant stressors. Following each feed restriction experiment described in Task 3, the remaining green and white sturgeon from each age class will be subjected to swimming performance assessments and to thermal and/or salinity challenges to assess their physiological responses. A suite of physiological measurements will be made integrating across biological levels of organization from the whole organism to sublethal indices of physiological stress at tissue and cellular levels. These experiments will be conducted in the Fish Conservation Physiology Laboratory at UC Davis.

Part I: Sturgeon larvae (19 DPH) stress tests

Swimming performance methodology: The effects of nutritional status on the resting (RMR) and active metabolic rates (AMR) of sturgeon will be determined using oxygen consumption measurements (Cech 1990) and Blazka-type or Brett-type swimming respirometers. A minimum of 12 fish per species and feeding treatment will be assessed. Following all experiments, fish will be weighed (wet weight ± 0.1 g), and measured (total length ± 0.1 cm). The scope for activity (SFA) will be calculated as SFA=AMR-RMR, (Heath 1990). To initiate a U_{crit} determination, fish will be placed individually into a swimming respirometer (5 L total volume, Loligo Systems, Denmark) and acclimated at a low swimming velocity (3 cm·sec^{-1}) for 1 hour to allow for orientation to the current direction. Water velocity will then be increased in a stepwise fashion in 10 cm·sec^{-1} increments every 20 minutes. Fatigue is established when the fish impinges against the back screen 3 consecutive times after being reintroduced to the current. U_{crit} is calculated using the following formula from Brett (1964):

$$U_{crit} = U_i + \left(\frac{t_i}{t_{ii}} \times U_{ii} \right)$$

where U_i is the highest speed fish swim for the full time period (cm·sec^{-1}), U_{ii} is the incremental speed increase (cm·sec^{-1}), t_i is the time the fish swims at the final speed (minutes), and t_{ii} is the prescribed period of swimming per speed (20 minutes). If the cross sectional area of the fish is >10% of the area of the swimming chamber, the calibrated water velocities will be corrected for solid blocking effects according to the calculations described by Bell and Terhune (1970).

Thermal challenge: The critical thermal maximum (CTMax) of green and white sturgeon will be determined as an index of whole-organism thermal tolerance (n=3 fish per rearing tank for a total of 18 individuals assessed per feeding treatment). CTMax trials will be performed as described in Fangue et al. (2006), using loss of righting response as an experimental endpoint. Briefly, water temperature will be increased at a rate of 0.3ºC per minute and the temperature at which fish were unable to right themselves within 3 seconds of being gently flipped with a rounded probe will be recorded as their individual CTMax. Immediately following loss of righting response, fish will be removed from the test chamber, weighed and measured, and returned to tanks at their acclimation temperature for recovery.

In order to assess the sublethal effects of heat stress, sturgeon larvae will be exposed to an experimental heat shock treatment. As a pre-heat shock control, fish will be sampled directly from the holding tanks. Because sturgeon larvae are so small, 5 individuals will be pooled per sample for an overall sample size of n=6 (derived from 30 individuals) per feeding treatment. The remaining fish will be exposed to increasing temperatures at a rate of 0.3ºC per minute until the heat shock temperature of 26ºC is reached. Following the 4-hour heat shock, fish will be returned to their acclimation temperature (18ºC) and sampled after recovery periods of 0, 1, 2, and 4-hour recovery at 18ºC. A subset of fish that were not heat shocked will be transferred to a recovery tank for 4 hours and sampled (n=6) as a handled control. This time course of thermal exposure and recovery agrees with those previously established by Deng et al. (2009) for sturgeon showing detectable elevations in Hsp protein levels. Fish will then be sacrificed using a lethal dose of MS-222 and liver, white muscle, and gill tissue will be dissected and immediately placed into liquid nitrogen. Tissue samples will be stored at -80°C until analysis.

Liver, gill, and muscle levels of Hsp 90, 70, 60, and 30 will be determined by SDS - PAGE and Western blotting as described by Deng et al. (2009) for sturgeon. The same supernatant prepared for Hsp level determinations will be used to determine caspase 3/7 activity (an index of apoptosis) using the Apo-ONE Homogeneous Caspase-3/7 Assay kit (Promega Corporation, Madison, WI). Fluorescence activity with an excitation wavelength of 485 nm and an emission wavelength of 530 nm will be read by a Spectra Max M2 micro plate reader (Molecular Devices Corporation, Sunnyvale, CA). Caspase activity will be expressed as fluorescence/µg protein. Liver TUNEL assay will be performed using laser scanning-cytometry after DNA strand break labeled by the APO-BRDU kit (Phoenix Flow Systems, San Diego, CA).

Salinity challenge: It is very unlikely that sturgeon larvae of this size would be exposed to brackish or sea water in their rearing habitats, thus no salinity challenge will be conducted with this age class.

Part II: Sturgeon fingerling (3.5-6.5 months old) stress tests

Swimming performance methodology: Resting (RMR) and active (AMR) metabolic rates as well as U_{crit} determinations will be made as described previously except that a larger swimming tunnel (30 L) will be used. In addition, sturgeon fingerlings fed 4 different feeding treatments will be given a standardized exercise challenge. This methodology will be used to determine the patterns of metabolic fuel use and energetic reserves supporting exercise metabolism. Determining patterns of metabolic fuel use is particularly relevant for older stages of sturgeon as these fish are preparing for migration. Sturgeon will be introduced to the swimming chamber for a 1-hour acclimation, and swimming speed will be incrementally increased over the first 30 min until a speed representing 80% U_{crit} is reached (determined for each feeding treatment and fish length above). Fish will then swim at this speed for an additional 1.5 h. Control fish will be kept in a swim tunnel with circulating, aerated water for 3 hours at a velocity of 3 $cm \cdot sec^{-1}$. At the end of the swimming period, a lethal dose of ethyl p-amino-benzoate (benzocaine, Sigma-Aldrich, Oakville, ON, Canada; stock solution made by dissolving 62.5 g of benzocaine in 500 mL ethanol) will be introduced to the tunnel. Fish will then be quickly sampled for blood plasma, white muscle, and liver. Tissues will be immediately frozen with aluminum blocks pre-cooled in liquid nitrogen, and sampling time will be within 1 minute of benzocaine addition. Metabolites of interest include ATP, phosphocreatine (PCr), protein, glycogen, glucose, total lipid, and intramuscular triacylglycerol (IMTG). The analytical techniques to determine concentrations of key metabolites have been described in detail in Fangue et al. 2008.

Thermal challenge: The CTMax of sturgeon fingerlings will be determined as previously described, except the fingerlings will be large enough to obtain sufficient blood samples for measurement of stress indicators as individuals and therefore pooling of fish will not be required. The experimental heat shock treatment will also follow previous methods. Hematocrit will be determined in fresh blood using an Adams Micro-Hematocrit II centrifuge (Becton Dickinson, Sparks, MD). Plasma glucose, protein, and TAG concentrations will be determined as described earlier. Plasma cortisol will be determined according to the method by Duncombe (1964) and using a radio-immunoassay kit (Baxter Travenol Diagnostics, Dada, MA). After the blood sample, the fingerlings will be euthanized by cervical dislocation, and their mucus, white muscle, gill, heart, spleen, and part of the liver will be dissected, frozen in liquid nitrogen, kept at -80°C for future determinations of Hsp 90, 70, 60, 30 levels, caspase 3/7 activity, and for TUNEL assays as previously described.

Salinity challenge: Salinity tolerance will be assessed using methodologies described by Tashjian et al. (2007). Briefly, 20 sturgeon from each feeding treatment will be acutely transferred from freshwater to 1 of 5 salinity challenges (8, 16, 20, 24, and 32 $g.L^{-1}$) or to 0 $g.L^{-1}$ as a handling control. Seawater will be made by mixing aerated well water with Instant Ocean artificial sea salts (Aquarium Systems, Mentor, OH), and the salinity will be measured with a refractometer. Exposures will be conducted in 675 L fiberglass tanks with recirculating filtration systems. Temperature will be held constant at 18°C ± 0.5°C. Fingerlings will not be fed during the salinity challenge and ammonia levels will be monitored to ensure values are below 0.5 $mg.L^{-1}$. Mortality will be recorded at 3, 6, 12, 24, 72, and 120 h following acute salinity transfer. High mortality rates are expected at the two highest salinity treatments within the first 24 h (Amiri et al. 2009) while the 8 and 16 $g.L^{-1}$ exposures should be non-lethal (Tashjian et al. 2007).

To assess the sublethal physiological response to salinity challenge, 36 sturgeon will be acutely exposed to 0 (control), 4, 8, or 16 $g.L^{-1}$ for 120 h and 6 individuals from each group will be sampled at 24 h intervals. This exposure protocol was chosen to encompass the range of salinities sturgeon fingerlings would encounter while migrating to the SFBD and has been used in juvenile white sturgeon previously to investigate salinity tolerance and recovery of osmoregulatory balance (Amiri et al. 2009). However, should we find significant mortality in any of our groups at 8 or 16 $g.L^{-1}$ (tolerance assessment above) we will adjust our protocol based on these findings. Several physiological salinity stress indicators will be measured including plasma hematocrit (packed red-cell volume), lactate, and glucose concentrations using standard methods (Wedemeyer et al. 1990). Plasma osmolarity will be determined using a 5100B vapor- pressure osmometer (Wescor, Logan, UT), plasma Na^+, K^+ ion concentration will be measured using a IL 343 flame photometer (Instrumentation Laboratory, Lexington, MA) and plasma Cl^- concentration will be determined using a CMT-10 chloride titrator (Radiometer, Cleveland, OH). To determine muscle hydration, a piece of dorsal epaxial muscle will be weighed before and after drying to determine percent water. The right 4 gill arches will be sampled and frozen at -80°C for later determination of Na^+, K^+-ATPase (NKA), v-type H^+ATPase (VHA), and caspase 3/7 enzyme activities. The left 4 gill arches and a small piece of liver and kidney tissue will be fixed in 10% phosphate-buffered formalin for tissue microarray (TMA) construction (Lima and Kultz 2004) following established protocols for sturgeon (Sardella and Kultz 2009). Using TMA's, the abundance of NKA, VHA, Na^+ K^+ $2Cl^-$ cotransporter, Hsp 60, 70, and 90, and ubiquitin will be quantified as described by Sardella and Kultz (2009).

Temperature & Salinity challenge test:

It is clear that variation in environmental temperatures as well as environmental salinities can have widespread effects on the physiology of fishes. It has also been shown in teleost fishes that the effects of combined stressors are often greater than when either stress is imposed singly (Gonzalez and McDonald, 2000; Sardella et al, 2004). In sturgeon, we have very little information about how salinity and temperature stress may interact to compromise physiological function. Recent work, however, has shown a subtle but significant interaction between salinity acclimation and thermal tolerance limits in 60 g green sturgeon (Sardella et al. 2008) suggesting that to fully appreciate the ecological and management implications of changing salinities and climate warming on SFBD species, we will need to pursue the investigation of multiple and potentially synergistic stressors further.

As a first step towards investigating simultaneous increases in temperature and salinity, we will simulate an extreme exposure for sturgeon migrating through the SFBD. Measurements of metabolic rates and swimming performance will be made following an overnight acclimation to an increase in temperature (18 to 26°C) and an increase in salinity (zero to 30 $g.L^{-1}$), to simulate a tidal cycle in the SFBD system. Fish will be gradually exposed to increasing experimental temperature and salinity over 6 hours and held for an additional 8-hour acclimation period before measurements will be made. This methodology has been used previously for stress testing in sturgeon exposed to contaminant stress (Tashjian et al. 2007), adding to the comparative value of standardizing these methodologies. Tissue samples will be obtained and stress indicators measured as described previously.

Part III: Sturgeon juvenile (10-13 months old) stress tests

Assessments of active and swimming metabolic rates, U_{crit}, standardized exercise challenge, thermal and salinity tolerance, sublethal exposures to heat shock and salinity transfer, and multiple stressor testing will be conducted as described in Task 4, Part II. These data will allow us to compare potential sensitivity differences between age classes of sturgeon.

Task 5: Data Management/ Statistical Analysis/ Modeling (Leader, Kebreab)

The experimental design is flexible in the sense that only factors of sturgeon species (green and white) and lifestage (30 mg, 30 g, 100 g fish) are fixed prior to the initiation of the experiment. Hence, when results from the first treatment batch (i.e. green sturgeon of 30 mg fed at 25, 50, 75 and 100% of optimal feeding) are obtained, then it is possible to reformulate the feeding regimen for improving the data resolution for the modeling part (see organizational chart). If this is necessary then feeding strategy is altered by doubling the number of feeding level (e.g., 30, 40, 50, 60, 70, 80, 90 and 100% of optimal feeding), which will improve the resolution of the responses (growth rate, feed conversion and mortality). We consider a unified strategy for designing and analyzing dose-response studies as outlined by Pinheiro et al. (2006), including the testing of our feeding regime and the selection of one or more doses to take into further development. The methodology combines the advantages of multiple comparisons and modeling approaches, consisting of a multi-stage procedure (Pinheiro et al. 2006). However, acknowledging that the dose response relationship may be different at later stages of growth and difference may exist between the species of sturgeon. Hence, the flexibility in experimental setup combined with statistical method outlined above should insure that optimal data are obtained for developing the structural equation model as outlined below. All response variables collected from the feed restriction and stress test will be subjected to analysis of variance as part of screening the final data. The statistical model includes the main effect of feed restriction, stress tolerance, stage of growth, and sturgeon species and the corresponding interaction terms (Snedecor and Cochran, 1956). The statistical analysis will be undertaken in the statistical package SAS (SAS Institute, Cary, NC) utilizing the GLM procedure.

In order to combine all the information generated in the current proposal, a general framework is proposed. The framework is based on a structural equation model. Structural equation modeling is a multivariate statistical method that allows evaluation of a network of relationships between manifest and latent variables (See the path diagram below, Figure 3). In this statistical technique, pre-conceptualizations that reflect the research questions and existing knowledge of system structure create the framework for model development, while both direct and indirect effects and measurement errors are considered (Lee 2007). Our study introduces a Bayesian structural equation modeling methodology that has the flexibility to (a) translate fairly complicated physiological phenomena (growth and development) and express them as functions of several conceptual environmental factors (salinity and temperature) and the nutritional status (body composition and blood variables); (b) link the conceptual factors of interest with observed variables (growth rate, feed efficiency, mortality) by explicitly acknowledging that none of those perfectly reflect the underlying property (model uncertainty); and (c) test both direct and indirect paths of this physiological structure and identify the importance of their role (Austin 2007).

In this proposal, a Bayesian approach to structural equation model will be adopted that has several advantages over the classical methods (e.g., maximum likelihood, generalized and weighted least squares). For example, Bayesian structural ecological equation model has the ability to incorporate prior knowledge about the parameters and more effectively treat unidentified models (Lee 2007). In addition, the assumptions used to determine the latent variable metrics can be treated stochastically and can provide additional insight into the physiological structures. The modeling process does not rely on asymptotic theory, a feature that is particularly important when the sample size is small and the classical estimation methods are not robust (Lee 2007). Markov Chain Monte Carlo samples are taken from the posterior distribution, and as a result the procedure works for all sample sizes. The Bayesian nature of the framework provides more realistic estimates of the existing knowledge/predictive uncertainty by taking into account both the uncertainty about the parameters and the uncertainty that remains when the parameters are known.

The structural equation model can be used to identify, assess and quantify risk factors, explaining mortality rates in green and white sturgeon because the sturgeon responses collected in task 3 and 4 are used to parameterize the structural equation model. This model will provide a set of tools for natural resource managers to assess management strategies in the context of global climate change by predicting future population trends.

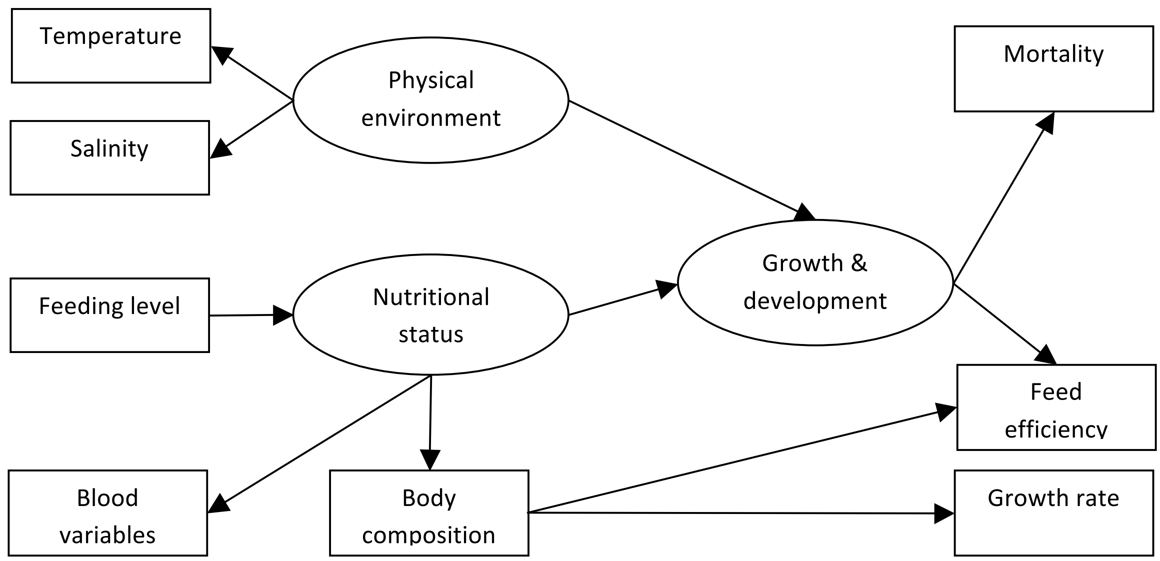

Figure 3: Path diagram of the proposed model for modeling growth and development of sturgeon. Rectangular and oval boxes represent observed (with error) and latent (unobserved) variables, respectively.

4. Feasibility –

Our proposal focuses on assessing physiological tolerances in both green and white sturgeon faced with a rapidly changing environment under differential nutritional status. These species will likely respond differentially to stressors in the SFBD system therefore effective management will rely on our understanding of each species, independently. Sturgeon were chosen because they are appropriate for the ecosystem management and restoration goals of the Delta Science Program to protect at-risk native species and those species of importance for food and recreation. White sturgeon juveniles are reliably available yearly in May-June from local fish farmers (see letter of support from Sterling caviar) making research on this species highly feasible. The opportunity to work with green sturgeon is unique and made possible through the long standing breeding program established in Dr. Doroshov's laboratory. Green sturgeon are usually available every March-April. Should this proposal be funded as scheduled in December 2010, we will have two spawning seasons to obtain green sturgeon juveniles. In the unlikely event that green sturgeon do not successfully spawn in Spring 2011, we will focus on whites in the first year.

This proposal builds on previously conducted feed restriction and physiological stress studies on white and green sturgeon in Prof. Hung's laboratory as well as in the Fish Conservation Physiology lab (formerly Prof. Joseph Cech's lab, but now the laboratory of Prof. Nann Fangue) establishing successful methodologies and equipment for the proposed experiments. The integrative approaches proposed in these studies have been well-established by us or our colleagues with their utility in sturgeon research demonstrated in the peer-reviewed scientific literature (Mayfield and Cech 2004; Allen and Cech 2007; Sardella et al. 2008; Fangue et al. 2006; 2008; 2009). Because all biological systems are noisy, appropriate quantitative tools are necessary for handling and quantifying biological variability. Recent studies on growth in other species have shown that models, which specifically account for multiple sources of biological variability are preferable (Porter et al. 2010, Strathe et al. 2010b). Furthermore, similar growth modeling possibilities have also been applied to fish data (Dumas et al. 2010). Structural equations models have become increasingly popular for describing effects of nutrition, genotypes and gender on the utilization of metabolizable energy for protein and lipid deposition in farm animals. Azevedo et al. (2005) presented an application of the methodology for quantifying energy utilization in fish. The modeling framework has recently been updated, estimating population variability in utilization of dietary energy (Strathe et al. 2010a). The new statistical framework has shown to be superior to the previous framework(s) by estimating population variability in the structural parameters, describing maintenance requirements and the efficiency of utilizing metabolizable energy above maintenance for protein and lipid deposition. Hence, the proposed modeling work fits well into the already established track record of the UC Davis Sustainable Agriculture Modeling Laboratory. All hardware and software necessary to conduct the modeling analysis is already in place.

5. Relevance to the Delta Science Program

The **relevance of this proposal** to the Delta Science Program's 2010 Proposal Solicitation Package is three fold. First, we have chosen to study white and green sturgeon, both of which are species of interest in the SFBD system with green sturgeon listed as threatened. Secondly, our study is integrative and considers the impact of multiple environmental variables, potentially sensitive early life-stages which we know very little about ecologically, and includes studies at multiple levels of biological organization from molecules up to the modeling of population level responses. Finally, this proposal is cross-cutting addressing two of the Delta Science Program's priority research topics: <u>Topic 1:</u> Native Fish Biology and <u>Topic 2:</u> Food-Web Interactions with Native species.

The SFBD system is one of the most anthropogenically impacted aquatic systems in the world with many species in decline. It is our view that making accurate predictions about the effects of global climate change on the physiological performance of sturgeon in the SFBD is linked to understanding the interactions between multiple, relevant environmental variables and their physiological effects. Our results will have broad appeal to SFBD managers, but also to animal physiologists, climate change biologists, and certainly conservation biologists. The application of physiological tools in the conservation of many threatened fishes is undergoing resurgence worldwide (Cooke et al. 2008; Hofmann and Gaines 2008; Roessig et al. 2004) and nowhere is this more important than for studies of declining California native fishes (Lund et al. 2010; Moyle et al. 2008). The results of this work will be presented at Regional and International meetings and published in aquatic conservation and physiological journals providing a mechanism to share Delta Science research with a broad range of scientific audiences.

6. Qualifications

Serge Doroshov, Ph.D. is a professor in the Department of Animal Science at UC Davis. Doroshov is a reproductive physiologist and an expert in reproduction, embryogenesis and vitellogenesis of finfishes, particularly in sturgeon. His current research focus is the study of green sturgeon maturation and spawning in captivity and the development of techniques to accurately stage female sturgeon for spawning, during their extended final maturation stage. Joel Van Eenennaam has been a research associate in the Doroshov lab for over 25 years and has extensive experience in the spawning of sturgeon and the development of husbandry techniques. He will be responsible for providing sturgeon progenies (Task 2) for the proposed tasks of this project.

Silas S.O. Hung, Ph.D. is a professor in the Department of Animal Science at UC Davis. Hung is a fish nutritionist and toxicologist and an expert in nutrition of finfishes, particularly, of sturgeon. His current research focuses on the use of restricted feeding to manipulate the nutritional status and to study the effects of restricted feeding on cellular, biochemical, and biological markers such as heat shock proteins, muscle RNA/DNA, liver glycogen and lipid, plasma protein, lipid, and glucose, whole body protein, lipid, and energy, and specific growth rate, feed efficiency, and mortality. Hung has worked on nutrition and feeding of fish for more than 30 year with the last 27 years concentrated on white sturgeon. These include determining the nutrient requirements and utilization as well as optimum feeding rates of white sturgeon. In the last 10 years, Hung has also conducted toxicology experiments on splittail and green and white sturgeon funded by the CalFed Science Program. Mr. Seung-hyung Lee is an incoming Ph.D. student in the Hung lab and has a M.S. degree in fish nutrition. Mr. Lee has published 13 scientific papers in different areas of fish nutrition and he has the appropriate skill to conduct nutrition experiments with fishes and analyze samples collected in Task 3.

Nann A. Fangue, Ph.D. is an assistant professor in the Wildlife, Fish, and Conservation Biology Department at UC Davis. Fangue is an environmental physiologist and an expert is stress physiology of aquatic organisms, particularly fishes. Her current research focus is the use physiological approaches in a conservation context to address questions of whether aquatic organisms have sufficient physiological capacity or plasticity to maintain successful performance in the face of anthropogenic environmental perturbations such as climate change. Research in the Fangue lab utilizes molecular, cellular, and whole-organism measures of organismal performance interpreted in an ecological context to elucidate the connections between environment, physiology, and ecosystem function. Fangue has expertise in the use of swimming performance, and salinity/thermal tolerance challenges to evaluate physiological performance in fishes and has published in all of these areas. Robert Coalter is an incoming Ph.D. student in the Fangue lab and has the appropriate skill set to design and conduct experimental manipulations with fishes as well as to analyze samples collected in Task 4.

Ermias Kebreab, Ph.D. is a professor of sustainable agriculture in the Animal Science Department at UC Davis. Kebreab is an integrative biologist and has a long experience in developing nutrition-based mathematical models in farm animals. His current research focus is development and application of mathematical models for prediction of greenhouse gas emissions from animals and excreta. The models are used to investigate the effect of climate change on various farming systems. Research in the Kebreab lab is mostly quantitative and aims to describe biological systems in mathematical terms to determine nutrient utilization and requirement in several species of animals. Kebreab has extensively published in all of these areas. Anders Strathe, Ph.D. is a post-doctoral fellow in the Kebreab lab and has the appropriate skill set to analyze experimental results and develop the ecological framework described in Task 5.

7. Literature Cited

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Visser, M.E., 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. Proceedings of the Royal Society B, 275: 649-59.

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Watanabe, T., and Kiron, V. 1994. Prospects in larval fish dietetics. Aquaculture, 124:223-251.

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Yin, M.C., and Blaxter, J.H.S. 1987. Temperature, salinity tolerance, and buoyancy during early development and starvation of Clyde and north Sea herring, cod, and flounder larvae. J. Exp. Mar. Biol. Ecol. 107:279-290.

Young, P.S. and Cech, J.J. Jr., 1993. Improved growth, swimming performance, and muscular development in exercise-conditioned young-of-the-year striped bass (*Morone saxatillis*). Canadian Journal of Fisheries and Aquatic Sciences, 50:703-707.

UNIVERSITY OF CALIFORNIA, DAVIS

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June 29, 2010

Delta Science Program
Research Proposal Evaluation Panel

Dear Research Proposal Evaluation Panel members:

I enthusiastically support the research proposal "Ecological Performance of Fishes in an Ever-changing Estuary: Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon," submitted in response to your PSP 2010 by Professors Doroshov, Hung, Fangue, and Kebreab! I have read this proposal, and its strengths include its focus on two native fishes in the Bay-Delta ecosystem, diversity of experimental approaches addressing key physiological and food-web-related ecological mechanisms, testing of explicitly stated hypotheses on early life-history stages, and integration of measurements into appropriate models to assist natural-resource managers. The proposed research project's experimental manipulation of nutritional and fish-condition status (via graded, quantitative ration restrictions) simulates food-web disruptions in Bay-Delta ecosystems (e.g., from global-climate-change-related effects and associated survival advantages bequeathed to competing invasive species, environmental hypoxia or pollution-related mortalities of prey organisms, or low productivity from nutrient entrapment/diversion via established benthic organisms or water diversions). These manipulations comprise a new approach that addresses the results of relatively rapid food-web changes in the Bay-Delta ecosystem that have been observed in the recent past or can be anticipated in the near future, especially with likely global-climate shifts and possible changes in water-circulation patterns.

The proposed project's measurements of stress and well-being will identify specific functions in the green and white sturgeon affected by the graded ration-restriction levels. The degrees of stress regarding these functions will guide managers' assessments of sturgeon population vulnerabilities associated with measured or anticipated changes in environmental temperature or salinity. Furthermore, the managers' resulting corrective actions (e.g., aeration of released reservoir-sourced water of a suitable temperature to minimize hypoxic zones) will be designed to support specific (stressed) functions in these important fishes. The great diversity of measurements, from organismal to molecular, strengthens one's confidence in their accurately assessing the ration/environmental effects on these sturgeons. Finally, I would expect that many, useful findings will result from this collaboration and that many students will be trained in research areas critical to California's Bay-Delta ecosystem.

Sincerely,

Joseph J. Cech, Jr.
Professor Emeritus

May 14, 2010

The proposed study, "Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon" by Doroshov, Van Eenennaam, Hung, Fangue and Kebreab is an important study. This is both in terms of resource management, given the challenges many sensitive fish species such as sturgeon will experience with global warming, but the applications are directly applicable to sturgeon aquaculture worldwide. Sturgeon populations worldwide are under increased poaching pressure on top of already damaging environmental stresses which will only increase. A good understanding of environmental stressors on both wild and captive populations is important.

Sterling Caviar, due to it's close proximity to UC Davis is willing to donate 10,000 white sturgeon fry as an in kind match to this study. With a value of $0.75 each, this is a $7,500 match to this study.

I would urge all reviewers to strongly support this study.

Sincerely,

Peter Struffenegger

Peter Struffenegger
Manager

A subsidiary of Stolt Sea Farm Inc. 9149 East Levee Road, Elverta, CA 95626
Telephone: (916) 991-4420 Facsimile: (916) 991-4334

2010 PSP SIGNATURE PAGE

The applicant for this proposal must submit the signature form by printing it, having it signed, scanning the signed form, and uploading the scanned document by using the "upload" button on the signature page form on our website. If you do not have access to a scanner, you may submit your signed form via FAX to (916) 445 - 7311. Please send only one form per FAX transmission.

Failure to sign and submit this form, by the submission deadline, will result in the application not being considered for funding. The Primary Contact for this proposal will receive e-mail confirmation as soon as this signature page has been processed.

By signing below, I declare that:

- All representations in this proposal are truthful;
- I am authorized to submit the application on behalf of applicant (if applicant is an entity or organization);
- I have read and understand the conflict of interest section in the main body of the PSP and waive any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent provided in this PSP, and
- I have read and understood all attachments of this PSP.

Proposal Title:	Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon
Lead Investigator:	Nann Fangue
Organization:	Davis, California University of
Proposal #:	2010.01-0090

Signatory for the applicant organization: **Suzanne Iwatate**
Contracts and Grants Analyst

(Please print the name of the signatory)

Signature

6/23/10

Date

Nann A. Fangue, Ph.D.

Address
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Education
B.S. University of West Florida, Pensacola, 1999 (Marine Biology)
M.S. University of West Florida, Pensacola, 2001 (Biology)
Ph.D. University of British Columbia, Vancouver, 2007 (Zoology)

Positions
Assistant Professor, University of California, Davis, September 2009-present
NSF Postdoctoral Research Fellow, University of California, Santa Barbara, 2007-2009
Adjunct Instructor of Biology, University of West Florida, Pensacola, 2002
Research Consultant, Benedict Engineering Company, Pensacola, 2001

Publications

Fangue, N.A., M.J. O'Donnell, M.A. Sewell, P.G. Matson, A.C. MacPherson. and G.E. Hofmann. A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnology and Oceanography Methods, *In Press*

O'Donnell, M.J., A.E. Todgham, M.A. Sewell, L.M. Hammond, K. Ruggiero, **N.A. Fangue**, M.L. Zippay and G.E. Hofmann. 2010. Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. Marine Ecology Progress Series. 398: 157-171.

Fangue, N.A., J.E. Podrabsky, L.I. Crawshaw and P.M. Schulte. 2009. Countergradient Variation in Temperature Preference in Populations of Killifish *Fundulus heteroclitus*. Physiological and Biochemical Zoology. 82(6): 776-786.

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Rummer, J.L., **N.A. Fangue**, H.L. Wallman, B.N. Tiffany, K.J. Fitchett, S. Galleher, A. Kirkpatrick, C.M. Pomory, and W.A. Bennett. 2009. Effects of Physiological Tolerance to Hyperthermia and Hypoxia on Species Richness and Distribution of Rockpool Fishes of Loggerhead Key, Dry Tortugas National Park. Journal of Experimental Marine Biology and Ecology. 371: 155-162.

Sloman, K.A., M. Mandic, A.E. Todgham, **N.A. Fangue**, P. Subrt, and J.G. Richards. 2008. The Response of the Tidepool Sculpin, Oligocottus maculosus, to Hypoxia in Laboratory, Mesocosm and Field Environments. Comparative Biochemistry and Physiology Part A. 149: 284-292.

Fangue, N.A., M. Hofmeister, and P.M. Schulte. 2006. Intraspecific Variation in Thermal Tolerance and Heat Shock Protein Gene Expression in Common Killifish, *Fundulus heteroclitus*. The Journal of Experimental Biology. 209: 2859-2872.

Tiffany, B.N., **N.A. Fangue,** and W.A. Bennett. Destruction of a Pygmy Octopus Population Following a Harmful Algal Bloom in a Northwest Florida Bay. American Malacological Bulletin. 21(1-2): 11-15.

Fangue, N.A., and W.A. Bennett. Thermal Tolerance Responses of Laboratory-acclimated and Seasonally-acclimatized Atlantic Stingray, *Dasyatis sabina.* Copeia. 2003(2): 315-325.

Fangue, N.A., K.E. Flaherty, J.L. Rummer, G. Cole, K.S. Hansen, R. Hinote, B.L. Noel, H. Wallman, and W.A. Bennett. 2001. Temperature and Hypoxia Tolerance of Selected Fishes from a Hyperthermal Rockpool in the Dry Tortugas, with Notes on Diversity and Behavior. Caribbean Journal of Science. 37(1-2): 81-87.

Synergistic activities:

1. Co-Instructor for the Ocean and Carbon Biogeochemisty workshop on Ocean Acidification, Woods Hole, Massachusetts, 2009.
2. Carbon Biogeochemistry Workshop Participant, Bergen, Norway, 2009.
3. Co-Instructor for the Three Seas Program, 2008 & 2009, Northeastern University & USC Wrigley Institute.
4. Invited speaker to the 'Girls in Ocean Science Teen Conference', 2008, 2009
5. Participant in the NSF New Investigators Workshop, 2006.
6. Participant in the NSF Antarctic Biology Course, 2005 (PI: Dr. Donal Manahan, University of Southern California).

Research Training:

Undergraduate honors thesis advisees: 2007 Timothy Healy and Edward Joude, **2006** Talia Gurwitz, **2005** Jensen Wong, **2004** Julia Wierzkowski and Kevin Eade, **2003** Milica Mandic

M.S. advisees: **2008** Ivy Marr

Ph.D. advisees: 2010 Emily Martinez (in progress)

Recent Collaborators:

Joseph J. Cech, Jr. (UC Davis), Frank Loge (UC Davis), Eugenio de J. Carpizo Ituarte (U Autónoma de Baja California), Kevin T. Fielman (Auburn University), Michael O'Donnell (UC, Santa Barbara), Mary A. Sewell (University of Auckland), Lars Tomanek (California Polytechnic State Univeristy), Jason Podrabsky and Larry Crawshaw (Portland State University), Katherine Sloman (University of Plymouth, UK), Jeffrey G. Richards (U of British Columbia)

Graduate Advisor: Dr. Patricia M. Schulte, Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4

Postdoctoral Advisor: Dr. Gretchen E. Hofmann, Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106

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

POSITIONS
Fish Biologist, Institute of Freshwater Reservoirs, Academy of Sciences, Borok, 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, Rome: 1975 - 1977; Visiting Professor, School of Fisheries, University of Washington, Seattle: 1977; Associate Professor, Department of Animal Science, University of California, Davis: 1978 - 1983; Director, Aquaculture and Fisheries Program, University of California, Davis: 1995 – 1998; Professor, Department of Animal Science, University of California, Davis: 1984 - present.

SELECTED PUBLICATIONS
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.

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 (Acipenser transmontanus). *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.

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Gisbert, E., J.J, Cech Jr., and S.I. Doroshov. 2001. Routine metabolism of larval green sturgeon (*Acipenser medirostris Ayres*). *Fish Physiology and Biochemistry* 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, Acipenser transmontanus. *Aquaculture* 201: 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.

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*). *Aquatic Living Resources* 16:77-89.

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., 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.

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Werner, I., J. Linares-Casenave, J.P. Van Eenennaam, and S.I. Doroshov. 2007. The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (*Acipenser medirostris*). Environmental Biology of Fishes 79: 195-200.

Gessner, J., J.P. Van Eenennaam, and S.I. Doroshov. 2007. North American green and European Atlantic sturgeon: comparisons of life histories and human impacts. Environmental Biology of Fishes 79: 397-411.

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Fontana, F., L. Congiu, V.A. Mudrak, J.M. Quattro, T.I.J. Smith, K. Ware, and S.I. Doroshov. 2008. Evidence of hexaploid karyotype in shortnose sturgeon, Genome 51: 113-119.

Allen, P.J., J.A. Hobbs, J.J. Cech, Jr., J.P. Van Eenennaam, and S.I. Doroshov. 2009. Using trace elements in pectoral fin rays to assess life history movements in sturgeons: estimating age at initial seawater entry in Klamath River green sturgeon. Transactions of the American Fisheries Society 138: 240-250.

Palumbo, A.J., M.S. Denison, S.I. Doroshov, and R.S. Tjeerdema. 2009. Reduction of vitellogenin synthesis by an aryl hydrocarbon receptor agonist in the white sturgeon (Acipenser transmontanus). Environmental Toxicology and Chemistry 28: 1749-1755.

Lu, X., M. Webb, M. Talbott, J. Van Eenennaam, A. Palumbo, J. Linares-Casenave, S. Doroshov, P. Struffenegger, and B. Rasco. 2010. Distinguishing ovarian maturity of farmed white sturgeon (Acipenser transmontanus) by fourier transform infrared spectrodcopy: a potential tool for caviar production management. Journal of Agricultural and Food Chemistry 58: 4056-4064.

Salutation: Prof.
Last Name: Hung
First Name: Silas
Title: Professor
Organization: UC Davis − Department of Animal Science
Position: Co−PI
Responsibilities: tasks 4

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

Appointments
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

Active Membership in Professional Society
Comparative Nutrition Society, 2004-present
Society of Environmental Toxicology and Chemistry, 2004-present
World Aquaculture Society, 2004-present
World Sturgeon Conservation Society, 2004-present

Adjunct/ Visiting 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.

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

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

Selected Publications in the Last Five Years

Teh, S.J., Deng, D.F., Werner, I., Teh, F.C., and 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.

Deng, D.F., Hemre, G-I., Storebakken, T., Shiau, S.Y., and 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. and 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. 25:2618-2622.

Tashjian, D.H., Teh, S.J., Sogomonyan, A., and Hung, S.S.O. 2006. Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquatic Toxicology. 79:401-409.

Tashjian, D., Cech, J.J.Jr., and Hung, S.S.O. 2007. The influence of dietary L-Selenomethionine exposure on the survival and osmoregulatory capacity of white sturgeon in fresh and brackish water. Fish Physiology and Biochemistry 33:109-119.

Deng, D.F., Hung, S.S.O., and Teh, S.J. 2007. Selenium depuration: Residual effects of dietary selenium on Sacramento splittail (*Pogonichthys Macrolepidotus*). Science of the Total Environment. 377:224-232.

Ricca, M.A., Miles, A.K., Anthony, R.G., Deng, X., and Hung, S.S.O. 2007. Effect of lipid extraction on analyses of stable carbon and nitrogen isotopes in a coastal marine food web. Canadian Journal of Zoology 85:40-48.

Greenfield, B.K., Teh, S.J., Ross, J.R.M., Hunt , J., Zhang, G.H., Davis, J.A., Ichikawa, G., Crane, D., Hung, S.S.O., Deng, D.F., Teh, F.C., and Green, P.G. 2008. Contaminant concentrations and histopathology of Sacramento splittail (*Pogonichthys macrolepidotus*) from the Sacramento-San Joaquin River Delta, California. Archives of Environmental Contamination and Toxicology 55:270-281.

Deng, D.F., Wang, C.F., Lee, S.H., Bai, S.C. and Hung, S.S.O. 2009. Feeding rates affect heat shock protein levels in liver of larval white sturgeon (*Acipenser transmontanus*). Aquaculture 287:223-226.

Bakke, A.M., Tashjian, D.H., Wang, C.F., Lee, S.H., Bai, S.C. and Hung, S.S.O. 2010. Competition between selenomethionine and methionine absorption in the intestinal tract of green sturgeon (*Acipenser medirostris*). Aquatic Toxicology 96:62-69.

Rigby, M.C., Deng, X., Grieb, T.M., Teh, S.J. and Hung, S.S.O. 2010. Effect Threshold for Selenium Toxicity in Juvenile Splittail, *Pogonichthys macrolepidotus* A. Bulletin of Environmental Contamination and Toxicology. 84:76-79.

Lee, S.H., Lee, J.H., Bai, S.C. and Hung, S.S.O. 2010. Evaluation of the dietary toxic level of selenium (Se) in juvenile olive flounder, *Paralichthys olivaceus*. Journal of the World Aquaculture Society 41:245-254.

CURRICULUM VITAE
Ermias Kebreab

EDUCATION

1998	Ph.D, Integrated Biology, University of Reading (United Kingdom)
1991	M.Sc., Integrated Biology, University of Reading (United Kingdom)
1987	B.Sc. University of Asmara (Eritrea) Biology/Math

POSITION AND EMPLOYMENT HISTORY

2009 - present	*Full Professor, Sesnon Endowed Chair in Sustainable Agriculture* Department of Animal Science, University of California, Davis, CA.
2007-2009	*Associate Professor, Canada Research Chair in Modeling Sustainable Agriculture Systems* Department of Animal Science, University of Manitoba, Winnipeg, MB.
2003- 2006	*Adjunct Professor* Department of Animal and Poultry Science, University of Guelph, Guelph, ON.
1998- 2003	*Post Doctoral Research Fellow* School of Agriculture, University of Reading, UK.
1992-1994	*Department Head* Department of Plant and Animal Sciences, University of Asmara, Eritrea.
1987-1989	*Lecturer* Department of Arid Zone Agriculture, University of Asmara, Eritrea.

AWARDS

2009	Merit award in Research, University of Manitoba, Canada
2008	Early Career Achievement Award, American Society of Animal Science
2006	Young Scientist Award, Canadian Society of Animal Science
2005	Senior Research Fellowship, Wageningen University, The Netherlands

PUBLICATIONS (last 4 years)

1. Ellis, J.L., A. Bannink, J. France, **E.Kebreab** and J.Dijkstra. 2010. Prediction of enteric methane production by dairy cows in whole farm models. Global Change Biology (in press).
2. Huang, Q., O. Wohlgemut, N. Cicek, J. France and **E. Kebreab**. 2010. A mechanistic model for predicting methane emissions from unstirred anaerobic manure storage systems. Canadian Journal of Animal Science (in press).
3. Dijkstra, J., S. Lopez, A. Bannink, M.S. Dhanoa, **E. Kebreab**, N. E. Odongo, M.H. Fathi Nasri, U.K. Behera, D. Hernandez-Ferrer and J.France. 2010. Evaluation of a mechanistic lactation model using cow, goat and sheep data. Journal of Agricultural Science (in press).
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Proposal # 90

Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

Delta Science Program
2010 PSP Final Review Panel Meeting
January 19-20, 2011

2010 Final Review Panel – Summary of Review

Proposal # 90

Proposal Title: Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

Lead Primary Investigator: Nann Fangue

Applicant Organization: University of California, Davis

Amount Requested: $446,690

Panel Findings:

Relevance to Topic Areas: The study is relevant to enhancing our understanding of Native Fish Biology and Ecology and to the wider goal of basic research to foster stewardship of the SFBD region.

Quality of the Proposed Research: Final Panel Reviewers rated the overall quality of proposal as Sufficient, Above Average and Above Average.

Main Summary Comments of Reviewers: The proposal is based on a clear conceptual model and is hypothesis driven. It will contribute importantly to our understanding of two key species and the scope they have for responding to future stresses related to climate change and food web modifications in the SFBD; however, Panel members questioned whether or not the research would produce information useful for the restoration or conservation of the two species. While the laboratory methods outlined in the proposal are sound and will produce publications and a better understanding of sturgeon physiology, the immediate benefits to management seem less well defined. The information the project would produce, in terms of lab responses and condition estimates, would be difficult to relate to the field. The overall approach could be improved to provide direct management benefits if it were to directly involve an assessment of the general condition of wild sturgeon within the system to better justify the need for refinements to the existing studies. Measures such as condition indices or bioimpedance analysis could be used to assess the general condition and nutritional status of wild fishes without killing the fish. This could be used as a basis for judging the relevance of the lab studies.

Funding Category: Above Average

External Technical Reviews

Proposal 0090: Review 1

Proposal Title: Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

Proposal Number: 0090

Proposal Applicant: Davis, California University of

The reviewer has made no 'accept comment' about whether or why (s)he will Review this Proposal.

Project

comments	One of the real strengths of this proposal is that it is very much hypothesis driven and the broad and specific goals and hypotheses are stated. In the broad sense, the goal of the proposal is to address whether early life stages of green and white sturgeon possess the physiological capacity to survive and thrive when faced with the potentially novel environmental challenges associated with anthropogenic climate change. The specific hypotheses tested in this proposal are 1) Higher nutritional status is positively correlated with tolerance to thermal and salinity stressors and to these stressors in combination. 2) Sensitivity to environmental stressors is age dependent with younger stages of sturgeon showing increased sensitivity. 3) Green and white sturgeon species, with the same nutritional status, will respond differently to environmental stressors. The authors have done a thorough literature review highlighting what is known about sturgeon stress physiology, especially with regard to salinity and temperature change, and it is clear there are large knowledge gaps which desperately need filling before accurate predictions can be made regarding the effect of projected changes in salinity and temperature on these two very sensitive species. While a tremendous amount of knowledge is ultimately required, this project makes a very important first step and in this reviewers' opinion, the proposed approach will provide answers to some of the most pressing questions and the full scale project is justified. There is no doubt that research arising from the proposed project will generate a great deal of novel information which will equip environmental managers with the tools needed to make predictions about the impact of climate change on these two very sensitive species, white and green sturgeon.
rating	Superior

Background

comments	The proposal is very well written and organized and a conceptual model of the proposed approach is very clear and consistent with the information required to meet the goals of the study. The background information provided is thorough and exhaustive and clearly indicates the large knowledge gaps that exist in our understanding how projected changes in the Bay-Delta region may

	affect white and green sturgeon, two species that are currently very susceptible. The choice of sub-lethal physiological indicators to assess the performance of sturgeon exposed to different environmental and nutritional treatments is a novel approach that will be very informative and is justified in this proposal.
rating	Superior

Approach

comments	The researchers have clearly put in great thought to this aspect of the proposal. As described above the proposed approach is well designed to meet the specific objectives that have been outlined in the proposal. Figure 2 simply and effectively demonstrates the relative responsibilities of each PI and how they will be intricately associated. It is indicated in the proposal that the PI will hold quarterly meetings, and write and submit semi-annual progress reports which will be important in integrating findings and updating and that scientific manuscripts resulting from this project will be prepared as soon as sufficient data are gathered. An annual meeting with all personnel involved will be held at the end of each year, and participants will present an overview of achievements accomplished during the current year and propose a detailed work plan for the subsequent year. This level of communication will be integral to optimizing findings from the proposed research and disseminating this information rapidly to environmental managers.
rating	Superior

Feasibility

comments	The described approach is well documented as indicated above, and consistent with the proposed objectives. Given the experience and track records of the PI and Co-PI's (see further elaboration on this point below), I feel that the project is technically feasible with a very high likelihood of success.
rating	Superior

Relevance To The Delta Science Program

comments	This proposal is directly relevant to Delta Science Program's 2010 Proposal Solicitation Package in that it addresses two of the programs priority research topics, namely Topic 1: Native Fish Biology and Topic 2: Food-Web Interactions with Native species. The proposal focuses upon two species of interest to the San Francisco Bay-Delta system, white sturgeon and green sturgeon, the latter of which is listed as threatened. This proposed study addresses the potential of multiple, simultaneously combined stressors relevant to the SFBD system;

	temperature and salinity. Furthermore, studies to assess the effect of these environmental variables on these species is done at multiple life history stages, to increase the chances of finding the time at which these species may be most sensitive, and integrates responses from the molecular to the whole animal level. Thus, this study is highly integrative in many ways. Based upon my understanding, the results of this proposal will be very useful to Delta resource managers and policy makers.
rating	Superior

Qualifications

comments	Although the lead PI (N. Fangue) is relatively junior, she has an impressive track record at this stage of her career and has the support of more established researchers as Co-PI's whom have a great deal of experience with what used to be CalFed. Given the quality of this research proposal, there is every reason to believe that she has the abilities to oversee and run this research proposal and disseminate this information quickly and to the best journals in the field (Task 1). The procurement of experimental animals (Task 2) for use in tasks 3 & 4 is lead by S. Doroshov who has a great deal of experience in sturgeon husbandry and both white and green sturgeon will be provided for these experiments from the captive white and green sturgeon stocks, at sturgeon farms and the UC Davis Center for Aquatic Biology and Aquaculture (CABA), respectively. S. Doroshov is a leader in the field of sturgeon culture, experimentation and conservation. Generation of sturgeon on different feed rations to generate fish of differing nutritional status (Task 3) which will be central for experiments will be lead by S. Hung, a renowned expert in the field, again with a very strong track record in this area and with a great potential to rear fish on the proposed diets. N. Fangue is charged with overseeing Task 4: Physiological Tolerance to Key Environmental Stressors which is her specific field of expertise. She has a great deal of experience with conducting the physiological challenges (swim performance trials and thermal tolerance studies in particular for which she already has an international reputation) and is experienced with most of the proposed sampling and analytical methodology. While she does not have lot of experience with salinity tolerance. Finally, Task 5, Data Management/ Statistical Analysis/ Modeling, will be conducted by E. Kebreab who also has a strong publication record in the proposed statistical and modeling approaches to be used to analyze the data. In short, the intellectual and technical expertise, along with the required infrastructure appear to be in place to ensure completion of a very interesting study.
rating	Superior

Overall Evaluation Summary Rating

comments	This is a very well written and interesting proposal to address two topics in the Delta Science PSP 2010 Topic 1: Native Fish Biology and Ecology, and Topic 2: Food web effects on key Delta species: where nutritional status will be assessed on physiological tolerance of white and green sturgeon to key environmental stressors relevant to the SFBD. The proposal is hypothesis driven and clearly outlines data gaps that need to be filled in order to predict the effect of projected changes in the abiotic and biotic environment in the years to come. The manipulation of the nutritional status of fish simulates food-web disruptions in the Bay-Delta ecosystems, which combined with measurements of sub-lethal disturbances at different developmental stages following exposure to relevant changes in temperature and salinity should be invaluable in making predictions on populations of green and white sturgeon in the Bay-Delta system. The PI and Co-PI's are each experts in tasks to which they have been assigned and the infrastructure at UC Davis is in place to conduct the proposed experiments. The time-line and proposed costs are reasonable and based upon the past record of the PI's and Co-PI's the information will be disseminated rapidly and appropriately.
rating	Superior

Proposal 0090: Review 2

Proposal Title: Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon

Proposal Number: 0090

Proposal Applicant: Davis, California University of

The reviewer has made no 'accept comment' about whether or why (s)he will Review this Proposal.

Project

comments	This study has ambitious goals: to estimate the effects of changing nutritional factors on the ability of two sturgeon species to withstand environmental stresses. The concept is a tad circular in that already experienced and also expected environmental changes will not only stress these species in general, but will also stress and change their food sources, while they're nutritional status is being used as a metric to examine their ability to withstand stress. This is not fatal, only complicated and it bears keeping in mind when analyzing results. Another way of looking at it is that nutrition and environmental stresses are both moving targets, resulting in endless possible permutations.
rating	Above Average

Background

comments	Yes, the conceptual model is made clear in the very useful Figure 1, which hierarchically integrates a whole lot of diverse information. Review of climate change effects and sturgeon species life histories are a bit cursory but adequate. However, the strength of this section was with the team's expertise--the descriptions of the roles of nutritional status, environmental effects on physiological responses, and the quantification of nutritional and thermal responses.
rating	Above Average

Approach

comments	Tasks are very well described, with clear lines of authority and responsibility. The project hangs around three reasonable hypotheses, none of which are highly detailed or provocative but which all serve to provide a framework to this highly complex study plan. The quarterly meetings seem essential to keep all these elements moving forward in concert. The graded series of life stages is a plus, given the likelihood of different vulnerabilities at those stages.

	The critical thermal tolerance and salinity tolerance and interaction experiments both seem considerably more extreme than what would be experienced in nature (at least in their speed of onset), which may provide a clear response spectrum but don't seem realistic to me, especially for mobile creatures that can adjust distributionally. It would have been be good if the investigators had discussed the relationship between these challenges and conditions in the real world. Inclusion of the apparently cutting edge structural equation model has the potential to distill what will appear to be a great deal of noise down to to some sort of signals. I found mention of the possible importance of a new bivalve (Asian clam) as a source of food to be a potentially important topic. There is circumstantial evidence in the Hudson River that the almost endless supply of non-native zebra mussels may have assisted the major recovery of shortnose sturgeon there. It could be that a suddenly bountiful wild food source might offset some of the negative effects identified in isolation in these lab-based studies. Finally, I'm sure the team will report their experimental results but how will they use them to forecast future effects given the vagaries of environmental change in the system. How far out on a limb can they or should they go? It is up to the investigators to try and make all this as useful as possible to the Delta's resource managers.
rating	Superior

Feasibility

comments	I see no reason why any of the proposed work is infeasible. It is a large-scale project but broken down into numerous individual components. The young fish apparently are available,the individual researchers know what they are doing, and the budget is not ungenerous. Despite inclusion of thestructural effects model, documentation of the study becomes noticeably weaker at the data analysis end, both technically and conceptually.
rating	Sufficient

Relevance To The Delta Science Program

comments	The proposed study is relevant (as the investiagtors point out) to two of the Delta Science Program's Priority Research Topic List. These are Native Fish Biology and Ecology and, Food Web effects on Key Delta Species. The case for their relevance is well made.
rating	Above Average

Qualifications

comments	The qualifications of the investigators are appropriate to the goals of the project. Fangue is relatively fresh to her position and the Delta landscape but she is joined by experienced UC Davis-based professionals such as Doroshov and Hung, and another newcomer, Kebreab, who has a good track record from elsewhere. Moreover, the faculty of UC Davis have considerable experience managing these large CALFED projects and have published productively from that source of support.
rating	Above Average

Overall Evaluation Summary Rating

comments	The proposed study includes an unusually high number of metrics and thus, will produce quite a lot of data. Given that much of the data will be associated across a range of endpoints including many with sublethal effects, many different levels of effects will be seen seen, from nothing to severe. This is a strength of the proposal but it will make final analysis difficult and nuanced. Indeed, conflicting signals may be detected and needed to be sorted out. I'm all for more rather than less information but I suspect it may not boil down to a few clear take-home messages. The great hope here is the structural equation model, which seems to show promise for this application. It is safe to say that no matter how many of the findings are actually applicable to the management of the Delta, at minimum, a great deal more will be learned about basic sturgeon physiologies. In keeping with those comments, it's not altogether clear that much can be done with the resultant information, other than having a clearer sense of what the fate of these populations will be. Environmental change, both in expected and unexpected forms (i.e., Ever-Changing Estuary) is hitting the Delta in pronounced fashion and the trajectories of these two sturgeon populations likely will be altered, but can anything be done to mitigate these changes from the information this study will provide. I'm not sure, and I don't feel that is reason not to do the study; in fact, it is a question that is germane to much environmental work.
rating	Above Average

Final Review Panel

Assigned Panelists Reviews

Proposal Number:	0090
Proposal Title:	Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon
Proposal Applicant:	Davis, California University of
Amount Requested:	$446,690
Primary Investigator:	Nann A. Fangue, UC Davis

FRP primary Reviewer's Evaluation Summary and Rating

Provide a brief explanation of your summary and rating.

Comments:

Purpose	This is a complicated multi investigator project designed to experimentally and by modeling determine relative effects of nutritional status on ecological performance of sturgeon in a changing environmental climate within the delta. The proposal strongly emphasizes the potential for future declines in sturgeon populations because of climate change that will induce stresses related to warmer temperatures and changing salinity patterns influenced by climate induced changes to coastal oceanography and rainfall.
Background/Conceptual Models	This proposal builds on a history of physiological studies of a variety of species important in the SFBD system including sturgeons. Much of the research proposed here represents refinements to existing knowledge on tolerances of sturgeon to salinity and temperature and assessment of swimming performance as a metric of ability to tolerate stress. In other words, as the proposal points out and the citation indicate studies of physiological tolerance, swimming performance, thermal tolerance and salinity tolerance have been conducted before. The justification for continued research along these lines is that it will provide refined assessment of physiological responses that will account for likely changes in nutritional status of sturgeons in the system because of declining food webs and likely future changing salinity and temperature conditions within the Delta. One could argue that it is not just within the Delta as coastal conditions will change with influences of climate on coastal upwelling that are already being experienced.
Approach	The laboratory methods proposed here are for the most part reasonable and the participants are well versed in

	what it will take to accomplish the project goals. One exception is the extremes of exposure conditions that may provide quick endpoints and may be standard but are unlikely to mimic field exposures to stressful conditions as well as one might like. It would have been nice to see some field element incorporated into this project that would be able to either apply it directly to resident populations or gauge the relevance of the results for fish in the field. The modeling effort proposes to bridge the gap between the lab and field and promises to provide a set of tools that will allow resource managers to assess management strategies in the context of global climate change by predicting future population trends but it is not clear how that well would work with extreme exposure scenarios. I am also not sure how a model can predict outcomes in the field without considering factors outside the scope of this proposal such as migration patterns, spawning frequency, recruitment success, movement outside the system and nutritional status upon entering the system.
Feasibility	This project is technically feasible given that this line of research has a history within the Delta and also is based upon tried and true laboratory methods. Although it is relevant to Delta science, you can also say that this is a broadly based set of issues of importance to multiple species that exist within similar estuarine systems all along the west coast that will be similarly impacted by climate change.
Relevance	There is no doubt that this project will generate lots of data and publishable results but it is less clear that is will translate directly to a better understanding of the effects of environmental conditions in the Bay Delta system on sturgeon. Immediate management implications are not well justified given that strategies for dealing with climate change impacts may be limited, especially for population of species spawning at the southern end of their range.
Qualifications	This is a highly qualified team, well equipped and well trained to conduct this research.
Summary Comments	While the laboratory methods outlined in the proposal are sound and will produce publications, theses and a better understanding of sturgeon physiology, the immediate benefits to management seem less well defined. The overall approach could be improved to provide direct management benefits if it were to directly involve an assessment of the general condition of sturgeon within the system to better justify the need for refinements to the existing studies.

Please identify your overall ranking for this proposal:

– Superior
– Above Average
x Sufficient
– Inadequate

Proposal Number:	0090
Proposal Title:	Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon
Proposal Applicant:	Davis, California University of
Amount Requested:	$446,690
Primary Investigator:	Nann A. Fangue, UC Davis

FRP secondary Reviewer's Evaluation Summary and Rating

Provide a brief explanation of your summary and rating.

Comments:

Purpose	This proposal examines the ecological performance of two key fishes, green and white sturgeon, in the SFBay Estuary that face ever-changing environmental stressors, especially global climate change factors including temperature and salinity stress on species and their food webs. The proposal addresses Native Fish Biology and Ecology (Topic 1) and Food web effects on key Delta species (Topic 2).
Background/Conceptual Models	Background information is well developed for the most part and reflects the expertise of the PIs in their assigned tasks
Approach	The approach is sound and the research plan is generally well written and based on a conceptual plan with clear objectives, testable hypotheses and well defined tasks. I appreciate the focus on early life history stages and the incorporation of a gradation of three sizes/ages in the experimental design. Changing requirements with ontogeny is often an overlooked aspect of such studies. The modeling section is unclear and unfamiliar or may not be sufficiently developed. One outside reviewer also identified the data analysis section as weak. In this regard I am not sure that statistical designs discussed in Task 3 are entirely appropriate or how they will be analyzed statistically. Such details are lacking. One reviewer was concerned about the circularity of using food limitation as a means of assessing stress and of conducting stress tests (heat and salinity shocks) that go well beyond the environmental conditions the fish are likely to experience in the near term.
Feasibility	The Pis are accomplished at their given tasks in this proposal so the feasibility of the proposal is high and

	the likelihood of success is very good for meeting the objectives.
Relevance	The research will make relevant contributions to our understanding of early life history stages of two important species and will contribute greatly to the information base needed to manage sturgeons successfully in the SFBD.
Qualifications	The PIs are all highly qualified and well published in their respective fields. They are a mix of junior and senior talents. The most junior is the lead-PI on the proposal, which is well written and organized, suggesting that she is ready to handle the administrative aspects of the project as well as her research task and she has two senior researchers, Drs. Doroshov and Hung, experienced in managing similar large undertakings to assist her as necessary.
Summary Comments	The proposal is based on a clear conceptual model and is hypothesis driven. It will contribute importantly to our understanding of two key species and the scope they have for responding to future stresses related to climate change and food web modifications in the SFBD. Climate change will likely effect temperature, salinity and food webs in the long term, but food webs are being changed or stressed in the short term on a regular basis with frequent invasions of exotic species.

Please identify your overall ranking for this proposal:

– Superior
X Above Average
– Sufficient
– Inadequate

Proposal Number:	0090
Proposal Title:	Ecological Performance of Fishes in an Ever-changing Estuary: The Effects of Nutritional Status on Environmental Stress Tolerance in Sturgeon
Proposal Applicant:	Davis, California University of
Amount Requested:	$446,690
Primary Investigator:	Nann A. Fangue, UC Davis

FRP secondary Reviewer's Evaluation Summary and Rating

Provide a brief explanation of your summary and rating.

Comments:

Purpose	This study seeks to understand interacting effects of changing nutritional status and climate (temperature and salinity) on the physiological stress tolerance of green and white sturgeon. The authors present three general hypotheses—higher nutritional status is positively correlated with tolerance to environmental stressors, sensitivity to environmental stressors is age dependent, and green and white sturgeon respond differently to environmental stressors. This study is timely, as primary productivity has changed dramatically in the SFBD in recent decades, with the potential to negatively impact native fishes. Green and white sturgeon are species of conservation concern, and they are especially sensitive to anthropogenic alterations due to their long life expectancies and late sexual maturity. Findings from this study will add to knowledge gaps about the resiliency of early life stages of these species when faced with environmental stress.
Background/Conceptual Models	The conceptual model is clearly shown as a diagram and supported well with background text. The authors do an exceptional job of introducing complicated topics in a succinct manner—ranging from effects of climate change on the SFBD system, sturgeon biology and ecology, nutritional status of young sturgeon, and measurements of physiological responses of fish. The necessary information is provided and documented to understand the proposed work.
Approach	The approach and scope of work are well designed and appropriate for meeting the objectives of the project. The PIs have assembled a team with an array of expertise, and they clearly explain who is responsible

	for each task and how the tasks will be managed and coordinated. I particularly like the emphasis on modeling in this project.
Feasibility	The approach appears technically feasible, given the expertise of the PIs. The emphasis placed on coordination among the PIs, such by having annual meetings, will help guarantee that researchers remain on track and that information is shared and synthesized as it becomes available, thus ensuring the success of this project.
Relevance	Study is an excellent fit to Topic 1: Native fish biology and ecology, and it also includes Topic 2: Food web effects on key Delta species. The information will be useful to policy makers, and as stated by the authors, will help link ecosystem health with management decisions in the SFBD system.
Qualifications	This is a nice team of researchers that includes an early-career scientist and investigators with progressively greater experience in their fields. They bring a range of expertise, including environmental physiology, reproductive physiology, fish nutrition and toxicology, mathematical modeling, and rearing of sturgeon. In addition, the team will include a Ph.D. student and a post-doctoral fellow who have already been selected based on their existing skill sets.
Summary Comments	The authors made an extremely convincing argument for the importance of this research. I was impressed with their ability to make such a complex topic clear to a reader unfamiliar with this ecosystem. The lead PI is early in her career and has an excellent record of success so far. I expect that under her leadership, this project will be successfully implemented with important findings for managers and researchers in the Bay-Delta system and beyond.

Please identify your overall ranking for this proposal:

X Superior
– Above Average
– Sufficient
– Inadequate