FINAL PROJECT REPORT FINAL REPORT #: (5)
CALFED SCIENCE PROGRAM PROJECT NUMBER: 1035
PROJECT TERM: NO-COST EXTENSION UNTIL DEC-31-2010
PROJECT TITLE:

CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

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Funding Source: CALFED
Project Location: UC Davis and San Francisco Bay / Sacrameto River Delta

Brief Description of Project:
This project seeks to analyze the effects of the pollutant stressor selenomethionine (SeMet), singly and in combination with methyl mercury (MeHg), temperature, and salinity stresses on the biochemistry, physiology, and overall fitness of different life stages of sturgeon. Because white sturgeon (*Acipenser transmontanus*) and green sturgeon (*Acipenser medirostris*) both occur in the San Francisco (SF) Bay−Delta this study will focus on both species. We aim to better understand the mechanistic basis for bioaccumulation of SeMet and MeHg, and unraveling key elements of the molecular response to such pollutant stresses in sturgeon. Our research will significantly enhance the depth of knowledge about the stress biology of sturgeon and allow further development of life history models by performing a series of integrated tasks. These tasks will test the overall hypothesis that specific stress proteins in combination with biochemical, cellular, and physiological parameters a) indicate exposure of sturgeon to specific types and defined combinations of stresses and b) allow us to deduce molecular mechanisms and biological processes of stress adaptation in sturgeon. We will breed sturgeon and obtain samples from laboratory acclimation experiments and biopsies from wild sturgeon in the field. Using these samples we will quantify the toxicokinetics, bioaccumulation, and chronic toxicity of selenium when present alone or in combination with MeHg in the diet. Moreover, we will quantify effects of chronic exposure to dietary SeMet stress, singly and in combination with MeHg, salinity, and temperature stresses on swimming performance, resting metabolism, and hematology. Furthermore, we will identify novel bioindicators and entire proteome signatures and biochemical pathways affected by the stressors outlined above. These 'stress-signatures' will be compared to the proteome of sturgeon biopsies obtained in the field to deduce exposure history in field specimen. By knowing the proteins and biochemical pathways associated with stressful levels of selenium, mercury, salinity, and temperature we can identify physiological functions that are affected by such stresses in endangered fish species such as sturgeon. After identifying such proteins we will construct tissue microarrays (TMAs) from sturgeon tissues to enable robust, stressor-specific, and high-throughput bio-monitoring field assays in the future and population-level modeling approaches.

Primary Objective to be Achieved:

BUDGET SUMMARY (All tasks should exactly match those identified in the project Scope of Work.)
This is an updated budget as approved by CALFED last year after funding was restored. It takes into account a no-cost extension from May01, 2010 – Dec31, 2010.

TASK/ SUBTASK	% COMPLETE (BY DOLLARS)	AMOUNT INVOICED (CURRENT FISCAL YEAR)	AMOUNT INVOICED TO DATE (ALL YEARS)	PROJECTED EXPENDITURES (NEXT 6 MONTHS)
Task 1	46%	0	2572	3053
Task 2	100%	0	87147	0
Task 3	100%	0	118070	0
Task 4	100%	0	107559	0
Task 5	N/A	0	0	0
Task 6	93%	123132	353890	25188
Task 7	55%	0	3103	2521
Totals		**123132**	**669238**	**30762**

INDIRECT COSTS ARE INCLUDED IN TASK-SPECIFIC EXPENSES.

ACHIEVED OBJECTIVES, FINDINGS, CONTRIBUTIONS, AND PROJECT-WIDE STATUS: (BY TASK)

This section should be a cummulative overview of the activities performed to date and include both current and past information for each task. Please list all new information at the top of each task section so that it is clear which information is the most recent. At the end of the project, this section will serve as a full historical record of all activities performed on the project.

TASK 1:

- Meetings were held in which monthly progress was evaluated and coordinated with all PIs and key personnal present (except for Marty Gingras who was unable to attend due to scheduling conflicts).
- A semi-annual progress report was generated
- Problems, results, and future directions / improvements were discussed

- Data collected during the first year of the project were presented by all Co-PIs for all tasks and possibilities for packaging and publication of those data were discussed
- Unexpectly high costs of Hg and Se monitoring during our experiments and strategies for coping with these costs were discussed
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- Meetings were held to discuss and cope with funding freeze due to state budget, including animal care, keeping personnel trained with the tasks of this project employed on emergencty funds, etc.
- Joint publications were discussed and prepared and submitted
- Final reports for tasks 2, 3, and 4, which expired after 2 years before the budget was reinstated wwere prepared and added to this report.
-
- Meetings were held to consolidate work beuing done on this project and to collate publications that have resulted so far. Publications in preparation or in press have also been identified as well as unfinished work that will likely also result in publications.
- The final report was prepared and submitted.

  - **PROJECT STATUS:** Task 1 has been completeled in its entirety.

TASK 2:

PROCUREMENT OF EXPERIMENTAL ANIMALS (TASK 2, PART 1)

- In March 2007 We succeeded in the first captive breeding of green sturgeon reared at the UC Davis facilities (year class 1999, originated from the Klamath River brood stock). Ovulation was induced in three females, but only one produced high quality eggs. Several thousands normal larvae and juveniles resulting from this cross were used in Tasks 2-5.

- Three progenies of white sturgeon were obtained from the nearby commercial sturgeon farms (Sterling Caviar, Wilton, CA and Lazy Q Fish Ranch, Dixon, CA). Fertilized eggs were incubated at the UC Davis aquatic facilities, and the hatched larvae were then distributed to the other investigators. Data were collected on brood fish size and age, fertilization and hatching rates.

- During January – March 2008 we sampled green sturgeon broodstock held at the UC Davis facilities and identified ripe males suitable for spawning. Three maturing females were identified by nuclear migration in the oocytes (polarization index) and in vitro oocyte maturation assays. Two females did not respond with ovulation to injections of gonadotropin releasing homone, due to preovulatory arrest in egg maturation and onset of follicular atresia. One female continued normal development and will be resampled at the end of March.

- During April 2008 we attempted to spawn the third female but it also did not ovulate and appeared to have begun the process of follicular atresia. Two progenies of white sturgeon were obtained from a nearby commercial sturgeon farm (Sterling Caviar, Wilton, CA). Fertilized eggs were incubated at the UC Davis aquatic facilities, and the hatched larvae were distributed to the other investigators.
- **Task 2 has been completed in its entirety. The objective to spawn green sturgeon and provide hatched larvae for this project was achieved during spring 2007. This was the first recorded successful spawning of green sturgeon reared in captivity. The selenium and temperature stress experiments were completed with three progenies of white sturgeon and one progeny of green sturgeon. Our findings indicate that green sturgeon larvae are more sensitive to the selenium and temperature stresses. The main results are outlined below.**

TEMPERATURE STRESS AND NOTOCHORD DEFORMITY IN LARVAL STURGEON (TASK 2, PART 2)

- In June 2008 we carried out an experiment to determine the effect of thermal stress on survival and notochord development of white sturgeon larvae. The experiment was replicated using two white sturgeon progenies (Task2, Part2). Newly hatched larvae (Stage 36) were acclimated to 18 ºC (control) and 22, 24, 26, 28 and 30 ºC at a rate of 2 ºC·h^{-1} in triplicate tanks (40 larvae·tank^{-1}), and subsequently reared at constant temperatures until completion of yolk sac absorption (Stage 45). We recorded daily morphological abnormalities and mortalities, sampled larvae for weigth and length at stages 36 and 45, and collected samples for histology (pending analysis), proteome analysis (pending analysis) and photography.

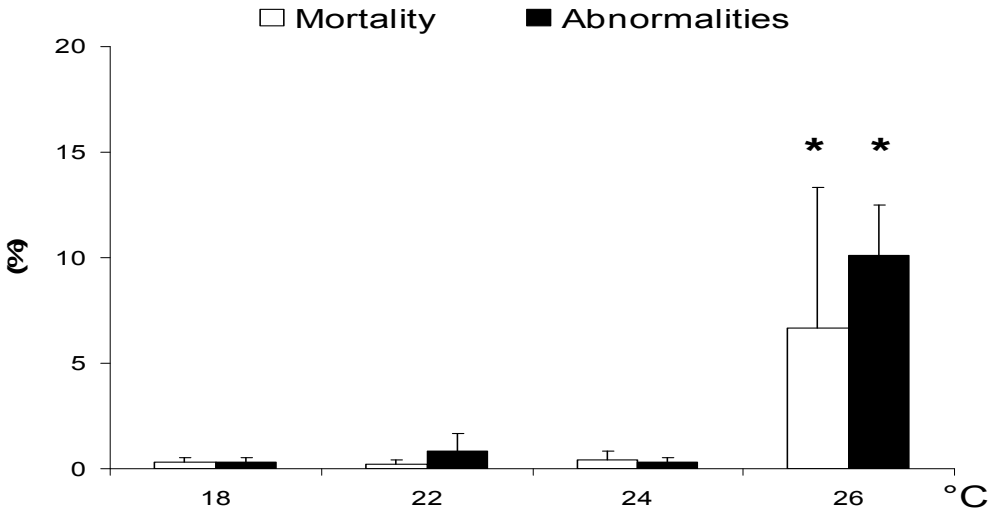


Fig. 1: Mean ± sem mortality and incidence of kyphosis at experimental endpoint of white sturgeon larvae reared at 18 – 26 ºC from hatch to completion of yolksac resorption. Asterisks denotes significant difference between treatment groups and control (18 ºC), $p<0.05$.

- Temperatures ≥26 ºC significantly increased the mortality and the proportion of stage 45 survivors exhibiting kyphosis, forward bending of notochord (see figure below). Temperature treatments 28 and 30 ºC were lethal for both progenies, whereas 26 ºC treatment was sub-lethal.
- Maximum proportion of kyphotic larvae were observed at 26 ºC. At 28 and 30 ºC, larvae never reached stage 45, exhibited impaired yolk utilization and severe kyphosis. A small proportion (< 0.5%) of kyphotic larvae reared at 26-30 ºC also exhibited yolk sac and pericardial edema accompained with hemorrage in the vasculature of one or more of the following regions: yolk sac, heart, ventral and lateral aspects of the head (see photo plate below).

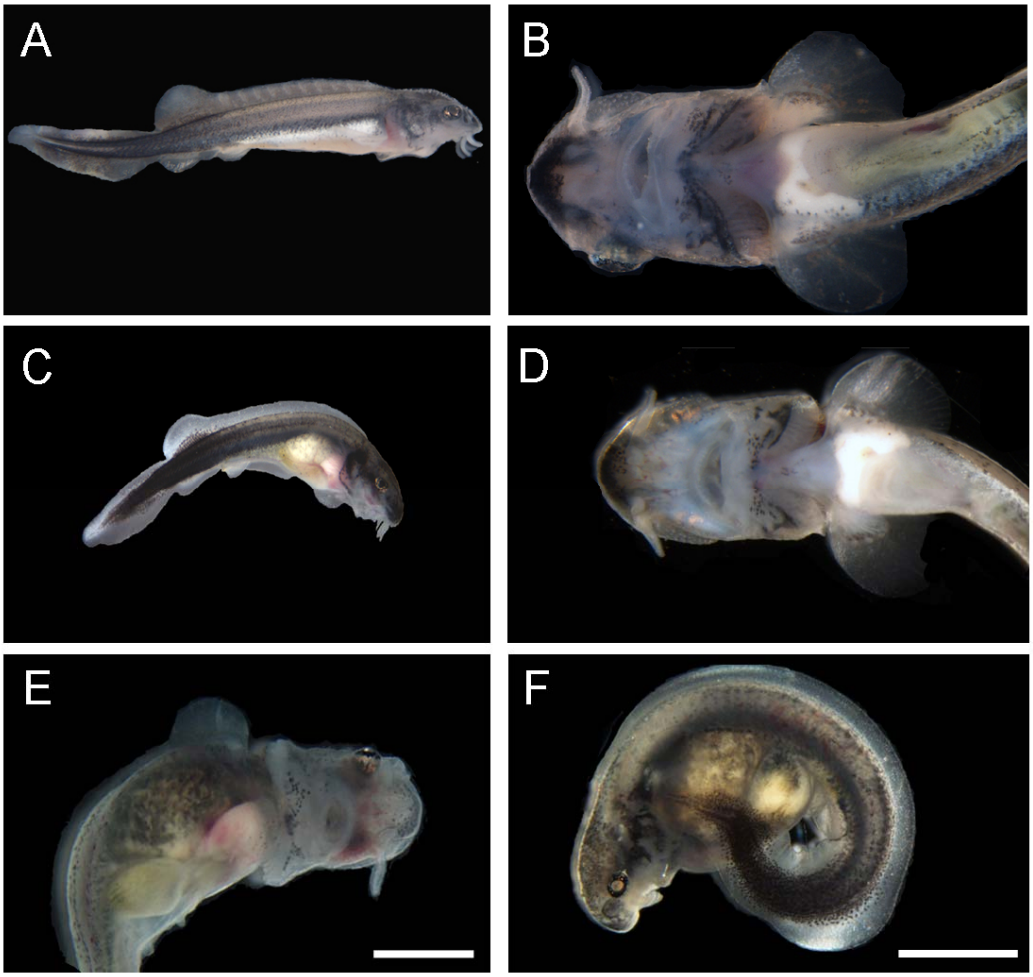


Fig. 2: Stage 45 larvae from control treatment (A, B). Four days post-acclimation (dpa) and Stage 45 larvae reared at 26 ºC (C, D). Larvae from 28 ºC treatment exhibiting kyphosis, heamorrage, and edema at 4 dpa (E). Severe kyphosis and microphthalmia exhibited by a larvae 2 dpa to 30 ºC (F). Bars: 5 mm (A, C, E) and 2.5 mm (B, D, F).

- Maximum proportion of kyphotic larvae were observed after 1-2 dpa at 26-30 ºC and at 3-4 days at 22-24 ºC (See figure below). Peak in proportion of kyphotic larvae observed at 22-26 ºC was followed by a gradual decline with most larvae recovering by stage 45. Even a slight dregree of kyphosis precluded larvae from forward swimming and swim-up, reducing ability of larvae to avoid

predation, to migrate to nursing areas and to forage. No malformed larvae were observed in the 18 ºC control group.

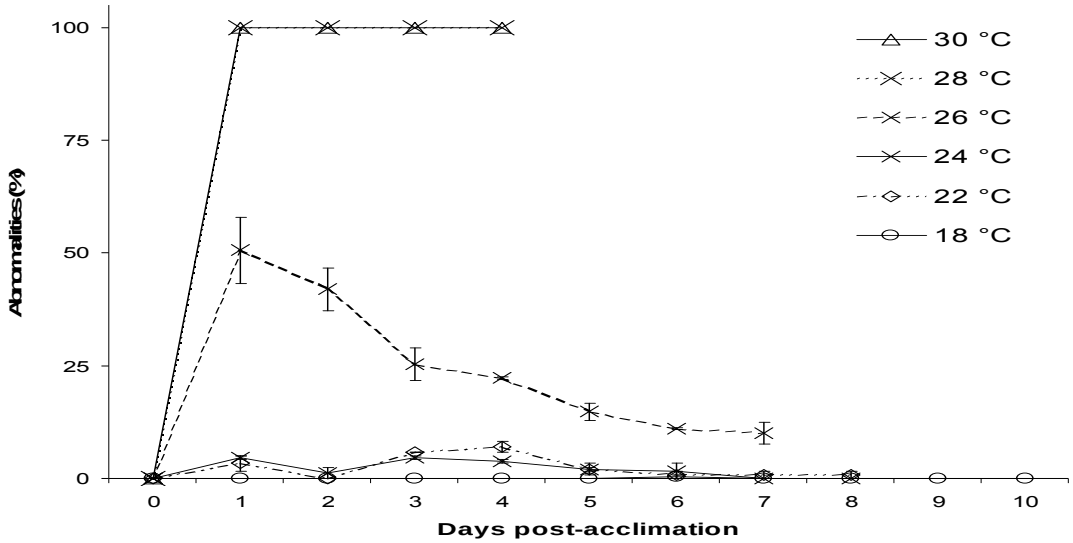


Fig. 3: Average (mean ± sem, n=2 progeny) daily proportion of abnormal larvae reared from Stage 36 to completion of yolk-sac resorption (18-26 ºC) or until 100% larvae sucumb (28-30 ºC).

- Progeny 1 larvae were significantly larger at stage 36 ($p < 0.01$; t-test on log transformed data) and at stage 45 ($p < 0.01$; ANOVA on log transformed data) than progeny 2 larvae (see table). Eventhough there were significant size differences between progenies, effect of temperature on end point larvae survival and incidence of axial malformations were similar for both progenies ($p = 0.43$ and $p = 0.99$ respectively).

		Progeny 1		Progeny 2	
	Temperature	W (mg)	DW (mg)	W (mg)	DW (mg)
Stage 36	18 ºC	27.9 ± 0.4	11.6 ± 0.1	20.7 ± 0.2	7.9 ± 0.1
Stage 45	18 ºC	63.9 ± 0.4	8.2 ± 0.1	46.1 ± 0.5	5.3 ± 0.1
	22 ºC	66.6 ± 0.6	8.0 ± 0.1	46.4 ± 0.3	5.2 ± 0.1
	24 ºC	65.8 ± 1.1	8.0 ± 0.2	44.5 ± 0.3	5.2 ± 0.1
	26 ºC	65.8 ± 1.0	8.3 ± 0.2	44.5 ± 0.3	5.6 ± 0.1

Table 1: Mean ± sem (n=3) larvae weight (W) and dry weight (DW) at stage 36 and at experimental end point (Stage 45) for two white sturgeon progeny.

- **SELENIUM AND TEMPERATURE STRESS IN LARVAL STURGEON (TASK 2, PART 3)**
  - We conducted four experiments on temperature and selenium stresses in larval sturgeon. Three progenies of white sturgeon and one progeny of green sturgeon were used for these experiments. Each experiment started with larval stage 36 (newly hatched), with the endpoint at stage 45 (full absorption of yolk).
  - The experiments were factorial and included temperature (normal 18 ºC and high 26 ºC) and selenium (putative and 8 ppm burden, dw) treatments. The excess organic selenium was introduced with microinjected Seleno-L-Methionine (Sigma-Aldrich, St Louis, MO). The

percent mortality and abnormality was recorded daily and the samples for proteome analysis, histology, and selenium body burden were collected at the stage 45 endpoint. The high temperature at putative selenium affected survival of green sturgeon but not of white sturgeon. The selenium treatment affected survival of both species. The highest average mortality 11% of white sturgeon was observed in the high temperature x selenium treatment. The selenium-treated green sturgeon larvae had a high mortality at both temperature levels. Both species exhibited stressor-specific abnormalities: bent notochord at high temperature and edema in selenium treatment (Table).

Treatment	Non-injected		L-Met		Se-L-Met	
	18°C	26°C	18°C	26°C	18°C	26°C
WS	<1	1	<1	1	**7 ***	**11 ***
GS	1	**12 ***	**12 ***	**16 ***	**61 ***	**60 ***

Table 2: Percent mortality (means of 3 replications in 3 progenies of white sturgeon, WS, and one progeny of green sturgeon, GS). Asterisks show significant difference ($P < 0.05$) compared non-injected control at 18°C, for each species.

- Both species exhibited abnormalities: bent notochord at high temperature and edema in selenium treatment. Combination of bent notochord and edema was observed in the high temperature x selenium treatment (see Figure below). The daily count of abnormal larvae on the bottom of tank revealed effect of temperature and selenium treatment on time course and peak of abnormalities.

- The temperature x selenium and temperature alone stresses resulted in early sharp peaks of abnormalities, whereas selenium stress alone (Se-L-Met at 18°C) occurred slowly (see Figure below).

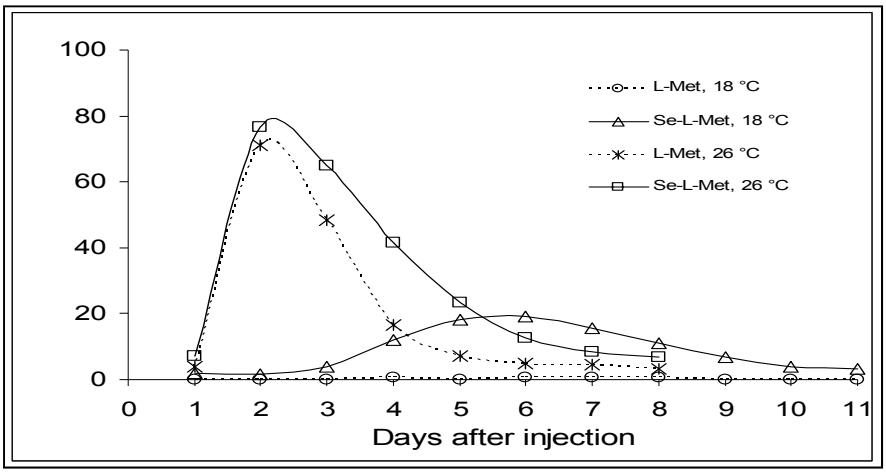


Fig. 4: Figure legend for above figure: Percent abnormal larvae of white sturgeon in control (L-Met, 18°C), temperature stress (L-Met, 26°C), selenium stress (Se-L-Met, 18°C), and selenium x temperature stress (Se-L-Met, 26°C) treatments. Most white sturgeon larvae recovered at the end of the experiment.

Fig. 5: Figure legend for above figure: White (left) and green (right) sturgeon larvae at Stage 45. Top row: normal larvae in non-injected and L-Met control. Middle row: abnormal larvae with edema in the Se-L-Met x 18°C treatment. Bottom row: larvae of white sturgeon in the Se-L-Met x 26°C treatment (left) and green sturgeon in the L-Met x 26°C treatment (right). Scale bar 5 mm.

- Selenium burden in whole larvae at stage 36 and 45 was determined by inductively coupled plasma (ICP) atomic emission (California Animal Health and Food Safety; California Veterinary Diagnostic laboratory system, toxicology laboratory, University of California, Davis). Putative selenium concentration of Stage 36 GS larvae was 1.6 times greater than in Stage 36 WS larvae (see table below). Putative selenium concentration of Stage 45 larvae reared at 18 and 26 ºC was similar ($p > 0.05$) than at Stage 36 for both species.

	Stage 36	Stage 45	
		18 ºC	26 ºC
White sturgeon (n = 3 progeny)	2.1 + 0.1	2.4 ± 0.1	2.0 ± 0.1
Green sturgeon[1]	3.4 ± 0.2	3.2 ± 0.1	2.8 ± 0.1

Table 3: Mean ± sem putative selenium concentration ($\mu g \cdot g^{-1}$ DW) of WS and GS larvae. [1] At stage 36 n=2 and at stage 45 n=3 replicates).

- At experimental end-point, Se-L-Met injected larvae had significantly greater selenium burden than larvae from non-injected and L-met groups (ANOVA, $p < 0.01$ for GS and WS). Thus, indicating microinjection of Se-L-Met into yolk sac of stage 36 larvae is an efficient method to mimic maternal transfer of selenium to progeny (see table below). Although we only had a single GS progeny for experimentation, our results also suggest that WS Stage 45 larvae can

assimilate significant more selenium derived from Se-L-Met (57.0% and 49.6 % at 18 and 26 ºC respectively) than GS stage 45 larvae (11.7% and 8.8% at 18 and 26 ºC respectively).

- Reduced Se-L-Met assimilation capacity of GS larvae and limited capacity of larvae to metabolize (excrete) Se-L-Met could explain the increased sensitivity of GS larvae to Se-L-Met treatment (61 and 60 % mortality at 18 and 26 ºC respectively) as compared to WS (7 and 11% mortality at 18 and 26 ºC respectively).
- GS and WS Stage 45 larvae reared at 26 ºC had significantly (ANOVA, $p < 0.05$) lower Se concentration than larvae reared at 18 ºC across all injection treatments (see table below).

			Non-injected	L-Met	Se-L-Met
White sturgeon (n = 3 progeny)	Stage 36	18 ºC	2.1 ± 0.1	2.1 ± 0.1	8.6 ± 1.3
	Stage 45	18 ºC	2.4 ± 0.2	2.3 ± 0.1	7.6 ± 0.3
		26 ºC	2.0 ± 1.5	2.0 ± 1.8	6.8 ± 1.0
Green sturgeon (n = 3 tank)	Stage 36 [1]	18 ºC	3.2	3.5	7.3
	Stage 45	18 ºC	3.1 ± 0.1	3.3 ± 0.1	3.8 ± 0.3
		26 ºC	2.9 ± 0.1	2.8 ± 0.1	3.4 ± 0.2

Table 4: Selenium concentration of GS and WS larvae ($\mu g \cdot g^{-1}$ DW) at 24h post-injection (Stage 36) and experimental endpoint (Stage 45). [1] Measurement based on a 100 larvae sample.

- **PROJECT STATUS:** Task 2 has been completed as planned. Much of this project was hinging on the successful spawning of green sturgeon in captivity. We achieved this for the first time in this species, which provided the animals needed for part of the study.
- **The following publications have resulted from the completion of this task:**
  - **Van Eenenaam J.P., Linares-Casenave J., Muguet J.-B. & Doroshov, S.I. (2008) Induced spawning, artificial fertilization, and egg incubation techniques for green sturgeon. North Am. J. Aquaculture 70: p.434–445.**
  - **Werner I., Linares-Casenave J., Van Eenennaam J.P. & Doroshov, S.I. (2007) The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (Acipenser medirostris). Environ. Biol. Fish 79: p.191–200**

TASK 3:

- **SELENIUM DISTRIBUTION, SPECIATION, AND DEPURATION OF SEMET WITH AND WITHOUT ADDITIONAL METHYLMERCURY (TASK 3 OBJECTIVE A)**

We have completed a preliminary study on assimilation efficiency of SeMet in green sturgeon. This study is needed to design the experiment in Objective a) Part I to determine the tissue distribution, speciation, and depuration of SeMet when present alone or in combination with methyl mercury (MeHg) in the diet.

- Absorption of selenomethionine (SeMet) by green sturgeon is similar to white sturgeon. Furthermore, the absorbed SeMet are mainly incorporated in the plasma protein.
- Green sturgeon is much more sensitive to SeMet chronic toxicity than white sturgeon.
- Growth rate but not survival rate was significantly depressed in SeMet chronic toxicity but vise versa in MeHg chronic toxicity in green sturgeon.

- **Objective a: to establish a kinetic modle SeMet absorption, distribution and depuration in sturgeon**

Absorption of selenomethionine (SeMet) by green sturgeon is similar to white sturgeon. Furthermore, the absorbed SeMet are mainly incorporated in the plasma protein. Assimilation efficiency of SeMet by green sturgeon is very high approaching 100%.

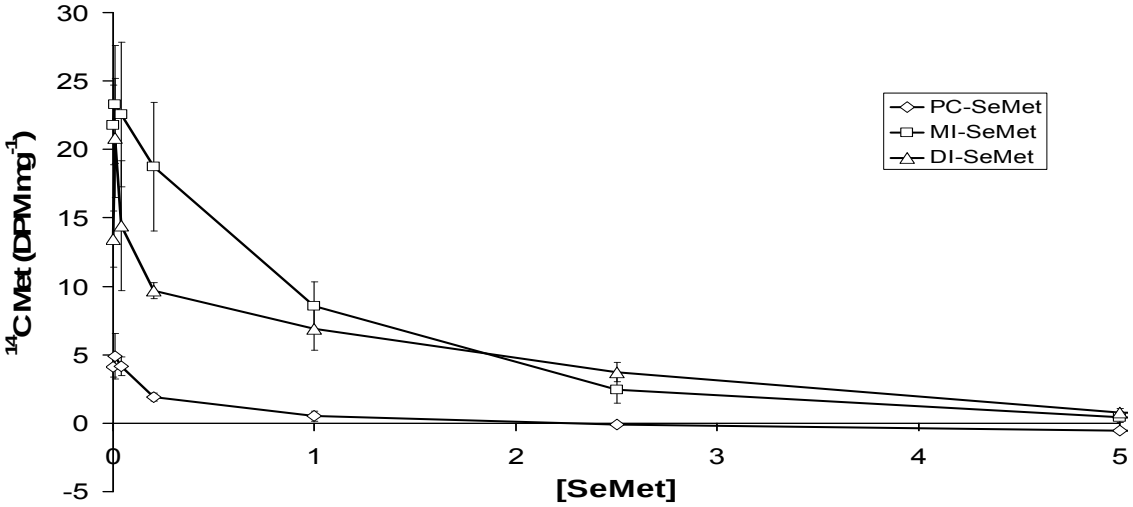


Fig. 6: Absorption of selenomethionine (SeMet) by green sturgeon is inhibited by methionine. Using the everted gut sleeve technique, we observed a competitive inhibition of uptake between selenomethionine and methionine in the pyloric caecum (PC), mid intestine (MI), and distal intestine (DI, also referred as spiral valve) in green sturgeon (See figure below).

- This result suggested that we need to consider the feeding status of the sturgeon before and after we administrate the SeMet into esophagus when we design our gelatin capsule feeding experiments for objective a). We are working on obtaining the Animal Care and Maintenance Protocol to conduct preliminary trials in this summer.
-

  1. Toxic threshold of dietary SeMet: The toxic threshold of SeMet is around 20 mg Se/kg diet for green sturgeon and around 40 mg Se/kg diet for white sturgeon.

  2. Toxic threshold of dietary MeHg: Toxic threshold of MeHg is between 20-40 mg Hg/kg diet for green sturgeon and 40-80 mg Hg/kg diet for white sturgeon which is higher than toxic threshold of SeMet (10-20 mg Se/kg diet). Furthermore, the toxic threshold of MeHg in sturgeon is much higher than the ecologically reverent concentration of 0.1-0.5 mg Hg/kg diet. It also takes longer to manifest toxic symptoms of MeHg (8 weeks) than SeMet (2 weeks) in sturgeon.

  3. Green sturgeon is more sensitive to SeMet or MeHg toxicity than white sturgeon.

4. Body composition reponsed differently between green and white sturgeon fed diets with the same four concentrations of SeMet. In general, sturgeon fed diets with higher SeMet had lower whole body lipid and energy content but higher moisture content where as ash content were not that different. The lower of body lipid in sturgeon fed diets with higher SeMet are more pronounced and showed up earlier (4 instead of 8 weeks) in green than white sturgeon. Whole body protein were not affected by dietary SeMet concentration at 4 and 8 weeks in white sturgeon but they were lower lower in green sturgeon at 8 weeks.

► Proximate composion in green and white sturgeon fed MeHg diets

There was no significant body composition difference among treatments in both green and white sturgeon fed fortified MeHg diets. However, moisture contents of green sturgeon were higher than those of white sturgeon in each Hg treatment groups (Table 3 & 4). Inversely, crude protein and lipid, and energy contents were significantly higher in white sturgeon than those in green sturgeon (Table 3 & 4).

► MeHg body burden

There were significant differences ($p < 0.05$) among treatments in total Hg burdens in all tissues (whole body, liver, kidney, white muscle, gill, heart) (Table 5 & 6). Mercury concentrations in all 6 tissues significantly increased in a dose-response manner, and the highest Hg accumulations were found in the kidney of both sturgeons fed 80 µg Hg/mg diet, except for green sturgeon at week 8 due to mortality (Table 5 & 6). The highest linear correlation between tissue and dietary Hg concentration was found in white muscle (r = 0.998, p<0.0001), followed by gill (r = 0.978, p<0.0001), kidney (r = 0.957, p<0.0001), heart (r = 0.948, p<0.0001), and liver (r = 0.943, p<0.0001).

There was a difference in Hg burdens between green and white sturgeon, even though the difference was not always significant. Kidney, white muscle, gill and heart of white sturgeon generally showed higher Hg concentrations than those of green sturgeon, and vice versa in liver. Mercury concentrations in kidney, white muscle, gill, and heart of white sturgeon fed 80 µg Hg/mg diet were significantly higher than those of green sturgeon.

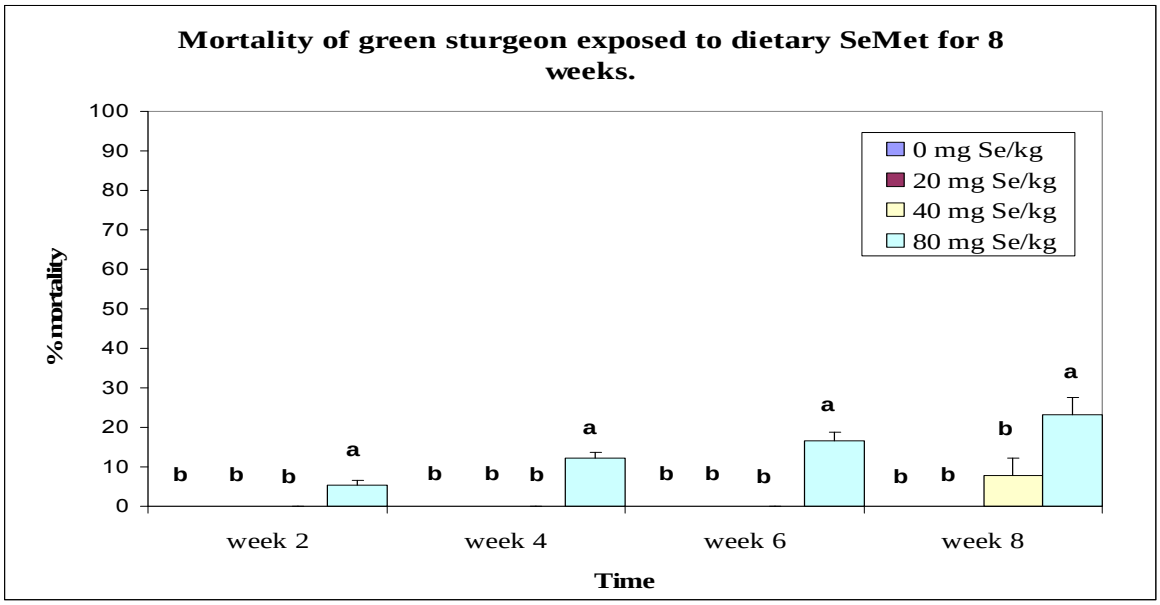


Fig. 7: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

No Mortality in white sturgeon exposed to dietary Se for 8 weeks occurred.

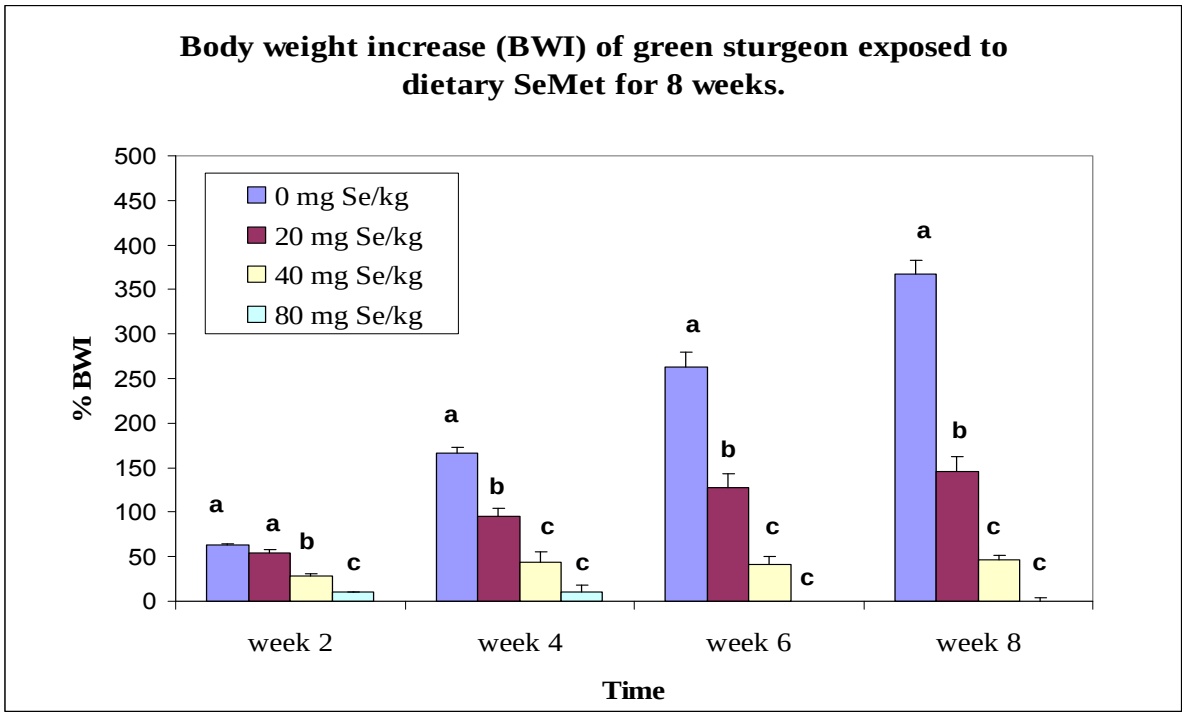


Fig. 8: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

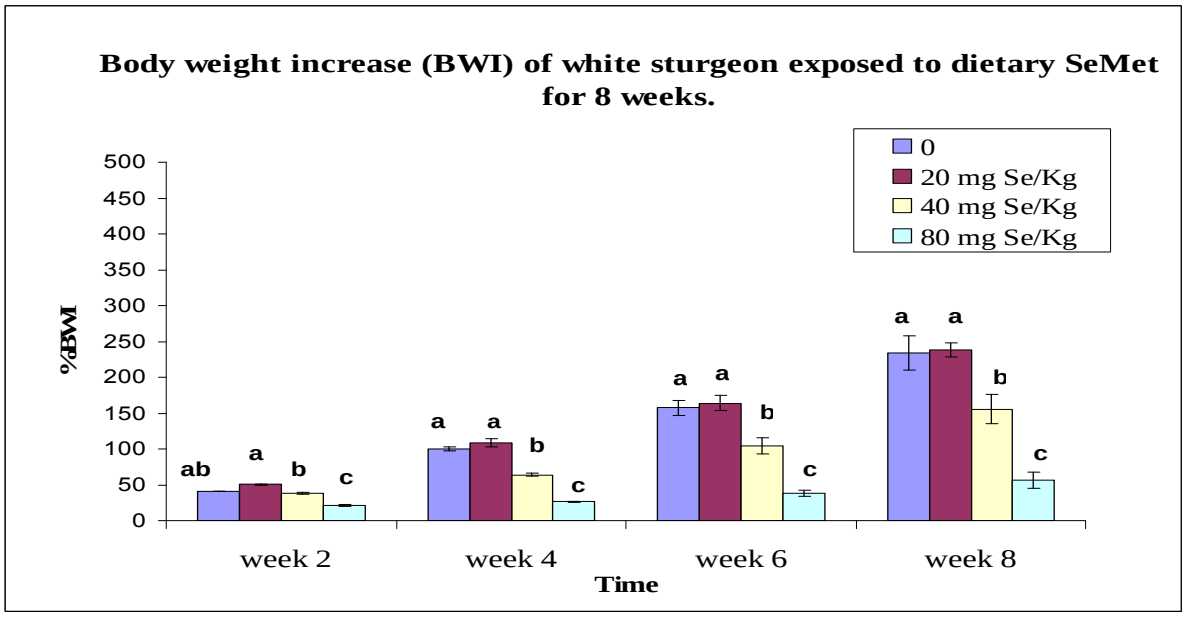


Fig. 9: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

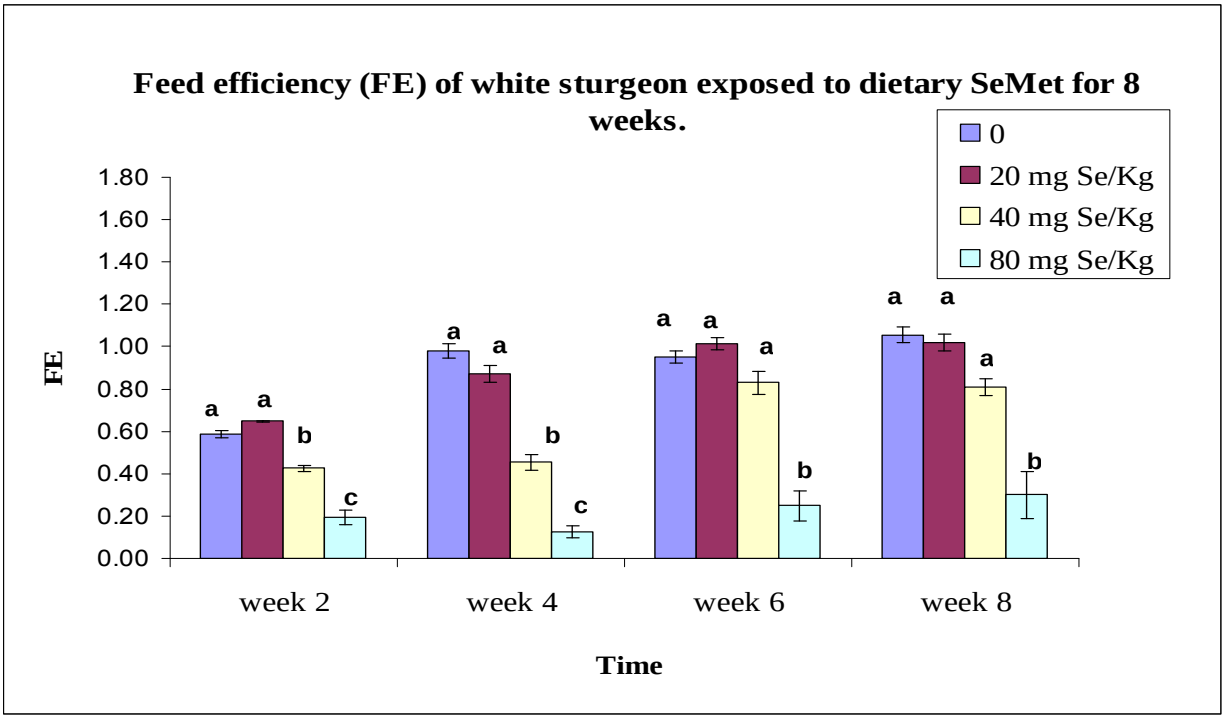


Fig. 10: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

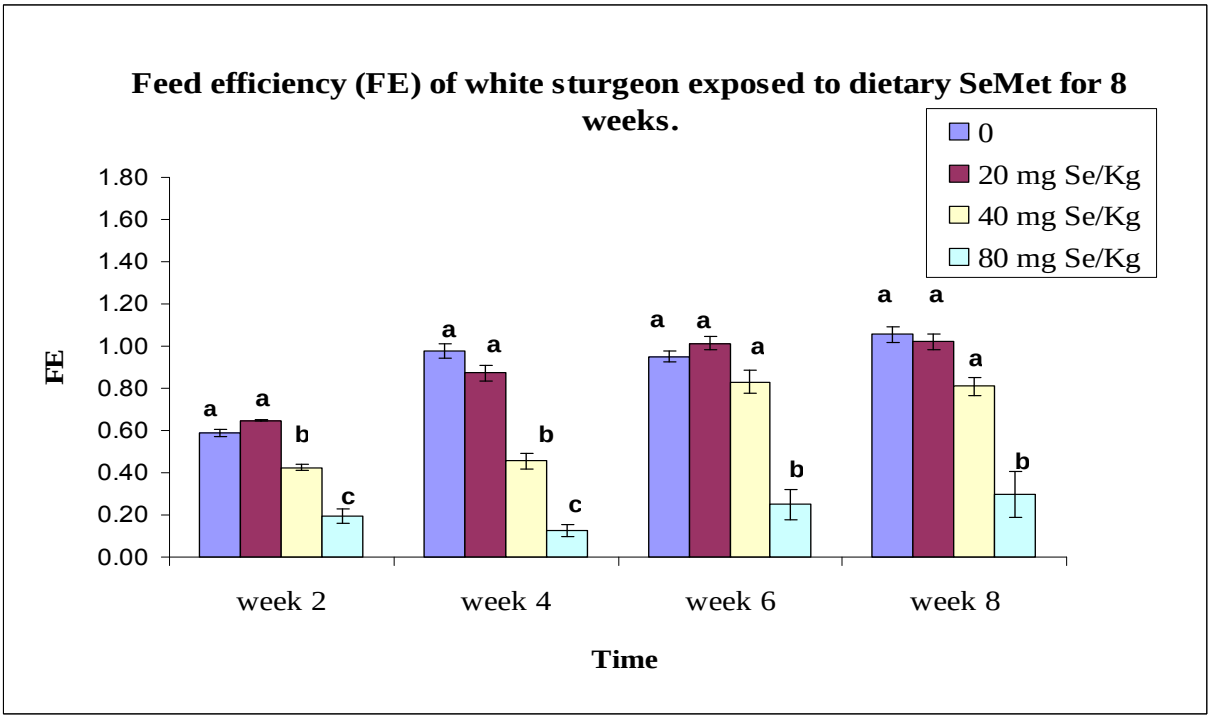


Fig. 11: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

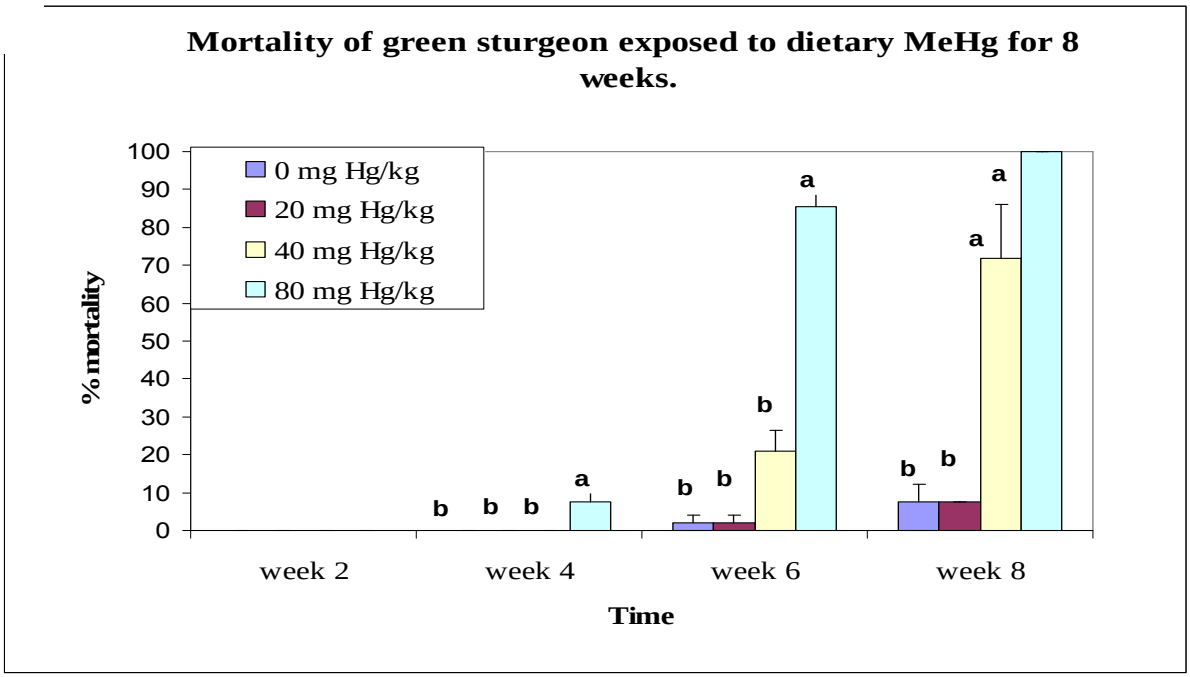


Fig. 12: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

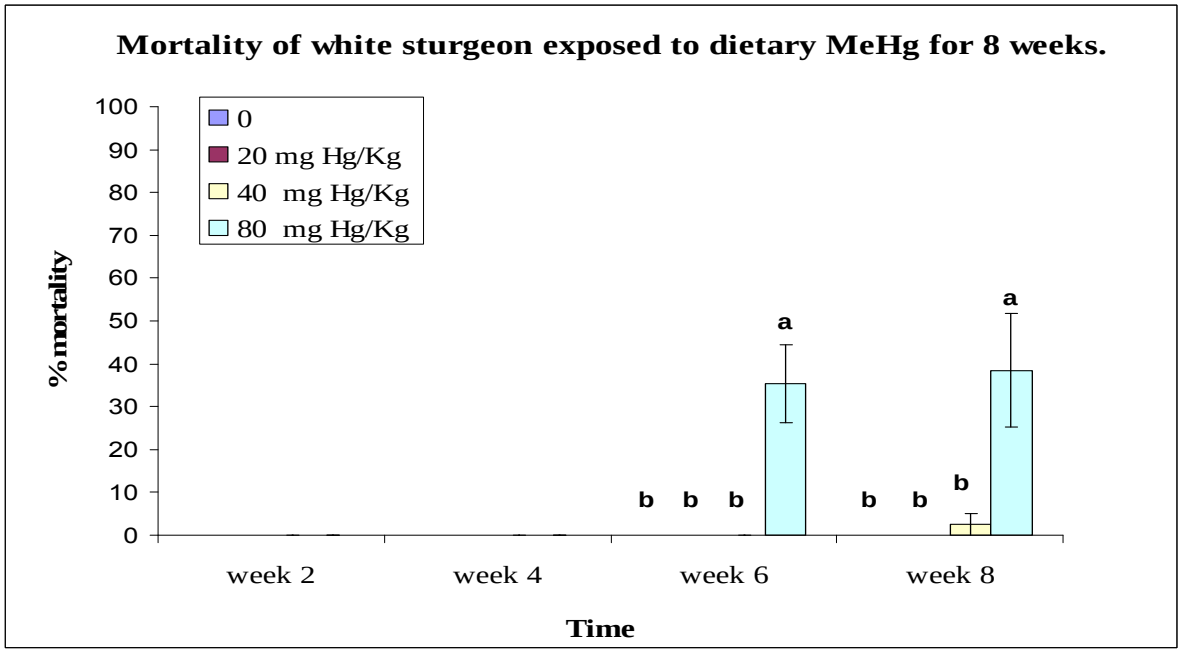


Fig. 13: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P <0.05.

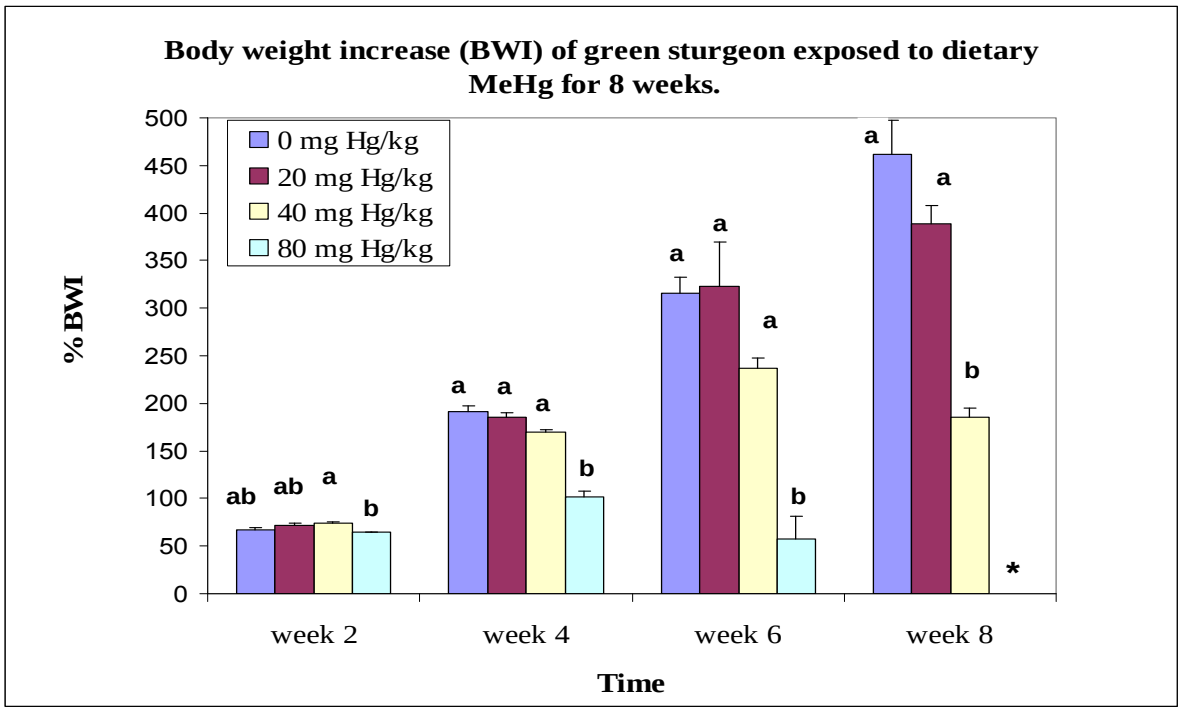


Fig. 14: * No data due to mortality and tissue sampling. Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

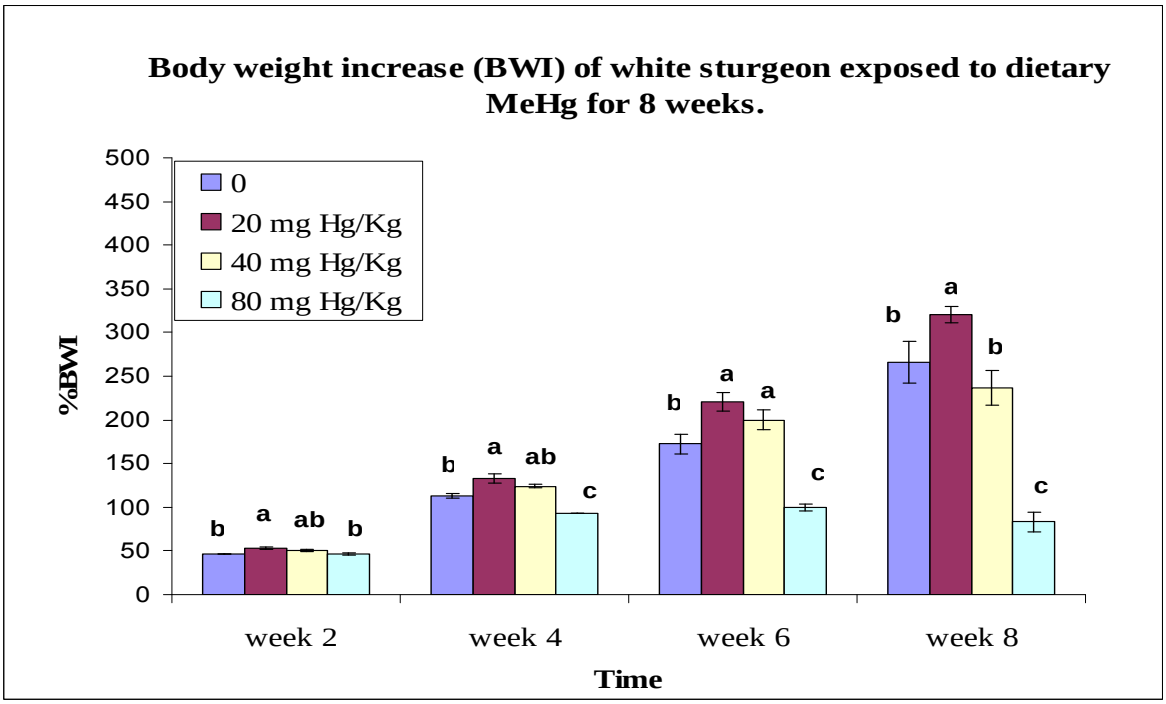


Fig. 15: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

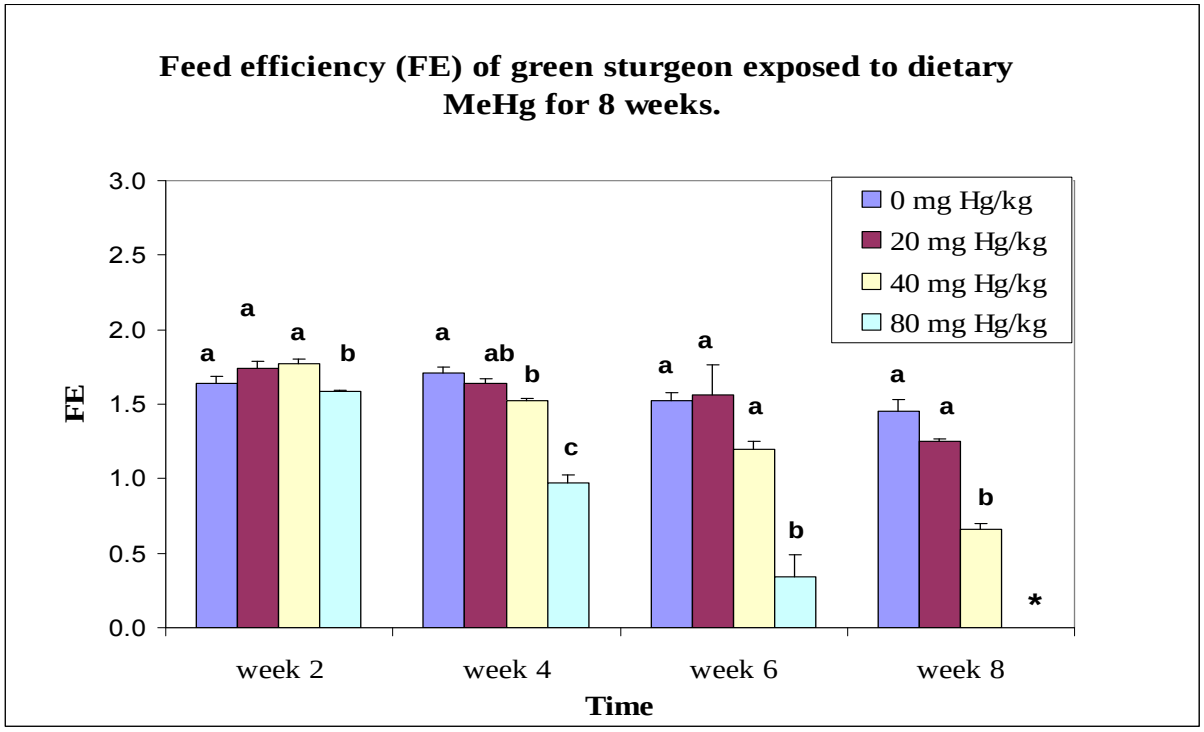


Fig. 16: * No data due to mortality and tissue sampling. Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

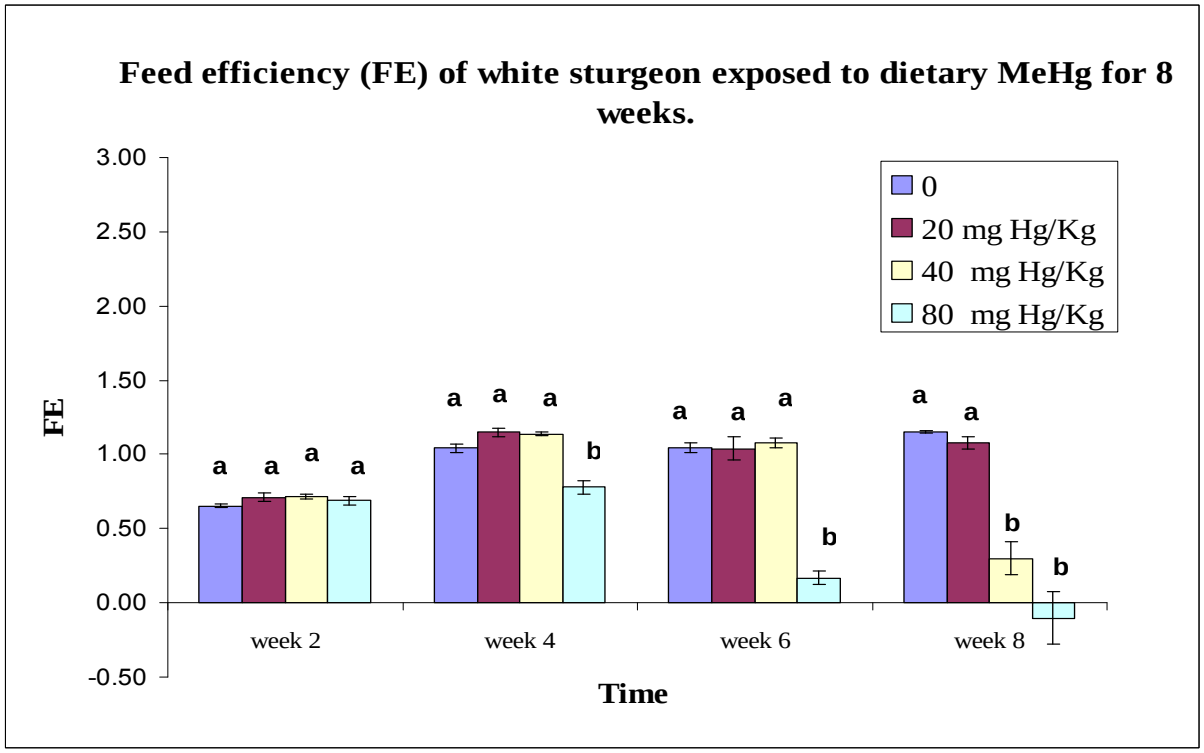


Fig. 17: Values represent mean ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

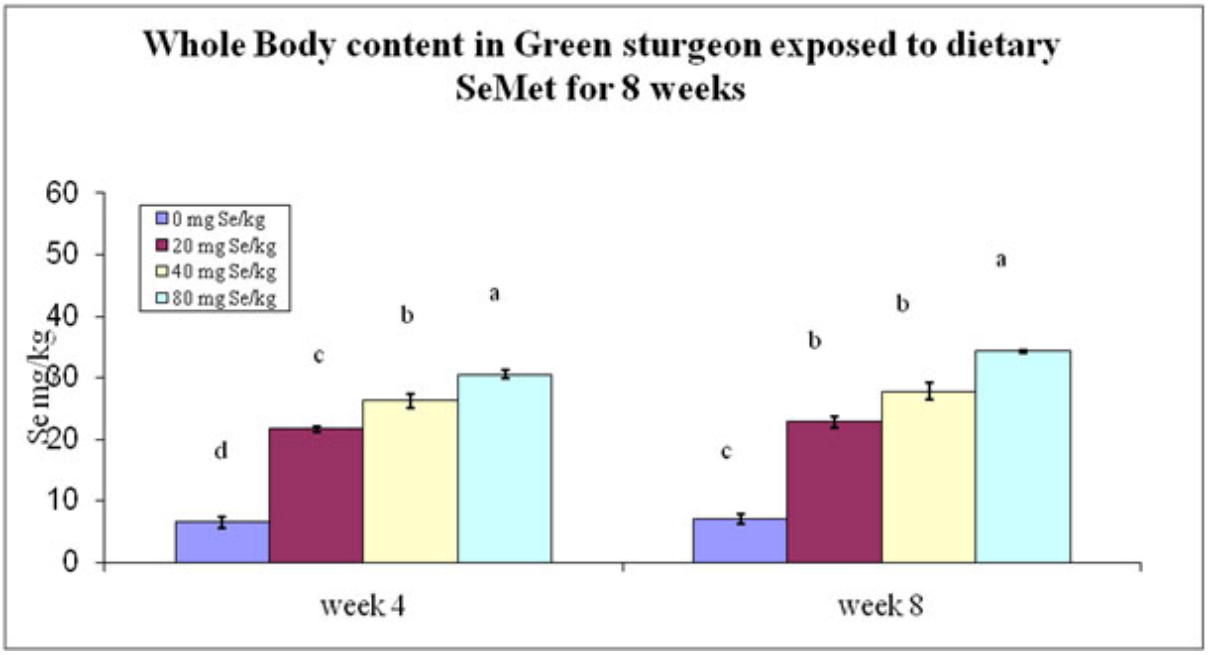


Fig. 18: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

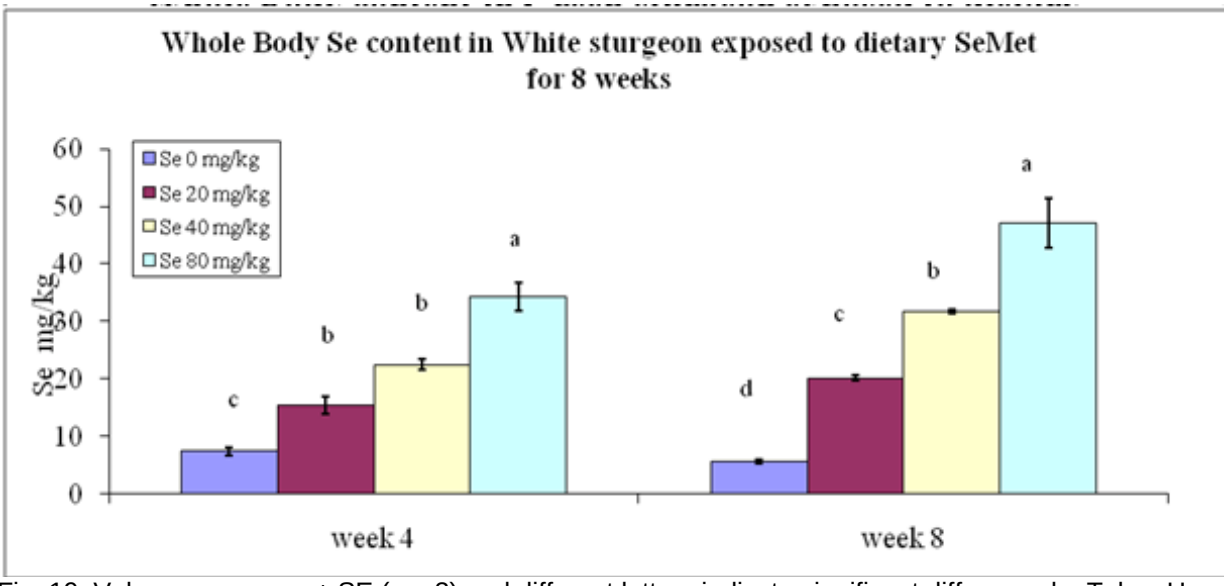


Fig. 19: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

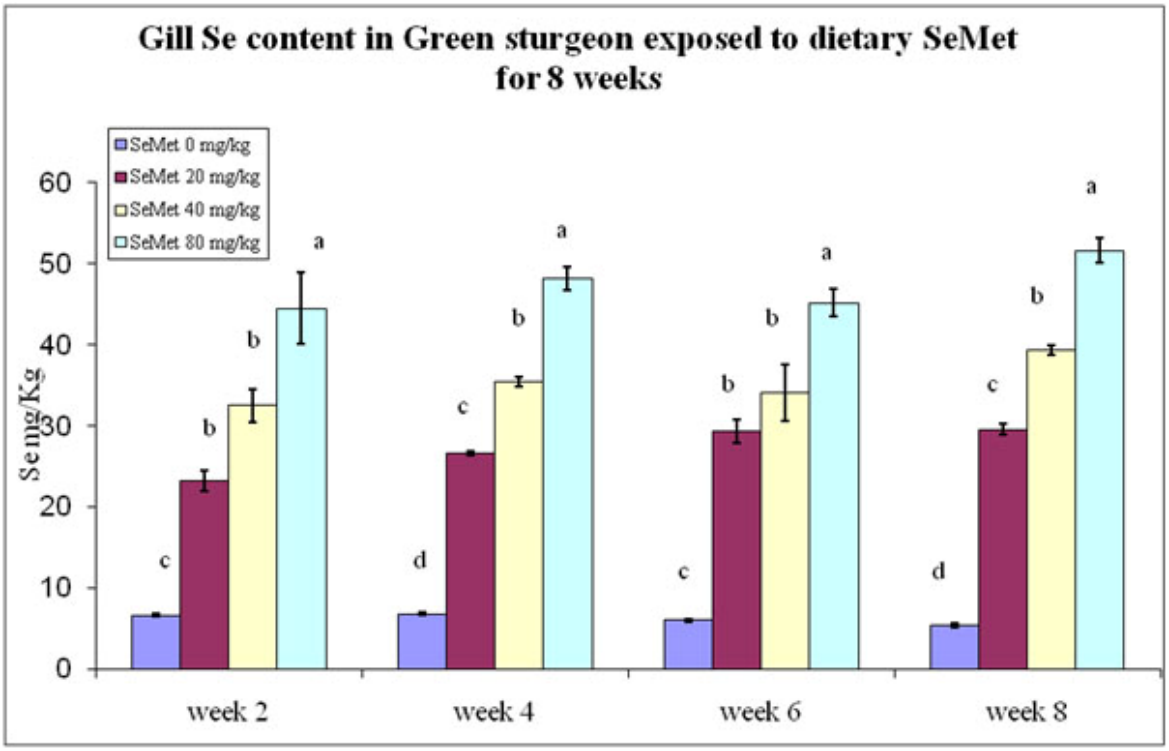


Fig. 20: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

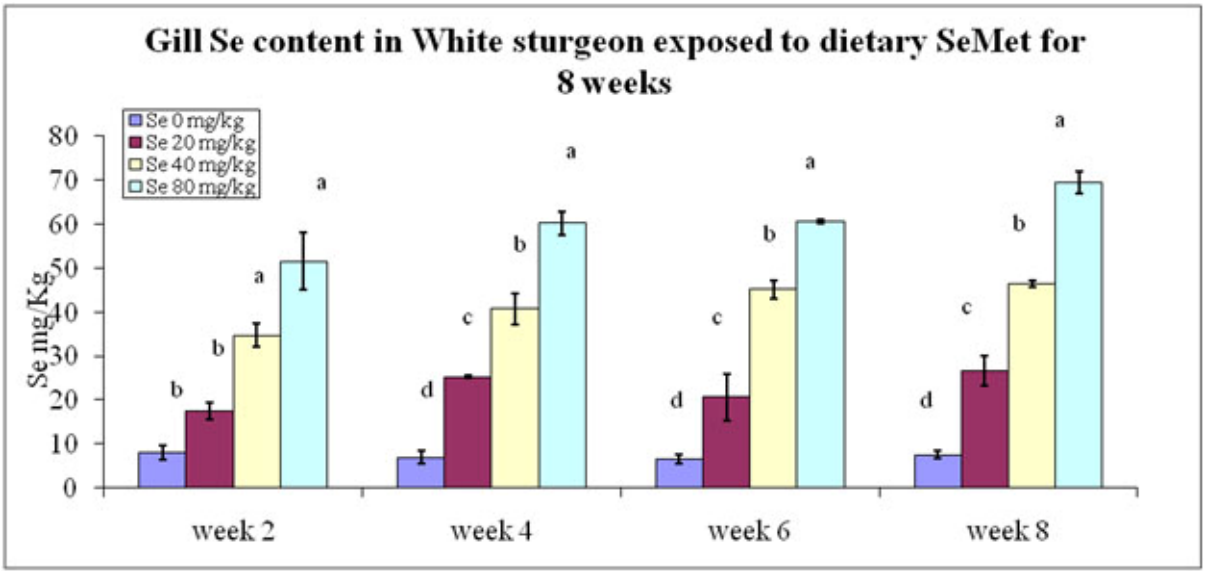


Fig. 21: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

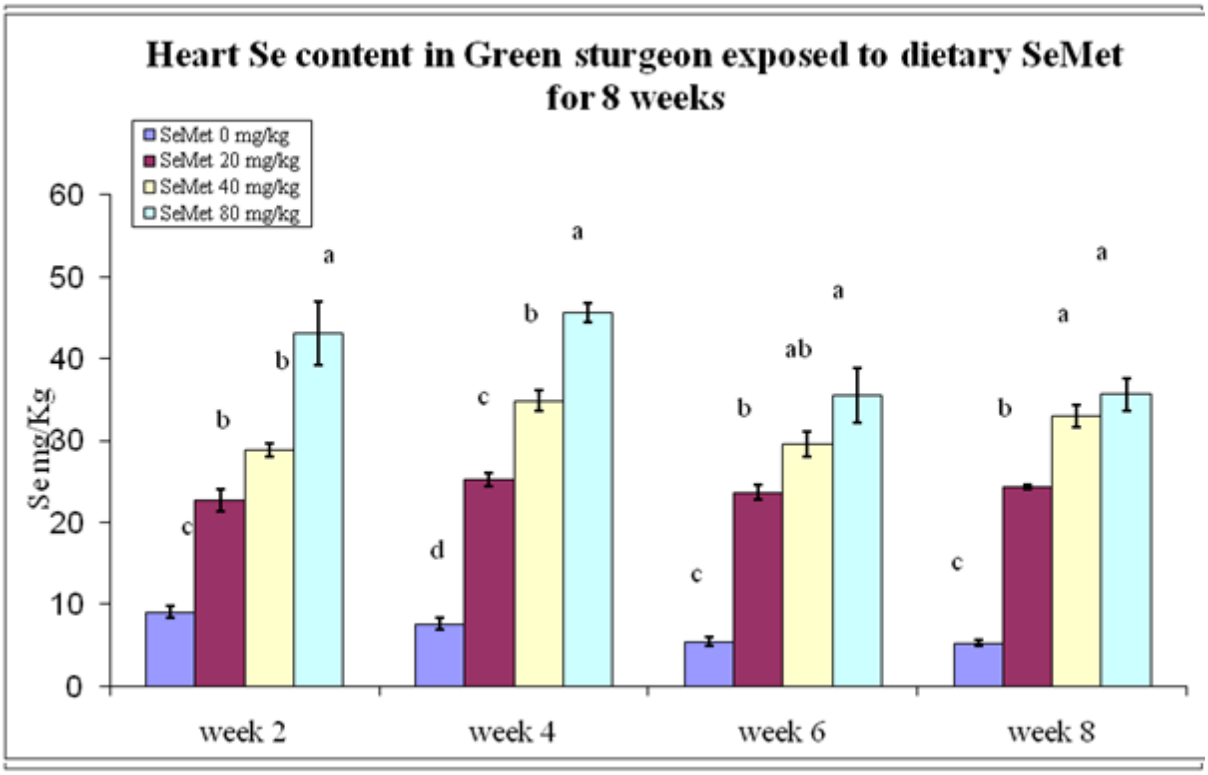


Fig. 22: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

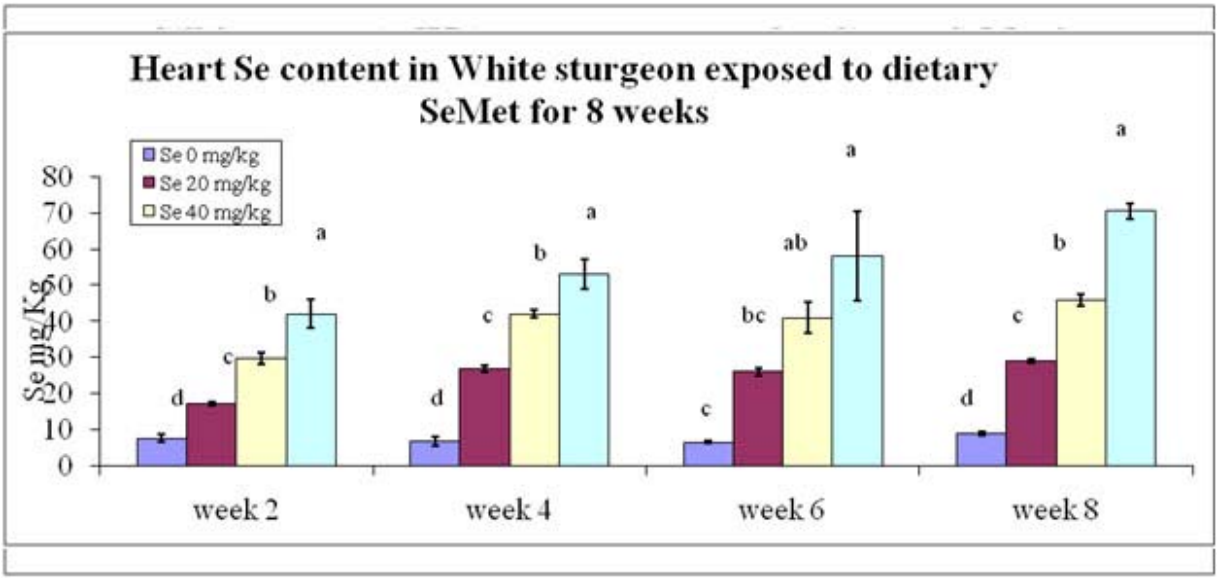


Fig. 23: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

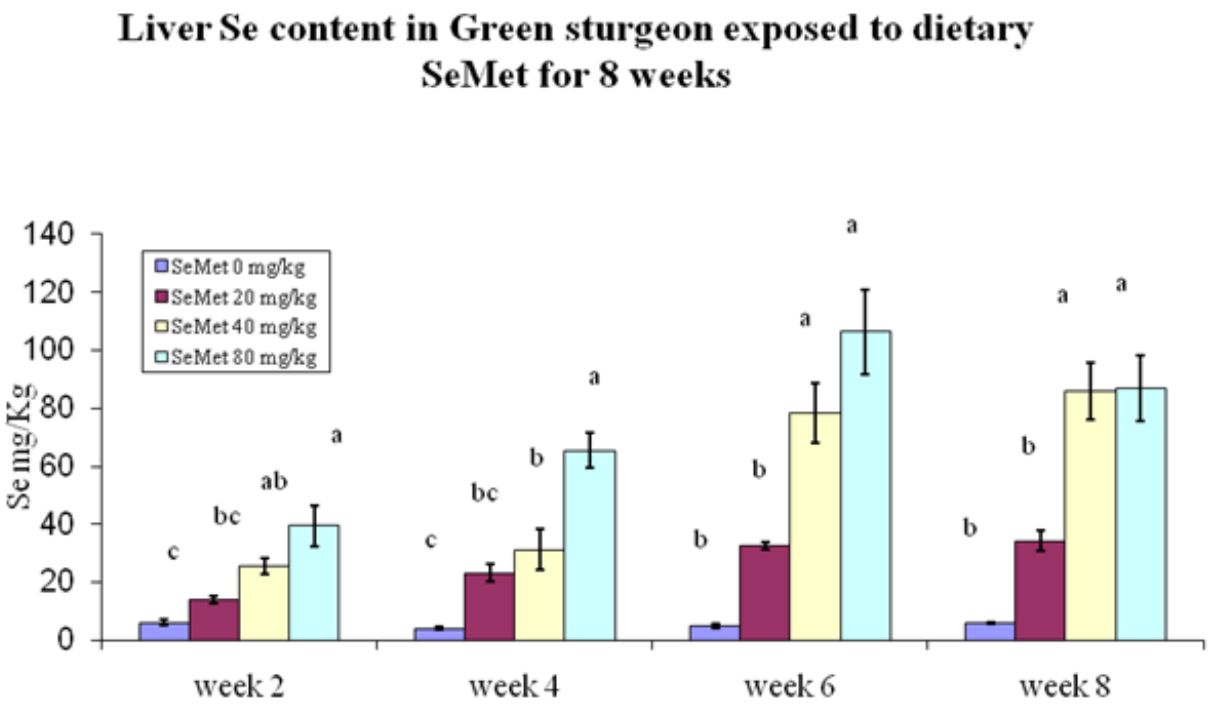


Fig. 24: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

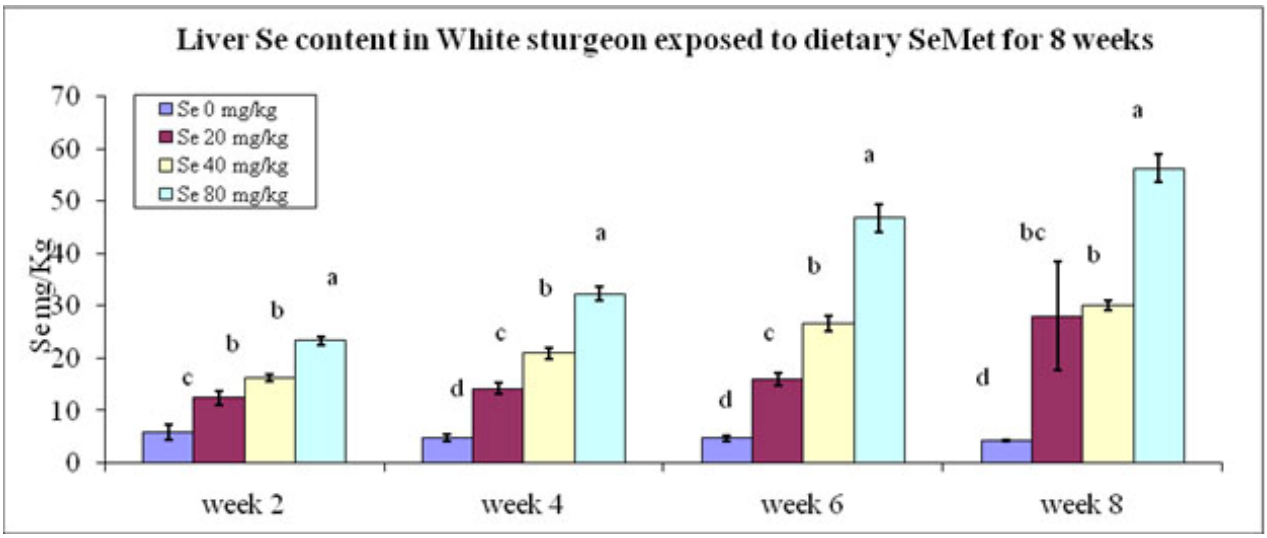


Fig. 25: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

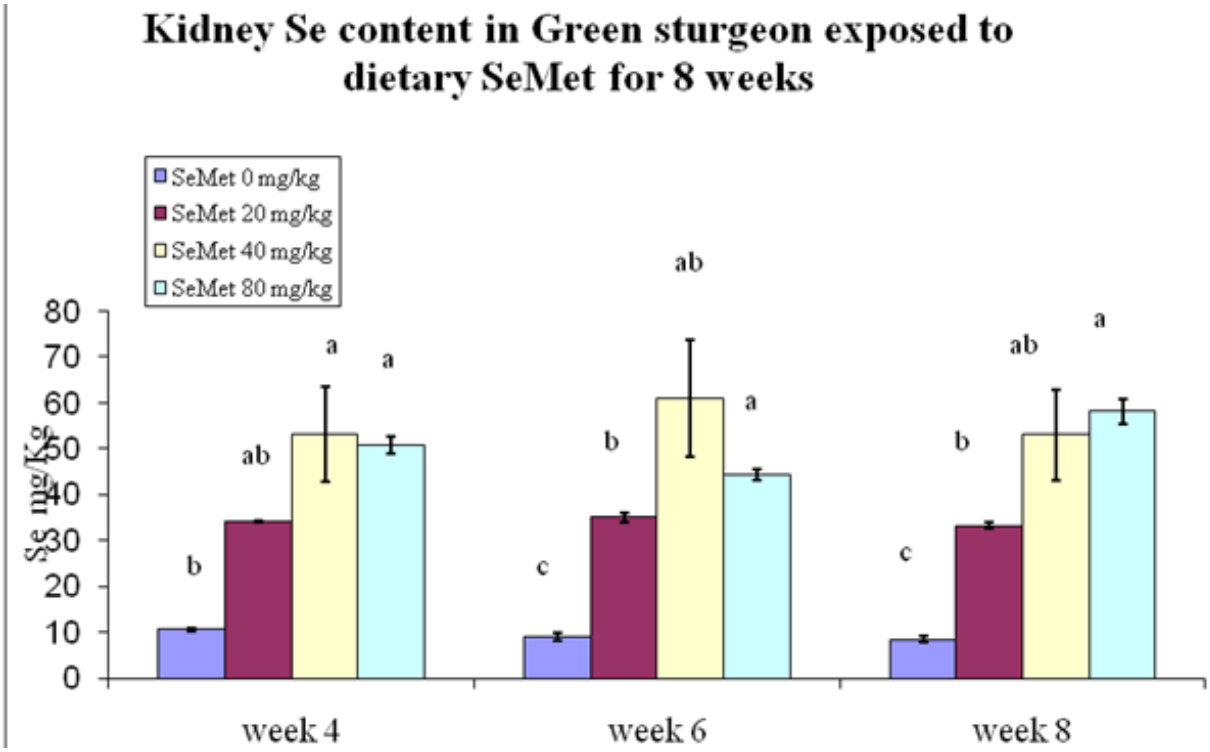


Fig. 26: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

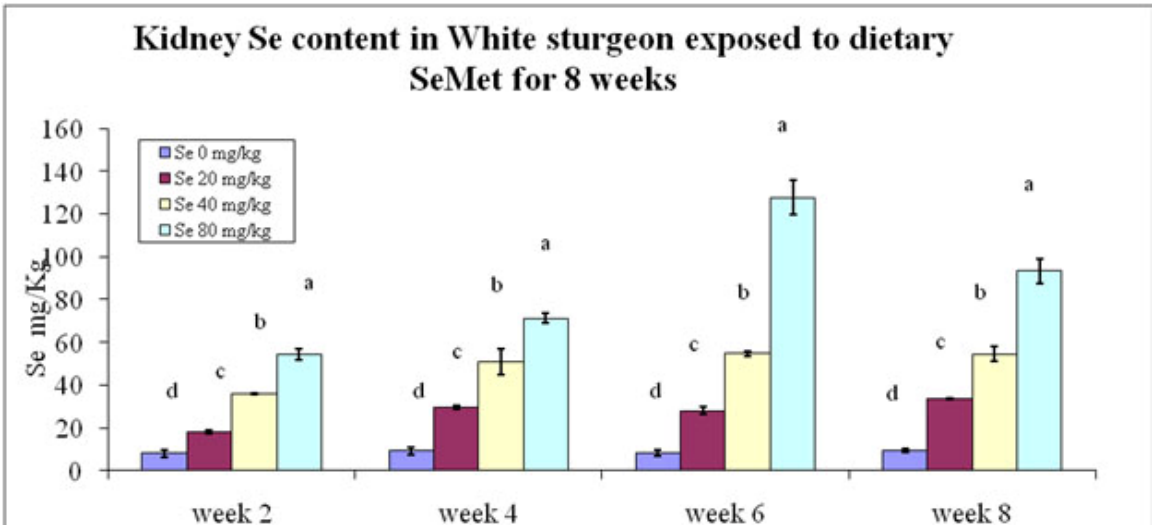


Fig. 27: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

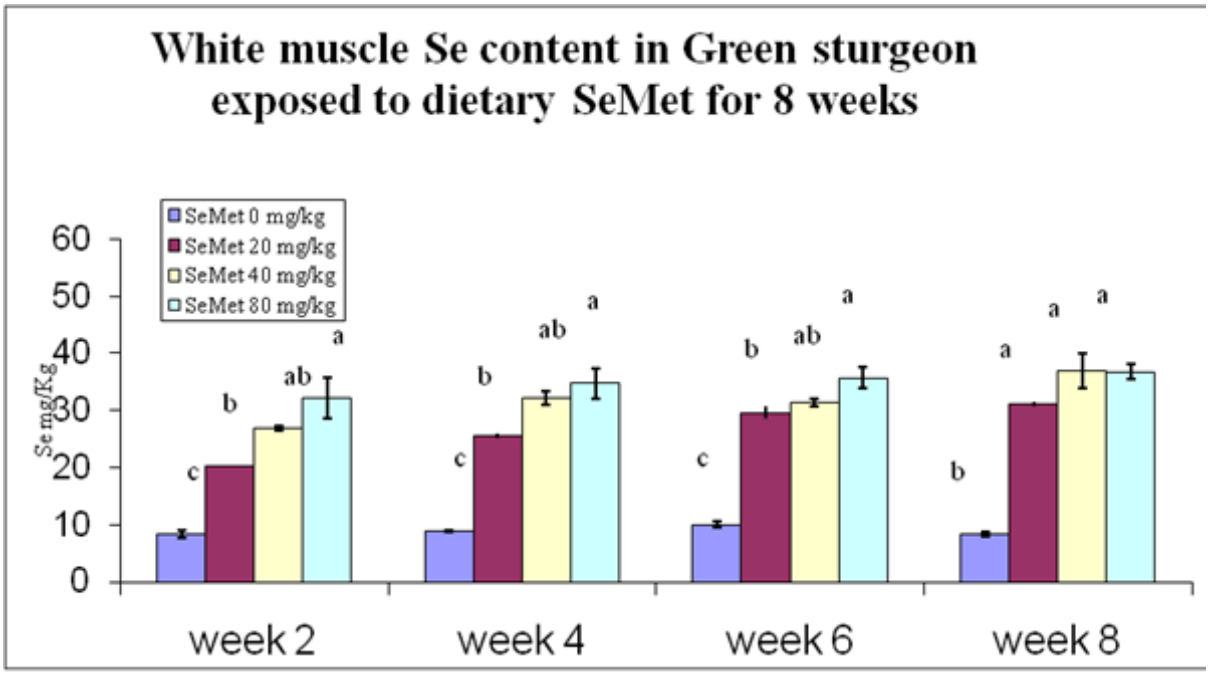


Fig. 28: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

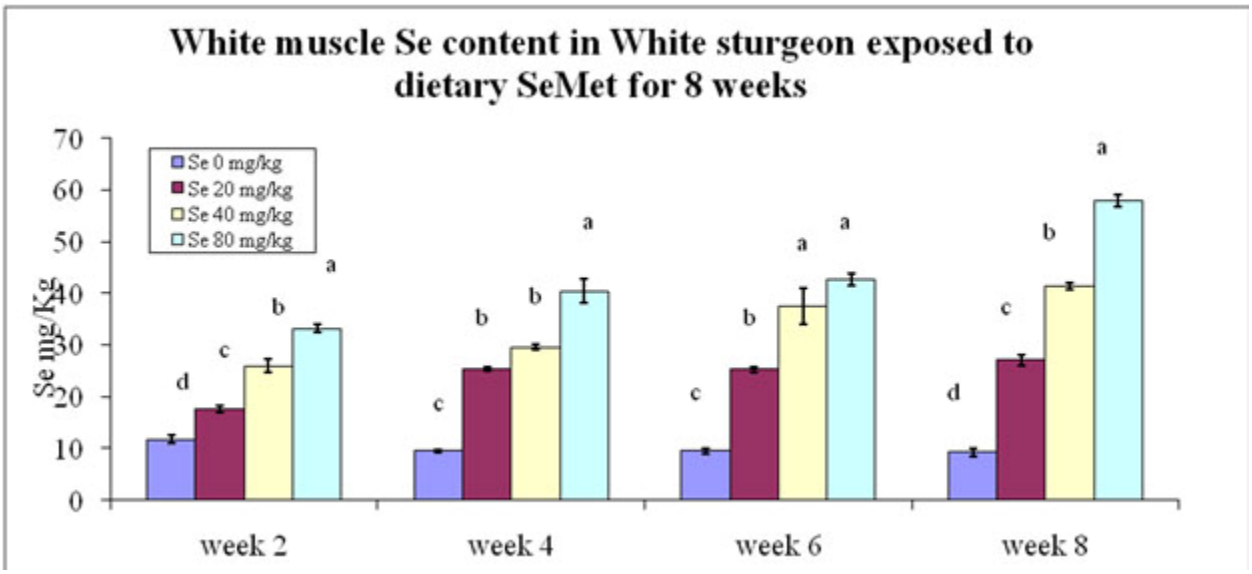


Fig. 29: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

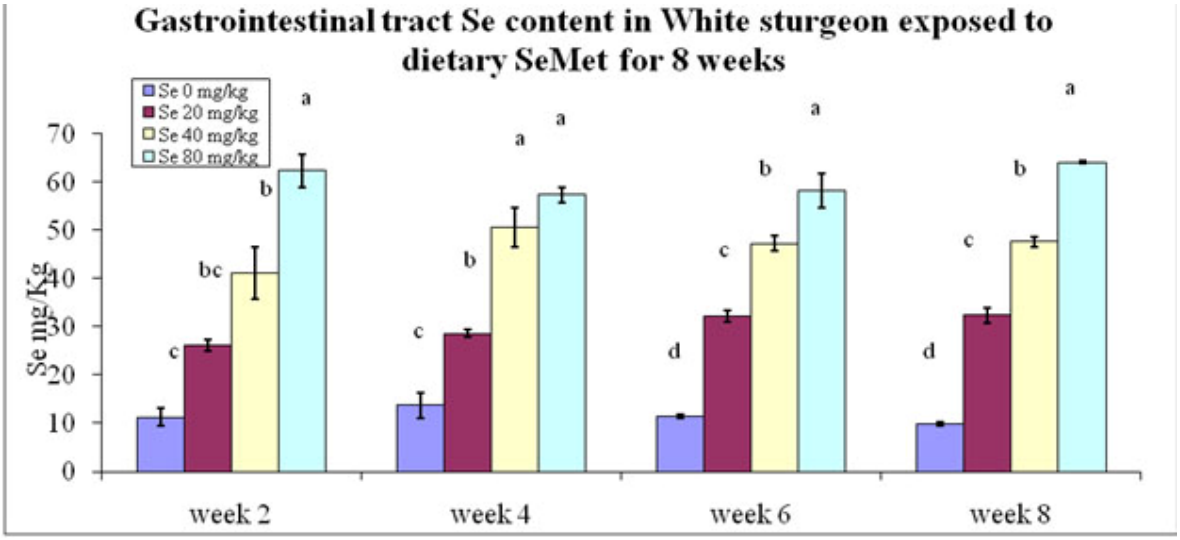


Fig. 30: Values are means ± SE (n = 3) and different letters indicate significant difference by Tukey Honestly Significant Different Test at P < 0.05.

We have also completed two growth trials determining the effects of feeding rate on heat shock protein expression in green sturgeon and the optimum feeding rate of white sturgeon. Information from these two studies is needed to determine the appropriate feeding rate in the growth trials in objective b mentioned above. They also provide baseline information on growth performances and heat shock protein expression when compare to results from task 4 Part I. We have submitted two abstracts to the World Aquaculture Society Meeting, May 19-24, 2008 in Busan, Korea (See abstracts below). Partial support from the CalFed will be acknowledged in our presentations and when we submit our manuscripts. We currently are analyzing the tissue samples obtained from the four growth trials, and these include the whole body proximate composition, tissue burden of Se or Hg, oxidative status of tissues, histopathological examination, etc.

- **LONG-TERM TOXICITY OF SELENIUM WITH AND WITHOUT ADDITIONAL METHYLMERCURY (TASK 3 OBJECTIVE B)**
    - Part I We have completed the growth trial of chronic toxicity of SeMet in green sturgeon and are currently conducting the growth trial of SeMet chronic toxicity in white sturgeon. The growth trial on chronic dietary SeMet toxicity in white sturgeon was completed. We have completed the Se tissue burden of green and white sturgeon exposed to chromic dietary SeMet toxicity.
    - We have completed the whole body proximate analyses of fish in these two studies (Table 1 & 2) since our last report. We are conducting histopathological examinations currently and we have already prepared over 300 samples (350 parafin blocks and 800 tissue slides) for histopathological examinations including fixation, dehydration, embedding in parafin, sectioning, and stainning with hematoxylin/eosin and PAS. We are currently investigating pathological lesions under compound microscopes and planning to complete the examination until the end of July 2010.
    - Part II We have completed the growth trial of chronic toxicity of MeHg in green sturgeon and we are currently conducting the growth trial of MeHg chronic toxicity in white sturgeon. The growth trial on chronic dietary MeHg toxicity in white sturgeon was also completed. Abstracts were presented at the 5th Biennial CALFED Science Conference.

growth trial on chronic dietary MeHg toxicity in white sturgeon was also completed. Abstracts were presented at the 5th Biennial CALFED Science Conference.

- We have completed the whole body proximate analyses of fish in these two studies (Table 3 & 4) since our last report. We have also completed the tissue burdens of mercurry in the two experiments (Table 5 & 6). We are conducting histopathological examinations currently and we have already prepared about 300 samples (350 parafin blocks and 800 tissue slides) for histopathological examinations including fixation, dehydration, embedding in parafin, sectioning, and stainning with hematoxylin/eosin and PAS. We are currently investigating pathological lesions under compound microscopes and planning to complete the examination until the end of July 2010.

We have also completed two growth trials determining the effects of feeding rate on heat shock protein expression in green sturgeon and the optimum feeding rate of white sturgeon. Information from these two studies is needed to determine the appropriate feeding rate in the four growth trials in Part I and II mentioned above. They also provide baseline information on growth performances and heat shock protein expression when compared to results from task 4 Part I.

OPTIMUM FEEDING RATES OF JUVENILE WHITE STURGEON FROM 53 TO 88 DAYS-POST-HATCH

Five 1-week trials were conducted to determine the effects of feeding rates on growth performances and body proximate compositions of white sturgeon juvenile from 53 to 88 days-post-hatch which corresponded to 6 to 10 weeks after initiation of feeding. Six feeding rates with four replications were used in the five trials and the feeding rates were 3.0-8.0%, 2.0-7.0%, 1.0-6.0%, 1.0-6.0% body weight (bw)/day (d) (in 1% increment) for the first four trials whereas feeding rates were 0.8-4.0% bw/d at 0.8% increment for the last trial. Number of fish per replicate tanks and the initial body weight of the fish were 60, 45, 30, 30, and 21 and 2.8±0.1, 4.5±0.4, 8.5±0.7, 10.0±0.7, and 13.4±1.0 g, respectively, for the five growth trials. The juveniles were kept at 18-19ºC in small circular tanks (66 cm diameter and xx cm water height) and fed a commercial salmonid soft-moist feed using automatic feeders. The proximate composition of the feed was xx% moisture, xx% crude protein, xx% lipid, and xx% ash. Mortality rates in the trials were zero or very low and unrelated to feeding rates. Specific growth rates (SGR), feed efficiencies, and body compositions were significantly ($P<0.05$) affected by the feeding rates. Broken-line analysis on SGR indicated the optimum feeding rates of white sturgeon juvenile were 6.5±0.4, 4.8±0.2, 4.2±0.1, 3.8±0.2, and 3.8±0.1 % bw/d for the 6, 7, 8, 9, and 10 weeks after initiation of feeding, respectively.

EFFECTS OF FEEDING RATES ON GROWTH PERFORMANCES AND RESPONSES TO TEMPERATURE STRESS IN JUVENILE GREEN STURGEON

Green sturgeon (*Acipenser medirostris*) is listed as a species of special concern by the California state and a threatened species by the federal government of USA. However, information on the nutrition and feeding of green sturgeon is limited. The objective of the current study is to estimate the optimal feeding rates and to investigate the effect of feeding rates on their body compositions, RNA/DNA, and stress response to temperature challenge in juvenile green sturgeon.

Four growth trials (one week/trial) with six feeding rates each were carried out on green sturgeon at 5, 6, 7, and 8 weeks after initiation of exogenous feeding. The six feeding rates (% body weight day^{-1}) were 2.5-15.0 at 2.5 increment for trial I, 1.25-7.50 at 1.25 increment for trial II, and 2.0-7.0 at 1.0

increment for trials III and IV. Four replicates with 50 fish per replicate for trials I, II, III and 30 fish per replicate for trial IV were randomly assigned to each feeding rates. Average initial body weights of the juveniles were 1.6, 2.6, 5.1, and 7.5 g, respectively, for trials I–IV. The fish were raised at 18.5°C in circular tanks and fed a commercial salmonid soft-moist diet (moisture, 14%; protein, 51%; lipid, 12%). At the end of each trial, 5 fish from each tank were subjected to a temperature shock at 28ºC for 4 hours. Stress responses were evaluated based on heat shock protein (hsp70) expressions in liver, muscle and heart before and after the fish were exposed to the temperature shock.

Mortalities were low and unrelated to feeding rates. Specific growth rate and feed efficiency were significantly ($P<0.05$) affected by the different feeding rates. The optimal feeding rates were 7.1, 5.8, 4.3 and 5.2% body weight day^{-1}, respectively, for fish during the 5th, 6th, 7th, and 8th weeks based on broken line analysis on specific growth rate. Lower feeding rate generally inhibited hsp70 expression in tissue both at the basal and induction levels. Heat shock significantly induced hsp70 expression in tissues and the induction was shown to be tissue and age dependent. Our results indicated that optimal feeding is important not only for fish growth but also for their tolerance to environmental stress. Data on proximate composition and RNA/DNA will be discussed.

Trial	Feeding rate (%)	Initial weight (g)	Optimal feeding rate (%)
I (5th week)	2.5, 5.0, 7.5, 10.0, 12.5, and 15	1.6	7.1
II (6th week)	1.25, 2.50, 3.75, 5.0, 6.25, and 7.5	2.6	5.8
III (7th week)	2.0, 3.0, 4.0, 5.0, 6.0, and 7.0	5.1	4.3
IV (8th week)	2.0, 3.0, 4.0, 5.0, 6.0, and 7.0	7.5	5.2

Table 5: Optimal feeding rate

- **PROJECT STATUS:** Task 3 was completed and resulted in the following publications that describe the outcome of this task in more detail**:**
    - **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.**

TASK 4

- **TASKS COMPLETED:**
    - Abstract for presentation of results of this study have been submitted and accepted for CALFED Science Conference (see appendix).
    - Muscle tissue determinations of total Hg (representative of MeHg concentrations) were completed for green and white sturgeon samples obtained from physiology experiments.
    - Preparation of muscle tissue samples for selenium analysis by DANR Lab, UC Davis.
    - Instrumentation for analysis of plasma electrolytes, osmolality, as well as liver glycogen determinations have been serviced and the analysis of these samples is currently being conducted.
    - Optomotor video tapes are currently being reviewed and scored for both green and white sturgeon performance.

- Tissue samples for proteomic determinations have been transferred to the appropriate laboratory and staff..
- Setup experimental apparatus and instrumentation in preparation for conclusion of white sturgeon growth trials: projected end of growth trial October 25, 2007.
- Completed physiology experiments on green sturgeon post-MeHg & selenium feeding/growth trials
- Decision to include plasma collection and analysis for plasma electrolytes and osmolality, in addition to tissue sample collection
- Completed construction and test trials of respirometers, swimming flume, thermal-stress experimental set-up, salinity-stress experimental set-up.
- Prepared supplies and reagents for tissue analysis of mercury content.

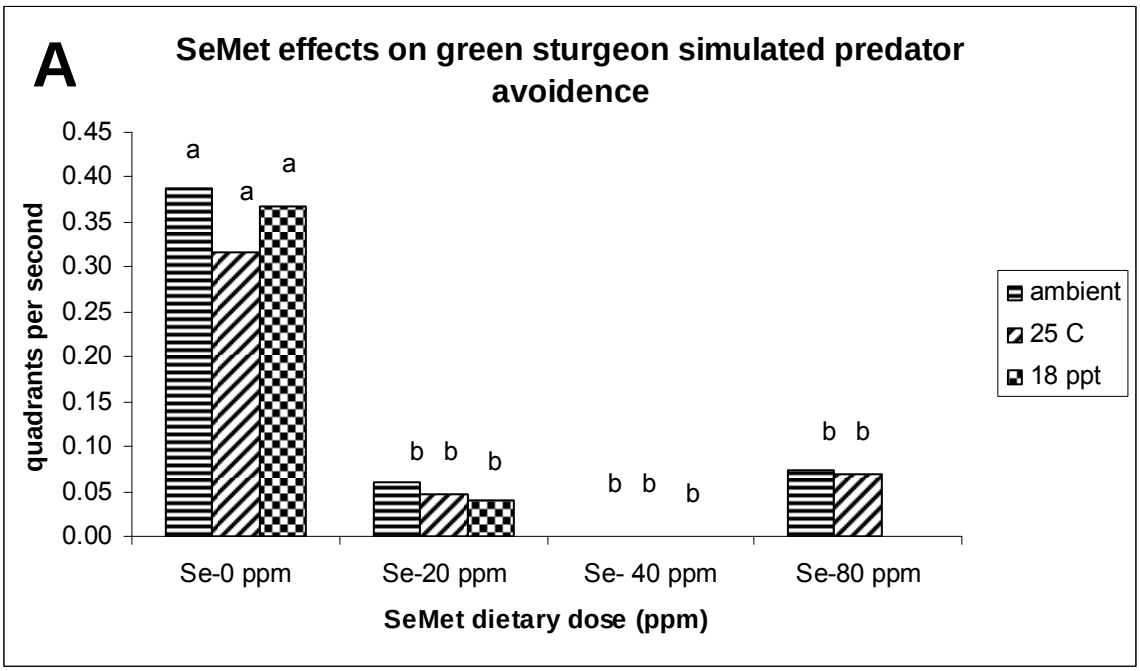
- Task 4 experimental trials with white sturgeon juveniles were completed. We obtained the juvenile white sturgeon exposed to several doses of either methylmercury (MeHg) or selenomethionine (SeMet) from Task 3 growth experiments. These fish were used to determine effects of MeHg or SeMet on their simulated predator avoidance ability, swimming performance, optomotor response, and metabolic rates (routine and active). In addition to these bioenergetic measures under Task 3 growth-experiment conditions (19º C & 0 ‰ salinity), we also determined the effects of elevated temperatures (to 25º C) and salinity (to 18 ‰) on the these bioenergetic measures. In these experiments, white sturgeon juveniles acclimated to19º C were subjected to either an increase in temperature (to 25º C) or salinity (to 18 ‰), over a 12-hour period. White sturgeon juveniles were then held under these conditions for the duration of the bioenergetic experiments (≈ 56 hours).
- At the experiments' conclusions tissue (muscle, liver, and gill) samples were obtained for Task 6 proteomic determinations, Task 4 liver glycogen measures, and determinations of tissue burdens of SeMet and MeHg. Also plasma samples were collected, from the 18 ‰ challenged fish, for plasma ion (Cl-,, Na+, K+) and osmolality determinations.
- Analyses of tissue samples from white and green sturgeon Task 4 experiments currently are being conducted for the determination of body burdens of MeHg (via total Hg) and SeMet (via total Se) to examine the relationships between body burdens and bioenergetic performance measures.
- Analytical procedures for liver glycogen determinations are being optimized for sturgeon liver tissue samples with characterization of a pooled-sturgeon liver sample from non-experimental fish, for use as a standard.
- Data reduction, scoring, and analyses for 2007 experiments were conducted for optomotor and metabolic experiments.
- All treated green sturgeon performed poorly when compared to controls
- Significant differences in critical swimming velocity in treated fish when compared to controls

- Our experiments have already shown that green sturgeon are much more sensitive to selenium toxicity compared to white sturgeon. The levels of selenium that significantly and adversely affect green sturgeon are physiologically relevant in that they are in the range observed in the SF Bay Delta. This is not the case for methylmercury and was entirely unexpected. Thus, we hypothesize that the selective decline of green sturgeon relative to white sturgeon in the SF Bay delta ecosystem is primarily a result of selenium toxicity. We are now working on rigorously testing this hypothesis, which, if confirmed would have specific and manageable implications on what kind of measures would be required for green sturgeon conservation.
- Dr. Brian Sardella who received a postdoctoral fellowship from CALFED and works in the Kültz-Lab has conducted experiments to determine c**ritical Thermal Maxima of Green Sturgeon;** Critical thermal maxima were measured for green sturgeon acclimated to three salinities (0, 10, 24 ppt). This experiment produced data on the CTMax of green sturgeon as well as the changes in

- Dr. Brian Sardella also performed an experiment to simulate the effects of higher precipitation rate that is predicted to result from global climate change. Green sturgeon that were acclimated to 24 ppt salinity were exposed to a flush of freshwater that resulted in a 0 ppt salinity after 3 h. The effects of the rapid salinity drop were monitored over 24 h. This experiment was repeated with fish exposed to 15 ppt. The flush of freshwater resulted in changes in plasma ion status that did not recover within the 24 h period. Gill, muscle, liver, and intestine were all collected in order to later assess the stress response in these animals. Gills were also preserved for immunohistochemistry.

Task 4 Notes/Other

- Analysis of Task 4 simulated predator avoidance and swimming performance revealed an unanticipated sensitivity of green sturgeon juveniles to dietary SeMet. Although, in some experiments, animal numbers were reduced due to mortalities from the unanticipated high sensitivity green sturgeon exhibited towards SeMet exposure, a clear trend of adverse, dose-response effects as noted. This, while green sturgeon control (0 ppm additional SeMet) fish showed no significant differences among the environmental challenges, all other dietary exposures (20, 40, 80 ppm SeMet) generated significant declines in both simulated predator avoidance and swimming performance in green sturgeon. Tellingly, we were unable to determine a no effect concentration (NOEC), for SeMet, for juvenile green sturgeon exposure to dietary SeMet. These data show that juvenile green sturgeon bioenergetics are being compromised, from dietary selenium exposures, at environmentally relevant concentrations found in the SFBD. (Task 4: Figure 1 A & B).
- Task 4 data on white sturgeon simulated predator avoidance and swimming performance showed a trend of declining performance with increasing SeMet exposure. Simulated predator avoidance trial data show significant declines in performance at concentrations above 40 ppm when combined with additional stressors (i.e., increased temperature and salinity). These results demonstrate that white sturgeon juveniles have a much higher tolerance to SeMet than do green sturgeon juveniles except perhaps, after exposure to 18 ‰ salinity.. (Task 4 Figure 2 A & B)
- Task 4 MeHg data show similar responses in both white and green sturgeon juveniles with a non-significant trend of green sturgeon juveniles exhibiting slightly greater sensitivity. However, both of these species apparently tolerate MeHg concentrations far above those found in the SFBD. Note that this is in direct contrast to the environmentally relevant concentrations of SeMet that have been found to adversely impact green sturgeon. (Task 4 Figure 3 A&B; Task 4 Figure 4 A&B)
- From our bioenergetic data, it appears that we will be unable to use white sturgeon juveniles as a direct surrogate for green sturgeon juveniles, a species that is currently listed as threatened, in conducting SeMet toxicity testing, regarding responses to environmental stressors found in the SFBD.



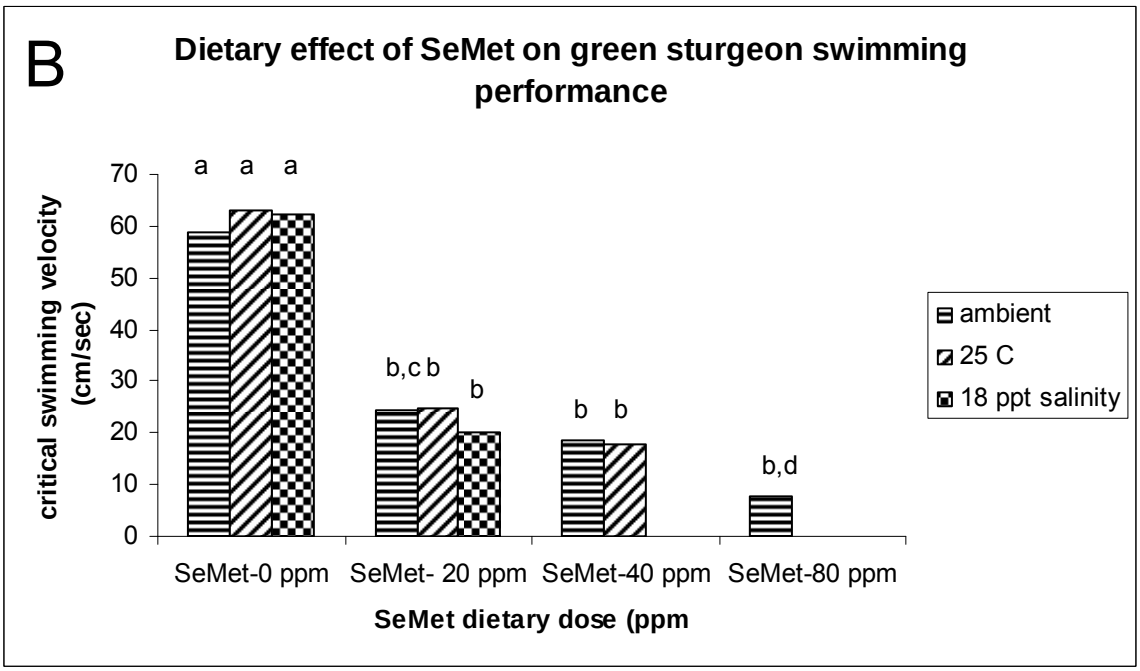
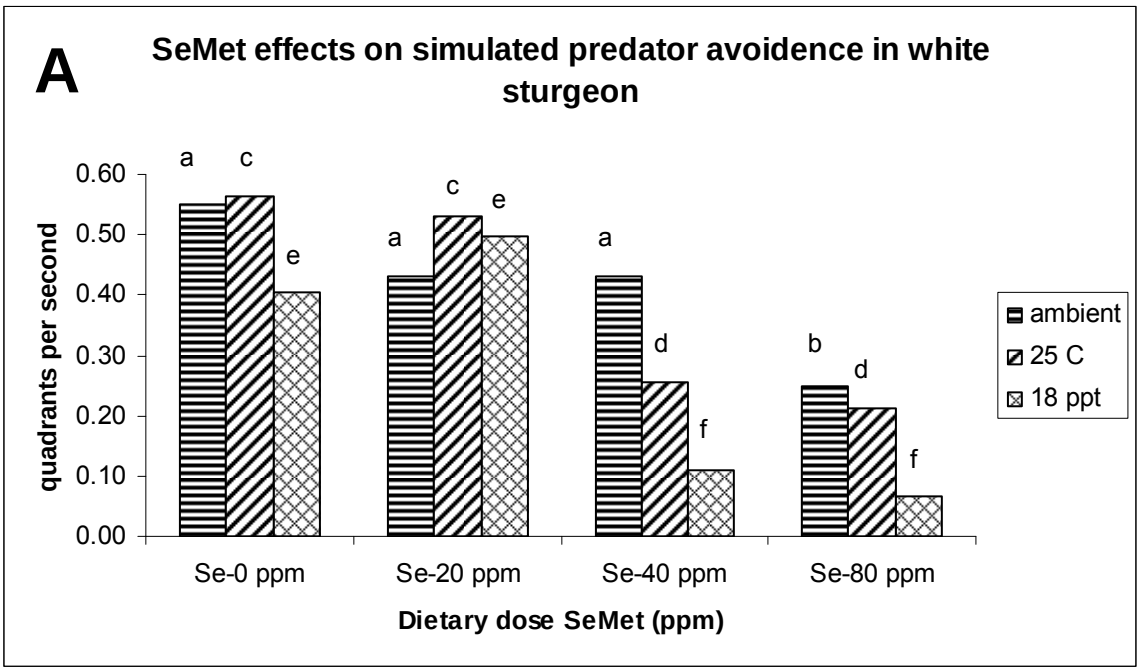


Fig. 31: Marked decrease in the response of green sturgeon to a simulated predator. All treatment groups differed significantly from the control group across all experimental conditions (ambient [19C], 25 C, and 18 ppt salinity). In columns showing letters, the data were so low that a histogram bar is indistinguishable from the x-axis. B) There were significant differences between the control group treatments and all SeMet treatment groups (20, 40, & 80 ppm SeMet).There was a significant difference between the ambient SeMet-20 ppm group and the ambient SeMet-80 ppm group.



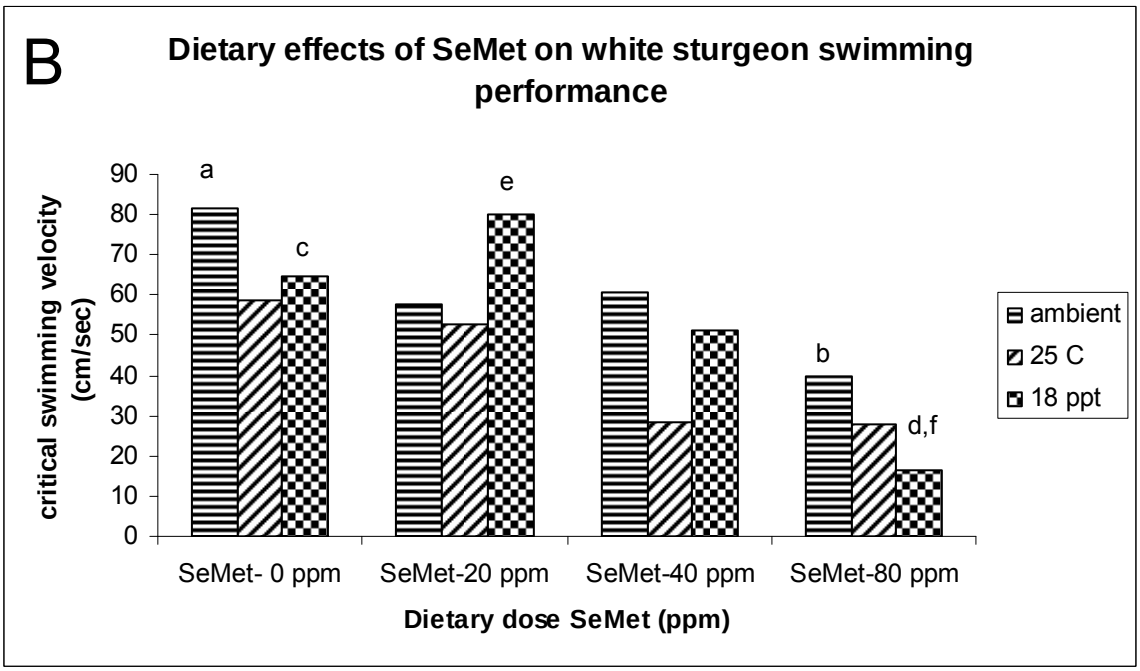
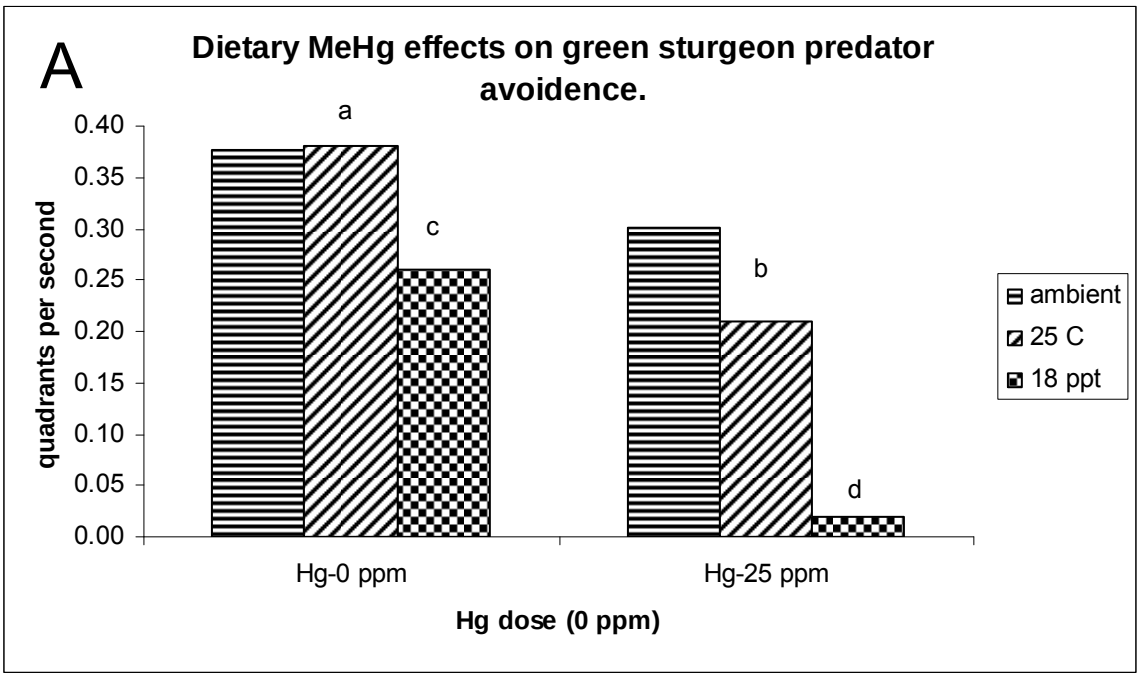


Fig. 32: No significant differences between the ambient control group, 20 ppm SeMet, and 40 ppm SeMet with significant differences found in the 80 ppm SeMet treatment. There were no significant differences in the 25ºC control group and the 20 ppm SeMet group with significant differences found between the 40 and 80 ppm SeMet groups. There were no significant differences found between the 18 ppt control and 18 ppt 20 ppm SeMet groups but significant differences were observed between the 40 and 80 ppm Se Met groups at 18 ppt. B) Significant differences between the ambient control group and the SeMet-80 ppm group. There were also significant differences between the 18 ppt SeMet control group and the 80 ppm SeMet group as well as the 18 ppt 20 ppm SeMet group and the 18 ppt SeMet-80 ppm group.



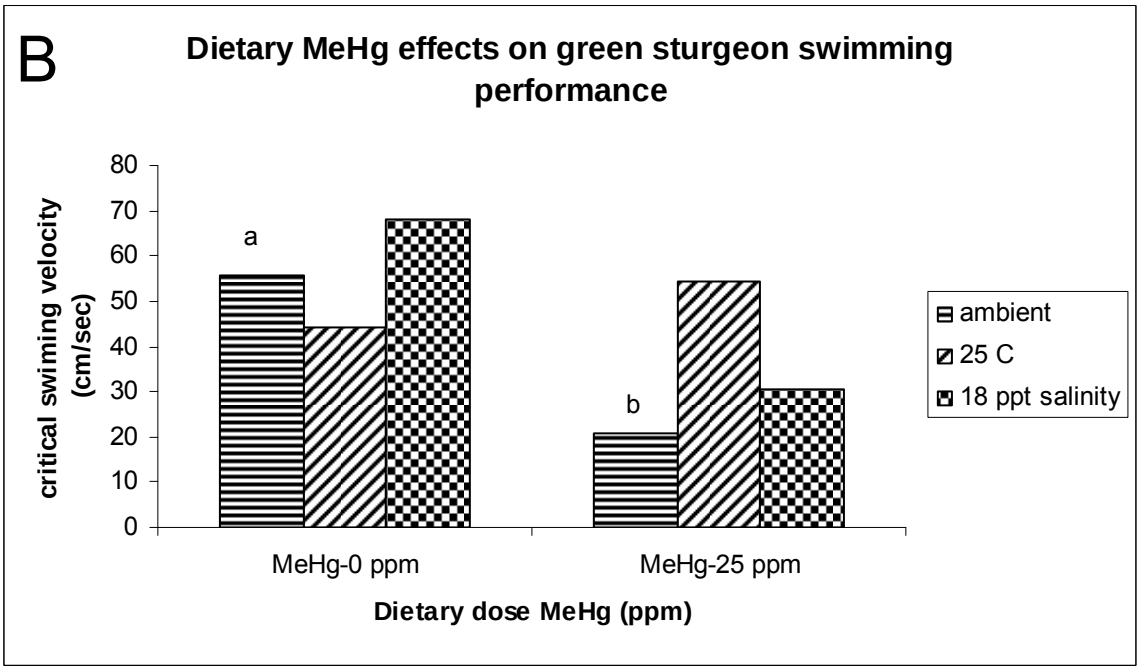
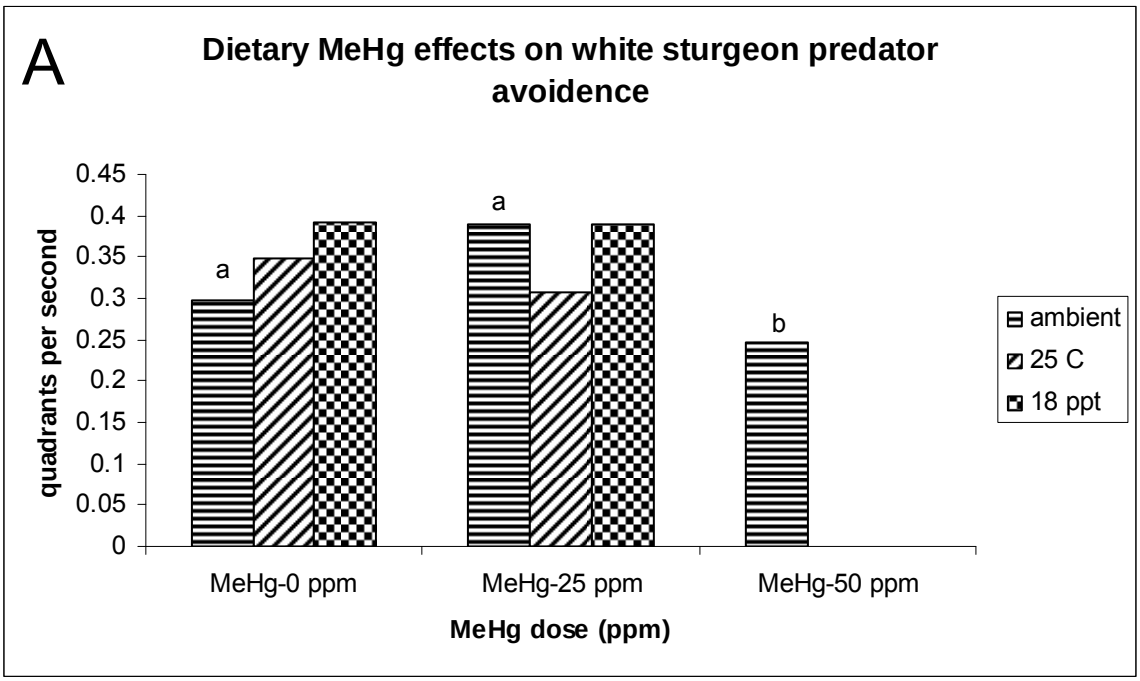


Fig. 33: Significant differences were observed between the 25º C controls and the 25º C MeHg-25 ppm. A significant difference was also noted between the 18 ppt control group and the 18 ppt MeHg-25 ppm group. B) There was a significant difference between the ambient control group and the MeHg-25 ppm group. A slight significant difference was noted between the 18 ppt treatment group and the MeHg-25 ppm exposed group.



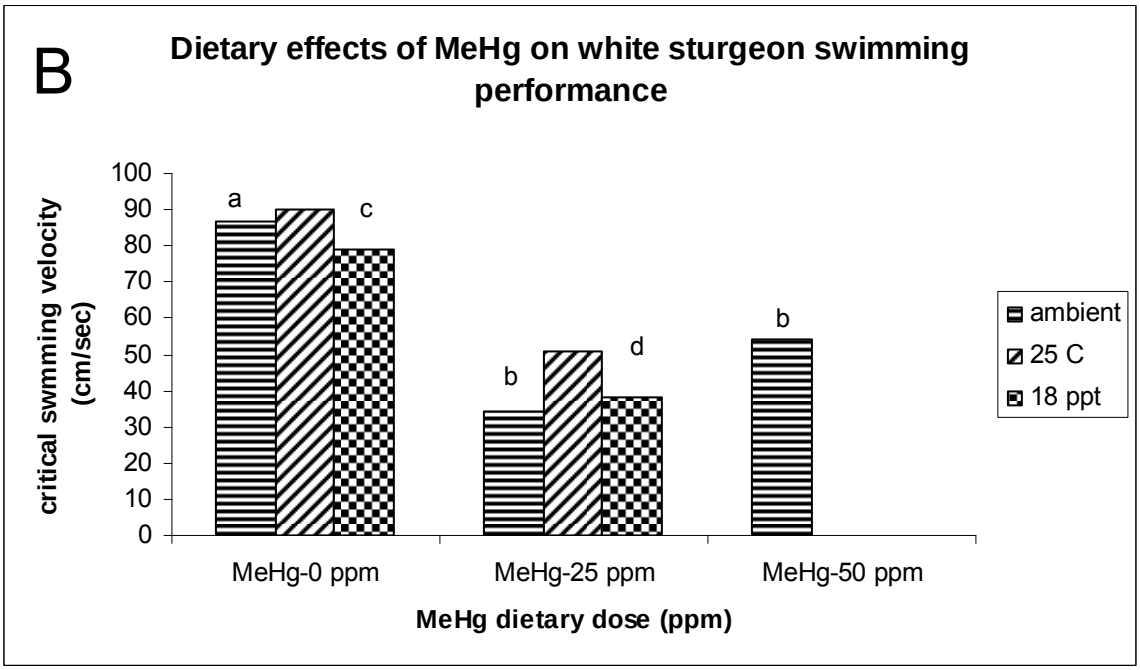


Fig. 34: Significant differences were noted between ambient control and MeHg-25 ppm and the ambient MeHg-50 ppm group. B) Significant differences were noted between ambient controls and the MeHg exposed fishs swimming performance. There was a significant difference between the control 18 ppt group and the MeHg-25 ppm treatment group.

PROJECT STATUS: The experiments under task 4 have been completed. The following publication resulted from this task:

- **Allen, P.J., Cech. J.J., Jr., Kültz, D. (2009) Mechanisms of seawater acclimation in a primitive, anadromous fish, the green sturgeon. J. Comp. Physiol. B 179: 903-920.**

TASK 5

- Biopsies of white muscle and gill were collected from 45 white sturgeon from three locations in the Sacramento River at Knights Landing, Suisun Bay, and San Pablo Bay in collaboration with CDFG.
- Biopsies of white muscle and gill were collected from these sturgeon collaboration with CDFG. For some fish only gill biopsies were collected.
- More field collections were carried out in three different areas of Suisun Bay and San Pablo Bay in collaboration with CDFG and gill biopsies of white sturgeon prepared for further analysis.
- Collection of biopsies from white sturgeon is straight-forward but collection of biopsies from wild green sturgeon was not feasible since very few green sturgeon are encountered by salvaging operations of CDFG and those few that are encountered are likely stressed due to high selenium in the SF Bay Delta (according to our initial results in lab acclimation experiments); thus, any gill or muscle biopsies would further weaken these animals and are not advisable. Fortunately, we were able to spawn and rear green sturgeon in captivity for the first.

- **PROJECT STATUS:** Sample collection for Task 5 was completed but the analysis of these samples is incomplete. The samples are stored at -80°C and we will pursue their analysis when alternative funds and personnel become available in the future.

TASK 6

- Optimization of protein extraction and two-dimensional protein gels for sturgeon larvae provided by task 2.
- Installation and fitting of nanoUPLC-MS spotter setup for preparing sturgeon proteins for mass spectrometry analysis; This equipment was custom-configured and tuned for two-dimensional liquid chromatography and MALDI spotting of protein digests originating from sturgeon larvae;
- Optimization of in-solution tryptic digestion of proteins extracted from sturgeon larvae;
- Calibration of nanoUPLC-MS spotter for peptide digests from sturgeon larvae samples;
- **Proteomic Analysis of Temperature and Salinity Stress in White Sturgeon:**
    - Six groups of white sturgeon were exposed to a range of temperatures and salinities. Sturgeon were acclimated to three salinities (0, 10, and 24 ppt) at both control (18°C) and experimental temperature (25°C) for one week. Tissues were sampled and frozen for use in two-dimensional gel proteomic analysis. Sturgeon gills have been processed for 2D gels, and the first dimension separation has been conducted, second dimension separation is now being conducted.
- **Development of a potential screening test for temperature/salinity stress.**
    - Green sturgeon that had been acclimated to three salinities (0, 10, and 24 ppt) were then exposed to 25, 50, and 75% of the salinity-based CTMax for one week. There was complete mortality in the 75% groups, so freshwater-acclimated sturgeons were exposed to a 60% challenge instead and they survived the acclimation period. Gill and muscle tissue were taken to build a tissue microarray that can quantify the expression of various stress proteins including those discovered during the execution of task 6. Tissues that were collected are currently in preparation

for building tissue microarrays. All muscle and gill tissues are already embedded in parraffin to maximize their preservation.

- We have optimized 2D gels for sturgeon gill and larvae samples and analyzed sturgeon proteins separated by 2D electrophoresis using trypsin in-gel digestion and mass spectrometry (see figure below).

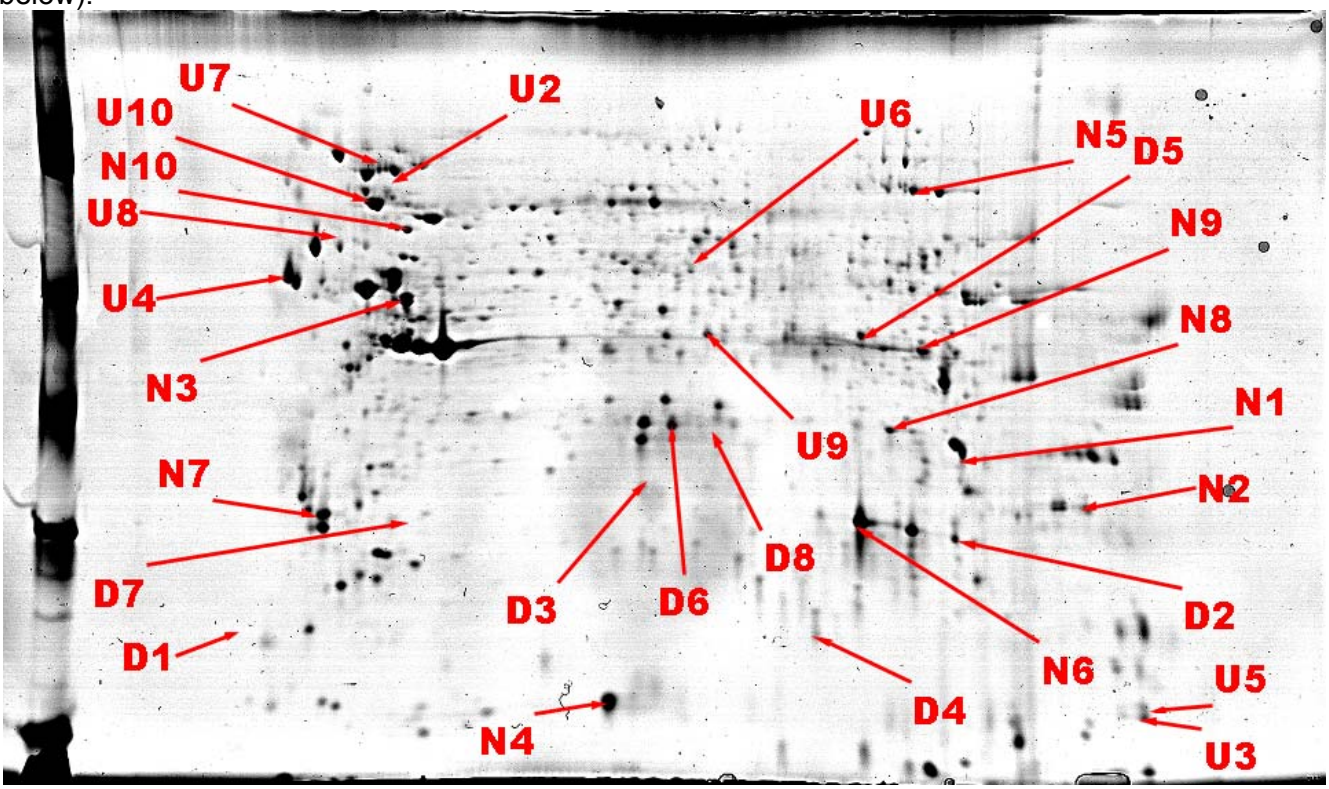


Fig. 35: Two-dimensional protein gel showing proteins extracted from green sturgeon larvae. Labeled spots represent proteins upregulated (U) or downregulated (D) in response to selenium exposure. N = Normalization spots for quantitative analysis.

- We have also setup a workflow for proteomics using the new nanoUPLC + MALDI spotter and optimized it for analyzing the sturgeon gill and larval proteome. Optimization included finding an optimal salt gradient and optimal retention times for maximum resolution (maximum peak capacity was achieved using a 250 mm x 75 µm column. Optimal sample extraction was achieved in 6M urea / 0.1% Rapigest followed by in-solution digestion with immobilized trypsin. A representative example of a strong cation exchange fraction of a sturgeon gill sample (100mM Ammonium Formate / 5% Acetonitrile) is shown in the figure below.

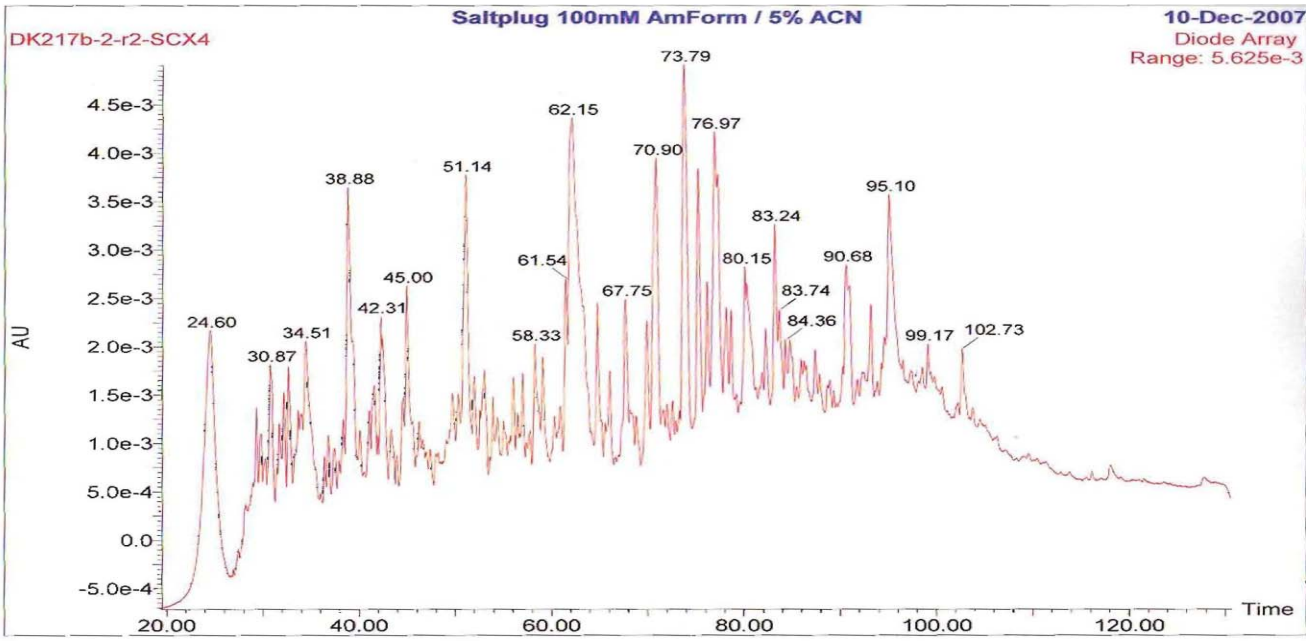


Fig. 36: Strong cation exchange (SCX) fraction of 100mM Ammonium formate for a sturgeon gill sample separated by subsequent reversed phase nano-ultraperformance liquid chromatography. Peaks represent tryptic peptides from highly abundant proteins.

- Using 6M urea and 0.1% Rapigest during in-solution trypsin digestion of gill extracts yields the maximum peak area (largest peptide amount, see figure below).

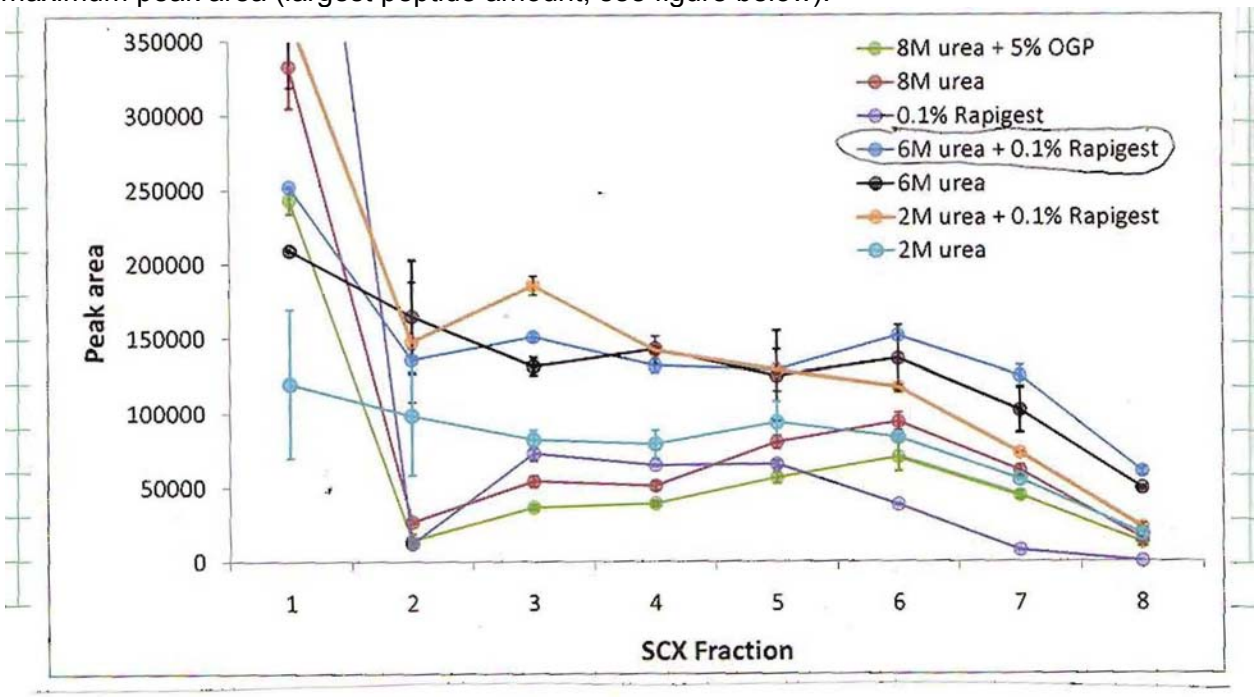


Fig. 37: Peak area of peptides from proteins that were digested using different urea and detergent concentrations.

- Using 6M urea and 0.1% Rapigest during in-solution trypsin digestion of gill extracts and also yields the maximum peak number (most peptides, see figure below).

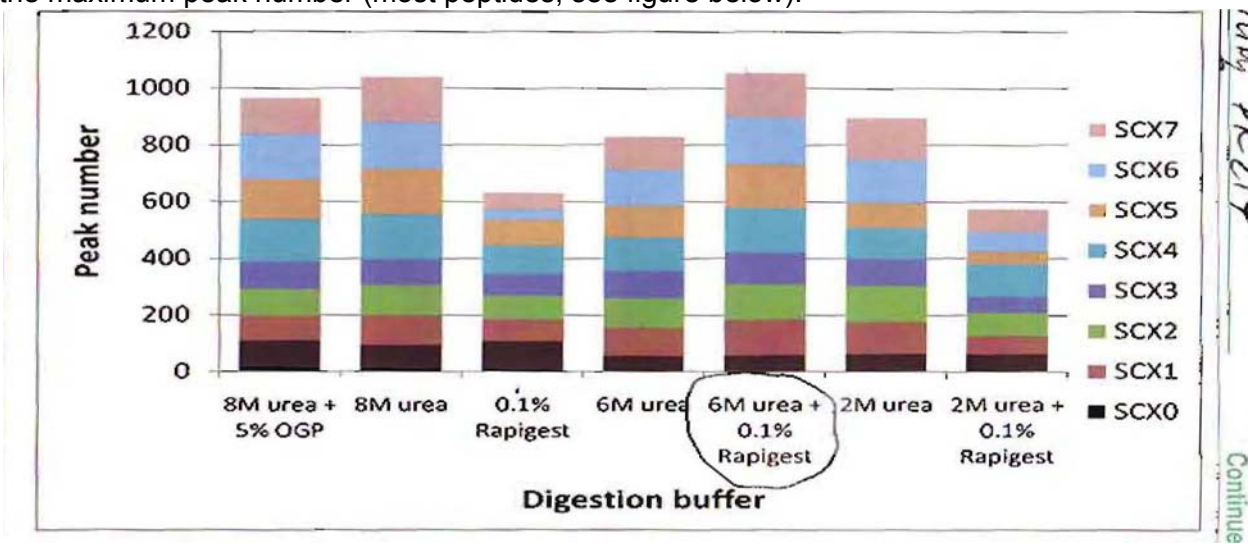


Fig. 37: Peak number of peptides from proteins that were digested using different urea and detergent concentrations.

- Heat maps of peptide peaks eluted from nanoUPLC and analyzed by MALDI-ToF/ToF mass spectrometry were generated using 4700 Peak Explorer and MSight software packages. We will use such heat maps to identify peptide peaks that differ between sturgeon treatment groups.

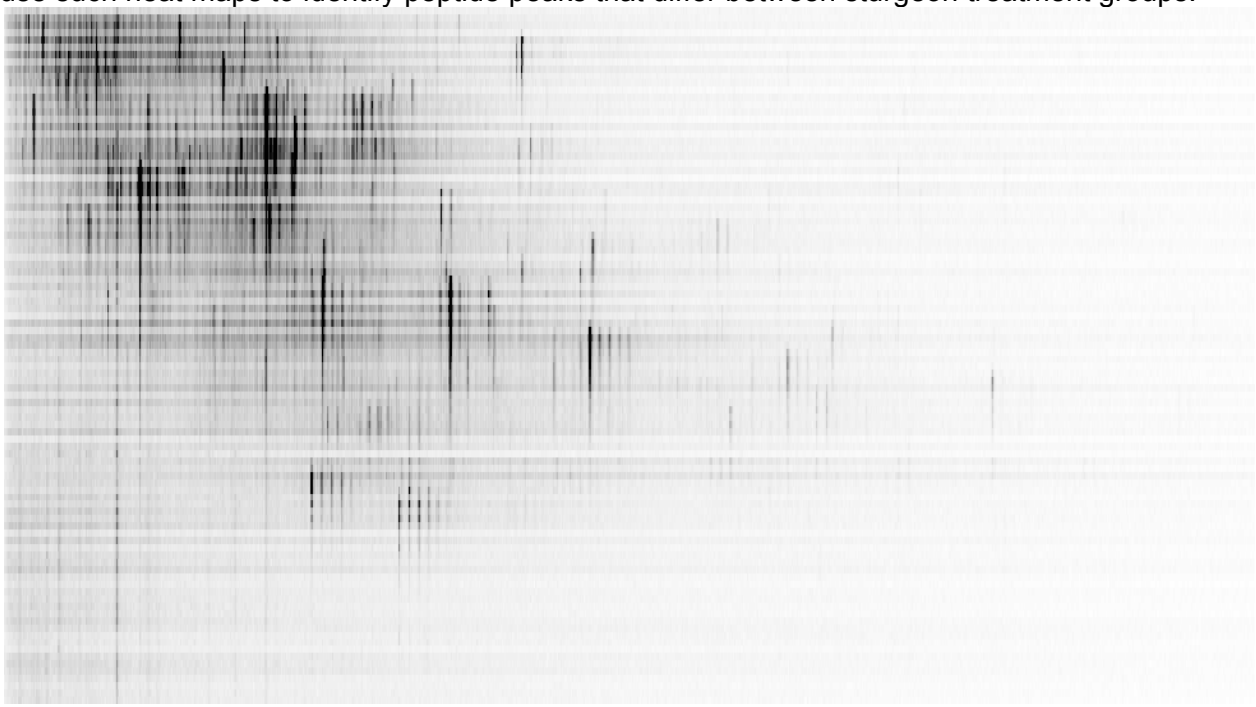


Fig. 38: Heat map showing peptide peaks eluted from strong cation exchange fraction 3 of a sturgeon gill sample tryptic digest over a retention time period of 120 min (Y axis) and mass range of 600 to 2600 Dalton (x axis).

White and green sturgeon larvae proteomics.

For white sturgeon (WS), 180 larvae were homogenized individually in RIPA buffer. After centrifugation, the supernatant was aliquoted and kept at -80° until analysis. For green sturgeon (GS), 44 larvae were homogenized individually in the same way.

A first set of 2D gels was performed with all individuals from a same condition pooled together. This first analysis could highlight some high differences of protein expression but is not statistically significant and need confirmation. Eleven cm IPG strips of a pH range from 3 to 10 were used for the isoelectric focusing, allowing the separation of acidic proteins as well as basic proteins. For the second dimension, a classical 12% polyacrylamide gel allowed the separation of proteins from about 14 to 200 kDa. The gels were then stained with a colloidal coomassie blue solution, scanned with a flatbed densitometer and analysed with the software Delta2D.

For WS larvae, an average of 565 spots were detected per gel while a total of 763 were observed for the GS larvae. From them, 15 and 18% of spots, respectively, showed a minimum 2-fold modified expression profile for organisms exposed to selenium compared to unexposed, whatever the temperature. Among these spots, only 12 (WS) and 6% (GS) were identically over or under-expressed under both temperature (18 and 26°C), indicating that the temperature significantly modulates the selenium effect on protein expression profile. In Fig 1, we can observe typical 2D gels from WS and GS with modified spots encircled.

A first attempt of protein identification has been done for a few spots from WS proteome. Spots n° 1493 and 1591 could not be identified by the use of a MALDI 4700 Proteomics Analyzer (Applied Biosystems). However, 2 highly abundant proteins (but unmodified) were unambiguously identified, giving evidence that the technique and the homology approach to compare micro-sequences can lead to reliable identifications. Those proteins are beta actin and isocitrate dehydrogenase, both identified by homology with Danio rerio (Fig 2.).

Those encouraging results however necessitate confirmation with several replicates. For this purpose, we performed a second set of 2D gels. For WS, we ran 36 gels in the same conditions as described above. However, each gel separated the proteins from a pool of 3 individuals from the same exposure group. A total of 3 replicates per group were analysed. As the larvae came from 3 progenies, a total of 9 replicates per experimental condition were used. We decided to first focus on 4 experimental conditions: injected with L-Methionine at 18°C (LMET18); injected with Seleno-L-Methionine at 18°C (SE18); injected with L-Methionine at 26°C (LMET26); injected with Seleno-L-Methionine at 26°C (LMET26). Other groups (uninjected and deformed) were kept for further analysis. When the gel was not of the best quality, a second run has been done. The best gel was kept for each group. All gels have been scanned and the analysis using Delta 2D is still in progress. A hierarchical cluster analysis will help to distinguish the specific biomarkers betwen conditions.

For GS larvae, 24 2D gels were performed. As we get less individuals per condition, we decided to keep them separated. One individual was run per gel, a total of 6 individuals per condition (see the conditions above). The analysis is still in progress.

We have performed the cluster analysis for GS and WS larvae and identify the protein of interest with the help of a MALDI 4700 proteomics analyser.

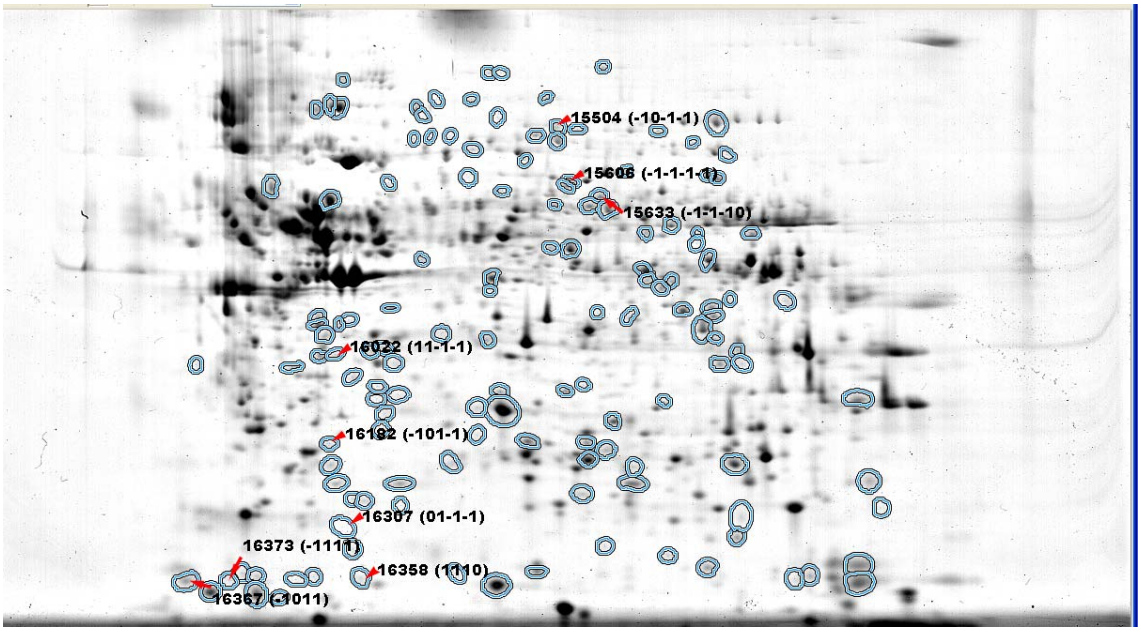


Fig. 39. Typical 2D gel from white sturgeon larvae pool of 9 individuals. pH range 3-10 NL; 12% acrylamide. Spots with a minimum 2-fold over or under-expression in larvae exposed to selenium compared to controls, whatever the temperature (circles).

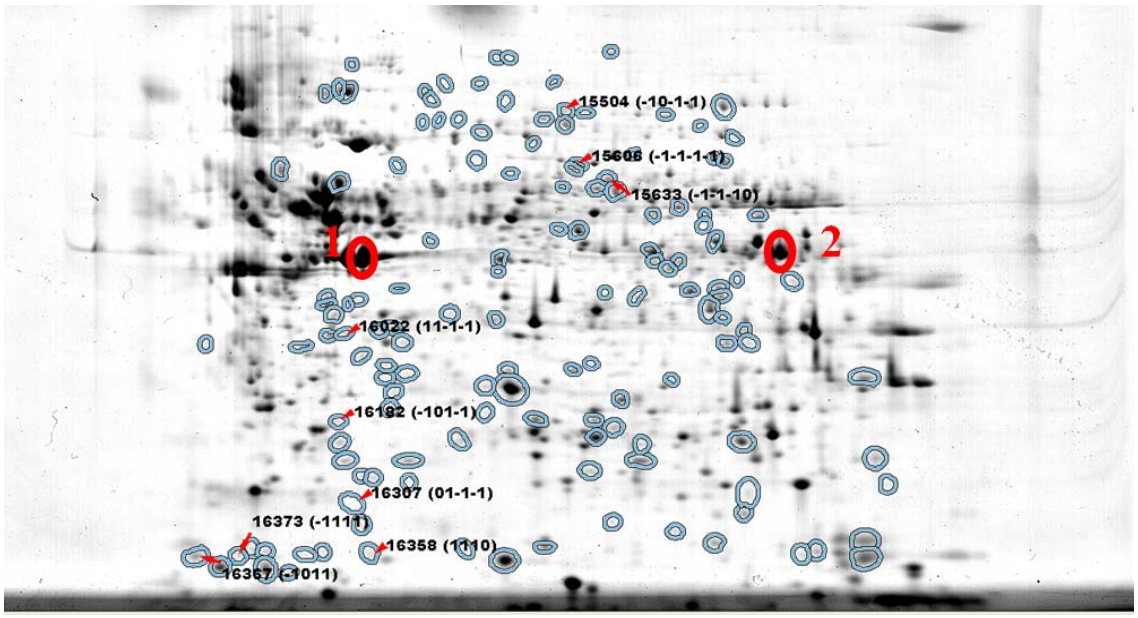


Fig. 40. Identification of beta actin (red circle n° 1) and isocitrate dehydrogenase (red circle n° 2).

A postdoc in my lab (Frederic Silvestre) has finished proteomic analysis of green and white sturgeon larvae exposed to selenium and temperature stress. Several new protein biomarkers of selenium and temperature stress have been discovered.

A graduate student in my lab (Rachel Madison) has identified spots for protein biomarkers of dietary selenium in gills from juvenile (ca. 6 months old) green and white sturgeon on two-dimensional gels. She is

currently working to sequence the corresponding spots on her gels using mass spectrometry to learn what these proteins are that change significantly in response to dietary selenium on her 2D gels. This is the central focus of her PhD thesis project and I expect her to finish this analysis and generate a paper from it by the end of the year.

A laboratory technician in my lab (Romina Sacchi) has analyzed gill samples from juvenile (ca. 8 month old) green and white sturgeon that were exposed to dietary selenium and mercury stress with additive temperature and salinity stress to simulate effects of pollution + climate change. This was done (and is still being done) using a new proteomics workflow that I have developed in my lab. This new workflow is gel-free and based on LCMSn. It will allow for much higher throughput and I expect that we can finish this analysis by the end of the project period.

We have identified multiple proteins by mass spectrometry that are indicative of exposure to different levels of selenium stress in gill epithelium of green and white sturgeon. These proteins are potential biomarkers of exposure to selenium stress. This work is still not fully complete but we continue working on this project because it represents the PhD thesis project for one of my graduate students (achel Madison). We plan on generating two additional publications (one for white and one for green sturgeon) that will contain the folliowing data:

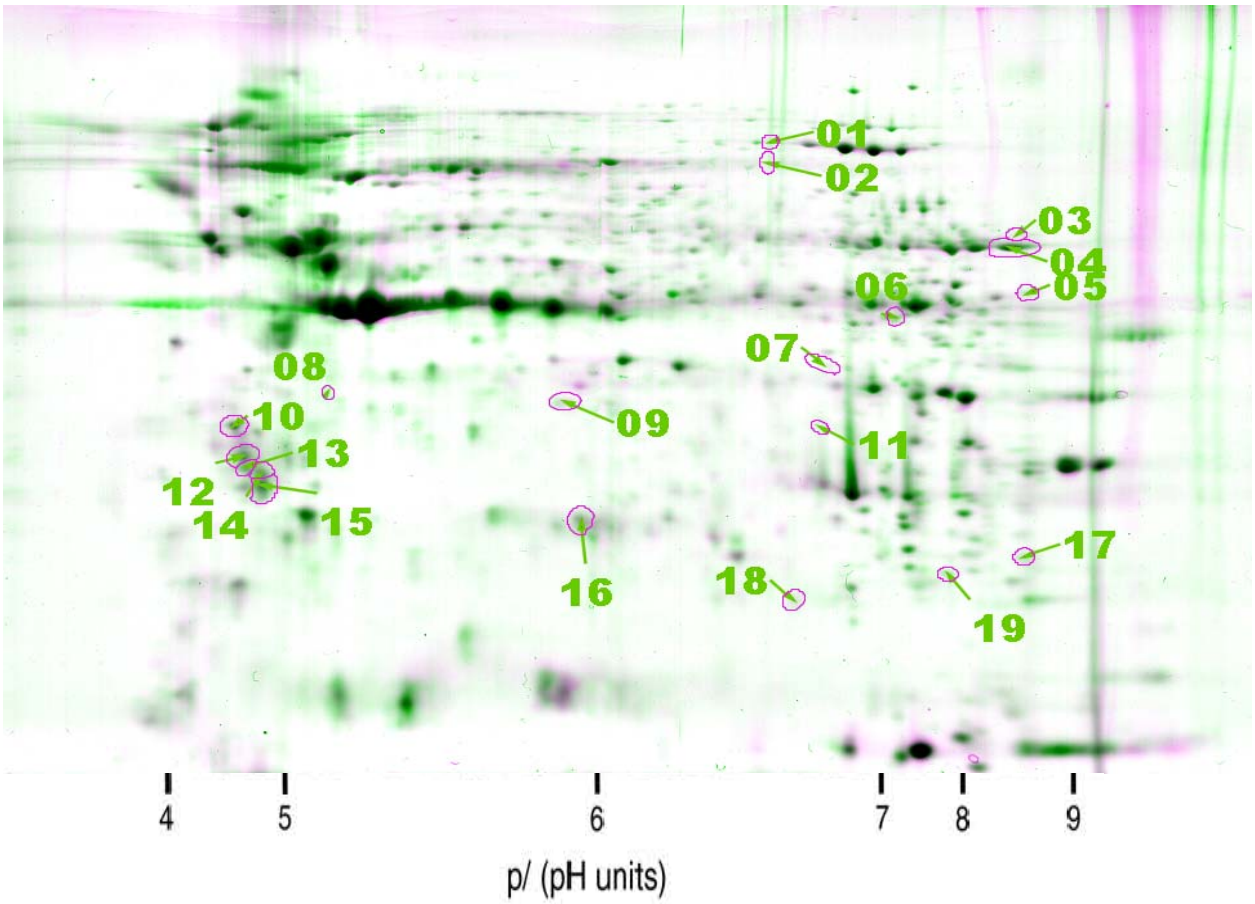


Figure 41: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 20 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after four weeks.

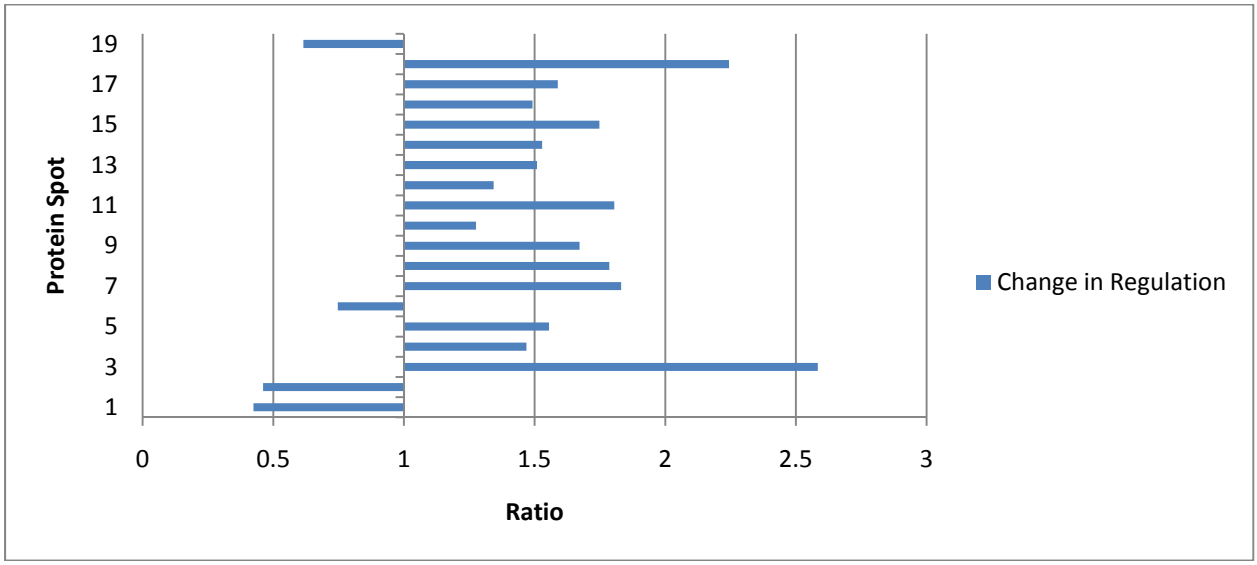


Table 6: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 20 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel above at four weeks.

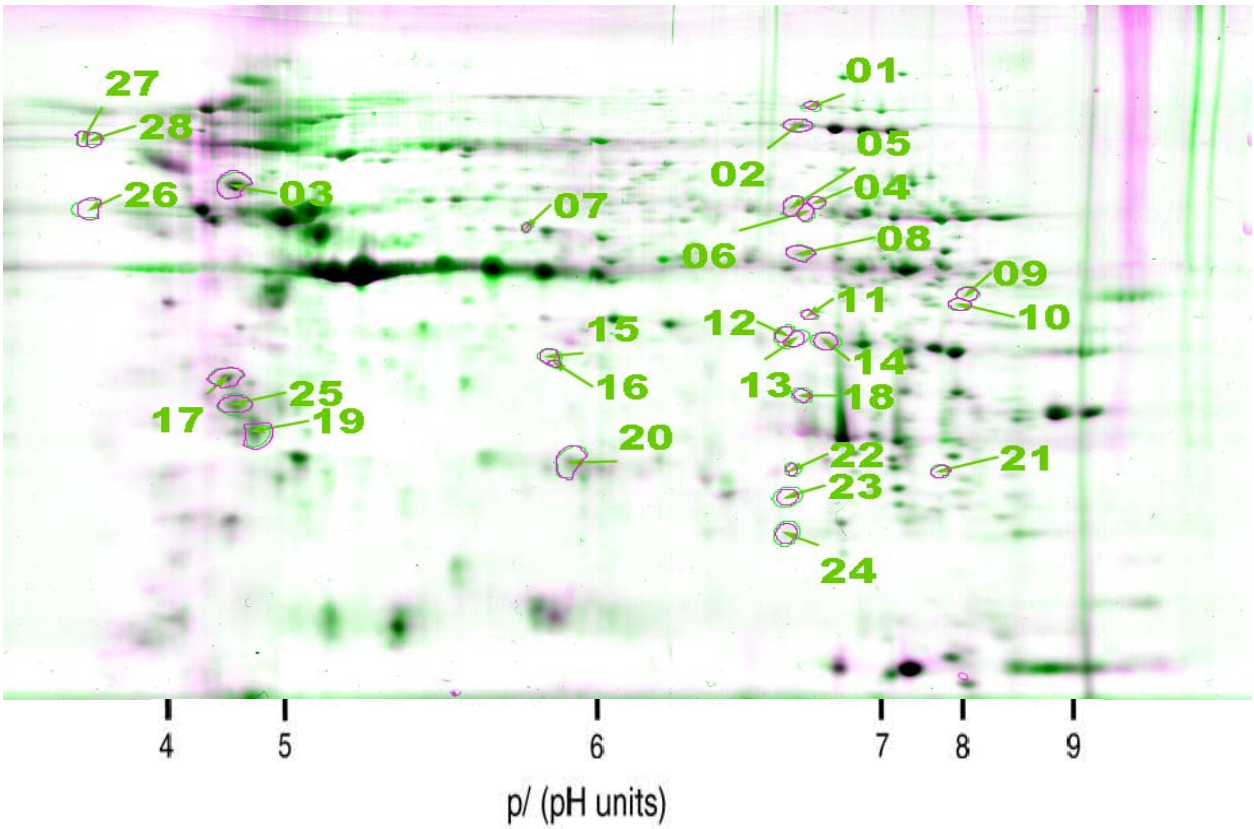


Figure 42: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 40 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after four weeks.

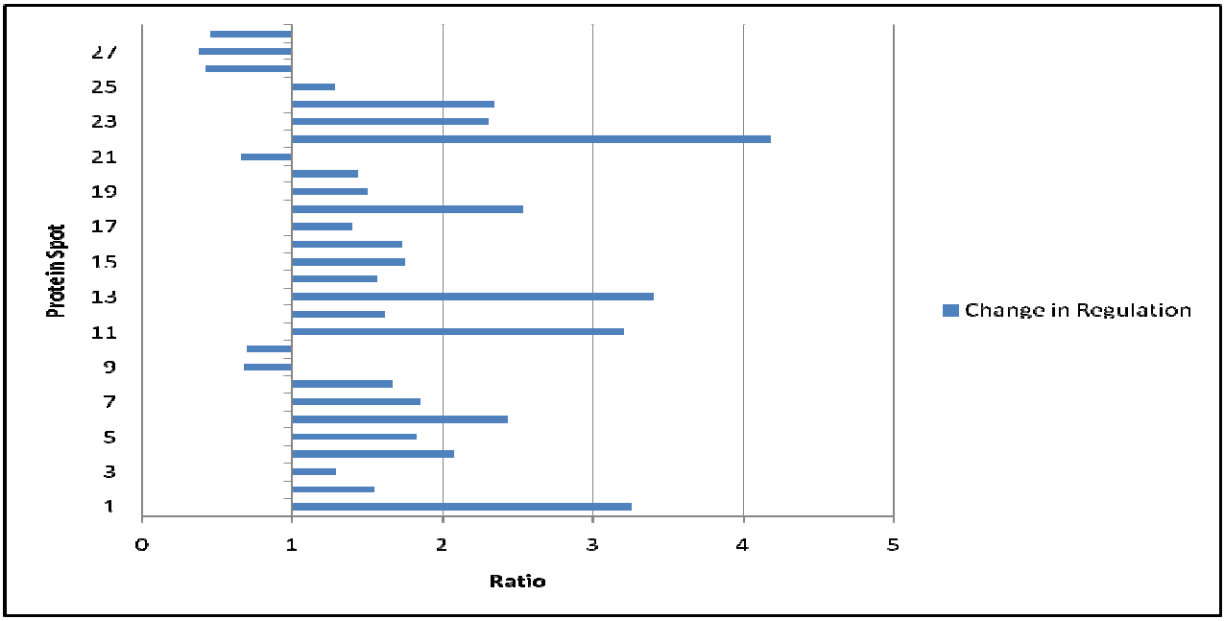


Table 7: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 40 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel above at four weeks.

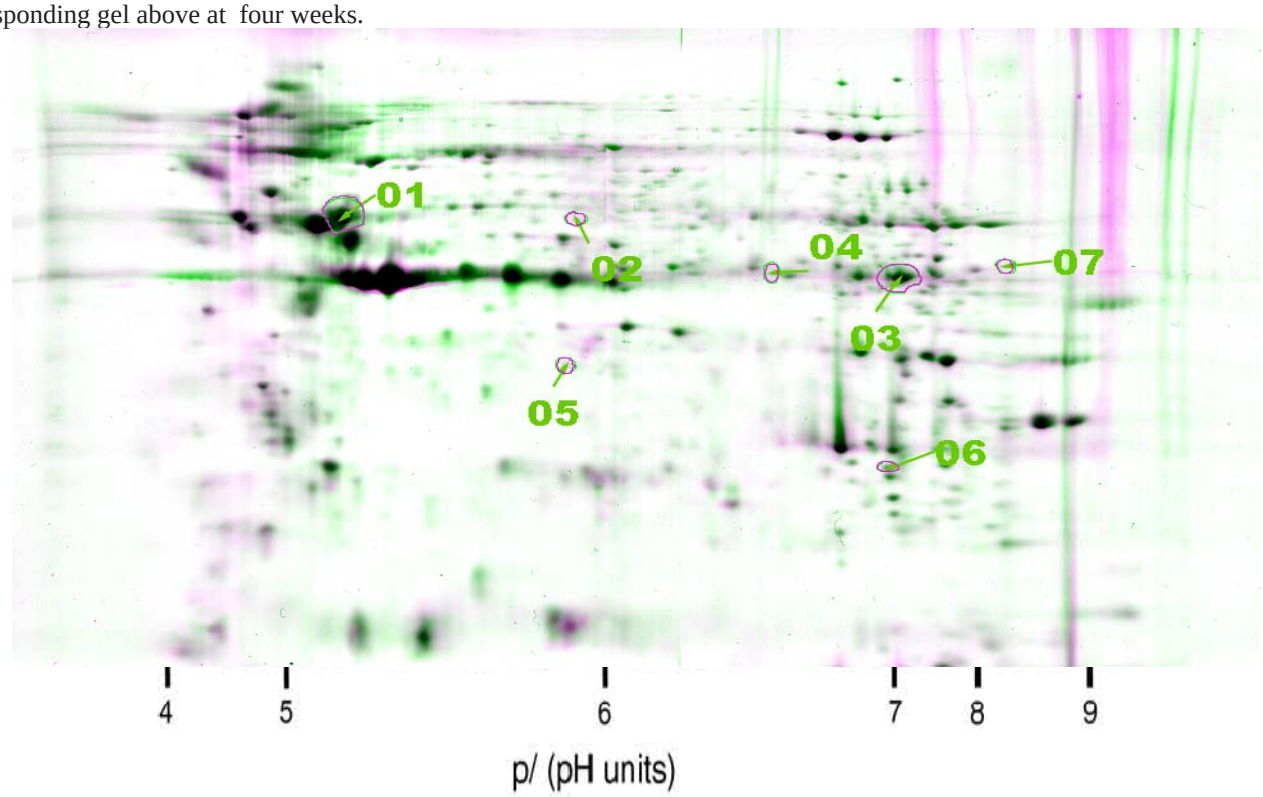


Figure 43: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 80 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after four weeks.

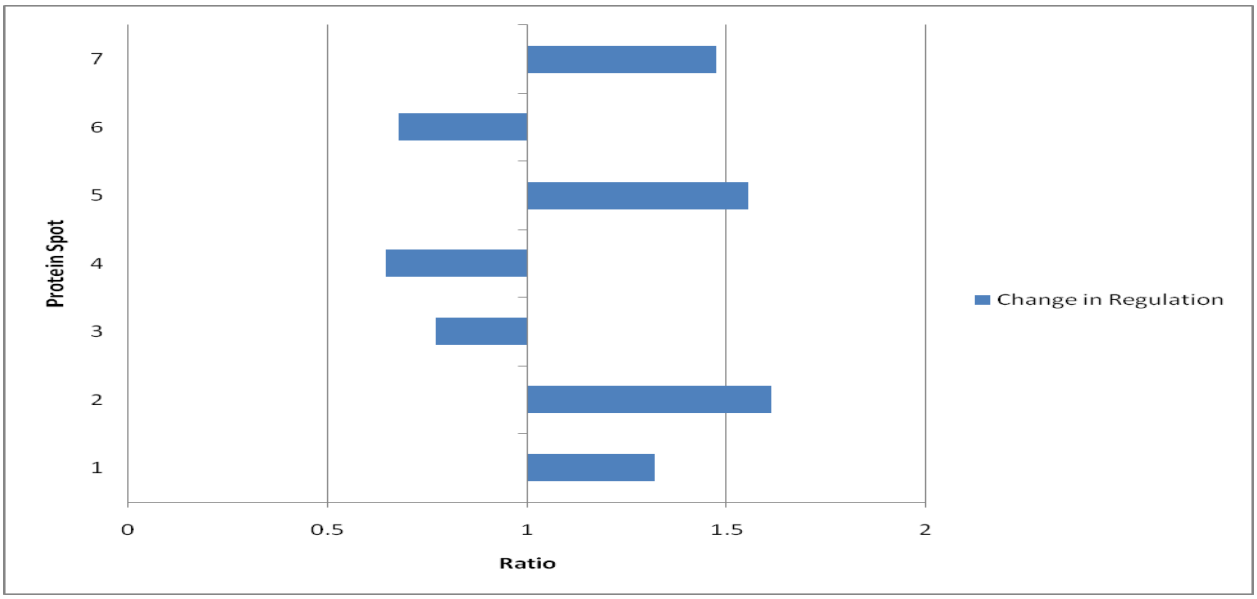


Table 8: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 80 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel at four weeks.

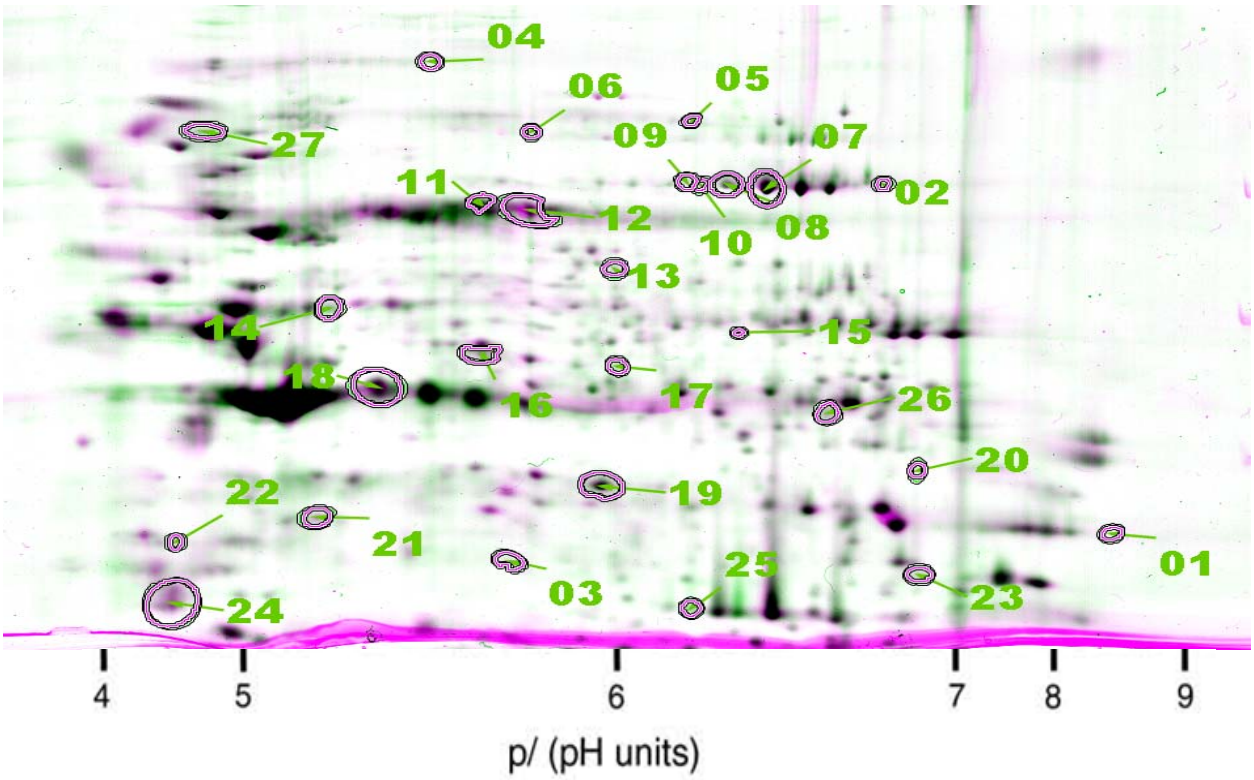


Figure 44: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 20 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after eight weeks.

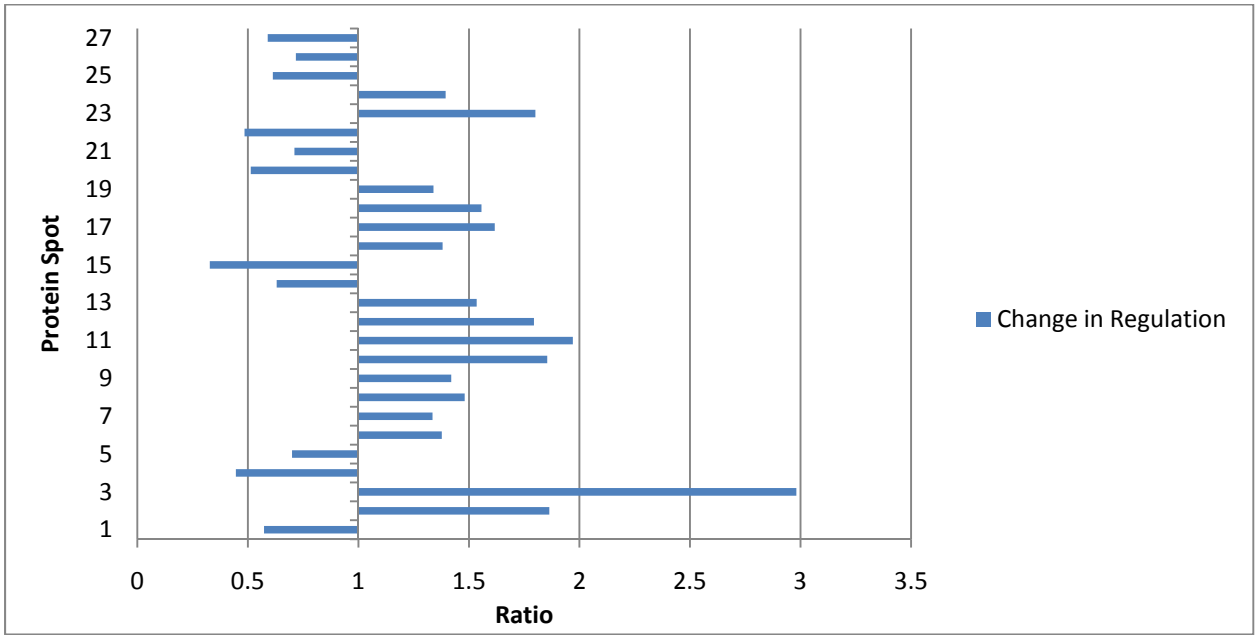


Table 9: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 20 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel above at eight weeks.

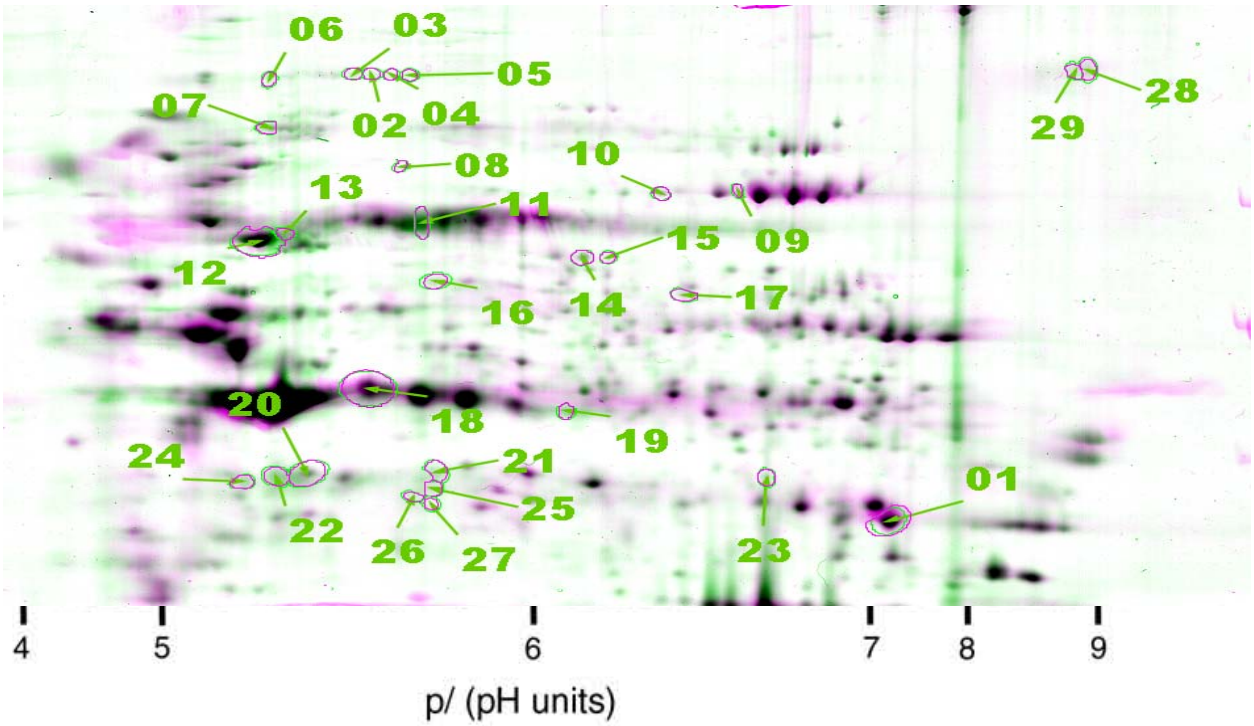


Figure 45: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 40 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after eight weeks.

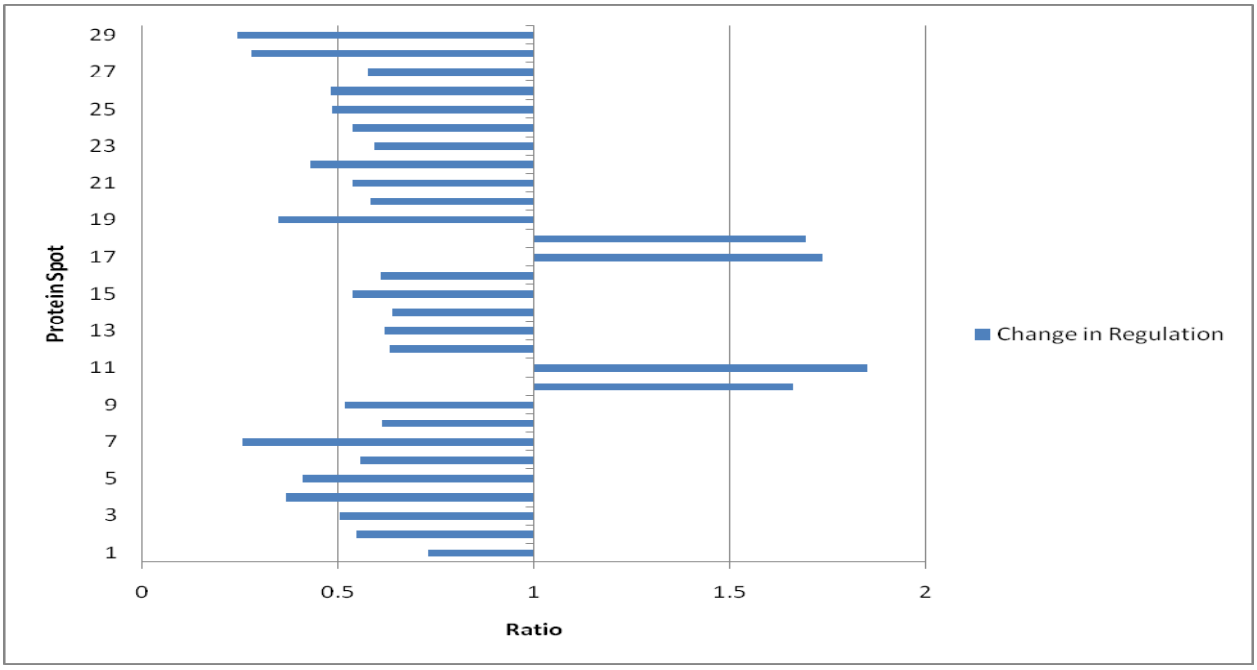


Table 10: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 40 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel above at eight weeks.

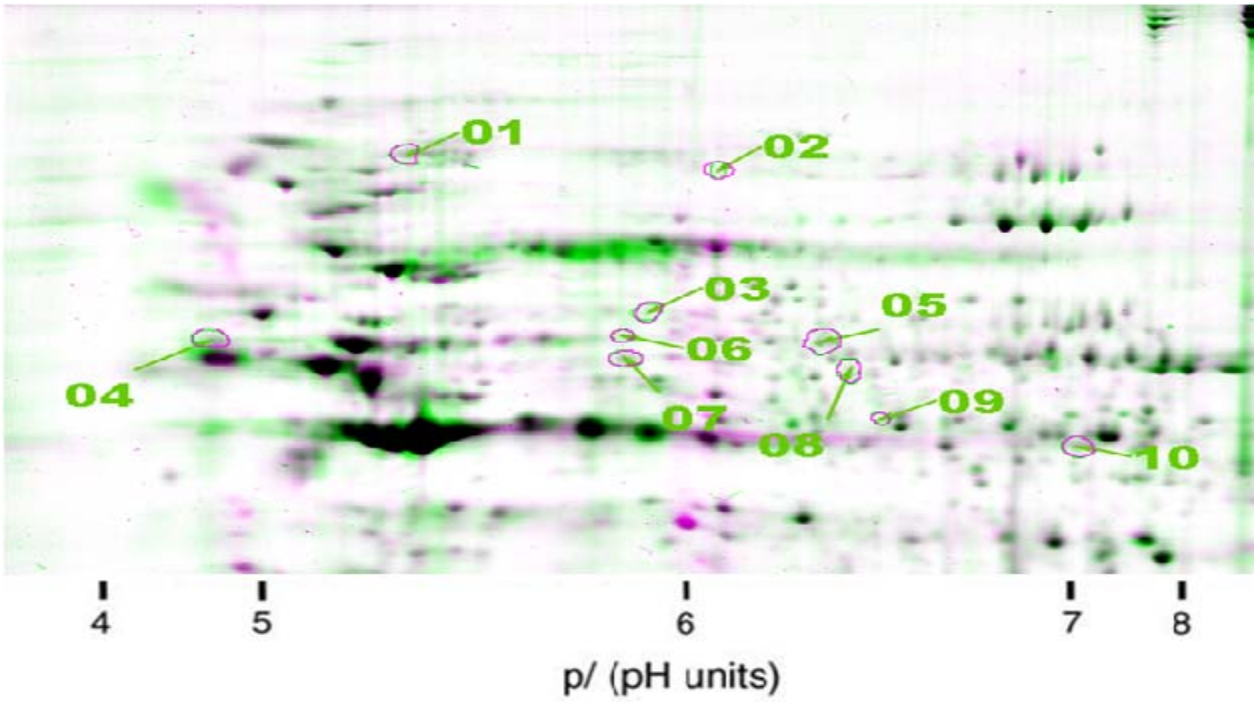


Figure 46: Two-dimensional gel image showing relative protein expression from juvenile white sturgeon after an exposure to 80 mg/kg Se added to the diet (green) versus the control (pink). The numbers in the gels correspond to spots with significant variations in expression following exposure after eight weeks.

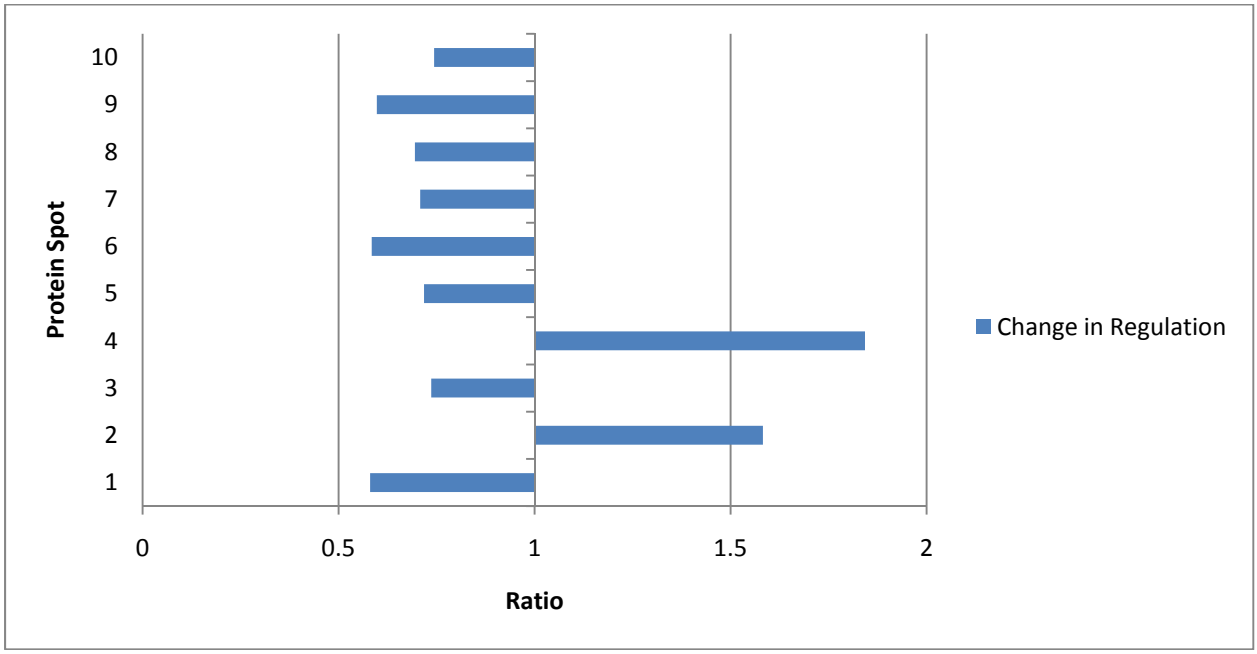


Table 11: Ratios of relative protein expression from juvenile white sturgeon after an exposure to 80 mg/kg Se added to the diet versus the control. A ratio below 1 represents a downregulation of the protein expression in the treatment. A ratio above 1 represents an upregulation of the protein expression in the treatment. Protein spot numbers correspond to the numbers in the corresponding gel above at eight weeks.

Table 12 (below): Proteomics Results from searching mass spectra against NCBI and Swiss Prot Databases.

Wk	Se (mg/kg)	Spot	Protein Name	Species	Accession #	MW	PI	MS Score	C.I.%	MS/MS Score	C.I.%
8	80	5	Luc7l2 protein	M. musculus	gi|12805661	14679	12.05	71	93.442		
8	80	7	arginine and glutamate rich 1	B. taurus	gi|84000355	33196.8281	10.35	74	96.861		
8	40	18	Tropomyosin 3, gamma isoform 2	R. norvegicus	gil29336093	29244.8	4.75	187	100	158	100
8	20	13	Predicted: Similar to SFRS4	G. gallus	gil118101604	51294.5	11.35	90	99.919		
8	20	17	Predicted: Similar to cytoplasmic beta-actin isoform 1	M. mulatta	gil109149170	39526.6	5.82	95	99.975	68	99.993
8	20	18	Tropomyosin 3, gamma isoform 2	Rattus norvegicus	gil29336093	29244.8	4.75	187	100	158	100
8	20	22	Guanine nucleotide binding protein (G Protein), beta polypeptide 2-like-1	D. rerio	gil152013098	35584.7	7.6	217	100	193	100
8	20	24	thyroid hormone receptor protein 3	X.tropicalis	gi|82524635	105691.023	10.17	118	100		

8	20	3	PREDICTED: Similar to peptidyl-prolyl isomerase-G (Cyclophilin G) isoform 3	C. familiaris	gil74004499	56715.3	10.77	179	100		
8	20	9	peptidyl-prolyl isomerase G	X.tropicalis	gi|47575780	85930.6719	10.35	146	100		
4	80	1	Tubulin, alpha 1A	R.norvegicus/D. rerio	gil38328248	50815.9	4.94	398/404	100	357	100
4	80	3	isocitrate dehydrogenase 2 (NADP+) mitochondrial	D.rerio/O.mossambicus	gil41054651	50934.7	8.35	108	99.999	96	100
4	80	5	high molecular weight basic nuclear protein	P.americanus	gi|1066104	38079.8516	11.38	114	100		
4	80	6	Proteasome (prosome, macropain) subunit alpha type 6	R.norvegicus/X. laevis	gil8394076	27838	6.34	178	100	157	100
4	80	7	Unknown (protein for IMAGE:4824581)	H. sapiens	gi|71051451	66662.9766	12.15	170	100		
4	40	13	PREDICTED: hypothetical protein	M. domestica	gil126343903	23666.9	10.66	100	99.992		
4	40	15	high molecular weight basic nuclear protein	P. americanus	gi|1066104	38079.8516	11.38	114	100		
4	40	19	thyroid hormone receptor associated protein 3	X.tropicalis	gi|82524635	105691.023	10.17	118	100		
4	40	22	Wu:fc32f04 protein	D. rerio	gil27881943	78110.6	9.72	130	100		
4	40	23	suppressor of variegation 4-20 protein-like-1	D. rerio	gil113206669	15726.8	10.38	108	99.999		
4	40	24	PREDICTED: similar to RIKEN cDNA 3110050K21	M.domestica	gi|126337731	188682.203	9.51	86	99.802		
4	40	4	novel protein	D. rerio	gil94733941	126646.5	5.18	118	100		
4	20	1	peptidyl-prolyl isomerase G	X.tropicalis	gi|47575780	85930.6719	10.35	146	100		
4	20	11	SAFB-like, transcription modulator, isoform CRA_a	H. sapiens	gi|119597956	92599.8203	9.84	94/150	100		
4	20	15	thyroid hormone receptor associated protein 3	X.tropicalis	gi|82524635	105691.023	10.17	118	100		
4	20	18	PREDICTED: similar to RIKEN cDNA 3110050K21	M.domestica	gi|126337731	188682.203	9.51	86	99.802		
4	20	2	Si:dkey-67c22.2 protein	D. rerio	gi|58701975	62752.9805	11.66	98	99.987		
4	20	3	RNA binding protein	H. sapiens	gi|5821151	137074.594	12.38	142/124	100		
4	20	5	Unknown (protein for	H. sapiens	gi|71051451	66662.9766	12.15	170	100		

			IMAGE:4824581)								
4	20	8	splicing factor, arginine/serine-rich 6	X. laevis	gi|148224986	76474.1016	11.81	143	100		
4	20	9	high molecular weight basic nuclear protein	P. americanus	gi|1066104	38079.8516	11.38	114	100		

Table 12 (continued, above): Proteomics Results from searching mass spectra against NCBI and Swiss Prot Databases.

The effect of environmental salinity on thermal tolerance.

We investigated how environmental salinity affects the upper thermal tolerance of green sturgeon. Sturgeon were acclimated to salinities representing freshwater (FW), estuary water (EST) or San Francisco Bay water (BAY) at 18 ºC, and their Critical Thermal Maximum (CTMax) was measured by increasing temperature by 0.3 ºC/min until branchial ventilation ceased. Ventilation rate initially increased with temperature, peaked at 26-28 ºC, and then briefly declined before ultimately ceasing (Fig. 1). CTMax was 34.2±0.09 ºC in EST-acclimated fish, with FW and BAY-acclimated fish CTMax at 33.7±0.08 and 33.7±0.1 ºC, respectively.

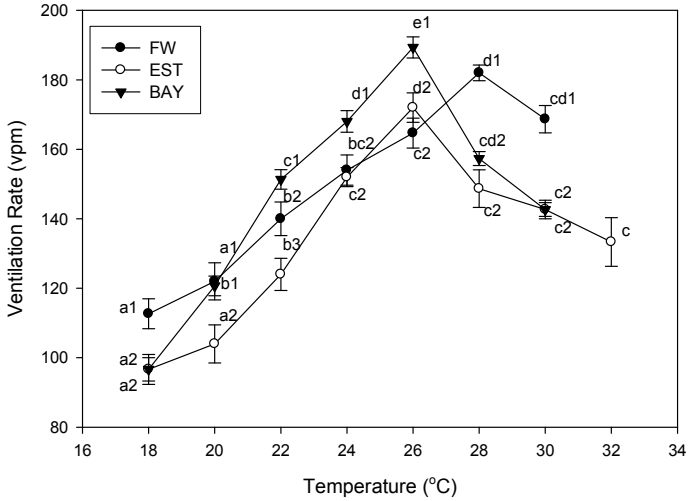


Figure 47. The effect of increasing temperature on green sturgeon ventilation rate. Letters indicate significant difference with time (p<0.05).

The initial increase in ventilation rate was linear, reaching peak rates at 26 °C in EST and BAY sturgeon, and at 28 °C in FW sturgeon. We calculated the temperature coefficient (Q_{10}) for ventilation rate in each group across the range of temperatures from control to that which elicited the peak in ventilation rate (18-26 ºC for EST and BAY; 18-18 ºC for FW). The Q_{10} value represents the factor by which biological rates increase as a result of a 10 ºC temperature increase (Hochachka and Somero, '02). For ectotherms, a Q_{10} near 2 is expected when an organism is within normal physiological limits (Hochachka and Somero, '02). Our Q_{10} values for the increase in ventilation rate were approximately two in all groups, although the range of temperatures eliciting this response was greater in FW-acclimated sturgeon by a full 2 ºC (Figure 2). The Q_{10} values for ventilation rate following the peak temperature (down slope; all < 1) indicated that the temperature increase was resulting in damage to the system (Hochachka and Somero, '02). FW-acclimated sturgeon were able to maintain a normal physiological function to a higher peak temperature relative to EST and BAY fish, and had a greater range of temperature tolerance before damage to the

system occurred. Despite the better performance of FW-acclimated sturgeon in functional tolerance, CTMax estimates assessed by cessation of ventilation were highest in the EST-acclimated sturgeon.

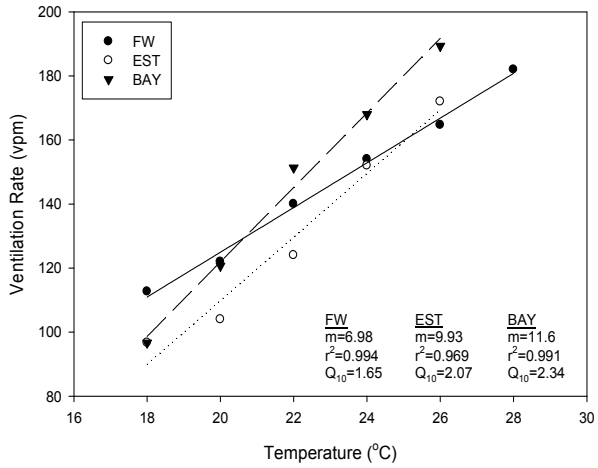


Figure 48. Regression analysis of functional temperature range (Q_{10}~2)

The collapse of osmoregulatory mechanisms was evident in the EST and BAY groups after exposure to near-lethal temperature. Temperature increases have been shown to have deleterious effects on osmotic balance, which are mediated by altering enzyme kinetics (Handeland and others, '00), varying phospholipid membrane fluidity/integrity (Johnston and Cheverie, '85), and/or altering metabolic rate (Hayton and Barron, '90; Barron and others, '87). Plasma osmolality was significantly increased following CTMax exposure in the EST and BAY groups (Figure 3), indicating an increased osmotic gradient across the gill as blood flow and gill ventilation rate increased. These effects have been shown to exacerbate the effects of the osmo-respiratory compromise at the gill, and result in greater ion flux (Gonzalez and Mcdonald, '94; Gonzalez and McDonald, '92; Randall and others, '72; Sardella and Brauner, '07). FW-acclimated sturgeon were able to maintain plasma osmolality during CTMax exposure, although ventilation rate was dramatically increased. The greater leakiness in the hyperosmotic-acclimated groups (EST and BAY) likely contributed to the loss of osmotic balance as ventilation rate increased, and may have played a role in the shorter functional range of temperatures for these groups compared to FW.

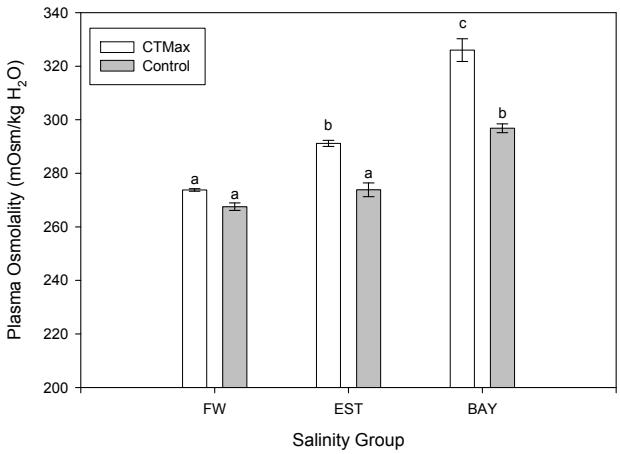


Figure 49. The effect of CTMax test on plasma osmolality. Letters note significant difference.

As these abiotic factors become more unstable, the population dynamics and distribution of estuarine inhabitants will eventually be determined by their level of thermal tolerance and/or osmoregulatory capability. Salinity and temperature combinations that sturgeon are not able to acclimatize to will lead to elevated physiological stress. For example, in this study juvenile sturgeon that were of the age/size class typically found in FW were exposed to higher salinities, and as a result, had a shorter range normal physiological function as temperature was increased. Such physiological stresses resulting from changing abiotic conditions may subsequently reduce fitness and/or force relocation to sub-optimal regions within the system, both of which may negatively affect population dynamics and distribution of an already threatened species.

Osmoregulation and ionoregulation in green sturgeon

We acclimated juvenile green sturgeon to 0 (FW), 15 (EST), and 24 g/l (BAY) at 18 ºC for two weeks and measured the physiological and biochemical responses with respect to osmo- and ionoregulatory mechanisms. The purpose of this study was to investigate the osmo- and ionoregulatory abilities of the green sturgeon using a comprehensive investigation of physiological and biochemical variables. We measured plasma osmolality and muscle hydration to assess the level of osmotic stress resulting from salinity acclimation, as well as the activities of Na^+, K^+-ATPase (NKA) and v-type H^+-ATPase (VHA) and caspase 3/7. Finally we used tissue microarrays (TMA) to assess the abundance of several proteins in the branchial epithelium, such as NKA, VHA, Na^+, K^+, $2Cl^-$ co-transporter (NKCC), heat shock proteins (HSP) 60, 70, and 90, and ubiquitin. HSPs and ubiquitin are good indicators of cellular stress and were used as indicators for damaged proteins being salvaged or destroyed, respectively.

Plasma osmolality in EST- and BAY-acclimated sturgeon was elevated relative to FW-acclimated sturgeon (Figure 3a; $p<0.01$), but there was no difference in muscle water content (Figure 3b) or abundance of stress proteins (not presented).

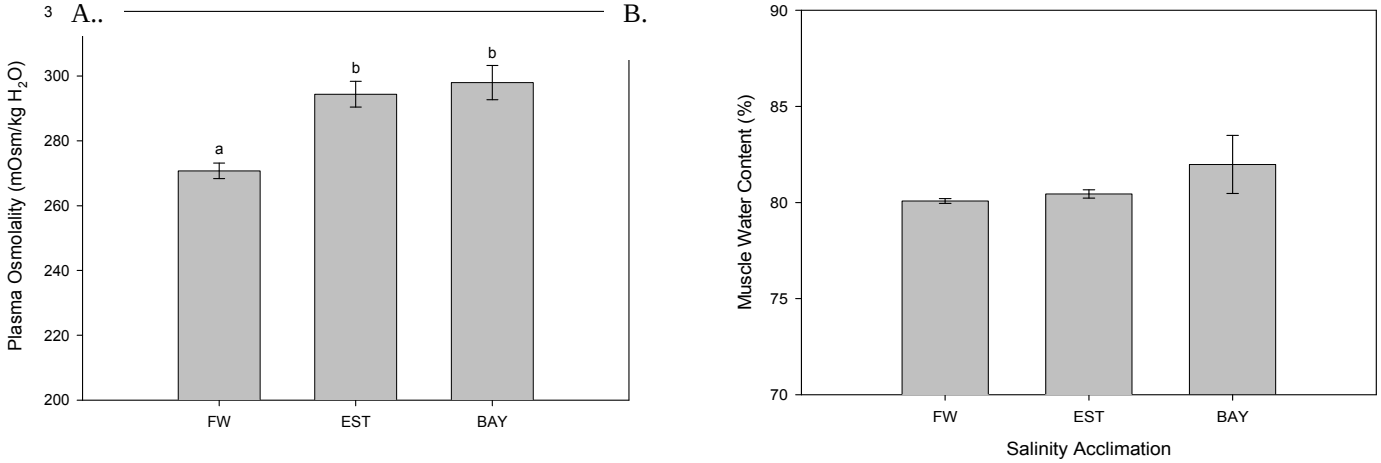


Figure 50. The effect of salinity on a) plasma osmolality and b) muscle water content. Letters note significant difference.

Na^+, K^+-ATPase (NKA) activity was also unchanged, but abundance within MRCs was greater in BAY-acclimated sturgeon ($p<0.01$). FW-acclimated sturgeon had the greatest NKA abundance when assessed at the level of the entire tissue ($p<0.01$). The Na^+, K^+, $2Cl^-$ co-transporter (NKCC) was present in FW-acclimated sturgeon (Figure

5a, but the overall abundance was lower relative to sturgeon in EST or BAY water (Figure 5b; $p<0.01$) where this enzyme is crucial to hypoosmoregulation.

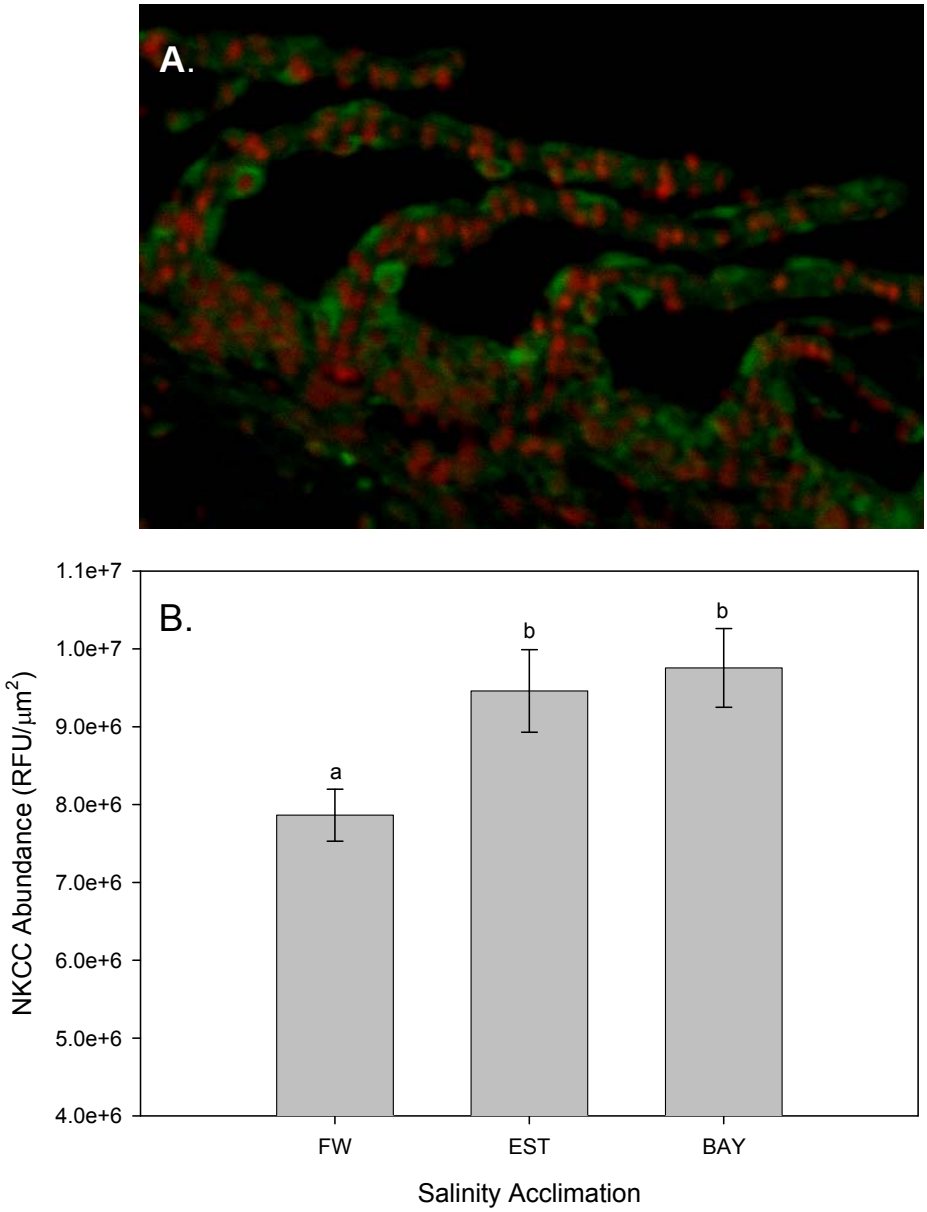


Figure 51. A) Fluorescently labeled NKCC (green) in FW-acclimated sturgeon gill. B) The effect of salinity on NKCC abundance. Letters note significant difference.

The role of NKCC has been well defined and when present on the basolateral membrane of MRCs has been characterized as part of the mechanism for ion excretion in SW-acclimated fishes (Marshall, '02). In this model, the Na^+ gradient produced by NKA is used to drive two Cl^- into the MRC where they diffuse though an apical Cl^- channel and into the environment (Hiroi and McCormick, '07; Marshall, '02). Much like the NKA-labeled slides, NKCC could be used to define MRCs along both the filament and lamellae. NKCC was present within the MRCs of sturgeon

regardless of acclimation salinity, although the abundance was greatest in EST- and BAY-acclimated fish (Fig. 5b). It is not surprising that the EST- and BAY-acclimated sturgeon had a greater abundance of NKCC as the function of this transporter is crucial to hypoosmoregulation. The presence of this transporter in sturgeon that had been reared and held in FW may indicate that at this age/size, sturgeon are pre-acclimating to SW, or at least for entry into water of variable salinity such as an estuary. Similar results have been observed in the anadromous Atlantic salmon (*Salmo salar*) where there was essentially no difference between FW and SW individuals with respect to NKCC abundance (Hiroi and McCormick, '07). Furthermore, injection of cortisol and growth hormone, which have been associated with salmonid smoltification, resulted in an upregulation of NKCC in FW-acclimated Atlantic salmon (Pelis and McCormick, '01). There have been previous discussions of how age/size affects SW-readiness in sturgeon (McKenzie and others, '01; McEnroe and Cech, '85; Rodriguez and others, '03; Allen and Cech, '07), and the age of initial expression of NKCC may provide some insight into the precise age at which sturgeon become SW-ready.

The effects of global climate change.

The San Francisco Bay-Sacramento River Delta watershed represents California's primary hydrologic system and has crucial roles in agriculture, municipal water supply, and maintenance of fish and wildlife (Knowles and Cayan, '02; Knowles and Cayan, '04). Recently the threat posed by global climate change has become a much greater concern, as the predicted changes in hydrological and meteorological factors will further complicate maintenance and/or restoration of the already-threatened species like the green sturgeon. Increased temperature will have dramatic effects on the SF Bay-Delta, the main deleterious effect being a shift in precipitation type from snow to rain, and subsequently less water storage within the snow pack (Knowles and Cayan, '02; Knowles and Cayan, '04). This will result in a larger and less steady salinity range within the estuarine habitats, confounding the direct effect of warmer temperature. A higher percentage of precipitation falling as rain will also mean higher water flow and decreased salinity in the estuary throughout the winter months. The opposite problem will result during the spring and summer; smaller snow pack and reduced flow will result in greater variation in salinity and temperature with daily and monthly tidal cycles (Knowles and Cayan, 2002; Knowles and Cayan, 2004). Salinity and temperature are important determinants of water quality and population dynamics. While shifts in theses variables will have deleterious effects on spawning success, development, growth, and survival of many fish species in the SF Bay-Delta area, physiological systems are likely to be even more sensitive, and their upregulation will be used as valuable predictive bioindicators of stress well in advance of effects at the population level.

The purpose of this study was to simulate these variables in a controlled setting and characterize their effects on juvenile green sturgeon. To simulate the 'winter scenario', fish acclimated to 24 g/l (BAY) or 15 g/l (EST) were exposed to a 3 h salinity decrease from either salinity to 0 g/l (FW) and monitored for 24 h. To simulate the 'summer scenario', fish acclimated to FW were exposed to a fluctuation in salinity between 0-24 g/l oscillating every 6 h for 24 h. We monitored survival of the fish and sampled throughout to investigate which sub-lethal indicators of osmotic stress were prevalent and to what degree sturgeon were capable of acclimating to the salinity variation.

We did not observe any sturgeon mortality during these experiments. Sturgeon did not appear stressed and were active throughout the 24 h exposures. Although survival was 100%, there were substantial sub-lethal effects, in particular within the FW-acclimated sturgeon that were exposed to the tidal cycle fluctuation.

Winter Scenario

Despite a rapid drop in plasma osmolality (Figure 6), there were no changes in the abundance of stress proteins in the gills. HSP 60, 70, and 90 all remained unchanged as did the abundance of ubiquitin. It is reasonable to conclude that the drop in osmolality was within a tolerable range for sturgeon, but within the BAY-acclimated group maintenance within this tolerable range was at the cost of NKA activation and possibly cellular rearrangement.

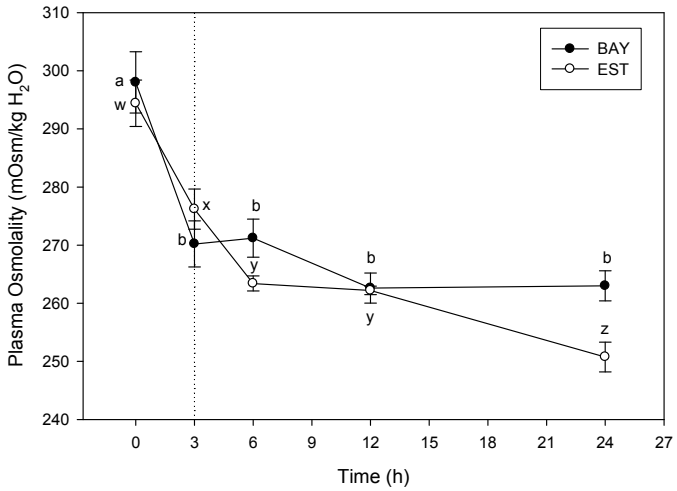


Figure 52. The effect of rapid salinity decrease on the plasma osmolality of EST and BAY-acclimated green sturgeon. Letters note significant difference.

Summer Scenario

Exposure of green sturgeon to a repeated fluctuation in salinity was much more detrimental at a sub-lethal level. Sturgeon lost control of plasma osmolality when salinity was at its highest pints, with the second increase resulting in a greater relative loss as opposed to the first (Figure 7a). The increases in plasma osmolality were 11.0 and 18.0% greater than values at 0 h at 6 and 18 h, respectively. At 6 h, the increase in osmolality corresponded with a drop in muscle water content (Figure 7b), which indicates that the muscle water was drawn out osmotically (Brauner and others, '92). This was not observed at 18 h, which similar to the winter scenario data may indicate a defense of the muscle compartment, but here it is more likely that the muscle was taking on ions from the plasma and thus water could not be drawn out. This is only speculative, but given the overall level of stress present at 18 h it seems more likely.

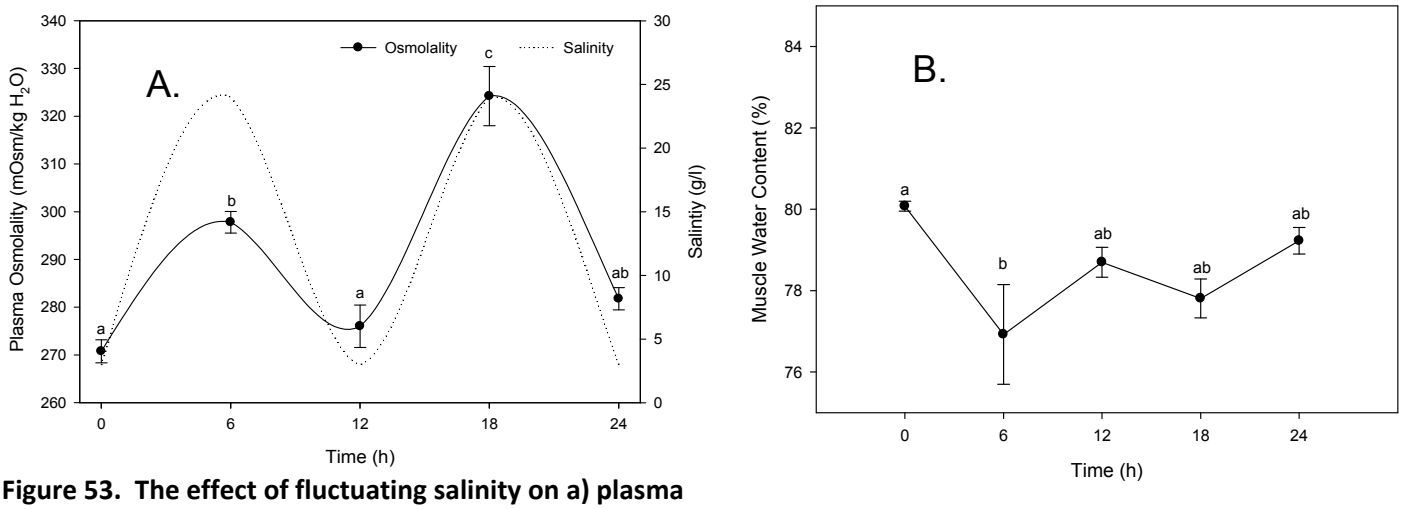


Figure 53. The effect of fluctuating salinity on a) plasma osmolality and b) muscle water content. Letters not significant differences.

- We achieved to setup and optimize a workflow for proteomic analysis of proteins extracted from sturgeon larvae; A new nanoUPLC-MALDI spotter setup was successfully custom-configured and calibrated for the purpose of protein identification by mass spectrometry and quantitative whole-proteome analysis in this type of tissue;

- During the previous six months we have optimized two-dimensional gels, in-gel and in-solution digestions, and two-dimensional ultra-performance chromatography with sturgeon gill and larvae samples. We have also optimized the sample preparation procedures for these tissues to be analyzed using proteomics methods. We are now in a position to start processing samples collected in tasks 2, 3, and 4 using these methods to screen for proteins that represent good biomarkers of selenium stress, mercury stress, temperature stress, and salinity stress.

  PROJECT STATUS: The experiments under task 6 have been completed. Most of the results have been published (see below). However, the proteome analysis of green and white sturgeon gill tissue is still in progress and will be finished soon as part of the PhD thesis project of Rachel Madison. We will use alternative funds for finishing this analysis (i.e. validating up- and down-regulated spots identified using mass spectrometry).The following publication resulted from this task:

1. **Sardella, B. A., E. Sanmarti, and D. Kültz, 2008. The critical thermal maximum of green sturgeon*Acipenser medirostris*) and the effect of acclimation salinity. J Exp Zool Part A Ecol Genet Physiol. 2008 Oct 1;309(8):477-83.**
2. **Zhang, Y.; Doroshov,S.; Famula, T.; Conte, F.; Kültz, D.; Linares-Casenave, J.; Van Eenennaam, J.; Struffenegger, P.; Beer, K. & Murata, K. (2010) Husbandry and dietary effects on sturgeon (Acipenser transmontanus) farmed for caviar. J. Appl. Ichthyol. submitted: -.**
3. **Silvestre, F.; Linares-Casenave, J.; Doroshov, S.I. & Kültz, D. (2010) A proteomic analysis of green and white sturgeon larvae exposed to heat stress and selenium. Sci.Total Env. 408: 3176-3188.**
4. **Sardella,B.A. & Kültz,D. (2009) Osmo- and ionoregulatory responses of green sturgeon (Acipenser medirostris) to salinity acclimation. J. Comp. Physiol. B 179(3): 383-390.**

TASK 7

- Data generated in all tasks were analyzed and discussed;
- Organization of data from all tasks into publishable units was discussed and elements identified that would complete publishable units.
-

- The following full publications (in addition several abstracts not listed here have been published) resulting from this project have been coordinated in task 7, submitted, and published (or are currently in press):

1. Van Eenenaam J.P., Linares-Casenave J., Muguet J.-B. & Doroshov, S.I. (2008) Induced spawning, artificial fertilization, and egg incubation techniques for green sturgeon. North Am. J. Aquaculture 70: p.434–445.
2. Werner I., Linares-Casenave J., Van Eenennaam J.P. & Doroshov, S.I. (2007) The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (Acipenser medirostris). Environ. Biol. Fish 79: p.191–200
3. Sardella, B. A., E. Sanmarti, and D. Kültz, 2008. The critical thermal maximum of green sturgeon*Acipenser medirostris*) and the effect of acclimation salinity. J Exp Zool Part A Ecol Genet Physiol. 2008 Oct 1;309(8):477-83.

4. 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.
5. 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.
6. Zhang, Y.; Doroshov,S.; Famula, T.; Conte, F.; Kültz, D.; Linares-Casenave, J.; Van Eenennaam, J.; Struffenegger, P.; Beer, K. & Murata, K. (2010) Husbandry and dietary effects on sturgeon (Acipenser transmontanus) farmed for caviar. J. Appl. Ichthyol. In press.
7. Silvestre, F.; Linares-Casenave, J.; Doroshov, S.I. & Kültz, D. (2010) A proteomic analysis of green and white sturgeon larvae exposed to heat stress and selenium. Sci.Total Env. 408: 3176-3188.
8. Allen, P.J., Cech. J.J., Jr., Kültz, D. (2009) Mechanisms of seawater acclimation in a primitive, anadromous fish, the green sturgeon. J. Comp. Physiol. B 179: 903-920.
9. Sardella,B.A. & Kültz,D. (2009) Osmo- and ionoregulatory responses of green sturgeon (Acipenser medirostris) to salinity acclimation. J. Comp. Physiol. B 179(3): 383-390.

PROJECT STATUS: Task 7 has been completed.

An update on publications is posted at http://kueltzlab.ucdavis.edu/Publications.cfm.

DELIVERABLES:

INITIAL PROJECT SUMMARY
4 PROGRESS REPORTS
7 PEER-REVIEWED PUBLICATIONS IN SCIENTIFIC JOURNALS

PERSONNEL CHANGES:

NONE

CONTRACT MODIFICATIONS:

NONE