Project 5
Brine Shrimp Kinetic Studies

SUBCONTRACT WITH:	University of Miami
PRINCIPAL INVESTIGATOR:	Dr. Martin Grosell
CONTRACT VALUE:	$159,956
SCHEDULE:	March 20, 2006 through November 20, 2007 (elapsed time: 8 months)

Project Objectives

The overarching main objective of the present proposal is to provide reliable predictions of selenium accumulation in *Artemia franciscana* under conditions realistic for the populations residing in the Great Salt Lake (GSL), Utah.

This general objective will be addressed by pursuing the following specific objectives:

1) Determine the influence of salinity on selenium uptake and feeding rate by *Artemia fransiscana*
2) Determine selenium uptake rates in *Artemia franciscana* from dissolved selenium concentrations in artificial Great Salt Lake (GSL) water (uptake kinetics).
3) Determine dietary selenium intake and subsequent selenium assimilation efficiency in *Artemia franciscana* fed a diet of selenium loaded algae cells (*Dunaliella viridis*).
4) Determine selenium elimination rates from *Artemia franciscana* following selenium accumulation from elevated ambient concentrations.
5) Model selenium accumulation in *Artemia franciscana* based on the results from objectives 1-3 to provide predictions of selenium accumulation during realistic exposure scenarios.
6) Determine the "knee" of the dissolved selenium accumulation rate curve in *Artemia franciscana*.
7) Investigate possible regulation of selenium accumulation in *Artemia franciscana* during prolonged exposure to selenium.

Introduction and Background

Selenium has long been recognized as a reproductive toxicant[7; 17] causing teratogenesis and chick mortality in birds[2] and the primary avian exposure pathway for selenium is the diet[1; 14]. Consequently, environmental regulations for selenium ought to aim at maintaining selenium concentrations below effect thresholds in avian prey organisms. As such, a tissue residue criterion (TRC) rather than traditional water-quality criteria has

recently been proposed for selenium by the USEPA[20]. A TRC approach however, is sensitive to variation in bioaccumulation of the element in question which potentially varies with site specific conditions (water and sediment chemistry) and other environmental stressors. Furthermore, bioaccumulation is often species specific and may be subject to homeostatic control, complicated uptake kinetics and excretion or elimination in the organisms of interest [1; 4; 5; 15; 19]. The Great Salt Lake, Utah is an important staging and breeding area for high numbers of migratory waterfowl and shorebirds. The high salinity of the Great Salt Lake (3-10 times that of seawater) limits the aquatic fauna and the highly abundant brine shrimp, *Artemia franciscana* is the largest aquatic predator and serves as one of the principle avian food sources[6; 13]. Because of the unusual water chemistry in the Great Salt Lake, standard water quality criteria do not apply and a TRC approach is currently applied for selenium discharge from the Kennecott copper smelting facility. Using an estimated dietary effect threshold for avifauna of 5 mg/kg dw[12; 18] and *in situ* measurements of total water selenium concentrations and corresponding concentrations in brine shrimp[3], the current water quality discharge limit is set at 27 µg/l. The *in situ* measurements from the Kennecott copper smelting facility[3] outflow provide a site relevant foundation for the establishment of discharge limits but are associated with some uncertainty. This uncertainty is a consequence of relatively limited field derived data which consists of water selenium concentrations below 5, around 30 and >80 µg/l, concentrations which appear to bracket the "knee" in the selenium accumulation curve[3]. The data forming the basis for the current discharge limit were analyzed by simple linear regression (which errs on the conservative side) yielding predicted brine shrimp selenium concentrations of 5 mg/kg dw at 27 µg/l. While the statistical approach is conservative in nature, it is important to recognize that the data set is small, exposure times are uncertain and life stages of the brine shrimp varied. Considerable error may therefore be associated with this estimated "safe" level.

Controlled laboratory studies are needed to address this uncertainty to better define the relationship between ambient selenium concentrations and concentrations of accumulated selenium in brine shrimp.

General Experimental Approach

The brine shrimp, *Artemia franciscana* and the GSL indigenous algae, *Dunaliella viridis* will be used for the experimental efforts outlined below. Brine shrimp cysts from the GSL are obtained from M & M Suppliers, Bothell, WA and will be hatched and raised in the laboratory in natural seawater from Bear Cut, Florida and in artificial GSL water at reduced salinity prior to experiments performed on adults. The male/female ratio in the brine shrimp culture will be determined on a regular basis with a target ratio of 1:1. Current male:female ratio is 46:54% in the University of Miami cultures. *Dunaliella viridis* was originally obtained from University of Wyoming and is currently cultured in the Grosell lab in artificial GSL water (see appendix A for composition). Selenium will be added as either stable or radioactive sodium-selenate in all experiments, as selenate is the main form in the GSL (recent USGS data) and is the principal form discharged from Kennecott[3]. Arrangements have been made for collection of algal samples from the GSL for selenium speciation and samples of selenium exposed algae from the present experiments will be obtained for selenium analysis. Note that the selenium speciation

analysis is not part of the present workplan or contract value. All experiments will be conducted at room temperature (22°C) in artificial GSL water at a salinity to be determined based on results from experiments addressing objective 1. Algae fed to Artemia will be pre-concentrated by centrifugation.

Selenium accumulation in Brine shrimp and *D. viridis* will be determined using a radioisotopic approach employing the gamma emitting Se-75 radioisotope. An isotopic approach offers multiple advantages including very high sensitivity and resolution, no background and relatively low analytical cost at high throughput. The low analytical cost and high throughput analysis avoids matrix interferences from the highly saline GSL water and limits the need for selenium analyses using hydride generation. It is important to note that the use of natural GSL water for uptake and accumulation experiments would require hydride generation analysis. To avoid these expensive and challenging analyses, artificial GSL water will be employed to the studies outlined below unless otherwise mentioned.
In brief, the isotopic approach relies on careful measurements of total selenium concentrations and radioactivity from Se-75 spiked selenium stock solutions providing the specific activity (SA) expressed in counts per minute (cpm)/µg selenium. From the SA of stock solutions used to spike exposure media, total selenium concentrations in exposure media (artificial GSL water), algae cells and Brine shrimp can be calculated from measurements of gamma radioactivity alone as follows:

Eq 1: Amount of selenium (µg) = radioactivity of sample (cpm)/SA of stock solution (cpm/µg)

The isotopic approach was used to generate the data presented in figure 1 which illustrates the high sensitivity of the method allowing us to determine selenium accumulation in the sub-picogram/individual level in individual young adult Brine shrimp at an average mass of 0.4 mg/individual.

Note that the approaches involved in addressing the overall objective of the present project will adhere to the present work plan but that experimental strategies may be adjusted as feedback from experiments are obtained. Significant deviations from the outlined experiments will be presented for approval.

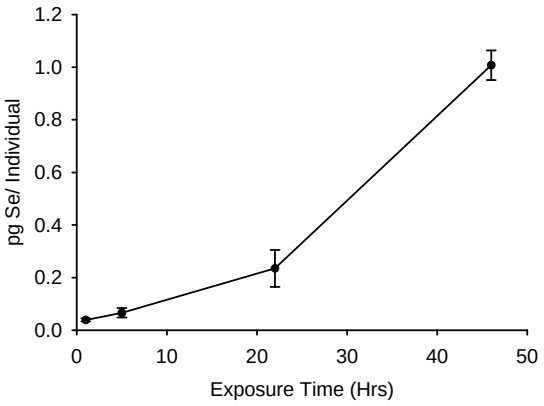

Figure 1. Selenium accumulation in *Artemia franciscana* during a 48 hour exposure to 13 ng/l selenate-75. Values are expressed as picogram/individual (wet weight). Mean ± SEM, n=10.

Activities

SPECIFIC APPROACHES AND EXPECTED RESULTS FOR INDIVIDUAL OBJECTIVES

Objective 1

Initial experiments are aimed to determine the best suited salinity for subsequent experiments. Competitive interactions with SO_4^{2-} lead to an expected reduced selenium uptake rate at higher salinities while elevated salinity likely results in increased energetic demand by brine shrimp for osmoregulatory purposes. An increased energetic demand at higher salinities may result in higher feeding rates and thus higher accumulation of selenium from dietary exposures. The salinity in the GSL varies considerably and since selenium uptake from both water and diet likely differs with salinity the need to evaluate effects of salinity on uptake rates seems eminent. Uptake of selenium from the water at ~1 µg Se/L will be determined (as outlined below) in adult brine shrimp acclimated to 100 and 160 ppt artificial GSL water. In addition, feeding rates will be determined for adult brine shrimp acclimated to these two salinities. For these feeding rate determinations, groups of brine shrimp will be fed a single meal of *D. viridis* and the decline in *D. viridis* cells over time will be followed to determine feeding rates. These experiments will be performed in triplicate. Once these experiments have been completed, a brief memorandum summarizing the experiment will be submitted so that a decision on a single salinity for further experiments may be made by the science panel. Subsequent experiments will be performed on this one salinity only.

Objective 2

Determination of selenium uptake rates in Brine shrimp from dissolved selenium concentrations in artificial Great Salt Lake (GSL) water (uptake kinetics).

Adult Brine shrimp (12-15 individuals per group) will be placed in 25 ml artificial GSL medium in 50 ml PYREX glass beakers gently aerated to ensure oxygenation and mixing and exposed to selenium-75 (selenate) for 24 hours. In initial experiments accumulation of total ammonia will be measured at the end of the 24-h exposure period to ensure adequate water quality. Expectations are that total ammonia concentrations will remain far below toxic values. Should these measurements indicate substantial ammonia accumulation, greater exposure GSL water volume will be considered. Note that the chosen exposure volume represents a compromise between sufficient water quality for the experimental animals and practical concerns associated with use of large amounts of radioactivity. The density in our current brine shrimp culture range from 10 to 20 individuals per 25 ml. For these experiments a total of 8 selenium concentrations ranging from 0 to 100 µg Se/L (representing GSL environmentally relevant concentrations) will be tested. In the preliminary study presented in Fig 1 accumulation rates seems constant during the first 24 hours of exposure but this will be verified for brine shrimp at 100 ppt by sampling at 2, 4, 8 and 24 hours. The Brine shrimp will be allowed 10 minutes to recover from handling (plastic Pasteur pipettes) prior to isotope addition. An initial water sample will be collected 15 minutes after isotope addition and again immediately prior to exposure termination at 24 hours. At this point individual Brine shrimp will be collected, rinsed three times in isotope free media to remove

selenium loosely associated with the surface and their mass will be determined to the nearest 100 µg. Subsequently, individual Brine shrimp and samples of the exposure media will be analyzed for gamma radioactivity. Food will be withheld for the 24-h isotope exposure. The high and medium exposures will also be performed in filtered Great Salt Lake water to demonstrate the utility/comparability of the artificial GSL water. Note that for these experiments, natural GSL water is required at a time (and volume) yet to be specified and that the total selenium concentration in this natural GSL water must be determined by hydride generation. The collection, shipment and total selenium analysis associated with this aspect of the project is not covered by the present work plan and budget and it is assumed that water and analytical results are provided to the University of Miami by Utah Division of Water Quality.

Saturation type kinetics is expected for these experiments showing disproportional uptake rates as a function of elevated dissolved selenium. Hence, one expects to see less proportional selenium accumulation with increasing exposure concentration, but an increase in absolute mass. This assumption will be evaluated.

Objective 3

Determine dietary selenium intake and subsequent selenium assimilation efficiency in Brine shrimp fed a diet of selenium loaded algae cells (*D. viridis*).

For the determination of dietary intake and selenium assimilation efficiency adult Brine shrimp will be fed a single meal of *D. viridis* labeled with radioactive Se-75. A range of three selenium concentrations in *D. viridis* will be tested. The concentrations will be aimed at bracketing the selenium concentrations observed in algae in the GSL and will be achieved by exposure of algae to nominal selenium concentrations of 3, 15 and 50 µg/l. Algae medium exposure concentrations are expected to vary but will be monitored and adjusted as needed to reach the above target concentrations as best as possible. Note that selenium concentrations in the algae medium (and the algal cells) will be measured. The media selenium concentration will be monitored daily and will be adjusted by selenium addition to maintain target selenium concentrations in the media. Algae will be harvested when maximal culture density is achieved or when sufficiently high selenium concentrations in the algae are reached.

Note that the objective is not to determine relationships between media selenium concentrations and algae selenium concentrations, but between selenium uptake via the diet by brine shrimp. We bring this to the attention of the Science Panel as you may wish to comment on this aspect of the proposal. This proposed approach was taken after reviewing the work of Dr. Marjorie Brooks and recognizing that the Science Panel is now receiving total selenium data on suspended solids (brine shrimp diet) collected in the GSL. For purposes of modeling accumulation of selenate in brine shrimp, it is not necessary to obtain kinetic data on algae. This significantly reduces the work load, cost and time required and avoids complications associated with how to accurately measure uptake and depuration kinetics in algal cells that are frequently dividing.

A total of 3 selenium loaded algae diets will be fed to adult Brine shrimp and the ingested selenium dose will be determined by analyzing individual Brine shrimp for Se-75 radioactivity during consumption and immediately after the algae diet has been consumed. Subsequently, the Brine shrimp fed the Se-75 labeled diet will be allowed to

depurate ingested but unabsorbed selenium overnight after which a sub sample of individuals will be reanalyzed for Se-75 radioactivity. Uncontaminated algal diet will be provided during this depuration period to ensure depuration of nonabsorbed algae from the intestinal tract. The difference between the initial measurements and the measurements of depurated Brine shrimp will allow for the determination of assimilation efficiency (AE) and the initial Se-75 accumulation rate will provide the ingestion rate (IR). From the measured selenium concentration in the algae diet, and the accumulated selenium in brine shrimp immediately after feeding, estimates of algae ingestion rate will be determined. Together these parameters will determine dietary selenium uptake rates at different algae concentrations.

Reduced or perhaps constant selenium assimilation efficiency in Brine shrimp with increasing algae selenium concentrations is expected from these experiments which will translate to either a saturation-type relationship or linear increase in accumulation with increasing algae selenium concentration, respectively. In either case, the data collected will allow for predictive dietary accumulation modeling.

Objective 4

Determine selenium elimination rates from Brine shrimp following selenium accumulation from elevated ambient concentrations.

Selenium elimination from adult Brine shrimp will be determined in animals which accumulated selenium from the dissolved and from the dietary phase (separate exposure scenarios). In both cases, adult *Artemia* will be exposed to selenium at the higher end of the concentration range employed under objectives 2 and 3 to result in substantial selenium accumulation after which they will be transferred to clean GLS media and fed an uncontaminated diet. Animals will be subsampled at regular intervals after termination of selenium exposure until a convincing (statistically significant) depuration has been observed.

A linear or exponential decay of selenium concentrations in the adult Brine shrimp after termination of exposure is expected and no difference between animals accumulating selenium from the water or the diet is predicted based on past experience. However, it is important to determine this assumption and to calculate the elimination rate constants.

Objective 5

Model selenium accumulation in Brine shrimp based on the results from objectives 2-4 to provide predictions of selenium accumulation during realistic exposure scenarios.

The ultimate goal of objectives 2-5 is to provide predictions of steady state selenium concentrations (ss[Se]) in GSL Brine shrimp which will be modeled as described recently[15] according to the following equation:

Eq 2: ss[Se] = (uptake rate from dissolved phase x diss[Se]) + (AE x IR x diet[Se])/Se elimination rate + growth

where, "diss[Se]" is the water selenium concentration, "AE" and "IR" are assimilation efficiency and ingestion rate, respectively and "diet[Se]" is the algae selenium

concentration. Growth is expected to have minimal influence on steady state concentrations during exposure to elevated ambient selenium. Note that the "uptake rate from dissolved phase", "AE" and "IR" and "Se elimination rates" are determined as described in objectives 2, 3 & 4 respectively.

We anticipate that the "uptake rate from dissolved phase" will be a non-linear component and anticipate the same may be true for "AE" and, but are uncertain regarding the "Se elimination rate".

Objective 6

Determine the "knee" of the dissolved selenium accumulation rate curve in *Artemia*.

Common for the uptake of many non-metal and metallic[9] inorganic elements is a low capacity-high affinity carrier mediated uptake system and a low affinity but high capacity transport system which combine to result in a "hockey stick" shaped relationship between uptake rate and ambient concentration (fig. 2). The low capacity-high affinity system acts to protect against metal uptake until the carrier capacity is exhausted. At this point the high capacity transport system dominates and carries larger amounts of metal into the organism. Assuming elimination remains relatively constant, the organism begins to accumulate the metal. This combination of transport systems was recently demonstrated for selenite and the freshwater algae *Chlamydomonas reinhardtii*[16].

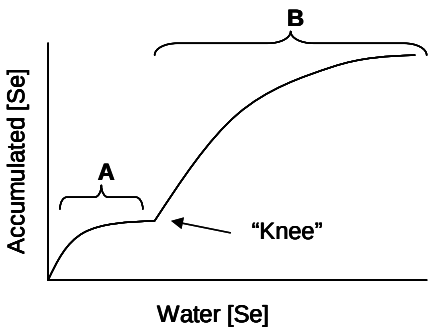

Figure 2. Expected selenium accumulation in *Artemia franciscana* as a function of ambient selenium concentrations. "A" denotes a high affinity, low capacity uptake system, while "B" denotes the low affinity, high capacity system. The transition between these two uptake pathways are often referred to as the "knee" in a "hockey stick relationship".

To identify the threshold concentration at which the low affinity-high capacity system begins to dominate the selenium uptake rates, Brine shrimp will be exposed to a range of dissolved selenium concentrations spanning beyond the environmentally relevant concentrations employed to address objective 2. Initial experiments will be performed as range finders with relatively large increments of concentration increase (for example 50, 100, 200, 400 µg/l) after which an experiment with a narrower series of exposures will be performed to better define the threshold ("knee"). Note that uptake kinetics for concentrations < 100 µg/l are determined under objective 2 and that exposure concentrations under objective 2 and the present objective may be adjusted as we obtain feedback from conducted experiments. Experimental procedures will otherwise be similar to those described under objective 1.

Objective 7

Investigate possible regulation of selenium accumulation in Brine shrimp during prolonged exposure to selenium.

It is entirely possible that Brine shrimp exerts regulatory control of accumulated selenium concentrations by acclimating to elevated ambient selenium to reduce selenium uptake. Such acclimation responses would influence ss[Se] as predicted from the model developed as objective 5.

This possibility will be evaluated by comparing Se-75 uptake rates in two groups of adult Brine shrimp, one non-acclimated control group and one exposed to an elevated selenium concentration (27 µg/l) for a two-week period. The two-week exposure will be conducted using non-radio labeled selenium and selenium uptake rates in both the control and the selenium pre-exposed group will subsequently be evaluated using Se-75 labeled selenium. This approach has been employed by the PI previously to distinguish between background levels and recently accumulated levels of toxicants[8; 10; 11].

A reduced or unaltered selenium accumulation is expected in selenium acclimated compared to control Brine shrimp. The data will either prove or disprove this assumption.

Deliverables

1. Prior to executing work, Data Quality Objectives (DQOs) will be prepared and reviewed by the Great Salt Lake (GSL) Science Panel. DQOs should address how this experiment will address DQOs for Project 5. Harry Ohlendorf will assist in preparing the DQOs. Principal investigators will participate in study team meetings and conference calls on an as-needed basis.
2. Standard Operating Procedures will be prepared that define experimental protocol to be followed. The SOPs should be forwarded for review via defined communication lines.
3. All work will follow UDWQ's Quality Assurance Plan protocol to the extent relevant for the present experimental work.
4. Brief progress reports will be provided after 3 and 6 months from project notice to proceed and a final report will be provided at 8 months after notice to proceed. Final report will document activities, methods, assumptions, data, recommendations, and conclusions completed as part of this task. All draft reports should be reviewed by and discussed with Dr. Buchwalter prior to submission to project team. One manuscript intended for publication in the peer-reviewed literature is anticipated from these studies but is not included as a deliverable to UDWQ.

Schedule

The table below indicates the anticipated rate of project progress.

	Time after project initiation (months)							
	1	2	3	4	5	6	7	8
Objective 1 (5%)	X							
Objective 2 (10%)	X							
Objective 3 (10%)		X						
1st progress report (10%)			X					
Objective 4 (10%)			X					
Objective 5 (10%)				X	X			
2nd progress report (10%)						X		
Objective 6 (10%)					X			
Objective 7 (10%)						X		
Draft complete report (10%)							X	
"Cleanup" experiments							X	
Final report (5%)								X

Table indicates % effort associated with each of the 7 objectives and various reporting tasks. "X" indicates anticipated time of completion of individual tasks.

Assumptions

1. The Grosell lab at the University of Miami is fully licensed and equipped to perform Se-75 experiments and a reliable supplier of Se-75 (as selenate) has been identified. It is assumed that supply and cost of Se-75 does not change dramatically during the completion of the present project.

2. It is not unlikely that results from the above experiments may identify additional needed experiments. It is assumed that such additional experiments are subject to adjustment of contract value and time schedule.

3. For experiments involving natural GSL water, analysis of non-radioactive selenium in this natural GSL water is required. It is assumed that GSL water and analyses of selenium concentrations are provided to University of Miami free of charge as the associated cost has not been incorporated in the budgeted contract value.

4. It is assumed that the principle investigator has the right to publish findings obtained from the present project in international peer-reviewed scientific journals. The principal investigator is committed to obtaining input from the GSL science panel and CH2MHILL on data presentation and manuscript preparation prior to submission. Specifically, the GLS science panel and CH2MHILL will have an opportunity to comment on manuscripts prior to submission.

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18. **Skorupa JP, Morman SP et al**. Guidelines for interpreting selenium exposure of biota associated with nonmarine aquatic habitats. *US Fish and Wildlife service national irrigation water quality program unnumbered report* 1996.

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Appendix A

	Artificial GSL (g/L)	Algae Media (g/L)
NaCl	69.306	52.596
$MgCl_2$ ($6H_20$)	14.378	1.500
$MgSO_4$ ($7H_20$)	11.734	0.500
KCl	3.580	0.200
$CaCl_2$ ($2H_20$)	0.200	0.200
$NaHCO_3$	0.223	0.043
$CaSO_4$ ($2H_20$)	0.540	--
KNO_3	--	1.000
KH_2PO_4	--	0.035
Trace Metals	--	10 ml/L
Iron Solution	--	10 ml/L