

**Note: The enclosed draft report was submitted in November  
2007. Dr. Grosell expects to complete his final reports  
shortly.**

## Final report for the “Brine Shrimp Kinetics Study, Project 5”

### Summary

#### Introduction

Selenium has long been recognized as a reproductive toxicant<sup>15,31</sup> causing teratogenesis and chick mortality in birds<sup>2</sup> and the primary avian exposure pathway for selenium is the diet<sup>1,26</sup>. 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<sup>39</sup>. 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<sup>1,6,38,9,28</sup>. 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<sup>24,12</sup>. 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<sup>22,35</sup> and *in situ* measurements of total water selenium concentrations and corresponding concentrations in brine shrimp<sup>5</sup>, the current water quality discharge limit is set at 27 µg/l. The *in situ* measurements from the Kennecott copper smelting facility<sup>5</sup> 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 consist of water selenium concentrations below 5, around 30 and >80 µg/l, concentrations which appear to bracket the “knee” in the selenium accumulation curve<sup>5</sup>. 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 experiments were performed to address this uncertainty and to better define the relationship between ambient selenium concentrations and concentrations of accumulated selenium in brine shrimp. The overarching main objective pursued in the present study was 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 was addressed by pursuing the following specific objectives:

- 1) Determine the influence of salinity on selenium uptake and feeding rate by *Artemia franciscana*.
- 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.

## Materials and Methods

### *Organisms*

The algae *Dunaliella viridis* which is indigenous to the GSL was used in the present project and was obtained as a gift from Marjorie Brooks (then at the University of Wyoming). Cultures of *D. viridis* were maintained in artificial GSL medium (Table 1) for the present project and subcultures were raised in appropriate selenium concentrations as described below. The brine shrimp, *Artemia franciscana* were obtained as cysts from M&M Suppliers, Bothell, WA and were hatched in natural seawater from Bear Cut Florida and maintained in bulk culture in 73.5 ppt artificial GSL water. *Artemia* were acclimated to higher salinity artificial GSL waters for a period of at least 48 hours prior to experimentation. *Artemia* were maintained in mass culture in 4 individual aerated 10 gallon tanks with partial water renewal as necessary and were fed commercially available dried algae daily. Specifically, 1 gram of Wardley Premium Algae Discs (Secaucus, NJ) was homogenized in 20 mls of deionized water and offered to *artemia* according to culture density. Feeding amount was adjust such that *artemia* were able to completely clear the water between feedings.

### *General experimental procedures*

Only adult (>4 mg whole animal wet weight) *artemia* were used in the present experiments and a radioisotope labeling procedure was employed to facilitate fast, yet accurate measurements of low levels of selenium in a highly saline matrix. Radiolabeled (<sup>75</sup>Se) selenium stock solutions were prepared in deionized water for individual experiments and the exact <sup>75</sup>Se radioactivity and total selenium concentrations were determined in these stock solutions by gamma detection and graphite furnace atomic absorption, respectively (see below). From the ratio of <sup>75</sup>Se radioactivity and total selenium concentration in the stock solutions (specific activity; SA) and corresponding <sup>75</sup>Se radioactivity from artificial GSL water, algae and *artemia*, selenium concentrations were determined as:

$$^{75}\text{Se radioactivity (cpm)} / \text{SA (cpm}/\mu\text{g Se)} = \mu\text{g Se}$$

	Artificial GSL for culturing (g/L)	Artificial GSL for experiments (g/L)	Algae Media (g/L)
NaCl	50.960	69.306	52.596
MgCl <sub>2</sub> (6H <sub>2</sub> O)	10.572	14.378	1.500
MgSO <sub>4</sub> (7H <sub>2</sub> O)	8.628	11.734	0.500
KCl	2.632	3.580	0.200
CaCl <sub>2</sub> (2H <sub>2</sub> O)	0.147	0.200	0.200
NaHCO <sub>3</sub>	0.164	0.223	0.043
CaSO <sub>4</sub> (2H <sub>2</sub> O)	0.397	0.540	--
KNO <sub>3</sub>	--	--	1.000
KH <sub>2</sub> PO <sub>4</sub>	--	--	0.035
Trace Metals	--	--	10 ml/L
Iron Solution	--	--	10 ml/L

**Table 1.** Composition of artificial GSL media and *D. viridis* culture medium

#### *Analytical procedures*

Gamma activity arising from the Se-75 isotope was detected by a Packard, Cobra II auto gamma counter D5003 using a counting window from 60-467 keV. Total selenium concentrations in stock solutions were determined by graphite furnace atomic absorption (Varian, 220Z). A sample injection volume of 10 µl and 10 µl of modifier (1 mg Ni/ml) were co-injected with deionized water for a total injection volume of 25 µl. Following evaporation, an ashing step of 1000 °C for 8 seconds preceded the atomization step of 2600 °C. Absorbance recorded from samples was compared to the absorbance obtained from automatically generated dilutions of a certified selenium standard (Aldrich) to determine selenium concentrations.

Algal dry weight was determined by filtering 15 mls of an algae culture of known cell density (determined by cell counting on a hemacytometer) through a pre-weighed 47 mm glass microfibre filter (Whatman) then rinsing the filtered cells with 10 mls of 0.5 M ammonium formate. The filters were then dried for 24 hrs at 80 °C then re-weighed. Dry weight was estimated by dividing mass of the dried algae by the number of algae cells in the 15 ml sample.

*Artemia* dry weight was determined by sampling and gently blotting dry 12 adult *artemia* on a paper towels then placing them on 1 cm<sup>2</sup> pieces of pre-weighed aluminum foil. These *artemia* were weighed to determine wet weights, then dried for 24 hours at 80 °C and re-weighed to determine dry weights.

#### *Data presentation and statistical evaluation*

Data is reported as means ± SEM throughout. Non-linear regressions were performed using SigmaPlot version 8.0 and statistical comparisons were performed using Student's two-tailed *t*-tests.

#### *Influence of salinity of selenium uptake from the water and feeding rates (objective 1)*

To examine the effect of salinity on selenium uptake from the water 24-hour selenium uptake was measured in individual artemia at 100 and 160 g/L GSL medium at  $1.75 \pm 0.05$  and  $1.83 \pm 0.08$   $\mu\text{g Se/L}$  respectively. Measurements were performed in triplicate treatments each with 15 adult artemia and otherwise as outlined in the SOP provided below. A 24-hour exposure duration was chosen based on experiments demonstrating linear Se accumulation for at least 24 hours (Fig.1) and initial experiments demonstrated that Se exposure concentrations remained relatively constant over at least 24 hours of exposure (Fig. 2).

Feeding rates were also determined in adult artemia fed *D. viridis* at 100 and 160 g/L GSL medium. Adult artemia (n= 15) in 30 mls of GSL media were offered a density of  $93.4 \cdot 10^6$  *D. viridis* cells and cell density was monitored every 10 minutes for a total of 30 minutes by measuring the absorbance of water samples at 750 nm. Preliminary experiments revealed a good correlation between cell count (cells/mm<sup>2</sup>) and absorbance (Fig. 3). The feeding rate experiments were performed in triplicate and otherwise as described in the SOP.

*Determination of selenium uptake rates in brine shrimp from dissolved selenium concentrations in GSL water (Objective 2 & 6)*

Based on findings from the salinity experiments above, all subsequent experiments were performed at 100 g/L. Adult artemia (n=15) were placed in 25 ml artificial GSL medium in 50 ml PYREX glass beakers gently aerated to ensure oxygenation and mixing and were exposed to <sup>75</sup>Se (as selenate) for 24 hours. Artemia were allowed to recover from handling for 10 min prior to isotope addition and water samples were obtained from the exposure medium 15 minutes after isotope addition and immediately prior to exposure termination at 24 hours. After 24 hours of exposure, individual artemia were collected from the exposure medium and rinsed three times in isotope free media to remove <sup>75</sup>Se loosely associated with the surface. Individual artemia were blotted dry on paper towels and their wet weight determined to the nearest 100  $\mu\text{g}$  prior to <sup>75</sup>Se radioactivity determination.

*Determination of dietary selenium intake and subsequent selenium assimilation efficiency in artemia fed a diet of selenium loaded algae cells (Objective 3).*

*D. viridis* cultured in presence of different selenium concentrations served as the dietary source of selenium for artemia. *D. viridis* was cultured under constant light at 18°C in artificial GSL media (table 1) in gently aerated Erlenmeyer flasks and media selenium concentrations were monitored daily and adjusted as necessary by addition of <sup>75</sup>Se stock solutions or selenium free media to elevate or reduce media selenium concentrations, respectively. The time required to reach steady state selenium concentrations in *D. viridis* was determined in an initial 40-day experiment and subsequent exposures were 21 days in duration. In addition to daily monitoring of media selenium concentrations, algal density and algal selenium concentrations were determined in algal cells sampled from the cultures and rinsed in <sup>75</sup>Se free medium prior to <sup>75</sup>Se detection.

*D. viridis* was harvested for artemia feeding studies at day 20-21 of exposure at which point steady state was achieved. Algae raised at four different media selenium concentrations ranging from 1.2 to 60.4  $\mu\text{g Se/L}$  were used in the present study. Algae were isolated by centrifugation in a microcentrifuge at 8000 rpm after which the

radioactive supernatant (algae media) was discarded and cells were rinsed by resuspension in selenium free media followed by additional centrifugation and media replacement. Algal density in this concentrated cell suspension was determined using a Bright-Line Hemacytometer (Hausser Scientific, Horsham PA) for direct counting using a light microscope.

For each algae selenium concentration, a total of 20 adult artemia were placed in 4 L of GSL medium in a 5 L plastic beaker gently aerated to ensure oxygen saturation and continuous mixing and suspension of algal cells. A total of  $37 \cdot 10^6$  cell/L were added to the 4 L of GSL media and artemia were allowed to feed for 60 minutes after which they were removed, rinsed and transferred alive to a gamma counting vial containing 3 ml GSL media. This protocol was chosen from initial experiments to prevent depletion of algal cell density during the feeding experiments and to allow for accurate determination of ingestion rates. Experiments monitoring dietary selenium ingestion during a 90 minute period revealed a gut passage time of around 60 minutes (Fig 4) and subsequent feeding experiments were restricted to this duration. Gamma counting of individual artemia was conducted immediately and then individual artemia were transferred to 15 ml falcon tubes containing 10 ml of selenium free GSL medium. The artemia were subsequently fed a non-radioactive algae diet to allow for depuration of unassimilated food overnight after which individual artemia were rinsed and transferred to fresh gamma counting vials containing 3 ml of GSL media in preparation for a second gamma counting. Following this second gamma radioactivity determination, artemia wet weight was determined as above.

Fecal matter from the 15 ml falcon tubes was collected and its  $^{75}\text{Se}$  content was determined via gamma counting.

Assimilation efficiency was determined as the ratio of assimilated  $^{75}\text{Se}$  to ingested  $^{75}\text{Se}$ . The ingestion rate of individual artemia in these studies was determined from the  $^{75}\text{Se}$  accumulated during the 60 minutes of feeding and the corresponding selenium concentration in algal cells.

*Determination of selenium elimination rates from artemia following selenium accumulation from elevated ambient concentrations (objective 4).*

Selenium elimination rate constants were determined for artemia exposed to water-borne and dietary selenium. For the waterborne exposure a total of 30 adult artemia were exposed to  $72 \mu\text{g Se/l}$  for 48 hours without feeding while dietary selenium accumulation in 20 adult artemia was ensured by a 1 hour exposure to  $^{75}\text{Se}$  containing algae. Following the initial exposure individual artemia were rinsed (3 times for the water-borne exposure and once for the dietary exposure) and placed in 3 ml GSL medium in individual gamma counting vials for  $^{75}\text{Se}$  determination. After  $^{75}\text{Se}$  counting, artemia were placed in 50 ml falcon tubes containing 30 ml GSL medium each and were fed daily. Following this initial  $^{75}\text{Se}$  determination, measurements were performed on a regular basis for a minimum of 20 days allowing for more than 50% depuration of the initial  $^{75}\text{Se}$  levels.

*Investigate possible regulation of selenium accumulation in artemia during prolonged exposure to selenium (objective 7).*

The potential influence of prolonged exposure on selenium uptake rates from the water and on assimilation efficiency for dietary exposures was evaluated. To examine uptake

rates after long term selenium exposure a group of adult artemia were exposed to  $2.87 \pm 0.14 \mu\text{g Se/L}$  for 14 days. To avoid  $^{75}\text{Se}$  accumulation in this group of selenium pre-exposed artemia, these organisms were exposed to non-radioactive selenium. A parallel group of artemia was exposed to identical conditions using radio-labeled  $^{75}\text{Se}$  in the water to allow for measurements of selenium concentrations during the 14 days of exposure. Exposure concentrations were adjusted in both these groups of artemia according to  $^{75}\text{Se}$  measurements in the radio-labeled group. The  $^{75}\text{Se}$  group acted simply as a parallel surrogate to the non-radioactive 14-day exposure to ensure constant and characterized exposure concentrations. In addition to these two groups of artemia, a third group was maintained under control conditions without selenium added. All three groups consisted of 20 adults maintained in 1L gently aerated GSL media (100 g/L) and were fed *D. viridis* daily 3-4 hours prior to adjustments of Se exposure concentrations. After 14 days of exposure, uptake rates from  $^{75}\text{Se}$  containing GSL medium were determined for the controls and for the artemia exposed to non-radioactive selenium. These  $^{75}\text{Se}$  uptake rate experiments were performed at  $2.55 \pm 0.11 \mu\text{g Se/L}$  according to procedures outlined for waterborne experiments elsewhere.

To examine the potential influence of prolonged exposure to dietary selenium on subsequent assimilation efficiencies, *D. viridis* were raised in the presence (resulting in  $2.68 \mu\text{g Se/g}$  dry weight) and absence of selenium. Two algae cultures were raised in presence of selenium, one in which  $^{75}\text{Se}$  was employed and one containing the same concentration of non-radioactive selenium. The culture medium selenium concentrations were adjusted in parallel in the two cultures based on measurements of  $^{75}\text{Se}$  in the radioactive medium to ensure constant exposure conditions. In parallel with these two selenium containing cultures, a selenium free control algae culture was raised simultaneously. All algae cultures were maintained for a minimum of 20 days to ensure steady state selenium concentrations.

Two groups of 30 adult artemia were maintained in 1 L gently aerated GSL (100 g/L) and were fed daily with algae raised in presence or absence of unlabelled selenium (not  $^{75}\text{Se}$ ). During the 14 days of exposure, exposure beakers were siphoned daily and water was replaced twice weekly.

After these 14 days of exposure to either control or non-radioactive selenium loaded algae ( $2.68 \mu\text{g Se/g}$  dry weight), 25 individuals from each group were transferred to 4 L of GSL media and ingestion rate as well as assimilation efficiency were determined as above using a  $^{75}\text{Se}$  labeled algae culture ( $3.73 \mu\text{g Se/g}$  dry weight).

## **Results**

### *Effects of salinity*

As predicted selenium uptake from the water was reduced at 160 ppt compared to 100 ppt (Fig 5). However, in contrast to expectations, elevated salinity (160 ppt) resulted in an apparent reduced feeding rate compared to that seen at 100 ppt (Fig 6). All subsequent experiments were performed at 100 ppt.

### *Selenium uptake by artemia from the water*

A general trend of increasing selenium uptake rates with increasing ambient selenium concentrations was observed in experiments exposing adult artemia to a range of

selenium concentration in GSL media for 24-hour periods (Fig. 7). Upon closer examination, however, a saturation pattern is observed for selenium concentrations below  $10 \mu\text{g/L}$  ( $((660.2 \cdot C_w)/(1.20 + C_w))$ ) after which selenium uptake rates appear to increase in proportion to ambient concentrations. At the highest concentration tested a tendency for reduced selenium accumulation was observed. A near perfect linear fit describes selenium uptake at ambient selenium concentrations below  $2.5 \mu\text{g/L}$  equivalent of a  $K_u$  of  $0.211 \text{ L/g dry weight/day}$ .

#### *Selenium accumulation in Dunaliella viridis exposed to elevated media selenium.*

An unexpected tri-phasic pattern of selenium accumulation in *D. viridis* was observed during an initial 40-day exposure to  $2.17 \mu\text{g Se/L}$  characterized by an initial rapid increase in algal selenium concentrations followed by apparent depuration and subsequent stabilization (Fig. 8). Steady state selenium concentrations in *D. viridis* appear to be reached in approximately 20 days and subsequent algae selenium loading experiments were performed over this exposure period.

An additional four experiments employing different media selenium concentrations were performed at concentrations ranging from an average  $1.2$  to  $60 \mu\text{g Se/L}$  and all exhibited a similar pattern of fast initial accumulation followed by depuration and stabilization (Fig. 9). Our radio-isotopic approach allowing for rapid detection of selenium concentrations in the exposure media and prompt adjustments ensured relatively stable exposure concentrations during the 20 days of culturing (Fig. 10). Considering the algae selenium concentrations after 21 days of culturing a less than linear increase in cell selenium concentrations as a function of ambient selenium was observed pointing to lower bioconcentration factors at higher ambient concentrations (Fig. 11).

Comparing the growth rates, as indicated by absorbance at  $750 \text{ nm}$  throughout 21 days (Fig. 12), of algae cultures at different selenium concentrations to growth rates in absence of added selenium revealed highest growth rate at  $\sim 18 \mu\text{g/L}$ . The lowest growth rate was observed in absence of added selenium and it appeared that the highest employed selenium concentration ( $60 \mu\text{g/L}$ ) tended to reduce growth of *D. viridis* somewhat (Fig 13).

#### *D. viridis ingestion rate, dietary selenium intake and assimilation efficiency in artemia*

The 1-hour feeding experiments revealed feeding rates of  $0.185 \text{ g/g (dry weight)/day}$  and demonstrated increasing selenium ingestion and assimilation with increasing algae selenium concentrations (Fig. 14). Selenium assimilation efficiency showed a 2<sup>nd</sup> order exponential decay equation with a minimum assimilation efficiency of  $74\%$  at higher dietary selenium concentrations and a near  $100\%$  at low selenium concentrations (Fig. 15).

#### *Selenium elimination rates constants*

The possibility of distinct elimination rates for selenium accumulated from the water and the diet was considered. For water-borne selenium, an initial rapid elimination was observed during the first 24 hours following termination of exposure. From day 1 and onward, a simple exponential decay equation describes selenium concentrations in artemia well ( $r^2=0.99$ ) with a  $6.79\%$  daily selenium loss (Fig. 16). The dietary selenium, elimination rates of  $7.37\%$  per day remained constant at least during the first 14 days of

depuration after which an apparent reduction in elimination rates is evident (Fig. 17). Despite initial accumulated selenium concentrations approximately 6-fold higher in water-borne compared to dietary exposures, elimination rates appeared slightly lower for water-borne selenium.

#### *Influence of prolonged exposure on selenium uptake rates.*

Two weeks of exposure to elevated, yet environmentally relevant selenium concentrations tended to reduce selenium uptake rates from the water and assimilation efficiency from the diet. Water-borne exposure to selenium resulted in an apparent 23% reduction in subsequent <sup>75</sup>Se labeled selenium uptake although this difference escapes statistical significance (Fig. 18). Similarly, prolonged exposure to dietary selenium concentrations of environmental relevance resulted in a modest (not statistically significant) reduction in subsequent assimilation efficiency from 73.5 to 68.6% equivalent to a 7% reduction in total dietary selenium assimilation (Fig. 19).

### **Discussion**

Initial experiments revealed that 24 hours of exposure to water-borne selenium resulted in linear accumulation in artemia and revealed that exposure concentrations remained constant during this period. Furthermore, it was revealed that 60 minutes of duration for feeding experiments is appropriate for determination of ingestion rates and quantification of selenium ingestion and subsequent assimilation efficiency.

No mortality was observed during selenium uptake experiments and less than 10% mortality was observed in depuration experiments in which repeated handling of individual artemia likely was the cause of mortality.

#### *Influence of salinity*

In agreement with expectations, increased GSL salinity from 100 to 160 ppt resulted in a significant reduction in selenium uptake by artemia. Although it is unknown which component(s) of the ionic matrix in the GSL medium is responsible for this observation it appears likely that sulfate is the anion competing with selenium uptake. Early studies demonstrated a direct antagonistic relation between sulfate and selenium uptake in plants<sup>23</sup> and several subsequent studies have revealed that elevated sulfate protects against acute selenium toxicity in algae as well as aquatic organisms, including artemia in freshwater and hypersaline environments<sup>13,7,34</sup>.

In contrast, elevated dietary selenium intake (feeding rate) was expected at 160 compared to 100 ppt. Elevated salinity can be expected to be associated with an increased metabolic demand from osmoregulatory processes and such an elevated metabolic cost was expected to be associated with higher feeding rates and thus higher dietary selenium intake in artemia fed *ad lib*. While the reason for apparently reduced selenium ingestion at higher salinities is unknown, the brief duration of the feeding experiments allows for the conclusion that feeding rate is lower at 160 ppt compared to 100 ppt and that ingested/assimilated selenium levels likely are not influenced directly by ambient sulfate levels which might be ingested with food.

#### *Selenium uptake from the water*

Selenium uptake from the water displayed a complex pattern of saturation kinetics at concentrations below 10 µg Se/L followed by a sharp increase in selenium uptake rates with a threshold somewhere between 10 and 20 µg Se/L. In addition it appears that selenium uptake is down regulated at concentrations above 40 µg Se/L, although this later observation is based on a single high selenium concentration. In the following, “high affinity, low capacity system” will refer to the selenium uptake at concentrations below 10 µg Se/L and “low affinity, high capacity system” will refer to the uptake pathways dominating at higher concentrations.

The apparent saturation pattern at relatively low selenium concentrations indicates that selenium is taken up from the water, presumably via the respiratory surface, via protein carriers in epithelial cells. Saturation uptake patterns have also recently been reported for freshwater algae exposed to selenate<sup>14</sup>. Although it seems that high sulfate concentrations may interfere with selenium uptake, the specificity of this putative selenium uptake system is not known but transporters with high specificity for selenium are known from mammalian systems and from plants<sup>4,36,30</sup>. The apparent affinity constant for the high affinity, low capacity selenium uptake system ( $K_m$ ) which denotes the ambient concentration at which the transport system is half saturated is 1.2 µg Se/L. The significance of this becomes clear when one considers the range of selenium concentrations normally observed in the GSL (0.297 to 0.899 µg Se/L, Brad Marden unpublished data). Regardless of the nature of the selenium transporters responsible for this high affinity transport system, variations in ambient selenium concentrations within the range normally observed in the GSL will greatly influence the selenium uptake rates by this transport system.

The low affinity, high capacity system dominates at selenium concentrations exceeding those observed in open GSL waters and thus are not a factor for steady state selenium concentrations in GSL artemia.

A situation of an apparent dual carrier uptake system with distinct transport characteristics is not unprecedented and has been observed for copper in the freshwater rainbow trout<sup>19</sup>. Like selenium, copper is an essential micronutrient which is potentially highly toxic and therefore it is not surprising that these two elements might share this unusual uptake pattern.

The apparent reduction in selenium uptake at the highest concentration tested could be a consequence of down regulation of the low affinity, high capacity selenium uptake system but the highest selenium concentration tested is orders of magnitude lower than concentrations considered to be acutely toxic to artemia<sup>13</sup>. Furthermore, the highest tested selenium concentration falls well above concentrations relevant for the GSL and uncertainty associated with the reason for apparent reduced uptake at this concentration is of no consequence for predictions on steady state selenium concentrations in GSL artemia.

#### *Selenium accumulation in D. viridis*

The careful characterization of selenium accumulation on *D. viridis* for the purpose of providing a natural diet for the study of dietary selenium uptake in artemia under conditions relevant to the GSL revealed a complicated pattern of selenium accumulation. An initial increase in cellular selenium concentration in algae cultured in presence of selenium was expected but the clear depuration of cellular selenium concentrations from

algae cells despite continued exposure to constant ambient selenium concentrations was not anticipated. An obvious possible explanation for this pattern, growth dilution, can be dismissed based in continued low cellular selenium concentrations during the last 20 days of the 40-day culture period during which cell density remained relatively constant. During this period, net growth was minimal but no increase in cellular selenium concentrations was observed and values remained much below peak concentrations observed around day 5-8 of culturing. Furthermore, calculations of specific growth rate in the algae culture (daily % increase in cell density) revealed that growth rates were also high during the initial rapid accumulation phase observed during the first week or so of culture. A final observation of lack of correlation between algal cellular selenium concentrations and specific growth rate also argues against growth dilution as an explanation for the observed selenium depuration during continued exposure. Two possible explanations remain which may account for the observed reduction in cellular selenium concentrations during continued exposure. For one, reduced selenium uptake as a negative feedback to elevated cellular selenium concentrations combined with constant growth and selenium elimination would result in reduced cellular selenium concentrations. A second possibility is that selenium elimination is stimulated by elevated cellular selenium concentrations which, even when combined with constant uptake would result in reduced cellular selenium concentrations. Obviously, a combination of reduced uptake and stimulated excretion cannot be dismissed as a possibility. While activation of a selenium export system is the only way to account for selenium excretion, reduced uptake could potentially be accounted for by a down regulation (reduction in numbers) of selenium uptake proteins or be explained by cellular excretion of substances rendering ambient selenium less available for cellular uptake. This latter explanation could be highly important in algal culture situations where cell densities are extremely high compared to natural situations but might be less important under natural conditions. In contrast, a down regulation of selenium uptake proteins would have the same effect in algal cultures as in natural algae populations. The bioconcentration factors for *D. viridis* at steady state were  $2.23 \cdot 10^3$ ,  $2.16 \cdot 10^3$ ,  $1.87 \cdot 10^3$  and  $1.31 \cdot 10^3$  at 1.2, 3.6, 17.8 and 60.4  $\mu\text{g Se/L}$ , respectively (calculated from the data in Fig 11) and thus adhere to what appears to be a general pattern of reduced bioconcentration factors with increasing exposure concentrations<sup>29</sup>. An important consequence of the dynamic Se accumulation pattern over time in *D. viridis* is that bioconcentration factors will differ depending on what point in time during exposure the algal selenium concentrations are considered. The above bioconcentration factors compare favorably to an overall bioconcentration factor for seston in the GSL of  $0.71 \cdot 10^3$  (based on mean concentrations from 2006, Marden, unpublished), although they are somewhat higher. A possible explanation for the higher bioconcentration factors for *D. viridis* under laboratory conditions compared to the field may reflect that seston from the GSL is comprised in part of organic material without cellular metabolic activity and thus selenium concentrating processes.

#### *Trophic selenium transfer to artemia*

Gut retention time for artemia fed *D. viridis* is 60 minutes and ingestion rates at the cell densities employed for the present study were 0.021 g algae dry weight/day/g artemia wet weight, which is equivalent to 0.185 g algae dry weight/day/g artemia dry weight. This

ingestion rate is comparable to feeding rates reported for marine zooplankton including copepods and mysids although slightly lower than the reported range from 0.33 and 0.44 g algae dry weight/day/g artemia dry weight<sup>28</sup>.

The assimilation efficiencies determined in the present study were not constant across exposure concentrations. To the best of our knowledge no studies to date have considered the influence of dietary exposure concentrations on selenium assimilation efficiency and it is generally assumed to be constant regardless of concentration for metals in general<sup>28</sup>. The exponential decay equation describing the relationship between dietary selenium concentration and assimilation illustrates that selenium assimilation efficiency in artemia fed *D. viridis* ranges from 75% at high concentrations to 100% at very low selenium concentrations. This relationship is in agreement with the saturation pattern observed for uptake of selenium from the water and strongly suggests that intestinal selenium uptake is mediated by specific transport pathways which become limiting for uptake at higher selenium concentrations. The assimilation efficiencies observed in the present study (>75%) compare favorably with earlier reports ranging from 30-86%<sup>27,33,37,40</sup> but cannot be assumed to be constant across exposure concentrations. The assimilation efficiencies determined as part of the present study represent a suspension feeding/algae relationship which is directly relevant to the GSL and considers algae in steady state with respect selenium concentrations. While using algae at steady state represents a realistic situation for chronic exposures, it is unknown how factors like cell density and thus feeding rate, and seston rather than pure algae as a food source might influence dietary selenium assimilation.

#### *Selenium elimination by artemia*

Considering first elimination of selenium accumulated from the water, an 80% depuration was obtained during a 20 day period with an initial rapid selenium loss during the first 24 hours following termination of exposure. An elimination rate constant of 6.79%/day was determined from fitted exponential decay curves based on data points collected after the initial rapid depuration phase. Similar observations of rapid initial elimination of metals have been reported previously and are believed to be associated with dissociation of surface bound metal. The rapid initial elimination phase was not considered when deriving elimination rate constants because it most likely does not reflect the physiology of organisms chronically exposed in natural environments<sup>8,10,11</sup>.

Considering next the elimination of dietary selenium originating from a *D. viridis* diet, a near 80% depuration was also obtained approximately 20 days after ingestion of a <sup>75</sup>Se labeled algae diet. The elimination rate constant for dietary selenium was 7.37%/day and thus tended to be slightly higher than the 6.79%/day observed for water borne selenium. From both the water-borne and dietary selenium elimination experiments it appears that elimination rate constants are independent of accumulated selenium concentrations which is consistent with the majority of earlier studies. Although slightly different, the elimination rate constants observed in the present study for water-borne and dietary selenium are in agreement with elimination rate constants reported for many other invertebrates for a number of different metals<sup>28</sup>.

#### *A model to predict steady state selenium concentrations in artemia (Objective 5)*

The development of a model to predict steady state selenium concentrations in *artemia* under conditions relevant to the GSL was inspired by the DYMBAM model approach<sup>32</sup>. In brief, the differential equations describing this model have been solved to determine selenium concentrations at steady state (constant metal concentration in the organism,  $C_{ss}$ ) as:

$$C_{ss} = [(k_u \cdot C_w) + (AE \cdot IR \cdot C_f)] / (k_e + g)$$

where:

$k_u$  = is the uptake rate constant from water

$C_w$  = water-borne selenium concentration

AE = dietary assimilation efficiency

IR = ingestion rate

$C_f$  = dietary selenium concentration

$k_e$  = elimination rate constant

g = growth dilution

The approach presented in the following deviates slightly from the original DYMBAM model in that it considers the two (slightly) different  $k_e$ 's, one for water-borne Se ( $k_{ew}$ ) and one for dietary Se ( $k_{ef}$ ) discussed above.

Thus the principal model developed for the steady state selenium concentrations in brine shrimp in the GSL is as follows:

$$Ss[Se] = ((k_u \cdot C_w) / k_{ew}) + ((AE \cdot IR \cdot C_f) / k_{ef})$$

Note that growth dilution “g” is omitted from the model since it has been developed for adult *artemia*.

In addition to this deviation, uptake rate from the water ( $k_u$ ) is considered in two different ways: in scenario I a traditional  $k_u$  is used as in previous reports whereas in scenario II it is reflected by a Michaelis-Menten kinetics equation (Fig. 20). These two different scenarios result in slightly different predicted steady state selenium concentrations and are both presented in the following. Furthermore, our observations of varying assimilation efficiency (AE) depending on dietary Se concentrations prompted the use of an equation rather than a constant to describe AE.

The constants/equations used for the developed  $ss[Se]$  model are listed in Table 2.

Parameter	Water-borne		Dietary
	Scenario I	Scenario II	
$K_u$	0.211	$(660.2 \cdot C_w)/(1.20 + C_w)$	-
$C_w$	Input variable		-
$k_{ew}/k_{ef}$	0.0679	0.0679	0.0737
AE	-	-	$(74.97 + 26.54^{-0.1088C_f}) \cdot 10^{-2}$
IR	-	-	0.185
$C_f$	-	-	Input variable

**Table 2.** Individual model parameters for the ss[Se] model. Uptake parameters ingestion rates are expressed per g dry weight

**Waterborne exposure  $((k_u \cdot C_w)/k_{ew})$ :**

Uptake:

Scenario I  $(k_u \cdot C_w)$ :

The traditional uptake rate constant ( $k_u$ ) can be determined from the near-linear part of the uptake kinetics curve to be 0.211 l/g dry weight/day) and applies to ambient selenium concentrations  $< 2.5 \mu\text{g/L}$  since the uptake kinetics curve is only linear below this concentration (Fig 20).

Scenario II:

From the Michaelis-Menten saturation kinetics applying to selenium concentrations below  $10 \mu\text{g/l}$  an uptake rate constant (or equation rather, Fig. 20) was determined to be:

$$k_u = (660.2 \cdot C_w)/(1.2 + C_w), r^2 = 0.92$$

Note that the constant in scenario I is considerably higher than previously reported  $k_u$ 's for pelagic crustaceans ranging from 0.024-0.027<sup>33,37</sup> but is in good agreement with  $k_u$ 's for estuarine macro invertebrates<sup>3</sup> and that it is determined for low selenium concentrations relevant for the GSL. Employing scenario II for higher concentrations reveals numbers in closer agreement with the above mentioned previous values.

Elimination: The rate constant of loss ( $k_e$ ) relevant to selenium accumulated from water-borne exposure are discussed above.

Steady state Se concentrations (Ss[Se]) in artemia arising from water-borne exposures in artificial GSL water (the first part of the ss[Se] model above) can be estimated using the following equations:

$$\text{Scenario I: Water-borne ss[Se]} = ((0.211 \cdot C_w)/0.0679)$$

$$\text{Scenario II: Water-borne ss[Se]} = [((660.2 \cdot C_w)/(1.2 + C_w)]/ 0.0679$$

**Dietary exposure  $((AE \cdot IR \cdot C_f)/k_{ef})$ :**

Uptake: The assimilation efficiency (AE) is normally assumed to be constant in DYMBAM models regardless of dietary metal concentration. However, the present project identified that assimilation efficiency decreases with increasing dietary selenium concentrations and that it adheres to an exponential decay equation:  $(74.97 + 26.54 \cdot 0.1088^{\text{Se}f})$  which is used to predict AE in the present ss[Se] model.

#### *Conversion from artemia wet weight to dry weight*

The water content of adult artemia used in the present investigation was  $88.6 \pm 0.5\%$  (n=12).

#### *Predicted steady state selenium concentrations (ss[Se])*

Tables 3 & 4 show artemia steady state selenium concentrations according to the two scenarios described above for water-borne selenium, combined with the dietary contribution. Highlighted values represent mean measured concentrations from the GSL (mean selenium concentrations in the GSL during the period from April to December 2006; data provided by Brad Marden) and corresponding predicted ss[Se] according to the scenarios described above. Measured total selenium concentrations in artemia from the GSL range from 0.5 to 3.3 with a mean of 1.185  $\mu\text{g Se/g dry weight}$  and the model predictions of 2.51 and 3.89 for scenarios I and II respectively are thus in general agreement. Note that for the GSL selenium concentrations observed in April – December 2006, scenario I is the recommended model.

The model predictions and measurements of artemia selenium concentrations reported by Brad Marden are in good agreement with measured selenium concentrations in artemia collected from the GSL in 2002 which range from 2.86 to 3.38  $\mu\text{g Se/g dry weight}$  for artemia collected in open GSL water<sup>5</sup>. However, in the study by Brix and co-workers little if any effect of ambient selenium on artemia selenium concentrations was observed at concentrations below 30  $\mu\text{g Se/L}$ . The “knee” in the accumulation curve appears to somewhere between 30 and 80  $\mu\text{g Se/L}$  for field collected artemia<sup>5</sup> which is somewhat higher than the 10-20  $\mu\text{g Se/L}$  observed in the present study. A similar pattern was observed by Brooks in a report for Kennecott Utah Copper, Inc which report the “knee” in laboratory studies of selenium exposed artemia to be around 50  $\mu\text{g Se/L}$  and artemia selenium concentrations of around 2-3  $\mu\text{g Se/g dry weight}$  at concentrations below this threshold. Using a conservative approach, fitting a linear relationship between artemia and water selenium concentrations from field collected samples Brix and co-workers suggested that 5 mg Se/kg dry weight in artemia would not be reached until ambient selenium concentrations reached 27  $\mu\text{g Se/L}$ <sup>5</sup>. The models developed as part of the present study are not suited to evaluate ambient concentrations as high as 27  $\mu\text{g Se/L}$  and should not be used to consider situations of selenium concentrations above 2.5 and 10  $\mu\text{g Se/L}$  for scenarios I and II, respectively. However, both model scenarios agree that artemia steady state concentrations of 5 mg/kg will be reached at concentrations considerably below 27  $\mu\text{g Se/L}$ . The reason(s) for this discrepancy is unknown but it is possible that field collected artemia were not at steady state with respect to selenium concentrations due to limited residence time in the local environment sampled.

Interestingly, both field collected and laboratory reared artemia display the “knee” in the accumulation curve although at slightly different exposure concentrations. From the present study one can conclude that this shape of the accumulation curve can be ascribed to uptake from the water rather than the diet. This conclusion is based on the proportionality of algae selenium accumulation in relation to media selenium concentrations and artemia algae ingestion rate which is constant across the tested dietary selenium concentrations. These observations combined cannot account for a disproportional increase at higher selenium concentrations. In contrast, uptake from the water shows an accumulation pattern similar to that observed in GSL collected artemia (although with different thresholds) with an apparently disproportional increase in accumulated selenium above the “knee”.

An interesting observation arising from model predictions made possible through the present study is that water-borne selenium uptake contributes significantly to steady state concentrations in GSL artemia. Using the mean selenium concentrations for seston and water collected from the GSL above, water contributes 64% of the steady state selenium concentrations (model scenario I). This conclusion is supported by the observation that uptake from the water likely dictates the accumulation pattern with increasing ambient concentration as water-borne uptake displays the hockey stick-shaped patterns observed for selenium accumulation in artemia collected from the GSL.

The  $K_u$  determined as part of the present study for low ambient selenium concentrations is high compared to previous reports which likely explains the relatively high water-borne contribution to steady state selenium concentrations in artemia.

However, it should be noted that since uptake from the water and assimilation efficiencies are not strict linear functions of selenium concentrations the relative contribution of the two uptake pathways will depend on environmental conditions and selenium concentrations.

#### *Acclimation – reduced selenium uptake?*

Reduced metal uptake and elevated metal excretion has been observed during prolonged exposure to essential elements<sup>18,17,16,21,20,25</sup> and serves to maintain stable tissue levels despite elevated environmental concentrations. Considering the essentiality of selenium, homeostatic control of selenium in artemia is likely and might involve both reduced uptake and elevated elimination. When predicting steady state concentrations using biodynamic models, such physiological responses may go unnoticed and could result in overestimation of steady state concentrations. The approach employed to determine selenium elimination in the present study involved a brief exposure but several weeks of depuration measurements which likely would have captured and included any influence of adjustments to serve homeostatic control. In contrast, the uptake measurements, dietary as well as water-borne, were performed over 1-24 hours using artemia not previously exposed to selenium. The possibility of reduced uptake from the water or reduced dietary selenium assimilation efficiency in artemia following prolonged selenium exposure was therefore examined in the present study. For both water-borne uptake and dietary assimilation efficiency, the predicted reductions following prolonged exposure were observed but were statistically insignificant. Although no statistical significance was noted, both water-borne uptake and dietary assimilation efficiency tended to drop as

predicted but only to a modest extent. The combined effect of these reductions in selenium uptake likely would not exceed a 10-20% reduction in predicted steady state selenium concentrations in artemia. This potential effect on steady state concentrations is not currently represented by the models (scenario I or II) but the models could be adjusted to accommodate for this effect should it be desired.

### **Conclusions**

It appears that selenium accumulation regardless of the route of uptake is lower at higher salinities. Although the generality of this observation across a wider range of salinities remains to be demonstrated it appears that steady state selenium concentrations, all other factors being equal, may correlate negatively with ambient salinity.

Algae exposure time is of great importance for apparent bioconcentration factors as algae (at least *D. viridis*) display a complex selenium accumulation pattern over time.

At steady state, *D. viridis* display a negative correlation between selenium bioconcentration factors and exposure concentration.

Homeostatic control of selenium in *D. viridis* is suggested by the reduced cellular selenium concentration during continued exposure, a reduction which cannot be accounted for by growth dilution

Selenium uptake from the water displays saturation kinetics at low ambient concentrations (<10 µg Se/L) with a high affinity constant and relatively high  $K_u$ . At higher concentrations, a low affinity, high capacity uptake system contributed to a “hockey stick-shaped” accumulation pattern.

Selenium assimilation efficiency by artemia is not constant. Near 100% assimilation efficiency applies to low *D. viridis* selenium concentrations while 75% is relevant for higher concentrations.

A developed set of DYMBAM-type models allows for predictions of steady state selenium concentrations in artemia under conditions relevant to the GSL. Model predictions are in good agreement with measured values from the GSL and other laboratory studies.

The models ascribe water-borne uptake as a significant contribution to steady state selenium concentrations in artemia.

Acclimation (likely to occur during prolonged exposure) possibly results in a modest reduction of selenium uptake from both water-borne and dietary sources.

### **Recommendations**

The reason for the reduced cellular selenium concentration in *D. viridis* during continued exposure remains unknown and it is uncertain if such a pattern would apply under natural conditions. It is advised that the two possible explanations for reduced selenium uptake

(reduced number of selenium transporters versus excretion of substances rendering selenium less available for uptake) are examined experimentally.

Furthermore, it is desirable to examine if selenium taken up during the early phases of algae growth and accumulation is more or less amendable to trophic transfer to artemia.

The above modeling effort and conclusions are based on experiments performed at a single and high algae cell density and a uniform, single species algae diet. None of these conditions are completely realistic for the GSL. Algae densities are always below the densities employed in the present study and seston rather than pure algae communities are the natural food source for artemia in the GSL. A lower cell density might result in a lower feeding rate, which in turn may result in higher assimilation efficiency. Furthermore, seston rather than pure algae diets might reduce assimilation efficiency. The combined influence of these possible factors on dietary selenium uptake is impossible to accurately predict without further studies.

The isotopic approach has proven very effective for fast feedback on exposure concentrations and thus for the maintenance of constant exposure concentrations and for determination of selenium uptake and accumulation in a cost effective manner. In addition, the resolution and sensitivity of isotope measurements is superior to that of other analytical techniques. However, this technique is not without potential drawbacks. From a biodynamic modeling perspective it is assumed that  $^{75}\text{Se}$  uptake, internal distribution and subsequent elimination reflects all components of selenium homeostasis and that it is in perfect equilibrium with internal selenium stores present in the organisms prior to isotope exposure. While this is not a problem for uptake rate measurements from the water or for dietary uptake measurements from a chronically exposed diet as used in the present study, it may influence elimination rate constant determination. The extensive duration of the depuration measurements in the present study were aimed at limiting this potential problem but it is not known for certain if  $^{75}\text{Se}$  elimination truly reflects overall selenium elimination. A set of validation experiments comparing DYMBAM model predictions from isotope measurements to actual measured total selenium concentrations in artemia held under identical conditions would address this uncertainty.

**Table 3. Scenario I: total ss[Se] ( $\mu\text{g Se/g dry weight}$ )**

Water borne [Se]	Dietary [Se]										
	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
0		0.50	1.00	1.50	1.99	2.47	2.95	3.42	3.89	4.35	4.81
0.2	0.62	1.13	1.62	2.12	2.61	3.09	3.57	4.04	4.51	4.98	5.44
0.4	1.24	1.75	2.25	2.74	3.23	3.71	4.19	4.66	5.13	5.60	6.06
0.6	1.86	2.37	2.87	3.36	3.85	4.33	4.81	5.28	5.75	6.22	6.68
0.8	2.49	2.99	3.49	3.98	4.47	4.95	5.43	5.91	6.38	6.84	7.30
1.0	3.11	3.61	4.11	4.60	5.09	5.58	6.05	6.53	7.00	7.46	7.92
1.2	3.73	4.23	4.73	5.23	5.71	6.20	6.68	7.15	7.62	8.08	8.54
1.4	4.35	4.85	5.35	5.85	6.34	6.82	7.30	7.77	8.24	8.70	9.16
1.6	4.97	5.48	5.97	6.47	6.96	7.44	7.92	8.39	8.86	9.33	9.79
1.8	5.59	6.10	6.60	7.09	7.58	8.06	8.54	9.01	9.48	9.95	10.41
2.0	6.21	6.72	7.22	7.71	8.20	8.68	9.16	9.63	10.10	10.56	11.03

**Table 4. Scenario II: total ss[Se] ( $\mu\text{g Se/g dry weight}$ )**

Water borne [Se]	Dietary [Se]										
	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
0		0.50	1.00	1.50	1.99	2.47	2.95	3.42	3.89	4.35	4.81
0.2	1.39	1.89	2.39	2.89	3.37	3.86	4.34	4.81	5.28	5.74	6.20
0.4	2.43	2.93	3.43	3.93	4.42	4.90	5.38	5.85	6.32	6.78	7.24
0.6	3.24	3.74	4.24	4.74	5.23	5.71	6.19	6.66	7.13	7.59	8.05
0.8	3.89	4.39	4.89	5.39	5.87	6.36	6.84	7.31	7.78	8.24	8.70
1.0	4.42	4.92	5.42	5.92	6.40	6.89	7.37	7.84	8.31	8.77	9.23
1.2	4.86	5.37	5.86	6.36	6.85	7.33	7.81	8.28	8.75	9.22	9.68
1.4	5.23	5.74	6.24	6.73	7.21	7.70	8.18	8.66	9.12	9.59	10.05
1.6	5.55	6.06	6.56	7.05	7.54	8.02	8.50	8.98	9.44	9.91	10.37
1.8	5.83	6.34	6.84	7.33	7.82	8.30	8.78	9.25	9.72	10.19	10.65
2.0	6.08	6.58	7.08	7.57	8.06	8.54	9.02	9.50	9.97	10.43	10.89

## Reference List

1. Adams, W. J., Brix, K. V., Cothorn, K. A., Tear, L. M., Cardwell, R. D., Fairbrother, A., and Toll, J. E. (1998): Assessment of selenium food chain transfer and critical exposure factors for avian wildlife species: Need for site specific data. In Little, E.E., Delonay, A.J., Greenberg, B.M., eds, *Environmental Toxicology and Risk Assessment 7 American Society for testing and materials, Philadelphia, PA*, 312-342.
2. Adams, W. J., Brix, K. V., Edwards, M., Tear, L. M., DeForest, D. K., and Fairbrother, A. (2003): Analysis of field and laboratory data to derive selenium toxicity thresholds for birds. *Environ.Toxicol.Chem.* **22**, 2020-2029.
3. Alquezar, R., Markich, S. J., and Twining, J. R. (2007): Uptake and loss of dissolved Cd-109 and Se-75 in estuarine macroinvertebrates. *Chemosphere* **67**, 1202-1210.
4. Bridges, C. C. and Zalups, R. K. (2005): Molecular and ionic mimicry and the transport of toxic metals. *Toxicology and Applied Pharmacology* **204**, 274-308.
5. Brix, K. V., DeForest, D. K., Cardwell, R. D., and Adams, W. J. (2004): Derivation of a chronic site-specific water quality standard for selenium in the great salt lake. Utah, USA. *Environ.Tox.Chem.* **23**, 606-612.
6. Brix, K. V., Toll, J. E., Tear, L. M., DeForest, D. K., and Adams, W. J. (2005): Setting site-specific water-quality standards by using tissue residue thresholds and bioaccumulation data. Part 2. Calculating site-specific selenium water-quality standards for protecting fish and birds. *Environ.Toxicol.Chem.* **24**, 231-237.
7. Brix, K. V., Volosin, J. S., Adams, W. J., Reash, R. J., Carlton, R. G., and McIntyre, D. O. (2001): Effects of sulfate on the acute toxicity of selenate to freshwater organisms. *Environ.Toxicol.Chem.* **20**, 1037-1045.
8. Buchwalter, D. B., Cain, D. J., Clements, W. H., and Luoma, S. N. (2007): Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. *Env.Sci.Tech.* **41**, 4821-4828.
9. Croteau, M. N. and Luoma, S. N. (2005): Delineating copper accumulation pathways for the freshwater bivalve *Corbicula* using stable copper isotopes. *Environ.Toxicol.Chem.* **24**, 2871-2878.
10. Croteau, M. N., Luoma, S. N., Topping, B. R., and Lopez, C. B. (2004): Stable metal isotopes reveal copper accumulation and loss dynamics in the

freshwater bivalve corbicula. Environmental Science & Technology **38**, 5002-5009.

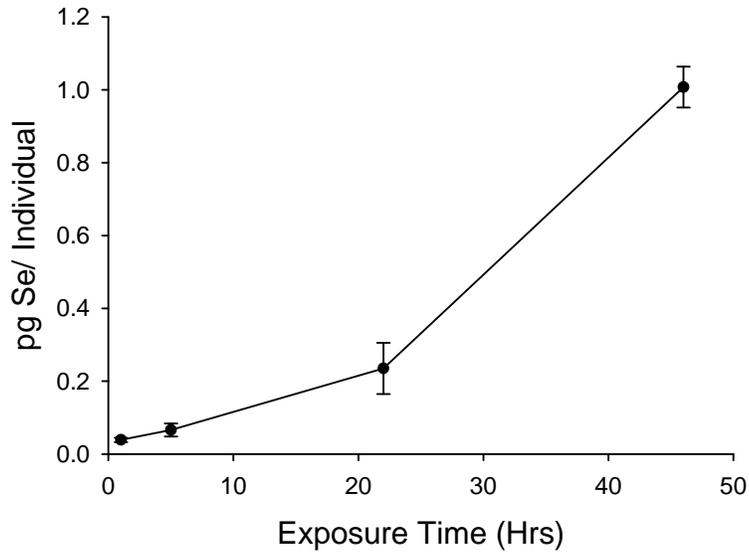
11. Cutshall, N. (1974): Turnover of Zn-65 in Oysters/S. Health Physics **26**, 327-331.
12. Domagalski, J. L., Orem, H. P., and Eugster, H. P. (1989): Organic geochemistry and brine composition in Great Salt Lake, Mono, and Walker lakes. Geochim.Cosmochim.Acta **53**, 2857-2872.
13. Forsythe, B. L. and Klaine, S. J. (1994): The Interaction of Sulfate and Selenate (Se+6) Effects on Brine Shrimp, Artemia Spp. Chemosphere **29**, 789-800.
14. Fournier, E., Adam, C., Massabuau, J. C., and Garnier-Laplace, J. (2006): Selenium bioaccumulation in Chlamydomonas reinhardtii and subsequent transfer to Corbicula fluminea: Role of selenium speciation and bivalve ventilation. Environ.Toxicol.Chem. **25**, 2692-2699.
15. Franke, K. W. and Tully, W. C. (1935): A new toxicant occurring naturally in certain samples of plant foodstuffs. V. Low hatchability due to deformities in chicks. Poult.Sci. **14**, 273-279.
16. Grosell, M., Boetius, I., Hansen, H. J. M., and Rosenkilde, P. (1996): Influence of preexposure to sublethal levels of copper on Cu-64 uptake and distribution among tissues of the European eel (*Anguilla anguilla*). Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology **114**, 229-235.
17. Grosell, M., Hansen, H. J. M., and Rosenkilde, P. (1998): Cu uptake, metabolism and elimination in fed and starved European eels (*Anguilla anguilla*) during adaptation to water-borne Cu exposure. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology **120**, 295-305.
18. Grosell, M., McGeer, J. C., and Wood, C. M. (2001): Plasma copper clearance and biliary copper excretion are stimulated in copper-acclimated trout. American Journal of Physiology-Regulatory Integrative and Comparative Physiology **280**, R796-R806.
19. Grosell, M. and Wood, C. M. (2002): Copper uptake across rainbow trout gills: mechanisms of apical entry. Journal of Experimental Biology **205**, 1179-1188.
20. Grosell, M. H., Hogstrand, C., and Wood, C. M. (1997): Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). Aquat.Toxicol **38**, 257-276.
21. Grosell, M. H., Hogstrand, C., and Wood, C. M. (1998): Renal Cu and Na excretion and hepatic Cu metabolism in both Cu acclimated and non

- acclimated rainbow trout (*Oncorhynchus mykiss*). *Aquat.Toxicol* **40**, 275-291.
22. Heinz, G. H., Hoffman, D. J., and Gold, L. G. (1989): Impaired reproduction of mallards fed an organic form of selenium. *J.Wildl.Manage* **53**, 418-428.
  23. Hurd-Karrer, A. M. (1938): Relation of sulfate to selenium absorption by plants. *Am.J.Bot.* **25**, 666-675-
  24. Jehl, J. R. (1994): Changes in saline and alkaline lake avifaunas in western North America in the past 150 years. In J.J.Jehl & N.K.Johnson (Eds), *A century of avifaunal change in Western Nort America.Studies in Avian biology.Cooper Ornithological Society* 258-272.
  25. Kamunde, C., Grosell, M., Higgs, D., and Wood, C. M. (2002): Copper metabolism in actively growing rainbow, trout (*Oncorhynchus mykiss*): interactions between dietary and waterborne copper uptake. *Journal of Experimental Biology* **205**, 279-290.
  26. Lemly, A. D. (1999): Selenium transport and bioaccumulation in aquatic ecosystems: A proposal for water quality criteria based on hydrological units. *Ecotoxicology and Environmental Safety* **42**, 150-156.
  27. Luoma, S. N., Johns, C., Fisher, N. S., Steinberg, N. A., Oremland, R. S., and Reinfelder, J. R. (1992): Determination of Selenium Bioavailability to A Benthic Bivalve from Particulate and Solute Pathways. *Environmental Science & Technology* **26**, 485-491.
  28. Luoma, S. N. and Rainbow, P. S. (2005): Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science & Technology* **39**, 1921-1931.
  29. McGeer, J. C., Brix, K. V., Skeaff, J. M., DeForest, D. K., Brigham, S. I., Adams, W. J., and Green, A. (2003): Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment. *Environ.Toxicol.Chem.* **22**, 1017-1037.
  30. Miyauchi, S., Srinivas, S. R., Fei, Y. J., Gopal, E., Umapathy, N. S., Wang, H., Conway, S. J., Ganapathy, V., and Prasad, P. D. (2006): Functional characteristics of NaS2, a placenta-specific Na+-coupled transporter for sulfate and oxyanions of the micronutrients selenium and chromium. *Placenta* **27**, 550-559.
  31. Poley, W. E., Moxon, A. L., and Franke, K. W. (1937): Further studies on the effects of selenium poisoning on hatchability. *Poult.Sci.* **16**, 219-225.

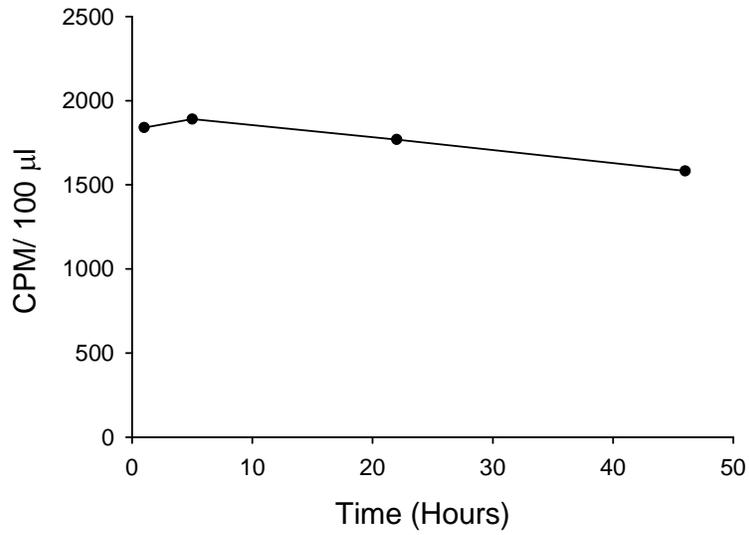
32. Schlekot, C. E. and Luoma, S. N. (2002): Dietary metal exposure and toxicity to aquatic organisms: Implications for ecological risk assessment. In Coastal and Estuarine Risk Assessment. Newman, M., Ed., CRC press: Boca Raton, FL.
33. Schlekot, C. E., Purkerson, D. G., and Luoma, S. N. (2004): Modeling selenium bioaccumulation through arthropod food webs in San Francisco Bay, California, USA. *Environ.Toxicol.Chem.* **23**, 3003-3010.
34. Shrift, A. (1954): Sulphur-selenium antagonism. I. Anti-metabolic action of selenate on the growth of *Chlorella vulgaris*. *Am.J.Bot.* **41**, 223-232.
35. Skorupa, J. P., Morman, S. P., and Sefchick-Edwards, J. S. (1996): Guidelines for interpreting selenium exposure of biota associated with nonmarine aquatic habitats. US Fish and Wildlife service national irrigation water quality program unnumbered report
36. Sors, T. G., Ellis, D. R., Na, G. N., Lahner, B., Lee, S., Leustek, T., Pickering, I. J., and Salt, D. E. (2005): Analysis of sulfur and selenium assimilation in Astragalus plants with varying capacities to accumulate selenium. *Plant Journal* **42**, 785-797.
37. Stewart, A. R., Luoma, S. N., Schlekot, C. E., Doblin, M. A., and Hieb, K. A. (2004): Food web pathway determines how selenium affects aquatic ecosystems: A San Francisco Bay case study. *Environmental Science & Technology* **38**, 4519-4526.
38. Toll, J. E., Tear, L. M., DeForest, D. K., Brix, K. V., and Adams, W. J. (2005): Setting site-specific water-quality standards by using tissue residue criteria and bioaccumulation data. Part 1. Methodology. *Environ.Toxicol.Chem.* **24**, 224-230.
39. U.S.Environmental Protection Agency (2002): Draft aquatic life water quality criteria for selenium. Washington, DC.
40. Wang, W. X., Fisher, N. S., and Luoma, S. N. (1996): Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. *Marine Ecology-Progress Series* **140**, 91-113.

SOPs

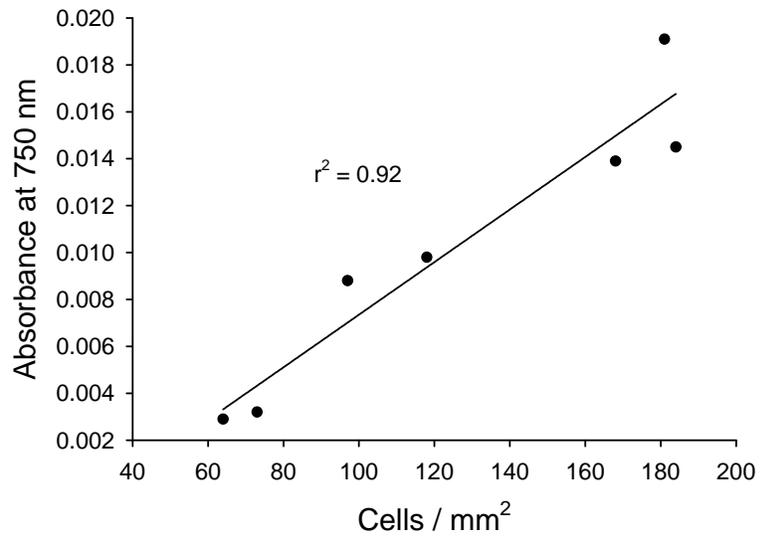
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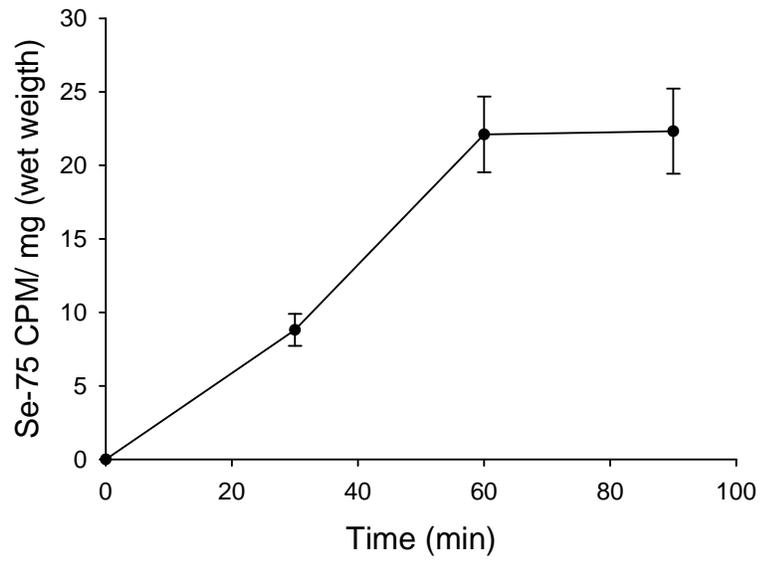
**Fig. 1.** Selenium accumulation in artemia during a 48 hour exposure to 13 ng Se/l as <sup>75</sup>Se labeled selenate. Values are expressed as pg Se individual. Mean ± SEM, n=10.



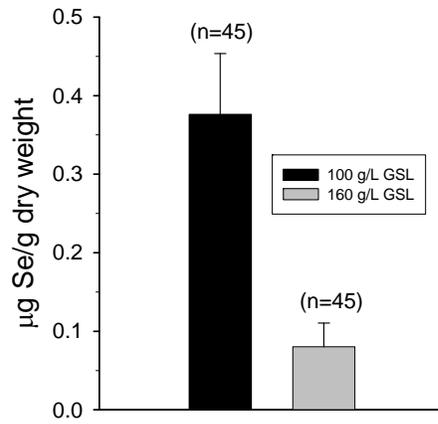
**Fig. 2.** Levels of  $^{75}\text{Se}$  in GSL medium during a 48 hour exposure of artemia to 13 ng Se/L as selenate under conditions employed in the present study (see text for details).



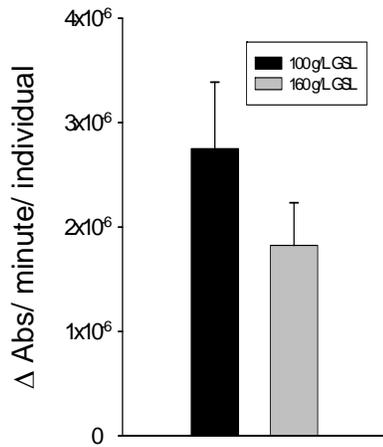
**Fig. 3.** Correlation between absorbance at 750 nm and cell count. See text for further details.



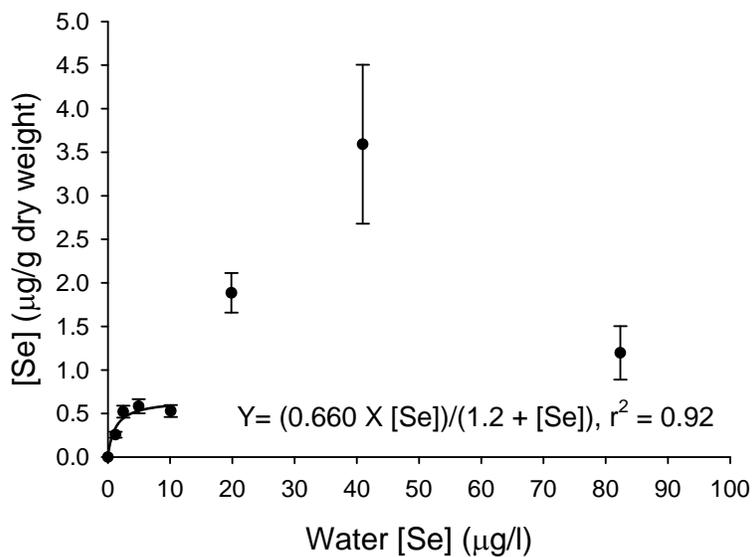
**Fig. 4.** Selenium accumulation in individual artemia during a 90 minute feeding trail during which artemia were fed at a cell density of approximately 20 million cells/L.



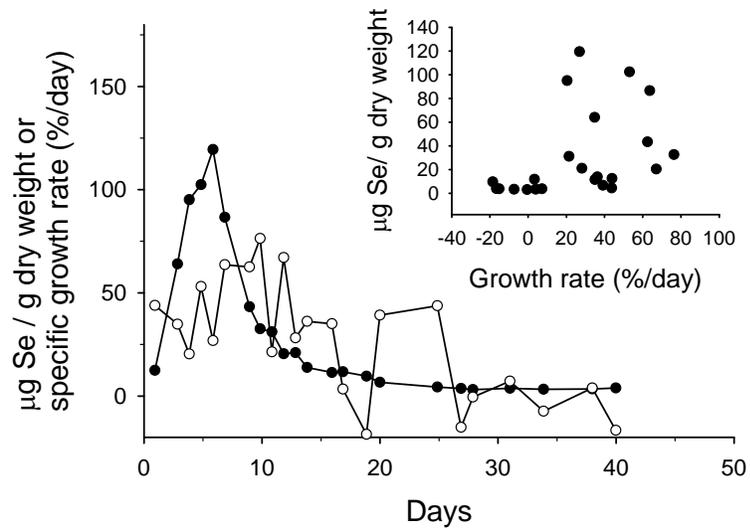
**Fig. 5.** Influence of salinity on  $^{75}\text{Se}$  uptake in adult artemia during a 24-hour exposure to selenium in GSL medium



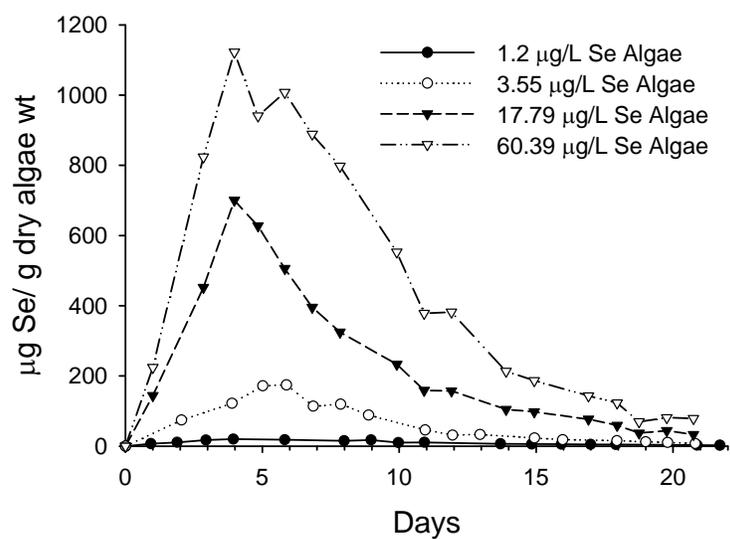
**Fig. 6.** Average feeding rates determined in three separate experiments from the depletion of algal cells evident from change in absorbance at 750 nm (cell density)



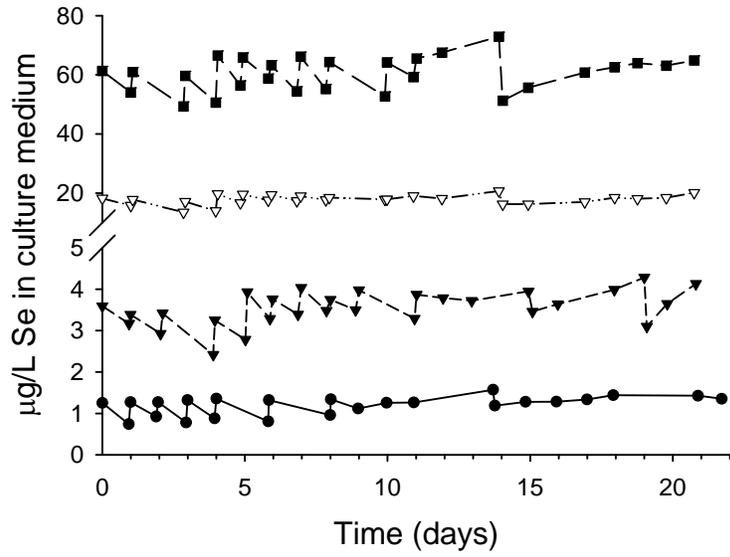
**Fig. 7.** Selenium accumulation in individual adult *Artemia* as a function of ambient selenium concentrations following a 24 hour exposure. Note the apparent saturation kinetics at concentrations below 10 µg Se/L and the clear disproportional increase in uptake at concentrations above 10 µg Se/L identifying the “knee” to be between 10 and 20 µg Se/L.



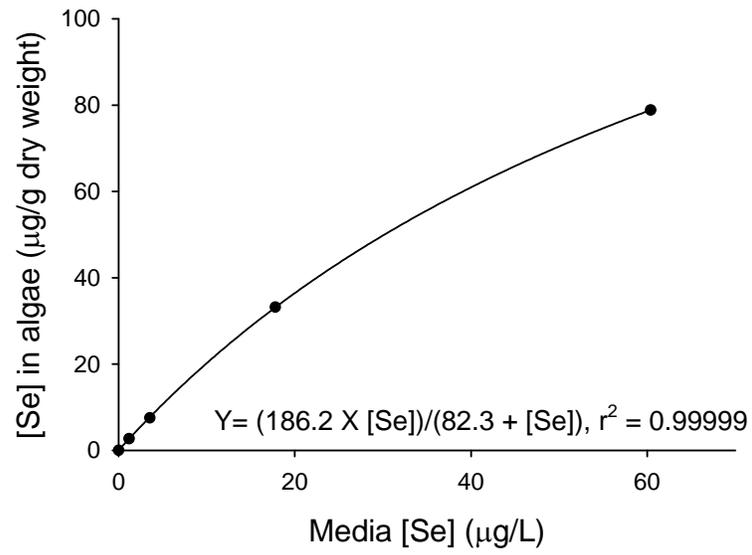
**Fig. 8.** Selenium concentration in *D. viridis* (dark circles) and specific growth rate (open circles) during 40 days of culturing in the presence of 2.17  $\mu\text{g Se/L}$ . **Insert:** Selenium concentration in algae as a function of specific growth rate



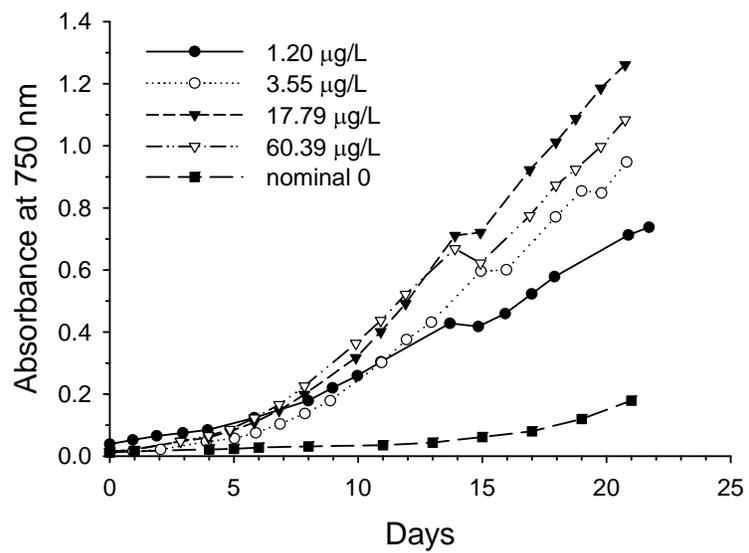
**Fig. 9.** Time course of selenium accumulation in *D. viridis* raised at four different selenium concentrations. Average media selenium concentrations are indicated on the figure and measured media selenium concentrations throughout the 21 days of culturing are reported in Fig. 10.



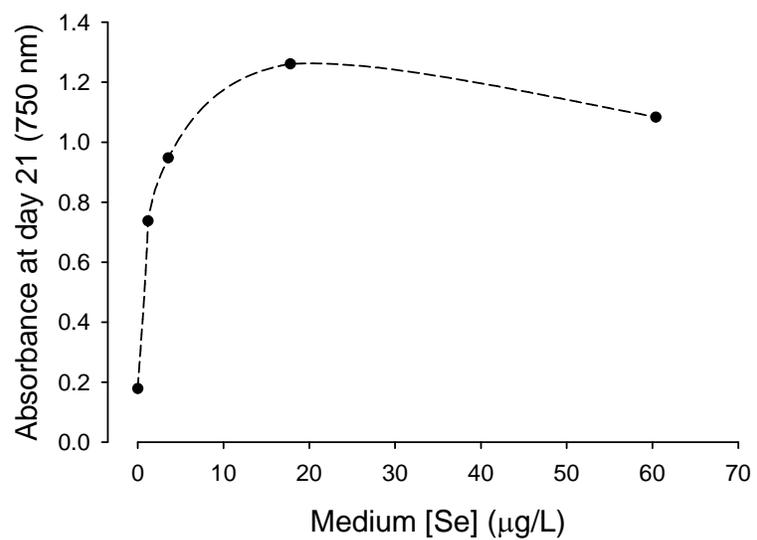
**Fig. 10.** Media selenium concentrations during 21-day culturing of *D. viridis*. The near daily fluctuations represents uptake or excretion from the algae cells and corresponding adjustments of exposure concentrations by addition of selenium stock solution or selenium-free culture medium as appropriate.



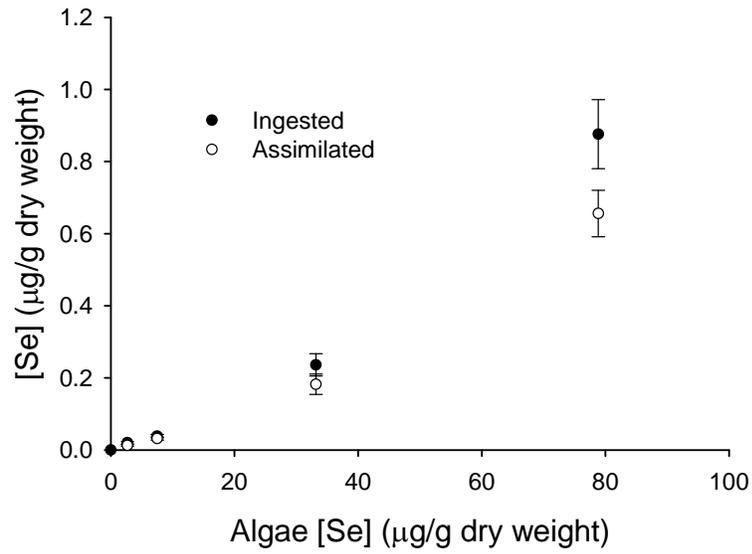
**Fig. 11.** Steady state selenium concentrations in *D. viridis* after 21 days of exposure as a function of media selenium concentrations



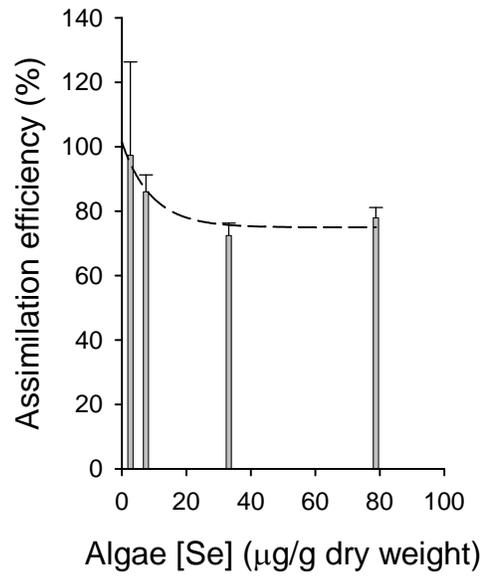
**Fig. 12.** *D. viridis* growth in culture media of different selenium concentrations.



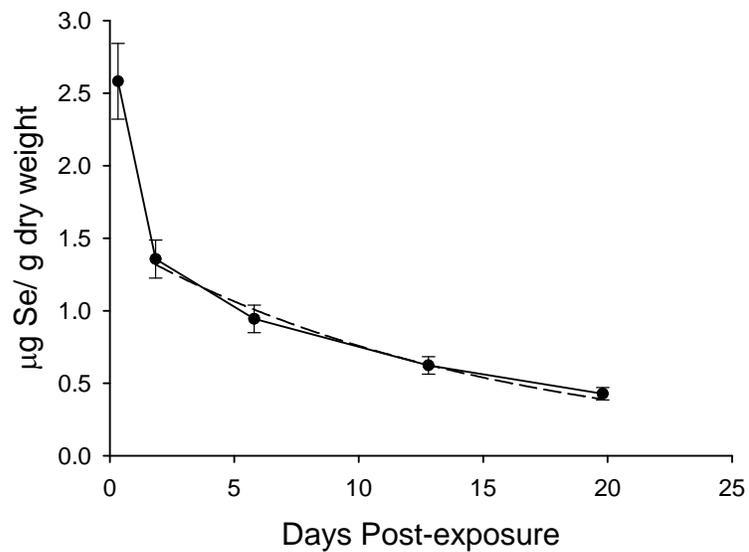
**Fig. 13.** Cell density after 21 days of culturing at different selenium concentrations.



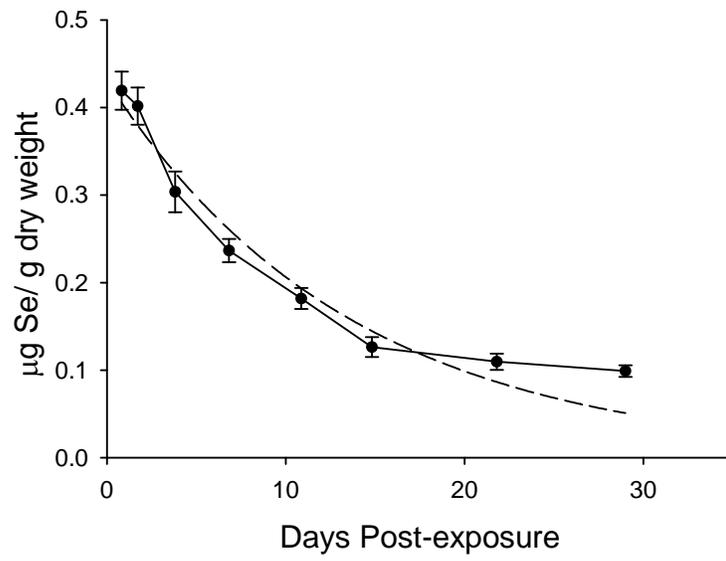
**Fig. 14.** Ingested (dark symbols) and assimilated (open symbols) selenium in individual artemia following a 60 minute feeding at four different algae selenium concentrations



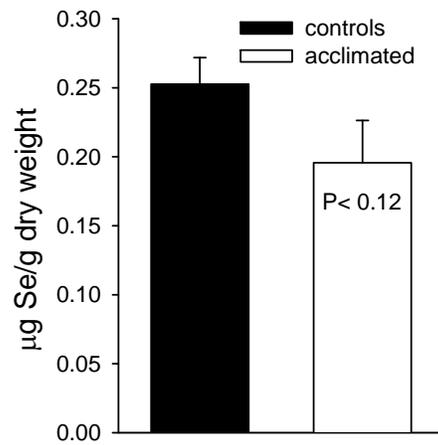
**Fig. 15.** Assimilation efficiencies for four different algae selenium concentrations calculated from the observations presented in Fig. 14. Data adhere to an exponential decay equation (see text for details),  $r^2 = 0.95$ .



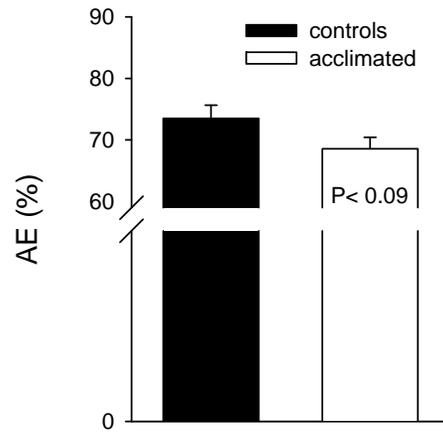
**Fig. 16.** Elimination of selenium from adult artemia exposed to water-borne selenium ( $72 \mu\text{g Se/L}$ ) for 48 hours. An elimination rate constant of 0.0679 was obtained from the fitted curve,  $r^2=0.99$ .



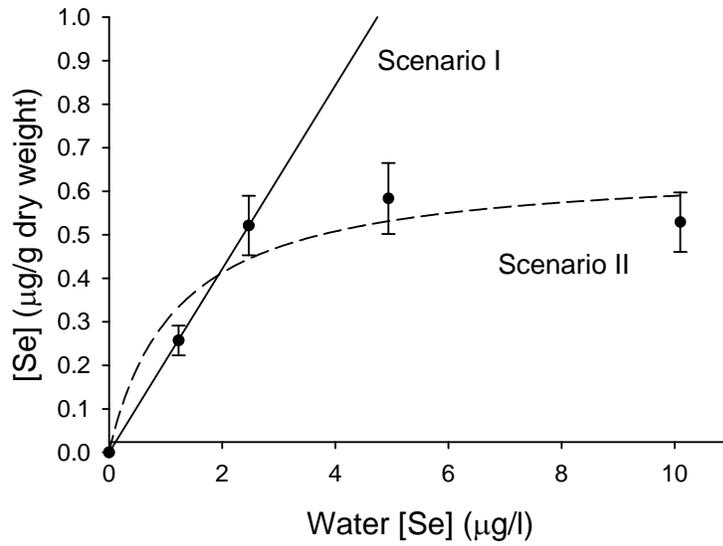
**Fig. 17.** Elimination of selenium from adult artemia exposed to dietary selenium for 24 hours. An elimination rate constant of 0.0737 was obtained from the fitted curve,  $r^2 = 0.95$ .



**Fig. 18.** Selenium uptake from the water at  $2.55 \pm 0.11 \mu\text{g Se/L}$  in control Artemia and Artemia acclimated to  $2.87 \pm 0.14 \mu\text{g Se/L}$  for 14 days.



**Fig. 19.** Assimilation efficiency from algae containing 3.73  $\mu\text{g}$  Se/g dry weight in control artemia and in artemia acclimated to dietary selenium at 2.68  $\mu\text{g}$  Se/g dry weight for 14 days.



**Fig. 20.** Subset of data presented in Fig. 7. focusing on low ambient selenium concentrations. A traditional linear  $K_d$  applies to exposure concentrations below 2.5 µg Se/L but saturation kinetics apply to a wider range of concentrations from 0 to 10 µg Se/L.