U. S. DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Evaluation of the Toxicity of Selenium to White and Green Sturgeon

Prepared by
Dr. William N. Beckon

Sacramento, California
March 2012

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Abstract

Fish of the genus *Acipenser* (sturgeon) are likely to be among the most vulnerable to selenium exposure in the San Francisco Estuary because these fish feed predominantly on benthic invertebrates, including the Asian clam, *Corbula amurensis*. This clam is an efficient bioaccumulator of selenium. The best data available for the most sensitive endpoint for sturgeon come from studies in which the survival of larvae was monitored following micro-injection of organic selenium (L-selenomethionine) into the yolk sacs of newly hatched larvae. Benchmark larval selenium concentrations from these studies were translated, by means of regressions, to selenium concentrations in the tissue and diet of adult white and green sturgeon. This analysis indicates that white and green sturgeon are among the most sensitive of fish to adverse effects of selenium, with the listed green sturgeon being the more sensitive of these two species. These levels of sensitivity evidently put sturgeon at substantial risk at current levels of exposure in the San Francisco Bay area. Selenium concentrations in food items of sturgeon in the San Francisco Bay area are almost always high enough that they may cause at least 10 percent mortality in hatchling green sturgeon (≥3.58 µg/g), and they are frequently high enough that they may cause at least 10 percent mortality among hatchling white sturgeon (≥10.8 µg/g) as well. Below is a summary of benchmark concentrations of selenium derived here for the diets, whole body, muscle, and eggs of these two sturgeon species.

Summary Table of Selenium Benchmarks

Effect Level	Concentration of selenium (µg/g dry wt.) corresponding to effect level (proportion affected)											
	5%	10%	5%	10%	5%	10%	5%	10%				
Species	**Se in diet of sturgeon**		**Se in sturgeon (whole body)**		**Se in sturgeon muscle**		**Se in sturgeon eggs**		**Effect**	**Form of selenium**	**Exposure**	**Data source**
White sturgeon	5.45	10.8	2.55	3.86	3.55	5.56	4.67	6.83	larval mortality	L-selenomethionine	microinjection of larvae	Linville 2006
	39.3	48.4	8.51	9.65	13.1	15.0	14.0	15.8	larval abnormalities	selenized yeast	maternal diet	Linville 2006
Green sturgeon	2.51	3.58	1.59	1.97	2.13	2.69	3.03	3.70	larval mortality	L-selenomethionine	microinjection of larvae	Linares-Casenave et al. 2010
	8.22	10.1	3.27	3.71	4.65	5.33	5.87	6.59	larval abnormalities	selenized yeast	maternal diet*	Linares-Casenave et al. 2010, Linville 2006

*Green sturgeon maternal diet effect levels were calculated from white surgeon maternal dietary exposure larval developmental defects (Linville 2006) adjusted for relative species sensitivity using the ratio of EC10s for peak larval abnormalities from the white and green sturgeon microinjection experiments of Linares-Casenave *et al*. (2010).

Benchmark values listed in this table are shown in boldface type in the following text and graphs where the derivations are explained and illustrated.

Introduction

The San Francisco Bay, San Pablo Bay, and Suisun Bay are listed as impaired by selenium under 303(d) of the Clean Water Act. Of the wildlife that live in, pass through, or are dependent on, these bays, benthic (bottom-dwelling) fish of the genus *Acipenser* (sturgeon) are likely to be among the most vulnerable to selenium. This is because these large, long-lived fish feed predominantly on benthic invertebrates (McKechnie and Fenner 1971; Radtke 1966; Ritz 2010), and since 1986 the San Francisco Bay benthic community has experienced a massive invasion of an Asian clam, *Corbula amurensis*, (Carlton et al. 1990; Nichols et al. 1990) that is a particularly efficient bioaccumulator of selenium (Linville *et al*. 2002).

Two species of sturgeon are found in the San Francisco Bay estuary: white sturgeon (*Acipenser transmontanus*) and green sturgeon (*Acipenser medirostris*). White sturgeon in the San Francisco Bay estuary congregate in Suisun and San Pablo Bays where they remain year-round except for a small fraction of the population that moves up the Sacramento River, and to a lesser extent the San Joaquin River, to spawn in late winter and early spring (Kohlhorst *et al*. 1991). Thus, many individuals of this species remain year-round in a region of the San Francisco Bay estuary where they are exposed to dietary items (*Corbula amurensis*) that exceed selenium concentrations of 10 µg/g (dry weight) much of the time (Linville *et al*. 2002, Kleckner *et al.* 2010). Linares *et al*. (2004) found concentrations of selenium as high as 46.7 µg/g in gonads of 39 white sturgeon captured in the San Francisco Bay. Kroll and Doroshov (1991) reported that developing ovaries of white sturgeon from San Francisco Bay contained as much as 71.8 µg/g selenium (dry weight); several times the concentration in fish eggs (20 µg/g dry weight) that Lemly (1996) suggested would cause a high level of hazard from reproductive impairment.

Green sturgeon are more marine-oriented than white sturgeon, but also congregate in coastal bays and estuaries (Adams *et al.* 2007). Furthermore, green sturgeon in this area are federally listed as threatened under the Endangered Species Act.

Therefore, in view of their status as well as their particular vulnerability to selenium, sturgeons must be pivotal species in establishing regulatory guidelines that would be broadly protective of wildlife in the San Francisco Bay area. The purpose of this report is to provide an analysis of recent sturgeon data that may be relevant to developing such guidelines.

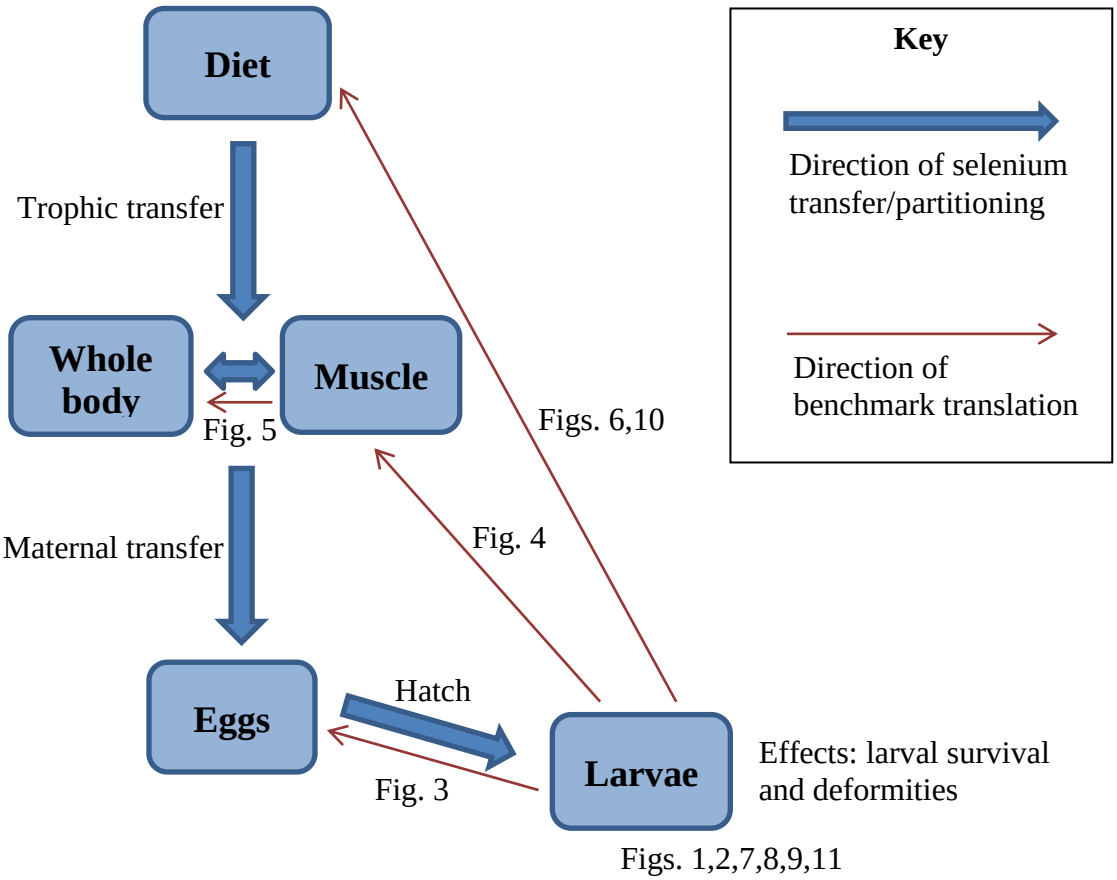
This analysis focuses on the "most sensitive endpoint", the ecologically relevant adverse effect that occurs at the lowest concentrations of selenium, relative to the concentrations at which other effects or "endpoints" start to occur. This focus is consistent with the approach used by the USEPA Region 9 Biological Technical Assistance Group for establishing toxicity reference values (CDTSC HERD 2002). It also conforms with prudence and reason; the most sensitive endpoint should trump other effects levels in developing protective guidelines.

Key studies by Linville (2006), Linares-Casenave *et al.* (2010), Silvestre et al. (2010), and Tashjian *et al.* (2006) provide white and green sturgeon larval and egg toxicity data. Linville (2006) also provides dietary, female muscle and maternal transfer data for white sturgeon while Tashjian *et al.* (2006) provides whole body and muscle data for white sturgeon. However, data on green sturgeon dietary, muscle and whole body is not currently available. To the best of our

knowledge, the best data available for the most sensitive endpoint for sturgeon come from studies in which the survival of larvae was monitored following micro-injection of organic selenium (L-selenomethionine) into the yolk sacs of newly hatched larvae (Linville 2006, Linares-Casenave *et al.* 2010, Silvestre et al. 2010). Benchmark larval selenium concentrations from these studies can be translated, by means of regressions, to selenium concentrations in the tissue and diet of adult sturgeon that deposit eggs from which the larvae with those concentrations would hatch. For rarer species, such as green sturgeon, for which there are insufficient data to derive species-specific regressions, regressions from a surrogate species of the same genus (i.e. white sturgeon) can be used (USEPA 1995). Use of such surrogates and regressions is a protective approach based on best available information. It is an approach that EPA employs extensively in setting water quality standards (e.g. USEPA 2004).

The following flow diagram is provided to show the relationship of the larval effect levels and their translations to selenium in sturgeon diet and tissue.

Flow Diagram of Translation Procedures. White and green sturgeon 5% and 10% larval effect values are determined from microinjection and maternal transfer studies. These are then translated to selenium concentrations in egg, muscle, whole body, and diet using regressions presented in each of the figures noted.



White Sturgeon (*Acipenser transmontanus*)

At the University of California, Davis, Linville (2006) studied several effects of selenium on white sturgeon, including the toxicity of selenium to larvae after hatching and before the larvae begin to actively feed. During this period of about two weeks, the larval fish obtain nutrition (and toxicants) from a yolk sac. Linville (2006) exposed these larvae to elevated selenium in two ways: (1) by microinjection of L-selenomethionine into the yolk sacs just after hatch and (2) by exposing mother fish to dietary selenium (selenized yeast) for up to six months before they deposited the eggs, thereby transferring selenium from the mother to the larvae mainly via the yolk sac ("maternal transfer"). We first provide a larval toxicity regression to determine 5% and 10% effect levels for white sturgeon based on the Linville (2006) microinjection study. These effect levels are then translated to egg, muscle, whole body, and diet using other Linville (2006) data and Tashjian et al. (2006). Then we use results from the Linville (2006) maternal transfer study to determine 5% and 10% effect levels via maternal transfer and then perform similar translation of those effect levels to egg, muscle, whole body and diet.

White Sturgeon Exposure by Microinjection

Linville's (2006) analysis focused mainly on developmental defects in the larvae, but the data she provided show that larval mortality in the microinjection treatments was the most sensitive endpoint of those she investigated (at the 5% and 10% effect levels, mortality occurs at lower selenium concentrations than defects; compare Figures 1 and 2).

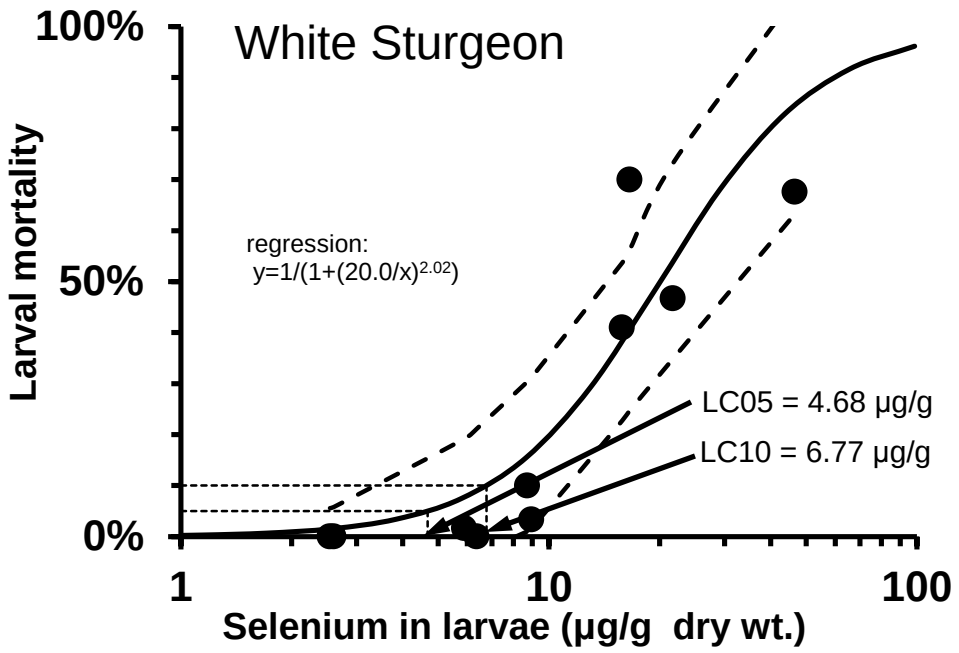


Figure 1. Mortality of white sturgeon larvae microinjected with L-selenomethionine in the yolk sac just after hatching (developmental stage 36). Selenium was measured in a subsample of larvae one day after injection. Mortality is the proportion that died by the time of yolk depletion, when the fish is ready to start eating (stage 45). The data are from Table 3-15 in Linville (2006). Dashed lines indicate 95% confidence boundaries. LC05 and LC10 are the concentrations of selenium at which 5 and 10 percent (respectively) mortality is expected.

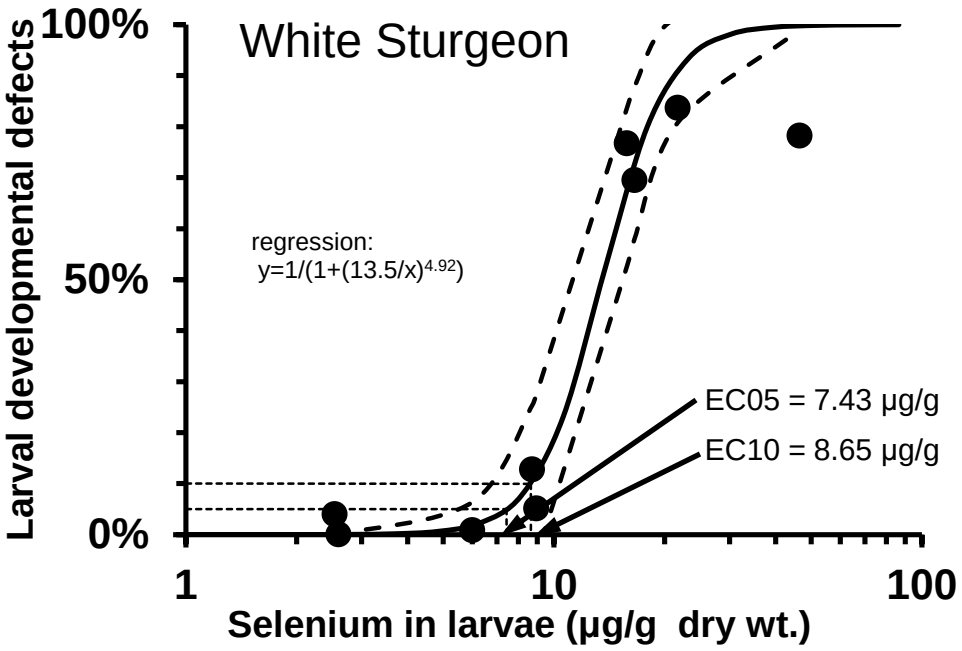


Figure 2. Incidence of larval edema and/or skeletal deformities among white sturgeon larvae microinjected with L-selenomethionine in the yolk sac just after hatching. Data are from Tables 3-11 and 3-13 in Linville (2006). EC05 and EC10 are the concentrations of selenium at which 5 and 10 percent (respectively) rates of adverse effects are expected.

This agrees with the relative sensitivities of bird eggs to selenium, because bird egg hatchability is a more sensitive endpoint than teratogenesis (embryonic deformities) (Ohlendorf 2003), and bird egg hatchability is the avian equivalent of larval mortality (death by the time the yolk is fully depleted and feeding begins) in fish.

White Sturgeon Larval Benchmarks – Microinjection

A standard log-logistic model (Beckon et al. 2008; Ritz 2010) of the larval mortality data yield LC05 and LC10 (the concentrations of selenium in larvae corresponding to 5% and 10% mortality) of 4.68 µg/g and 6.77 µg/g, respectively (Figure 1). As far as we know, field data on larval concentrations of selenium are not available; therefore, these larval benchmark concentrations are shown here only for the purpose of anchoring the following calculations of more useful benchmarks in eggs, muscle, whole body, and diet.

White Sturgeon Egg Benchmarks – Microinjection

In another experiment, Linville (2006) fed gravid female white sturgeon selenized yeast for up to six months before they deposited their eggs, and she measured selenium in the eggs and in the larvae that hatched from those eggs. Therefore, the larval benchmark selenium concentrations from Figure 1 (from microinjection experiments) can be translated into corresponding egg concentrations (Figure 3). This translation indicates that selenium concentrations of **4.67** µg/g and **6.83** µg/g in white sturgeon eggs correspond to 5% and 10% mortality rates (respectively) in the larvae that hatch from those eggs.

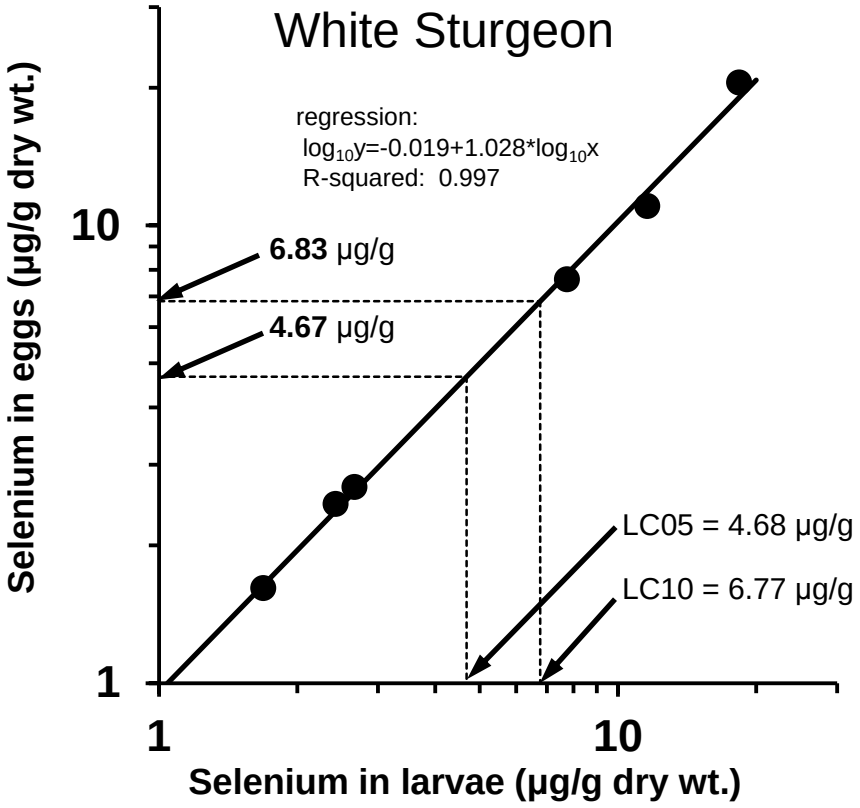


Figure 3. Relationship between selenium in white sturgeon eggs and in the larvae (stage 36) that hatch from the eggs. The data are from Tables 3-18 in Linville (2006).

White Sturgeon Muscle Benchmarks – Microinjection

The benchmark larval selenium concentrations from Figure 1 translate into maternal muscle selenium concentrations of **3.55** µg/g (5% mortality) and **5.56** µg/g (10% mortality) using a conversion equation based on data from Linville's (2006) work (Figure 4). Evidently, white sturgeon in the San Francisco estuary commonly exceed these benchmarks. In 46 white sturgeon (ages 4-18 years) caught in the San Francisco estuary from 2002 to 2004 (Linares *et al.* 2004), mean selenium concentration in muscle was 6.59 µg/g dry wt., above the 10% mortality benchmark. Among these sturgeon, selenium concentrations increased with age; therefore, it is likely that individuals older than 18 years (the age at which reproduction was beginning to occur in these wildcaught sturgeon) have even higher concentrations of selenium. This suggests that among white sturgeon in the San Fransico estuary, there may be more than 10% risk of mortality due to selenium.

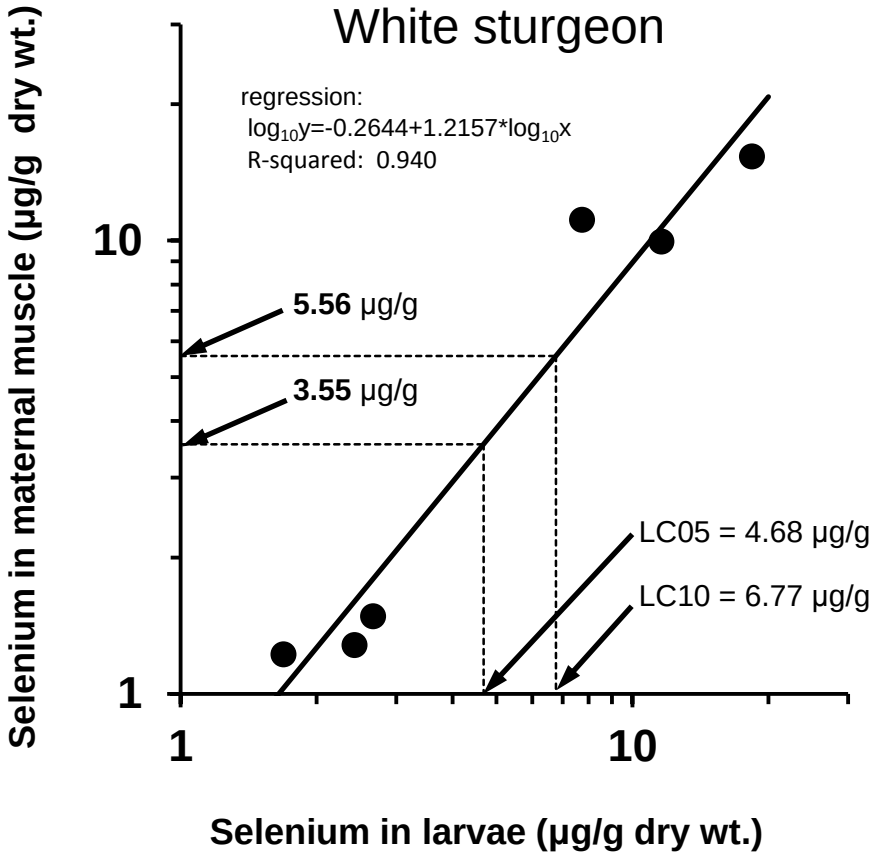


Figure 4. Conversion from larval (stage 36) selenium to selenium in muscle tissue of the mother before depositing eggs. The data are from Table 3-18 in Linville (2006)

White Sturgeon Whole Body Benchmarks – Microinjection

These muscle concentrations (above) translate into maternal whole body selenium concentrations of **2.55** µg/g (LC05) and **3.86** µg/g (LC10) using a conversion equation based on data from a Tashjian *et al.* (2006) study (Figure 5). See the **Discussion** section for more details on this conversion step.

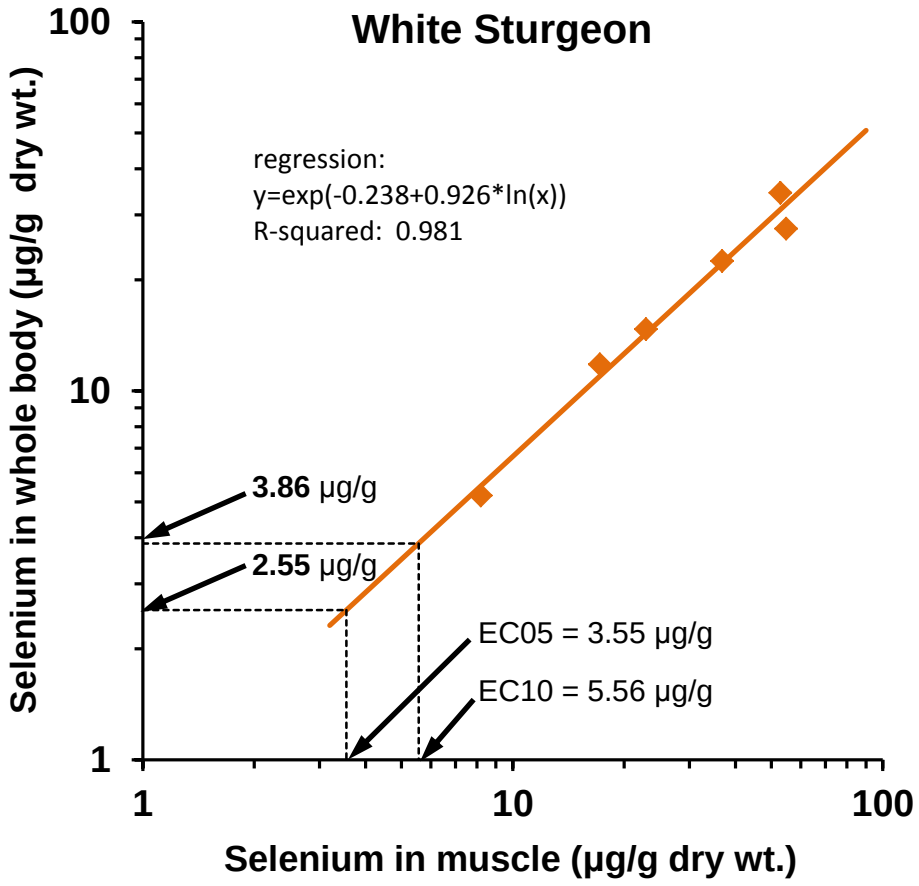


Figure 5. Relationship between muscle and whole-body concentrations of selenium in young white sturgeon (initially weighing about 30 g) exposed for 56 days to dietary selenium in the form of L-selenomethionine. Data are from Tables 2 and 3 in Tashjian *et al.* (2006).

White Sturgeon Dietary Benchmarks – Microinjection

The larval benchmark selenium concentrations from the microinjection experiment shown in Figure 1 can be translated directly into maternal dietary selenium concentrations by using data from the study by Linville (2006). In this study, she fed gravid female white sturgeon selenized yeast for up to six months before they deposited their eggs. She then measured selenium in the larvae that hatched from those eggs. These data indicate that selenium concentrations of **5.45** µg/g and **10.8** µg/g in the food of adult female sturgeon correspond to 5% and 10% mortality rates (respectively) in the larvae they produce (Figure 6). See the **Discussion** section for more information on this translation. Diet items (i.e. *Corbula amurensis*) in the San Francisco Bay estuary exceed selenium concentrations of 10.8 µg/g (dry weight) much of the time (Linville *et al*. 2002, Kleckner *et al.* 2010) indicating that these selenium benchmarks are environmentally relevant in this system.

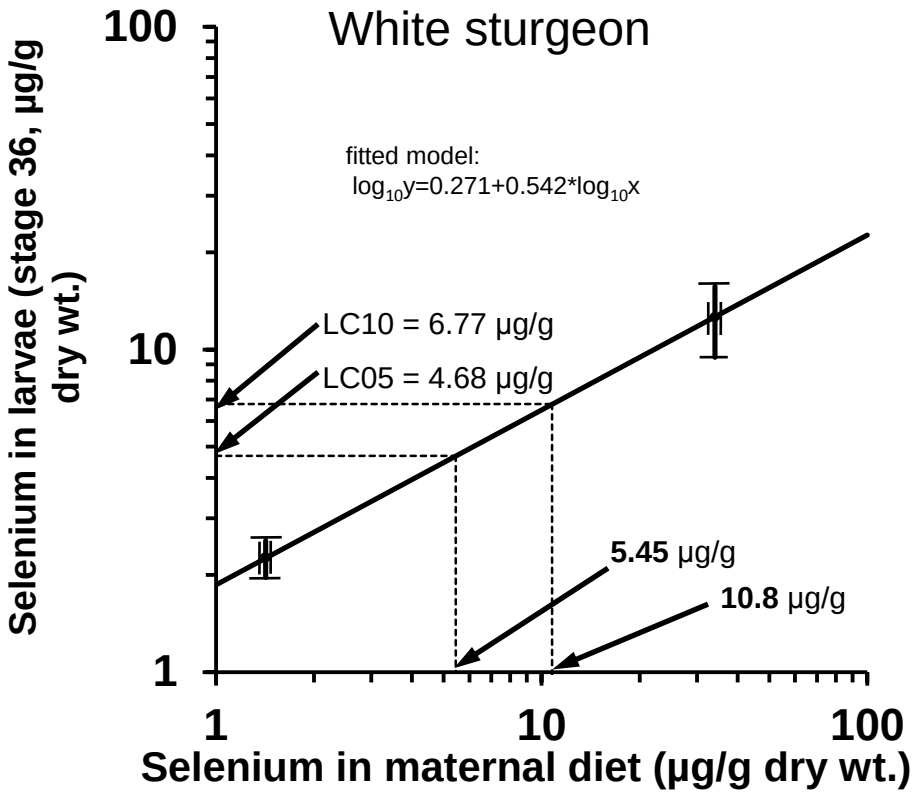


Figure 6. Relationship between selenium in the diet (selenized yeast) of gravid female white sturgeon and selenium in the larvae (stage 36) that hatch from the eggs that these females produced. Data for the fitted model are from Tables 3-1 and 3-5 in Linville (2006). Whiskers indicate standard error bars. Larval effect levels (LC05 and LC10) are from Figure 1.

White Sturgeon Exposure by Maternal Transfer

In the experiments of Linville (2006), adverse effects of selenium on larval white sturgeon occurred at lower concentrations in microinjection treatments than in experiments in which larvae were exposed to elevated selenium solely by "maternal transfer" (selenium deposited in the yolk of developing eggs by mother fish that had been exposed to dietary selenium) (compare Figures 2 and 7). However, although the most sensitive known endpoint is the "controlling" endpoint for protection of the species, here we include analysis of the maternal transfer experiment, because this appears to be a more natural mode of exposure (but see **Discussion**). In Linville's (2006) maternal transfer experiment, the most sensitive endpoint for larvae was developmental abnormality rather than mortality (compare Figures 7 and 8). Developmental abnormalities are an equally valid determination of adverse effect or "take" in endangered species management decisions; therefore, in this experiment we focus on these developmental defects.

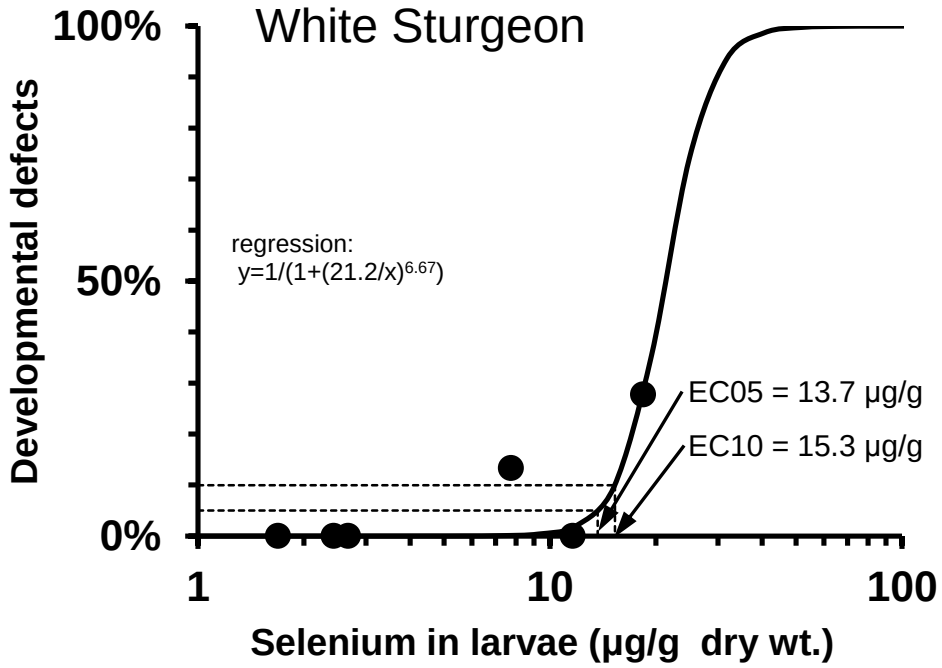


Figure 7. Incidence of larval edema and/or skeletal deformities among larvae from eggs of female white sturgeon exposed to dietary selenium in the form of selenized yeast. The data are from Table 3-18 in Linville (2006).

White Sturgeon Larval Benchmarks – Maternal Transfer

The EC05 and EC10 (the concentrations of selenium in larvae corresponding to 5% and 10% abnormalities, that is, edema and/or spinal curvature) are 13.7 µg/g and 15.3 µg/g, respectively (Figure 7).

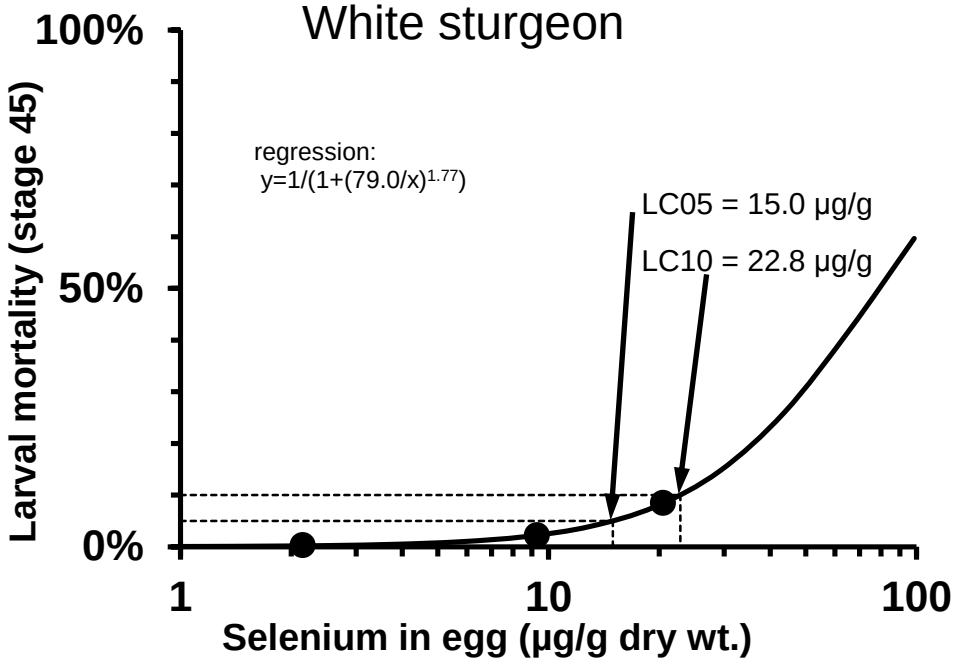


Figure 8. Mortality among larvae (Stage 45) from eggs of female white sturgeon exposed to dietary selenium as selenized yeast. The data are from Table 3-14 in Linville (2006).

White Sturgeon Egg Benchmarks – Maternal Transfer
These larval selenium concentrations translate into egg concentrations of **14.0** µg/g (EC05) and **15.8** µg/g (EC10), using the equation of Figure 3.

White Sturgeon Muscle Benchmarks – Maternal Transfer
The above larval concentrations translate into maternal muscle concentrations of **13.1** µg/g (EC05) and **15.0** µg/g (EC10), using the conversion equation of Figure 4. Of the 46 white sturgeon (ages 4-18 years) caught in the San Francisco estuary from 2002 to 2004 (Linares *et al.* 2004) some individuals approached, but none exceeded the EC05 benchmark (maximum selenium concentration in muscle: 12.4 µg/g), but see the discussion under **White Sturgeon Muscle Benchmarks – Microinjection** above.

White Sturgeon Whole Body Benchmarks – Maternal Transfer
These muscle concentrations translate into whole body selenium benchmark concentrations of **8.51** µg/g (EC05) and **9.65** µg/g (EC10), using the equation of Figure 5.

White Sturgeon Dietary Benchmarks – Maternal Transfer
The larval benchmarks (above) translate into maternal diet selenium concentrations of **39.3** µg/g (EC05) and **48.4** µg/g (EC10) using the equation of Figure 6. These benchmarks are above the maximum concentrations of selenium in diet items (*Corbula amurensis*) collected in San Francisco estuary from 1995 to 2010 (22 µg/g), but see **White Sturgeon Dietary Benchmarks – Microinjection** above**.**

Green Sturgeon (*Acipenser medirostris*)

Since 2006, green sturgeon in the San Francisco Bay-Delta area have been federally listed under the Endangered Species Act as threatened (71 FR 17757). Therefore, any regulatory benchmark established by a federal agency, such as the U. S. Environmental Protection Agency, must be reviewed by the National Oceanic and Atmospheric Administration (NOAA) to determine whether that benchmark is protective of the species.

Recently, groups of researchers at the University of California, Davis, have investigated the effects of selenium and methylmercury on both green and white sturgeon in various conditions of salinity and temperature (Kaufman et al. 2008; Linares-Casenave et al. 2010; Silvestre et al. 2010; Walker 2009). Dietary exposure experiments showed that green sturgeon are "much more sensitive to selenium" than white sturgeon at environmentally relevant concentrations of selenium (Kaufman et al. 2008), but data from these experiments are not yet publicly available. However, data from selenium micro-injection experiments are available: in tabular form in the poster of Linares-Casenave *et al.* (2010) and in graphical form in the published paper of Silvestre *et al.* (2010). The data from these microinjection experiments confirm that the yolk sac larvae of green sturgeon are more sensitive to selenium than those of white sturgeon (compare Figures 1 and 9).

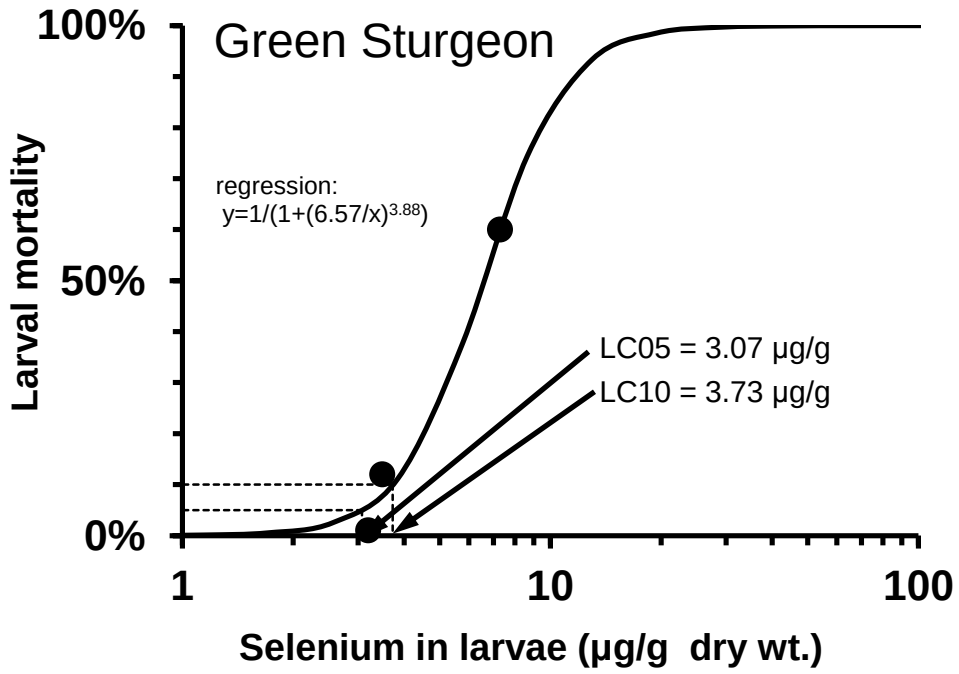


Figure 9. Mortality of green sturgeon larvae microinjected with L-selenomethionine in the yolk sac just after hatching (developmental stage 36). Selenium was measured one day after injection. Mortality is the proportion that died by the time of yolk depletion, when the fish is ready to start eating (stage 45). The data are from Tables 1 and 2 in Linares-Casenave *et al.* (2010), also shown in Table 1 and Figure 1 in Silvestre *et al.* (2010).

We first provide a larval toxicity regression to determine 5% and 10% effect levels for green sturgeon based on the Linares-Casenave et al. (2010) microinjection study. These effect levels are then translated to egg, muscle, whole body, and diet using the white sturgeon data from Linville (2006) and Tashjian et al. (2006) as a surogate. Then we use results from the Linares-Casenave et al. (2010) green and white sturgeon microinjection study to determine a sensitivity ratio and apply it to the Linville (2006) maternal transfer study to calculate green sturgeon 5% and 10% effect levels via maternal transfer. As for the white sturgeon we then perform similar translation of those effect levels to egg, muscle, whole body and diet for the green sturgeon.

Green Sturgeon Larval Benchmarks – Microinjection

A standard log-logistic model of the green sturgeon microinjection data yield larval LC05 and LC10 (the concentrations of selenium in larvae corresponding to 5% and 10% mortality) of 3.07 µg/g and 3.73 µg/g, respectively (Figure 9).

Green Sturgeon Egg Benchmarks – Microinjection

To the best of our knowledge, as yet there are no data relating selenium in eggs and larvae for green sturgeon. Therefore, we use the equation for white sturgeon (Figure 3) to translate larval benchmark selenium concentrations to corresponding selenium concentrations in the eggs from which the larvae hatch. Accordingly, selenium concentrations of **3.03** µg/g and **3.70** µg/g in green sturgeon eggs correspond to 5% and 10% mortality rates (respectively) in the larvae that hatch from those eggs.

Green Sturgeon Muscle Benchmarks – Microinjection

To the best of our knowledge, no data specific to green sturgeon are yet available to translate these larval selenium concentrations into concentrations in the muscle of the mother that produced the larvae. Therefore, we use the white sturgeon data referenced above (Figure 4) for the best available approximations of these translations in green sturgeon. Accordingly, the 5% and 10% larval benchmarks translate into maternal muscle selenium concentrations of approximately **2.13** µg/g and **2.69** µg/g, respectively.

Green Sturgeon Whole Body Benchmarks – Microinjection

To the best of our knowledge, no data specific to green sturgeon are yet available to translate the above muscle selenium concentrations into concentrations in the whole body of the mother that produced the larvae. Therefore, we use the white sturgeon data referenced above (Figure 5) for the best available approximations of these translations in green sturgeon. Accordingly, the muscle benchmarks translate into maternal whole body (dry weight) selenium concentrations of about **1.59** µg/g (LC05) and **1.97** µg/g (LC10).

Green Sturgeon Dietary Benchmarks – Microinjection

To the best of our knowledge, no trophic transfer data specific to green sturgeon are yet available to translate larval selenium concentrations or maternal tissue concentrations into concentrations in the diet of the mother that produced the eggs from which the larvae hatched. Therefore, we use the white sturgeon data referenced above (Figure 6) for the best available approximation of the appropriate translation. Accordingly, the green sturgeon larval benchmark selenium concentrations from Figure 9 translate into selenium concentrations of approximately **2.51** µg/g (EC05) and **3.58** µg/g (EC10) in the food of adult female green sturgeon (Figure 10). Almost all of the diet items (*Corbula amurensis*) collected in the San Francisco Bay estuary from 1995 to 2010 exceeded these benchmark concentrations (Kleckner *et al.* 2010).

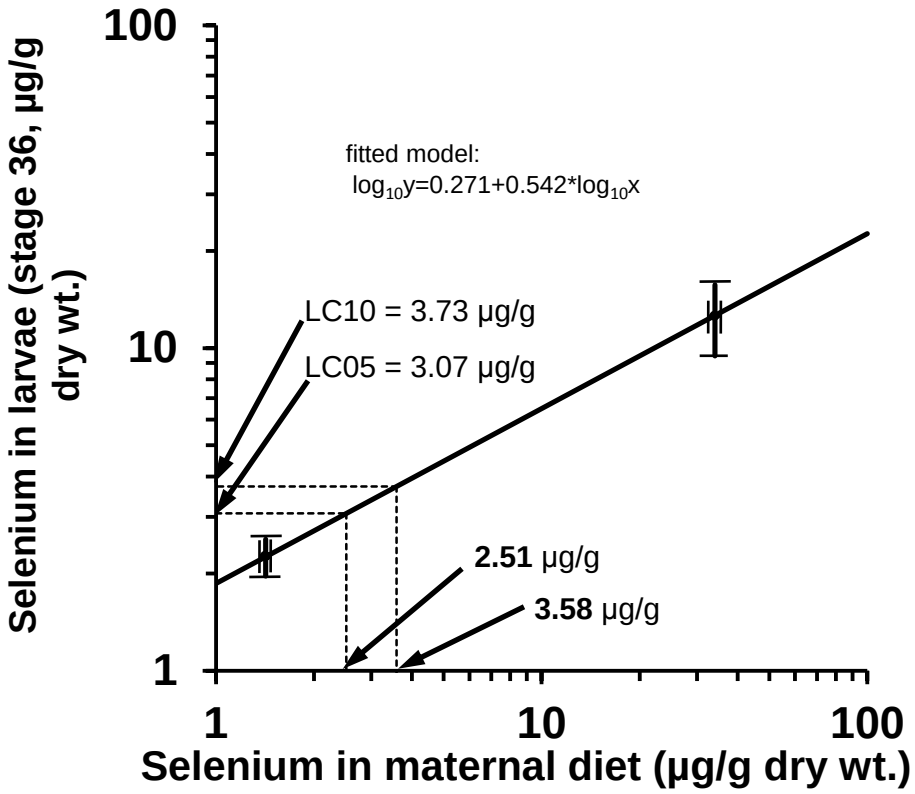


Figure 10. Translation of selenium effect levels for larval green sturgeon (from Figure 9) into corresponding selenium concentrations in maternal diet using trophic and maternal transfer data for white sturgeon are from Linville (2006) (see Figure 6).

Green Sturgeon Exposure by Maternal Transfer – calculated from relative sensitivity

As far as we know, for green sturgeon there has been no maternal transfer selenium toxicity experiment comparable to Linville's (2006) experiment with white sturgeon (Figures 7 and 8). However, if we assume that the relative sensitivity of green and white sturgeon would be the same for naturally transferred selenium as for micro-injected selenium, then we can calculate a larval selenium sensitivity ratio for green and white sturgeon, and use this ratio to estimate, for green sturgeon, selenium benchmarks that correspond to those for maternally transferred selenium in white sturgeon. Such a ratio can be obtained from the micro-injection experiments

of Linares-Casenave *et al.* (2010) on both green and white sturgeon (Figure 11). The ratio of EC10's (the sensitivy ratio, white to green) is 10.49/4.49 = 2.34.

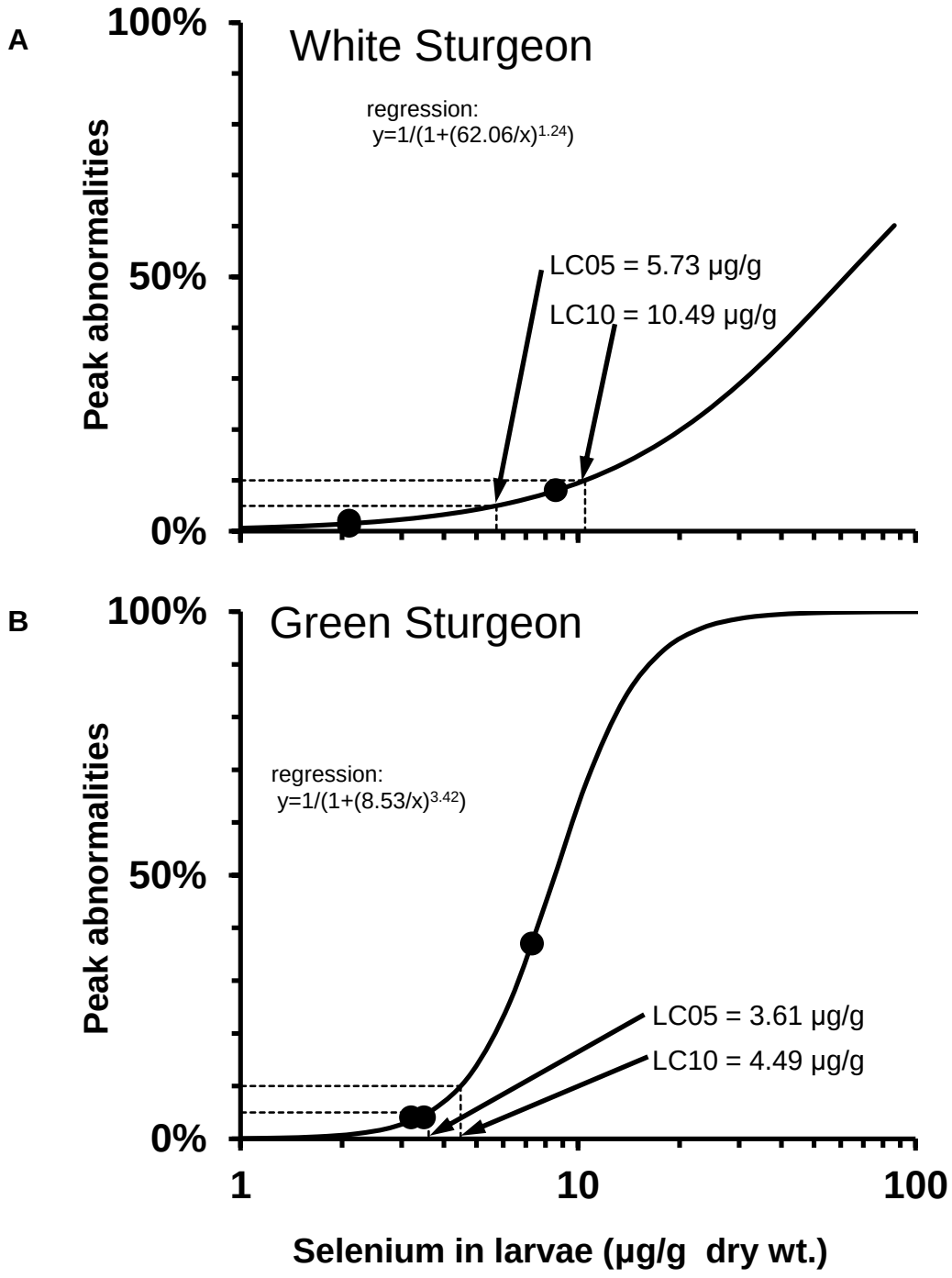


Figure 11. Incidence of larval abnormalities among larvae from eggs of (A) white sturgeon and (B) green sturgeon microinjected with L-selenomethionine in the yolk sac just after hatching. Data are from Tables 1 and 2 in Linares-Casenave *et al.* (2010).

In Figure 11, the effect or "endpoint" used is the peak incidence of abnormalities rather than the incidence of abnormalities at the end of the experiment. This is because transient abnormalities effectively immobilize developing larvae (Serge Doroshov pers.com.). This may cause adverse effects during larval development that are not represented by the incidence of abnormalities at the end of larval development. In the wild, transient abnormalities resulting in immobilization are likely to result in mortality due to predation or the inability to escape from poor habitat conditions. Even the peak incidence of abnormalities may not fully represent the full consequences of transient abnormalities, because some individual larvae may be affected by transient abnormalities only at some time other than the time of peak abnormalities in the group of larvae. Any adverse effects on those larvae are not counted in the analysis presented here. Full accounting of these adverse effects would require tagging and tracking the condition of each individual larva.

Green Sturgeon Larval Benchmarks – Maternal Transfer

The above sensitivity ratio applied to the white sturgeon maternal transfer benchmark selenium concentrations in larvae (Figure 7) yield the following corresponding green sturgeon benchmark larval selenium concentrations: larval EC05 = 5.84 µg/g (= 13.7 µg/g /2.34) and EC10= 6.54 µg/g (=15.3 µg/g /2.34).

Green Sturgeon Egg Benchmarks – Maternal Transfer

Corresponding benchmark selenium concentrations for green sturgeon eggs are calculated from the larval concentrations as they were for the injection experiment above using Figure 3. The resulting egg benchmarks are EC05 = **5.87** µg/g and the EC10 = **6.59** µg/g.

Green Sturgeon Muscle Benchmarks – Maternal Transfer

Corresponding benchmark selenium concentrations for green sturgeon muscle are calculated from the larval concentrations as they were for the injection experiment above using Figure 4. These translations yield maternal muscle benchmarks of EC05 = **4.65** µg/g and EC10 = **5.33** µg/g.

Green Sturgeon Whole Body Benchmarks – Maternal Transfer

Corresponding benchmark selenium concentrations for green sturgeon whole body are calculated from the larval concentrations as they were for the injection experiment above using Figure 5. These translations yield maternal whole body benchmarks of EC05 = **3.27** µg/g and EC10 = **3.71** µg/g.

Green Sturgeon Dietary Benchmarks – Maternal Transfer

Corresponding benchmark selenium concentrations for the diet of green sturgeon are calculated from the larval concentrations as they were for the injection experiment above using Figure 10. This translation produces green sturgeon maternal diet benchmarks of EC05 = **8.22** µg/g and EC10= **10.1** µg/g.

Discussion

The whole-body LC10 selenium concentration derived here for white sturgeon (3.86 µg/g) is similar to the toxicity threshold of 4 µg/g suggested by Lemly (1996) and used by the U. S. Fish and Wildlife service for warmwater fish (Beckon *et al.* 2010). Green sturgeon are more susceptible to selenium toxicity; they appear to be about as sensitive to selenium as the more sensitive coldwater fish (Salmonids). The whole-body LC10 derived here for green sturgeon (1.97 µg/g) is close to the LC10 of 1.84 µg/g for juvenile Chinook salmon, and the EC10 of 2.19 µg/g for juvenile rainbow trout (Beckon *et al.* 2010). The dietary LC05 and LC10 derived here for green sturgeon (2.51 µg/g and 3.58 µg/g respectively) bracket the concern threshold of 3 µg/g used by the U. S. Fish and Wildlife service (Beckon *et al.* 2010).

Because adult sturgeon are very large, it is difficult to analyze their tissue for selenium on a whole-body basis (requiring homogenization of the entire body). Therefore, as far as we know, data are not available for muscle/whole-body selenium regressions in adults of these species. The best available data for such a regression (Figure 5) came from only juvenile white sturgeon data (Tashjian *et al*. 2006). However, this regression is in good agreement with a generic muscle/whole-body relationship based on a much larger data set comprising several different fish families (Figure 12). Furthermore, the relationship is fairly robust regardless of the gender or reproductive condition of the fish (Figure 13) despite the suggestion (Osmundson & Skorupa 2011) that selenium is differently partitioned among tissues depending on reproductive status. Therefore, it is likely that the juvenile muscle/whole-body relationship adequately represents the muscle/whole-body relationship of adult white and green sturgeon, and that the modest extrapolation of this relationship in this analysis (Figure 5) is reasonable.

Because of the meager data available to translate from larval selenium concentrations to maternal dietary concentrations of selenium (Figures 6 and 10), the choice of model is critical for interpolating between the data points. This translation incorporates two stages: trophic transfer (from maternal diet to maternal tissue) and maternal transfer (from maternal tissue to larval tissue). The exponential model (straight line on a log-log graph) used here for the combined transfer is valid if both transfer stages are adequately represented by exponential models. As far as we know, no one has questioned the applicability of such a model to maternal transfer, but it has been suggested that organisms actively, homeostatically regulate their internal concentrations of selenium (Brix *et al.* 2005). Such homeostatic regulation would result in trophic transfer functions that are better represented by "hockey stick" models, which include a plateau region in which internal tissue concentrations are purportedly held constant as exposure increases. If such regulation occurs, it would invalidate the straight-line exponential model, enabling mother sturgeon to consume more selenium without elevating their internal selenium concentrations or those of their eggs and larvae. However, a broad survey of field and laboratory bioaccumulation and trophic transfer data offers no support for such homeostatic regulation (for other bioaccumulative metals as well as for selenium). Rather the data strongly support continuous, non-homeostatic regulation, with trophic transfer functions that are well described by exponential models (Beckon 2010, Appendix II). Therefore, it is likely that the fitted straight line in Figures 6 and 10 (describing an exponential model) is suitable for translations of concentrations of selenium between maternal diet and larvae. Because only two data points are available in the published literature for this relationship (Linville 2006), regression is not

possible; instead, the model was fitted manually so as to pass through those two points. The resulting model should be reasonably stable because each of the data points represents the average of three replicates (different cohorts from three different females) of 60 to 90 individual larvae per replicate.

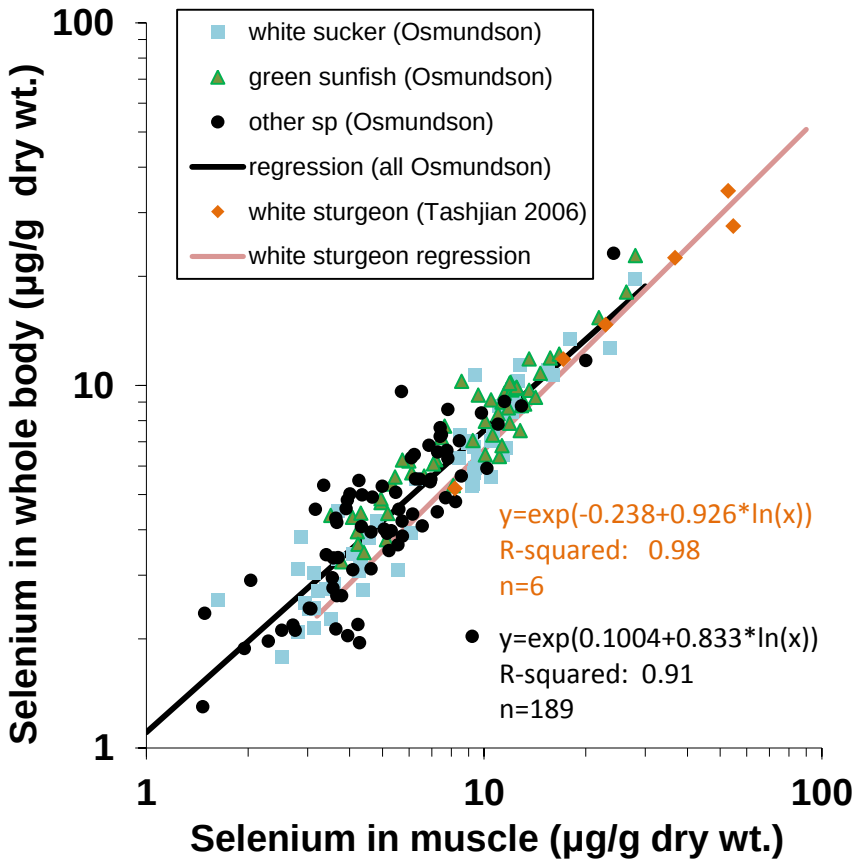


Figure 12. Relationship between muscle and whole-body concentrations of selenium in young white sturgeon (Tashjian *et al.* 2006) compared with the relationship between muscle plug and whole-body concentrations of selenium in representatives of several families of fish caught in the Gunnison and Colorado Rivers and associated tributaries (data from Osmundson *et al.* 2007, Osmundson & Skorupa 2011).

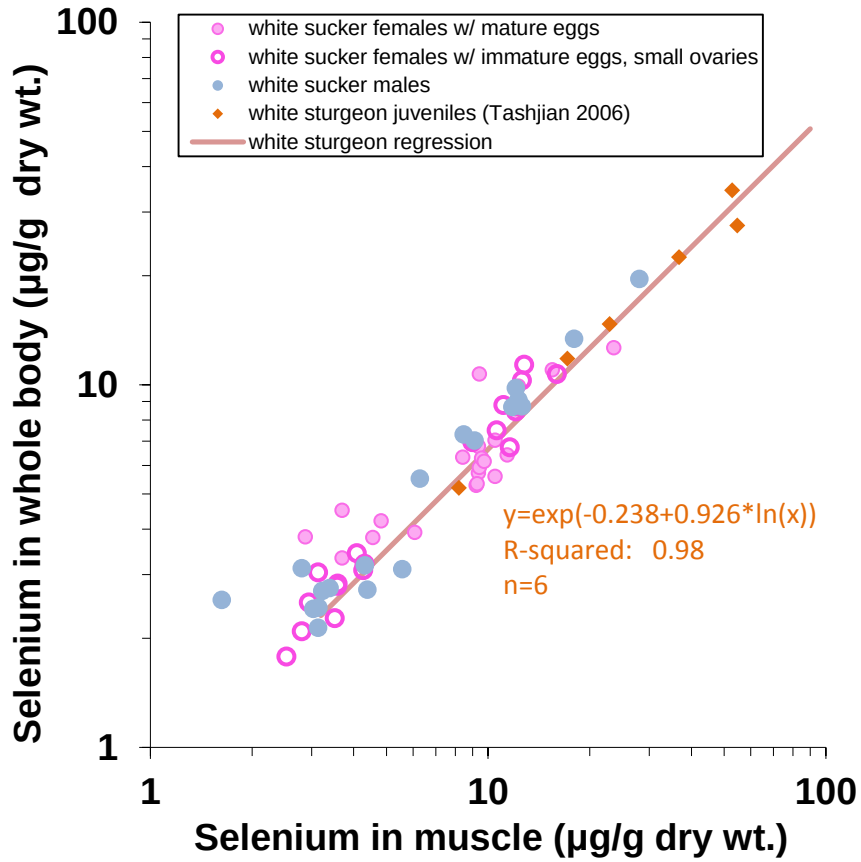


Figure 13. Relationship between muscle and whole-body concentrations of selenium in young white sturgeon (Tashjian *et al*. 2006) compared with the relationship between muscle plug and whole-body concentrations of selenium in white suckers (*Catastomus commersoni*) segregated by gender and reproductive condition (data from Appendix I in Osmundson & Skorupa 2011).

For comparisons of the sensitivities of different species and endpoints to selenium, here we use EC10s rather than the more traditional EC50s. This is because EC10 is an effect level that is closer to levels that may be protective of species. When concentration-response curves have substantially different slopes (as in Figures 1 and 2, and Figure 11), then sensitivity comparisons based on EC50s would poorly represent relative sensitivities at effect levels of EC10 or below.

It should be noted that the green sturgeon experiment analyzed here (Linares-Casenave *et al.* 2010, Silvestre *et al.* 2010) was performed on the projeny of a single individual (Obtaining multiple individuals for experimentation was not practical because the species is listed as threatened in this area). Therefore, this analysis may underestimate or overestimate the sensitiviy of the population of green sturgeon from which this individual came. However, these are the best data available for green sturgeon at this time.

In the experiments of Linville (2006), adverse effects of selenium on larval white sturgeon occurred at lower concentrations in microinjection treatments than in experiments in which larvae were exposed to elevated selenium solely by maternal transfer. If this apparent difference is real, it may be related to the fact that in the microinjection experiments, the selenium is introduced suddenly as a free amino acid (L-selenomethionine) that is readily available for assimilation by the developing larvae, whereas maternally transferred selenium is incorporated into proteins that must be digested by the larvae before assimilation (Linville 2006). Heinz *et al.* (2011) offered a similar explanation for his observation that methylmercury chloride injected into mallard eggs is more terratogenic than the same concentration of methylmercury maternally deposited in the egg. However, at the present time, for selenium in white sturgeon larvae, maternal transfer data are more limited (one experiment, two treatments) than microinjection data (multiple experiments, each with several treatments). Additionally, although the maternal transfer mode of exposure may seem to be more natural than injection of selenomethionine into larvae, in the maternal transfer experiments of Linville (2006), the mother fish were fed selenium in the form of selenized yeast. Sturgeon do not feed on selenized yeast in the wild, and there are indications that experiments based on exposure to dietary selenized yeast may underestimate selenium assimilation and toxicity (Heinz *et al.* 1996). For these reasons, until more data confirm the apparent difference in sensitivity to these two modes of exposure, and clarify reasons for it, prudence and caution impel us to use the data from the microinjection experiments as the basis for protective guidelines.

It may be that our analysis of the maternal transfer experiment of Linville (2006) underestimates the potential hazard of trophic and maternal transfer of selenium because of the relative youth of the captive-reared female sturgeon used in that experiment. White sturgeon captured in the wild from the San Francisco Bay/Estuary region exhibit a clear pattern of increasing selenium bioaccumulation with increasing age (Linares *et al.* 2004); individuals just beginning to reproduce in the wild (17 years old) had more than twice the selenium concentrations in muscle than the concentrations in the age class (6 years old) that were used in the Linville (2006) experiment. Still older (>17 years), fully reproductive individuals in the wild are likely to accumulate even higher levels of selenium.

It may be that the most sensitive endpoint for selenium effects on sturgeon is neither larval mortality nor larval abnormalities. Possibly these fish are even more sensitive to selenium after the larvae have finished yolk sac absorption and begin exogenous feeding, as seems to be the case for at least some salmonids (Beckon 2007, Beckon *et al.* 2010). To the best of our knowledge, sturgeon at this very sensitive stage have not yet been tested; Tashjian *et al.* (2006) tested a later, potentially less sensitive juvenile stage. Fish such as sturgeon could also be very sensitive to selenium effects on behavior, which could be important in the wild for predator avoidance, foraging, or reproductive success. For example, Hopkins *et al.* (2003) documented a substantial reduction in swimming speed, probably at least partly attributable to selenium, in benthic lake chubsucker (*Erimyzon sucetta*) exposed to coal ash. In green and white sturgeon, the effects of selenium on metabolic rates, growth, swimming performance, and avoidance of predators have been studied (Kaufman et al. 2008; Walker 2009), but the data have not yet been published. Until data on sublethal reproductive and behavioral effects such as these are published, to the best of our knowledge, the data used here are the best available for the most sensitive endpoint currently known for these fish.

The analysis presented here focuses only on the single most sensitive known endpoint. In reality, mortality and non-lethal endpoints are additive. That is, in addition to the mortality of developing embryos directly caused by selenium, a further proportion of surviving individuals suffers from other adverse effects of selenium: non-lethal effects (such as spinal deformities and impaired growth), indirect lethal effects (such as elevated vulnerability to predation due to mobility impairment caused by transitory edema, or other behavioral impairment), and later lethal effects (such as mortality among early stage juveniles). For these reasons, the analysis presented here probably underestimates the full extent of adverse effects of selenium on sturgeon. Therefore, benchmarks based on all adverse effects would likely be lower (more stringent) than those derived here on the basis of the most sensitive endpoint only.

Although uncertainty in data analysis has been viewed as an argument for relaxing protective standards, in reality, uncertainty may require greater stringency. Agencies that implement the Endangered Species Act are expected to "provide the benefit of the doubt" to listed species (USFWS & NOAA 2004).

A strict interpretation of the "benefit of the doubt" guidance would suggest that, in modeling dose-response relationships, rather than a central-tendency model (least squares regression: solid line in Figures 1 and 2), some lower (more stringent) confidence bound should be used for deriving a protective guideline. Alternatively, a "safety factor", "uncertainty factor", "modifying factor" (USEPA 1993), and/or "margin of safety" could be included in the analysis ("*Margins of safety are essential to any health related environmental standards if a reasonable degree of protection is to be provided against hazards which research has not yet identified."* [Senate Committee on Public Works, Report No.91-1196 (1970), pp.9-10]). However, in the case of selenium, care must be exercised in implementing any such factor or margin of safety, because selenium is essential at low concentrations, and there is a very narrow range between essentiality and toxic excess (*e.g*., Figures 3B and 5B in Beckon *et al.* (2008)). Larval mortality in sturgeon may be caused by deficiency of selenium as well as by toxic excess of selenium. An excessively large uncertainty factor could push a benchmark concentration into the zone of deficiency. No factor providing any margin of safety is included in the derivation of benchmark selenium concentrations presented here. However, the above analysis, based on the more sensitive microinjection route of exposure (rather than the maternal transfer route of exposure), provides conservative benchmarks founded on the best information currently available.

If selenium deficiency can occur in larval sturgeon, then data spanning a full range of concentrations from deficiency to toxic excess would best be represented by a biphasic model (Beckon *et al*. 2008). We know of no data as yet available relating larval survival of sturgeon to selenium in the zone of tissue concentrations that are likely to cause deficiency—probably < 1 µg/g (whole body dry weight), based on experiments with juveniles of other species (Beckon *et al.* 2010). Therefore, for the available data, the simple log-logistic model used here (Figures 1, 2, 7, 8, 9 and 11) should be adequate.

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Appendix I
Data and Methods

Log-logistic models were fitted to dose-response relationships by least squares non-linear regression, using the "nls" and "nls2" functions in the "R" statistical program. Confidence (95%) bounds were determined for non linear models using the "nls2", "predict", and "as.lm.nls" functions in "R". Linear models for translations of tissue concentrations were determined using the "lm" function in "R" (except for Figures 11 and 12, as noted below).

White sturgeon (*Acipenser transmontanus*)

Figure 1

Selenium effect on mortality of microinjected white sturgeon larvae. Data source: Table 3-15 in Linville (2006).

Treatment	Selenium in larvae (µg/g dry wt.)	Larval mortality
Study 1 non inj	2.6	0
Study 1 sham inj	2.54	0
Study 1 low	15.8	0.41
Study 1 medium	21.7	0.467
Study 1 high	46.6	0.676
Study 2 non inj	6.36	0
Study 2 sham inj	5.89	0.0167
Study 2 low	8.74	0.1
Study 2 medium	8.97	0.0334
Study 2 high	16.56	0.7

```
Formula: y ~ 1/(1 + (e/x)^b)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
e  20.0490     3.0848   6.499 0.000188 ***
b   2.0233     0.6224   3.251 0.011683 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1392 on 8 degrees of freedom

Number of iterations to convergence: 9
Achieved convergence tolerance: 4.346e-06
```

Figure 2

Selenium effect on defects of microinjected white sturgeon larvae. Data source: Tables 3-11 and 3-13 in Linville (2006).

Treatment	Selenium in larvae (µg/g dry wt.)	Edema and/or spinal curvature
Study 1 non inj	2.6	0
Study 1 sham inj	2.54	0.0395
Study 1 low	15.8	0.7669
Study 1 medium	21.7	0.8366
Study 1 high	46.6	0.7821
Study 2 low	8.74	0.1273
Study 2 medium	8.97	0.0508
Study 2 high	16.56	0.6949
Study 2 non/sham inj	6	0.0085

```
Formula: y ~ 1/(1 + (e/x)^b)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
e  13.5260     0.8507  15.900 9.44e-07 ***
b   4.9174     1.1663   4.216  0.00395 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.09781 on 7 degrees of freedom

Number of iterations to convergence: 9
Achieved convergence tolerance: 7.008e-06
```

Figure 3

Selenium in white sturgeon eggs and larvae. Data source: Table 3-18 in Linville (2006).

```
Call:
lm(formula = log10(egg) ~ log10(larva), data = WhiteSturgeonLinvile3_18)

Residuals:
        1         2         3         4         5         6
 0.013976 -0.008056  0.009141 -0.033171  0.031295 -0.013184
```

```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  -0.01930    0.02187  -0.882    0.427
log10(larva)  1.02763    0.02711  37.909 2.89e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.02548 on 4 degrees of freedom
Multiple R-squared: 0.9972,      Adjusted R-squared: 0.9965
F-statistic:  1437 on 1 and 4 DF,  p-value: 2.892e-06
```

Figure 4

Maternal transfer of selenium in white sturgeon. Data source: Tables 3-18 in Linville (2006).

Treatment	Selenium in maternal muscle (µg/g dry wt.)	Selenium in larvae (µg/g dry wt.)
Control C3	1.28	2.43
Control C4	1.22	1.69
Control C5	1.48	2.67
Treatment T1	9.93	11.6
Treatment T2	15.3	18.4
Treatment T3	11.1	7.75

```
Call:
lm(formula = log10(muscSe) ~ log10(larvaeSe), data = WhiteSturgeonLinville3_18)

Residuals:
       1        2        3        4        5        6
-0.09717  0.07372 -0.08385 -0.03273 -0.08856  0.22859

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)     -0.2644     0.1237  -2.137  0.09937 .
log10(larvaeSe)  1.2157     0.1533   7.930  0.00137 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1441 on 4 degrees of freedom
Multiple R-squared: 0.9402,      Adjusted R-squared: 0.9252
F-statistic: 62.88 on 1 and 4 DF,  p-value: 0.001369
```

Figure 5

Selenium in muscle and whole body in young white sturgeon. Data source: Tables 2 and 3 in Tashjian *et al.* (2006).

Selenium in muscle (µg/g dry wt.)	Selenium in whole body (µg/g dry wt.)
8.2	5.2
17.2	11.8
22.9	14.7
36.8	22.5
52.9	34.4
54.8	27.5

```
Call:
lm(formula = log(WB) ~ log(musc), data = WhiteSturgeonTTF)

Residuals:
       1        2        3        4        5        6
-0.06139  0.07235  0.02715  0.01373  0.10234 -0.15419

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.23765    0.21398  -1.111 0.328997
log(musc)    0.92565    0.06399  14.467 0.000133 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1051 on 4 degrees of freedom
Multiple R-squared: 0.9812,      Adjusted R-squared: 0.9766
F-statistic: 209.3 on 1 and 4 DF,  p-value: 0.0001327
```

Figure 6

Trophic and maternal transfer in white sturgeon. Data source: Tables 3-1 and 3-5 in Linville (2006).

Selenium in maternal diet (µg/g dry wt.)	Selenium in larvae (stage 36, µg/g dry wt.)
1.42	2.26
34.04	12.58

Figure 7

Developmental defects in white sturgeon larvae exposed to selenium via maternal diet. Data source: Table 3-18 in Linville (2006).

Treatment	Selenium in larvae (µg/g dry wt.)	Developmental defects
Control C3	2.43	0
Control C4	1.69	0
Control C5	2.67	0
Treatment T1	11.6	0
Treatment T2	18.4	0.2778
Treatment T3	7.75	0.1333

```
Formula: defects ~ 1/(1 + (e/larvaeSe)^b)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
e   21.246      4.084   5.202  0.00651 **
b    6.666      8.410   0.793  0.47230
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.06662 on 4 degrees of freedom

Number of iterations to convergence: 65
Achieved convergence tolerance: 9.34e-06
```

Figure 8

Mortality in white sturgeon larvae exposed to selenium via maternal diet. Data source: Table 3-14 in Linville (2006).

Grouped exposure category	Selenium in egg (µg/g dry wt.)	Larval mortality (stage 45)
low	2.15	0.0027
med	9.31	0.022
high	20.5	0.0842

```
Formula: Mortality ~ 1/(1 + (e/SeEggs)^b)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
e 78.98147    3.86639   20.43   0.0311 *
b  1.76984    0.06086   29.08   0.0219 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.00103 on 1 degrees of freedom

Number of iterations to convergence: 3
Achieved convergence tolerance: 7.521e-06
```

Green sturgeon (*Acipenser medirostris*)

Figure 9

Selenium effect on mortality of microinjected green sturgeon larvae. Data source: Javier Linares-Casenave, et al. poster (2010).

Treatment	Selenium in larvae (µg/g dry wt.)	Larval mortality
Se-L-Met	7.3	0.6
L-Met	3.5	0.12
Non-Inj	3.2	0.01

```
Formula: y ~ 1/(1 + (e/x)^b)

Parameters:
  Estimate Std. Error t value Pr(>|t|)
e   6.5661     0.4116  15.952   0.0399 *
b   3.8791     0.9478   4.093   0.1526
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.06239 on 1 degrees of freedom

Number of iterations to convergence: 5
Achieved convergence tolerance: 6.219e-06
```

Figure 10

Data and fitted model are the same as in Figure 5

Figure 11A

Selenium effect on peak abnormalities of microinjected green sturgeon larvae. Data are from Tables 1 and 2 in Linares-Casenave et al. 2010.

Treatment	Selenium in larvae (µg/g dry wt.)	Peak abnormalities
Se-L-Met	8.6	0.08
L-Met	2.1	0.02
Non-Inj	2.1	0.01

S-PLUS 6 analysis:

```
Formula: Abnorm ~ 1/(1 + (e/SeLarvae)^b)

Parameters:
     Value Std. Error t value
e 62.06060  26.492800 2.34255
b  1.23579   0.249522 4.95262

Residual standard error: 0.00707107 on 1 degrees of freedom

Correlation of Parameter Estimates:
       e
b -0.985
```

Figure 11B

Selenium effect on peak abnormalities of microinjected white sturgeon larvae. Data are from Tables 1 and 2 in Linares-Casenave et al. 2010.

Treatment	Selenium in larvae (µg/g dry wt.)	Peak abnormalities
Se-L-Met	7.3	0.37
L-Met	3.5	0.04
Non-Inj	3.2	0.04

S-PLUS 6 analysis:

```
Formula: Abnorm ~ 1/(1 + (e/SeLarvae)^b)

Parameters:
    Value Std. Error t value
e 8.52964   0.130246 65.4887
```

b 3.42169 0.202209 16.9216
Residual standard error: 0.00820243 on 1 degrees of freedom
Correlation of Parameter Estimates:
e
b -0.755

Figure 12

Selenium in muscle and whole body in representatives of several families of fish caught in the Gunnison and Colorado Rivers and associated tributaries. Data sources: Barbara Osmundson pers. com., Osmundson *et al.* (2007), Osmundson & Skorupa (2011). For comparison, the white sturgeon data and regression from Figure 5 are also shown (orange color).

ID	Species	Selenium in whole body (µg/g dry wt.)	Selenium in muscle (µg/g dry wt.)
BSW-WS3	WS	3.81	2.88
BSW-WS4	WS	4.21	4.83
BSW-WS7	WS	3.33	3.7
BSW-WS8	WS	4.51	3.7
2PW-WS1	WS	6.32	8.42
2PW-WS2	WS	6.77	9.36
2PW-WS3	WS	10.99	15.5
2PW-WS4	WS	12.65	23.6
MWB-WS2	WS	5.72	9.37
MWB-WS1	WS	8.32	11.5
2MW-WS1	WS	3.92	6.07
2MW-WS2	WS	3.79	4.56
2FL-WS1	WS	9.92	12.3
2FL-WS3	WS	5.28	9.23
WS-FL-1	WS	10.72	9.44
WS-FL-2	WS	5.92	9.44
WS-FL-3	WS	7.03	10.5
WS-FL-4	WS	6.4	11.4
WS-FL-5	WS	6.27	9.57

WS-FL-6	WS	5.33	9.29
WS-FL-8	WS	6.15	9.75
WS-FL-9	WS	5.59	10.5
AC-WSWB1	WS	8.8	11.1
AC-WSWB2	WS	8.71	12.1
MW-WS2	WS	11.37	12.8
MW-WS6	WS	10.7	16
MW-WS7	WS	8.44	12.1
PS2-WS1	WS	6.95	8.99
PS2-WS3	WS	7.49	10.6
PS2-WS4	WS	10.28	12.6
PS2-WS5	WS	6.73	11.6
GV1-WS1	WS	2.09	2.81
GV1-WS4	WS	1.78	2.53
GV1-WS6	WS	3.21	4.31
GV1-WS8	WS	2.27	3.52
GV1-WS9	WS	3.08	4.27
GV1-WS10	WS	3.04	3.14
GV1-WS13	WS	2.79	3.58
GV1-WS14	WS	2.51	2.95
GV1WS15	WS	3.43	4.09
GV1WS16	WS	2.83	3.59
WS-FL-7	WS	3.1	5.58
WS-FL-10	WS	5.51	6.29
2FL-WS2	WS	7.02	9.13
2FL-WS4	WS	7.3	8.47
2FL-WS5	WS	2.41	3.04
BSW-WS1	WS	2.72	4.39
BSW-WS6	WS	2.7	3.23
BSW-WS10	WS	2.55	1.63
2HW-WS1	WS	19.6	28.1
AC-WS2	WS	9.8	12.1
MW-WS1	WS	8.69	11.8
MW-WS4	WS	8.73	12.6
MW-WS5	WS	9.08	12.3
AC-WSWB3	WS	13.4	18
GV1-WS2	WS	3.12	2.81
GV1-WS5	WS	2.43	3.15
GV1-WS7	WS	2.14	3.14
GV1WS11	WS	3.18	4.32
GV1WS12	WS	2.75	3.4
AC-GS1	GS	10.8	14.7
ACOGS1	GS	22.8	28.1
ACO-GS3	GS	8.79	12.9
RW-GS1	GS	15.37	21.9
LW-GS2	GS	4.75	4.95
LW-GS4	GS	5.74	6.11
LW-GS6	GS	4.43	5.19
2MW-GS2	GS	3.75	5.14
BSW-GS2	GS	11.9	15.7

2PW-GS1	GS	6.43	10.1
2HW-GS1	GS	9.51	11.5
FL-GS-1	GS	9.13	10.5
GS-FL-8	GS	6.24	7.2
GS-FL-9	GS	7.04	9.26
GS-FL10	GS	7.72	7.65
FL-GS11	GS	6.19	5.99
FL-GS12	GS	10.2	12
FL-GS14	GS	9.71	12.1
FL-GS16	GS	9.88	12.5
SW-GS2	GS	7.18	7.49
MW-GSF5	GS	8.99	11.3
MW-GSF6	GS	9.7	13.6
MW-GSF7	GS	8.89	13.2
MW-GSF8	GS	9.81	12.4
MW-GSF9	GS	9.87	12.5
HW-GS1	GS	10.27	8.59
PS2-GS1	GS	5.34	5.34
PS2-GS2	GS	10.14	11.9
PS2-GS3	GS	11.83	13.6
GV1GS1	GS	3.26	3.79
GV1GS2	GS	3.95	4.22
GV1GS3	GS	4.33	4.08
GV2GS1	GS	3.65	4.23
GV2GS2	GS	6.22	5.74
GV2GS3	GS	3.46	4.41
GV3-GS1	GS	4.38	3.52
GV3-GS2	GS	5.59	5.45
GV3-GS6	GS	4.87	4.95
GV3-GS9	GS	4.44	4.32
BSW-GS1	GS	7.96	10.1
ACO-GS2	GS	7.87	11.9
MWB-GS1	GS	6.36	11.1
MWB-GS2	GS	8.67	11.8
MWB-GS3	GS	8.34	11
GS-FL-1	GS	6.08	7.08
GS-FL-2	GS	5.62	6.65
GS-FL-3	GS	18.1	26.4
FL-GS-5	GS	9.4	9.62
GS-FL-6	GS	12.2	16.7
SW-GS1	GS	5.29	8.12
SW-GS3	GS	7.3	10.6
MW-GSF1	GS	9.28	14.2
MW-GSF2	GS	6.82	11.3
MW-GSF4	GS	7.5	12.8
SC-FMS1	FMS	3.1	4.09
SC-FMS2	FMS	2.63	3.79
FL-FMS1	FMS	4.48	7.28
FL-FMS2	FMS	3.5	5.23
FL-FMS3	FMS	2.95	3.56

FL-FMS4	FMS	4.42	6.15
FL-FMS5	FMS	3.12	4.63
32-FMS1	FMS	2.19	4.23
32-FMS5	FMS	1.95	4.28
UCS-FMS1	FMS	2.76	3.57
RP-FM3	FMS	4.22	5.72
RP-FM4	FMS	4.55	5.6
FL-BHS3	BHS	1.97	2.3
FL-BHS4	BHS	1.3	1.47
FL-BHS5	BHS	2.42	3.07
PW3-BHS1	BHS	5.62	8.57
FL-BHS11	BHS	3.91	5.16
FL-BHS1	BHS	2.11	2.52
FL-BHS2	BHS	2.18	2.72
RP-BH1	BHS	2.11	2.76
RP-BH2	BHS	2.43	3.04
UCS-BHS1	BHS	2.13	3.64
PW3-CP1	CCP	11.7	20
PW3-CP2	CCP	4.78	8.24
PW3-CP3	CCP	4.1	6.56
PW3-CP4	CCP	6.29	7.81
CL-CP1	CCP	23.12	24.2
RP-CC1	CCP	6.32	6.1
32-C-1	CCP	4.01	5.06
FL-CP-1	CCP	5.91	10.2
FL-CP-2	CCP	9.02	11.5
FL-BT2	BT	5.02	4.01
RFL-BT1	BT	4.55	3.17
FL-BT1	BT	5.52	6.27
FL-BT11	BT	4.3	3.64
CR-RTC5	RTC	6.44	6.22
CR-RTC6	RTC	6.83	6.87
CR-RTC7	RTC	8.4	9.84
CR-RTC9	RTC	4.08	4.34
CRRTC10	RTC	5.27	5
FL-RTC1	RTC	6.55	7.29
JARVRTC	RTC	5.51	6.96
RP-RT1	RTC	7.23	7.42
UCS-RTC3	RTC	3.84	5.74
UCS-RTC4	RTC	3.63	5.56
CR-BG-1		8.78	12.9
FL-BH3	BBH	7.31	7.49
FL-BH4	BBH	4.83	3.94
FL-BH1	BBH	5.47	4.26
FL-BH2	BBH	7.64	7.42
FL-BH8	BBH	8.59	7.82
FL-BH9	BBH	9.61	5.7
RFL-BH1	BBH	6.61	7.77
RFL-BH2	BBH	2.03	9.22
HW-BH1	BBH	4.92	4.67

HW-BH2	BBH	5.3	3.35
LCBH-1	BBH	2.9	2.04
BH-FL2	BBH	3.94	4.63
BH-FL-3	BBH	4.57	3.91
2PW-BH1	BBH	4.99	4.36
FL-CCF1	CCF	3.97	5.31
PW3-CC1	CCF	3.34	3.58
FL-CCF2	CCF	3.41	3.41
RFL-CF1	CCF	2.63	3.67
UCS-CC1	CCF	2.04	3.95
?	CCF	1.88	1.95
CRCF-1	CCF	3.35	3.7
32	CCF	2.35	1.49
CL-LMB1		7.03	8.45
CL-SMB1		5.42	6.93
CL-SMB2		4.19	3.67
GVICSMB3		5.07	5.48
GVICSMB5		4.9	7.7
CRRSMB1		5.51	6.45
CRRSMB7		7.82	11

Linear least squares regression performed on natural-log-transformed data using Microsoft Excel:

SUMMARY OUTPUT
muscle to WB

Regression Statistics	
Multiple R	0.908382
R Square	0.8251578
Adjusted R Square	0.8242229
Standard Error	0.2307081
Observations	189

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	46.97407909	46.97408	882.5361	9.86504E-73
Residual	187	9.953306565	0.053226		
Total	188	56.92738565			

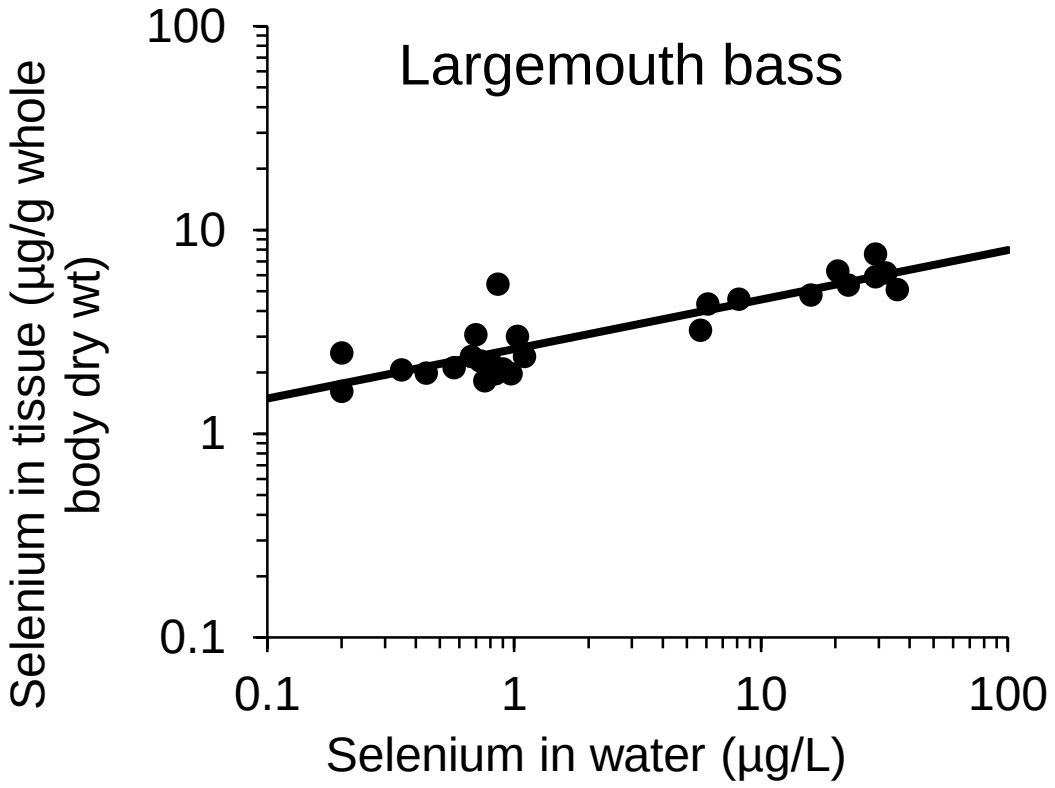
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.1003801	0.055508758	1.808365	0.072156	-0.009123739	0.209884
X Variable 1	0.8326865	0.028029496	29.70751	9.87E-73	0.77739187	0.887981

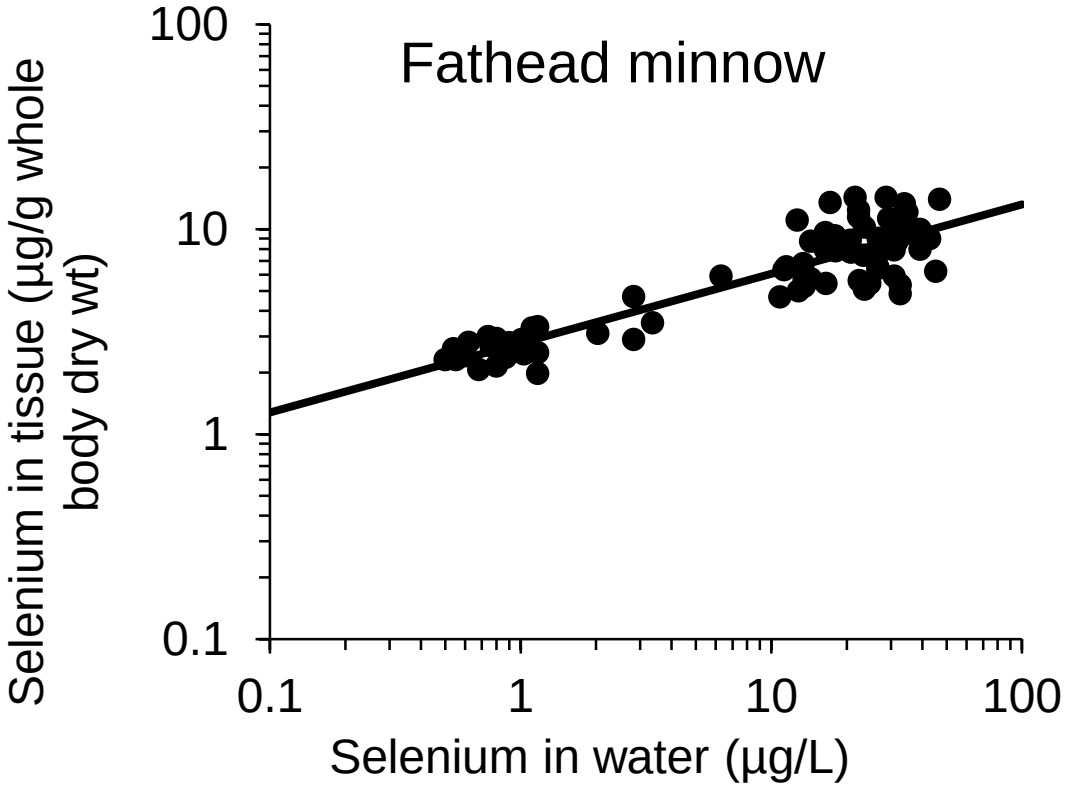
Figure 13

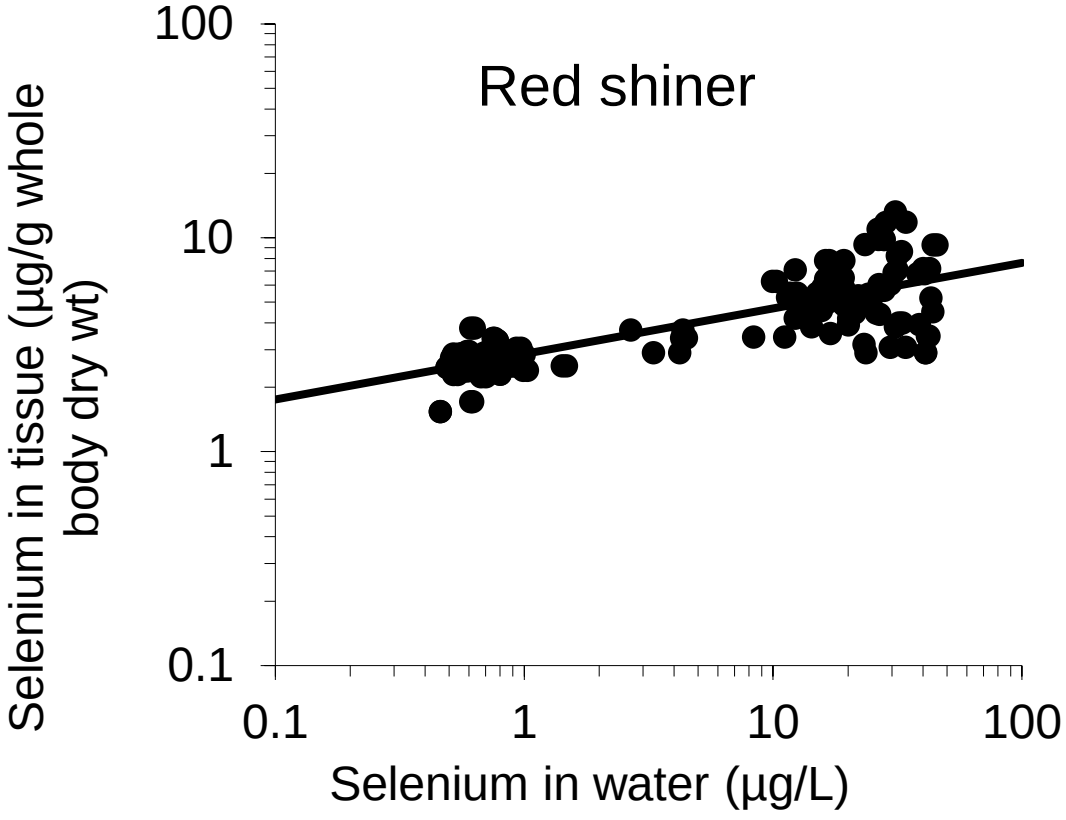
Selenium in muscle and whole body in white sucker caught in the Gunnison and Colorado Rivers and associated tributaries. Data source: Osmundson & Skorupa (2011). The white sucker data are the same as in Figure 12, but here separated by gender and reproductive condition of females. For comparison, the white sturgeon data and regression from Figure 5 are also shown (orange color).

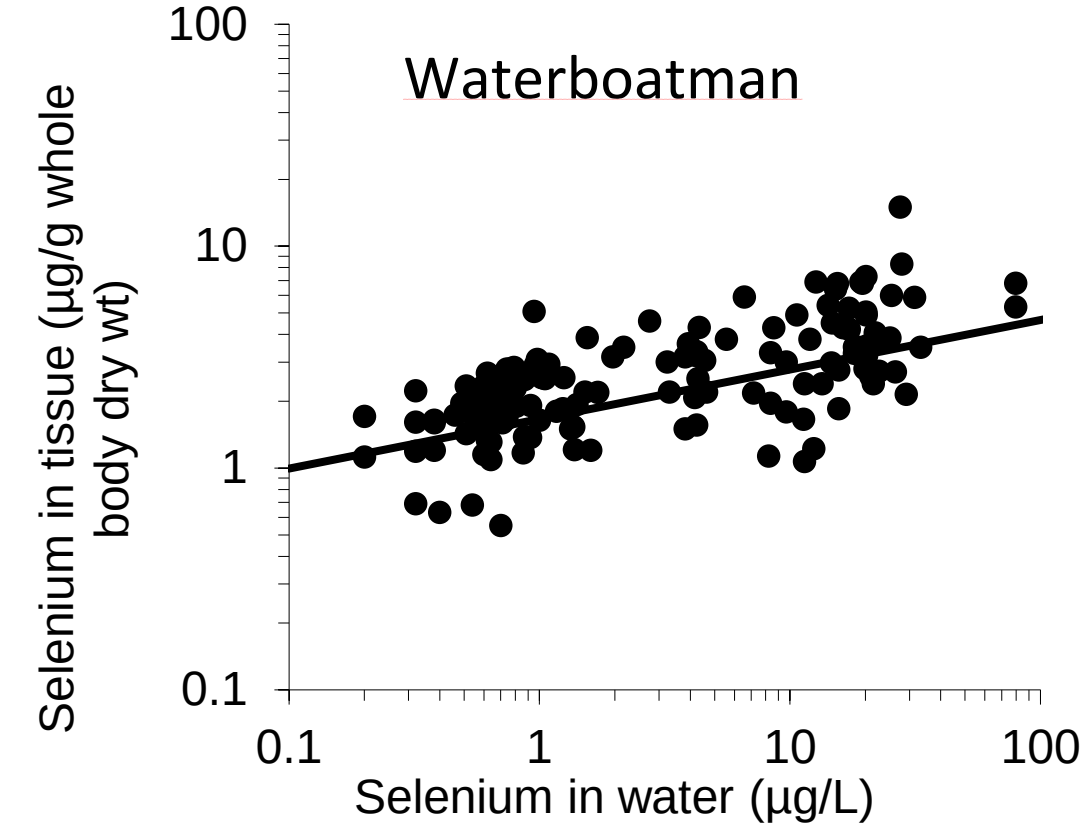
Appendix II

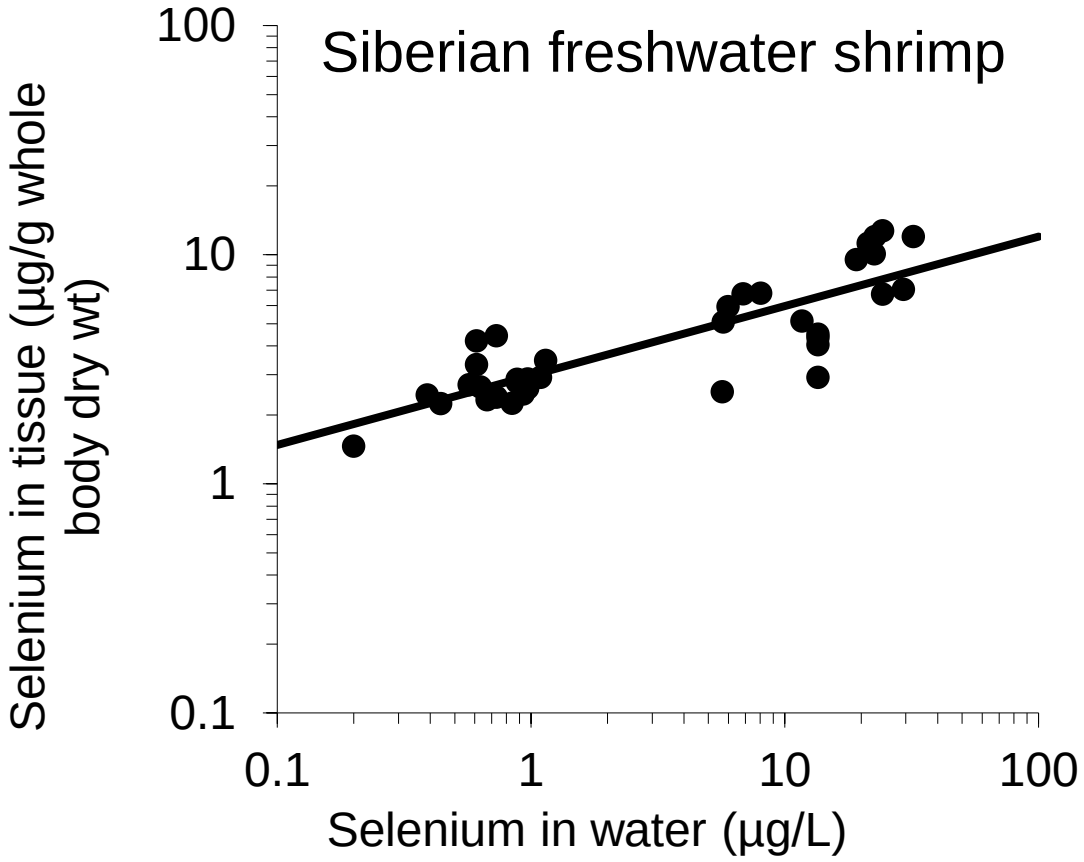
It has been suggested that homeostatic regulation of selenium in food chains can be discerned in bioaccumulation across several steps of trophic transfer (Brix *et al.* 2005), requiring that at least one trophic transfer exhibit a "hockey stick" function. If such homeostatic regulation occurs, it would invalidate the exponential model (straight line on a log-log graph) used in Figures 6 and 10. Below are five examples of the best species-specific and location-specific bioaccumulation relationships currently available. All these examples show field data from Beckon *et al.* (2010), incorporating estimates of lag time; all are well characterized by exponential models, and exhibit no evidence of homeostatic regulation at any link in the food chain. These results are consistent with the model used in Figures 6 and 10.



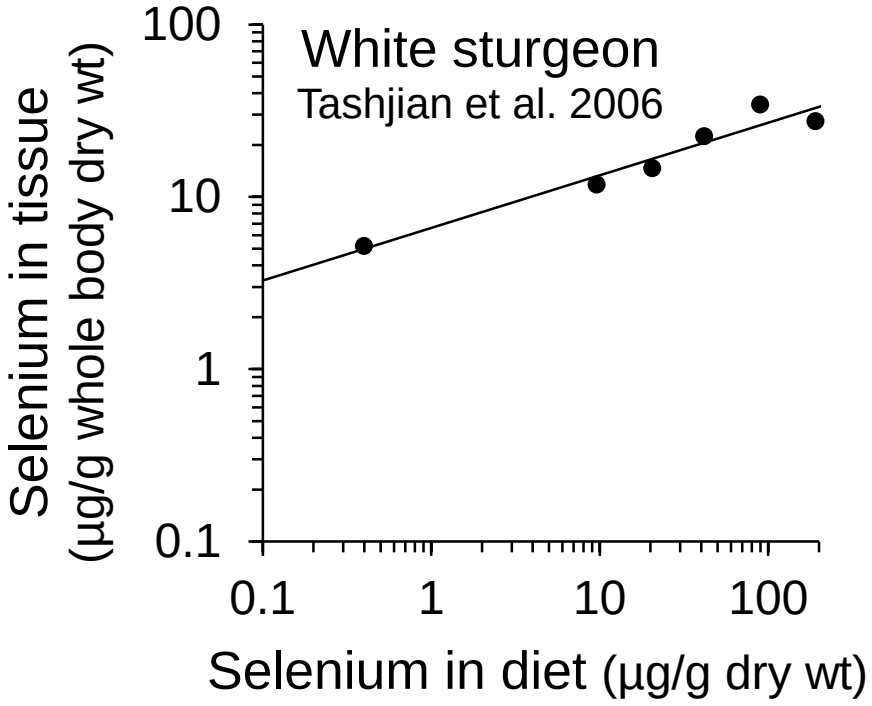

Fathead minnow
Selenium in tissue (µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in water (µg/L)

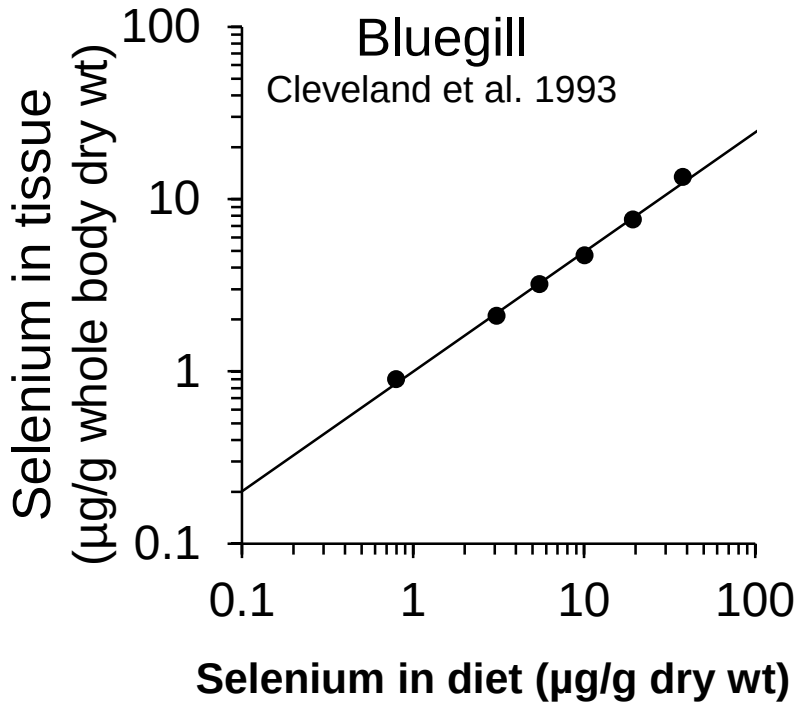

Red shiner
Selenium in tissue (µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in water (µg/L)

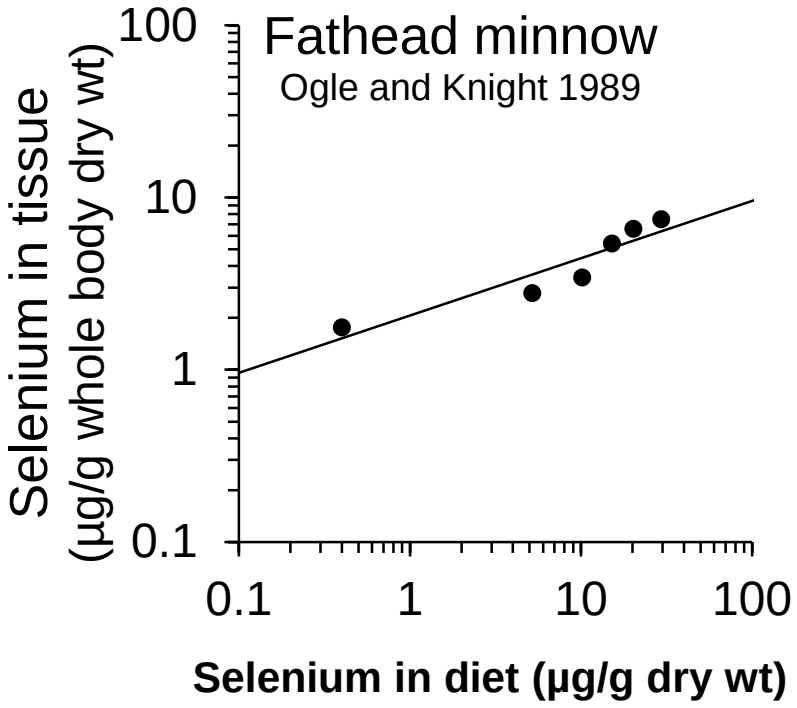

Waterboatman
Selenium in tissue (µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in water (µg/L)

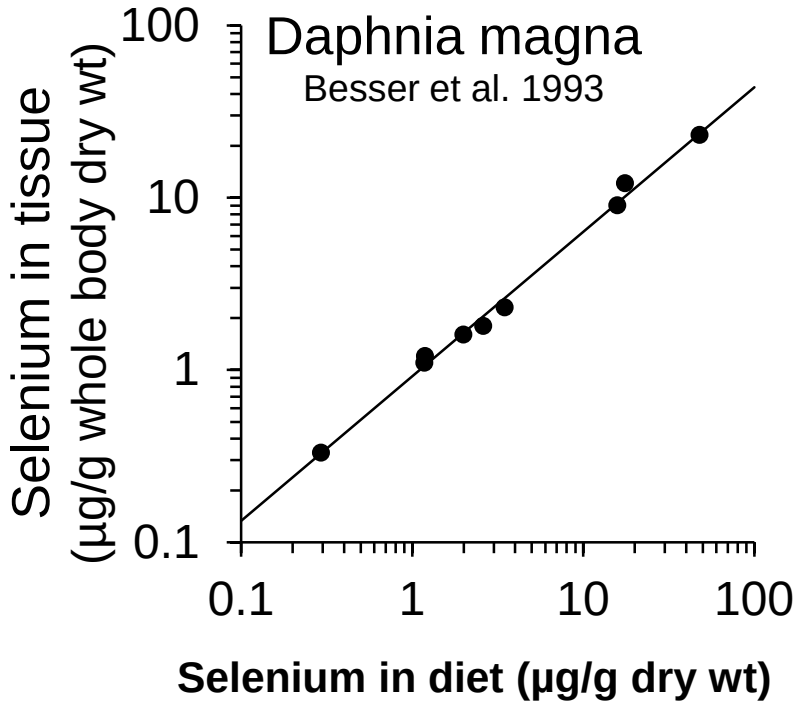

Siberian freshwater shrimp
Selenium in tissue (µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in water (µg/L)

The following four graphs show the best available data from laboratory studies of trophic transfer of selenium across a single link in the food chain. All support the conclusion that trophic transfer of selenium does not involve homeostatic regulation but is well represented by exponential functions (straight lines on log-log graphs), consistent with the model used in Figures 6 and 10.

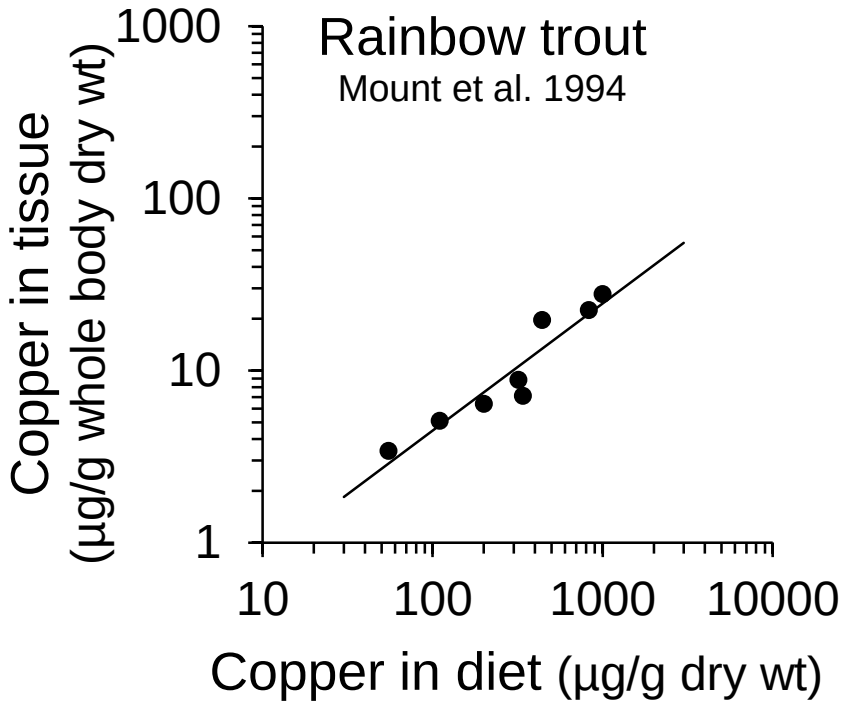


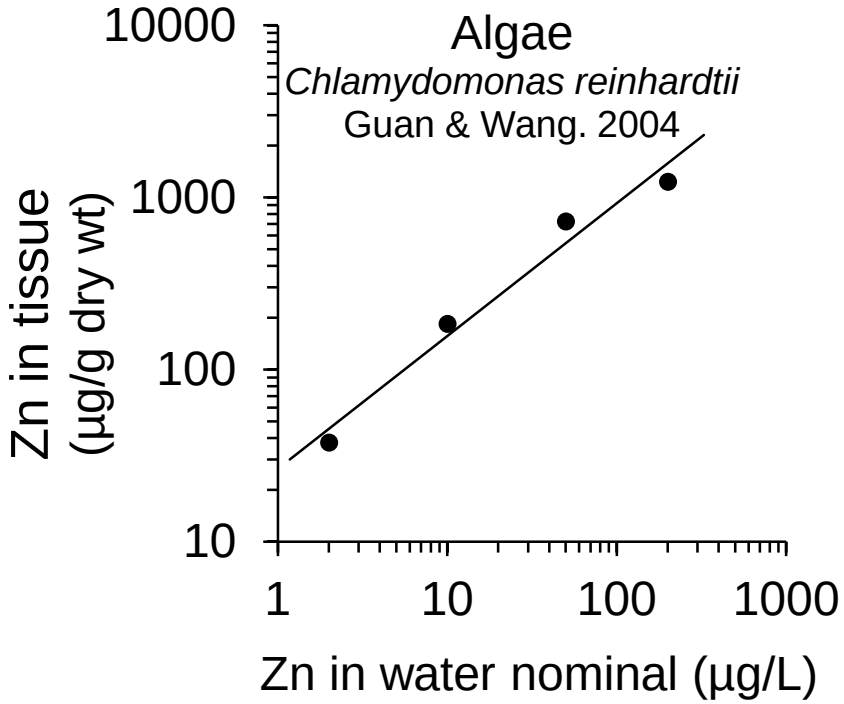


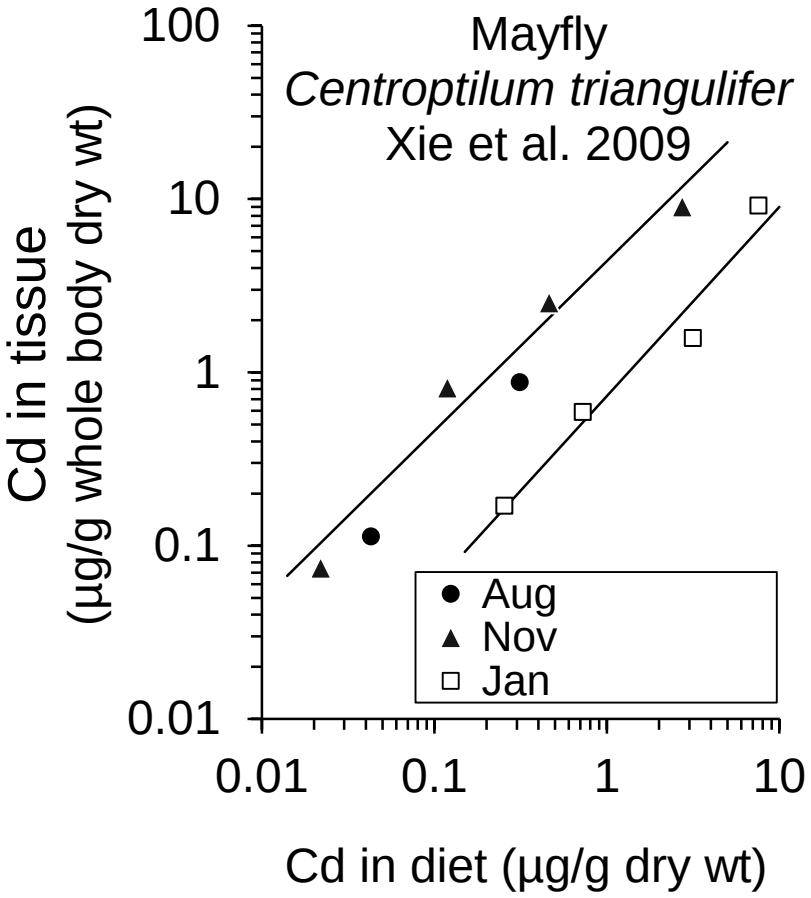

Fathead minnow
Ogle and Knight 1989
Selenium in tissue
(µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in diet (µg/g dry wt)

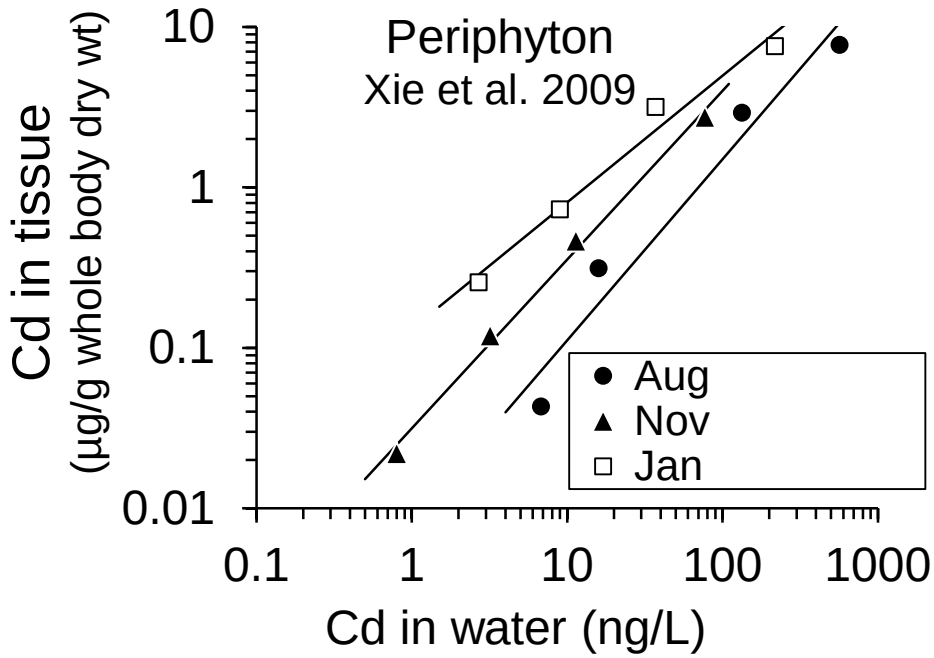

Daphnia magna
Besser et al. 1993
Selenium in tissue
(µg/g whole body dry wt)
100
10
1
0.1
0.1
1
10
100
Selenium in diet (µg/g dry wt)

The following four graphs show the best available data on trophic transfer functions for metals, both essential (copper and zinc) and nonessential (cadmium). These data suggest that the above conclusion for selenium is generalizable to trophic transfer of metals: there is no evidence of homeostatic regulation of these elements anywhere in the food chain; trophic transfer functions are well represented by exponential models.






Mayfly
Centroptilum triangulifer
Xie et al. 2009
Cd in tissue
(µg/g whole body dry wt)
100
10
1
0.1
0.01
0.01
0.1
1
10
Aug
Nov
Jan
Cd in diet (µg/g dry wt)


Periphyton
Xie et al. 2009
Cd in tissue
(µg/g whole body dry wt)
10
1
0.1
0.01
0.1
1
10
100
1000
Aug
Nov
Jan
Cd in water (ng/L)

References (Appendix II only)

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Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environ Toxicol Chem* 12:57–72.

Brix KV, Toll JE, Tear LM,. DeForest DK, Adams WJ. 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.

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