GRASSLAND BYPASS PROJECT

7 Biological Effects

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Abstract

In the fifth year of operation of the Grassland Bypass Project (GBP), contaminant concentrations in whole-body fish and invertebrates collected at sampling sites in Mud Slough below the outfall of the San Luis Drain (SLD) exceeded thresholds of Concern and Toxicity. The overall hazard of selenium to the ecosystem (Lemly's index) continued to be high in this reach of Mud Slough.

In Salt Slough, where drainwater has been removed by the GBP, average selenium concentrations in fish and invertebrates have remained at No-Effect levels since the latter half of 1998, with the exception of a single logperch (5 mg/kg) collected in June 2001. Lemly's index of selenium hazard to the Salt Slough aquatic ecosystem rose from "low" in WY 2000 to "moderate" in WY 2001 due to a small increase in the maximum concentration of selenium measured in Salt Slough water.

In the San Joaquin River both upstream (Site G) and downstream (Site H) of Mud Slough discharge, selenium concentrations in whole-body fish remain below the Concern threshold of 4 mg/kg (dry weight). Selenium concentrations in all invertebrates collected from the San Joaquin River site upstream of the Mud Slough discharge (Site G) during the fifth year of GBP operation remained below the 3 mg/kg (dry weight) threshold of Concern for invertebrates as prey items. However, the average concentration of selenium in invertebrate samples collected downstream of the Mud Slough discharge (Site H) reached the threshold of Concern for the first time since monitoring began in 1993. The concentrations of selenium in fish muscle tissue collected at both sites remained below the 2 mg/kg (wet weight) limited consumption guideline.

The selenium concentrations in all bird eggs collected in the Salt Slough area were within the No-Effect range. The selenium concentration in a black phoebe egg collected from a nest under a bridge across the SLD exceeded the level of Concern. The Toxicity threshold was exceeded by two killdeer eggs collected along the SLD adjacent to the filled Kesterson Reservoir.

Selenium concentrations in seeds collected at all sites in WY 2001 were below the 3 mg/kg (dry weight) threshold of Concern as diet for birds, with the exception of a sample of swamp timothy seed heads collected at Site D, just below the SLD outfall.

Boron concentrations in seed samples from the banks of Salt Slough were below the threshold of Concern. The boron concentration in samples collected from Mud Slough sites below the SLD outfall were all above the 30 mg/kg (dry weight) threshold of Concern. Two of three seed samples collected along Mud Slough above the outfall (Site C) were above the threshold of Concern.

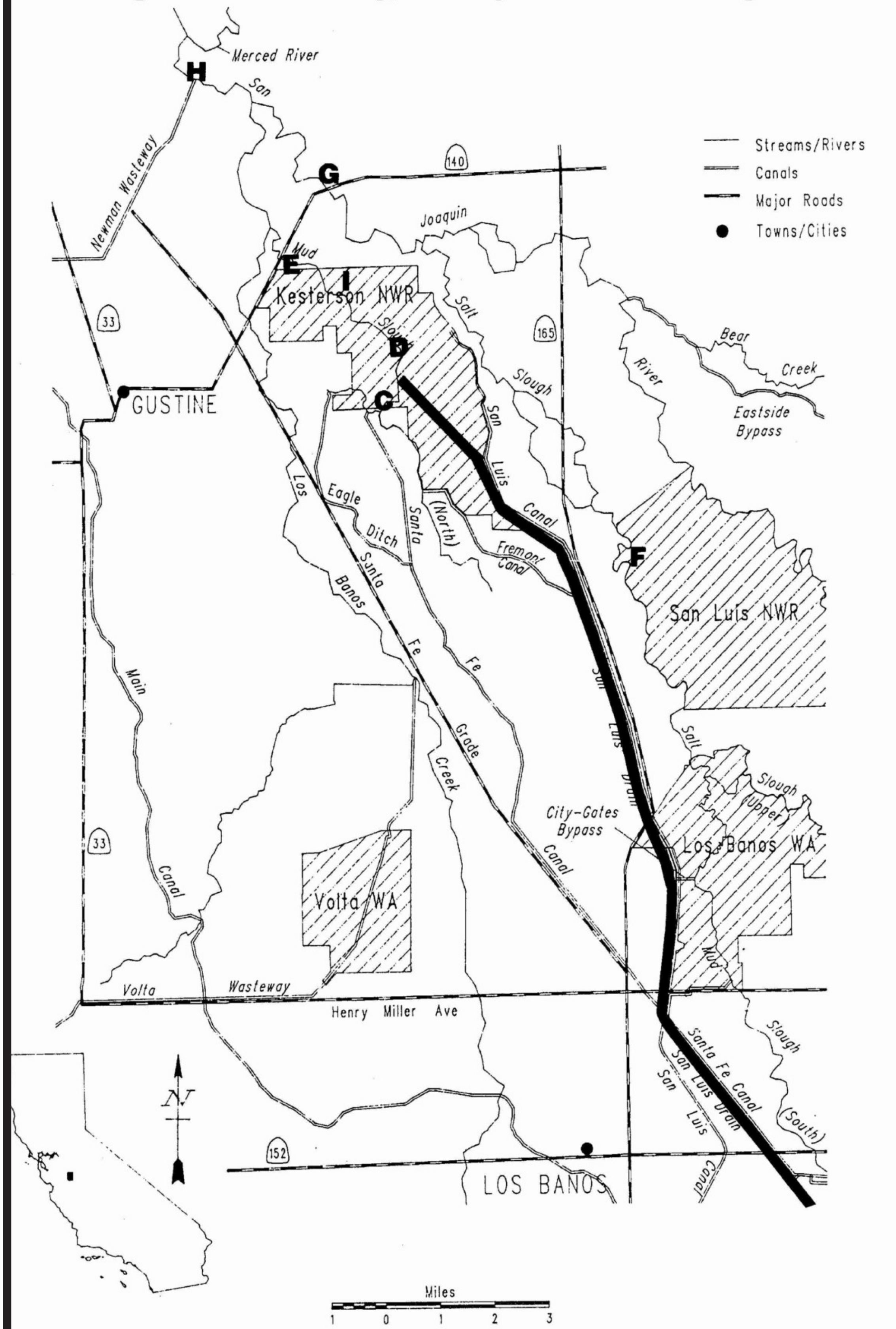
Introduction

Project History

In 1985 the SLD was closed due to deaths and developmental abnormalities of waterbirds at a reservoir in the Kesterson National Wildlife Refuge at the terminus of the SLD. The SLD, constructed by the U.S. Bureau of Reclamation (USBR), had been conceived as a means to dispose of agricultural drainwater generated from irrigation with water supplied by the federal Central Valley Water Project. However, due to environmental concerns and budget constraints, the SLD had never been completed as originally planned. The constructed portion of the SLD had been used only to convey subsurface agricultural drainwater from the Westlands Water District in the western San Joaquin Valley. Farms in the adjacent Grassland Drainage Area (GDA) never used the SLD, but discharged subsurface drainwater through wetland channels in the Grassland Water District, San Luis National Wildlife Refuge Complex, and the China Island Unit of the North Grasslands Wildlife Area (Refuges) to the San Joaquin River. This drainwater contains elevated concentrations of selenium, boron, chromium, and molybdenum, and high concentrations of various salts (CEPA, 2000) that disrupt the normal ionic balance of affected aquatic ecosystems (SJVDP, 1990b). In addition, unknown concentrations of agricultural chemical residues (fertilizers and pesticides that do not readily adsorb to soil) may contaminate this drainwater.

Discharge from GDA farms continued to contaminate Refuge water delivery channels after closure of the SLD and Kesterson Reservoir in 1986. To address this problem, a proposal to use a portion of the SLD and extend it to Mud Slough, a natural waterway in the Refuges, was implemented by the USBR in September 1996 with support from other federal and state agencies (USBR, 1995; USBR and SL&D-MWA 1995; USBR et al., 1995). This project, known as the Grassland Bypass Project (GBP), diverts agricultural drainwater from the GDA into the lower 28 miles of the SLD and thence into the lower portion of Mud Slough (about six miles). The GBP has removed drainwater from more than 90 miles of wetland water supply channels, including Salt Slough, and allows the Refuges full use of water to create and restore wetlands on the Refuges. The GBP, as currently implemented, continues to adversely affect the northernmost six

Figure 1 Grassland Bypass Project biota monitoring sites



miles of Mud Slough and the reach of the San Joaquin River between Mud Slough and the Merced River. However, as phased-in load reduction goals are achieved by GDA farmers, these adverse effects are expected to be reduced. An essential component of the GBP is a monitoring program that tracks contaminant levels and effects in water, sediment, and biota to ensure that the overall effect of the GBP is not a net deterioration of the ecosystems in the area.

Contaminants of Concern

In the aftermath of the deaths and developmental abnormalities of birds at Kesterson Reservoir in the early 1980s, studies definitively traced the cause to selenium in the agricultural subsurface drainwater in the reservoir (Suter, 1993). Because of this, and because of the well-known history of death, teratogenesis, and reproductive impairment caused by selenium in agricultural drainwater elsewhere (reviewed in Skorupa, 1998), the primary contaminant of concern in this monitoring program is selenium. Other inorganic constituents of potential toxicological interest in drainage water include boron, molybdenum, arsenic and chromium (Klasing and Pilch, 1988; SJVDP, 1990a; CVRWQCB, 1998).

Selenium Ecological Risk Guidelines

The assessment of the risks that selenium poses to fish and wildlife can be difficult due to the complex nature of selenium cycling in aquatic ecosystems (Lemly and Smith, 1987). Early assessments developed avian risk thresholds through evaluating bird egg concentrations and relating those to levels of teratogenesis (developmental abnormalities) and reproductive impairment (Skorupa and Ohlendorf, 1991). In 1993, to evaluate the risks of the Grassland Bypass Project on biotic resources in Mud and Salt Sloughs, a set of Ecological Risk Guidelines based on selenium in water, sediment, and residues in several biotic tissues were developed by a subcommittee of the San Luis Drain Re-Use Technical Advisory Committee (CAST, 1994; Engberg, et.al., 1998). These guidelines (as recently modified: Table 1) are based on a large number of laboratory and field studies, most of which are summarized in Skorupa et al. (1996) and Lemly (1993). In areas where the potential for selenium exposure to fish and wildlife resources exists, these selenium risk guidelines can be used to trigger appropriate actions by resource managers, regulatory agencies, and dischargers. For the GBP the

Table 1. Recommended Ecological Risk Guidelines for Selenium Concentrations.

Medium	Effects on	Units	No Effect	Concern	Toxicity
Warmwater Fish (whole body)	fish growth/condition/survival	mg/kg (dry weight)	< 4	4-9	> 9
Vegetation (as diet)	bird reproduction	mg/kg (dry weight)	< 3	3-7	> 7
Invertebrates (as diet)	bird reproduction	mg/kg (dry weight)	< 3	3-7	> 7
Sediment	fish and bird reproduction	mg/kg (dry weight)	< 2	2-4	> 4
Water (total recoverable Se)	fish and bird reproduction (via foodchain)	µg/L	< 2	2-5	> 5
Avian egg	egg hatchability	mg/kg (dry weight)	< 6	6-10	> 10

Notes
- These guidelines, except those for avian eggs, are intended to be population based. Thus, trends in means over time should be evaluated. Guidelines for avian eggs are based on individual level response thresholds (e.g., Heinz, 1996; Skorupa, 1998)
- A tiered approach is suggested with whole body fish being the most meaningful in assessment of ecological risk in a flowing system.
- The warmwater fish (whole body) Concern threshold is based on adverse effects on the survival of juvenile bluegill sunfish experimentally fed selenium enriched diets for 90 days (Cleveland et al., 1993). It is the geometric mean of the "no observable effect level" and the "lowest observable effect level."
- The Toxicity threshold for warmwater fish (whole body) is the concentration at which 10% of juvenile fish are killed (DeForest et al., 1999).
- The guidelines for vegetation and invertebrates are based on dietary effects on reproduction in chickens, quail and ducks (Wilber, 1980; Martin, 1988; Heinz, 1996).
- If invertebrate selenium concentrations exceed 6 mg/kg then avian eggs should be monitored (Heinz et al., 1989; Stanley et al., 1996).

selenium risk guidelines have been divided into three levels: No Effect, Concern, and Toxicity.

In the No Effect range risks to sensitive species are not likely. As new information becomes available it should be evaluated to determine if the No Effect level should be adjusted. Since the potential for selenium exposure exists, periodic monitoring of water and biota is appropriate.

Within the Concern range there may be risk to sensitive species, and contaminant concentrations in water, sediment, and biota should be monitored on a regular basis. Immediate actions to prevent selenium concentrations from increasing should be evaluated and implemented if appropriate. Long-term actions to reduce selenium risks should be developed and implemented. Research on effects on sensitive or listed species may be appropriate.

Within the Toxicity range, adverse affects are more likely across a broader range of species, and sensitive or listed species would be at greater risk. These conditions will warrant immediate action to reduce selenium exposure through disruption of pathways, reduction of selenium loads, or other appropriate actions. More detailed monitoring, studies on site-specific effects, and studies of pathways of selenium contamination may be appropriate and necessary. Long-term actions to reduce selenium risks should be developed and implemented.

The guidelines (except those for avian eggs) are intended to be population based. Therefore they should be used for evaluating population means rather than contaminant concentrations in individuals.

Warmwater Fish

The warmwater fish guidelines (Table 1) refer to concentrations of selenium in warmwater fish that adversely affect the fish themselves. The original 1993 fish guidelines have been replaced by explicitly "warmwater fish" guidelines in recognition of the evidence from the literature that coldwater fish (salmon and trout) are more sensitive to selenium than warmwater fish and that GBP monitoring data available is limited to warmwater fish. Although a coldwater fish guideline is not proposed here, a discussion of selenium effects on coldwater fish is provided in this section since the best information currently available happens to be very site-specific to the GBP area (Merced River and downstream San Joaquin River).

The Concern threshold for warmwater fish has been kept at about 4 mg/kg (all fish data are whole body, dry weight). Experimental data reported in the literature may be interpreted to support a range of thresholds around this value. In particular, bluegill sunfish dietary and waterborne toxicity data in Cleveland et al. (1993) can be used to support warmwater fish Concern thresholds of 3.3 mg/kg, 3.4 mg/kg, 3.9 mg/kg, or 5.9 mg/kg. Bluegill sunfish are warmwater fish that are found in the sloughs in the GBP area, and the Cleveland et al. (1993) study yielded the best available data on warmwater fish toxicity applicable to GBP.

Cleveland et al. (1993) found no adverse effects after 59 days of exposure to concentrations of dietary selenium that resulted in a bluegill tissue concentration of 2.7 mg/kg no observable effect concentration (NOEC). Fifty nine days of exposure to dietary concentrations that resulted in tissue concentrations of 4.2 mg/kg lowest observable effect concentration (LOEC) caused a significant increase in mortality relative to controls. Following the USEPA method (Stephan et al., 1985) employed by DeForest et al. (1999), the tissue threshold is calculated as the geometric mean of the NOEC and the LOEC. Application of the USEPA procedure to these data yields a Toxicity threshold of 3.4 mg/kg. A similar analysis of a water-borne selenium exposure experiment (Cleveland et al., 1993) yields a threshold value of 3.3 mg/kg.

Other data in Cleveland et al. (1993) may be interpreted to support a threshold closer to 4 mg/kg or a threshold of 5.9 mg/kg. The experiments of Cleveland et al. (1993) suggest that selenium concentrations in fish tissues do not reach equilibrium until at least 90 days of dietary exposure (Figure 3 in Cleveland et al., 1993). This appears consistent with the finding, summarized below, that in the field, selenium concentrations in fish are best predicted by water concentrations averaged over the entire period of one to seven months prior to the date the fish is sampled. In deriving a tissue threshold, there then appears to be some support for using the relationship between dietary concentration and tissue concentration at 90 days rather than 59 days. After 90 days of dietary exposure bluegill with a tissue selenium concentration of 3.3 mg/kg did not exhibit adverse effects that were significantly greater than controls, but bluegill with a tissue concentration of 4.6 mg/kg experienced significantly increased mortality. Bluegill with a tissue concentration of 7.5 mg/kg had three times the mortality of controls, but that difference in mortality was not statistically significant at the 95% level of confidence (Table 4 and Figure 3 in Cleveland et al., 1993). However, the condition factor (a measure of weight relative to length) of the fish at 7.5 mg/kg, was significantly worse than controls. Depending on whether or not the significant mortality at a tissue concentration of 4.7 mg/kg is treated as anomalous, the LOEC would be either 4.7 mg/kg or 7.5 mg/kg. Corresponding thresholds would be 3.9 mg/kg (geometric mean of 3.3 mg/kg and 4.6 mg/kg) or 5.9 mg/kg (geometric mean of 4.6 mg/kg and 7.5 mg/kg) respectively. Given the range of possible threshold values discussed above, the Concern threshold of 4 mg/kg listed in Table 1 was not changed from the original 1993 threshold. However, considering that these data do not include adverse effects on reproduction which may be affected at lower concentrations, this threshold may not be fully protective of sensitive warmwater fish species.

The Toxicity threshold for warmwater fish (whole body) of 9 mg/kg is recommended by DeForest et al. (1999). In the analysis of DeForest et al. (1999) the threshold represents an EC_{10}, that is, the concentration at which 10 percent of fish are affected. DeForest et al. (1999) excluded some toxicity data from their analysis that could support a lower threshold (Cleveland et al., 1993). Also, reproductive impairment may occur at lower selenium concentrations, but too few data are available to do a similar analysis on this effect. Therefore, this Toxicity threshold may not be fully protective of sensitive warmwater fish species.

Coldwater Fish

Testing fall run chinook salmon from the Merced River, Hamilton et al. (1990) found that salmon fry growth was significantly reduced compared to controls after 30 and 60 days of being fed a diet (containing mosquitofish from the SLD) having a selenium concentration of 3.2 mg/kg dry weight. After 90 days of that diet, the selenium concentration in the salmon fry averaged 2.7 mg/kg whole body, dry weight. This fish tissue concentration was the lowest observable effect concentration (LOEC). The no observable effect concentration (NOEC) in salmon fry tissue was 0.8 mg/kg. Following the USEPA method (Stephan et al., 1985) employed by DeForest et al. (1999), the tissue threshold is calculated as the geometric mean of the NOEC and the LOEC. This procedure applied to the Hamilton et al. (1990) SLD data yields a threshold of 1.5 mg/kg (geometric mean of 0.8 and 2.7 mg/kg). It should be noted that this threshold may incorporate the interacting effects of other toxic constituents of drainwater that may have been assimilated by the SLD mosquitofish that were used as feed in the Hamilton, et al. (1990) experiments. Furthermore, at the time of these experiments (1985), the SLD held agricultural drainwater from the Westlands, an area adjacent to the Grasslands area. Therefore, although these are the most site-specific selenium toxicity data available, these data may not perfectly match the current risk of toxicity to coldwater fish in the San Joaquin River due to agricultural drainwater from the GBP. Although the sloughs affected by the GBP have coldwater beneficial uses designated by the Central Valley Regional Water Quality Control Board, the fish community principally consists of warmwater species. A temporary barrier is installed seasonally across the San Joaquin River to exclude chinook salmon (a coldwater species) from these sloughs and from the San Joaquin River upstream of its confluence with the Merced River. Additionally, any application of the coldwater fish risk guidelines should take into account the fact that many coldwater fish are anadromous, and therefore feed in the selenium-contaminated portion of the San Joaquin River for a limited period of time— a brief period in their juvenile stage as they migrate downstream to the ocean.

A Toxicity threshold for coldwater fish (whole body) of 9 mg/kg has been recommended by DeForest et al. (1999). In the analysis by DeForest et al. (1999) the Toxicity threshold represents an EC_{10}, that is, the concentration at which 10 percent of fish are affected. DeForest et al. (1999) excluded site-specific and longer term data (Hamilton et al., 1990) which could support lower thresholds. For example, to derive their Toxicity threshold for coldwater fish, DeForest et al. (1999) used only the 60 day growth data in Hamilton et al. (1999); they disregarded the 90 day mortality data in Hamilton et al. (1999) that would have yielded a Toxicity threshold (corresponding to 10% mortality) of 1.7 mg/kg. In addition, the DeForest et al. (1999) analysis focused on growth and mortality. Reproductive impairment may occur at lower selenium concentrations, but too few data are available to do a similar analysis on this effect. Therefore, this threshold may not fully protect sensitive coldwater fish species.

Vegetation and Invertebrates

The guidelines for vegetation (as diet) and invertebrates (as diet) refer to selenium concentrations in plants and invertebrates affecting birds that eat these items. These guidelines are mainly based on experiments in which seleniferous grain or artificial diets spiked with selenomethionine were fed to chickens, quail or ducks resulting in reproductive impairment (Wilber, 1980; Martin, 1988; Heinz, 1996). The Concern threshold for vegetation is 3 mg/kg (dry weight) and the Toxicity threshold is 7 mg/kg. The invertebrate Concern threshold and Toxicity threshold are the same as those for vegetation.

Water

Fish and wildlife are much more sensitive to selenium through dietary exposure from the aquatic food chain than by direct waterborne exposure. Therefore the guidelines for water reflect water concentrations associated with threshold levels of food chain exposure (Hermanutz et al., 1990; Maier and Knight, 1994), rather than concentrations of selenium in water that directly affect fish and wildlife. The Concern threshold is 2 μg/L and the Toxicity threshold is 5 μg/L.

Sediment

As with water, the principal risk of sediment to fish and wildlife is via the aquatic food chain. Therefore the sediment guidelines are based on sediment concentrations as predictors of adverse biological effects through the food chain (USFWS, 1990; Van Derveer and Canton, 1997). The Concern threshold for sediment (dry weight) is 2 mg/kg and the Toxicity threshold is 4 mg/kg.

Bird Eggs

Bird eggs are particularly good indicators of selenium contamination in local ecosystems (Heinz, 1996). However, the interpretation of selenium concentrations in bird eggs in the GBP area is complicated by the proximity of contaminated and uncontaminated sites and by the variation in foraging ranges among bird species. Relative to the guidelines originally used for the GBP, the guidelines used here for bird eggs have been revised upward based on recent studies of hatchability of ibis, mallard, and stilt eggs (Henny and Herron, 1989; Heinz, 1996; USDI-BOR/FWS/GS/BIA, 1998). The Concern threshold has been raised from 3 to 6 mg/kg dry weight, and the Toxicity threshold has been raised from 8 to 10 mg/kg dry weight.

Selenium Ecological Risk Index

Several years after the risk guidelines were developed for the GBP, Lemly (1995, 1996) published a risk index designed to provide an estimate of ecosystem-level effects of selenium. Lemly's assessment procedure sums the effects of selenium on various ecosystem components to yield a characterization of overall hazard to aquatic life. The procedure involves determining an index of toxicity for each component, then adding these indexes together to yield a single index, often known as the Lemly Index.

In contrast to the ecological risk guidelines outlined in Table 1, the component indexes of the Lemly Index are based on maximum contaminant concentrations rather than means. Therefore, the Lemly Index is sensitive to brief spikes in contaminant levels, but is unaffected by prevailing contaminant levels. Furthermore, the Lemly Index is strongly dependent on sampling periods and sampling frequency, yet Lemly provided no sampling protocol. For these reasons, there is a need to develop a new protocol and index that replaces Lemly's categorical rating format (low, medium, high) with a direct estimate of the probability of adverse effects (e.g.10%+ probability of reproductive impairment). Despite the weaknesses of the Lemly Index, we continue to use it for comparative purposes as long as it remains the best available overall index of the ecological risk of selenium.

Boron Ecological Risk Guidelines

The dietary and tissue concentrations of boron associated with toxic effects on fish and wildlife are not as well known as for selenium. The effects of dietary exposures and waterborne exposures (without dietary exposures) are known for some taxa (Table 2), but there are as yet no definitive data associating tissue concentrations with adverse effects in fish and invertebrates. Boron concentrations as low as 0.1 mg/l in water may adversely affect reproduction of sensitive fish species (review in NIWQP, 1998).

Methods

The role of the California Department of Fish and Game (CDFG) and the USFWS in this interagency program is to implement the bio-monitoring portion of the Compliance Monitoring Program. The methods used by the CDFG and USFWS are described in the Quality Assurance Project Plan for Use and Operation of the Grassland Bypass Project (QAPP; Entrix, Inc., 1997). These methods are also based on standard operating procedures described in Standard Operation Procedures for Environmental Contaminant Operations (USFWS, 1995) and standards used by the other agencies participating in the compliance monitoring program. Deviations from the QAPP that have occurred since 1996 will be discussed later in this section.

To obtain baseline data for this Project, the USFWS began sampling in March 1992, after the reuse of the SLD was initially proposed by the USBR in 1991. The

Table 2. Recommended Ecological Risk Guidelines for Boron Concentrations.

Medium	Effects on	Units	No Effect	Concern	Toxicity
Water	fish (catfish and trout embryos)	mg/L	< 5	5-25	> 25
Water	invertebrates (*Daphnia*)	mg/L	< 6	6-13	> 13
Water	vegetation (crops and aquatic plants)	mg/L	< 0.5	0.5-10	> 10
Waterfowl diet	duckling growth	mg/kg (dry weight)		> 30	
Waterfowl egg	embryo mortality	mg/kg (dry weight)	<1		>30

Notes
- Water guidelines for invertebrates are based on the "no observed adverse effects level" and "lowest observed adverse effects level" for Daphnia magna (Lewis and Valentine 1981; Gersich 1984).
- Waterfowl diet guidelines are based on mallard ducks (Smith and Anders 1989).
- The waterfowl egg no effect level is based on poultry data from Romanoff and Romanoff (1949) and San Joaquin Valley field data for reference sites (R. L. Hothem and D. Welsh; J. P. Skorupa et al.).
- The waterfowl egg Concern and Toxicity thresholds are based on Smith and Anders (1989), Stanley et al. (1996), and the "order-of-magnitude rule of thumb" (toxicity at about 10 times background concentrations).
- The US Environmental Protection Agency's suggested no adverse response level for drinking water is 0.6 mg/L.

CDFG began sampling in August of 1993. USFWS and CDFG sampling plans before the reopening of the SLD and the early drafts of the monitoring plan were mutually influencing. Therefore, methods used by both agencies before the final approval of the QAPP are, except for a few minor differences, identical to the methods ultimately approved by the Data Collection and Reporting Team. The sampling schedule, though, as discussed below, now follows a regular timetable.

Matrices Sampled

Samples of the biota were collected at each site and analyzed for selenium and boron. Aquatic specimens were collected with hand nets, seine nets and by electrofishing. Mosquitofish (*Gambusia affinis*), inland silversides (*Menidia beryllina*), red shiners (*Cyprinella lutrensis*), fathead minnows (*Pimephales promelas*), carp (*Cyprinus carpio*), white catfish (*Ameiurus catus*), and green sunfish (*Lepomis cyanellus*) were the principal species of fish collected. Waterboatmen (family: Corixidae), backswimmers (family: Notonectidae), and red crayfish (*Procambarus clarkii*) were the principal invertebrates collected. Separation of biological samples from unwanted material also collected in the nets was accomplished by using stainless steel or Teflon sieves, and glass (or enamel) pans pre-rinsed with de-ionized water then native water. To the extent possible, three replicate, composite samples (minimum 5 individuals totaling at least 2 grams for each composite) of each primary species listed above were collected, but other species were also collected. Fish species were analyzed as composite whole-body samples except as noted below. Estimates of a conversion factor for relating selenium concentration in skeletal muscle (M) to whole-body concentrations (WB) range from M=0.6 x WB for many freshwater fish (Lemly and Smith, 1987) to M=0.045+1.23 x WB for bluegills and M=-0.39+1.32 x WB for largemouth bass (Saiki et al., 1991).

Between 1992 and 1999, frog tadpoles occasionally collected from Mud Slough and Salt Slough sites were archived. In 1999 these archived samples were analyzed. Additional samples were collected and analyzed from these sites in 2000 and 2001.

Analyses of fish samples collected from the San Joaquin River sites and Mud Slough (Sites E, G, and H) were prioritized to first meet the objectives of the Compliance Monitoring Plan (Section 4.5.1.4). Supplemental fish samples were analyzed only when baseline biota target species and sample sizes could not be obtained.

In WYs 1999, 2000, and 2001 several samples of fish and invertebrates submitted for analysis were of insufficient mass to permit individual measurement of the water content (percent moisture) of the sample, a measurement used to calculate the dry weight selenium concentration in the sample. For these samples (designated with asterisk on the graphs), an average percent moisture was calculated from the percent moisture measurements of comparable samples in the closest possible conditions of sampling location, time, species, and size of organism. This average percent moisture was used to calculate the dry weight selenium concentration. Selenium concentrations discussed in text and displayed in figures below are averages of composite sample concentrations except for bird eggs and except where otherwise stated.

The seed heads of wetland plants that provide food for waterfowl were collected along the sloughs in the late summer of the years 1995-2001. Much of this plant material was archived until analyzed in the years 2000 and 2001.

Waterfowl and/or shorebird eggs, depending on availability, were collected from areas adjacent to Mud Slough and the SLD in the spring of each year from 1996 through 2001. In addition, in 1992 snowy egret and black-crowned night heron eggs were collected at East Big Lake, which has served as a reference sampling site for the USFWS. Bird eggs were analyzed individually, and the results are discussed and displayed below as individual concentrations and geometric means.

Graphs of whole-body and avian egg selenium concentrations presented in this report include indications of the threshold concentrations delimiting the risk ranges listed above (Table 1). The threshold between the No Effect zone and the Concern zone is indicated by a horizontal line of short dashes; the Toxicity threshold is marked on each graph by a horizontal line of long dashes.

All biota samples were kept on ice or on dry ice while in the field then kept frozen to 0° C during storage and shipment. For all samples, after freeze drying, homogenization, and nitric-perchloric digestion, total selenium was determined by hydride generation atomic absorption spectrophotometry and boron was determined by inductively coupled (argon) plasma spectroscopy.

Sampling Sites

Between 1992 and 1999 biological samples have been collected from two sites on Salt Slough, five sites on Mud Slough, two sites in the SLD, two sites on the San Joaquin River, and one reference site that does not receive selenium-contaminated drainwater (East Big Lake). Beginning in 1995, sampling efforts were concentrated on the seven sites (Figure 1) identified in the Compliance Monitoring Plan: four sites on Mud Slough (C, D, E, and I), one on Salt Slough (F) and two San Joaquin River sites (G and H). Site C is located upstream of where the Grassland Bypass discharges into Mud Slough. Site D is located immediately downstream of the discharge point. Site I is a small, seasonally flooded backwater area fed by Mud Slough and is located approximately 1 mile downstream from Site D. Site E is located further downstream where Mud Slough crosses State Highway 140. To assess the mitigative effects of drainwater removal from Salt Slough, one sample point, Site F, is located on the San Luis National Wildlife Refuge approximately 2 miles upstream of where State Highway 165 crosses Salt Slough. Site G is located on the San Joaquin River at Fremont Ford, upstream of the Mud Slough confluence, while Site H is located on the San Joaquin River 200 meters upstream of the confluence of the main branch of the Merced River, downstream of the Mud Slough confluence. Sites C, D, F, and I are monitored by the USFWS while CDFG monitored Sites E, G, and H.

During the WY 2001, biological sampling in Mud Slough was moved from Site I to a new site (Site I2) about 0.5 km upstream of Site I. The new site has a larger, more permanent backwater area.

Sampling Times

Baseline sampling conducted by the USFWS occurred monthly during the spring and summer of 1992 and then less frequently during 1993 and 1994. Baseline sampling by CDFG occurred during the summer and fall of 1993 and then resumed in the spring of 1996. Between 1992 and 1995 sampling by either the CDFG and the USFWS occurred at least once every season. Experience and interagency discussions led to the identification of four sampling times based on historic water use and drainage practices and on seasonal use of wetland resources by fish and wildlife. Biota sampling since 1995 has been synchronized to occur during the months of November, March, June, and August. Since 1996, avian eggs have been collected in May and June.

Statistical Analysis

Student's 2-tail t-tests were used to compare means of concentrations for groups of samples collected at different times at the sampling sites (unpaired samples with unequal variances).

Selenium Hazard Assessment

The protocol proposed by Lemly (1995, 1996) was used to estimate the overall hazard of selenium to the ecosystems affected by the GBP. The implementation of the protocol presented here incorporates data for water from Central Valley Regional Water Quality Control Board and data for sediment from the USBR in addition to biological data collected by the USFWS, CDFG, and CH2M HILL. In accordance with Lemly's protocol, the assessments use the highest (rather than the mean) concentrations of selenium found in each of the ecosystem components (Appendix A).

Data from the biological sampling in November 1996, shortly after GBP initiation, were excluded from the WY 1997 hazard assessments because temporarily extremely high concentrations of selenium in some fish may have been due to those fish having been flushed out of the previously stagnant, evapo-concentrated SLD. Very high levels of selenium in the water associated with storm flows were not excluded because elevated concentrations persisted long enough (especially in February 1998) potentially to affect the ecosystem adversely.

Concentrations of selenium in fish eggs were estimated from whole-body concentrations using the conversion factor (fish egg selenium = fish whole-body selenium x 3.3) recommended in Lemly (1995, 1996).

In this report, care has been taken to ensure that Lemly index for the area potentially adversely affected by the Grassland Bypass Project incorporates only contaminant levels that are due to this project. Therefore, although Figure 31 displays selenium concentrations in killdeer eggs collected along the San Luis Drain in the Kesterson Reservoir area, those data are not used in the calculation of the Lemly index because of the possibility that some of the most elevated selenium concentrations in eggs are due to killdeer foraging in areas of the Kesterson Reservoir residually contaminated by selenium from Westlands area farms predating this project.

Site E (lower Mud Slough) and the San Joaquin River (SJR) sites (G and H) cannot be rated as to overall hazard of selenium because not all media have been collected to assess these sites. Further confounding the evaluation at these sites is the prevalence of introduced fish species with broad environmental tolerances and the limited catch of invertebrates during WY 1999 and WY 2000.

Departures from the Compliance Monitoring Plan and Quality Assurance Project Plan

To ensure reliable and consistent data, the USFWS and the CDFG followed the procedures specified in the Compliance Monitoring Plan and the Quality Assurance Project Plan (QAPP) with the exceptions listed below.

External quality assurance samples (QAPP Appendix A, Section 7) were not submitted to analytical labs with GBP biological samples before January of 1998. External quality assurance samples are biological materials (e.g. powdered chicken egg, shark liver) with certified concentrations of the analytes of concern (selenium, boron), supplied by third party laboratories. The analyte concentrations in these samples are known to the agencies submitting the samples, but not known to the laboratory doing the analysis. This blind test of laboratory analytical precision supplements the internal quality control procedures of the analytical laboratory. Internal quality control protocols specified in the QAPP (procedural blanks, duplicate samples, and spiked samples) have been followed throughout the history of GBP biological sampling.

The USFWS used stainless steel (rather than Teflon) strainers for sorting small fish (QAPP Appendix A, Section 4.7).

For some species at some locations it has not been practical at some times to collect the full target minimum numbers of individuals and/or mass per sample that are specified in the Compliance Monitoring Plan (Section 4.5.1.4) and the QAPP (Appendix A, Section 4.5).

From 1992 through 1997 all biological samples collected by the USFWS (except bird eggs in 1996 and 1997) were analyzed by Environmental Trace Laboratory at the University of Missouri in accordance with the QAPP (Appendix A, Section 6.1). Bird egg samples collected in 1996 and 1997 were analyzed at Trace Element Research Laboratory (TERL) at Texas A & M University, a USFWS contract laboratory. All biological samples collected in 1998 were analyzed at TERL. TERL is subject to the same performance standards as Environmental Trace Substance Laboratory, therefore, the GBP quality assurance objectives (QAPP Table 1) apply to analytical results from TERL. All biological samples were analyzed at the Water Pollution Control Laboratory of the CDFG in Rancho Cordova, California, after this laboratory was screened and approved by the GBP Quality Control Officer.

Seine net mesh size was increased from 3/16 inch to 1/4 inch after the first two pre-Project collections in 1993 from sampling sites E, G, and H (QAPP Appendix A, Section 4.6). This change in sampling gear resulted in significant declines in catch abundance of smaller forage fish without altering diversity of representative assemblages. Data collected from 1993 sampling efforts at these sites were not included in making quantitative spatial or temporal comparisons between sites unless otherwise noted. At Sites C, D, I, and F, 1/8 inch mesh seines were used from 1992 through 1998. Since 1999 a 3/16 inch mesh bag seine has been used at these sites in place of the 1/8 inch mesh bag seine that was previously used by the USFWS.

As discussed previously, biological sampling in Mud Slough was moved from Site I to Site I2, a new site about 0.5 km upstream with a larger, more permanent backwater area.

Results

Salt Slough (Site F)

Fish (Whole-Body)

Salt Slough is a principal wetland water supply channel from which drainwater has been removed by the GBP. Concentrations of selenium in Salt Slough fish composite samples declined during the first year of operation of the GBP but have stabilized since then at levels well below the Concern threshold (Figures 2 and 3), with the exception of March 1998 when concentrations rose in the aftermath of storms that resulted in substantial releases of drainwater into Salt Slough. The average of all composite samples of fish at this site in WY 2001 was 2.6 mg/kg (n=51), substantially below the warmwater fish Concern threshold (4 mg/kg), significantly below the pre-Project average (5.3 mg/kg, n=66; p<0.0001), but not different from the average for the previous year (2.6 mg/kg, n=70; p=0.7). In June 2001 the selenium concentration (5.0 mg/kg dry weight, calculated from wet weight using average percent moisture of 79.3%) in a single 1.8 gram logperch (*Percina caprodes*) exceeded the Concern threshold for warmwater fish (4 mg/kg).

Tadpoles

Frog tadpoles (mainly bullfrog, *Rana catesbeiana*) have been collected only occasionally in the GBP area. Results suggest that in Salt Slough, selenium concentrations in tadpoles, as in fish and invertebrates, declined after implementation of the GBP (Figure 4). A composite sample of four bullfrog tadpoles collected in Salt Slough in August 1999 had about half the selenium concentration (2.6 mg/kg) of a single bullfrog tadpole collected in March 1993 (5.8 mg/kg). Selenium concentrations appeared to rise in the summer of 2000 (2.9 mg/kg in a composite sample of three bullfrog tadpoles in June 2000 (7.5 mg/kg in a composite sample of three tadpoles, and 2.3 mg/kg in a single, 19 g frog in August 2000), but returned to lower levels in the summer of 2001 (3.8 mg/kg in a single, 0.4 g tadpole in June 2001; 2.5 mg/kg in a composite sample of 13 tadpoles in August 2001). However, sample sizes are very small for drawing conclusions about year-to-year trends.

Invertebrates

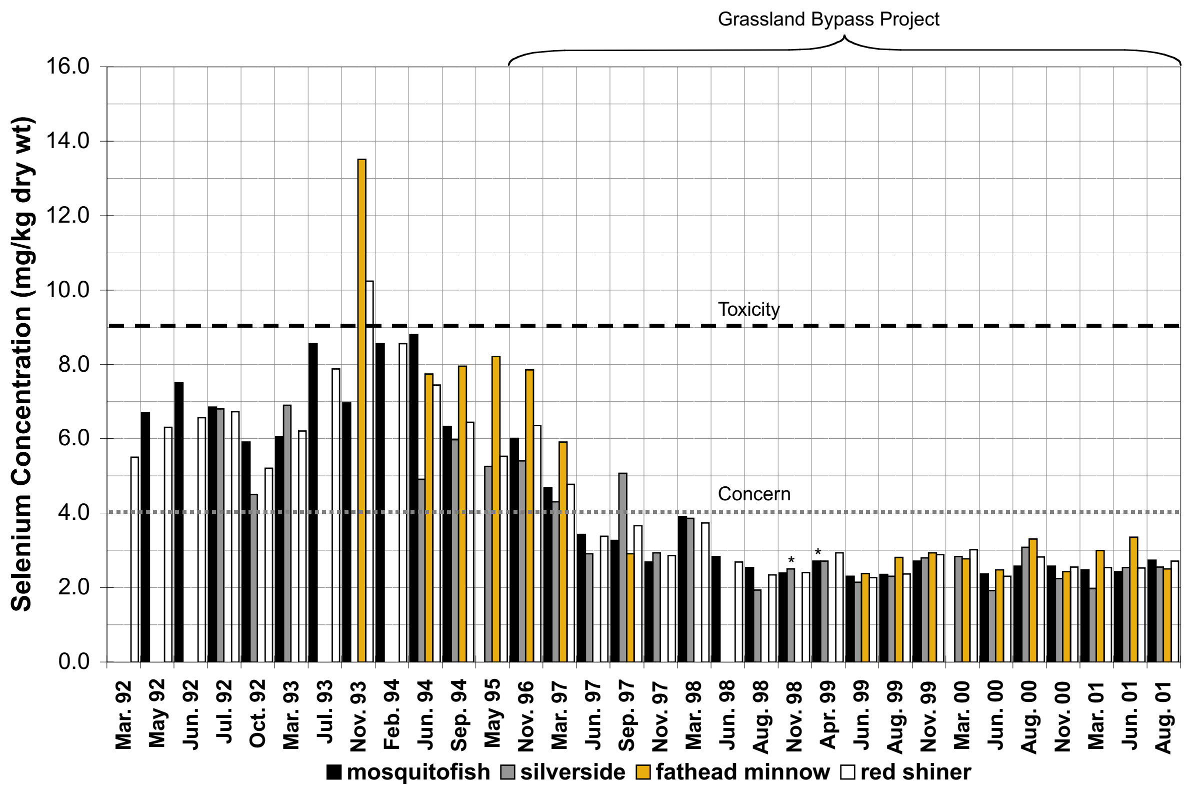
During WY 2001, selenium concentrations in invertebrates collected from Salt Slough (Figure 5) remained within the range of concentrations associated with no known adverse effects (<3 mg/kg) on animals that eat invertebrates. The mean concentration of selenium in all invertebrate samples collected in WY 2001 (2.2 mg/kg, n=9) was significantly below (p<0.00001) the pre-Project mean (4.4 mg/kg, n=27), but not significantly different (p=0.63) from the WY 2000 mean (2.1 mg/kg, n=5).

Mud Slough 0.4 km above SLD Outfall (Site C)

Fish (Whole-Body)

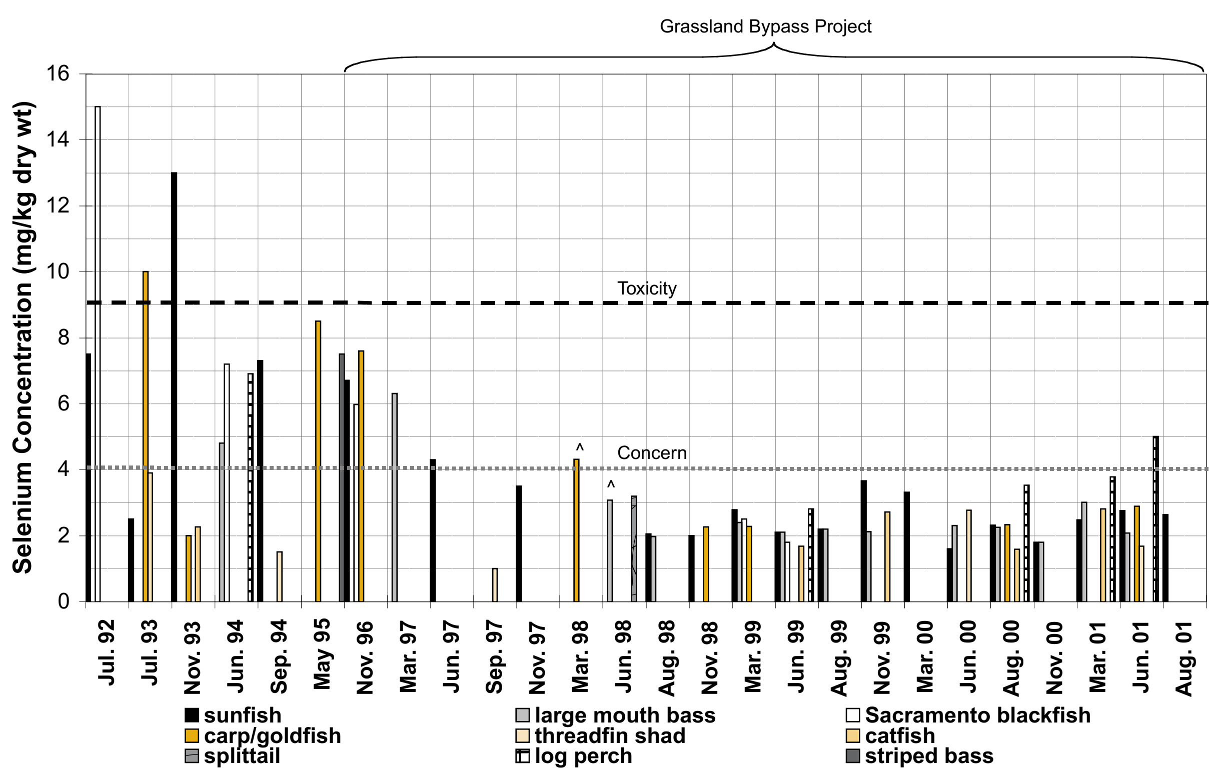
During the fifth year of operation of the GBP, average selenium concentration in fish just above the SLD (3.0 mg/kg, n=63) remained the same as in the previous year (3.0 mg/kg, n=65, p=0.84) and not significantly different (p=0.2) from the pre-Project average at this site (2.8 mg/kg, n=37; Figures 6 and 7). The warmwater fish Concern threshold (4 mg/kg; see Table 1) was exceeded by the average selenium concentrations in fathead minnow and/or red shiner composite samples in every sampling period during the water year (November 2000 one composite fathead minnow sample 6.7 mg/kg, average of three composite red shiner samples 4.7 mg/kg; March 2001 one composite fathead minnow sample 4.4 mg/kg; June 2001 one composite fathead minnow sample 5.0 mg/kg; August 2001 average of three red shiner composite samples (6.35 mg/kg). Elevated average selenium concentrations in some samples at this site may be due to the influence of individual fish swimming upstream from the more contaminated reach of Mud Slough below the discharge of the San Luis Drain.

Figure 2. Selenium in small fish in Salt Slough (Site F).
Each bar represents an average of composite samples.



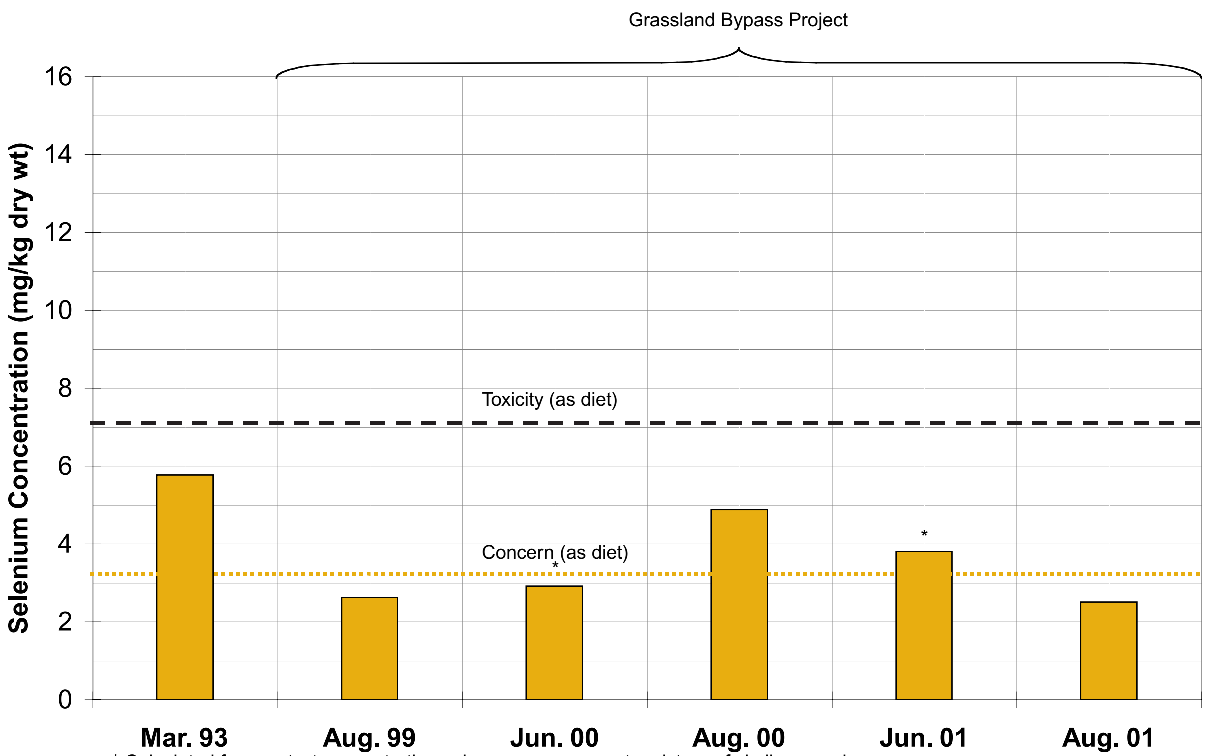
* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 3. Selenium in medium-size fish in Salt Slough (Site F).



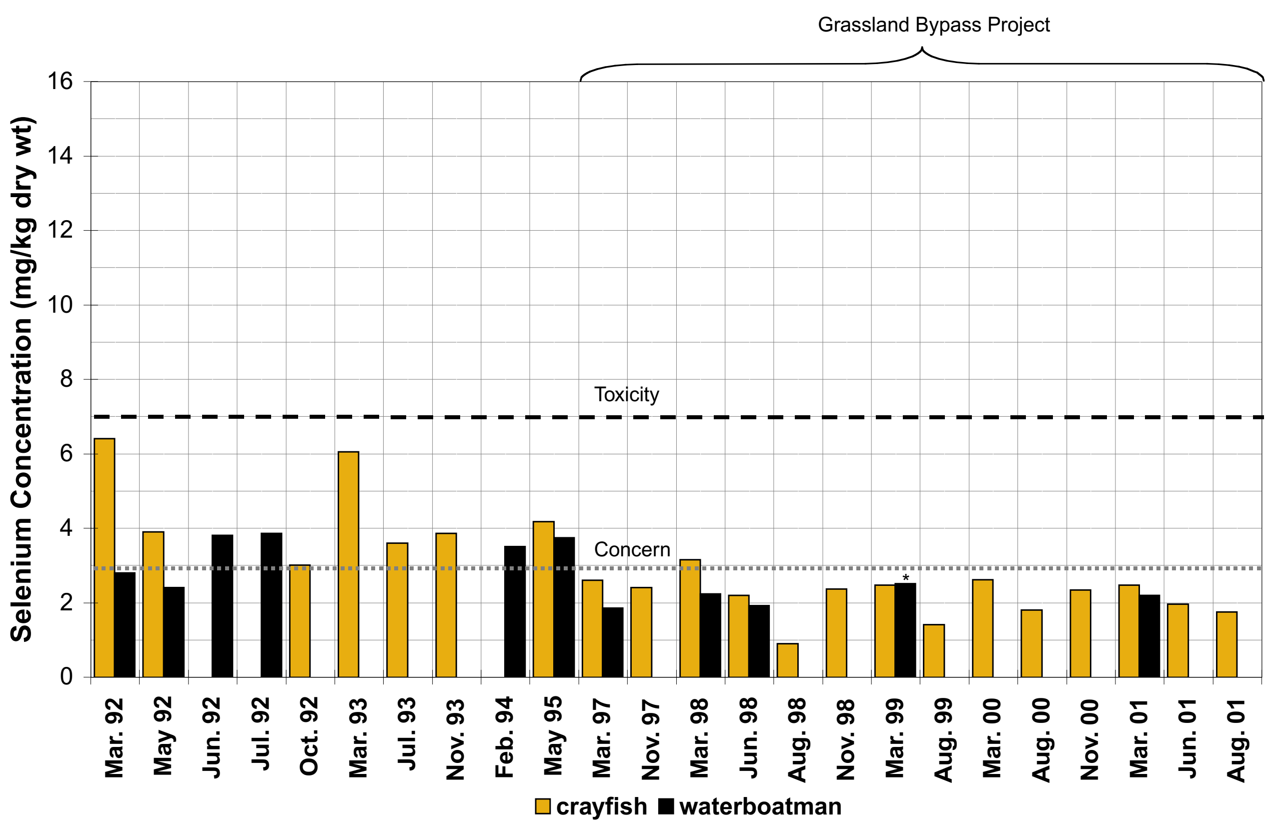
^muscle only

Figure 4. Selenium in bullfrogs/tadpoles in Salt Slough (Site F).



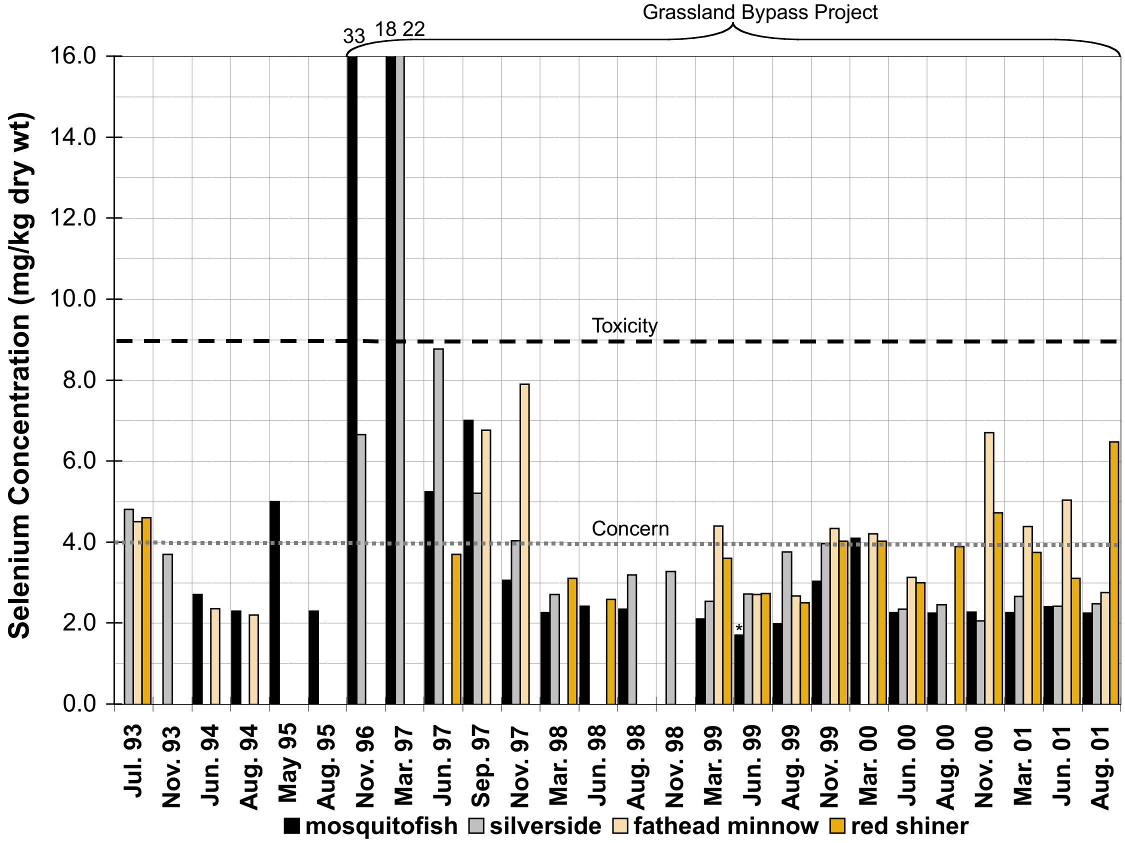
* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 5. Selenium in invertebrates in Salt Slough (Site F).



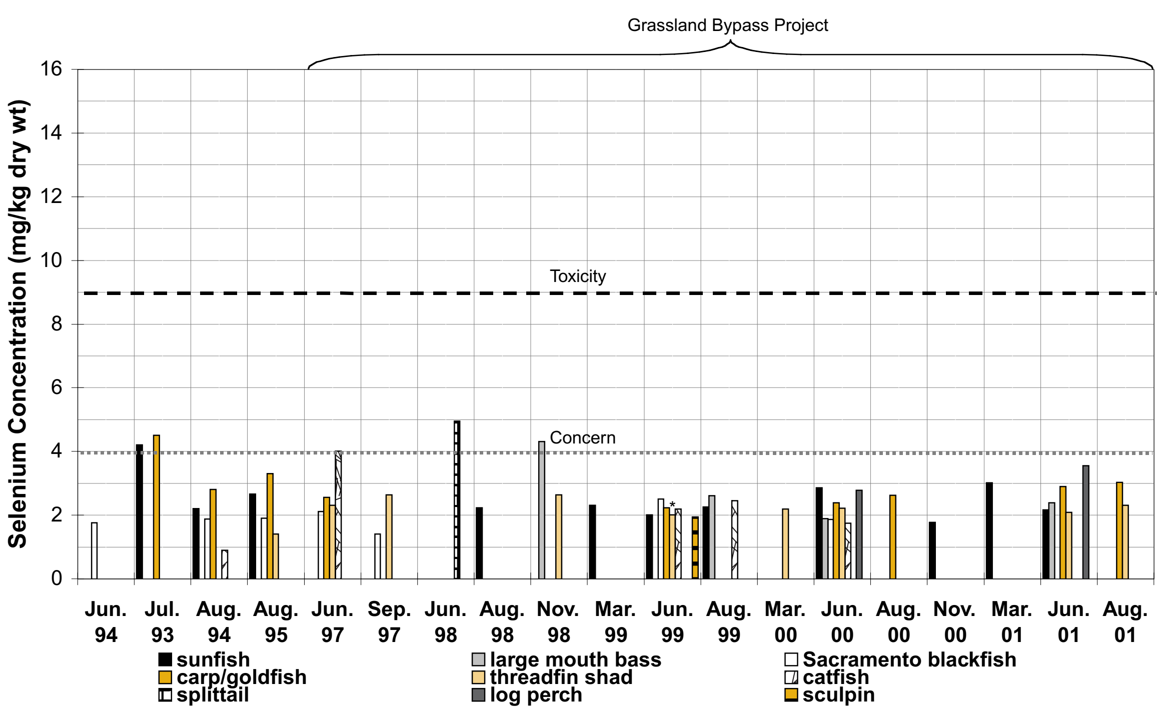
* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 6. Selenium in small fish in Mud Slough above the San Luis Drain discharge (Site C).



* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 7. Selenium in medium-size fish in Mud Slough above the San Luis Drain discharge (Site C).



* Calculated from wet wt concentration using average percent moisture of similar samples.

Tadpoles

At Site C, a single bullfrog tadpole was collected in March 2001. The selenium concentration in this sample (3.5 mg/kg) was in the middle of the range of concentrations in tadpole samples collected previously at this site (Figure 8), above the threshold of Concern (3 mg/kg) for dietary effects on birds that may forage on tadpoles. No tadpoles were collected at this site prior to WY 1999.

Invertebrates

In the fifth year of operation of the GBP, selenium concentrations in invertebrates at Site C remained generally about the same as in previous years, below the Concern threshold (Figure 9). The average concentration in all invertebrate composite samples in WY 2001 was 1.8 mg/kg (n=14), not significantly different (p=0.23) from the previous year (1.7 mg/kg, n=10), and not significantly different (p=0.56) from the pre-Project average (2.0 mg/kg, n=15).

Mud Slough 0.2 km below SLD Outfall (Site D)

Fish (Whole-Body)

At Site D, about 200 m below the SLD outfall, the average selenium concentration in fish (7.3 mg/kg, n=42) increased significantly (p<0.0001) above the average for the previous year (5.0 mg/kg, n=39) and significantly (p<0.0001) above the pre-Project mean (3.8 mg/kg, n=67; Figures 10 and 11). As in previous years, within WY 2001, selenium concentrations in fish exhibited significant (p<0.00001) seasonal variation in addition to the secular year-to-year increase (November 2000-March 2001 average: 3.7 mg/kg, n=11; June-August 2001 average: 8.6 mg/kg, n=31).

Tadpoles

A single 11-gram composite sample of four bullfrog tadpoles collected in March at this site had a selenium concentration of 4.0 mg/kg (Figure 12). Tadpoles have only be collected occasionally in Mud Slough below the San Luis Drain outfall, and selenium concentrations have always been within the range that is of concern as diet for birds that prey on aquatic vertebrates (3-7 mg/kg).

Invertebrates

Average selenium concentration in invertebrate samples at Site D (4.4 mg/kg, n=8) increased significantly (p=0.037) in the fifth year of operation of the GBP compared to the previous year (2.2 mg/kg, n=2; Figure 13). However this may be due to seasonal differences and the exigencies of sampling at a site where invertebrates have been scarce throughout the history of the GBP monitoring program. In the fifth year of the GBP, no invertebrates where collected here in November, but in the previous year, the only invertebrates collected in sufficient numbers to analyze were collected in November, a time of seasonally low selenium concentrations.

Mud Slough 1.5 km below SLD Outfall (Site I/I2)

During the fifth year of the GBP, biological monitoring was moved from the original Site I to a new site, designated I2, that better serves the purpose for which Site I was chosen. Site I was originally selected to represent backwater conditions along the adversely affected reach of Mud Slough. This site was located along Mud Slough about 1.5 km downstream of the SLD outfall where high winter and spring flows in Mud Slough often overtop the slough channel to form a broad, shallow backwater on the east side of the slough. In the early years of monitoring for the GBP, biological monitoring was done at Site I only when this backwater condition coincided with regular monitoring times for the other sites. Therefore, this site was only monitored occasionally. However, this site represented a better measure of the effects of the GBP on Mud Slough biota than Site D because it was further from the diluting influence of aquatic organisms swimming downstream from the cleaner reach of Mud Slough above the outfall of the San Luis Drain. Therefore, from 1999 to 2000, monitoring at this site was increased to four times per year, matching the monitoring at the other biological monitoring sites for this project; monitoring was conducted in the main channel of Mud Slough at this site in addition to or instead of the backwater, depending on backwater conditions. Meanwhile, National Wildlife Refuge staff helped locate a new backwater site along Mud Slough that typically remains inundated throughout the year. This backwater, Site I2, is located about 1 km downstream of the outfall of the San Luis Drain. Monitoring at Site I2 began in March 2001.

Figure 8. Selenium in tadpoles in Mud Slough above the San Luis Drain discharge (Site C).

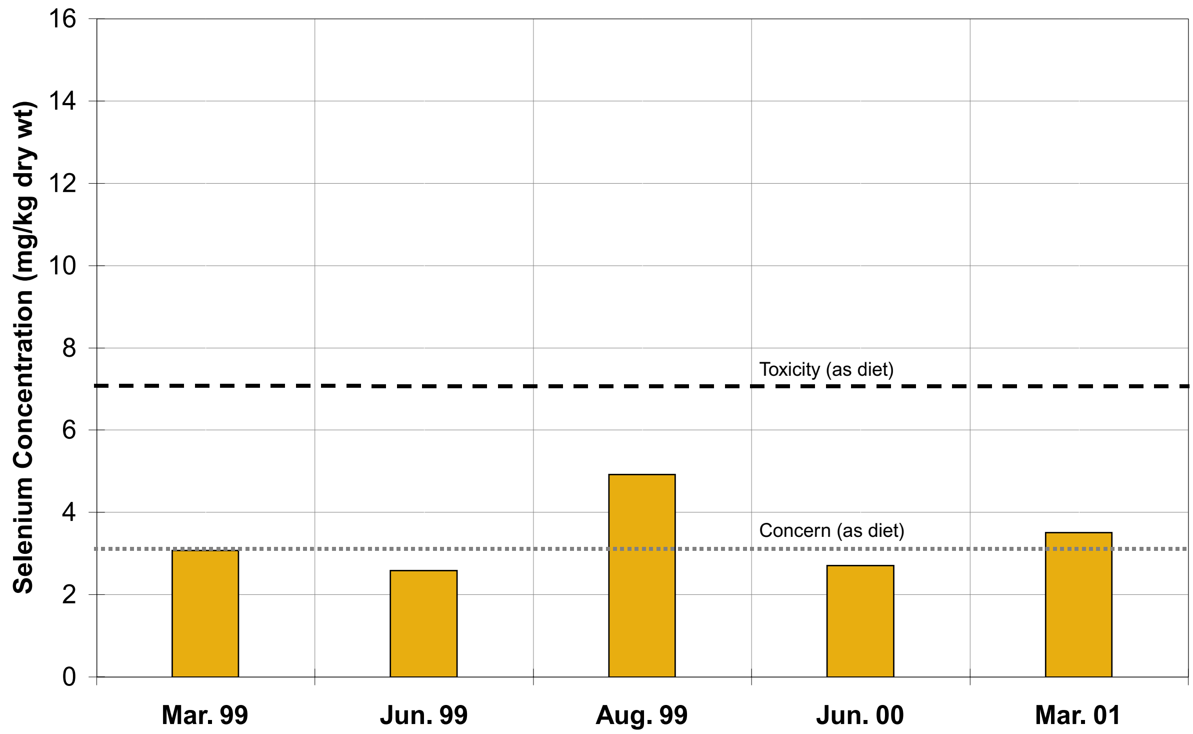
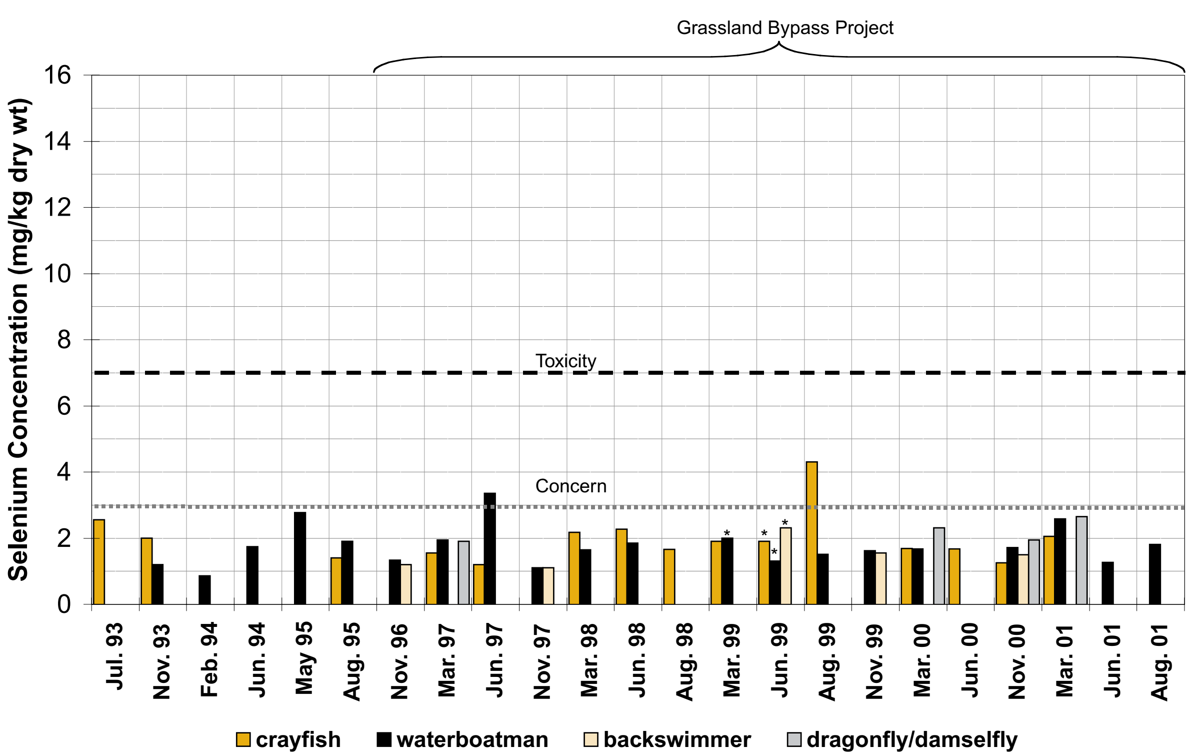


Figure 9. Selenium in invertebrates in Mud Slough above the San Luis Drain discharge (Site C).



* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 10. Selenium in small fish in Mud Slough below the San Luis Drain discharge (Site D).

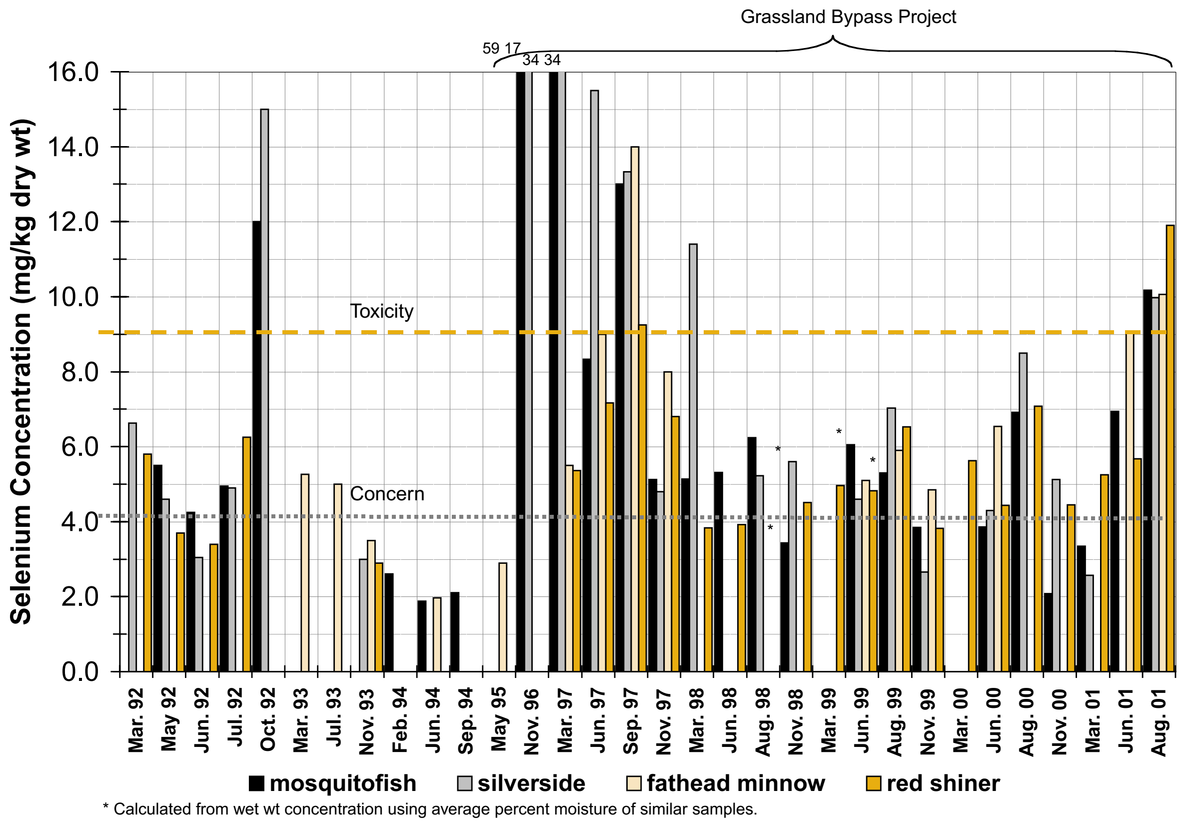


Figure 11. Selenium in medium-size fish in Mud Slough below the San Luis Drain discharge (Site D).

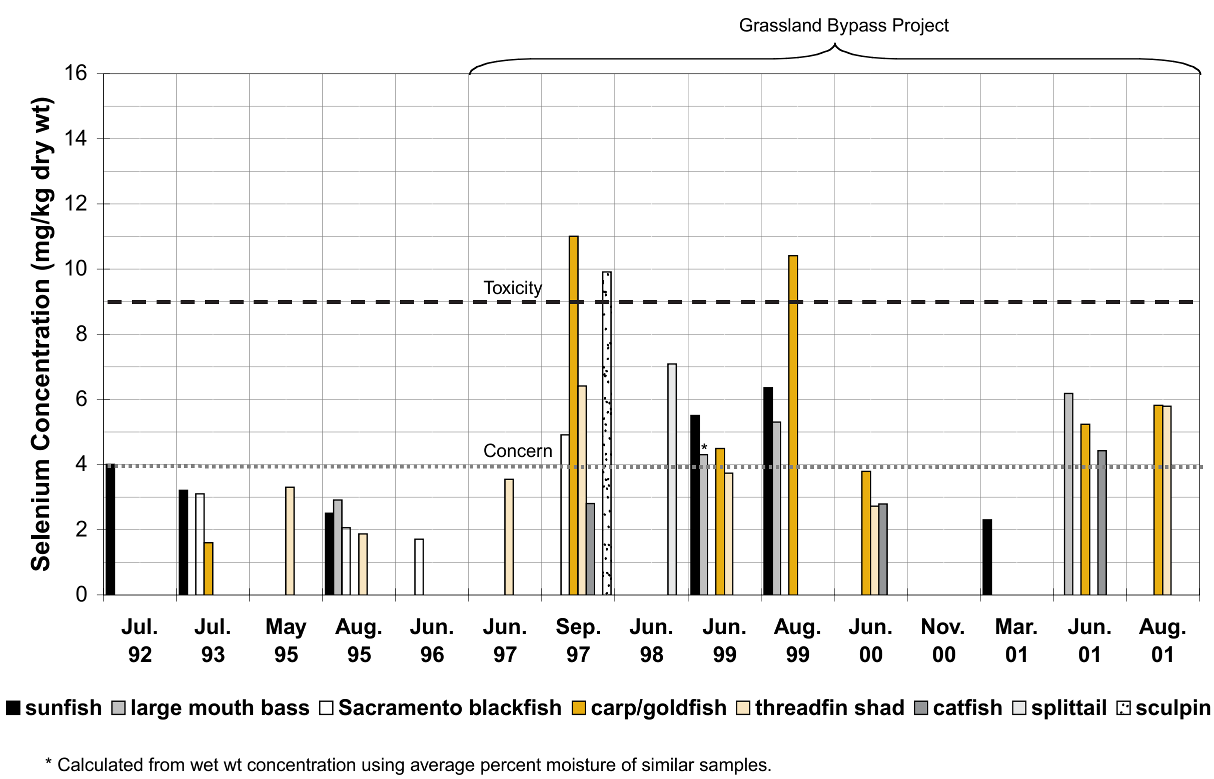


Figure 12. Selenium in tadpoles in Mud Slough below the San Luis Drain discharge (Site D).

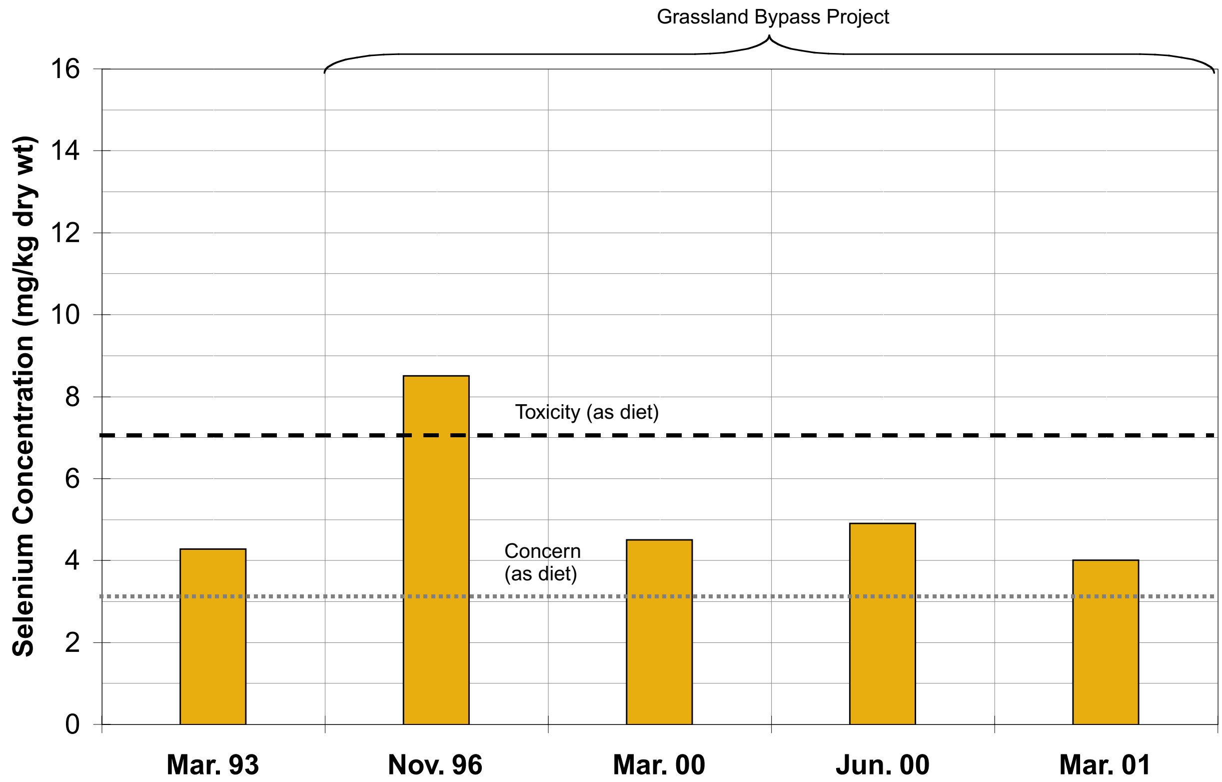
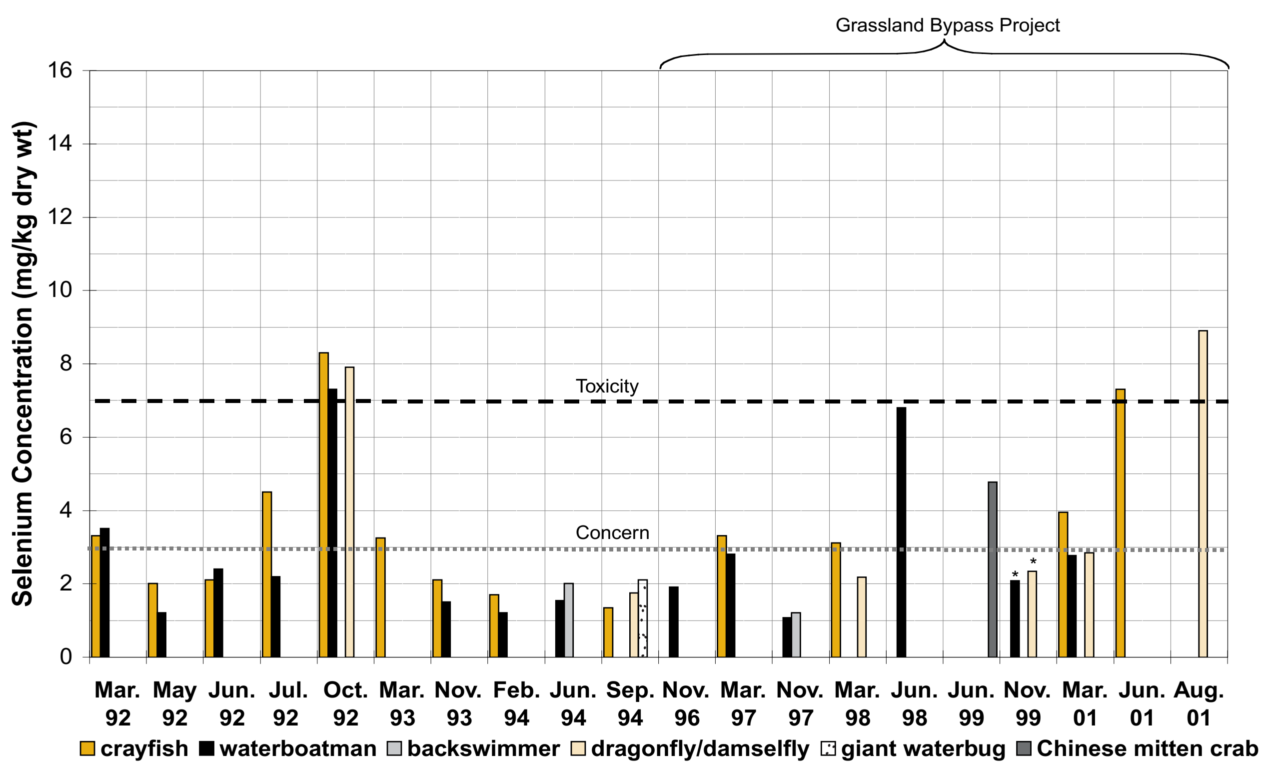


Figure 13. Selenium in invertebrates in Mud Slough below the San Luis Drain discharge (Site D).



* Calculated from wet wt concentration using average percent moisture of similar samples.

Fish (Whole-Body)

At Sites I and I2, average selenium concentration in fish (9.2 mg/kg, n=59) increased significantly (p=0.0002) in the fifth year of the GBP compared to the previous year (7.0 mg/kg, n=47; Figures 14 and 15). Some of this apparent increase could be due to the change in sites from I to I2; however, comparison of the December/November monitoring, which was conducted at the same site (I) both years, suggests a real increase (December 2000 average 6.2 mg/kg, n=6; November 1999 average 5.1 mg/kg, n=7, p=0.078). Furthermore, the year-to-year increase echoes a similar increase upstream at Site D (Figure 10). As at Site D and at Site I in previous years, selenium concentration exhibited a marked seasonal increase (p<0.000001) from early spring (March average 5.1, n=16) to late summer (August average 14.3, n=19). In August at Site I2, selenium concentrations in all fish samples were elevated well into the Toxicity zone both for effects on warmwater fish themselves (>9 mg/kg) and as diet for piscivorous birds (>7 mg/kg).

As in the previous year at Site I, in WY 2001 at Site I2, significantly greater bioaccumulation of selenium appeared to occur compared to Site D (in August 2001, mean of all fish at Site I2: 14.3 mg/kg, n=19; at Site D: 10.0 mg/kg, n=16, p<0.00001). This may in part be a real effect due to more efficient bioaccumulation in the backwater conditions at Site I2. However, it is likely that a principal reason is that composite samples of fish and invertebrates collected at Site D include substantial numbers of individuals that have moved downstream from the cleaner reach of Mud Slough above the outfall of the Drain, thereby diluting the measurements of the effects of drainwater on the biota at Site D.

Tadpoles

Tadpoles have not been collected at this site.

Invertebrates

Average selenium concentration in all invertebrates collected at Sites I and I2 during the fifth year of operation of the GBP (5.1 mg/kg, n=13) was not significantly different (p=0.8) from that of Site I in the previous year (5.6 mg/kg, n=7; Figure 16). However, as with Site D, so few invertebrates can be collected here in sufficient numbers for analysis that year to year comparisons are problematic. All invertebrate samples collected at this site had selenium concentrations above the threshold of Concern for birds that would forage on these invertebrates (3 mg/kg). As with fish at this site, and both fish and invertebrates upstream at Site D, there is significant (p=0.003) cyclic seasonal variation in selenium concentrations in invertebrates at this site (March 2001 average 4.4 mg/kg, n=6; June-August average 6.1 mg/kg, n=5).

Mud Slough at Highway 140 (Site E)

Site E is located in lower Mud Slough downstream from Sites D and I2 but upstream from the confluence of Mud Slough with the San Joaquin River. This site represents the lower, portion of the reach of Mud Slough that is adversely affected by the operation of the Project. At this point along Mud Slough, within the flood plain of the San Joaquin River, flows are slower and more spread out, and flood waters of the San Joaquin River periodically back up into slough, providing some flushing. Selenium in whole body fish and invertebrate samples collected at this site in 1999, 2000 and 2001 confirm the trend of increasing concentrations that is evident at Sites D, I, and I2. Samples at this site are collected by the California Department of Fish & Game.

Fish (Whole-Body)

Selenium concentrations in composite samples of whole-body fish collected during WY 2001 ranged from 6.5 to 13.7 mg/kg (dry weight). The average selenium concentration in whole-body fish (μ= 9.2 mg/kg, n=12) increased significantly (p<0.002[1]) above the average for the previous year (5.9 mg/kg, n=16) and significantly (p<0.000) above the pre-Project average (2.5 mg/kg, n=20; Figure 17). As in previous years, selenium concentrations in fish exhibited seasonal variation in addition to the year-to-year increase (November 2000-March 2001 average: 7.3 mg/kg, n=12; June-August 2001 average: 11.1 mg/kg, n=12). All samples collected in August 2001 exceeded the Toxicity threshold of 9 mg/kg (dry weight).

Invertebrates

Crayfish were not as difficult to catch at this site during WY 2001 as in previous years. Seven composite samples of crayfish collected at this site during WY 2001 had an average selenium concentration of 6.1 mg/kg (dry weight), within the Concern range (3 - 7 mg/kg dry weight) for invertebrates (Figure 18). This was significantly higher than the average selenium concentration of crayfish caught at this site during WY 2000 (μ=2.6, n=4, p<0.002). In August 2001, the average selenium concentration in sampled crayfish was 5.85 mg/kg (dry weight, n=3), exceeding the Concern threshold (4 mg/kg, dry

Figure 14. Selenium in small fish in a Mud Slough backwater below the Drain discharge (Sites I and I2).

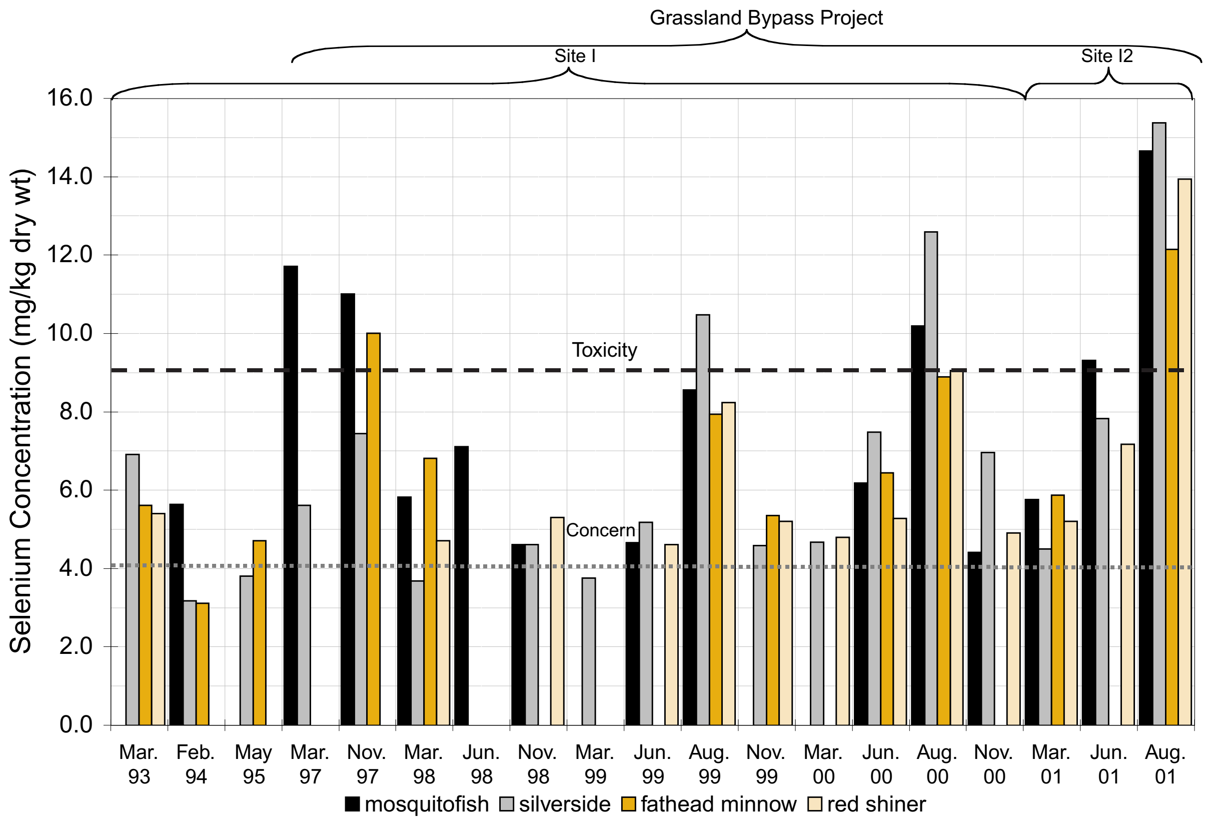


Figure 15. Selenium in medium-size fish in a Mud Slough backwater below the Drain discharge (Sites I and I2).

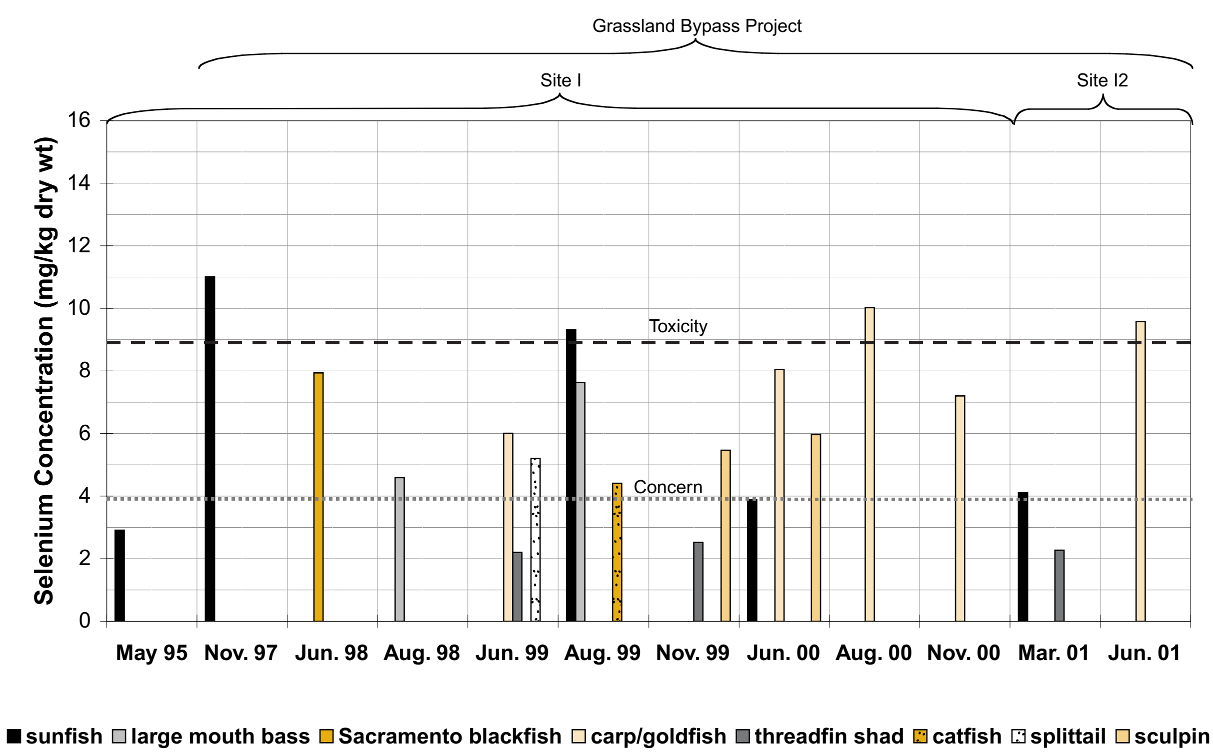
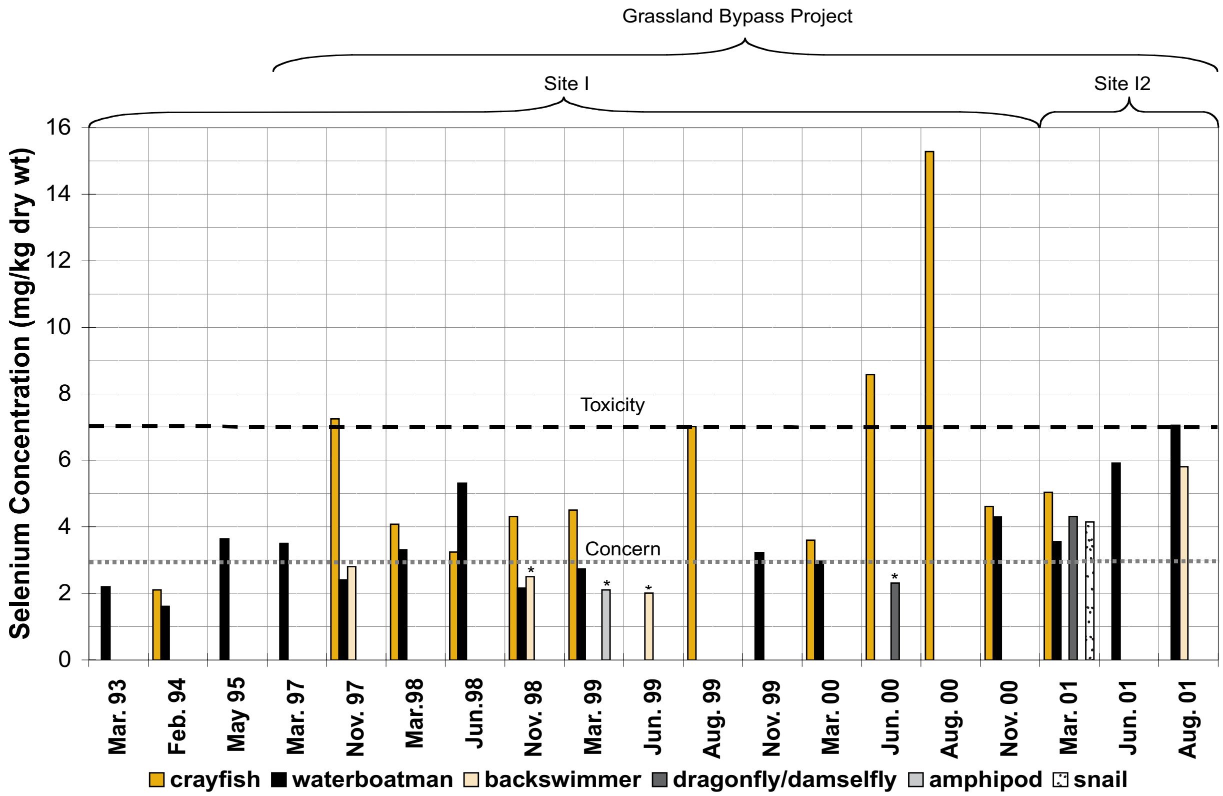
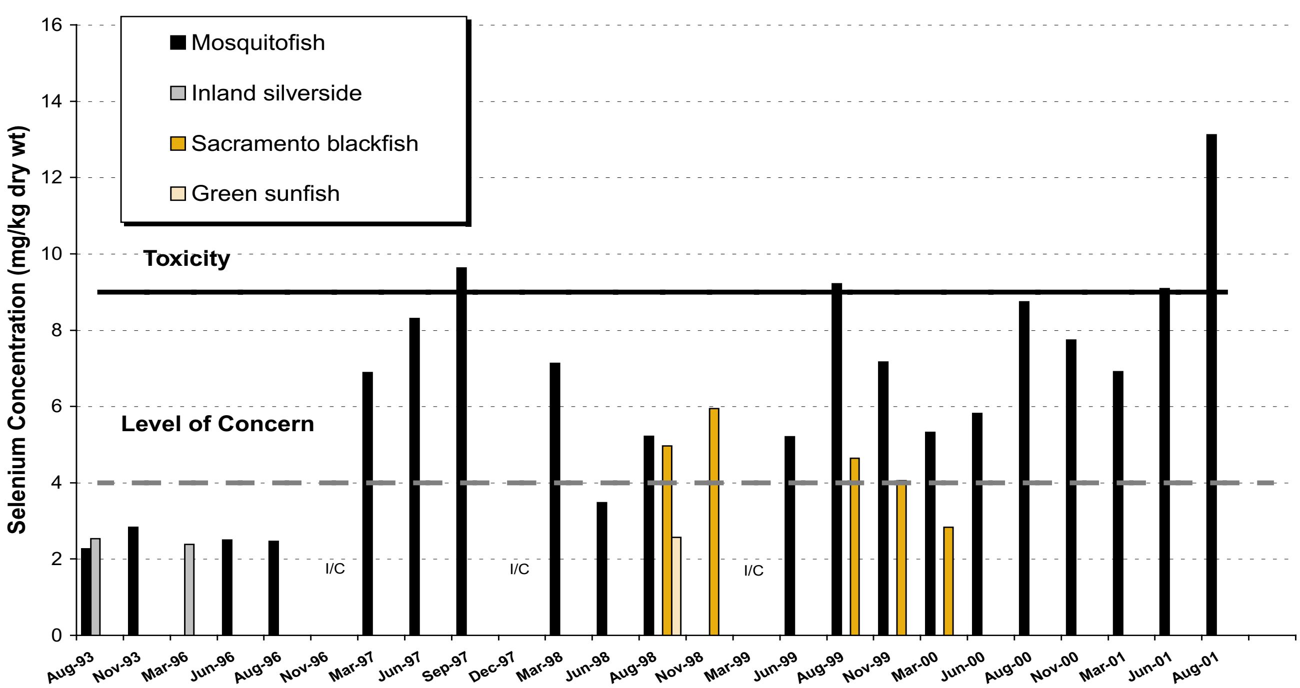


Figure 16. Selenium in Invertebrates in a Mud Slough backwater below the Drain discharge (Sites I and I2).



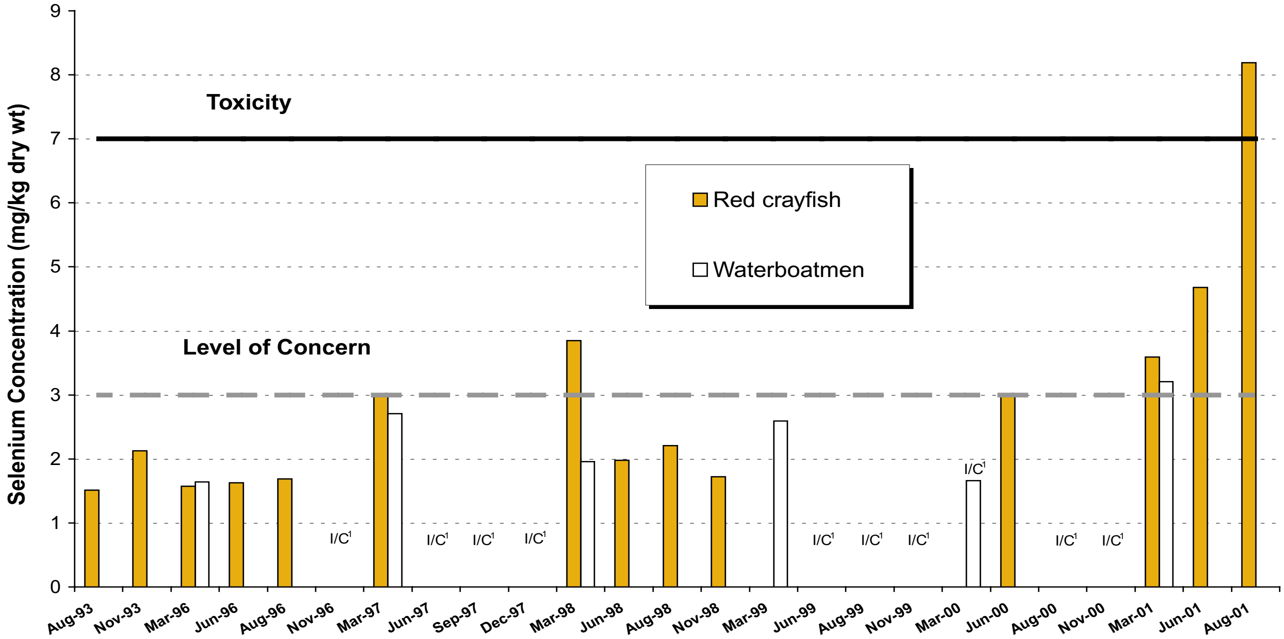
* Calculated from wet wt concentration using average percent moisture of similar samples.

Figure 17. Selenium Concentrations in Whole-Body Fish Tissue from Mud Slough at Hwy 140 (Site E).



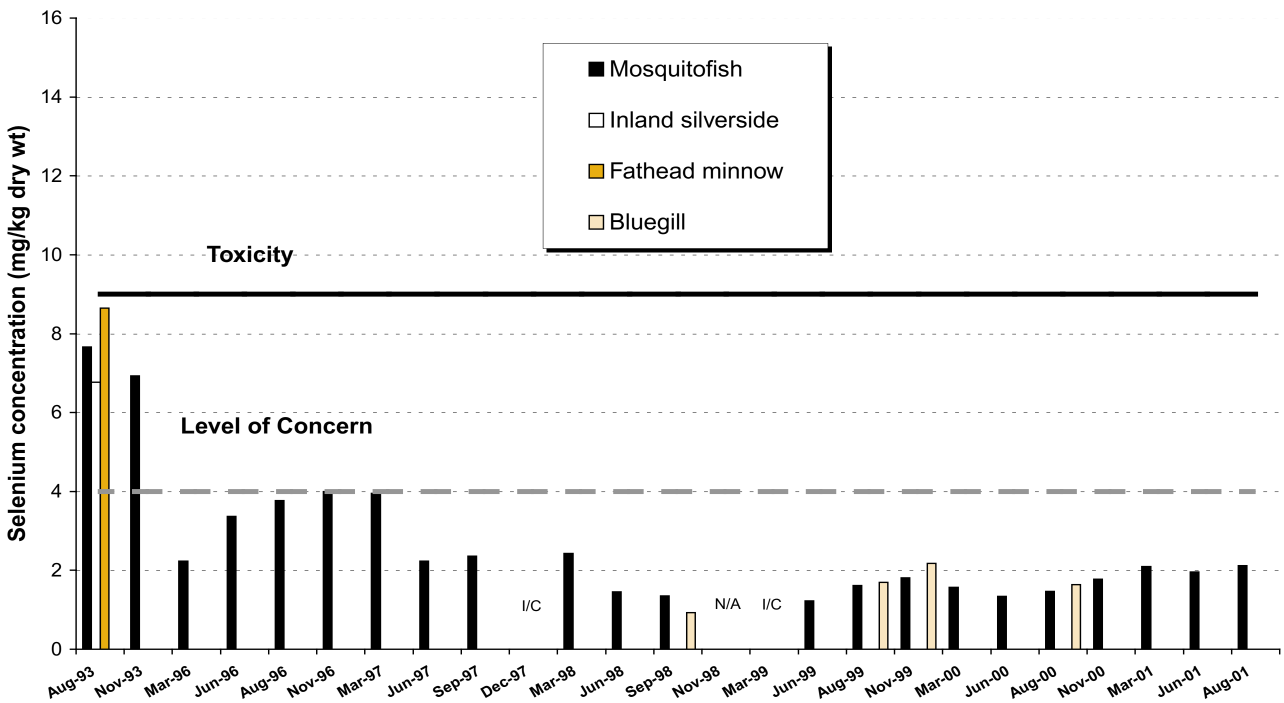
I/C: Insufficient Catch of Targeted Fish Species for Adequate Sample

Figure 18. Selenium Concentration in Invertebrates from Mud Slough at Hwy 140 (Site E).



I/C[1]: Insufficient Catch of Red Crayfish for Adequate Quarterly Sample

Figure 19. Selenium Concentrations in Whole-Body Fish Tissue from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).



I/C: Insufficient Catch of Targeted Fish Species for Adequate Sample
N/A: No Analysis of Collected Samples

weight). Composite samples of waterboatmen collected during March 2001 were slightly above the threshold of Concern (3 mg/kg dry weight), with an average selenium concentration of 3.2 mg/kg (dry weight, n=3). In prior years, annual samples of waterboatmen were below the 3 mg/kg threshold of Concern.

San Joaquin River at Fremont Ford (Site G)

Site G is located is located on the San Joaquin River upstream of the Mud Slough confluence. This site represents the reach of the San Joaquin River that no longer receives agricultural drainwater from the GDA as a result of the GBP.

Fish (Whole-Body)

Similar to the first four years of GBP operation, selenium concentrations in composite samples of fish collected from this site continued to reflect removal of selenium-laden drain water. Selenium concentrations in composite samples of whole-body mosquitofish collected during WY 2001 ranged from 1.6 to 2.4 mg/kg (dry weight), remaining well below the Concern threshold (4 mg/kg dry weight) for warmwater fish (Figure 19). Average selenium concentration for all whole-body fish collected in WY 2001 (μ=2.0, n=12) was higher than that in the previous year (WY 2000, μ=1.6, n=15, $p<0.000$), but less than 4 mg/kg (dry weight) Concern threshold. Selenium concentrations in whole-body fish have decreased significantly from pre-project levels (μ=5.6 mg/kg dry weight, n=21, $p<0.000$), and have consistently been below the Concern range (4–9 mg/kg dry weight).

Invertebrates

Selenium concentrations in all invertebrates from this site were higher than WY 2000 and previous years since project operations began (Figure 20). The nine composite samples of crayfish were collected during WY 2001 had selenium concentrations ranging from 0.9 to 2.4 mg/kg (dry weight), remaining below the 3 mg/kg (dry weight) threshold of Concern for invertebrates as prey items. The average concentration of selenium in crayfish caught at this site during WY 2001 was 1.46 mg/kg (dry weight, n=9) which was significantly greater than the average of crayfish caught during WY 2000 (μ=0.4 mg/kg dry weight, n=8, $p<0.010$). However, the WY 2001 average concentration was significantly less than the pre-project concentration of 3.5 mg/kg (dry weight, n=9, $p<0.004$).

Similar to crayfish, waterboatmen collected from this site during WY 2001 were well below the 3 mg/kg (dry weight) threshold of Concern, with an average selenium concentration of 1.4 mg/kg (dry weight).; this level has consistently remained below the threshold of Concern during all water years since Project operations began.

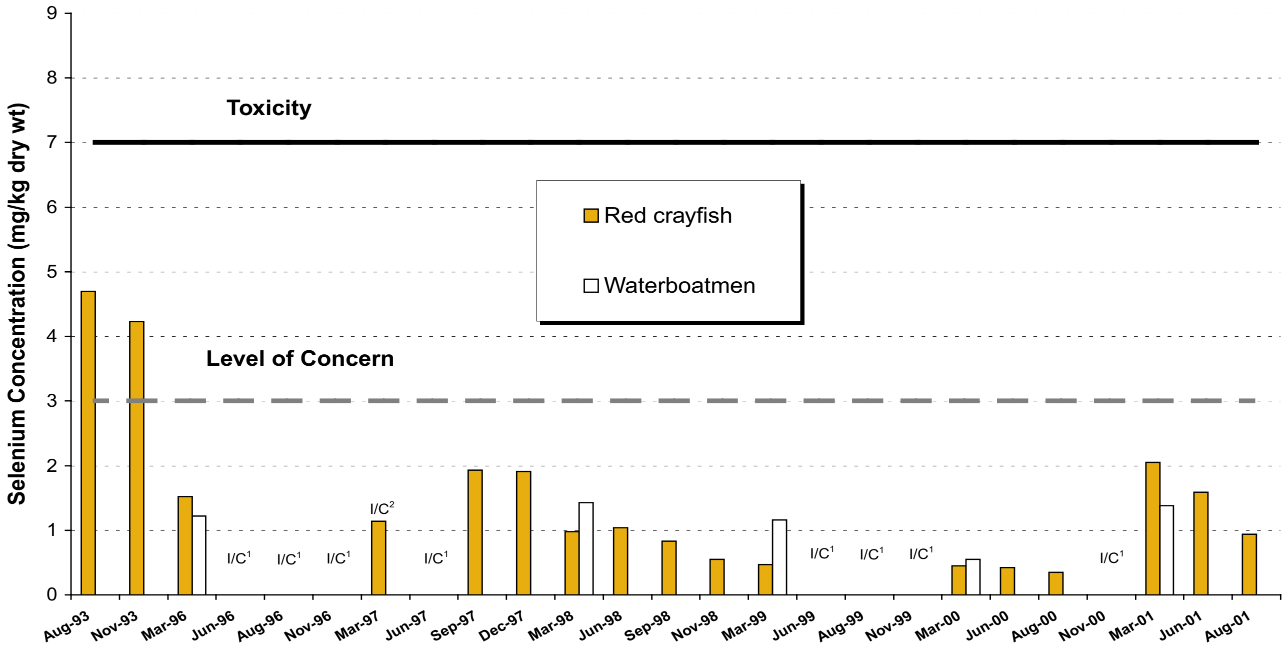
San Joaquin River Below Mud Slough (Site H)

Site H is located at Hills Ferry on the San Joaquin River about two miles downstream of the Mud Slough confluence. This site represents the reach of the San Joaquin River most strongly influenced by agricultural drain water discharged by the GBP. One of the environmental commitments of the GBP is that it will not worsen water quality in the San Joaquin River. For practical reasons of year-round accessability, the site was located just upstream of the Merced River confluence; Merced River waters have relatively low concentrations of selenium. It is likely that many of the fish and invertebrates collected at Site H have moved into this area after foraging within the Merced River and other cleaner reaches of the San Joaquin River. Additionally, seasonally high flows in the Merced River can enter the San Joaquin River upstream of Site H, temporarily diluting the load of contaminants there. Due to these confounding influences on selenium body burdens, selenium concentrations in fish and invertebrate tissues collected at this site may not be well correlated with water concentrations of selenium at this site.

Fish (Whole-Body)

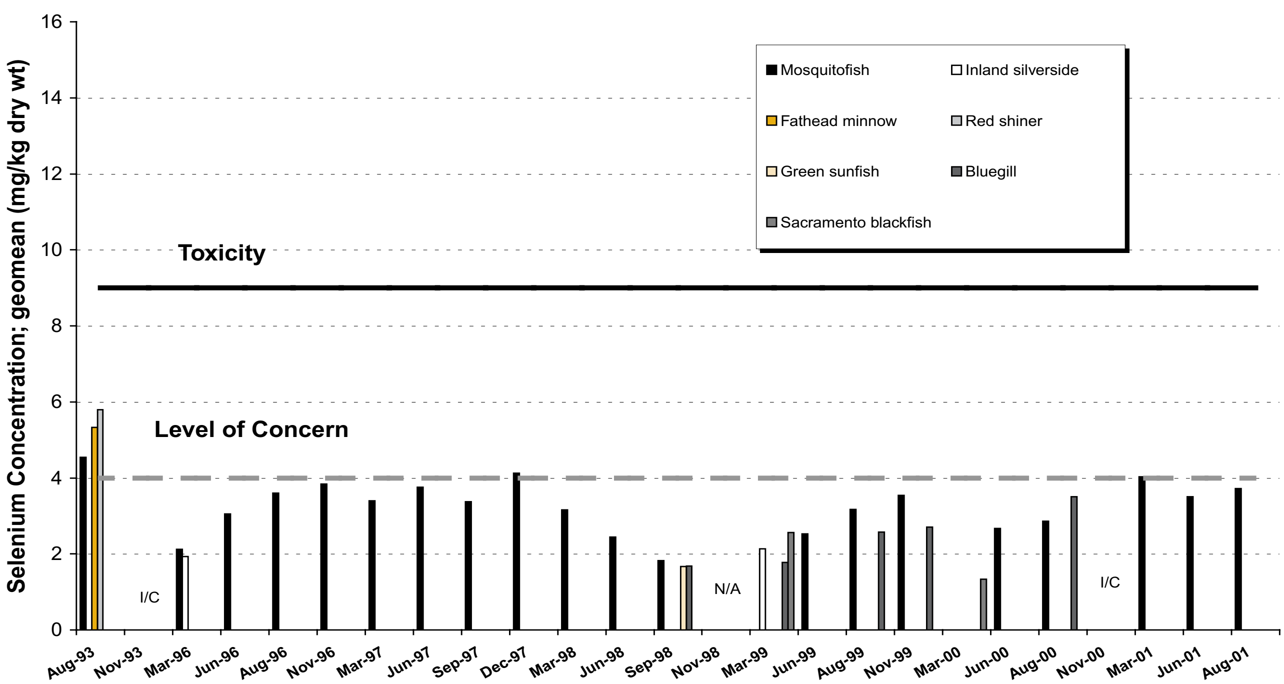
Selenium concentrations in nine composite samples of whole-body mosquitofish collected during WY 2001 averaged 3.8 mg/kg (dry weight), slightly below the 4 mg/kg (dry weight) threshold of Concern for warmwater fish (Figure 21). The average was greater than selenium concentrations in fish collected during WY 2000 (μ=2.9 mg/kg, n=16, $p<0.001$). Two composite samples collected in March 2001 exceeded the 4 mg/kg (dry weight) threshold of Concern. Despite this, selenium concentrations in composite whole-body fish samples throughout the five years of GBP operation have generally remained below the 4 mg/kg (dry weight) threshold of Concern and are not significantly different than concentrations in fish collected before the GBP began in 1996 (μ=3.78, n=21, $p<0.925$).

Figure 20. Selenium Concentration in Invertebrates from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).



I/C[1]: Insufficient Catch of Red Crayfish for Adequate Quarterly Sample
I/C[2]: Insufficient Catch of Waterboatmen for Adequate Annual Sample

Figure 21. Selenium Concentrations in Whole-Body Fish Tissue from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).



I/C: Insufficient Catch of Targeted Fish Species for Adequate Sample
N/A: No Analysis of Collected Samples

Invertebrates

Selenium concentrations in three composite samples of red crayfish collected from this site in WY 2001 ranged from 3.3 mg/kg to 3.5 mg/kg (dry weight), with an average of 3.4 mg/kg, which is above the 3 mg/kg (dry weight) threshold of Concern associated with known adverse effects on higher order consumers (Figure 22). The average selenium concentration in two composite samples of waterboatmen collected in WY 2001 was 1.38 mg/kg (dry weight), which was not significantly higher than the average selenium concentration of waterboatmen collected in WY 2000 (μ=0.6 mg/kg dry weight, n=3, p=0.06). The selenium concentration in all three invertebrate samples caught at this site in March 2001 exceeded the threshold of Concern of 3 mg/kg (dry weight). This was the first time such levels have been measured since monitoring began here in 1993.

Fish Communities Assessment

Fish communities assessment was conducted to describe fish assemblages based on species richness, abundance and community structure. Fish populations were sampled in Mud Slough at Highway 140 (Site E), San Joaquin River at Fremont Ford (Site G), and San Joaquin River below Mud Slough (Site H). Fish assemblages from these sites were compared both spatially and temporally to see if conditions for fish species in the San Joaquin River improved and conditions in Mud Slough degraded. We sampled in August and November 1993 and March, June, and August/September of the years 1996 - 2001. As the Grassland Bypass Project began operation in September 1996, this sampling schedule provided a before-and-after picture of the fish communities at these sites. Only data collected with standardized sampling methodologies and effort were analyzed.

Table 3 is a compilation of the 31 fish species (n=18,946) that have been collected at these sites during 5 pre-project and 16 post-project sampling events. Native fish represented 2% of the catch by number (n=423) and 27% of the catch by species.

Only four native species were caught during the 2001 WY at the three sites. One splittail, one Sacramento sucker, and two Sacramento blackfish were collected at the Site G in March 2001. No Chinook salmon were caught this year at either site. Annual spring abundance of fry and survival of juvenile chinook salmon in the delta appear to be influenced by river flow rate and temperature; survival and abundance decreased as flow rates decreased and temperatures increased (Kjelson, Raquel, and Fisher 1982 and Brandes and McLain 2000).

Sacramento blackfish continue to be the most abundant native fish throughout the study. The most common non-native fish are mosquitofish, inland silversides, fathead minnow, and carp.

We ran simple linear regression on trophic types caught at each site during the period 1993 to 2001. Invertivores and omnivores were dominant at all three sites and had complementary distributions. No time trend is evident in invertivores and omnivores at Site E (Figure 23). There appears to be an increase in invertivores and a decrease in omnivores caught at Site G (Figure 24). There appears to be a slight decrease in invertivores and an increase in omnivores at Site H (Figure 25).

Based on linear regression, there appears to be a slight decrease in the total anomalies observed at Sites E and H for the various groups of fishes at each site (Figure 26).

During September and October 1997, about one year after implementation of the GBP, Saiki (1998) sampled fish at 13 sites in the Grassland area. These sites correspond to locations he had surveyed more than a decade earlier (Saiki 1986). Some of his sample sites were the same as, or close to, GBP monitoring sites, but others were located in areas not monitored by the GBP. The SLD was the only site in the area that lacked bluegill and goldfish, and overall, fewer species of fish were found in the SLD than at any other site. However, Saiki did not find any significant difference in community structure related to the proportion of drainwater present. To explain this, he noted that all waterways in the area are overwhelmingly dominated by introduced species having broad environmental tolerances. Saiki's findings are consistent with those of the GBP biological monitoring program.

After 5 years of Project operation, current methods of assessing fish species assemblages cannot distinguish any significant temporal or geographic pattern of variation in fish community structure attributable to the Project.

Assessment of Risk to Public Health from Consumption of Fish

During the fifth year of project operation, selenium concentrations in all carp fillets from Site E ranged from 0.9 to 1.9 mg/kg (wet weight, n=9), below the 2 mg/kg health screening level (Figure 27).

Selenium concentrations in carp fillets collected at Sites G (μ=0.5 mg/kg wet weight, n=60) and H (μ=0.7 mg/kg wet wt, n=57) on the San Joaquin River were well below the 2 mg/kg health screening level and have remained this level throughout all five years of GBP operations (Figures 28 and 29).

Table 3. Fishes collected from Grassland Bypass Project Stations E, G, and H in decreasing order of numerical abundance.

Species	Number	Origin	Trophic Classification	Tolerance
Mosquitofish, *Gambusia affinis*	7,139	Introduced	I	T
Inland silverside, *Menidia beryllina*	3,311	Introduced	I	M
Fathead minnow, *Pimephales promelas*	2,058	Introduced	O	T
Carp, *Cyprinus carpio*	1,873	Introduced	O	T
Red shiner, *Cyprinella lutrensis*	816	Introduced	O	T
White catfish, *Ameiurus catus*	806	Introduced	I/P	T
Bluegill, *Lepomis macrochirus*	626	Introduced	I	T
Goldfish, *Carassius auratus*	346	Introduced	O	T
Largemouth bass, *Micropterus salmoides*	318	Introduced	P	T
Threadfin shad, *Dorosama petenese*	303	Introduced	I	M
Green sunfish, *Lepomis cyanellus*	250	Introduced	I/P	T
Redear sunfish, *Lepomis microlophus*	233	Introduced	I	M
Sacramento blackfish, *Orthodon microlepidotus*	211	Native	O	T
Channel catfish, *Ictalurus punctatus*	197	Introduced	I/P	M
Splittail, *Pogonichthys macrolepidotus*	109	Native	O	M
Bigscale logperch, *Percina macrolepida*	77	Introduced	I	T
Black crappie, *Pomoxis nigromaculatus*	53	Introduced	I/P	M
Sacramento sucker, *Catostomus occidentalis*	26	Native	O	M
Prickly sculpin, *Cottus asper*	25	Native	I	M
Striped bass, *Morone saxatilis*	25	Introduced	P	M
Spotted bass, *Micropterus punctulatus*	24	Introduced	P	M
Sacramento pikeminnow, *Ptychocheilus* grandis	22	Native	I/P	M
Brown bullhead, *Ameiurus nebulosus*	21	Introduced	I/P	T
Chinook salmon, *Oncorhynchus tshawytscha*	21	Native	I	I
Smallmouth bass, *Micropterus* dolomieui	19	Introduced	I/P	M
American shad, *Alosa sapidissima*	9	Introduced	I	M
Black bullhead, *Ameiurus melas*	8	Introduced	I/P	T
White crappie, *Pomoxis annularis*	8	Introduced	I/P	T
Hitch, *Lavinia exilicauda*	4	Native	O	M
Tule perch, *Hysteocarpus traski*	4	Native	I	I
Warmouth, *Lepomis gulosus*	2	Introduced	I	M
Golden Shiner, *Notemigonus crysoleucas*	1	Introduced	I	M
Riffle sculpin, *Cottus gulosus*	1	Native	I	M

Totals		18,946	
Introduced	24 species	18,523	98%
Native	9 species	423	2%

Trophic Classification: O=Omnivore, I=Invertivore, P=Piscivore, I/P=Invertivore/Piscivore
Tolerance to environmental degradation: I=Intolerant, M=Moderately Tolerant, T=Tolerant

Figure 22. Selenium Concentration in Invertebrates from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).

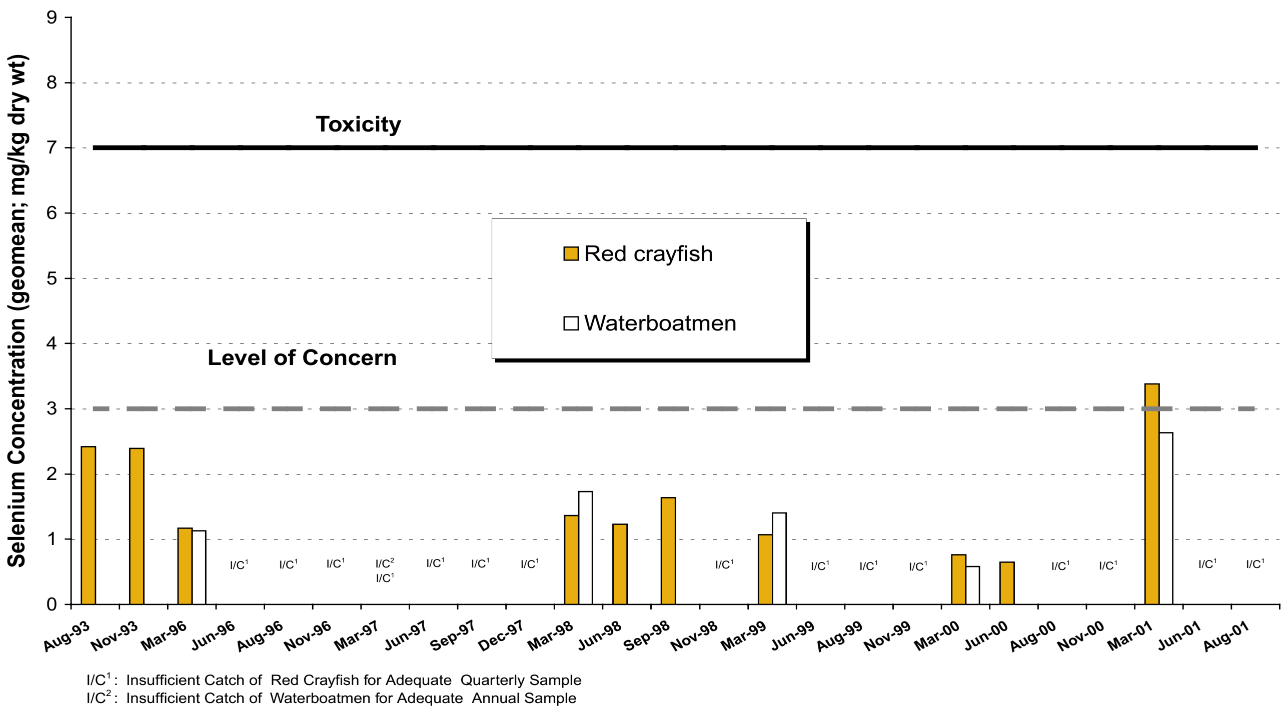


Figure 23. Percent abundance of trophic classifications over time at Site E.

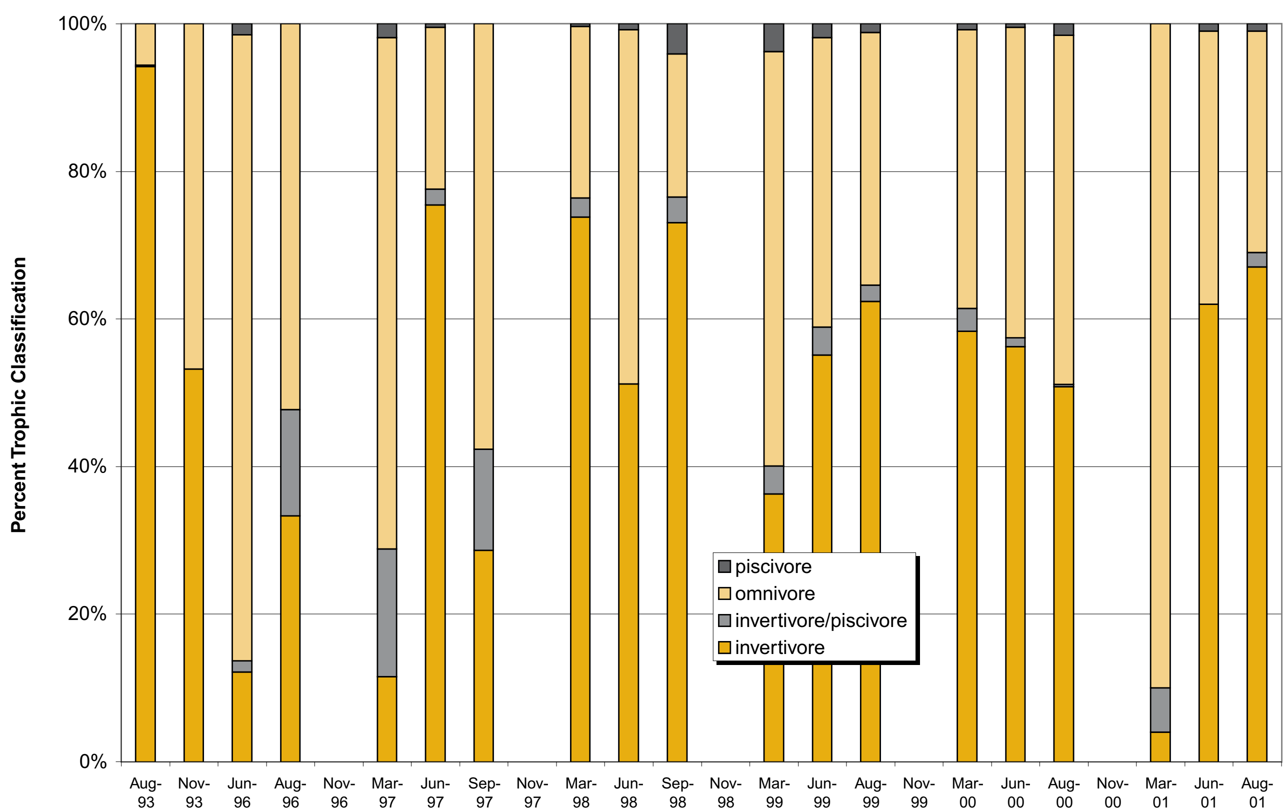


Figure 24. Percent abundance of trophic classifications over time at Site G.

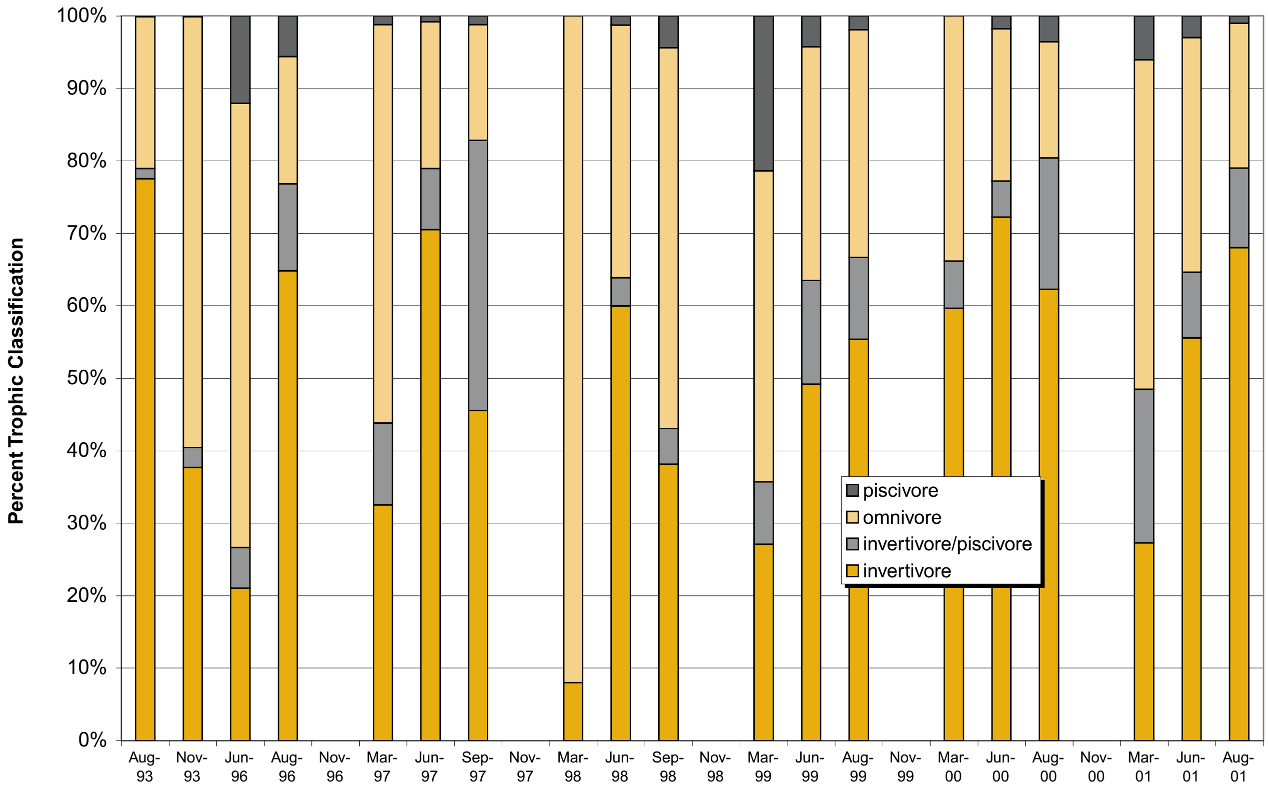


Figure 25. Percent abundance of trophic classifications over time at Site H.

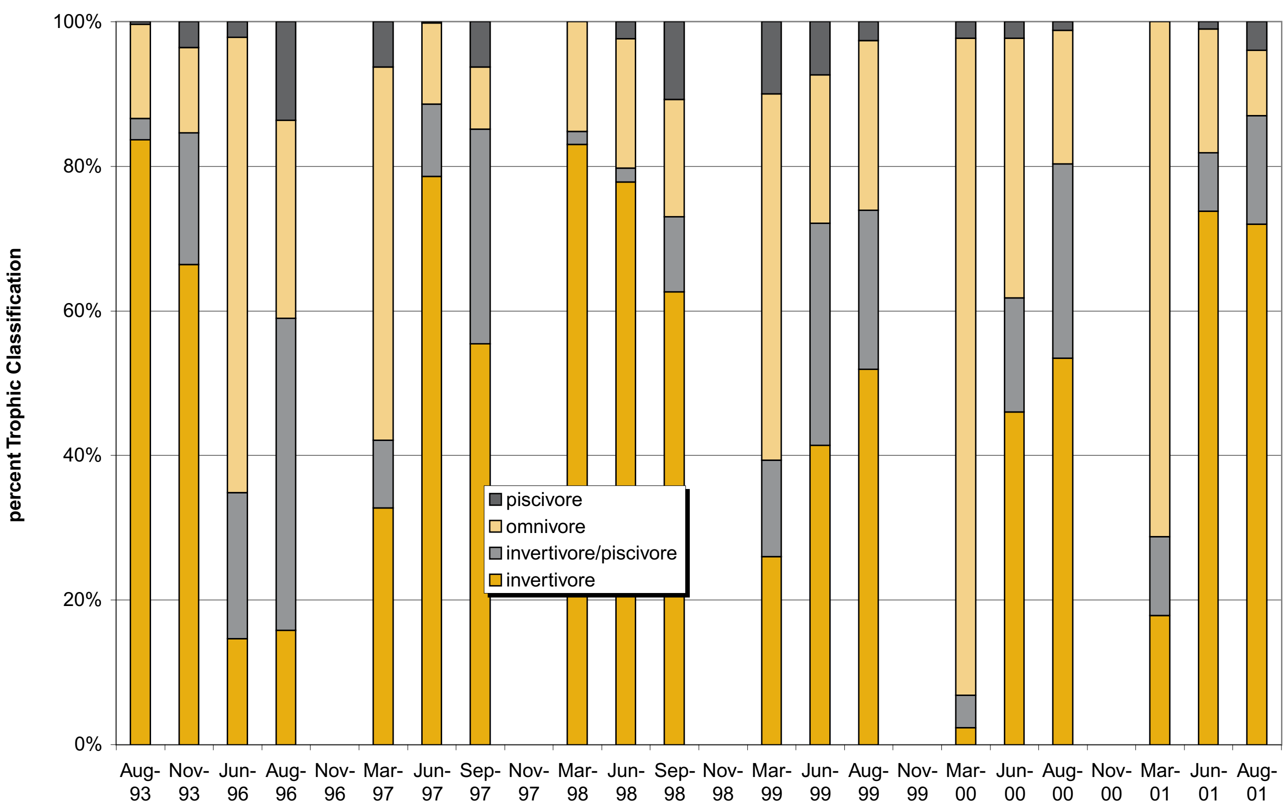
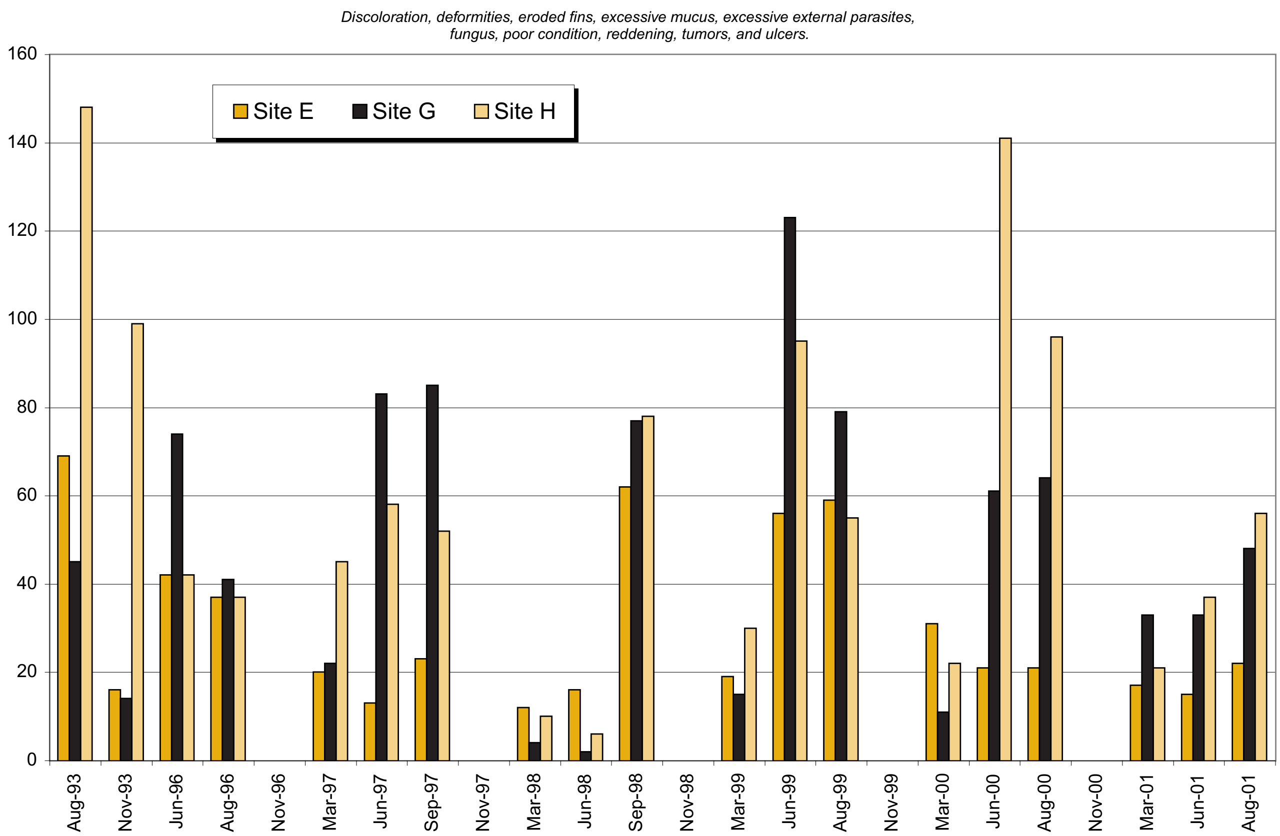


Figure 26. Total Anomalies in all Fish Species Caught from Sites E, G, and H.



Selenium in Plants

Composite samples of plant material that provides preferred forage for waterfowl (seed heads) have been collected in late summer for several years, but funding has been adequate to analyze some of these materials for selenium in the last two years (Figure 30). In WY 2001, the highest selenium concentrations found in water-side plants were from samples collected along Mud Slough downstream of the San Luis Drain (Sites D and I2). All samples were well below the threshold of Concern for reproductive effects on waterfowl due to dietary exposure (3 mg/kg) except a composite sample of swamp timothy seed heads (3.5 mg/kg) collected from the banks of Mud Slough below the San Luis Drain outfall (Site D). These data suggest that birds in this area are generally at greater risk due to eating invertebrates and fish than from eating plants.

The concentrations of selenium in knotgrass (*Paspalum disthum*) seed heads collected by CDFG at Sites E, G, and H were well below the 3 mg/kg (dry weight) threshold of Concern. The average concentration of selenium in three composite samples of seeds collected at Site E was 1.94 mg/kg (dry weight) during WY 2001. This average was greater than the average of seed samples collected during WY 2000 (μ=0.9, n=3,$p<0.150$) and before the GBP (μ=0.3, n=3, $p<0.071$). The average concentration of selenium in composite samples of seed collected at Sites G and H were 0.19 mg/kg (dry weight) and 0.26 mg/kg (dry weight), respectively. These averages were not significantly different than the average selenium concentration of seeds collected before the GBP ($p<0.184$ and $p<0.543$, respectively).

Selenium in Bird Eggs

A single egg was randomly collected and analyzed from each of 23 bird nests in the Grassland area in 2001 (Figure 31). Species sampled included killdeer, black-necked stilt, gadwall, wood duck, cinnamon teal, kestrel, barn swallow, black phoebe and Redwing blackbird. The selenium concentrations in all eggs collected in the Salt Slough area (San Luis Unit) were within the “No-Effect” range of concentrations (<6 mg/kg). Selenium concentrations in eggs analyzed from the Mud Slough area (geometric mean 4.5 mg/kg, n=10) were significantly ($p=0.02$, t-test performed on log-transformed concentrations) higher than those analyzed from the Salt Slough area (geometric mean 1.9 mg/kg, n=13). One Mud Slough area egg (black phoebe: 7.0 mg/kg) was in the Concern

Table 4. Aquatic Hazard Assessment of Selenium in Mud and Salt Slough Areas.

	BEFORE PROJECT 1995-Sept. 1996		SINCE PROJECT WY1997		WY1998		WY1999		WY2000		WY2001	
	concentration	hazard	concentration	hazard	concentration	hazard	concentration	hazard	concentration	hazard	concentration	hazard
Mud Slough below Drain outfall												
Water	19.4 µg/l	high	79.6 µg/l	high	104.0 µg/l	high	50.7 µg/l	high	66.0 µg/l	high	50.8 µg/l	high
Sediment	0.4 µg/g	none	0.76 µg/g	none	2.0 µg/g	low	4.8 µg/g	high	4.4 µg/g	high	3.5 µg/g	moderate
Invertebrates	1.6 µg/g	none	3.3 µg/g	low	11 µg/g	high	7.0 µg/g	high	15.3 µg/g	high	7.1 µg/g	high
Fish eggs	14.2 µg/g	moderate	56.1 µg/g	high	34.2 µg/g	high	39.6 µg/g	high	46.5 µg/g	high	54.8 µg/g	high
Bird eggs	3.12 µg/g	minimal	4.4 µg/g	minimal	6.6 µg/g	low	10 µg/g	low	5.1 µg/g	low	7.0 µg/g	low
TOTAL SCORE	13	moderate	16	high	21	high	23	high	23	high	22	high
Salt Slough												
Water	37.8 µg/l	high	3.4 µg/l	moderate	5.1 µg/l	high	1.5 µg/l	minimal	1.7 µg/l	minimal	2.1 µg/l	low
Sediment	0.8 µg/g	none	0.94 µg/g	none	2.1 µg/g	low	0.93 µg/g	none	0.68 µg/g	none	0.77 µg/g	none
Invertebrates	4.7 µg/g	moderate	2.6 µg/g	minimal	3.15 µg/g	low	2.8 µg/g	minimal	2.7 µg/g	minimal	0.7 µg/g	minimal
Fish eggs	28.1 µg/g	high	17.8 µg/g	moderate	12.9 µg/g	moderate	11.2 µg/g	moderate	14.5 µg/g	moderate	12.5 µg/g	moderate
Bird eggs	5.2 µg/g	low	3.6 µg/g	minimal	3.72 µg/g	minimal	2.7 µg/g	none	4.9 µg/g	minimal	4.0 µg/g	minimal
TOTAL SCORE	**18**	**high**	**13**	**moderate**	**17**	**high**	**10**	**low**	**11**	**low**	**12**	**moderate**

Notes:
Hazard scale for components (water, sediment, etc.): none (no hazard),1; minimal, 2; low, 3; moderate, 4; high 5.
Hazard scale for total score (sum of component scores): none, 5; minimal 6-8; low, 9-11; moderate, 12-15; high, 16-25.

range (6-10 mg/kg) and two Mud Slough area eggs (killdeer: 15.6 and 26.3 mg/kg) were above the Toxicity threshold (10 mg/kg).

Aquatic Hazard Assessment of Selenium

To provide an estimate of ecosystem-level effects of selenium, Lemly (1995, 1996) developed an aquatic hazard assessment procedure that sums the effects of selenium on various ecosystem components to yield a single characterization of overall hazard to aquatic life. Lemly's procedure applied to Mud Slough downstream of the SLD outfall indicated that the hazard to aquatic life in the affected portion of Mud Slough continued to be "high" in WY 2001 (Table 3). In the Salt Slough area, the Lemly index rose from "low" in WY 2000 to "moderate" in WY 2001 (Table 3) due to a small increase in the maximum concentration of selenium measured in water (1.7 µg/L on 15 March 2000; 2.1 µg/L on 1 March 2001). Because the Lemly index is based on maximum concentrations, it is highly sensitive to data "outliers." A Lemly index was not determined for San Joaquin River sites due to lack of sufficient sample of invertebrates and because bird eggs, one component of the index, were not sampled there.

Boron in Plants

Samples of seed heads from plants (knotgrass, smartweed, swamp timothy, bullrush sedge) collected in August 2001 from Sites C, D, E, I2, F, G, and H were analyzed for boron.

At Site C, two of three samples (23.2, 57.2, and 115.0 mg/kg) exceeded the threshold of Concern for boron in plants as diet (30 mg/kg, Table 2). At Sites D and I2 all samples exceeded the threshold of Concern (Site D: 58.2, 48.6, and 152 mg/kg; Site I2: 50.1 and 57.9 mg/kg). At Site F, all samples (20.9, 28.9, and 19.6 mg/kg) were in the No-Effect zone.

The average concentration of boron in knotgrass (*Paspalum distichum*) seed heads in three composite samples collected by CDFG at Site E was 48 mg/kg (dry weight). This was above the 30 mg/kg (dry weight) level of Concern. The average concentration of boron in knotgrass seed heads collected at Sites G (µ=8.3, n=3) and H (µ=13.7, n=3) were well below the level of Concern.

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Figure 27. Se Concentration in Fish Muscle Tissue from Mud Slough at Hwy 140 (Site E).

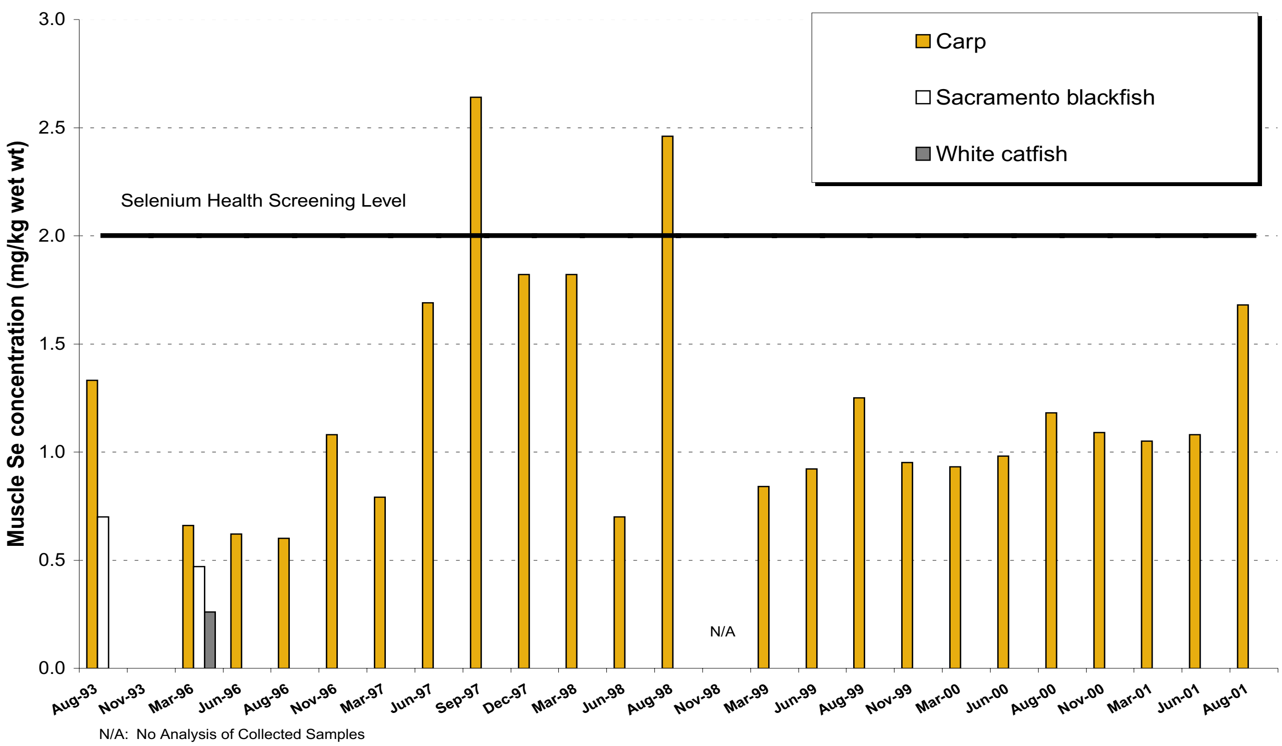


Figure 28. Se Concentration in Fish Muscle Tissue from the San Joaquin River Upstream of the Mud Slough Confluence (Site G).

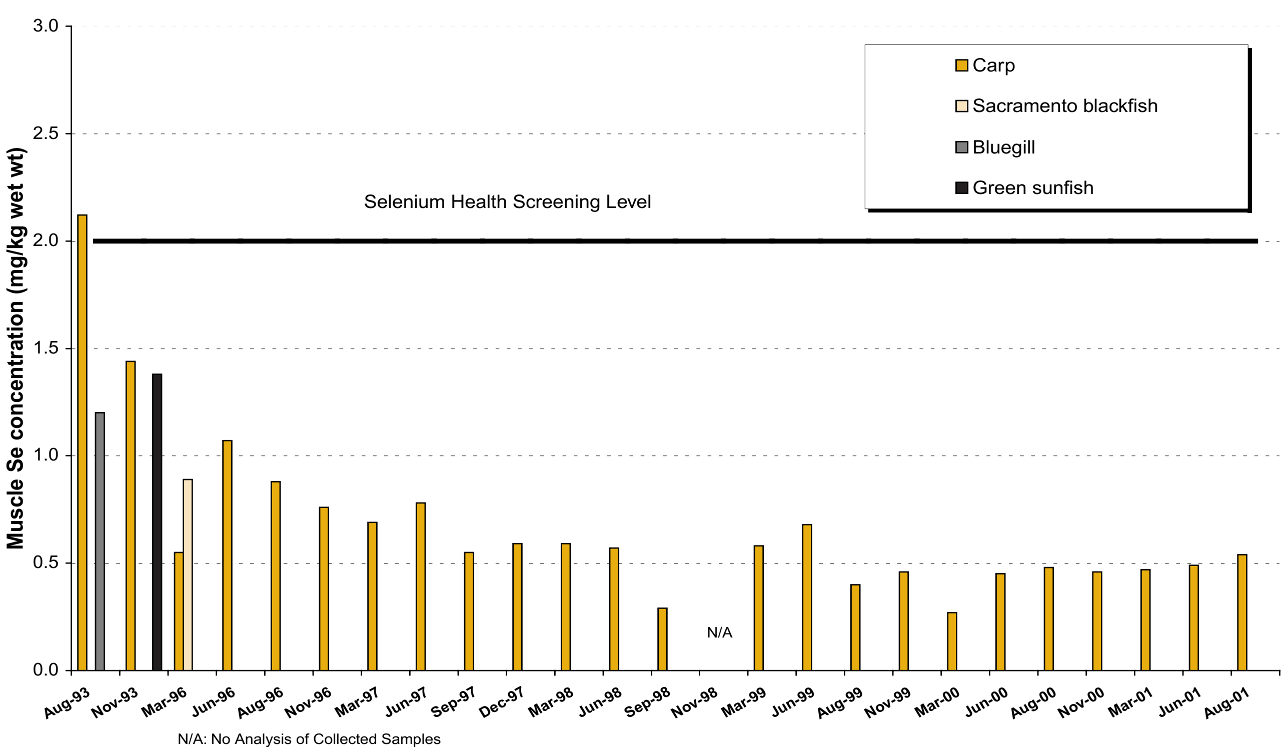
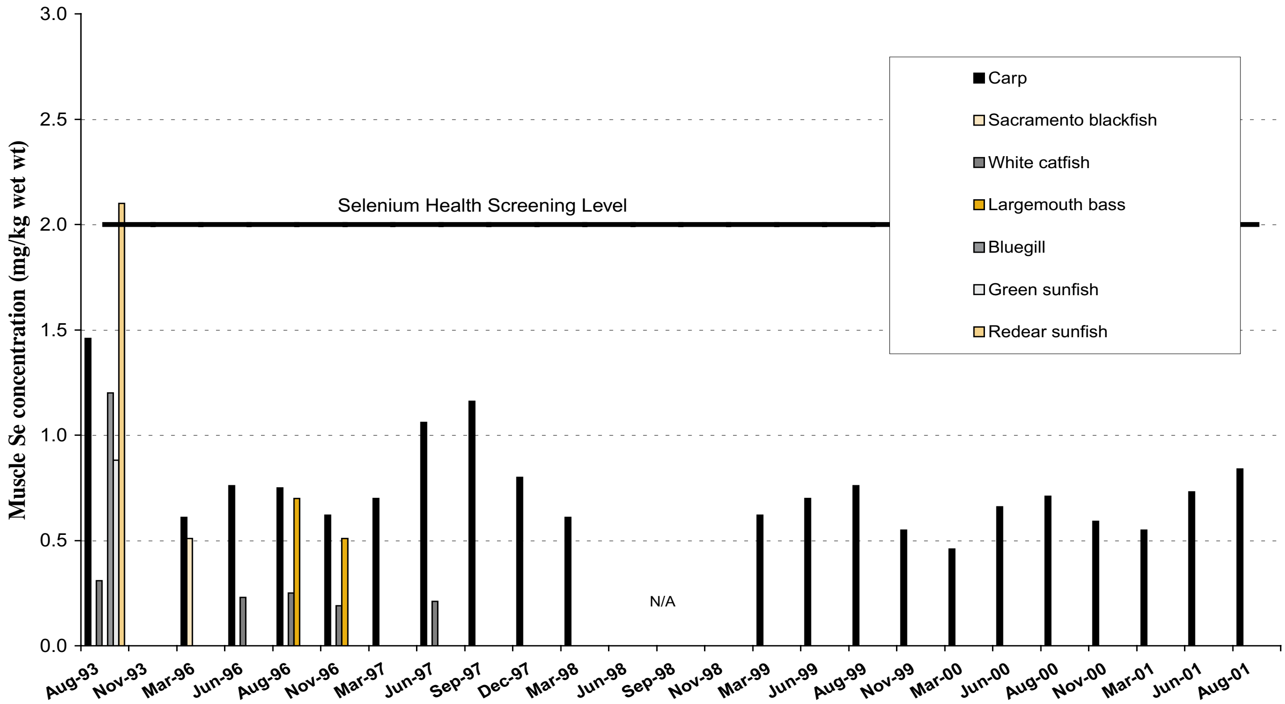


Figure 29. Selenium Concentration in Fish Muscle Tissue from the San Joaquin River Downstream of the Mud Slough Confluence (Site H).



N/A: No Analysis of Collected Samples

Figure 30. Selenium in plants (seed heads where applicable).

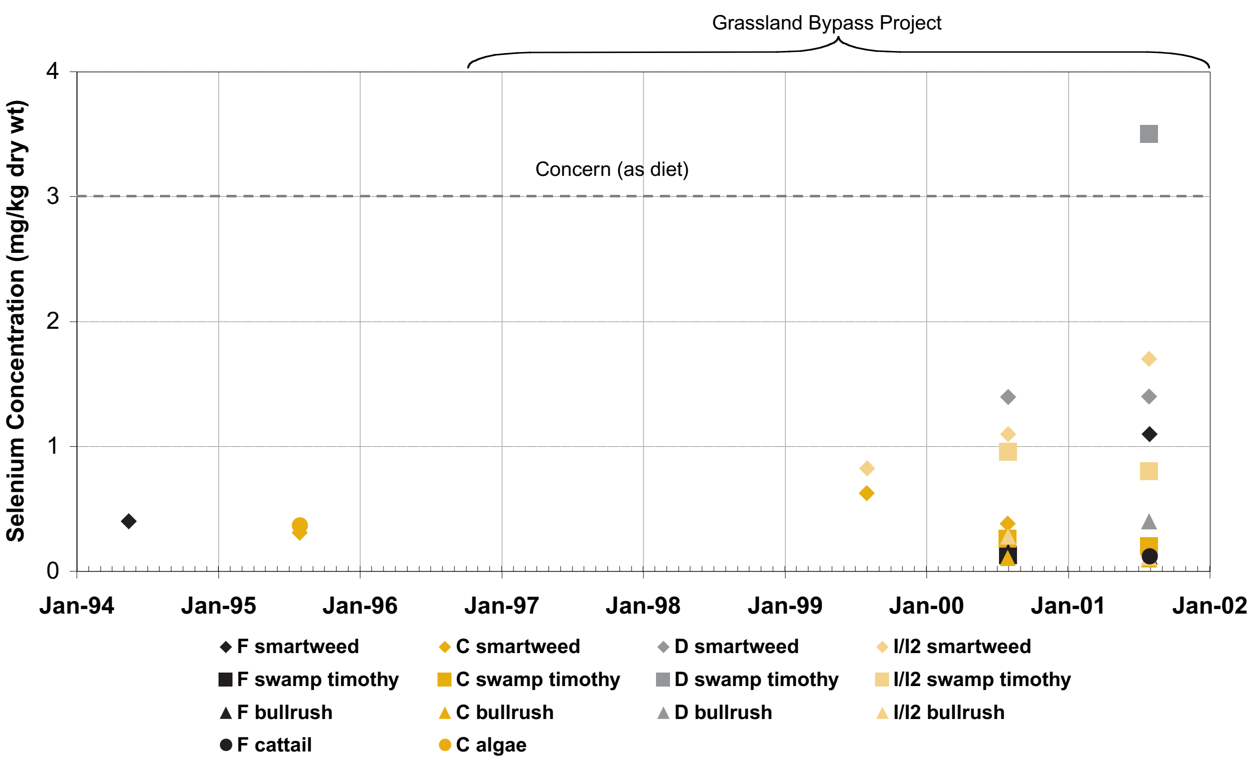
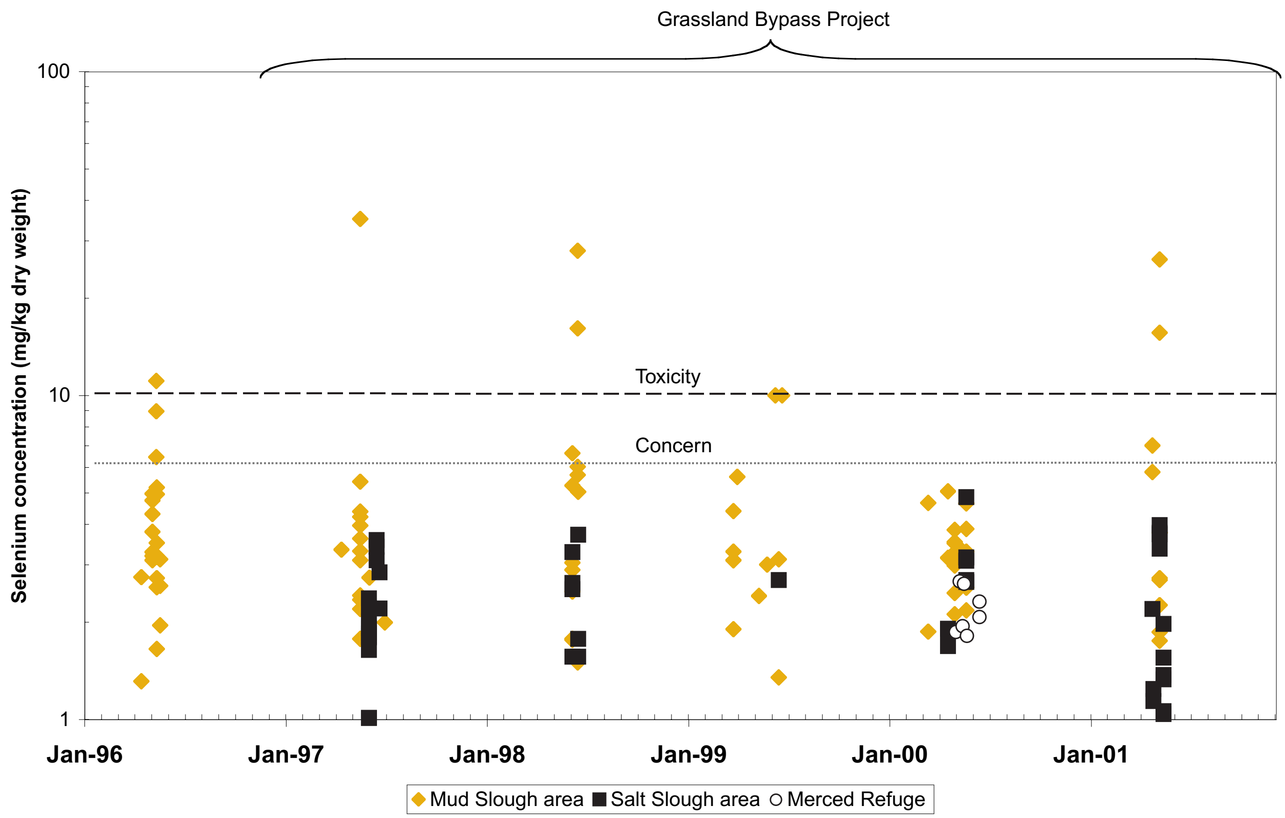


Figure 31. Selenium in bird eggs.



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