Chapter 7

BIOLOGICAL EFFECTS OF THE GRASSLAND BYPASS PROJECT

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ABSTRACT

In its eighth and ninth years of operation (2004 and 2005), the Grassland Bypass Project continued to reduce the risk of selenium toxicity in the ecosystem from which the Project removed agricultural subsurface drainwater, with continued elevated risk in waterways into which the drainwater has been discharged by the Project. In Salt Slough, where drainwater has been removed by the Project, selenium concentrations in fish and invertebrates remained largely below thresholds of concern. The overall selenium hazard (Lemly index) to the Salt Slough ecosystem rose from low to moderate. In Mud Slough below the outfall of the San Luis Drain (SLD), selenium concentrations in fish and invertebrates continued generally to exceed thresholds of concern; average concentrations have not dropped as loads and concentrations of selenium in water in Mud Slough have declined. The Lemly index of selenium hazard to the aquatic ecosystem remained high in this area. Selenium concentrations did not exceed the 2 mg Se/kg (wet weight) human Health Screening Level in seventeen of nineteen composite samples of carp muscle tissue collected in Mud Slough below the SLD in 2004 and 2005.

After dramatically increasing in numbers at some sites in 2003, the invasive Siberian freshwater shrimp (Exopalaemon modestus), became firmly-established as a major component of aquatic ecosystems at all monitoring sites in 2004 and 2005. This species evidently bioconcentrates selenium more efficiently than other aquatic invertebrates, and may be contributing to the persistence of elevated concentrations of selenium in the biota as loads of selenium discharged into Mud Slough have been generally declining.

In the San Joaquin River upstream of the Mud Slough confluence (Fremont Ford), selenium concentrations in whole-body fish and invertebrates remained below the threshold of concern. The selenium concentrations in carp muscle tissues collected at this site during 2004 and 2005 remained below the 2 mg Se/kg human Health Screening Level.

In the San Joaquin River downstream of the Mud Slough confluence (Hills Ferry), selenium concentrations in whole-fish exceeded the threshold of concern in eight of 24 samples collected in 2004 and 2005. Selenium concentrations in red crayfish collected from this site were less than the 3 mg Se/kg concern threshold in twelve of thirteen composite samples. The concentrations of selenium in all samples of carp muscle tissue collected at this site were below the 2 mg Se/kg human Health Screening Level.

Selenium concentrations in all seed samples collected along Salt Slough and along Mud Slough upstream of the SLD discharge remained entirely below levels of concern as diet for waterbirds; along Mud Slough downstream the outfall of the SLD, concentrations of selenium in seeds remained largely below levels of concern. Boron concentrations most seed samples (four of seven) collected from along Salt Slough were below the threshold of concern as diet for waterbirds; along Mud Slough, concentrations of boron in seeds were above the threshold of concern. Along the San Joaquin River above the Mud Slough confluence (near Fremont Ford) and below the confluence with the Merced River (Hills Ferry), the concentrations of boron in all samples of seeds were above the 10 mg Se/kg threshold of concern in 2004 and all samples collected in 2005 exceeded the 30 mg Se/kg level of toxicity.

INTRODUCTION

Project History

In 1985, the San Luis Drain (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 agricultural drainwater from Westlands Water District in the western San Joaquin Valley.

Farms in the adjacent Grassland Drainage Area (GDA) never used the SLD, but discharged agricultural 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).

Discharge of agricultural drainwater from GDA farms was unaffected by the closure of the SLD, and drainage continued to contaminate Refuge water delivery channels after the closure of the SLD and Kesterson Reservoir. 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 SLDMWA 1995; USBR et al., 1995). This project, known as the Grassland Bypass Project (GBP), diverts agricultural drainwater from GDA farms 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 rights to create and restore wetlands on the Refuges.

The GBP continues to contaminate the northernmost six 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 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 affected by the GBP.

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 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 proposed 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 selenium risk guidelines have been divided into three threshold 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 species sensitive to elevated contaminant concentrations in water, sediment, and biota, and 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.

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 4 mg Se/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 Se/kg, 3.4 mg Se/kg, 3.9 mg Se/kg, or 5.9 mg Se/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 Se/kg (NOEC). Fifty nine days of exposure to dietary concentrations that resulted in tissue concentrations of 4.2 mg Se/kg (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 Se/kg. A similar analysis of a water-borne selenium exposure experiment (Cleveland et al., 1993) yields a threshold value of 3.3 mg Se/kg.

Other data in Cleveland et al. (1993) may be interpreted to support a threshold closer to 4 mg Se/kg or a threshold of 5.9 mg Se/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 Se/kg did not exhibit adverse effects that were significantly greater than controls, but bluegill with a tissue concentration of 4.6 mg Se/kg experienced significantly increased mortality. Bluegill with a tissue concentration of 7.5 mg Se/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 Se/kg, was significantly worse than controls. Depending on whether or not the significant mortality at a tissue concentration of 4.7 mg Se/kg is treated as anomalous, the LOEC would be either 4.7 mg Se/kg or 7.5 mg Se/kg.

Corresponding thresholds would be 3.9 mg Se/kg (geometric mean of 3.3 mg Se/kg and 4.6 mg Se/kg) or 5.9 mg Se/kg (geometric mean of 4.6 mg Se/kg and 7.5 mg Se/kg) respectively. Given the range of possible threshold values discussed above, the concern threshold of 4 mg Se/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 that may occur 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 Se/kg is recommended by DeForest et al. (1999). In the analysis of DeForest et al. (1999) the threshold represents an EC10, 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 Se/kg dry weight. After 90 days of that diet, the selenium concentration in the salmon fry averaged 2.7 mg Se/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 Se/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 Se/kg (geometric mean of 0.8 and 2.7 mg Se/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 in their juvenile stage as they migrate downstream to the ocean.

A toxicity threshold for coldwater fish (whole body) of 9 mg Se/kg has been recommended by DeForest et al. (1999). In their analysis, the toxicity threshold represents an EC10, 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 to10% mortality) of 1.7 mg Se/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 Se/kg (dry weight) and the Toxicity threshold is 7 mg Se/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 Se/kg and the toxicity threshold is 4 mg Se/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 individual 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 Se/kg dry weight, and the toxicity threshold has been raised from 8 to 10 mg Se/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

Agency Responsibilities

The role of the California Department of Fish and Game (CDFG) and the United States Fish and Wildlife Service (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 (USBR 2001). 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 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 electro fishing. 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=0.6xWB for many freshwater fish (Lemly and Smith, 1987) to M=0.045+1.23xWB for bluegills and M=-0.39+1.32xWB 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 G and H) and Mud Slough (Sites C, D, I2 and E) 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 WY 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 1995-2005. This plant material was archived for later analysis.

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 2003. 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 Zero degrees centigrade 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 were 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 I2), one on Salt Slough (F) and two San Joaquin River sites (G and H). Site C is located upstream of where the SLD discharges into Mud Slough. Site D is located immediately downstream of the discharge point. Site I2 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 I2 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 persistent 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. CDFG staff conducted baseline sampling during the summer and fall of 1993 and then resumed in the spring of 1996. Between 1992 and 1995 sampling by either CDFG or USFWS staff 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 and CDFG. 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 (**Tables 4-7**).

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).

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.

Departures from the Monitoring Plan and Quality Assurance Project Plan

To ensure reliable and consistent data, the USFWS and the CDFG followed the procedures specified in the 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 Substance 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 beginning in 1999 have been 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 earlier, 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)

Salt Slough is a principal wetland water supply channel from which drainwater has been removed by the GBP.

Selenium in fish

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 generally below the 4 mg Se/kg threshold of concern for warmwater fish (**Figures 2A-2E**).

In the period 2004-2005, of the 58 composite samples of fish collected at this site, only two samples (61 male mosquitofish 4.2 mg Se/kg; two black bullhead 4.9 mg Se/kg) exceeded the concern threshold for warmwater fish (4 mg Se/kg, **Table 1**). The average selenium concentration in all 58 composite samples was 2.6 mg Se/kg, well under the 4 mg Se/kg threshold of concern (Table 1) and significantly ($p<0.0000001$) less than the pre-project average of 6.7 mg Se/kg (n=77), but the same as the average for all composite samples collected here from 1998-2003 (2.6 mg Se/kg, n=285).

Selenium in invertebrates

In 2004-2005, selenium concentrations in almost all of the 19 composite invertebrate samples collected from Salt Slough (**Figure 2F**) remained within the range of concentrations associated with no known adverse effects (<3 mg Se/kg) on wildlife that eat invertebrates. The only exceptions were two composite samples of Siberian freshwater shrimp (3.3 mg Se/kg, n=6, in August 2004, and 4.2 mg Se/kg, n=4, in August 2005). The mean concentration of selenium in all invertebrate samples collected during this period (1.9 mg Se/kg, n=19) was significantly below (p<0.0000001) the pre-project average (4.4 mg Se/kg, n=27), but not significantly different (p=0.9) from the average for the period 1998-2003 (2.0 mg Se/kg, n=51).

Selenium in plants

Selenium concentrations in all composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Salt Slough in August 2004 and August 2005 (**Figure 2G**) were well below the dietary threshold of concern (3 mg Se/kg, see **Table 1**). Not shown in Figure 2G are five samples that were below detection or reporting limits.

Boron in plants

Three of the seven composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Salt Slough in 2004 and 2005 had boron concentrations (**Figure 2H**) above the dietary threshold of concern (30 mg Se/kg, see **Table 2**).

Mud Slough upstream of the San Luis Drain discharge (Site C)

This sampling location, about 400 m upstream of the outfall of the SLD, was intended to serve as a kind of reference site, representing the baseline conditions in Mud Slough that would prevail in lower Mud Slough (North) were it not for drainwater discharges into lower Mud Slough due to the Grassland Bypass Project. However, evidence has emerged that this site, though upstream from the SLD discharge, is close enough to the discharge point that fish samples at this site are affected by upstream movement of fish from the downstream drainwater. Evidence for this can be seen in the very high concentrations of selenium in mosquitofish and silversides sampled at this site as well as Site D (just downstream of the discharge) in the months immediately after the opening of the Grassland Bypass Project in October 1996 (compare **Figures 3A** and **3B** with **Figures 4A** and **4B**). There is no known reason for such a spike in selenium in fish at this site apart from the hypothesis that some selenium-laden fish moved upstream from the discharge of the San Luis Drain.

Selenium in fish

Following the pulse of selenium in fish in the immediate aftermath of the initial discharge of the San Luis Drain into Mud Slough (see above), selenium concentrations in fish at this site stabilized from 1998 through 2002. Except red shiners and fathead minnows, most species of fish remained at levels generally below the threshold of concern for warmwater fish (4 mg Se/kg, **Figures 3A, 3E**). In 2003, the average selenium concentration in all fish sampled at this site (3.84 mg Se/kg, n=62) rose significantly (p=0.02) above the previous year average (3.21 mg Se/kg, n=57). In 2004 and 2005 the average (3.23 mg Se/kg, n=51; and 3.62 mg Se/kg, n=55 respectively) declined, but remained somewhat elevated relative to previous years. The average selenium concentration in fish collected in the period 2003-2005 (3.58 mg Se/kg) was significantly higher (p=0.002) than in 1998-2002 (3.10 mg Se/kg). The increase in average selenium concentration in fish coincides with an increase in selenium in invertebrates evidently due to an invasive species of freshwater shrimp (see below). This suggests that this exotic species is adversely affecting the aquatic ecosystem due to its greater propensity to bioaccumulate selenium relative to other invertebrates.

Selenium in invertebrates

Unlike fish, invertebrates at Site C, above the discharge of San Luis Drain, seem to have been uninfluenced by that discharge (**Figure 3F**). This may be because they are more localized than fish.

After selenium concentrations in invertebrates collected at Site C increased in 2003 to a higher average level (2.15 mg Se/kg, n=15) than at any time since monitoring began in 1993, the average concentration in 2004 increased even further to 2.89 mg Se/kg (n=12). The average in 2005 (2.08 mg Se/kg, n=17) declined somewhat, but remained above pre-2003 levels. The average for the period 2003-2005 (2.31 mg Se/kg, n=44) was significantly higher (p=0.004) than for the period 1998-2002 (1.72 mg Se/kg, n=56).

This increase appears to have been driven by rapidly increasing numbers of a recently-arrived east Asian palaemonid shrimp known as the Siberian freshwater shrimp, *Exopalaemon modestus*. Since it appeared in the lower Sacramento River in 2000 (Hieb *et* *al*. 2002), populations evidently have exploded in rivers upstream of the delta. By 2003 it became one of the most common invertebrate species seined at this location in Mud Slough. The propensity of Siberian freshwater shrimp to bioaccumulate selenium evidently is higher than that of other aquatic arthropods in the area (**Figure 3F**).

Selenium in plants

Selenium concentrations in all composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Mud Slough at this site in 2004 and 2005 (**Figure 3G**) were well below the dietary threshold of concern (3 mg Se/kg, see **Table 1**).

Boron in plants

Boron concentrations in composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Mud Slough at Site C in 2004 and 2005 (**Figure 3H**) were generally above the dietary threshold of concern (30 mg Se/kg, see **Table 2**). Elevated boron in plants at this site may be due to the proximity of this site to the old Kesterson Reservoir. Site C is within about 50 meters of the northern levee of the northern-most cell of the reservoir. Although the reservoir has not been used to store drainwater since it was closed more than 20 years ago, residual boron from historic drainwater storage may still contaminate groundwater in the area.

Mud Slough just below San Luis Drain discharge (Site D)

This sampling location, about 200 m downstream of the outfall of the SLD, was intended to represent the effects of discharged drainwater on the biota of Mud Slough. However, this site is even closer than the upstream site (Site C) to the point where the San Luis Drain discharges in to Mud Slough. Therefore, evidence that contaminated fish swim upstream to Site C (see above) also suggests that relatively clean fish from above the discharge point swim downstream and are likely to be included among the fish seined at Site D. Consequently, composite samples collected at this site are effectively diluted by clean fish, and do not represent the full effects of drainwater discharged by the Grassland Bypass Project.

Selenium in fish

Prior to the commencement of the Grassland Bypass Project in the fall of 1996, a “flip-flop” system of agricultural drainwater management was in operation, which alternately routed drainwater through Mud Slough and Salt Slough. This management pattern is reflected in pre-project sampling of fish at this site: higher selenium concentrations in fish in 1992 and early 1993 followed by lower concentrations from late 1993 to 1996 (**Figures 4A**-**4E**). Immediately following the opening of the SLD, selenium concentrations in mosquitofish and silversides reached very high levels (**Figures 4A** and **4B**), probably as a result of the discharge of some individuals of these species from the SLD itself when the first conveyance of Grassland Area drainwater flushed previously stagnant resident water from the SLD. Over the next several months in 1997, selenium concentrations in composite fish samples dropped, evidently as a consequence of death, dispersal and depuration of these highly contaminated individuals. Thereafter, from 1998 through the most recent 2005 sampling results, selenium concentrations in fish have stabilized at this site, with most composite samples having concentrations at levels of concern (4-9 mg Se/kg, see **Table 1**). The average concentration of fish composite samples in 2004-2005 (6.0 mg Se/kg, n=79) was not significantly different (p=0.53) from the average of all samples from 1998-2003 (6.2 mg Se/kg, n=230), but remained significantly (p=0.000004) higher than the pre-project average (4.0 mg Se/kg, n=70).

The invasion of the Siberian freshwater shrimp has not had a clear effect on the fish at this site as it has at the upstream monitoring site (Site C, see above). This may be because of lower populations of the shrimp at this site, suggested by the substantially lower numbers collected here than at Site C (for example, in 2005, 193 individuals were collected at Site C while only 36 individuals were collected at Site D). In addition, it appears that the greater propensity of the shrimp to bioaccumulate selenium, relative to other invertebrates, is more pronounced at lower environmental exposures to selenium (compare **Figures 2F** and **3F** (lower selenium) with **Figures 4F** and **5F** (higher selenium)).

Selenium in invertebrates

Invertebrates have been relatively difficult to collect in numbers at Site D since the SLD began discharging drainwater into Mud Slough. The slough in this reach is generally steep-sided, deep, and fast-flowing. Scouring minimizes streamside emergent vegetation, reducing food and cover for invertebrates.

While loads of selenium discharged into Mud Slough from the SLD have declined substantially since the beginning of the GBP (see Chapter 2 of this report), and concentrations of selenium in water at this site have trended downward somewhat (see Chapter 4 of this and previous reports), selenium in invertebrates at this site has not declined but rather may have increased (**Figure 4F**). The average selenium concentration in composite invertebrate samples collected at this site in 2004-2005 (4.0 mg Se/kg, n=26) was slightly, though not significantly (p=0.33) higher than average of samples collected from the beginning of the project to the end of 2003 (3.4 mg Se/kg, n=33). For all invertebrate samples analyzed from this site since the beginning of the project (November 1996 through 2005) the average selenium concentration was 3.6 mg Se/kg, which is above the concern threshold (3 mg Se/kg) for dietary exposure of fish and wildlife.

The invasion of the Siberian freshwater shrimp (see above) may have contributed to maintaining elevated concentrations of selenium in invertebrates. A single Siberian freshwater shrimp was collected at this site in March 2003, but not until November 2003 was this species collected here in sufficient numbers to be analyzed for selenium. As elsewhere in the Grassland area, Siberian freshwater shrimp here seem to bioaccumulate selenium to higher levels than other aquatic arthropods (**Figure 4F**). However, the difference between their tendency to bioaccumulate and those of other aquatic arthropods may be reduced in such sites as this one, where selenium concentrations in the entire aquatic ecosystem are particularly elevated.

Selenium in plants

Selenium concentrations in most composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Mud Slough at this site in 2004 and 2005 (**Figure 4G**) were below the dietary threshold of concern (3 mg Se/kg, see **Table 1**). However, in August 2004 the concentration of selenium in a sample of sedge seeds reached 6.7 mg Se/kg, approaching the threshold of toxicity for selenium as diet for fish and wildlife (7 mg Se/kg, see **Table 1**).

Boron in plants

Boron concentrations in composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of Mud Slough at Site D in 2004 and 2005 (**Figure 4H**) were at or above the dietary threshold of concern (30 mg Se/kg, see **Table 2**). At this site, as at Site C, boron in plants may be elevated due to the proximity of this site to the old Kesterson Reservoir. Site D is about 200 meters down slope from the northern levee of the northern-most cell of the now-dry reservoir. More than 20 years ago, when Kesterson Reservoir was used to store subsurface agricultural drainwater from Westlands Water District, the most evapo-concentrated, high-boron drainwater ended up in this northern sector of the reservoir.

Mud Slough backwater 1.5 km below San Luis Drain discharge (Site I/I2)

Site I2 is intended to be a better representation of the adverse effects of bioaccumulative drainwater contaminants than Site D, because it consists of a backwater such as is thought to increase selenium assimilation into aquatic food chains. In addition, it is located farther downstream from the cleaner reach of Mud Slough upstream of the outfall of the SLD. Therefore, the concentrations of contaminants in mobile aquatic organisms collected here are less likely to be diluted effectively by feeding in nearby cleaner water.

Selenium in fish

With the exception of two samples of threadfin shad, all composite samples of fish collected at Site I2 in 2004 and 2005 had concentrations of selenium (**Figures 5A – 5E**) at levels of concern (4-9 mg Se/kg) or toxicity (>9 mg Se/kg). The average selenium concentration in fish in 2004-2005 (8.3 mg Se/kg, n=97) was close to the toxicity threshold of 9 mg Se/kg and significantly (p=0.0000003) above the pre-project average (4.5 mg Se/kg n=13), but was not significantly different (p=0.5) from the average for the period 1998-2003 (8.1 mg Se/kg, n=254). As at Site D, selenium concentrations in fish at this site have not declined as selenium loads in the slough have declined over the life of the GBP.

Selenium in invertebrates

As at Site D, selenium concentrations in invertebrates at this site have not declined as selenium loads in Mud Slough have trended downward since the start of the GBP (**Figure 5F**). Rather, the average selenium concentration in all invertebrates collected at Site I2 in 2004-2005 (5.6 mg Se/kg, n=29) was higher, though not significantly higher (p=0.2) than the previous project average (1997-2003: 4.8 mg Se/kg, n=65). As elsewhere, the continued high, and possibly increasing, concentrations of selenium in invertebrates at this site may be due in part to the invasion of the Siberian freshwater shrimp (**Figure 5F**).

All but three of the 29 invertebrate samples collected at this site in 2004-2005 had selenium concentrations above the threshold of concern for birds that might forage on these invertebrates (3 mg Se/kg). Eleven samples had selenium concentrations above the dietary toxicity threshold of 7 mg Se/kg.

Selenium in plants

Selenium concentrations in all composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of the Mud Slough backwater at this site in 2004 and 2005 (**Figure 5G**) were below the dietary threshold of concern (3 mg Se/kg, see **Table 1**).

Boron in plants

Boron concentrations in composite samples of waterfowl forage plant material (seed heads) that were collected along the banks of the Mud Slough backwater at Site D in 2004 and 2005 (**Figure 5H**) were above the dietary threshold of concern (30 mg Se/kg, see **Table 2**). This site is down slope from the old Kesterson Reservoir, and may be influenced by a plume of residual boron, a legacy of the boron-enriched, evapo-concentrated drainwater previously stored in the reservoir.

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 with the San Joaquin River. This site represents the lower reach of the slough that is contaminated by the operation of the Project. This point along Mud Slough is within the flood plain of the San Joaquin River, so flows are slower and more spread out. In 2005, higher flows in the slough and the San Joaquin River resulted in generally lower concentrations than in 2004.

Selenium in fish

The average concentration of selenium in 21 composite samples of whole-body mosquitofish (*Gambusia affinis*) collected during 2004 and 2005 ranged from 4.0 to 16.4 mg Se/kg (dry weight). Six of twelve samples collected in 2004 exceeded the toxicity threshold of 9 mg Se/kg (dry weight); however, all nine samples collected in 2005 were above the 4 mg Se/kg level of concern but below the toxicity threshold. The data are shown in **Figure 8A**.

The average concentration of selenium in 2004 was 10.4 mg Se/kg and in 2005 it was 5.3 mg Se/kg. The average concentration of selenium in the mosquitofish collected during both years was significantly higher than the concentration of samples collected before the Grassland Bypass Project began in 1996 ($n=12$, $\mu=2.52$, $p<0.001$).

Selenium in invertebrates

Four of five composite samples of red crayfish (*Procambarus clarkii*) collected at Site E in 2004 had selenium concentrations above the 7 mg Se/kg (dry weight) threshold of toxicity for invertebrates as diet for waterfowl (**Figure 8B**). In 2005, none of the seven composite samples exceeded the 7 mg Se/kg level of toxicity; five samples collected exceeded the 3 mg Se/kg threshold of concern and two samples were less than 3 mg Se/kg (dry weight).

The average concentration of selenium in five composite samples of red crayfish collected during 2004 was 8.6 mg Se/kg (dry weight); the 2005 average was 3.6 mg Se/kg. These concentrations were significantly higher than the 1.7 mg Se/kg (dry weight) average selenium concentration in crayfish caught at this site before 1996 ($n=15$, $p=<0.001$).

Many Siberian freshwater shrimp, *Exopalaemon modestus,* were collected at this site in 2004 and 2005. The average concentration of selenium in 36 specimens caught in 2004 was 9.7 mg Se/kg (dry weight). In 2005, 84 shrimp were collected with an average selenium concentration of 6.9 mg Se/kg (dry weight). It is interesting to note that the average wet weight concentration was the same in both years (1.6 mg Se/kg).

The concentration of selenium in waterboatmen collected from this site during 2004 was 4.1 mg Se/kg (dry weight), above the 3 mg Se/kg (dry weight) concern threshold. Only one composite sample was collected in 2005, and it had a selenium concentration of 2.8 mg Se/kg (dry weight).

Selenium in plants

Selenium concentrations in six composite samples of waterfowl forage plant material (seed heads) that were collected at this site in 2004 and 2005 were below the 3 mg Se/kg (dry weight) threshold of concern. The average concentration of selenium in three samples collected in March 2004 was 1.03 mg Se/kg, and the average of samples collected in March 2005 was 0.35 mg Se/kg.

Boron in plants

Boron concentrations in six composite samples of waterfowl forage plant material (seed heads) collected at Site E in 2004 and 2005 were above the 30 mg/kg dietary threshold of toxicity. The average concentration of boron in three samples collected in March 2004 was 74 mg/kg, and the average of samples collected in March 2005 was 54 mg/kg.

San Joaquin River at Fremont Ford (Site G)

Site G is located at Fremont Ford on the San Joaquin River upstream of the Mud Slough confluence. This site represents the reach of the San Joaquin River that is no longer contaminated with agricultural drainwater from the Grassland Drainage Area as a result of the GBP. Water in the river at this place is dominated by runoff from local refuges ad wetlands and farmland outside the GDA.

Selenium in fish

Similar to prior years of GBP operation, selenium concentrations in composite samples of whole-body fish collected from this site during 2004 and 2005 continued to reflect removal of selenium-laden drain water. Selenium concentrations in 24 composite samples of whole-body mosquitofish collected during 2004 and 2005 ranged from 1.0 to 2.2 mg Se/kg (dry weight), below the concern threshold (4 mg Se/kg dry weight) for warmwater fish (**Figure 9A**).

The average concentration of selenium in mosquitofish collected during 2004 was 1.9 mg Se/kg (dry weight); the average in 2005 was 1.5 mg Se/kg. Both are significantly less than the pre-project average concentration of selenium of 4.7 mg Se/kg (dry weight) measured in eighteen samples ($p<0.001$). Selenium concentrations in whole-body mosquitofish have consistently been below the 4 mg Se/kg (dry weight) threshold of concern since the GBP began September 1996 (**Figure 9A**).

Selenium in invertebrates

Selenium concentrations in all invertebrates collected from this site during 2004 and 2005 continued to be well below the threshold of concern (**Figure 9B**). The selenium concentrations ranged from 0.4 to 3.5 mg Se/kg (dry weight) in 35 composite samples. Only one sample of shrimp collected in August 2005 exceeded the 3 mg Se/kg (dry weight) threshold of concern for invertebrates as prey items.

The average concentration of selenium in nine composite samples of red crayfish collected during 2004 was 1.2 mg Se/kg (dry weight). In 2005, the average concentration was 1.1 mg Se/kg in five composite samples. Both years' were significantly less than the pre-project level of 3.5 mg Se/kg dry weight ($n=9$, $p<0.001$).

More than 225 Siberian freshwater shrimp, *Exopalaemon modestus,* were collected at this site in 2004 and 2005. The average concentration was 2.3 mg Se/kg. The concentration of selenium in all composite samples of shrimp were less than the 3 mg Se/kg level of concern, except for one sample collected in August 2005.

No waterboatmen were collected from this site during 2004 and 2005.

Selenium in plants

Selenium concentrations in six composite samples of waterfowl forage plant material (seed heads) that were collected at this site in 2004 and 2005 were below the 3 mg Se/kg (dry weight) threshold of concern. The average concentration of selenium in three samples collected in March 2004 was 0.11 mg Se/kg, and the average of samples collected in March 2005 was 0.08 mg Se/kg.

Boron in plants

Boron concentrations in three composite samples of waterfowl forage plant material (seed heads) collected at Site G in 2004 were 12 mg/kg, just above the 10 mg/kg level of concern. The average concentration of boron in three samples collected in March 2005 was 119 mg/kg, well above the 30 mg/kg dietary threshold of toxicity. We do not know the reason for this increase.

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 accessibility, the site was located just upstream of the Merced River confluence; Merced River waters have relatively low concentrations of selenium. It is possible that some of the fish and invertebrates collected at Site H have moved into this area after foraging within the Merced River and other less contaminated 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.

Selenium in fish

The selenium concentrations in seven of twelve composite samples of whole-body mosquitofish collected during 2004 exceeded the 4 mg Se/kg (dry weight) concern threshold for warmwater fish. During 2005, only one of twelve samples exceeded the level of concern, possibly due to much higher flows in the river. No samples exceeded the 7 mg Se/kg (dry weight) level of toxicity in 2004 and 2005. The average concentration of selenium in 2004 was 4.6 mg Se/kg (dry weight) and 2.5 mg Se/kg in 2005 (**Figure 10A**). With the exception of 2002, average selenium concentrations in composite samples throughout the all nine years of GBP operation have generally remained below the 4 mg Se/kg (dry weight) concern threshold, although 21of the 113 individual composite samples collected during this period exceeded the concern threshold; none have exceeded the 9 mg Se/kg (dry weight) level of toxicity. The average selenium concentration in 2004 was 4.0 mg Se/kg, and in 2005 it was 2.5 mg Se/kg. Both year's averages were significantly different ($p=0.04$ and <0.001) from the average selenium concentration in mosquitofish collected before the GBP began in 1996 ($n=24$, $\mu=3.8$).

Selenium in invertebrates

Selenium concentrations in seven composite samples of red crayfish collected from this site during 2004 and 2005 ranged from 2.0 to 4.36 mg Se/kg (dry weight). Five samples collected in 2004 were above the 3 mg Se/kg (dry weight) concern threshold associated with known adverse effects on higher order consumers (**Figure 10B**); none exceeded the 7 mg Se/kg level of toxicity. Only one sample of red crayfish was caught in March 2005 with 2.2 mg Se/kg (dry weight) of selenium; this was less than the 3 mg Se/kg level of concern.

The concentration of selenium in one composite sample of water boatmen, collected March 2004, was 2.7 mg Se/kg (dry weight), below the 3 mg Se/kg level of concern.

The average concentration of selenium in seven composite samples of red crayfish caught during 2003 was 2.36 mg Se/kg (dry weight). This average was the same as the previous water year ($n=8$, $\mu=2.39$, $p=0.94$). The 2003 concentration of selenium was not statistically different than the concentration measured in nine samples collected before the project began in 1996 ($\mu=2.08$ mg Se/kg, $p=0.45$).

More than 235 Siberian freshwater shrimp, *Exopalaemon modestus,* were collected at this site in 2004 and 2005. The average concentration of selenium was 4.9 mg Se/kg (dry weight) in 2004, and 3.7 mg Se/kg in 2005. The concentrations of selenium in all composite samples of shrimp were more than the 3 mg Se/kg level of concern, and none exceeded the 7 mg Se/kg (dry weight) level of toxicity.

Selenium in plants

Selenium concentrations in six composite samples of waterfowl forage plant material (seed heads) that were collected at this site in 2004 and 2005 were below the 3 mg Se/kg (dry weight) threshold of concern. The average concentration of selenium in three samples collected in March 2004 was 0.31 mg Se/kg, and the average of samples collected in March 2005 was 0.13 mg Se/kg.

Boron in plants

Boron concentrations in three composite samples of waterfowl forage plant material (seed heads) collected at Site G in 2004 were 16 mg/kg, just above the 10 mg/kg level of concern. The average concentration of boron in three samples collected in March 2005 was 90 mg/kg, well above the 30 mg/kg dietary threshold of toxicity. We do not know the reason for this increase that also occurred upstream in the river at Site G.

Fish Community Assessment

Fish community assessments are conducted to describe 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. Samples are typically collected in March, June, August /September, and November/December of the years 1996 – 2005. Only data collected with standardized sampling methodologies and effort were analyzed.

Table 3 is a compilation of the 43 fish and invertebrate species (n = 40,768), that have been collected at these sites during five pre-project and 36 Project sampling events. Thirteen species of native fish have been caught, representing only four percent of the catch by number (n = 1,588).

The native species were:

California roach (*Hesperoleucus symmetricus*)	39
Chinook salmon (*Oncorhynchus tshawystcha*)	22
Hardhead (*Mylopharodon cocncephalus*)	6
Hitch (*Lavinia exilicauda*)	14
Pacific staghorn sculpin (*Leptocottus armatus*)	89
Prickly sculpin (*Cottus asper*)	35
Riffle sculpin *(Cottus golosus*)	1
Sacramento blackfish (*Orthodon macrolepidotus*)	255
Sacramento pikeminnow *(Ptychocheilus grandis*)	28
Sacramento splittail (*Pogonichthys macrolepidotus*)	125
Sacramento sucker (*Catostomus occidentalis*)	47
Speckled dace (*Rhinichthys osculus Carrington*)	15
Tule perch (*Hysteocarpus traski*)	912

The CDFG operates a fish screen at Site H to prevent fall-run salmon from moving upstream to the sampling sites for this project.

Tule perch were the most abundant native fish at the three sites throughout the study. The most common non-native fish are mosquitofish (*Gambusia affinis*), inland silversides (*Menidia beryllina*), carp (*Cyprinus carpio*), and fathead minnow (*Pimephales promelas*).

No time trends are apparent in fish species assemblages during the period 1993 to 2005at Sites E, G, and H (**Figures 11** - **13**). Omnivores were dominant at Site E and invertivores were dominant at Sites G and H in the San Joaquin River. No time trend is evident in total anomalies for the various groups of fishes at each site (**Figure 14**).

After nine years of Project operation, no clear pattern of temporal or geographic variation in fish community structure attributable to the Project has emerged. However, current methods of assessing fish species assemblages may lack the power to detect all but the most pronounced alterations in community structure.

Assessment of Risk to Public Health from Consumption of Fish

A public health advisory on consumption of fish is in effect for the Grasslands area (OEHHA 2001):

> *Because of elevated selenium levels, no one should eat more than four ounces of fish from the Grassland area, in any two-week period. Women who are pregnant or may become pregnant, nursing mothers, and children age 15 and under should not any eat fish from this area.*

To assess current human health risks due to selenium in gamefish, carp (*Cyprinus cario*) were collected from Mud Slough (Site E) and the San Joaquin River (Sites G and H). Samples of skinless fillets from these fish were analyzed for selenium and compared with the 2 mg Se/kg wet weight interim internal guidance and screening level for selenium established by the Office of Environmental Health Hazard Assessment (OEHHA).

The concentration of selenium in twelve composite samples of carp collected at Site E during 2004 ranged from 0.8 to 2.5 mg Se/kg (wet weight). One sample of carp muscle tissue collected in June 2004 exceeded the 2 mg Se/kg (wet weight) health screening level for selenium. In 2005, the concentration of selenium ranged from 0.4 to 2.1 mg Se/kg (wet weight). Only one sample collected in August 2005 exceeded the screening level. This indicates that selenium in carp in Mud Slough below the outfall of the SLD may represent a continuing risk for human consumption (**Figure 15**). However, that risk has not changed notably during the nine years of GBP operation. During this period, 16 of 93 samples of carp muscle collected at Site E exceeded the screening level, whereas none of the 14 samples of carp tissue collected at Site E before the Grassland Bypass Project began in October 1996 exceeded the screening level. The average concentration of selenium in carp tissue samples collected at Site E in 2004 and 2005 were significantly higher than the average of fourteen samples collected prior to the beginning of the project in 1996 (μ=0.82 mg Se/kg).

The average concentrations of selenium in all carp fillets collected at Sites G in 2004 and 2005 (μ=0.6 mg Se/kg wet wt, n=24) and H (μ=0.5 mg Se/kg wet weight, n=24) on the San Joaquin River remained well below the 2 mg Se/kg health screening level throughout all years of GBP operations (**Figures 16** and **17**).

Selenium in amphibians

Since the beginning of the GBP there has not been a strong relationship between the selenium concentrations in samples of tadpoles (entirely or almost entirely bullfrog, *Rana catesbeiana*) and the amount of seleniferous drainwater in ambient water (**Figure 6**). For example the average selenium concentration in composite samples collected at Site D, below the outfall of the SLD (4.4 mg Se/kg, n=8) is not significantly higher (p=0.25) than the average in samples collected at Site F on Salt Slough (3.5 mg Se/kg, n=12).

Selenium in bird eggs

Of the 16 bird eggs collected in the Grassland area in 2004-2005 (**Figure 7**), only one egg (11.8 mg Se/kg), collected March 2005 along the SLD, exceeded the toxicity threshold for avian eggs (10 mg Se/kg; see **Table 1**). All other eggs had selenium concentrations below the threshold of concern (6 mg Se/kg). Note that 15 eggs collected in 2003, but not previously analyzed, have now been analyzed and are shown in **Figure 7**. These data have been used to update the Lemly index for 1993 (see below).

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. Because the Lemly index is based on maximum concentrations, it is sensitive to data “outliers.” However, it remains the best selenium hazard index available at this time.

Lemly's procedure applied to Mud Slough downstream of the SLD outfall indicated that the hazard to aquatic life continued to be "high" in 2004 and 2005 (**Table 4**). In the Salt Slough area, the Lemly index rose from “low” in 2003 to "moderate" in 2004, and remained “moderate” in 2005 (**Table 4**).

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.

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TABLES

FIGURES

Table 1. Recommended Ecological Risk Guidelines for Selenium Concentrations

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

Notes:
1/ 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)
2/ A tiered approach is suggested with whole body fish being the most meaningful in assessment of ecological risk in a flowing system.
3/ 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."
4/ The toxicity threshold for warmwater fish (whole body) is the concentration at which 10% of juvenile fish are killed (DeForest et al., 1999).
5/ The guidelines for vegetation and invertebrates are based on dietary effects on reproduction in chickens, quail and ducks (Wilber, 1980; Martin, 1988; Heinz, 1996).
6/ If invertebrate selenium concentrations exceed 6 mg/kg then avian eggs should be monitored (Heinz et al., 1989; Stanley et al., 1996).

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**	**> 10**	**>30**

Notes:
1/ 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).
2/ Waterfowl diet guidelines are based on mallard ducks (Smith and Anders 1989).
3/ 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 Welsh; J. P. Skorupa et al.).
4/ 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).
5/ The US Environmental Protection Agency's suggested no adverse response level for drinking water is 0.6 mg/L.

Table 3. Summary of Community Assessment data

Common name	Scientific Name	2003 report	Origin	Trophic Classification	Tolerance to environmental degradation
Mosquitofish	*Gambusia affinis*	18,151	Introduced	I	T
Inland silverside	*Menidia beryllina*	3,396	Introduced	I	M
Carp	*Cyprinus carpio*	2,859	Introduced	O	T
Fathead minnow	*Pimephales promelas*	2,216	Introduced	O	T
Red shiner	*Cyprinella lutrensis*	1,585	Introduced	O	T
White catfish	*Ameiurus catus*	1,513	Introduced	I/P	T
Bluegill	*Lepomis macrochirus*	940	Introduced	I	T
Threadfin shad	*Dorosama petenese*	610	Introduced	I	M
Largemouth bass	*Micropterus salmoides*	470	Introduced	P	T
Goldfish	*Carassius auratus*	429	Introduced	O	T
Green sunfish	*Lepomis cyanellus*	420	Introduced	I/P	T
Redear sunfish	*Lepomis microlophus*	307	Introduced	I	M
Channel catfish	*Ictalurus punctatus*	276	Introduced	I/P	M
Sacramento blackfish	*Orthodon microlepidotus*	223	Native	O	T
Warmouth	*Lepomis gulosus*	215	Introduced	I	M
Splittail	*Pogonichthys macrolepidotus*	115	Native	O	M
Bigscale logperch	*Percina macrolepida*	101	Introduced	I	T
Pacific staghorn sculpin	*Leptocottus armatus*	74	Native	I/P	M
Black crappie	*Pomoxis nigromaculatus*	58	Introduced	I/P	M
Spotted bass	*Micropterus punctulatus*	52	Introduced	P	M
Striped bass	*Morone saxatilis*	52	Introduced	P	M
Brown bullhead	*Ameiurus nebulosus*	47	Introduced	I/P	T
Red crayfish	*Procambarus clarkii (Scapulicambarus)*	43	Introduced	O	T
Shrimp species		39	Introduced	O	
Smallmouth bass	*Micropterus dolomieui*	37	Introduced	I/P	M
Sacramento sucker	*Catostomus occidentalis*	31	Native	O	M
Prickly sculpin	*Cottus asper*	30	Native	I	M
Chinook salmon	*Oncorhynchus tshawytscha*	27	Native	I	I
Sacramento pikeminnow	*Ptychocheilus grandis*	21	Native	I/P	M
Black bullhead	*Ameiurus melas*	18	Introduced	I/P	T
Bullfrog	*Rana catesbeiana*	16	Introduced	O	T
Golden Shiner	*Notemigonus crysoleucas*	16	Introduced	I	M
Hitch	*Lavinia exilicauda*	14	Native	O	M
American shad	*Alosa sapidissima*	13	Introduced	I	M
White crappie	*Pomoxis annularis*	11	Introduced	I/P	T
California roach	*Hesperoleucus symmetricus*	7	Native	I	
Tule perch	*Hysteocarpus traski*	4	Native	I	I
Pumpkinseed	*Lepomis gabbosus linaeas*	2	Introduced	I	M
Riffle sculpin	*Cottus gulosus*	1	Native	I	M
Total		34,439			

Data Source: California Department of Fish and Game

Notes:

Trophic Classification:
- O - omnivore
- I - invertivore
- P - piscivore
- I/P - invertivore/piscivore

Tolerance to environmental degradation:
- I - intolerant
- M - moderately tolerant
- T - tolerant

Table 4. Aquatic Hazard Assessment of Selenium in Mud and Salt Slough (Lemly Index)

		BEFORE PROJECT			GRASSLAND BYPASS PROJECT Phase I														
		1995 - Sept. 1996			WY1997			WY1998			WY1999			WY2000			WY2001		
	Units	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale
Mud Slough below Drain outfall																			
Water	µg/L	19	high	5	80	high	5	104	high	5	50.7	high	5	66	high	5	51	high	5
Sediment	µg/g	0.4	none	1	0.8	none	1	2	low	3	4.8	high	5	4.4	high	5	3.5	moderate	4
Invertebrates	µg/g	1.6	none	1	3.3	low	3	11	high	5	7	high	5	15.3	high	5	7.1	high	5
Fish eggs	µg/g	14.2	moderate	4	56.1	high	5	34.2	high	5	39.6	high	5	46.5	high	5	54.8	high	5
Bird eggs	µg/g	3.1	minimal	2	4.4	minimal	2	6.6	low	3	10	low	3	5.1	low	3	7.0	low	3
TOTAL HAZARD SCORE			**Moderate**	**13**		**High**	**16**		**High**	**21**		**High**	**23**		**High**	**23**		**High**	**22**
Salt Slough																			
Water	µg/L	38	high	5	3	moderate	4	5.1	high	5	1.5	minimal	2	1.7	minimal	2	2.1	low	3
Sediment	µg/g	0.8	none	1	0.9	none	1	2.1	low	3	0.93	none	1	0.7	none	1	0.8	none	1
Invertebrates	µg/g	4.7	moderate	4	2.6	minimal	2	3.15	low	3	2.8	minimal	2	2.7	minimal	2	0.7	minimal	2
Fish eggs	µg/g	28.1	high	5	17.8	moderate	4	12.9	moderate	4	11.2	moderate	4	14.5	moderate	4	12.5	moderate	4
Bird eggs	µg/g	5.2	low	3	3.6	minimal	2	3.72	minimal	2	2.7	none	1	4.9	minimal	2	4.0	minimal	2
TOTAL HAZARD SCORE			**High**	**18**		**Moderate**	**13**		**High**	**17**		**Low**	**10**		**Low**	**11**		**Moderate**	**12**

Table 4. Aquatic Hazard Assessment of Selenium in Mud and Salt Slough (Lemly Index) cont.

		GRASSLAND BYPASS PROJECT Phase II											
		2002+ October 1, 2001 - December 31, 2002			Calendar Year 2003			Calendar Year 2004			Calendar Year 2005		
	Units	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale	Maximum Selenium concentration	Lemly Aquatic hazard	Hazard Scale
Mud Slough below Drain outfall													
Water	µg/L	55	high	5	48	high	5	48.9	high	5	36.6	high	5
Sediment	µg/g	8.5	high	5	7.8	high	5	7.5	high	5	6.4	high	5
Invertebrates	µg/g	7.5	high	5	10.5	high	5	12.97	high	5	12.7	high	5
Fish eggs	µg/g	51.5	high	5	53.2	high	5	54.6	high	5	48.8	high	5
Bird eggs	µg/g	3.2	minimal	2	5.62	Low	3	4.74	minimal	2	11.8	Low	3
TOTAL HAZARD SCORE			**High**	**22**		**High**	**23**		**High**	**22**		**High**	**23**
Salt Slough													
Water	µg/L	1.1	minimal	2	1.3	minimal	2	1.1	minimal	2	1.5	minimal	2
Sediment	µg/g	0.7	none	1	0.75	none	1	0.64	none	1	1.5	minimal	2
Invertebrates	µg/g	2.4	minimal	2	2.5	minimal	2	3.32	Low	3	4.21	moderate	4
Fish eggs	µg/g	13.8	moderate	4	11.6	moderate	4	10.6	moderate	4	11.6	moderate	4
Bird eggs	µg/g	2.7	none	1	1.46	none	1	5.00	minimal	2	5.86	Low	3
TOTAL HAZARD SCORE			**Low**	**10**		**Low**	**10**		**Moderate**	**12**		**Moderate**	**15**

Notes: Table prepared by US Fish and Wildlife Service, Sacramento.

Hazard Scale:		
	5	high
	4	moderate
	3	low
	2	minimal
	1	none

TOTAL HAZARD SCORE	
	High 16 - 25
	Moderate 12 - 15
	Low 9-11
	Minimal 6-8
	None 0-5

Table 5. Maximum contaminant concentration data used for the Lemly Index (Table 4) for Calendar Year 2003- Revised

Mud Slough below the San Luis Drain (Sites D and I2)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	10-Jul-03	Site D	48 µg/L	weekly grab		CVRWQCB
Sediment	6-Nov-03	Site I2	7.8 µg/g	3 - 8 cm		USBR
Invertebrates	18-Nov-03	Site I2	10.5 µg/g	red crayfish	6	USFWS
Fish eggs (*)	19-Aug-03	Site D	53.23 µg/g	red shiner	14	USFWS
Bird eggs	14-May-03	Along SLD	5.62 µg/g	barn swallow	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Salt Slough (Site F)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	27-Feb-03	Site F	1.3 µg/L	weekly grab sample		CVRWQCB
Sediment	1-Jun-03	Site F	0.75 µg/g	whole core		USBR
Invertebrates	20-Aug-03	Site F Salt Slough at boat ramp	2.5 µg/g	Red crayfish	2	USFWS
Fish eggs (*)	20-Aug-03	Site F Salt Slough at boat ramp	11.55 µg/g	western mosquitofish	42	USFWS
Bird eggs	14-May-03	San Luis Unit	1.46 µg/g	wood duck	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Table 6. Maximum contaminant concentration data used for the Lemly Index (Table 4) for Calendar Year 2004

Mud Slough below the San Luis Drain (Sites D and I2)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	8-Apr-04	Site D	48.9	weekly grab		CVRWQCB
Sediment		Site I2	µg/g	3 - 8 cm		USBR
Invertebrates	17-Aug-04	Site I2	12.97	waterboatman	~300	USFWS
Fish eggs (*)	15-Mar-04	Site D	54.6	Mississippi silverside	2	USFWS
Bird eggs	1-Jun-04	Mud Slough area	4.74	mallard	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Salt Slough (Site F)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	19-Feb-04	Site F	1.1 µg/L	weekly grab sample		CVRWQCB
Sediment		Site F	µg/g	whole core		USBR
Invertebrates	17-Aug-04	Site F Salt Slough at boat ramp	3.32 µg/g	Siberian freshwater shrimp	6	USFWS
Fish eggs (*)	16-Mar-04	Site F Salt Slough at boat ramp	10.6 µg/g	red shiner	95	USFWS
Bird eggs	1-Jun-04	San Luis Unit	5.00 µg/g	American bittern	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Table 7. Maximum contaminant concentration data used for the Lemly Index (Table 4) for Calendar Year 2005

Mud Slough below the San Luis Drain (Sites D and I2)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	5-Dec-05	Site D	36.6 µg/L	weekly grab		CVRWQCB
Sediment		Site I2	µg/g	3 - 8 cm		USBR
Invertebrates	24-Aug-05	Site I2	12.7	Siberian freshwater shrimp	5	USFWS
Fish eggs (*)	22-Jun-05	Site I2	48.8	common carp	109	USFWS
Bird eggs	29-Mar-05	Along SLD	11.8	killdeer	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Salt Slough (Site F)

Media	Sample Date	Location	Value	Sample Type	Sample Size	Data Source
Water	24-Feb-05	Site F Salt Slough at Highway 165	1.5 µg/L	weekly grab sample		CVRWQCB
Sediment		Site F	µg/g	whole core		USBR
Invertebrates	24-Aug-05	Site F Salt Slough at boat ramp	4.21 µg/g	Siberian freshwater shrimp	4	USFWS
Fish eggs (*)	5-Jun-05	Site F Salt Slough at boat ramp	11.6	western mosquitofish	150	USFWS
Bird eggs	21-Jun-05	San Luis Unit	5.86 µg/g	killdeer	1	USFWS

(*) fish egg selenium = fish wholebody selenium x 3.3

Figure 1. Grassland Bypass Project biota monitoring site

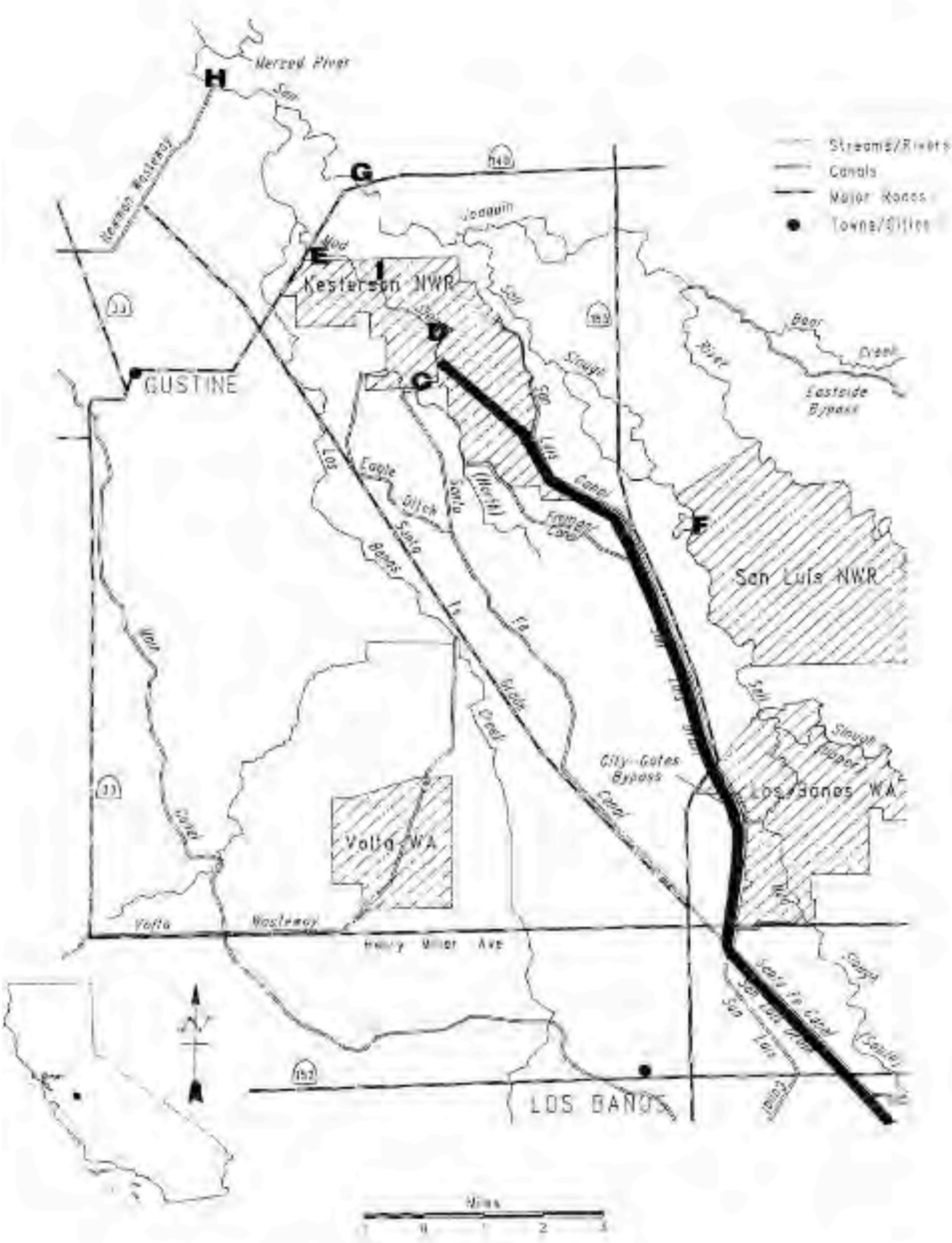

Figure 2A. Selenium in mosquitofish in Salt Slough (Site F)

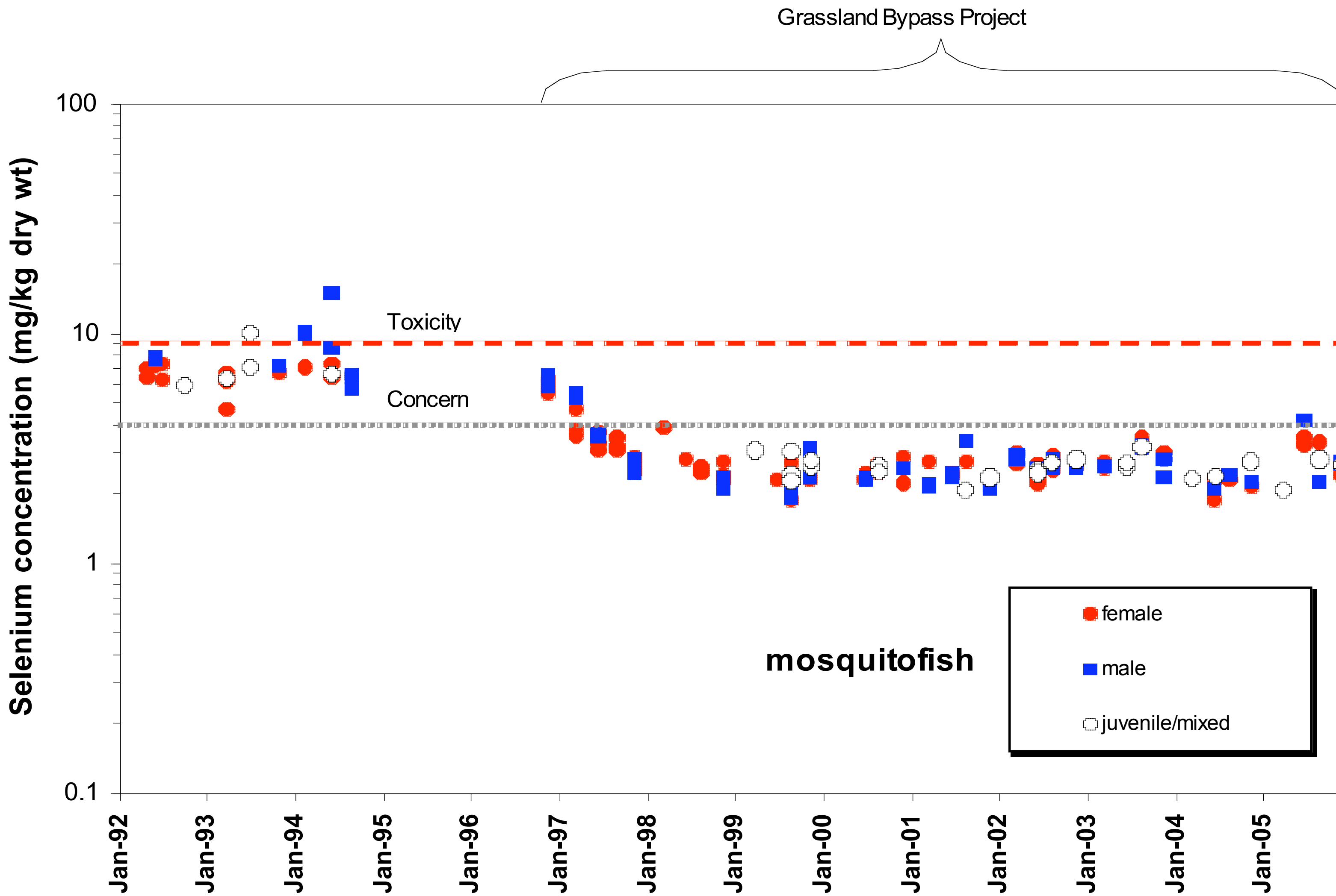

Figure 2B. Selenium in Mississippi silversides in Salt Slough (Site F)

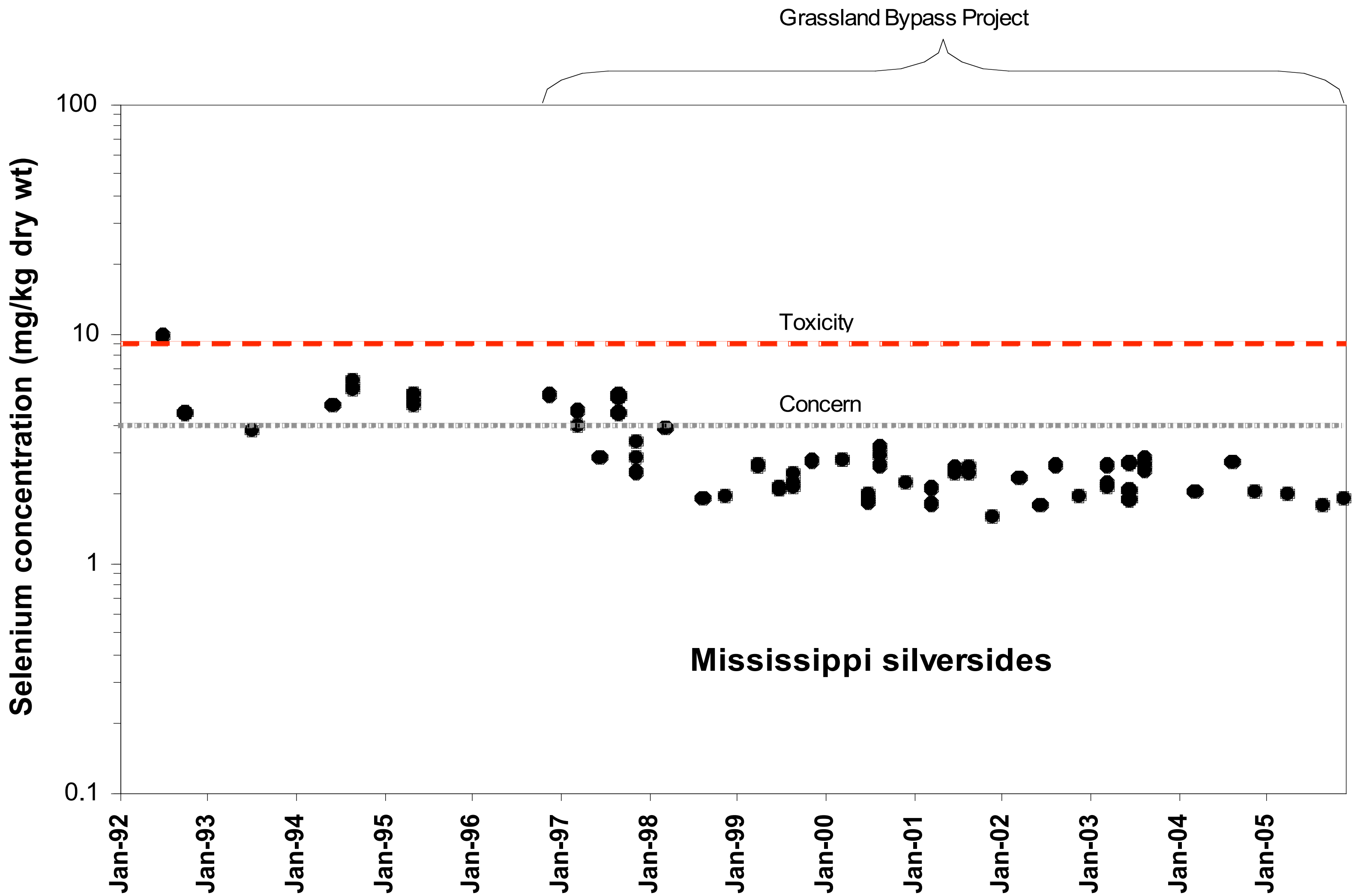

Figure 2C. Selenium in minnows in Salt Slough (Site F)

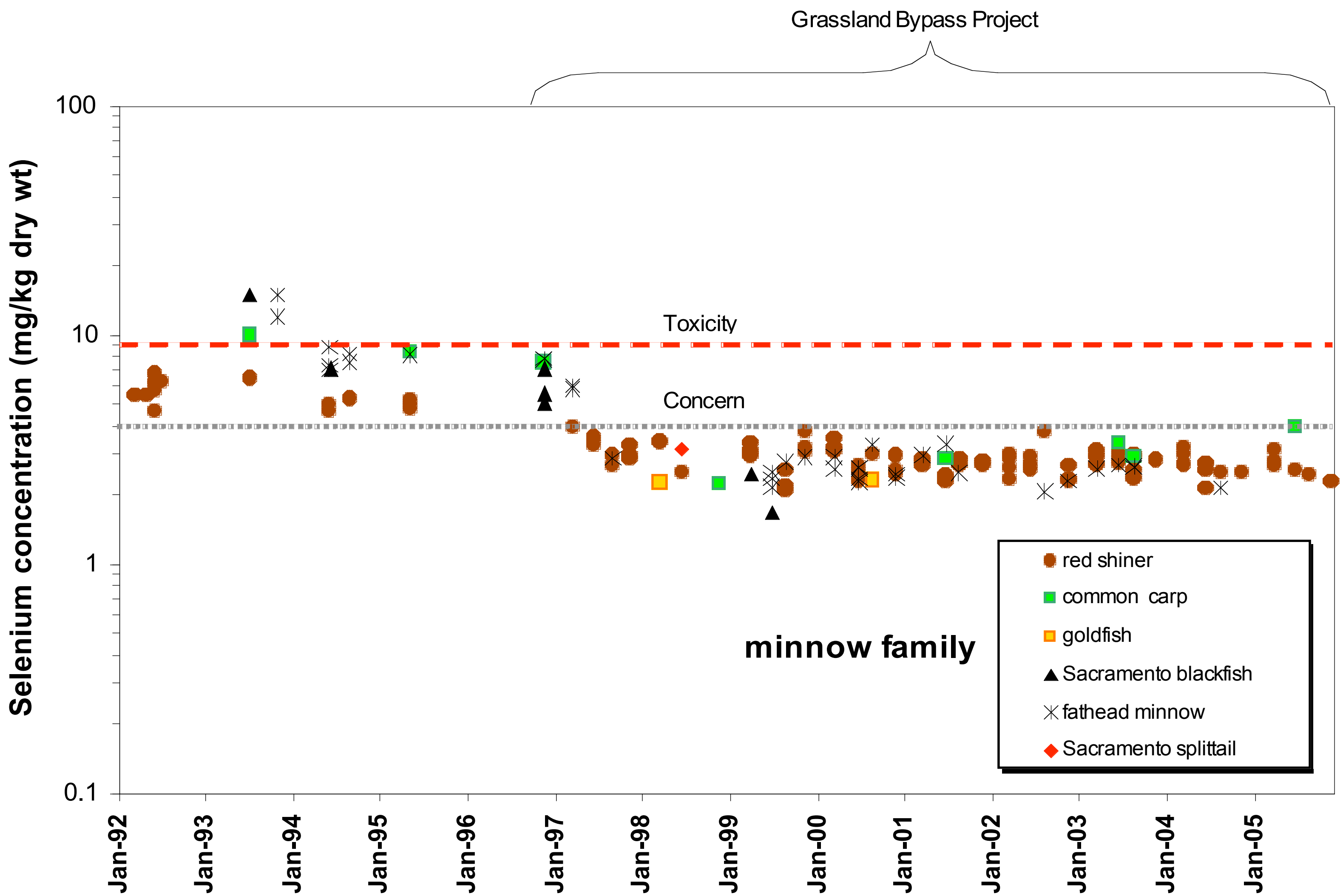

Figure 2D. Selenium in sunfish and bass in Salt Slough (Site F)

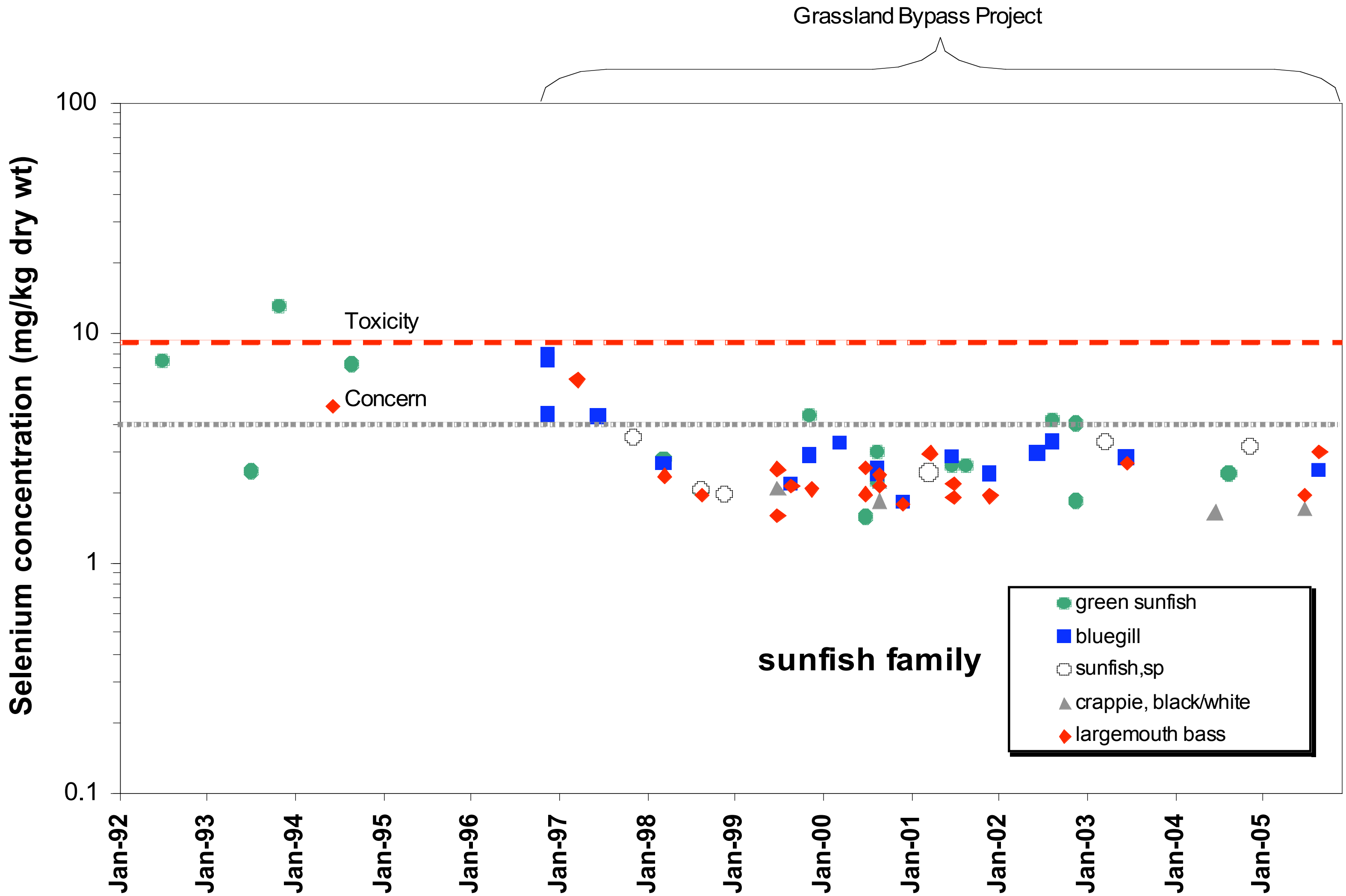

Figure 2E. Selenium in various fish in Salt Slough (Site F)

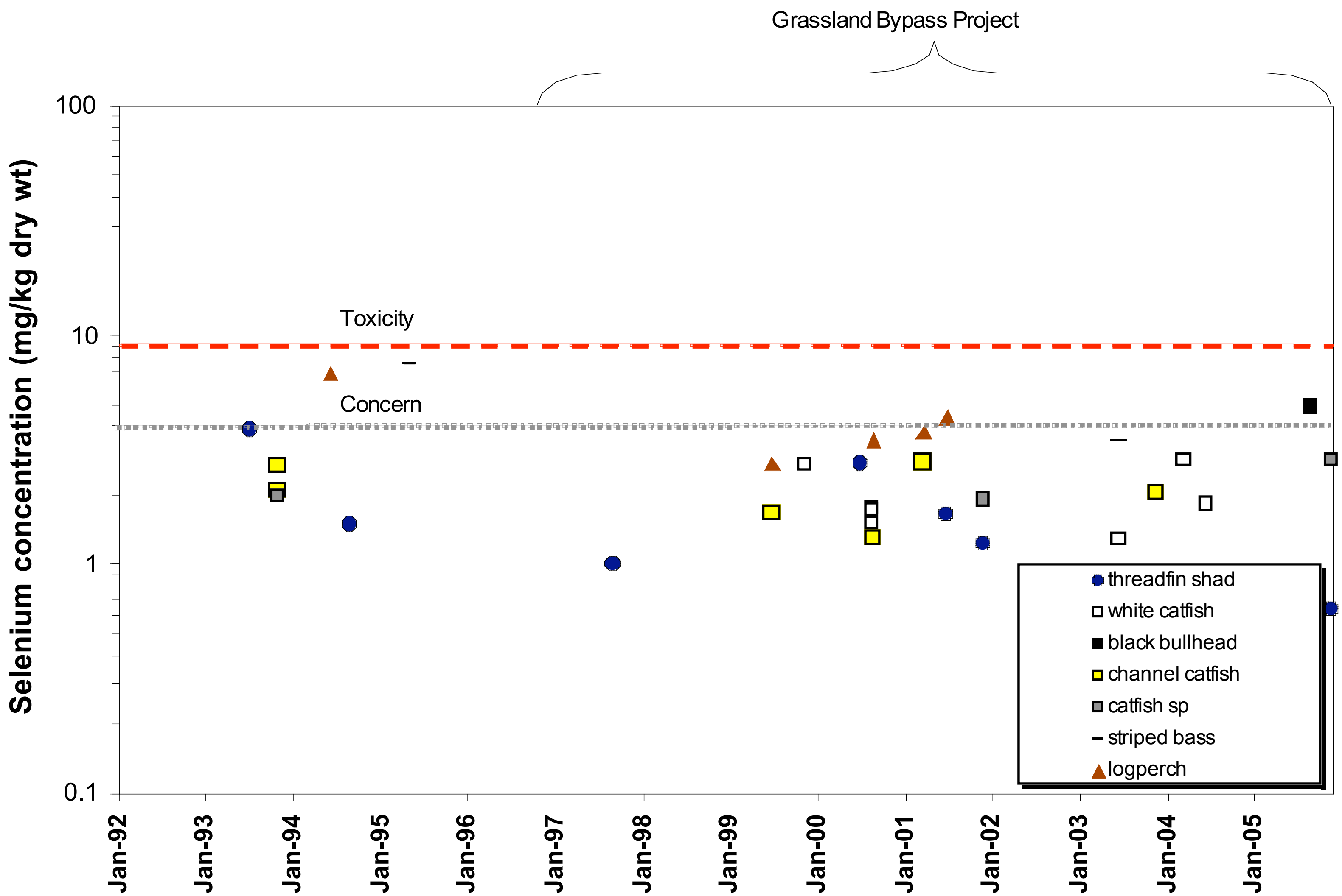

Figure 2F. Selenium in invertebrates in Salt Slough (Site F)

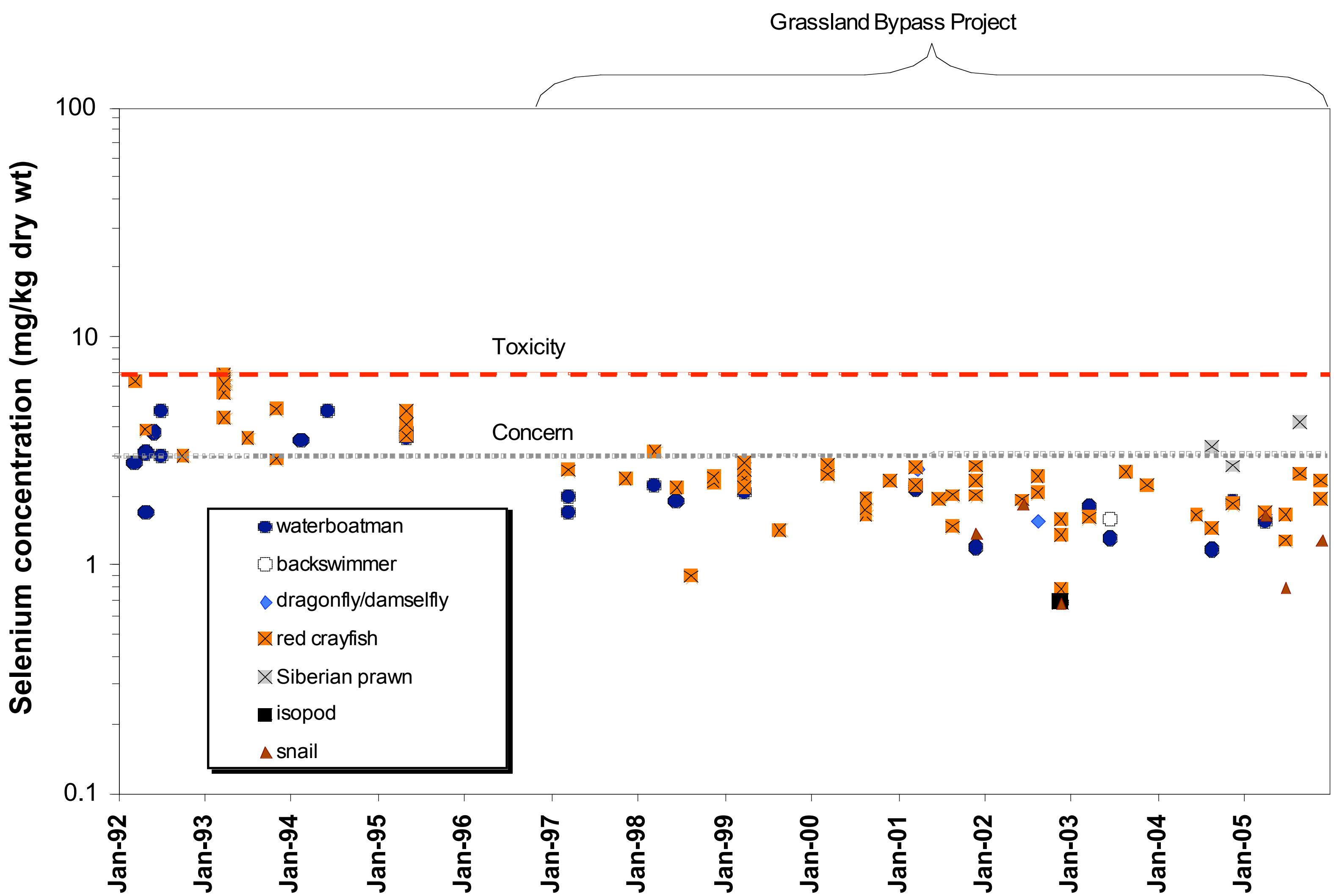

Figure 2G. Selenium in plants along Salt Slough (Site F)

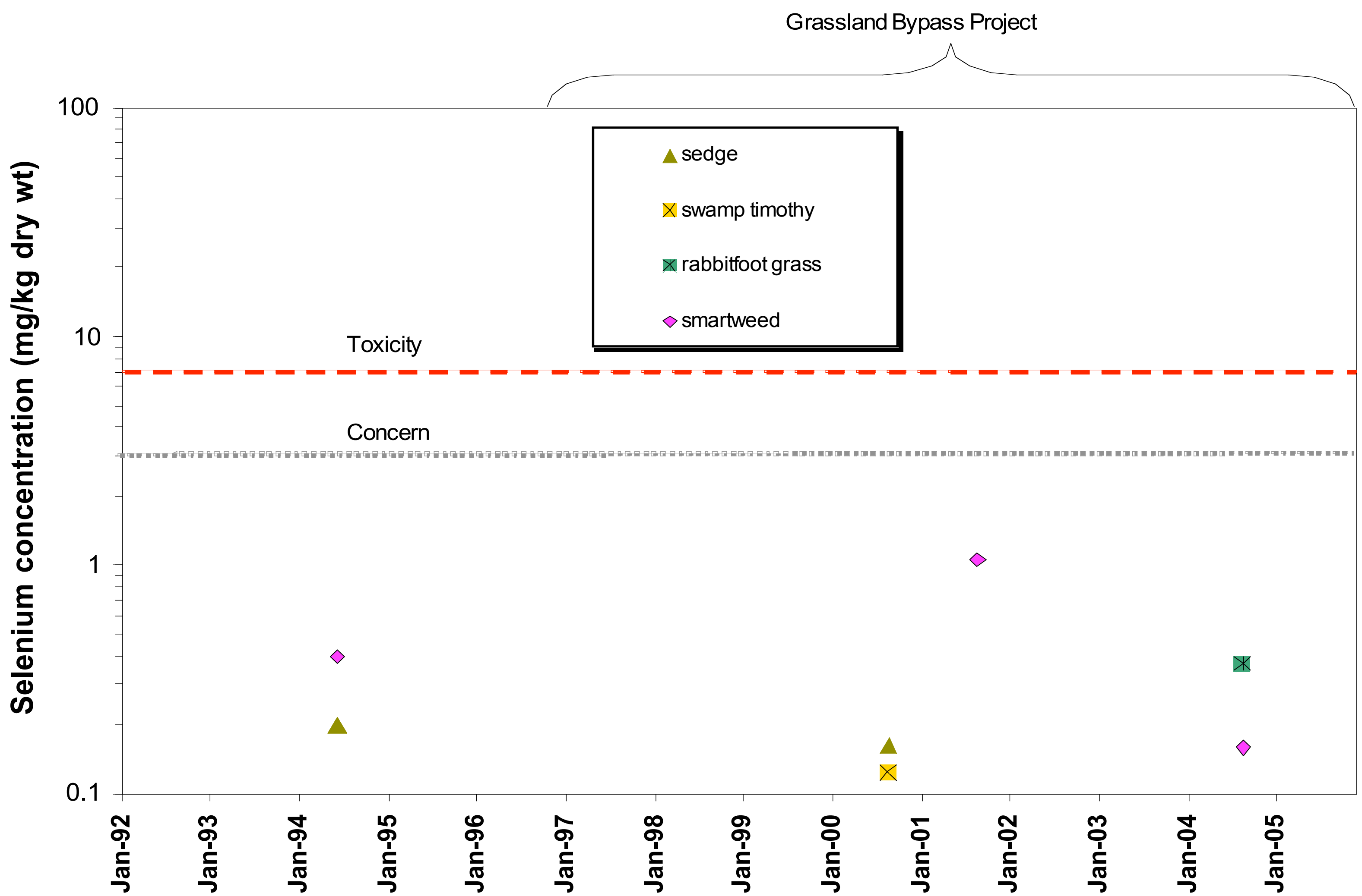

Figure 2H. Boron in plants along Salt Slough (Site F)

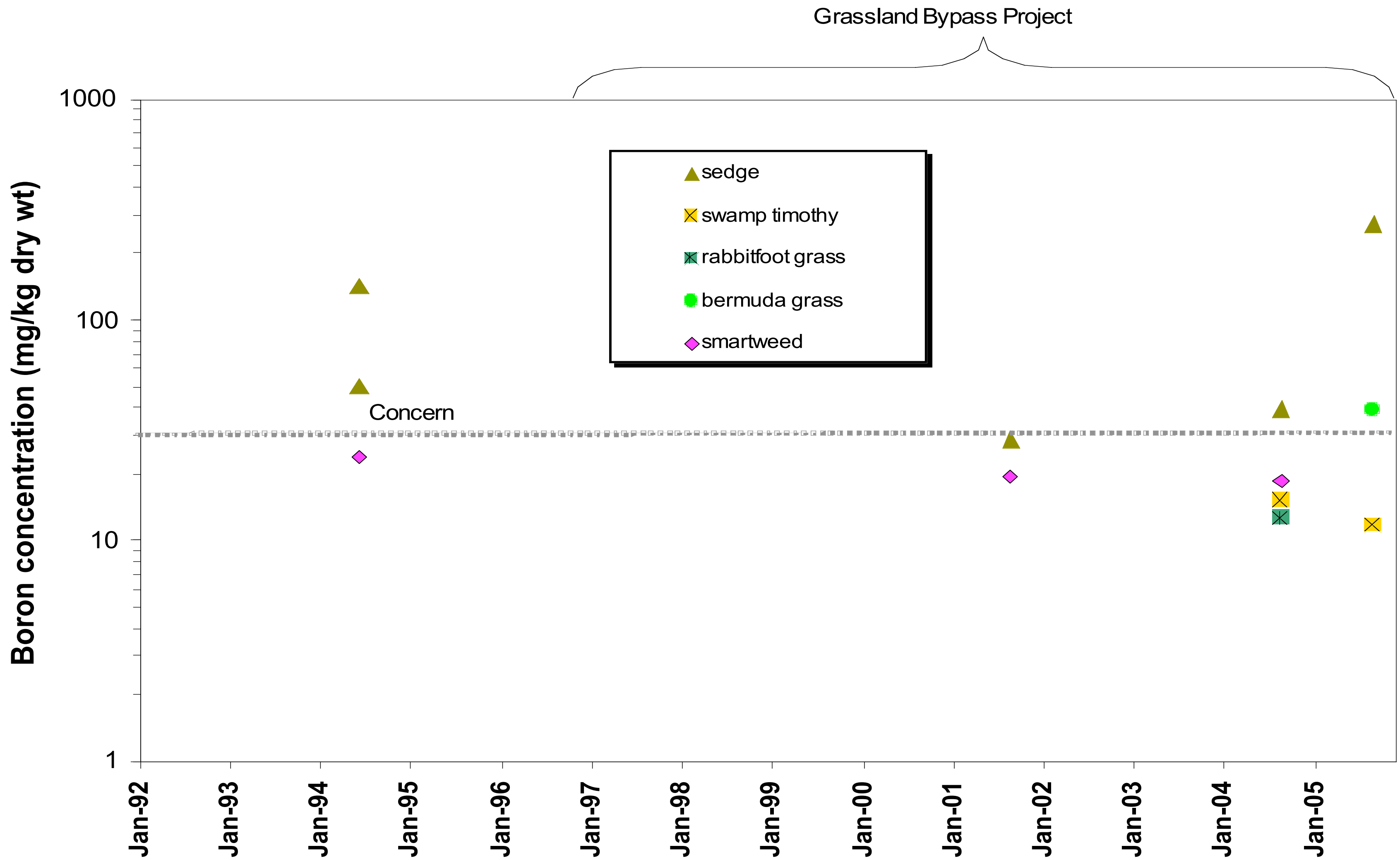

Figure 3A. Selenium in mosquitofish in Mud Slough above the San Luis Drain discharge (Site C)

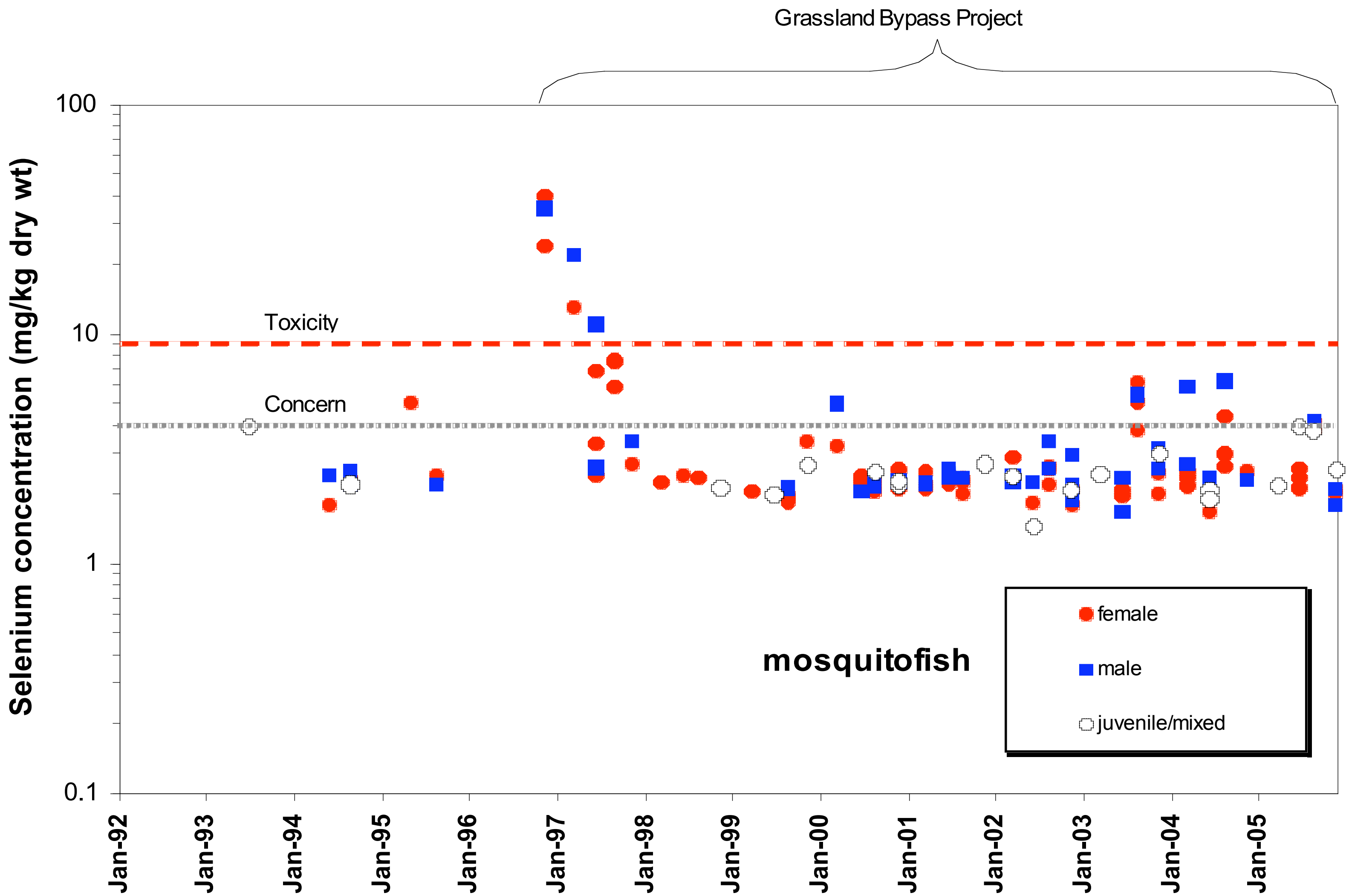

Figure 3B. Selenium in Mississippi silversides in Mud Slough above the San Luis Drain discharge (Site C)

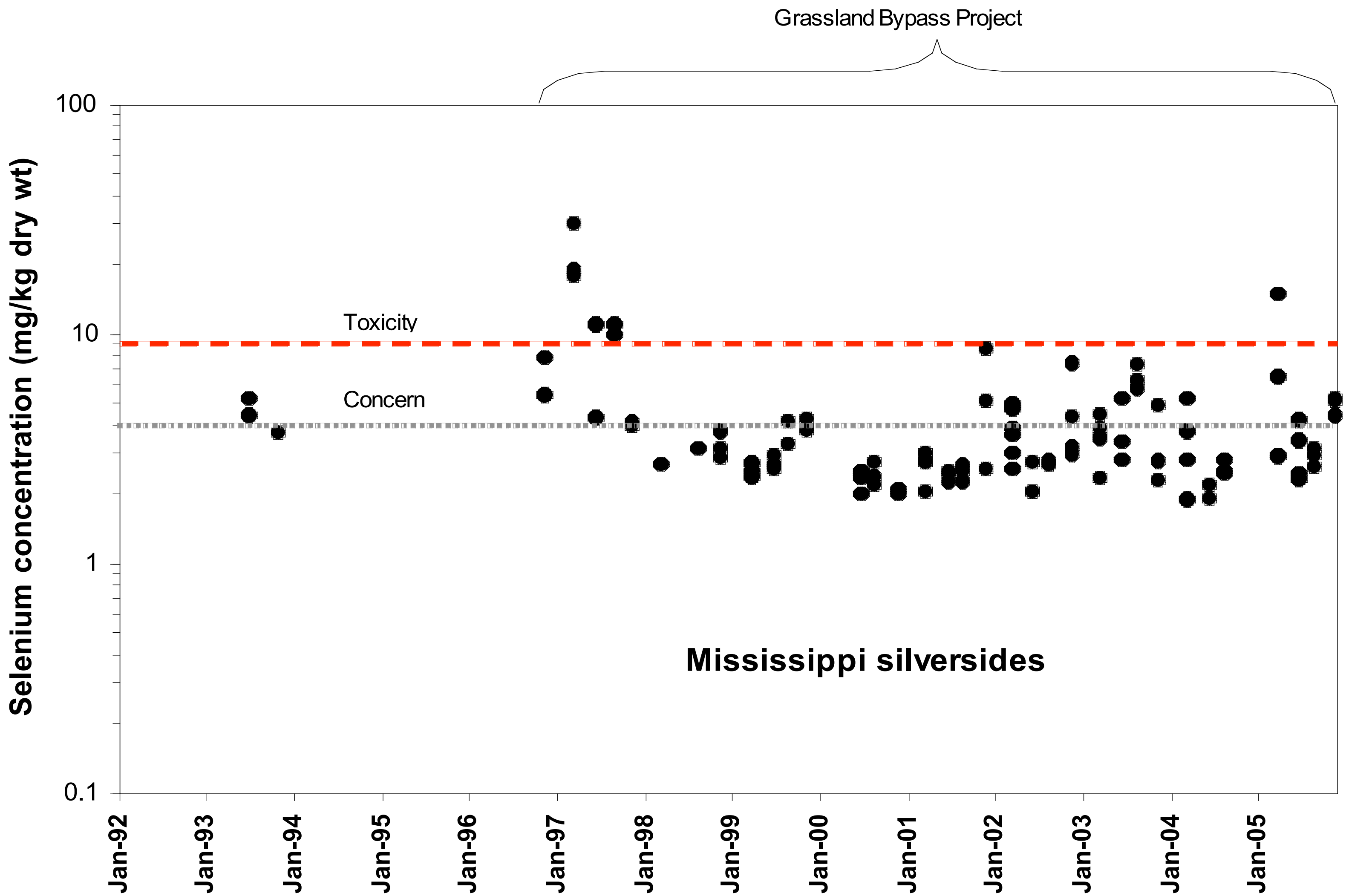

Figure 3C. Selenium in minnows in Mud Slough above the San Luis Drain discharge (Site C)

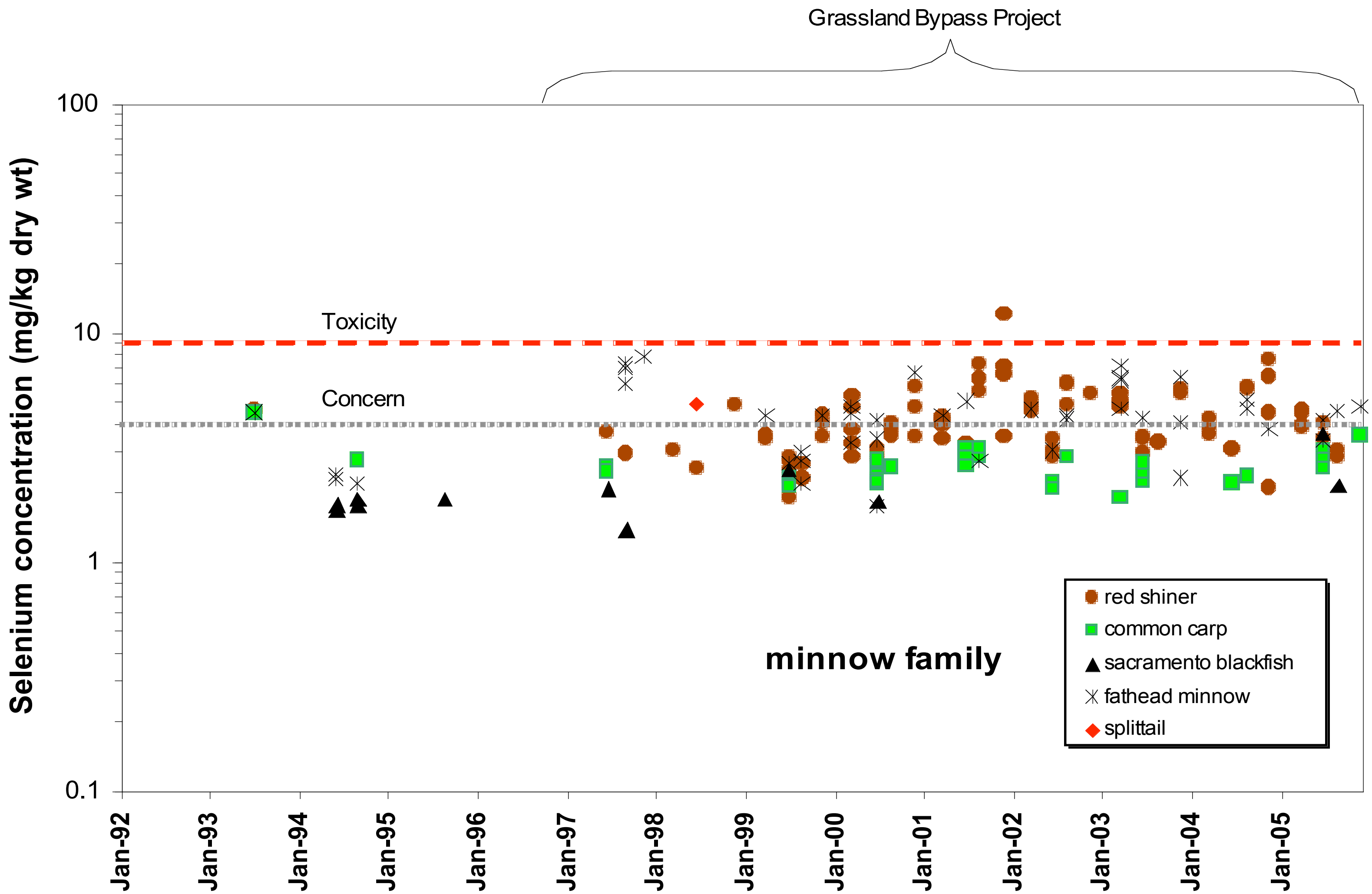

Figure 3D. Selenium in sunfish and bass in Mud Slough above the San Luis Drain discharge (Site C)

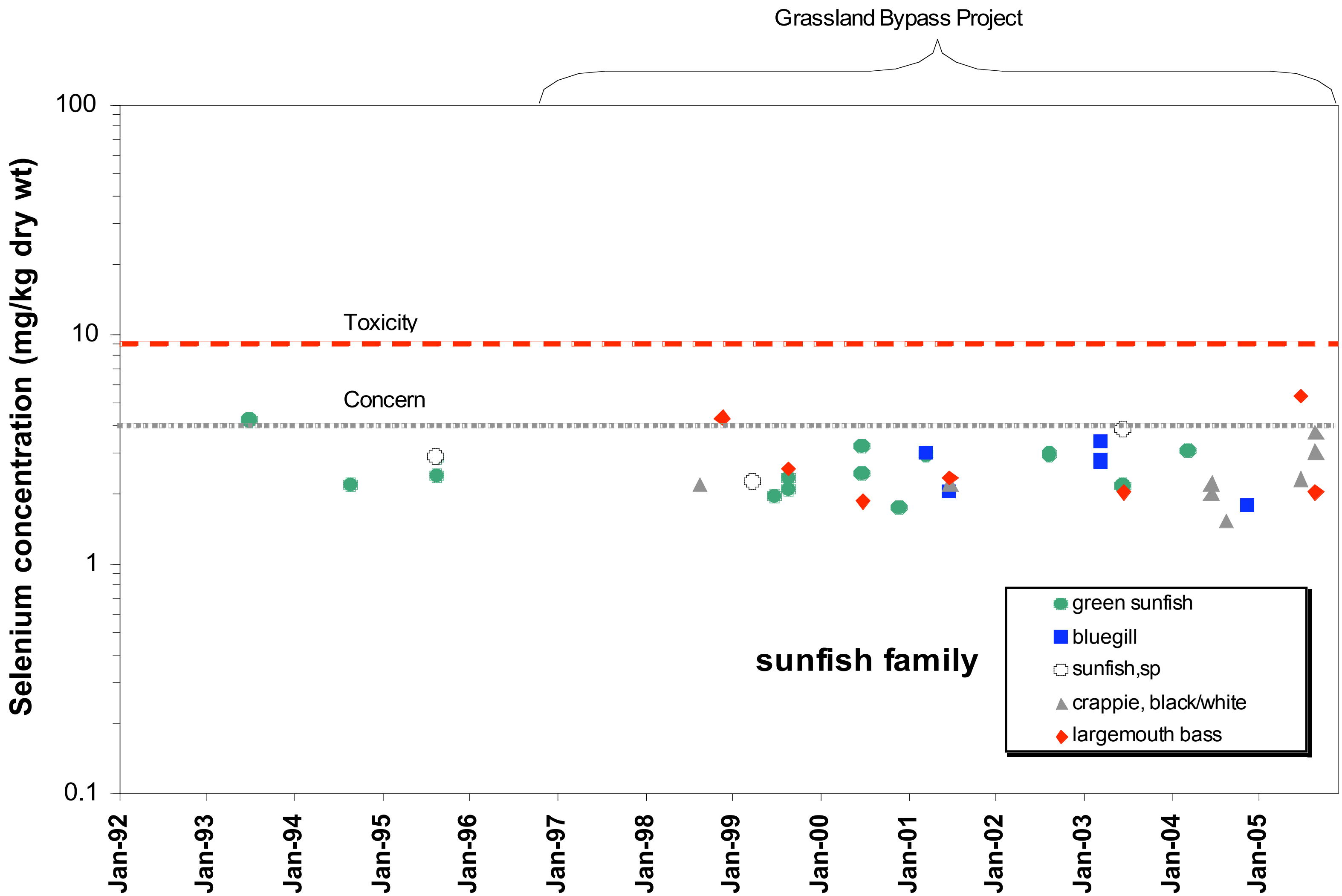

Figure 3E. Selenium in various fish in Mud Slough above the San Luis Drain discharge (Site C)

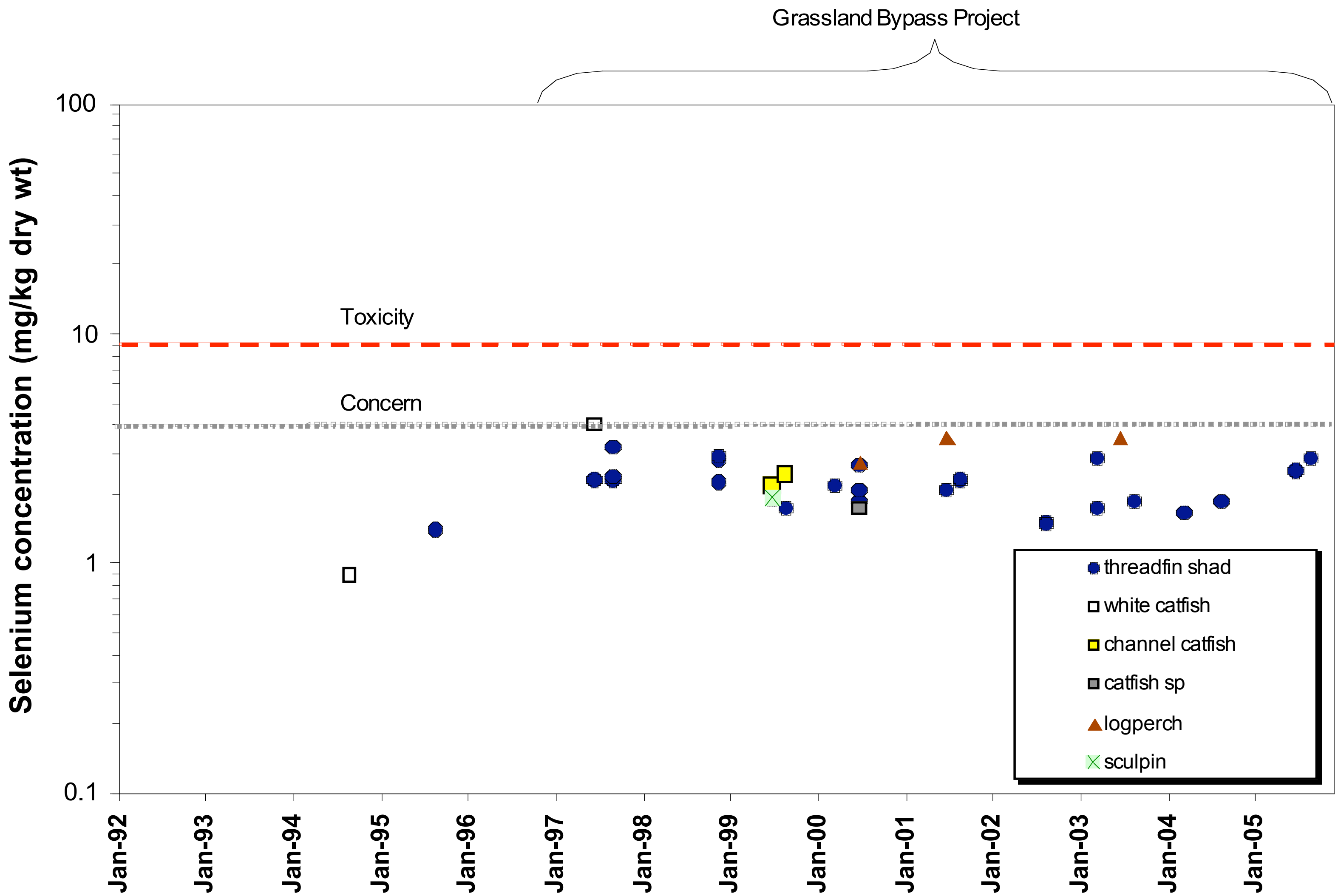

Figure 3F. Selenium in invertebrates in Mud Slough above the San Luis Drain discharge (Site C)

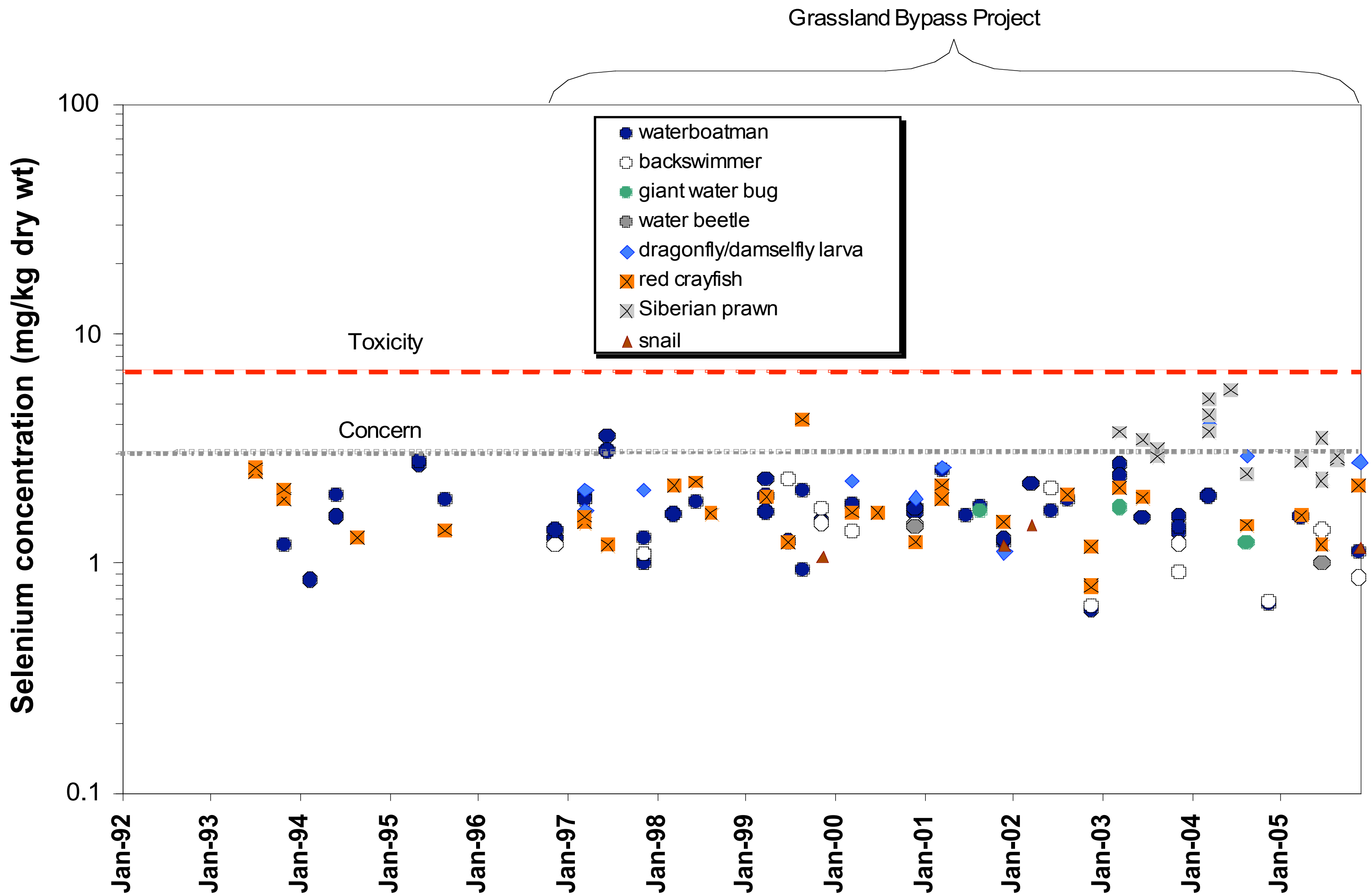

Figure 3G. Selenium in plants along Mud Slough above the San Luis Drain discharge (Site C)

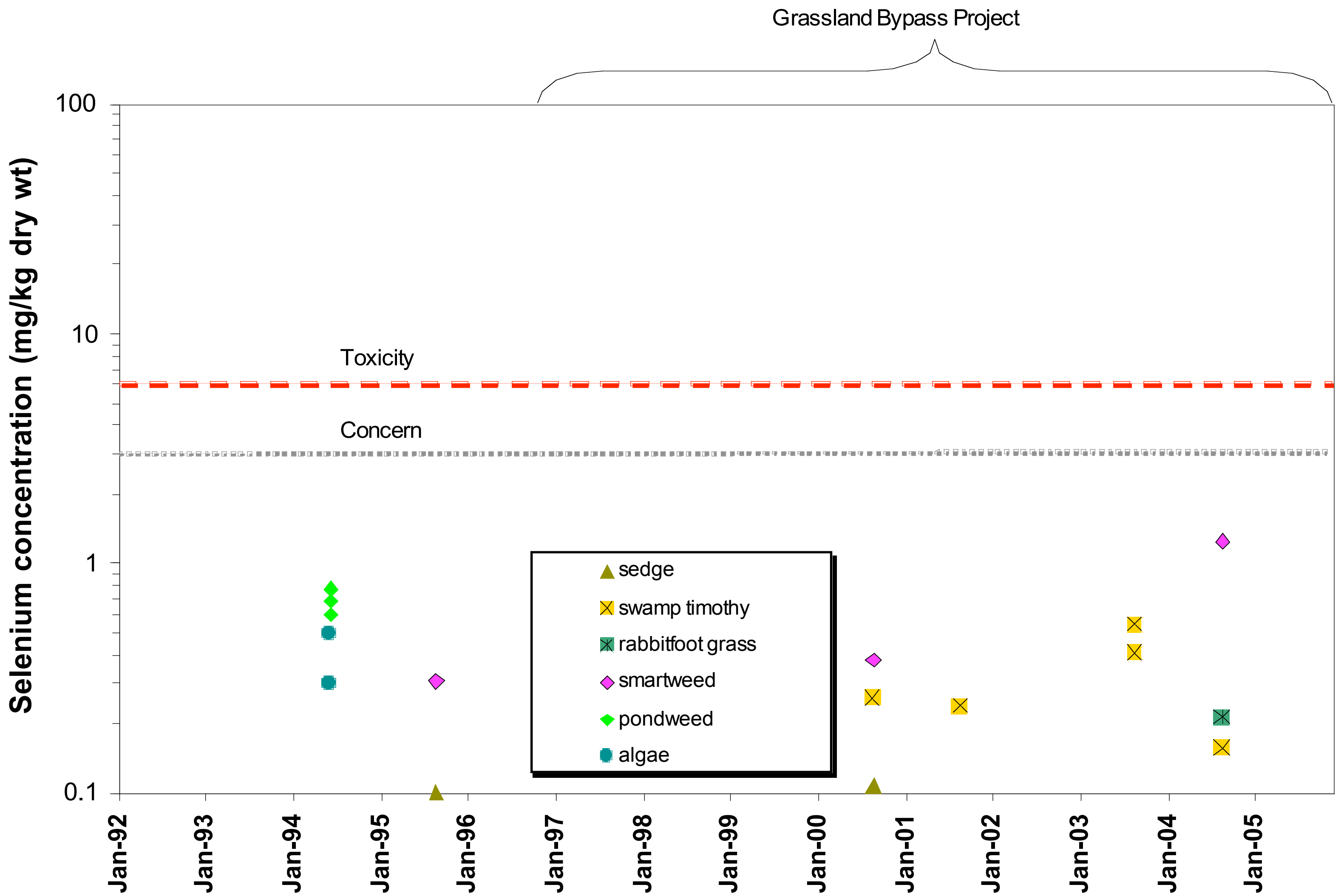

Figure 3H. Boron in plants along Mud Slough above the San Luis Drain discharge (Site C)

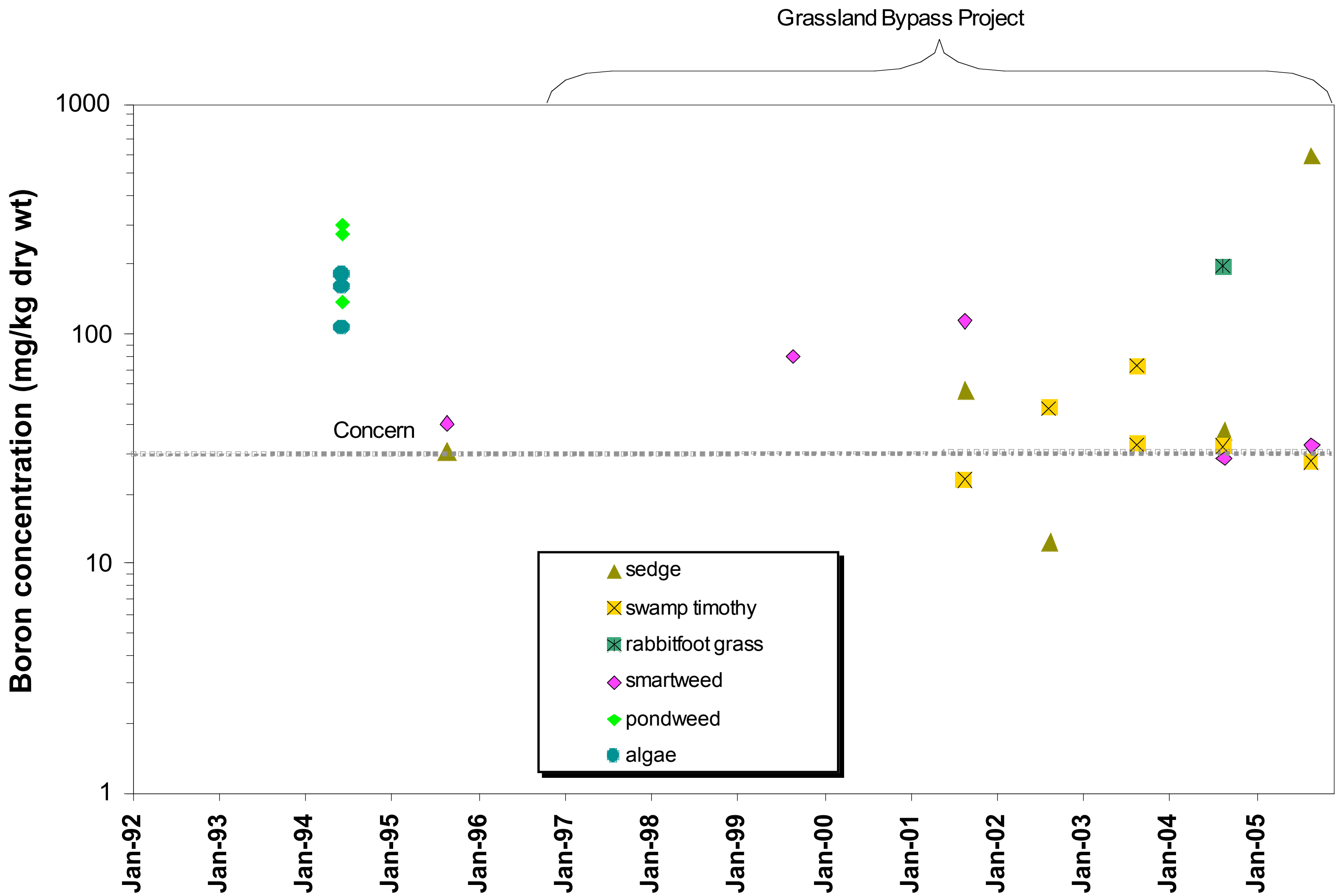

Figure 4A. Selenium in mosquitofish in Mud Slough below the San Luis Drain discharge (Site D)

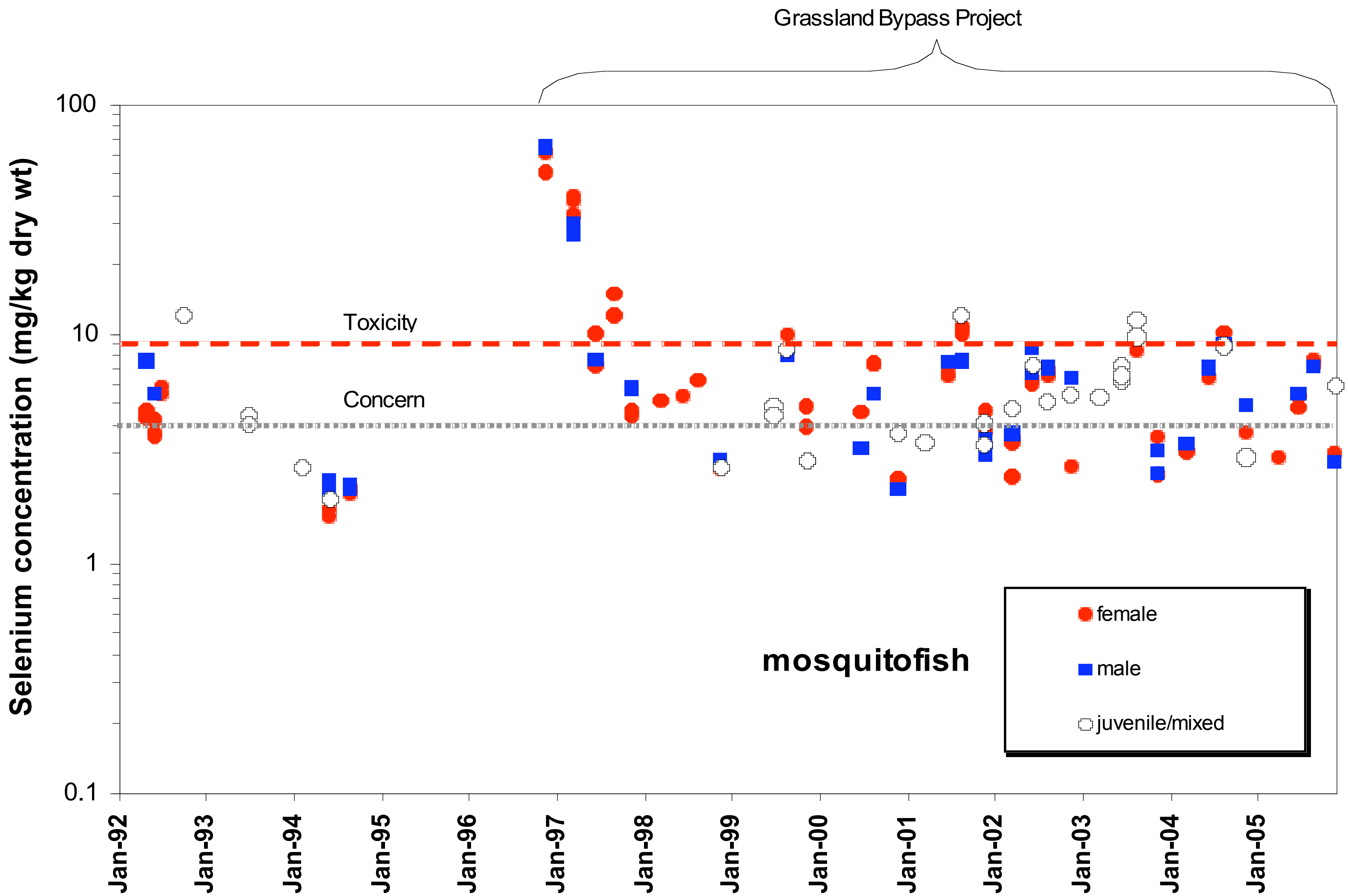

Figure 4B. Selenium in Mississippi silversides in Mud Slough below the San Luis Drain discharge (Site D)

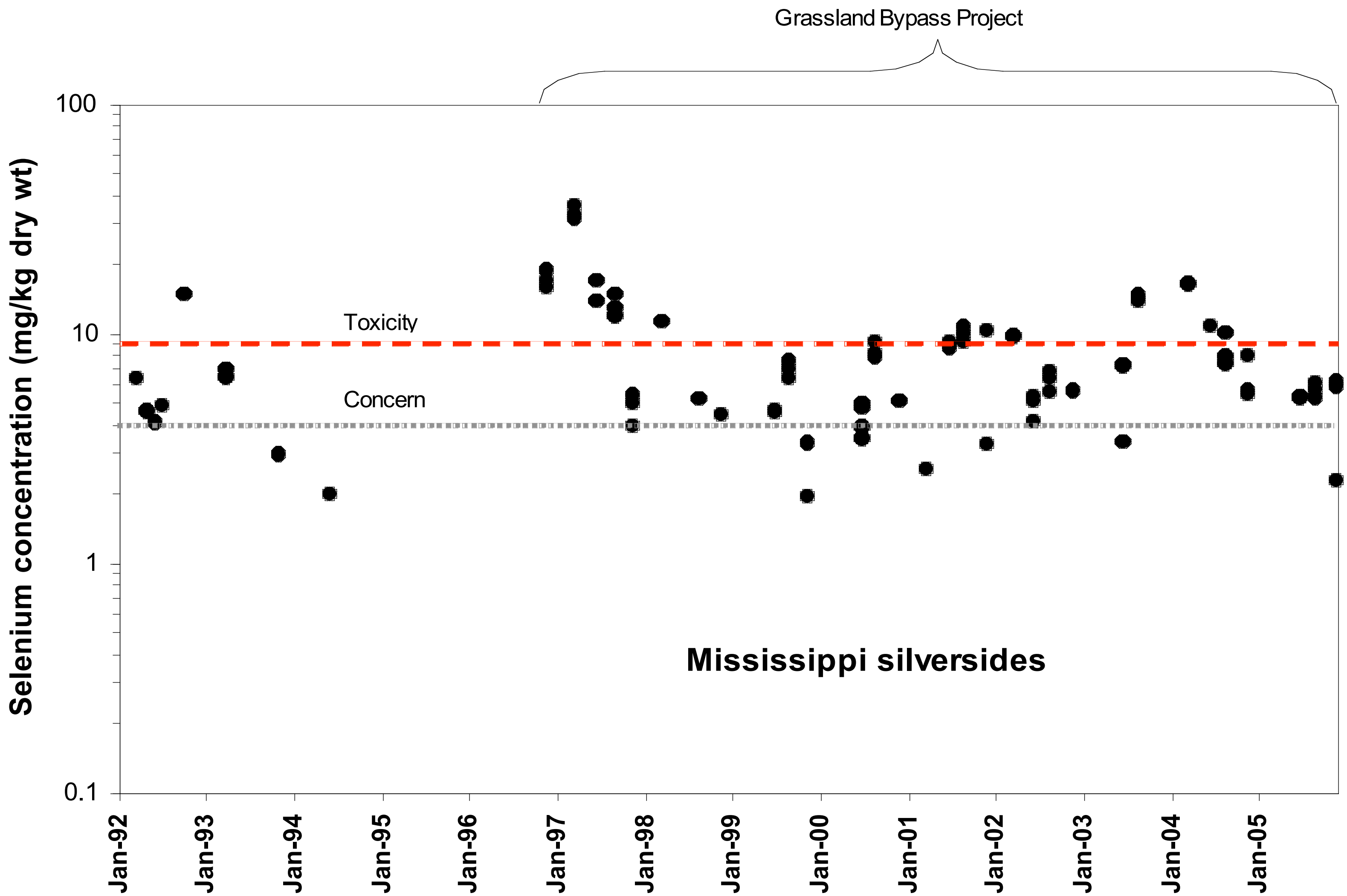

Figure 4C. Selenium in minnows in Mud Slough below the San Luis Drain discharge (Site D)

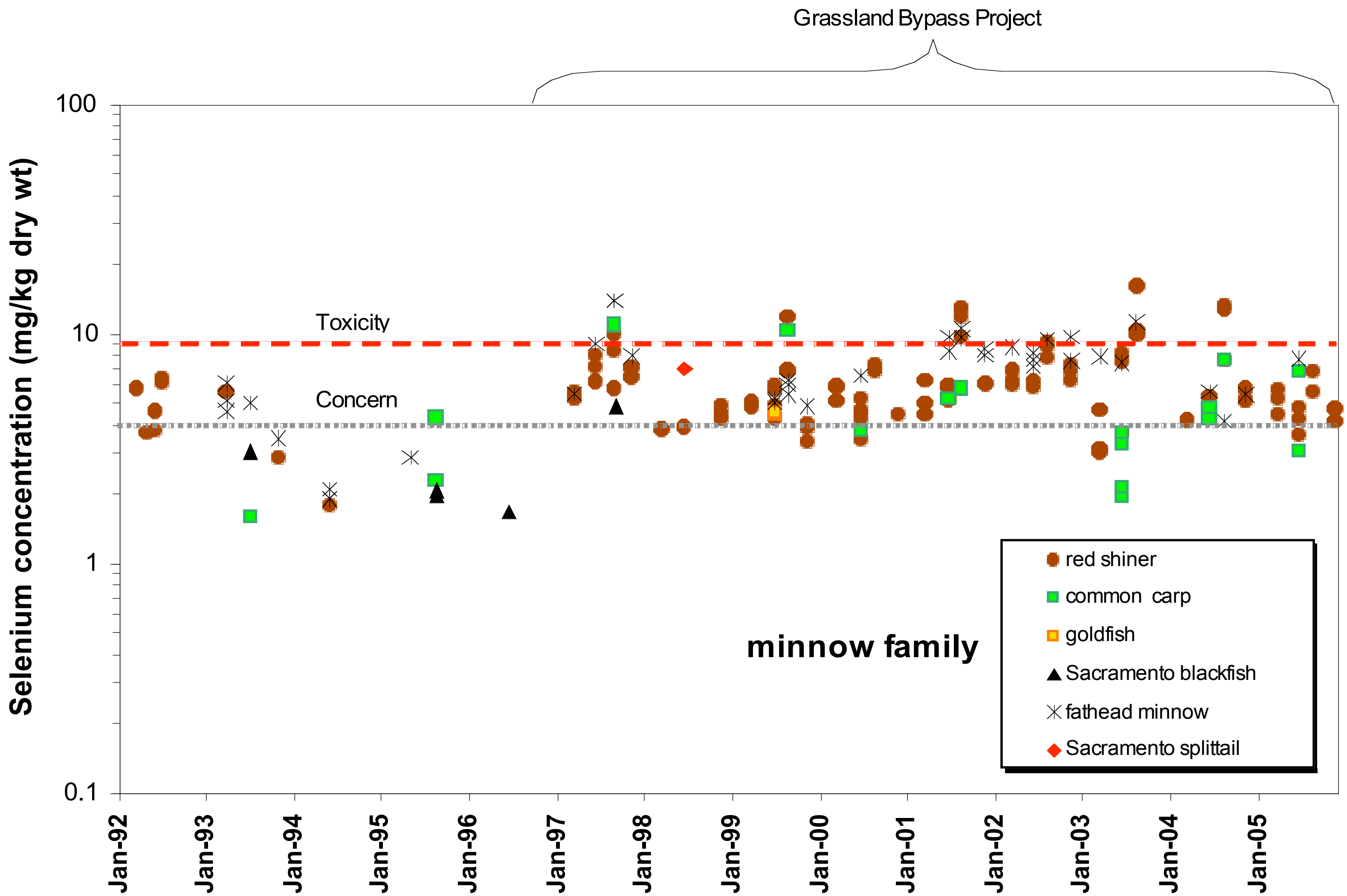

Figure 4D. Selenium in sunfish and bass in Mud Slough below the San Luis Drain discharge (Site D)

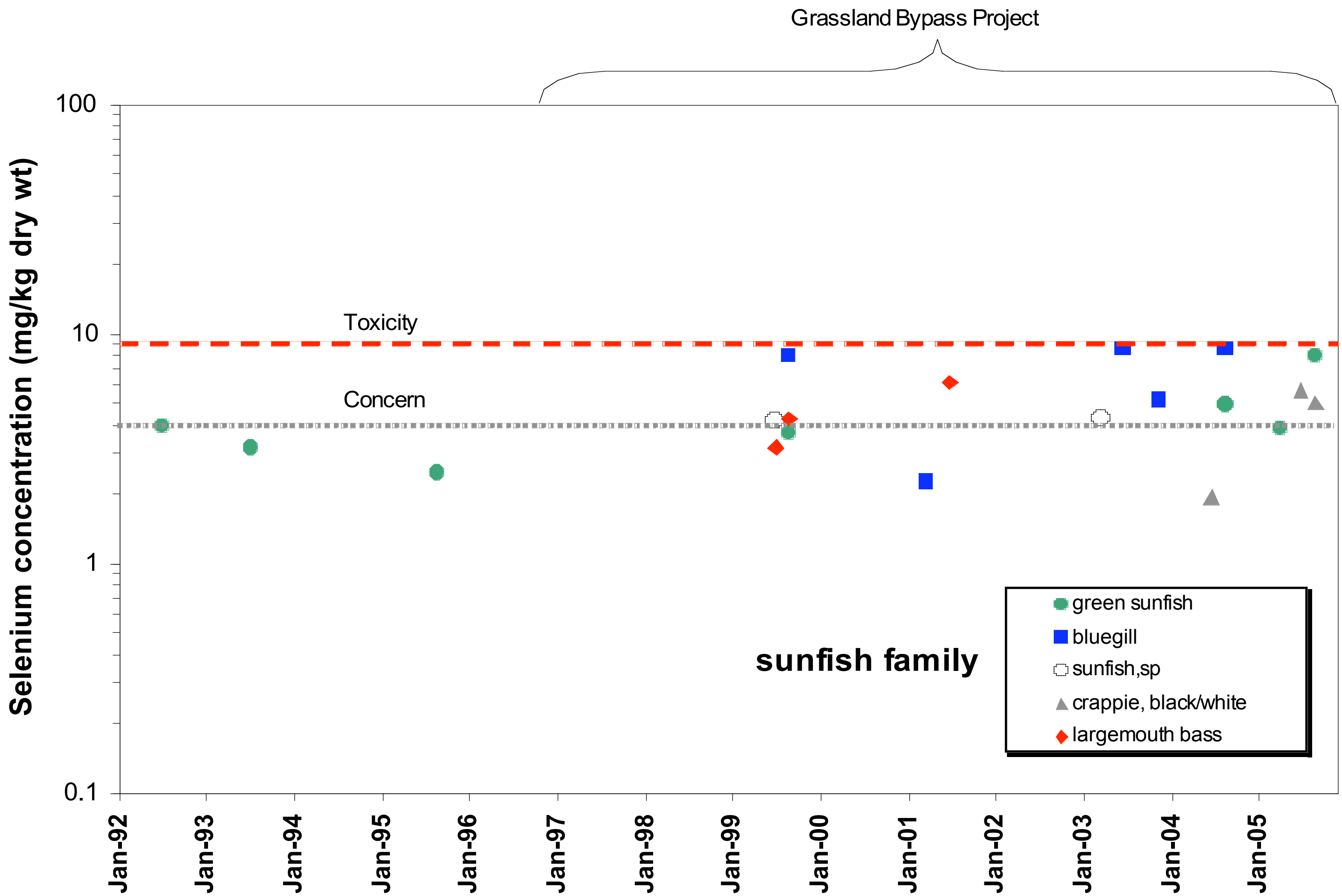

Figure 4E. Selenium in various fish in Mud Slough below the San Luis Drain discharge (Site D)

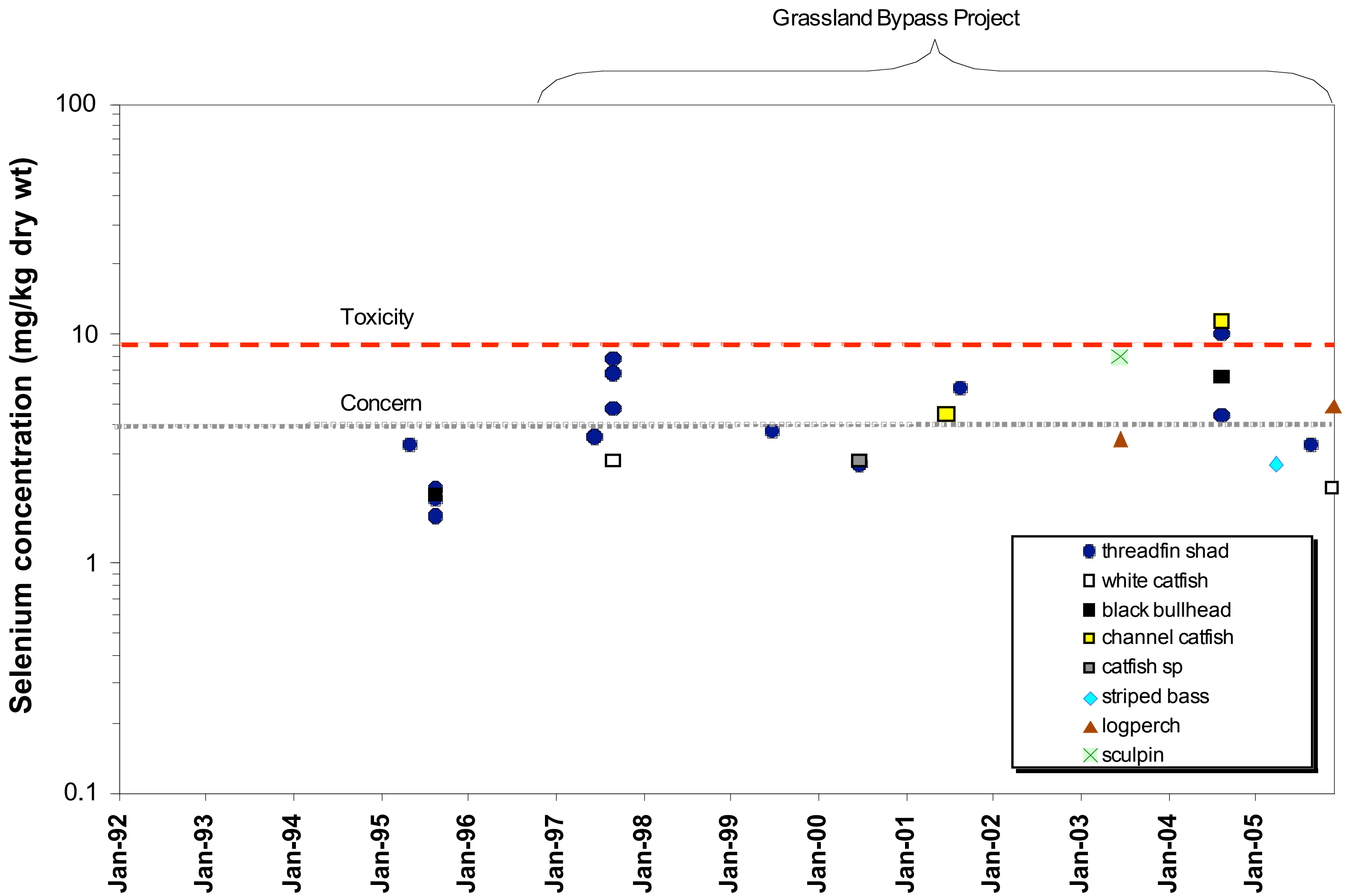

Figure 4F. Selenium in invertebrates in Mud Slough below the San Luis Drain discharge (Site D)

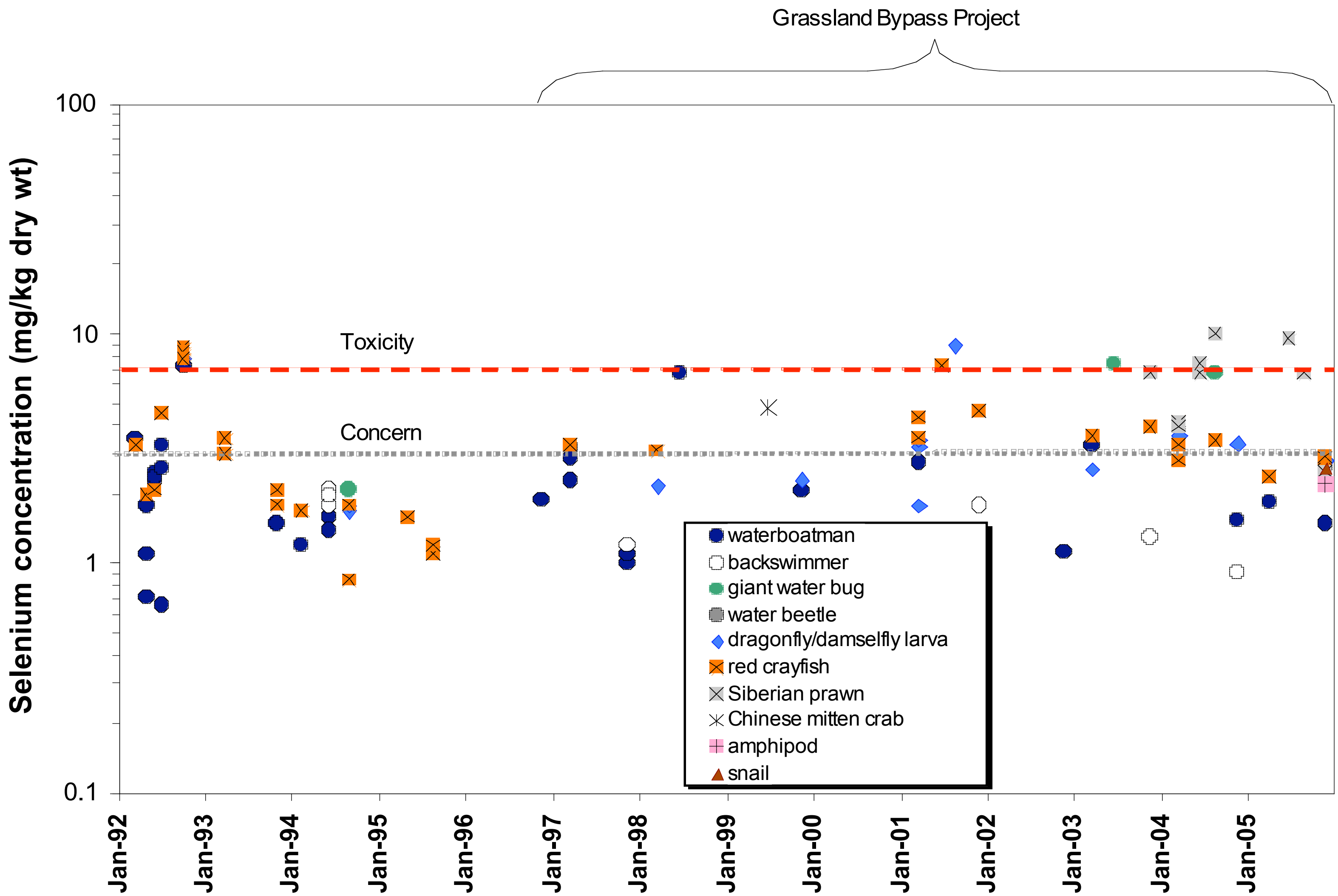

Figure 4G. Selenium in plants along Mud Slough below the San Luis Drain discharge (Site D)

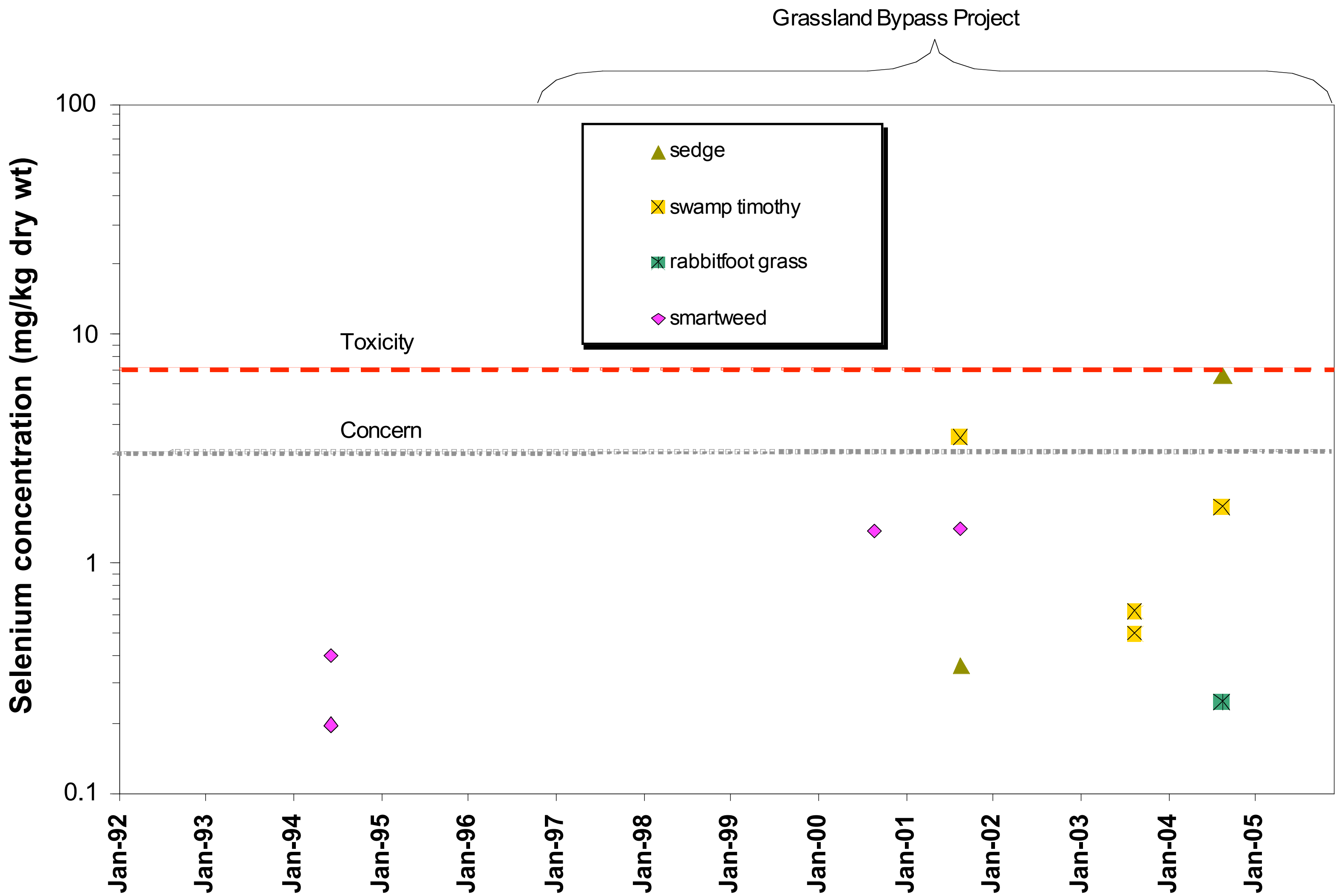

Figure 4H. Boron in plants along Mud Slough below the San Luis Drain discharge (Site D)

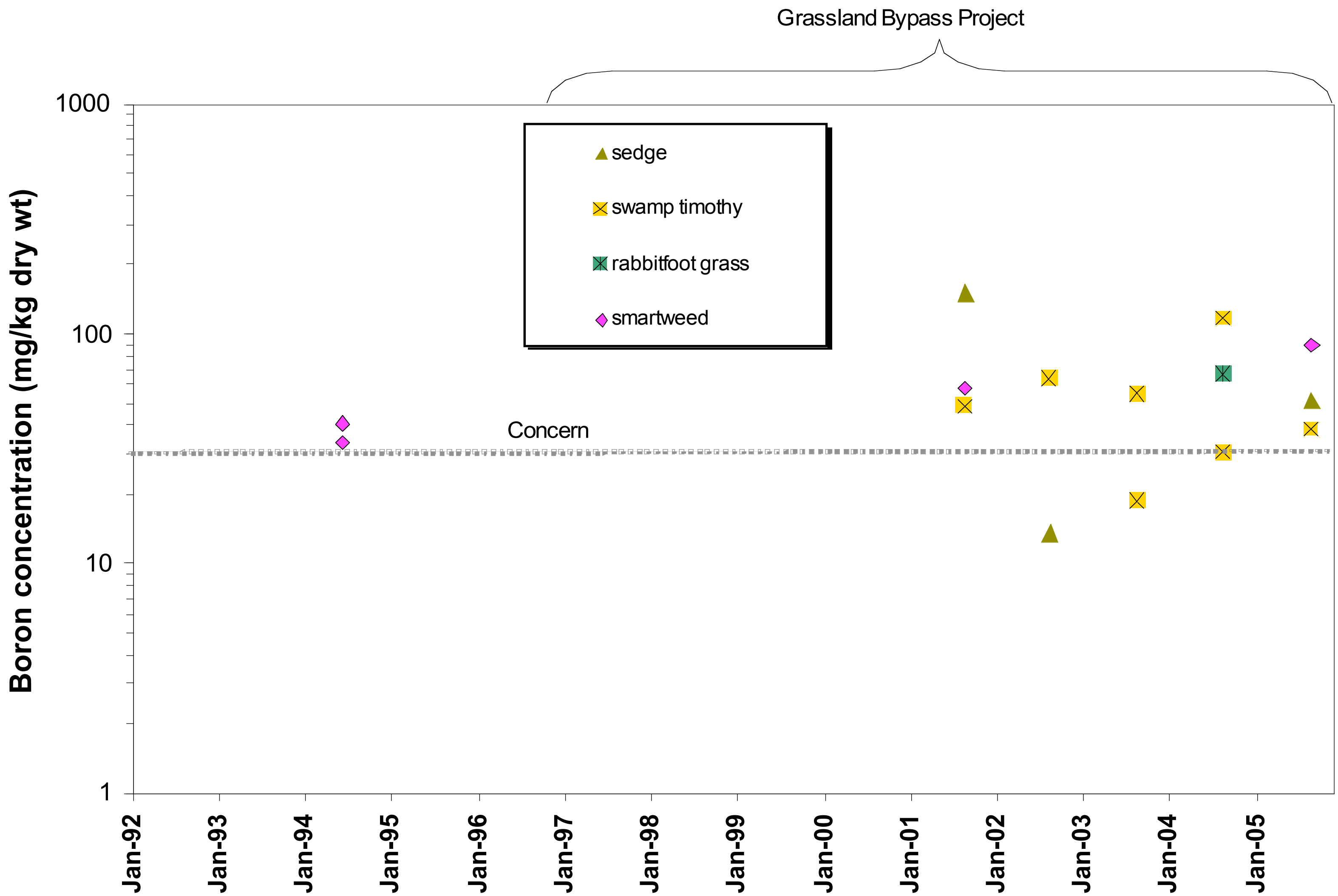

Figure 5A. Selenium in mosquitofish in Mud Slough backwater below the Drain discharge (Sites I and I2)

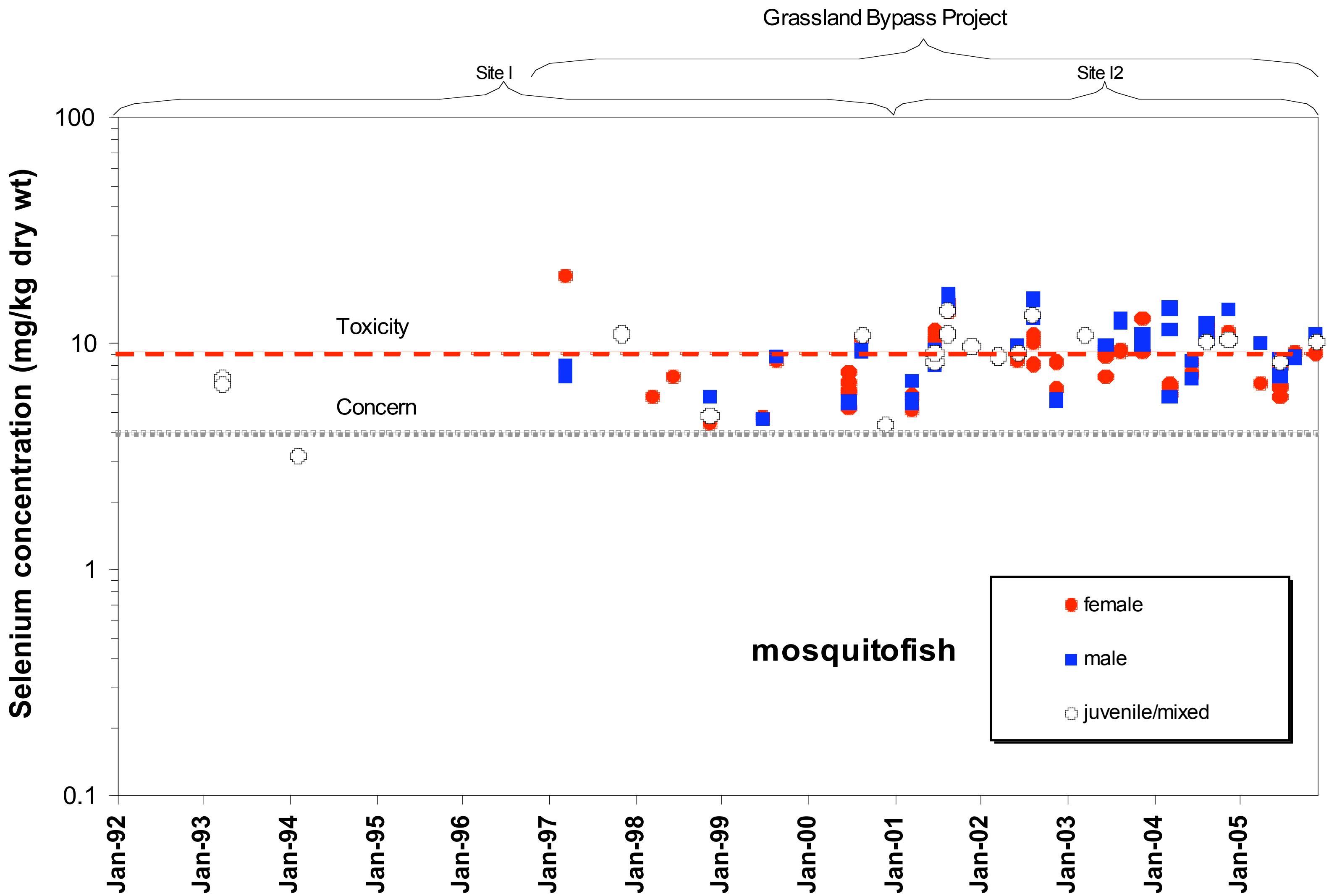

Figure 5B. Selenium in Mississippi silversides in Mud Slough backwater below the Drain discharge (Sites I and I2)

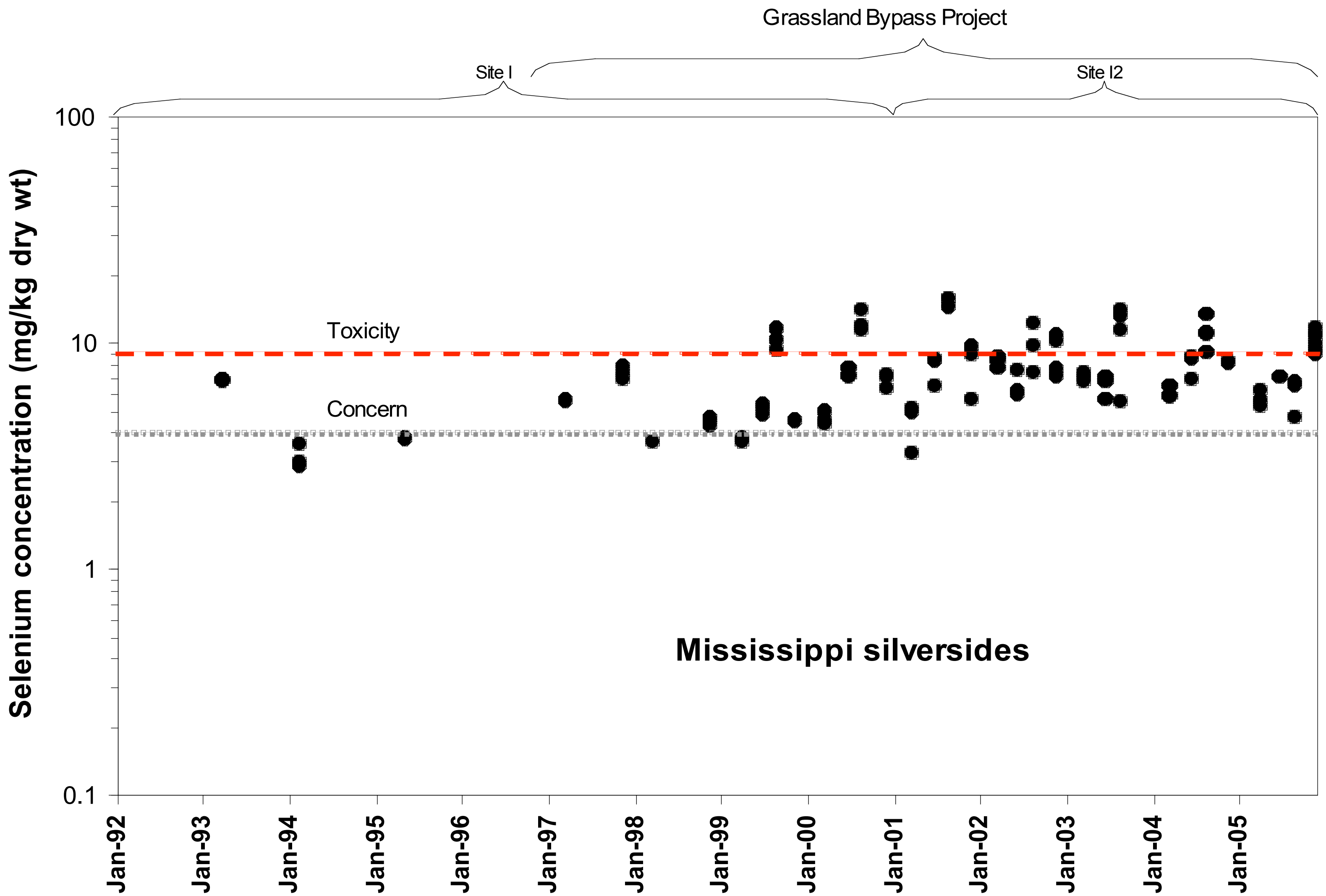

Figure 5C. Selenium in minnows in Mud Slough backwater below the Drain discharge (Sites I and I2)

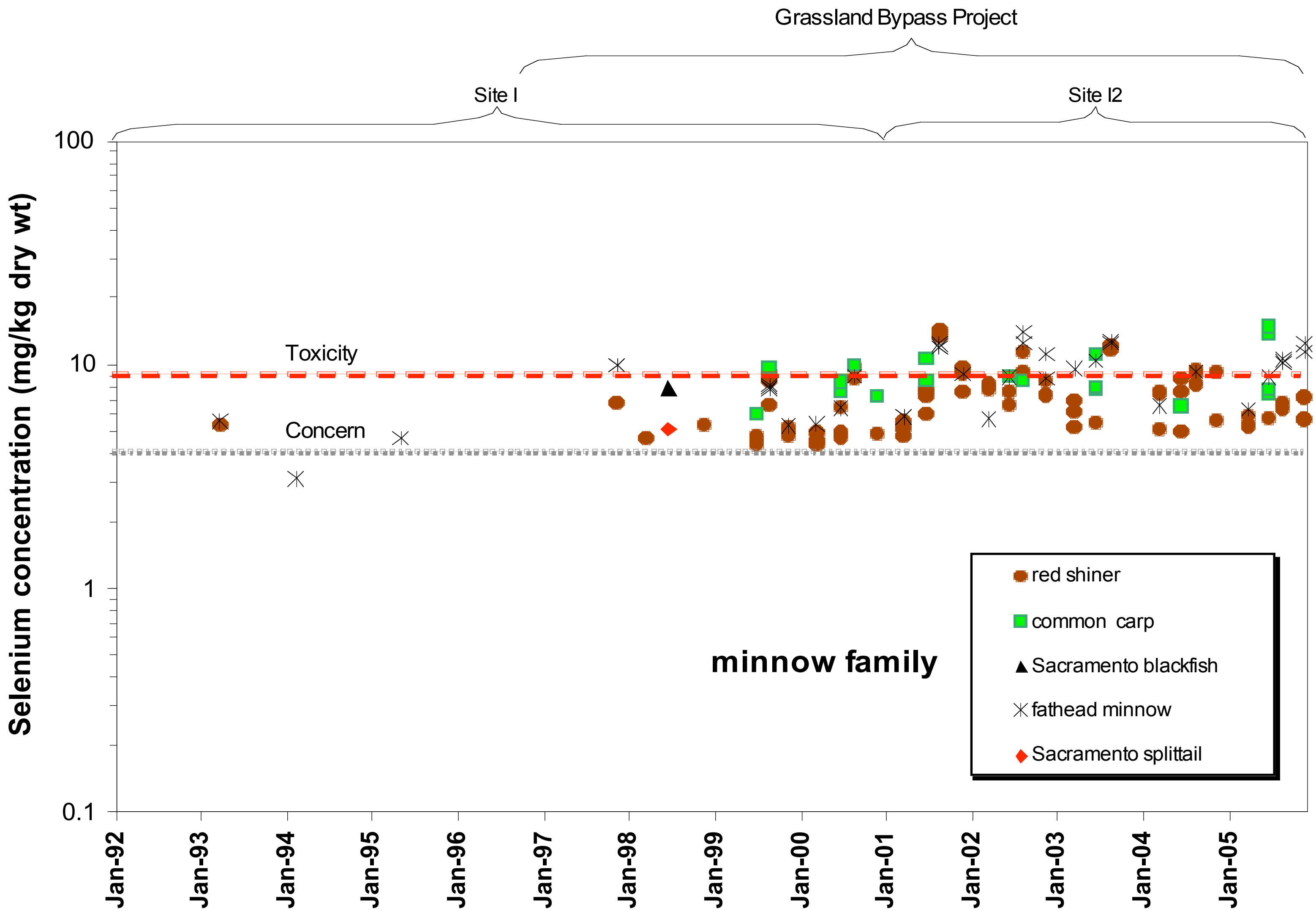

Figure 5D. Selenium in sunfish and bass in Mud Slough backwater below the Drain discharge (Sites I and I2)

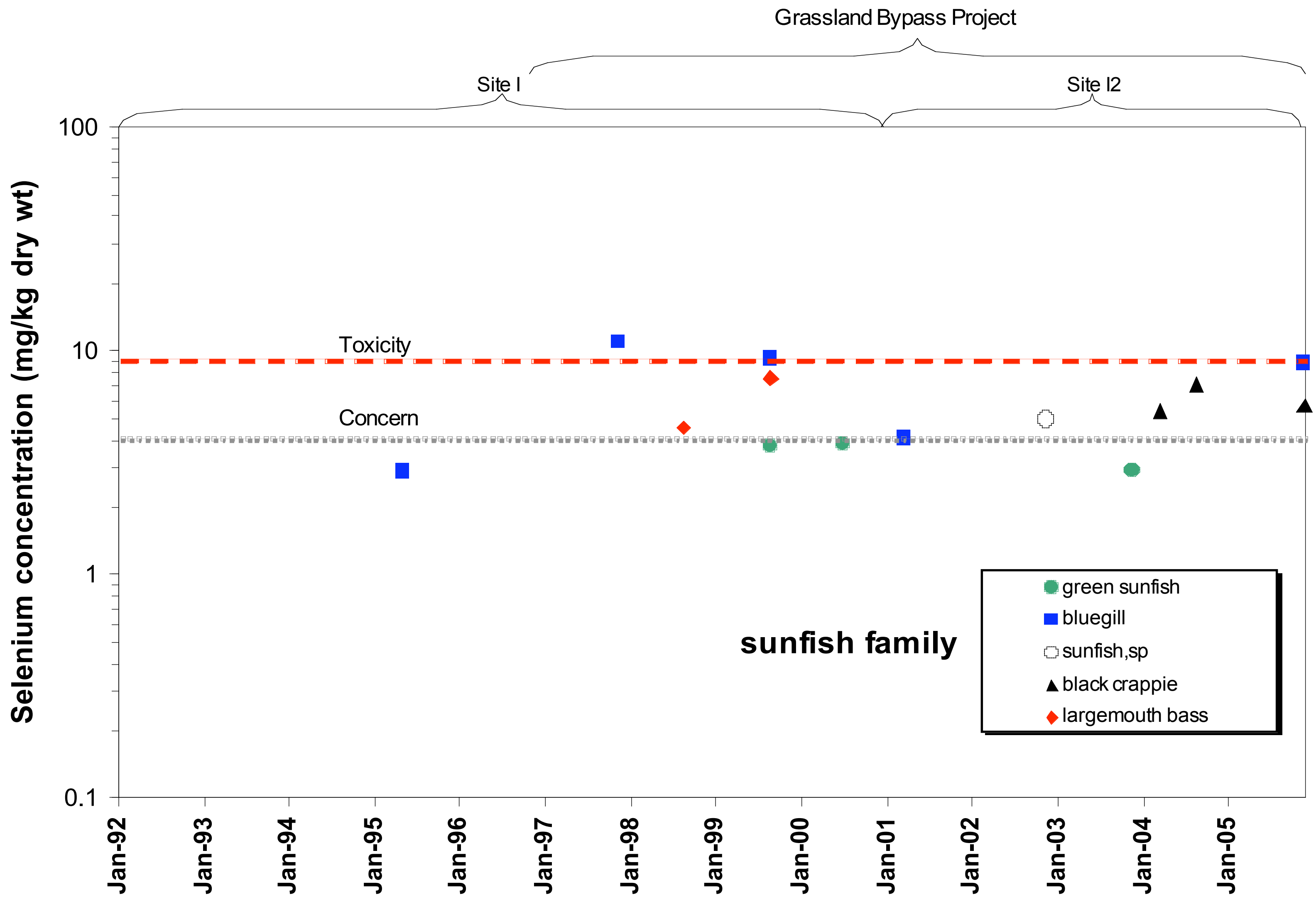

Figure 5E. Selenium in various fish in Mud Slough backwater below the Drain discharge (Sites I and I2)

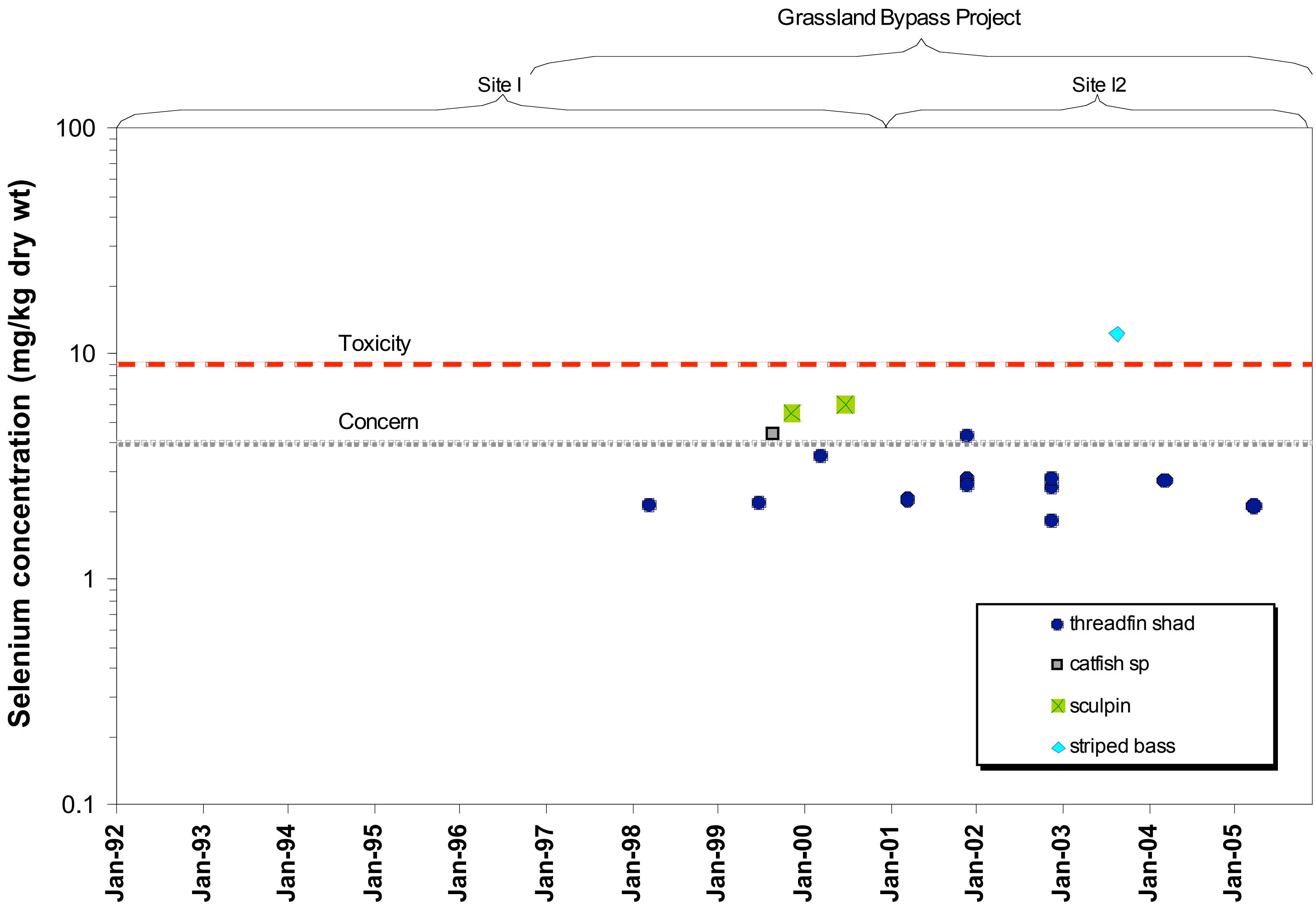

Figure 5F. Selenium in invertebrates in Mud Slough backwater below the Drain discharge (Sites I and I2)

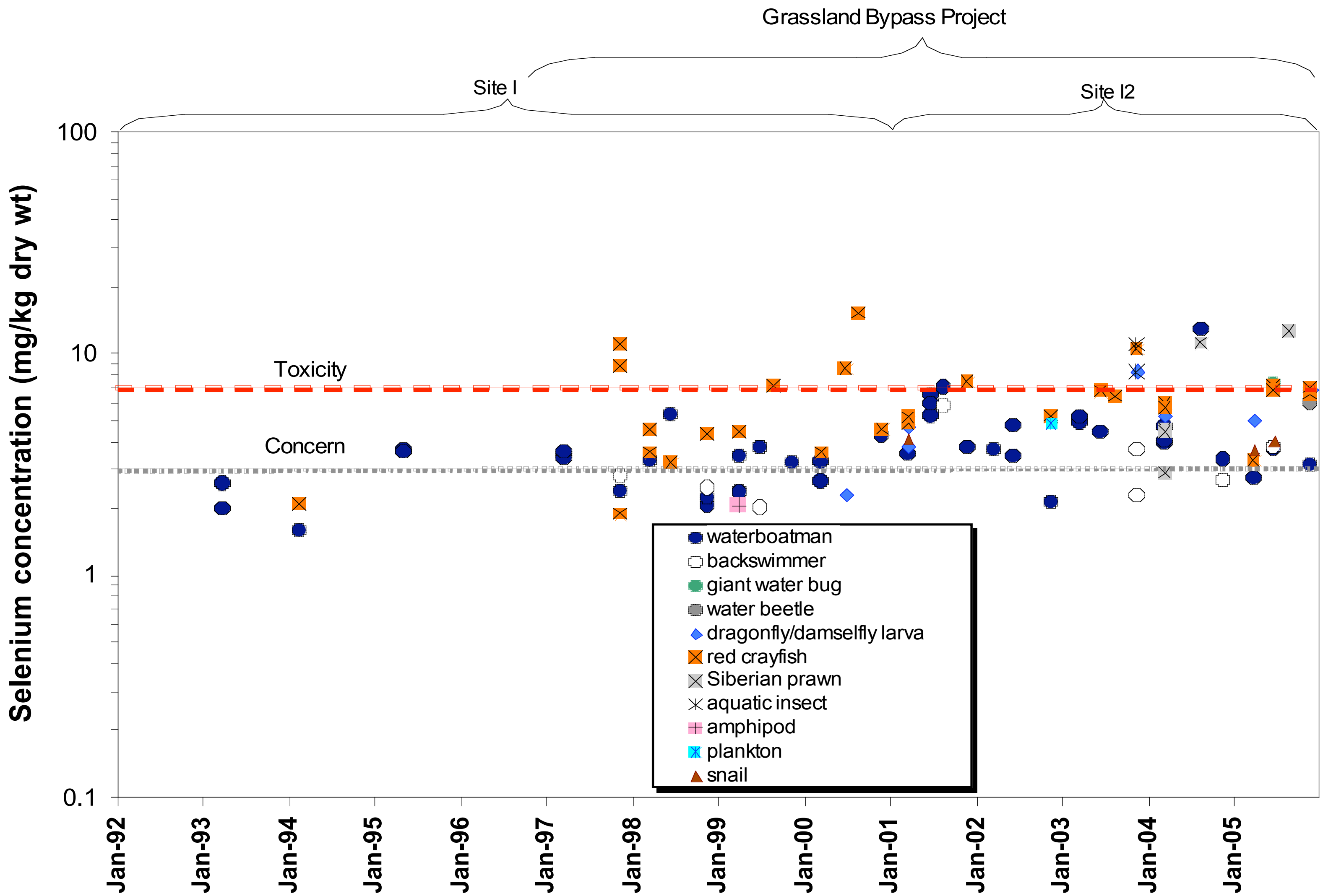

Figure 5G. Selenium in plants along Mud Slough backwater below the Drain discharge (Sites I and I2)

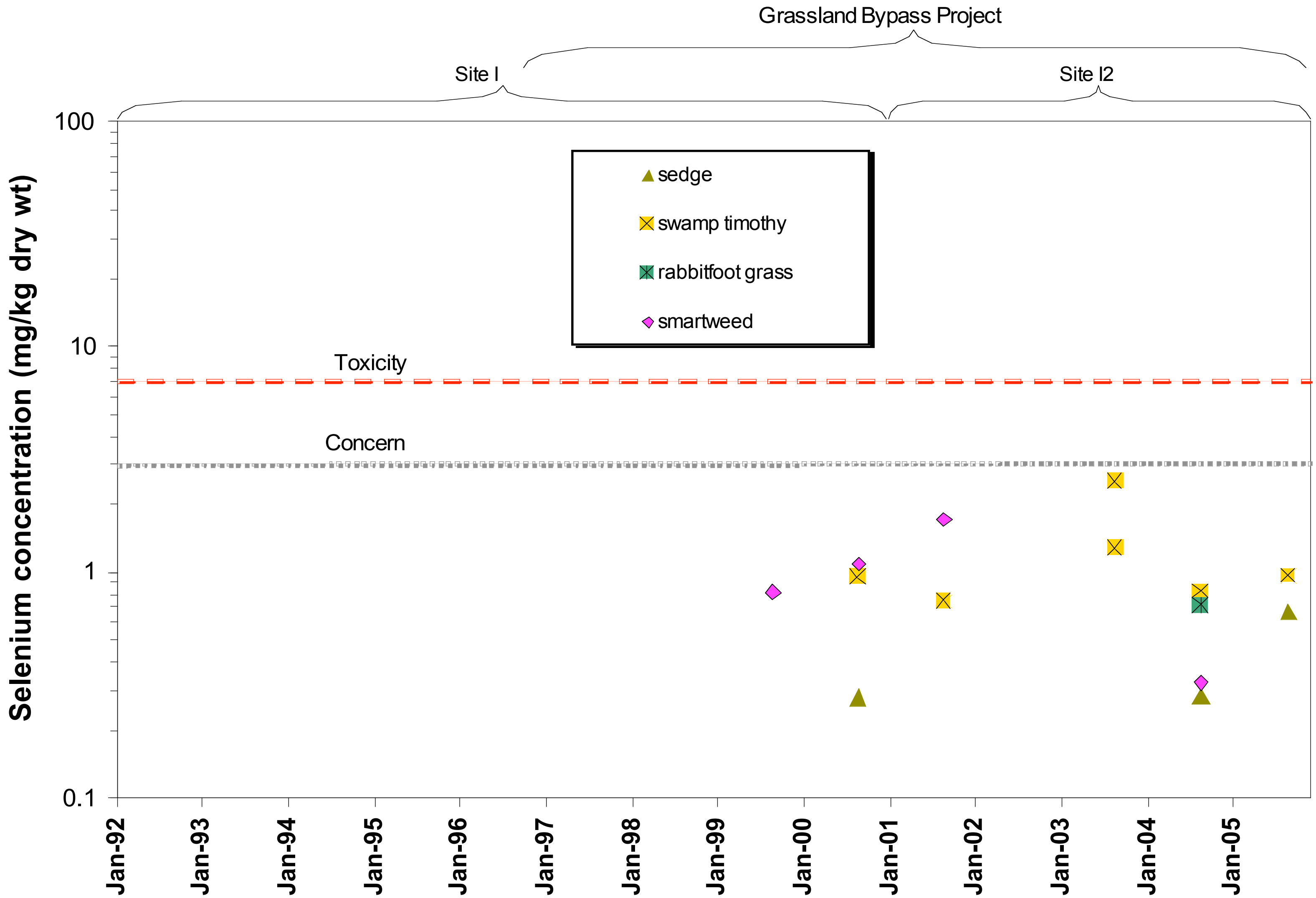

Figure 5H. Boron in plants along Mud Slough backwater below the Drain discharge (Sites I and I2)

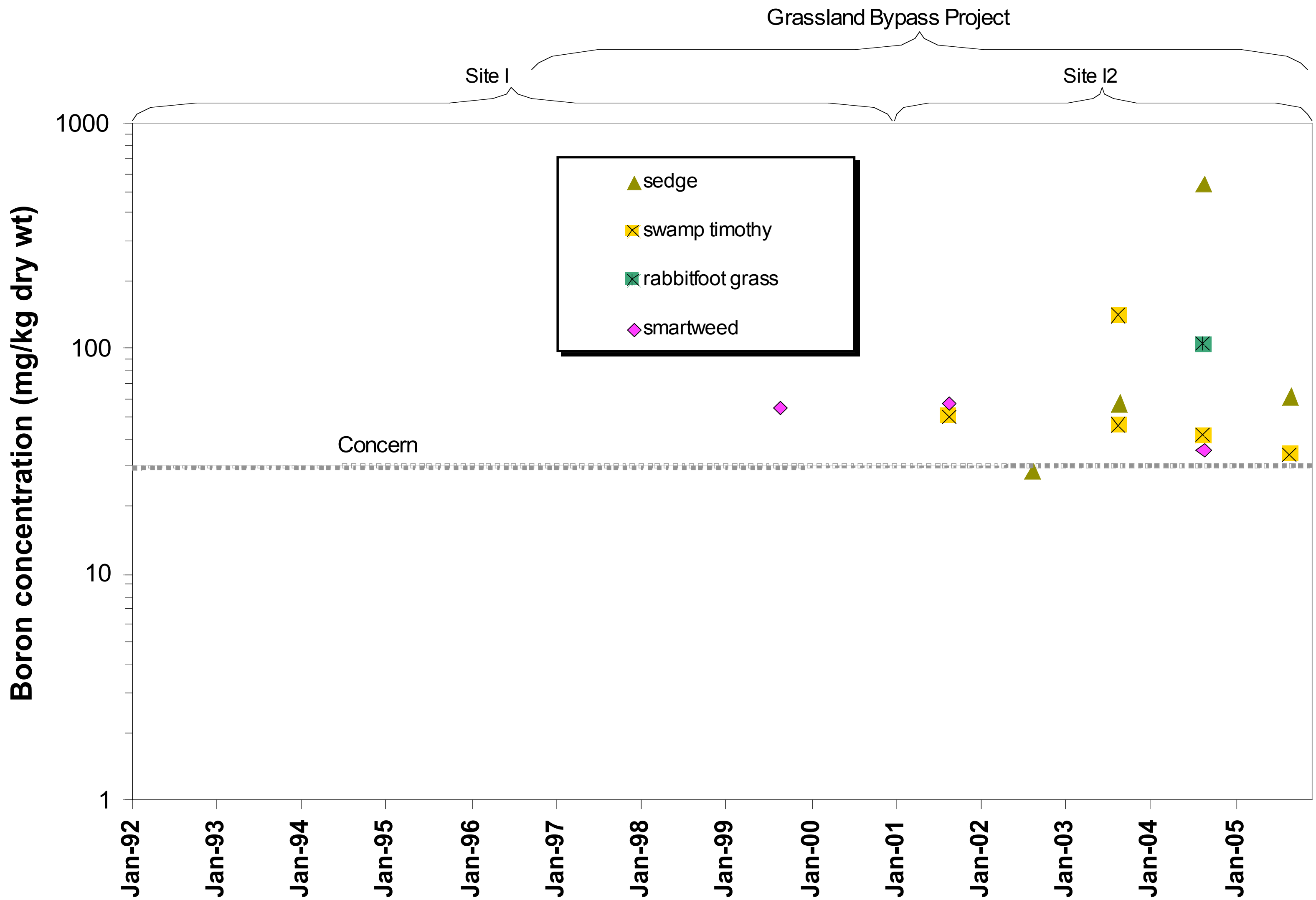

Figure 6. Selenium in frog tadpoles at all sites

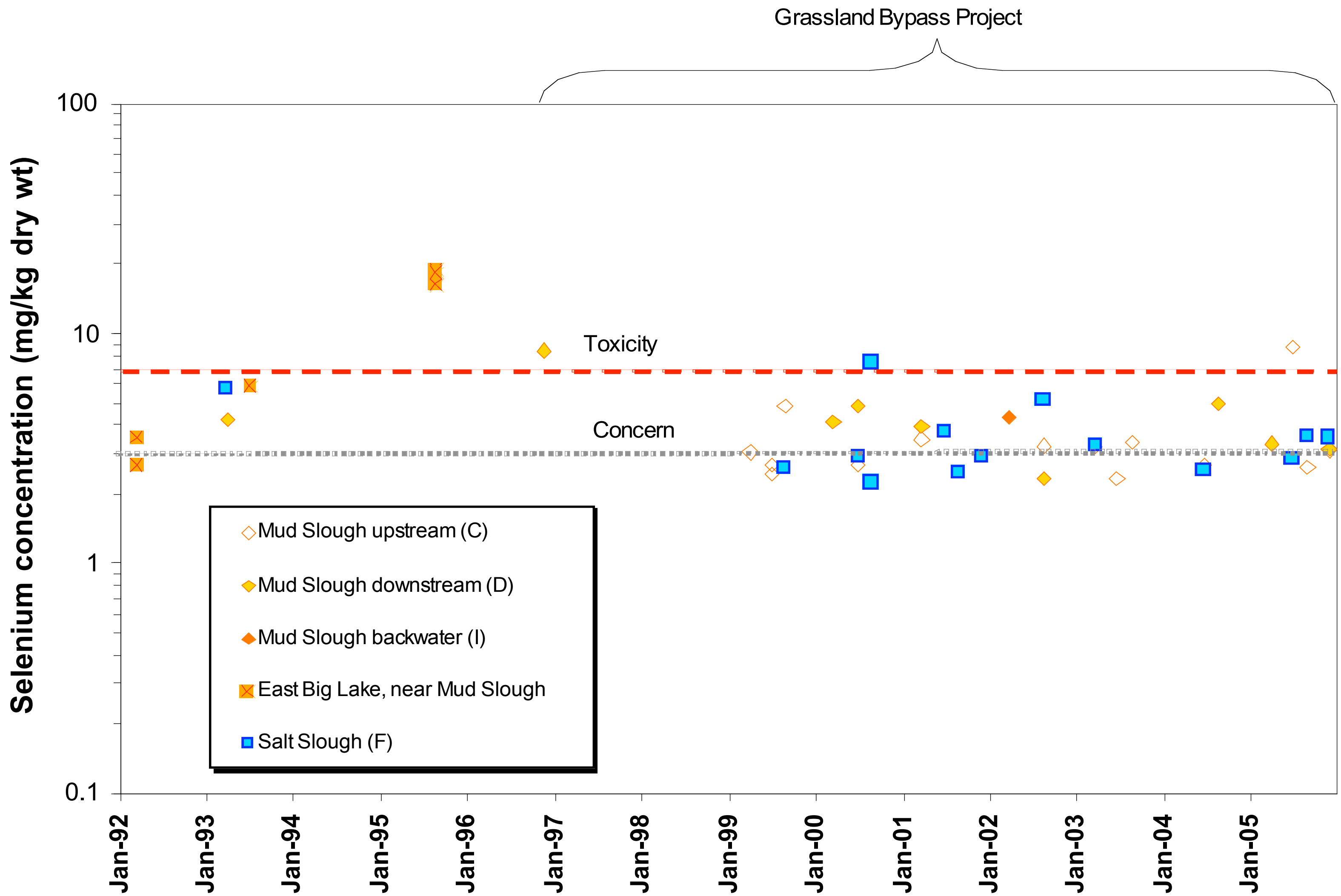

Figure 7. Selenium in bird eggs at all sites

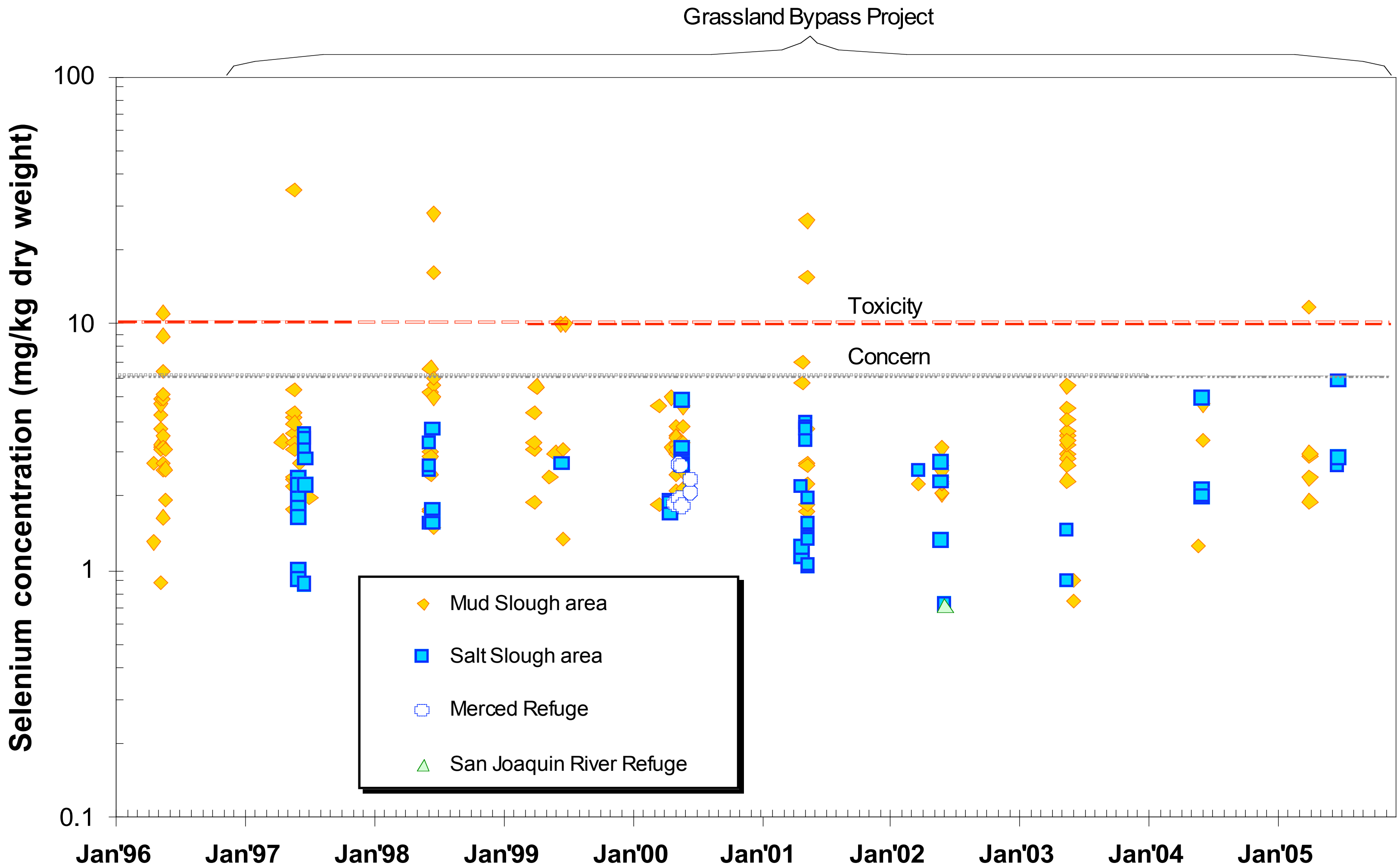

Figure 8A. Selenium Concentration in Whole-body Mosquitofish from Mud Slough at Hwy 140 (Site E).

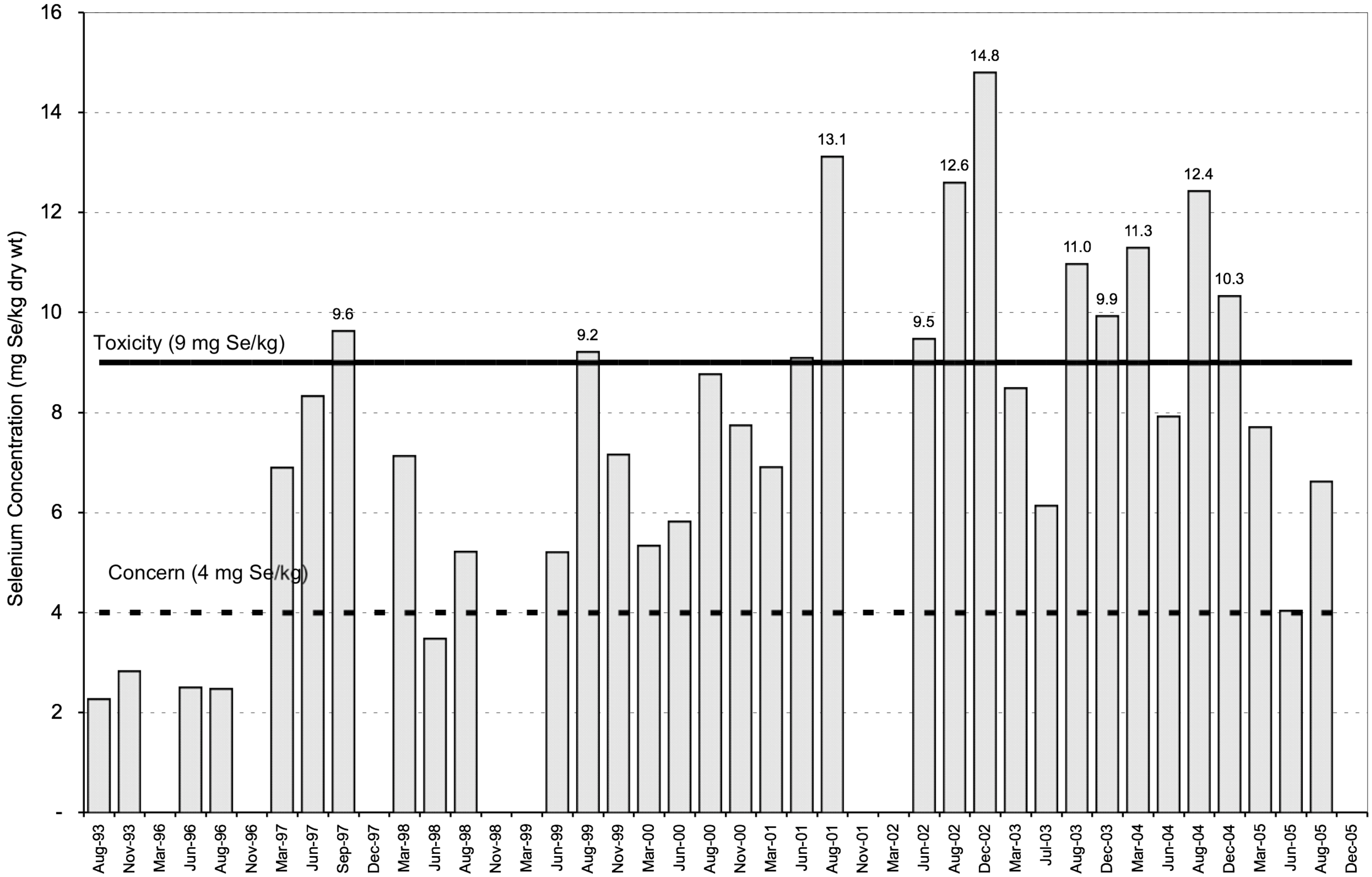

Figure 8B. Selenium Concentration in Invertebrates from Mud Slough at Hwy 140 (Site E).

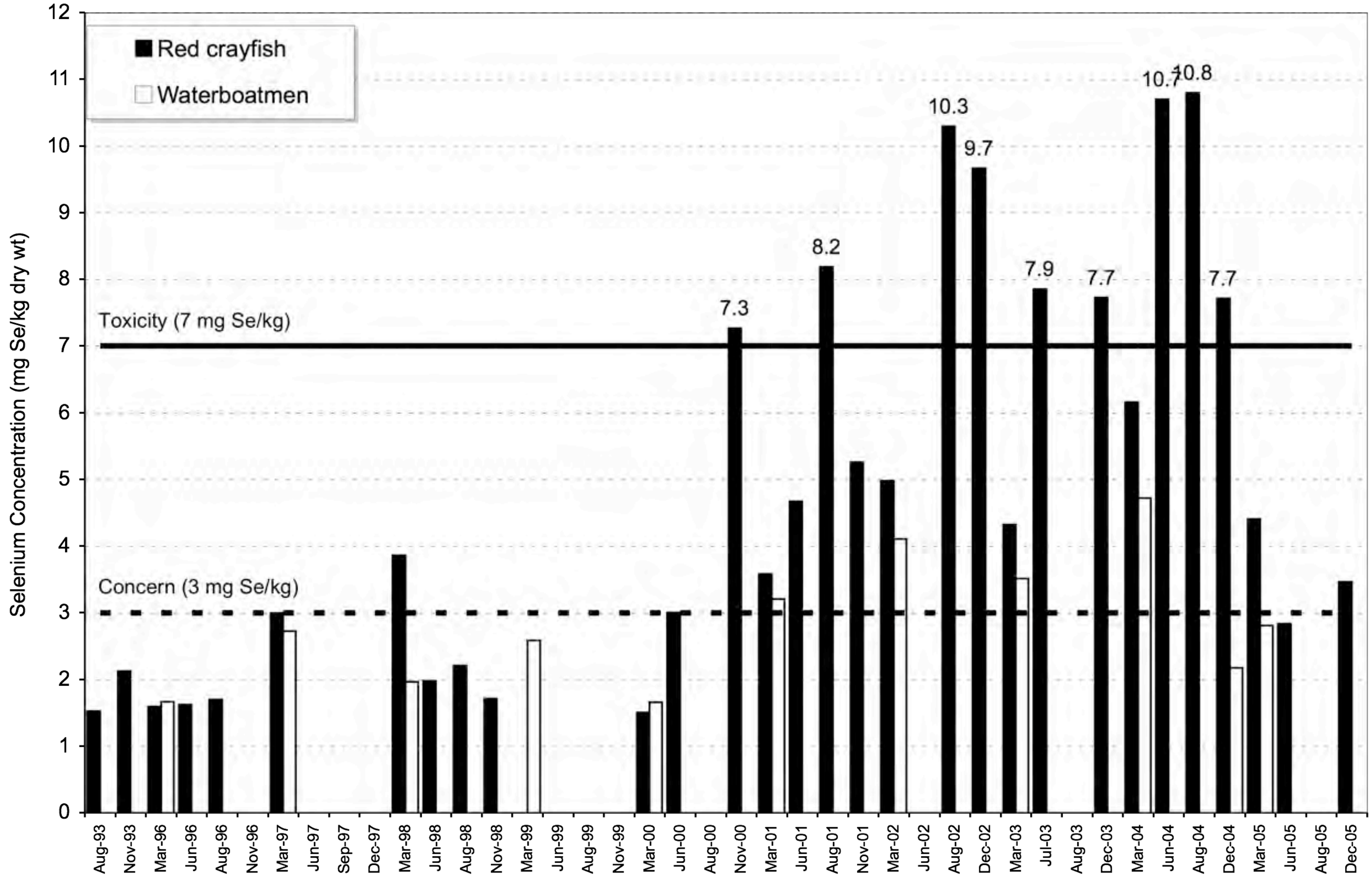

Figure 9A. Selenium Concentrations in Whole-Body Fish Tissue from the San Joaquin River at Fremont Ford, California (Site G).

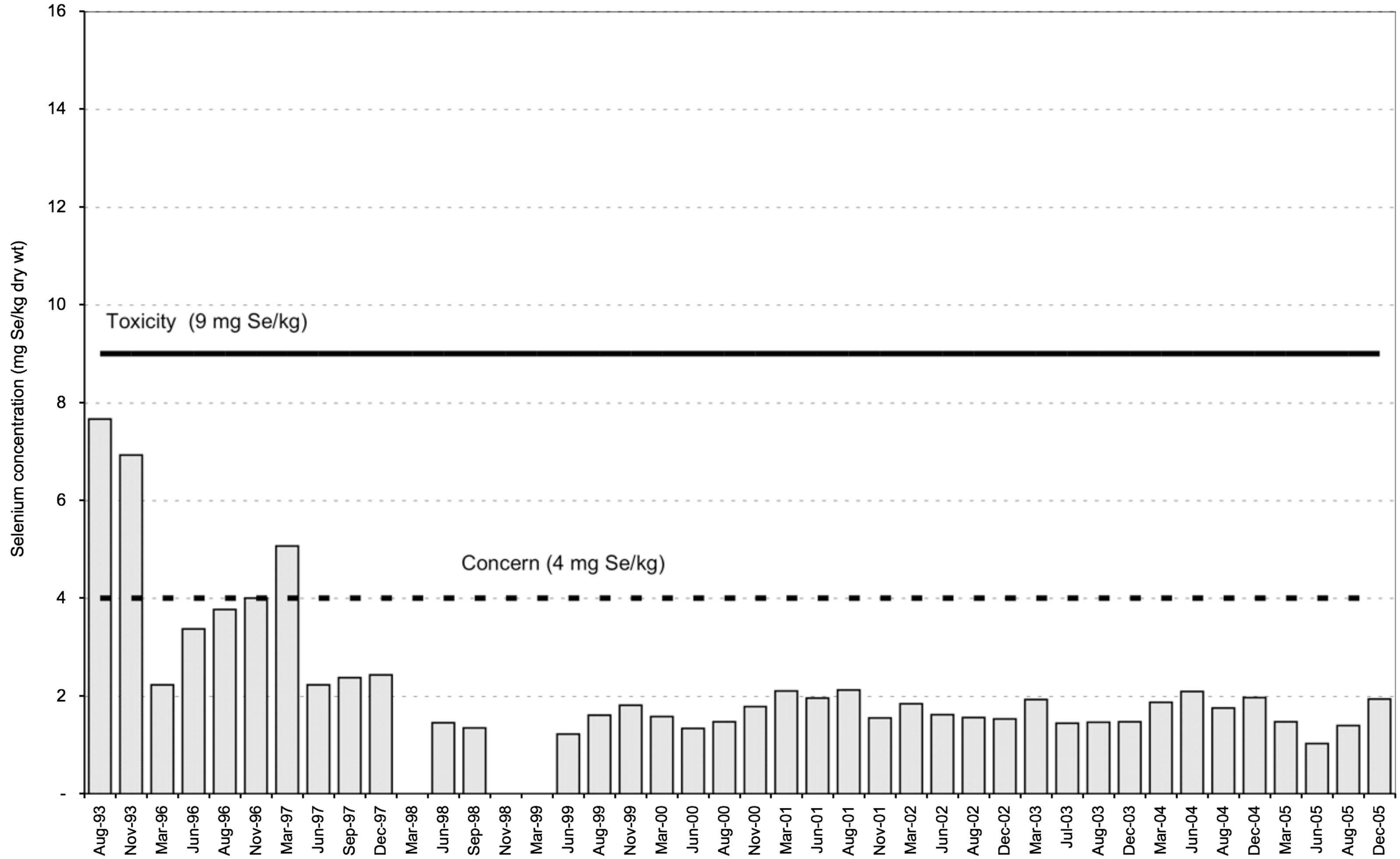

Figure 9B. Selenium Concentration in Invertebrates from the San Joaquin River at Fremont Ford, California (Site G).

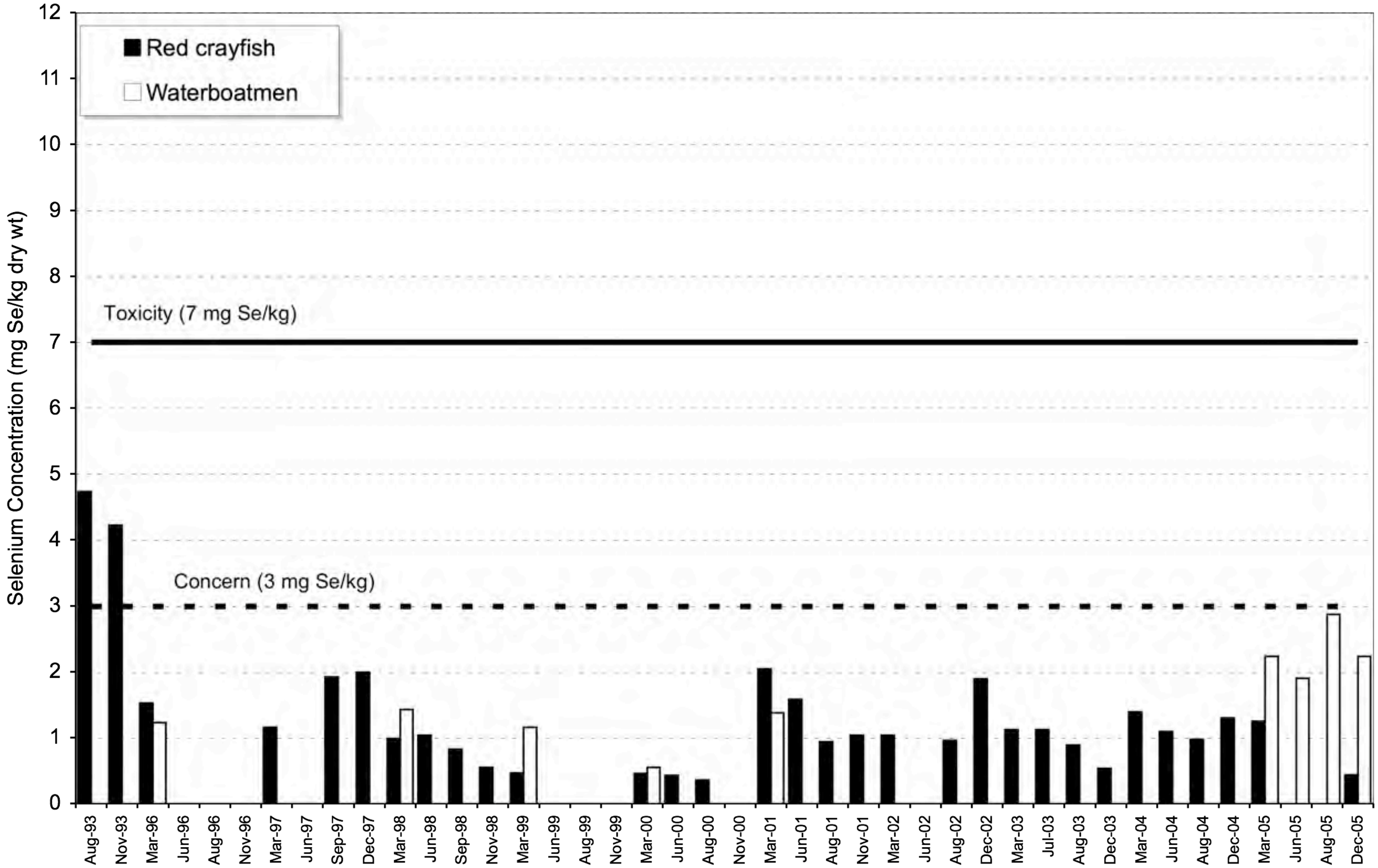

Figure 10A. Selenium Concentrations in Whole-Body Fish Tissue from the San Joaquin River at Hills Ferry, California (Site H).

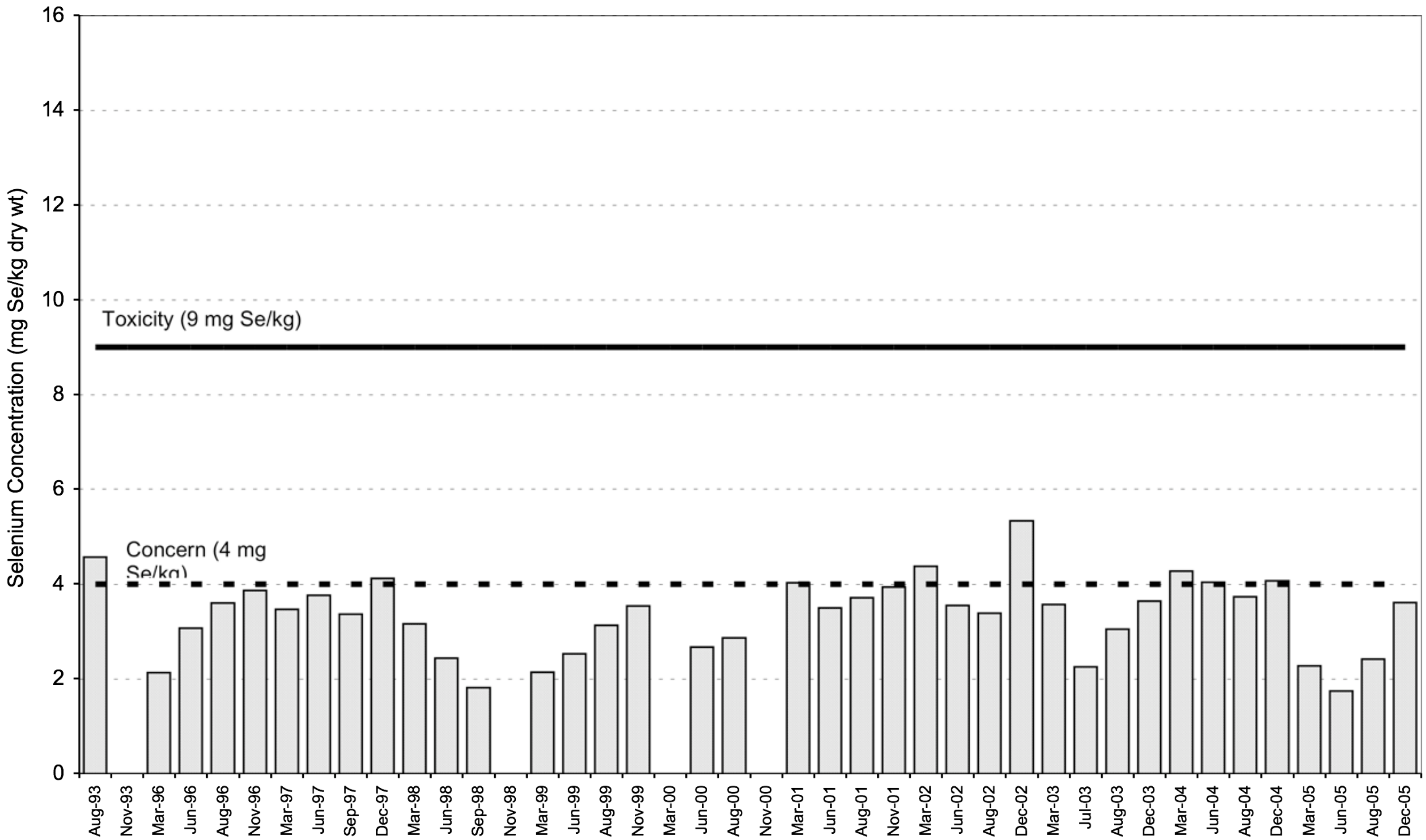

Figure 10B. Selenium Concentration in Invertebrates from the San Joaquin River at Hills Ferry, California (Site H).

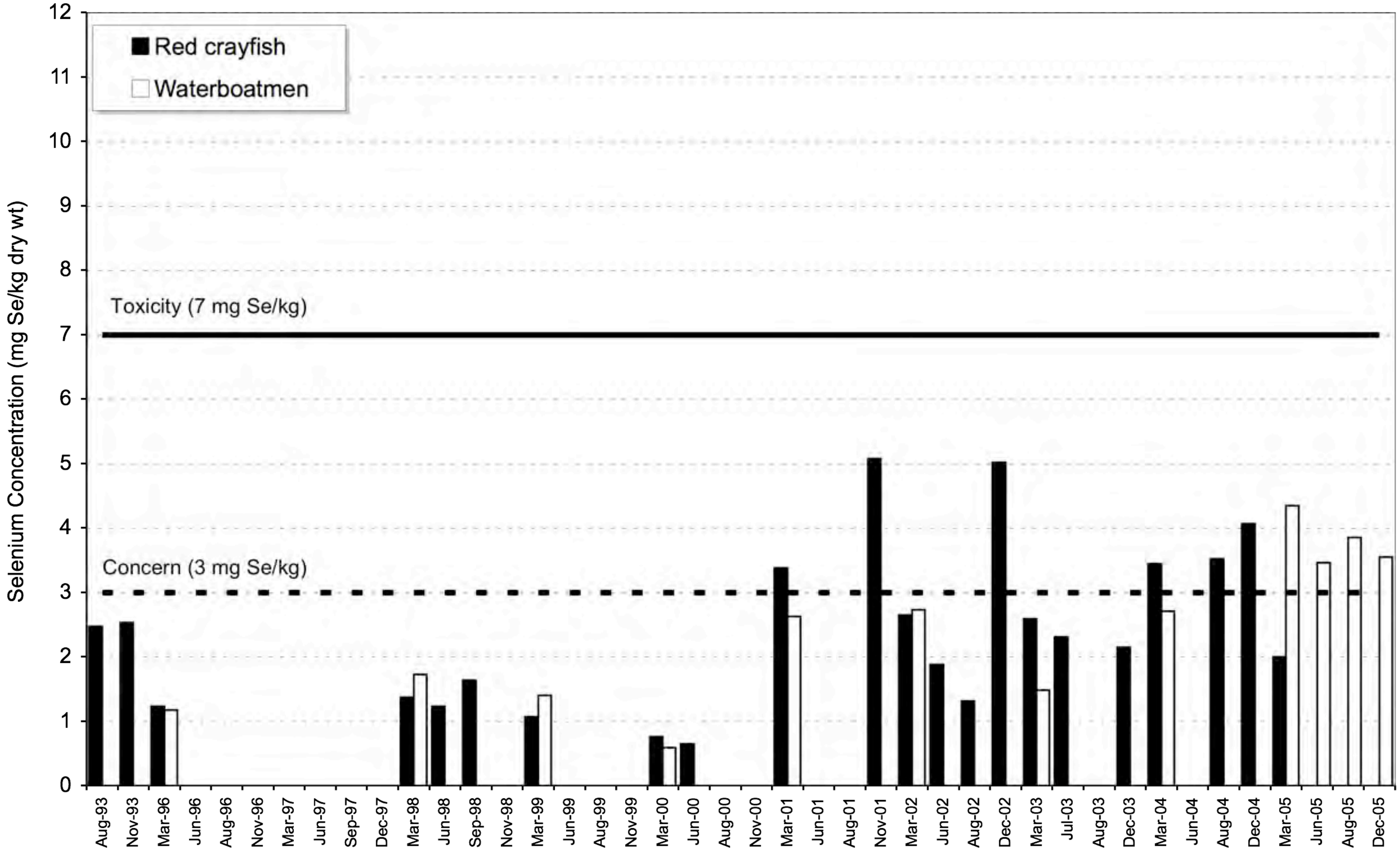

Figure 11. Percent abundance of trophic classifications over time in Mud Slough at Hwy 140 (Site E), August 1993 – December 2005

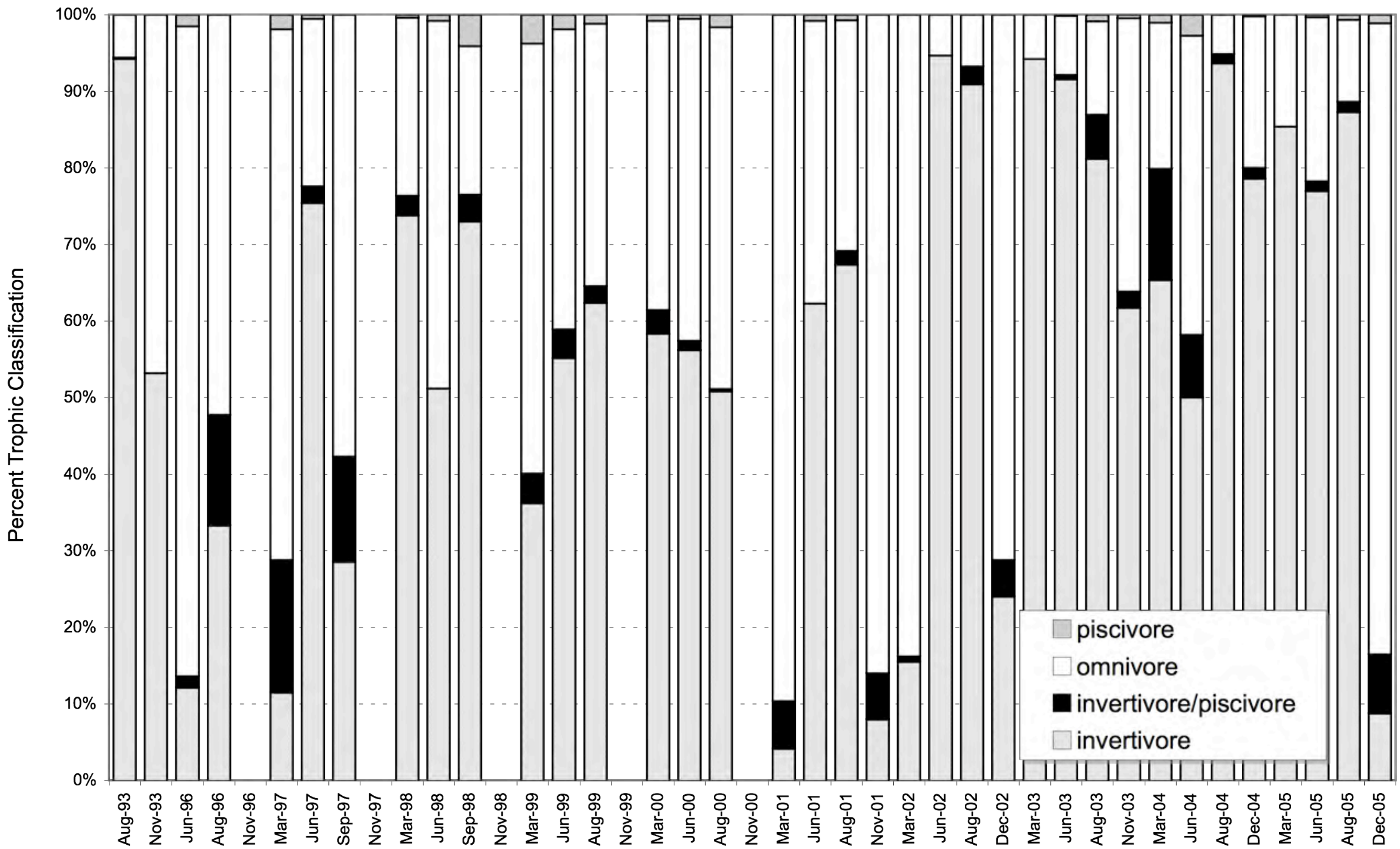

Figure 12. Percent abundance of trophic classifications over time in the San Joaquin River at Fremont Ford (Site G), August 1993 – December 2005

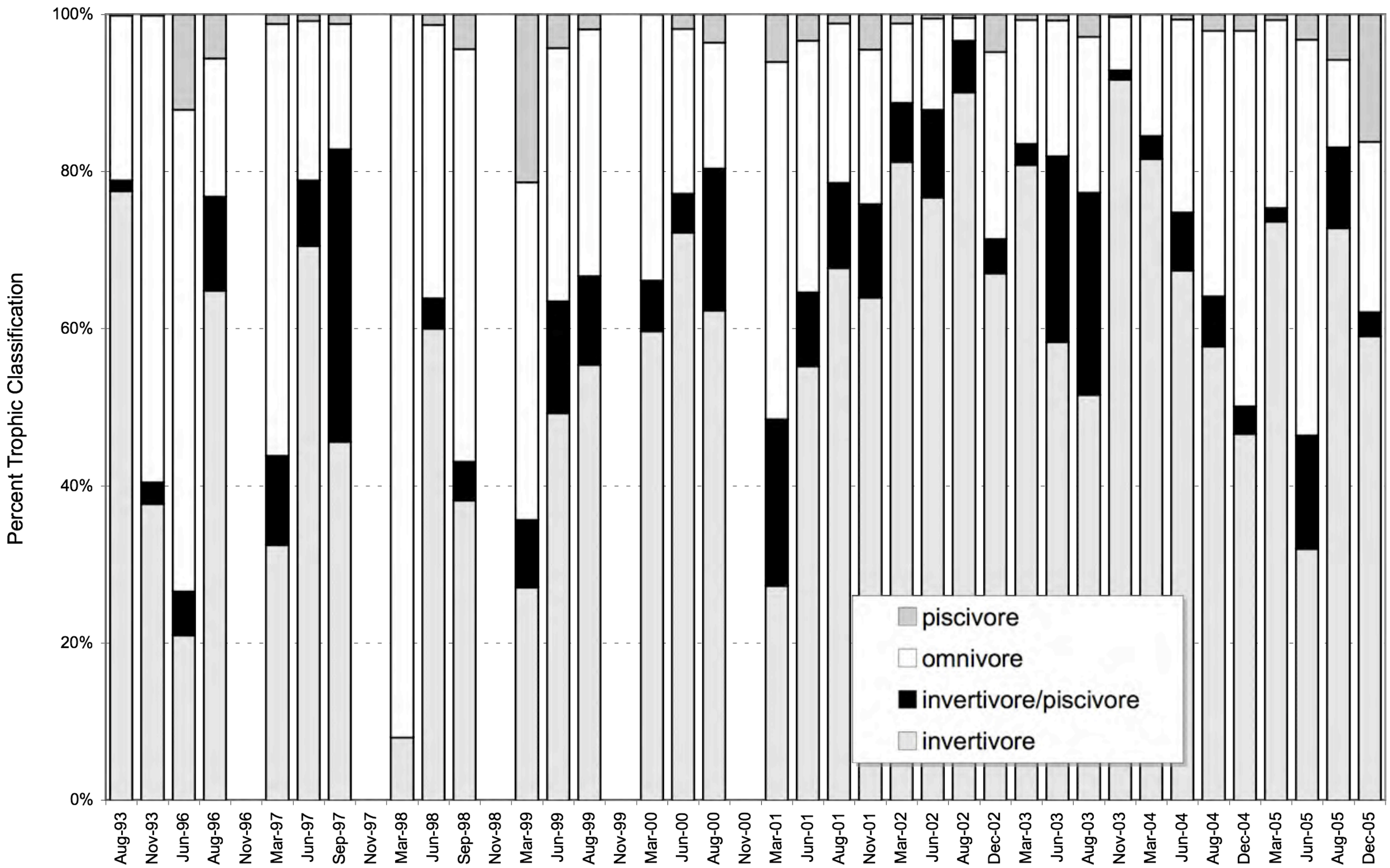

Figure 13. Percent abundance of trophic classifications over time in the San Joaquin River at Hills Ferry (Site H), August 1993 – December 2005

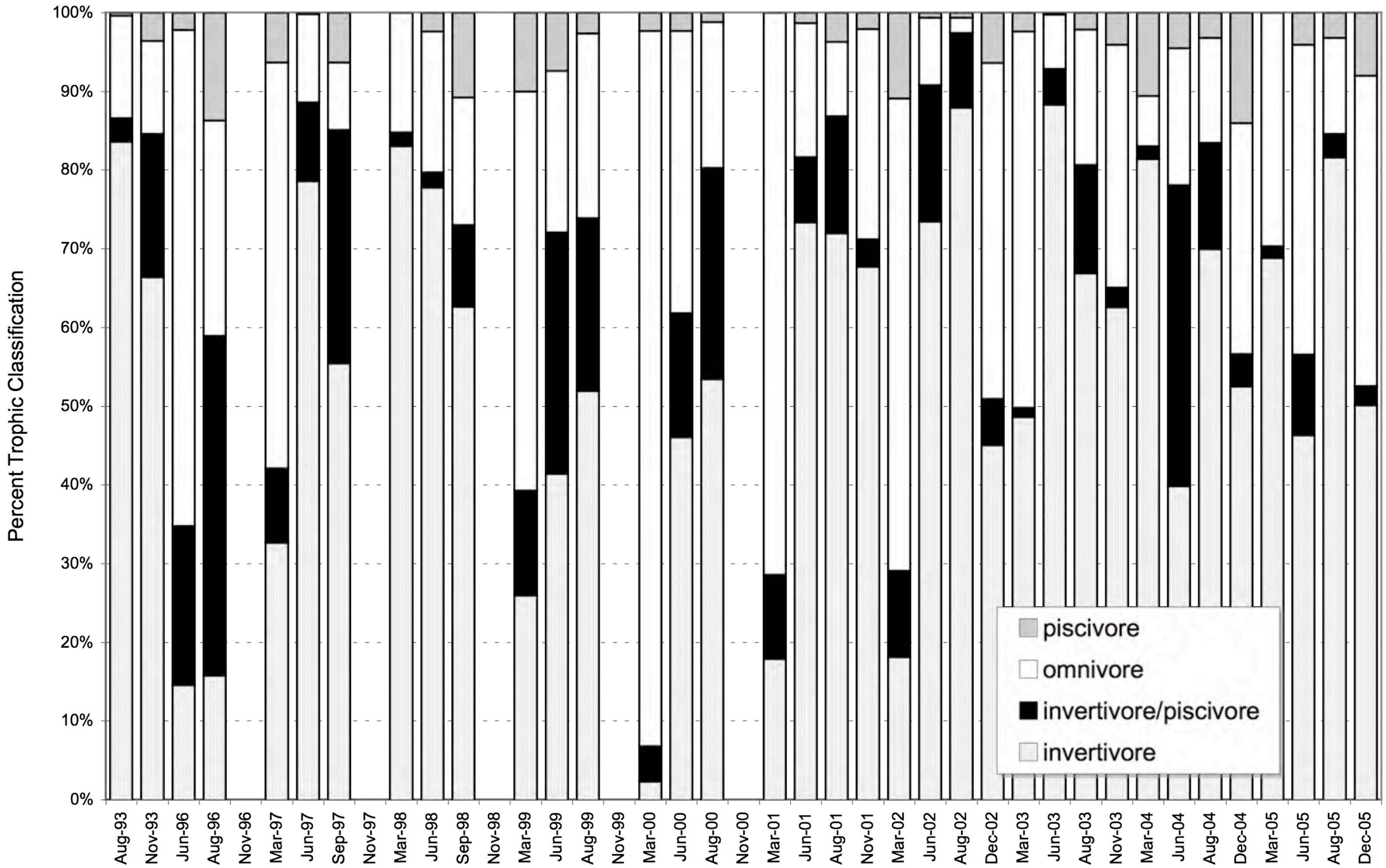

Figure 14. Observed Anomalies in all Fish Species Caught in Mud Slough and the San Joaquin River, August 1993 – December 2005

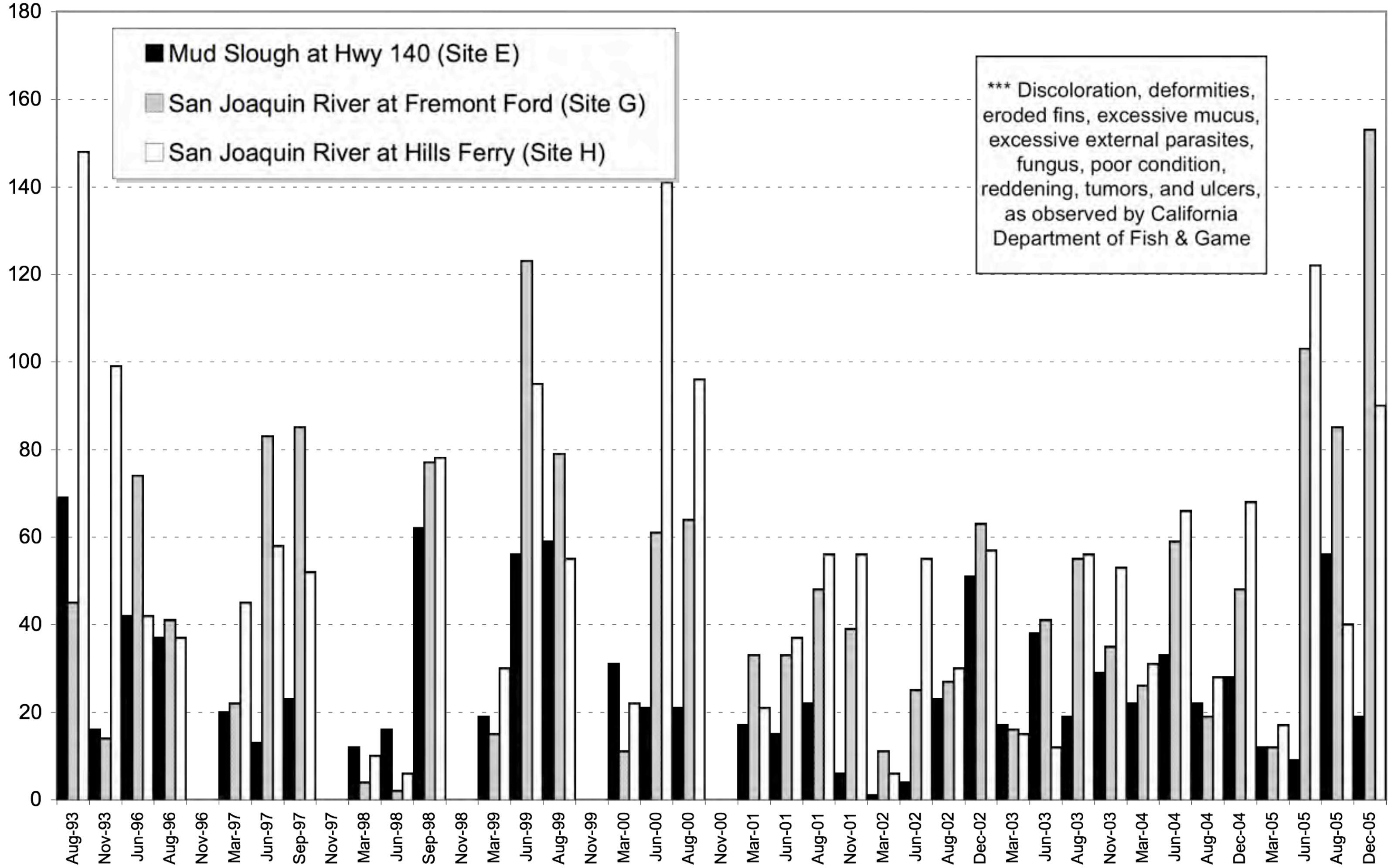

Figure 15. Selenium Concentrations in Carp Muscle Tissue from Mud Slough at Hwy 140 (Site E).

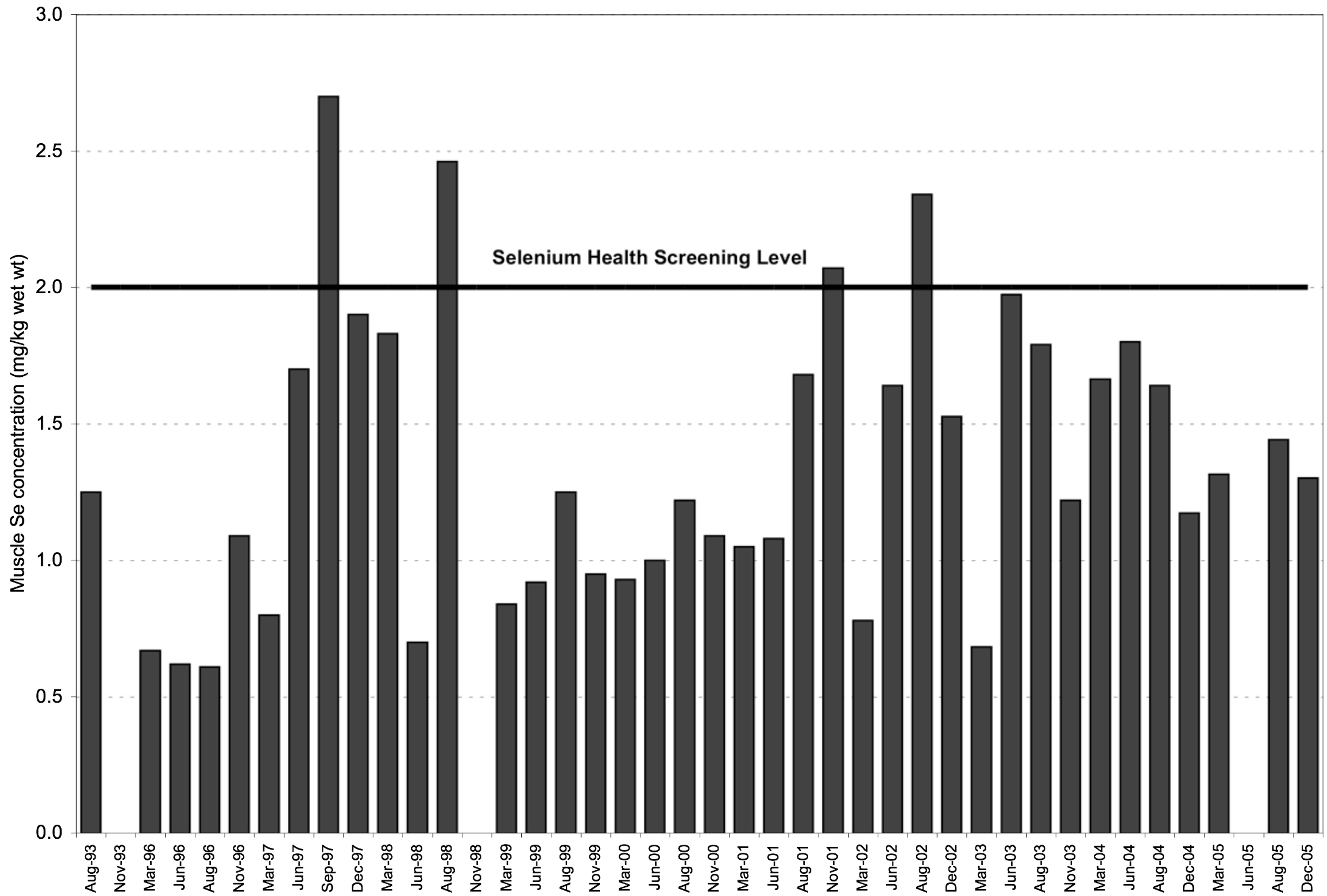

Figure 16. Selenium Concentrations in Fish Muscle Tissue from the San Joaquin River at Fremont Ford (Site G).

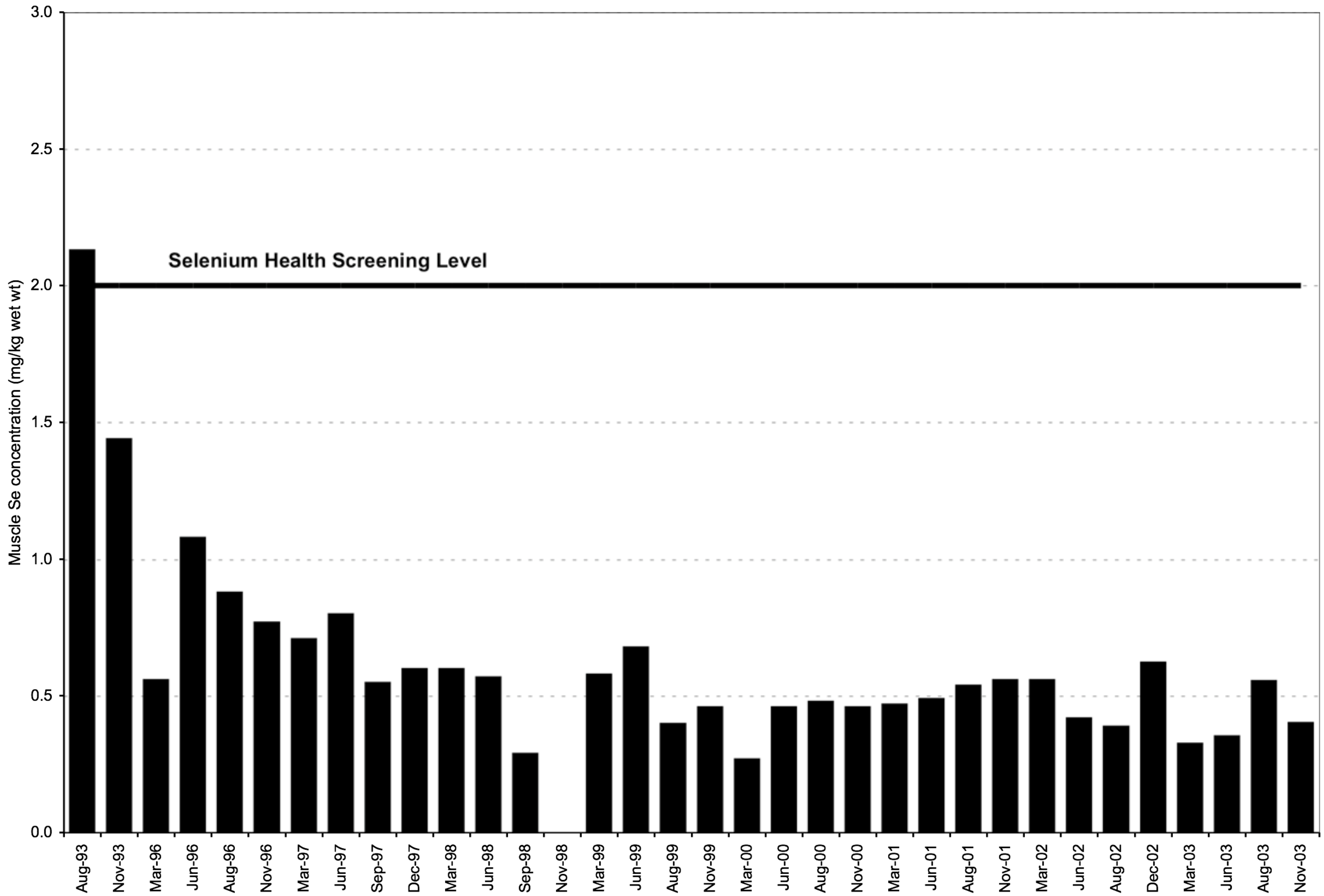

Figure 17. Selenium Concentrations in Fish Muscle Tissue from the San Joaquin River at Hills Ferry (Site H).