OW-2004-0019

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Washington, D.C. 20240

ADDRESS ONLY THE DIRECTOR
FISH AND WILDLIFE SERVICE

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Stephan L. Johnson, Administrator
U.S. Environmental Protection Agency
Water Docket
Mailcode: 4101T
1200 Pennsylvania Ave. NW.
Washington, DC 20460
Attention Docket ID No. OW-2004-0019

May 19, 2005

Dear Mr. Johnson,

The U.S. Fish and Wildlife Service (USFWS) respectfully submits the following public comment package in response to the U.S. Environmental Protection Agency's (EPA) request for scientific information, data, and views pertaining to the "*Draft Aquatic Life Criteria Document for Selenium*" (Federal Register 69(242):75541-75546; December 17, 2004). Selenium is a particularly potent environmental stressor for fish and wildlife, and USFWS scientists (often in collaboration with the U.S. Geological Survey's Biological Resources Division (BRD), EPA's Office of Research and Development (ORD), and university researchers), have produced a substantive portion of the scientific record documenting the ecotoxicology of selenium through a combination of field and laboratory research. For example, publications by current and former FWS scientists comprise thirty-five percent (81 of 228) of the literature cited in a recent review of the ecotoxicology of selenium published in the "*Handbook of Ecotoxicology*" (Hoffman et al. 2003).

The USFWS examined EPA's Draft Criteria Document, associated documents posted on EPA's selenium web site (http://www.epa.gov/waterscience/criteria/selenium), and documents found in EPA's e-docket for the selenium proposal, Docket ID No. OW-2004-0019 (http://www.epa.gov/edocket). The USFWS has identified technical concerns regarding the Draft Criteria Document (EPA 2004). We are aware of the exceptional complexity of selenium chemistry, its environmental dynamics and partitioning, and its biological effects, and the USFWS realizes the difficulty in deriving Ambient Water Quality Criteria for selenium. The USFWS appreciates EPA's substantial allocation of expertise and other resources in producing the Draft Criteria Document (EPA 2004) and pulling together an enormous and diverse base of scientific information into a single document for review by the wider scientific community. Although our comment package focuses on what the USFWS has identified as technical concerns in the Draft Criteria

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Document (EPA 2004) there was much in the document that the USFWS viewed as appropriate, such as the preference for a tissue-based chronic criterion and the recognition that Lemly's (1993) winter-stress study is environmentally relevant in addition to those conducted under thermoneutral laboratory conditions.

General Comments and Recommendations:

Documentation and background for our comments and recommendations are found in the attached appendices. All tissue values for selenium cited in this letter are on a dry weight basis unless noted otherwise.

The USFWS agrees with the conceptual basis and scientific soundness of tissue-based criteria for bioaccumulative pollutants. The basic components for scientific tissue-based criteria are: (1) the tissue-based numerical value must be scientifically defensible; (2) detailed sampling protocol for measuring the tissue number, and (3) detailed guidance for translating measured tissue numbers back to water-based numerical values since several Clean Water Act (CWA) programs are designed for implementation of water-based numerical values.

The guidelines employed to draft the proposed acute criteria for selenium (Stephan et al. 1985) are recognized both within EPA and throughout the scientific community as not being most relevant for application to highly bioaccumulative pollutants (e.g., Reiley et al. 2003). For proposed acute criteria of a bioaccumulative pollutant, one needs to know toxic risks for fish and wildlife based on their dietary exposures and the risk posed by exposure to the proposed water concentration. Although an acute excursion may be very short-lived in the water column, for bioaccumulative pollutants, the food web effects last much longer (e.g., Maier et al. 1998). The USFWS recommends the USEPA consider bioaccumulation as part of a multipathway exposure in the acute criterion. The USFWS realizes it may be necessary to collect data to evaluate the toxic risks to fish and wildlife based on their dietary exposures.

The USFWS recommends the USEPA employ an effects target level for the chronic criterion which is consistent with that of acute criteria. The USFWS has concluded the proposed selenium chronic criterion of 7.91 ug/g in whole body fish tissue exceeds an LC-20 effects target level. In the study cited by EPA as the basis for the 7.91 ug/g proposal (i.e., Lemly 1993), the lowest observed adverse effects (tissue) concentration (LOAEL) was 5.85 ug/g. The USFWS recommends EPA replace the chronic value of <7.91 ug/g for the winter-stress study (Lemly 1993) with a chronic value of <5.85 ug/g. Furthermore, the USFWS notes because 5.85 ug/g appears to be an LC-40 concentration, a tissue-based chronic criterion in the 4-5 ug/g range may be scientifically warranted and would also be consistent with wildlife toxicity data.

The USFWS recommends EPA give consideration to a new strategy on both water column and tissue based approaches. A national generic safety-net water criterion of 2 ug/L, as has been recommended (DuBowy 1989; Peterson and Nebeker 1992; Sweet 2002) and could be combined with a fish tissue-based criterion for site-specific

implementation. The monitoring of water concentrations in discharges could continue without increased expense of biotic sampling and translation of those sample results back to a water basis. Dischargers would be required to do biotic sampling intermittently (not a routine monitoring burden) on fish tissue relative to the fish tissue criterion. Only when the water column criterion and the fish tissue criterion are both exceeded, or the fish tissue criterion alone, would a full site-specific analysis including development of inter-media translation factors be necessary. Exceedance of the water criterion alone would not require any action. Hamilton (2002) reported a mixed strategy was being employed for mercury criteria in Australia and Canada. Because mercury, like selenium, is a bioaccumulative pollutant, valuable information may be garnered from the Australian and Canadian experiences.

The USFWS is confident that with modifications a revised version of the Draft Criteria Document (EPA 2004) will serve as a scientifically sound basis for updating the national selenium criteria. The USFWS appreciates the opportunity to review and provide comments on this document and looks forward to a continued close working relationship on selenium criteria to achieve our respective Agency's mutual goals and responsibilities for scientifically sound environmental protection and stewardship.

Sincerely,

Everett F. Wilson

Everett F. Wilson
Chief Division of Environmental Quality

Attachments:

Technical Comments

Documentation and background for our comments and recommendations are included below. All tissue values for selenium cited in this and following sections are cited on a dry weight basis unless noted otherwise.

1. ***Acute Criteria*:** The standard guidelines (Stephan et al. 1985) were not developed with highly bioaccumulative pollutants in mind. This is illustrated by referring to EPA's Figure 1 (EPA 1989:8), where bioaccumulation is not considered in the acute criterion flow paths, Reiley et al. (2003) viewed the standard guidelines for deriving water quality criteria as problematic as they give minimal consideration to such concerns. Both the mode of action and critical body residues are affected by the bioaccumulation of a pollutant. Similarly, the expert panel noted: "Little consideration is given to multipathway exposure, leaving criteria to reflect uptake from the water only." The USFWS believes that for highly bioaccumulative compounds in order to develop meaningful acute criteria the potential for residual food chain effects, from even brief acute excursions, must be considered. The USFWS looks forward to working with EPA to develop a model to accomplish this difficult task.

2. ***Fish Tissue-based Chronic Criterion:*** The USFWS agrees with the conceptual basis and scientific soundness of tissue-based criteria for bioaccumulative pollutants. The basic components for scientific tissue-based criteria are: (1) the tissue-based numerical value must be scientifically defensible; (2) it needs to be accompanied by a detailed sampling protocol for measuring the tissue number, and (3) it must be accompanied by detailed guidance for translating measured tissue numbers back to water-based numerical values since several Clean Water Act (CWA; 33 U.S.C. 1251 *et seq.*) programs are designed for implementation of water-based numerical values.

The USFWS concluded the proposed tissue value of 7.91 ug/g selenium (parts per million; EPA 2004) is not protective of fish or aquatic-dependent wildlife. In the study cited in the Draft Criteria Document (EPA 2004) as the basis for the 7.91 ug/g proposal (i.e., Lemly 1993), the lowest observed adverse effects (tissue) concentration (LOAEL) was <5.85 ug/g, and this value appears to be an LC-40 (see Attachments 1 and 2). Based on linear extrapolation, an underestimate of effects levels as these curves are exponential, the USFWS has concluded the 7.91 ug/g was greater than an LC-50 for the Lemly (1993) experiment because response curves for selenium are typically very steep (i.e., Lemly 2002; Holm et al. 2003). EPA's standard practice for deriving acute water quality criteria is to divide Final Acute LC-50 Values by a factor of 2 to approximate an LC-01 level of protection (e.g., EPA Water Quality Standards Academy Participant's Manual 1999; Reiley et al. 2003; Keating 2003). The USFWS agrees that a 1-5% effect level is an appropriate target level for setting adequately protective water quality criteria. The USFWS concluded that the Lemly (1993) study demonstrates an EC-20 tissue value for bluegill that is less than 5.85 ug/g. Based on this data and other data presented later in this review the USFWS believes that a tissue concentration less than 5 ug/g would provide an appropriate level of protection, not only for aquatic organisms but also for wildlife. As noted in other sections of this analysis other data also suggests a lower concentration. The USFWS would like to work with USEPA to assist them in developing a protective concentration.

2(a). ***Sampling Guidance:*** EPA states (Federal Register 69(242):75541-75546): "*Because EPA has not yet made decisions on the form or value of its final water quality criteria for selenium, EPA has not yet developed implementation procedures.*" The USFWS believes EPA should promulgate a final tissue-based chronic criterion with developed implementation procedures. The USFWS further notes that in conjunction with this criterion an EPA-approved analytical method for whole-body fish tissue may require promulgation.

2(b). ***Implementation Guidance:*** EPA's proposed tissue-based criterion is founded on the whole-body selenium concentration in juvenile bluegill associated with over-wintering mortality. However, when dealing with a mortality endpoint, and the sampling of surviving fish, it is difficult to get a true measure of tissue selenium due to "survivor-bias" (see Seiler et al. 2003). EPA suggested adult fish tissues should be monitored as they will not be affected by the criterion value and thereby survivor-bias would be avoided (Federal Register 69(242):75541-75546). However, the criterion value would be expected to kill at least twenty percent of juvenile fish; thereby biasing the pool of surviving fish available for tissue monitoring (i.e., introducing survivor bias). The dietary habits, and therefore exposure to selenium, are very different for many species of juvenile and adult fish. This is compounded by the additional summer/fall screening value of whole-body selenium. EPA proposes the monitoring of adult fish as a check on whether exposure at those seasons may exceed the proposed criterion value due to winter-stress syndrome. However, these effects would be expected in juvenile fish (Lemly 1993), but not in adult fish.

EPA's outside formal peer reviewers brought up the issue of implementation guidance and how technically complex many of the implementation issues were likely to be (see peer review comments from reviewers Canton, Lemly, Moller, and Reash; Selenium Docket Document No.s OW-2004-0019-0019 thru OW-2004-0019-0023). In response EPA states (in part): "*We agree that implementation guidance is essential, and needs to address a range of issues, from tissue sampling to BAF calculations. Implementation of selenium criteria will be addressed in a separate publication.*" Elsewhere in response to peer reviewers, EPA states: "*...we recognize that in practice* [i.e., site-specific modifications] *would not be easy to implement in the absence of an EPA protocol.*" In reference to a recommended tissue-based criterion for methylmercury, EPA states: "This the first time EPA has issued a water quality criterion expressed as a fish and shellfish tissue value rather than as a water column value. EPA recognizes this approach differs from traditional water column criteria, and will pose implementation challenges" (EPA 2002:5). The methylmercury precedent serves to reinforce the conclusion a scientifically sound implementation protocol should precede or coincide with promulgation of tissue-based water quality criteria.

The USFWS understands EPA will likely undertake an effort to develop implementation guidance in the near future, as EPA repeatedly noted in response to outside peer reviewers (see EPA responses to peer reviewers Canton, Lemly, Moller, and Reash; Selenium Docket Document No.s OW-2004-0019-0019 thru OW-2004-0019-0023). It is difficult to assess the proposed chronic criterion without the implementation guidance, as the success of the criterion is dependent on an accurate, representative sampling of the target populations in the receiving water. It is possible some states and/or dischargers will prefer to develop site-specific water-based standards. This will require development of bioaccumulation factors (BAFs).

EPA has begun to define the implementation procedures (e.g. whole body sampling vs. tissues, adult vs. juvenile), but other aspects of how the criterion will be used are not well described. Technical implementation issues needing to be addressed include; species selection, age of the fish, development of site-specific bioaccumulation factors, survivor bias, fishless waters, sample locations, and appropriate tissue.

Species Selection: When selecting a species to monitor for regulation of selenium discharges, it is important to consider not only the chemical sensitivity, but also to consider the candidate species life history aspects, which contribute to their vulnerability. Species with long life cycles and low reproductive rates are often more vulnerable to increases in mortality than species with short life cycles and high reproductive rates. These characteristics are important when assessing the potential adverse effects of selenium to threatened and endangered aquatic species. Information on selenium sources, speciation, exposures, site-specific characteristics, lag effects, and integration of ecological effects, must be taken into consideration.

Fishless Waters: Implementing a fish tissue-based chronic criterion is problematic for fishless waters. EPA suggests the possibility of applying the criterion to invertebrate tissue where invertebrate samples are obtained in place of fish samples (Federal Register 69(242):75541-75546). However, in fishless waters, invertebrates are not eaten by fish, but rather become food for aquatic-dependent wildlife. As EPA notes, their proposed criterion was not derived with intent to protect wildlife (Federal Register 69(242):75541-75546).

Bioaccumulation Factors (BAF's): The proposed tissue-based chronic criterion will be problematic for the development of an NPDES permit limit for new discharges. EPA notes "where translation from the tissue benchmark to a water concentration is needed, a bioaccumulation factor (BAF), which may vary substantially from site to site, would need to be established" (Federal Register 69(242):75541-75546). There are difficult technical obstacles to determining representative BAF's required for site-specific standards. The BAF is not a fixed number that can be applied universally. This value is usually dependent upon the concentration of selenium in the water column (cf., McGeer et al., 2003), and thus will vary with temporal and spatial factors affecting water column concentrations. These problems may not be insurmountable (Toll et al., 2005), but considerable time and effort will likely be needed to develop site-specific BAF's.

Alternative Approaches: The USFWS recognizes a tissue-based chronic criterion may be difficult to implement. The USFWS recommends EPA give consideration to a strategy based on both water column and tissue based approaches. A national generic safety-net water criterion of 2 ug/L, as has been recommended (DuBowy 1989; Peterson and Nebeker 1992; Swift 2002) and could be combined with a fish tissue-based criterion for site-specific implementation. For the majority of waters nationwide, permitting and other CWA activities could continue without increased expense of biotic sampling and translation of those sample results back to a water basis. Dischargers could be required to do biotic sampling intermittently (not a routine monitoring burden) on fish tissue relative to the fish tissue criterion. Only when the water column criterion and the fish tissue criterion are both exceeded, or the fish tissue criterion alone, would a full site-specific analysis including development of inter-media translation factors be necessary. Exceedance of the water criterion alone would not require any action. The tissue-based criterion would also be used in the 303(d) listing process. The USFWS notes other advantages of a transitional mixed strategy are to allow collection

of data, which may alleviate uncertainties, both with tissue criteria values and difficulties implementing the criteria. A mixed strategy would have to be developed more fully, but the USFWS believes the concept has merit and recommends EPA give further consideration to this. Hamilton (2002) reported a mixed strategy was being employed for mercury criteria in Australia and Canada. Because mercury, like selenium, is a highly bioaccumulative pollutant, valuable information may be garnered from the Australian and Canadian experiences.

3. ***Analysis of the Protection of Reproductive Endpoints:*** The proposed chronic criterion value of 7.91 ug/g selenium on a whole-body fish tissue basis was developed from EPA's interpretation of an over-wintering survival endpoint (Lemly 1993). Reproductive endpoints are normally considered the most sensitive fish and wildlife biological effects endpoints for selenium (e.g., EPA 2004). Also, winter stress, may not be pertinent to water bodies in climatologically mild regions, nor to coldwater species of fish (Moller 2002; but see Mebane 2005). Therefore, it is necessary to evaluate what the proposed criterion would imply for gravid ovaries/eggs of fish. Also, EPA suggests tissue monitoring would be based on sampling adult tissue (Federal Register 69(242):75541-75546). A regression to relate selenium in bluegill ovaries to selenium in bluegill whole-body tissue was developed and presented in the Draft Criteria Document (EPA 2004:Appendix H), but is employed only to translate fish exposure data from studies for fish ovaries to a whole-body tissue basis so all species chronic values can be reported as whole-body tissue equivalents.

The question of whether a whole-body tissue concentration of 7.91 ug/g selenium would be protective of reproductive endpoints in fish is not addressed in the Draft Criteria Document (EPA 2004). Alternative interpretations of the relevant literature have produced guidelines for reproductive toxicity thresholds ranging from 10-17 ug/g for fish ovaries/eggs (Lemly 1996; DeForest et al. 1999). Using the equation developed by EPA (2004), at 7.91 ug/g whole-body selenium, ovaries would be expected to contain 17 ug/g selenium. However, one set of data (17 of the 23 data pairs; Hermanutz et al. (1996)) used to develop the regression were converted from wet weight to dry weight values without having the percent moistures for the samples, producing inaccurate dry weight values. Doroshov et al.(1992) reported for bluegill that the percent moisture in ovaries varies widely (59.6 – 80.2%) depending on the annual cycle of gonadal development (Gonadal Somatic Index, GSI %). The corrected conversion factor for the Hermanutz et al. (1996) data may be from 2.48 to 5.00 times the wet weight; an uncertainty which can not be resolved. This leaves the six data pairs from Coyle et al. (1993) for valid dry weight basis comparison of whole-body selenium versus ovary selenium in bluegill. One of Coyle et al.'s (1993) treatments resulted in a whole-body selenium concentration of 7.2 ug/g selenium in adult tissue. They found 7.2 ug/g whole-body selenium translated to 25 ug/g ovary selenium in reproductively active female bluegill. Because this exceeds the reproductive toxicity threshold range of 10-17 ug/g, it is reasonable to conclude a whole-body chronic criterion of 7.91 ug/g selenium would not be protective of reproductive endpoints. Doroshov et al. (1992) reported on a reproductive tissue (eggs) toxicity study that yields a chronic value of 12.7 ug/g for bluegill. They did not measure whole-body selenium, however data from Coyle et al (1993), associated 12.5 ug/g in ovary with 4.9 ug/g whole-body; (see also EPA 2004:Appendix H), suggesting a whole-body criterion of 5 ug/g or less would be required to protect bluegill reproductive endpoints.

Although the data for bluegill are limited, relatively few data exist for other species to assess reproductive protectiveness of the proposed chronic criterion. The Draft Criterion Document (EPA

2004:Table 4) reports the reproductive chronic value for rainbow trout was estimated at less than 6 ug/g whole-body selenium. USFWS data for bullhead and catfish from the Colorado River system (USFWS, Grand Junction, CO; written communication) reveal these species commonly exhibit ovaries with selenium concentrations ten-fold the whole-body concentration (e.g., 5.46 ug/g whole-body and 54.2 ug/g ovary). Available data for bluegill and rainbow trout indicate a whole-body tissue-based chronic criterion for selenium would have to be lower than 6 ug/g to be protective of reproductive endpoints for these fish.

4. ***Aquatic-Dependent Wildlife:*** The present chronic criterion for selenium is 5 ug/L (EPA 1987; 2002). Scientists assessing aquatic-dependent wildlife have concluded a range of 1-3 ug/L is required to be protective of wildlife (e.g., DuBowy 1989; Peterson and Nebeker 1992; Sample et al. 1996; Van Derveer and Canton 1997; Skorupa 1998). An analysis of the National Irrigation Water Quality Program (NIWQP) database conducted by Dr. William Beckon (SWG) suggests the proposed tissue criterion may be comparable, to a 7 ug/L water criterion (Figure 1), thus increasing the risk for wildlife.

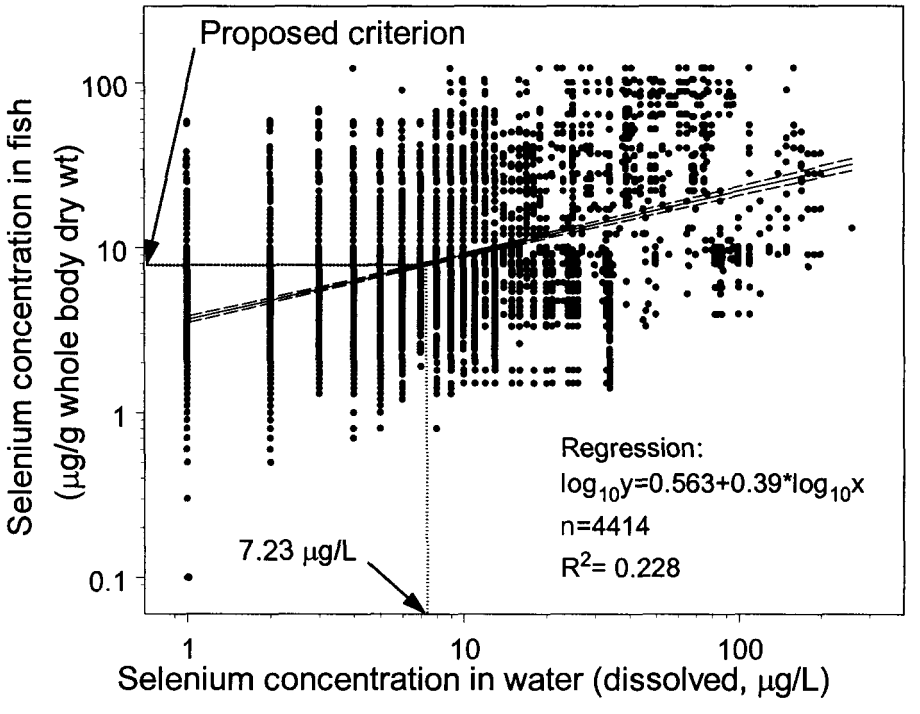

Figure 1.

Selenium in temporally and spatially matched samples of water and fish collected by the National Irrigation Water Quality Program from 26 areas of 14 states in the western United States (Seiler and Skorupa 2001).

National Contaminants Biomonitoring Program (NCBP) data show that the 85th percentile value for fish whole-body tissues has varied between 2.5-3.0 ug/g selenium nationwide with less than five percent of individual sampling sites rising to the level of even 4 ug/g (Walsh et al. 1977; May and McKinney 1981; Lowe et al. 1985; Schmitt and Brumbaugh 1990). The level of selenium loading in fish tissues will be mirrored closely by co-occurring aquatic invertebrates (e.g., May et al. 2001; Swift 2002) important to the diets of aquatic-dependent wildlife such as breeding waterfowl. The USFWS queried the NIWQP biota database (Seiler and Skorupa 2001; Seiler et al. 2003) and summarized the spatial and temporal matched samples of fish and aquatic invertebrates from sampling sites where whole-body fish tissue averaged between 5 and 10 ug/g selenium. The implied invertebrate-to-fish concentration factors from this dataset ranged from 0.67 to 1.36 (Attachment 3). These results suggest the selenium content of aquatic invertebrates would fall in the range of 5.8-11.8 ug/g. This dietary exposure range

for mallards would correspond with an EC20 to EC85 range of effects based on reproductive toxicity (e.g., Ohlendorf 2003). The query results also suggested a central tendency for the implied concentration factors of 1.1 (Attachment 3). Thus, 7.91 ug/g in whole-body fish tissue translates to 7.2 ug/g in aquatic invertebrates. This estimate exceeds the upper 95% statistical confidence boundary (6.64 ug/g) of the dietary EC20 for mallards and equals the EC40 (Ohlendorf 2003). Fish whole-body tissue containing 7.91 ug/g selenium would allow levels of aquatic food chain contamination to exceed the dietary EC20 for reproductive toxicity in mallards (>95%), with an estimate of an EC40.

5. *Threatened and Endangered Species:* There are about one hundred species of aquatic-dependent wildlife in the United States listed as threatened and endangered pursuant to the Endangered Species Act (ESA; 16 U.S.C. 1531 *et seq.*) which is roughly equal to the number of listed species of fish. There are no promulgated national "Wildlife Criteria" for selenium. The California Toxics Rule wildlife criterion process cited by EPA (Federal Register 69(242):75541-75546; December 17, 2004), has been initiated and is projected to require a minimum of five more years to produce a wildlife criterion recommendation (EPA, written comm.). Promulgation of USFWS recommended tissue-based chronic criterion using present practices for acute criteria (i.e., LC-01) would be consistent with the purposes and goals of the CWA/ESA Memorandum of Understanding (MOA) between EPA, FWS, and NOAA-Fisheries (formerly National Marine Fisheries USFWS; see Federal Register 66(36):11202-11217; February 22, 2001).

6. Data Screening and Analyses: Appropriate studies should be included for analyses such as the Hamilton et al. studies; the Beyers and Sodergren studies of razorback sucker (though Beyers and Sodergren studied a less sensitive life stage; see Hamilton, In Press); the Hamilton and Palace (2001) critical review of the Kennedy et al. (2000) study; and including the Hamilton et al. (1990) 90-day results based on performance of controls.

The USFWS is concerned about bias in the Draft Criteria document due to wet to dry weight conversion factors. Conversions of data from wet weight to dry weight basis and vice-versa were done using inaccurate percent moistures which leads to a 25% overestimation of chronic values for whole-body analyses. In one case, calculation of wet weight data from an unpublished manuscript (Hermanutz et al. 1996) was done using a percent moisture value, derived from other studies, whereas a published paper from the same study (Swift 2002) employs the value from the cited study.

The USFWS is concerned about the potential bias in the Draft Criteria Document by not accounting for the hormetic status of selenium. EPA should use a hormetic model rather than the sigmoid logistic model for regressions in deriving a chronic criterion for selenium (EPA 2004:59), especially for data sets that span optimum and deficiency side of selenium exposures. (e.g. Hilton et al. 1980, cited at EPA 2004:I-13). Hormetic models are available (e.g., Brain and Cousens 1989; Van Ewijk and Hoekstra 1993; Svendsgaard 1993; Bailer and Oris 1997; Devidas et al. 1993) and widely used (Schabenberger et al. 1999; Stephenson et al. 2000; Chèvre et al. 2002). However, a non-hormetic statistical model is used to estimate LC and EC 20's.

Specific examples are presented below for Chinook salmon and rainbow trout.

1. Laboratory exposure of juvenile Chinook salmon to dietary selenium: (Hamilton et al. 1990, cited in EPA (2004: Appendix I-5)

This experiment indicates juvenile Chinook salmon with the proposed chronic criterion tissue concentration of 7.91 ug/g whole-body selenium would experience 59 percent mortality after 90 days of exposure.

The EPA analysis included 60-day results but excluded the 90-day results of this study of fall run Chinook salmon (*Oncorhynchus tshawytscha*) juveniles because "control survival declined significantly" during the final 30 days of experiment. However, the survival of all treatments declined substantially during the period, exhibiting a clear concentration-response relationship, with about 30 percent baseline mortality not attributable to selenium (Figure 2a). This general decline may have been caused by some unknown health problem, but it also may have been due in part to the physiological stress Chinook salmon of this strain experience during this developmental period as they undergo the genetically programmed osmoregulatory changes associated with the normal pattern of migration from freshwater breeding areas to the ocean (Scott Foott, Project Leader, California-Nevada Fish Health Center; James Smith, Project Leader, Red Bluff Fish and Wildlife Office; personal communication). The diet of the control group included a low concentration of selenium (1.0 μg/g) intended to represent background exposure. Thus, the control group effectively constituted the low end of a spectrum of exposures rather than a distinctive zero-exposure treatment. The model suggests the "controls" may have been slightly deficient in selenium (Figure 2a). The control survival rate of 66.7 percent accorded well with hormetic concentration-response models (Brain and Cousens 1989). Additionally, mortality due to selenium might be expected to increase during this period if the effects of dietary exposure involve some lag time associated with assimilation and incorporation of selenium into enzymes or tissues (Beckon In Prep.).

The results of this study indicate the proposed criterion of 7.91 μg/g (whole body dry weight) would result in 59 percent mortality of young Chinook salmon attributable to selenium (Figure 2a). The fish tissue criterion would need to be lowered to 2.5 μg/g to reduce mortality to 20%. These projections are based on a standard hormetic model while a sigmoid logistic model used by in the Draft Criteria Document (EPA 2004) does not account for hormesis. Sigmoid models indicate the proposed criterion would cause 64.5 percent mortality in young Chinook salmon (Figure 2b), and a criterion of 1.0 μg/g (whole body dry weight) would be needed to limit selenium-caused mortality to 20 percent.

The experiment included two parallel series of dietary selenium treatments. One set was spiked with seleno-DL-methionine (SeMet), the other set, was mosquitofish collected from the San Luis Drain (SLD), which carried seleniferous agricultural drainwater from a subsurface tile drainage system in the Westlands Water District in the San Joaquin Valley of California. The Draft Criteria Document (EPA 2004) suggests the SLD diets may have included other contaminants, such as pesticides, which may have contributed to the adverse chronic effects measured in this experiment. The data indicate, once selenium is incorporated into fish tissue, there is no difference in the tissue concentration-response relationship due to the different selenium (SLD or SeMet) sources. The experiment indicates the other contaminants effects were not detected. Therefore, all data from both diet series were used in the analysis presented here (Figure 3). The experimental data demonstrate assimilation into tissue of salmon larvae was more efficient with the SLD diet. This however, may be due to the

racemic mix of SeMet isomers used in the spiked diet rather than interaction with other contaminants.

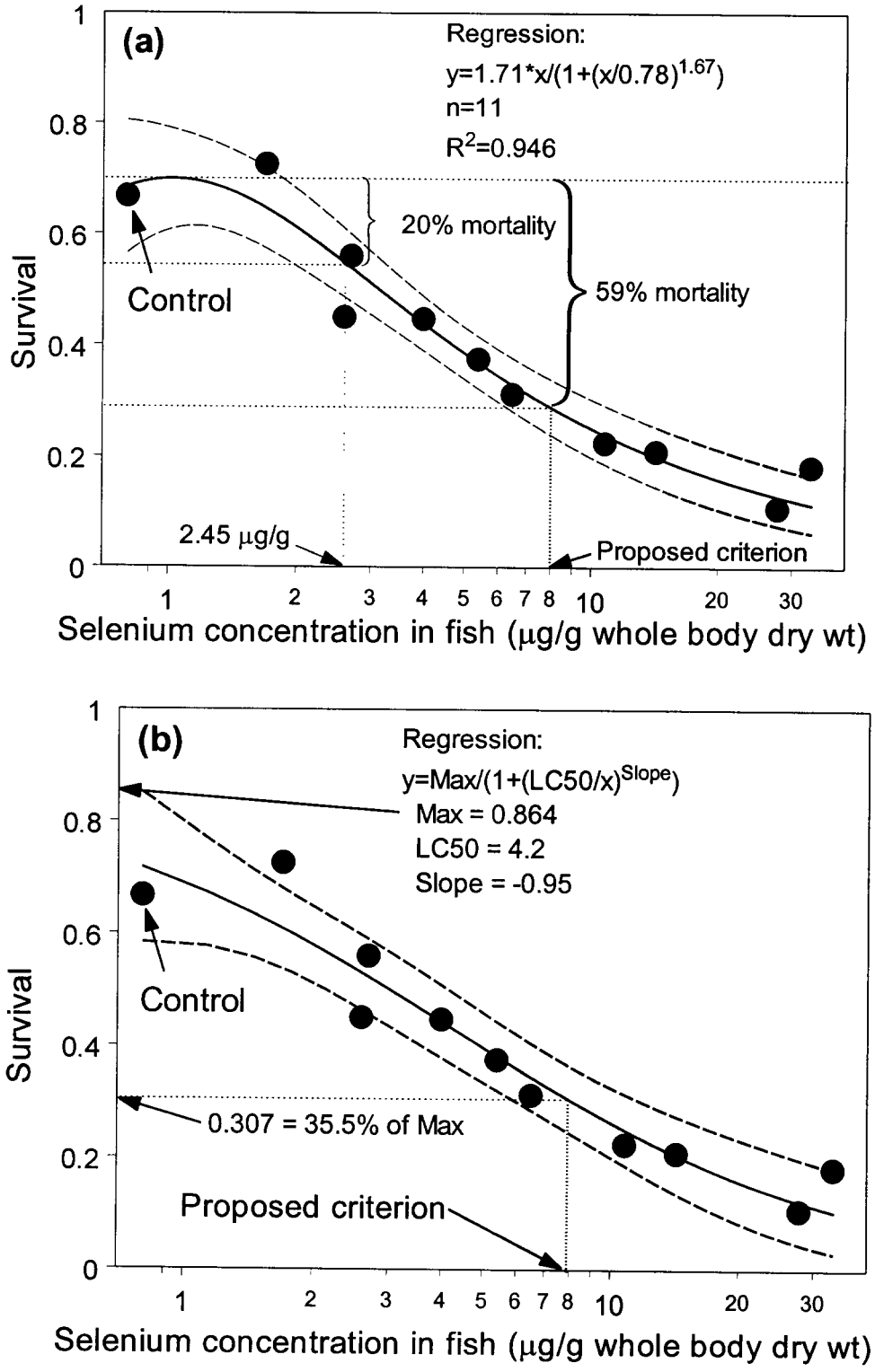

Figure 2. Survival as a function of selenium concentration in tissue of juvenile Chinook salmon after 90 days of exposure to dietary selenium. Hormetic model (a) and logistic model (b) fitted by least squares regression. Dashed lines indicate 95% confidence bands around the regressions in this and following figures.

Guidelines currently used in the U. S. do not address "controls" for hormetic substances. Furthermore, these guidelines explicitly apply only to waterborne, not dietary, exposure (ASTM 2004).

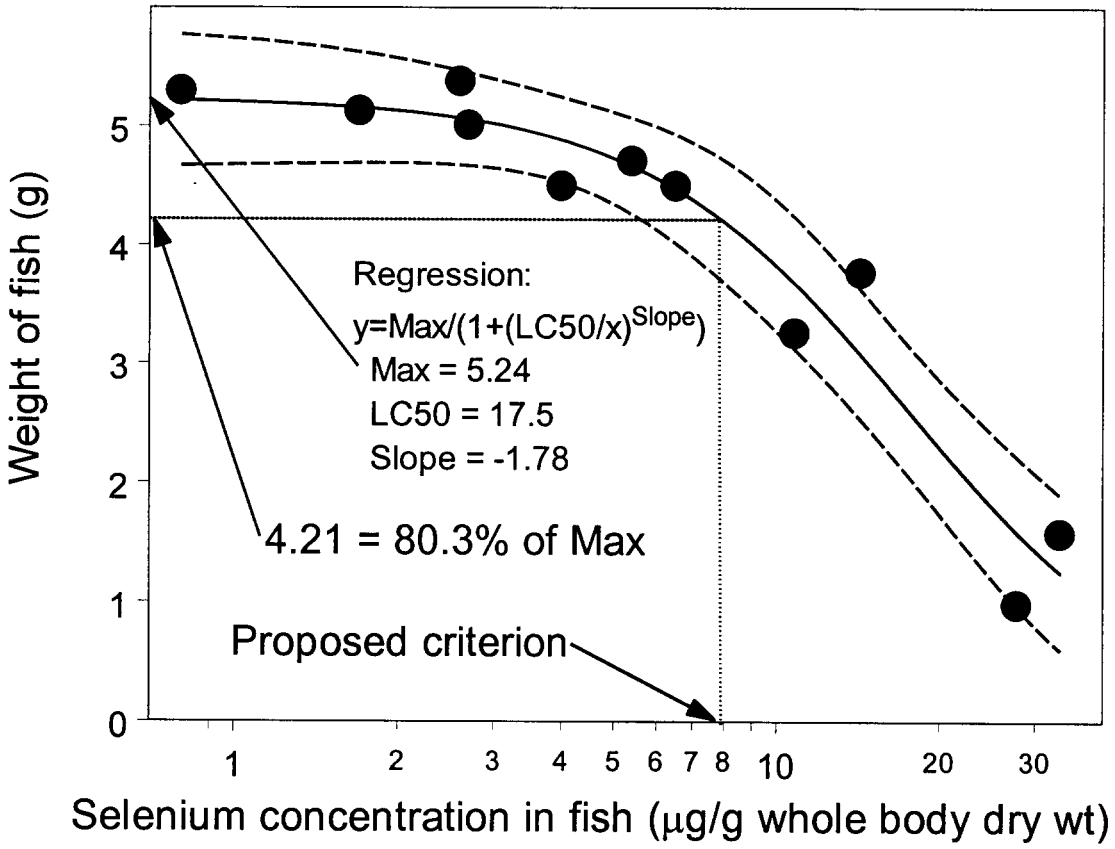

Figure 3. Juvenile fall run Chinook salmon weight 90 days after swim up, in fresh water with dietary exposure to selenium.

The surviving juvenile Chinook salmon at 90 days after swimup exhibit 20 percent weight loss due to selenium (Figure 3). Other studies performed on salmonds mirror those results and confirm the sensitivity of salmonids to selenium.

EPA failed to consider another major component of the Hamilton et al. (1990) study. A separate experiment of Hamilton et al. (1990) reared Chinook salmon fingerlings in reconstituted brackish water with dietary exposure to selenium for 120 days. These fingerlings were then challenged by 10 days of emersion in reconstituted seawater. The results indicate proposed chronic criterion concentration of selenium in salmon rearing in brackish water will result in 2.3 percent reduction in growth within 120 days (Figure 4), and upon entering the ocean will experience an additional 15 percent mortality within 10 days, due to selenium (Figure 5).

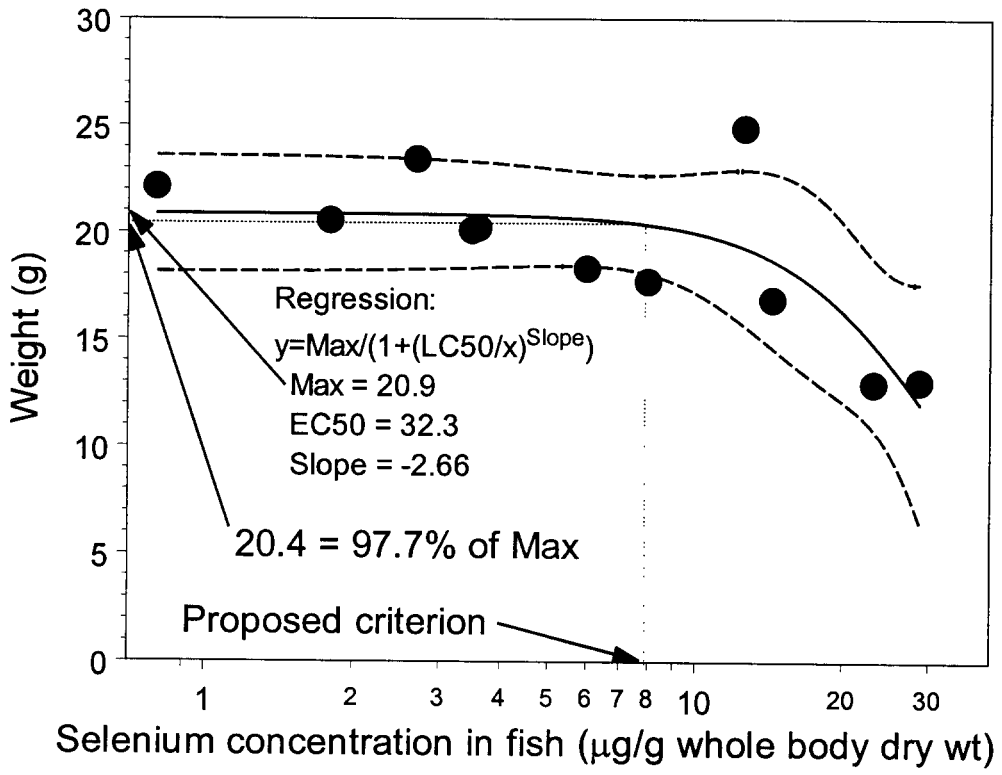

Figure 4. Juvenile fall run Chinook salmon weight after 120 days of rearing in brackish water with dietary exposure to selenium.

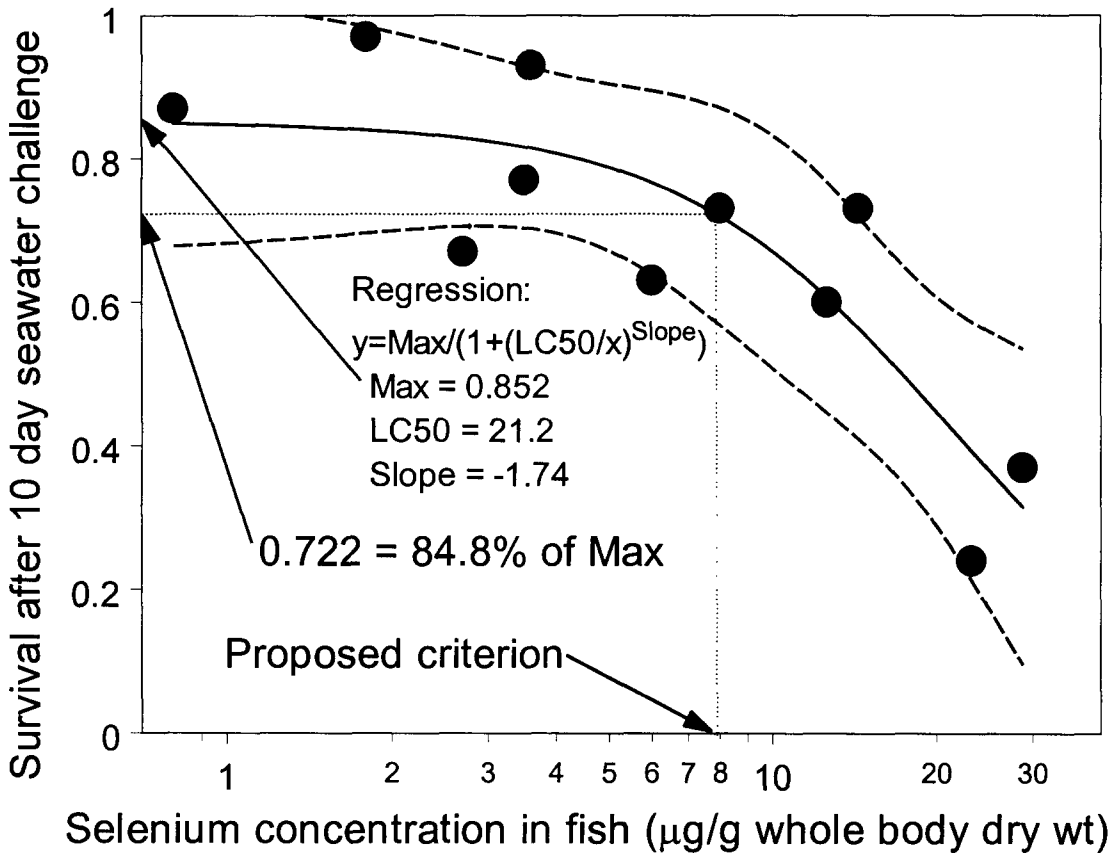

Figure 5. Survival of juvenile fall run chinook salmon after 10 day seawater challenge following rearing for 120 days in brackish water with dietary exposure to selenium.

2. Effects of selenium on fry of rainbow and brook trout exposed in streams in Alberta, Canada: (Holm 2002 and Holm et al. 2003, cited in the Draft Criterion Document (EPA 2004: Appendix I-15).

This study indicates female rainbow trout in the wild with the proposed criterion concentration of selenium in their (whole-body) tissue would produce eggs and swimup stage fry with 44.2 percent mortality. Among the 55.8% swimup survivors, 96 percent would suffer edema and 42 percent would have craniofacial deformities. To protect rainbow trout at an EC_{20} level, this study calls for a criterion of 3.51 µg/g whole-body dry weight.

Data for regressing egg selenium concentration against adult muscle selenium concentration in rainbow trout are displayed on a linear-scaled graph rather than on a log-log scaled graph (EPA 2004:I-23). This led to using an incorrect regression method to minimize the influence of a single datum (1.9 µg/g muscle wet weight). However, plotting the data on a log-log scale reveals this datum is not an outlier. The lowest egg selenium concentration datum (0.01 µg/g), is an extreme outlier (Figure 6). This datum is questionable as it is below typical analytical detection limits. Pending confirmation of this datum, it should be omitted from the regression (Figure 7).

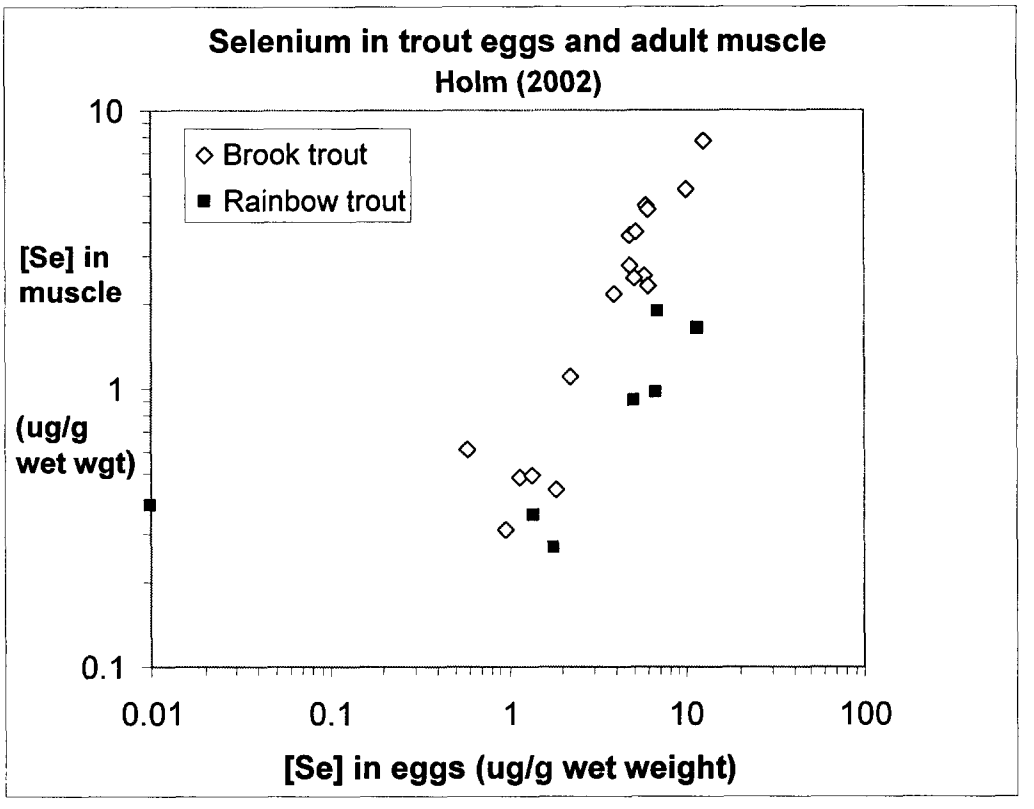

Figure 6. Relationship between maternal muscle and egg selenium concentrations in rainbow and brook trout from streams in northeastern Alberta (Holm 2002 and pers com).

Because contaminant concentrations are log normally distributed (Ott 1990) least squares regression should be performed on log-transformed concentrations (Figure 7).

The Draft Criterion Document (EPA 2004) used Holm's (2002) rainbow trout data to project selenium concentration in brook trout muscle from brook trout eggs even though better data for brook trout egg-muscle selenium relationship were available (Figure 6, Holm 2002, Holm et al. 2003).

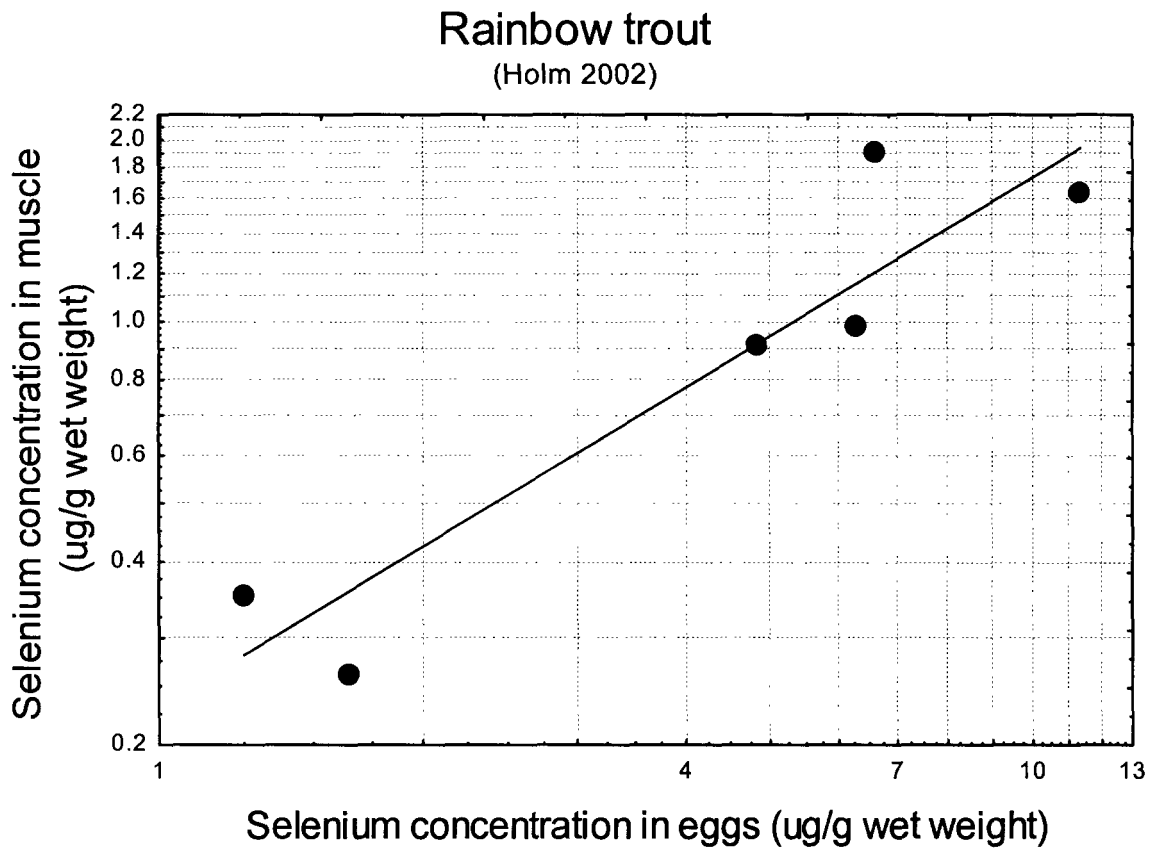

Figure 7. Regression performed on log-transformed Holm (2002) data excluding questionable outlier.

Using the regression in Figure 7, the percent moisture used by the EPA converting dry weight to wet weight (EPA 2004:I-23), and the muscle-whole body regression Equation I (EPA 2004:58), adult female rainbow trout would produce eggs with a selenium concentration of 12.47 µg/g wet weight at the criterion tissue concentration of 7.91 µg/g.

The Draft Criterion Document (EPA 2004:I-16) states a logistic curve could not be fitted to the 2001 rainbow trout edema data shown in "Holm Figure 3." However, a standard logistic curve can be fitted to these data, and shows the proposed tissue criterion will result in more than 80 percent edema in rainbow swimup fry (Figure 8).

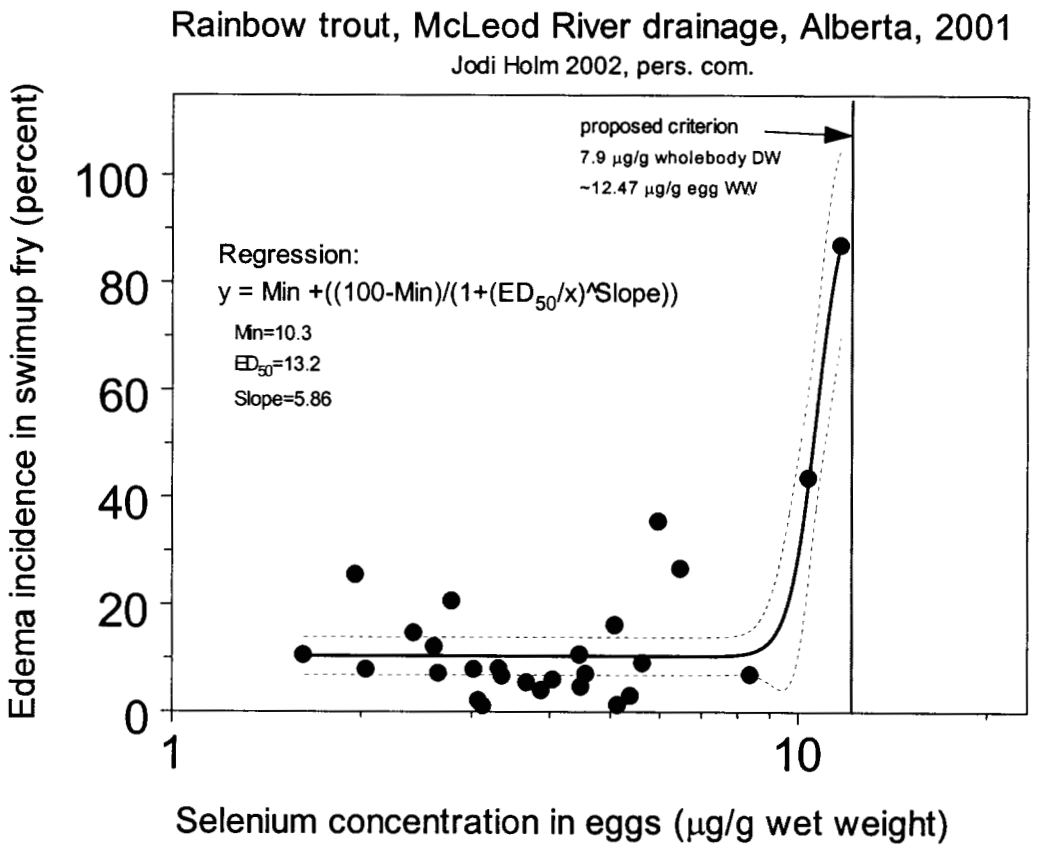

Figure 8. Relationship between selenium in rainbow trout eggs and edema in surviving swimup fry, data from the year 2001 only.

Inclusion of data from years 2000 and 2002 of the same study (Holm pers. com.) extends the regression, projecting 96 percent of the surviving swimup fry will suffer edema, 86.5 percent attributable to selenium in addition to a baseline of 9.5 percent (Figure 9).

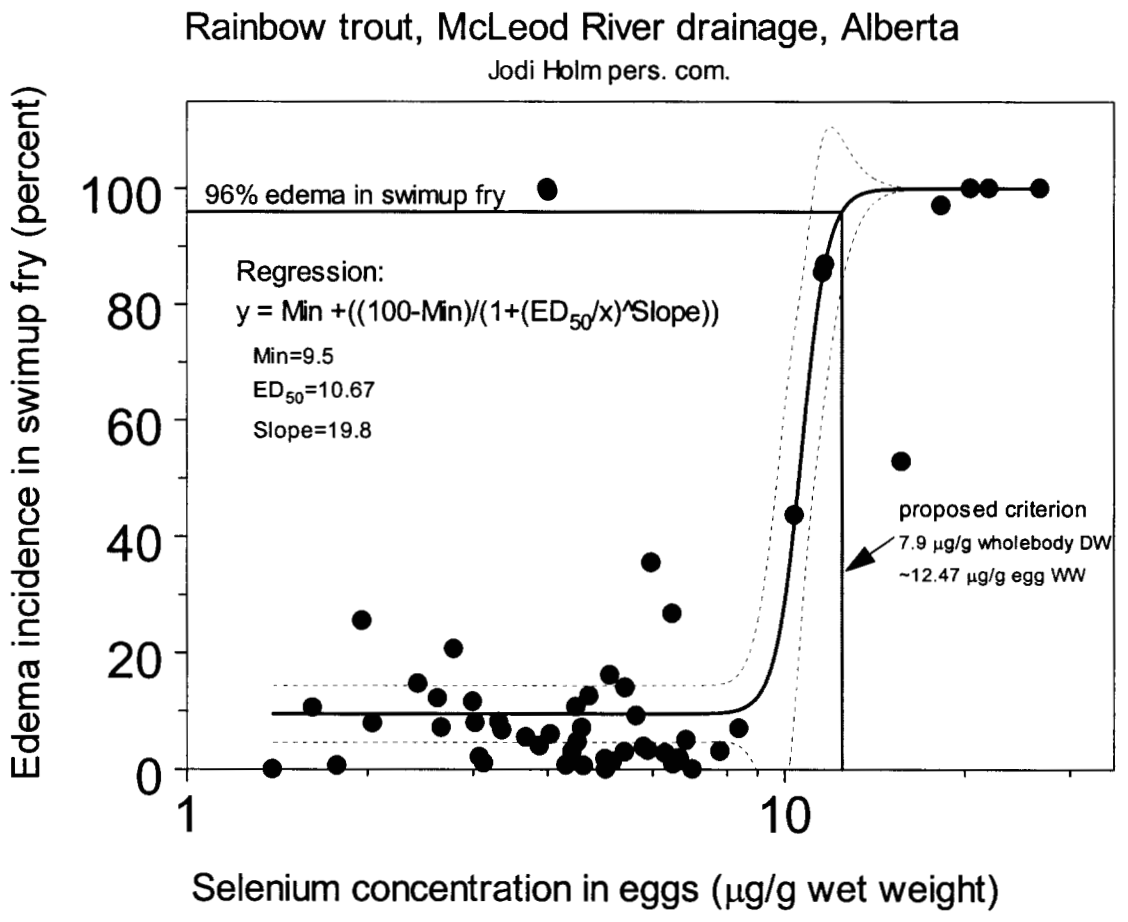

Figure 9. Relationship between selenium in rainbow trout eggs and edema in surviving swimup fry. Data from the years 2000-2002.

Edema is only one of a number of gross defects caused by selenium and measured in this study. For example, 42 percent of the surviving fry will have craniofacial deformities, 32 percent attributable to selenium (Figure 10, Holm 2002, Holm et al. 2003, Holm pers.com.).

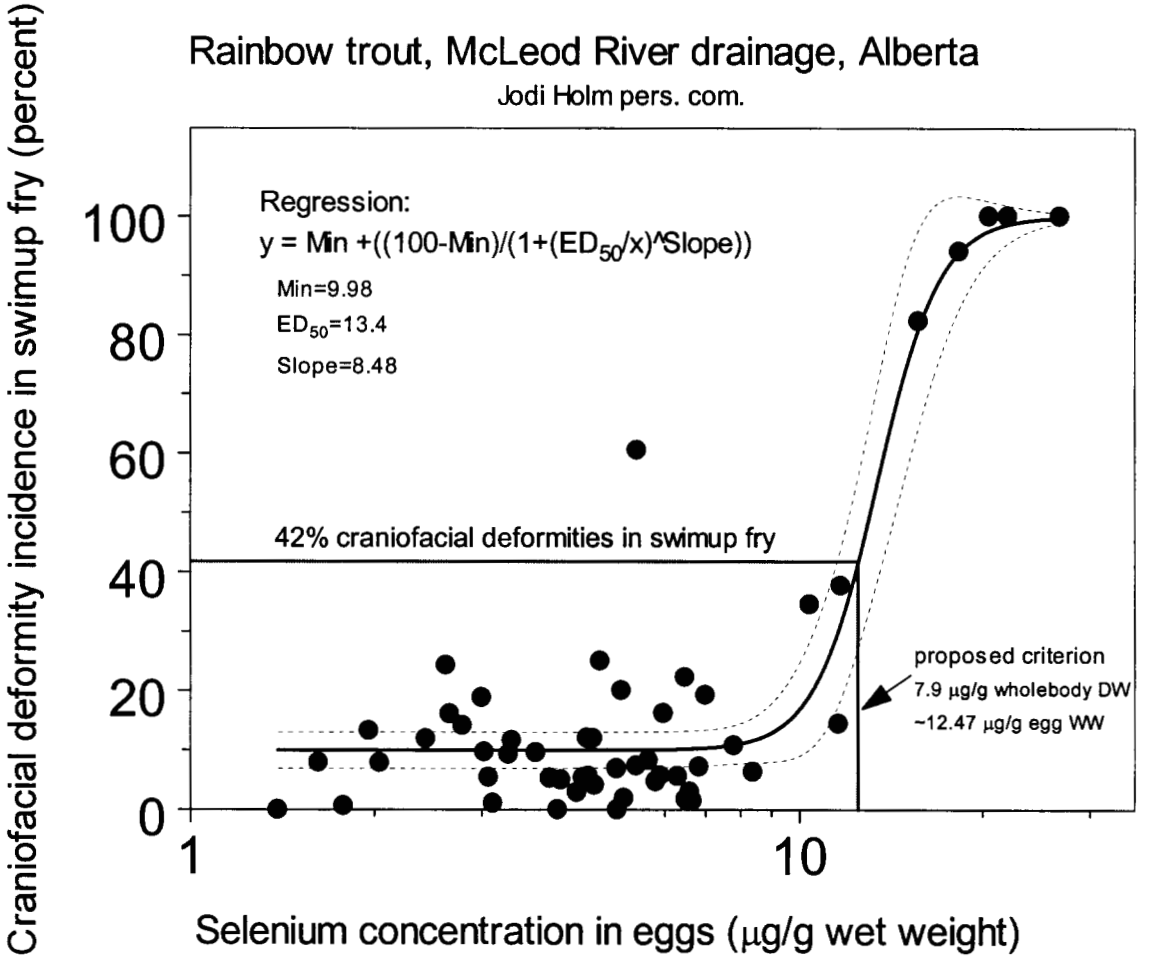

Figure 10. Relationship between selenium in rainbow trout eggs and craniofacial deformities in surviving swimup fry. Data from the years 2000-2002.

Furthermore, all these defects could only be assessed in the fry that survived to reach the swimup stage. Analysis of mortality data from the same study indicates rainbow trout would produce eggs experiencing 44.2 percent mortality at swimup stage with the proposed criterion concentration (7.91 µg/g whole-body dry weight), (Figure 11).

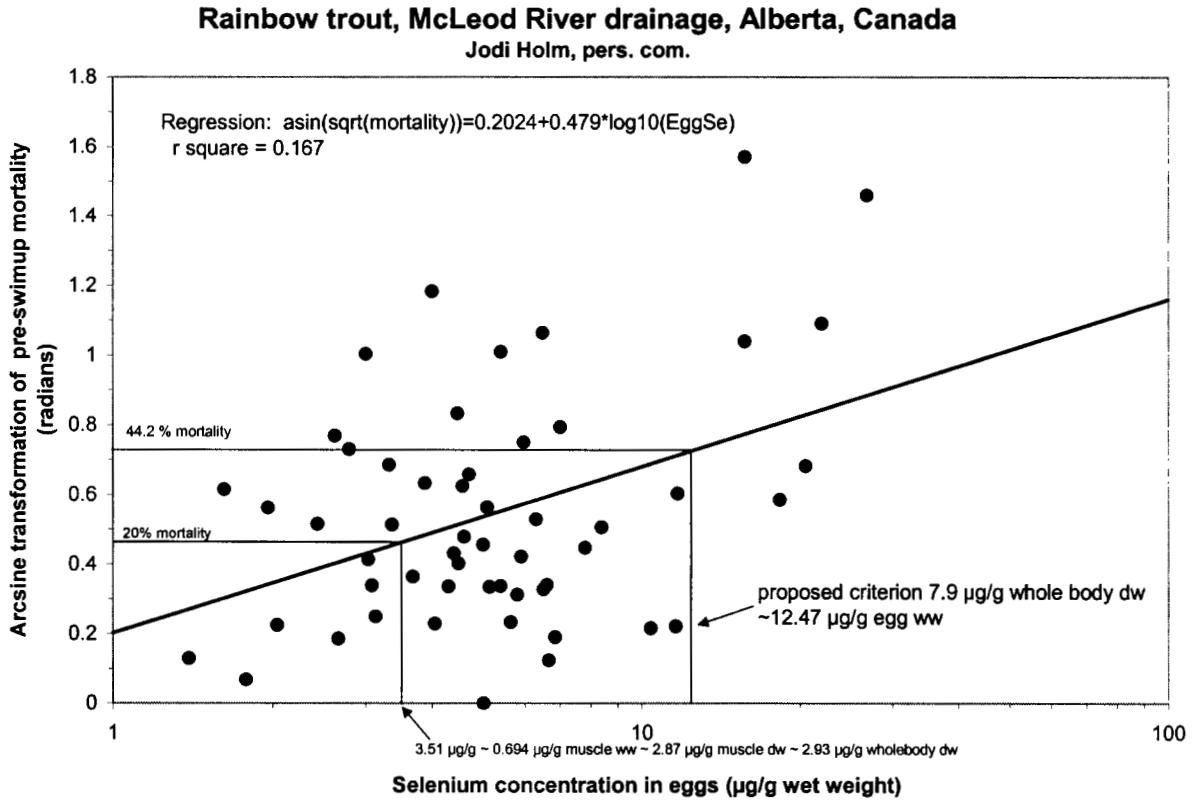

Figure 11. Relationship between selenium in rainbow trout eggs and mortality of eggs and fry by swimup stage. The arcsine transformation is applied to mortality data, as appropriate for linear regressions with percents or proportions (Sokol and Rohlf 1981). Data from the years 2000-2002.

Applying the EC_{20} benchmark is used in the Draft Criterion Document (EPA 2004) for regression analyses, and using the regression in Figure 7, the rainbow trout mortality data from this study yield a species maximum chronic value of 2.93 µg/g (Figure 11).

3. Laboratory exposure of juvenile rainbow trout to sodium selenite-spiked diet: (Hilton et al. 1980, cited in EPA 2004: Appendix I-14)

This experiment indicates the proposed chronic criterion in young rainbow trout will impair growth by at least 86 percent. The proposed criterion in prey of young rainbow trout will impair growth by 34 percent. Because the form of selenium used in this feeding experiment was inorganic selenium (rather than organo-selenium), the Hilton et al. (1980) data are not suitable for deriving a chronic criterion (cf., Heinz et al. 1987); but these data do provide an excellent example of the hormetic nature of selenium.

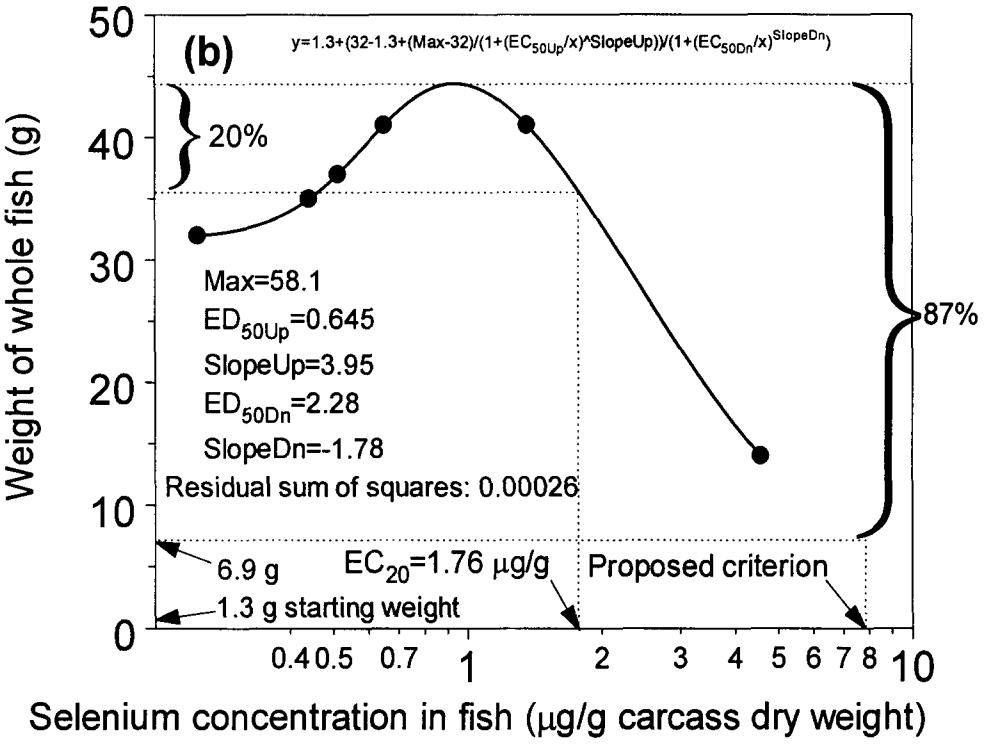

Figure 12. Average weights of juvenile rainbow trout after 20 weeks of exposure to diets spiked with sodium selenite (Hilton et al. 1980), with least squares regression using (a) a standard hormetic model (Brain and Cousens 1989)[, and (b) an improved general hormetic model (Beckon et al. In Prep.) with the assumption the baseline weight was the weight measured at the lowest treatment concentration of selenium. In both models it was assumed at very high selenium concentrations, the fish would have remained at the initial average weight of 1.3 g. Carcass concentrations are from Fig. 2 of Hilton et al. 1980.

The Draft Criteria Document (EPA 2004) selected only data for liver concentrations of the rainbow trout rather than the carcass selenium concentrations for their analysis of selenium in the experiment. The liver concentrations were converted to whole-body concentrations using a linear regression based on bluegill data (EPA 2004: 57-58, I-14). The liver-carcass data from this experiment compared to the bluegill liver-whole body data used by the Draft Criteria Document (EPA 2004) show elevated exposures to selenium. Rainbow trout sequester selenium in their livers to a greater extent than bluegill sunfish by an order of magnitude. Therefore, the bluegill sunfish-based conversion is inappropriate. Each carcass selenium concentration reported in this experiment is a combined value from three to six whole fish and three to four fish from which the liver (about 1% of body weight) and kidneys had been removed. Therefore, carcass selenium concentrations are a good approximation of whole-body concentrations. Using the carcass data from Fig. 2 of Hilton et al. (1980), this experiment indicates juvenile rainbow trout that reach the proposed criterion concentration by exposure for 20 weeks to dietary selenium in the form of sodium selenite will

experience at least an 86 percent reduction in weight relative to the weight they would gain if their exposure were optimal (Figure 12). Applying EPA's EC_{20} procedure to a hormetic model of these data yields a chronic value of 1.98 or 1.76 µg/g (Figure 12). These are the best data presently available for rainbow trout.

The Federal Register notice for the Draft Criterion Document (Federal Register 69(242):75541-75546) states that EPA took into consideration dietary exposure for aquatic life. This should include the effect of selenium concentrations in prey tissue on aquatic predators, e.g. the effect of selenium concentrations in small fish on the bigger fish that eat the small fish. However, the Draft Criterion Document (EPA 2004) does not include such analysis. Analysis of the data included in Draft Criteria Document (EPA 2004: I-14) for effects of selenium in the diet of juvenile rainbow trout on their weight (Hilton et al. 1980) indicates that if these fish feed on tissue at the criterion level (in the form of sodium selenite), they will suffer a reduction in growth of about 34 percent (Figure 13). Because the form of selenium administered to the fish in this experiment was sodium selenite, this analysis may underestimate the adverse effects of the more bioavailable organic forms of selenium.

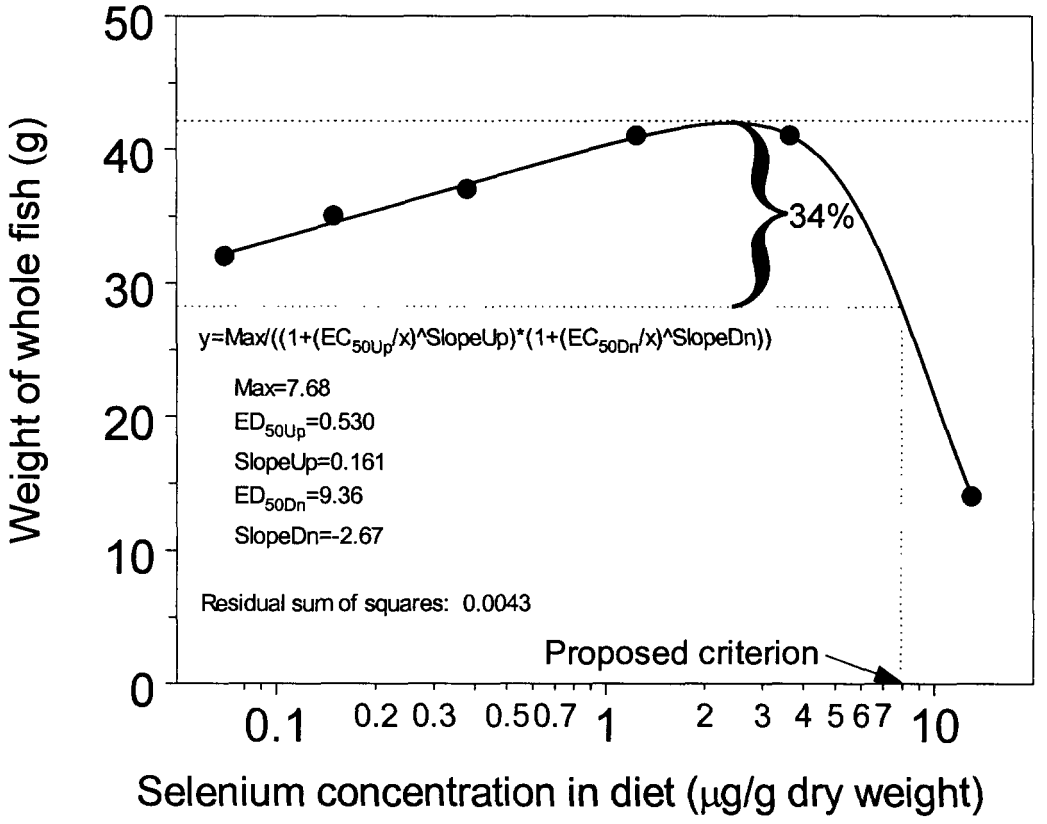

Figure 13. Average weights of juvenile rainbow trout after 20 weeks dietary exposure to sodium selenite (Hilton et al. 1980). A hormetic model is fitted to the data by least squares non-linear regression (Beckon et al. In Prep.).

Wet Weight/Dry Weight Conversions: Most of the wet weight to dry weight conversions for tissue concentrations of selenium are calculated with inappropriate estimates of percent moisture. For example, much of the most crucial analyses are focused on data from studies of bluegill, yet bluegill whole-body tissue was assumed to contain 80% moisture based on a single twenty-year-old Federal

Register citation (EPA 1985) which was meant to apply to edible filet tissue (not whole-body tissue) and is not taxon-specific for bluegill. However, taxon-specific data for percent moisture in bluegill whole-body tissue have been published (e.g., Saiki and May 1988:73.0% moisture; Saiki et al. 1992:74.7% moisture; Welsh and Maughan 1994:74.3%) and are consistent to an applied value of 75% moisture for fish whole-body tissue (e.g., Lemly 1996; Swift 2002; Holm et al. 2003; Hamilton 2004). In addition, national databases support the application of 75% moisture for fish whole-body tissue in the absence of taxon-specific information. National Contaminant Biomonitoring Program (NCBP) data (Walsh et al. 1977; May and McKinney 1981; Lowe et al. 1985; Schmitt and Brumbaugh 1990) yielded pooled estimates of 72% percent moisture in fish whole-body tissue (n=591 samples) in 1978-1981 and 74% (n=315 samples) in 1984. Data from the National Irrigation Water Quality Program for 57 species of freshwater fish revealed median percent moisture for whole-body tissue of 74.5% (Attachment 4).

The use of 80% moisture introduces a systematic 25% bias in the direction of overestimating species chronic values. For example, simply by using the appropriate percent moisture, the species chronic value EPA estimated from the intensely examined and re-analyzed study of bluegill by Hermanutz et al. (1996:Study II) would change downward from 12.12 ug/g to 9.70 ug/g (EPA 2004:81). Additionally, the tissue to tissue translation regressions are affected similarly which widely propagates inaccuracies related to percent moisture through much of the Draft Criteria Document's (EPA 2004) analyses.

7. *Significant New Data*: USFWS recently discovered a significant body of relevant data that we were previously unaware of and that we believe EPA has also not previously considered. The Department of Animal Science at the University of California-Davis conducted studies on the reproductive toxicity of selenium to Channel Catfish (*Ictalurus punctatus*) and bluegill (*Lepomis macrochirus*) for the California State Water Resources Control Board. The studies and their results are documented extensively in a final report to the State Water Board titled, "*Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River*" and is co-authored by Serge Doroshov, Joel Van Eenennaam, Christine Alexander, Erik Hallen, Howard Bailey, Kevin Kroll, and Camilo Restrepo (i.e., Doroshov et al. 1992; we provide a copy as Attachment 5 with the permission of Dr. Doroshov).

Channel Catfish Study: "Bioaccumulation of Selenium in Broodstock of Channel Catfish (*Ictalurus punctatus*) and its Effect on Reproduction" -- The objective of the channel catfish study was... "*...to determine effect of selenium bioaccumulation and yolkborne selenium concentration on the reproductive performance of broodstock and survival of resulting progeny.*" In summary selenium treatments did not affect vitellogenesis and ovarian development. Liver tissues exhibited rapid bioaccumulation and ovary tissue exhibited more delayed bioaccumulation of selenium. Eggs produced by treated females averaged 3.17 to 17.40 ug/g Se (dry wgt.) as compared to 2.85 ug/g Se in control eggs. Spawning response of experimental groups ranged from 23 to 40% with no statistically significant dose trend. Similarly, no significant differences were found for the endpoints of weight and relative weight of spawned egg masses. Fertilization success estimated at 48 hours was similar in all experimental groups.

Pre-hatch embryo mortality was significantly elevated in the two highest treatment groups of eggs which averaged 6.34 and 17.40 ug/g Se (dry wgt.). These two treatment groups exhibited 32% and

96% embryo mortality compared to 10% in controls and 5% (consistent with hormesis) in the lowest treatment group. Morphometrics of embryos that hatched did not differ between treatments. In the high treatment group only one of three egg batches produced hatchable embryos and most of those died between hatching and swim-up stage. The reported NOEC for egg selenium was 3.2 ug/g (dry wgt.) and the reported LOEC was 6.3 ug/g (dry wgt.). The LOEC on a control-adjusted basis was equivalent to about a 25% level of adverse effect (embryo death).

If these results are taken at face value they would indicate that Channel Catfish are more sensitive to selenium than any of the species currently included in EPA's database. Direct interpretation, for EPA's purposes, is complicated by two factors. First, whole-body selenium concentrations were not measured. Employing EPA's translation equation for ovary tissue, and the LOEC concentration of 7 ug/g for ovary tissue in this study, yields a whole-body equivalency value of 3.26 ug/g Se. EPA's translation equation is based on bluegill data with unknown relevance to channel catfish. However, EPA does not confine its use of translation equations to the taxa whose data are the basis for the equations (EPA 2004).

Secondly, Se treatments in this study consisted of exposure to seleno-L-methionine via injection directly into the bloodstream of broodstock fish rather than via dietary exposure. The authors contend that with respect to deposition of selenium into adult tissues and eggs, the only difference between this exposure route and a dietary exposure route would be the bypassing of gut transmission of selenium to the bloodstream. If the authors' contention is correct, then the form of selenium in the catfish eggs would be no different than studies based on dietary exposure to seleno-L-methionine.

Independent of potential uncertainty related to route of exposure, and more important from USFWS' perspective, is the fact that significant pre-hatch embryo mortality occurred in this experiment. This is unusual for fish studies. Doroshov et al. (1992) point out that catfish produce relatively large and yolky eggs. Therefore, unlike most other fish taxa, catfish embryos complete major organogenesis and draw more substantively upon yolkborne selenoproteins **before** they hatch. Dr. Doroshov suggests (Pers. comm.) that this different progression for selenium exposure is categorically more sensitive. With that very plausible possibility in mind, USFWS notes that EPA's database does not include reproductive endpoint data for any taxon reproductively comparable to catfish (i.e., that produce catfish-like eggs). Given the small number of fish species tested to date, and the possibility that the most sensitive reproductive category of fish is as yet unrepresented, we note that conservative treatments of the limited data we have would therefore be scientifically well justified.

Bluegill Study: "Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (*Lepomis macrochirus*)" -- The objective of the bluegill study was to…"…*determine tissue selenium concentrations in adult bluegill, Lepomis macrochirus Rafinesque, critical to normal reproduction.*" In summary, treatment diets contained measured concentrations of 5.5, 13.9, and 21.4 ug/g Se (as seleno-L-methionine; dry wgt.). Control diets with nominally paired additions of L-methionine for each Se-L-methionine treatment level contained measured concentrations of 1.1, 1.6, and 1.2 ug/g Se (dry wgt.).

No apparent differences were observed in fish behavior. No significant differences in fork length or body weight were measured. Testes accumulated less selenium compared to ovaries, but liver

accumulation was similar in both sexes. The dry matter content of ovaries was highly variable and related to the reproductive cycle. No histological differences were observed for post-spawning gonad tissues. No difference in fertilization success were measured.

Larval effects (edema) were observed for the 13.9 and 21.4 ug/g Se dietary treatments. For the 21.4 ug/g Se treatment more than 95% of larvae died before day 16 post-hatch. Low (5.5 ug/g Se) and medium (13.9 ug/g Se) treatments exhibited only slight increase in larval mortality to day 16. Overt larval abnormalities were observed, but were not clearly related to dose levels. For the endpoint of reproductive failure, and based on egg tissue, the reported NOEC and LOEC selenium concentrations were 8.3 and 19.5 ug/g Se (dry wgt.). That would yield a chronic value for this study of 12.7 ug/g.

Again, whole-body selenium concentrations were not measured. Ovary concentrations were measured, but only for stripped ovaries, which the authors report as likely lowering the selenium content of the ovaries. Thus, to translate the results of this study to a whole-body equivalency, 12.7 ug/g Se would have to be viewed as the best measure of selenium concentrations for unstripped ovaries. Using EPA's ovary translation equation, the whole-body equivalency chronic value would be 5.9 ug/g Se (dry wgt.). As presented earlier in these review comments, USFWS prefers to compare the egg chronic value from this study directly to data from Coyle et al. (1993) because EPA's translation regression is based largely on data from Hermanutz et al. (1996) for which there was no scientifically defendable basis for making wet weight to dry weight conversions. Based on data for bluegill presented by Coyle et al. (1993), who directly measured dry weight concentrations, a chronic value of 12.7 ug/g Se (dry wgt.) in eggs/ovary would be equivalent to a whole-body chronic value of about 4.9 ug/g Se (dry wgt.).

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ATTACHMENT 1.

LEMLY WINTER-STRESS STUDY
RAW DATA

Treatment Day	Cold +Se Fish at Start	Cold + Se Fish at End	Cold + Se Fish Exposure Days	Cold +Se Dead Fish	Daily Survival	Cumulative Mortality	Cold Control Fish at Start	Cold Control Fish at End	Cold Control Fish Exposure Days	Cd Control Dead Fish	Daily Survival	Cumulative Mortality
1	210	210	210	0	1	0	70	70	70	0	1	0
2	210	210	210	0	1	0	70	70	70	0	1	0
3	210	210	210	0	1	0	70	70	70	0	1	0
4	210	210	210	0	1	0	70	70	70	0	1	0
5	210	210	210	0	1	0	70	70	70	0	1	0
6	210	210	210	0	1	0	70	70	70	0	1	0
7	210	210	210	0	1	0	70	70	70	0	1	0
8	210	210	210	0	1	0	70	70	70	0	1	0
9	210	210	210	0	1	0	70	70	70	0	1	0
10	210	210	210	0	1	0	70	70	70	0	1	0
11	210	210	210	0	1	0	70	70	70	0	1	0
12	210	210	210	0	1	0	70	70	70	0	1	0
13	210	210	210	0	1	0	70	70	70	0	1	0
14	210	210	210	0	1	0	70	70	70	0	1	0
15	210	210	210	0	1	0	70	70	70	0	1	0
16	210	210	210	0	1	0	70	70	70	0	1	0
17	210	210	210	0	1	0	70	70	70	0	1	0
18	210	210	210	0	1	0	70	70	70	0	1	0
19	210	210	210	0	1	0	70	70	70	0	1	0
20	210	209	209.5	1	0.995238	0.004762	70	70	70	0	1	0
21	209	209	209	0	1	0.004762	70	70	70	0	1	0
22	209	209	209	0	1	0.004762	70	70	70	0	1	0
23	209	209	209	0	1	0.004762	70	70	70	0	1	0
24	209	209	209	0	1	0.004762	70	70	70	0	1	0
25	209	209	209	0	1	0.004762	70	70	70	0	1	0
26	209	209	209	0	1	0.004762	70	70	70	0	1	0
27	209	209	209	0	1	0.004762	70	70	70	0	1	0
28	209	209	209	0	1	0.004762	70	70	70	0	1	0
29	209	209	209	0	1	0.004762	70	70	70	0	1	0
30	209	209	209	0	1	0.004762	70	70	70	0	1	0
31	209	209	209	0	1	0.004762	70	70	70	0	1	0
32	209	209	209	0	1	0.004762	70	70	70	0	1	0
33	209	209	209	0	1	0.004762	70	70	70	0	1	0
34	209	208	208.5	1	0.995215	0.009524	70	70	70	0	1	0
35	208	208	208	0	1	0.009524	70	70	70	0	1	0
36	208	208	208	0	1	0.009524	70	70	70	0	1	0
37	208	208	208	0	1	0.009524	70	70	70	0	1	0
38	208	208	208	0	1	0.009524	70	70	70	0	1	0
39	208	208	208	0	1	0.009524	70	70	70	0	1	0
40	208	207	207.5	1	0.995192	0.014286	70	70	70	0	1	0
41	207	207	207	0	1	0.014286	70	70	70	0	1	0
42	207	207	207	0	1	0.014286	70	70	70	0	1	0
43	207	207	207	0	1	0.014286	70	70	70	0	1	0
44	207	207	207	0	1	0.014286	70	70	70	0	1	0
45	207	207	207	0	1	0.014286	70	70	70	0	1	0
46	207	206	206.5	1	0.995169	0.019048	70	70	70	0	1	0
47	206	205	205.5	1	0.995146	0.02381	70	70	70	0	1	0
48	205	205	205	0	1	0.02381	70	70	70	0	1	0
49	205	205	205	0	1	0.02381	70	70	70	0	1	0
50	205	204	204.5	1	0.995122	0.028572	70	70	70	0	1	0
51	204	204	204	0	1	0.028572	70	70	70	0	1	0

52	204	201	202.5	3	0.985294	0.042858	70	69	69.5	1	0.985714	0.014286
53	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
54	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
55	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
56	201	201	201	0	1	0.042858	69	69	69	0	1	0.014286
57	201	199	200	2	0.99005	0.052381	69	69	69	0	1	0.014286
58	199	197	198	2	0.98995	0.061905	69	69	69	0	1	0.014286
59	197	196	196.5	1	0.994924	0.066667	69	69	69	0	1	0.014286
60	196	195	195.5	1	0.994898	0.071429	69	69	69	0	1	0.014286
Whole-body Se = 5.85 ug/g			30 fish removed						10 fish removed			
61	185	165	165	0	1	0.071429	59	59	59	0	1	0.014286
62	165	165	165	0	1	0.071429	59	59	59	0	1	0.014286
63	165	165	165	0	1	0.071429	59	59	59	0	1	0.014286
64	165	163	164	2	0.987879	0.082684	59	59	59	0	1	0.014286
65	163	162	162.5	1	0.993865	0.088312	59	59	59	0	1	0.014286
66	162	162	162	0	1	0.088312	59	59	59	0	1	0.014286
67	162	162	162	0	1	0.088312	59	59	59	0	1	0.014286
68	162	160	161	2	0.987654	0.099567	59	59	59	0	1	0.014286
69	160	156	158	4	0.975	0.122078	59	59	59	0	1	0.014286
70	156	155	155.5	1	0.99359	0.127706	59	59	59	0	1	0.014286
			3825	20					1421.5	1		
			5.23 mortalities per 1,000 Exp. Days						0.703 mortalities per 1,000 Exp. Days			
71	155	153	154	2	0.987097	0.138961	59	59	59	0	1	0.014286
72	153	152	152.5	1	0.993464	0.144589	59	59	59	0	1	0.014286
73	152	146	149	6	0.960526	0.178355	59	59	59	0	1	0.014286
74	146	145	145.5	1	0.993151	0.183983	59	59	59	0	1	0.014286
75	145	142	143.5	3	0.97931	0.200866	59	59	59	0	1	0.014286
76	142	141	141.5	1	0.992958	0.206494	59	59	59	0	1	0.014286
77	141	137	139	4	0.971631	0.229005	59	59	59	0	1	0.014286
78	137	137	137	0	1	0.229005	59	59	59	0	1	0.014286
79	137	136	136.5	1	0.992701	0.234632	59	59	59	0	1	0.014286
80	136	136	136	0	1	0.234632	59	59	59	0	1	0.014286
81	136	136	136	0	1	0.234632	59	59	59	0	1	0.014286
82	136	135	135.5	1	0.992647	0.24026	59	59	59	0	1	0.014286
83	135	134	134.5	1	0.992593	0.245888	59	59	59	0	1	0.014286
84	134	134	134	0	1	0.245888	59	59	59	0	1	0.014286
85	134	133	133.5	1	0.992537	0.251515	59	59	59	0	1	0.014286
86	133	133	133	0	1	0.251515	59	59	59	0	1	0.014286
87	133	132	132.5	1	0.992481	0.257143	59	59	59	0	1	0.014286
88	132	130	131	2	0.984848	0.268399	59	59	59	0	1	0.014286
89	130	130	130	0	1	0.268399	59	59	59	0	1	0.014286
90	130	128	129	2	0.984615	0.279654	59	59	59	0	1	0.014286
91	128	128	128	0	1	0.279654	59	59	59	0	1	0.014286
92	128	128	128	0	1	0.279654	59	59	59	0	1	0.014286
93	128	127	127.5	1	0.992188	0.285282	59	59	59	0	1	0.014286
94	127	127	127	0	1	0.285282	59	59	59	0	1	0.014286
95	127	127	127	0	1	0.285282	59	59	59	0	1	0.014286
96	127	126	126.5	1	0.992126	0.290909	59	59	59	0	1	0.014286
97	126	126	126	0	1	0.290909	59	59	59	0	1	0.014286
98	126	124	125	2	0.984127	0.302165	59	59	59	0	1	0.014286
99	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
100	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
101	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
102	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
103	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
104	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286

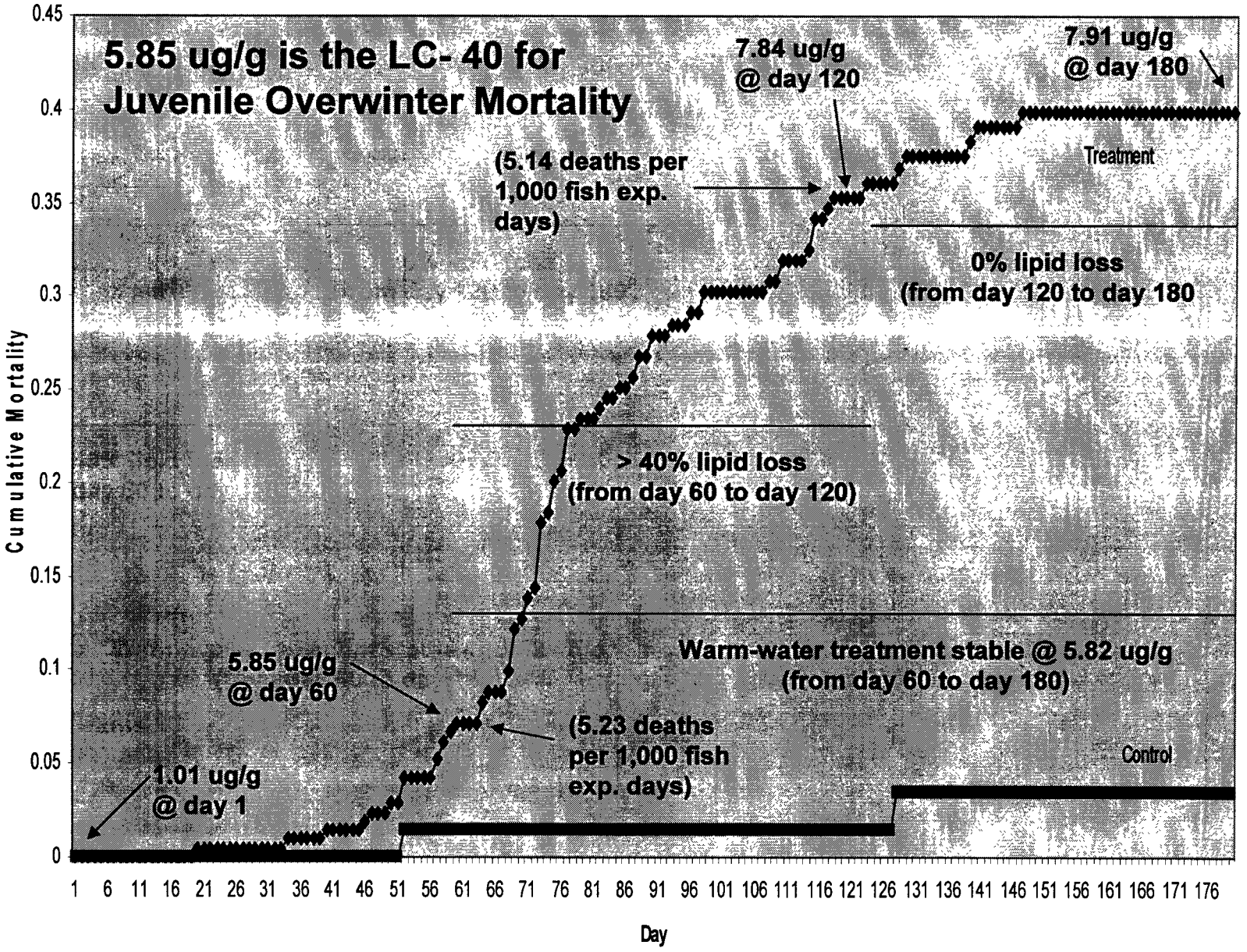
105	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
106	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
107	124	124	124	0	1	0.302165	59	59	59	0	1	0.014286
108	124	123	123.5	1	0.991935	0.307792	59	59	59	0	1	0.014286
109	123	123	123	0	1	0.307792	59	59	59	0	1	0.014286
110	123	121	122	2	0.98374	0.319048	59	59	59	0	1	0.014286
111	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
112	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
113	121	121	121	0	1	0.319048	59	59	59	0	1	0.014286
114	121	120	120.5	1	0.991736	0.324676	59	59	59	0	1	0.014286
115	120	117	118.5	3	0.975	0.341559	59	59	59	0	1	0.014286
116	117	117	117	0	1	0.341559	59	59	59	0	1	0.014286
117	117	116	116.5	1	0.991453	0.347186	59	59	59	0	1	0.014286
118	116	115	115.5	1	0.991379	0.352814	59	59	59	0	1	0.014286
119	115	115	115	0	1	0.352814	59	59	59	0	1	0.014286
120	115	115	115	0	1	0.352814	59	59	59	0	1	0.014286
Whole-body Se = 7.84 ug/g			30 fish removed						10 fish removed			
121	85	85	85	0	1	0.352814	49	49	49	0	1	0.014286
122	85	85	85	0	1	0.352814	49	49	49	0	1	0.014286
123	85	84	84.5	1	0.988235	0.360428	49	49	49	0	1	0.014286
124	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
125	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
126	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
127	84	84	84	0	1	0.360428	49	49	49	0	1	0.014286
128	84	83	83.5	1	0.988095	0.368042	49	48	48.5	1	0.979592	0.034403
129	83	82	82.5	1	0.987952	0.375656	48	48	48	0	1	0.034403
130	82	82	82	0	1	0.375656	48	48	48	0	0.034403	0.034403
			2141.5	11					1136.5	1		
			5.14 mortalities per	1,000 Exp.	Days				0.88 mortalities per	1,000 Exp.	Days	
131	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
132	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
133	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
134	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
135	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
136	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
137	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
138	82	82	82	0	1	0.375656	48	48	48	0	1	0.034403
139	82	81	81.5	1	0.987805	0.38327	48	48	48	0	1	0.034403
140	81	80	80.5	1	0.987654	0.390884	48	48	48	0	1	0.034403
141	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
142	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
143	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
144	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
145	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
146	80	80	80	0	1	0.390884	48	48	48	0	1	0.034403
147	80	79	79.5	1	0.9875	0.398498	48	48	48	0	1	0.034403
148	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
149	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
150	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
151	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
152	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
153	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
154	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
155	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
156	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
157	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403

158	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
159	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
160	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
161	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
162	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
163	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
164	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
165	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
166	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
167	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
168	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
169	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
170	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
171	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
172	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
173	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
174	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
175	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
176	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
177	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
178	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
179	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
180	79	79	79	0	1	0.398498	48	48	48	0	1	0.034403
Whole-body Se = 7.91 ug/g			**30 fish removed**						**10 fish removed**			
TOTALS			**25321.5**	**71**		**0.398498**			**10,619.0**	**2**		**0.034403**

Note: Exposure Days were quantified based on the assumption that fish died at mid-day.

36

Attachment 2: Lemly winter-stress results



ATTACHMENT 3.

MATCHED SAMPLES OF FISH AND AQUATIC INVERTEBRATES FROM SAMPLING SITES WHERE THE FISH TISSUE SAMPLES AVERAGED 5-10 ug/g SELENIUM, DRY WEIGHT

Location	Invertebrate Selenium	Fish Selenium	Implied Concentration Factor
Colorado	4.8 ug/g	5.3 ug/g	1.10
Utah	4.4	6.0	1.36
Utah	4.4	5.2	1.18
Utah	8.2	10	1.22
Utah	8.4	9.4	1.12
Utah	7.6	5.7	0.75
Utah	6.9	6.7	0.97
Montana	4.8	6.1	1.32
Montana	9.2	5.3	0.67
Median Concentration Factor			1.12
Average Concentration Factor			1.08

Source: National Irrigation Water Qualtiy Program biota database (4, 76)

ATTACHMENT 4.
Summary of Percent Moisture Data From NIWQP Database for Fish Whole-body Samples (asce

Fish Species Common Name	Percent Moisture	No. of Samples
Tahoe Sucker	68.4	1
Goldeye	69.3	25
Cutthroat Trout	69.7	5
Flannelmouth Sucker	70.3	175
Longnose Sucker	70.4	39
Mountain Whitefish	70.6	6
Shorthead Redhorse	70.9	74
Utah Chub	71	11
Speckled Dace	71	193
Gizzard Shad	71.3	9
Sauger	71.4	5
Longnose Dace	71.4	8
Bluehead Sucker	71.8	54
Squawfish	72	1
River Carpsucker	72.5	9
Sacramento Perch	72.6	2
Flathead Chub	72.9	32
Northern Squawfish	73.1	1
Brown Trout	73.2	51
Redear Sunfish	73.8	1
Hitch	73.8	7
Bairdiella	74	5
Utah Sucker	74.1	6
Sailfin Molly	74.1	6
Rainbow Trout	74.2	39
Yellow Perch	74.3	50
Channel Catfish	74.4	78
White Sucker	74.5	82
Walleye	74.5	15
Smallmouth Bass	74.6	21
White Bass	74.6	6
Tui Chub	74.8	36
Sunfish	75	3
Pumpkinseed	75.2	1
Green Sunfish	75.2	60
Red Shiner	75.2	14
Largemouth Bass	75.4	14
Common Carp	75.8	165
Mottled Sculpin	75.8	34
Roundtail Chub	75.9	100
Stonecat	76	4
Redside Shiner	76	1
White Crappie	76.1	7
Plains Killifish	76.1	6
Bluegill	76.1	5
Black Crappie	76.2	23

Brook Stickleback	76.6	13
Brassy Minnow	76.8	4
Fathead Minnow	77.6	97
Mosquitofish	77.6	54
Bullhead	78.3	23
Northern Pike	78.4	7
Sacramento Blackfish	79	2
Longjaw Mudsucker	80.2	1
Black Bullhead	80.8	75
Brown Bullhead	81.6	5
Northern Redbelly Dace	82.9	1

nding order)

Notes

25th Percentile Value = 72.25
25th Percentile Value = 72.25

Genus Lepomis

50th Percentile Value

Genus Lepomis
Genus Lepomis
Genus Lepomis

75th Percentile Value
75th Percentile Value
Genus Lepomis

EPA uses 80% moisture for all species!!

[ATTACHMENT 5]

FINAL REPORT (DRAFT)

Development of Water Quality Criteria for Resident Aquatic Species of the San Joaquin River.

for

State Water Resources Control Board

State of California

Contract Number 7-197-250-0

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PART I. Bioaccumulation of Selenium in Broodstock of Channel Catfish (Ictalurus punctatus) and its effect on reproduction.

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A. INTRODUCTION

Selenium has been implicated in reproductive failure of fish and waterfowl in polluted aquatic systems. In particular, certain areas of lower San Joaquin River were severely affected by selenium pollution. State Board Order 85-1 addressed waterfowl problems at Kesterson Reservoir resulting from selenium laden water discharged to the facility. State Board staff and San Joaquin River Basin Technical Committee developed water quality criteria for nine constituents and proposed objectives for three of them, selenium, boron, and molybdenum. The toxicity data upon which the criteria and objectives are based are not adequate in that site-specific toxicity data were generally not available. The objective of this contract is to provide additional selenium toxicity data for two resident species of fish, channel catfish and bluegill.

Species investigated in the first part of the study is channel catfish, Ictalurus punctatus. The objective was to determine effect of selenium bioaccumulation and yolkborne selenium concentration on the reproductive performance of broodstock and survival of resulting progeny. Commonly, in experiments with small size laboratory fish, selenium bioaccumulation is induced by dietary treatment. However, due to the large size of catfish broodstock and the prolonged period required for ovarian bioaccumulation we utilized an alternative selenium delivery method: by repeated injections of selenoaminoacid. Introduction of exogenous selenium directly into the blood stream bypasses the assimilation via gut absorption.

B. MATERIALS AND METHODS

1. Broodstock

Channel catfish broodstock were obtained from farm ponds (Fishery Inc., Galt, California) in January-March 1989. Fish were visually sexed and females 3-4 year old, ranged in body weight from 1.1 to 3.4 kg, average weight 2 kg. During the experimental treatment before spawning, the fish were held in six foot diameter (1400 L volume) fiberglass tanks located outside. The initial stocking density was 60 fish (120 kg) per tank. Tanks were supplied with flow-through water with temperature ranging 13-21°C. Water quality parameters are summarized in Appendix 1. Fish were fed Silvercup trout diets (Murray Elevators, Utah), 1 to 2 % body weight per day. Separate tanks were used for each experimental treatment. The additional stock of sexed males was obtained before the spawning season, in May. Males were not treated and were kept in separate tanks.

2. Experimental Protocol.

2.1. Range Finding

This study was conducted in January-February 1989. Three untreated females were sampled on Day 0 (muscle, liver, ovaries, and blood plasma). The remaining fish were injected with L-methionine (L-Met) or seleno-L-methionine (Se-L-Met, Sigma Chemical) at dose levels 0.25, 2.5, and 25 mg/kg body weight. On Day 14, one fish from each control dose and 2 fish from each selenium treatment were sampled. Half of the remaining fish (3 in control and 2 in each selenium treatment) were injected a second time, similar to the first dose. The other half did not receive a second injection. All females were sampled on Day 28, half were injected once (Day 0) and the other half were injected twice (Days 0 and 14). Tissue samples were analyzed for selenium concentrations.

2.2. Bioaccumulation Treatment

Treatment by intramuscular injections was initiated on March 14 (Day 0). Populations were randomly assigned to six tanks. Six untreated females were sacrificed and sampled on Day 0. All remaining females received biweekly injections of L-Met and Se-L-Met at doses 0.02, 0.2, and 2.0 mg/kg body weight. Six injections total were given in each treatment on Days 0, 14, 28, 42, 56, and 70 (Table 1). On each day 2 fish from each control and 3 fish from each selenium treatment were randomly sampled for ovarian and liver selenium burden (actual number of sampled fish or analyzed tissue varied from 1 to 4). The last sampling was conducted on Day 84 (June 5).

2.3. Spawning

The remaining females (all injected 6 times) were used for spawning with untreated males. Spawning trials were conducted from June 5 to June 24 in five consecutive sessions, with 12 randomly chosen females (3 control and 3 in each selenium treatment dose) in each session (Table 1). Spawned females were sacrificed and sampled for tissue selenium analysis. The egg masses were weighed, treated in iodophore (10%) to prevent fungal infection and placed in catfish hatchery incubators. A core sample was taken for selenium analysis. At 48 hours after fertilization, two additional samples were taken from each egg mass: one for microscopic examination of fertilization success, and another for the embryo-larval bioassay. To separate the eggs from their adhesive matrix, samples were bathed in a 1% sodium sulfite solution. Bioassays

were conducted with two replications for each progeny (except for one with no replication) for 28 days. Survived fry were counted, weighed and measured. A more detailed description of each procedure follows.

Table 1. Treatment/spawning schedules, and number of sampled catfish females. Step: INJ - sampling and injection of remaining fish with treatment dose; SPAWN - spawning and sampling.

DATE	DAY	STEP	CONTROL L-Met mg/kg			TREATMENT Se-L-Met mg/kg		
			0.02	0.2	2.0	0.02	0.2	2.0
Mar 14	0	INJ	6 (untreated)					
Mar 28	14	INJ	2	2	2	3	3	2
Apr 11	28	INJ	2	2	2	3	3	2
Apr 25	42	INJ	2	2	2	3	3	3
May 9	56	INJ	2	2	1	3	3	2
May 23	70	INJ	2	2	2	1	1	1
Jun 5	84	-	2	4	3	2	2	1
Jun 7	86	SPAWN	1	1	1	3	3	2
Jun 11	90	SPAWN	1	1	1	3	3	3
Jun 15	94	SPAWN	1	1	1	3	3	3
Jun 19	98	SPAWN	1	1	1	3	3	3
Jun 24	103	SPAWN	1	1	1	3	3	3
		Total:	17	19	17	30	30	25

2.4. Tissue Sampling and Selenium Analysis

Fish were selected by random numbers. Approximately 15 ml of blood was collected by vacutainer, plasma was separated by centrifugation at 3000 rpm, distributed in plastic vials and stored frozen at -20°C. Fish was sacrificed with a blow to the head, weighed and measured. Tissue samples were divided into two subsamples, weighed, rinsed in distilled water, and frozen in plastic bags for selenium analysis. White muscle samples were collected from the filet on the left side of the fish (skin and red muscle tissue were removed). Duplicate tissue samples were used for selenium analysis on a wet weight basis, and for dessication (lyophilization) to determine dry matter content for

conversion of selenium concentrations to dry weight (both wet and dry weight data were used for data analysis). All selenium analyses were conducted by California Veterinary Diagnostic Laboratory System, Veterinary Medicine, UC Davis. Samples were analyzed by the ICP atomic emission, using hydride generation. Analytical detection limit of method is 0.005 µg/g for tissue selenium. The details of this method and the quality control protocol are described in the report of CVDLS (Ardans et al.,1988).

2.5. Plasma Protein Phosphorus Analysis

Plasma alkali-labile protein phosphorus (ALPP) measures relative concentration of plasma yolk precursor, vitellogenin. The technique is based on precipitation of plasma proteins, liberation of protein (mainly vitellogenin) -bound phosphorus, and measuring phosphorus concentrations by colorimetry. Plasma ALPP concentrations increase with progression of vitellogenesis (synthesis of vitellogenin by liver and deposition of proteins into the egg yolk) and decrease around spawning time. With some minor modifications, we used the technique described by Wallace and Jared (1968) and de Vlaming et al.(1984).

2.6. Spawning Induction Procedure

Fish were injected IM with human chorionic gonadotropin (hCG), females with 1800 IU and males with 600 IU per kg body weight. Each pair was put in a rectangular 6 x 2 x 2 foot tank supplied with flow-through water at a constant temperature 26°C. Each tank had a spawning container. Fish were allowed to spawn for 72 hours, and containers were observed at regular intervals for mating and spawning. Date and time of oviposition were recorded. The egg mass was weighed and placed in a water bath of 10% iodophore solution for 2 minutes and then put in a wire basket in a standard paddlewheel catfish egg incubator.

2.7. Embryo-Larval Bioassay

Two replicate samples of thirty eggs from each egg mass were placed into a glass petri dish (100x15mm) and submerged into a 21 L rectangular glass aquaria, supplied with constant flow of underground water from a campus well (Hardness 225-300 mg/L $CaCO_3$). Temperature was maintained constant within the range 24 - 26°C. Other water quality parameters are summarized in the Appendix 2. Incubation of eggs and rearing of fry continued for 28 days. The aquaria were examined daily and mortalities were removed and recorded. Starting from the completion of yolk sac absorption and swimup stage (Days 10-11), fry were fed ad libitum a

commercial salmon diet (Biodiet, Bio-Products Inc). The fry were starved for 24 hours before final sampling on Day 28. Wet body weight was measured on an electronic balance (0.01 mg) and total length was measured on a measuring board (1 mm).

3. Data Analysis

Relative weight of ovaries and liver were expressed as gonadosomatic (GSI) or hepatosomatic (HSI) indices, in percent of whole body weight. All proportion data were transformed into the arcsine-roots before statistical analysis. Selenium concentrations were transformed into $\log_{10}$ values.

Differences between control groups, and between pooled control and treatment groups were tested by one-way analysis of variance and Dunnett's procedure, at the probability level 95%. Linear regression analysis was used to examine relationships between selenium concentrations in different tissues. For the estimation of LC_{50}, we used the trimmed Spearman-Karber method.

C. RESULTS

1. Range Finding

The females injected with 25 mg Se-L-Met/kg BW died from acute selenosis (edema, paralysis, and strong odor) within four hours after injection. Controls, 0.25 and 2.5 mg/kg selenium treatments were not affected: they survived to Day 28 and were sampled on Days 14 and 28. Extra fish injected with intermediate Se-L-Met doses (6.2; 10.0, and 17.5 mg/kg) survived for 8 days, 27 hours, and 18 hours after injection, respectively. These fish also suffered acute selenosis. A single female injected with dose 4.4 mg/kg died in 6 days after injection, with extensive hemorrhages of fins and skin. Death of this fish might have been caused by transportation and handling.

Tissue selenium concentrations of sampled fish are shown in Table 2. There was no significant effect of control treatment (carrier amino acid) on tissue selenium level. Concentrations of selenium in liver, muscle and plasma of fish sampled on Day 14 exhibited significant increase after one injection of 2.5 mg/kg Se-L-Met. Liver and muscle selenium concentrations in this treatment group remained significantly elevated on Day 28. Fish that received two consecutive injections of 2.5 mg/kg Se-L-Met exhibited significantly elevated selenium levels in all four tissues sampled, including the ovaries. Selenium treatment dose 0.25 mg/kg did not result in bioaccumulation, although plasma

selenium concentration was slightly elevated compared with control. We concluded from the range-finding experiment that Se-L-Met injections at doses higher than 2.5 mg/kg produce acute effect on catfish broodstock, and the selenium bioaccumulation is likely to occur at the dose range 0.25-2.5 mg/kg.

2. Reproductive Indices and Plasma Protein Phosphorus (ALPP)

Data on GSI and HSI in fish sampled biweekly during the 84 day period of treatment are shown in Table 3. The GSI increased from 6% at Day 0 (March) to 7-12% on Day 84 (June). The HSI exhibited some increase during the sampling period (Days 28-56) and decreased before spawning (Day 84). Overall changes in GSI and HSI reflect normal reproductive profile of channel catfish female. No significant differences were detected between control and treatment groups, for both GSI and HSI (analysis of variance). However, there was substantial individual variability in the ovarian growth (characteristic of farmed catfish broodstock), and small sample size may not be adequate to detect the effect of selenium treatment.

Samples of plasma ALPP included more fish, particularly in control (Table 4). Data show increase in plasma vitellogenin level during April-May (Days 28-56), with no significant differences between control and treatment groups. In summary, observations on GSI, HSI, and plasma protein phosphorus suggest that selenium treatment did not affect vitellogenesis and ovarian development.

Table 2. Tissue selenium concentrations (µg/g, wet weight) in range-finding experiment with channel catfish. Fish were injected once, on Day 0 (x1), or twice, on Day 0 and Day 14 (x2). Data are means and SEM. Asterisks indicate treatments significantly different from their respective controls (L-Met). All control doses (0.25-25 ppm) are pooled).

TREATMENT	GONADS	LIVER	MUSCLE	PLASMA
	Sampled on Day 0:			
Untreated (n=3)	1.65 ±0.13	1.45 ±0.05	0.15 ±0.01	0.22 ±0.02
	Sampled on Day 14:			
L-Met (n=3)	1.76 ±0.20	1.95 ±0.25	0.12 ±0.01	0.26 ±0.01
Se-L-Met 0.25 (n=2)	1.93 ±0.21	2.25 ±0.08	0.15 ±0.01	0.26 ±0.02
Se-L-Met 2.5 (n=2)	3.13 ±0.96	5.17 ±0.38 *	0.66 ±0.06 *	0.78 ±0.07 *
	Sampled on Day 28:			
L-Met (n=6)	2.02 ±0.46	1.92 ±0.20	0.14 ±0.01	0.22 ±0.01
Se-L-Met 0.25 (x1) (n=2)	0.92 [1)]	2.59 ±0.08	0.15 ±0.01	0.28 ±0.01
Se-L-Met 0.25 (x2) (n=2)	1.79 ±0.15	3.02 [1)]	0.20 ±0.01	0.32 ±0.01
Se-L-Met 2.5	2.41 ±0.04	4.87 ±0.15 *	0.67 ±0.07 *	0.53 ±0.01

(x1) (n=2)				
	*	*	*	*
Se-L-Met 2.5 (x2) (n=2)	4.79 ±1.27	9.55 ±0.51	1.59 ±0.12	1.27 ±0.36

1) n=1

Table 3. Gonadosomatic (GSI) and hepatosomatic (HSI) indices of channel catfish. Data are x ± s.e.m., sample size in parentheses.

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		GSI		
0	5.9 ±1.0 (6)	- sample on Day 0, untreated fish		
14	5.3 ±1.5 (6)	3.6 ±1.0 (3)	2.5 ±1.8 (3)	6.9 ±0.5 (2)
28	N/A	1.6 ±0.9 (2)	6.2 ±0.7 (3)	3.8 ±2.4 (2)
42	5.4 ±1.4 (6)	6.3 ±0.8 (3)	5.4 ±1.6 (3)	3.3 ±1.5 (3)
56	6.7 ±1.5 (5)	5.4 ±1.7 (3)	7.5 ±1.4 (3)	1.6 ±0.7 (2)
70	7.3 ±0.7 (6)	6.5 (1)	10.0 (1)	5.2 (1)
84	8.1 ±1.6 (9)	8.1 ±1.3 (2)	11.9±1.0 (2)	7.0 (1)
		HSI		
0	0.9 ±0.1 (6)	- sample on Day 0, untreated fish		
14	1.0 ±0.1 (6)	1.1 ±0.1 (3)	0.8 ±0.1 (3)	1.1 ±0.3 (2)
28	N/A	1.1 ±0.2 (2)	1.9 ±0.2 (3)	1.1 ±0.2 (2)
42	1.2 ±0.1 (6)	1.2 ±0.1 (3)	1.4 ±0.2 (3)	1.2 ±0.1 (3)
56	1.0 ±0.1 (5)	1.3 ±0.1 (3)	1.4 ±0.2 (3)	0.9 ±0.1 (3)

70	1.2 ±0.1 (6)	1.0 (1)	1.2 (1)	1.1 (1)
84	1.1 ±0.1 (9)	1.1 ±0.1 (2)	1.1 ±0.1 (2)	1.1 (1)

Table 4. Plasma protein phosphorus (ALPP, µg/ml) of channel catfish. Data are x ± s.e.m., sample size in parentheses.

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
0	14 ± 3 (6)	- sample on Day 0, untreated fish		
14	18 ± 2 (9)	19 ± 3 (3)	9 ± 4 (3)	24 ± 13 (2)
28	58 ± 4 (9)	26 ±10 (2)	77 ±16 (3)	26 ± 16 (2)
42	64 ± 6 (9)	61 ± 7 (3)	51 ±10 (3)	58 ± 11 (3)
56	32 ± 5 (7)	34 ± 8 (3)	48 ± 4 (3)	34 ± 10 (2)
70	33 ± 6 (9)	42 (1)	21 (1)	11 (1)
84	41 ± 7 (9)	19 ± 2 (2)	37 ± 7 (2)	30 (1)

3. Tissue Selenium in Bioaccumulation Treatments

Ovarian and liver selenium concentrations are shown in Tables 5 and 6. Treatment 2.0 mg/kg Se-L-Met resulted in significant selenium bioaccumulation in the ovarian tissue after two injections (Day 28), and in the liver after the first injection (Day 14). Treatment 0.2 mg/kg resulted in significant increase

of liver selenium level after 6 weeks and 3 injections (Day 42). Data for the ovary in this treatment were less consistent: significantly elevated ovarian selenium levels were observed on Days 28, 56, and 84 for the wet weight tissue, and only on Day 84 for the dry weight. The discrepancies may relate to procedural errors, but most likely they resulted from individual variation in stages of gonadal development and different dry matter content, associated with vitellogenesis.

Treatment 0.02 mg/kg produced no significant effect on selenium bioaccumulation, although sample means were consistently higher than in control, and in one case (liver, Day 28) there was detectable ($P<0.05$) difference between control and treatment. Average concentrations of bioaccumulated selenium were similar between ovarian and hepatic tissues (Table 5 and 6). In summary, data indicate that repeated injections of 0.2 and 2.0 mg/kg Se-L-Met elicited rapid bioaccumulation response in liver, and delayed response in ovary. In this respect, the results were similar with observations in the range-finding experiment (see Table 2).

Table 5. Selenium content of catfish ovaries (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		Wet Weight		
0	1.59±0.14(6)	- sample on Day 0, untreated fish		
14	1.50±0.14(6)	1.55±0.05(3)	1.32±0.10(3)	1.80±0.06(2)
28	1.33±0.06(6)	1.37±0.04(3)	1.94±0.17(3)*	5.40±0.80(2)*
42	1.45±0.14(6)	1.64±0.10(3)	2.26±0.15(3)	9.19±1.87(3)*
56	1.48±0.07(5)	1.82±0.13(3)	2.13±0.10(3)*	12.45±0.88(2)*
70	1.42±0.07(6)	1.56 (1)	2.14 (1)	8.20 (1)
84	1.23±0.10(3)	1.62±0.03(2)	2.46±0.07(2)*	9.74 (1)

Dry Weight

0	3.99±0.42(6)	- sample on Day 0, untreated fish		
14	4.43±0.81(6)	4.38±0.27(3)	7.00±1.85(3)	4.03±0.01 (2)
28	3.29±0.17(6)	5.70±1.83(3)	4.88±0.44(3)	29.66±13.74(2) *
42	4.32±0.76(6)	4.05±0.30(3)	6.01±0.70(3)	31.04±8.06 (3) *
56	3.96±0.34(5)	4.72±0.53(3)	5.25±0.29(3)	59.80±19.51(3)
70	3.47±0.18(6)	3.76 (1)	4.95 (1)	22.40 (1)
84	3.00±0.28(3)	3.93±0.14(2)	5.80±0.22(2) *	23.58 (1)

Table 6. Selenium content of catfish liver (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

DAY	TREATMENT			
	L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
		Wet Weight		
0	2.15±0.16(6)	- sample on Day 0, untreated fish		
14	1.63±0.22(6)	1.60±0.05(3)	2.48±0.34(3)	3.90±0.32(2) *
28	1.11±0.09(6)	1.84±0.33(3) *	1.40±0.18(3)	6.11±0.71(2) *
42	1.39±0.12(6)	1.65±0.25(3)	1.94±0.06(3) *	8.14±0.23(3) *
56	1.29±0.06(5)	1.59±0.37(3)	1.87±0.05(3) *	10.75±0.25(2)
70	1.29±0.06(6)	1.29 (1)	1.29 (1)	9.37 (1)
84	1.29±0.17(3)	1.54±0.05(2)	2.10±0.08(2) *	10.30 (1)

Dry Weight

0	9.17±0.72(6)	- sample on Day 0, untreated fish		
14	6.57±1.04(6)	5.97±0.27(3)	10.60±1.49(3)	16.56±2.36(2)*
28	4.15±0.40(6)	7.22±1.47(3)*	5.55±0.80(3)	25.90±3.07(2)*
42	5.45±0.50(6)	6.83±1.22(3)	7.93±0.31(3)*	34.53±1.29(3)*
56	4.85±0.23(5)	5.75±1.29(3)	7.07±0.19(3)*	43.37±0.37(3)*
70	5.22±0.24(6)	5.17 (1)	5.12 (1)	41.67 (1)
84	5.73±0.81(3)	6.01±0.15(2)	8.86±0.25(2)*	39.78 (1)

4. Selenium in Eggs and Tissues of Spawned Females

Spawning trials were conducted in five consecutive sessions, during the interval of time 16 to 33 days after the last, sixth, injection (see Table 1). The analysis of variance revealed that ovarian and liver selenium concentrations did not exhibit significant changes over time, e.g. there was no detectable tissue depuration during overall spawning. Therefore, observations for all female tissues and fertilized eggs were pooled within each treatment. Data analysis shows significantly elevated selenium concentrations in 0.2 and 2.0 mg/kg Se-L-Met treatments, for all sampled tissues (Table 7). Treatment 2.0 mg/kg exhibited 5 times higher selenium concentrations compared with control. No detectable effect was observed in 0.02 mg/kg treatment. Concentrations of selenium were highest in liver, intermediate in the ovary, and lowest in fertilized eggs, with significant linear relationships between different tissues. Linear regressions for the ovarian/liver and egg/liver selenium residues are shown in Figure 1.

Table 7. Selenium content of spawned catfish females and fertilized eggs (µg/g). Data are x ± s.e.m., sample size in parentheses. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test). First and second rows for each tissue are concentrations on wet and dry weight bases.

TREATMENT			
L-Met (pool)	Se-0.02	Se-0.2	Se-2.0
Liver			
1.63±0.13 (15)	1.93±0.15 (15)	3.08±0.26 (15)*	8.80±0.79 (13)*
6.79±0.43 (15)	7.34±0.69 (15)	12.54±0.94 (15)*	34.30±3.61 (13)*
Ovary			
0.99±0.10 (15)	1.31±0.18 (15)	1.91±0.17 (15)*	5.56±0.62 (13)*

5.72±0.81 (15)	5.65±1.14 (15)	7.02±0.37* (15)	25.97±3.08* (13)	

Eggs

0.46±0.04 (6)	0.55±0.04 (6)	0.99±0.04* (5)	2.96±0.31* (3)
2.85±0.15 (6)	3.16±0.17 (6)	6.34±0.35* (5)	17.40±1.61* (3)

5. Spawning Performance

Fifteen females were used for spawning trials in the pooled control and each of the 0.02 and 0.2 mg/kg treatments, and 13 females were used in 2.0 μg/kg treatment (one female jumped out of tank and was lost for spawning). Spawning response ranged from 23-40% (Table 8). There was no statistically significant difference in spawning response between control and selenium treatments (Fisher's Test, P>0.05). The trend of decreasing spawning response in higher dose selenium treatments, seen in Table 8, may be due to a higher proportions of fish with underdeveloped ovaries in the 2.0 mg/kg treatment group. No significant differences in weight and relative weight of spawned egg masses were detected between control and treatment groups. Fertilization success estimated at 48 hours was similar in all groups (Table 8).

Table 8. Spawning performance of channel catfish. Data are x ± s.d. (sample size is number of spawned females). L-Met and Se-dose are control and treatment.

	TREATMENT			
	L-Met	Se-0.02	Se-0.2	Se-2.0
Number of spawned females	6	6	5	3
Spawning response (%)	40	40	33	23
Weight of egg mass (g)	409 ±131	372 ±46	474 ±91	600 ±14

Weight of egg mass (%-body weight)	18 ± 5	24 ± 2	24 ± 5	28 ± 1
Fertilization success (%)	49 ±19	69 ±14	60 ±12	61 ±19

6. Embryo-Larval Survival and Growth

Average cumulative mortalities for each treatment are shown in Figure 1. Major mortalities were observed during the first week of bioassays. Hatching in all treatments occurred on Day 6 after fertilization, and the swimup stage (onset of exogenous feeding) on Days 11-12. High mortalities (>90%) were observed in 2.0 mg/kg selenium treatment before hatching. Only one out of three egg batches in this treatment produced hatchable embryos, and most of them died between hatching and swim-up stage. Embryos and newly emerged larvae had pale yellow color, contrasting with orange-red coloration of normal embryos (possibly, circulatory system or blood pigments were affected, but no microscopic examination was conducted). Treatment 0.2 mg/kg Se-L-Met also exhibited substantial mortality before hatching and some additional die-off during the swimup stage. Lowest mortality was observed in 0.02 mg/kg selenium treatment.

The analysis of survival was conducted for three intervals: from Day 0 to hatching, from hatching to Day 28, and from fertilization to Day 28 (Table 9). Selenium treatment 2.0 mg/kg exhibited significantly lower survival in each interval, compared with control. Survival in treatment 0.2 mg/kg was significantly different from control only for the interval between hatching and Day 28. Length and body weight of fry sampled on Day 28 did not differ between control and treatments. Substantial differences in survival and tank densities between control and two highest selenium treatments might have affected growth end points (Table 9). No differences between treatments were observed in weight-length relationship. Observations from all treatments fitted the common linear regression: Log(Weight) = 0.438*(Length) + 0.966 (R^2=0.933, N=660).

Table 9. Survival and body size of catfish embryos and fry in bioassays with progenies of treated females. Data are x ± s.e.m. for pooled observations on each progeny. Asterisks denote significant difference between control, L-Met, and treatment, Se (Dunnett's test).

	TREATMENT			
	L-Met	Se-0.02	Se-0.2	Se-2.0
Number of tested progenies	5	6	4	3
Survival (%):				
Fertilization - hatching	86 ± 5	95 ± 2	74 ± 10	7 ± 5*
Hatching - 28 days	90 ± 3	95 ± 1	68 ± 8*	4 ± 3*
Fertilization - 28 days	78 ± 7	90 ± 2	63 ± 10	2 ± 1*
Body size (28 d):				
	(n=230)	(n=296)	(n=146)	(n=3)
Length (mm)	30.0 ±0.1	30.0 ±0.1	29.8 ±0.1	30.3 ±0.1
Wet weight (mg)	200 ± 24	197 ± 21	194 ± 17	204 ± 28

7. Relationship between Maternal Selenium and Survival of Progenies.

Data used for the analysis of LC_{50} are shown in Table 10 and in Figure 3. In general, bioassay mortality rates were in good correspondence with tissue selenium levels. LC_{50}'s for liver and fertilized egg selenium were 11.5 and 6.3 µg/g (dry weight), respectively. Based on observed responses in three selenium treatments, average tissue concentrations in treatments 0.02 and 0.2 mg/kg may approximate empirical NOEC and LOEC values. Maximum acceptable range and LC_{50}' for selenium residues in different tissues are summarized in Table 11.

Table 10. Maternal tissue selenium concentrations (µg/g, d.w.) and mortality in bioassays, from fertilization to 28 days (data

used for LC_{50} analysis). N - number of live embryos on Day 0, r - mortality on Day 28, p - proportions.

Female/progeny identification	Selenium concentration		Mortality		
	Liver	Eggs	N	r	P
L-Met-0.02	4.47	2.49	60	2	0.033
Se-0.02	4.50	3.02	30	2	0.067
Se-0.02	5.42	2.64	60	13	0.217
Se-0.02	5.94	3.25	60	7	0.117
Se-0.02	6.77	3.70	60	5	0.083
Se-0.02	7.44	3.52	60	2	0.033
Se-0.02	7.88	2.82	60	5	0.083
Se-0.20	9.52	6.60	60	9	0.150
Se-0.20	10.13	6.82	60	15	0.250
Se-0.20	13.02	6.06	60	41	0.683
Se-2.00	12.50	19.06	60	57	0.950
Se-2.00	31.70	18.96	60	60	1.000
Se-2.00	33.96	14.19	60	60	1.000

Table 11. Maximum acceptable tissue selenium concentrations and LC_{50} selenium concentrations in maternal tissues, for 28 day survival of progeny. Selenium concentrations are µg/g, d.w.

TISSUE	MATC RANGE		LC_{50} (95% CL)
	NOEC (Se-0.02)	LOEC (Se-0.2)	
Liver	7.3	12.5	11.5 (10.9-12.1)

Eggs (fert)	3.2	6.3	7.7 (7.2-8.4)
Ovary	5.6	7.0	N/A

D. DISCUSSION AND RECOMMENDATIONS

The effect of accumulated ovarian selenium on reproduction has been investigated in bluegill (see Part II of this report). Information on the selenium effect on catfish is limited to field observations and experimental works on nutrition (Gatlin and Wilson, 1984), pathology (Ellis et al., 1937), and mercury metabolism (Jorgensen and Heisinger, 1987). This report provides the first experimental evidence for reproductive effect of elevated tissue selenium in channel catfish female. Bioaccumulation of selenium in broodstock tissue after six consecutive injections of 0.2 and 2.0 mg/kg Se-L-Met did not appear to affect gonadal development, spawning and egg fertility. However, fertilized eggs had significantly elevated selenium burden and survival of embryos before and soon after hatching was significantly reduced. The injection dose 2.0 mg/kg was lethal for the offsprings, and a dose 0.2 mg/kg reduced fry survival.

Catfish produce relatively large (3-3.4 mm) and yolky eggs. Their embryos complete major organogenesis during a relatively long period of embryonic development before hatching (Armstrong, 1962). High mortalities observed in the selenium treatments may be associated with utilization of yolkborne selenoproteins during the embryonic growth and the excessive selenium in embryonic circulation. Early life stages of fish appear to be much more sensitive to selenium, compared with adults. The LC_{50} 8 ppm was reported for newly hatched larvae of zebrafish exposed to waterborne inorganic selenium (Niimi and LaHam, 1975, 1976).

The tissues of channel catfish and closely related species were analyzed for selenium content in several selenium-polluted areas. Sager and Cofield (1984) and Woock and Summers (1984) reported selenium concentrations 12 µg/g in liver and 9-10 µg/g in the ovaries (wet weight) of channel catfish sampled in Hyco Reservoir, North Carolina. These concentrations are similar with 2.0 mg/kg Se-L-Met treatment in our study, which produced a lethal effect on the catfish embryos. Even higher selenium concentrations (26 µg/g, wet weight) have been found in muscle tissue of catfish in Belew Lake (Cumbie and Van Horn, 1978). Field data collected by Lemly (1985) indicate that channel catfish were not found in the lake after 1977, suggesting complete reproductive failure.

Muscle tissue from limited number of channel catfish from selenium-polluted areas of San Joaquin River had low selenium concentrations, 0.26-0.52 µg/g wet weight (CDFG, 1987, 1988). However, the catfish livers collected from the same areas and analyzed by California Veterinary Diagnostic Laboratory, UC Davis, ranged in selenium level 4-24 µg/g dry weight (Ardans et al.1988), with about 30 percent of samples above LC_{50} value estimated by our study.

The acute effect of yolkborne selenium on the offsprings of channel catfish indicates that biomonitoring program in selenium-polluted areas of San Joaquin River (such as Mud Slough North, Salt Slough, and confluence with the Merced River) should be focussed on reproduction; the most sensitive to selenium, part of the life cycle. In sampling programs, the seasonality of the ovarian cycle and vitellogenesis in catfish should be considered to obtain reliable information on the potential effect of yolkborne selenium level. Catfish initiate vitellogenesis in early fall, but the ovarian growth and vitellogenin synthesis are particularly intense in early spring. Vitellogenesis is completed by mid or late spring, and spawning takes place in late spring or early summer. Females with GSI 9-10 % have completed or are close to completion of vitellogenesis and should be most suitable for sampling.

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We appreciate cooperation and assistance of California Veterinary Diagnostic Laboratory System in the analyses of tissue selenium. Catfish broodstock was supplied by the Fishery, Inc. Aquaculture and Fishery Program, UC Davis provided important support in aquatic facility management and animal maintenance. State Board Staff, Laura Valoppi, Nancy Richard, and Victor de Vlaming contributed to this study by providing helpful advice and suggestions.

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G. LIST OF FIGURES

Figure 1. Top: Relationship between selenium concentrations of ovary and liver in catfish females. Data are log-transformed values. Regression equation: $Y = 0.923*X - 0.067$ ($R^2=0.810$, d.f.=52). Outliers are marked by "x".
Bottom: Relationship between selenium concentrations of fertilized eggs and livers of spawned catfish females. Regression equation: $Y = 0.647*X - 0.057$ ($R^2=0.895$, d.f.=16). Outliers are marked by "x".

Figure 2. Average cumulative mortalities in embryo-larval bioassays with progenies of catfish females from different treatments. Control is pooled L-Met treatments, Se is Se-L-Met treatment with respective dose (injections mg/kg body weight).

Figure 3. Relationships between mortalities of catfish in bioassay (from fertilization to Day 28) and selenium concentrations (ppm, d.w., log) in fertilized eggs (top) and livers of treated females (bottom). C is control with highest selenium concentrations, L - treatment Se-0.02, M - Se-0.2, H - Se-2.

H. APPENDICES

Appendix 1. Water Quality Parameters in Broodstock Tanks (data are averages and ranges in 9 tanks).

Date	T°C	D.O.mg/L	pH	TAN mg/L
4-4-89	19.8	7.3	7.9	0.3
	17.0 - 23.0	4.9 - 8.0	7.6 - 8.1	0.2 - 0.5
4-5-89	19.9	7.1	8.0	0.3
	18.5 - 22.0	6.7 - 7.6	8.0 - 8.1	0.2 - 0.4
4-6-89	20.5	7.2	7.9	0.4
	18.0 - 23.0	6.4 - 7.5	7.8 - 7.9	0.3 - 0.4
4-7-89	19.6	7.6	7.6	0.0
	18.0 - 22.0	7.2 - 7.9	7.6 - 7.6	0.0 - 0.2
4-8-89	19.5	8.3	7.7	0.3
	18.0 - 21.0	7.5 - 9.9	7.7 - 7.8	0.3 - 0.4
4-10-89	19.7	7.8	N/A	N/A
	18.0 - 25.0	7.6 - 8.0		
4-14-89	18.8	8.1	7.7	0.3
	13.0 - 21.0	7.8 - 8.6	7.8 - 8.7	0.3 - 0.4
4-17-89	19.2	8.1	N/A	N/A
	16.0 - 21.0	7.8 - 8.5		
4-21-89	21.3	8.1	7.7	0.3
	17.0 - 20.0	7.5 - 8.5	7.6 - 7.7	0.2 - 0.3
4-24-89	17.8	8.0	N/A	N/A
	15.0 - 20.0	7.6 - 8.3		
4-28-89	18.5	8.3	7.6	0.4
	17.0 - 20.0	8.1 - 8.5	7.6 - 7.7	0.3 - 0.4
5-1-89	19.2	8.2	N/A	N/A
	17.5 - 21.5	7.9 - 8.5		
5-5-89	20.2	7.8	7.7	0.4
	19.0 - 23.0	7.4 - 8.1	7.6 - 7.7	0.3 - 0.4
5-8-89	19.7	8.4	N/A	N/A
	18.0 - 22.0	8.1 - 8.6		

Appendix 1 (Continued)

5-12-89	18.4	8.3	7.7	0.2

	14.0 - 21.0	7.5 - 8.7	7.6 - 7.7	0.1 - 0.4
5-15-92	19.5 18.0 - 21.0	7.9 7.5 - 8.6	N/A	N/A
5-19-89	18.8 15.0 - 20.5	8.5 8.1 - 8.8	7.8 7.7 - 7.8	0.3 0.2 - 0.4
5-22-89	18.9 18.0 - 20.0	8.5 8.3 - 8.8	N/A	N/A
5-26-89	N/A	8.1 8.0 - 8.3	7.7 7.6 - 7.8	0.2 0.1 - 0.3
5-30-89	N/A	8.0 7.7 - 8.3	N/A	N/A
6-9-89	N/A	6.6 5.7 - 7.4	7.9 7.8 - 7.9	0.4 0.2 - 0.5
6-12-89	N/A	7.0 6.5 - 7.8	N/A	N/A
6-15-89	N/A	7.2 6.2 - 7.8	8.1 8.0 - 8.1	0.4 0.3 - 0.5
6-19-89	N/A	7.1 6.9 - 7.3	N/A	0.3 0.2 - 0.4

Appendix 2. Water Quality Parameters in Larval Bioassay System (data are averages and ranges of 30 aquaria).

Date	T°C	D.O.mg/L	pH	TAN mg/L
6-27-89	25±1 through all period	7.5 7.3 - 7.6	8.2 8.2 - 8.3	0.3 0.1 - 0.4
6-30-89		7.7 7.5 - 7.8	8.2 8.2 - 8.4	0.3 0.3 - 0.4
7-3-89		7.7 7.4 - 7.9	8.2 8.2 - 8.5	0.3 0.3 -0.4
7-6-89		7.5 7.4 - 7.8	8.2 8.2 - 8.3	0.3 0.2 - 0.4
7-11-89		7.4 6.8 - 7.7	8.2 8.1 - 8.2	0.3 0.3 - 0.4
7-15-89		7.7 7.7 - 7.6	8.1 8.0 - 8.1	0.3 0.3 - 0.4
7-18-89		7.7 7.2 - 7.8	8.2 8.1 - 8.3	0.3 0.3 - 0.4

Appendix 3. A. Dry matter content of channel catfish tissues (observed in broodstock females).

TISSUE	DRY MATTER (%) x ± s.e.m. (n)	Coeff. of variation (%)
Liver	24.75 ± 0.16 (135)	7.5
Ovary	34.79 ± 0.83 (114)	25.6
Egg mass	16.56 ± 0.44 (20)	11.8

B. Conversion factors for selenium content from wet (X) to dry weight (Y). Data are from regression equations with zero intercepts.

LIVER (n=134): $Y = 4.124*X$ (R^2=0.994, S.ERR.= 0.843)

EGG MASS (n=20): $Y = 5.923*X$ (R^2=0.981, S.ERR.= 0.711)

OVARY: (all fish with weight of ovaries < 50g are deleted)

During vitellogenesis (n=58) $Y = 2.498*X$ (R^2=0.993, S.ERR.= 0.310)

Before spawning (n=21) $Y = 2.851*X$ (R^2=0.991, S.ERR.= 0.646)

PART II. Bioaccumulation of Dietary Selenium and its Effects on Growth and Reproduction in Bluegill (Lepomis macrochirus).

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I. APPENDICES 59

A. INTRODUCTION

State Board Order 85-1 addressed waterfowl problems at Kesterson Reservoir resulting from selenium laden water discharged to the facility and directed the formation of the San Joaquin River Basin Technical Committee. One of the tasks of this committee was to develop proposed water quality objectives for constituents of agricultural drainage. State Board staff developed water quality criteria for nine constituents based on available toxicity data. The Technical Committee proposed objectives for three of these constituents, selenium, boron and molybdenum. The toxicity data upon which the criteria and objectives are based are not adequate in that site-specific toxicity data were generally not available. As a result, the Technical Committee recommended that additional site-specific toxicity data be developed to refine these water quality criteria and objectives. The objective of this contract is to provide additional selenium toxicity data to be used to refine the criteria and objectives already developed.

The aim of this study was to determine tissue selenium concentrations in adult bluegill, Lepomis macrochirus Rafinesque, critical to normal reproduction. Bioaccumulation of selenium was induced by dietary selenomethionine treatments applied during the periods of gonadal growth and spawning. We examined the effect of selenium on gonadal development, fertilization, early development and survival of progeny to age 30 days. Two studies were conducted: 1) a selenium bioaccumulation study aimed to evaluate the effect of dietary treatments on tissue selenium burden and gonadal development, and 2) a reproductive performance study aimed at evaluating the effect of tissue selenium concentrations, on the survival of offspring. Both experiments contained six groups of fish: three control groups (diet supplemented with L-Methionine), and three treatment groups (diet supplemented with Se-L-Methionine). The experiments were started in the fall 1990 and completed during the summer 1991. Work was conducted at UC Davis Aquatic Center (Aquaculture and Fisheries Program) and in the Department of Animal Science.

Previous work indicates that bioaccumulation of environmental selenium in reproductive tissue, not detrimental (at least, not lethal) to adult fish, may cause reproductive failure. Reduced population recruitment in selenium-contaminated Belews Lake was documented by sampling at several locations in 1974 through 1977

(Cumbie and Horn, 1979). By the end of sampling period, virtually no juveniles of several species, including bluegill, were found in the polluted locations. Muscle selenium concentrations in adult fish from polluted areas ranged from 10 to 50 ppm compared to a 0.5 to 7.0 ppm (wet weight) from non-polluted locations. Changes in selenium tissue burden of adults were consistent with the disappearance of juveniles.

Sorensen et al (1982, 1983, 1984) investigated the histopathology of Centrarchids collected from the selenium-polluted lakes. The authors described some atretic changes in the vitellogenic oocyte, but the major pathological changes were observed in the kidney, liver and pancreatic tissues of the adults.

Gillespie and Baumann (1986) presented strong evidence for associating the potential route of selenium reproductive effects with maternal egg yolk. They conducted artificial cross-inseminations of wild bluegill collected from lakes with high and low waterborne selenium. Crosses were performed between the parents that had high and low tissue selenium burdens, corresponding to waterborne selenium. The tissue selenium of males did not affect survival of progeny, but selenium levels of females did correlate with larval survival. The ovarian selenium concentrations 7-8 ppm (wet weight) resulted in high larval mortality.

Woock et al (1987) investigated the effects of bluegill broodstock exposure to dietary and waterborne selenium on the survival of progenies. Dietary treatment with up to 30 ppm selenomethionine had no effect on spawning and hatching success; however, elevated or complete larval mortality were observed in 13 and 30 ppm selenomethionine treatments, respectively. This reproductive effect was clearly confirmed by a recent study of the National Fisheries Contaminant Research Center, Missouri (Lemly, 1990). In the waterborne (inorganic) and dietary (organic) selenium treatments, the tissue selenium burden of the broodstock and fertilized eggs, exhibited correlations with dose-dependent increases. High selenium concentrations did not affect gonadal development and natural spawning of treated broodstocks, but all larvae hatched in the high-dose selenium treatment, died before exogenous feeding.

These studies suggest that detrimental effects of selenium on reproduction is, most likely, due to its bioaccumulation in the oocyte yolk during vitellogenesis, and utilization of selenium-saturated yolk during embryonic and early larval development. The effect of selenium bioaccumulation in the egg yolk was experimentally confirmed in our study with channel catfish

broodstock injected with selenoaminoacid during vitellogenesis (Part I of this report).

B. MATERIALS AND METHODS

1. Source of fish

Two different populations of bluegill were used for observations on selenium bioaccumulation (A) and spawning performance (B). Population A included 250 fish obtained from Rainbow Ranch Fish Farm, Kelseyville, California. These fish were held in 1400 L and 6400 L, flow-through outdoor tanks, at water temperatures ranging from 18° to 22°C. The fish were fed Silver Cup #4 trout diet (Murray Elevators, Utah) ad libitum three times daily. After a 32 day weaning period, 194 fish were transferred into the laboratory tanks. The average body weight was 113 g (range 30-220 g). Due to large variation in individual size and poor expression of secondary sex characters, mixed sex-cohorts were used in the experiments, and the sex ratio was assumed to be 1:1.

Population B included 45 females and 50 males obtained from Chico Game Fish Farm. Females averaged 106 g in body weight (range 65-250 g), and males 164 g (range 80-289 g). These fish were reliably sexed (McComish, 1968), and maintained in outdoor 1400 L foot flow-through tanks for 56 days before initiation of the experiments under similar to population A conditions, but in a different tank.

2. Experimental Design

Population A and fin-clipped females of population B were moved into indoor facilities in November, 1989 and randomly assigned to 6 tanks, each receiving one of the following nominal dietary treatments through the end of the experiment: L-Met-8, 18, and 28 ppm (controls); Se-L-Met-8, 18, and 28 ppm (3 selenium treatments). Males from population B were held in separate indoor tanks and received untreated diets until the start of the spawning season.

Population A was randomly sampled on Days 0, 30, 58, 86, and 114. Sampling from each tank was done by sacrificing fish and examining gonads until 3 females were sampled (the number of males varied). After the last sampling, on Day 114, all remaining fish of population A were removed to outdoor tanks, fed with untreated diets, and sacrificed on Day 144, to examine tissue depuration.

In March (day 120 from the initiation of treatment) treated females and untreated males of population B were paired in tanks to obtain natural spawning. These trials had limited success, and natural spawning was replaced with hormonal induction of ovulation and fertilization in vitro.

Fish were maintained in treatment tanks, and both females and males were fed treatment diets. In May-July females were examined by in vivo ovarian catheterization for ripeness, and ready-to-spawn fish were induced to ovulate, stripped of ova, and their eggs were fertilized in vitro by semen from two randomly chosen males from the same treatment tank (males exhibited natural spermiation). Spawned females were necropsied within one hour after stripping. Males were kept in tanks for repeated spawning, and were necropsied at the end of the spawning season.

Fertilized eggs from each individual mating were sampled for fertilization success, selenium content, and two live subsamples were randomly removed: one for the embryo-larval 30-day bioassay, and another for observations on larval development during the first 5 days after hatching. More detailed descriptions are provided in further sections.

3. Feed Preparation

Trout chow was supplemented with either L-methionine or seleno-L-methionine to achieve nominal selenium concentrations of 8, 18, and 28 mg/kg in the diets. The purity of both the L-Methionine and the Se-L-Methionine was 99% (Sigma Chemical Company, St. Louis, Missouri). The Silver Cup mash contained minimum 38 % crude protein and 10 % fat, and maximum 4 % crude fiber and 12 % ash. The dietary mash contained 2.93 ppm residual selenium (ICP-atomic emission, Veterinary Diagnostic Laboratory, UC Davis). Experimental diets were prepared by mixing premix containing supplemented amino acids, cellulose, and dietary mash, with water, herring oil, and dietary mash. 150 g of cellulose and the appropriate amount of Se-L-met (calculated based on the proportion of selenium in the molecular weight of the amino acid) were placed into a vortex mixer and mixed for 20 minutes. The cellulose mixture was combined with 600 g of mash, mixed for 20 minutes. 13.5 kg of mash was combined with premix and mixed for twenty minutes. 750 g of herring oil was added and mixing continued for 20 more minutes. 1800 ml of distilled water was added and mixed for 5 more minutes. Diets were cold extruded to form one eighth inch pellets and dried overnight in a forced air drier. Prepared diets were sealed in plastic bags and stored at -20°C. Three samples were collected during the experiment (11/9/89, 12/5/89, 2/27/90) and analyzed for selenium content.

Observed selenium concentrations were approximately 25% lower than targeted concentrations. The selenium concentrations in three control diets ranged from 1.2 to 1.6 μg/g, and the treatment diets had a selenium content of 5.5, 13.9, and 21.4 μg/g (Table 1).

Table 1. Selenium concentrations and moisture content of the experimental diets.

	Nominal Concentration of Selenium (μg/g)	Moisture (percent)	Actual Selenium concentration (dry weight) (μg/g) mean ± sd (n=3)
	-	14.32	1.17 ± 0.25
L-met	-	16.33	1.60 ± 0.49
(control)	-	15.59	1.23 ± 0.19
	8	13.03	5.52 ± 0.75
Se-L-met	18	16.52	13.93 ± 1.55
(treatment)	28	15.11	21.41 ± 1.92

4. Experimental Protocol

4.1. Rearing and sampling

Fish were put into the experimental tanks on November 6, 1989 at an initial stocking density of 31 per tank (round fiberglass tanks, diameter 130 cm, water volume 355 L). Tanks were supplied with flow-through well water, at a rate of 7-8 L/min. Water was degassed and aerated in a stripping column, and distributed to rearing tanks by gravity from a holding container supplied with a quartz water heater. The water source was an underground well which had the following water quality parameters: pH = 7.6, Alkalinity = 220 mg/L $CaCO_3$, Hardness = 140 mg/L $CaCO_3$, Sulfate = 40 mg/L, Nitrate = 2 mg/L, Boron = 0.46 mg/L, Calcium = 35 mg/L, Sodium = 31 mg/L. Tanks were housed in a room with luminescent light. The initial photoperiod was 10 L: 13 D, with a water temperature of 19 °C. Daily photoperiod was gradually increased from December through February to 15 L, with a concomitant increase of water temperature to 26°C (Figure 1). The adjustments

were made to stimulate the bluegill gonadal cycle, based on information of Kaya and Hasler (1972). Fish were fed ad libitum three times a day, at a daily rate of 1-3 percent of their total body weight, depending on temperature. Bioaccumulation (Population A) fish were sampled in November, December, January, February, and March. With few exceptions, each treatment sample included 3 females and 1-3 males. On Day 114, the last sampling, only two remaining females were sampled in the 8 ppm L-methionine control and the 13.9 ppm selenium treatment, where the proportions of males were higher than expected. Population A fish, remaining in tanks after Day 114 sampling (3 females in pooled control, and 6 females and 5 males in 5.5 and 21.4 ppm selenium treatments) were removed for depuration sampling.

Population B fish were not sampled during the experimental treatment before spawning, but a Day 0 sample was collected in November. Starting in March, photoperiod was increased to 16 L, and maintained through the spawning season. Water quality parameters were recorded throughout the period of rearing and spawning, and are provided in Appendix 1.

4.2. Spawning

Paired broodfish for natural spawning were placed in rectangular tanks (122cm x 64cm x 58cm) constructed from marine plywood and supplied with water from the same system as the rearing tanks. Water flow rates were 1.0 L/min and water depth was maintained at 46cm. After about one month, there had been no natural spawning. Literature data indicated that our 26°C spawning temperature was at the lower level of the optimum range 26-30 C° (Kaya and Hasler, 1972; Banner and Hyatt, 1975). Banner and Hyatt (1975) also reported that daily temperature cycling was favorable for final gonadal maturation and spawning of bluegill.

We began a daily temperature cycle in April, continuing for eight weeks. The spawning tanks were allowed to cool down during the evening and night to 20° C and then warmed up during the day to 27-28° (by turning the heaters on at 8 am). Since no spawning was observed after two weeks of temperature cycle, we decided to catheterize females to determine the stage of ovarian maturity. A polyethylene tubing (1.14 mm ID) was inserted into the females oviduct, and the ovarian eggs were removed by aspiration. Collected eggs were examined under a dissecting scope and egg diameter was measured in each sample. In all females, we observed 4 to 5 distinct clutches of vitellogenic follicles, and all females had clutches of mature follicles, recognized by the coalesced oil globule and transparent yolk (Figure 2). It was also apparent that the most advanced clutches periodically undergo atretic changes, without ovulation and oviposition. The

administration of exogenous hormone was used to induce spawning. Six females were injected IM with 0.03 μg/kg body weight of synthetic mammalian LHRH analog (des Gly 10, D-Ala 6, LHRH ethylamide), dissolved in a physiological saline carrier. After 48 hours only one female spawned. All males were milting. Catheterization of the remaining five females revealed that they had ovulated. In fact, two of the females had freely flowing eggs when netted and removed from the tank for catheterization. About 10 mls of eggs from one female were collected in a petri dish and inseminated by hand-stripped semen, to examine egg quality. At one hour postfertilization, fertilization success was 75 percent (16-32 cell stage). We concluded that broodfish were capable of normal ovulation and spermiation, but unknown environmental or behavioral factors inhibited spawning. Selenium treatments were not a factor, since the response was similar in both control and treatment groups.

Since reliable natural spawning was not available, we developed and applied a standard induced spawning procedure as follows. All females were catheterized weekly. A 1.14 mm (ID), 1.57 mm (OD) polyethylene tubing was inserted 2-3 cm into the female oviduct and the eggs were removed by aspiration. Eggs were placed in a petri dish containing Leibovitz L-glutamine cell culture media (Sigma Chemical Company, St. Louis, Missouri), and examined under a dissecting scope. Females with ripe eggs (Figure 2) were selected for spawning, whereas those with immature follicles or with ripe atretic follicles, were re-sampled weekly, until they were found in the proper ovarian stage. Ripe females were injected with 0.1 μg/ml LHRHa at approximately 8:00 am, and 24 hours later a second dose, of the same concentration, was given. They were examined every two hours, beginning 7 hours after the second injection. Ovulation was evident when freely flowing eggs were released upon gentle pressure to the lower abdomen. Naturally spermiating males were available during the entire spawning season. The following standard procedure of in vitro fertilization was performed at each spawning. About 10-15 ml of ova were stripped into a 100 x 15 mm plastic petri dish. Two males were removed, from the same treatment tank, and a single drop of milt from each male was stripped onto the top half of the petri dish. The milt was then rinsed off the petri dish into the dish containing the eggs, with 40 ml of the spawning tank inlet water. The mixture was gently stirred for two minutes, and then rinsed three times with clean water. Time of fertilization was recorded.

Fertilized eggs were placed in a Lab-Line Incubator at 26 ± 0.5°C in a 1.5 liter (21 cm x 15.5 cm x 5 cm) pyrex glass tray filled with 1 liter of water. The eggs were distributed over the bottom of the glass tray in a single layer using a gentle swirling motion. They quickly adhered to the bottom of the tray. At one hour postfertilization, a subsample of 100 eggs was collected by

placing a plastic grid containing 60 (2.6 cm x 2.1 cm) rectangles underneath the glass trays, and ten eggs from each of ten randomly selected rectangles were collected with a plastic pipet to examine fertilization success. Developing embryos (at 8-32 cell stage) were counted under a dissecting scope.

In 10-12 hours after fertilization (to allow completion of epiboly) approximately 400 eggs were removed from the tray and stocked in a 1 L beaker. Beakers were placed in the incubation cabinet, for observations on larval development. In addition, 90 eggs were transferred into the larval bioassay system. The remaining eggs were weighed and frozen for selenium analyses.

4.3 Larval Development

Four hundred eggs, placed in the incubation cabinet at 26°C, were used for observations on larval development. After hatching, 100 larvae were randomly transferred into a new 1 L beaker containing 500 ml of water. Dissolved oxygen concentrations were measured each day, followed by a renewal of 80% water. Samples were collected daily during the next 5 days. Ten larvae were randomly pipetted from the beaker, into a petri dish, and examined under a dissecting scope. The numbers of normal, abnormal and dead were recorded. The larvae were anesthetized, preserved in 10% phosphate buffered formalin, and later examined for total length, and the oil globule and yolk sac cross-sectional optical areas. Measurements were conducted by point-count image analysis, using a darkfield dissecting microscope with camera lucida, and a Nikon Microplan II image analyzer with microcomputer interface (accuracy 0.01mm).

In addition, approximately three thousand eggs from three females in one selenium treatment (Se-L-Met 28 ppm) and two females in the respective control were placed in separate 1.5 L beakers. Several thousand larvae were sampled at 4 days posthatch, from each female, and analyzed for selenium content.

4.4 Larval Bioassay

Ninety fertilized eggs from each female were placed in groups of approximately 30 eggs into three separate 1-L Nalgene Tri-pour beakers with screened windows (Nitex, 250 μm). Three beakers were suspended in a common 15 L circular fiberglass tank, housed in a recirculating system with temperature control and biological filtration. All components of the systems were made of either fiberglass or PVC. Temperature was maintained at 26° $\pm$ 0.5° C by quartz heaters and YSI thermostats. Water was supplied at the surface of each tank at a rate of 400-500 ml/min. Artificial

photoperiod was 14 L.

Hatching was observed in 24 hours, and feeding with rotifers was initiated at swim-up stage, 4 days after hatching. On Day 16, the survived larvae were counted in each beaker and released in the common tank, where rearing was continued until Day 30 on brine shrimp nauplii. On Day 30, fry were euthanized, counted, weighed (0.1 mg, Mettler AE-100) and measured (0.1 mm). Samples of fry were frozen for selenium analyses.

The following standard protocol was used for monitoring the larval bioassay. Beakers and tanks were checked three times daily for dead embryos and larvae. Beakers were removed one at a time, draining off approximately 2/3 the beaker's volume in the process. Care was taken not to impinge the larvae on the screen during draining. Beakers were placed on a light box, and sediments and dead animals were pipetted out and recorded. When all three beakers were on the light box, food was added. When the beakers were returned to the tank, their relative positions were shifted to randomize the effect of their location. During the period 4-12 days posthatch, each beaker received a 5 to 10 ml suspension of rotifers (concentration 900-1800/ml) three times a day (Appendix 2). The feeding rate was adjusted as necessary to maintain approximately ten rotifers per 1 ml of beaker volume. During the morning and evening feedings, the rotifers were supplemented with a 4 ml concentrate of Selenastrum capricornutum, in order to provide a diet for the uneaten rotifers remaining in the beaker. Once the larvae were large enough to consume brine shrimp nauplii, 8-12 drops of a freshly hatched Artemia suspension were added to each beaker at each feeding, starting on days 8-9 posthatch. Rate of additional feeding with nauplii was adjusted downwards if the uneaten nauplii were present on the bottom of the beaker, indicating that the feeding rate was too high.

Once all larvae were feeding on Artemia nauplii (12-14 days post hatch, or 14-16 days post fertilization) and they had been transferred from the beakers to the tank, they were no longer fed rotifers. In general, 25 ml of an Artemia suspension was given to each tank three times a day. Rate of feeding was adjusted in each tank to maintain an approximate concentration of 3 nauplii per 1 ml at 10 min after each feeding.

After the larvae were released into the tank, uneaten food and wastes were siphoned from the tanks daily. Daily records of embryonic and larval mortalities in beakers and tanks were used for estimation of cumulative mortalities to Day 30. However, small numbers of embryos and larvae were not accounted for by mortality records, and the final analysis of bioassay survival was based on counted numbers of eggs stocked, larvae survived to Day 16 in each beaker, and those survived to Day 30 in the common tank.

Water temperature was measured three times daily in one of the tanks of the system. Dissolved oxygen (YSI Model 58), electrical conductivity (YSI Model 33), hardness (Hach kit) and alkalinity (Hach kit) were measured in the system sump weekly. Total ammonia nitrogen (Hach kit) and Ph (Nestor probe) were measured weekly in one of the three beakers, tanks, and sump. When the conductivity of the water in the recirculating system increased to 800 μmos, water was siphoned out of the sump, allowing fresh well water in, for about 2 hours.

5. Laboratory Methods

5.1 Necropsy and Sample Preparation

All necropsied fish were measured for body and eviscerated carcass weight (accuracy 0.01 g), and the fork length (1 mm). Liver and gonads were dissected and weighed (0.01 g). Wet weight of organs was used to calculate hepatosomatic (HSI = 100 x liver weight/body weight) and gonadosomatic (GSI = 100 x gonads weight/body weight) indices. A strip of the dorsal muscle (4-6 cm length) was dissected and separated from the skin. Samples of liver, gonad, muscle, eggs, larvae, and 30-day old juveniles were weighed and frozen (-20 °C) in Whirl-Pak bags for subsequent selenium analysis. In addition, blood was collected from the caudal vein, with 22 gauge needles and heparinized vacutainers, plasma was separated by centrifugation and frozen (-20°C) in plastic vials for the analysis of protein phosphorus. Gonadal samples were preserved in 10% phosphate-buffered formalin for histological analysis.

5.2 Selenium Analysis

Total selenium was determined by fluorimetry, using methods described by Brown and Watkinson (1977), and Whetter and Ullrey (1978). Wet tissue was desicatted by lyophilization, and stored at -40°C.

Gonad and liver samples (0.1-0.3 g dry weight) were digested with 5 mL of concentrated HNO_3 and 2 mL $HClO_4$. Digestion was carried out at 150°C for 1.5 hr and then at 210°C for 1.25 hr. Reduction of selenate to selenite was accomplished by adding 3 mL of 6 N HCl to the cooled solution and returning to the digestion block for 7 min at 160°C (modification of A.Jacobson and R. Burau, UC Davis, pers.comm). The solution was cooled before adding 2.5 mL of EDTA (0.016 M). The resulting solution was adjusted to pH 1.0 using cresol red indicator, 10M NH_4OH, and 6 M HCl sequentially.

Samples were diluted to a final volume of 25 ml with 0.1 M HCl and compared to selenite liquid standards (0.001-0.010 ppm), a tissue standard (bovine 1577A, NIST, SRM), and blanks using a Perkin-Elmer 650-15 fluorimeter. All reagents were of analytical grade and selenium free. Replicate tissue samples and liquid standard measurements were within 10% accuracy, the recovery of liquid standards was 85-115%. The bovine liver standard (NIST standard 1577A = 0.71 ug/g was analyzed eight separate times, yielding a concentration of 0.739 $\pm$ 0.076 μg/g (mean and s.d.). Inter-laboratory validation was performed at the end of the study on 30 randomly chosen tissue samples (liver and gonad) in the analytical laboratory of the Department of Fish and Game (Stockton, California) by hydride generation atomic absorption (HGAA). Selenium concentrations of samples, analyzed in the two laboratories, exhibited correlations of 0.966 and 0.912 for gonadal and liver samples, respectively. Data are provided in Appendix 3.

5.3 ALPP (plasma protein phosphorus)

Plasma vitellogenin concentrations were evaluated by measurement of alkali-labile protein phosphorus (ALPP), which is an indirect but appropriate method to measure yolk precursor. Previous studies with numerous teleost species demonstrated that the plasma vitellogenin molecule contains nearly all plasma protein phosphorus, and ALPP profile correlates with vitellogenin secretion (Wallace and Jared, 1968; Emmersen and Petersen, 1976; Hori et al , 1979; Nath and Sundararaj, 1981). The laboratory procedure utilized in this study was similar to that described by Wallace and Jared (1968), and de Vlaming et al (1984).

5.4 Histology

Samples of gonadal tissue were dehydrated in a series of alcohol, cleared in xylenes, embedded in paraffin and sectioned at a thickness of 5 microns. Slides were stained with hematoxylin and eosin, and with periodic acid- Schiff stain (PAS), using procedures described in Sheehan and Hrapchak (1980). Slides were examined under a compound scope for the staging of development and atretic changes in the ovarian follicle.

6. Statistical Methods

Comparisons between control and treatments were conducted by Dunnett's procedure; one-tailed test, at α = 0.05 was used (Dunnett, 1955). Three control groups were pooled when the analysis of varience did not reveal significant differences

between all groups (P>0.05). Tissue selenium concentrations were transformed to log_{10} values before performing ANOVA tests. The proportional data (fertilization, survival, abnormalities) were transformed into the arcsine-roots. The relationships between selenium concentrations in different tissues were computed by linear regression analysis. Evaluation of larval bioassay results was based on the survival from egg stocking to 16 and 30 days after fertilization. Continuation of larval rearing in the common tank after their pooling on Day 16 affected final results in some trials due to beaker effect. The proportions of survived larvae in three beakers were compared post-factum by Chi-square analysis, and the assays with heterogenous beaker survival were deleted from the analysis of 30 day endpoint (Appendix 4 and 5). The LC_{50} of maternal tissue and egg yolk selenium for resulting progenies were computed by the Spearman-Karber method (Hamilton et al., 1977; Gelber et al., 1985. Log-transformed tissue selenium concentrations were used. Abbott's correction (Finney, 1971) for control mortality was utilized. The LC_{50} values give an approximate estimation of the acute effect of maternal tissue selenium, and are not intended for regulatory use.

C. RESULTS

1. Bioaccumulation (population A)

1.1 Survival and growth

Only two fish out of the total population 236 died during the course of the experiment (one in the medium selenium treatment and one in the control group).

No apparent difference was observed in fish behavior between the control and treatment groups. Most fish in all experimental treatments fed well on the prepared diets. Swimming activity, as well as feeding, increased as the temperature and day length increased.

Changes in fork length and body weight of both sexes are shown in Tables 2 through 5. No significant differences between control and treatment groups were observed at any sampling time. Control groups gained approximately 1-2 cm in fork length and 50 % in body weight. No such gain was observed in females from the medium and high dose selenium treatments, but statistical comparison of growth was not possible due to the small sample size.

Table 2. Fork length (cm) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	15.80 ± 0.49 (4)			
30	16.08 ± 0.49 (9)	16.47 ± 1.16 (3)	14.63 ± 0.45 (3)	15.20 ± 0.55 (3)
58	16.56 ± 0.55 (9)	17.87 ± 0.81 (3)	14.23 ± 0.94 (3)	15.37 ± 0.39 (3)
86	14.75 ± 0.46 (6)	16.40 ± 0.44 (3)	15.60 ± 1.21 (3)	16.07 ± 1.09 (3)
114	17.45 ± 0.34 (8)	17.17 ± 0.68 (3)	15.70 ± 0.10 (2)	16.00 ± 0.93 (3)
Depuration				
142	17.17 ± 0.60 (3)	16.57 ± 0.38 (3)	N/A	16.10 ± 0.55 (3)

N/A = not available.

Table 3. Fork length (cm) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	18.27 ± 0.37 (6)			
30	18.25 ± 0.43 (6)	18.07 ± 0.64 (3)	15.20 ± n/a (1)	17.73 ± 0.93 (3)
58	18.30 ± 0.25 (5)	N/A	18.57 ± 0.07 (3)	17.40 ± n/a (1)
86	16.95 ± 0.53 (6)	18.37 ± 0.23 (3)	18.77 ± 0.86 (3)	19.30 ± 0.20 (2)
114	18.50 ± 0.49 (8)	16.50 ± n/a (1)	19.53 ± 0.15 (3)	17.35 ± 1.55 (2)
Depuration				
142	18.70 ± n/a	18.95 ± 0.18		18.93 ±

0.56	(1)	(2)	N/A	(3)

N/A = not available.

Table 4. Body weight (g) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	80.05 ± 7.55 (4)			
30	88.51 ± 10.84 (9)	99.04 ± 27.43 (3)	64.25 ± 8.08 (3)	74.24 ± 9.87 (3)
58	98.47 ± 10.19 (9)	138.44 ± 23.11 (3)	61.90 ± 15.13 (3)	70.77 ± 6.94 (3)
86	68.82 ± 8.44 (6)	106.54 ± 14.89 (3)	85.07 ± 23.52 (3)	86.87 ± 16.51 (3)
114	131.93 ±	135.37 ±	96.15 ±	100.03 ±

	8.84 (8)	18.40 (3)	1.45 (2)	18.43 (3)
Depuration				
142	145.63 ± 14.18 (3)	113.45 ± 8.53 (3)	N/A	102.43 ± 11.67 (3)

N/A = not available.

Table 5. Body weight (g) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium Diet (ppm) 5.5	13.9	21.4
0	149.08 ± 0.37 (6)			
30	140.56 ± 10.78 (6)	140.97 ± 15.94 (3)	71.02 ± n/a (1)	122.72 ± 16.99 (3)
58	134.00 ± 6.61 (5)	N/A	153.37 ± 4.46 (3)	131.65 ± N/A (1)
86	112.03 ± 12.34 (6)	148.47 ± 5.91 (3)	154.33 ± 19.86 (3)	182.55 ± 11.65 (2)

114	162.41 ± 13.43 (8)	121.70 ± n/a (1)	190.47 ± 5.54 (3)	136.35 ± 38.85 (2)
Depuration				
142	171.50 ± n/a (1)	190.10 ± 23.70 (2)	N/A	176.03 ± 16.28 (3)

N/A = not available.

1.2 GSI, HSI and Plasma ALPP

There was dramatic increase in GSI of broodstock, particularly in females, from February (Day 86) to March (Day 114) (Tables 6 and 7). This increase reflects rapid ovarian and testicular growth in response to elevated rearing temperature and increased photophase. No statistically significant differences were observed between selenium treatments and control.

Changes in female and male HSI were less evident but, in general, followed the same pattern as GSI (Tables 8 and 9). On Day 86, female HSI values in the 13.9 and 21.4 ppm selenium treatments were significantly higher than in control groups, and the mean values of HSI in these treatments were also higher on Day 114, although not at a significant level (Table 8).

Plasma protein phosphorus (ALPP) in females was elevated on Day 114 and 142 (Table 10). Two selenium treatments (5.5 and 13.9 ppm) exhibited significantly higher ALPP values on Days 86 and 114, compared with controls. However, there were no consistent trends, indicating effect of selenium treatment on vitellogenesis.

Bluegill males exhibited unusually high plasma ALPP concentrations (Table 11). In the majority of teleost fish plasma ALPP of males remains below 10 μg/ml throughout the reproductive cycle, whereas we observed concentrations above 50 μg/ml in some samples. However, the spontaneous synthesis of vitellogenin in male fish was reported in the literature.

Table 6. GSI (percent) of females (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test for each row do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.06 ± 0.04 (4)			
30	1.12 ± 0.06 (9)	1.06 ± 0.12 (3)	1.19 ± 0.09 (3)	1.26 ± 0.05 (3)
58	1.24 ± 0.04 (9)	1.11 ± 0.04 (3)	1.22 ± 0.10 (3)	1.16 ± 0.02 (3)

86	1.20 ± 0.05 (6)	1.20 ± 0.15 (3)	1.30 ± 0.05 (3)	1.29 ± 0.03 (3)
114	7.98 ± 0.42 (8)	7.32 ± 0.69 (3)	6.44 ± 0.42 (2)	9.06 ± 1.25 (3)
Depuration				
142	7.26 ± 0.51 (3)	8.01 ± 1.28 (3)	N/A	6.71 ± 0.64 (3)

Table 7. GSI (percent) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	0.34 ± 0.05 (6)			
30	0.39 ± 0.05 (6)	0.36 ± 0.13 (3)	0.25 ± (1)	0.22 ± 0.05 (3)
58	0.35 ± 0.05 (5)	N/A	0.37 ± 0.10 (3)	0.47 ± (1)

86	0.39 ± 0.08 (6)	0.45 ± 0.10 (3)	0.55 ± 0.17 (3)	0.68 ± 0.03 (2)
114	1.10 ± 0.09 (8)	0.60 ± (1)	1.05 ± .09 (3)	0.99 ± 0.67 (2)
Depuration				
142	1.48 ± (1)	1.20 ± 0.21 (2)	N/A	1.10 ± 0.11 (3)

Table 8. HSI (percent) of females (population A). Data are means ± s.e.m. (n). Asterisks denote significant differences between selenium treatments and control (Dunnett's test).

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.37 ± 0.23 (4)			
30	1.25 ± 0.10 (9)	1.48 ± 0.05 (3)	1.24 ± 0.13 (3)	1.42 ± 0.05 (3)
58	1.50 ± 0.12	1.87 ± 0.08	1.23 ± 0.28	1.26 ± 0.03

	(9)	(3)	(3)	(3)
86	1.01 ± 0.11 (6)	1.35 ± 0.05 (3)	1.39 ± 0.07* (3)	1.39 ± 0.06* (3)
114	1.59 ± 0.12 (8)	1.46 ± 0.08 (2)	1.72 ± 0.27 (3)	1.71 ± 0.10 (3)
Depuration				
142	1.45 ± 0.26 (3)	1.37 ± 0.11 (3)	N/A	1.52 ± 0.18 (3)

Table 9. HSI (percent) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	1.50 ± 0.14 (6)			
30	1.29 ± 0.13 (6)	1.13 ± 0.07 (3)	1.34 ± (1)	1.08 ± 0.13 (3)

58	1.44 ± 0.08 (5)	N/A	1.40 ± 0.08 (3)	2.61 ± (1)
86	1.10 ± 0.05 (6)	1.23 ± 0.08 (3)	1.30 ± 0.09 (3)	1.12 ± 0.10 (2)
114	1.36 ± 0.06 (8)	1.57 ± (1)	1.20 ± 0.05 (3)	1.47 ± 0.03 (2)
Depuration				
142	1.71 ± (1)	1.48 ± 0.05 (2)	N/A	1.25 ± 0.03 (3)

Table 10. Plasma ALPP (ug/ml) of females (population A). Data are means ± s.e.m. (n). Asterisks denote significant differences between the selenium treatments and control (Dunnett's test).

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	58.5 ± 7.9 (4)			
30	25.4 ± 6.3 (9)	16.0 ± 4.0 (3)	6.0 ± 0.0 (1)	20.7 ± 4.7 (3)
58	23.7 ± 2.8	33.0 ± 8.4	17.0 ± 3.1	16.0 ± 2.0

	(9)	(3)	(3)	(3)
86	16.5 ± 4.0 (6)	26.0 ± 1.5 (3)	41.7 ± 4.4* (3)	16.7 ± 2.4 (3)
114	40.3 ± 0.8 (8)	67.0 ± 11.7* (3)	41.0 ± 6.0 (2)	47.7 ± 5.0 (3)
Depuration				
142	64.0 ± 18.1 (3)	118.0 ± 16.7 (3)	N/A	49.3 ± 9.2 (3)

Table 11. Plasma ALPP (μg/ml) of males (population A). Data are means ± s.e.m. (n). The analysis of variance and Dunnett's test do not reveal significant differences between control and treatment groups.

Sample Day	Control Pooled	Selenium in Diets (ppm) 5.5	13.9	21.4
0	56.8 ± 9.7 (6)			
30	36.5 ± 7.4 (6)	25.3 ± 4.8 (3)	20.0 ± (1)	65.0 ± 5.0 (3)

58	28.0 ± 5.6 (5)		28.0 ± 6.7 (3)	6.0 ± (1)
86	10.8 ± 2.2 (6)	26.3 ± 8.8 (3)	23.3 ± 8.7 (3)	24.0 ± 1.0 (2)
114	37.8 ± 11.0 (8)	12.0 ± (1)	29.3 ± 7.9 (3)	58.0 ± 46.0 (2)
Depuration				
142	13.0 ± (1)	18.0 ± 9.0 (2)	N/A	13.2 ± 2.7 (3)

1.3 Gonadal development

Microscopic examination of the ovarian and testicular histological sections did not reveal abnormalities in any experimental treatments.

At the beginning of the experiment (in November), ovaries contained 50% immature and 50% previtellogenic follicles, approaching the onset of vitellogenesis (medium to large cytoplasmic vacuoles and chorion in the process of differentiation). These proportions were estimated by observing 10 separate fields of one slide at 20x.

During the next two samplings (12-5-89, 1-2-90), the proportion of previtellogenic and early vitellogenic follicles remained unchanged, but vitellogenic oocytes were gradually increasing in size.

During January (1-30-90), the proportion of early vitellogenic oocytes increased to 70-80 % , and an estimated 10-20% of the oocytes were in phase of active vitellogenesis (cytoplasm contained yolk globules).

In February (2-27-90 sample) vitellogenesis progressed rapidly. Over 90% of the ovarian follicles contained late vitellogenic oocytes that almost doubled in size and had cytoplasm filled with large yolk platelets, in some cases fused in yolk spheres.

At last sampling (3-27-90), the majority (90%) of the gonad contained large vitellogenic oocytes. In addition, there were about 10% degenerating follicles, recognized by the disintegration of yolk platelets and cytoplasm. These atretic follicles were observed in all control and treatment groups.

Spermatogenesis in the males followed a similar rate of development. During the first three months (11-7-89, 12-5-89, 1-2-90 samplings), testicular tissue contained 50-60% cysts with spermatogonia and the remaining cysts had spermatocytes in the early phase of meiotic proliferation. In sample 1-30-90, the more advanced meiotic stages (secondary spermatocytes, and spermatids) were found towards the main duct of the lobule testis. Cysts with gonial cells were still predominant around the periphery of the testis. During the next month (2-27-90) spermatogenesis accelerated, and testes contained large cysts with mature spermatozoa. At the end of March, testicular ducts were filled with free spermatozoa.

1.4 Tissue selenium concentrations

Practically all treatment groups of females at each sampling had significantly higher tissue selenium concentrations, compared with controls. Female gonads and livers exhibited a 5-20 times increase in selenium concentrations, reflecting dietary selenium dose and exposure time (Table 12). At each sampling, mean tissue selenium concentrations exhibited significant correlations ($r = 0.923$ to 0.999, $d.f.=2$) with dietary selenium levels. After one month depuration (feeding regular diet), the selenium concentrations in female gonads and liver decreased 20-40 percent, but remained at significantly higher levels, compared with the control group (Table 12). Testes appeared to accumulate less selenium, compared with
the ovaries, but liver accumulation was similar in both sexes. The selenium concentrations in male gonads and liver decreased after one month of depuration (Table 13).

Moisture content of gonadal and liver tissues is presented in Appendix 6, allowing the conversion of dry weight selenium values to wet weight. Liver dry matter content exhibited little change during different sampling times, however dry matter content of gonadal tissues changes with an increase of GSI: in the ovary, dry matter content increases during vitellogenesis; in the testis, dry matter decreases during spermatogenesis.

Table 12. Tissue selenium concentrations of bluegill females (μg/g, dry weight). Data are means, s.e.m., (n). Asterisks denote significant difference between control and treatments (Dunnett's test).

Days on Feed	Control (pooled)	DIETS Se-5.5	Se-13.9	Se-21.4
		OVARY		
0	1.88 ± .16 (4)	-	-	-
29	2.30 ± .45 (9)	3.91 ± .23 (3)	4.91 ± 1.22* (3)	10.30 ± 0.78* (3)
57	2.24 ± .13	5.55 ± .11*	8.06 ± 1.63*	19.24 ± 0.61*

	(9)	(3)	(2)	(3)
85	2.65 ± .24 (6)	7.07 ± .64* (3)	20.20 ± 3.50* (3)	31.83 ± 9.38* (3)
113	2.17 ± .05 (8)	10.89 ± 1.83* (3)	26.17 ± 0.07* (2)	40.32 ± 2.44* (3)
Depuration 141	2.76 ± .10 (3)	6.31 ± 0.64* (3)	N/A	32.12 ± 2.20* (3)
		LIVER		
0	3.07 ± .40 (4)	-	-	-
29	3.23 ± .37 (9)	5.78 ± .38* (3)	8.19 ± 1.84* (3)	19.07 ± 0.41* (3)
57	2.64 ± .16 (9)	5.40 ± 2.69* (3)	18.92 ± 2.79* (3)	25.37 ± 2.57* (3)
85	3.75 ± .14 (6)	.10 ± 1.41* (3)	29.12 ± 3.47* (3)	36.10 ± 7.02* (3)
113	2.51 ± .32 (8)	NA[1]	22.75 ± 2.96*	40.68 ± 2.14* (2)
(3)				
Depuration 141	3.15 ± .80 (3)	9.25 ± 1.24* (3)	N/A	27.24 ± 2.28* (3)

NA[1]) Concentration was 8.83 ± 1.09* (3). However, sample was analyzed 4 days after digestion.

Table 13. Tissue selenium concentrations of bluegill males (μg/g, dry weight). Data are means, s.e.m., (n). Asterisks denote significant difference between control and treatments (Dunnett's test).

Days on Feed	Control (pooled)	DIETS Se-5.5	Se-13.9	Se-21.4
		TESTIS		
0	2.78 ± .11 (6)	-	-	-

29	3.20 ± .36 (6)	6.07 ± 1.13 (3)	15.72 (1)	8.58 ± 1.53* (3)
57	3.26 ± .68 (5)	N/A	12.91 ± 2.61* (3)	7.19 (1)
85	4.43 ± .84	4.94 ± .59 (6)	15.24 ± 1.98* (3)	24.79 ± 6.52* (2)
113	2.65 ± .21	9.87 (8)	16.38 ± 0.71* (1)	29.70 ± 5.02* (2)
Depuration				
141	4.94 (1)	6.06 ± 0.28 (2)	N/A	18.70 ± 1.59 (3)
		LIVER		
0	3.80 ± .33 (6)	-	-	-
29	3.29 ± .48 (6)	5.64 ± .25 (3)	12.75 (1)	18.01 ± 3.82* (3)
57	2.76 ± .37 (5)	N/A	22.67 ± 3.67* (3)	41.56 (1)
85	4.68 ± .45 (6)	10.93 ± 1.46* (3)	21.68 ± 2.21* (3)	29.47 ± 7.66* (2)
113	4.10 ± .37 (8)	14.32 (1)	24.28 ± 4.54* (3)	52.47 ± 5.23* (2)
Depuration				
141	7.02 (1)	9.93 ± 0.40 (2)	N/A	25.69 ± 4.58 (3)

2. Spawning Performance (population B)

2.1 Gonadal Histology

Histological examination of the broodstock gonads after spawning did not reveal any differences between control and treatment groups. Ovaries of spawned females contained previtellogenic, vitellogenic, mature and post-spawned follicles. Testes contained mature spermatozoa, and the next generation of spermatogonia along the periphery of the testicular cross-section. There were no apparent atretic changes in the ovaries and testes, except for the advanced (overripe) ovarian follicles, usually observed in bluegill females.

2.2 Ovulatory Response and Fertilization Success

Individual ovulatory response, latency, and egg fertility are shown in Appendix 7. Adequate ovulatory response was scored "1", based on the observed quality of ovulation and egg fertility. Lack of ovulation (no eggs stripped) or poor ovulation (overripe eggs, or eggs with little ovarian fluid) were scored as "0". One female (13.9 ppm selenium treatment) did not respond to hormonal stimulation, but this fish also had the smallest gonads at necropsy. Two injected females and one non-injected female (all in the 28 L-methionine control group) spawned naturally. Only 7 females (1 or 2 in each treatment except the high selenium) were given an ovulation score of 0, and no larval bioassay was conducted for these females. There was no clear treatment effect to the poor ovulations: in fact, the high-dose selenium treatment exhibited all normal ovulations.

The proportions of ovulatory females with score "1" ranged from 50-86 percent in control groups and 71-100 percent in the selenium treatments, with no significant differences between control and treatment groups. Overall ovulatory success was 81.6% (Table 14). Latency time exhibited small variation (9-12 hours), and control and treatment groups did not significantly differ as well. Mean fertilization success was 71 % in control and 76 % in selenium treatment groups, with no significant difference between control and treatments (Table 14).

Table 14. Ovulatory response, latency, and egg fertility of bluegill females, administered LHRHa. Data are means and standard deviations.

Treatment	N	Ovulation (scored "1") (n/percent)	Latency (hours)	Fertilization (percent)
Control:				
08	6	5 / 83.3	8.8 ± 1.0	78 ± 16.6
18	7	6 / 85.7	10.2 ± 2.8	69 ± 15.9

28	4	2 / 50.0	10.6 ± 2.6	58 ± 16.7
Pooled:	17	13 / 76.5	9.7 ± 2.4	71 ± 17.4
Selenium Treatment:				
5.5	7	5 / 71.4	11.5 ± 1.9	76 ± 7.9
13.9	7	6 / 85.7	10.8 ± 2.2	75 ± 14.1
21.4	7	7 / 100	11.7 ± 3.3	76 ± 8.3
repPooled :	21	18 / 85.7	11.3 ± 2.7	76 ± 10.5
Control & Selenium Treatment Pooled:	38	31 / 81.6	10.7 ± 2.7	74 ± 14.1

2.3 Observations on Early Development

Observations on early development (from hatching to Day 5 posthatch) revealed a severe effect of the 21.4 ppm selenium treatment on larval development. Systemic edema and underdevelopment of the lower jaw were evident in all larvae from this treatment on Day 3 posthatch, and there was complete mortality by day 5, except for two progenies where 10% of the larvae appeared normal. These abnormalities were not observed in control groups and in the 5.5 pm maternal selenium treatment. However, 3 out of 6 progenies from 13.9 selenium treatment exhibited 10 to 20 percent larvae with abnormalities similar to high selenium treatment (Appendix 8). The average proportions of larvae with edema were 5 ± 2 percent in 13.9 ppm, and 95.7 ± 2.7 percent in 21.4 ppm treatment (x ± SD), significantly different from the pooled control and 5.5 ppm selenium treatment (Table 15).

Yolk sac and oil globule absorption were affected by selenium treatment (Table 16). Areas of larval yolk sac and oil globules were significantly larger on Days 3 and 4 in 13.9 ppm selenium treatment, and on Days 2, 3 and 4 in 21.4 ppm selenium treatments. These abnormalities are shown in microphotographs, taken during the four consecutive days of posthatch development (Figure 3).

Total length of larvae was similar in all experimental groups on Day 1 posthatch, but significantly smaller in all three

selenium treatments on Day 2 posthatch. During the Days 3 and 4 the significant difference in length was observed only between control and the 21.4 ppm selenium treatment (Table 17). The utilization of the yolk sac for embryonic and larval growth might have been influenced by all selenium treatments, but persistent effect was observed only at the high dose. Throughout the period of observation on early development, water temperature was constant (26 ± 0.5 °C), and the dissolved oxygen in beakers never fell below 5 ppm (Appendix 9).

Table 15. Proportions of 5 day-old larvae with edema in different treatments. "n" is a number of progenies examined. Asterisks denote significant difference between control and treatments (Dunnett's test).

TREATMENT	n	PROPORTIONS (x ± SD)
Control (pooled)	14	0.000 ±
Se - 5.5	5	0.000 ±
Se - 13.9	6	0.050 ± 0.020 *
Se - 21.4	7	0.957 ± 0.027 *

Table 16. Cross-sectional optical areas (mm^2) of the yolk sac and oil globules in larvae from pooled control and selenium treatments at Days 1 through 4 posthatch. Asterisks denote significant difference between each selenium treatment and pooled control (analysis of variance and Dunnett's test). Sample size for each progeny was 10 larvae. Data are mean ± s.d. Table "n" values show the number of examined progenies.

	YOLK AREA (mm^2)			
	Day 1	Day 2	Day 3	Day 4

Treatment				
Pooled Controls (n=14)	.460 ± .052	.315 ± .043	.105 ± .018	.060 ± .016
Se - 5.5 (n=5)	.450 ± .044	.324 ± .033	.103 ± .021	.061 ± .014
Se - 13.9 (n=6)	.464 ± .041	.324 ± .032	.145 ± .043*	.080 ± .021*
Se - 21.4 (n=7)	.457 ± .046	.425 ± .031*	.298 ± .040*	.137 ± .040*

	OIL GLOBULE AREA (mm^2)			
Treatment	Day 1	Day 2	Day 3	Day 4
Pooled Controls (n=14)	.097 ± .014	.068 ± .013	.030 ± .009	.010 ± .005
Se - 5.5 (n=5)	.096 ± .012	.065 ± .011	.030 ± .006	.010 ± .003
Se - 13.9 (n=6)	.093 ± .013	.070 ± .011	.037 ± .008*	.014 ± .006*
Se - 21.4 (n=7)	.096 ± .016	.076 ± .013*	.060 ± .012*	.031 ± .007*

Table 17. Total larval length in pooled control and selenium treatments, at Day 1 through 4 posthatch. Data are means ± s.d. Asterisks denote significant difference between each treatment and control (analysis of variance and Dunnett's test). Sample size is 10 larvae for each progeny. Table "n" show number of progenies examined.

Treatment	TOTAL LENGTH (mm) Day 1	Day 2	Day 3	Day 4
Pooled Controls (n=14)	4.09 ± .12	4.62 ± .14	5.06 ± .13	5.32 ± .14
Se - 5.5 (n=5)	4.10 ± .12	4.56 ± .15*	5.08 ± .11	5.29 ± .16
Se - 13.9 (n=6)	4.10 ± .13	4.56 ± .14*	5.06 ± .15	5.30 ± .19
Se - 21.4 (n=7)	4.05 ± .15	4.48 ± .13*	4.90 ± .14*	5.18 ± .10*

2.4 Survival and Growth of Larvae in Bioassays

Three bioassays in control and 5 in selenium treatments were excluded from the analysis of 30-day survival because of significant difference in the proportions of survived larvae in three beakers by Day 16 (Appendix 4). However, survival to Day 16 was analyzed for most of the progenies, by pooling data for two beakers and deleting one beaker with the significantly different survival rate. One control progeny, 18-3-C, was entirely omitted (Appendix 5).

Survival in the bioassays is summarized in Table 18. Control survival was 70-80 percent, and the analysis of variance and Dunnett's test did not reveal significant difference between three control groups. Mean survival in selenium treatments was lower than the control (range 2.5 - 65 percent), but statistically significant difference was observed only in the 21.4 ppm treatment, where more than 95 percent of larvae died before Day 16. Cumulative mortality curves for control and each treatment are shown in Figure 4. Major mortality occurred between days 5 and 8 after fertilization (Days 3-6 after hatching) in selenium treatment 21.4 ppm, with very few individuals survived to

metamorphosis. Low and medium selenium treatments exhibited only slight increase in larval mortality that was, in general, similar with control. A compressed developmental period of high mortality in the 21.4 ppm selenium treatment indicates acute effect of maternal selenium treatment on larval development during the endogenous feeding phase.

Data on fry body size and proportions of abnormalities at age 30-days, are summarized in Table 19. Both total length and body weight were significantly smaller in the 21.4 ppm selenium treatment, compared with the pooled control. The condition factor of fry ($100xBW/TL^3$) ranged from 1.612 to 1.659 and did not differ between control and treatment groups. The proportions of abnormal juveniles appeared to be elevated in low and high selenium treatments, but statistical analysis did not reveal significant differences between control and treatment groups. Abnormalities observed in 30-day old fry might have been caused by both developmental defects and environmental factors. In contrast to edema observed at yolk sac stage, they did not discriminate selenium treatments. Water quality parameters in the bioassay system are given in Appendix 10.

Table 18. Survival of bluegill larvae in the bioassays. "n" is the number of progenies tested in each treatment. Asterisks denote significant difference between control and treatment means (Dunnett's test).

Treatment	Proportion Alive Mean	Std. Dev.	n
Control:			
Survival, Day 16			
08	0.753	0.100	5
18	0.711	0.085	5
28	0.805	0.107	5
Survival, Day 30			
08	0.730	0.110	5
18	0.688	0.087	5
28	0.715	0.045	3
Control and treatments:			
Survival, Day 16			
Pooled control	0.756	0.099	15
Se-5.5	0.649	0.251	5
Se-13.9	0.626	0.235	6
Se-21.4	0.025*	0.035	7
Survival, Day 30			
Pooled control	0.710	0.085	13
Se-5.5	0.519	0.265	3
Se-13.9	0.644	0.034	3
Se-21.4	0.025*	0.035	7

Table 19. Body size of 30-day old bluegill juveniles and proportions of the individuals with spinal and head deformities, and non-inflated swimbladders. Data are means and standard deviations. "n" is the number of progenies tested in each

treatment. Asterisks denote significant differences between pooled control and selenium treatments (Dunnett's test).

Treatment (n)	Total Length (mm)	Weight (mg)	Abnormalities (percent)
Control:			
08 (5)	19.2 ± 0.6	116 ± 14	6.0 ± 8.6
18 (6)	19.2 ± 0.9	119 ± 15	8.8 ± 8.7
28 (5)	18.8 ± 1.7	106 ± 37	3.5 ± 4.8
Control and treatments:			
Pool.Cont.(16)	19.1 ± 1.2	114 ± 24	6.3 ± 7.9
Se-5.5 (5)	19.9 ± 1.2	133 ± 27	15.0 ± 5.8
Se-13.9 (6)	19.3 ± 0.8	119 ± 16	7.2 ± 3.1
Se-21.4 (4)	16.6 ± 2.5*	81 ± 37*	25.0 ± 43.3

2.5 Tissue Selenium Concentrations

Tissue selenium concentrations of the broodfish and resulting progenies are shown in Table 20. Concentrations in female livers were similar with those of fish from population A on Day 113 (Table 12). Concentrations of postspawned (stripped) ovaries were lower, compared with maturing ovaries, indicating that some selenium is lost with the ovulated eggs. Selenium concentrations of somatic and reproductive tissues were significantly elevated in all treatments, compared with controls, except for the male liver in the 5.5 ppm treatment (Table 20). Highest levels were observed in female liver, and the lowest in female muscle and testes.

Selenium concentrations of eggs, sampled 24 hours after fertilization, were lower than those in the liver, but higher than concentrations in the muscle and stripped ovary. Only three progenies (all from 21.4 ppm selenium treatment) were sampled at the yolk sac stage. Their selenium concentrations were high and similar to the concentration in fertilized eggs. However, 30 day old juveniles exhibited low (not different from control) selenium concentrations in all maternal treatments, indicating that

selenium was metabolized during a period between the end of endogenous feeding and metamorphosis (Table 20). Rotifers and brine shrimp nauplii, used as larval feed, had low selenium concentrations (0.5 μg/g, dry weight).

Egg selenium concentrations exhibited highly significant correlations with those of liver, muscle, and ovary in spawned females ($r = 0.947$, 0.947, and 0.957, respectively, DF=36). The regression analysis with log-transformed data revealed significant ($P<0.05$) relationships between selenium concentrations in fertilized eggs and those in liver, muscle and ovary of respective mothers (Figures 5a, 5b, 6a). Female liver, muscle, ovary, and fertilized eggs selenium levels exhibited significant linear relationships with dietary selenium (Figure 6b, equations are based on mean values, reported in Table 20).

Table 20. Tissue selenium concentrations in broodstock bluegill and their progenies (μg/g, dry weight). Data are means $\pm$ s.e.m. (n). Asterisks denote significant difference between control and treatment (Dunnett's test).

TISSUE	CONTROL (pooled)	Se-5.5	Se-13.9	Se-21.4
		TREATMENTS		
		MALES		
LIVER	4.07 ± 0.23 (9)	6.94 ± 1.58 (3)	20.46 ± 3.46* (3)	31.63 ± 1.75* (3)
TESTIS	1.87 ± 0.11 (9)	3.64 ± 0.47* (3)	9.96 ± 0.45* (3)	15.25 ± 0.45* (3)
		FEMALES		
LIVER	4.00 ± 0.26 (20)	12.33 ± 1.09* (7)	25.98 ± 4.28* (7)	47.60 ± 4.11* (7)
MUSCLE	1.47 ± 0.14 (20)	5.80 ± 0.79* (7)	10.41 ± 2.02* (7)	23.64 ± 2.04* (6)
OVARY	2.23 ± 0.11 (20)	6.34 ± 0.47* (7)	14.10 ± 2.62* (7)	30.63 ± 3.23* (7)
		PROGENIES		
EGGS[1]	2.81 ± 0.14 (19)	8.33 ± 0.63* (7)	19.46 ± 3.83* (6)	38.39 ± 3.14* (7)
LARVAE[2]	N/A	N/A	N/A	35.30 ± 4.16 (3)
FRY[3]	1.48 ± 0.11 (16)	1.25 ± 0.02 (5)	1.37 ± 0.06 (5)	1.46 ± 0.03 (2)

1) 24 hours after fertilization.
2) 4 days after fertilization.
3) 30 days after fertilization.

2.6 The Effect of Maternal Tissue Selenium on Larval Mortality in Bioassays

The proportions of larvae with edema and delayed resorption of the yolk sac indicates a significant negative effect of maternal selenium treatment 13.9 ppm on larval development. The effect was not observed in the 5.5 ppm treatment. Therefore, dietary selenium concentrations 5.5 and 13.9 ppm, and their respective mean selenium concentrations in female tissues and fertilized eggs can be treated as NOEC and LOEC values for reproductive failure. Data in Table 20 show the intervals of maximum acceptable tissue selenium concentrations.

Nonparametric Spearman-Karber method was used for all estimations of LC_{50} since the tolerance distribution substantially deviated from normal (due to high mortality in one observation from 5.5 ppm selenium treatment and low mortalities in two observations with 13.9 ppm, data in Table 21).

The resulting NOEC, LOEC and LC_{50} values for maternal tissue selenium are given in Table 22. LC_{50}'s for all tissues are within their observed MATC ranges. Muscle tissue has the lowest LC_{50} and LOEC values, and liver tissue exhibits the highest values. Data in Appendix 6 can be used to convert these values from dry to wet weight of tissue.

Table 21. Thirty day bioassay mortalities and tissue selenium concentrations in respective females. "n" is number of eggs on Day 0, "r" is mortality on Day 30, "p" is proportions. One control treatment with highest selenium concentration was used to estimate LC_{50} for respective tissue.

Progenies	n	r	p	Selenium, µg/g, dry weight (female) Ovary	Liver	Muscle	Eggs
08-2C	89	17	0.191	1.95	4.04	2.25	3.54
18-4C	85	17	0.200	2.38	5.03	0.95	3.25
5.5-1S	85	64	0.753	7.72	14.89	7.07	11.49
5.5-2S	90	42	0.467	5.55	7.06	5.80	8.31
5.5-6S	85	19	0.224	4.06	10.49	1.41	6.18
13.9-1S	90	29	0.322	3.94	7.54	2.75	8.55
13.9-3S	87	34	0.391	21.82	34.74	15.44	22.06
13.9-6S	87	31	0.356	20.40	36.82	16.58	30.20
21.4-1S	88	87	0.989	29.90	38.02	NA	44.02
21.4-2S	90	89	0.989	45.82	33.96	31.10	36.31
21.4-3S	86	79	0.919	27.24	59.01	17.28	25.21
21.4-4S	88	88	1.000	23.18	62.71	27.40	52.18
21.4-5S	90	90	1.000	32.64	55.25	24.00	42.40
21.4-6S	86	86	1.000	37.63	48.14	24.66	38.47
21.4-7S	88	82	0.932	18.02	36.10	17.42	30.12

Table 22. MATC and LC_{50} (95% CL) tissue selenium concentrations for reproductive success of bluegill.

TISSUE	Selenium, µg/g, dry weight NOEC	LOEC	LC_{50}
Liver	12.3	26.0	17.4 (15.7-19.4)
Eggs	8.3	19.5	16.4 (15.0-18.0)
Ovary (stripped)	6.3	14.1	10.8 (9.7-12.1)
Muscle	5.8	10.4	10.1 (9.0-11.3)

D. DISCUSSION

1. Bioaccumulation of selenium in somatic and reproductive

tissue.

Previous observations on bioaccumulation of dietary selenomethionine in fish tissue indicate that organic selenium is readily incorporated in the somatic and reproductive tissues, and has a long retention time (Hilton et al., 1980; 1982; Hodson and Hilton, 1983; Kleinow and Brooks, 1986; 1986a). Tissue saturation in the fathead minnow and bluegill was observed during a period of exposure from 2 weeks to 3 months (Bertram and Brooks, 1986; Lemly, 1982; Ogle and Knight, 1989). Our data are in general agreement with these reports. Several field observations indicate that the ovarian and testicular tissues may reach selenium concentrations similar with liver, and higher than those in the muscle or carcass (Cumbie and Van Horn, 1978; Sager and Cofield, 1984; Gillespie and Baumann, 1986; Sorenson, 1991).

Bioaccumulation of selenium in the gonads of fish depends on stage of gonadal development e.g. proliferation of germ cells in males and vitellogenesis in females. In addition, gonadal tissue of fish undergoes substantial changes in dry matter content during the reproductive cycle. Absence of information on stage of gonadal development (GSI) or dry matter content makes it difficult to interpret many field observations.

Bioaccumulation of selenium in the carcass, ovary and testis of bluegill broodstock, experimentally exposed to dietary selenomethionine and waterborne inorganic selenium, was recently reported by National Fisheries Contaminant Research Center, Missouri (Lemly, 1990). Ovaries and testes concentrated more selenium, compared with the carcass, but the final ovarian concentrations were higher compared with testes. Higher concentration of selenium in the egg yolk of vitellogenic oocytes could account for differences in gonadal bioaccumulation between the two sexes.

High concentration of selenium in maturing ovary and significant relationships between selenium bioaccumulation in liver, ovary and freshly spawned eggs suggest that selenium is stored in the egg yolk proteins, as was indicated by the analyses of yolk protein fractions in white sturgeon, Acipenser transmontanus from the Sacramento - San Joaquin Estuary (Kroll and Doroshov, 1991). The potential routes of selenium transfer may include vitellogenin synthesis in the liver, selenium-binding proteins, and incorporation of free selenium forms into the oocyte during the pre-ovulatory egg hydration phase.

2. Effect of Tissue Selenium on Reproductive Performance

Inspite of high selenium concentrations in somatic and reproductive tissues of bluegill from dietary treatments, and

potential subtle effects on growth and health of the broodstock in the high selenium treatment, we did not observe negative influence of selenium treatment on gametogenesis. The vitellogenic phase of the ovarian development was similar in all treatments, characterized by the increases of GSI analogous to the reproductive profile of wild bluegill stock (James, 1946). Natural spawning in our trials was adversely affected by conditions in the water system, but females in all treatments responded to hormonal injection with ovulation and produced eggs of high fertility; males were spermiating naturally throughout period of spawning. The researchers of NFCRC (Lemly, 1990) did not observe inhibition of natural spawning in bluegill in the high-dose (33.3 μg/g) dietary selenomethionine treatment. Other studies did not reveal negative effects of chronic dietary or waterborne selenium treatments on gonadal development, natural spawning, fertility, and hatchability of bluegill or fathead minnow (Gillespie and Baumann, 1986; Woock et al, 1987; Ogle and Knight, 1989; Schultz and Hermanutz, 1990; Lemly, 1990).

The experiments of Gillespie and Baumann (1986), Woock et al (1987), and Lemly (1990) indicated that the early larval mortality of bluegill correlates with selenium accumulated in spawned eggs. Freshly spawned eggs of bluegill in this study had selenium concentrations proportional to other maternal tissues and the dietary treatment dose. Furthermore, the newly hatched yolk sac larvae from the high selenium treatment had high selenium concentrations, similar to freshly spawned eggs, whereas the same progenies survived to metamorphosis exhibited low selenium concentrations, similar to the control. Therefore, the selenium stored in the egg yolk should be metabolized during yolk sac absorption.

The greatest impact of bioaccumulated selenium on embryonic and larval development can be anticipated in late embryonic or early larval stages, when the rates of yolk sac utilization increase compared to early embryonic development. Toetz (1966) provides detailed information on the utilization of yolk by the bluegill embryos. He indicates that only a small amount of yolk, mainly non-proteins are utilized before hatching, and 90 percent of the yolk proteins are utilized by larvae between hatching and 4th day posthatch, concomitant with observed abnormalities and larval mortality in selenium treatments. It appears that selenium-enriched yolk does not affect early phase of embryonic development, but produces major impact during yolk sac utilization for growth.

We observed almost 100% bioassay mortalities in the high selenium treatment, with maternal selenium concentrations of 48 μg/g in liver, 24 μg/g in muscle, and 38 μg/g in fertilized eggs. The effects of low and medium selenium treatments were more variable:

the average survival was less than in the control, but this decrease was not statistically significant. However, larvae in medium selenium treatment exhibited edema and delay of yolk sac resorption. This sublethal effect was observed at selenium tissue concentrations ranged from 10 to 26 µg/g (muscle and liver, respectively).

Similar acute effect of tissue selenium on bluegill larvae at high concentrations was reported by NFCRC (Lemly, 1990). However, sublethal concentrations of tissue selenium were not elucidated. Lemly and Smith (1987) proposed a value 12 µg/g (dry weight) of whole body selenium residue as a threshold level for reproductive failure in centrarchids, which approximately corresponds to LOEC values in this study. Ogle and Knight (1989) did not observe reproductive failure in the fathead minnow at the concentrations of selenium 8 µg/g in female's muscle and 11 µg/g in the ovaries (dry weight). Schultz and Hermanutz (1990) reported distinct reproductive failure in the same species at ovarian concentrations of 6 µg/g (wet weight), which corresponds to 23-26 µg/g selenium on a dry weight basis. Literature data supports a sublethal selenium concentration of maternal tissues to be within the range of 10-20 µg/g (dry weight).

The negative effect of yolk selenium on offspring can be manifested differently by fish species with different size of eggs and different rates of embryonic development. For example, our data for channel catfish indicate a significantly lower LC_{50} value for broodstock liver and spawned eggs, compared with the bluegill. However, even at twice lower selenium concentration in the yolk of channel catfish, the total selenium burden of one egg in this species is approximately 20 times greater due to the dramatic difference in egg size. In addition, the lethal effect of yolk selenium is expressed in catfish mainly before hatching due to the prolonged period of embryonic development and greater utilization of yolk by the embryo. It suggests the LC_{50} for maternal tissue selenium may be species-specific, reflecting different reproductive strategies.

The pathological mechanism of selenium effect on embryos and larvae is not clear. Larval abnormalities in bluegill appeared to be a result of functional disorders, rather than developmental defects. The most characteristic symptom was systemic edema, and an enlarged yolk sac. The inferior growth of fry survived to metamorphosis in the high selenium treatment may reflect an inadequate nutrient supply during endogenous feeding.

Similar reproductive failures have been observed in birds. Olson (1986) induced embryonic defects in chicken by selenium injections. Heinz et al (1987) have shown that dietary selenomethionine at concentrations of 10 µg/g or higher impairs

hatching success in mallards. Olendorf et al (1986) and Hoffman et al (1988) observed selenium-induced embryonic deformities in aquatic birds at concentrations above 28 μg/g in the mother's liver and above 9 μg/g in the eggs.

Data of NFCRC (Lemly, 1990) and our study confirm dietary dose-dependent bioaccumulation of selenium in broodstock gonadal tissue. We examined the relationship between dietary selenium levels and average selenium residues of fertilized eggs in our and NFCRC studies (estimated from graphs of Lemly, 1990). Data fit the same linear regression, where dietary selenium concentrations from 6 to 15 μg/g correspond to 95% CL of LC_{50} in fertilized eggs. Thus, continuous exposure of bluegill females to dietary selenium levels above 6 ppm (dry weight) during vitellogenesis can impair reproduction. For comparison, normal dietary selenium concentrations for livestock and cultured fish do not exceed 1-2 ppm, and selenium concentrations about 3-4 are considered dangerous (NRC, 1983). Lemly (1990) reports field sampling of bluegill and food organisms from the San Joaquin River basin. In several selenium -polluted areas, concentrations of selenium in bluegill carcasses ranged from 3-8 μg/g, and in food (chironomid larvae, amphipods, detritus) 3-22 μg/g (dry weight).

E. RECOMMENDATIONS FOR FURTHER STUDIES AND A BIOMONITORING PROGRAM

This study, as well as others, have utilized large intervals between selenium treatment doses because the information on selenium bioaccumulation in maturing ovary and critical levels in the egg yolk was absent. As a result, NOEC and LOEC values within the range of sublethal effect can be further narrowed. For example, it may be useful to conduct experiments using dietary selenium ranging from 5 - 15 ppm with smaller intervals between the doses. The 30 day larval bioassay may be unnecessary. Sensitive endpoints may be achieved by examining proportions of yolk-sac larvae with edema and evaluating survival, larval defects and body length at two weeks after hatching. Histopathological examination of larvae may add significant resolution to these end points.

Bioaccumulation and critical concentrations of selenium in the ovary and resulting eggs may differ in different species of fish, depending on vitellogenesis, egg size, and rates of early development. Pilot studies with other key species affected by selenium may be necessary. The experimental findings should be validated by field sampling, taking into consideration the reproductive biology of species (seasonality and duration of vitellogenesis, gonadosomatic index, and spawning time). Some of these sampling programs have already been conducted in the San Joaquin River (Saiki and May, 1988).

To utilize tissue selenium concentrations in a biomonitoring program with bluegill, fish should be sampled at the end of gonadal growth or at the start of the spawning season. For bluegill stocks in San Joaquin River basin, optimal sampling time will be during March or April, or when water temperature reaches approximately 20-22°C (see James, 1946, for detailed description of bluegill gonadal cycle). The GSI higher than 8 % indicates completion of gonadal growth in bluegill female. Only adult mature fish will be important to sample. Fork length and body weight of mature females in our study were 17 cm (range 15-22 cm) and 123 g (range 74-256 g). Average wet weight of liver (whole organ), muscle and ovary samples were 1.1, 6.2, and 8.1 g, respectively. Liver may be a less convenient tissue to sample in bluegill, because of the small size of this organ. Muscle is easy to dissect, and this sample is adequate for several analyses.

F. ACKNOWLEDGMENTS

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H. LIST OF FIGURES

Figure 1. Photoperiod and temperature regimes in the bluegill rearing system. The studies were conducted from November, 1989 to August, 1990. Arrows indicate the days fish were sampled in the bioaccumulation study. The spawning period is indicated by a horizontal bar. Temperature data are means reported for all tanks in °C.

Figure 2. Bluegill eggs removed by catheterization. A) typical population of ovarian follicles at different stages of development (original magnification 6x). B) three stages of development; the top left is early vitellogenic follicle, top right is an overriped, atretic egg, the bottom two are the eggs completed vitellogenesis (original magnification 12x). C & D) follicles in the process of final ovarian maturation, exhibiting partial fusion of oil droplets and yolk platelets (original magnification 12x). E & F) eggs with coalesced oil globule (original magnification 25x).

Figure 3. Microphotographs of the representative larvae from the control and each selenium treatment at Days 1-4 posthatch. Only head and body regions are shown. The oil globule is located posterior to yolk sac. Note enlarged oil globules and yolk sacs in maternal selenium treatments 13.9 and 21.4 ppm on Days 3 and 4, and edema in treatment 21.4 ppm.

Figure 4. Cumulative daily mortalities in 16 (top) and 30-day (bottom) larval bioassays.

Figure 5a. Relationship between the egg and liver selenium concentrations in spawned bluegill females. Equation for log-transformed data: $Y= -0.036 + 0.921 * X$ ($R^2=0.897$)

5b. Relationship between the egg and muscle selenium concentrations in spawned bluegill females. Equation for log-transformed data: $Y= 0.242 + 0.938 * X$ ($R^2=0.898$)

Figure 6a. Relationship between the egg and ovary selenium concentrations in spawned bluegill females. Equation for log-transformed data: $Y= 0.101 + 0.989 * X$ ($R^2=0.916$)

6b. Relationships between selenium concentrations of maternal tissues and dietary concentrations. Equations are based on mean values reported in Table 20, and dietary selenium 1.33 (control), 5.52, 13.93, and 21.41 ppm (treatments). All regressions are significant ($P<0.05$)

$$Y \text{ (liver)} = 0.194 + 2.112 * X \quad (R^2=0.982)$$

$$Y\ (\text{eggs}) = -1.025 + 1.732 * X \quad (R^2=0.971)$$
$$Y\ (\text{ovary}) = -1.140 + 1.369 * X \quad (R^2=0.951)$$
$$Y\ (\text{muscle}) = -0.613 + 1.037 * X \quad (R^2=0.939)$$

I. APPENDICES

Appendix 1. Water quality parameters in the spawning system.

Means and ranges of total ammonia nitrogen values for the spawning tank system. All values are given as mg/L.

Dates	Mean	Range
11/6 - 11/27/89	0.46	0.43 - 0.49
11/28 - 12/18/89	0.41	0.38 - 0.45
12/19 - 1/8/90	0.65	0.54 - 0.73
1/9 - 1/29/90	0.56	0.44 - 0.68
1/30 - 2/19/90	0.62	0.58 - 0.69
2/20 - 3/12/90	0.63	0.49 - 0.72
3/13 - 4/2/90	0.62	0.58 - 0.70
4/3 - 4/20/90	0.63	0.43 - 0.71
4/21 - 5/11/90	0.45	0.40 - 0.55
5/12 - 6/1/90	0.48	0.42 - 0.54
6/2 - 6/22/90	0.40	0.35 - 0.45
6/23 - 7/13/90	0.41	0.35 - 0.46
7/14 - 8/6/90	0.38	0.35 - 0.42

Means and ranges of pH for the spawning tank system.

Dates	Mean	Range
11/6 - 11/27/89	8.3	8.2 - 8.6
11/28 - 12/18/89	8.2	8.1 - 8.4
12/19 - 1/8/90	8.4	8.2 - 8.6
1/9 - 1/29/90	8.5	8.2 - 8.7
1/30 - 2/19/90	8.3	8.1 - 8.5
2/20 - 3/12/90	8.4	8.2 - 8.6
3/13 - 4/2/90	8.3	8.2 - 8.4
4/3 - 4/20/90	8.3	8.2 - 8.6
4/21 - 5/11/90	8.5	8.3 - 8.8
5/12 - 6/1/90	8.2	8.1 - 8.5
6/2 - 6/22/90	8.4	8.3 - 8.5
6/23 - 7/13/90	8.5	8.4 - 8.7
7/14 - 8/6/90	8.7	8.5 - 9.2

Appendix 1. (continued)

Means and ranges of water temperatures for the spawning tank system. All values are given as ° C

Dates	Mean	Range
11/6 - 11/27/89	18.9	18.7 - 19.1
11/28 - 12/18/89	18.6	18.5 - 18.7
12/19 - 1/8/90	18.6	18.0 - 18.9
1/9 - 1/29/90	20.6	19.8 - 22.2
1/30 - 2/19/90	23.8	22.4 - 24.8
2/20 - 3/12/90	25.3	24.6 - 25.7
3/13 - 4/2/90	25.5	24.7 - 26.2
4/3 - 4/20/90	25.2	19.2 - 27.3
4/21 - 5/11/90	25.3	19.3 - 26.7
5/12 - 6/1/90	26.8	19.3 - 28.9
6/2 - 6/22/90	27.1	26.8 - 28.9
6/23 - 7/13/90	27.3	27.0 - 27.8
7/14 - 8/6/90	27.4	27.3 - 28.8

Means and ranges of dissolved oxygen for the spawning tank system. All values are given as mg/L.

Dates	Mean	Range
11/6 - 11/27/89	8.3	7.8 - 8.8
11/28 - 12/18/89	8.4	8.0 - 8.6
12/19 - 1/8/90	8.6	8.1 - 9.1
1/9 - 1/29/90	8.4	7.8 - 9.3
1/30 - 2/19/90	7.1	6.3 - 7.6
2/20 - 3/12/90	7.3	5.0 - 8.2
3/13 - 4/2/90	7.4	6.3 - 8.2
4/3 - 4/20/90	7.6	6.3 - 8.3
4/21 - 5/11/90	6.7	5.6 - 7.6
5/12 - 6/1/90	6.5	5.6 - 7.6
6/2 - 6/22/90	6.9	5.4 - 7.7
6/23 - 7/13/90	7.1	6.2 - 7.7
7/14 - 8/6/90	7.0	6.2 - 8.2

Appendix 2. Culture of Algae and Rotifers

Selenastrum capricornutum was raised in large batch cultures as a food source for rotifers. Glass water bottles (18.9 L) were used for the batch cultures. Heavy aeration kept the media mixed and the algal cells suspended. Fifteen liters of media (see table) were used in each bottle. Cool white fluorescent lights were placed above and next to the bottles. The remainder of the exposed surfaces in the batch culture area was covered in reflective metallic sheeting to maximize lighting efficiency. Temperatures ranged from 20 °C to 28 °C. The media was inoculated with *S. capricornutum* isolates (100 ml) that had been transferred under sterile conditions and reared in 500 ml Erlenmeyer flasks on a shaker table under continuous illumination (400 foot candles). Cultures were allowed to incubate for 5 to 7 days. After incubation, the algae was settled in large refrigerated vats. The resulting concentrated algae was used to feed the rotifers.

Brachionus sp. cysts (Florida Aqua Farms, Dade City, FL) were hatched in well water in 2 L aerated beakers. Six of these beakers were maintained as pure cultures and used to inoculate large batch cultures. The large cultures were grown in 18.9 L glass bottles containing 15 L of well water. Heavy aeration was used to keep the rotifers suspended in the water column. The rotifers were fed algae daily at a concentration of 100,000 to 300,000 cells/ml of rotifers. When the rotifer concentration reached 30 rotifers/ml, 5 L could be harvested per container per day. Five liters of well water plus the day's allotment of algae were added to replace the harvested rotifers. Approximately every 30 days the batch cultures were drained, the bottles washed and bleached, and new cultures started. This procedure was done to minimize contamination with fungi, ciliates and other zooplankton, and the accumulation of organic detritus and the buildup of algal growth on the sides of the containers.

Table. Algal media composition.

Ingredient	Amount used
Nusalts, Type 1 (Argent, Redmond, WA)*	3.75 g
Filtered Sea Water (0.45 micron)	1.5 L
Glass distilled water	13.5 L

*Guillard f2 medium

Appendix 3. Selenium Analyses Inter-Laboratory Validation
Inter-laboratory validation of selenium values for the same tissue samples, analyzed at UC Davis (fluorimetry) and the Department of Fish and Game (hydride generation atomic absorption). Values are ug/g dry weight.

Gonad Tissue		Liver Tissue	
DFG (Y)	UCD (X)	DFG (Y)	UCD (X)
1.9	2.1	4.3	1.0
28.0	26.1	25.0	26.9
31.0	45.8	41.0	34.0
10.0	15.0	11.0	10.7
2.8	2.3	3.0	1.8
17.0	20.4	32.0	45.2
39.0	38.4	16.0	13.8
2.9	2.5	12.0	12.9
2.6	2.3	18.0	18.6
6.5	8.0	14.0	2.1
30.0	32.6	2.8	4.5
6.5	8.6	30.0	28.7
25.0	26.3	16.0	14.9
5.4	5.5		
2.4	2.4		

Linear Correlations
Between The Two Measurments:

$r = 0.966$ $(P<0.05)$	$r = 0.912$ $(P<0.05)$
$Y = 0.603 + 0.848x$	$Y = 4.357 + 0.783x$

Figure 1. UC Davis and Fish & Game selenium analyses for gonad (top) and liver tissue (bottom). Data points are measured values and the regression line fits the equation from Appendix 4.

Appendix 4. Survival of bluegill larvae in three different beakers kept in one tank to Day 16 (before pooling). Asterisks denote heterogenous survival, deleted from the analysis of survival to Day 30. Progenies are coded by dose level (8,18,28) and female number.

		NUMBER ALIVE		SURVIVAL
Progenies	Beakers	Day 0	Day 16	(p)
CONTROLS:				
8-1	A	26	21	
	B	28	20	
	C	29	26	0.807
8-2	A	31	26	
	B	29	21	
	C	29	25	0.809
8-3	A	30	21	
	B	29	18	
	C	29	23	0.705
8-5	A	30	25	
	B	29	25	
	C	30	25	0.843
8-6	A	30	19	
	B	30	16	
	C	30	19	0.600
18-1	A	28	12	
	B	29	19	
	C	28	19	0.588
18-2	A	30	21	
	B	30	24	
	C	29	22	0.753
18-3	A	30	18	
	B	33	9	0.429 *
18-4	A	28	21	
	B	29	22	
	C	28	26	0.812

18-5	A	31	23	
	B	31	20	
	C	29	18	0.670

Appendix 4. (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	Day 16	SURVIVAL (p)
CONTROLS:				
18-6	A	30	25	
	B	29	21	
	C	30	19	0.730
28-2	A	30	10	
	B	30	25	
	C	30	25	0.667 *
28-3	A	25	19	
	B	22	14	
	C	23	17	0.714
28-5	A	25	20	
	B	27	22	
	C	27	25	0.848
28-7	A	31	19	
	B	28	27	
	C	28	26	0.828 *
28-8	A	30	20	
	B	29	22	
	C	29	18	0.682
Se TREATMENTS				
8-1	A	23	9	
	B	18	4	
	C	44	11	0.282
8-2	A	30	15	
	B	30	18	
	C	30	16	0.544
8-3	A	29	16	
	B	31	27	

	C	29	28	0.798 *
8-4	A	29	21	
	B	28	4	
	C	31	19	0.500 *

Appendix 4 (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	Day 16	SURVIVAL (p)
8-5	A	27	24	
	B	29	21	
	C	29	26	0.835
18-1	A	30	18	
	B	30	26	
	C	30	21	0.722
18-2	A	30	24	
	B	30	24	
	C	30	16	0.711 *
18-3	A	29	19	
	B	30	19	
	C	28	19	0.655
18-4	A	28	20	
	B	27	14	
	C	30	0	0.400 *
18-5	A	30	3	
	B	29	7	
	C	27	15	0.291 *
18-6	A	29	23	
	B	29	24	
	C	29	22	0.793
28-1	A	29	0	
	B	29	0	
	C	30	1	0.011
28-2	A	30	0	
	B	30	1	
	C	30	0	0.011
28-3	A	28	0	

	B	29	5	
	C	29	2	0.081
28-4	A	27	0	
	B	30	0	
	C	31	0	0.000

Appendix 4. (continued).

Progenies	Beakers	NUMBER ALIVE Day 0	SURVIVAL Day 16	(p)
28-5	A	30	0	
	B	30	0	
	C	30	0	0.000
28-6	A	29	0	
	B	29	0	
	C	28	0	0.000
28-7	A	30	1	
	B	30	2	
	C	28	3	0.068

Appendix 5. Pooled survival of bluegill larvae in bioassays to Days 16 and 30. Significantly different beakers were removed from Day 16 end-point.

PROGENIES	NUMBER ALIVE Day 0	Day 16	Day 30	SURVIVAL (p) Day 16	Day 30
CONTROL:					
8-1	83	67	67	0.807	0.807
8-2	89	72	72	0.809	0.809
8-3	88	62	57	0.705	0.648
8-5	89	75	72	0.843	0.809
8-6	90	54	52	0.600	0.578
18-1	85	50	50	0.588	0.588
18-2	89	67	67	0.753	0.753
18-4	85	69	68	0.812	0.800
18-5	91	61	61	0.670	0.670
18-6	89	65	56	0.730	0.629
28-2	60	50	1)	0.833	1)
28-3	70	50	50	0.714	0.714
28-5	79	67	60	0.848	0.759
28-7	56	53	1)	0.946	1)
28-8	88	60	59	0.682	0.670
SELENIUM TREATMENTS:					
8-1	85	24	21	0.282	0.247
8-2	90	49	48	0.544	0.533
8-3	60	55	1)	0.917	1)
8-4	60	40	1)	0.667	1)
8-6	85	71	66	0.835	0.776
18-1	90	65	61	0.722	0.678
18-2	60	48	1)	0.800	1)
18-3	87	57	53	0.655	0.609
18-4	55	34	1)	0.618	1)
18-5	59	10	1)	0.169	1)
18-6	87	69	56	0.793	0.644
28-1	88	1	1	0.011	0.011
28-2	90	1	1	0.011	0.011

28-3	86	7	7	0.081	0.081
28-4	88	0	0	0.000	0.000
28-5	90	0	0	0.000	0.000
28-6	86	0	0	0.000	0.000
28-7	88	6	6	0.068	0.068

1) due to different survival in three pseudoreplications (beakers) before pooling survived larvae in one tank, data for 30 day survival were discarded. The survival to 16 days in beakers is based on two beakers.

Appendix 6. Percent dry weight in female and male tissue analyzed for selenium content. Data are means and standard deviations.

	Bioaccumulation Study					
	Day 0	Day 29	Day 57	Day 85	Day 113	Day 141
FEMALE TISSUE						
Liver	30.5 ± 1.5	28.8 ± 2.3	29.8 ± 1.7	28.4 ± 1.7	30.1 ± 1.3	31.9 ± 1.1
Ovary	21.8 ± 1.1	19.9 ± 1.9	19.8 ± 1.3	21.0 ± 1.6	36.7 ± 0.7	40.4 ± 14.5
GSI(%)	1.06 ± .08	1.15 ± .16	1.20 ± .11	1.24 ± .13	7.86 ± 1.41	7.33 ± 1.35
MALE TISSUE						
Liver	31.4 ± 3.1	30.8 ± 3.5	30.1 ± 1.3	28.6 ± 1.9	30.1 ± 1.1	33.1 ± 1.2
Testis	21.3 ± 5.2	22.4 ± 6.8	15.5 ± 4.3	16.8 ± 2.9	18.0 ± 1.6	20.9 ± 1.9
GSI(%)	1.50 ± .35	0.33 ± .14	0.37 ± .12	0.48 ± .21	1.04 ± .35	1.20 ± .21

	Reproductive Study	
	Day 0	At Spawning
FEMALE TISSUE		
Muscle	-----	21.3 ± 2.7
Eggs (10-12 hours postfertilization)	-----	17.0 ± 1.8
Larvae (4 days	-----	17.6 ± 1.3

posthatch)

Liver	29.8 ± 1.5	26.1 ± 2.0
Ovary	36.2 ± 2.9	26.1 ± 4.8
GSI(%)	5.63 ± 2.79	6.35 ± 2.18
MALE TISSUE		
Liver	31.0 ± .87	30.1 ± 4.8
Testis	16.1 ± .34	15.2 ± .94
GSI(%)	1.16 ± .11	1.34 ± .31

Appendix 7. Latency (time from hormonal injection) to stripping, ovulation score and fertility at one hour postfertilization data for all control treatments.

ID	Latency (hrs)	Eggs Scored (n)	Fertility (%)	Ovulation Score (0/1)	Comments
08-1C	10.2	95	73.7	1	
08-2C	9.4	128	87.5	1	
08-3C	9.0	103	78.6	1	
08-4C	9.0	120	0.0	0	overripe eggs
08-5C	7.5	96	93.8	1	
08-6C	8.0	72	45.8	1	
18-1C	13.7	109	67.9	1	
18-2C	7.0	140	92.9	1	
18-3C	10.5	109	74.3	1	
18-4C	14.0	138	43.5	1	
18-5C	8.5	105	52.4	1	
18-6C	7.5	85	71.8	1	
18-7C	11.0	54	5.6	0	"dry ovulation"
28-1C	na	61	na	na	natural spawn, no bioassay
28-2C	na	130	na	na	injected & natural spawn
28-3C	na	143	na	na	natural spawn
28-4C	13.0	98	19.4	0	overripe eggs
28-5C	13.2	110	74.5	1	
28-6C	8.0	105	26.7	0	overripe eggs
28-7C	na	na	na	na	injected & natural spawn, siphoned out posthatch larvae for bioassay
28-8C	8.0	102	41.2	1	

na = not available

Appendix 7. (continued).

ID	Latency (hrs)	Eggs Scored (n)	Fertility (%)	Ovulation Score (0/1)	Comments
08-1	12.0	108	69.4	1	
08-2	10.0	112	70.5	1	
08-3	15.0	140	89.3	1	
08-4	10.4	132	68.2	1	
08-5	11.0	97	22.7	0	dry ovulation
08-6	10.0	90	77.8	1	
08-7	10.0	123	0.0	0	dry ovulation
18-1	14.0	122	68.9	1	
18-2	13.5	112	80.4	1	
18-3	10.0	121	58.7	1	
18-4	10.0	97	54.6	1	
18-5	8.0	126	95.2	1	
18-6	9.0	114	82.5	1	
18-7	na	na	na	0	no ovulation
28-1	10.0	101	80.2	1	
28-2	18.0	98	80.6	1	
28-3	14.4	100	81.0	1	
28-4	8.0	102	55.9	1	
28-5	13.0	118	76.3	1	
28-6	9.5	101	79.2	1	
28-7	9.0	67	73.1	1	

na = not available

Appendix 8. Percent abnormal (edema) out of ten larvae sampled each day.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
8-1C	0	0	0	0	0
8-2C	0	0	0	0	0
8-3C	0	0	0	0	0
8-5C	0	0	0	0	0
8-6C	0	0	0	0	0
18-1C	0	0	0	0	0
18-2C	0	0	0	0	0
18-3C	0	0	0	0	0
18-4C	0	0	0	0	0
18-5C	0	0	0	0	0
18-6C	0	0	0	0	0
28-5C	0	0	0	0	0
28-7C	0	0	0	0	0
28-8C	0	0	0	0	0
8-1	0	0	0	0	0
8-2	0	0	0	0	0
8-3	0	0	0	0	0
8-4	0	0	0	0	0
8-6	0	0	0	0	0
18-1	0	0	0	0	0
18-2	0	0	0	0	0
18-3	0	0	0	10	10
18-4	0	0	0	20	10
18-5	0	0	0	0	0
18-6	0	0	0	10	10
28-1	0	0	100	100	all dead

28-2	0	0	100	100	all dead
28-3	0	0	90	80	90% dead*
28-4	0	0	100	100	all dead
28-5	0	0	100	100	all dead
28-6	0	0	100	100	all dead
28-7	0	0	90	90	90% dead*

* 10% were normal swim-up stage.

Appendix 9. Dissolved oxygen data for the beakers in which the larvae were reared for five days and sampled daily for measurements of total length, oil and yolk areas. All values are given as mg/L.

Treatment	Posthatch	Day 1	Day 2	Day 3	Day 4	Day 5
8-1C	5.9	6.5	6.5	6.8	6.1	6.1
8-2C	6.1	5.9	6.5	5.9	5.9	6.4
8-3C	5.9	6.5	6.4	6.1	6.5	6.9
8-5C	7.0	6.9	7.2	7.0	7.4	7.1
8-6C	6.4	7.0	7.2	7.4	7.5	7.1
18-1C	5.9	6.5	6.1	5.9	6.5	7.1
18-2C	6.0	6.1	5.9	5.9	6.1	6.5
18-3C	5.9	6.1	5.8	6.4	7.0	6.9
18-4C	6.8	6.9	7.0	6.9	6.9	7.1
18-5C	6.8	7.1	7.1	6.8	7.0	7.0
18-6C	7.2	6.5	7.1	6.9	7.4	7.1
28-5C	6.1	5.9	6.6	7.0	6.2	5.9
28-7C	6.5	6.8	6.1	6.0	7.1	6.0
28-8C	7.6	6.9	7.1	6.9	7.0	7.1
8-1	6.5	5.8	6.2	6.2	5.8	6.9
8-2	5.9	6.7	7.0	6.6	6.5	6.9
8-3	6.8	5.9	6.5	6.1	6.5	7.1
8-4	6.8	6.1	5.8	6.9	6.5	6.1
8-6	5.9	6.5	7.1	6.5	6.9	7.0
18-1	6.9	6.5	6.9	6.2	6.0	6.9
18-2	6.5	5.9	5.9	6.4	7.1	7.0
18-3	5.9	6.1	5.8	6.1	5.9	6.5
18-4	5.9	6.1	6.4	7.1	6.9	6.5
18-5	7.1	7.4	6.9	7.2	7.1	7.1
18-6	7.4	6.8	7.1	7.2	7.5	7.0

28-1	5.9	6.1	6.4	6.4	7.0	7.1
28-2	6.0	6.2	5.9	6.4	6.1	6.9
28-3	6.1	6.5	6.4	5.9	6.4	6.1
28-4	5.9	6.5	5.9	6.7	6.9	7.0
28-5	7.2	6.9	7.1	7.0	6.8	7.0
28-6	6.9	7.4	7.1	6.9	7.4	7.2
28-7	6.9	7.2	7.1	7.1	6.8	7.5

Appendix 10. Weekly water quality parameters in the larval rearing system.

Daily Water Temperature (°C)

Dates	Mean	Range
4/11 - 5/2/90	25.5	24.8 - 25.9
5/3 - 5/10/90	25.3	24.3 - 26.2
5/11 - 5/22/90	25.5	24.6 - 26.6
5/23 - 6/7/90	25.5	24.5 - 26.5
6/8 - 6/20/90	25.7	24.8 - 26.7
6/21 -7/1/90	26.0	25.8 - 26.1
7/2 - 7/8/90	26.3	25.0 - 26.8
7/9 - 7/15/90	26.6	26.3 - 27.1
7/16 - 7/22/90	26.4	25.8 - 27.2
7/23 - 7/29/90	26.2	26.0 - 26.2
7/30 - 8/5/90	26.2	25.8 - 26.5
8/6 - 8/12/90	26.1	25.8 - 26.2
8/13 - 8/19/90	26.1	25.8 - 26.2
8/20 - 8/26/90	26.1	25.8 - 26.2
8/27 - 9/2/90	26.0	25.8 - 26.1
9/3 - 9/6/90	26.0	26.0 - 26.1

Appendix 10. (continued) Weekly water quality parameters in the larval rearing system.

Date	Conductivity (umos)	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)	Total Ammonia Nitrogen (mg/L)
4/11/90	470	8.0	9.2	273.6	239.4	0.51
4/18/90	590	8.0	9.1	273.6	290.7	0.50
4/25/90	640	7.7	8.9	290.7	290.7	0.62
5/2/90	600	6.9	8.8	na	342.0	0.63
5/10/90	720	7.4	8.4	na	342.0	0.55
5/11/90	800	7.8	8.8	273.6	393.3	0.62
5/12/90	650	7.7	8.6	256.5	256.5	0.54
6/6/90	720	7.4	9.0	290.7	342.0	0.45
6/20/90	800	7.8	9.1	na	393.3	0.46
6/27/90	650	7.7	9.0	256.5	256.5	0.47
7/4/90	720	7.7	9.0	273.6	307.8	0.29
7/11/90	650	7.7	9.0	222.3	290.7	0.33
7/18/90	750	7.7	8.9	239.4	273.6	0.38
7/24/90	700	8.5	8.9	239.4	273.6	0.45
7/31/90	750	8.6	8.8	239.4	239.4	0.50
8/7/90	800	8.6	8.9	239.4	239.4	0.44
8/15/90	550	8.5	8.6	239.4	256.5	0.50
8/24/90	550	8.5	8.8	239.4	273.6	0.43
8/31/90	800	8.4	8.8	239.4	273.6	0.40
9/6/90	780	8.4	8.7	239.4	273.6	0.38