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# A Teratogenic Deformity Index for Evaluating Impacts of Selenium on Fish Populations

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**This paper describes a method for using teratogenic deformities in fish as the basis for evaluating impacts of selenium contamination. Teratogenic deformities are reliable bioindicators of selenium toxicosis in fish. They are produced in response to dietary exposure of parent fish and subsequent deposition of selenium in eggs. There is a close parallel between selenium concentrations in eggs, incidence of teratogenic deformities in larvae, and magnitude of reproductive failure. Using these relationships, an index was developed for teratogenic-based assessment of impacts to fish populations. The index is composed of three ratings that signify increasing levels of terata-induced population mortality: 1, negligible impact (<5% population mortality); 2, slight to moderate impact (5–20% population mortality); 3, major impact (>20% population mortality). Each rating is based on the anticipated population-level impact of the corresponding degree of mortality. Teratogenic-based impact assessment provides a conclusive cause–effect linkage between the contaminant and the fish. It is particularly useful for verifying selenium-induced impacts on reproductive success because poor reproduction can be caused by many things—i.e., fluctuating water levels, nest predation, food shortages, poor recruitment, etc. The index given here should be a useful tool for evaluating the effect of selenium on fish populations. Moreover, application of this technique may save considerable time and money by identifying the most efficient use of manpower and funds early in the assessment process.** 

## INTRODUCTION

Selenium presents an interesting paradox in the field of aquatic toxicology because it is both a nutrient and a poison. As a nutrient, it is required in the diet of fish at concentrations of about 0.1–0.5 $\mu$g/g dry weight (Hodson and Hilton, 1993; Gatlin and Wilson, 1984; hereafter, all dietary and tissue concentrations are reported on a dry weight basis). It is necessary for proper formation and functioning of glutathione peroxidase, which is a major cellular antioxidant enzyme (Heisinger and Dawson, 1983; Bell *et al.,* 1986). This enzyme protects cell membranes from damage or lysis due to lipid peroxidation. Without adequate selenium, normal cellular and organ metabolism break down because of peroxides produced as a by-product of digestion. Symptoms of selenium deficiency in fish include reduced growth, anemia, exudative diathesis, muscular dystrophy, and increased mortality (Poston *et al.,* 1976; Bell and Cowey, 1985; Bell *et al.,* 1985; Gatlin *et al.,* 1986). Thus, the beneficial effects of proper selenium in the diet of fish are firmly established.

At dietary concentrations of only 7–30 times those required (i.e., >3 $\mu$g/g), selenium becomes a poison. Some of the major toxic effects are due to a simple principle of cell biology. From a biochemical perspective selenium is very similar to sulfur, and cells do not discriminate well between the two when carrying out one of their key functions—protein synthesis. When present in excessive amounts, selenium is erroneously substituted for sulfur in proteins that are being formed inside the cells. Sulfur-to-sulfur linkages (ionic disulfide bonds) are necessary in order for protein molecules to coil into their tertiary (helix) structure which, in turn, is necessary for proper functioning of the protein, either as a cellular building block or as a component of enzymes. Substitution of selenium for sulfur disrupts the normal chemical bonding, resulting in improperly formed and dysfunctional proteins or enzymes (Diplock and Hoekstra, 1976; Reddy and Massaro, 1983; Sunde, 1984).

Thresholds for dietary selenium toxicity in fish are easily reached and exceeded in contaminated aquatic systems. For example, selenium released in wastewater from a coal-fired electric generating station contaminated Belews Lake, North Carolina, to the extent that fish were consuming 20–80 $\mu$g/g selenium (Cumbie and Van Horn, 1978; Lemly, 1985). Naturally occurring selenium leached from soils due to agricultural irrigation in California bioaccumulated in wetlands to concentrations of over 100 $\mu$g/g in fish food organisms (Lemly *et al.,* 1993; Lemly, 1994). Both of these sites experienced massive poisoning of fish and wildlife (Lemly, 1997a). These events emphasize how severe the environmental impacts of excessive selenium can be. Moreover, in a field setting fish accumulate some selenium from water through their gills, which increases the risk that concentrations in tissues may reach toxic levels.

Excessive selenium can cause a wide variety of toxic effects at the biochemical, cellular, organ, and system levels (So-



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rensen, 1986). This paper examines the most prominent outward manifestation of selenium toxicosis—teratogenic deformities—and presents an index for using this pathological symptom as a diagnostic tool to assess impacts on fish populations in contaminated aquatic habitats.

## OCCURRENCE AND PERSISTENCE OF TERATOGENIC EFFECTS

Teratogenic deformities in fish are a permanent pathological marker of selenium poisoning. They are congenital malformations that occur due to excessive selenium in eggs. The process begins with the diet of parent fish. Excess dietary selenium (>3 $\mu$g/g) causes elevated concentrations of selenium to be deposited in developing eggs, particularly the yolk. When eggs hatch, larval fish rapidly utilize the selenium-contaminated yolk, both as an energy supply and as a source of protein for building new body tissues. Hard and soft tissues may be deformed if the molecular structure of the protein building blocks has been distorted due to substitution of selenium for sulfur. Some tissues may not be generated at all, resulting in missing body parts.

The prevalence of teratogenic deformities increases rapidly once selenium concentrations in eggs exceeds 10 $\mu$g/g. Hatchability of eggs is not affected by elevated selenium even though there may be a high incidence of deformities in resultant larvae and fry, and many may fail to survive (Gillespie and Baumann, 1986; Coyle *et al.,* 1993). The time for induction of teratogenesis is when larval fish are relying on their attached yolk sac for nourishment and development. Once external feeding begins, the potential for teratogenic effects declines and is soon lost. Feeding excessive selenium (up to lethal levels) to fry or juvenile fish as they are growing will not cause teratogenic malformations to occur (Hamilton *et al.,* 1990; Cleveland *et al.,* 1993). Moreover, dietary selenium levels sufficient to load eggs beyond teratogenic thresholds (diet: 5–20 $\mu$g/g) do not cause teratogenesis in, or otherwise generally affect the health or survival of, parent fish (Coyle *et al.,* 1993). Thus, the teratogenic process is strictly an egg–larvae phenomenon. Because of these relationships, teratogenesis can be a very subtle, but important, cause of reproductive failure in fish. Entire populations may disappear with little evidence of ''toxicity'' since major impacts to early life stages can be taking place at the same time that adult fish appear healthy (Cumbie and Van Horn, 1978; Lemly, 1985).

Mortality of larval fish can be high if the teratogenic defects are severe enough to impair critical body functions (Woock *et al.,* 1987). However, in some cases the abnormalities may not be life threatening and the malformations can persist into juvenile and adult life stages (Lemly, 1993). This is likely restricted to locations where there is little threat from predators since all but the most subtle deformities would probably compromise a fish's ability to feed and avoid predators. Thus, in assessing the prevalence of teratogenic defects, it is important to focus on the earliest life stages, i.e., newly emerging larvae and young fry.

## SYMPTOMS OF TERATOGENESIS

Teratogenic deformities can occur in most, if not all, hard or soft tissues of the body. However, some of the most conspicuous (consequently, the most diagnostic) are found in the skeleton, fins, head, and mouth. These typically involve (1) lordosis—concave curvature of the lumbar region of the spine; (2) scoliosis—lateral curvature of the spine; (3) kyphosis—convex curvature of the thoracic region of the spine resulting in a ''humpback'' condition; (4) missing or deformed fins; (5) missing or deformed gills or gill covers (opercle); (6) abnormally shaped head; (7) missing or deformed eyes; and (8) deformed mouth. Several of these symptoms are presented in Figs. 1–3.

In general, a careful fish-in-hand inspection is sufficient to diagnose any of the major teratogenic deformities. However, careful examination with the aid of a dissection microscope is needed to make the diagnosis for larvae and fry, small species (e.g., small cyprinids, poeciliids, etc.), or in situations when it is necessary to tabulate all of the subtle, less overt symptoms (e.g., slightly deformed fins, opercles, etc.). This is particularly true for larval fish. Some of their undeveloped features could erroneously be considered a defect when, in fact, they are a consequence of a premature life stage, not selenium teratogenesis. However, this is not a serious concern because larval fish have distinctive patterns of development that quickly become apparent to the investigator looking for teratogenesis. With a

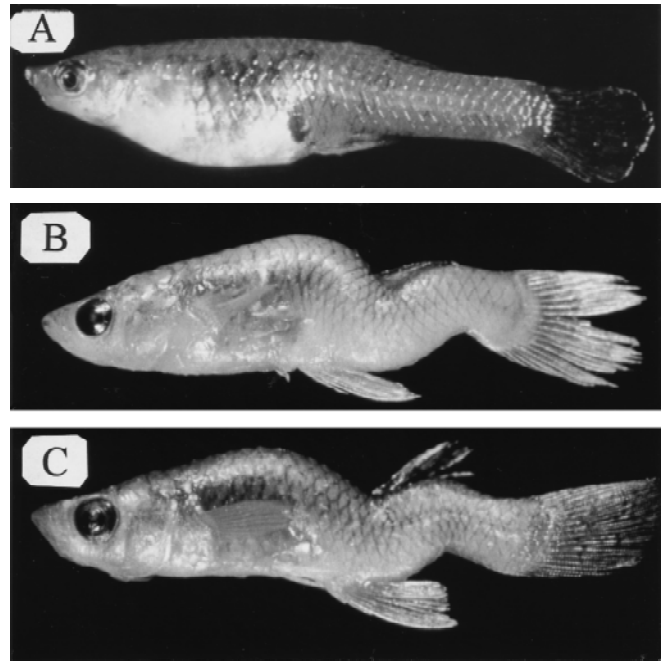


**FIG. 1.** Normal (A) and teratogenic (B, C) adult mosquitofish (*Gambusia affinis*) exhibiting dorsoventral deformation (kyphosis and lordosis) of the spine.


**FIG. 2.** Teratogenic (A) and normal (B) juvenile red shiners (*Notropis lutrensis*). The teratogenic effect is scoliosis, or lateral curvature of the spine.

bit of hands-on experience, the true teratogenic defects are easily distinguishable, even in young fish.

There are some other symptoms of selenium poisoning that may be confused with teratogenic effects. These are generally thought to represent acute toxic responses to high doses or tissue concentrations of selenium—they are not true teratogenic effects. The most common of these symptoms are (1) edema—swollen and distended abdomen due to accumulation of fluid in the visceral cavity; (2) exopthalmus (bulging or protruding eyes) due to accumulation of fluid in the eye sockets; and (3) cataracts, which appear as a white coating on the eyes. All of these symptoms may be present concurrently, along with the true teratogenic effects. Particular care must be exercised when examining larval fish. Edematous larvae with distended abdomens are a common occurrence (Bryson *et al.*, 1984; Gillespie and Baumann, 1986; Pyron and Beitinger, 1989). This condition may progress to, or be associated with, the expression of terata but the edema itself does not constitute a teratogenic defect. However, severe edema is usually accompanied by deformity of the spine (most often lordosis) or soft tissues in the abdomen (Fig. 4). The prevalence of edema and terata can be virtually the same (Schultz and Hermanutz, 1990), or quite different (Hermanutz *et al.*, 1992). Thus, one should not assume a 1:1 relationship. Reasonable caution—i.e., close inspection and comparison with normal larvae—will prevent inaccurate diagnoses.

In order to draw a conclusion of selenium-induced teratogenesis, the visual indicators and symptoms (deformities) must be corroborated with the presence of elevated concentrations of selenium in tissues. Concentrations in the range of 10–20 $\mu$g/g or greater (whole-body homogenate) would be sufficient to confirm the diagnosis. This corresponds to concentrations of about 6–12 $\mu$g/g in muscle (fillets), or 20–40 $\mu$g/g in visceral tissues, including the liver. Although measurement of tissue concentrations is essential, it is not necessary to conduct extensive surveys on hundreds of fish. Analysis of six samples per fish species (e.g., six adults or juveniles with teratogenic deformities or six composites for teratogenic larvae/fry) is sufficient.

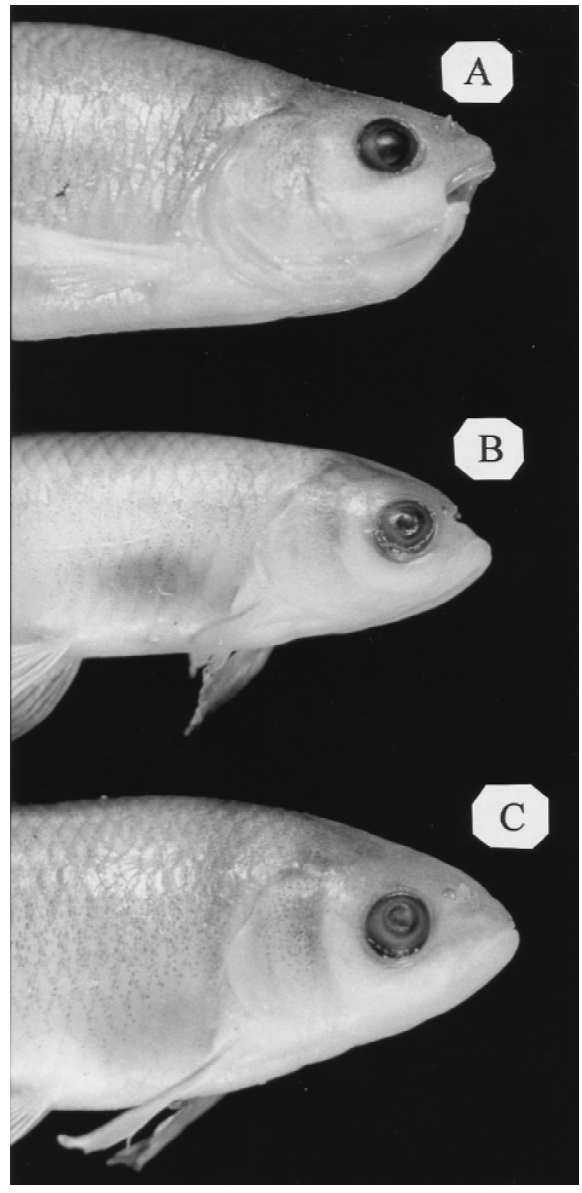


**FIG. 3.** Teratogenic (A, B) and normal (C) juvenile red shiners (*N. lutrensis*). Teratogenic effects presented include deformity of the mouth and lower jaw (A), upper portion of the head (B), and pectoral fins (B).

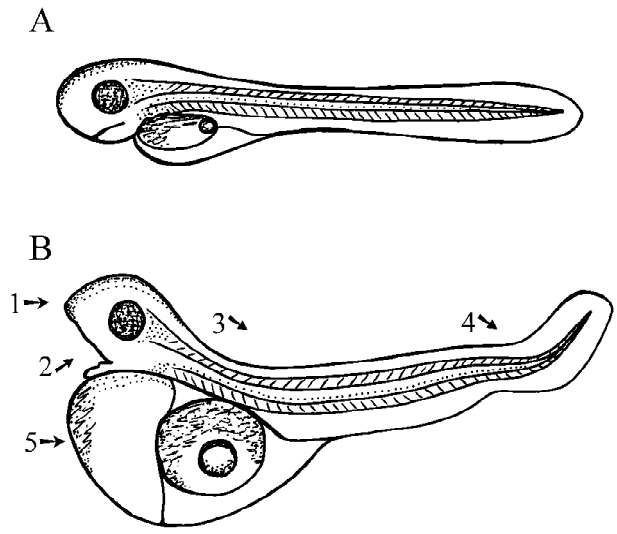


**FIG. 4.** Typical appearance of larval fish at about 2–4 days after hatching. (A) Normal larvae exhibiting yolk absorption nearing completion and straight, developing spine. (B) Abnormal development due to selenium-induced terata: (1) deformed, pointed head; (2) deformed, gaping lower jaw; (3) kyphosis (curvature of the thoracic region of the spine); (4) lordosis (concave curvature of the lumbar and/or caudal regions of the spine). Other symptoms of selenium poisoning that usually accompany terata include (5) edema (swollen, fluid-filled abdomen) and delayed yolk absorption.

## IMPLICATIONS FOR ASSESSING IMPACTS TO FISH POPULATIONS

Resource managers dealing with aquatic systems known or suspected of being contaminated with selenium usually face a dilemma over how to proceed with hazard assessment. A typical course of action is to initiate a monitoring program for measuring selenium in water, sediments, and biota. Such efforts will reveal the level and extent of contamination but the information gained will also raise two very important questions: (1) what do the concentrations of selenium mean with regard to potential biological impacts, and (2) is there any evidence of effects at the site? Unless these questions are answered the resource manager cannot accurately assess risks or develop a prudent plan for reducing or mitigating hazards.

The utility of using teratogenic deformities as a tool for assessing impacts to fish populations becomes apparent when one considers the direct linkage with mortality and reproductive impairment. Concentrations of selenium in tissues can be used to suggest a cause–effect linkage but there must be other evidence to confirm that actual toxic impacts have occurred. Teratogenesis is a direct expression of selenium toxicity and it is a clear marker of cause–effect. It can be used to evaluate and predict impacts to fish populations without having to expend large amounts of time and money on contaminant monitoring which, in itself, leaves important questions unanswered.

## AN INDEX FOR TERATA-BASED ASSESSMENT

A considerable amount of data are available for assessing or predicting the impact of selenium-induced teratogenesis on fish populations. Laboratory studies provide important information on the relationships between egg concentrations of selenium, prevalence of teratogenic deformities in larvae, and associated mortality. These studies are of three types: (1) those in which captive adult fish were fed selenium-laden diets or exposed to high-selenium water and then allowed to spawn in indoor tanks (Bryson *et al.*, 1984, 1985a,b; Woock *et al.*, 1987; Pyron and Beitinger, 1989); (2) those in which outdoor artificial streams were dosed with waterborne selenium, providing for exposure of adult fish to natural food-chain selenium prior to spawning (Schultz and Hermanutz, 1990; Hermanutz, 1992; Hermanutz *et al.*, 1992); and (3) those in which adult fish were taken from selenium-contaminated aquatic habitats and spawned artificially, i.e., eggs and milt were removed and mixed, and the resultant hatch was monitored (Gillespie and Baumann, 1986).

The field data come from Belews Lake, North Carolina. This lake was impounded in the early 1970s to serve as a cooling reservoir for a large coal-fired electric generating station (2250-MW generating capacity). Fly ash produced by the power plant was disposed in a settling basin, which released selenium-laden effluent containing 100–200 $\mu$g/liter (about 80% selenite) in return flows to the lake. This selenium bioaccumulated in aquatic food chains and within 2 years the fishery of Belews Lake began to decline because of reproductive failure. Of the 20 species of fish originally present in the reservoir, 16 were entirely eliminated (which included all of the primary sport fish), 2 were rendered effectively sterile but persisted as aging adults, 1 was eliminated but adults managed to recolonize to a limited extent from a relatively uncontaminated headwater area (but did not reproduce), and 1 was unaffected. This pattern of selenium contamination from the power plant and resultant poisoning of fish persisted from 1974 to 1985 (Lemly, 1985). In late 1985, under mandates from the State of North Carolina, the power company changed operations for fly ash disposal and selenium-laden effluent no longer entered the lake. Since that time, selenium levels have fallen and the fishery has begun to recover but sediments and associated aquatic food chains remain moderately contaminated and there are residual effects on the fishery, including persistent teratogenic deformities (Lemly, 1997b).

Teratogenic assessment was used to evaluate the reproductive success of fish and determine the degree of impact in Belews Lake in 1975, 1978, 1982, 1992, and 1996 (Lemly, 1993, 1997b). The 1975 survey was conducted during the period of initial selenium contamination of the reservoir, before the fishery experienced serious decline. This was the only survey made when the original assemblage of fish species was still present in the lake. This data set is quite informative because it documents levels of teratogenesis in a wide range of species that represent various feeding modes and trophic positions. Selenium concentrations in whole-body fish samples (juveniles and adults) were high (40–65 $\mu$g/g), as was the prevalence of teratogenic deformities (up to 55%). Teratogenesis was present in all of the 19 species examined. Surveys conducted in 1978

and 1982 yielded similar results although only 4 fish species remained in 1978, and 6 in 1982. Selenium concentrations were very high (up to 130 μg/g) and were closely paralleled by the occurrence and prevalence of teratogenic deformities, which ranged up to 70% (juvenile and adult fish). The survey in 1992 indicated that gradual recovery was taking place but there were still only 9 of the original 20 species present, and numerical abundance was quite low. Concentrations of selenium in fish had fallen to 11–20 μg/g and the incidence of teratogenesis did not exceed 11% (juveniles and adults). Fish were successfully reproducing and it was soon possible to collect larval fish for examination. Further recovery of the fishery was evident in 1996. All of the major sport fish had reestablished and were successfully reproducing. Tissue concentrations of selenium had fallen to 5–10 μg/g and teratogenic deformities were 6% or lower (larvae, fry, juveniles, and adults).

Relationships between the amount of selenium in fish tissues, prevalence of teratogenesis, and associated mortality are presented in Figs. 5 and 6. These figures represent a compilation of all field and laboratory data on teratogenic effects (e.g., field studies such as Lemly, 1993, and laboratory studies such as Woock *et al.*, 1987). The prevalence of teratogenic deformities is dependent on tissue concentrations of selenium—more selenium results in more frequent terata. However, the association follows an exponential function rather than a linear relationship. In natural populations of juveniles and adult centrarchids (i.e., not laboratory studies) the inflection point for the function occurs in the range 40–50 μg/g. At these concentrations, about one-fourth of the fish exhibit teratogenesis (Fig. 5). Beyond the inflection point, relatively small increases in selenium cause substantial increases in terata. The maximum observed frequency is 70% for individuals with body burdens of selenium in the range 70–90 μg/g. The exponential function

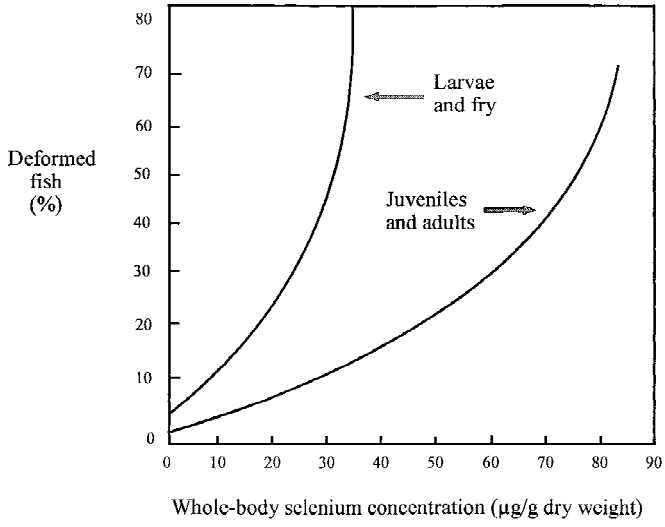


**FIG. 5.** Relationship between whole-body concentrations of selenium and prevalence of teratogenic deformities in fish.

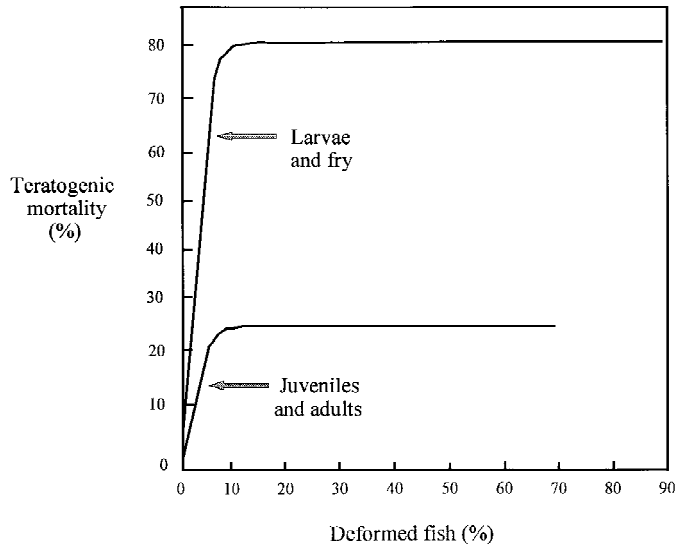


**FIG. 6.** Relationship between prevalence of deformities and incidence of teratogenic mortality in fish.

holds for larval centrarchids as well but the selenium concentrations for the inflection point and maximum are much lower. For example, up to 80% deformities result at tissue concentrations of only about 30–40 μg/g.

The relationship between teratogenesis and mortality is of primary importance in developing an assessment index. Whereas the prevalence of terata is influenced by tissue concentrations of selenium, the degree of mortality from terata is not, i.e., about 80% of teratogenic larval fish die regardless of their body burden of selenium (Fig. 6). This suggests that there is a maximum body burden for generation of lethal terata. Saturation beyond this maximum by additional selenium has little impact. Mortality is nearly constant for juvenile and adult fish, as well, but the magnitude is not nearly as great as that for larvae—only about 25% of teratogenic juvenile and adult fish die. This is probably a reflection of simple mathematics and, to some extent, the severity of terata—i.e., the 20% or so of teratogenic larvae that survive will make up the teratogenic juvenile/adult population. Although terata persist, they may no longer be as life threatening as those in younger fish.

The difference in mortality between life stages indicates that larval fish should be the priority for assessing or predicting population-level impacts of selenium because it is more likely that teratogenic mortality will be expressed in this life stage. Moreover, persistence of deformities into the juvenile and adult life stages may only occur in special circumstances where natural predation has been sharply reduced or eliminated (Lemly, 1993). Ideally, all life stages should be examined, with the focus placed on larval fish.

Both the laboratory and the field data indicate a close parallel between selenium concentrations, incidence of teratogenic deformities, and magnitude of reproductive failure in fish. Using these relationships, an index was developed for teratogen-

ic-based assessment of impacts to fish populations (Table 1). This index is composed of three ratings that signify increasing levels of terata-induced population mortality: 1, negligible impact (<5% population mortality); 2, slight to moderate impact (5–20% population mortality); 3, major impact (>20% population mortality). Each rating is based on the anticipated population-level impact of the corresponding degree of mortality, i.e., little effect is expected with <5% mortality but substantial effects may occur with >20% mortality. Population mortality is calculated in four simple steps: (1) determine the percentage of teratogenic fish and the percentage of normal fish in the total sample, (2) multiply the percentage of teratogenic fish by the expected mortality rate (80% for larvae, 25% for juveniles and adults) to estimate the percentage of fish that will survive, (3) add the percentage of normal fish and the percentage of surviving teratogenic fish, (4) subtract this sum from 100%—the result is population mortality, which will be less than teratogenic mortality. For example, 20% teratogenic larvae with 80% mortality translates to 16% population mortality; 20% teratogenic juveniles/adults with 25% mortality translates to 5% population mortality. Because of the differences in teratogenic mortality between larval and juvenile/adult fish, age-specific indices were developed. As discussed previously, persistence of terata in older life stages (and thus accurate evaluation) can be heavily influenced by predation; thus, the index for juveniles and adults may have limited application.

The terata–mortality relationships are based on data for two fish families—Centrarchidae (bass, sunfish) and Cyprinidae (minnows). The resultant index for impacts may or may not be directly applicable to cold- or cool-water families such as Salmonidae (e.g., trout, salmon) or Esocidae (e.g., pike, muskellunge). However, extrapolation to other families of fish is probably not necessary since centrarchids and cyprinids have characteristics that make them a good indicator or sentinel for other species, i.e., they are sensitive to selenium, include nationally important sport fish species, and occupy most of the aquatic habitats in the continental United States (Lee *et al.*, 1980; Lemly, 1993).

**TABLE 1**
**Index for Evaluating the Impact of Selenium-Induced Teratogenic Mortality on Fish Populations**

| Fish life stage | % With terata | % Population mortality[a] | Index rating | Anticipated impact |
|---|---|---|---|---|
| Larvae or fry | <6 | <5 | 1 | Negligible |
| | 6–25 | 5–20 | 2 | Slight to moderate |
| | >25 | >20 | 3 | Major |
| Juveniles or adults | <20 | <5 | 1 | Negligible |
| | 20–80 | 5–20 | 2 | Slight to moderate |
| | >80 | >20 | 3 | Major |

[a] Mortality is expressed as a percentage of the total fish population, not teratogenic mortality. For example, 20% larvae with terata translates to 16% population mortality because up to 20% of those with terata would be expected to survive to adulthood (e.g., only about 80% of teratogenic larvae die).

The index can be applied to virtually any aquatic habitat because it consists of impact-based assessment. Impacts (terata) are a function of selenium concentrations in fish eggs. Conditions responsible for getting selenium into fish eggs—bioaccumulation in aquatic food chains and consumption of contaminated diets by parent fish—can be highly variable from location to location and are influenced by such things as hydrology and landform (amount and timing of precipitation; stream, lake, or wetland), chemical form of selenium (selenate, selenite, organoselenium), and timing and amount of selenium inputs relative to spawning periods (Lemly and Smith, 1987). Consequently, the potential hazard (likelihood of toxic impacts) of selenium to fish and wildlife is also highly variable (Lemly, 1995, 1996). However, the index is based on a measure of existing impact (terata), not potential hazard. As such, terata are an expression of the sum total of parental exposure, regardless of the temporal, spatial, or chemical variations that may exist from site to site. Thus, the applicability of the index is not influenced by local environmental conditions that affect selenium dynamics and biological uptake. It makes no difference whether the system is a fast-flowing stream in which selenate predominates and bioaccumulation is low or a terminal wetland experiencing high bioaccumulation from selenite—population-level impacts are indicated only if a sufficient amount of terata exist.

## EXAMPLE ASSESSMENTS

(1) Collect and examine 500 larval fish using ichthyoplankton sampling techniques; assess the prevalence of teratogenic deformities and measure selenium concentrations in six composite samples of teratogenic individuals. The investigation reveals that 15% have terata and the associated selenium concentrations are 10–15 $\mu$g/g. The expected population-level mortality is 12% (85% normal + 3% surviving teratogenic = 88% total survival), resulting in an index rating of 2. Conclusion—slight to moderate impact on the population due to teratogenic effects of selenium.

(2) Collect and examine 300 juvenile and 200 adult fish. Assess the prevalence of teratogenic deformities and measure selenium concentrations in individuals with terata (whole-body; 6 juveniles and 6 adults). The investigation reveals that 8% have terata and the associated selenium concentrations are 20–30 $\mu$g/g. The expected population-level mortality is 2% (92% normal + 6% surviving teratogenic = 98% total survival), resulting in an index rating of 1. Conclusion—negligible impact on the population due to teratogenic effects of selenium.

(3) Sample 1000 larval, 200 juvenile, and 100 adult fish. Determine the prevalence of teratogenic deformities and measure selenium concentrations in teratogenic individuals (6 composite samples for larvae, 6 individuals for juveniles and adults). The investigation reveals that 35% of larvae have terata and 3% of juveniles and adults have terata; selenium

concentrations are 10–30 $\mu$g/g. The expected population-level mortality is 28% for larvae (65% normal + 7% surviving teratogenic = 72% total survival) and 0.6% for juveniles and adults (97% normal + 2.4% surviving teratogenic = 99.4% total survival). Resulting index ratings are 3 for larvae and 1 for juveniles/adults. Conclusion—major impact on the population due to teratogenic effects of selenium on larvae.

## CONCLUSIONS

Teratogenic deformities are reliable bioindicators of selenium toxicosis in fish. They are produced in response to dietary exposure of parent fish and subsequent deposition of selenium in eggs. Toxicity of waterborne and dietary selenium to adult fish is variable, with organic forms such as selenomethionine being most readily bioaccumulated and toxic, followed by selenite and selenate. However, once ingested, selenium is biochemically processed and incorporated into egg proteins primarily as seleno–amino acids, e.g., selenomethionine, selenocystine, etc. If concentrations are sufficiently high, deformed embryos develop due to dysfunctional proteins and enzymes. Thus, terata are produced by a rather uniform process regardless of what chemical form(s) of selenium the parent fish was exposed to. Consequently, the sensitivity and applicability of the assessment index are unaffected by variations in the environmental mixture of selenium species.

Teratogenic-based impact assessment provides a conclusive cause–effect linkage between the contaminant and the fish. It is particularly useful for verifying selenium-induced impacts on reproductive success because poor reproduction can be caused by many things—i.e., fluctuating water levels, nest predation, food shortages, poor recruitment, etc. The index given here should be a useful tool for evaluating the effect of selenium on fish populations. Moreover, application of this technique may save considerable time and money by identifying the most efficient use of manpower and funds early in the assessment process.

## ACKNOWLEDGMENT

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