6 Selenium Toxicity to Aquatic Organisms

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6.1 INTRODUCTION

This chapter addresses the characteristics and nature of organic Selenium (Se) toxicity to aquatic organisms, based on the most current state of scientific knowledge. As such, the information contained in this chapter relates to the toxicity assessment phase of aquatic ecological risk assessments. While the inorganic forms of Se (e.g., selenate, selenite) can be toxic at concentrations in the 10^2 µg/L range via waterborne exposures, dietary exposure to mg/kg concentrations of organic Se poses a greater hazard to certain classes of aquatic biota, such as fish and birds (Skorupa and Ohlendorf 1991; USEPA 1998).

In terms of organic Se toxicity to aquatic biota, this chapter specifically addresses

- mechanisms of organic Se toxicity;
- most relevant/indicative toxicity endpoints;
- comparative sensitivity of organic Se to various aquatic species (and factors influencing this relative sensitivity);
- factors that modify organic Se toxicity;
- linkages between organic Se toxicity at the suborganismal and organismal level to population-level impacts;

- considerations and recommendations for the design and conduct of site-specific effects studies;
- uncertainties associated with Se toxicity; and
- future research needs.

6.2 MECHANISMS OF ACTION

6.2.1 Selenium Essentiality

Selenium was first recognized as an essential element in 1957 (Mayland 1994) and is a key component of a variety of functional selenoproteins in all living organisms, except for higher plants and yeasts (Hesketh 2008). Selenium-containing proteins fall into 3 categories: 1) proteins into which Se is incorporated nonspecifically (mainly as selenomethionine), 2) specific Se-binding proteins, and 3) enzymes that incorporate selenocysteine (the 21st amino acid) into their active site (Patching and Gardiner 1999; Behne and Kyriakopolis 2001; Reilly 2006; Hesketh 2008). Currently characterized selenoproteins catalyze oxidation–reduction reactions (glutathione peroxidases and thioredoxin reductases), activate, or inactivate thyroid hormone (iodothyronine deiodinases), mediate the synthesis of selenocysteine (selenophosphate synthetase), or are involved in Se transport (selenoprotein P) (Behne et al. 2000; Reilly 2006). Selenium is also required for thioredoxin reductase activity, which is involved in DNA synthesis, oxidative stress defense, and protein repair (Arner and Holmgren 2000). In addition, there are at least 20 other selenoproteins identified in vertebrates whose functions remain unclear (Hesketh 2008).

Despite being an essential trace element at dietary concentrations of 0.1 to 0.5 mg Se/kg dry weight (dw) (Mayland 1994), a significant aspect of the toxicological hazard associated with Se is the narrow margin between essentiality and toxicity. In fish, Se toxicity has been reported to occur at dietary concentrations only 7 to 30 times greater than those considered essential for proper nutrition (i.e., > 3 mg Se/kg dw) (Hilton et al. 1980; Hodson and Hilton 1983). In poultry, dietary Se concentrations of less than 0.3 mg/kg dw are considered below the range adequate for good adult health and reproduction, 3 to 5 mg/kg dw are considered high, and above 5 mg/kg dw are considered toxic. In eggs, the tipping point between essentiality and toxicity shifts upward such that Se concentrations lower than 1 mg/kg dw in eggs may indicate inadequate Se in the maternal diet (Puls 1988; Table 6.1). Additional information on physiological requirements is provided in Chapter 5 (Section 5.2.1).

In addition, although Se has important roles in antioxidant defenses at normal dietary levels, at elevated exposure levels it can become involved in the generation of reactive oxygen species, resulting in oxidative stress with increasing exposure. As discussed below, oxidative stress is a key mechanism of toxicity in vertebrate animals.

6.2.2 Selenium Toxicity

Although uncertainties remain, there is a large and growing body of knowledge regarding the toxicity of Se to aquatic biota. Oviparous (egg-laying) vertebrates appear to be the most sensitive taxa, and this section focuses on mechanisms of Se toxicity in these animals. The sequence of mechanistic events involved in Se toxicity

TABLE 6.1
Data Illustrating the Range of Assessment Values for Effects of Egg Se Concentrations in Birds

Status[a]	Concentration (mg Se/kg, dw)	Effects	Comments	References
Adequate	0.66–5.0 (0.20–1.5 ww)	Nutritional needs are met for poultry	Lower dietary concentrations are marginal or deficient, and diets must be fortified	Puls 1988
High	5.0–16 (1.5–5.0 ww)	Levels are excessive and upper end of range may be toxic to poultry	Poultry are relatively sensitive to effects of Se	Puls 1988
Toxic	>8.2 (>2.5 ww)	Reduced egg hatchability and teratogenic effects in embryos/chicks	Poultry are relatively sensitive to effects of Se	Puls 1988
Background	Mean < 3.0 (typically 1.5–2.5); individual eggs <5	None	Concentrations may be higher in some marine birds (Section 6.5.4)	Ohlendorf and Harrison 1986; Skorupa and Ohlendorf 1991; USDOI 1998; Eisler 2000
Reproductive impairment	7.7 (about 2.3 ww)	EC10 for reduced egg hatchability	Based on results of one laboratory study with mallards, assuming hormetic effects	Beckon et al. 2008
Reproductive impairment	9.0	EC8.2 for impaired egg hatchability	Based on results of one laboratory study with mallards, using linear regression analysis	Lam et al. 2005
Reproductive impairment	12 (95% CI = 6.4–16)	EC10 for reduced egg hatchability	Based on results of six laboratory studies with mallards, using logistic regression analysis	Ohlendorf 2003
Reproductive impairment	12 (95% CI = 9.7–14)	EC10 for reduced egg hatchability	Based on results of six laboratory studies with mallards, using hockey stick analysis	Adams (pers. comm.; see Ohlendorf 2007)
Reproductive impairment	14	EC11.8 for reduced clutch viability	Based on results of extensive field studies of black-necked stilts	Lam et al. 2005
Teratogenicity	13–24	Threshold for teratogenic effects on population level	Sensitivity varies widely by species	Skorupa and Ohlendorf 1991

TABLE 6.1 (CONTINUED)
Data Illustrating the Range of Assessment Values for Effects of Egg Se Concentrations in Birds

Status[a]	Concentration (mg Se/kg, dw)	Effects	Comments	References
Teratogenicity	23	EC10 for teratogenic effects in mallard	Mallard is considered a "sensitive" species	Skorupa 1998b; USDOI 1998
Teratogenicity	37	EC10 for teratogenic effects in stilt	Stilt is considered an "average" species	Skorupa 1998b; USDOI 1998
Teratogenicity	74	EC10 for teratogenic effects in American avocet	Avocet is considered a "tolerant" species	Skorupa 1998b; USDOI 1998

[a] 65–80% moisture, varying with species and incubation stage; 70% moisture (i.e., factor of 3.3) used for approximate conversion

Note: Values in the first 3 rows (Puls 1988) are based on domestic poultry rather than wild species.

Source: From information contained in Ohlendorf and Heinz (in press).

to oviparous vertebrates, from molecular to biochemical to subcellular/cellular to individual to population levels of biological organization, is described below.

6.2.2.1 Cellular Mechanisms of Se Toxicity

It has long been thought that the primary initiating event behind the ability of elevated Se concentrations to cause embryo toxicity and teratogenicity comes from its propensity to substitute for sulfur, while protein synthesis is occurring during organogenesis within the embryo. Indeed, while there is a strong body of scientific literature documenting organo-Se residues presumably bound to protein within the eggs and embryos of oviparous vertebrates, there is growing evidence that oxidative stress is also likely to play a role in Se-related teratogenesis.

6.2.2.1.1 *Selenium Substitution for Sulfur in Amino Acids: Importance as Mechanism of Toxicity Uncertain*

Until recently, researchers had focused on substitution for sulfur as the mechanism for Se toxicity in oviparous vertebrates. Specifically, it was thought that Se obtained from the diet transferred maternally to the developing embryo and assimilated, in place of sulfur, into structural and functional proteins during embryonic development. Since the normal tertiary structure of protein molecules depends upon the formation of S-S linkages, substitution of Se for S in protein synthesis could result in improperly folded or dysfunctional proteins such as enzymes (Diplock and Hoekstra 1976; Reddy and Massaro 1983; Sunde 1984; Maier and Knight 1994). Resultant deformities were believed to result from this nonspecific substitution (Lemly 1997a). However, this proposed mechanism of toxic action has been questioned as discussed below. Definitive studies investigating the mechanistic importance of oxidative stress remain to be conducted in egg-laying vertebrates.

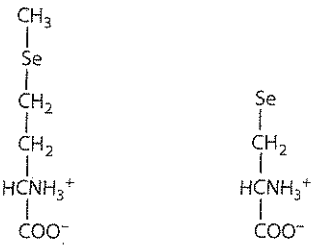

FIGURE 6.1 Schematic structures of the amino acids capable of containing Se: selenomethionine and selenocysteine.

Selenium can be incorporated into two possible amino acids, selenomethionine and selenocysteine. The formation of each relative to their sulfur analogues, methionine and cysteine, appears to use sulfur pathway enzymes and to depend on the relative concentration of Se in the cell (Allan et al. 1999). However, subsequent incorporation of these amino acids into proteins is concentration-dependent only for selenomethionine (Schrauzer 2000). The Se moiety in selenomethionine is insulated by the terminal methyl group in the amino acid structure (Figure 6.1) and so, not surprisingly, substitution of methionine with selenomethionine does not appear to alter either the structure or function of proteins (Yuan et al. 1998; Mechaly et al. 2000; Egerer-Sieber et al. 2006). Conversely, selenocysteine incorporation into proteins is highly regulated at the ribosomal level, by the UGA codon that specifies selenocysteinyl-tRNA (Stadtman 1996). Thus, proteins requiring Se for their structure or function specifically incorporate selenocysteine in the polypeptide via the mRNA sequence. Evidently, cysteine and selenocysteine can randomly be substituted only in some bacteria and plants (Allan et al. 1999). Thus, it appears that neither selenocysteine, which is controlled by the mRNA sequence, nor selenomethionine in which the Se is shielded by the terminal methyl group affect protein structure or function.

6.2.2.1.2 Oxidative Stress Mechanism

More recently, oxidative stress has been proposed as the initiating event of embryo mortality and teratogenic effects from several chemicals (Wells et al. 1997, 2009; Kovacic and Somanathan 2006), including avian species exposed to Se (Hoffman 2002; Spallholz and Hoffman 2002). Interaction with the tripeptide glutathione is apparently critical to propagating oxidative stress in Se-exposed organisms through a variety of mechanisms (Spallholz et al. 2004). Below, we first discuss the normal antioxidative process involving glutathione. Glutathione acting with the enzyme glutathione peroxidase is an intracellular antioxidant with tremendous reducing power that maintains antioxidant enzyme systems. As stated above, the enzyme that catalyzes this critical reaction is glutathione peroxidase (GPx-Se), which contains Se:

$$\text{GPx-Se} + 2\text{GSH} + \text{R-O-OH} \rightarrow \text{GSSG} + \text{H2O} + \text{ROH} + \text{GPx-Se} \quad (1)$$

Where GSH is the reduced form of glutathione, R-O-OH is a peroxide substrate, ROH is an alcohol product, and GSSG is the oxidized form of glutathione. Intermediate steps, which are not shown, regenerate the glutathione peroxidase (Equation 1). Normally, the ratio of GSH: GSSH is 500: 1 (Stryer 1995).

In a review of the avian Se toxicity literature, Hoffman (2002) documented that exposure to Se caused lower ratios of reduced GSH to oxidized GSSG and increased indices of oxidative cell damage. In feeding studies, mallard ducks (*Anas platyrhynchos*) exposed to elevated levels of selenomethionine as both ducklings (Hoffman et al. 1989, 1991a, 1992a,b, 1996) and adults (Fairbrother and Fowles 1990) demonstrated elevated plasma and hepatic GPx-Se activity as well as increased tissue Se concentrations. The mallard studies also demonstrated that there exists a dose-dependent increase in the hepatic ratio of GSSG to GSH. Such increased GSSG relative to GSH in the presence of elevated Se, apparently increased hydroperoxides responsible for the observed increase in hepatic lipid peroxidation, measured as thiobarbituric-acid reactive substances (TBARS). Consistent results have been obtained in rat (LeBoeuf et al. 1985) and fish models (Holm 2002; Miller et al. 2007; Atencio et al. 2009).

In other instances, glutathione can react with some forms of Se to produce selenopersulfides and thiyl radicals (Spallholz and Hoffman 2002). Selenopersulfides spontaneously produce superoxide anion in the presence of oxygen, or they may react with additional glutathione, producing hydrogen selenide, eventually giving rise to elemental Se and again producing superoxide anion (Lin and Spallholz 1993). Thiyl radicals may react with glutathione to form glutathione disulfide radicals (Arteel and Sies 2001).

The chemical speciation of Se is complex (Chapter 4), and not all forms of Se are capable of associating with glutathione and generating oxidative stress (Spallholz and Hoffman 2002). In fact, the predominant form of Se in the eggs of oviparous vertebrates, selenomethionine, is not highly reactive with glutathione (Spallholz et al. 2001; Spallholz and Hoffman 2002). However, in vivo metabolism of selenomethionine and/or selenocysteine to more reactive Se forms, including methylselenol, could potentiate oxidative stress (Sunde 1997; Miki et al. 2001; Wang et al. 2002; Fan et al. 2002; Palace et al. 2004). In eggs, concentration-dependent incorporation of elevated selenomethionine from exposed adults and subsequent enzymatic cleavage into reactive metabolites in the developing embryo is hypothesized to initiate generation of reactive oxygen species and development of oxidative stress. Furthermore, it has been hypothesized that oxidative stress may be involved in pericardial and yolk sac edema in rainbow trout (*Oncorhynchus mykiss*) embryos exposed to elevated Se (Palace et al. 2004) in a similar manner and etiology as some organic contaminants (Bauder et al. 2005).

Some authors convincingly argue the case for oxidative stress being the mode of action in teratogenesis (Wells et al. 2009). They successfully used rat and mouse models to describe examples where effects from organic xenobiotics well known for teratogenicity (e.g., thalidomide) are ameliorated when embryos are simultaneously exposed to antioxidants (e.g., Vitamin A). However, to date, we are not aware of comparable work conducted with Se and oviparous vertebrates. Currently, there is primarily correlative evidence of oxidative stress and incidence

of terata after embryo exposure to Se, and much less in terms of cause–effect relationships.

Aside from oxidative stress related to glutathione homeostasis, a few studies have examined the effect of Se on other antioxidant enzymes and nonenzymatic vitamins in fish. Li et al. (2008) reported a decline in the hepatic activity of superoxide dismutase, which neutralizes the reactive oxygen species superoxide anion, in Japanese medaka (*Oryzias latipes*) exposed to waterborne selenite or nanoparticle Se. Holm (2002) reported that concentrations of the antioxidant vitamins E (tocopherol) and A (retinol) were slightly lower among rainbow trout fed diets enriched with selenomethionine (10 or 20 mg/kg dw) for 302 days. Vitamin E may have been lower because of greater production of Se-dependent glutathione peroxidase, which metabolizes lipid-peroxyl radicals, accounting for the ability of Se to reduce metabolic requirements for vitamin E (Ursini et al. 1985). Histopathological lesions in the livers of splittail (*Pogonichthys macrolepidotus*) fed a selenized yeast diet containing 57.6 mg Se/kg dw exhibited cytoplasmic protein droplets and fatty vacuolar degenerations that the authors speculated could be due to lipid peroxidation (Teh et al. 2004). Miller et al. (2007) found that exposures of juvenile rainbow trout to subacute (to 160 µg Se/L) concentrations of waterborne selenite for 30 days did not alter antioxidant enzyme activities or lipid peroxidation levels. Determining the importance of oxidative stress resulting from Se exposure to different species and life stages is a pressing research need.

6.2.2.1.3 Mechanism of Suppressed Immune Function

Although Se is a well-known antioxidant with positive effects on the immune system (Koller et al. 1986), it has the potential to adversely affect the immune system at elevated concentrations in mammals or birds. Mammals have a slightly reduced immune response at elevated dietary exposures of selenomethionine, sodium selenate, or sodium selenite (Raisbeck et al. 1998). Bird immunity appears to be less sensitive to Se exposure. For example, selenomethionine in drinking water decreased some aspects of mallard immune response, while sodium selenite had no effect (Fairbrother and Fowles 1990). American avocet (*Recurvirostra americana*) chicks hatched from eggs collected from ponds with elevated Se and arsenic (As) concentrations showed reduced responses in some aspects of their immune systems, but elevated activity in others (Fairbrother et al. 1994). Mallard chicks hatched from eggs of ducks feeding in streams contaminated with Se demonstrated increased mortality following infection with duck hepatitis virus (Fairbrother et al. 2004).

The immunocompetence of adult common eiders (*Somateria mollissima*) was impaired when they were fed a diet containing 60 mg Se/kg dw (Franson et al. 2007). Interestingly, thymus glands were absent and cell-mediated immunity was reduced in this group of birds. However, humoral immunity was enhanced in eiders fed a lower concentration of Se (20 mg/kg dw) (Franson et al. 2007). In field-collected birds, cell-mediated immunity was positively correlated, and the ratio of heterophils to lymphocytes was negatively correlated with hepatic Se over a range of concentrations from 9 to 76 mg/kg dw (Wayland et al. 2002). Collectively, the results of the eider studies suggest that Se may enhance immunocompetence at low levels of supplementation or over a range of normal dietary levels in the wild, but that it can impair immunocompetence at elevated dietary levels.

6.2.2.2 Toxicodynamics

6.2.2.2.1 Incorporation of Se into Vitellogenin

Maternal deposition of Se into eggs and its subsequent assimilation by the developing embryo is the key vector for determining the reproductive effects of Se in oviparous vertebrates. However, there are important differences among species in reproductive strategy, reproductive physiology, pattern of oogenesis, biochemical and physical properties of their eggs, and behavior that may affect the deposition of Se into eggs. Vertebrate eggs vary considerably in their anatomical and biochemical composition (Blackburn 1998, 2000; Romano et al. 2004). As discussed below, there appear to be multiple physiological pathways for maternal transfer of Se.

6.2.2.2.2 Fish

Fish exhibit a remarkable range of reproductive strategies, from semelparous species that spawn only once in their lifetime to iteroparous species that spawn multiple times during their lifetime. Even among iteroparous species, strategies may range from taking many years to reach sexual maturity and spawning only every 2 to 3 years, to spawning every year or even multiple times each year (Mommsen and Walsh 1988; Rinchard and Kestemont 2005). While a comprehensive evaluation of the effect of these various reproductive strategies on susceptibility to Se-induced reproductive toxicity has not been conducted, some inferences can be made based on the timing and duration of oogenesis.

In fish, the primary yolk precursor is vitellogenin (VTG), a phospholipoglycoprotein synthesized in the liver under the regulation of the hypothalamic–pituitary–gonadal–liver endocrine axis (Arukwe and Goksøyr 2003). Vitellogenin is exported from the liver, transported in the blood, and incorporated into the developing ovarian follicle by receptor-mediated endocytosis (Kime 1998). In the follicle, VTG is enzymatically cleaved into the primary yolk proteins lipovitellin and phosvitin (Arukwe and Goksøyr 2003). These sulfur-containing proteins can also contain Se, and not surprisingly, Se binding to VTG has been demonstrated in fish (Kroll and Doroshov 1991).

The duration and relative amount of VTG deposited into developing oocytes, and hence the potential for Se incorporation, depends on the reproductive strategy of the fish species in question. For many salmonid fish species, vitellogenesis can occur over several months prior to spawning with a relatively large amount of energy-rich yolk being invested (Estay et al. 2003). As a result, for salmonids, the dietary intake of Se immediately prior to spawning may not have a major impact on egg Se concentrations. Instead, Se from tissue storage sites, including the liver and muscle, will likely contribute proportionally more Se to the oocytes in these fish. In fish species that spawn multiple times in one season, the period of oogenesis can be highly variable with oocyte maturation occurring well before, immediately prior to, or even during the spawning season (Rinchard and Kestemont 2005). In these cases, the immediate diet may be more important for supplying nutrients and trace elements, including Se, for maternal transfer to the developing oocytes. The efficiency of transfer from maternal tissues to eggs is also highly variable among fish species. In their review, deBruyn et al. (2008) report that regression slopes for Se concentrations in muscle versus eggs vary widely among eight fish species, with rainbow trout exhibiting the highest egg

muscle ratios, and brook trout (*Salvelinus fontinalis*), the lowest ratio. However, the underlying biochemical reasons for the variable efficiency of Se transfer from tissues to eggs remains uncertain.

6.2.2.2.3 Amphibians and Reptiles

Amphibians and squamate reptiles (lizards and snakes) also incorporate proteins derived from VTG in a similar manner to fish (Unrine et al. 2006). Presumably this means that these groups also mobilize Se from storage tissues to eggs in a similar manner to that described above for fish. Little is known about the mechanisms of maternal transfer of Se in amphibians and reptiles, but their vast diversity in reproductive biology and life history provides opportunities for important comparative research on Se exposure and effects.

Oviparous amphibians produce anamniotic eggs that are most similar in structure to fish eggs (Duellman and Trueb 1986). Like fish, amphibian species vary dramatically in their annual fecundity, ranging from several offspring/year to >80,000/year (Duellman and Trueb 1986) and, as a result, they vary considerably in the proportion of energy, nutrients, and contaminants that they allocate to progeny. Amphibian embryos often undergo development in water where they hatch and transition to a larval stage (i.e., amphibians with complex lifecycles), but others forego the aquatic stage and undergo direct development in the terrestrial environment (e.g., Plethodontid salamanders). Such differences in maternal provisioning and developmental patterns obviously have important implications for understanding maternal transfer of Se and any resultant effects. In the only two studies examining maternal transfer of Se in amphibians, females transferred approximately 28% to 53% of their preoviposition body burden to their eggs (Hopkins et al. 2006; Bergeron, Bodinof, Unrine, and Hopkins, unpublished data).

Similar to amphibians, reptiles (turtles, crocodilians, lizards, snakes, and tuatara) span an incredibly broad range of reproductive strategies, from oviparity to true viviparity (Tinkle and Gibbons 1977; Shine 1985; Thompson et al. 2000). Oviparous species produce an amniotic egg, most similar to that of birds. Although several studies demonstrate maternal transfer of Se in various oviparous reptiles (Nagle et al. 2001; Hopkins et al. 2004, 2005a,b; Roe et al. 2004), selenomethionine is also transferred from low (invertebrate) to high trophic levels (western fence lizard, *Sceloporus occidentalis*) under controlled conditions. During trophic transfer, considerable Se partitions within the lizards' developing follicles and eggs (Hopkins et al. 2005a; Unrine et al. 2007a). Selenium is transported to the egg by vitellogenin, but also via two previously undescribed egg proteins (Unrine et al. 2006).

Among vertebrates, squamate reptiles provide fruitful opportunities for understanding the mechanisms of maternal transfer in placental and nonplacental vertebrates because closely related species (congenerics) span the full spectrum of oviparity to viviparity (Shine 1985; Thompson et al. 2000). Just as squamates have been adopted as models for studying the evolution of viviparity (e.g., Tinkle and Gibbons 1977), similar comparative approaches could prove invaluable for understanding mechanisms of maternal Se transfer.

6.2.2.2.4 Birds

In birds, Se-containing proteins and Se effects differ distinctly from those of fish, amphibians, and squamate reptiles. In contrast to fish, most of the Se in an avian egg is

found in the albumin, and therefore the developing chick takes up most of the egg Se before hatching and before yolk sac resorption. For example, in the domestic chicken (*Gallus gallus*) Se is incorporated in ovalbumen, conalbumin, globulin, ovomucoid, and flavoprotein (Jacobs et al. 1993; Davis and Fear 1996). Consequently, also unlike fish, one of the most sensitive toxic endpoints for bird reproduction is reduced egg viability because of hatching failure among full-term, fertile eggs. Because the yolk sac may not be completely metabolized until several days posthatch, the Se dose received from yolk sac resorption can decrease growth rates of newly hatched chicks (Fairbrother et al. 1994) and cause direct chick mortality (Williams et al. 1989; Marn 2003). More research on posthatch reproductive effects (or lack thereof) associated with yolk sac resorption in avian chicks is highly warranted.

The ratio of Se in albumin versus yolk in avian eggs collected in the wild corroborates the ratio observed in controlled feeding studies that supplemented the female's diet with selenomethionine but is not consistent with controlled studies that supplemented the diet with inorganic forms of Se (Latshaw and Osman 1975; Latshaw and Biggert 1981; Moksnes and Norheim 1982; Heinz et al. 1987, 1990; Santolo et al. 1999; Detwiler 2002). Unlike fish, most of the Se in avian eggs is mobilized exogenously from the diet rather than endogenously from maternal tissue (Heinz 1996; DeVink et al. 2008a). Consequently, under controlled feeding conditions (i.e., uniform dietary exposure), there is no laying-order effect (i.e., differences among eggs within the same clutch) as would be expected if an endogenous pool of Se were being increasingly depleted with the production of each successive egg (Heinz 1996). Thus, Se in bird eggs is representative of a relatively short-term (few days) snapshot of a female's dietary exposure during ovulation; moreover, females also return to laying Se-normal ("clean") eggs within days to weeks of switching to a Se-normal diet, depending on the starting point (Heinz 1996). One implication of avian egg Se being derived primarily from the diet over a discrete time window is that eggs within a single clutch in nature can contain Se concentrations that vary to the extent that the female's dietary exposure varies during the ovulation of one egg to the next. For example, among 31 completed 4-egg black-necked stilt (*Himantopus mexicanus*) clutches, total within-clutch variation was typically <1 mg Se/kg dw for clutches averaging <7 mg Se/kg dw (Joe Skorupa, USGS, personal communication). However, variability increased as the mean Se content of each clutch increased, probably reflecting a heterogeneous spatial distribution of Se within an aquatic system with a broad range of Se concentrations (i.e., "hot spots" become more pronounced and the chance of a feeding hen moving in and out of hot spots during ovulation increases). In the most severe case, a clutch that averaged 62 mg Se/kg dw exhibited a 100 mg/kg dw spread between the low egg (9.9 mg Se/kg dw) and the high egg (110 mg Se/kg dw). However, another clutch that averaged 62 mg Se/kg dw exhibited a spread of only 11 mg/kg dw between the low (55 mg Se/kg dw) and high (66 mg Se/kg dw) egg (Skorupa unpublished data). Within-clutch variability can also be exacerbated by landscape scale movement between re-nesting attempts if the first nesting attempt is terminated early because of egg predation, nest flooding, or other sources of early nest failure. Cases of extreme within-clutch variability (such as the 100 mg/kg dw example above) are probably the result of hen movement through a hot spot between successive nesting attempts.

Although captive studies have noted depuration of Se from the liver of laying mallards that were induced to produce artificially excessive numbers of eggs (30 or more as opposed to a normal clutch size of 6 to 8 eggs; Ohlendorf and Heinz, in press), Paveglio et al. (1992) reported that liver Se in field-collected breeding female and male mallards did not follow the pattern observed in captive studies. Given the exogenous source of most avian egg Se, egg production should not normally be a major pathway for excretion of the endogenous pools of tissue Se in breeding females.

6.3 RELEVANT TOXICITY ENDPOINTS

6.3.1 Diagnostic Indicators of Se Toxicity

As indicated earlier, the most important toxicological effects of Se in fish arise following maternal transfer of Se to eggs during vitellogenesis, resulting in Se exposure when hatched larvae undergo yolk absorption. During this life stage (fry), permanent developmental anomalies (e.g., spinal curvatures, missing or deformed fins, and craniofacial deformities) and other effects (e.g., edema) in fish can be related to elevated Se in eggs (Hodson and Hilton 1983; Lemly 1993a; Maier and Knight 1994; Hamilton 2003) (see Figure 6.2 for examples of Se-induced terata in larval fish). Although certain other natural and anthropogenic factors can result in deformities of the spine, fins, and craniofacial structures (Section 6.3.2.1), these terata have been considered diagnostic for Se toxicity (Maier and Knight 1994; Lemly 1997a). As discussed below, various forms of edema (e.g., edema of the pericardium or yolk sac) can arise from exposure to other xenobiotics such as polycyclic and halogenated aromatic hydrocarbons; however, this response is also prevalent in larval fish exposed to elevated concentrations of Se in yolk. In birds, embryonic deformities accompanied by substantively elevated egg Se are perhaps the most unequivocal basis for diagnosing reproductive selenosis (Ohlendorf 1989, 2003; Heinz 1996). However, impaired egg hatchability occurs at distinctly lower egg Se levels than embryo teratogenesis and is therefore a more sensitive effects endpoint (Skorupa 1999).

6.3.2 Types and Severities of Deformities and Edema

6.3.2.1 Fish

In contrast to birds, Se generally does not affect fertility and hatching rates in fish (Gillespie and Baumann 1986; Coyle et al. 1993; Holm et al. 2005; Muscatello et al. 2006). Hermanutz et al. (1992) observed a statistically significant reduction in the hatching rate of Se-exposed bluegill sunfish (*Lepomis macrochirus*) relative to the control, but this effect was atypical in Se toxicity studies. Rather, teratogenesis, edema, and/or larval mortality following hatch are the most sensitive endpoints in fish.

The frequency of teratogenic deformities in early developmental stages of fish is the most useful indicator of Se toxicity. Teratogenesis is a direct expression of Se toxicosis and represents the sum total of parental exposure, regardless of temporal, spatial, or chemical variations in Se exposures. Terata represent a measure of existing, rather than potential, hazard and can be subtle but important causes of recruitment failure in fish populations. Significant loss of the early life stages of a fish

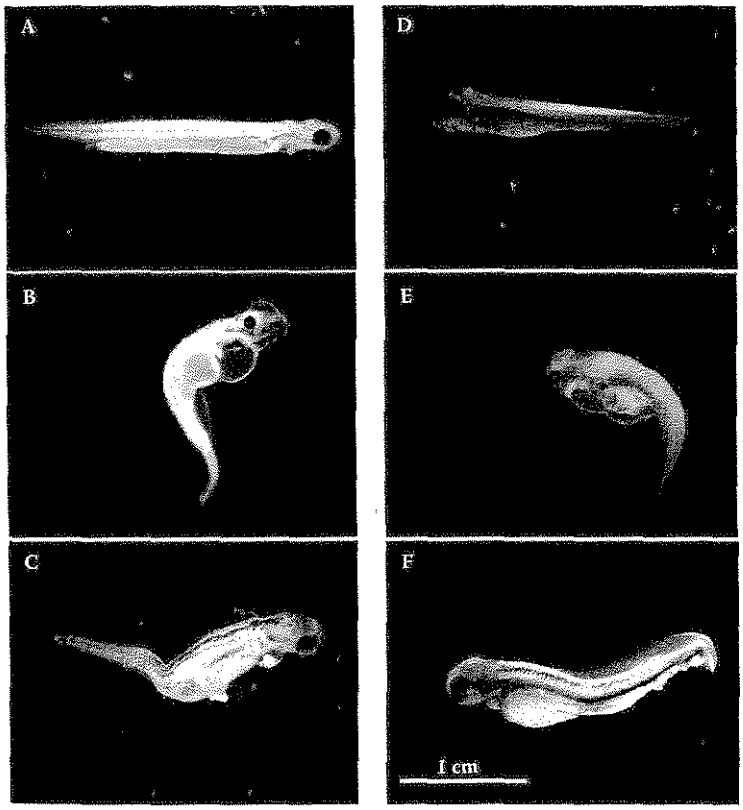

FIGURE 6.2 Examples of Se-induced deformities in larval white sucker (A–C) and northern pike (D–F). Plates A and D are larvae originating from reference sites showing normal morphology. Plate B shows pericardial and craniofacial edema and spinal curvature (scoliosis). Plate C shows spinal curvature (scoliosis) and shortened pelvic fin. Plate E shows craniofacial deformity, microphthalmia, pericardial and yolk sac edema, and spinal curvature (kyphosis). Plate F shows spinal curvature (kyphosis and lordosis) and craniofacial deformity. (Adapted with permission from Muscatello JR. 2009. Selenium accumulation and effects in aquatic organisms downstream of uranium mining and milling operations in northern Saskatchewan. PhD Dissertation. Saskatoon: University of Saskatchewan (Canada).)

population can occur at the same time that adult fish appear healthy. An index for evaluating the impact of Se-induced terata on population mortality (Lemly 1997a) predicts that when <6% terata appear in larvae or fry, less than 5% mortality and negligible impact would result to the population. However, when terata are quantified at rates of between 6% to 25%, mortality would be 5% to 20%, with a slight to moderate impact, and >25% terata would correspond to >20% population loss and a major impact on populations. These relationships are based on data from two families of fishes (Centrarchidae and Cyprinidae), and several studies have questioned the applicability of this index to cold-water fish species, including salmonids and esocids (Kennedy et al. 2000; Holm et al. 2005; Muscatello et al. 2006).

There are at least three ways of determining and categorizing deformities. Simple frequency analysis is scored as either presence or absence for a given category of deformity (e.g., presence of spinal deformity). Graduated severity index (GSI) methods assign a numerical value based on the severity of a given deformity (i.e., 1 = mild, 2 = moderate, and 3 = severe), most effectively with a predetermined criterion established for assignment to each score. Finally, morphometric analyses attempt to make actual quantifiable measures of a given category of deformity, such as the angle of spinal column diversion, or the volume of edematous fluid accumulated or degree of jaw shortening. Holm et al. (2003) evaluated each of these methods by repeated measurement of preserved rainbow trout and brook trout fry. Graduated severity index and frequency analysis provided similar information, but the GSI analysis detected increases in the severity of deformities that simple frequency analysis could not. Morphometric analysis did not provide better information than the previous two methods; however, it required far more analysis effort and specialized analytical instrumentation. Kennedy et al. (2000), also used a GSI approach and a subsequent recommendation has supported the use of this type of analysis (McDonald and Chapman 2009). Quality assurance and quality control (QA/QC) considerations for assessing larval deformities in fish are discussed in Text Box 6.1.

Studies of deformity rates are often hampered by data gaps in the basal rates of deformity in each of the categories (Villeneuve et al. 2005). Clearly, factors other than Se toxicity can lead to the development of larval deformities. For example, skeletal and craniofacial deformities can be influenced by genetic factors (Alfonso et al. 2000), parasite infections (Villeneuve et al. 2005), vitamin or amino acid deficiencies (Dabrowski et al. 1996; Villeneuve et al. 2005), organic contaminants (Mehrle et al. 1982; Tillitt and Papoulias 2002), and elevated water temperatures (Sfakianakis et al. 2006; Georgakopoulou et al. 2007). Spinal deformities can arise from failure of the swim bladder to inflate (Daoulas et al. 1991; Chatain 1994) or when fish develop in high water velocities (Sfakianakis et al. 2006). Spinal deformities associated with vitamin deficiencies, parasitic infections, or contaminants appear at numerous locations along the vertebral column, as is also the case for Se-induced deformities in fish. Conversely, spinal deformities associated with elevated water velocity or swim bladder inflation failure tend to manifest at a consistent spinal location in affected populations (Divanach et al. 1997). Finally, sampling artifacts, including the effects of electrical shock during collection (EVS and PLA 1998) or "packing effects" (where larvae are shaped by other organisms or objects in the fixative) during preservation (Kingsford et al. 1996), influence the final enumeration of skeletal abnormalities.

Baseline deformity rates of 2% to 5% occur in salmonids spawned in the laboratory (Gill and Fisk 1966; Werner et al. 2005); slightly higher rates occur in fish spawned in the wild (Kennedy et al. 2000; de Rosemond et al. 2005; Holm et al. 2005). Villeneuve et al. (2005) reported baseline deformity rates of several Cyprinids and a Catostomid species, as follows: 7% for pikeminnow (*Ptychocheilus oregonensis*); 6% in redside shiner (*Richardsonius balteatus)*; 17% in large-scale sucker (*Catostomus macrocheilus*); 8% in peamouth (*Mylocheilus caurinus*); and 13% in chiselmouth (*Acrocheilus alutaceus*) from reference locations in the Willamette River, Oregon. Skeletal, craniofacial, and finfold deformities were generally <10% in northern pike (*Esox lucius*) collected from cold water reference locations in northern

TEXT BOX 6-1: QA/QC FOR ASSESSMENT OF LARVAL FISH DEFORMITIES

Although a standard operating procedure for conducting deformity (frequency) analysis of field-derived fish larvae has recently been published (Muscatello 2009; Janz and Muscatello 2008), a standardized and validated methodology including quality assurance/quality control (QA/QC) has not yet been formally adopted to assess the frequency and severity of larval fish (or other life stage) deformities. To minimize subjectivity, decrease uncertainty, and provide robust interpretation, we support the following recommendations for Se-induced larval deformity assessments from several studies (Muscatello 2009; Janz and Muscatello 2008; McDonald and Chapman 2009), including

- use of a two-way ANOVA experimental design for embryo incubations (for details, see Muscatello et al. 2006; Muscatello 2009);
- euthanization using overdose of appropriate anesthetic (e.g., 3-aminobenzoic acid [MS-222]) prior to fixation in preservative;
- blind and nonsequential labeling of treatment groups;
- development and application of an a priori framework for deformity analysis;
- internal QC checks to quantify the influence of sample preservatives, observer drift or multiple observers; and
- an external QC check of a minimum of 10% of all larval fish.

Although a standardized, validated methodology is preferred, future reproductive studies with larval fish should, at a minimum, include raw deformity data and assessment, details on all QA/QC elements, and an explicit uncertainty analysis.

Issues with preservatives: Larvae for deformity analysis are typically preserved in formalin together with various buffering agents (Kennedy et al. 2000; Muscatello et al. 2006; de Rosemond et al. 2005) or the Davidson's solution (Holm et al. 2005; Rudolph et al. 2008). Larvae may also be transferred between solutions (e.g., Saiki et al. 2004; transfer from Davidson's solution to isopropyl alcohol). Larval fish morphology (length and weight) is altered by long-term preservation but has not been comprehensively quantified (Paradis et al. 2007; Cunningham et al. 2000; Fey 1999; Fisher et al. 1998). A rigorous evaluation of the effects of preservatives (including both type and duration) on Se-related deformities in larval fish is a clear research need. Larvae should be initially assessed for deformities before preservation, at least in a subsample of larvae. Also, larval fish must be euthanized with an anesthetic overdose before preservation in fixative, since the absence of this step can cause artefactual skeletal curvatures (Janz and Muscatello 2008; Muscatello 2009).

Saskatchewan (Muscatello et al. 2006; Muscatello 2009; Muscatello and Janz 2009). Finally, up to 5% of fish produced in the aquaculture industry have some form of spinal deformity (Andrades et al. 1996). Therefore, and in agreement with Lemly (1997a), deformity rates of less than 5% are not likely ecotoxicologically relevant.

Not all types of deformities have the same ecological relevance. While abnormalities may not be lethal, their persistence in the general population is only likely where there is little threat from predators (Lemly 1997a). It is generally agreed that vertebral deformities are potentially the most critical because they impact the ability of fish to swim to avoid predators or obtain food. Laboratory-reared sea bass (*Dicentrarchus labrax*) that exhibited kyphosis were also lethargic and unresponsive to visual and auditory stimuli. More important, they grew more slowly and did not contribute to population health because their mortality rates were high (Koumondourous et al. 2002). Aside from decreased survival, carp (*Cyprinus carpio*) with spinal deformities exhibited lower growth, further supporting the notion of reduced fitness in affected fish (Al-Harbi 2001). Fernandez et al. (2008) reviewed the literature and found that the operculum complex, premaxilla, maxilla, and dentary bones were the cranial structures most commonly affected when Se-induced deformities were detected. Opercular deformities are often characterized as minor relative to other types of deformities, but Al-Harbi (2001) noted that in a cultured population of carp, while opercular deformities did not impair swimming performance, affected fish exhibited lower growth. Any assessment of Se-induced effects should consider that opercular deformities, in particular, have been linked with disruption of vitamin A and C metabolism and exposure to other contaminants (Lindesjoo and Thulin 1992; Lindesjoo et al. 1994; Fernandez et al. 2008).

One of the most prevalent, and contentious, effects from Se exposure is the appearance of edema in early life stages of fish. Edema is not a true terata because it can be transient and reversible and does not occur solely at the embryo-larval stage (Lemly 1993a). The appearance of edema has also been linked with exposure to organic chemicals (Barron et al. 2004; Billiard et al. 1999). Selenium-related edema in the developing embryo may be mediated by oxidative stress (Palace et al. 2004). Edematous effects from Se toxicity are difficult to dismiss because of strong associations between edema and elevated Se concentrations in fish eggs in a number of studies (Gillespie and Baumann 1986; Pyron and Beitinger 1989; Holm et al. 2005; Muscatello et al. 2006) and because edema is often one of the most sensitive (Muscatello and Janz 2009) and prevalent endpoints (Gillespie and Baumann 1986; Woock et al. 1987; Holm et al. 2005). Pyron and Beitinger (1989) reported that nearly all edematous fathead minnow larvae that were produced by adults exposed to Se did not survive longer than 7 days posthatch. However, Hermanutz (1992) reported that larval fish with edema survived to the juvenile stage in outdoor artificial streams. Additional evaluations regarding the ability of early life stages with edema to survive in the field are required to establish the potential ecological relevance of edema as a marker of Se exposure.

6.3.2.2 Birds

In birds, as in fish, the incidence of embryonic deformities is a commonly measured endpoint. The process for determining whether Se exposure may be implicated in embryonic deformities can be straightforward; often embryos are examined for the

presence or absence of deformities and Se concentrations are then measured in those resulting embryos (Seiler et al. 2003). In other instances, a subset of eggs from nests located at a Se-contaminated site is collected and analyzed for Se, and the incidence of deformities in embryos is documented for that site (Ohlendorf et al. 1988; Ohlendorf and Hothem 1995).

Among mallard embryos, a spatulate upper bill is a Se-specific deformity (O'Toole and Raisbeck 1997, 1998). Seiler et al. (2003) illustrated the consistency of this type of deformity across different species of duck embryos and across field study sites (Figure 6.3). Although the exact nature of embryo deformities and the order in which they express themselves may vary among species (e.g., eyes first in shorebirds, bills first in ducks; Skorupa, personal observation), they consistently involve the reduction or

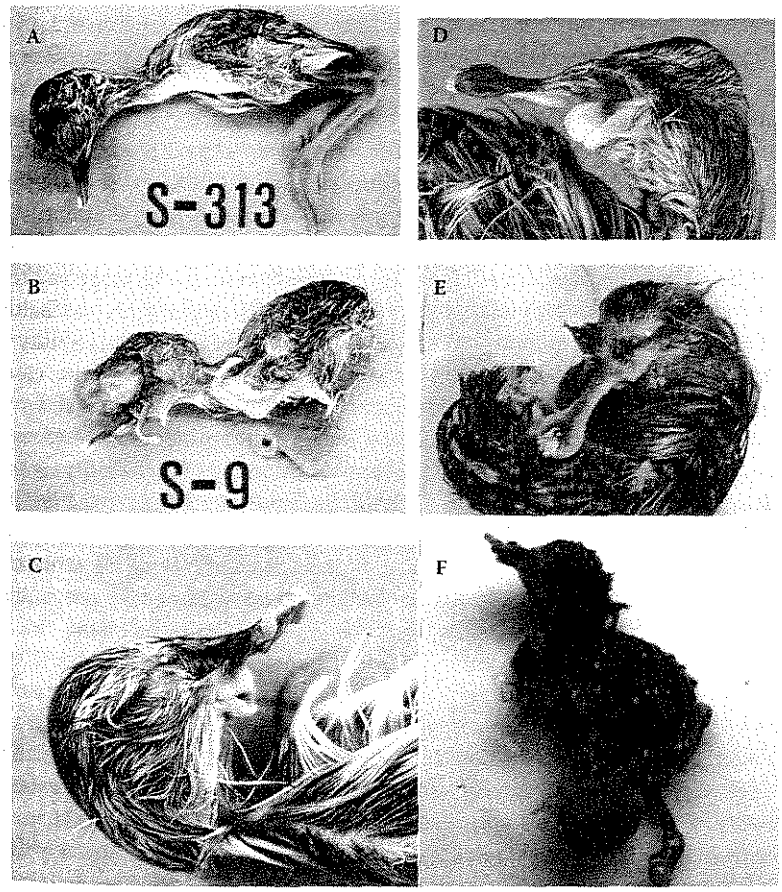

FIGURE 6.3 Examples of Se-induced deformities in bird embryos. (A) Normally developed black-necked stilt; (B) black-necked stilt with missing eyes, malformed bill, limb deformities and exencephaly; (C) gadwall and (D) northern pintail with arrested development of lower bill, spoonbill narrowing of upper bill, and missing eyes; (E) redhead with spoonbill narrowing of upper bill; and (F) American avocet with club foot and malformed bill (adapted from Seiler et al. 2003).

absence of eyes, and/or the reduction or malformation of the upper, lower, or both bills (beak), and/or the reduction or malformation of the limbs, especially the lower limbs (Hoffman and Heinz 1988; Ohlendorf et al. 1988). At very high exposure concentrations, other uncommon deformities (such as the brain protruding from the eye socket) become expressed. Seiler et al. (2003) documented Se-induced embryo deformities in shorebirds. Deformed embryos almost never hatch and likely die quickly in those rare cases when they do hatch. During 2 decades of monitoring more than 5,000 shorebird nests in the Tulare Basin (California), and despite the documentation of hundreds of deformed embryos, only 2 deformed hatchlings were documented, and both had no eyes, were incapable of feeding, and when placed in water could swim only in circles (Skorupa, personal observation). Thus, deformities must be assessed by collecting eggs that are far enough along in incubation to yield embryos and not by surveying hatched broods. Surveying only hatched broods represents a classic case of survivor bias.

Nonteratogenic embryo mortality occurs at substantively lower egg Se concentrations than are required to induce embryo deformity. In one controlled feeding experiment with mallards, a 7 mg Se/kg dietary exposure caused >30% embryo mortality due to impaired egg hatchability but no teratogenic, embryo deformities (Stanley et al. 1996). Based on experimental data, Ohlendorf (2003) estimated the EC10 for *egg hatchability* to be 12 mg Se/kg in mallard eggs in contrast to an EC10 of 23 mg Se/kg for *embryo deformities* in field-collected duck eggs (Seiler et al. 2003). The gap between the EC10 for hatchability and for teratogenesis is even larger based on Beckon et al.'s (2008) estimated EC10 for mallard egg hatchability of 7.7 mg Se/kg. However, compared to teratogenesis, impaired egg hatchability is less Se-specific and more easily induced by many kinds of stressors. Therefore, causation for impaired egg hatchability in the field can be more difficult to establish with high confidence. Artificial incubation of field-collected eggs is one method of reducing the uncertainty of causation potentially associated with egg hatchability as an endpoint (Peakall and Fox 1987; Smith et al. 1988; Hoffman 1990; Skorupa 1999; Henny et al. 2001). A clear exposure–response relationship can also increase diagnostic confidence for field hatchability data, because the effects of confounding stressors and natural stochastic variation should not normally co-vary with egg Se concentration.

6.3.3 Mortalities

6.3.3.1 Fish

Adult fish can accumulate sublethal Se concentrations that can cause mortality of their offspring via maternal Se transfer to eggs. This is a severe manifestation of the maternal Se transfer that can result in larval deformities and edema, as discussed above. At sufficiently high Se concentrations in eggs, larvae are unable to survive. Moreover, larvae that initially survive with severe deformities and/or edema will die if these effects impact their ability to adequately feed or escape predators. Hatchability of fish is generally not affected by Se (Gillespie and Baumann 1986; Coyle et al. 1993; Holm et al. 2005; Muscatello et al. 2006), but Rudolph et al. (2008) observed 100% egg mortality at egg Se concentrations ≥86.3 mg/kg dw. In general, larval mortality is not a diagnostic indicator of Se toxicity because the endpoint is not Se specific and most biological surveys would not detect larval mortality (with

the exception, perhaps, of a large die-off following a spill). Of course, the absence of early life stages in itself may indicate Se toxicity. At sufficiently high Se concentrations and under the right conditions, adult fish mortality can also be observed. Hermanutz et al. (1992) exposed adult bluegill sunfish to Se in experimental streams dosed with selenite to concentrations of 10 and 30 µg/L. The streams contained well-developed assemblages of fish food organisms, so an environmentally relevant dietary exposure pathway was simulated. After a 356-d exposure, adult survival was significantly reduced from 99% in the control fish to 84% in the 10 µg Se/L treatment and to 0% in the 30 µg Se/L treatment. In this same study, however, effects on offspring (i.e., larval edema and deformities) were a more sensitive endpoint than adult mortality. Therefore, chronic dietary Se exposures can result in adult mortality under certain conditions, and embryo mortality may occur at extremely high egg Se concentrations, but larval mortality resulting from maternal Se transfer is the most sensitive life stage for the mortality end point.

6.3.3.2 Birds

Reproductive impairment is considered the most sensitive indicator of Se toxicity to birds (Ohlendorf 2003; Seiler et al. 2003). The hatchability of eggs incubated to full term is a frequently measured endpoint in birds. Relating egg hatchability to egg Se concentrations is a complicated process, in the field. By the time a clutch of eggs hatches, only the failed eggs remain for chemical analysis, a biased subset of all eggs. An alternative approach is to randomly select a single egg from a clutch for chemical analysis and to use the measured concentration of Se in the egg as a representation of the Se concentrations in all eggs in the clutch. The Se concentration in the egg is then related to the hatchability of the uncollected sibling eggs whose fate must be monitored by several visits to the nest. A similar approach is used in experimental studies in which Se-dosed diets are fed to captive birds. In the case of field studies, suitable reference areas must be included in the study design, whereas in captive feeding trials, a Se-adequate diet must be fed to control birds. In the case of field studies as opposed to feeding trials, the possibility of extreme within-clutch egg Se variability (cf. Section 6.2.2.2.4) must be considered. However, for large sample sizes, such random error should affect the precision but not the accuracy of exposure–response statistical relationships.

For mallards, it has been clinically demonstrated that much higher dietary exposure to Se is required to induce substantive adult mortality than is required to induce substantive embryo mortality (Heinz 1996; O'Toole and Raisbeck 1997, 1998). Accordingly, only one example of substantive Se-induced adult mortality under field conditions has been documented as opposed to numerous cases of embryo mortality (Skorupa 1998a). Specifically, significant Se-induced adult mortality among American coots (*Fulica americana*) occurred at the Kesterson Reservoir (California) (Ohlendorf et al. 1988), possibly due to their greater reliance on aquatic herbivory, as compared to the co-occurring species of water birds (DuBowy 1989).

From a bioenergetic perspective, DuBowy (1989) illustrated that, even if there was no potency difference, the herbivorous diet would be more dangerous. Vegetation has such a low caloric content that coots must consume far more vegetation than do

birds feeding on calorie-rich invertebrates to meet their metabolic caloric requirements. As a consequence, coots likely ingested more Se per day than other bird species, even if the concentration of Se in the coot diet was lower than the concentration in co-occurring bird species' diets. This example illustrates that ultimately it is the mass loading of Se that an animal ingests, and not the concentration that determines the dose. However, in most cases, dietary concentrations are an accurate surrogate for mass loading. One noteworthy indicator of severe adult poisoning among Kesterson coots was alopecia (loss of feathers; Ohlendorf et al. 1988), which has also been induced clinically among Se-dosed adult mallards (Albers et al. 1996; O'Toole and Raisbeck 1998).

6.4 COMPARATIVE SENSITIVITY OF AQUATIC ORGANISMS TO Se

6.4.1 Bacteria

Bacteria are extremely tolerant of metals and metalloids, and bacteria generally have tremendous tolerance for Se. This tolerance may stem from the ability of some bacteria to sequester selenite in insoluble nodules (Sarret et al. 2005). In addition, bacteria are able to eliminate Se through dissimilatory reduction, while anaerobic bacteria can excrete elemental Se as nanospheres (Oremland et al. 2004). Possibly because of chelation with organic acids, siderophores, and phenols produced by bacteria in the rhizosphere, bacteria increase the efficiency of Hg and Se uptake by wetland plants (Pilon-Smits 2005). Bacteria in aquatic systems accumulate approximately twice as much Se as do phytoplankton, and neither appears to be impaired by its Se uptake (Baines et al. 2004).

6.4.2 Algae and Plants

Greater concentrations of Se enter marine food webs because some algae have tremendous tolerance for Se, acquiring relatively high tissue burdens without any apparent effect (Baines and Fisher 2001). For example, algae can bioconcentrate Se to a greater extent than any other trophic level. Accumulation can range by 4 to 5 orders of magnitude among algal species when exposed to 40 to 355 ng/L selenite. Chlorophytes typically accumulated the least Se, whereas prymnesiophytes, prasinophytes, and dinoflagellates had the greatest enrichments. The Se by volume per cell of diatoms and cryptophytes can vary by >2 orders of magnitude (Baines and Fisher 2001). However, within species, the Se cell concentrations are not dose dependent and typically vary by only 2- to 3-fold despite exposure to selenite concentrations with as great as 30-fold differences. As would be expected for an essential nutrient, the greatest accumulation occurs at lower concentrations, meaning that algae take up relatively more Se at low concentrations to maintain a consistent body burden (Baines and Fisher 2001; Baines et al. 2004). Clearly, biological mechanisms have evolved that provide algal species with enhanced Se tolerance through elimination or sequestration. They can efficiently volatize selenomethionine as dimethylselenide (Neumann et al. 2003), sequester it

as nonprotein seleno-amino acids such as methylselenocysteine (Brown and Shrift 1982), or accumulate it in an insoluble form as Se^0, which has a relatively low toxicity (Wilber 1980). Formation of Se^0 can occur via a reduction reaction from selenocysteine by the selenolyase enzyme, or from selenite (Garifullina et al. 2003). The high tolerance of algal species for Se is of concern because phytoplankton can concentrate Se to an extent that can cause toxicity at higher trophic levels, even at a selenite concentration as low as 0.04 µg/L (Baines and Fisher 2001; Baines et al. 2004).

6.4.3 Protozoans

Relative to the information available on the effects of Se on bacteria, algae, and plants (above), there is a paucity of information on biological effects on protozoans (i.e., nonphotosynthetic unicellular organisms, including free-living amoebae, zooflagellates, and ciliates), despite the fact that these organisms are effective models for evaluating aquatic toxicity (Lynn and Gilron 1993). Two early laboratory studies evaluated the behavioral (swimming speed), growth, and survival effects of Se on the ciliate, *Tetrahymena pyriformis* (Bovee 1978; Bovee and O'Brien 1982). These studies indicated slightly stimulated growth of *T. pyriformis* at Se water concentrations ranging between 5 and 15 µg/L. Moreover, Se inhibited swimming speed of the ciliate at 5 µg/L and stopped it completely at 30 µg/L. Based on comparative experiments with the ciliate, Bovee (1978) concluded that selenite was more toxic than selenous acid, and that overall growth and survival effects were evident in this species at Se water concentrations of >20 µg/L.

In a subsequent laboratory study using a microbial food web model to investigate the potential accumulation of Se, Sanders and Gilmour (1994) reported that population growth rates of the ciliate, *Paramecium putrinum*, were not inhibited when exposed to Se concentrations (as dissolved selenite or selenate) lower than 1,000 µg/L. This study further concluded, based on 5-day feeding experiments with the ciliate and bacteria, that Se was primarily taken up through the diet, and that biomagnification of Se did not occur at the microbial level. In laboratory microcosm experiments, Pratt and Bowers (1990) reported that protozoan species richness could be reduced by 20% when exposed to concentrations of Se >80 µg/L.

There is a high degree of uncertainty with respect to our understanding of potential toxicity of Se to protozoans, based on the relatively small database. Future research should focus on the establishment of acute and chronic water Se concentration thresholds for protozoans, with potential standardized endpoints relating to behavior, growth, and survival.

6.4.4 Macroinvertebrates

Macroinvertebrates have typically only been considered dietary sources of Se to higher trophic levels, in part based on Lemly's (1993b) statement that prey organisms can remain unaffected even when they accumulate relatively high Se body burdens. Cases of major adverse effects to fish and water birds (e.g., Belews Lake, Hyco Reservoir, Kesterson Reservoir) have not coincided with evidence of major adverse

effects on macroinvertebrate communities. However, sensitive species within such communities cannot be ruled out. It was concluded by deBruyn and Chapman (2007) that Se may cause toxic effects in some freshwater invertebrate species at concentrations considered "safe" for their predators. Recent studies with the mayfly *Centroptilum triangulifer* report that dietary exposure to 15 to 30 mg Se/kg dw resulted in a 38% reduction in fecundity with significant maternal transfer of Se to eggs (Conley and Buchwalter, North Carolina State University, unpublished). There is also evidence of Se toxicity to planktonic invertebrates in marine ecosystems at environmentally realistic concentrations (Anastasia et al. 1998; Fisher and Hook 2002). Thus, although Se appears not to have community-level impacts to macroinvertebrates, it may adversely affect sensitive species within those communities.

6.4.5 Fish

Since deformed fish and the loss of fish species observed at Belews Lake were linked to Se contamination in the late 1970s, there has been considerable analysis of Se effects on fish. Following the first full year between 1975 and 1976 that the generating units of the power plant began operation at Belews Lake (and the ash pond effluents reached maximal Se loading), largemouth bass (*Micropterus salmoides*), redbreast sunfish (*Lepomis auritus*), and pumpkinseed (*Lepomis gibbosus*) populations crashed (Van Horn 1978; Appendix A). Other centrarchids, including bluegill sunfish, showed dramatic declines in their population by 1977 (Barwick and Harrell 1997). Bluegill sunfish was the focus of early effects testing with Se at the Belews Lake site and another water body receiving effluent from a coal-fired power plant, Hyco Lake. Sexually mature bluegill females and males collected from the Se-enriched Hyco Lake and reference lakes were cross-fertilized and their offspring evaluated for effects (Bryson et al. 1984, 1985a,b; Gillespie and Baumann 1986). Offspring associated with maternal exposure to Se had reduced larval survival and malformations such as edema. This important finding established that Se was maternally transferred to the eggs and that effects in embryos and larvae were considerably more sensitive than effects in adults.

As described above, field investigations discovered a number of fish species that are sensitive to Se. However, field studies also indicate that certain species are relatively insensitive to Se. Following the extirpation of 16 species from Belews Lake due to Se contamination, four species remained: fathead minnow, common carp, eastern mosquitofish (*Gambusia holbrooki*), and black bullhead (*Ameiurus melas*; Lemly 1985). Similarly, following the declines of *Lepomis* spp., largemouth bass, crappie (*Pomoxis annularis*), yellow perch (*Perca flavescens*), and sucker (*Catostomus*) species in Hyco Lake, green sunfish (*Lepomis cyanellus*), satinfin shiner (*Notropis analostanus*), gizzard shad (*Dorosoma cepedianum*), eastern mosquitofish, and redbelly tilapia (*Tilapia zillii*) dominated the fish community (Crutchfield 2000). The western mosquitofish (*Gambusia affinis*) is also relatively insensitive based on reproductive studies by Saiki et al. (2004). Different fish species can thus have differential sensitivity to Se.

When evaluating Se toxicity, it is important to distinguish reproductive from nonreproductive threshold effects for fish because of distinctions in exposure routes.

In reproductive studies, female fish are exposed to Se and the Se is maternally transferred to their eggs during vitellogenesis (see Section 6.2.2.2). In laboratory (Doroshov et al. 1992; Coyle et al. 1993; Hardy et al. 2009) and mesocosm (Hermanutz et al. 1996) studies, parent fish were exposed to Se via the diet and water or diet only. After spawning, the embryos and larvae were monitored for effects. More often, the reproductive effects of Se have been assessed by the field collection of gametes from sites with elevated Se levels and from reference sites (Kennedy et al. 2000; Holm et al. 2005; Muscatello et al. 2006; GEI 2008a; Rudolph et al. 2008; Muscatello and Janz 2009; NewFields 2009). Eggs are most often fertilized in the field and then transported to the laboratory, where embryos and larvae are monitored for effects. In contrast, the larval and juvenile fish in nonreproductive testing had no preexposure to Se from their mother but were fed a series of Se concentrations. Growth and survival were the typical endpoints in such juvenile exposure studies.

The comparative sensitivity of the reproductive endpoints, the EC10 values based on Se concentrations in the egg or ovary, are reasonably similar (Table 6.2). The range of EC10 (or equivalent threshold) values for the fish species shown are within a factor of 1.4 (17 to 24 mg Se/kg dw in eggs; Figure 6.4). Due to testing limitations, lack of response, or excessive variability in response to Se, effect concentrations could not be determined for some studies. One study not listed in Table 6.3 (GEI 2008a) compared larval malformations from fathead minnows collected from streams with elevated Se concentrations and spawned in the laboratory to those of laboratory-reared fathead minnows. Although a wide range of Se concentrations were measured in the female fathead minnows (2 to 47 mg/kg dw whole body) an effect level could not be estimated due to the considerable variation in the endpoint showing the greatest response to Se (graduated severity index of the malformations). The 8 species in Table 6.2 with EC10 values represent both cold water and warm water fish. The overall similarity in reproductive effect levels for these eight species suggests little difference in Se sensitivity between warm water and cold-water fish species. However, further studies are required to confirm this observation.

Fewer EC10 values were determined for nonreproductive endpoints, but their 2-fold range in effect concentration is not particularly large (Table 6.3). Bluegill sunfish and chinook salmon (*Oncorhynchus tshawytscha*) were the more sensitive species with a concentration for winter stress in bluegill at 5.85 mg Se/kg dw whole body and an EC10 for decreased growth in juvenile chinook salmon at 7.34 mg Se/kg dw. As may be the case with the reproductive endpoints, the effect concentration for larval growth in fathead minnows was the greatest, at 51.4 mg Se/kg dw whole body. The relatively higher reproductive and non-reproductive effect values for fathead minnows are consistent with field observations at Belews Lake where fathead minnows were 1 of 4 remaining species after Se contamination extirpated 16 relatively sensitive species (see above).

Hamilton et al. (2005a, 2005b, 2005c) and Beyers and Sodergren (2001a,b) also evaluated Se toxicity to razorback suckers (*Xyrauchen texanus*), but due to uncertainties in whether effects observed in the Hamilton et al. (2005a,b,c) studies could be solely attributed to Se (cf USEPA 2004) and discrepancies in the Se effects levels from the Hamilton et al. (2005a,b,c) and Beyers and Sodergren (2001a,b) studies, Se toxicity data for razorback sucker are not included here.

TABLE 6.2
Data Illustrating the Range of Assessment Values for Reproductive Effects of Se in Fish

Species	Reference	Toxicological Endpoint	Effect conc'n, mg Se/kg dw[a]
Oncorhynchus mykiss Rainbow trout	Holm et al. 2005	EC10 for skeletal deformities	21.1 E
Oncorhynchus clarki Cutthroat trout	Kennedy et al. 2000	NOEC for embryo/larval deformities and mortality	>21.2 E
Oncorhynchus clarki Cutthroat trout	Hardy et al. 2009	NOEC for embryo/larval deformities	>16.04 E
Oncorhynchus clarki Cutthroat trout	Rudolph et al. 2008	EC10 for alevin survival	17–24.1 E
Salvelinus fontinalis Brook trout	Holm et al. 2005	NOEC for craniofacial deformities	>20.5 E
Salmo trutta Brown trout	NewFields 2009	EC10 for larval survival	17.7 E
Esox lucius Northern pike	Muscatello et al. 2006	EC10 larval deformities	20.4 E
Pimephales promelas Fathead minnow	Schultz and Hermanutz 1990	LOEC for larval edema and lordosis	<24 O
Lepomis macrochirus Bluegill sunfish	Bryson et al. 1984	LOEC for larval mortality	<49.65 O
Lepomis macrochirus Bluegill sunfish	Bryson et al. 1985a	Swim-up larvae	<30; >9.1 O
Lepomis macrochirus Bluegill sunfish	Gillespie and Baumann 1986	Larval survival	<46.30 O
Lepomis macrochirus Bluegill sunfish	Doroshov et al. 1992	EC10 larval edema	21.2 E
Lepomis macrochirus Bluegill sunfish	Coyle et al. 1993	EC10 for larval survival	24.10 E
Lepomis macrochirus Bluegill sunfish	Hermanutz et al. 1996	NOAEC and LOAEC for larval survival, edema, lordosis, and hemorrhaging	14.0; 42.1 O
		NOAEC for larval survival, edema, lordosis, and hemorrhaging	≥16.3 O
Micropterus salmoides Largemouth bass	Carolina Power & Light 1997	Threshold for larval mortality and deformities	19 O

Note: Concentrations (dw = dry weight) of Se in fish tissues (E = egg, O = ovary) relative to endpoints.

[a] Effect concentrations based on measured or estimated Se concentration in egg or ovary tissues.

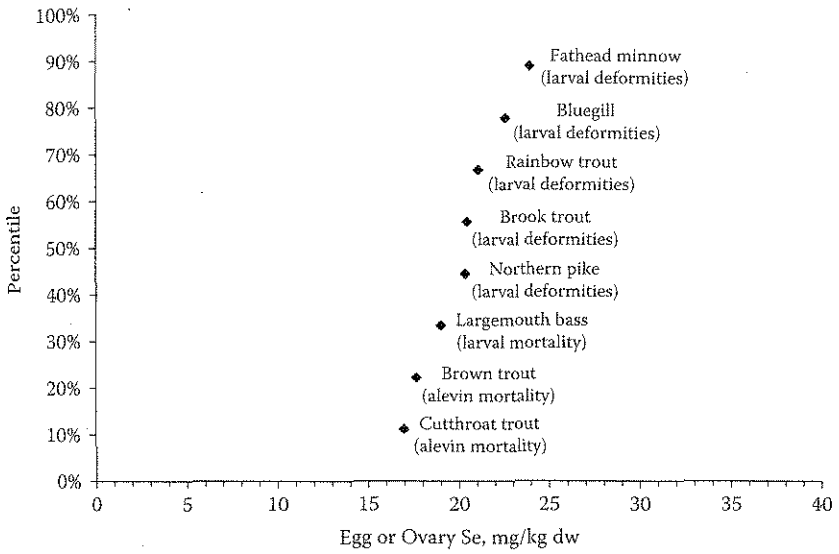

FIGURE 6.4 Distribution of egg- or ovary-based EC10 values (or comparable values if EC10 values could not be calculated). The value shown for brook trout is a NOEC because an EC10 could not be determined (from Holm et al. 2005). The value shown for bluegill is the geometric mean of EC10 values for larval edema (from Doroshov et al. 1992) and larval deformities (from Coyle et al. 1993). The value shown for fathead minnows is an unbounded LOEC associated with 25% larval edema and lordosis (from Schultz and Hermanutz 1990). See Table 6.2 for all toxic effect concentrations.

As discussed above, Se toxicity studies with fish can be broadly classified as either 1) maternal transfer studies in which effects are evaluated in the offspring of Se-exposed fish or 2) dietary Se exposure studies in which effects are evaluated in juveniles. Because these exposure routes are so fundamentally different, from a risk assessment perspective it is important to understand which exposure route is most environmentally relevant and sensitive. Relative sensitivity cannot be inferred by comparing tissue-based toxicity thresholds due to potential differences in bioaccumulation between adults in the maternal transfer studies and juveniles in the direct toxicity studies (DeForest 2008). To truly compare relative sensitivity, the exposure (i.e., dietary Se) concentrations must be compared. Laboratory toxicity data are necessary to make this comparison because in field-based exposure studies the Se concentration in the diet is unknown.

The only species for which both maternal transfer (Woock et al. 1987; Doroshov et al. 1992; Coyle et al. 1993) and juvenile toxicity studies (Cleveland et al. 1993; Lemly 1993c; McIntyre et al. 2008) have been conducted is bluegill sunfish (razorback sucker studies discussed above (Beyers and Sodergren 2001a, 2001b; Hamilton et al. 2005a, 2005b, 2005c) are excluded). As shown in Figure 6.5, the concentration–response relationships were similar among the three maternal transfer studies,

TABLE 6.3
Data Illustrating the Range of Assessment Values for Nonreproductive Effects of Se in Fish

Species	Reference	Toxicological Endpoint	Chronic Value, mg Se/kg dw[a]
Acipenser transmontanus White sturgeon	Tashjian et al. 2006	EC10 juvenile growth	15.08 WB
Oncorhynchus tshawytscha Chinook salmon	Hamilton et al. 1990	EC10 juvenile growth (mosquitofish diet)	11.14 WB
		EC10 juvenile growth (SeMet diet)	7.354 WB
Oncorhynchus mykiss Rainbow trout	Hilton and Hodson 1983	Juvenile growth NOAEC	21.0 L
	Hicks et al. 1984	LOAEC	71.7 L
Oncorhynchus mykiss Rainbow trout	Hilton et al. 1980	Juvenile survival and growth NOAEC	40 L
		LOAEC	100 L
Pogonichthys macrolepidotus Sacramento splittail	Teh et al. 2004	Juvenile deformities NOAEC	10.1 M
		LOAEC	15.1 M
Pimephales promelas Fathead minnow	Bennett et al. 1986	Chronic value for larval growth	51.40 WB
Lepomis macrochirus Bluegill sunfish	Lemly 1993c	LOAEC juvenile mortality at 4 °C	<7.91 WB
		Threshold prior to "winter stress"	5.85 WB
		NOAEC juvenile mortality at 20 °C	>6.0 WB
Lepomis macrochirus Bluegill sunfish	McIntyre et al. 2008	EC10 juvenile survival at 4 °C *Lumbriculus* diet	9.27 WB
		EC10 juvenile survival at 9 °C *Lumbriculus* diet	14.00 WB
		NOAEC juvenile survival at 4 °C	>9.992 WB
		Selenomethionine in Tetramin diet	
Lepomis macrochirus Bluegill sunfish	Cleveland et al. 1993	NOAEC for juvenile survival	>13.4 WB
Morone saxitilis Striped bass	Coughlan and Velte 1989	LOAEC for survival of yearling bass	<16.2 M

Note: Concentrations (dw = dry weight) of Se in fish tissues (WB = whole body; M = muscle; L = liver) relative to endpoints.

[a] All chronic values based on measured Se concentration in whole body, muscle tissues, or liver.

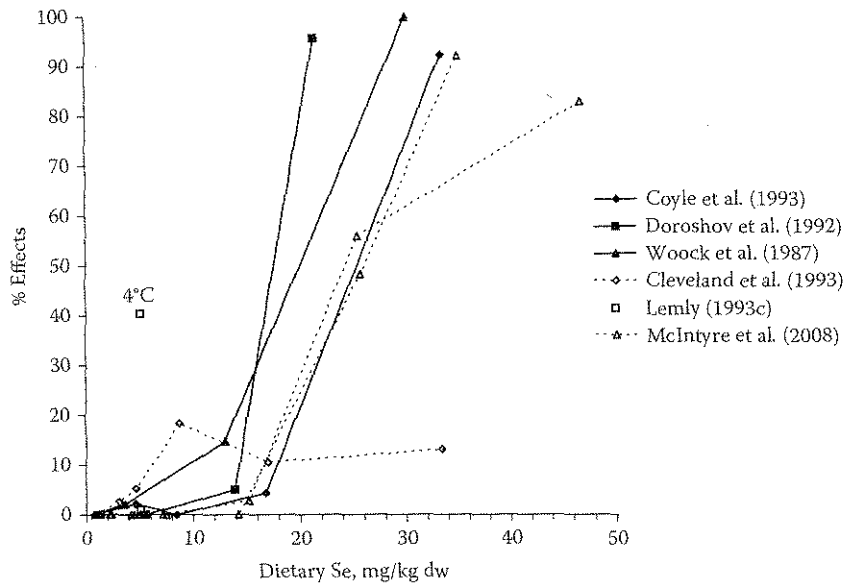

FIGURE 6.5 Effects of Se on bluegill sunfish larvae (from maternal transfer; solid lines) and juveniles (from direct dietary exposures; dashed lines) as a function of dietary Se.

with little effect (larval mortality, edema) up to a dietary threshold of approximately 12 mg Se/kg dw, followed by a rapid increase to a 90% to 100% effect level at concentrations above approximately 21 mg Se/kg dw. The juvenile results were more variable. The toxicity data from Cleveland et al. (1993) did not show the same rapid increase in Se toxicity, and McIntyre et al. (2008) reported a pattern similar to the maternal transfer studies (i.e., it appears that significant juvenile mortality does not begin to occur until dietary Se concentrations reach approximately 14 to 15 mg/kg dw; Figure 6.5). The single data point from Lemly (1993c) at 4 °C suggests high mortality at a dietary concentration of 5 mg Se/kg dw, but a concentration-response could not be evaluated because only a single Se treatment was used.

Overall, the limited data preclude strong conclusions on the relative sensitivity of maternal transfer versus dietary uptake by juveniles. The data for bluegill sunfish suggest that maternal transfer is more toxic or similarly toxic to direct juvenile ingestion. However, more studies are needed to develop predictive capability.

6.4.5.1 Selenium Concentration Relationships between Fish Tissues

Se concentrations in different tissues can be highly variable among species, as shown in Figure 6.6 for egg and muscle Se (deBruyn et al. 2008). Therefore, tissue–tissue relationships should not be used generically to derive tissue-based Se toxicity thresholds. Regression equations for specific species can estimate Se concentrations in one tissue from measurements in another. However, even within a species tissue–tissue extrapolations should ideally be site-specific because individuals show considerable intraspecific variation in the ratio between egg/ovary and whole-body Se.

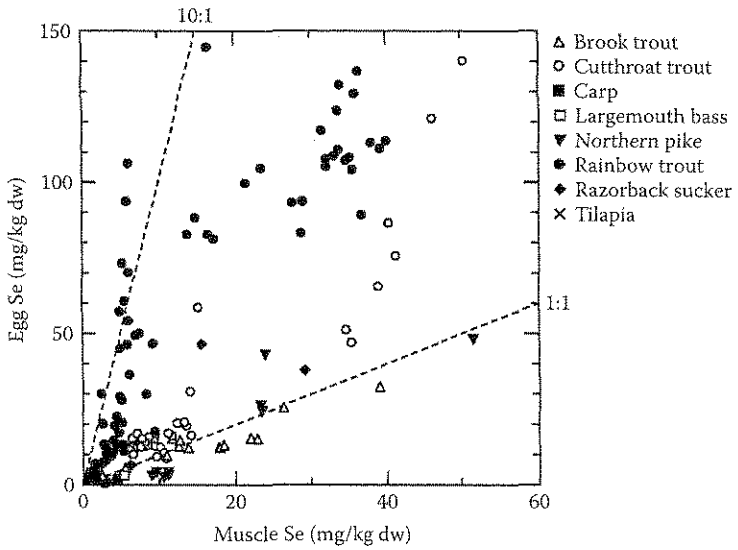

FIGURE 6.6 Egg Se concentrations relative to muscle Se concentrations for 8 fish species (from deBruyn et al. 2008).

For example, the ratio of egg to whole-body Se for black bullhead ranged from 3.1 to 27.9 (average 9.1; $n = 24$) (Osmundson et al. 2007). On the other hand, some species such as bluegill and green sunfish show minimal variation in this relationship (Figure 6.7). After a species-specific tissue–tissue relationship has been developed, any of the candidate tissues should be a reliable surrogate for early life stage Se exposure. If no species-specific tissue–tissue relationship is available, it is not possible to use adult tissue Se to reliably estimate potential early life stage exposure.

6.4.6 Amphibians

Although amphibians appear to be an extremely ecologically and toxicologically vulnerable class of vertebrates, and pollution has been implicated in some amphibian population declines (Stuart et al. 2004; Hopkins 2007; Wake and Vredenburg 2008), the effects of Se on amphibians are largely unknown. All amphibian Se effects studies currently available were based on complex mixtures, of which Se was only one component. In these situations causal relationships with Se are difficult to establish, but some observed effects are similar to those found in other vertebrates exposed to Se.

When exposed to coal fly ash (containing a complex mixture of trace elements enriched with Se) amphibian larvae (tadpoles) appear to efficiently accumulate Se in their tissues (Unrine et al. 2007a), probably because of their close association with the benthos and their tendency to ingest particulates while grazing biofilms. Associated with elevated concentrations of Se in their tissues, amphibian larvae exhibited increased incidence of axial malformations (Hopkins et al. 2000), similar to those described for fish exposed to Se in Belews Lake (North Carolina;

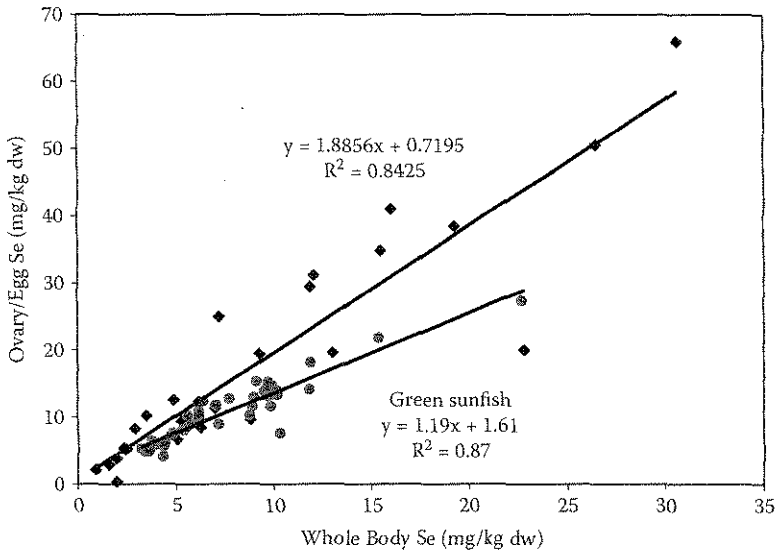

FIGURE 6.7 Ovary/egg Se concentrations relative to whole-body Se concentrations for bluegill sunfish (data compiled from Osmundson et al. 2007; Doroshov et al. 1992; Coyle et al. 1993; Hermanutz et al. 1996) and green sunfish (Osmundson et al. 2007; Doroshov et al. 1992).

Appendix A). Deformations of the keratinized mouthparts of amphibian larvae also occur (Rowe et al. 1996, 1998), and synchrotron X-ray fluorescence demonstrated colocalization of high Se concentrations in these deformed areas (Punshon et al. 2005). Spinal and oral abnormalities affected swimming and feeding performance (Hopkins et al. 2000; Rowe et al. 1998). Additional studies on amphibian larvae with elevated whole-body Se concentrations documented reductions in growth (Rowe et al. 1998; Snodgrass et al. 2004, 2005), altered predator avoidance capabilities (Raimondo et al. 1998), reduced larval survival (Rowe et al. 2001; Snodgrass et al. 2004, 2005; Roe et al. 2006), and altered time and size at metamorphosis (Snodgrass et al. 2004, 2005; Roe et al. 2006). Studies are needed to determine to what extent Se is responsible for these aberrations.

Unlike most trace elements, Se is retained in amphibian tissues as they undergo metamorphosis (Snodgrass et al. 2003, 2004, 2005). This has important implications for amphibian health during this critical life history transition and during sensitive early terrestrial life stages. Thereafter, terrestrial life stages of amphibians can continue to accumulate Se from invertebrate prey, which are often closely associated with the aquatic environment (Roe et al. 2005; Hopkins et al. 2006). Only two studies have considered the effects of Se on adult amphibians. In the first study, Hopkins et al. (1997, 1998, 1999a) demonstrated that adult male toads (*Bufo terrestris*) with elevated Se and other elements in their tissues exhibited abnormal hormonal profiles during the breeding season. More recently, Hopkins et al. (2006) found that adult narrow-mouthed toads (*Gastrophryne carolinensis*) maternally transfer elevated concentrations of Se (up to 100 mg/kg dw) to their eggs. Compared

to reference conditions, eggs from the contaminated site displayed significant reductions in hatching. In addition, there was a higher prevalence of malformed hatchlings from the contaminated site, most of which also exhibited abnormal swimming behavior. Importantly, craniofacial abnormalities, which may be diagnostic of Se teratogenicity, were most common in hatchlings at the contaminated site. This work strongly suggests that additional studies of Se maternal transfer and embryotoxicity are needed and that future studies should be designed to describe Se concentration–response relationships to facilitate comparisons to fish and birds.

6.4.7 Reptiles

Much like amphibians, little is known about the effects of Se on reptiles. Field and laboratory studies on water snakes (*Nerodia fasciata*) demonstrate that elevated concentrations of Se and other contaminants (e.g., As and Cd) are accumulated from ingestion of amphibian and fish prey in a coal ash–contaminated site (Hopkins et al. 1999b, 2001, 2002, 2005a). At lower levels of Se accumulation, exposure to seleniferous prey had no effect on growth, survival, overwinter survival, or metabolism (Hopkins et al. 2001, 2002). However, about one-third of snakes at these same exposure levels exhibited histopathological abnormalities, most notably liver necrosis (Ganser et al. 2003). At higher levels of tissue accumulation (mean liver Se ~140 mg Se/kg dw) in the field, snakes exhibited abnormally high respiratory rates, suggesting significant energetic costs associated with exposure (Hopkins et al. 1999b). At the same field site, maternal transfer of Se was examined in both turtles (red-eared sliders, *Trachemys scripta*) and American alligators (*Alligator mississippiensis*). Despite enormous differences in feeding ecology between these two species (i.e., alligators are top predators that even eat adult turtles), both species maternally transferred approximately 7.5 mg Se/kg dw to their eggs (Nagle et al. 2001; Roe et al. 2004). For comparison, common grackles (*Quiscalus quiscala*), eastern mosquitofish, and narrow-mouthed toads from the same site maternally transferred approximately (means) 6 mg Se/kg, 16 mg Se/kg, and 44 mg Se/kg dw, respectively (Bryan et al. 2003; Staub et al. 2004; Hopkins et al. 2006). This observation further supports the concept that Se does not biomagnify; the majority of food web enrichment in Se occurs at the lowest trophic levels, with comparable exposure possible for secondary (e.g., *Trachemys scripta*) and tertiary (e.g., *Alligator mississippiensis*) consumers (Chapter 5). Neither of these studies on reptiles was designed to rigorously quantify relationships between Se exposure, maternal transfer, and reproductive effects. However, anecdotal observations suggested that hatching success was consistently low for 3 years in the adult alligator monitored at the contaminated site (Roe et al. 2004).

In the laboratory, snakes and lizards have been exposed to Se in isolation from other contaminants, but primarily to study trophic transfer and bioaccumulation. In a simplified laboratory food chain, lizards receiving ~15 mg Se/kg dw for 98 days accumulated whole-body concentrations of ~10 mg Se/kg and exhibited no changes in food consumption, growth, or survival (Hopkins et al. 2005b; Unrine et al. 2006, 2007a). Reproductive effects were not examined, but females partitioned about 33% of their total Se burden into their yolked follicles. Likewise, snakes raised on an experimental diet containing 10 and 20 mg Se/kg dw (as seleno-DL-methionine) for

10 months showed no adverse effects on food consumption, growth, and body condition (Hopkins et al. 2004). Although only limited conclusions can be drawn regarding reproductive effects from this study, available evidence suggested that fewer females exposed to elevated dietary Se were reproductively active. Among individuals that did reproduce, maternal transfer of ~22 mg Se/kg dw to eggs did not adversely affect hatching success or malformation frequency. Clearly, additional studies adopting similar approaches, but with a primary focus on reproductive effects, are critical to advancing our understanding of the sensitivity of reptiles compared to birds and fish (Hopkins 2000, 2006).

6.4.8 Birds

The results of field and captive-feeding studies indicate widely variable responses among species of birds to in ovo Se exposure. Field studies in areas receiving irrigation drain water in the western United States have examined the incidence of embryonic deformities in ducks, black-necked stilts, and American avocets and related such information to embryonic Se concentrations (Seiler et al. 2003). Terata considered in that study included major structural deformities that were overtly obvious upon superficial examination. They were limited to major deformities of the eyes, bill, or limbs, whereas nonstructural abnormalities like hydrocephaly and generalized edema were not considered. There were no significant differences between mallards and other duck species, which included gadwalls (*Anas strepera*), pintails (*Anas acuta*), and redheads (*Aythya americana*) in the relationship between embryonic Se concentrations and the frequency of deformities, justifying the pooling of these species in further analyses. The comparison among ducks, black-necked stilts, and American avocets revealed that ducks were more sensitive to in ovo Se exposure than black-necked stilts, which were, in turn, more sensitive than American avocets (Figure 6.8). Fifty percent probabilities of teratogenic effects were calculated to occur at concentrations of 30, 58, and 105 mg Se/kg egg for ducks, stilts, and avocets, respectively. Corresponding Se concentrations related to a 10% probability (i.e., EC10) of teratogenesis were 23, 37, and 74 mg Se/kg, respectively. The results of the Seiler et al. (2003) study suggested that black-necked stilts are about twice as sensitive as avocets and that ducks are about 3.5 times as sensitive as avocets to the teratogenic effects of Se. Teratogenic effects of Se have been reported for killdeer (*Charadrius vociferus*) (Ohlendorf 1989; Skorupa 1998b). Based on data compiled from additional studies (Skorupa, unpubublished data) and controlled for equivalent exposure at a concentration range high enough to quantify a response for the insensitive avocet, killdeer show a degree of sensitivity intermediate between that of avocets and black-necked stilts (Figure 6.8).

Teratogenesis is a less sensitive measure of selenosis in birds than is the hatchability of their eggs when incubated to full term. Captive studies have been conducted in which mallards were fed diets containing various levels of Se (Heinz et al. 1987, 1989; Stanley et al. 1994, 1996; Heinz and Hoffman 1996, 1998). Depending on how the data in those studies were analyzed, it was calculated that a 10% hatch failure rate would correspond with egg Se concentrations (in dry weight) of 7.7 mg/kg (Beckon et al. 2008), 12 mg/kg (Adams et al. 2003), or 12.5 mg/kg (Ohlendorf 2003) (Table 6.4). Field data for balck-necked stilts also support the conclusion that egg inviability, which

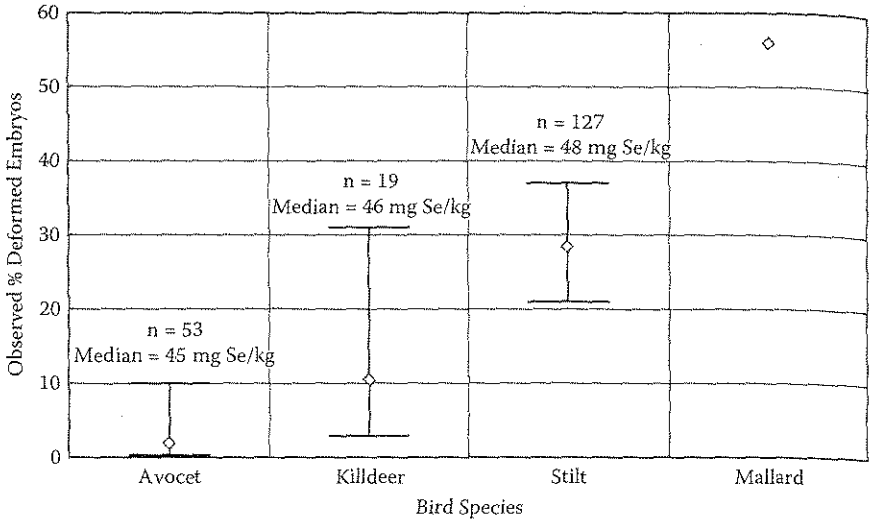

FIGURE 6.8 Comparative embryonic sensitivity of waterbird species to Se based on eggs containing 40 to 60 mg Se/kg. Whiskers are the binomial 95% confidence limits. Data for avocets, killdeer, and stilts are from Skorupa, unpublished field data; comparable field data for mallards in the 40 to 60 mg Se/ kg exposure range are unavailable, the point plotted here for mallards is from laboratory data in Heinz et al. 1989.

is expressed as clutch inviability if at least one egg in the clutch did not hatch, is a more sensitive endpoint that teratogenesis. Clutch-wise EC10s for black-necked stilts, again depending on how the data were analyzed, have been reported to correspond with egg Se concentrations (in dry weight) of 6 to 7 mg/kg (USDOI 1998) and 14 mg/kg (EC11.8; Lam et al. 2005). Adams et al. (2003) used the equation from Skorupa (1998b) to relate the probability of a given egg Se concentration resulting in an inviable egg or an inviable clutch. They then derived EC10 values ranging from 21 to 31 mg/kg dw for stilt egg inviability (Table 6.4). Because the studies of stilt and mallard egg hatchability are based on different sampling units (affected clutches for stilts and affected eggs for mallards), estimates of EC10 values across these two species for egg hatchability do not necessarily reflect relative sensitivity (Skorupa 1999). The apparent overlap in mallard egg inviability EC10s and stilt clutch-wise EC10s suggests that mallards and stilts may be similarly sensitive to Se; however, the analysis of Adams et al. (2003) suggests stilts may be less sensitive. Mallards and black-necked stilts are clearly more sensitive to Se than the American avocet, for which hatchability does not begin to decline until Se concentrations in eggs exceed 60 mg Se/kg (USDOI 1998; Table 6.4). In a captive-feeding study, hatchability of black-crowned night-heron (*Nycticorax nycticorax*) eggs was not adversely affected by dietary exposure to selenomethionine, resulting in a mean concentration of 16.5 mg Se/kg dw in eggs, suggesting that this species is less susceptible to reproductive impairment than mallards (Smith et al. 1988; Table 6.4). However, because an effect-threshold was not established in that study, heron susceptibility relative to that of more tolerant species like the American avocet remains unknown. In red-winged blackbirds (*Agelaius phoeniceus*), the estimated threshold

TABLE 6.4
Hatchability of Bird Eggs in Relation to Se Concentrations in Eggs

Species	Concentration (mg Se/kg dw)	Effect	Considerations	References
Mallard	7.7–15	Hatchability EC10	Based on analysis of results from 5 to 6 captive-feeding studies	Adams et al. 2003 Ohlendorf 2003, 2007 Beckon et al. 2008
Black-necked stilt	6–7	Threshold point for hatchability effects	Field study where eggs were randomly selected from each clutch and Se hatch success compared to that of group with a lower range of Se concentrations	USDOI 1998
	21–31	Hatchability EC10	Same field study as above but different data analysis approach used	Adams et al. 2003
Black-crowned night heron	16.5	NOAEL	Captive feeding study—mean egg Se concentration for group fed Se in their diet	Smith et al. 1988
American avocet	60	Low bound of a concentration range associated with reproductive impairment in 20% of clutches	Field study	USDOI 1998
Red-winged blackbird	22	Threshold for adverse effects	Field study examined hatchability of eggs incubated to full term	Harding 2008
Eastern screech owl	37	5% hatchability of incubated eggs (adjusted to hatchability of control eggs)	Captive study in which parent birds were fed diet containing 13.2 ppm Se (wet Wt)	Wiemeyer and Hoffman 1996
American kestrel	25	Hatchability NOAEL	Captive study in which parents were fed a diet containing 12 ppm Se (dry wt)	Santolo et al. 1999

for reproductive impairment was approximately 22 mg Se/kg dw egg (Harding 2008; Table 6.4). Thus, it appears that reproductive impairment at the EC10 level may begin in the range 10 to 20 mg Se/kg dw in eggs of several species. However, some species, such as the American avocet and possibly the black-crowned night-heron, remain unaffected at higher Se concentrations.

Mechanisms that might explain why a given concentration of Se is more likely to cause reproductive impairment in some species than in others have not yet been

elucidated. Hoffman et al. (2002) examined sublethal effects and oxidative stress in stilts and avocets from two Se-contaminated sites and a reference site. Oxidative stress was greater in avocets from the most contaminated site than in those from the other sites, while oxidative stress in stilts was not noticeably higher at the most contaminated site. Selenium concentrations in avocet hatchlings at the most contaminated site averaged about 30 mg Se/kg, whereas stilt embryos averaged 21 mg Se/kg at that site. Despite the greater concentrations of Se in avocets than in stilts, it remains surprising that oxidative stress appeared less severe in the latter species given that it is about twice as sensitive as the former to the teratogenic effects of Se. Thus, in the case of these 2 species, there is no evidence that their relative susceptibility to Se-induced teratogenicity can be explained by inter-specific differences in the potential of Se to produce oxidative stress.

The documented inter-specific variability in sensitivity of reproductive impairment to Se exposure suggests that predicting the severity of toxic effects in an array of species in a field situation based on the results of laboratory toxicity tests using different "indicator" species may be fraught with uncertainty. Moreover, as evidenced from the disparity between stilts and avocets, even closely related species can be differentially susceptible to Se. The assessment of Se risk to aquatic-dependent species of birds would be improved by a better understanding of the physiological and biochemical mechanisms underlying embryotoxicity of Se in birds.

6.4.9 Mammals

Mammalian species that rely on water for their food such as mink (*Mustela vison*), otter (*Lutra canadensis*), muskrat (*Ondatra zibethicus*), raccoon (*Procyon lotor*), and beaver (*Castor canadensis*) also have the potential for significant Se accumulation via dietary exposure in aquatic settings. Studies conducted in both contaminated (Clark 1987; Clark et al. 1989) and non-contaminated (Wren 1984; Wren et al. 1986; Gamberg et al. 2005) settings clearly demonstrate the ability of these semiaquatic mammals to accumulate Se.

In the 1984 studies on Kesterson National Wildlife Refuge (California), Clark (1987) collected 332 mammalian organisms comprising 10 different species. Of those species, muskrats comprised 18 organisms split between Kesterson (n = 11) and a reference site (Volta Wildlife area, n = 7). Average liver Se concentrations for Kesterson organisms were as high as 32.1 mg/kg dw, while Volta liver concentrations were 1.5 mg/kg dw. By contrast, other rodent species such as the house mouse (n = 18; *Mus musculus*), western harvest mouse (n = 45; *Reithrodontomys megalotis*), and California ground squirrel (n = 5; *Spermophilus beecheyi*), whose diet consisted principally of vegetation not associated with pond water, had liver concentrations of 14.5, 15.3, and 1.82 mg Se/kg dw, respectively. However, other small mammals not often considered "aquatic-dependent," such as the California vole (*Microtus californicus*), deer mouse (*Peromyscus maniculatus*), and the ornate shrew (*Sorex ornatus*), also had elevated Se levels in liver with the shrew having the highest average concentration of 92.7 mg/kg dw (n = 8). After noting these elevated concentrations in small mammals, Clark et al. (1989) also collected raccoons from Kesterson (n = 8) and Volta (n = 8) to investigate accumulation and potential effects on a tertiary mammalian predator. Selenium concentrations in blood, liver, hair, and feces from animals trapped on

Kesterson averaged 2.61 mg/L, 19.9, 28.3, and 21.6 mg Se/kg dw, respectively, compared to concentrations of 0.27 mg/L, 1.69, 0.93, and 1.05 mg Se/kg dw in the same tissues collected from Volta raccoons. Gamberg et al. (2005) documented Se concentrations in the tissues of mink from noncontaminated areas of the Canadian Yukon. Average total Se concentrations ($n = 98$) found in kidney, liver, and brain were 7.45, 4.5, and 1.70 mg Se/kg (converted from wet weight concentrations using % moisture data provided in the paper). In a relatively pristine setting in an undisturbed watershed in south central Ontario (Canada), Wren (1984) reported similar low Se concentrations in the liver, kidney, intestine, and muscle of raccoon, beaver, and otter.

Regarding sensitivity of mammals, none of the documented incidences of elevated Se concentrations in tissues indicated any negative impacts on the organisms. The most quantitative studies conducted were the Clark (1987) and Clark et al. (1989) studies completed on Kesterson National Wildlife Refuge. In Clark (1987), 88 California voles were captured on Kesterson and 89 were captured on Volta. Sex ratios, condition, organ weights, and reproductive status were observed and were not significantly different for most of the endpoints. Interestingly, no pregnant females were found on Kesterson (0/50), whereas 12 out of 29 females were found pregnant on Volta. The author attributed this finding not to Se, but rather to other factors, such as diet, that resulted in different reproductive schedules between the two sites. In the raccoon study (Clark et al. 1989), body condition, blood parameters, histopathology, and evidence of pregnancy were investigated in the animals. No effects were noted, and one pregnant female from Kesterson was trapped. In ecological settings such as Kesterson, aquatic-dependent mammalian species appear unaffected by high Se exposure despite extirpation of fish populations and severe effects on avian waterfowl (Ohlendorf et al. 1986). In fact, studies (e.g., Wren et al. 1986; Khan and Wang 2009) suggest that Se in the tissues of wild mammals has beneficial effects in the binding of mercury, thereby reducing its impacts to the organism. It should be noted, however, that relative to the avian and fish literature, very little quantitative, robust data exist that rigorously examine the effects of Se in wild mammalian species; moreover, no controlled dosing experiments were found. From the available toxicity data for laboratory rodents, the most sensitive endpoint subsequent to Se exposure appears to be growth (USEPA 2007). This subtle endpoint would be difficult to document in field studies with wild species.

One likely explanation for the lower sensitivity of mammals compared to other vertebrates, such as fish and birds in these Se-contaminated settings, is the differences they have in the ratio of essentiality and toxicity compared to fish and birds. As stated at the beginning of this chapter, that ratio might range from 7 to 30 for fish and birds (but typically less than 10), while this ratio in mammals is greater. NRC (2006) reviewed nutritional adequacy and toxicity for several elements for laboratory rodents (Watson 1996) and small ruminants. For laboratory rodents, they recommended a diet with 0.15 mg Se/kg as adequate, whereas a diet containing 5.0 mg Se/kg may lead to effects on growth in weanling pups (i.e., 33-fold difference). The recommendation for cervids and other ruminants for adequate Se in the diet is 0.25 mg/kg^{-1}, whereas 5.0 mg/kg^{-1} in food is the maximum tolerable level (20×). No data exist for wild aquatic-dependent mammalian species, but of these two ratios, the species of most potential concern (i.e., small to midsize mammals) the ratio of 33-fold difference between essentiality and toxicity is likely the most pertinent.

6.4.10 Comparative Sensitivity Summary and Implications for Se Threshold Development

Fish and birds are generally the most sensitive taxa to Se in aquatic systems; the sensitivity of reptiles and amphibians is less understood. There are several options for evaluating whether environmental concentrations of Se at a site are potentially toxic. For example, Se may be measured in environmental media, including water, sediment, fish, or bird diets, fish tissue, and bird tissue. Further, different tissues may be analyzed. Tissues commonly analyzed in fish are whole body, muscle, liver, ovaries, and eggs. Overall, there is a clear consensus that tissue Se is the most reliable indicator of toxic effects in the field (Chapter 7). Previous sections have discussed the many site-specific factors that influence Se speciation and bioaccumulation, such as variable intake of Se-rich foods, factors that ultimately dictate whether the Se in an aquatic system is toxic for fish or birds. However, by measuring Se in fish or bird tissues, site- and species-specific variation in Se bioaccumulation can be determined. Over the past 15 years, several studies have recommended tissue-based Se benchmarks for fish and birds (Lemly 1993b, 1996a; USDOI 1998; DeForest et al. 1999; Hamilton 2002; Ohlendorf 2003; Adams et al. 2003; Chapman 2007). Moreover, the USEPA (2004) has developed a draft fish tissue-based Se criterion. Although there is not always consensus on the benchmarks recommended, there is consensus that tissue-based Se benchmarks are the most appropriate medium for linking Se concentrations to toxicity. The next step is to develop toxicity studies that directly relate Se toxicity to the internal Se concentration in the organism.

For fish and birds, the most appropriate tissue for linking Se concentrations with toxicity is still under debate. As discussed above, for both fish and birds, the critical exposure route and endpoint for Se toxicity is maternal transfer of Se to the eggs and subsequent effects on either the developing larvae (fish) or embryo (birds). As such, egg Se appears to be the most appropriate tissue for linking fish or bird Se exposure to toxicity. For fish, likely Se benchmarks are eggs and ovaries because interspecific egg- and ovary-based Se EC10 values are reasonably consistent across species tested to date (Figure 6.4). For birds, use of egg Se rather than dietary Se is beneficial because many birds are highly mobile, and egg Se is a direct reflection of Se exposure during the critical reproductive period. The use of these toxicity thresholds in site-specific evaluations is discussed further in Section 6.7.1.

6.5 FACTORS THAT MODIFY Se TOXICITY

6.5.1 Interactions

Table 6.5 summarizes the known interactions of Se with other factors (e.g., metals/metalloids, biotic and abiotic stressors). Interactions with other trace elements (e.g., As, Cd, Cu, Pb, Hg, S, Tl, Sb, Pb, Zn) are typically antagonistic, although there are three exceptions, as noted below. At extremely high concentrations, these antagonistic effects may be reduced or nonexistent (Stanley et al. 1994).

The most well-known antagonistic reactions occur between organic Hg (methyl Hg [meHg]) and organic Se; both reduce the bioavailability and thus the toxicity of

TABLE 6.5
Selenium Interactions with Other Factors

Factor	Organism	Interaction	Reference
Arsenic (As)	Chicken	As reduced Se toxicity (e.g., effects on growth, egg production and weight, hatching success)	Hill 1975; Howell and Hill 1978; Thapar et al. 1969
	Mallard	As improved hatching success and reduced embryo mortality due to Se	Stanley et al. 1994
		As reduced duckling mortality, growth, and hepatic lesions due to Se; Se similarly reduced As toxicity	Hoffman et al. 1992a
	As plant hyperaccumulator	Se alleviated As oxidative stress and improved As uptake essential to this plant	Srivastava et al. 2009
	Rat	As reduced Se toxicity	Moxon 1938; Dubois et al. 1940; Palmer et al. 1983
		Synergistic toxicity of As and organic Se	Kraus and Ganther 1989
	Dog	As prevented Se poisoning in dogs	Rhian and Moxon 1943
Boron (B)	Mallard	Bo and Se synergistically suppressed growth, altered blood protein and, with restricted dietary protein, decreased survival	Hoffman et al. 1991b
		No interaction with adult health, reproductive success, duckling growth and survival, tissue residues	Stanley et al. 1996
Cadmium (Cd)	Chicken	Cd reduced Se toxicity	Hill 1975; Howell and Hill 1978
	Rat	Se reduced liver damage, more so in combination with Zn	Jihen et al. 2008, 2009
	Cabbage and Lettuce	Se together with Zn reduced Cd absorption by roots	He et al. 2004
Copper (Cu)	Chicken	Cu reduced Se toxicity	Hill 1975; Howell and Hill 1978
Lead (Pb)	Cabbage and Lettuce	Se together with Zn reduced Cd absorption by roots	He et al. 2004
Mercury (Hg)	Chicken	Hg reduced Se toxicity	Hill 1975; Howell and Hill 1978
	Cricket	Se increased survival and growth	Ralston et al. 2006, 2008
	Aquatic oligochaete	Se reduced meHg bioaccumulation	Nuutinen and Kukkonen 1998

(*continued*)

TABLE 6.5 (CONTINUED)
Selenium Interactions with Other Factors

Factor	Organism	Interaction	Reference
	Four waterbird species	Se may act as a binding site for demethylated Hg in bird livers, reducing the potential for secondary toxicity	Eagles-Smith et al. 2009
	Fish, birds, other fauna	Se reduced meHg toxicity	Cuvin-Aralar and Furness 1991; Belzile et al. 2006; Yang et al. 2008
	Fish	Se reduced bioaccumulation of meHg in fish	Paulsson and Lindbergh 1989; Southworth et al. 1994, 2000; Chen et al. 2001; Peterson et al. 2009
	Mallard	Antagonistic effects in adults, but additive or synergistic in embryos	Hoffman and Heinz 1998; Heinz and Hoffman 1998
Sulfur (S)	Fish, invertebrates	S reduces Se bioaccumulation	Hansen et al. 1993; Bailey et al. 1995; Ogle and Knight 1996; Riedel and Sanders 1996
Tellurium (Te)	Chicken	Tl reduced Se toxicity	Hill 1975; Howell and Hill 1978
Tin (Sb)	Chicken	Sb reduced Se toxicity	Hill 1975; Howell and Hill 1978
Lead (Pb)	Chicken	Pb reduced Se toxicity	Hill 1975; Howell and Hill 1978
Zinc (Zn)	Rat	Zn and Se together provide more protection against Cd-induced liver damage than either alone	Jihen et al. 2008, 2009
Cyanobacterial algal bloom	Tilapia	Se reduced algal toxicity (oxidative stress and histological lesions)	Atencio et al. 2009
UV radiation	Plant	Se protects against UV-reduced plant growth	Hartikainen and Xue 1999
Oxidative stress	Chinook salmon	Dietary α-tocopherol + ascorbic acid decreased oxidative stress, but Se and Fe did not	Welker and Congleton 2009

Note: That both organic and inorganic Se interactions are considered.

the other, possibly via the formation of metabolically inert mercury selenides (HgSe; Ralston et al. 2006; Yang et al. 2008; Khan and Wang 2009; Peterson et al. 2009). The one exception of additive or synergistic effects in mallard embryos (Heinz and Hoffman 1998) despite antagonistic effects in adult mallards (Hoffman and Heinz 1998) remains unexplained. For other trace elements, antagonistic reactions between As and Se are also well documented. The single exception (Kraus and Ganther 1989) also remains unexplained. Two studies examined the interactive effects of Se and B

on mallards and found opposing results: Hoffman et al. (1991b) reported synergistic toxicity, while Stanley et al. (1996) did not. Selenium decreased toxicity to tilapia due to an algal bloom (Atencio et al. 2009) and toxicity to a plant due to UV-exposure (Hartikainen and Xue 1999). However, it had no effect on oxidative stress in chinook salmon (Welker and Congleton 2009).

With the possible exception of interactions between organic Se and methyl Hg, the mechanisms and extent of antagonistic reactions between Se and other factors (e.g., other elements, and biotic and abiotic stressors) are unclear. Further, there are a few studies showing synergistic, not antagonistic, interactions that remain unexplained.

6.5.2 Nutritional Factors

Interactions of Se with other stressors has been shown to result in increased or decreased toxicity, depending on the particular stressors involved (Section 6.5.1; Table 6.5). Nutritional factors can also significantly influence Se toxicity. Selenium appears to enhance the nutritional uptake of other essential elements by at least some plants. For example, He et al. (2004) observed that the addition of Se and Zn to soils increased the uptake of the essential elements Fe, Mn, Cu, Ca, and Mg by Chinese cabbage and lettuce, with positive effects on growth. Reduced dietary protein interacts with increased Se exposure to increase Se toxicity to mallards (Hoffman et al. 1991b, 1992a,b). Similarly, dietary restriction of protein increases Se toxicity in growing chickens and mammals (Combs and Combs 1986). Conversely, increased dietary protein reduced Se toxicity in rats (Gortner 1940). Increased dietary protein reduced Se toxicity in chickens in one study (El-Begearmi and Combs 1982) but in another study showed no effect (Hill 1979), possibly because different types of protein impart different levels of protection against Se toxicity (Levander and Morris 1970). Variations in available dietary protein and types of protein may therefore influence the toxicity of Se to water birds and mammals and likely also influence the toxicity of Se to other vertebrates.

In contrast to the beneficial effects of increased dietary protein, excess dietary carbohydrate enhanced dietary Se toxicity in rainbow trout (Hilton and Hodson 1983). The mechanisms of increased toxicity due to carbohydrates and of decreased toxicity due to protein are unknown, as are the extent of these demonstrated effects to different organisms than those tested.

6.5.3 Tolerance

Chapman (2008) summarizes the extensive research in the literature pertaining to metals tolerance, including both heritable genetic adaptation (i.e., modifications of tolerance by changes in heritable genetic material) and physiological acclimation (i.e., shifting of tolerance within genetically defined limits by up-regulating existing processes). Selection for metals-resistant populations, resulting in inheritable genetic adaptations, can occur following long-term exposure to elevated metals concentrations. Shorter-term exposures can result in reduced metals uptake and increased detoxification mechanisms to deal with metal exposure without selection for a metal-tolerant population. Tolerance is largely metal specific, and resistance to one stressor

does not necessarily confer resistance to other stressors. In addition, there are energetic costs to acclimation; reduced energy available for growth or reproduction may also result in some level of reduced fitness. Genetic adaptation can involve reduced genetic diversity via selective pressure eliminating the least fit (i.e., most sensitive) organisms but may or may not involve energetic costs.

The possibility of Se tolerance in aquatic organisms has been suggested (e.g., for westslope cutthroat trout [*Salmo clarkii*] by Kennedy et al. 2000), but remains to be convincingly demonstrated. However, Se tolerance has been demonstrated in terrestrial organisms. For example, plants showing hypertolerance to Se (i.e., Se hyperaccumulators) are the result of long-term evolutionary selective pressures in naturally Se-enriched environments (Chiang et al. 2006), which protects them from herbivory and pathogens but has also led to the evolution of tolerant herbivores (Freeman et al. 2006; Quinn et al. 2007). Acclimation and adaptation are normal responses of organisms to adjust the boundaries of their ecological niches in order to maximize their chances to survive and reproduce.

The potential for Se tolerance in aquatic biota such as fish is an important research need. Such research could involve, for instance, parallel toxicity tests of suspected "tolerant" species (e.g., collected from areas with highly elevated Se concentrations) and intolerant species (e.g., collected from areas with relatively low Se concentrations typical of background conditions). Undertaking such tests, particularly interspecific experimental designs, would be powerful for testing site-specific adaptation versus acclimation of populations.

6.5.4 Marine versus Freshwater Environments

Some marine animals bioaccumulate much greater concentrations of Se than freshwater species without teratogenic effects on offspring (Muir et al. 1999). Higher concentrations of Se have been observed across many taxa from bacteria to brine shrimp (*Artemia* spp.) to marine mammals to seabirds (Dietz et al. 2000; Brix et al. 2004; Oremland et al. 2004). An important exception appears to be fish, which apparently do not bioaccumulate greatly higher concentrations of Se in uncontaminated waters than do freshwater fish (Stewart et al. 2004; Campbell et al. 2005; Kelly et al. 2008; Burger et al. 2007; McMeans et al. 2007). While accumulations to elevated levels can occur in long-lived, piscivorous fish such as tunas, swordfish, and marlins (Nigro and Leonzio 1996; Eisler 2000; Table 6.6), it is unclear whether Se is impacting these wild populations.

Se accumulation to elevated levels in the tissues of many marine species without apparent *ill* effect is an interesting observation, because it suggests that there are fundamental distinctions in the essentiality and toxicity of Se in animals adapted to seawater and to hyper- rather than hypo-osmotic waters. High Se uptake by primary producers and high assimilation efficiency or feeding rates as discussed in Chapter 5 explain higher tissue concentrations of Se in marine species, but do not clarify the exact mechanisms that mitigate toxicity. Potential explanations for greater Se tolerance include complexation with, and detoxification of, Hg (Koeman et al. 1973; Eagles-Smith et al. 2009). Given that Se is central to antioxidant mediation, it is not surprising that Se requirements could be greater in marine species; however, mechanisms, and the extent and interaction with other stressors such as UV radiation (which poses a greater

TABLE 6.6
Selenium Tissue Concentrations in Wild Marine and Freshwater Species

Organism	n	Location	[Se] mg/kg dw (Mean ± SEM)	Reference
		Seabirds		
Common eiders (*Somateria mollissima*), nesting females	40	North shore and islands of Beaufort Sea, Alaska	36.1 ± 1.7 B	Franson et al. 2004
Common eiders	20	North shore and islands of Beaufort Sea, Alaska	2.28 ± 0.09 E	Franson et al. 2004
Common eiders, males	30	Yukon-Kuskokwim Delta (Y-K Delta), Aleutian Islands, Saint Lawrence Island, Alaska	9.29[a] (2.00–21.2) L	Stout et al. 2002
Common eiders, females	21	Y-K Delta, Aleutian Islands, Saint Lawrence Island	7.85[a] (2.50–44.0) L	Stout et al. 2002
King eiders (*Somateria spectabilis*), males	33	Barrow, AK, USA	34.5[a] (14.3–93.0) L	Stout et al. 2002
King eiders, females	21	Barrow, AK, USA	27.6[a] (9.60–63.1) L	Stout et al. 2002
Steller's eiders (*Polysticta stelleri*), males	4	Barrow, Togiak, and Kotzebue, AK, USA; Russia	25.6 (13.0–56.8) L	Stout et al. 2002
Steller's eiders, females	6	Barrow, Togiak, and Kotzebue, AK, USA; Russia	17.6[a] (8.18–31.4) L	Stout et al. 2002
Spectacled eiders (*Somateria fischeri*), males	28	Y-K Delta, Barrow, and Saint Lawrence Island, AK, USA; Russia	124[a] (35.4–401) L	Stout et al. 2002
Spectacled eiders, females	10	Y-K Delta, Barrow, and Saint Lawrence Island, AK, USA; Russia	43.5[a] (4.98–235) L	Stout et al. 2002
Spectacled eiders, males	2	Saint Lawrence Island and Togiak, AK, USA	76.0[a] (75 to 77) L	Henny et al. 1995
Spectacled eiders, females	1	Saint Lawrence Island, AK, USA	35.0 L	Henny et al. 1995
White-winged scoters (*Melanitta fusca*), males	8	Cape Yakataga, Cape Suckling, AK, USA	21.7[a] (12 to 75) L	Henny et al. 1995
Black-legged kittiwakes (*Rissa tridactyla*)	63 (21)	Prince Leopold Island, Canada	3.52[a] ± 0.26 E	Braune 2007
Black-legged kittiwakes	10 (1)	Prince Leopold Island, Canada	36.2 L	Braune and Scheuhammer 2008

(*continued*)

TABLE 6.6 (CONTINUED)
Selenium Tissue Concentrations in Wild Marine and Freshwater Species

Organism	n	Location	[Se] mg/kg dw (Mean ± SEM)	Reference
Northern fulmars (*Fulmarus glacialis*)	93 (31)	Prince Leopold Island, Canada	4.12[a] ± 0.15 E	Braune 2007
Northern fulmars	10 (1)	Prince Leopold Island, Canada	34.4 L	Braune and Scheuhammer 2008
Thick-billed murres (*Uria lomvia*)	90 (30)	Prince Leopold Island, Canada	2.65[a] ± 0.10 E	Braune 2007
Thick-billed murres	29 (4)	Ivujivik, Salluit, Coats Island, Prince Leopold Island, Canada	5.68 ± 0.17 L	Braune and Scheuhammer 2008
Black guillemots (*Ceppus grylle*)	15 (2)	Ivujivik, Prince Leopold Island, Canada	9.95 ± 0.85 L	Braune and Scheuhammer 2008
Black guillemots	10	North water polynya	15.85 ± 5.96 L	Campbell et al. 2005
Black-winged scoters (*Melanitta perspicillata*), females	3	Cape Yakataga, AK, USA	22.1[a] (14 to 32) L	Henny et al. 1995
Glaucous gulls (*Larus hyperboreus*)	7 (2)	Akpatok Island, Coats Island, Canada	14.40 ± 5.20 L	Braune and Scheuhammer 2008
Glaucous gulls	9	North water polynya	12.65 ± 1.90 L	Campbell et al. 2005
Thayer's gull (*Larus thayeri*)	1	North water polynya	15.78 ± nr L	Campbell et al. 2005
		Marine Mammals		
Porpoise: *Phocoena phocoena*	3	Dutch coast of North Sea		
Dolphins:				
Tursiops truncates	3	A dolphinarium		
Delphinus delphis	2	New Zealand	24.52[a]	
Lagenorhynchus obscures (species pooled)	1	New Zealand	(2.0 to 266.4) L	
Sotalia guianensis	2 (1)	Surinam		Koeman et al. 1973
Common seal (*Phoca vitulina*)	16	Wadden Sea, Dutch coast of North Sea	46.71[a] (2.3 to 416.3) L	Koeman et al. 1973
Ringed seal (*Phoca hispida*)	9	North water polynya	33.93 ± 22.38 L	Campbell et al. 2005
Polar bears (*Ursus maritimus*) M:F = 5:7	13	East Baffin Island, Canada	30.50 ± 12.49[b] L	Rush et al. 2008
M:F = 3:1	13	Lancaster Sound, Canada	47.89 ± 12.49[b] L	Rush et al. 2008

TABLE 6.6 (CONTINUED)
Selenium Tissue Concentrations in Wild Marine and Freshwater Species

Organism	n	Location	[Se] mg/kg dw (Mean ± SEM)	Reference
M:F = 7:5	12	Northern Baffin Island, Canada	38.73 ± 12.65[b] L	Rush et al. 2008
M:F = 8:3	11	Southeast Beaufort Sea, Canada	72.66 ± 12.82[b] L	Rush et al. 2008
M:F = 5:6	11	Southeast Hudson Bay, Canada	10.99 ± 12.82[b] L	Rush et al. 2008
1994 to 1999 M:F = 0:6	6	Chukchi Sea, AK, USA	16.58 ± 10.29[b] L	Rush et al. 2008
1983 to 2000 M:F = 19:27	46	Avanersuaq, Greenland	18.65 ± 12.22[b] L	Rush et al. 2008
1983 to 2000 M:F = 40:42	82	Ittoqqortoormiit, Greenland	16.15 ± 11.22[b] L	Rush et al. 2008
		Marine Fish		
Leopard shark (*Triakis semifasciata*)	2	North San Francisco Bay, CA, USA	4, 10 L	Stewart et al. 2004
Pacific sleeper shark (*Somniosus pacificus*) M:F = 7:7	14	Prince William Sound, AK, USA	1.81 ± 0.09 L	McMeans et al. 2007
Greenland shark (*Somniosus microcephalus*) M:F = 14:10	24	Cumberland Sound, Greenland	1.72 ± 0.10 L	McMeans et al. 2007
Starry flounder (*Platichthys stellatus*)	3	North San Francisco Bay, CA, USA	7[c] (3 to 13) L	Stewart et al. 2004
Yellowfin goby (*Acanthogobius flavimanus*)	12 (3)	North San Francisco Bay, CA, USA	2[c] (1 to 7) L	Stewart et al. 2004
Arctic cod (*Boreogadus saida*)	2	North water polynya	5.13 ± nr L	Campbell et al. 2005
Pacific cod (*Gadus macrocephalus*)	16	Nikolski (Umnak I), AK, USA	4.63[a] (2.78 to 7.62) L	Burger et al. 2007
Pacific cod	6	Adak Island, AK, USA	5.29[a] (3.30 to 8.49) L	Burger et al. 2007
Pacific cod	77	Amchitka Island, AK, USA	4.66[a] (0.94 to 13.32) L	Burger et al. 2007
Pacific cod	42	Kiska Island, AK, USA	4.33[a] (0.23 to 12.42) L	Burger et al. 2007
Japanese tunas, 4 species	nr	Japan	33.3 to 50.0 L	Eisler 2000
Tuna (*Thunnus thynnus*)	nr	Southern Tyrrhenian Sea, Italy	10 L	Nigro and Leonzio 1996

(*continued*)

TABLE 6.6 (CONTINUED)
Selenium Tissue Concentrations in Wild Marine and Freshwater Species

Organism	n	Location	[Se] mg/kg dw (Mean ± SEM)	Reference
		Marine Fish		
Swordfish (*Xiphias gladius*)	nr	Southern Tyrrhenian Sea, Italy	19 L	Nigro and Leonzio 1996
Black marlin (*Makaira indica*)	nr	nr	4.7 to 45.0 L	Eisler 2000
Striped bass (*Morone saxitilis*)	nr	nr	1.0 to 4.3 L	Eisler 2000
		Anadromous Fish		
Striped bass, adult (*Morone saxatilis*)	15	North San Francisco Bay, CA, USA	12[c] (8 to 14) L	Stewart et al. 2004
Striped bass, juvenile	16	North San Francisco Bay, CA, USA	13[c] (12 to 14) L	Stewart et al. 2004
Atlantic salmon (*Salmo salar*) chinook salmon (*Oncorhynchus tshawytscha*) coho salmon (*Oncorhynchus kisutch*)	110	8 British Columbia (Canada) salmon farms	0.67 ± 0.01[c] M	Kelly et al. 2008
Wild chinook, coho, chum (*Oncorhynchus keta*) sockeye (*Oncorhynchus nerka*) chum (*Oncorhynchus keta*) pink (*Oncorhynchus gorbuscha*)	91	Coastal British Columbia, Canada	0.57 ± 0.02[c] M	Kelly et al. 2008
		Freshwater Fish		
White sturgeon (*Acipenser transmontanus*)	15	North San Francisco Bay, CA, USA	25[c] (13 to 32) L	Stewart et al. 2004
Sacramento splittail (*Pogonichthys macrolepidotus*)	12 (3)	North San Francisco Bay, CA, USA	13[c] (7 to 20) L	Stewart et al. 2004
Bluegill sunfish (*Lepomis macrochirus*)	3 to 5 (1)	San Joaquin River Basin, CA, USA	1.85[a] (0.46 to 5.28) W	Saiki et al. 1992
Common carp (*Cyprinus carpio*)	3 to 5 (1)	San Joaquin River Basin, CA, USA	2.05[a] (0.57 to 4.28) W	Saiki et al. 1992
Mosquito fish (*Gambusia affinis*)	3 to 5 (1)	San Joaquin River Basin, CA, USA	1.63[a] (0.53 to 3.44) W	Saiki et al. 1992

TABLE 6.6 (CONTINUED)
Selenium Tissue Concentrations in Wild Marine and Freshwater Species

Organism	n	Location	[Se] mg/kg dw (Mean ± SEM)	Reference
		Freshwater Fish		
Largemouth bass (*Micropterus salmoides*)	3 to 5 (1)	San Joaquin River Basin, CA, USA	2.31[a] (0.92 to 5.25) W	Saiki et al. 1992

[a] Geometric mean (range).
[b] Converted from wet weight, assuming 70% moisture content.
[c] Median and (25th to 75th percentile).
Note: nr = not reported. WB = whole body, B = blood, E = egg, L = liver, dw = dry weight. If samples were pooled, the number of pools is shown in parentheses. Unless specified, gender or sex ratios were not reported. Data are representative rather than all inclusive.

threat in marine waters with low spectral absorbance compared to freshwater systems; Johannessen et al. 2003) warrant further investigation in oceanic ecosystems.

Selenium plays an important role in salt tolerance. It is well known that biota, from bacteria to mammals, use organic compounds to osmoregulate in highly saline environments. Such compounds are called "compatible solutes" because they do not inhibit cell macromolecules or function. Some organic osmolytes contain Se. The most extreme example occurs in the salt-tolerant plant, *Astragalus bisulcatus*, which has a nutritional requirement for Se and has been observed to accumulate tissue concentrations of Se > 500 mg/g. *Astragalus* accumulates approximately 80% of its Se as methylselenocysteine (Brown and Shrift 1982). This is the same presumed osmolyte that has been identified in the Se-tolerant diamondback moth (*Plutella xylostella*) and its parasite, the Se-tolerant wasp (*Diadegma insulare*) (Freeman et al. 2006). In fish, most osmolytes are neutral free amino acids such as taurine and glycine, small carbohydrates, such as myo-inositol, and methylamines, such as trimethylamine oxide (Fiess et al. 2007). Interestingly, bivalves surrounding deep-sea thermal vents contain elevated concentrations of thiotaurine but only in tissues containing sulfur-fixing endosymbionts (Brand et al. 2007), suggesting that Se could serve in the place of sulfur. However, to our knowledge, it has not been demonstrated that bivalves or marine vertebrates enzymatically fix Se as components of organic osmolytes. As part of their osmoregulatory physiology, many marine animals have salt glands, which provide them with an additional excretory pathway to maintain intracellular homeostasis. Higher concentrations of metals and Se in the tissues of salt glands suggest that this organ is also an excretory pathway for Se (Burger et al. 2000). Future research needs include manipulative testing of this correlative relationship as well as investigation of whether bivalves or marine vertebrates enzymatically fix Se as a component of organic osmolytes.

Marine birds differ from freshwater birds in the way in which they partition Se among different tissues. This difference may have consequences for the relative susceptibilities

of marine and freshwater birds to Se-mediated reproductive toxicity. Some marine birds accumulate relatively high concentrations of Se in their livers, but Se concentrations in their muscles, blood, and eggs are similar to those in freshwater birds. For example, hepatic Se concentrations in several species of marine birds that spend all, or most, of their lives, at sea averaged 37 and 44 mg/kg dw, respectively, whereas hepatic Se concentrations in several species of freshwater birds averaged only 11 mg/kg dw. Moreover, apparently natural Se concentrations as high as 300 mg/kg dw have been recorded in some marine birds. Conversely, Se concentrations in the eggs of marine and freshwater bird species are comparable; marine species average about 4 mg Se/kg dw in eggs, whereas freshwater species average just under 3 mg Se/kg dw (Ohlendorf and Harrison 1986; Bischoff et al. 2002; Braune and Simon 2004; Braune and Scheuhammer 2008; Braune et al. 2002; DeVink et al. 2008b; Grand et al. 2002; Harding et al. 2005; Scheuhammer et al. 1998, 2001; Skorupa and Ohlendorf 1991; Trust et al. 2000). Franson et al. (2007) fed common eiders (a marine bird) a commercial diet containing either 20 or 60 mg Se/kg dw as selenomethionine. Mean Se concentrations in liver and muscle and peak levels in blood of birds fed the low and high Se diets were as follows (mg/kg dw): 351 and 735, 85 and 88, and 14 and 17, respectively. In comparison, when mallards, a species found most often in freshwater ecosystems, were fed a commercial diet containing either 25 or 60 mg Se/kg dw as selenomethionine, their livers contained Se concentrations only about 25% as high as those in eiders (98 and 200 mg/kg on the low- and high-Se diets, respectively), whereas concentrations in their muscles and blood were similar to those in eiders (O'Toole and Raisbeck 1997). It remains unknown how seabirds appear to preferentially store Se in their livers when compared to freshwater birds. Nevertheless, this preferential storage of Se in livers by marine birds compared to freshwater birds coupled with the similarity between the two groups of birds in Se levels in blood may be one way in which marine birds appear able to avoid excessive in ovo exposure to Se, even when consuming diets with relatively high naturally occurring Se concentrations. Although marine birds may differ from freshwater birds in tissue partitioning of dietary Se to liver and eggs, thus affording a greater degree of protection to the former group from reproductive problems associated with excessive exposure to dietary Se, the higher concentrations of Se in livers of marine birds may have consequences for toxic effects in adults. The frequency of occurrence and severity of liver and feather lesions were similar between common eiders fed diets containing 20 and 60 mg Se/kg dw and mallards fed diets containing 25 and 60 mg Se/kg dw as selenomethionine (O'Toole and Raisbeck 1997; Franson et al. 2007).

6.5.5 Winter Stress Syndrome

Fish species that experience prolonged winter periods are at risk of overwinter mortality due to many natural factors such as low temperature (i.e., thermal stress), low oxygen (i.e., hypoxia/anoxia), reduced food availability (i.e., starvation), increased predation, disease, and parasitism (reviewed in Hurst 2007). Most of these factors act more severely in smaller fish due to size-dependent changes in surface: volume relationships (Post and Parkinson 2001). Since survival of fish beyond the first year of life is a critical determinant of future year class strength, significant overwinter mortality of early life stages (e.g., young-of-the-year) can cause a "recruitment bottleneck"

and negatively impact fish population dynamics (Post and Parkinson 2001; Hurst 2007). Thus, factors that increase the frequency of overwinter mortality of fish can negatively influence the sustainability of fish populations. In aquatic ecotoxicology, the term *winter stress syndrome* has been proposed to describe the potential for contaminants to potentiate overwinter mortality (Lemly 1996b). The three conditions noted by Lemly (1996b) for winter stress syndrome to occur are 1) the presence of a metabolic stressor (natural or anthropogenic), 2) cold water temperatures, and 3) the response of fish to cold with reduced activity and foraging. Importantly, the potential that a given stressor will lead to winter stress syndrome depends on its propensity to increase metabolism. Metabolic stressors can include such variables as exposure to inorganic or organic chemicals, parasites, altered pH, or elevated concentrations of suspended sediment. The presence of multiple metabolic stressors increases the probability of occurrence of winter stress syndrome (Lemly 1993c).

The winter stress syndrome hypothesis is based on a laboratory study in which juvenile bluegill sunfish were exposed for 180 days to dietary and waterborne Se under either summer or winter conditions (Lemly 1993c). Winter conditions of low water temperature (4 °C) exacerbated the toxicity of Se, indicated by increased mortality, decreased condition factor (weight-at-length) and decreased energy (lipid) stores. Lemly's (1993c) laboratory study has recently been replicated except for photoperiod, with similar results overall (McIntyre et al. 2008). The juvenile bluegill sunfish studies (Lemly 1993c; McIntyre et al. 2008) indicated that Se likely causes increased metabolism, which may occur, in part, through oxidative stress (Spallholz and Hoffman 2002; Palace et al. 2004). Thus, Se has the potential to cause winter stress syndrome in field settings.

Although the concept of winter stress syndrome is a scientifically sound hypothesis, it has rarely been tested explicitly under field conditions of elevated Se, or other chemical, exposures (Janz 2008). Higher lipid metabolism and lower triglyceride levels in fish experiencing chronic metal exposure in the fall relative to fish inhabiting uncontaminated lakes have been reported (Levesque et al. 2002). Other studies investigated aspects of the winter stress syndrome hypothesis in several native fish species inhabiting areas receiving complex metal mine effluents containing elevated Se (Bennett and Janz 2007a,b; Kelly and Janz 2008, 2009; Weber et al. 2008; Driedger et al. 2009). In these studies, juvenile fish were collected just prior to ice-on and immediately following ice-off from lakes and creeks receiving discharges from uranium mining (Bennett and Janz 2007a,b) and base metal (copper or nickel) mining (Driedger et al. 2009) operations in northern Canada. Measures and indicators of growth (length, weight, condition factor, muscle RNA:DNA ratio, muscle proteins) and energy storage (whole-body lipids, whole-body triglycerides, liver triglycerides, and liver glycogen) were determined in fish collected along gradients of exposure and from reference sites. Based on the winter stress syndrome concept, it was hypothesized that fish collected in spring from all sites (both exposure and reference) would exhibit decreases in growth and energy storage measures compared to the previous autumn and that these measures would be decreased to a greater extent in juvenile fish collected from exposure sites. In contrast to these hypotheses, juvenile northern pike, burbot (*Lota lota*), fathead minnow (*Pimephales promelas*), creek chub (*Semotilus atromaculatus*), and white sucker (*Catostomus commersoni*) collected from exposure

sites generally exhibited similar or greater growth and energy stores in spring when compared to the previous autumn, in comparison with reference sites (Bennett and Janz 2007a,b; Driedger et al. 2009). Only slimy sculpin (*Cottus cognatus*) exhibited changes in energy stores (whole-body triglycerides) that were consistent with the winter stress syndrome hypothesis (Bennett and Janz 2007b). In contrast, the majority of these species collected from reference sites exhibited overwinter decreases in energy stores that were consistent with the overwinter fish biology literature (Hurst 2007). In these studies, Se residues were also measured in selected species. Whole-body Se concentrations in juvenile fathead minnows and white sucker ranged from 11 to 43 mg/kg dw (Driedger et al. 2009) and in juvenile northern pike muscle ranged from 17 to 23 mg/kg dw at exposure sites (Kelly and Janz 2009). At both study locations, Se was the predominant element consistently elevated in fish tissues.

Further work investigating whether winter stress syndrome occurs under field conditions of Se exposure is needed to fully assess the hypothesis (Janz 2008). As Lemly (1993c) notes, basic knowledge of life history characteristics and feeding ecology, particularly for juvenile fish, would allow identification of potentially vulnerable fish species in temperate regions of the world. Unfortunately, there are few studies with direct observations and concrete conclusions regarding the feeding ecology of juvenile fishes. It is possible that winter stress syndrome is most important in species at the northern limit of their ranges, and future studies should focus on this aspect. Knowledge of local fish community ecology is essential when assessing the potential importance of overwinter mortality in aquatic ecotoxicological investigations of Se.

Limited evidence suggests that winter stress may occur in avian species as well (e.g., chickens [Tully and Franke 1935]). Heinz and Fitzgerald (1993) exposed mallard ducks ($n = 5$) to diets supplemented with 0, 10, 20, 40, and 80 mg Se/kg dw for 16 weeks (November 16–March 7) in outdoor pens. By week 8, all the ducks in the 80 mg Se/kg dose level died. Ninety-five percent of the ducks exposed to 40 mg Se/kg died after 11 weeks. After 16 weeks, 75% of the birds exposed to the 20 mg Se/kg diet survived, while none died at the 0 and 10 mg Se/kg dose levels. The authors observed the majority of mortalities between December 26 and January 11, when temperatures were consistently below freezing. Following necropsy, these animals were extremely emaciated with no body fat. In similar studies (Heinz et al. 1987, 1989, 1990) conducted during spring and summer, little to no mortality occurred in mallards exposed to diets supplemented with 10 to 32 mg Se/kg. The authors noted that in this and other studies birds fed the elevated Se diets became sick and did not eat as much food, which ultimately resulted in emaciation and death. However, they argued that the increased energy demands of cold weather probably forced the ducks to eat more Se-treated food, leading to effects faster than would occur in warm weather.

6.5.6 Comparison of Fish Early Life Stages, Juveniles, and Adults

The larval life stage of an anadromous fish species may not receive a large Se dose via maternal transfer, but it could begin to feed on an elevated Se diet if rearing occurs in an area with elevated Se concentrations. Therefore, although maternal transfer is often considered to be the classic pathway by which early life stages are exposed to Se, dietary Se exposures alone by young juvenile fish are important

in some situations. Hamilton et al. (1990), for example, hypothesized that juvenile chinook salmon feeding in nursery areas of the Sacramento–San Joaquin Delta (California), an area with elevated food-chain Se due to irrigation in the San Joaquin river system, could be adversely affected by Se when they undergo parr-smolt transformation prior to migration to the sea. To test this, the authors conducted 90-d dietary organic Se exposures and observed a dose-dependent reduction in juvenile survival and growth. In a 10-d seawater challenge test following 120-d organic Se exposures in brackish waters, they observed a dose-dependent reduction in smolt survival. Elevated Se levels apparently caused a physiological imbalance that impaired performance during the seawater challenge test (Hamilton et al. 1990), but the precise mechanism for this effect is unclear.

In another juvenile fish study, Vidal et al. (2005) exposed juvenile rainbow trout to dietary selenomethionine for 90 days. In addition to growth, they measured the reduced and oxidized glutathione and thiobarbituric acid reactive substance levels in the livers of the trout to assess oxidative damage caused by Se. Lipid peroxidation and GSH:GSSG ratios were unchanged by all Se treatments (dietary exposures up to 18 mg Se/kg dw), suggesting that mechanisms other than oxidative stress caused the observed toxicity.

6.5.7 Population-Level Linkages (Fish and Birds)

In practice, establishing cause–effect linkages between toxic effects at the individual organism level and adverse impacts on populations is challenging and has rarely been demonstrated in the field of ecotoxicology. However, as discussed in Chapter 3, there have been several examples of significant declines of fish (e.g., the Belews Lake incident) and water bird (e.g., the Kesterson Reservoir incident) populations caused by Se contamination of aquatic ecosystems. These impacts were primarily due to reproductive failure resulting from embryo- and early-life-stage mortality of fish (mainly due to deformities) and birds (mainly due to failed hatchability). Based on extensive data available for the Belews Lake fish population collapses (Appendix A), Lemly (1997a) proposed a "teratogenicity index" that relates the proportion of deformed fish to an adverse population impact. To our knowledge, Lemly's (1997a) deformity index has not been applied to other aquatic ecosystems contaminated with Se. Nevertheless, it has provided one of the clearest linkages to date between individual and population level effects in ecotoxicology and illustrates the significant potential hazard posed by Se in aquatic ecosystems. Linkages between organic Se toxicity and population-level impacts are discussed further in Section 6.6.

6.6 LINKAGES BETWEEN Se TOXICITY AT SUBORGANISMAL AND ORGANISMAL LEVELS TO POPULATION- AND COMMUNITY-LEVEL IMPACTS

6.6.1 Potential Population and Community-Level Impacts

Selenium, when in excess of nutritional levels, can be toxic at multiple levels of ecological organization. Lower trophic level organisms (primary producers and most primary consumers) are less sensitive to Se toxicity than higher trophic level organisms,

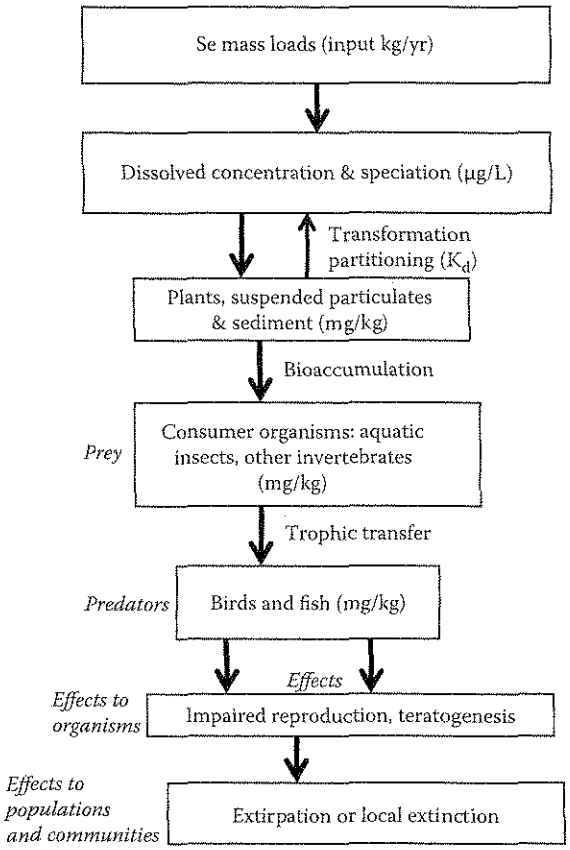

FIGURE 6.9 Conceptual model describing linked factors that determine the effects of Se on ecosystems. (After Presser and Luoma 2006.)

although as noted in Section 6.5, this has not been fully established. Thus, since lower trophic-level organisms are relatively insensitive to Se toxicity, they act as vectors transferring Se to more vulnerable organisms such as fish, birds, and potentially also to oviparous amphibians and reptiles. Selenium has the potential to adversely impact ecologically and toxicologically vulnerable fish, waterbird, amphibian, and reptile populations and cause profound ecosystem changes (Figure 6.9). For example, Se enrichment of reservoir environments (Chapter 3; Appendix A) provide classic examples of adverse effects through different levels of biological organization; they effectively comprise integrated whole-ecosystem "experiments" of trophic transfer, resulting in a cascade of population and community impacts (e.g., Gillespie and Baumann 1986; Sorensen 1991; Lemly 2002). Recovery from these impacts has also been documented once Se sources were eliminated (Lemly 1997b; Crutchfield 2000; Finley and Garrett 2007).

6.6.1.1 Fish

In contrast to reservoirs or other contained lentic environments, some lotic environments have few or no adverse effects, despite elevated Se concentrations. This is the case even when the species exposed in the field are the same or closely related to those demonstrating adverse effects in laboratory settings at lower Se tissue concentrations. Appendix B illustrates an extreme example in streams from the Great Plains region of southeast Colorado. Those streams had greatly elevated Se concentrations in water and biota, relative to effects-thresholds for fish. The elevated Se was apparently long-standing and predominantly of natural origin via the regional geology and groundwater. One minnow species, the central stoneroller (*Campostoma anomalum*), was common in a stream with a mean Se concentration of 418 µg/L and with whole-body tissue concentrations of 20 to 50 mg Se/kg dry weight. Appendix B includes speculation on several plausible factors that might explain this species' persistence in a stream with those extremely high Se concentrations, including 1) whether the low organic carbon and high sulfate in sediments limited the accumulation of Se in sediments and subsequent trophic transfer to fish, 2) whether central stonerollers were inherently less sensitive to Se than better studied species, and/or 3) whether the reproductive strategy of central stonerollers and perhaps other small-bodied Great Plains fishes that have evolved under harsh conditions makes their populations more resilient than less fecund, longer-lived fish species (Matthews 1987).

Data from Thompson Creek (Idaho) illustrate the difficulty of detecting responses in stream fish communities exposed to moderately elevated Se concentrations. Thompson Creek is a cold-water, mountain stream with elevated Se concentrations from groundwater input originating in waste dumps for overburden from a large, open-pit molybdenum mine. Species richness and abundance of fish and benthic communities in the stream have been monitored for about 20 years at fixed monitoring points, using consistent methods. Since Se contamination was detected in the late 1990s, Se residues in sediments, organic detritus, invertebrates, and the dominant fish species, shorthead sculpin (*Cottus confusus*) and cutthroat/rainbow trout hybrids ("trout") were measured annually for 7 years (CEC 2004; GEI 2008b).

At Thompson Creek, trout whole-body Se residues ranging from 4 to 14 mg Se/kg dw had no apparent relation to trout density. Sculpin densities were negatively related to whole-body Se residues over a range of 9 to 18 mg Se/kg dw, although the relationship was not statistically significant ($p = 0.12$) (Figure 6.10). In contrast, in a tributary and adjacent pond with water concentrations ranging from 30 to 35 µg Se/L, trout collected in 2000 had average whole-body concentrations of about 13 mg Se/kg dw and, when the site was resurveyed in 2003, no trout could be found (Mebane 2000; CEC 2004). These limited observations suggest that in a small, closed population, whole-body tissue residues approximately 2-fold higher than laboratory thresholds of 6 mg Se/kg dw (DeForest et al. 1999) could contribute to a local extirpation.

The Thompson Creek long-term monitoring record also illustrates how natural variability in fish densities can make all but persistent population declines difficult to detect through field monitoring. Over the 20-year period of record at the upstream reference site along Thompson Creek, the median year-to-year variation

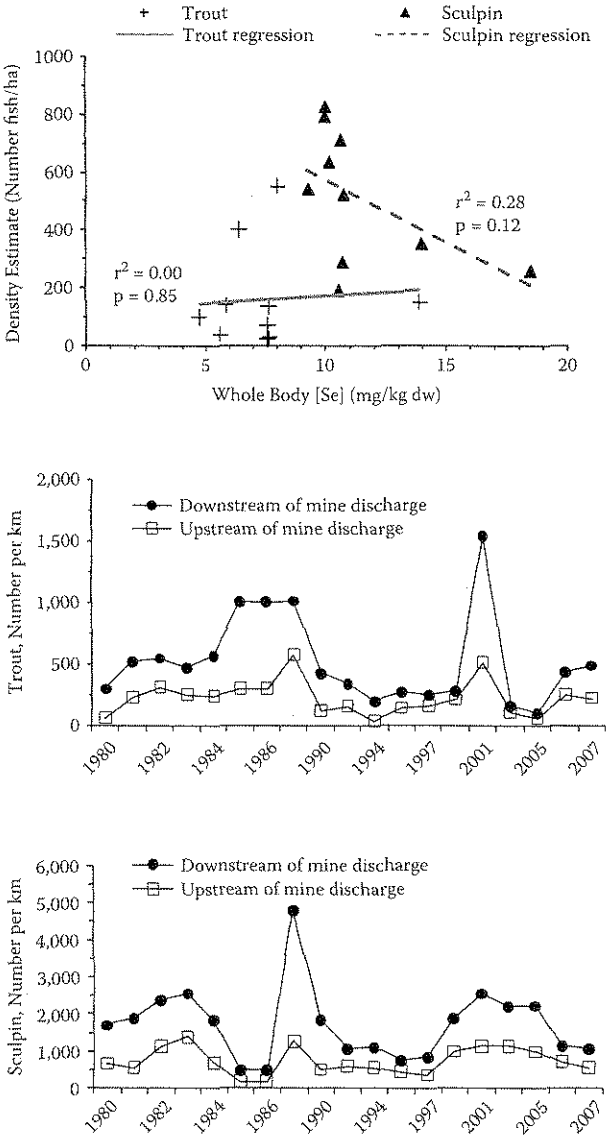

FIGURE 6.10 Whole-body Se residues (dw; A) and variability in density estimates for trout (B) and sculpin (C) in Thompson Creek, Idaho. (Data from GEI Consultants 2008b, 2008c; Canton and Baker 2008.)

in trout densities was 36% (mean 76%) and ranged from 1 to 260%. With sculpin, the median year-to-year variation in densities was only 22% (mean 70%) but ranged from 1 to 590% (Figure 6.10B,C; GEI 2008b).

6.6.1.2 Birds

For birds, an avian population's ability to tolerate even low rates of reproductive impairment from evolutionarily novel sources, such as anthropogenically mobilized Se, is strongly contingent on how well the population has adjusted to historic evolutionary pressures such as nest predation (Terborgh 1989). Evaluating the linkage between Se-induced individual toxicity and population-level implications requires demographic modeling or long-term field monitoring. Detecting a Se signal is most feasible for closed populations such as fish populations in reservoirs, lakes, and ponds. Detecting a Se signal in bird populations is challenging because they are highly mobile, often accessing resources across entire continents, and their populations are almost always demographically open. Consequently, modeling and field investigation of population level effects are most appropriate at the landscape scale and require meta-population analyses.

The mere perpetual presence from year-to-year of normal densities of breeding water birds at a Se-contaminated study site is not evidence for lack of population-level effect. For example, the abundance of breeding water birds at Kesterson Reservoir did not crash (or even notably decline) despite the complete reproductive failure of several species (Ohlendorf 1989). Because the bird populations at Kesterson were demographically open, even though Kesterson was indisputably a demographic sink, immigration from unaffected source populations substituted for the lost productivity at Kesterson. In this respect, Se exposure of breeding birds may often be more forgiving at the population level than for fish because flight links them to a much larger meta-population and a larger effective demographic landscape. However, the landscape-level relationship between demographic source and demographic sink subpopulations can be complex and can lead to counter-intuitive outcomes. In other words, populations are structured by actual habitat quality (e.g., low value of the invertebrates as food due to selenosis) rather than perceived habitat quality (e.g., high level of aquatic invertebrate productivity). In such cases, a seemingly attractive sink site (such as Kesterson was) can ultimately drain source populations dry. The critical landscape level impact is then geographically removed from the contaminated site itself.

Basic demographic modeling of potential population-level impacts from Se exposure in wild birds requires data relating tissue levels of Se to overwinter survivorship, and posthatch mortality. These data are meager. Winter stress syndrome has been reported for Se-exposed birds in two studies (Tully and Franke 1935; Heinz and Fitzgerald 1993). Similarly, there has been only one rigorous (i.e., radio telemetry) field study of Se effects on posthatch survivorship of water bird chicks (Marn 2003). Posthatch survivorship might be critical because experimental (Heinz 1996; Fairbrother et al. 2004) and nontelemetered observational field studies (Williams et al. 1989) suggest that posthatch mortality can be a larger component of overall Se-induced reproductive impairment than embryo mortality (see discussion in Section 6.2.2.2.3 and in Seiler et al. 2003). For example, Marn (2003) studied the least Se-sensitive species of waterbird, the American avocet, according to current

data, and yet clearly documented a Se effect on posthatch survivorship of chicks. Until more exposure–response information is produced for the endpoints of overwinter survivorship and posthatch chick survivorship, the basis for demographic modeling of potential population-level effects in birds will be weak.

6.6.2 Population Recovery

Recovery of populations and communities from Se stress is a counterpart to the preceding examples of case studies on studying population- or community-level effects. The following discussion focuses on lentic fish, since population-level effects with birds are difficult to interpret because of the opportunity for immigration from other populations, as discussed above. The case study of Belews Lake not only provides an example of Se impacts on fish populations, it also provides data for evaluating the recovery of a severely impacted system after Se discharges to the lake were eliminated in 1985. Selenium-contaminated water was first discharged into Belews Lake in fall 1975, and in 1976 no centrarchids in the 0–64 mm size range were found and only 8 black and flat bullheads were found in the projected young-of-the-year size range (Cumbie and Van Horn 1978). Based on fish sampling conducted in 1977, 1980, and 1981, diversity ranged from 7 to 13 taxa and estimated biomass ranged from 5.67 to 15.02 kg/ha (Barwick and Harrell 1997). From 1984 through 1994 diversity ranged from 14 to 22 taxa and biomass ranged from 36.39 to 79.66 kg/ha (Barwick and Harrell 1997). In 1984 fish taxa and biomass were dominated by Se-tolerant species (e.g., green sunfish, common carp), but by 1994 fish biomass was dominated by more Se-sensitive species, such as bluegill sunfish and largemouth bass (Barwick and Harrell 1997). Overall, based on taxa diversity and standing stock estimates, fish populations in Belews Lake had generally recovered by the mid-1990s (Finley and Garrett 2007).

Correspondingly, Se concentrations in fish tissue have declined as fish populations in Belews Lake recovered. In 1976, Se concentrations in composite muscle samples collected from areas of the lake with elevated aqueous Se were on the order of 7.96 to 22.3 mg Se/kg ww (31.8 to 89.2 mg Se/kg dw assuming 75% moisture) and in 1977 Se concentrations in composite muscle samples ranged from 6.32 to 54.6 mg Se/kg ww (25.3 to 218 mg Se/kg dw assuming 75% moisture) (Cumbie and Van Horn 1978). By 1992, maximum muscle Se concentrations were 2.6, 3.8, and 3.2 mg Se/kg ww (10.4, 15.2, and 12.8 mg Se/kg dw assuming 75% moisture) for catfish, green sunfish, and bluegill sunfish, respectively (Barwick and Harrell 1997). Similarly, Finley and Garret (2007) reported that median estimated whole-body Se concentrations in carp, redear sunfish, and crappie were approximately 22, 17, and 18 mg Se/kg dw, respectively, in 1994–1996, and approximately 9, 10, and 9 mg Se/kg dw in 2004–2006. Lemly (1997b) also reported substantial reductions in fish tissue Se concentrations from pre-1986 compared to 1996. For example, Se concentrations in bluegill eggs ranged from 40–133 mg Se/kg dw prior to 1986 and from 3–20 mg Se/kg dw in 1996. Similar reductions were observed for other fish species. The reductions in Se concentrations in fish tissue at Belews Lake and the corresponding recovery of fish populations supports the hypothesis that Se toxicity at the organism level has a direct

relationship to population-level impacts. In the late 1970s and early 1980s, Se concentrations in fish tissues exceeded all known levels demonstrated to cause toxicity, while by the mid-1990s, Se concentrations in fish tissues approached or were below recommended toxicity thresholds.

However, reductions in environmental Se concentrations or persistent residual contamination in tissues do not necessarily indicate biological recovery or the lack thereof.

Differing recovery trajectories have been demonstrated for different ecosystem components in the reservoir studies. In both Belews Lake and Hyco Lake, recovery was relatively fast (~2 to 3 years) for overall fish assemblage biomass as well as recolonization by fish species that were previously extirpated from the reservoir. However, the relative composition of the fish assemblages was markedly different from that pre-exposure or reference areas (Lemly 1997b; Crutchfield 2000). Some 20 years after major reductions in Se loading were implemented in Belews Lake, the fish community composition was largely stabilized, approaching a new equilibrium (Finley and Garrett 2007). This emphasizes the general challenge of defining recovery and the limitations of the concept of ecosystem "equilibrium."

6.6.3 Factors That May Confound Linking Se Fish Tissue Residues to Population-Level Impacts

As discussed in the previous section, effects thresholds derived from fish tissues are logically more relevant to predicting effects in aquatic ecosystems than thresholds based on abiotic media (water, sediment) because tissue-based thresholds bypass the confounding influences of variable exposures and bioavailability to the organism (Chapter 7). However, factors such as density compensation and the role of refugia may make population impacts difficult to detect.

6.6.3.1 Density Compensation

Density compensation in this context refers to patterns where densities of organisms increase, food and space become limiting, and individuals begin to suffer from lack of resources or from competition and interference. When habitats are overcrowded, competition for food and space intensifies, causing individuals to expend more energy foraging or defending territories, which may result in lower growth or displacement to suboptimal habitats. Such shifts in energy allocation can reduce overwinter survival and increase the risk of being captured and eaten. In a declining population, as densities thin, overcrowding is relaxed, resulting in compensatory increases in growth, survival, and the overall chance of reproductive success. Thus, density compensation for overcrowding will tend to stabilize populations and lessen extinction risks as populations decline. In contrast to these compensatory effects, when habitats are undercrowded, population declines may lead to further declines in population growth (also referred to as Allee or depensation effects). For example, when spawning adults are scarce, they may not find each other or expend more energy finding each other and reduce the probability of fertilization (Chapman 1966; Liermann and Hilborn 2001; Rose et al. 2001). Thus, depending on the compensatory capacity, loss of individual organisms may not result in commensurate effects to the overall population abundance.

Modeling of cutthroat trout populations in tributaries of the upper Snake River (Idaho) has demonstrated the theoretical potential for density dependence to compensate for substantial juvenile mortality. Using functions fit to toxicity test data from the literature, Van Kirk and Hill (2007) projected decreased prewinter survival and growth of juveniles due to Se exposure. Population-level effects of Se were simulated by relating growth reductions to reduced fecundity and by extrapolating the Se-survival to reduced population size. However, because juvenile survival in trout is highly density-dependent, particularly during winter when individuals compete for limited concealment cover, trout populations may compensate for increased juvenile mortality via reduced density-dependent effects. Van Kirk and Hill (2007) suggested that population-level effects would be lower than individual-level effects until juvenile mortality rates exceeded 80%.

In another example of fish populations compensating for large juvenile mortalities, a toxic algal bloom along the coast of southern Norway killed an average of 60% of age-0 cod (*Gadus morhua*) populations with no effects on the cod population persisting beyond 3 years (Chan et al. 2003). If fish populations compensated for additive mortality to early life stages at rates close to those estimated by Van Kirk and Hill (2007), the release from density-dependent inhibition could mitigate fatal early life stage deformities caused by Se. However, density dependence is controversial because it is notoriously difficult to reliably estimate from even well-studied populations, and unreliable estimates can seriously underestimate the risks of population decline or extinction (Barnthouse et al. 1984; Rose et al. 2001). Indeed, Ginzberg et al. (1990) caution that even when working with plausible estimates of density dependence, "by choosing the model of density dependence carefully, one can achieve any quasi extinction risk desired." A prime example of the result of overgeneralizing the mitigating effects of density compensation, are marine fisheries that have become depleted under management plans that recommended annual harvests of approximately 25% of the population (Myers et al. 1997).

Further, other trout stream populations may have substantially less compensatory reserve than that estimated for the Snake River cutthroat trout populations. The cutthroat trout populations modeled by Van Kirk and Hill (2007) compensated for Se-induced mortality because this additional mortality occurred in winter before the density-dependent effects. If density-dependent mortality occurred in juvenile trout not in winter but rather during spring or summer when low flows and high temperatures were a limiting factor, this compensatory ability could be greatly reduced, if not lost completely (Elliott 1989; Van Kirk and Hill 2007).

6.6.3.2 Role of Refugia

Hydrologically intact stream networks provide important habitat linkages, reduce extinction risks through metapopulations, and reduce time to recovery from disturbances among many other important stream and landscape ecological functions. Stream networks also provide important access to refugia from naturally occurring or anthropogenic hazardous conditions such as high summer water temperatures or

low flows (Sedell et al. 1990; Poole et al. 2004). These connections undoubtedly add resilience to some fish populations. With birds, as discussed in Section 6.6.1.2, immigration from unaffected demographic source populations would likewise mitigate declines in abundance at smaller scales.

Palace et al. (2007) have shown characteristic deformities related to elevated Se concentrations in larval rainbow trout derived from adults that were captured downstream from a coal mining operation in Alberta (Canada). Deformities such as these might be expected to reduce recruitment in the population and overall population numbers, but adult rainbow trout are still present in the affected streams. One suggestion for this apparent contradiction is that adult fish migrate to the affected system from nearby areas with background Se concentrations. Analyses of concentrations of Se in annual growth zones of otoliths (calcified structures in the inner ear of fish) suggested that fish from the mine-impacted system are recent immigrants from nearby reference streams not receiving Se-bearing effluent (Palace et al. 2007). In general, while such movements may allow stream fish to reduce risks from elevated Se, they complicate efforts to relate experimental effects of elevated Se to field populations. Some larger-bodied fishes such as suckers and the larger salmonids may move over 100 km seasonally. Smaller stream-resident salmonids or centrarchids commonly move on the order of 100 m to several km, whereas some small-bodied fish may only move on the order of 10 m (Munther 1970; Hill and Grossman 1987; Gibbons et al. 1998; Munkittrick et al. 2001; Baxter 2002; Schmetterling and Adams 2004). It follows that small-bodied fishes such as sculpin and small cyprinids or percids may be more locally vulnerable to elevated Se in streams and more indicative of local effects than more motile species.

The challenge and difficulty of reliably extrapolating effects from laboratory experiments to populations or ecosystems has been long recognized and is by no means unique to Se (Chapman 1983; Suter et al. 1985). Unambiguous identification of ecosystem level effects due to Se is rare in ecotoxicology. The Hyco and Belews Lake (North Carolina) power plant cooling reservoirs had been operated with a history of systematic baseline and ongoing chemical and biological monitoring with testing facilities and staff scientists onsite (Chapter 3; Appendix A). These Experimental Reservoir Area settings essentially provided before-after-control-impact and recovery study designs. The reservoirs were closed, dammed systems with limited opportunity for emigration or migration to refugia. In these settings, profound Se-related, population-level impacts were clearly apparent. In contrast, in interconnected streams motile fish species may freely migrate in and out of areas with Se enrichment. Large, readily captured species common to streams in western North America such as suckers, fluvial trout, and mountain whitefish (*Prosopium williamsoni*) often have annual ranges of hundreds of kilometers (Baxter 2002). Further, the hydrology of streams is usually more variable than that of power plant cooling reservoirs. Spates and drought features of stream environments often contribute to highly variable stream fish populations. This combination of high natural variability, compensating factors, and the generally less efficient trophic transfer of Se in lotic versus lentic systems (Orr et al. 2006) makes population- or community-level effects of Se difficult to detect.

6.7 SITE-SPECIFIC STUDIES FOR DETECTING PRESENCE OR ABSENCE OF Se EFFECTS

A general challenge in evaluating risks from elevated Se in aquatic environments is the difficulty of extrapolating effects from laboratory to field settings and generalizing between different field settings (Lemly and Skorupa 2007; McDonald and Chapman 2007; Ohlendorf et al. 2008). A tiered assessment approach is recommended, starting with relatively low-cost monitoring of exposure and comparison to screening benchmarks (e.g., water or tissue concentrations). Lemly and Skorupa (2007) recommended proceeding to a Se management plan that includes loading reductions if Se tissue benchmarks are exceeded, without necessarily investing time and resources into site-specific toxicity testing or population assessment. Ohlendorf et al. (2008) focus on best practices for site-specific assessment of bioaccumulation and trophic transfer of Se in aquatic ecosystems. McDonald and Chapman (2007) recommend that, if tissue residue benchmarks are exceeded, more definitive risks should be evaluated through reproductive toxicity testing of fish collected from the site of concern, and/or assessment of fish populations in the area of interest. This section assesses the use of reproductive toxicity testing and/or resident population assessment at higher tiers of a site-specific evaluation relative to the appropriate toxicity endpoint, using fish as an example.

6.7.1 Selenium Exposure Measurement

To evaluate whether environmental concentrations of Se at a site are at potentially toxic levels, Se can be measured in a variety of abiotic (sediments, water) and biotic (diet, tissue) media. However, as noted herein (Section 6.5.10), tissue Se is the most reliable predictor of toxic effects (Lemly 1993b, 1996a; USDOI 1998; DeForest et al. 1999; Hamilton 2002; Ohlendorf 2003; Adams et al. 2003; USEPA 2004; Chapman 2007). Further, estimates of risk with the lowest uncertainty are derived from measurements of Se in ovaries and/or eggs (Chapter 7).

6.7.2 Reproductive Effects Testing

McDonald and Chapman (2007) and Janz and Muscatello (2008) recommend reproductive toxicity testing conducted by capturing spawning fish from exposure and reference sites, collecting gametes for fertilization, rearing the fertilized eggs to the swim-up fry stage, and examining the fry for prevalence of deformities. This approach provides highly relevant site-specific information regarding Se effects. While relatively few species have been tested in this manner (n = 8; Figure 6.4), thresholds of effects for mortality or deformities of early life stage fish have been remarkably similar across those species when expressed as a factor of egg or ovary concentrations. Effects thresholds for 8 species in Figure 6.4, as egg or ovary Se concentration, ranged over a factor of 1.4, from 17 to 24 mg/kg dw. If the species of interest at a site have previously been tested, obtaining site-specific data on concentrations of Se in ovaries or eggs can provide useful estimates of risk without undertaking site-specific reproductive toxicity testing. If similar species have been

tested, uncertainty is probably within about a factor of 2 (i.e., not unreasonable for initial screening). However, the number of species tested to date remains small, thus it is possible that untested species may have lower or higher thresholds than the range shown in Figure 6.4. Clearly, properly designed and executed species-specific studies are the best way to reduce uncertainties in risk estimates if one is not sure of the relative sensitivities of the species of interest. As discussed below in Section 6.7.3, additional considerations are required to assess the possible population impact of any reproductive toxicity noted in fish species sampled from natural systems.

Other than the fathead minnow, we are not aware of reproductive toxicity testing with small-bodied fish (Figure 6.4). Small-bodied fish would be expected to experience greater exposures to locally elevated contaminant concentrations than more motile, larger-bodied fish species with lower site fidelity such as salmonids or catostomids (Gibbons et al. 1998; Munkittrick et al. 2001). For instance, sculpin may only move tens of meters or less over their lifetimes, while even "stream resident" salmonids typically travel hundreds of meters to tens of kilometers, and larger salmonids move much greater distances (Schmetterling and Adams 2004).

Thus, reproductive testing of small-bodied species is recommended, but may require pilot studies to determine husbandry requirements. Geckler et al. (1976) successfully conducted chronic toxicity tests with progeny from field-collected darter (Percidae) and minnow (Cyprinidae) species. Besser et al. (2007) were able to induce laboratory spawning and conduct early-life stage tests with mottled sculpin (*Cottus bairdi*) that were collected shortly before their normal spawning period and held for a short period of time under conditions similar to those in their native streams. However, they found that Ozark sculpin (*C. hypselurus*), which were similarly handled, failed to produce viable eggs, as did shorthead sculpin, which were collected before their spawning season and held overwinter in the laboratory. These studies suggest that some, but not necessarily all, field-collected small-bodied fish may be amenable to laboratory spawning and testing, particularly if sexually mature brood stock are collected shortly before their normal spawning period and held for a short period of time under conditions similar to those in their native streams. Because of their higher site fidelity, shorter life spans, and usual abundance, small-bodied fish may be useful sentinel species for monitoring effects of Se in streams and rivers, and the available data set on reproductive effects of Se should be expanded to these species.

6.7.3 Monitoring and Assessing Fish Populations

Field assessments of fish (and other relevant, potentially Se-affected) populations are an intuitive approach to evaluating whether elevated Se concentrations could be linked to community-level impacts in aquatic ecosystems. Technical details relevant to the design of monitoring programs for detecting effects of Se are available elsewhere (Environment Canada 2002; Guy and Brown 2007; Johnson et al. 2007; Ohlendorf et al. 2008). This subsection focuses on general principles, using fish as an example.

Linking effects observed during field monitoring of fish populations to causal factors can be challenging. Selenium toxicity to early life stages may not be reflected

at the population level because of density compensation or immigration from other source populations (Section 6.6). There are practical limitations in using field monitoring to reliably detect and link apparent population-level effects to elevated Se or other stressors. Further, monitoring programs and statistical tests are not customarily designed to detect noneffects. However, such biomonitoring can be optimized to deal with Se exposure. Monitoring and accompanying statistics are customarily intended to detect effects with a given likelihood of falsely detecting an effect when no true effect is present (Type I error, α). Natural variability and measurement error inherent to biomonitoring programs make statistically based comparative procedures necessary to detect adverse effects; traditionally, these have been significance or null hypothesis tests. This approach has been repeatedly criticized across disciplines on logical grounds, but the practice has endured (e.g., Berkson 1942; McCloskey 1995; Johnson 1999; Newman 2008). Alternative approaches for evaluating trends or comparing sites that should be considered include comparing confidence intervals for nonequivalence or testing if linear trends are near zero (Parkhurst 2001; Dixon and Pechmann 2005; McGarvey 2007).

The companion problem of Type II errors (β, failing to detect adverse effects that are present) has been ignored in some monitoring studies. However, the Environment Canada (2002) Environmental Effects Monitoring program seeks to balance risks of misguided remediation or unnecessary expenses resulting from spurious results (Type I error) with risks of undetected ecosystem degradation (Type II error) by setting $\alpha = \beta$, with both at 0.1 or less. In practice, tests that incorporate statistical power must also specify a priori the size of effect that they are trying to detect.

For a monitoring variable, selecting the magnitude of difference that distinguishes between a biologically important or negligible effect is an important aspect of monitoring since effect size allows ranking of the importance of impacts or alternative outcomes as well as hypotheses testing. However, selecting the effects magnitude of interest for a monitoring variable is not a trivial problem. The stipulation of an effect size threshold is a judgment about biology, not simply a statistical or procedural decision, and relies on many underlying explicit or implicit judgments about the biological importance of an effect of a nominated magnitude. Because of the difficulty in selecting broadly applicable criteria for what constitutes a biologically significant effect among different species and populations, some authors have argued that the selection of "critical" sizes for effects monitoring may need to be made by subjective consensus of those with relevant expertise (Mapstone 1995; Reed and Blaustein 1997; Munkittrick et al. 2009).

In the absence of a regulatory definition that designates critical effect sizes for interpreting monitoring results, three general approaches have been suggested (Munkittrick et al. 2001, 2009):

1) Select an arbitrary difference from reference conditions, such as two standard deviations (SD) from the mean, or other statistical extremes of the reference condition, such as the 5th or 10th percentile.
2) Use a predetermined difference that constitutes a change of significant magnitude to cause concern for the endpoint (e.g., a 25% decline).
3) Attempt to define statistically significant differences of smaller magnitudes.

The first approach selects effect sizes by quantifying natural variability under reference conditions, and by basing the effect size on exceedance of some statistic such as two standard deviations from the mean or the region of data outside of 90% to 95% of the possible observations under reference conditions (Kilgour et al. 2007; Munkittrick et al. 2009). Reference conditions may be defined more or less objectively, although the rules and criteria for defining reference conditions such as "natural," "least-disturbed," and "best attainable" are subjective and debatable (Mebane and Essig 2003; Stoddard et al. 2006). Documenting environmental variability under reference conditions is fundamental because the higher the variability, the less likely detection of trends becomes. Environmental variability is not simply stochasticity or measurement error, but often includes dynamic stabilities, that is, properties that vary in a repeated, reasonably predictable fashion. Examples include year-to-year differences in stream flows, temperatures, and thus, habitat suitability, seasonal differences, and spatial variability in abiotic factors that may influence fish populations (Luoma et al. 2001).

Variability introduced by flow regimes is universally important for design and interpretation of an effective monitoring program to detect effects of Se exposures in lotic water bodies. In all of the data sets reviewed in Table 6.7, stream flow variability had a major influence on the variability of fish populations. For instance, over the monitoring record for Thompson Creek (Idaho), stream flows ranged from 0.05 to 10 m^3/s (a factor of 200 difference) and annually ranged from 0.1 to 2 m^3/s (a factor of 20 difference) (GEI 2008b). Flood flows may scour fish habitats, and low flows may reduce habitat areas and cause displacement or direct mortality due to temperature extremes and reduced overwinter (and other) habitat. Depending on factors such as spawning timing and emergence, and resident or migratory life history, stream flow extremes may affect species that occupy the same streams during summer differently (e.g., Cunjak 1996; Waters 1999; Lobón-Cerviá 2009).

Because the prevalent adverse effect of Se in laboratory toxicity tests with fish is reproductive failure due to deformities in early life stage fish, monitoring relevant characteristics of fish populations is recommended. These characteristics include changes or differences in the age distribution and relative abundance of different age classes over time or from reference conditions. Young-of-year (age-0) fish would be the most directly relevant age class to target to detect reproductive failure. However, abundance estimates of age-0 fish are often more variable than those of older and larger fish (Table 6.7), and are likely influenced by high measurement error from variability in emergence timing and low capture efficiency. This may limit the effectiveness of detecting trends in the relative abundances of age-0 fish between sites or over time using routine methods (e.g., electrofishing or direct observation). Instead, adaptation of nonroutine methods that are specifically targeted for detecting trends in survival to emergence of early life stage fish such as fry emergence studies may be needed (Curry and MacNeill 2004).

Thus, for Se, detecting an effect requires monitoring of recruitment failure and, in some instances, species richness and composition. Recruitment failure is the logical population-level consequence of reproductive impairment. The general indication of recruitment failure in fish populations is a shift in the age distribution toward older and fewer fish. Recruitment failure may also be characterized by an increased growth rate in response to a decreased population size that lowers resource competition (Munkittrick

TABLE 6.7
Examples of Year-to-Year Variability of Fish Abundances from Long-Term Records of Reference Sites

Monitoring Endpoint	Year-to-Year Variability, as Median Coefficients of Variability, CV (Range of y-to-y CVs)	Average Fish Densities or Abundances (±SD)	The 2 SD Decline from the Mean Required to Detect Effects and Trigger Changes in Management Practices
Densities of age-1 cutthroat trout in an isolated population with no fishing or other observed disturbances, average year-to-year difference, 11-yr record, Dead Horse Canyon Creek, Oregon	30 (0–72)% (House 1995)	25 (± 6) fish/100 m^2	46%
Densities of age-0 cutthroat trout, average year-to-year difference, 11-yr record, Dead Horse Canyon Creek, Oregon	36 (7–111)% (House 1995)	16 (± 8) fish/100 m^2	100%
Abundance of age-1 brook trout in an open population with limited fishing and few other disturbances, average year-to-year difference, 14-yr record, Hunt Creek, Michigan	10 (5–48)% (McFadden et al. 1967)	1996 (± 317), number of individuals	32%
Densities of age-0 brook trout, average year-to-year difference, 14-yr record, Hunt Creek, Michigan	16 (7–80)% (McFadden et al. 1967)	4813 (± 983), number of individuals	41%
Densities of mottled sculpin, all ages, year-to-year difference, 12-yr record, Coweeta Creek, Georgia	22 (7–173)% (Grossman et al. 2006)	31 (± 13) fish/100 m^2	84%
Densities of mottled sculpin, all ages, year-to-year difference, 12-yr record, Ball Creek-A, Georgia	33 (0–61)% (Grossman et al. 2006)	34 (± 8) fish/100 m^2	46%
Densities of mottled sculpin, all ages, year-to-year difference, 12-yr record, Ball Creek-B, Georgia	50 (2–134)% (Grossman et al. 2006)	49 (± 18) fish/100 m^2	74%
Densities of shorthead sculpin, all ages, year-to-year difference, 19-yr record, Thompson Creek, Idaho, upstream of mining effluent	22 (0–590)% (Fig. 6.10; GEI 2008b)	737 (± 362) fish/km	98%
Densities of cutthroat/rainbow trout hybrids, all ages, year-to-year difference, 19-yr record, Thompson Creek, Idaho, upstream of mining effluent	35 (0–263)% (Fig. 6.10; GEI 2008b)	229 (±139) fish/km	100%

and Dixon 1989). Species richness and composition are effective measures for detecting community-level impacts of elevated Se, but only in locations where fish diversity is high (e.g., 20 to 30 species such as in reservoirs of the southeastern United States (Crutchfield 2000; Chapter 3; Appendix A)). However, this is not the case for many areas where elevated Se concentrations are a concern. For instance, in the Great Plains or in cold, temperate regions of North America, fish assemblages are depauperate and may have few native species at reference sites. In cold-water, mountainous streams, this limited fish assemblage is often dominated by sculpins and trout (Bramblett and Fausch 1991; Mebane et al. 2003; Bramblett et al. 2005).

Monitoring age distribution and abundance of fish populations may avoid diffusing resources on the plethora of measurement endpoints often collected in monitoring programs for nonspecific causes, or monitoring programs designed for other stressors such as urban wastewater or pulp mill effluents. Because adult fish can survive and appear healthy under chronic Se stress (Coyle et al. 1993; Lemly 2002), and the responses of macroinvertebrate communities to chronic Se stress are equivocal (Section 6.4), some measurement endpoints that are commonly used in other environmental settings may not necessarily be sensitive to effects of moderately elevated Se. Examples of such endpoints that thus may be of limited utility for detecting chronic Se stress include calculation of routine macroinvertebrate bioassessment metrics or biotic integrity indexes, and some common fish health measures such as fecundity, egg size, condition factors of adult fish, lipid content, liver size, gonadosomatic index, and gonad size.

In addition to determining the effect size and the likelihood of false positives (α, Type I errors), we must consider data variability that promotes Type II errors even when we monitor the correct aspects of the system. Data variability undermines the power of monitoring programs to detect changes. The variability in age-structured fish populations for several long-term studies with trout or sculpin in lotic environments are summarized in Table 6.7. In these studies, the median year-to-year variability ranged from 10% to 50%, and coefficients of variation (CVs) ranged from 0% to 590%. The "2 SD from the mean" approach to defining departure from reference conditions (Environment Canada 2002; Kilgour et al. 2007) would correspond to declines in abundances of about 32% to 100% (Table 6.7). These declines are large and variable enough to suggest avoiding using the "2 SD from the mean" approach to setting monitoring trigger effect sizes for assessing the status of field fish populations.

Ham and Pearsons (2000) evaluated the ability to detect change in eight salmonid populations based on annual abundance estimates over 9 to 15 years, using the equal error power scheme of setting $\alpha = \beta$, with both at 0.1. They found that, after 5 years, detectable effect sizes ranged from decreases of 19% to 79%. The smaller detectable effects occurred for the more abundant species, and the poorest trend detections occurred for the rare, but highly valued, species. Dauwalter et al. (2009) examined trends over time in inland trout populations in relation to temporal variability, effect size, error rates, and number of sampling sites. They found that, using the traditional error rate (α) of 0.05 at a single site with an average CV of 49%, it would take about 20 years to detect a 5% annual decline (i.e., an absolute decline of about 62% from initial abundance) with good power (1-β of 0.8). Using the median CV from Table 6.7 and relaxing α to 0.1, the power to detect an annual decline of 5% in 10 years (37% decline from initial abundance) would be about 0.55. Working with bream (*Abramis brama,* a cyprinid), Nagelkerke

and van Densen (2007) found that, in most cases, more than 6 years of monitoring would be required to detect a population decline of 15% per year, which roughly corresponded to a halving of the population size over 6 years. Working with data for many species from the English North Sea groundfish survey, Maxwell and Jennings (2005) also found that the power to detect declines in abundant species was much higher than for rare and vulnerable species and they often failed to detect declines of the magnitude that would lead to species being listed as endangered. Field et al. (2007) used Australian woodland bird census data sets to evaluate ability to detect a change of conservation status from "Least Concern" to "Vulnerable" (i.e., a decline in abundance of ≥ 30% over 10 years). They found that, although initially there was very low power to detect change for most species (<0.5), by the 10th year 4 species had reached their target power level of 0.9. For some of the less prevalent and more difficult to detect species, power to detect change started to rise more rapidly as time passed.

Examples of critical effect sizes used to determine population-level impacts in monitoring programs have ranged from 20% to the complete loss of dominant fish species (Table 6.8). However, several effect sizes were in the range of 20%–30%

TABLE 6.8
Examples of the Magnitude of Critical Effect Sizes Detected in or Used to Evaluate Fish Population Monitoring and Assessment Endpoints

Monitoring Endpoint	Effect Size and Notes
Wide variety of endpoints	25% less than reference conditions (Munkittrick et al. 2009)
Wide variety of endpoints	Values outside of the range of most reference conditions, such as values below the 5th or 10th percentile of the reference condition (Munkittrick et al. 2009)
Fish community or populations	20% reduction in fish species richness or abundance measured in the field (Suter et al. 1999)
Sentinel fish reproductive performance	50% decline in proportion of young-of-year fish from reference sites (Gray et al. 2002)
Fish condition factor	>10% change from reference condition (Kilgour et al. 2005)
Fish community	Loss of any dominant or nonrare species (Kilgour et al. 2007)
Fish abundance	15% decline/year over 6 years (Nagelkerke and van Densen 2007)
Species abundance	30–50% decline over 10 years or 3 generations specieswide could trigger a Red List "vulnerable" listing (IUCN 2006)
Species abundance	50–70% decline over 10 years or 3 generations specieswide would trigger a Red List "endangered" listing (IUCN 2006)
Species abundance	80–90% decline over 10 years or 3 generations specieswide would trigger a Red List "critically endangered" listing (IUCN 2006)
Fish assemblage (multiple metrics)	20% change in overall biological condition from reference conditions considered evidence of degradation, corresponding with the 10th percentile of reference condition (Meador et al. 2008)
Fish assemblage (multiple metrics)	Index scores 25% less than the highest biological condition scores for reference conditions more than minimally disturbed, corresponding with ~25th percentile of reference conditions (Mebane et al. 2003)

for different endpoints. As previously noted, the approach of using two standard deviations from the mean of reference conditions to define the range of acceptable conditions may yield very large allowable effect sizes, ranging from about 30% to 100% declines. Thus, determinations of acceptable conditions and critical effects sizes must be situation-specific rather than generic.

Given these considerations, and the fact that laboratory toxicity testing may not accurately reflect the natural environment, field monitoring programs need to be carefully designed to have any reasonable chance of detecting population-level impacts if these are truly occurring. Critical steps in the design and interpretation of field monitoring programs for Se ecotoxicology include the following:

- Increase power to detect trends by monitoring a network of comparable reference and exposure sites rather than single sites.
- Ensure adequate frequency and duration of monitoring.
- Assess and quantify major sources of natural variation.
- Select appropriate error rates (Type I and Type II errors).
- Determine *a priori* the critical effect size that constitutes a population-level impact.
- Because chronic effects of Se primarily affect recruitment, focus on differences in the relative abundance of age-0 and age-1 fish both temporally and spatially; this will involve different methods than typically used for monitoring fish populations.

Note that, as documented above, error rates and critical effect sizes should be situation specific. As such they should be determined based on a consensus of those with relevant technical expertise. Further, even robust monitoring programs may not be able to convincingly detect declining abundance trends until several years of data have been collected. Thus, data from field monitoring programs should not be used in isolation, but rather in a weight-of-evidence determination along with exposure (Section 6.7.1) and reproductive effects data (Section 6.7.2). Moreover, as discussed above, alternative statistical strategies are appropriate such as comparing confidence intervals for nonequivalence or testing if linear trends are near zero (Parkhurst 2001; Dixon and Pechmann 2005; McGarvey 2007).

6.8 UNCERTAINTIES AND RECOMMENDATIONS FOR FURTHER RESEARCH

While the field of Se toxicity has been highly productive and prolific in recent years, numerous uncertainties, questions and hypotheses still remain. Table 6.9 outlines key uncertainties related to Se toxicity to aquatic organisms and provides associated recommendations for further research in these areas.

TABLE 6.9
Uncertainties and Opportunities for Future Research Pertaining to Se Toxicity

Aspect	Uncertainty	Recommendations for Further Research
Cellular mechanisms of Se toxicity	Studies investigating effects of Se on immunocompetence.	Laboratory and field studies investigating potential effects of Se on immune function.
Toxicokinetics and toxicodynamics	In egg-producing vertebrates, the relationships between reproductive strategy (e.g., oviparity vs. ovoviviparity, synchronous vs. asynchronous egg development) and deposition of Se into eggs (i.e., amount and timing of Se deposition). Underlying reasons for large differences in transfer efficiencies from body tissues (e.g., liver, muscle) to eggs among species.	Identify potentially susceptible species with different reproductive strategies and evaluate relative Se bioaccumulation in eggs. Evaluate how different variables affect Se deposition into the eggs, such as timing of dietary Se exposure relative to vitellogenesis and number of spawns/clutches per season.
Factors modifying Se toxicity	With the possible exception of interactions between organic Se and meHg, the mechanism(s) and extent of antagonistic reactions between Se and other factors (e.g., other elements, biotic and abiotic stressors) are unknown. Further, there are a few studies showing synergistic, not antagonistic, interactions that remain unexplained.	Determine the mechanism(s), extent and significance of antagonistic reactions between Se and other factors (chemical, biotic, and abiotic) for fish, waterbirds, and amphibians.
Nutritional factors	The mechanism(s) by which dietary factors can increase or reduce Se toxicity remain unknown and the extent and significance of these modifications of Se toxicity are uncertain.	Determine the mechanism(s), extent, and significance of dietary-based variations in Se toxicity for fish, water birds and amphibians.
Tolerance	Can fish, waterbirds, amphibians, and aquatic reptiles become tolerant (acclimation and/or adaptation) such that population-level impacts do not occur in highly Se-contaminated aquatic environments? If so, what are the underlying mechanisms and potential costs of such tolerance?	Determine whether oviparous vertebrates can become tolerant such that organic Se toxicity is reduced or eliminated, the types of tolerance possible (i.e., physiological or genetic), for what organisms, and the implications of such tolerance (including energetic or other costs) to populations exposed to increasing Se concentrations. Possible research includes side-by-side toxicity tests of suspected "tolerant" species and intolerant species, or ideally intraspecific comparisons among populations from "low" versus "high" Se environments.

Comparative sensitivity (protozoans)	Understanding of potential toxicity of Se to protozoans, which is currently based on a very small database.	Establishment of acute and chronic water concentration thresholds for protozoans, with potential standardized endpoints relating to behavior, growth, and survival, would be an important advance.
Comparative sensitivity (macroinvertebrates)	Although macroinvertebrate communities do not appear impacted by elevated concentrations of Se, sensitive species within those communities may be adversely affected. Dietary exposure and maternal transfer of Se to eggs in invertebrates has had little study.	Comparisons of macroinvertebrate taxa observed in areas with elevated Se concentrations to taxa expected in reference conditions may indicate potentially Se sensitive taxa; controlled exposures would be necessary to make any definitive conclusions of sensitivity.
Comparative sensitivity (fish)	The relative sensitivity of fish based on diet-only juvenile exposures and maternal transfer exposures.	Additional laboratory studies investigating effects of diet-only Se exposures by juvenile and adult animals relative to maternal transfer studies, and expanding testing to little-studied species or potential "sentinel" species.
Comparative sensitivity (amphibians and reptiles)	The relative sensitivity of understudied taxonomic groups.	More laboratory and field studies investigating species differences in sensitivity of amphibians and reptiles to Se.
Population implications of fish deformities	In the wild, how strongly is the development of subtle deformities (e.g., mild or moderate edema) in fish early-life stages related to their survival to older age classes through reproduction? When is the occurrence of Se-induced deformities a predictor of ultimate mortality versus a transient effect that fish may recover from without incurring lasting impairment? What environmental co-factors are important (e.g., velocity or flow regimes; thermal regimes; predator, prey, competitive, interactions), species combinations	In the wild, tracking the survival rates of early life stage (ELS) fish with or without subtle deformities to recruitment as reproductively fit adults is logistically highly challenging. Newly hatched ELS fish are very small (<15 to about 25 mm), and by the time they grow to sizes large enough to tag (>≈ 50 mm) fish afflicted by deformities may have already been lost from the cohort. If for greater experimental control, survival of ELS fish with and without deformities were tracked in quasi-natural experimental stream mesocosms, achieving realistic conditions and stresses for ELS fish would be challenging (e.g., prey capture, predator avoidance, currents). Alternatively, indirect correlative approaches such as monitoring emergent fry for deformity rates, proportions of young-of-year fish, and age-class strength may be more feasible to carry out but interpretation may be complicated by natural variability or factors such as density compensation (Section 6.6.3).

(*continued*)

TABLE 6.9 (CONTINUED)
Uncertainties and Opportunities for Future Research Pertaining to Se Toxicity

Aspect	Uncertainty	Recommendations for Further Research
Linkage between individual effects and impacts on populations	In different environments (e.g., freshwater: lotic versus lentic; estuarine; marine).	Population-level studies in different settings with elevated Se levels may provide useful information on population dynamics, compensation, and perhaps recovery. Extensive field monitoring has been conducted in areas with elevated Se concentrations, but much of this work languishes as poorly accessible grey literature. Review and publication of these studies in the primary literature could provide valuable information on patterns of ecosystems responses to elevated Se.

6.9 SUMMARY

Selenium is an essential nutrient that is incorporated into functional and structural proteins as selenocysteine. Several of these proteins are enzymes that provide cellular antioxidant protection. A key aspect of the toxicity of Se is the extremely narrow range between dietary essentiality and toxicity. Another important aspect of Se toxicity is that, although it is involved in antioxidant processes at normal dietary levels, it can become involved in the generation of reactive oxygen species at higher exposures, resulting in oxidative stress. Toxicity results from dietary exposure to organic Se compounds, predominantly selenomethionine, and the subsequent production of reactive oxygen species.

Oviparous (egg-laying) vertebrates such as fish and waterbirds are the most sensitive organisms to Se of those studied to date. Toxicity can result from maternal transfer of organic Se to eggs in oviparous vertebrates. Eggs are an important depuration pathway for fish but less so for birds. The most sensitive diagnostic indicators of Se toxicity in vertebrates occur when developing embryos metabolize organic Se present in egg albumen or yolk. Certain metabolites of organic Se can become involved in oxidation-reduction cycling, generating reactive oxygen species that can cause oxidative stress and cellular dysfunction. Toxicity endpoints include embryo mortality (which is the most sensitive endpoint in birds), and a characteristic suite of teratogenic deformities (such as skeletal, craniofacial, and fin deformities, and various forms of edema) that are the most useful indicators of Se toxicity in fish larvae.

Relative species sensitivities are not well understood but may be related to differences in reproductive physiology (e.g., the pattern of oogenesis or relative number of Se-containing amino acids in yolk), dynamics of Se transfer from diet or body tissues to eggs (i.e., dose), and/or differences in the capacity to metabolize Se to reactive forms (i.e., reactive oxygen species). Importantly, embryo mortality and severe malformations (developmental abnormalities) can result in impaired recruitment of individuals into populations and have caused population reductions of sensitive fish and bird species. These established linkages between the molecular/cellular mechanism of toxicity (oxidative stress), effects on individuals (early life stage mortality and deformities), and negative effects on populations and community structure provide one of the clearest examples in ecotoxicology of cause–effect relationships between exposure and altered population dynamics.

Similar to other toxicants, many factors can modify the toxicological responses of organisms to Se. Selenium interacts with many other inorganic and organic compounds, both in the aquatic environment and *in vivo*, in a predominantly antagonistic fashion. Nutritional factors such as dietary protein and carbohydrate content can modify Se toxicity. Abiotic factors such as temperature also appear to be important modifying factors of Se toxicity in both poikilotherms and homeotherms. Differences among freshwater, estuarine, and marine environments in the toxicological responses of organisms to Se are important considerations but have not been studied in great detail. The ecology of a species, particularly feeding niche, is a critical aspect related to its vulnerability to Se because of differential prey accumulation of organic Se and dietary exposure routes. Considerations of spatial and temporal variation in diet are important factors to consider when assessing potentially susceptible species; effects tend to be site specific.

Among taxa, there is a wide range of sensitivities to Se. Algae and plants are believed to be the least sensitive organisms. Very few studies have investigated the sensitivity of bacteria to Se, although they appear to be insensitive. Protozoans have also been understudied, and further work is needed investigating Se toxicity in this taxon. Most species of invertebrates, which are essential components of aquatic food webs and a key vector for transfer of organic Se to higher trophic levels, are also relatively insensitive to Se. As discussed above, oviparous vertebrates appear to be the most sensitive organisms. Although fish and waterbird sensitivities are well documented, there are reasons to suspect that amphibians and reptiles with oviparous modes of reproductive strategy are also sensitive. Compared to oviparous vertebrates, aquatic-dependent mammals do not appear to be sensitive to dietary organic Se exposure, further illustrating the importance of oviparity in Se toxicity. Although there have been suggestions of tolerance to Se (physiological acclimation or genetic adaptation) in certain biota, it is not known whether this is an actual phenomenon.

Selenium enrichment of reservoir environments (e.g., Belews Lake, Hyco Lake, Kesterson Reservoir) provide classic examples of adverse effects occurring through different levels of biological organization, comprising integrated whole-ecosystem examples of trophic transfer resulting in population-level reductions of resident species. Recovery from adverse effects on fish populations occurred once Se sources were eliminated. However, population-level effects from Se in natural ecosystems are difficult to detect. This difficulty reflects differences in species sensitivity as well as food web complexities and demographics where population-level effects are suspected. Few such widespread impacts on populations as documented at Belews, Hyco, and Kesterson reservoirs have been definitively documented in other ecosystems; however, population-level effects have been suspected at several other sites, including San Francisco Bay and Lake Macquarie, Australia.

Inability to observe population-level effects in the field can occur even when the species exposed in the field are the same or closely related to those for which adverse effects have been demonstrated in laboratory settings at lower Se tissue concentrations. In addition, several studies of aquatic ecosystems with naturally elevated Se concentrations have reported unaffected aquatic communities. Although statistical considerations and normal fish population monitoring design can preclude detection of low level (<10%) field population effects, these examples illustrate the critical importance of considering ecological and environmental factors when investigating potential Se toxicity in aquatic ecosystems.

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