Chapter 16

Amphibian health as an indicator of wetland health

Regina M. Krohn and Danna M. Schock

Abstract

Wetlands are integral components of hydrological cycles and provide vital ecosystem services, including water purification and providing wildlife habitat. However, degradation and destruction of wetlands is a global issue that is increasing in rate and spatial scale. Assessing and monitoring the health of wetlands is best accomplished by examining several lines of evidence. Because wetlands are critical habitat for thousands of species of amphibians, they are often studied to gather information about wetland health. However, not all amphibian species are likely to provide useful information on wetland ecosystem health. There are more than 7400 species of amphibians, and ecology and physiology differ widely across the taxonomic class. These differences are further compounded by the ecologically distinct life stages that typify many amphibians (e.g. egg, aquatic tadpole, terrestrial adult lifecycles of many anurans). Environmental contamination is a major threat to wetland ecosystems, including the amphibians that inhabit them. Herein we focus on strengths and limitations of using the health of amphibians to assess wetland health, focusing especially on contamination of wetlands. We highlight the need for caution and explicit consideration of ecology using, as an example, a field study involving wood frog (*Rana sylvatica* = *Lithobates sylvaticus*) tadpoles reared under conditions associated with a controlled diluted bitumen spill into freshwater environments.

16.1 Introduction

Wetlands are areas of land that are saturated with freshwater or saltwater frequently and/or long enough that the underlying soils show evidence of being altered by water (e.g. anaerobic conditions, processes such as the accumulation of peat) and the vegetation and other biota associated with the area are tolerant of, or adapted to, wet environments (adapted from Ramsar, 2018). This broad definition of wetlands reflects the wide diversity of wetlands globally, which range from vast ocean-side estuaries to peatlands in the circumpolar boreal forest, as well as human-made wetlands such as rice paddies and naturalized borrow pits in agricultural landscapes (Ramsar, 2018). Ecosystem services provided by wetlands are diverse and vital, including water

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purification, carbon storage, wildlife habitat, and human food production (e.g. waterfowl that are hunted, naturally occurring plants that are gathered, production of agricultural crops such as rice). Unfortunately, both the quality and quantity of wetlands are declining (Albert et al., 2021; Ramsar, 2018). Cumulatively on a global scale, the area occupied by wetlands is greater than the area of Greenland but has declined substantially due to human activities. The true extent of the loss of wetlands due to human actions is difficult to quantify because human alterations of the landscape have been ongoing, and escalating in speed and geographic scale, for hundreds, if not thousands, of years (e.g. wetlands drained to create agricultural land). Where reliable data exist, more than one-third of the area of wetlands in some regions has been lost in less than 50 years (Ramsar, 2018). Declines in the quantity of wetlands are accompanied by declines in the quality of wetlands because of multipronged stressors such as aquatic invasive species, climate change, resource development, and pollution; these stressors often act on wetlands simultaneously.

Assessing and monitoring the health of wetlands is imperative to slowing, mitigating, and reversing the loss of wetlands and the services they provide. However, assessing the health of wetlands is complex because the ecology of wetlands is complex, and so too are the causes and consequences of declining wetland health. Consequently, multiple sources of evidence, each providing distinct information, are needed (e.g. Adams, 2003; Cairns et al., 1993). Here we discuss ways in which the health of amphibians can provide information about the health of wetlands – that is, the ways in which amphibian health can be used as an indicator of wetland health. Although there is vigorous and important debate in the literature about precise definitions of what '(bio)indicators' are and how they may or may not differ from 'sentinels' or 'biomarkers' (e.g. Burger, 2006; McCarty et al., 2002; Sewell and Griffiths, 2009), we are using the term 'indicator' here in an inclusive way to facilitate discussion about facets of amphibian biology that allow for their use as indicators of wetland health at different levels of biological organization. We also include comment and caveats with respect to the inferences that can be made about wetland health from assessment of amphibian health. Our chapter largely focuses on amphibian health from the perspective of environmental contaminants in wetlands. However, concepts we discuss also apply to the use of amphibian health for monitoring other aspects of wetland health.

16.2 Amphibians are biologically diverse – a frog is not a frog is not a frog

A phrase that may be familiar to many is that 'amphibians are canaries in a coalmine' (e.g. *Prince Edward News*, 2019; *Washington Post*, 2021). The phrase refers to the idea that amphibians provide early warnings about the health of their environment in the same way that canaries in cages were once carried by coalminers into mines to act as an indicator that deadly gases had accumulated, because the canaries were more sensitive to the gases than humans. However, the vast oversimplification of amphibian biology wrapped up in this phrase makes it misleading and problematic because it is used as justification for the use of amphibians as 'indicators' simply by virtue of a species' membership in the clade Amphibia.

There are currently 7,400+ recognized species of amphibians (i.e. members of Class Amphibia), from across three taxonomic Orders (frogs – Anura; salamanders – Caudata; caecilians – Gymnophiona). Amphibians are a biologically diverse taxon. They are found on all continents except Antarctica, in diverse ecosystems, from deserts to boreal forests, from

fast-moving alpine rivers to tropical cloud forests (IUCN Redlist, 2023; Vitt and Caldwell, 2013). Related to this diversity of habitats is a diversity of life history strategies. For example, modes of reproduction include the somewhat familiar 'biphasic lifecycle' whereby eggs are laid in water where the larvae grow, develop, and then go on to metamorphose into terrestrial animals. However, viviparity, ovoviviparity, and direct development (fully formed young that hatch from eggs, usually in species with entirely terrestrial lifecycles) also occur in all three taxonomic orders of amphibians (Vitt and Caldwell, 2013). These differences in reproduction have implications for exposure to, and depuration of, contaminants (e.g. maternal transfer of contaminants to offspring; Bergeron et al., 2010; Todd et al., 2011).

Globally more than 41% of amphibian species are threatened with extinction, including hundreds of species that are critically endangered or extinct in the wild; we are truly in the midst of a global amphibian decline crisis (IUCN Redlist, 2023; McCallum, 2007). However, more than 45% of amphibian species are not currently thought to be declining, and there are more than 1,800 species whose biology is too poorly understood at this time to gauge whether their populations are stable or not (IUCN Redlist, 2023). It is also worth noting that there are amphibian species that are formidable invasive species, wreaking havoc in their non-native ranges, including the decimation of local amphibian populations through competition, the spread of pathogens, and depredation (e.g. Garner et al., 2006; Kraus, 2015). A particularly notorious example is the American bullfrog (*Rana catesbiana* = *Lithobates catesbiana*), which is native to eastern North America but has been widely established (e.g. Jamaica, Brazil, South Korea, Japan) as a result of escapees from ranaculture facilities and from deliberate releases for well-intentioned but misguided biological control purposes.

Another aspect of amphibian biology that requires consideration is that the drivers of amphibian declines are diverse (Collins and Storfer, 2003) and have been ongoing for considerable periods of time – several decades or more in the case of environmental contamination, 1000+ years in the case of draining wetlands for agricultural and other human uses. It is possible, even probable, that some of the most sensitive species of amphibians were lost (regionally or globally) before issues were even recognized (Collins, 2010; Collins and Crump, 2009). Equally important and probable is that natural selection for resistance to stressors will have been taking place all the while, and complicating matters further is that amphibians may exhibit phenotypically plastic responses to stressors such as contaminants (e.g. Jones and Relyea, 2015). For these reasons, it is perhaps not surprising that Kerby et al. (2010) conducted a meta-analysis of toxicological studies and found that amphibians were not the most sensitive taxon for several categories of environmental contaminants, including various classes of pesticides. It should be noted, though, that the number of amphibian species used across the studies examined by Kerby et al. (2010) was limited to a handful.

As a final comment regarding the problematic nature of blanket statements about amphibians as 'canaries in a coalmine', it is instructive to consider that it is uncommon to encounter phrases indicating that 'all mammals are canaries in the coal mine' even though over a quarter of the ~6,000 recognized species of mammals are threatened with extinction (IUCN Redlist, 2023). The declines we are witnessing in mammals are alarming and are also part of the larger global biodiversity crisis (e.g. Albert et al., 2021). However, declarations are not routinely made about the utility of all mammals to serve as ecosystem health indicators. Indeed, there are vigorous debates about the utility of a single mammal species that has been extensively

studied, the American mink (*Neovison vison*), to act as a useful indicator of environmental contamination (e.g. Basu et al., 2007; Bowman et al., 2012; Bowman and Schulte-Hostedde, 2009). Perhaps mammals are not routinely inappropriately clustered together as indicators because the diversity of ecologies among mammals (e.g. primates versus ungulates) is more widely appreciated than it is for amphibians. In any event, it is untenable from an ecological standpoint to argue (or assume) that all 7,400+ species of amphibians are equally informative with respect to environmental health such as the effects of contaminants. It is imperative to explicitly consider the biology (e.g. physiology, ecology) of the particular species proposed as an indicator of wetland health because of the variability, including intraspecific variability, that permeates Class Amphibia.

16.3 Demonstrating causality – easier said than done, but (some) amphibians can help

For a species to be useful as an indicator of environmental health, it must be possible to demonstrate causality between the environmental stressor(s) and the effect(s) observed in the indicator species. However, causality can be difficult to demonstrate because multiple stressors are always simultaneously operating on natural systems and there can be direct and indirect interactions among stressors that mask or exacerbate effects. For example, Relyea (2004) demonstrated that the organophosphate insecticide malathion was moderately lethal to tadpoles of six North American anurans but became twice as lethal to gray treefrog tadpoles (*Hyla versicolor*) when they were simultaneously exposed to malathion and the chemical cues of the presence of one of their natural predators, the eastern newt (*Notophthalmus viridescens*). Environmental contamination can also interact with infectious diseases in intricate ways. For example, Bosch et al. (2021) exposed tadpoles of the common midwife toad (*Alytes obstetricans*) to different concentrations of microplastics and the highly pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (Bd). Among their findings was that tadpoles infected with Bd had lower accumulations of microplastics in their gut, likely because Bd damages tadpole mouthparts which interferes with the tadpoles' ability to ingest food – and microplastics. These are just two studies among many that demonstrate the importance of interactions among stressors. We recommend that study designs should allow for the detection of interactions wherever possible and, where not possible, interpretation of findings should explicitly consider what role(s) interactions among stressors may play.

Recognizing the ecological realities that complicate attempts to demonstrate straightforward cause-and-effect relationships, Adams (2003) proposed seven criteria, or lines of evidence, that indicate causality between suspected contaminant stressors and observed effects in aquatic ecosystems. When taken in concert in an 'eco-epidemiological approach', or 'weight-of-evidence approach', data that satisfy these criteria indicate causality between a putative stressor and its effects (Adams, 2003; Johnson et al., 2021). We highlight how amphibians are particularly well suited for providing evidence of causality for three of the criteria proposed by Adams (2003): (1) experimental evidence of causality, (2) biological plausibility of mechanisms linking suspected causes and effects, and (3) evidence of biological gradients in natural systems.

Experimental evidence of causality derives from controlled laboratory or mesocosm studies, and that evidence can be used to predict effects or to corroborate suspected cause–effect relationships observed in natural systems. This line of evidence is perhaps the most intuitive and best-known type of evidence used to link

causes and effects. The use of amphibians in experimental set-ups is facilitated by the relatively small size, egg-laying behavior, and tractable husbandry requirements of many species. Toxicological assays involve exposing amphibians (usually eggs or very young larvae of anurans) to different doses of chemicals of interest under standardized, highly controlled settings. These types of assay are a key source of evidence used by government agencies when establishing environmental protection regulations. The FETAX assay is one example of a widely used assay. This assay involves exposing the developing embryos of the African clawed frog (*Xenopus laevis*) to the substance of interest and then measuring effects such as growth, developmental, and mortality rates (e.g. Xu et al., 2019). Mesocosm studies allow for the introduction of more ecological realism and complexity into study designs while still controlling many of the variables that could confound results and frustrate interpretations. They also allow for experimental designs with sufficient sample sizes to meaningfully analyze complex data. The literature is replete with studies that have used amphibians in mesocosm set-ups to investigate interactions among environmental contaminants and other stressors (e.g. Boone and James, 2003; Sievers et al., 2018). The case study discussed later in our chapter, which is based on Krohn et al. (2021), is also a mesocosm study.

The second criterion outlined by Adams (2003), where (some) amphibians are well suited to provide evidence of causality, is with respect to the biological plausibility of proposed mechanisms that link cause and effect. One notable example is the effect of contaminants on amphibian metamorphosis, particularly anuran tadpole growth and development rates, which in turn determine time to, and size at, metamorphosis. The progression from fertilized egg to metamorphosed froglet follows predictable, well-described stages that can readily be measured and analyzed (e.g. Gosner, 1960; Nieuwkoop and Faber, 1994). Frog metamorphosis is regulated by the activity of the hypothalamus–pituitary–thyroid (HPT) axis, a neuroendocrine system that responds to stress and regulates metabolism. Consequently, contaminants that stimulate or downregulate any part of the HPT axis – that is, act as endocrine disruptors – are likely to affect metamorphosis in measurable ways (Carr and Patiño, 2011; Hersikorn and Smits, 2011). Melatonin, for example, accelerates metamorphosis in *X. laevis* tadpoles (Rose and Rose, 1998), while exposure to the fungicide triadimefon causes developmental delay (Li et al., 2016). Linking individual-level effects (e.g. size at metamorphosis) to population- or higher-level effects (e.g. decline or increase) is a challenge across broad disciplines of ecology, and one that has not yet been overcome with respect to disruptions of the HPT axis in amphibians (Carr and Patiño, 2011).

The third criterion outlined by Adams (2003) that we highlight in our chapter is with respect to demonstrating a biological gradient – that is, demonstrating that there is a spatial or temporal dose–response relationship between the stressor and effect. Studying animals with small home ranges greatly reduces ambiguity about where, when, and for how long the animals are exposed to a particular contaminated environment. In amphibians, home ranges and dispersal distances are usually much less than 1 km from their natal location, with many species spending their entire lives less than 100 m from their natal location (Smith and Green, 2005). Larvae of pond-breeding amphibians are even more fine-scaled, reliable indicators of local habitat conditions than their post-metamorphic counterparts. By comparing the health of amphibians across multiple locations that vary in distance from the putative source of contamination, it is possible to gather evidence of a causal relationship between stressors and effects (e.g.

Hayes et al., 2003). Demonstrating causality can be further strengthened by assessing effects at multiple levels of biological organization at each location in the study (e.g. molecular biomarkers of stress, bioaccumulation of contaminants, recruitment rates) because all effects can reasonably be attributed to local conditions. However, this is another situation where it is necessary to be aware of the biology of the species being proposed as an indicator, or at least to be explicit about the assumptions being made, because it is not rare for some individuals to make movements of up to ~10 km, particularly juvenile anurans in undisturbed habitat (Sinsch, 2014; Smith and Green, 2005).

The case study below makes use of frog tadpoles as indicators of contaminant bioaccumulation in the local habitat. It presents an example of how choosing to investigate contaminant accumulation in developing larvae may lead to very different conclusions if morphological changes during tadpole growth and metamorphosis are not considered.

16.4 Case study: changes in metal accumulation in anuran larvae during metamorphosis

Heavy metals and metalloids such as mercury, selenium, and arsenic are components of the Earth's crust and are naturally occurring in the environment. Although ecotoxic metal levels can be introduced into the environment through natural mobilization (e.g. weathering of metal-bearing rocks; Bradl, 2005), anthropogenic disturbances are making metals more biologically available, thereby creating threats to wildlife, ecosystem, and human health. For example, ore mining, the use of phosphate fertilizers, and metal-based pesticides all may lead to ecotoxic levels of heavy metals in aquatic environments. Endocrine-disrupting effects have been observed for some metals. For example, Wang et al. (2016) and Sun et al. (2018) observed delayed metamorphosis in Chinese toad (*Bufo gargarizans*) tadpoles exposed to copper and cadmium at levels observed in polluted environments, respectively. The authors also reported a reduction in body size of the tadpoles. Similarly, Lanctôt et al. (2016) reported that environmentally relevant concentrations of metals disrupted tadpole development as well as behavior in striped marsh frog tadpoles (*Limnodynastes peronii*) exposed to coalmine wastewater. The exposed larvae also had high tissue levels of cobalt, selenium, and arsenic.

In general, the available literature suggests that most amphibian species examined to date bioaccumulate environmental contaminants of concern, including heavy metals and organochlorine pesticides (e.g. Sparling et al., 2010). However, the extent to which amphibians bioaccumulate contaminants varies across taxonomic orders (e.g. Smalling et al., 2021), among species within the same order (e.g. Boczulak et al., 2017), and across life stages of the same species (e.g. Krohn et al., 2021; Roe et al., 2005). Studying the exposure of anuran tadpoles to metals and other contaminants can serve as an indicator of threats to wetland health in the following ways.

- Anurans with biphasic lifecycles may function as vectors for metals from aquatic to terrestrial systems. → Some metals, such as selenium and arsenic, may be transferred to terrestrial ecosystems while others are depurated during metamorphosis (Rowe, 2014).
- Bioaccumulation of metals in frog tadpoles, which are an integral part of the food web, can potentially expose species of organisms at higher trophic levels to toxic levels of contaminants.
- Lethal, but also sublethal, metal exposure and mixtures of contaminants pose a threat to anuran populations due to behavioral and developmental effects. → Heavy metals

can reduce predator avoidance in tadpoles, making them more susceptible to predation; this may also lead to increased intake of metals by the predator (Lefcort et al., 1998).

Crude oils mainly consist of hydrocarbons (~80%) plus other organic compounds and small amounts of metals that may contribute to the adverse effects of oil spills (Overton et al., 2016). For example, elevated levels of copper and lead were detected in feathers of sea birds for 3 years after the Prestige oil spill off the Spanish northwest coast (Moreno et al., 2011). Crude oil spills into marine environments have been studied extensively, at least partly because of the large volumes of oil released. However, important knowledge gaps exist with respect to crude oil spills in freshwater ecosystems, including information on the efficacy of clean-up methods (Lee et al., 2015). To address these knowledge gaps, a multidisciplinary Canadian team commenced in 2018 with a series of experimental diluted bitumen (dilbit) spills into lake shoreline enclosures at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) in Ontario, Canada. Bitumen is the form of petroleum extracted from oil sands deposits in northern Alberta, Canada, which is the third largest proven oil deposit in the world (Alberta Energy Regulator, 2023). Bitumen is viscous and must be diluted with lighter petroleum products (e.g. naphtha) in order for it to flow through pipelines (Government of Alberta, 2023). As part of this freshwater oil spill recovery study (FOReSt), effects of contaminants in wood frog (*R. sylvatica* = *L. sylvaticus*) tadpoles were investigated because contaminants related to oil and gas extraction can reduce survival of amphibians outright (e.g. Hersikorn et al., 2010), and lead to sublethal effects such as compromised immune function, endocrine disruption (e.g. Robert et al., 2019), and reduced growth (e.g. Pollet and Bendell-Young, 2000; Smalling et al., 2019).

A wood frog metamorphosis experiment was performed in an undisturbed shoreline of the experimental lake at the IISD-ELA prior to the oil spill experiment to acquire baseline data and validate the metamorphosis assay in the lake environment. Wood frogs were chosen as indicator organism because they are an abundant, nonthreatened species inhabiting the wetlands of the boreal forest. During this validation study, wood frog tadpoles were housed in wooden cages with mosquito net siding at a peat-organic shoreline of the experimental lake. Tadpoles were fed with frozen lettuce every other day until approximately 50% reached metamorphic climax, when front limbs emerge (Gosner stage 42 (G42)) (Gosner, 1960). At this time, several physiological endpoints were determined. Surprisingly, metal levels assessed in whole tadpoles showed a rapid decline in those that had reached metamorphic climax (within 24–48 h) (Krohn et al., 2021). With the exception of magnesium, selenium, zinc, and strontium, metals were several-fold lower in tadpoles at metamorphic climax. Differences were most pronounced for iron, lead, and manganese.

These observations indicated the importance of the developmental stage of frogs as indicator species when assessing contaminants, and led to further investigations of metal accumulation of tadpoles before and at metamorphic climax, and after exposure to dilbit in their habitat. For the dilbit spill experiment, shoreline enclosures (15 × 5 m) (Curry Industries, Winnipeg, Canada), were deployed in the experimental lake and sealed to the aquatic and terrestrial sediment/soil using a double row of sandbags. Additional booms were placed around the whole shoreline with enclosures. Seven kilograms of dilbit were weathered for 36 h in stainless steel evaporation pans over 25 cm of freshwater sourced from the experimental lake. After weathering, oil was retrieved from the surface of the water, immediately transported in glass jars to the site and applied to the water within

50 cm of the shoreline along the entire 5-m linear shoreline, estimated to result in a 1-mm oil film on the water. Two enclosures were left untreated as control systems. After 72 hours, sorbent pads were used to absorb the surface dilbit followed by micronutrient addition for enhanced natural recovery. After the clean-up, wood frog tadpoles were transferred to cages in the shoreline enclosures and fed with frozen kale until metamorphic climax (Figure 16.1). Some tadpoles were collected before metamorphic climax.

The digestive tract in premetamorphic anuran tadpoles can be tenfold the length of the tadpole (excluding the tail) and the so-called gut coil takes up most of the body volume. It is remodeled at metamorphosis to a shorter, more differentiated, organ comparable to a mammalian gastrointestinal tract (Ishizuya-Oka and Shi, 2005). *Xenopus laevis* digestive tracts shorten by about 75% within 8 days of the start of metamorphosis (Schreiber et al., 2005). Figure 16.2 shows photographs and microcomputer tomographic (mCT) scans of two boreal chorus frog tadpoles, one before metamorphic climax (G40) and one at metamorphic climax (G42). On the ventral mCT scans the gut coil in the premetamorphic tadpole is clearly visible while a much shorter, more differentiated, digestive tract is visible in the metamorphic tadpole. In boreal chorus frogs the gut coil volume was reduced by about 70% (unpublished observation) from stage G40–G42. While species-dependent differences exist, it is known that some metals concentrate in the tadpole gut coil. This is why in the dilbit spill experiment, premetamorphic tadpoles with and without the gut coil were analyzed for a 21-metal panel, as well as tadpoles at metamorphic climax including the digestive tract (G42) (Krohn et al., 2021). Interestingly, tadpoles reared in the control shoreline plots had lower metal levels than those from the validation experiment (undisturbed environment), which was likely due to the fact that, during that study, tadpole cages were positioned close to the sediment. Increased metal accumulation was likely due to ingestion of disturbed sediment during handling.

Figure 16.1 Cages with wood frog tadpoles in a shoreline enclosure at IISD-ELA, Ontario, Canada. Tadpole metal accumulation was determined in pre-and postmetamorphic tadpoles and premetamorphs without gut coil, in control and diluted bitumen spill sites. Photo: R. Krohn.

In both control and dilbit-exposed animals, whole premetamorphic tadpoles had higher metal levels than those tested without the gut coil, except for potassium, selenium, and zinc levels (strontium was not assessed in the spill study), which were not different, and magnesium, which was more concentrated when measured without the gut coil. These metals were also similar in tadpoles at metamorphic climax. Molybdenum, vanadium, and cadmium were significantly increased in dilbit-exposed tadpoles with gut coil when compared with control tadpoles. Molybdenum was also higher in dilbit-exposed premetamorphs without the gut coil as compared with control counterparts.

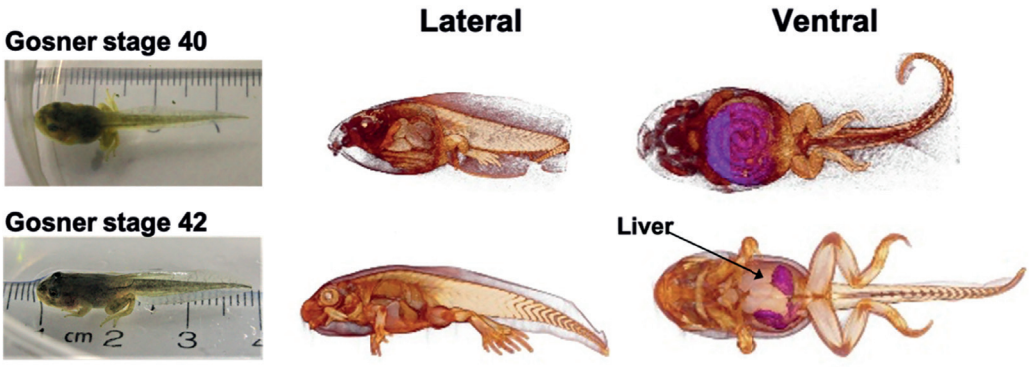

Figure 16.2 Gut coil remodeling and other morphological changes happen rapidly in boreal chorus frog (*Pseudacris maculata*) tadpoles after they reach metamorphic climax (tadpole shown at bottom). The digestive tract is highlighted in purple. At metamorphosis the liver moves to the ventral side. Photos: Ahmad Yaghi and Judit Smits, Dept. of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary; mCT images of boreal chorus frogs: Jason Pardo and Jason Anderson, Department of Comparative Biology & Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary.

Notably, mercury was the only element found to be significantly higher in dilbit-exposed metamorphs than in premetamorphs (with and without gut coil).

Another interesting finding was that tadpoles had much higher metal levels than those in the water they were reared in, and possibly accumulated more metals through the food source, which was frozen kale. Since higher levels of molybdenum, cadmium, and vanadium were observed in dilbit-exposed animals, it is conceivable that the kale adsorbed some of the metals from the water. Biosorption of metals into an aquatic plant has been reported previously (Keskinkan et al., 2004).

16.5 Conclusion

Wood frog tadpoles reared in shoreline plots for the experimental dilbit spill were housed in floating cages (see Figure 16.1), where they were not in contact with benthic plants or lake sediment. Tadpoles in their natural habitat ingest sediment and feed on plants and smaller invertebrates that may take up petroleum-related pollutants. As a result, their exposure to contaminants would likely be higher in nature than it was in the study. In addition, bitumen has the propensity to sink to the sediment within 1 week after a spill (Stoyanovich et al., 2019).

Metals accumulated mostly in the gut coil of premetamorphic wood frog tadpoles, with some exceptions of essential trace metals. In contrast, mercury was found to be highest in dilbit-exposed tadpoles at metamorphic climax, indicating higher tissue accumulation due to metabolic changes during metamorphosis. These findings highlight the importance of considering intraspecific differences in anurans generally, and the fact that studies measuring bioaccumulation must factor in the intestinal remodeling that occurs during metamorphosis. Speaking specifically with respect to wood frogs, these may serve as vectors of metals into terrestrial ecosystems for some metals, including mercury, zinc, and selenium.

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