Chapter 11
Effects of Environmental Change on Helminth Infections in Amphibians: Exploring the Emergence of *Ribeiroia* and *Echinostoma* Infections in North America

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Contents

Abstract Amphibians have long served as model organisms for studying animal physiology, vertebrate anatomy, and host–parasite interactions. Recently, however, the occurrence of precipitous declines in many amphibian populations and of severe limb malformations in others has catalyzed renewed efforts to understand the effects of parasites on amphibians. In this brief review, we examine the importance of two groups of trematodes that utilize amphibians as intermediate hosts: species in the genus *Ribeiroia* and the broader "echinostome" group which collectively includes the genera *Echinostoma* and *Echinoparyphium*. For each, we specifically explore the pathology resulting from infection, whether the parasite has recently increased in abundance or geographic range, and the biotic and abiotic factors likely to influence infection. Both groups of parasites can induce significant pathology in amphibian hosts. Exposure to *Ribeiroia* cercariae causes substantial increases in mortality and limb malformations in larval amphibians. These malformations, which include missing, malformed and extra limbs, may further reduce survival in amphibians; malformations are extremely rare in adult frogs, even following

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years in which they are abundant (>50%) among juvenile frogs. Similarly, the echinostomes, which colonize the kidneys of amphibians, can reduce the survival and increase the incidence of edema and renal failure, particularly in laboratory experiments. Recent surveys of National Wildlife Refuges across the USA suggest that both groups of parasites are widespread and sometimes extremely abundant (~1,000 metacercariae per frog). Infections appear to be most common along major rivers and bird flyways in the northern half of the country. While limited evidence suggests a recent increase in amphibian malformations and *Ribeiroia* infection, the paucity of available historical data precludes a definitive assessment of whether either parasite group has recently emerged. We discuss future approaches to this question and explore contemporary ecological changes known or hypothesized to influence patterns of infection, including changes in land use, increases in nutrient and pesticide runoff, decreases in community diversity and shifts in climate. Considering the documented pathologies of each parasite group, their widespread and often abundant infection patterns, and the ongoing declines observed in amphibian populations, we emphasize the urgent need for further study of *Ribeiroia* and echinostome infections in amphibians.

11.1 Introduction

For more than 50 years, amphibians have served as model hosts for studying the intricate nature of host–trematode interactions (Ingles 1933; Brandt 1936; Smyth and Smyth 1980; Prudhoe and Bray 1982). The first isolation of a trematode from an amphibian occurred in 1737, and a steady number of new species have been recovered over the subsequent centuries (Prudhoe and Bray 1982). The complex life cycles of many amphibians, which involve remarkable shifts in habitat (aquatic to terrestrial), diet (herbivory to carnivory), and morphology (tail to limbs), mirror the complexity of trematode life cycles. Correspondingly, amphibians can act as both intermediate and definitive hosts for a broad diversity of parasites. To date, hundreds of species of digenetic trematodes have been identified that depend on frogs, toads, or salamanders hosts (Prudhoe and Bray 1982). While the bulk of research has focused on amphibian as definitive hosts, amphibians also support larval stages (meso- and metacercariae) of more than 200 trematode species, and this almost certainly represents a considerable underestimate. Because metacercariae are often smaller than adult parasites, distributed throughout diverse regions of the host's body, and difficult to excyst and identify, they have traditionally received less attention than adult parasites. Often they have been ignored altogether.

More recently, renewed attention has focused on the potential importance of larval trematodes within amphibians. This trend is the result of several factors. First, while it was once supposed that trematode metacercariae caused little pathology in their intermediate hosts, waiting almost "patiently" in a resting form, there is growing recognition that this stage may be among the most pathogenic. Because

transmission of metacercariae to the definitive host often depends on predation (trophic transmission), there is strong selective pressure on trematodes to increase the susceptibility of intermediate hosts to predation. Examples of parasites that alter the susceptibility of intermediate hosts to predation by definitive hosts are evident across several phyla of parasites, including platyhelminths, nematodes, acanthocephalans, and apicomplexans (Moore 2002). These specific pathologies can include behavioral alteration, physical or developmental modification, and/or significant morbidity all of which can act as signals to definitive hosts of an easy meal. Among the trematodes, for instance, cercariae of *Euhaplorchis californiensis* encyst on the brain of killifishes by the thousands and induce erratic swimming behaviors which attract bird predators (Lafferty and Morris 1996). Recent experimental work by Shaw et al. (submitted) demonstrated that these metacercariae actually cause changes in monoaminergic brain chemical activities in killifish which can directly affect locomotory behavior.

Second, amphibians are now at the forefront of the global biodiversity crisis. With one-third of all species in decline and >100 species extinct or suspected extinct in the last 25 years, amphibians are considered the most imperiled class of vertebrates worldwide (Houlahan et al. 2000; Stuart et al. 2004). The causes of such declines are diverse, including habitat loss, infectious disease, invasive species, and climate change, and may interact through complex mechanisms (Blaustein and Kiesecker 2002). The emergence of the pathogen *Batrachochytrium dendrobatidis*, for example, has led to devastating population losses and extinction of amphibian populations worldwide, and is considered one of the greatest threats to global amphibian diversity (Johnson 2006; Skerratt et al. 2007). Gaining insight into how environmental drivers exacerbate disease threats to amphibians is a critical research area for amphibian conservation. By examining the environmental drivers that influence pathogenic trematode parasites, we can expand this field of study to address other types of amphibian disease as well.

Third and finally, trematode metacercariae have recently been linked to severe pathology in amphibians. Most notably, the digenean *Ribeiroia ondatrae* is a widespread and important cause of limb deformities in amphibians, including missing, extra, and misshapen limbs (Fig. 11.1a; Blaustein and Johnson 2003). These deformities have attracted international attention and generated considerable controversy over the likely causes and implications of the phenomenon (see Kaiser 1997, 1999; Souder 2000). Affected individuals suffer severely restricted mobility, and frequently die soon after metamorphosis. The link between *Ribeiroia* infection and malformations has been substantiated through extensive field surveys and controlled experiments, but the factors that control infection abundance and the apparent increase in deformities remain speculative (see later). Alongside *Ribeiroia*, other trematodes, including members of the echinostomatids (e.g., *Echinostoma* and *Echinoparyphium*), have been suggested to impair amphibian fitness. Because metacercariae aggregate in the kidneys of infected amphibians and may number several thousand within a single frog, these parasites have the potential to inhibit renal function and reduce survival (Fried et al. 1997; Schotthoefer et al. 2003; Skelly et al. 2006; Holland et al. 2007). As both *Ribeiroia* and the echinostomes

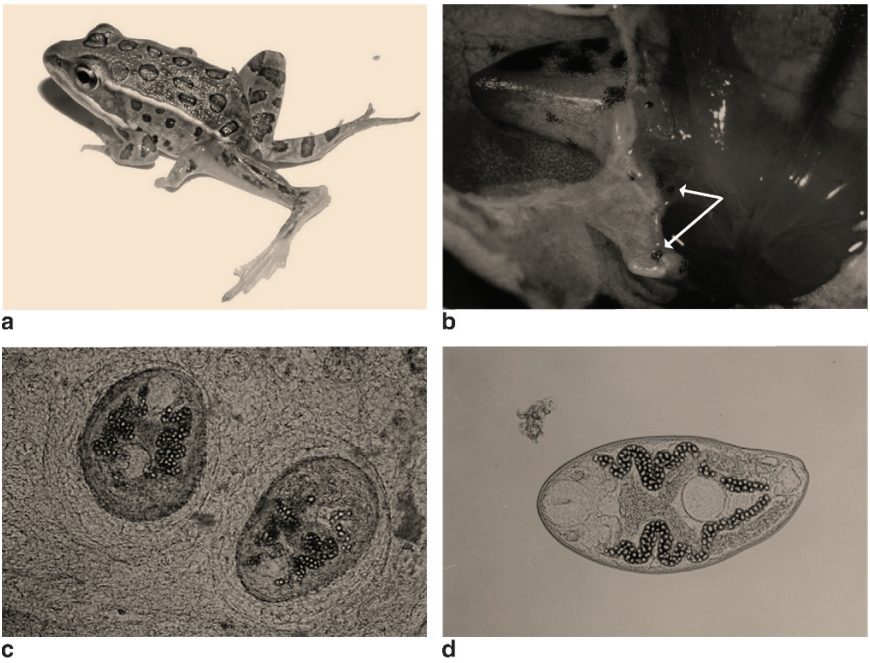

Fig. 11.1 (**a**) Representative malformation associated with *Ribeiroia* infection in a Northern leopard frog (*Rana pipiens*); (**b**) Dorsal view of hind limb and tail resorbtion area of a malformed frog; melanized metacercariae of *Ribeiroia* appear as brown cysts (see arrows); short projection on the left side represents a small supernumerary limb; (**c**) In situ view of two encysted *Ribeiroia* metacercariae within amphibian tissue; (**d**) Excysted metacercariae with visible esophageal diverticula

depend on predation of infected amphibians by vertebrate definitive hosts for transmission, pathology that increases the vulnerability of amphibians to predators could enhance transmission.

In this chapter, our objectives are to (1) review and assess the effects of *Ribeiroia* and *Echinostoma* on their amphibian hosts, (2) evaluate available evidence of an increase in the abundance, distribution, or pathology of each parasite, and (3) explore the environmental factors that influence amphibian infection levels and how they are expected to change in the future. We do not intend this as a comprehensive review for either parasite group, as such detailed information is beyond the scope of this chapter. Throughout, we refer readers instead to other sources for more comprehensive accounts of these groups' taxonomy, physiology, ecology, and pathology. It is our aim that this distillation will help catalyze additional research to address pressing gaps in our knowledge of *Ribeiroia* and *Echinostoma* and their ecological significance. In the sections that follow, we discuss the life cycles and pathology associated with each parasite, assess historical and contemporary evidence of emergence, and evaluate the most likely biotic and abiotic factors to drive

changes in infection. Because metacercariae isolated from the kidneys of amphibians are difficult if not impossible to identify on the basis of morphology alone, we suspect many previous investigations (including our own) have inadvertently confused members of the echinostomatids. Accordingly, we make no attempt here to differentiate among these groups, and elect instead to combine our discussions of *Echinostoma trivolvis*, *Echinostoma revolutum*, and *Echinoparyphium* spp. under the broad category of echinostomatids.

11.2 Overview of *Ribeiroia*

Trematodes in the genus *Ribeiroia* (Family Psilostomidae) have complex life cycles involving planorbid snails as first intermediate hosts (genera *Planorbella* and *Biomphalaria*), fish or amphibians as second intermediate hosts, and birds or mammals as definitive hosts (see Table 11.1). While this group's taxonomy will likely require revision, three species are currently recognized: *R. ondatrae*, *R. marini*, and *R. congolensis*. All share a number of morphological similarities, including distinctive esophageal diverticula (Fig. 11.1d; see Johnson et al. 2004 for a full review of this group). These species have been recorded from North and South America (*R. ondatrae*), the Caribbean (*R. marini*), and parts of Africa (*R. congolensis*). Because little is known about the species of *Ribeiroia* that occur outside the US, our focus here is exclusively on *R. ondatrae*, which has recently been linked to severe limb malformations in North American amphibians (Johnson et al. 1999; Blaustein and Johnson 2003; Sutherland 2005). Free-swimming cercariae released from infected snails colonize and infect larval amphibians, wherein they encyst in and around the developing limb buds (Fig. 11.1b,c). The resulting physical and/or chemical disruption of limb growth can lead to dramatic malformations, ranging from complete limb suppression to the induction of multiple extra limbs (Fig. 11.1a). These malformations may benefit the parasite by increasing the vulnerability of infected amphibians to definitive host predation (parasite manipulation). However, some reports suggest that the frequency and severity of these deformities may have increased in recent years (e.g., Johnson et al. 2003; Johnson and Lunde 2005), possibly in association with environmental change. Our goal is to explore the link between *Ribeiroia* and malformations, evaluate the potential importance of parasite-induced malformations for amphibians, and examine available evidence for a recent increase in infection and the resulting pathology.

11.2.1 History of Frog Deformities and the Link to Trematode Infection

Recent interest in amphibian malformations is often traced back to the Ney Pond near Henderson, Minnesota (e.g., Souder 2000). In August 1995, a group of middle school children discovered that nearly half of the emerging leopard frogs (*Rana*

Table 11.1 Hosts involved in the life cycles of *Riberoia ondatrae*, *Echinostoma trivolvis*, and *E. revolutum* in North America

Ribeiroia ondatrae			Echinostomatid species	
Life cycle stage	Common name[1]	Species	Common name	Species
First intermediate hosts	Ramshorn snails	*Biomphalaria obstructa* *Helisoma antrosum* *Planorbella campanulata* *Planorbella occidentalis* *Planorbella subcrenatum* *Planorbella tenue* *Planorbella trivolvis*	Ramshorn snail[2] Marsh pond snail[11,b]	*Planorbella trivolvis* *Lymnaea elodes*
Second intermediate hosts	**Fish:**		**Planarians[3]**	
	Rock bass	*Ambloplites rupestris*	**Molluscs:**	
	Yellow perch	*Perca flavescens*	Various pulmonate and prosobranch snails, mussels[3]	*Biomphalaria glabrata* *Planorbella spp.*
	Amphibians:			
	Northern cricket frog[13]	*Acris crepitans*	Ramshorn snails[11,b]	*Lymnaea spp.*
	Long-toed salamander	*Ambystoma macrodactylum*	Pond snails[11,b]	*Physa spp.*
	Blue-spotted salamander	*Ambystoma laterale*	**Fish:**	
	Tiger salamander	*Ambystoma tigrinum*	Various fishes[3]	
	Western toad	*Bufo boreas*	Various fishes[2,b]	
	American toad	*Bufo americanus*	**Amphibians:**	
	Pacific chorus frog	*Pseudacris regilla*	Northern cricket frog[4]	*Acris crepitans*
	Northern red-legged frog	*Rana aurora*	Blue-spotted salamander[13]	*Ambystoma laterale*
	Plains leopard frog[13]	*Rana blairi*	Long-toed salamander[13]	*Ambystoma macrodactylum*
	Cascades frog	*Rana cascadae*	Tiger salamander[5]	*Ambystoma tigrinum*
	American bullfrog	*Rana catesbeiana*	American toad[6]	*Bufo americanus*
	Green frog	*Rana clamitans*	Western toad[13]	*Bufo boreas*
	Colombia spotted frog	*Rana luteiventris*	Woodhouse's toad[13]	*Bufo woodhousei*
	Pickerel frog	*Rana palustris*	Pacific chorus frog[13]	*Pseudacris regilla*
	Northern leopard frog	*Rana pipiens*	Western chorus frog[5]	*Pseudacris triseriata*
	Oregon spotted frog	*Rana pretiosa*	Northern red-legged frog[13]	*Rana aurora*
	Mink frog	*Rana septentrionalis*	American bullfrog[7]	*Rana catesbeiana*
	Southern leopard frog[13]	*Rana sphenocephala*	Plains leopard frog[13]	*Rana blairi*
	Wood frog	*Rana sylvatica*	Green frog[7]	*Rana clamitans*

	Great Basin spadefoot	*Scaphiopus intermontanus*	Columbia spotted frog[13]	*Rana luteiventris*
	Rough-skinned newt	*Taricha granulosa*	Pickerel frog[13]	*Rama palustris*
	California newt	*Tarchica torosa*	Northern leopard frog[7]	*Rana pipiens*
			Mink frog[13]	*Rana septentrionalis*
			Southern leopard frog[13]	*Rana sphenocephala*
			Wood frog[13]	*Rana sylvatica*
			Gray treefrog[12]	*Hyla versicolor*
			Rough-skinned newt[13]	*Taricha torosa*
			Various amphibians[2,b]	
			Reptiles:	
Definitive hosts	**Birds:**	**Birds**	Freshwater turtles[3]	
	Cooper's hawk	*Accipiter cooperi*	Cooper's hawk[8]	*Accipiter cooperi*
	Western grebe	*Aechmophorus occidentalis*	Sharp-shinned hawk[8]	*Accipiter striatus*
	Wood duck	*Aix sponsa*	Northern goshawk[8]	*Accipiter gentilis*
	Mallard	*Anas platyrhynchos*	Northern saw-whet owl[8]	*Aegolius acadicus*
	Anhinga	*Anhinga anhinga*	Boreal owl[8]	*Aegolius funereus*
	Domestic goose	*Anser domesticus*	Common pintail[2]	*Anas acuta*
	Great egret	*Ardea alba*	American widgeon[2]	*Anas americana*
	Great blue heron	*Ardea herodias*	Domestic duck[2]	
	Great-horned owl	*Bubo virginianus*	Domestic duck[2,b]	*Anas borchas*
	Red-tailed hawk	*Buteo jamaicensis*	Cinnamon teal[2]	*Anas cyanoptera septentrionalium*
	Broad-winged hawk	*Buteo platypterus*	Blue-winged teal[2]	*Anas discors*
	Little blue heron	*Egretta caerulea*	Eurasian widgeon[2]	*Anas penelope*
	Redish egret	*Egretta rufescens*	Mallard[2]	*Anas platyrhynchos*
	Louisiana heron	*Egretta tricolor*	Black duck[2]	*Anas rubripes*
	American coot	*Fulica americana*	Australian black duck[2]	*Anas superciliosa rogersi*
	Domestic chicken	*Gallus domesticus*	Emden goose[2]	*Anser sp.*
	Common loon	*Gavia immer*	Graytag goose[2]	*Anser anser*
	Whooping crane	*Grus americana*	Magpie goose[2]	*Anseranus semipalmata*
	Bald eagle	*Haliaeetus leucocephalus*	Short-eared owl[8]	*Asio flammeus*
	Herring gull	*Larus argentatus*	Long-eared owl[8]	*Asio otus*
	California gull	*Larus californicus*	Lesser scaup[2]	*Aythya affinis*
	Common merganser	*Mergus merganser*	Redhead[2]	*Aythya americana*

(continued)

Table11.1 (continued)

Ribeiroia ondatrae			Echinostomatid species	
Life cycle stage	Common name[1]	Species	Common name	Species
	Osprey	*Pandion haliaetus*	Ring-necked duck[2]	*Aythya collaris*
	American white pelican	*Pelecanus erythrorhynchus*	Greater scaup[2]	*Aythya marila*
	Double-crested cormorant	*Phatacrocorax auritus*	Great-horned owl[2]	*Bubo virginianus*
	Red-necked grebe	*Podiceps grisegena*	Red-tailed hawk[8]	*Buteo jamaicensis*
	Pied-billed grebe	*Podilymbus podiceps*	Rough-legged hawk[2]	*Buteo lagopus*
	Mammals:		Broad-winged hawk[8]	*Buteo platypterus*
	Raccoon	*Procyon lotor*	Northern harrier[8]	*Circus cyaneus*
	Muskrat	*Ondatra zibethica*	Muscovy[2]	*Cairina moschata*
	Badger	*Taxidea taxus*	Domestic pidgeon[2,a]	
			Domestic pidgeon[2,b]	*Columba livia*
			Fulvous whistling duck[9]	*Dendrocygna bicolor*
			Peregrine falcon[8]	*Falco peregrinus*
			America kestrel[8]	*Falco sparverius*
			Domestic chicken[2,a]	
			Domestic chicken[2,b]	*Gallus domesticus*
			Blackheaded munia[2,a,b]	*Lonchura ferruginosa*
			Spotted munia[2,b]	*Lonchura punctulata*
			Little cuckoo dove[2,a,b]	*Macropygia ruficeps*
			Common scoter[2]	*Oidemia nigra*
			Long-eared owl[8]	*Otus asio*
			Java sparrow[2,b]	*Padda oryzivora*
			Osprey[2]	*Pandion haliaetus*
			American flamingo[2]	*Phoenicopterus ruber*
			Common grackle[2]	*Quiscalus quiscula*
			Great gray owl[8]	*Strix nebulosa*
			Barred owl[8]	*Strix varia*
			Mourning dove[2]	*Zenaida macroura*
			Mammals:	
			Dog[2,a]	*Canis familiaris*
			Guinea pig[2,a]	*Cavia porcellus*

Opossum[10]	*Didelphis virginiana*
Cat[2,a]	*Felis catus*
Golden hamster[2,a]	*Mesocricetus auratus*
House mouse[2,a]	*Mus musculus*
Muskrat[2]	*Ondatra zibethica*
Rabbit[2,a]	*Oryctolagus cuniculus*
Norway rat[2,a]	*Rattus norvegicus*
Pig[2,a]	*Sus scrofa*
Red fox[2]	*Vulpes vulpes*

Most of the life cycle information presented here for *R. ondatrae* was previously summarized in Johnson et al. (2004) (1) and life cycle information presented here for *Echinostoma* species was previously summarized in Huffman and Fried (1990) (2). Additional references are denoted by superscript numbers following the common names of hosts: (3) Kanev et al. (1995), (4) Beasley et al. (2005), (5) Johnson and McKenzie (unpublished data from Colorado), (6) Ulmer (1970), (7) McAlpine and Burt (1998), (8) Taft et al. (1993), (9) Forrester et al. (1994), (10) Alden (1995), (11) Sorensen et al. (1997), (12) Koprivnikar et al. (2006), (13) Sutherland and Johnson (unpublished)

[a] Indicates a host that was experimentally infected; all others are recorded from natural host infections

[b] Indicates a host species record for *E. revolutum*, while all other hosts listed in the echinostomatids column are records for *Echinostoma trivolvis*

pipiens) around this pond suffered severe limb malformations, including extra, missing, and misshapen limbs. Although some abnormalities are expected in any vertebrate population, this baseline frequency is generally low (0–5%), leading researchers to suspect that something unusual was happening at the pond. The intensity of the media's response to this discovery, coupled with the speed with which images and information were disseminated via the Internet, stimulated a surge of malformation surveys in North American amphibians, ultimately resulting in numerous new reports of deformed amphibians.

Many factors can cause malformations in amphibians (see Ouellet 2000 for a review), and it is likely that the current "phenomenon" is actually the product of multiple etiological factors. Nevertheless, growing evidence supports a link between *Ribeiroia* infection and high-frequency cases of amphibian limb deformities. The hypothesis that trematodes could cause amphibian malformations was first suggested by Sessions and Ruth (1990), who noted high concentrations of unidentified metacercarial cysts around the deformed limbs of Pacific treefrogs (*Pseudacris regilla*) and California long-toed salamanders (*Ambystoma macrodactylum croceum*) in California. More recently, a series of laboratory and field experiments have definitively tested this hypothesis in a variety of frog, toad, and salamander species. These experiments revealed that exposure to realistic levels of *Ribeiroia* cercariae causes elevated mortality and severe malformations (up to 100%) in larval amphibians, including extra limbs, skin webbings, bony triangles, and missing limbs in both the fore- and hind limbs (Johnson et al. 1999; 2001a; 2006; Kiesecker 2002; Stopper et al. 2002; Schotthoefer et al. 2003; Johnson and Hartson 2008). Importantly, however, the types and severity of malformations vary with parasite dosage (number of cercariae), the timing of exposure, and the amphibian species being exposed. More parasites generally translate into higher pathology, but only during certain periods of development (e.g., early larval stages) and in species that are susceptible to infection (Bowerman and Johnson 2003; Schotthoefer et al. 2003; Johnson and Hartson 2008). Field data further support the link between infection and malformations, and *Ribeiroia* has been connected to deformed amphibians in the Midwest, the Northeast, and especially the Western USA (Johnson et al. 2001b, 2002; Kiesecker 2002; Lannoo et al. 2003; Sutherland 2005; Johnson and Hartson 2008). Once again, higher average levels of *Ribeiroia* infection within a wetland are associated with higher frequencies of malformations, which can range from <5% to as high as >90% in metamorphosing amphibians.

Collectively, results of this growing body of research indicate that *Ribeiroia* is an important and widespread cause of amphibian deformities in the USA. It has even been recovered from many of the early reported "hotspot" malformation sites, such as the original Ney Pond and the enigmatic "CWB" pond, also in Minnesota (e.g., see Hoppe 2005; Sutherland 2005; Sutherland and Johnson, unpublished). This does not imply, however, that *Ribeiroia* infection explains all malformations in amphibians. *Ribeiroia* is unlikely to causes cases of predominantly or exclusively missing-legged frogs, as have been reported in some areas (Skelly et al. 2007). While *Ribeiroia* can and does cause missing and partially missing limbs in amphibians, these usually occur alongside a broad variety of other abnormality types, such as

skin webbings and bony triangles, such that parasite-induced outbreaks of *only* missing legged amphibians are rare. Extra limbs and digits may be a common form of pathology in select species (most notably *Pseudacris regilla*), but are uncommon or absent in many other species exhibiting parasite-induced malformations (Johnson et al. 2001a, 2002; Johnson and Hartson 2008).

Over the last 10 years, we have accumulated records of *Ribeiroia* and malformations from a broad range of locations and amphibian species in the USA. Much of this work has been in cooperation with scientists from the US Fish and Wildlife Service, who have collected and examined amphibians from National Wildlife Refuges across the country (Johnson et al., in press). Necropsies of animals from many of these refuges have revealed interesting patterns in the abundance and distribution of *Ribeiroia* and echinostomatids (see later). For example, within the National Wildlife Refuge survey, *Ribeiroia* was recovered from eight species of amphibians across 13 states, representing 14 of the 37 examined refuges. Many of these represent new host or locality records. The distributional pattern of *Ribeiroia* and of malformations corresponds roughly with the major flyways of migratory birds in the USA: the Pacific, the Mississippi, and the Atlantic flyways (Fig. 11.2). Intriguingly, however, records of *Ribeiroia* in amphibians are conspicuously absent from the southern USA below around 37° latitude. This is somewhat surprising given the diversity of birds in these areas, and in some cases the number of *Ribeiroia* records from birds (e.g., Forrester and Spalding 2003). It is not yet clear to what extent this pattern is truly representative of the parasite's distribution or owes instead to incomplete sampling, but it is interesting to note that deformed amphibians have also predominantly been recorded in the northern half of the USA (http://frogweb.nbii.gov/narcam/). These results also illustrate the broad range in infection levels exhibited by amphibians. While many populations exhibit low average infection abundances, other amphibians may support up to 1,000 metacercariae in a single frog, generally with a high corresponding frequency of severe malformations in the population (Fig. 11.3).

11.2.2 Are Parasite-Induced Malformations a Problem?

One of the most challenging and pressing questions to address is whether *Ribeiroia* infection and the resulting malformations represent a threat to affected amphibian populations. The answer to this question depends, in part, on whether infection and malformations are increasing (see below). Nevertheless, several compelling pieces of evidence suggest that *Ribeiroia*-induced pathology is problematic. First, based on laboratory exposure studies, even low numbers of *Ribeiroia* cercariae (10–50) cause high mortality in many amphibian species. For example, experiments with *P. regilla*, *B. americanus*, *B. boreas*, and *R. pipiens* have shown that exposure to as few as 25 cercariae causes 30–95% mortality in larval amphibians, often in <1 week. Invading cercariae penetrate amphibian tissue directly, with each parasite incurring an injury in its host. The combined effects of those injuries may be substantial

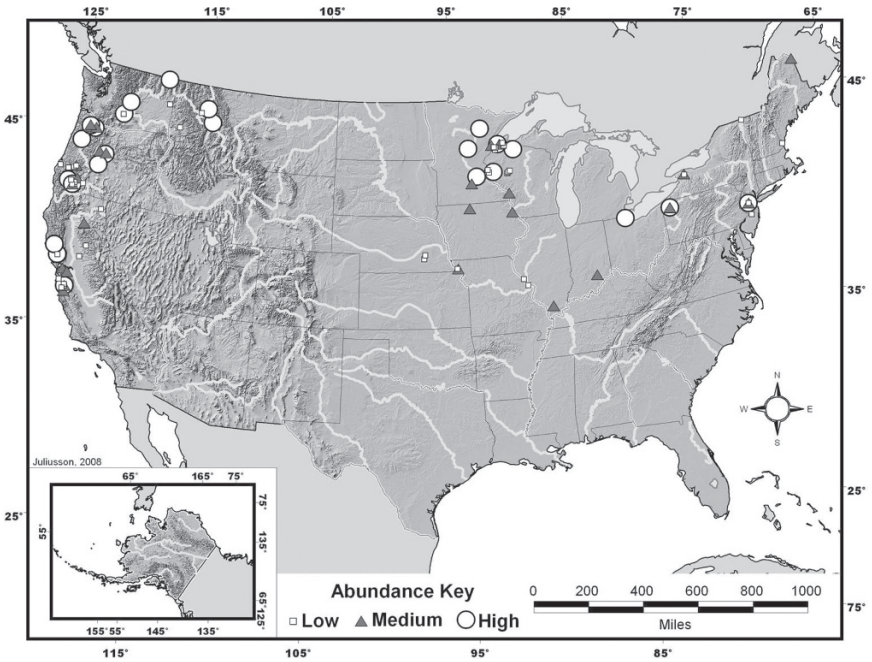

Fig. 11.2 Geographic distribution of *Ribeiroia* as determined from amphibian necropsies. Data represent a compilation of samples from USFWS National Wildlife Refuges and additional sampling on private lands. The size of each circle reflects the average infection abundance recorded in the sample (usually determined from a sample of 10 amphibians). In total, 16 amphibian species from 107 sites distributed across 20 states are included (1999–2007). Abundance values as follows: low (1-10 metacercariae per amphibian), medium (11–30 metacercariae), and high (31–135 metacercariae). Infection intensity for individual frogs ranged form 1 to 960

in early stage larvae. At wetlands that support *Ribeiroia*, one of us (PTJJ) has routinely observed dead and dying amphibian larvae with microhemorrhaging around the limbs characteristic of heavy cercarial penetration. In most cases, however, such mortality is difficult to observe and quantify given the rate of predation and decomposition in nature.

Second, many of the malformations induced by *Ribeiroia*, which typically affect the hind limbs, are almost certainly detrimental to amphibian survival. Missing, extra, or severely malformed limbs interfere with an animal's ability to swim, jump, obtain food, and, especially, to avoid predators. Following years in which >50% of metamorphic amphibians exhibited severe limb deformities, fewer than 2% of returning adults exhibited abnormalities, strongly suggesting that abnormal individuals are less likely to reach sexual maturity than their normal counterparts (Johnson et al. 2001b; Lunde and Johnson, unpublished). Those abnormalities that were observed in adults tended to be very minor. At a malformation hotspot in Minnesota, American toads (*B. americanus*) completely failed

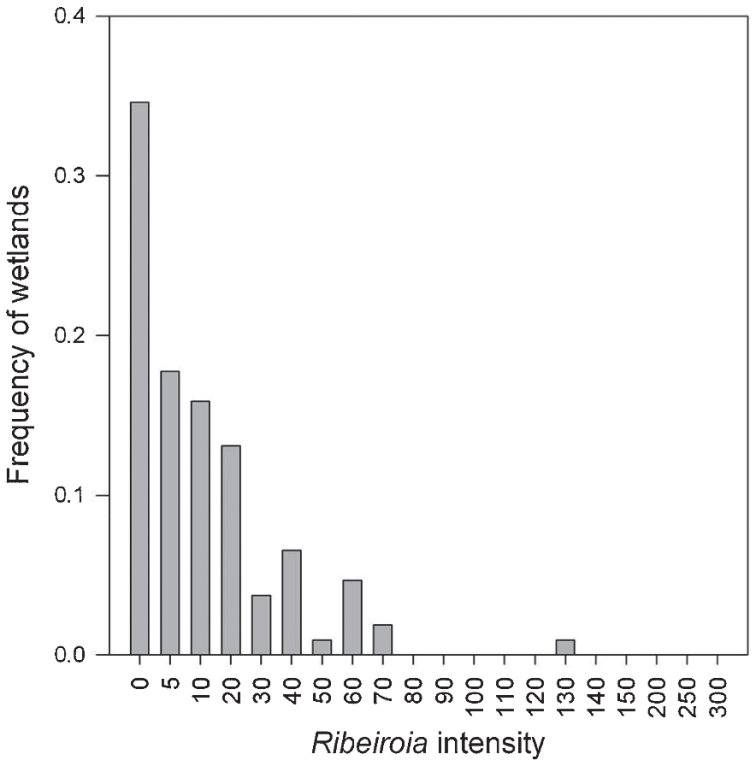

Fig. 11.3 Frequency distribution of *Ribeiroia* infection intensity among wetlands that support the parasite. Values represent the average level of infection within a wetland, as determined from the necropsy of ten individual amphibians. Data are combined among amphibian species and among years

to breed following two years of high-frequency (up to 80%) malformations in metamorphs (Johnson and Hartson 2008). Similarly, Hoppe (2005) noted the disappearance of several anuran species from a Minnesota wetland ("CWB") with high levels of *Ribeiroia* infection following several years of severe malformations. At Jette Pond, Montana, which boasts the longest history of malformations in the USA (1950s to present), three amphibian species have declined or disappeared, while *Ribeiroia* and malformations appear to have increased (see Johnson et al. 2003).

In all these cases, however, the role of infection and malformations in explaining such declines remains correlative and inconclusive. Definitive evidence linking *Ribeiroia* and population-level effects in affected amphibians remains lacking. A combination of long-term monitoring, mark-recapture data, and ecosystem-level manipulations (e.g., parasite removal or addition) is needed to address this issue.

Considering the widespread and abundant occurrence of *Ribeiroia*, as well as the diversity of amphibian species affected by malformations, we argue that population effects of conservation importance are likely, particularly when infection occurs in combination with additional stressors (e.g., habitat loss, pollution, introduced species, etc.).

11.2.3 Emergence of Ribeiroia and Malformations

The current and future importance of malformations for amphibian conservation depends on whether *Ribeiroia* represents an emerging infection. Owing to a lack of reliable historical data on infection patterns, this remains one of the most difficult questions to address adequately. For example, prior to 1999, we are aware of no published records documenting *Ribeiroia* infection within free-living amphibian populations. Beaver (1939) and Riggin (1956) each reported conducting experimental infections with *Ribeiroia* and larval amphibians, but did not comment on naturally occurring infections. Since 1999, however, *Ribeiroia* metacercariae have been recorded in more than 20 amphibian species, sometimes at relatively high abundance (Table 11.1; Fig. 11.3). Rather than signaling a recent host shift toward amphibians, this information most likely reflects a recording bias: trematode metacercariae are notoriously difficult to identify, particularly among preserved or frozen hosts, and many parasitologists focus on adult parasites. There are numerous records of *Ribeiroia* from definitive hosts between 1931 and 1999, collectively representing 40 species of birds and 4 species of mammals (Table 11.1). Thus, while unlikely to be an introduced parasite into the US, we must ask whether the abundance and distribution of *Ribeiroia* or the severity of amphibians' response to infection have recently changed. Several studies have reported an increase in the baseline frequency of abnormalities in amphibian populations (Hoppe 2000; Gray 2000; McCallum and Trauth 2003), but the role of changes in parasitic infection in these instances is unknown.

Alongside changes in baseline levels of abnormalities, an important question to address is whether the abundance or severity of "mass malformations" accounts, which involve deformities among >5% of the amphibian population (Johnson et al. 2003), have recently increased. Reports of mass malformations in amphibians occur in the historical literature, but they are exceptionally rare. Johnson et al. (2003) coupled examinations of malformed voucher specimens with contemporary resurveys to evaluate the role of *Ribeiroia* in historical mass malformation accounts. Their results indicate that *Ribeiroia* likely explained six of eight such historical cases of mass malformations in the USA, dating back to the 1940s. This suggests that parasite-induced malformations are not a new phenomenon, but does not address whether they have increased in number of cases, severity, or geographic distribution. Nevertheless, the authors suggested that several lines of evidence support the notion that parasite-induced malformations have increased. First, despite a long and intensive history of amphibian surveys, historical cases of mass malformations

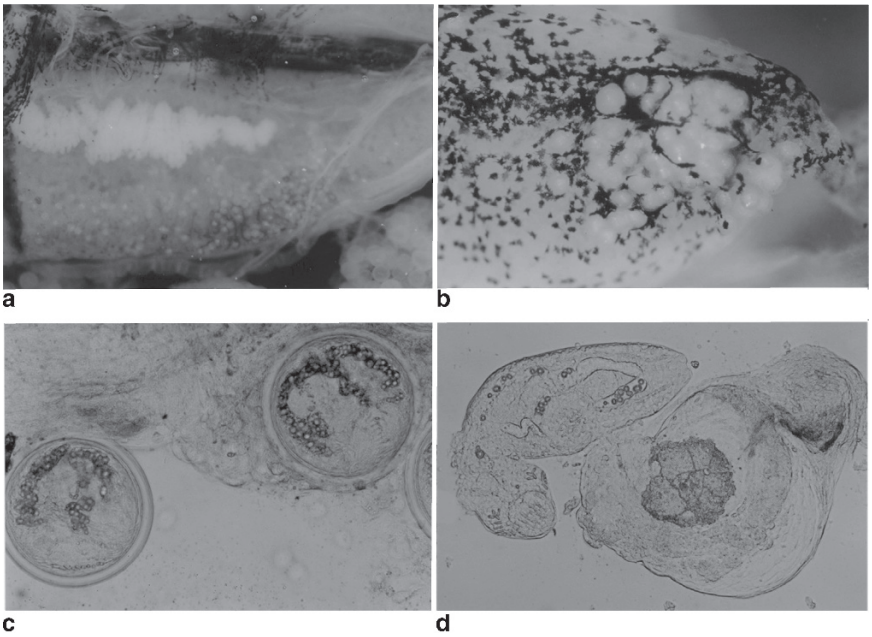

Fig. 11.4 (**a**) In situ view of echinostome infection within an amphibian kidney; metacercariae are visible as white cysts along the lower half of the kidney; (**b**) Close-up view of metacercariae within the kidney; (**c**) isolated encysted metacercariae; (**d**) excysted metacercaria next to its cyst (note collar spines)

in North American amphibians are extremely rare (less than 10 between 1947 and 1990). In contrast, more than 50 mass malformation sites associated with *Ribeiroia* have been recorded since 1996 (Fig. 11.3). Second, most mass malformations accounts (recent and historical) occurred in artificial or highly modified wetlands, such as farm ponds, cattle ponds, impoundments, or retention wetlands. *Ribeiroia* and its snail hosts (*Planorbella* spp.) often thrive in the nutrient-rich conditions of these environments, wherein they may be more likely to achieve elevated abundance. While *Ribeiroia* also occurs in natural wetlands, it typically exhibits a higher abundance that may be more likely to induce mortality and malformations in artificial wetlands (Johnson et al. 2002; 2003; Lannoo et al. 2003; Johnson and Lunde 2005). It is tempting to speculate that, prior to the widespread replacement of natural wetlands with artificial systems, which often differ dramatically in size, biota, hydroperiod, and water chemistry, high levels of *Ribeiroia* infection and the resulting malformations were less common than they are today. Again, however, extensive long-term data on sites with and without *Ribeiroia* are needed to identify the temporal trends in malformation frequency.

11.3 Overview of Echinostomes

Here, we focus on the echinostomes that utilize amphibians as intermediate hosts in their life cycles and compare and contrast them with *Ribeiroia*. While *Ribeiroia* infection in amphibians has been the subject of extensive field observations and ecological studies (see earlier), species of *Echinostoma* have proven excellent model organisms for laboratory studies of host–parasite relationships, ranging from physiology to immunology (Huffman and Fried 1990; Toledo et al. 2007). Given the ecological similarities of these two parasites in amphibians, we further aim to critically examine evidence for a possible rise in echinostome infection in response to the same drivers that influence *Ribeiroia*.

In North America, *Echinostoma trivolvis* is the most commonly reported echinostome that infects amphibians as second intermediate hosts (Fried and Graczyk 2004). Several other echinostomes that occur in North America and infect amphibians as intermediate hosts include *E. revolutum* and *Echinoparyphium* spp., but these are less commonly reported. Both molecular and morphological data support *Echinostoma* and *Echinoparyphium* as being distinct genera (Kostadinova et al. 2003). However, because they have relatively few recognizable features to separate them, especially as metacercariae, some workers believe that misidentification may be common (Sorensen et al. 1997; Fried et al. 1998). *Echinostoma revolutum* has a more prevalent distribution in Europe, Asia, and Africa but has been confirmed in the midwestern USA by Sorensen et al. (1997) using morphological traits and in Connecticut by Holland et al. (2007) using molecular techniques. The overall distribution of *E. revolutum* is not well understood, probably due, in part, to misclassification as *E. trivolvis* in some regions. *Echinoparyphium* species are known from snail hosts in North Carolina (Fried et al. 1998), Michigan, and California (Najarian 1954; Kanev et al. 1998), but they are also suspected to be frequently misidentified as *E. trivolvis* (Fried et al. 1998). Because of such difficulties in identification, we make no attempt here to differentiate among these groups and elect instead to combine our discussions of *Echinostoma trivolvis*, *Echinostoma revolutum*, and *Echinoparyphium* species under the broad category of echinostomes.

Similar to *Ribeiroia*, echinostomes require three hosts to complete their life cycles (Table 11.1). *Ribeiroia ondatrae* infects several genera of planorbid snails as first intermediate hosts, whereas echinostomes appear to be more specialized at this life cycle stage. *Echinostoma trivolvis* has only been reported from *Planorbella trivolvis*; trial infections with other planorbid species have failed, and miracidia from a Pennsylvania strain were infective to *P. trivolvis* from Pennsylvania, but not from Colorado (Fried et al. 1987). Similarly, miracidia of *Echinostoma revolutum* from Indiana infected *Lymnaea elodes*, but not *Lymnaea stagnalis, Physa gyrina*, or *Planorbella trivolvis* (Sorensen et al. 1997) (although Holland et al. 2007 reported *E. revolutum* from *P. trivolvis*). *Echinoparyphium flexum* infects *Lymnaea palustris* in North America (Najarian 1954), whereas *Echinoparyphium rubrum* infects *Physa gyrina* in Michigan and *Physa occidentalis* in California (Kanev et al. 1998).

With respect to second intermediate and definitive hosts, echinostomes are adept generalists. In addition to amphibians and fish, the echinostomes employ a diversity of snails as second intermediate hosts, as well as a few planarians and reptiles (see Table 11.1). Echinostome cercariae emerging from an infected snail thus have many potential second intermediate hosts to infect: they can (a) reinfect the same snail by encysting in the renal organs as metacercariae, (b) infect other snails of any number of species, or (c) encyst in the kidneys of fish, amphibians (Fig. 11.4), or reptiles (Fried and Graczyk 2004). Less commonly, encysted echinostomes can also be found within the body cavity or near the tail resorption site of metamorphosing amphibians (J. Koprivnikar, personal communication; Johnson, unpublished data). Such a range of second intermediate hosts increases the prospects in the trophic food web to cover many common prey animals taken by aquatic feeding birds. The use of snails as second intermediate hosts also simplifies the number of hosts required to complete the life cycle from three (e.g., *Ribeiroia*) to two (e.g., echinostomes). As a result, echinostomes are often more common among dabbling ducks (Anatidae) than is *Ribeiroia*, as these ducks are common consumers of gastropods (Benoy et al. 2002). Both *Ribeiroia* and echinostomes utilize a broad diversity of predatory birds, ranging from herons to owls to raptors, each of which commonly consume amphibians and fish. The hosts listed in Table 11.1 should not be regarded as complete, since many regions of North America have not been studied with regard to these parasites and specific training is needed to differentiate these parasite species, particularly at the larval stages.

11.3.1 Pathology and Patterns of Infection in Amphibians

Although echinostomes are relatively uncommon in the historical literature on amphibian parasites (e.g., Beaver 1937; Najarian 1952), many researchers have observed these parasites in amphibians in recent decades. Muzzall et al. (2001) reported "echinostomid-like metacercariae" from green frogs (*R. clamitans*) in southern Michigan. Skelly et al. (2006) and Holland et al. (2007) reported *Echinostoma revolutum* from green frogs in Connecticut. King et al. (2007) found echinstomes to be widely prevalent in Northern leopard frogs (*Rana pipiens*) in southern Quebec, while Koprivnikar et al. (2007a) reported echinostomes from snails in southern Ontario. In our own work with the National Wildlife Refuge survey, we recorded echinostomes in more than one-third of all examined amphibians ($n > 1{,}500$) from across the USA, including 16 species from 25 states. Again, many of these represent new host and/or locality records. All the 37 surveyed refuges supported at least one amphibian or wetland with infections by echinostomes. As found with *Ribeiroia*, the abundance of *Echinostoma* was greatest along the major bird flyways and in the temperate regions (Fig. 11.5). Echinostome infections exhibited a remarkable variation in intensity, ranging from 1 metacercariae per frog up to 2,750 metacercariae between the two kidneys (Fig. 11.6).

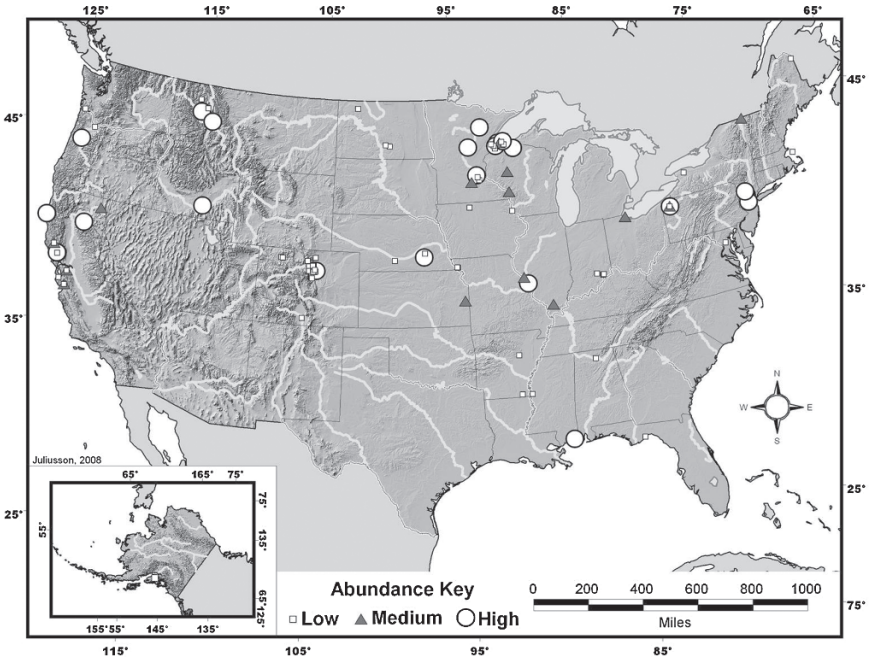

Fig. 11.5 Geographic distribution of echinostomes as determined from amphibian necropsies. Data represent a compilation of samples from USFWS National Wildlife Refuges and additional sampling on private lands. The size of each circle reflects the average infection abundance recorded in the sample (usually determined from a sample of 10 amphibians). In total, 16 amphibian species from 231 sites distributed across 30 states are included (1999–2007). Abundance values as follows: low (1–50 metacercariae per amphibian), medium (51–100 metacercariae), and high (101–1,960 metacercariae). Infection intensity for individual amphibians ranged from 1 to 2,750

Fig. 11.6 Frequency distribution of echinostome infection intensity among wetlands that support the parasite. Values represent the average level of infection within a wetland, as determined from the necropsy of ten individual amphibians. Data are from 231 wetlands combined among amphibian species and among years (1999–2007). Average infection intensity ranged from 1 to 1,960 metacercariae per amphibian

Within larval and postmetamorphic amphibians, the echinostomes encyst in the kidneys (Fig. 11.4). Whether such infection increases the susceptibility of amphibian hosts to definitive host predators is unknown. However, infection of vital organs such as kidneys can cause significant pathology at high parasite intensity (Martin and Conn 1990). Beaver (1937) was the first to observe the edema experienced by infected tadpoles and noted the pattern by which *E. trivolvis* cercariae systematically crawl across the body of a tadpole heading toward the cloaca, wherein they enter the host. Inside the cloaca, migrating cercariae enter the mesonephric ducts, crawl toward the kidneys and form a cyst wall, which becomes infective to definitive hosts within six hours (Fried et al. 1997). Fried et al. (1997) found that experimentally infected tadpoles (*Rana pipiens*) suffered edema, intensity-dependent mortality, and inhibited growth. Schotthoefer et al. (2003) made a significant contribution to the understanding of echinostome pathology in amphibians by considering the role of developmental stage in determining the response of tadpoles to parasite exposure. Early stage tadpoles (stage 25, Gosner 1960) exhibited the highest mortality following *E. trivolvis* exposure, suggesting that the developmental stage of the kidneys plays an important role in host response to infection. Early stage tadpoles only have small pronephroi (or "head kidneys"), and Schotthoefer et al. (2003) argued that these tiny, fragile organs are more susceptible to developmental disruption caused by encysting metacercariae relative to the fully developed mesonephroi of later stage tadpoles. They further noted that tadpoles became progressively less susceptible to infection with continued development toward metamorphosis. Working with *E. revolutum* infection in tadpoles of *Rana clamitans*, Holland et al. (2007) presented similar results linking host mortality and the loss of renal function. Corresponding histopathology of infected kidneys revealed signs of edema and granulomas surrounding metacercariae. Intriguingly, Thiemann and Wassersug (2000a) suggested that echinostome parasites have evolved a mechanism to reduce pathology and mortality risk within amphibian hosts: the bulk of cercariae colonize only one kidney within an individual host, thereby reducing the risk that overinfection leads to renal failure in both kidneys (vertebrates can survive with only one kidney). In support of this hypothesis, the authors noted a significant right side bias in echinostome infection in tadpoles of *Rana sylvatica* and *R. clamitans*. The generality of this infection pattern and the mechanisms producing it remain unknown.

In summary, the pathology of echinostomes in amphibians can be significant and, like *Ribeiroia*, varies with the developmental stage of the amphibian host and the level of cercarial exposure. However, the dosages of echinostome cercariae necessary to cause pathology are often greater than those observed for *Ribeiroia*. For example, while high mortality due to echinostome exposure has only been observed among early stage amphibian larvae (see Schotthoefer et al. 2003), even following relatively high cercarial exposures (100 cercariae), low-to-moderate dosages of *Ribeiroia* cercariae (e.g., 12–50) can induce high mortality across a wide range of developmental stages (Johnson and Hartson 2008; Johnson et al. 1999, 2001b; Schotthoefer et al. 2003). On the other hand, echinostome trematodes are extremely widespread in wetland habitats and are frequently abundant within amphibian hosts. Thus, while it is difficult to project the population level impacts

Fig. 11.7 Kidneys of a recently metamorphosed Pacific chorus frog (*Pseudacris regilla*) with a heavy echinostome infection. Note the large proportion of renal tissue with metacercariae (visible as small white cysts)

of echinostome parasites in amphibians, the combination of their high prevalence, high abundance, and pathogenic effects in laboratory studies suggest that, in some wetlands, their effects may be substantial. For example, Beasley et al. (2005) studied factors related to the declines of Northern cricket frogs (*Acris crepitans*) in Illinois and found all eight sites examined for parasites to have echinostomes present in juvenile frogs. In three of the eight sites, the prevalence of echinostomes in tadpoles and juvenile frogs was between 69% and 100%. In one of these sites, the majority of infected frogs had high-intensity infections where more than 50% of the kidney tissue was occupied by echinostome metacercariae as determined by histological sections (e.g., Fig. 11.7). This level of infection translates to individual tadpoles being exposed to multiple thousands of cercariae during their development in the aquatic environment. The following year, the authors observed a decrease in cricket frog recruitment in sites that had exhibited high echinostome infections, leading them to argue that high mortality due to infections was likely an important factor. Taken together with similar accounts for populations heavily impacted by *Ribeiroia* (discussed earlier), we feel these parasites warrant more attention in regions where they are highly abundant.

11.3.2 Emergence of Echinostoma

Finally, we ask whether echinostome infections have recently exhibited changes in abundance, in host usage, or in geographic distribution. Given the limited data available, we can only begin to address these questions here, and our goal is to suggest avenues of research that will advance this intriguing research area. Historical

records of echinostome infections in amphibians are few (Beaver 1937; Najarian 1952). However, it is not well understood to what extent previous researchers examined the kidneys for metacercariae. For example, Andrews et al. (1992) provide an extensive checklist of 51 species of digeneans reported from bullfrogs (*R. catesbeiana*) in North America, including many metacercariae and mesocercariae, but no echinostomes. It is difficult to use the rarity of historical records as a reliable indicator of past prevalence or abundance of echinostomes, similar to the limitations of the parasitology data for evaluating the historical abundance of *Ribeiroia*. But a few recent studies that have examined amphibian kidneys provide a strong signal that we should address this question more carefully. Beasley et al. (2005) observed extremely heavy echinostome infections in Northern cricket frogs where more than half the kidney tissues of tadpoles consisted of metacercariae. Similarly, Skelly et al. (2006) reported echinostome infections of up to 1,648 metacercarial cysts in a single green frog (*R. clamitans*) in Connecticut. There is no doubt that these high-intensity infections can cause extreme pathology and death, especially given that experimental infections of 100 cercariae led to high mortality in tadpoles (Schotthoefer et al. 2003). To more accurately address whether amphibians are experiencing more frequent and heavier echinostome infections, a comprehensive study of vouchered amphibians in museum collections is in order, particularly for sites with a long collection history. Though the current evidence for increasing levels of echinostomes is thin, it is noteworthy that, among our recent surveys, this group of parasites is the most common larval trematode encountered, often with 100% prevalence within the amphibian population and individual infections numbering in the several hundred.

11.4 Possible Causes of Parasite Emergence

Growing attention has recently focused on the question of how environmental change affects host–macroparasite interactions (Bradley and Altizer 2007; McKenzie and Townsend 2007; Johnson and Carpenter 2008). Because many of these parasites have complex or multihost life cycles, and different hosts vary in their responses to environmental changes, addressing this question is neither simple nor does it have a single answer. Ultimately, the response of a parasite will vary among types of parasites and types of environmental change, and the product of host abundance, host physiological condition, spatial and temporal interactions among hosts, the abundance and composition of the parasite community, and the effect of environmental conditions on the parasite's free-living stages (e.g., Lafferty and Holt 2003).

Investigations into this area have fallen largely into two distinct categories. On one hand, the diversity of macroparasite infection in a host species and community can be used as a biological indicator. Because of the complexity of helminth life cycles and their dependency on multiple interacting species across several trophic levels, the diversity of parasites is often indicative of a species-rich community of hosts (e.g., Hechinger et al. 2007). Simply stated, ecosystems with low

levels of disturbance can be expected to support more diverse host and parasite communities (Hudson et al. 2006). For example, Huspeni and Lafferty (2004) found that estuarine environments surrounded by development supported half as many parasite species as estuaries surrounded by natural areas. This difference was attributed to the reduced diversity of birds and other free-living species within impacted estuaries.

On the other hand, a second important issue is what controls the abundance of a macroparasite species within a community, particularly for parasites that cause severe pathology in their hosts. Many macroparasite infections are relatively benign at low levels. As infection abundance increases, however, so too does the likelihood of pathology and disease ("intensity-dependent" pathology). Thus, while the diversity of macroparasite species may decrease with certain forms of environmental change, the abundance of certain pathogenic species may increase, potentially increasing the risk of disease outcomes. In fact, the loss of many parasite species from a community following a disturbance may reduce competition among the remaining parasites, further contributing to an increase in parasite abundance. Parasite species richness and parasite species even-ness may therefore be uncorrelated or even negatively correlated, such that select parasites become over-represented in species-poor communities. We focus here on how environmental change can increase the abundance of pathogenic parasites.

With respect to *Ribeiroia* and echinostome infections in amphibians, several forms of environmental change have been shown or suggested to enhance infections. Collectively such changes alter the abundance or distribution of hosts, their susceptibility to infection, or the abundance and productivity of the parasite. Many of the mechanisms discussed as follows are not mutually exclusive or independent, and may interact to additively or synergistically influence patterns of infection:

1. Changes in land use and wetland characteristics

- Changes in patterns of trematode infection within amphibians ultimately result from shifts in surrounding land use and wetland characteristics. This forms the foundational context for the specific mechanisms discussed later. One particularly important form of land use change involves the destruction of natural wetlands and their replacement by artificial systems. As natural wetlands continue to be destroyed and altered, amphibians rely increasingly on artificial or highly modified wetlands to complete their breeding cycles, including farm ponds, stock ponds, retention systems, and other impoundments (Knutson et al. 2004). These artificial environments often exhibit strikingly different biotic and abiotic characteristics relative to their natural counterparts, and we discuss some of the important changes later.

2. Nutrient pollution

- Nutrient runoff from fertilizers, livestock, and erosion often leads to eutrophication in wetlands surrounded by agrarian or urbanized landscapes. The resulting increase in algal growth in freshwater environments may differentially favor some groups of freshwater snails, including the planorbids (Chase 2003; Johnson and Chase 2004). Johnson et al. (2002) and Johnson and Chase (2004)

reported field correlations among nutrient levels, snail host density, and *Ribeiroia* infection in amphibians. Skelly et al. (2006) suggested a similar pattern may apply to echinostome infections in amphibians from urban wetlands. They found that, while variable, the abundance of snails and echinostome infections in frogs were highest in urban wetlands. Recently, the relationship between nutrient inputs and trematode infection has been tested experimentally using outdoor mesocosms. By manipulating nutrient levels and the input of *Ribeiroia* eggs (e.g., bird activity), Johnson et al. (2007) found that eutrophication enhanced *Ribeiroia* infection in green frog larvae through two related mechanisms: nutrient-mediated increases in algal growth enhanced both the density of *Ribeiroia* infected snails and the per-snail production of *Ribeiroia* cercariae. Collectively, these changes caused a three- to five-fold increase in metacercerial abundance in co-occurring tadpoles.

- Importantly, however, agriculture and urban development can also affect definitive host activity. Forms of land use that depress activity of definitive hosts could reduce the abundance of parasite inputs (e.g., eggs) into aquatic systems, even while the density and susceptibility of snail hosts are maximized. Clearly this outcome depends on the type and severity of development and the particular needs of the definitive hosts. In some cases, eutrophication has been linked to increased colonization by vertebrate definitive hosts (e.g., Esch 1971; Wisniewiski 1958; Zander et al. 2002), whereas in others the loss of forested areas following development is associated with decreases in bird and mammal activity (e.g., King et al. 2007).

3. Pesticide contamination

- In addition to nutrients, wetlands surrounded by agriculture, industrial development, or residential areas often support a diverse mixture of pesticides and other contaminants. Because some contaminants can suppress immune function in amphibians, their exposure may increase the susceptibility of amphibian larvae to infection by trematode cercariae. Changes in tadpole behavior following contaminant exposure, such as reduced swimming activity, could further increase infection by parasites (Thiemann and Wassersug 2000b; Taylor et al. 2004). Kiesecker (2002) reported that amphibians in pesticide-contaminated ponds in Pennsylvania exhibited more *Ribeiroia* metacercariae and higher malformations than those in pesticide-free ponds. In complementary experiments, he showed that exposure to atrazine and malathion increased trematode infection success in wood frog (*Rana sylvatica*) larvae, possibly by reducing immune function (measured by eosinophil count). Other studies have also suggested a link between pesticide exposure and infections of amphibians by viruses (Forson and Storfer 2006) and other trematodes (Koprivnikar et al. 2007a). With respect to *Ribeiroia* and *Echinostoma*, however, more data are needed on the effects of contaminants on the parasites' free-living stages (e.g., miracidia and cercariae) and on snail hosts, including especially infected snails, which can be more sensitive than uninfected individuals (see also Koprivnikar et al. 2007b). Without such information, it is difficult to evaluate the ecological relevance of pesticide-parasite-amphibian interactions.

4. Loss of biodiversity

- The dilution effect hypothesis suggests that with decreases in host diversity, the abundance and pathology of some parasites will increase (e.g., Keesing et al. 2006). This hypothesis holds when more diverse communities support a greater relative abundance of low competency hosts that either resist infection or do a poor job of supporting parasites once infected. In this manner, diverse communities lead to more "wasted" transmission events, in which infectious stages of the parasites (e.g., cercariae) are eliminated by infecting less susceptible hosts. While most of this hypothesis has been developed with a focus on vector-borne infections (e.g., Lyme disease, West Nile virus), increasing evidence suggests that it may also apply to complex life cycle parasites with free-living infectious stages. Experimental evidence supports this principle in relation to infections of first intermediate hosts (e.g., snails) and second intermediate hosts (e.g., amphibians). Johnson et al. (2008) found that the addition of "decoy" snails, which were not susceptible to trematode miracidia, dramatically reduced infection in susceptible snails, even when the density of susceptible snail hosts remained constant among treatments. This ultimately caused a 40% reduction in snail infection prevalence and a 60% reduction in cercarial production. Similar results involving snail-miracidia infections have been reported by Chernin (1968), Mone and Combes (1986), and Kopp and Jokela (2007).
- With respect to amphibian infections, heterospecific amphibian communities containing larvae of both American toads (*B. americanus*) and gray treefrogs (*Hyla versicolor*) supported 40–60% fewer *Ribeiroia* metacercariae than did monospecific communities with *B. americanus* alone (Johnson et al. 2008). All communities were initially exposed to the same number of *Ribeiroia* cercariae. However, larval *H. versicolor* are largely resistant to infection, and cercariae that colonized these hosts were killed by the immune system. This led to a reduction in the total abundance of surviving metacercariae. Because the presence of *H. versicolor* also reduced infections in co-occurring toads, toads from the heterospecific treatments exhibited higher survival and fewer malformations than toads raised alone or with another toad (Johnson et al. 2008). Taken together, these results suggest that ongoing losses of aquatic biodiversity, including snails and especially amphibians, may influence patterns of trematode transmission and pathology. Artificial or modified wetlands often support lower community diversity than natural systems, which may therefore exacerbate infection levels via the dilution effect. It is important to recognize, however, that the applicability of the dilution effect to *Ribeiroia* and *Echinostoma* infections depends critically on the patterns of community assembly and community disassembly (Ostfeld and LoGiudice 2003). If, as has been suggested on coevolutionary grounds by Ostfeld and colleagues, low-diversity communities are dominated by highly competent hosts, with less competent hosts (dilution or decoy hosts) occurring with increasing diversity, then transmission will likely be maximized in species-poor communities. If, however, necessary hosts in the life cycles of either parasite are absent in low-diversity communities, transmission will be interrupted. Thus far, insufficient information is available on the (a) competency

of snail and amphibian hosts to each parasite and (b) the patterns of community disassembly in wetlands to assess the generality of the dilution effect under field conditions. Finally, while less commonly included in discussions of the dilution effect, it is important to recognize that nonhost species such as predators can also influence the abundance of infectious parasites in the environment through predation (see Schotthoefer et al. 2007; Thieltges et al. 2008).

5. Climate change

- Finally, forecasted changes in climate are expected to influence patterns of trematode infection and pathology in amphibians. Increases in minimum temperatures and the length of the growing season are likely to affect both the timing and maximal levels of infection. Despite growing interest in the effects of climate change on human and wildlife diseases (e.g., Harvell et al. 2002), the full complexity of host–parasite–climate interactions remains poorly understood. The pathology induced in amphibians by *Ribeiroia* and *Echinostoma* is stage dependent, such that early stage tadpoles are most vulnerable to mortality and malformations with progressively reduced vulnerability as development progresses toward metamorphosis (Bowerman and Johnson 2003; Schotthoefer et al. 2003; Holland et al. 2007). As a result, changes in the timing of cercarial release from snails could sharply affect pathology in amphibians. Because trematode maturation and cercarial production in snails are controlled largely by temperature (Poulin 2006), whereas amphibian breeding is often triggered by rainfall, forecasted increases in temperature and growing season duration could enhance amphibian exposure to trematodes early in their development when larvae are most vulnerable. Moreover, because of the high metabolic efficiency of trematodes relative to other invertebrates and vertebrates, any acceleration in amphibian growth rate will likely be overshadowed by the increase in trematode development (Poulin 2006). An extended growth season could also allow for a transition from a single snail infection cycle per year to multiple infection cycles per year, as found in wetlands exposed to thermal effluent (Marcogliese 2001). Models built by Kutz et al. (2005) for a protostrongylid nematode with a similarly complex life cycle suggested that even small increases in temperature (as little as 1°C) could increase infection from a 2-year cycle to a 1-year cycle. Limited empirical or experimental data are available to evaluate the effects of climate on *Ribeiroia* and *Echinostoma* infections, but we suspect the magnitude of such effects would be greatest in temperate regions where climate changes are expected to be pronounced and interactions between trematodes and amphibians are currently constrained by temperature.

11.5 Concluding Remarks

Emerging diseases include those that have expanded in prevalence, geographic distribution, host range, or pathology (e.g., CDC 1994; Friend et al. 2001). The causes of emergence are complex and may involve changes in the evolutionary and

ecological relationships between host(s) and pathogen. In many cases, definitive evidence of emergence is elusive, as historical baseline data for most pathogens (especially those of nonhuman hosts) are unavailable. Here, we explored available evidence to evaluate whether infections by *Ribeiroia* and *Echinostoma* in amphibians have recently increased. Owing to a lack of published historical information on these parasites in amphibians, this question remains difficult to answer definitively. Both parasites have only infrequently been recorded in amphibians historically (e.g., http://www.nhm.ac.uk/research-curation/projects/host-parasites/database/). While the rarity of published records of amphibians supporting either parasite is notable, particularly in contrast to their relative abundance in the last decade, significant biases exist in what parasites have been recorded in amphibians (e.g., adult worms over metacercariae). The apparent increase in records of mass amphibian malformations associated with *Ribeiroia* – a relatively obvious and severe form of pathology – is also suggestive of a recent increase in infection, but it is difficult to quantitatively compare historical and contemporary surveillance efforts to assess how large such a change might actually be (see Johnson et al. 2003). We suggest that examination of vouchered museum specimens, in combination with rigorous and long-term monitoring programs, holds the greatest promise in providing an adequate test of the emergence hypothesis.

Several characteristics of trematodes in the genera *Ribeiroia* and *Echinostoma* suggest they will be strongly influenced by environmental changes and may therefore be predisposed toward emergence. Despite their complex life cycles, both parasites can be considered relative generalists. Each can utilize an enormous diversity of second intermediate and definitive hosts, including a wide range of fishes, amphibians, birds, and mammals. Members of *Echinostoma* (but not *Ribeiroia*) can even infect snails as second intermediate hosts, which could be one contributing factor explaining the near ubiquity of echinostomes in amphibian habitats. This suggests that these parasites, which already occur in many wetland environments, will be influenced by changes in the abundance and composition of the host community. Importantly, *Ribeiroia* and *Echinostoma* can infect many species that thrive in disturbed or human-altered conditions, such as rats, raccoons, and some herons. The same principle apparently holds for their use of first intermediate hosts. Members of the genera *Physa* and *Planorbella* are common components of pond habitats, often achieving high densities in eutrophic, artificial wetlands (see Johnson et al. 2007). Thus, rather than eliminating these parasites, environmental changes could enhance their levels of infection by promoting such human-associated hosts.

Finally, because amphibians represent a vehicle of transmission between snail intermediate hosts and vertebrate definitive hosts, both *Ribeiroia* and *Echinostoma* may benefit by inducing pathology that enhances the predation risk of infected amphibians. This is particularly true for *Ribeiroia*, which can cause high levels of direct mortality among infected amphibian larvae, and surviving animals often develop severe malformations that are detrimental to long-term survival. Cercariae of *Echinostoma* are less pathogenic than those of *Ribeiroia*, but they can nevertheless cause impaired renal function, edema, and mortality at high dosages or during certain stages of amphibian development. The echinostomes are also incredibly widespread across the landscape and often locally abundant in amphibians,

suggesting their collective effects may be considerable. Because trematodes exist in a metacommunity dynamic, with mobile definitive hosts connecting wetlands that support the parasites, high amphibian mortality or population extirpation within a single pond is unlikely to dramatically affect parasite transmission (but it will take a larger toll on the amphibian hosts). Thus, a high abundance of *Ribeiroia* or *Echinostoma* has the potential to sharply affect the viability of amphibians, which are already threatened by numerous forms of environmental changes and are now considered the most threatened class of vertebrates worldwide.

Taken together, these characteristics suggest that current and future research should focus on evaluating ongoing changes in infection by these trematodes. While the exact trajectory of how their infections have changed in host range, geography, and abundance over time remains unavailable, environmental changes such as nutrient runoff, pesticide contamination, biodiversity loss and climate change all have demonstrated potential to enhance infection and/or pathology in amphibians. These changes occur through increases in snail host abundance, changes in parasite production, decreases in amphibian infection resistance, or by altering the transmission success among hosts. Based on the distributional data collected for each parasite in the USA, preliminary results are suggestive of higher infection along bird migratory routes and at more temperate latitudes. This latter trend is perhaps of particular concern, as temperate regions also tend to (a) support lower biodiversity, which may enhance transmission through the dilution effect, (b) be associated with high agricultural activity, leading to higher inputs of fertilizers and pesticides, and (c) be sharply affected by forecasted increases in temperature, which are expected to reduce winter duration and increase the number or intensity of parasite cycles per year. This "perfect storm" convergence underscores the importance of understanding the individual and combined effects of environmental changes on host–parasite dynamics, and of incorporating parasites into monitoring efforts aimed at amphibian conservation and restoration. We further advocate the need for greater molecular and morphological resolution of these groups, long-term studies of parasite abundance (preferably in conjunction with museum vouchers), and field-based manipulations to quantitatively assess the roles of multiple interacting drivers on parasite-induced pathology in amphibians.

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