Parasitology

cambridge.org/par

Effects of multiple stressors on northern leopard frogs in agricultural wetlands

David J. Marcogliese[1,2], Kayla C. King[3,4] and Kieran A. Bates[4]

[1]Aquatic Contaminants Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment and Climate Change Canada, St. Lawrence Centre, 105 McGill Street, 7th floor, Montreal, Quebec H2Y 2E7, Canada; [2]St. Andrews Biological Station, Fisheries and Oceans Canada, 125 Marine Science Drive, St. Andrews, New Brunswick E5B 0E4, Canada; [3]Department of Biology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec H3G 1M8, Canada and [4]Department of Zoology, University of Oxford, 11a Mansfield Road, Oxford OX1 3SZ, UK

Research Article

Cite this article: Marcogliese DJ, King KC, Bates KA (2021). Effects of multiple stressors on northern leopard frogs in agricultural wetlands. *Parasitology* **148**, 827–834. https://doi.org/10.1017/S003118202100038X

Received: 24 November 2020
Revised: 23 February 2021
Accepted: 24 February 2021
First published online: 9 March 2021

Key words:
Amphibians; atrazine; *Lithobates pipiens*; lysozyme; northern leopard frogs; oxidative stress; parasitism

Author for correspondence:
David J. Marcogliese,
E-mail: david.marcogliese@canada.ca

Abstract

Natural and anthropogenic stressors, including parasites and pesticides, may induce oxidative stress in animals. Measuring oxidative stress responses in sentinel species that are particularly responsive to environmental perturbations not only provides insight into host physiology but is also a useful readout of ecosystem health. Newly metamorphosed northern leopard frogs (*Lithobates pipiens*), a sentinel species, were collected from agricultural and non-agricultural wetlands exposed to varying concentrations of the herbicide atrazine. Significant effects of certain parasites' abundance and their interaction with atrazine exposure on frog oxidative stress were identified. Specifically, increased protein levels were detected in frogs infected with echinostome metacercariae. In addition, the nematode *Oswaldocruzia* sp. was significantly associated with increased thiol concentration and catalase activity. Significant parasite × atrazine interactions were observed for atrazine exposure and the abundance of *Oswaldocruzia* sp. on thiol, as thiol concentrations increased with parasite abundance at low atrazine localities and decreased in high atrazine wetlands. In addition, a significant interaction between the abundances of *Oswaldocruzia* sp. and gorgoderid trematodes on thiol concentrations was observed. These findings demonstrate that studies of oxidative stress on animals in natural ecosystems should account for the confounding effects of parasitism, particularly for amphibians in agricultural landscapes.

Introduction

It is well established that organisms are exposed to a multitude of biotic and abiotic stressors in their natural environments (Sih *et al.*, 2004; Holmstrup *et al.*, 2010). There is a growing recognition that cumulative stressors are important, not only for the health and well-being of individual organisms, but also their populations (Relyea, 2003; Szuroczki and Richardson, 2009; Blaustein *et al.*, 2011). Knock on or cascading effects subsequently can be detrimental not only to biological communities but to food webs and ecosystems, either directly or indirectly (Relyea and Hoverman, 2008; Blaustein *et al.*, 2011). Such combined effects are particularly relevant for anthropogenically impacted habitats. For example, wetlands in agricultural landscapes are affected by pesticides and other contaminants, eutrophication, fragmentation and landscape alteration, among others, in addition to any natural stressors which may exist there (Blaustein *et al.*, 2011). Indeed, synergistic interactions between environmental contaminants and natural stressors were found in over half of more than 150 studies reviewed across invertebrate and vertebrate taxa (Holmstrup *et al.*, 2010). One such natural stressor is parasitism. Parasitism can alter interactions with other stressors, either synergistically or antagonistically (Marcogliese and Pietrock, 2011; Sures *et al.*, 2017), and exposure to one stressor may affect vulnerability to the other (Morley *et al.*, 2006).

There are numerous examples of combined effects of parasites and contaminants causing enhanced mortality in a multitude of phylogenetically diverse animals compared to those exposed only to a single stressor (Marcogliese and Pietrock, 2011). However, more commonly impacts will be sublethal, and thus more difficult to detect (Marcogliese and Pietrock, 2011). Most studies have been conducted on fish (see Marcogliese and Pietrock, 2011; Sures *et al.*, 2017), with relatively fewer on amphibians. A few studies reported reduced growth and/or survival, and increased levels of malformations in anurans exposed to both pesticides and parasites compared to one or both stressors alone (Kiesecker, 2002; Koprivnikar, 2010; Jayawardena *et al.*, 2016), although results were variable in other studies (Jayawardena *et al.*, 2017). However, it is difficult to measure growth and survival in the field, and often researchers rely on non-specific biomarkers of animal health to evaluate the sublethal effects of different stressors, including parasites, on organisms (Marcogliese *et al.*, 2010; Marcogliese and Pietrock, 2011). For example, in a field study on bullfrogs (*Lithobates catesbeianus*), infection with >2 lungflukes (*Haematoloechus* sp.) affected acetylcholineserase activity, but the direction of the effect varied with the level of agricultural activity (Marcogliese *et al.*, 2009). Furthermore, not only differential blood cell counts were altered by the same parasite, but also in different directions, depending on agricultural activity.

© The Author(s), 2021. Published by Cambridge University Press

For more than two decades, ecotoxicologists have used oxidative stress as an indicator of general animal health (Di Giulio *et al.*, 1989; Martínez-Álavarez *et al.*, 2005), and more recently, ecologists have adopted these methods to examine stress responses in their study organisms in the field (Costantini, 2008; Monaghan *et al.*, 2009). Reactive oxygen species (ROS) are produced upon exposure to contaminants and inflammation. They are damaging to DNA, lipids and proteins when in excess (Storey, 1996; Sorci and Faivre, 2009). Organisms possess enzymatic mechanisms to deal with ROS, but oxidative stress occurs when redox signalling and control are disrupted and ROS production surpasses the organism's capacity to metabolize them (Winston and Di Giulio, 1991; Sies, 1997; Monaghan *et al.*, 2009). Oxidative stress can be evaluated by measuring substrates, enzymes and end-products involved in oxidative stress metabolism (Martínez-Álvarez *et al.*, 2005).

Oxidative stress responses can be induced by exposure to pesticides (Abdollahi *et al.*, 2004; Oruc *et al.*, 2004; Zhang *et al.*, 2004; Valavanidis *et al.*, 2006) in a variety of animals, including amphibians (Dornelles and Oliveira, 2014; Glinski *et al.*, 2018). However, infection with a diverse array of parasites also is known to induce oxidative stress in a variety of freshwater, marine and terrestrial organisms (Belló *et al.*, 2000; Neves *et al.*, 2000; Dautremepuits *et al.*, 2002*a*, 2002b, 2003; Marcogliese *et al.*, 2005, 2010; Gismondi *et al.*, 2012*a*, 2012*b*; Orledge *et al.*, 2012; Stumbo *et al.*, 2012; Lilley *et al.*, 2014; Dallarés *et al.*, 2016; Lacaze *et al.*, 2019; Akinsanya *et al.*, 2020*a*). Nevertheless, few studies examine oxidative stress in animals from contaminated habitats (but see Marcogliese *et al.*, 2005, 2010; Lacaze *et al.*, 2019; Akinsanya *et al.*, 2020*a*).

Furthermore, contaminants such as pesticides also may modulate the immune response in amphibians and other organisms, causing immunosuppression (Carey and Bryant, 1995; Carey *et al.*, 1999; Fournier *et al.*, 2005; Martin *et al.*, 2010; Rehberger *et al.*, 2017). Such a reduction in immune capacity may increase susceptibility to disease and parasites (Carey and Bryant, 1995; Rollins-Smith and Woodhams, 2012). Pesticides typically affect innate, non-specific immune responses (Rehberger *et al.*, 2017) such as lysozymes, which disrupt bacterial cell walls and are considered an important index of innate immunity in fish (Tort *et al.*, 2003; Saurabh and Sahoo, 2008; Uribe *et al.*, 2011). Lysozyme activity responds to stress (Bols *et al.*, 2001; Fatima *et al.*, 2007) and often is suppressed following exposure to contaminants (Saurabh and Sahoo, 2008). However, exposure to parasites may either increase or decrease lysozyme activity in fish (Álvarez-Pellitero *et al.*, 2008).

Among pesticides, atrazine is one of the most commonly used herbicides globally (Rohr and McCoy, 2010). A meta-analysis of ecotoxicological studies on fish and amphibians demonstrated that atrazine consistently diminished immune capacity in fish and frogs at ecologically relevant concentrations, either alone or in mixtures (Rohr and McCoy, 2010). The same synthesis linked exposure to atrazine at ecologically relevant concentrations to an increase in various measures of disease (Rohr and McCoy, 2010).

There exists widespread concern for declining populations, extirpations and extinctions of amphibians globally. Numerous causes have been put forward to explain these declines, including threats from pesticides and disease (Stuart *et al.*, 2004; Blaustein *et al.*, 2011), which, of course, may act in concert. Amphibians are known hosts for a wide diversity of parasites (Koprivnikar *et al.*, 2012; Bower *et al.*, 2018), regardless of habitat. Given their susceptibility to environmental changes and disease, amphibians also make useful sentinels that can provide insight into overall ecosystem function and health (Hopkins, 2007). Using different measures of oxidative stress in addition to a measure of the innate immune response, the effects of parasites and agricultural activity on the health of northern leopard frogs (*Lithobates pipiens*) were examined in agricultural wetlands. The following predictions were made: (1) exposure to high level of atrazine and/or certain parasites will induce an oxidative stress response, with potential interactive effects; and (2) exposure to high levels of atrazine and/or certain parasites will moderate lysozyme activity, with potential interactive effects.

Materials and methods

Sampling sites and frog collections

Sampling and subsequent analysis of parasites are described in King *et al.* (2007). There were seven sampling localities located in southwestern Quebec, Canada, which were categorized as low atrazine or high atrazine based on the highest atrazine concentration measured (means and ranges are presented in King *et al.*, 2007). Those with maximum measurements >0.10 $\mu g\,L^{-1}$ in either 2004 or 2005 were considered as high atrazine localities, while those with measures <0.10 $\mu g\,L^{-1}$ were considered as low atrazine localities (Table 1). The high atrazine localities were directly exposed to pesticide runoff, while the low atrazine localities were not. Atrazine use is relatively stable from 1 year to the next (Sass and Colangelo, 2006). Furthermore, atrazine use was consistent between 2001 and 2004–2005 at our study localities (King *et al.*, 2007, 2008), with the exception of a single locality (Île de la Commune) located in a provincial park, where atrazine was applied illegally in 2001, after which its use was subsequently halted. Thus, low atrazine localities included Étang John Sauro, Parc Le Rocher and Île de la Commune. High atrazine localities were Rivière St-François, Baie St-François, Ruisseau Fairbanks and Rivière Chibouet (Table 1).

The study species in our system was the northern leopard frog *L. pipiens*. Immature metamorph leopard frogs were collected by dip net or by hand from 26 July to 6 August 2004 (see King *et al.*, 2007). Collections were restricted to newly metamorphosed frogs ⩽45 mm snout-vent length (SVL) (Seburn and Seburn, 1998). The restricted sampling period and size range accounted for potential confounding factors including season, age, size and reproductive status, which might affect biomarker analyses (Martinez-Álvarez *et al.*, 2005). Frogs were killed in buffered 0.8% tricaine methane sulphonate (MS 222), kept on ice, and returned to the laboratory within a few hours where they were frozen at −20°C. Frogs were partially thawed prior to parasitological examination and spleens were removed and frozen at −80°C for subsequent biomarker analysis. Handling and treatment of animals were in accordance with the guidelines of the Canada Council on Animal Care.

Parasite analyses

Each frog was thawed, weighed to the nearest 0.1 g and SVL measured to the nearest mm. The frogs were examined using a stereomicroscope for macroparasites using standard parasitological techniques (King *et al.*, 2007). First, the frogs were examined externally. Then their eyes were removed and dissected. The body cavity and viscera were examined. Organs (brain, liver, gall bladder, heart and urinary bladder) were removed, squashed between glass plates and examined. The stomach and intestine were opened longitudinally and examined. They were then squashed between glass plates to detect worms in the tissue. The skin was removed from the flesh, which was thin-sliced, squashed between glass plates, and examined. A total of 156 frogs were examined for parasites. Sample sizes for each locality are reported in Table 1. Helminth parasites were identified to genus and, if possible, species based on descriptions from the

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

Table 1. Geographic coordinates of sampling localities, maximum atrazine concentration ($\mu g\,L^{-1}$), prevalence (%) of target parasite taxa and number of frogs used for each biomarker

Locality	Étang John Sauro	Parc Le Rocher	Île de la Commune	Rivière St-François	Baie St-François	Ruisseau Fairbanks	Rivière Chibouet
Location: Latitude Longitude	45°04.6′N 73°09.6′W	45°38.7′N 73°19.8′W	45°37.1′N 73°28.3′W	46°06.8′N 72 o54.8′W	46°05.4′N 72°56.5′W	45°01.0′N 73°21.3′W	45°47.4′N 72°49.5′W
Category	Low atrazine	Low atrazine	Low atrazine	High atrazine	High atrazine	High atrazine	High atrazine
Maximum atrazine concentration ($\mu g\,L^{-1}$)	0.04	0.03	0.06	0.13	0.80	0.35	3.70
Parasite	Prevalence (%)						
Echinostoma spp.	100	100	97	13	42	82	97
Gorgoderidae	15	5	97	3	32	63	96
Oswaldocruzia sp.	0	5	40	50	16	19	3
Rhabdias sp.	38	0	63	30	21	9	3
Biomarker	Number of frogs						
Protein	13	22	30	30	19	11	31
Thiols	13	22	30	29	19	11	30
Glutathione-S-transferase	13	17	29	30	15	30	27
Glutathione reductase	10	5	11	13	9	1	9
Catalase	12	12	18	18	16	5	17
Lysozyme	11	9	18	8	19	11	19

Parasite prevalence based on the maximum number of frogs included in biomarker analysis for each locality.

literature (Rau *et al.*, 1978; Prudhoe and Bray, 1982; McAlpine and Burt, 1998).

Biomarker analyses

All analyses were performed at the INRS – Institut Armand Frappier, Pointe-Claire, Quebec. Originally, 30 spleen samples were collected from each locality, but a laboratory accident resulted in the loss of a large number of samples. The final sample sizes are given in Table 1. Consequently, the results of glutathione reductase (GRd) were not retained due to small sample sizes at two localities. Once thawed, all samples were kept on ice throughout the preparation and analyses. Samples were processed and analysed as described in Dautremepuits *et al.* (2009). Frozen samples of spleen tissue were homogenized by suspending 0.2 g of tissue in 3 mL of phosphate buffer saline (PBS) (Dulbecco's, Sigma, USA) in a potter-pestle homogenizer (Sigma, USA). Homogenates were centrifuged at 4000 rpm for 30 min at 4°C. The supernatants were immediately analysed for antioxidant enzyme and immune activities. The total protein content (mg mL^{-1}) of each sample was measured with a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Canada).

The thiol glutathione (GSH) is an important antioxidant that neutralizes hydroxyl radicals (Di Giulio *et al.*, 1989; Monaghan *et al.*, 2009). Thiol (SH) groups were measured spectrophotometrically using the DTNB method. Four μL of the sample were added to 46 μL of phosphate buffer (0.2 ML, pH 6.8) and 50 μL of phosphate buffer containing 1 mM DTNB (5,5′-dithiobis-2-nitrobenzonic acid, Sigma, USA) in a 96-well tissue-culture plate. Absorbance was measured at 412 nm after 10 min of incubation at room temperature, using GSH (reduced glutathione) commercial solution (Sigma, USA) as a standard.

Glutathione *S*-transferase (GST) catalyzes reactions involving the GSH; measurement of GST activity was adapted from Habig *et al.* (1974). The reaction mixture consisted of 100 μL PBS (0.1 M, pH 6.5), 50 μL reduced GSH (Sigma, CA.) (1 mM), 25 μL H_2O, 10 μL 1-chloro-2,4-dinitro-benzene (CDNB) (Sigma, Ca.) (1 mM) and 15 μL of sample in a total volume of 200 μL. The change in absorbance was recorded at 340 nm during 5 min and the enzyme activity calculated as μmol of CDNB formed min^{-1} mg $protein^{-1}$ using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Catalase breaks down H_2O_2, an endproduct of oxidative stress (Di Giulio *et al.*, 1989). Catalase activity was assayed according to Claiborne (1985) and Giri *et al.* (1996). The assay mixture consisted of 190 μL PBS (0.05 M, pH 7.0), 100 μL hydrogen peroxide (Prolabo, CA) (0.01 M) and 10 μL of the sample in a final volume of 300 μL. Change in absorbance was recorded at 240 nm. Catalase activity was calculated as nmol H_2O_2 consumed min^{-1} mg $protein^{-1}$ using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}.

Lysozyme activity was measured by a turbidimetric assay (Studnicka *et al.*, 1986). A 10 μL sample was added to 200 μL of *Micrococcus lysodeikticus* (0.2 g L^{-1} in 0.05 M, pH 6.2 phosphate buffer) suspension and the decrease in absorbance was recorded at 450 nm by spectrophotometry (PowerWave X, Bio-Tek Instruments, Vermont, USA) for 30 min in a 96-well tissue culture plate (Sarstedt, USA). One unit of lysozyme activity was defined as the amount of enzyme that catalysed a decrease in absorbance of 0.001 min^{-1}. A commercial solution of lysozyme (Sigma, Canada) was used as a standard.

Statistical analyses

To determine if parasites or high/low atrazine exposure affected the expression of biomarkers in the spleen of leopard frogs and whether there was an interaction with the herbicide, criteria were established for further analyses. The same individual frogs were used for both parasites and biomarkers. To ensure an adequate sample size at each locality, only parasites present at six of the seven

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

localities were considered further. These included the trematodes *Echinostoma* spp. (metacercariae) and Gorgorderidae gen. (adults and larval stages), and the adult nematodes *Oswaldocruzia* sp. and *Rhabdias* sp. Adult gorgorderids infect the urinary bladder, whilst metacercariae encyst in the kidneys and muscle tissues. It is unclear whether the different stages cause different or similar pathologies, or indeed, any pathology at all, so they were combined (Koprivnikar *et al.*, 2012). For the purposes of analyses, localities were combined into high atrazine (Rivière St-François, Baie St-François, Ruisseau Fairbanks, Rivière Chibouet) and low atrazine (étang John Sauro, Parc le Rocher, Île de la Commune), based on the maximal concentration of atrazine measured in each system (King *et al.*, 2007).

To determine the effects of atrazine exposure and parasite abundance on biomarker concentration/activity separate linear mixed effects models were performed for each biomarker using the lme4 package (Bates *et al.*, 2015) in R version 3.6.0 (http://www.r-project.org/). We assessed the significance of parameters using the Satterthwaite method, implemented in the R package lmerTest (Kuznetsova *et al.*, 2017). Variables included high/low atrazine exposure, parasite abundance, pairwise interactions between high atrazine exposure and parasite abundance and pairwise interactions between each parasite as fixed effects. Host population was included as a random intercept term. Parasite abundance data were log10 + 1 transformed and protein, lysozyme and catalase concentration/activity were log10 transformed to improve normality and model fit. Two-way interactions were included in each model initially with non-significant interactions with the highest P value removed and the models re-run until only significant interaction terms remained ($P < 0.05$). Model fit was graphically assessed. Figures were produced using sjPlot (Lüdecke, 2021).

Terminology

The definitions of parasite population parameters are in accordance with Bush *et al.* (1997). Prevalence is the proportion of frogs infected with a particular species at a locality, expressed as a per cent. Abundance refers to the number of parasites in a host from a particular locality, be it infected or not. Intensity refers to the number of parasites of a given species in an infected host from a particular locality.

Results

Results of linear mixed effects models demonstrate that abundance of *Echinostoma* spp. was associated with a significant increase in protein concentration ($F_{(1,26.67)} = 6.68$, $P = 0.02$, Fig. 1, Supporting Information Table 1). Abundance of *Oswaldocruzia* sp. was associated with increased thiol concentration ($F_{(1,145.91)} = 10.92$, $P = 0.001$, Fig. 2, Supporting Information Table 2). However, the interactions of *Oswaldocruzia* sp.*High atrazine exposure and *Oswaldocruzia* sp.*Gorgorderidae gen. were associated with reduced thiol concentration (*Oswaldocruzia* sp.*High atrazine: $F_{(1,145.96)} = 7.96$, $P = 0.005$, Fig. 2, Supporting Information Table 2; *Oswaldocruzia* sp.*Gorgorderidae: $F_{(1,145.17)} = 9.03$, $P = 0.003$, Supporting Information Table 2). Lastly, abundance of *Oswaldocruzia* sp. was associated with increased catalase activity ($F_{(1,91.01)} = 10.35$, $P = 0.002$, Fig. 3, Supporting Information Table 3). Models for GST and lysozyme were not statistically significant (Supporting Information Tables 4 and 5).

Discussion

Our results failed to demonstrate an effect of herbicide exposure as a single predictor of oxidative stress, however we do demonstrate a clear interaction between high atrazine exposure and parasite abundance. Moreover, the abundance of certain parasites was associated with the levels of different biomarkers of oxidative stress. Thus, we fail to reject hypothesis one. We find no effect of atrazine levels or parasite exposure and their interaction on lysozyme, and therefore we reject hypothesis two. We show that frogs with high intensities of echinostomes possessed higher protein concentrations in spleen tissue. Frogs with high intensities of *Oswaldocruzia* sp. had greater catalase activity than those with lower infection intensities. Thiol levels were also

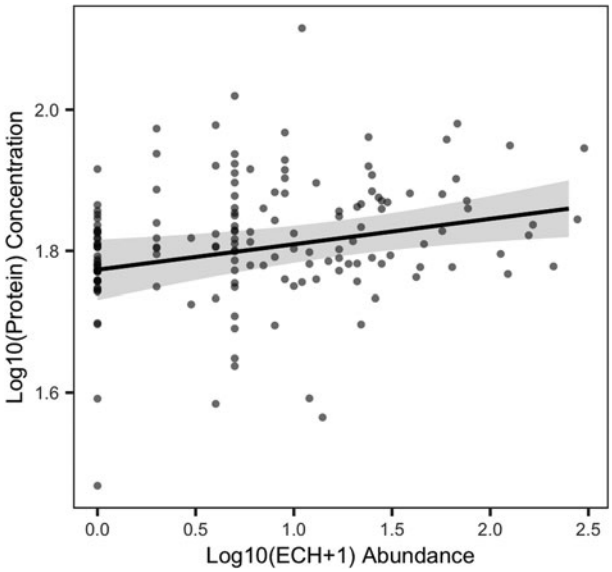

Fig. 1. Plot of log10 abundance of *Echinostoma* spp. (log10 ECH + 1) against log10 total protein concentration (mg g^{-1} F.W.) in spleens of northern leopard frogs (*Lithobates pipiens*) collected from agricultural and non-agricultural wetlands in Quebec, Canada in 2004. Results show linear mixed effects model predictions showing 95% confidence interval (shaded area) of marginal effects and raw data points.

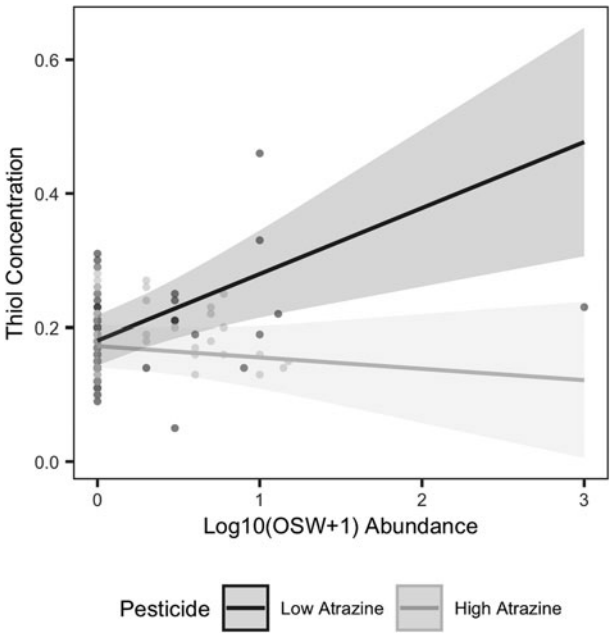

Fig. 2. Plot of log10 abundance of *Oswaldocruzia* sp. (log10 OSW + 1) against thiol concentration (U.I. × 10^{-3} mg^{-1} protein) in spleens of northern leopard frogs (*Lithobates pipiens*) collected from (A) low-atrazine and (B) high-atrazine wetlands in Quebec, Canada in 2004 and its interaction with atrazine exposure. Results show linear mixed effects model predictions showing 95% confidence interval (shaded area) of marginal effects and raw data points.

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

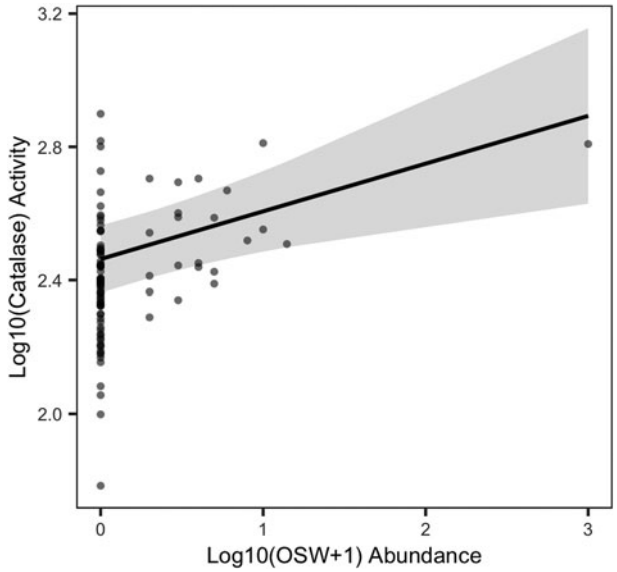

Fig. 3. Plot of log10 abundance of *Oswaldocruzia* sp. (log10 OSW + 1) against log10 catalase activity (nmol H_2O_2 min^{-1} mg $protein^{-1}$) in northern leopard frogs (*Lithobates pipiens*) collected from agricultural and non-agricultural wetlands in Quebec, Canada in 2004. Results show linear mixed effects model predictions showing 95% confidence interval (shaded area) of marginal effects and raw data points.

associated with the abundance of *Oswaldocruzia* sp. However, the direction of the relationship differed between frogs exposed to high atrazine concentrations and those exposed to low levels. Interactions also were observed between the abundance of *Oswaldocruzia* sp. and gorgoderids on thiol concentration. While we did not measure growth or other related parameters, oxidative stress can lead to decreased growth rates in amphibians (Szuroczki *et al.*, 2019) and thus reduced fitness. We caution that results should be interpreted with care, as there may be other unmeasured factors associated with the study system that could affect the biomarker responses.

Various parasites are known to induce oxidative stress in their host (see Introduction section for references). For example, parasites have been shown to induce higher catalase activity, a known biomarker of oxidative stress (Di Giulio *et al.*, 1989; Brodeur *et al.*, 2011), in fishes. These include carp (*Cyprinus carpio*) infected with the intestinal cestode *Ptychobothrium* sp. (Dautremepuits *et al.*, 2002*a*, 2002b, 2003), *Heterotis niloticus* infected with the intestinal acanthocephalan *Tenuisentis niloticus* (Akinsanya *et al.*, 2020*a*), and yellow perch (*Perca flavescens*) infected with the larval trematode *Diplostomum* spp. (Marcogliese *et al.*, 2010), although in the latter study effects were only observed in polluted waters, but not at reference localities. Infection with the parasitic isopod *Anilocra frontalis* increased catalase activity in the marine fish *Pomatoschistus microps*, but only at a higher acclimation temperature (Cereja *et al.*, 2018). In contrast, in amphibians, catalase activity was lower in African common toads (*Amietophyrnus regularis*) infected with the intestinal nematode *Amplicaecum africanum* from localities exposed to trace metal pollution but not those from a reference locality (Akinsanya *et al.*, 2020*b*). The later result was interpreted as depuration of metals in the anuran host due to bioaccumulation by the nematode (Akinsanya *et al.*, 2020*b*). Clearly, catalase activity depends not only on the host but varies with parasitic infection and environmental context.

Thiol levels in leopard frogs from reference localities were positively associated with the abundance of *Oswaldocruzia* sp., implying enhanced synthesis of antioxidants. This relationship was reversed in those frogs from high atrazine localities, suggesting potential environmental stress (Peña-Llopis *et al.*, 2003). GSH was also higher in *H. niloticus* infected with *T. niloticus* in a polluted lagoon in Nigeria (Akinsanya *et al.*, 2020*a*). Curiously, there was a significant interaction between *Oswaldocruzia* sp. and gorgoderids, associated with a reduction in thiol concentration. This implies that the effects of one parasite species may be moderated by a second infection (Bordes and Morand, 2009; Blaustein *et al.*, 2011, 2012).

There are no studies on oxidative stress in amphibians or reptiles infected with *Oswaldocruzia* sp. However, infections with *Oswaldocruzia* sp. and other intestinal nematodes can lead to host starvation, peritonitis and mortality at high intensities (Reichenbach-Klinke and Elkan, 1965). Larval *Oswaldocruzia filiformis* cause necrosis and atrophy of the stomach mucosa and epithelium (Hendrikx and van Moppes, 1983). Other intestinal nematodes induce oxidative stress in their hosts. For example, ring-necked pheasants (*Phasianus colchicus*) experimentally infected with *Heterakis gallinarum* displayed higher levels of lipid peroxidation in plasma after 8 weeks (Orledge *et al.*, 2012).

The abundance of echinostome trematodes was associated with higher protein levels. This result seems counterintuitive, as stress would be expected to lead to a decrease in proteins due to greater energy consumption in response (Dornelles and Oliveira, 2014). However, an increase in protein may reflect the activation of biochemical processes (Dautremepuits *et al.*, 2009). Larval echinostomes commonly infect the kidneys and are pathogenic in amphibians, especially at high intensities (Johnson and McKenzie, 2008; Koprivnikar *et al.*, 2012). Effects include oedema, reduced growth, kidney malfunction, pathology and mortality (Johnson and McKenzie, 2008). Thus, it is not surprising that they may affect oxidative stress. Other larval trematodes shown to induce oxidative stress in their hosts include the fish parasites *Apophallus brevis*, *Diplostomum* spp. and *Ornithodiplostomum ptychocheilus* (Marcogliese *et al.*, 2005, 2010; Stumbo *et al.*, 2012; Lacaze *et al.*, 2019).

Although we did not detect any direct effect of high atrazine exposure alone, pesticides, in general, are known to induce oxidative stress (Abdollahi *et al.*, 2004; Dornelles and Oliveira, 2014; Luschak, 2016). Bullfrog tadpoles experimentally exposed to atrazine experienced large increases in lipid peroxidation, a measure of damage resulting from oxidative stress, and a decrease in protein concentration, albeit at higher atrazine concentrations than at any of our sites (Dornelles and Oliveira, 2014). In studies of exposure to other pesticides, catalase activity increased in some, but not all, anurans (Costa *et al.*, 2008; Brodeur *et al.*, 2011; Li *et al.*, 2017). Notably, a recent synthesis suggests that results may vary with class of pesticides (Rumschlag *et al.*, 2019).

Contaminants can lead to a decrease in thiol concentration (van der Oost *et al.*, 2003). GSH is considered to be a biomarker of oxidative or environmental stress (Peña-Llopis *et al.*, 2003; Brodeur *et al.*, 2011; Hellou *et al.*, 2012). We found no effect of high atrazine exposure alone on total thiols in leopard frogs. Nor was any effect observed on GSH from other amphibians in agricultural landscapes (Brodeur *et al.*, 2011), although levels were reduced in tadpoles of *Bufo arenarum* (Venturino *et al.*, 2003). Curiously, high atrazine exposure changed the relationship between thiol concentration and abundance of *Oswaldocruzia* sp., demonstrating that effects of multiple stressors may be non-additive and unpredictable, rendering interpretation problematic (Sures *et al.*, 2017).

Numerous studies have shown higher GST activity in amphibians from agricultural localities exposed to other pesticides (Venturino *et al.*, 2003; Greulich and Pflugmacher, 2004; Attademo *et al.*, 2007), while others show reductions in GST activity (Brodeur *et al.*, 2011), and still, others show no effect (Brodeur *et al.*, 2012), as in our study. As stated above, effects may vary with pesticide class (Rumschlag *et al.*, 2019).

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

No evidence of additive or synergistic effects of pesticides and parasites on oxidative stress were observed in leopard frogs. In contrast, lipid peroxidation was induced in yellow perch from a polluted site, while it was higher still in those infected with the larval nematode *Raphidascaris acus* or high numbers of the larval trematode *A. brevis* at the same site suggesting additive effects (Marcogliese *et al.*, 2005). Similarly, GRd activity in gills, and catalase activity in the head kidney of yellow perch infected with *A. brevis* and *Diplostomum* spp. respectively, were intensity dependent at polluted localities, suggesting combined effects of pollution and parasitism on oxidative stress metabolism (Marcogliese *et al.*, 2010). It is likely that the combined effects of multiple stressors depend on the relative intensity of the different stressors, their toxicity and/or their pathogenicity. Results herein suggest that parasitism should be considered in any studies of oxidative stress in amphibians from different habitats, whether polluted or not.

Lysozymes are considered an important index of innate immunity in fish (Tort *et al.*, 2003; Saurabh and Sahoo, 2008; Uribe *et al.*, 2011). Although infections with parasites can modify the immune response (Martin *et al.*, 2010), effects of parasites on lysozyme activity are equivocal (Alvarez-Pellitero, 2008). Previous researchers have suggested that contaminants, and pesticides in particular, can decrease the immune response in amphibians (Carey and Bryant, 1995; Carey *et al.*, 1999; Fournier *et al.*, 2005; Saurabh and Sahoo, 2008; Mann *et al.*, 2009; Martin *et al.*, 2010; Rollins-Smith and Woodhams, 2012; Rumschlag *et al.*, 2019). Pesticides typically affect innate, non-specific immune responses (Rehberger *et al.*, 2017). However, while we did not detect any effects of exposure to atrazine on lysozyme activity, it represents only a small component of the innate immune response (Tort *et al.*, 2003; Magnadottir, 2010; Uribe *et al.*, 2011). Indeed, northern leopard frogs collected from some of the same localities as in this study had reduced numbers of splenocytes and phagocytic response in the agricultural wetlands (Christin *et al.*, 2013).

In conclusion, infection with certain parasites is associated with oxidative stress in northern leopard frogs. Specifically, abundances of larval echinostomes and the nematode *Oswaldocruzia* sp. were associated with oxidative stress, regardless of the habitat's agricultural status. Furthermore, interactions were detected between the abundance of *Oswaldocruzia* sp. and the degree of atrazine exposure. Lastly, an effect of the parasite × parasite interaction was detected between *Oswaldocruzia* sp. and gorgoderid abundance. In contrast, no effect of any parasite, or high atrazine exposure, or their interaction was observed on lysozyme activity. Clearly, environmental studies on oxidative stress and other biomarkers of animal health in amphibians from agricultural habitats should account for parasitism. Indeed, parasitism may affect biomarkers of animal health in any environment (Marcogliese and Pietrock, 2011; Sures *et al.*, 2017), but at this point in time it is difficult to predict with certainty which parasites will affect the biomarkers used in any particular host–parasite system, or how they will interact with different contaminants and other anthropogenic stressors.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003118202100038X

Acknowledgements. We thank Claire Dautremepuits for conducting the biochemical analyses.

Author contribution. DJM conceived and designed the study. DJM and KCK collected samples. KCK conducted parasitological examinations. KAB performed statistical analyses. DJM, KCK, and KAB wrote the article.

Financial support. Funding from Environment and Climate Change Canada's Pesticide Science Fund and the St. Lawrence Action Plan to DJM, a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship awarded to KCK at the time of data collection, and an EP Abraham Junior Research Fellow from St Hilda's College Oxford to KB all are gratefully acknowledged.

Conflict of interest. None.

Ethical standards. Handling and treatment of animals were in accordance with the guidelines of the Canada Council on Animal Care, and protocols were approved by Environment and Climate Change Canada's Animal Care Committee.

Data. Data are available from DJM upon request.

References

Abdollahi M, Ranjbar A, Shadnia S, Nikfar S and Rezale A (2004) Pesticides and oxidative stress: a review. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* **10**, RA141–RA147.

Akinsanya B, Ayanda IO, Fadipe AO, Onwuka B and Saliu JK (2020a) Heavy metals, parasitologic and oxidative stress biomarker investigations in *Heterotis niloticus* from Lekki Lagoon, Lagos, Nigeria. *Toxicology Reports* **7**, 1075–1082.

Akinsanya B, Isibor PO, Onadeko B and Tinuade A-A (2020b) Impacts of trace metals on African common toad, *Amietophrynus regularis* (Reuss, 1833) and depuration effects of the toad's enteric parasite, *Amplicaecum africanum* (Taylor, 1924) sampled within Lagos metropolis, Nigeria. *Heliyon* **6**, e03570.

Alvarez-Pellitero P (2008) Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. *Veterinary Immunology and Immunopathology* **126**, 171–198.

Álvarez-Pellitero P, Palenzuela O and Sitjá-Bobadilla A (2008) Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). *Parasitology International* **57**, 110–120.

Attademo AM, Peltzer PM, Lajmanovich RC, Cabagna M and Fiorenza G (2007) Plasma B-esterase and glutathione *S*-transferase activity in the toad *Chaunus schneideri* (Amphibia, Anura) inhabiting rice agroecosystems of Argentina. *Ecotoxicology* **16**, 533–539.

Bates D, Machler M, Bolker BM and Walker SC (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48.

Belló ARR, Fortes E, Belló-Klein A, Belló AA, Llesuy SF, Robaldo RB and Bianchini A (2000) Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. *Diseases of Aquatic Organisms* **42**, 233–236.

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS and Kats LB (2011) The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences* **1223**, 108–119.

Blaustein AR, Gervasi SS, Johnson PTJ, Hoverman JT, Belden LK, Bradley PW and Xie GY (2012) Ecophysiology meets conservation: understanding the role of disease in amphibian population declines. *Philosophical Transactions of the Royal Society of London, Series B Biological Sciences* **367**, 1688–1707.

Bols NC, Brubacher JL, Ganassin RC and Lee LEJ (2001) Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology* **25**, 853–873.

Bordes F and Morand S (2009) Parasite diversity: an overlooked metric of parasite pressure? *Oikos* **118**, 801–806.

Bower DS, Brannelly LA, McDonald CA, Webb RJ, Greenspan SE, Vickers M, Gardner MG and Greenlees MJ (2018) A review of the role of parasites in the ecology of reptiles and amphibians. *Austral Ecology* **44**, 433–448.

Brodeur JC, Suarez RP, Natale GS and Ronco AE (2011) Reduced body condition and enzymatic alterations in frogs inhabiting intensive crop production areas. *Ecotoxicology and Environmental Safety* **74**, 1370–1380.

Brodeur JC, Candioti JV, Soloneski S, Larramendy ML and Ronco AE (2012) Evidence of reduced feeding and oxidative stress in common tree frogs (*Hypsiboas pulchellus*) from an agroecosystem experiencing severe drought. *Journal of Herpetology* **46**, 72–78.

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.

Carey C and Bryant CJ (1995) Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environmental Health Perspectives* **103**(suppl. 4), 13–17.

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

Carey C, Cohen N and Rollins-Smith L (1999) Amphibian declines: an immunological perspective. *Developmental and Comparative Immunology* **23**, 459–472.

Cereja R, Mendonça V, Dias M, Vinagre C, Gil F and Diniz M (2018) Physiological effects of cymothoid parasitization in the fish host *Pomatoschistus microps* (Krøyer, 1838) under increasing ocean temperatures. *Ecological Indicators* **95**, 176–182.

Christin MS, Ménard L, Giroux I, Marcogliese DJ, Ruby S, Cyr D, Fournier M and Brousseau P (2013) Effects of agricultural pesticides on the health of *Rana pipiens* frogs sampled from the field. *Environmental Science and Pollution Research* **20**, 601–611.

Claiborne A (1985) Catalase activity. In Greenwald RA (ed.). *CRC Handbook of Methods in Oxygen Radical Research*. Boca Raton, USA: CRC Press, pp. 283–284.

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT and Kalinin AL (2008) Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original®. *Ecotoxicology* **17**, 153–163.

Costantini D (2008) Oxidative stress in ecology and evolution: lessons from avian studies. *Ecology Letters* **11**, 1238–1251.

Dallarés S, Moyá-Alcover CM, Padrós F, Cartes JE, Solé M, Castañeda C and Carrassón M (2016) The parasite community of *Phycis blennoides* (Brünnich, 1768) from the Balearic Sea in relation to diet, biochemical markers, histopathology and environmental variables. *Deep-Sea Research* **118**, 84–100.

Dautremepuits C, Betoulle S and Vernet G (2002a) Réponse au cuivre du système antioxydant de la carpe (*Cyprinus carpio*) saine ou parasitée par le cestode *Ptychobothrium* sp. *Bulletin de la Société de Zoologique de France* **127**, 303–312.

Dautremepuits C, Betoulle S and Vernet G (2002b) Antioxidant response modulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Biochimica et Biophysica Acta* **1573**, 4–8.

Dautremepuits C, Betoulle S and Vernet G (2003) Stimulation of antioxidant enzymes levels in carp (*Cyprinus carpio* L.) infected by *Ptychobothrium* sp. (Cestoda). *Fish and Shellfish Immunology* **15**, 467–471.

Dautremepuits C, Marcogliese DJ, Gendron AD and Fournier M (2009) Gill and head kidney antioxidant processes and innate immune system responses of yellow perch (*Perca flavescens*) exposed to different contaminants in the St. Lawrence River, Canada. *Science of the Total Environment* **407**, 1055–1064.

Di Giulio RT, Washburn PC, Wenning RJ, Winston GW and Jewell CS (1989) Biochemical responses in aquatic animals: a review of determinants of oxidative stress. *Environmental Toxicology and Chemistry* **8**, 1103–1123.

Dornelles MF and Oliveira GT (2014) Effect of atrazine, glyphosate and quinclorac on biochemical parameters, lipid peroxidation and survival in bullfrog tadpoles (*Lithobates catesbieianus*). *Archives of Environmental Contamination and Toxicology* **66**, 415–429.

Fatima M, Mandiki ANM, Douxfils J, Silvestre F, Coppe P and Kestemont P (2007) Combined effects of herbicides on biomarkers reflecting immune-endocrine interactions in goldfish. Immune and antioxidant effects. *Aquatic Toxicology* **81**, 159–167.

Fournier M, Robert J, Salo HM, Dautremepuits C and Brousseau P (2005) Immunotoxicology of amphibians. *Applied Herpetology* **2**, 297–309.

Giri U, Iqbal M and Athar M (1996) Porphyrin-mediated photosensitization has a weak tumor promoting effect in mouse skin: possible role of *in situ* generated reactive oxygen species. *Carcinogenesis* **17**, 2023–2028.

Gismondi E, Beisel J-N and Cossu-Leguille C (2012a) *Polymorphus minutus* affects antitoxic responses of *Gammarus roeseli* exposed to cadmium. *PLoS One* **7**, e41475.

Gismondi E, Rigaud T, Beisel J-N and Cossu-Leguille C (2012b) Effect of multiple parasitic infections on the tolerance to pollutant contamination. *PLoS One* **7**, e41950.

Glinski DA, Purucker ST, Van Meter RJ, Black MC and Henderson WM (2018) Endogenous and exogenous biomarker analysis in terrestrial phase amphibians (*Lithobates sphenocephala*) following dermal exposure to pesticide mixtures. *Environmental Chemistry* **16**, 55–67.

Greulich K and Pflugmacher S (2004) Uptake and effects of detoxification enzymes of cypermethrin in embryos and tadpoles of amphibians. *Archives of Environmental Contamination and Toxicology* **47**, 489–495.

Habig WH, Pabst MJ and Jakoky WB (1974) Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249**, 7130–7139.

Hellou J, Ross NW and Moon TW (2012) Glutathione, glutathione *S*-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic hosts. *Environmental Science and Pollution Reearch* **19**, 2007–2023.

Hendrikx WML and van Moppes MC (1983) *Oswaldocruzia filiformis* (Nematoda: Trichostrongylidae): morphology of developmental stages, parasitic development and some pathological aspects of infection in amphibians. *Zeitschrift für Parasitenkunde* **69**, 523–537.

Holmstrup M, Bindesbøl A-M, Oostingh GJ, Duschl A, Scheil V, Köhler H-R, Loureiro S, Soares AMVM, Ferreira ALG, Kienle C, Gerhard A, Laskowski R, Kramarz PE, Bayley M, Svendsen C and Spurgeon DJ (2010) Interactions between effects of environmental chemicals and natural stressors: a review. *Science of the Total Environment* **408**, 3746–3762.

Hopkins WA (2007) Amphibians as models for studying environmental change. *ILAR Journal* **48**, 270–277.

Jayawardena UA, Rohr JR, Navaratne AN, Amerasinghe PH and Rajakaruna RS (2016) Combined effects of pesticides and trematode infections on hourglass tree frog *Polypedates cruciger*. *EcoHealth* **13**, 111–122.

Jayawardena UA, Rohr JR, Amerasinghe PH, Navaratne AN and Rajakaruna RS (2017) Effects of agrochemicals on disease severity of *Acanthostomum burminis* infections (Digenea: Trematoda) in the Asian common toad. *Duttaphrynus melanostictus*. *BMC Zoology* **2**, 13.

Johnson PTJ and McKenzie VJ (2008) Effects of environmental change on helminth infections in amphibians: exploring the emergence of *Ribeiroia* and *Echinostoma* infections in North America. In Fried B and Toledo R (eds), *The Biology of Echinostomes. From the Molecule to the Community*. New York, USA: Springer-Verlag, pp. 249–280.

Kiesecker JM (2002) Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences of the USA* **99**, 9900–9904.

King KC, McLaughlin JD, Gendron AD, Pauli BD, Giroux I, Rondeau B, Boily M, Juneau P and Marcogliese DJ (2007) Impact of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. *Parasitology* **134**, 2063–2080.

King KC, Gendron AD, McLaughlin JD, Giroux I, Brousseau P, Cyr D, Ruby SM, Fournier M and Marcogliese DJ (2008) Short-term seasonal changes in parasite community structure in northern leopard froglets (*Rana pipiens*) inhabiting agricultural wetlands. *Journal of Parasitology* **94**, 13–22.

Koprivnikar J (2010) Interactions of environmental stressors impact survival and development of parasitized amphibian larvae. *Ecological Applications* **20**, 2263–2272.

Koprivnikar J, Marcogliese DJ, Rohr JR, Orlofske SA, Raffel TR and Johnson PTJ (2012) Macroparasite infections of amphibians: what can they tell us? *EcoHealth* **9**, 342–360.

Kuznetsova A, Brockhoff PB and Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* **82**, 1–26.

Lacaze E, Gendron AD, Miller JL, Colson T-LL, Giraudo M, Sherry JP, Marcogliese DJ and Houde M (2019) Cumulative effects of municipal effluent and parasite infection in yellow perch: a field study using high-throughput RNA-sequencing. *Science of the Total Environment* **665**, 797–809.

Li B, Ma Y and Zhang YUH (2017) Oxidative stress and hepatotoxicity in the frog, *Rana chensinensis*, when exposed to low doses of trichlorfon. *Journal of Environmental Science and Health, Part B. Pesticides, Food Contaminants, and Agricultural Wastes* **52**, 476–482.

Lilley TM, Stauffer J, Kanerva M and Eeva T (2014) Interspecific variation in redox status regulation and immune defence in five bat species: the role of ectoparasites. *Oecologia* **175**, 811–823.

Lüdecke D (2021) sjPlot: Data Visualization for Statistics in Social Science. Rpackage version 2.8.7. Available at https://CRAN.R-project.org/package=sjPlot (accessed 17 February 2021).

Luschak VI (2016) Contaminant-induced oxidative stress in fish: a mechanistic approach. *Fish Physiology and Biochemistry* **42**, 711–747.

Magnadottir B (2010) Immunological control of fish diseases. *Marine Biotechnology* **12**, 361–379.

Mann RM, Hyne RV, Choung CB and Wilson SP (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environmental Pollution* **157**, 2903–2927.

Marcogliese DJ and Pietrock M (2011) Combined effects of parasites and contaminants on animal health: Parasites do matter. *Trends in Parasitology* **27**, 123–130.

Marcogliese DJ, Gagnon Brambilla L, Gagné F and Gendron AD (2005) Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Diseases of Aquatic Organisms* **63**, 77–84.

Marcogliese DJ, King KC, Salo KM, Fournier M, Brousseau P, Spear P, Champoux L, McLaughlin JD and Boily M (2009) Combined effects of

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press

agricultural activity and parasites on biomarkers in the bullfrog, *Rana catasbeiana*. *Aquatic Toxicology* **91**, 126–134.

Marcogliese DJ, Dautremepuits C, Gendron AD and Fournier M (2010) Interactions between parasites and pollutants in yellow perch (*Perca flavescens*) in the St. Lawrence River, Canada: implications for resistance and tolerance to parasites. *Canadian Journal of Zoology* **88**, 247–258.

Martin LB, Hopkins WA, Mydlarz LD and Rohr JR (2010) The effects of anthropogenic global changes on immune functions and disease resistance. *Annals of the New York Academy of Sciences* **1195**, 129–148.

Martínez-Álvarez RM, Morales AE and Sanz A (2005) Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries* **15**, 75–88.

McAlpine DF and Burt MDB (1998) Helminths of bullfrogs (*Rana catesbeiana*), green frogs (*Rana clamitans*), and leopard frogs (*Rana pipiens*) in New Brunswick. *Canadian Field-Naturalist* **112**, 50–68.

Monaghan P, Metcalfe NB and Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters* **12**, 75–92.

Morley NJ, Lewis JW and Hoole D (2006) Pollution-induced effects on immunological and physiological interactions in aquatic host-trematode systems: implications for parasite transmission. *Journal of Helminthology* **80**, 137–149.

Neves CA, Santos EA and Bainy ACD (2000) Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Palemonidae) infected by *Probopyrus ringueleti* (Isopoda, Bopyridae). *Diseases of Aquatic Organisms* **39**, 155–158.

Orledge JM, Blount JD, Hoodless AN and Royle NJ (2012) Antioxidant supplementation during early development reduces parasite load but does not affect sexual ornament expression in adult ring-necked pheasants. *Functional Ecology* **26**, 688–700.

Oruc EO, Sevgiler Y and Uner N (2004) Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. *Comparative Biochemistry and Physiology – Part C: Toxicology & Pharmacology* **137**, 43–51.

Peña-Llopis S, Ferrando MD and Peña JB (2003) Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by *N*-acetylcysteine. *Aquatic Toxicology* **65**, 337–360.

Prudhoe S and Bray R (1982) *Platyhelminth Parasites of the Amphibia*. London, UK: Oxford University Press.

Rau ME, Doyle J and Gordon D (1978) Les parasites des animaux sauvages de Québec.2. Les parasites des grenouilles et des serpents de la région d'Île Perrot. *Naturaliste Canadien* **105**, 56–57.

Rehberger K, Werner I, Hitzfeld B, Segner H and Baumann L (2017) 20 years of fish immunotoxicology – what we know and where we are. *Critical Reviews in Toxicology* **47**, 509–535.

Reichenbach-Klinke H and Elkan E (1965) *The Principal Diseases of Lower Vertebrates. Book II. Diseases of Amphibians*. London, UK: Academic Press.

Relyea RA (2003) Predator cues and pesticides: a double dose of danger for amphibians. *Ecological Applications* **13**, 1515–1521.

Relyea RA and Hoverman JT (2008) Interactive effects of predators and a pesticide on aquatic communities. *Oikos* **117**, 1647–1658.

Rohr JR and McCoy KA (2010) A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. *Environmental Health Perspectives* **118**, 20–32.

Rollins-Smith LA and Woodhams DC (2012) Amphibian immunity. Staying in tune with the environment. In Demas GE and Nelson RJ (eds), *Ecoimmunology*. Oxford, UK: Oxford University Press, pp. 92–143.

Rumschlag SL, Halstead NT, Hoverman JT, Raffel TR, Carrick HJ, Hudson PJ and Rohr JR (2019) Effects of pesticides on exposure and susceptibility to parasites can be generalised to pesticide class and type in aquatic communities. *Ecology Letters* **22**, 962–972.

Sass JB and Colangelo A (2006) European Union bans atrazine, while the United States negotiates continued use. *International Journal of Occupational and Environmental Health* **12**, 260–267.

Saurabh S and Sahoo PK (2008) Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture Research* **39**, 223–239.

Seburn CNL and Seburn DC (1998) *Status Report: Northern Leopard Frog Rana Pipiens (Western Population)*. Ottawa, Canada: Committee on the Status of Endangered Wildlife in Canada.

Sies H (1997) Oxidative stress: oxidants and antioxidants. *Experimental Physiology* **82**, 291–295.

Sih A, Bell AM and Kerby JL (2004) Two stressors are far deadlier than one. *Trends in Ecology and Evolution* **19**, 274–276.

Sorci G and Faivre G (2009) Inflammation and oxidative stress in vertebrate host-parasite systems. *Philosophical Transactions of the Royal Society of London, Series B Biological Sciences* **364**, 71–83.

Storey KB (1996) Oxidative stress: animal adaptations in nature. *Brazilian Journal of Medical and Biological Research* **29**, 1715–1733.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL and Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. *Science (New York, N.Y.)* **306**, 1783–1786.

Studnicka M, Siwicki A and Ryka B (1986) Lysozyme level in carp (*Cyprinus carpio* L.). *Bamidgeh* **2**, 22–25.

Stumbo AD, Goater CP and Hontela A (2012) Parasite-induced oxidative stress in liver tissue of fathead minnows exposed to trematode cercariae. *Parasitology* **139**, 1666–1676.

Sures B, Nachev M, Selbach C and Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. *Parasites & Vectors* **10**, 65.

Szuroczki D and Richardson JML (2009) The role of trematode parasites in larval anuran communities: an aquatic ecologist's guide to the major players. *Oecologia* **161**, 371–385.

Szuroczki D, Koprivnikar J and Baker RL (2019) Effects of dietary antioxidants and environmental stressors on immune function and condition in *Lithobates* (*Rana*) *sylvaticus*. *Comparative Physiology and Biochemistry – Part A: Molecular & Integrative Physiology* **229**, 25–32.

Tort L, Balasch JC and Mackenzie S (2003) Fish immune system. A crossroads between innate and adaptive responses. *Immunologia* **22**, 277–286.

Uribe C, Folch H, Enriquez R and Moran G (2011) Innate and adaptive immunity in teleost fish: a review. *Veterinary Medecine* **56**, 486–503.

Valavanidis A, Vlahogianni T, Dassenakis M and Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety* **64**, 178–189.

Van der Oost R, Beyer J and Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* **13**, 57–149.

Venturino A, Rosenbaum E, Caballero de Castro A, Anguiano OL, Gauna L, Fonovich de Schroeder T and Pechen de D'Angelo AM (2003) Biomarkers of effect in toads and frogs. *Biomarkers* **8**, 167–186.

Winston GW and Di Giulio RT (1991) Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquatic Toxicology* **19**, 137–161.

Zhang J, Shen H, Wang X, Wu J and Xue Y (2004) Effect of chronic exposure of 2-4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere* **55**, 167–174.

https://doi.org/10.1017/S003118202100038X Published online by Cambridge University Press