Investigating the basis for pollutant guidelines regarding amphibians in stormwater retention ponds

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Amid an anthropogenic sixth mass extinction, amphibians are among the most threatened organism groups. This can be explained largely by the loss and fragmentation of natural wetlands due to agricultural activities and urbanisation. Stormwater retention ponds are designed both to provide flood control and to collect and purify runoff from impervious surfaces but could also serve as amphibian habitats. By their very nature, retention ponds tend to accumulate pollutants to a degree uncommon in natural wetlands and could therefore potentially act as ecological traps or sinks. This study aimed to sample previously generated amphibian ecotoxicological data and describe this data statistically as well as generate species sensitivity distributions (SSDs) with conservative hazard concentrations for pollutants commonly found in retention ponds. After data filtering, SSDs for a total of 13 pollutants were generated. Concentrations for 14 additional pollutants were established as the lowest shown to have detrimental effects or the highest producing no such effects. Several substances were predicted to cause negative effects at concentrations lower than those either measured in retention pond recipients or set as existing guidelines. This study points towards a clear lack of relevant data to construct comprehensive guidelines at present.

Key words: amphibians; ecotoxicology; pollutants; retention; stormwater; species sensitivity distribution

INTRODUCTION

The global flora and fauna are undergoing an anthropogenic sixth mass extinction. Commonly cited reasons are pollution, climate change, deforestation, habitat destruction and fragmentation (Dirzo et al., 2014; Ripple et al., 2017; Ceballos & Ehrlich, 2018). Globally, amphibians are the most threatened and rapidly vanishing vertebrates (Hoffman et al., 2010), estimated to be going extinct at a rate 200 times greater than the background extinction rate by conservative estimates (McCallum, 2007; Roelants et al., 2007) and by as much as 25,000 to 45,000 if species in imminent danger are considered (McCallum, 2007). The sensitivity of amphibians to pollutants and pathogens could be related to their permeable skin (up to 300 times more permeable to certain common pollutants compared to mammals), their aquatic-terrestrial life cycle which exposes them to threats from both

systems, and the simplicity of their immune system compared to many other vertebrates (Wake & Vredenburg, 2008; Quaranta, 2009).

Disease, habitat destruction, invasive species and pollutants in aquatic and terrestrial habitats have been cited as major threats to amphibians (Stuart et al., 2004; Hamer & McDonnell, 2008; Wake & Vredenburg, 2008; Hamer & McDonnell, 2010) and the complexity of their life cycle makes them particularly vulnerable to the effects of habitat loss (Becker et al., 2007). They are especially sensitive to habitat changes and fragmentation resulting from urban sprawl because they are dependent on resources from both aquatic and terrestrial environments and connections between wetlands and terrestrial habitats (Rubbo & Kiesecker, 2005; Price et al., 2006; Gagné & Fahrig, 2007). Amphibians need a minimum of interconnected suitable habitats in order to maintain and disperse populations and avoid ecological traps, and such

habitats decrease with increasing urbanisation (Ficetola & De Bernardi, 2004; Hermann et al., 2005; Cushman, 2006; Becker et al., 2007; Gardner et al., 2007; Windmiller et al., 2008). Indeed, it is widely accepted that urbanisation is one of the underlying reasons for the global decline of amphibian populations (Czech et al., 2000; Foley et al., 2005; Scheffers & Paszkowski, 2013) and a number of studies have shown negative correlations between urban land use and the abundance and diversity of many vertebrates (Czech & Krausman, 1997; Yahner, 2003; Rubbo & Kiesecker, 2005). Replacing terrestrial ecosystems with impervious surfaces leads to direct mortality in migrating organisms, changes in hydrological conditions, fragmentation of populations, and the presence of pollutants in aquatic systems (Collins et al., 2000; Pickett et al., 2001; Houlahan & Findlay, 2003; Radeloff et al., 2005; Rubbo & Kiesecker, 2005; McKinney, 2008).

Because non-urbanized and protected land constitutes a small part of major industrial countries, it stands to reason that conservation efforts cannot and should not be limited exclusively to exotic species and hotbeds of biodiversity (Le Viol et al., 2012). While traditionally focus has been firmly on natural environments, efforts exist aiming towards urban preservation and connecting natural and urban areas (Rosenzweig, 2003). Urban man-made structures may play a part as refuges in preserving less exotic species (Le Viol et al., 2009; Brand & Snodgrass, 2010) and act as corridors quite separately from their primary anthropocentric purpose (Le Viol et al., 2012). In areas where the loss of wetland and degree of urbanisation is significant, human-made bodies of water can act as a substitute, and retention ponds are common features which may supply amphibians with habitats for breeding (Bishop et al., 2000a; Scher & Thiéry, 2005; Ostergaard et al., 2008; Snodgrass et al., 2008; Simon et al., 2009; Hamer et al., 2012; Le Viol et al., 2012; Gallagher et al., 2014). Retention ponds are constructed to receive runoff from impervious surfaces (roads, sidewalks, parking lots, building sites, etc.) in order to provide both flood control and purification of water by sedimentation before it makes its way into natural recipients (Novotny, 1995; Bishop et al., 2000b). In many urban environments where the destruction of wetlands has been extensive, retention ponds can be among the only bodies of water able to support the complete amphibian life cycle (Hamer & McDonnell, 2008; Brand & Snodgrass, 2010) and in some areas, retention ponds have more suitable hydroperiods for amphibian breeding than surrounding natural wetlands (Gallagher *et al.*, 2014).

However, retention ponds are ultimately constructed to collect pollutants, which means that flora and fauna therein are exposed to such pollutants and may experience toxicological effects (Campbell, 1994; Helfield & Diamond, 1997; Bishop et al., 2000b; Casey et al., 2005; Massal et al., 2007; Snodgrass et al., 2008). Not being constructed for conservation purposes, screening is rarely carried out within retention ponds. Rather, such tests are carried out on concentrated runoff water flowing into the ponds and the natural recipients directly in connection with the ponds, to assess the effectiveness of pollutant removal. Pollutants which are commonly screened for include phosphorous (P), nitrogen (N), lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), chromium (Cr), nickel (Ni), suspended solids (SS), benzo[a]pyrene (BaP) and the 16 polycyclic aromatic hydrocarbons from the U.S. Environmental Protection Agency's (US EPA) priority pollutant list (PAH16). Mercury (Hg), oil fractions, and the prioritised pollutants of the EU's Water Framework Directive are sometimes analysed, but cost is often prohibitive (Larm, personal communication, January 5, 2020). Documented consequences of pollutant exposure on amphibians are both lethal and non-lethal and include but are not limited to stunted growth and development, increased frequency of deformities, disease, mortality and behavioural change (Bridges, 1999; Ortiz et al., 2004; Relyea, 2005; Griffis-Kyle, 2007; Karraker et al., 2008; Shinn et al., 2008; Snodgrass et al., 2008; Relyea, 2009; Brand et al., 2010).

It is of the utmost importance to establish whether the habitat offered by a given retention pond can be considered an ecological source, sink, or trap. A source habitat possesses favourable conditions for a taxonomical group which will lead to an increase in the size of the population which resides there, and this surplus of individuals will possibly migrate to other habitats. The opposite is an ecological sink, denoting a habitat which by its inability to support survival and reproduction decreases the size of populations residing there. If the negative effects on amphibian populations are large enough to affect their growth adversely despite being attracted to these habitats, these ponds can act as so-called ecological traps (Battin, 2004; Hamer & McDonnell, 2008; Snodgrass et al., 2008; McCarthy & Lathrop, 2011). Retention ponds in particular could act as ecological traps when the sudden habitat fragmentation and loss of natural wetlands caused by urbanisation makes them attractive to amphibians by virtue of being the only viable alternative, while simultaneously being polluted to a degree which makes them unable to sustain amphibian populations.

Analyses of water from aquatic habitats have shown that pesticides often occur in low concentrations but in complex mixtures (Munn et al., 2006; Daly et al., 2007; Gilliom et al., 2007). Pollutants which individually fall below the concentration threshold value for toxic effects may in a mixture together with other pollutants have a synergistic effect, where the impact of a mixture is greater than the sum of its individual parts (Cedergreen, 2014). It has been shown that while effects on some organism groups can be predicted additively from the substances in isolation, this may be less true for amphibians (Relyea et al., 2005; Rohr et al., 2006). Environmental guidelines are largely established using the standard taxonomical groups of algae, crustaceans, and fish, while amphibian data are more rarely utilised.

In the current landscape, there is a lack of studies evaluating and utilising the large amounts of recorded amphibian ecotoxicological data to investigate the basis for pollutant guidelines as they apply to retention ponds. The aim of this study is to rectify this lack, and this is to be accomplished by database sampling and subsequent descriptive statistics and construction of species sensitivity distributions (SSDs). While it will delve into the representation of Swedish species in the available data, its conclusions will not be regionally limited.

METHODS

Sampling

Sampling was carried out on the 6th of January 2020 by building a local SQL (Structured Query Language) version of the US EPA ECOTOX database. This was done using the *PostgreSQL* software (version 12.1; PostgreSQL Global Development Group, 2019), and subsequently the local database was accessed through the statistics software *R* (version 3.6.2; R Core Team, 2020), which was also used for statistical analysis. Instructions have been supplied by Szöcs (2016). The REACH database was considered but not sampled due to data not being traceable to its original

source and a lack of documentation regarding taxa, duration, and test conditions (Posthuma *et al.*, 2019). The local database was sampled using the query "Amphibians" as the "Species group" qualifier. Field descriptors used in the database are listed in Table 1.

TABLE 1. Field descriptors included in the database after initial sampling.

Field descriptor (unit)
CAS registry number
Chemical name
Species scientific name
Organism life stage
Organism age (days)
Exposure type
Test location
Concentration (mg/L)
Concentration unit
Concentration type
Effect measurement
Endpoint
Test duration (days)
Reference number
Publication year

Descriptive statistics

Unifying and expanding upon the chemical classes used by Kerby et al. (2010), Le Viol et al. (2012), Gallagher et al. (2014) and Wiklander (2017) in studies relating to retention pond pollutants, the classification of substances into 11 major categories as shown in Table 2 was used in the present study. Pesticides were further divided into subcategories depending on their primary organism targets, and all substances not fitting into any of the major categories used in this study were designated "other". While these substances are not expected to be found in retention ponds in appreciable concentrations, they were further classified to enable future statistical analysis on a per-need basis. As in the cited studies, the substance classes were defined by functional or structural characteristics.

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Chemical class (abbreviation)	Abbreviation	Explanation	
Metal	BAF	Bioaccumulation factor	
Polycyclic aromatic hydrocarbon (PAH)	BCF	Bioconcentration factor	
Polychlorinated biphenyl (PCB)	BCFD	BCF in the presence of dissolved organic matter	
Pesticide	EC	Effect concentration	
General pesticide	ED	Effective dose	
Acaricide	ET	Effect time	
Avicide	IC	Immobilisation concentration	
Fungicide	LC	Lethal concentration	
Herbicide	LD	Lethal dose	
Insecticide	LECT	Lowest effective concentration tested	
Molluscicide	LETH	No information found	
Nematicide	LOEC	Lowest observed effect concentration	
Per- or polyfluoroalkyl substance (PFAS)	LOEL	Lowest observed effect level	
Road salt	LT	Lethal time	
Nutrient	MATC	Maximum acceptable toxicant concentration	
Petrochemical	NOEC	No observed effect concentration	
Phenol	NOEL	No observed effect level	
Phthalate	ZERO	No information found	
Other			

TABLE 2. Chemical classifications applied after *initial sampling.*

A glossary of abbreviations describing varying endpoints is shown in Table 3. Data preparation sequentially followed the steps outlined in Table 4, adapted from de Zwart (2002) and Saouter *et al.* (2018).

Species sensitivity distributions

The purpose of a species sensitivity analysis is to determine the concentration of a substance at which most of the relevant species in a hypothetical habitat will not be adversely affected. The concentration used is normally the hazardous concentration where five percent of species are expected to experience negative effects (HC₅). To ascertain this value, log¹⁰transformed concentrations (NOEC, EC₅₀ etc.) are cumulatively plotted against rank assigned percentiles for said concentrations. Depending on the quality and quantity of the data, either parametric distributions or non-parametrically derived bootstrap distributions are fitted, and it is along this distribution the HC₅ is found (Wheeler *et al.*, 2002). The HC_5 is an analogue on a larger organizational scale to the lethal concentration (LC) or effect concentration (EC) commonly determined experimentally in laboratory settings for a given species and can be used to set an environmental quality criterion (EQC) or incorporate into an environmental risk assessment (ERA) in some countries.

While establishing acute LC₅₀ values (lethal concentration for 50% of the tested organisms) in laboratory conditions does not have a natural carryover to chronic population-level effects in the field (Vonesh & De la Cruz, 2002; Schmidt, 2004), pooling large amounts of laboratory data into species sensitivity distributions has been shown to result in threshold values which are generally protective of natural ecosystems (van Straalen et al., 2002) and SSDs have been associated with biodiversity impacts in the field (Posthuma & de Zwart, 2012). The set of species used may be those of a natural community, a representative selection, or а taxon.

TABLE 3. Glossary of endpoint abbreviations and their corresponding explanations.

TABLE 4. Data preparation for species sensitivity distributions (adapted and expanded from de Zwart, 2002 and Saouter et al., 2018).

Preparatory step
Unification of species name spelling

Resolving where possible entries only specified to class, genus, or order

Removal of entries not resolved to species level

Removal of entries with concentration units other than $\ensuremath{\text{mg/L}}$

Conversion of concentration to µg/L

Removal of entries lacking reported concentration

Removal of entries lacking reported endpoint

Removal of entries lacking reported duration

Removal of entries lacking reported chemical analysis

Removal of substances classified as "Other"

Designating data as acute or chronic

Acute: endpoint EC₅₀, LC₅₀ or IC₅₀; test duration \leq 96 hrs

Chronic: endpoint NOEC, LOEC, NOEL, NOAEC, MATC, EC₁₀₋₂₀, LC₁₀₋₂₀, IC₁₀₋₂₀; test duration >96 hrs

Removal of entries not fitting definitions for acute or chronic data

Removal of duplicate entries by inspection of references

An EQC or HC_x value can be determined by choosing the acceptable affected percentage of species on the y-axis and finding the corresponding pollutant concentration on the x-axis. Conversely, a measured or expected concentration on the x-axis can be used to determine the percentage of species affected on the y-axis; the potentially affected fraction (PAF) (van Straalen, 2002; Traas *et al.*, 2002). Fig. 1 shows a generalised SSD plot.

For the construction of SSDs the R package ssdtools (version 0.1.1; Thorley & Schwarz, 2018) was used. The package was used to fit distributions to the data using maximum likelihood estimation (MLE), with the Aikake information criterion (AIC) recommended for distribution selection. The Anderson-Darling goodness-of-fit test was used for comparisons between SSDs. This test measures vertical discrepancy in plotted cumulative density functions (Aldenberg *et al.*, 2002). Confidence limits were established by parametric bootstrapping with iterations set to 10,000.

For the sake of protectiveness, the lowest concentration was chosen when multiple entries existed for the same species. While it has been suggested that 10 species are the minimum for reliable estimates appropriate for regulatory use (Wheeler et al., 2002), all SSDs are reported in the interest of completeness.



FIG. 1. *Basic appearance of an SSD. Each dot represents a toxicity value for a species, the line a fitted distribution. From Posthuma* et al. (2002a).

Supplemental data

In cases where data were insufficient for the construction of SSDs, the choice was made to present the lowest concentrations available for the remaining relevant pollutants, such as NOEC, LC, and EC values. It is important to note that in contrast to an HC₅, these values do not include any probabilistic estimates which means that statistical uncertainties have not been considered. As a result, they are unsuited to be used as a basis for environmental guidelines, although they can be used to gain an approximate insight into amphibian sensitivities until such a time when statistical analysis can be carried out. For the sake of transparency, the total number of toxicity values available for each of these substances will also be reported, together with the effect type relevant for each concentration. Extracting and commenting on the lowest available concentration for every substance available in the data set after an initial sampling was likely to push the study beyond its intended scope. Instead, common sampling protocols used by StormTac AB and in studies carried out on behalf of the County Administrative Board of Skåne (Pirzadeh et al., 2015; Högstrand & Pirzadeh, 2018, StormTac AB, n.d.) were combined and compiled into a list of 97 substances and groups of substances. This list was still more comprehensive than what could reasonably be expected from any single screening protocol due to economic considerations and was used as a filter for the extraction of lowest concentrations. This list of substances and substance groups is presented in full in Appendix D (Table D1).

Guidelines and field measurements

To assess where the results of this study fall within an existing framework they will be compared to the guidelines for inland water put forth by the Swedish Agency for Marine and Water Management (SWaM) (2019) as well as measured concentrations in Swedish recipients downstream of retention ponds (Högstrand & Pirzadeh, 2018; StormTac AB, n.d.). In the case of the SWaM guidelines, comparisons will be made with the maximum allowed concentrations for inland surface waters or, where lacking for a given substance, mean annual concentration guidelines for inland surface waters. It should be noted that these guidelines and measurements are not directly comparable to withinpond pollutant loads, which would tend to be significantly higher, but they are the most reasonable

point of comparison when sampling has not been carried out within the relevant retention ponds (Larm, personal communication, January 5, 2020).

RESULTS

Descriptive statistics

The initial sampling resulted in 25,232 data points from 1,165 studies spanning the years 1925– 2019 and a total of 1,055 unique substances. In all, 59 genera and 177 species were present in the sampled data, after further resolving those specified only to class, order or genus, by inspection of the 53 relevant studies. The largest contributor to the data set (8,631 data points or 34%) was the Xenopus genus, chief among the species being the African clawed frog, *Xenopus laevis*. The second most represented genus was Lithobates (4,372 data points or 17%) with the most common species being the northern leopard frog, *Lithobates pipiens*.

Of the 13 Swedish amphibian species, 10 were represented in the data set, contributing 2,021 of the 25,232 data points (8%) and tested for 226 of the 1,055 substances (21%). All genera and species are listed in full in Appendix A (Table A1 and A2) together with their contribution to the data set expressed as the number of data points.

The most common chemical classes were found to be insecticides (156 or 15% of all substances), herbicides (122 or 12%), fungicides (78 or 7%), metals (70 or 7%), and those classified as "other" (464 or 44%). Dominant among substances classified as "other" were pharmaceuticals and personal care products (PPCP) totalling 152 (14% of all substances or 33% of the category other). All frequencies are shown in Fig. 3a.

Concerning the life stage of the tested amphibians, early stages were dominant with tadpoles (11,287 or 45% of all data points), embryos (4,790 or 19%), and larvae (3,134 or 12%) constituting the bulk of the data set. This is presented in Fig. 3b.

Shown in Fig. 3c are the types of effects measured in the sampled studies, where mortality (8,747 or 35% of all data points), growth (3,387 or 13%), development (3,107 or 12%), genetics (2,514 or 10%), morphology (1,477 or 6%), and enzymatic effects (1,345 or 5%) made up the bulk of the data set.

The most common endpoints used were NOEC (6,139 or 24% of all data points), LC (4,933 or 19%),

LOEC (4,148 or 16%), NOEL (1,810 or 7%), and LOEL (1,290 or 5%). These frequencies are shown in Fig. 3d.

The median test duration for all available data was 4 days, with the entire data set representing a range of 0 to 1,001 days. This has been visualised in Fig. 2a.

Regarding exposure type, renewal (13,112 or 52%) and static exposure (6,344 or 25%) made up most of the data set. All exposure types are shown in Fig. 2b.

As expected, nearly the entire data set consisted of laboratory tests (23,702 data points or 94%) as opposed to natural and artificial field experiments. The test location frequencies can be found in Fig. 2c.

Species sensitivity distributions

After filtering according to the specifications in Table 4, a total of 10,726 data points and 463 unique substances remained. 2,838 data points were assigned as acute (412 substances) and 5,955 as chronic (196 substances). A total of 18 SSDs were generated, of which eight were based on chronic data. The substances represented were ammonium, atrazine, cadmium, copper, DDT, dieldrin, diuron, endosulfan. endrin, glyphosate, mercury, pentachlorophenol (PCP), and zinc. The SSDs are presented in Fig. 4, 5 and 6, while larger versions complete with the species name labels for each included data point can be found in Appendix B. A summary of the key statistics and chosen distributions is presented in Table 5. Several of the pollutants were present in various forms such as salts and commercial formulations, and therefore multiple CAS registry numbers were represented in many of the final SSDs. These are shown in full in Appendix C (Table C1). Regarding goodness-of-fit, four of the SSDs did not fulfil the criterion of a sample size ≥ 7 required to calculate Anderson-Darling statistics (DDT, dieldrin, diuron, and PCP) although a model selection could still be performed using AIC.

Supplemental data

In addition to the constructed SSDs, data for an additional 14 pollutants were retrieved after the initial filtering process. These were 4-tert-Octylphenol (2.1 µg/L), alachlor (0.15 µg/L), benzene (76 µg/L), benzo[a]pyrene (33 µg/L), bisphenol A (2.3 µg/L), chromium (30 µg/L), fluoranthene (11 µg/L), isoproturon (1,300 µg/L), 2-methyl-4-chlorophenoxy acetic acid (MCPA) (1,300 µg/L), n-Nonylphenol (2.2 µg/L), perfluorooctanesulfonic acid (PFOA) (1,000 µg/L), simazine (1.2 µg/L), and trifluralin (200 µg/L). A summary of these data is shown in Table 6.

Guidelines and field measurements

Several of the potentially protective concentrations established fell below or close to either existing guidelines or concentrations measured in the field. Falling below one or both of these concentrations were atrazine, copper, diuron, and glyphosate. The HC_5 for atrazine was approximately 20 times lower than the SWaM guidelines, at 0.094 μ g/L compared to 2 μ g/L, while the measured concentration was 0.017 µg/L. Copper had an HC₅ of 0.3 μ g/L compared to the guideline of $0.5 \mu g/L$, while the measured concentration fell at $0.20 \ \mu g/L$. The HC₅ for diuron fell below the SWaM guideline by a factor of roughly 10 with 0.17 compared to 2 µg/L, but above the measured concentrations used in this study (0.04 μ g/L). Finally, the HC₅ for glyphosate fell below the SWaM guidelines by a factor of approximately 10, at 8 μ g/L compared to 100 μ g/L. The mercury HC₅ approached the measured concentrations, at 0.082 compared to 0.08 µg/L. Among the supplemental lowest concentrations derived from single data points, two substances fell below existing guidelines: Simazine (1.2 compared to 4 μ g/L) and alachlor (0.15 compared to 0.3 μ g/L). A comparison of HC5 values to existing guidelines and measured concentrations is outlined in Fig. 7.



FIG. 2. Visualisation of descriptive statistics. (a) Density plot showing the distribution of test durations in the data set. (b) Lollipop plot showing the number of data points categorised by exposure type. (c) Lollipop plot showing the number of data points categorised by test location.



FIG. 3. Visualisation of descriptive statistics. (a) Lollipop plot showing the number of data points categorised by substance class. (b) Lollipop plot showing the number of data points categorised by life stage. (c) Lollipop plot showing the number of data points categorised by effect group. (d) Lollipop plot showing the number of data points categorised by endpoint.



FIG. 4. Species sensitivity distributions. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅. (a) Ammonium chronic. (b) Atrazine acute. (c) Atrazine chronic. (d) Cadmium acute. (e) Cadmium chronic. (f) Copper acute.



FIG. 5. Species sensitivity distributions. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅. (a) Copper chronic. (b) DDT acute. (c) Dieldrin acute. (d) Diuron chronic. (e) Endosulfan chronic. (f) Endrin acute.



FIG. 6. Species sensitivity distributions. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.
(a) Glyphosate acute. (b) Glyphosate chronic. (c) Mercury acute. (d) Mercury chronic.
(e) Pentachlorophenol acute. (f) Zinc acute.

Substance	Acute/Chronic	Distribution	AD ^a	Species (n)	HC₅ (µg/L)
Ammonium	Chronic	Log-normal	0.285	15	560 (160–2,300)
A ++	Chronic	Log-normal	0.375	29	0.094 (0.02–0.50)
Atrazine	Acute	Gamma	0.490	13	570 (110–2,300)
Cadmium	Chronic	Gompertz	0.423	8	2.5 (0.89–6.5)
Caumum	Acute	Log-normal	0.363	14	15 (3.7–110)
Copper	Chronic	Log-normal	0.370	18	0.30 (0.060–2.2)
Сорреі	Acute	Burr type III	0.592	24	9.8 (4.1–24)
DDT	Acute	Gamma	n/a	6	78 (0.59–4,100)
Dieldrin	Acute	Gamma	n/a	6	6.9 (0.57–69)
Diuron	Chronic	Gamma	n/a	6	0.17 (0.000019–400)
Endosulfan	Chronic	Gamma	0.667	18	0.025 (0.0027–0.21)
Endrin	Acute	Burr type III	0.231	11	0.52 (0.060–4.0)
Clumbagata	Chronic	Gamma	0.967	35	8.0 (1.1–55)
Glyphosate	Acute	Burr type III	0.756	40	580 (360–950)
Mercury	Chronic	Gamma	0.523	8	0.082 (0.013–3.4)
Mercury	Acute	Gamma	0.586	15	1.0 (0.099–9.5)
Pentachlorophenol	Acute	Gamma	n/a	7	9.7 (1.1–75)
Zinc	Acute	Weibull	0.455	11	53 (4.4–720)

TABLE 5. Statistical summary of species sensitivity distributions; HC_5 reported with the lower and upper 95% confidence limit within parentheses.

^a Anderson-Darling test value for goodness-of-fit

TABLE 6. Supplemental data based on the lowest available concentrations.

Substance	Available values (<i>n</i>)	Conc. (µg/L)	Endpoint	CAS reg. no.	EPA ref. no.
4-tert-Octylphenol	33	2.1	NOEC (weight)	140669	119281
Alachlor	28	0.15	NOEC (metamorphosis)	15972608	85815
Benzene	11	76	EC ₁₀ (mortality)	71432	15418
Benzo[a]pyrene	15	33	NOEC (length)	50328	81628
Bisphenol A	207	2.3	NOEC (sex ratio)	80057	51029
Chromium	127	30	LC ₅₀ (mortality)	1333820, 7440473	4943
Fluoranthene	9	11	NOEC (movement)	206440	18947
Isoproturon	10	1,300	NOEL (ovulation rate)	34123596	117111
MCPA	14	1,300	NOEC (hormones)	94746	117111
n-Nonylphenol	69	2.2	NOEC (sex ratio)	25154523	51029
PFOA	23	1,000	NOEC (metamorphosis)	335671	176982
PFOS	35	50	NOEC (morphology)	1763231	179654
Simazine	40	1.2	NOEC (mortality)	122349	178653
Trifluralin	21	200	NOEC (morphology)	1582098	161203



FIG. 7. Dot plot showing HC_5 values as triangles (\blacktriangle) compared to SWaM guidelines as circles (\bullet) and Swedish measured concentrations as squares (\blacksquare). Note: Glyphosate (Gly) and endrin (Endr) lack measured concentrations. *Acute HC_5

This study has made it clear that there is a profound lack of ecotoxicological data for stormwater-relevant pollutants as they pertain to amphibians. This means that the construction of statistically derived guidelines for most such substances would be impossible at the time of writing, not least if one wishes to specifically investigate Swedish species, which make up a very small part of the data set.

DISCUSSION

Several of the pollutants in this study appear likely to exhibit some detrimental effects on amphibian communities at concentrations lower than or equal to existing guidelines or recipient concentrations measured in connection with Swedish retention ponds. The most support could be said to exist for those pollutants with chronic HC_5 values falling below either measured or guideline values: Atrazine, copper, diuron, and glyphosate (mercury fell close to the guidelines and practically matched the measured concentrations). In the case of zinc, data were insufficient for chronic SSD construction, and acute data will invariably produce (by varying orders of magnitude) higher and therefore less protective HC_x values (de Zwart, 2002). It is helpful, then, that both acute and chronic SSDs could be constructed for the three other transition metals included in this study: Copper, cadmium and mercury. In all three cases, the chronic HC_5 values were approximately one order of magnitude lower than their acute counterpart. If the fourth transition metal, zinc, were to follow the same trend, its chronic HC_5 value could reasonably be expected to fall below both measured values and existing guidelines.

Regarding such acute values, chronic SSDs were unable to be constructed for five of the substances included in this study. In addition to zinc, these were DDT, dieldrin, endrin, and PCP. It is likely that these would have chronic HC_5 values falling one or more orders of magnitude below those supplied here, and consequently below guidelines or measured concentrations. The most likely candidates

according to the present study even when applying a conservative one order of magnitude decrease in HC_5 are endrin and PCP. Overall, this study showed a difference between acute and chronic values ranging between approximately one and four orders of magnitude, with the smallest being cadmium and the largest being atrazine.

Due to the common practice of including a minimum of algae, crustaceans, and fish for the construction of overarching ecosystem wide SSDs and subsequent guidelines, many substances remain untested on other phylogenetic groups. If such guidelines are conservative enough to be protective of organism groups not included in the analysis, then the relative lack of representation is a non-issue, but the results of this study suggests that this cannot be assumed as a general rule.

It is worth noting that the acute and chronic SSDs for glyphosate, while including the largest number of data points, also had the lowest goodnessof-fit scores. Conversely, two of the SSDs with insufficient data points to calculate Anderson-Darling statistics, dieldrin and PCP, could upon a visual inspection be seen to have a very high goodness-of-fit. This is not surprising and highlights an important point: Goodness-of-fit measures have a weakness when it comes to large and small data sets. Only highly deviating data points and trends will be identified as an insufficient lack of fit when sample sizes are small enough, while large sample sizes almost without fail will produce a lack of fit which is statistically significant. This even when the deviation from the chosen distribution is decidedly modest and does not meaningfully impact the conclusions which can be drawn (Johnson & Wichern, 2007). Therefore, while the goodness-offit statistics reported in this study can be helpful as loose guidelines, their importance should not be overstated, neither as a strong support for distributions based on small data sets nor as a negation of distributions fitted using larger data sets.

Pollutants are only one of the filtering factors determining whether amphibians make use of any given retention pond as a habitat. Others include proximity to riparian zones and presence of vegetation cover, which is in turn dependent upon pond age (Birx-Raybuck *et al.*, 2010). Furthermore, studies on community ecology have pointed to pollutants chiefly having indirect rather than direct effects on amphibians (Relyea *et al.*, 2005; Relyea & Diecks, 2008), and traditional toxicity studies will invariably focus on the latter. As an example, the work done by Gallagher and colleagues (2014)

suggests that chloride, found in commonly administered road salts, could indirectly play an important role in the toxicity of metals and by extension the suitability of retention ponds as breeding habitats for more sensitive species. And while the concentration of a herbicide in a pond may be insufficient to affect amphibian mortality directly, it may be sufficient to reduce macrophyte coverage and thereby increase predation pressure both below and above water, as visibility increases. Pollutants may act as nodes in food chains and food webs, taking the role of predator or competitor and possibly causing trophic cascades and other, more subtle ecological effects. The SSD concept will never in and of itself address ecological interactions but can play a part in a larger framework with the goal of assessing ecosystem structure and function (Posthuma et al., 2002b). An ecotoxicological study will necessarily constitute one part of a larger, holistic approach to the evaluation of the suitability of retention ponds as amphibian habitats.

It should be noted that retention ponds which are expected to receive the greatest pollutant load due to their proximity to heavily urbanised areas, surrounded by large impervious surfaces, major traffic and construction work, could for these same reasons reasonably be expected to be the least likely to be used as amphibian habitats. There is good reason to assume that such ponds may simply be surrounded by too many other physical filters keeping amphibians at a distance, preventing them from acting as ecological traps due to their pollutant loads.

Normally, samples are taken from the undiluted runoff before it enters a pond as well as from the natural recipients downstream, to calculate the effectiveness of pollutant reduction. Samples are rarely if even taken in the ponds themselves, simply because their role as habitats is not a focal point. Nevertheless, if there is sufficient interest in this role, samples could be taken within-pond and compared to the results of this study. It must be kept in mind that pollutant load varies greatly during the year and between locations. In Sweden, most amphibians spawn between May and June, and as such the pollutant load during these months would be the most relevant to assess. It may be a necessary sacrifice to accept higher concentrations of in retention ponds pollutants to reduce concentrations in natural waters further downstream. This is, after all, the primary purpose of these structures, and while this study works under the assumption that retention ponds can function as

amphibian habitats, it is imperative to protect surrounding natural waters and ecosystems as well.

While the dominance of the model species X. *laevis* in the data set limits the number of substances for which SSDs can be constructed (many substances having been tested extensively on X. laevis but only additionally on a handful of other species), this dominance should not affect the quality of the SSDs. Only one data point representing this species can be present in any SSD, and therefore its prominence in the data set is not mirrored in the individual results. While some studies have shown phylogenetic differences between responses in amphibians exposed to chloride (Karraker et al., 2008; Collins & Russell, 2009; Gallagher et al., 2014), a meta-analytic review by Egea-Serrano and colleagues (2012) showed only weak differences in effect size between amphibian families, suggesting a general lack of correlation between pollutant sensitivity and phylogeny. There could nevertheless be value in-as suggested by Kerby and colleagues (2010)-implementing a more regional approach to regulatory testing. However, this is unusual due to budgetary considerations.

An addition to future studies could be the inclusion of alternate measures beyond concentrations in the water column. An important factor in relation to tadpoles is the pollutant concentration in sediment and food, both major exposure routes to young amphibians. Substances which tend to partition to sediment and fat tissue would end up in these compartments of the environment and thus be absent from a study like the present. Furthermore, the possible influence of biotic factors such as body size and amphibian genera on toxicity as well as abiotic factors including temperature, pH, and water hardness on the bioavailability of pollutants could be included within the scope of a larger study. Within the increased scope of such a study, possible ecological interactions, function and structure in retention ponds could be investigated by a more traditional pooling of several larger taxonomical groups and trophic levels into SSDs, as well as a subdividing into more granular functional and taxonomical groups. One obvious lack in the current amphibian ecotoxicological data is observed with phenols. When comparing large taxonomical units, Kerby and colleagues (2010) could show that if amphibians were particularly sensitive to any substance class compared to other groups, it was to phenols. Further insights gleaned from well-designed studies into the effects of phenols on different life stages could prove

invaluable, because these compounds are still underrepresented in amphibian toxicity data.

CONCLUSIONS

This study has highlighted the clear lack of ecotoxicological data for the construction of robust SSDs which could be used for guideline purposes regarding retention ponds' suitability as amphibian habitats. In fact, if a minimum of ten chronic toxicity values is set as the lower threshold for policy and guideline applicability, only five SSDs in the present study meet these requirements.

While it is obvious that more ecotoxicological data needs to be generated if amphibians' sensitivity to many common stormwater pollutants is to be evaluated, single-substance threshold values only give part of the picture. Further additions could be within-pond pollutant screening, analysis of synergistic pollutant effects, and considering effects on several trophic levels and the possible resulting interactions.

Some of the constructed SSDs could be used practically in policy creation and the development of guidelines geared towards amphibians. In the wider areas of conservation and urban ecology, these results also contribute with theoretical knowledge regarding the possibility of evaluating retention ponds as adequate habitats from a chemical standpoint. This could serve as a stark message to decision makers that a comprehensive set of guidelines cannot be created at present, but also as guidance towards future investigations building towards this goal.

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APPENDIX A

TABLE A1. Amphibian genera and their contribution to the data set.

Genus	Data points	Genus	Data points
Acris	107	Kassina	3
Adelotus	33	Leptodactylus	75
Agalychnis	189	Limnodynastes	144
Alytes	1	Lithobates	4,372
Ambystoma	793	Litoria	67
Anaxyrus	233	Microhyla	146
Boana	10	Notophtalmus	79
Bombina	59	Odontophrynus	6
Bufo	1,689	Osteopilus	49
Caudiverbera	6	Pelobates	58
Chioglossa	10	Pelodytes	6
Crinia	18	Pelophylax	1,173
Cynops	4	Physalaemus	71
Dendropsophus	12	Pleurodeles	243
Discoglossus	30	Polypedates	93
Duttaphrynus	200	Pseudacris	521
Elachistocleis	4	Pseudepidalea	191
Engystomops	2	Ptychadena	6
Epidalea	43	Quasipaa	18
Euphlyctis	173	Rana	2,041
Eurycea	20	Rhacophorus	2
Fejervarya	37	Rhinella	1,216
Gastrophryne	121	Scaphiopus	10
Glandirana	62	Scinax	69
Heleioporus	9	Silurana	563
Hoplobatrachus	104	Smilisca	7
Hyla	761	Spea	103
Hynobius	5	Triturus	248
Hypsiboas	170	Xenopus	8,631
Isthmohyla	7	Unspecified	109

TABLE A2. Amphibian species and their contribution to the data set.

Species	Data points	Species	Data points
Acris blanchardi	14	Centrolene prosoblepon	2
Acris crepitans	89	Chioglossa lusitanica	10
Acris gryllus	4	Crinia insignifera	16
Adelotus brevis	33	Crinia signifera	2
Agalychnis callidryas	189	Cynops pyrrhogaster	4
Alytes obstetricans	1	Dendropsophus microcephalus	1
Ambystoma barbouri	98	Dendropsophus minutus	11
Ambystoma gracile	68	Discoglossus galganoi	5
Ambystoma jeffersonianum	55	Discoglossus jeanneae	13
Ambystoma laterale	3	Discoglossus pictus	12
Ambystoma macrodactylum	38	Duttaphrynus melanostictus	200
Ambystoma maculatum	175	Elachistocleis bicolor	4
Ambystoma mavortium	6	Engystomops pustulosus	2
Ambystoma mexicanum	147	Epidalea calamita	43
Ambystoma opacum	37	Euphlyctis cyanophlyctis	44
Ambystoma punctatum	7	Euphlyctis hexadactylus	129
Ambystoma texanum	2	Eurycea bislineata	1
Ambystoma tigrinum	132	Eurycea wilderae	19
Anaxyrus americanus	27	Fejervarya limnocharis	30
Anaxyrus boreas	73	Fejervarya multistriata	7
Anaxyrus fowleri	42	Gastrophryne carolinensis	107
Anaxyrus terrestris	75	Gastrophryne olivacea	14
Anaxyrus woodhousii	16	Glandirana rugosa	62
Boana pardalis	10	Heleioporus eyrei	9
Bombina bombina	26	Hoplobatrachus rugulosus	8
Bombina orientalis	26	Hoplobatrachus tigerinus	96
Bombina variegata	7	Hyla chrysoscelis	199
Bufo americanus	488	Hyla cinerea	33
Bufo anderssoni	4	Hyla femoralis	3
Bufo arabicus	23	Hyla intermedia	62
Bufo boreas	5	Hyla japonica	3
Bufo bufo	686	Hyla meridionalis	6
Bufo canorus	8	Hyla squirella	30
Bufo cognatus	42	Hyla versicolor	425
Bufo fergusonii	22	Hynobius retardatus	5
Bufo gargarizans	74	Hypsiboas crepitans	6
Bufo japonicus	3	Hypsiboas pulchellus	164
Bufo maculatus	6	Isthmohyla pseudopuma	7
Bufo punctatus	60	Kassina senegalensis	3
Bufo quercicus	21	Leptodactylus latrans	73
Bufo terrestris	3	Leptodactylus ocellatus	2
Bufo woodhousei	145	Limnodynastes dorsalis	9
Bufo vulgaris	91	Limnodynastes peronii	120
Caudiverbera caudiverbera	4	Limnodynastes tasmaniensis	15

Lithobates areolatus	1	Ptychadena bibroni	6
Lithobates berlandieri	24	Quasipaa spinosa	18
Lithobates blairi	15	Rana arvalis	25
Lithobates catesbeiana	301	Rana aurora	39
Lithobates clamitans	772	Rana boylii	125
Lithobates grylio	71	Rana breviceps	9
Lithobates heckscheri	62	Rana brevipoda	45
Lithobates palustris	54	Rana cascadae	64
Lithobates pipiens	1,867	Rana catesbeiana	878
Lithobates septentrionalis	14	Rana chensinensis	7
Lithobates sphenocephalus	378	Rana cyanophlyctis	130
Lithobates sylvaticus	813	Rana dalmatina	95
Litoria adelaidensis	12	Rana limnocharis	117
Litoria aurea	4	Rana luteiventris	24
Litoria citropa	6	Rana muscosa	2
Litoria ewingi	4	Rana pretiosa	2
Litoria freycineti	11	Rana sierrae	39
Litoria moorei	21	Rana temporaria	400
Litoria raniformis	9	Rhacophorus arboreus	2
Microhyla ornata	141	Rhinella arenarum	1,114
Microhyla pulchra	5	Rhinella fernandezae	23
Notophthalmus viridescens	79	Rhinella granulosa	4
Odontophrynus americanus	6	Rhinella marina	74
Osteopilus septentrionalis	49	Scaphiopus couchii	8
Pelobates cultripes	58	Scaphiopus hammondii	1
Pelodytes ibericus	6	Scaphiopus holbrookii	1
Pelophylax esculentus	231	Scinax fuscovarius	43
Pelophylax nigromaculatus	243	Scinax nasica	7
Pelophylax perezi	127	Scinax nasicus	15
Pelophylax ridibundus	572	Scinax ruber	4
Physalaemus albonotatus	6	Silurana tropicalis	563
Physalaemus biligonigerus	6	Smilisca baudinii	7
Physalaemus centralis	21	Spea bombifrons	20
Physalaemus cuvieri	34	Spea intermontana	29
Physalaemus santafecinus	4	Spea multiplicata	54
Pleurodeles waltl	243	Triturus alpestris	1
Polypedates cruciger	60	Triturus boscai	13
Polypedates megacephalus	33	Triturus carnifex	73
Pseudacris crucifer	48	Triturus cristatus	101
Pseudacris regilla	356	Triturus helveticus	13
Pseudacris sierra	22	Triturus vulgaris	44
Pseudacris triseriata	95	Xenopus laevis	8,021
Pseudepidalea raddei	74	Xenopus tropicalis	501
Pseudepidalea viridis	117	Unspecified	295





FIG. B1. *Chronic SSD for ammonium. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC*₅*.*



FIG B2. Chronic SSD for atrazine. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B3. Acute SSD for atrazine. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B4. Chronic SSD for cadmium. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B5. Acute SSD for cadmium. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B6. Chronic SSD for copper. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B7. Acute SSD for copper. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B8. Acute SSD for DDT. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B9. Acute SSD for dieldrin. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B10. Chronic SSD for diuron. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B11. Chronic SSD for endosulfan. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B12. Acute SSD for endrin. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B13. *Chronic SSD for glyphosate. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC*₅*.*



FIG B14. Acute SSD for glyphosate. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC₅.



FIG B15. Chronic SSD for mercury. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B16. Acute SSD for mercury. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B17. Acute SSD for pentachlorophenol. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .



FIG B18. Acute SSD for zinc. The solid middle line represents the distribution prediction, while the solid outer lines represent the lower and upper 95% confidence limits. The dotted line shows the HC_5 .

APPENDIX C

Substance	Acute/Chronic	CAS reg. no.
Ammonium	Chronic	1066337, 6484522, 7783202, 12125029
Atra-in-	Chronic	1912249
Atrazine	Acute	1912249
Co drazio una	Chronic	10108642, 10325947
Cadmium	Acute	7440439, 10108642, 10124364, 10325947
C	Chronic	1317380, 7440508, 7447394, 7758987
Copper	Acute	1317380, 1332656, 7440508, 7447394, 7758987, 17599814
DDT	Acute	50293
Dieldrin	Acute	60571
Diuron	Chronic	330541
Endosulfan	Chronic	115297
Endrin	Acute	72208
Oh wala a sa sta	Chronic	1071836, 38641940
Glyphosate	Acute	1071836, 38641940, 70393850, 81591813
	Chronic	7487947
Mercury	Acute	7439976, 7487947
Pentachlorophenol	Acute	87865
Zinc	Acute	1314132, 7440666, 7646857, 7733020

TABLE C1. Complete list of CAS registry numbers of substances used in SSD construction.

APPENDIX D

Substance		
1,2-Dichloroethane	Cis-1,2-dichlorethene	Mercury
4-nonylphenol	Cobalt	Metribuzin
4-Tert-Octylphenol	Copper	Metsulfuron methyl
Acenaphthene	Cybutryn	Molybdenum
Acenaphthylene	DDT	Naphtalene
Alachlor	DEHP	Nickel
Aldrin	Dibenz(a,h)anthracene	Nitrogen
Aluminium	Dichlorprop	Nonylphenols
Ammonia	Diclofenac	PCB 101
Anthracene	Dieldrin	PCB 118
Arsenic	Diflufenican	PCB 138
Atrazine	Diuron	PCB 153
Barium	Endosulphan (alfa & beta)	PCB 180
BDE 209	Endrin	PCB 28
BDE 47	Ethinylestradiol	PCB 52
BDE 99	Fluoranthene	Pentachlorophenol
Bentazon	Fluorene	PFOA
Benzene	Glyphosate	PFOS
Benzo(a)anthracene	НСВ	Phenanthrene
Benzo(a)pyrene	HCH-alpha	Phosphorous
Benzo(b)fluoranthene	HCH-beta	Potassium
Benzo(k)fluoranthene	HCH-delta	Pyrene
Benzo[ghi]perylene	HCH-gamma	Simazine
Bisphenol A	Indeno[1,2,3-cd]pyrene	Sodium
Bronopol	Iron	Strontium
Cadmium	Isodrin	Terbutryn
Calcium	Isoproturon	Tetrachloroethylene
Chloralkanes	Lead	Tin
Chlorfenvinphos	Magnesium	Tributyltin
Chloridazon	Manganese	Trichloromethane
Chlorinated paraffins (C10-C13)	MCPA	Trifluralin
Chromium	Месоргор	Zinc
Chrysene		

TABLE D1. Complete list of substances used to filter for lowest available concentrations.