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Are deformation rates of anuran developmental stages suitable indicators for environmental pollution? Possibilities and limitations

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ABSTRACT

Among the multitude of reasons identified for amphibian decline, increased use of agrochemicals is suggested to contribute to amphibian population changes in industrialized countries. Contamination of breeding ponds with agrochemicals can provoke developmental effects. Deformation rates of tadpoles and metamorphs thus are expected to be high under agrarian land use. Deformation rates >5% is considered unnatural and implemented in a current amphibian monitoring guideline. We examined deformation rates and different endpoints at metamorphosis in Common frog (*Rana temporaria*) breeding sites (natural wetlands vs. studied waters under potential influence of agrochemicals). Deformation rates were not lower and metamorphosis success was not higher in natural wetlands, but deformation rates >5% were only found here. Rather natural abiotic factors led to higher deformation rates. Our study suggests that the 5%-threshold for unnatural deformation rates should be seen in a critical way until further evaluation if deformation rates of free living amphibians are suitable indicators for environmental pollution.

1. Introduction

Amphibian populations are globally declining at alarming rates (Alford and Richards, 1999; Houlahan et al., 2000; Mendelson et al., 2006; Stuart et al., 2008). Multiple factors, including tandem effects, are suggested to play a role and the reasons causing declines vary regionally and taxonomically (Collins and Storfer, 2003; Wake and Vredenburg, 2008). Destruction and degradation of suitable habitats are severe problems in many regions all over the world (Stuart et al., 2008), while for instance fungal disease emergence is remarkable for certain taxa and regions only (La Marca et al., 2005; Fisher et al., 2009). In industrialized countries, environmental contamination is supposed to be an important cause for population changes and agricultural activities heavily contribute to it (Boone et al., 2007; Stuart et al., 2008). Especially, the increasing pesticide use is suggested to have negative effects on biodiversity (Geiger et al., 2010), which in particular is applicable to amphibians (Brühl et al., 2011). Although proposed, the causality between amphibian declines and agrochemical use remains little studied (Mann et al., 2009; Wagner et al., 2013).

Ways of contacts with pesticides include direct and indirect expositions to contaminants, for instance, by direct over-spraying, wind drift or run-off, and all amphibian life stages can be affected (Oldham et al., 1997; Davidson, 2004; Davidson and Knapp, 2007; Brühl et al., 2011, 2013; Berger et al., 2013). Acute (e.g. Relyea, 2005; Shinn et al., 2013), chronic, delayed and indirect (e.g. Xu and Oldham, 1997; Howe et al., 2004; Relyea, 2009; Jones et al., 2010, 2011; Williams and Semlitsch, 2010; Boone et al., 2013) effects have been reported. Studies are mainly laboratory or mesocosm-based, but also results from in situ studies suggest negative impacts of agrochemicals on amphibians (Attademo et al., 2007; McCoy et al., 2008; Lajmanovich et al., 2010).

Various pesticides shape amphibians *in ovo* (Orton and Routledge, 2011) and lead to body deformations by teratogenic effects or those appearing during later larval development (Bridges, 2000; Gardiner et al., 2003; Lajmanovich et al., 2003). But so far, deformation studies mainly aimed at juvenile and adult amphibians and often deformations (especially limb malformations) can be well explained with predation, parasitism and abiotic interactions (Blaustein and Johnson, 2003; Johnson et al., 2003; Ankley et al., 2004; Taylor et al., 2005; Lannoo, 2008; Ballengeé and Sessions, 2009). Some observations could be linked to immune suppression due to agrochemicals (Kiesecker, 2002; Johnson et al., 2007;

Abbreviations: CSW, cultivated studied water bodies; DR, deformation rate; DRs, deformation rates; NSW, natural studied water bodies; VDI, Verein Deutscher Ingenieure (Association of German Engineers).

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Rohr et al., 2008). However, over 30 years after the description of a monitoring on malformations in tadpoles by Cooke (1981), little attention has been given to deformation rates (DRs) of freshly metamorphosed individuals (e.g. Veith and Viertel, 1993; Piha et al., 2006) and especially larvae. The studies concluded that a deformation rate (DR) >5% is unnatural.

The 'Association of German Engineers' (VDI) is publishing a series of monitoring handbooks dedicated to detect effects from genetically manipulated crops and their particular agrochemicals on biodiversity. Besides monitoring guidelines for other taxonomic groups like butterflies and moths or soil organisms, the VDI 4333 monitoring guideline aims at amphibians in the agrarian landscape in Austria, Germany and Switzerland. As published in its preliminary version ("Gründruck") (VDI, 2013), it uses tadpole DRs according to Cooke (1981) to determine effects potentially associable to herbicides. The guideline foresees 'obligate species' occurring in the agrarian landscape of most of Germany, one of which is the Common frog (*Rana temporaria*) (Böll et al., 2013).

We applied the procedure described in VDI 4333 with biologically meaningful modifications, to Common frogs in Germany. The purpose was to determine DRs and different parameters at metamorphosis in this species from breeding sites at different distances to agrarian land use. The results of the present work should be incorporated in the final version ("Weißdruck"). As potential alternative explanations to agrochemical use, we recorded several abiotic conditions (water chemistry and climatic variables) at breeding sites.

Our expectations were that (1) DRs and metamorphosis success correlated and that the first mentioned was higher and the second mentioned lower in water bodies in the agrarian landscape. Along with VDI 4333, we expected that (2) DR >5% only occurred at intensively agricultural used sites. Likewise, in accordance with Attademo et al. (2014), we expected (3) a correlative decrease in size and mass of metamorphs with distance to agrarian land use. Several studies have shown that agrochemicals can influence the time to metamorphosis, partly by disrupting the thyroid axis in tadpoles (e.g. Howe et al., 2004; Williams and Semlitsch, 2010). Hence, we expected that (4) time to metamorphosis differ in tadpoles from agricultural areas and in conspecifics from natural wetlands.

2. Materials and methods

2.1. Study sites

Field work was conducted at two localities between March and July 2013 in the Hunsrück low mountain ranges of Rhineland-Palatine, Germany, partly characterized by agricultural land use. The spatial monitoring design of VDI 4333 defines a set of studied waters with different magnitudes of exposure: the three most exposed breeding habitats and three additional, less exposed breeding habitats within a radius of up to 1000 m from the margins of a selected agricultural area (Böll et al., 2013; VDI, 2013). According to this, we chose the available five water bodies (i.e. *R. temporaria* breeding sites) around a high-fertilized hay meadow near the village of Waldweiler (49°36′ N, 06°48′ E, about 520 m a.s.l.; Fig. 1) and six breeding sites in the vicinity of a wheat field near the village of Mandern (49°35′ N, 06°45′ E, about 470 m a.s.l.; Fig. 2). With regard to surrounding land use in general, studied waters were defined as 'situated in the cultivated landscape' ('cultivated studied water bodies', CSW) when >50% within a 1-km radius were used for agriculture and settlement. If otherwise, they were defined as control references ('natural studied water bodies', NSW). Usually more than 70% of the surrounding land use of NSW was coniferous or mixed forest (Figs. 1 and 2). ArcMap 10 (Esri®) and current aerial photographs were used to classify land use, as described in

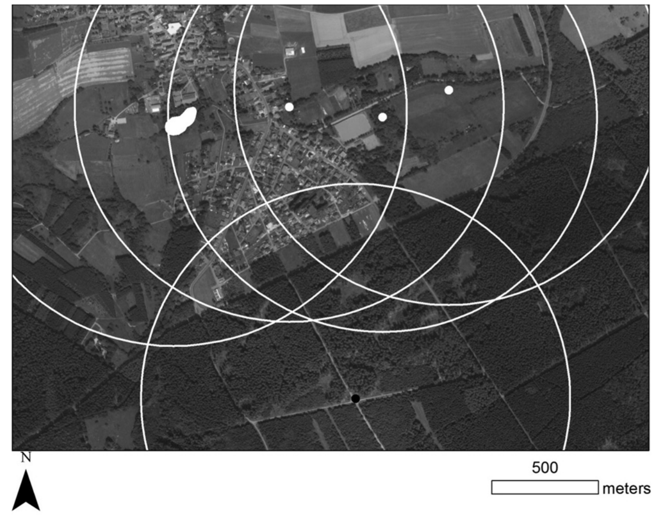

Fig. 1. Breeding sites of *Rana temporaria* around the village of Waldweiler. White dots indicate small water bodies, which were mainly surrounded by agricultural land use, likewise the white polygon indicates the studied pond situated in the cultivated landscape. The black dot indicates the natural studied water body from this site.

VDI 4333. All breeding sites were small and partly temporary water bodies; exceptions were each one CSW and NSW which were ponds (Table 1).

2.2. Tadpole examination

In accordance with VDI 4333, for DR determination we performed a weekly tadpole monitoring on Common frog larvae. This is a wide-spread species in Germany in which mating takes usually place in February or March, but due to cold weather conditions in 2013, main spawning events of the species in the study region were only at the beginning of April. Several thousands of eggs are laid by one female and various types of water bodies are exploited for tadpole development which lasts into summer (Schlüpmann and Günther, 1996).

VDI 4333 foresees a monitoring of early larval stages starting from developmental stage 25 (free swimming tadpoles with formed spiracles) up to 30 (hind limb bud development before toe

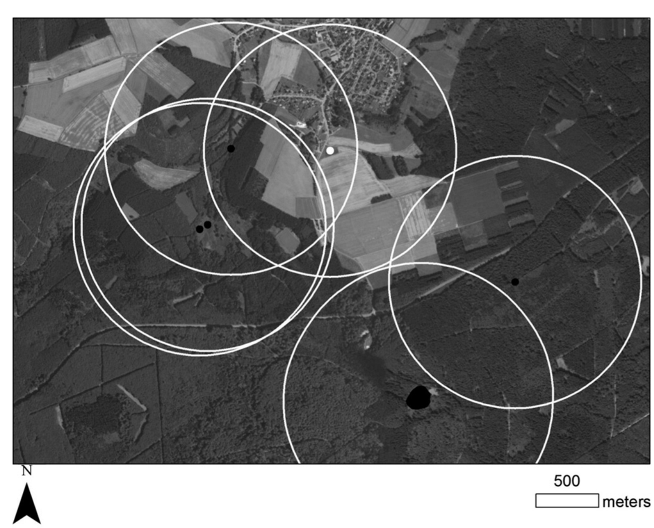

Fig. 2. Breeding sites of *Rana temporaria* around the village of Mandern. Black dots indicate small, remote water bodies, which were situated in the forest or on a natural wet meadow. Likewise, the black polygon indicates the studied woodland pond. The white dot indicates the studied retention basin, directly adjacent to agrarian land.