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Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands

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Abstract. In the Netherlands, the fire salamander (*Salamandra salamandra*) is at the edge of its geographic range and is restricted to three small populations in the extreme south of the country. Despite the species being listed as 'Endangered' on the national Red List, the situation was considered to be stable. However, from 2008 onwards dead individuals were seen on more than one occasion. A sharp decline in numbers has been observed since 2010 (96%; $P < 0.01$), but we were unable to attribute this to any known cause of amphibian decline, such as chytridiomycosis, ranavirus or habitat degradation. The present work describes this enigmatic decline, and we discuss these results in the context of possible causes.

Keywords: enigmatic decline, local extinction, mortality, *Salamandra salamandra*, terrestrial salamanders.

Introduction

Amphibians are experiencing declines globally, exemplified not only by population declines, but also by range reductions and extinctions of some species (Houlahan et al., 2000; Stuart et al., 2004; IUCN, 2012), and they may be part of a sixth major extinction event (Wake and Vredenburg, 2008), for which there is no single cause (Blaustein et al., 2011; Hof et al., 2011).

In the Netherlands, despite 50% of the amphibian species being on the Red List (Van Delft, Creemers and Spitzen-van der Sluijs, 2007), there were, until quite recently, no indications of an acute decline that could result in the extinction of a species from the country. Even when the two major infectious drivers of amphibian declines were present, *Batrachochytrium dendrobatidis* and ranavirus (Daszak, Cunningham and Hyatt, 2003; Spitzen-van der Sluijs et al., 2010; Kik et al., 2011) no specific decline has been observed.

However, the sudden, steep decline of the fire salamander (*Salamandra salamandra terrestris*) in the Netherlands is a new phenomenon. In the Netherlands, fire salamanders live at the very edge of their distribution and are confined to the old growth stages of deciduous forests on hillsides. Surface activity is limited to humid periods with night temperatures above 5°C. Mating usually occurs in autumn, the larvae are deposited from late winter until spring. In the Dutch populations, the animals mature at an age of 6 years in both males and females. Specimens can live for 20 years in this region (Gubbels, 2009). Listed as 'Endangered' on the national Red List, the species' range has decreased by 57% since 1950. This is probably due to the drying up of streams, or their canalisation and intensive cleaning. Additional threats are collection for the pet trade and the use of herbicides in surrounding agricultural land (Van Delft, Creemers and Spitzen-van der Sluijs, 2007; Gubbels, 2009).

Currently, the species is only known from two native populations and another small, introduced, non-native population, all three in the extreme south of the Netherlands. The

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largest population ('Bunderbos') has been monitored since 1971, and yielded high estimates of population densities in the most suitable area (350-500 individuals in 0.5 ha). It was estimated that the whole population comprised several hundred individuals (Gubbels, 2009). All populations have been monitored yearly since 1997 within the Network Ecological Monitoring framework (Goverse et al., 2006). From 2008 onwards, repeat findings of dead adults were recorded for the first time and from 2010 onwards, there was an extremely sharp decline in the number of sightings of living salamanders (fig. 1).

Even though amphibians are known for their large fluctuations in abundance (Green, 2003), and the fire salamander may have a strong dispersal capacity (Schmidt, Schaub and Steinfartz, 2007), the rapidity of the decline seemed more to be indicative of a disease or a toxin (Blaustein and Kiesecker, 2002). In this paper we analyse this enigmatic decline and we describe our attempts so far to elucidate its causes.

Materials and methods

The fire salamander currently has a very limited range in the Netherlands, being only present in the extreme south of the province Limburg. The largest native population is not far from the city of Maastricht in *Bunderbos* (N 50°54′51″, E 5°44′59″), an 8 km^2 area of brook valley woodland of which 144 ha is suitable habitat. Further to the east in *Vijlenerbos* (N 50°45′44″, E 5°56′33″; size: 6 km^2, 58 ha suitable habitat) the species is present near six small brooks in the valley of the river Geul. The non-native, introduced population in *Putberg* (N 50°51′17″, E 5°57′59″, size: 12 ha, 3 ha suitable habitat) occurs south of the city Heerlen. At all three sites, fire salamanders seem to occur in patches, thus not occupying the entire suitable habitat. The three areas are geographically isolated from each other, being 17-21 km apart, straight-line distance. The intervening land-

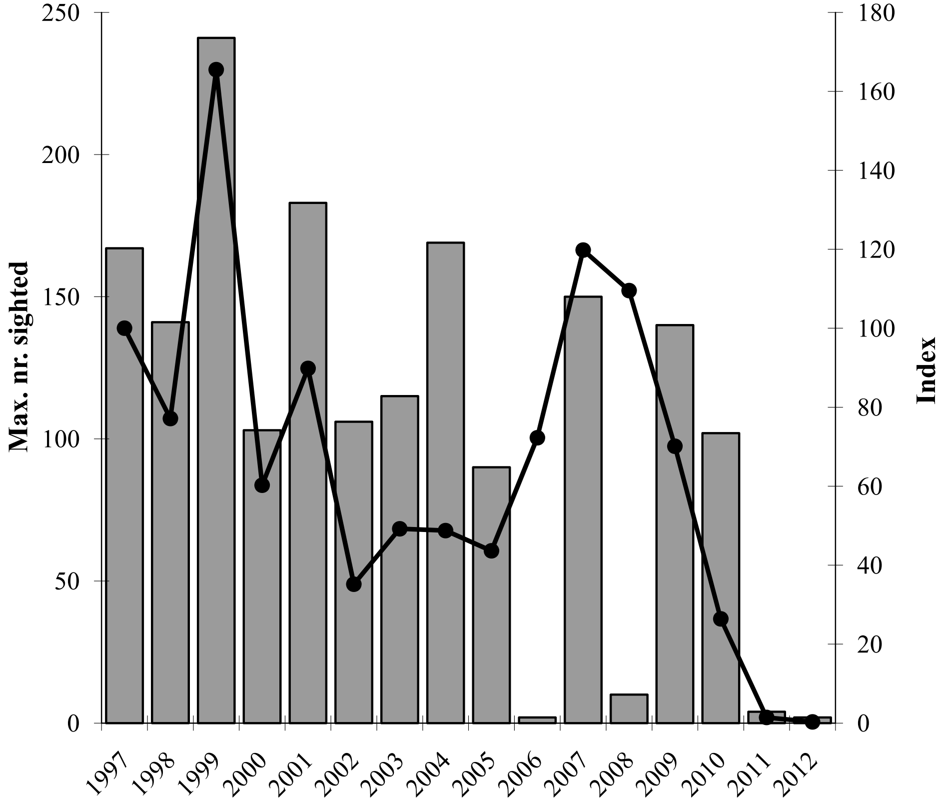

Figure 1. Maximum number of fire salamanders seen/year (bars) and the index (line) in the period 1997-2012. Index (calculated by TRIM) set at 100 at the start of the monitoring programme, shows a dramatic and continuing decline since 2008.

scape is unsuitable for the species, rendering migration between populations highly unlikely.

Standardized monitoring of amphibian populations and the calculation of population trends started in 1997. Transect counts are always done in the late evening or at night, under humid or wet conditions with temperatures $\geqslant 5°C$, according to the national standard (Groenveld, Smit and Goverse, 2011). Transect length ranges between 50-500 m (mean $\pm$ SD = 275 $\pm$ 162; median value 300 = m). The Bunderbos population has received the most attention. Between 1997-2007, volunteers monitored five transects four times yearly. One transect was situated too close to a railroad and for reasons of safety, monitoring was stopped during 2009 and continued the same year on three new transects at a safer distance nearby. For 'Bunderbos' there is no data available for 2006 and 2008. Monitoring effort was intensified by the volunteers due to their concern to the near absence of live animals and the discovery of dead ones. From 2009 onwards, covering all transects (7) were monitored between 4-30 times/year (mean $\pm$ SD = 18.9 $\pm$ 7.8). The transects in 'Vijlenerbos' (4) (mean $\pm$ SD = 4.1 $\pm$ 5.2 times/year; median value = 3) and 'Putberg' (1) (range: 0-1 times/year) have only been monitored irregularly.

Indices and trends were calculated using TRIM (Trends & Indices for Monitoring data), a statistical program based on log-linear Poisson regression designed for fauna monitoring data with missing values (Pannekoek and Van Strien, 2001; Van der Meij et al., 2009). The overall trend between 1997 and 2012 and the associated slope were calculated. Annual indices represent the yearly numbers as a percentage of the numbers in the first year (1997) of monitoring. If the standard deviation is below 0.02, the trends are considered to be reliable.

The indices are based on the estimated abundance of the species, calculated from the sum of the mean number of salamanders seen per transect per year, supplemented by the estimated values for the missing years as calculated by TRIM. The calculation of the population trend is based on the average change of the indices per year, for the three areas. The yearly indices and the trend are calculated for the whole period, taking serial correlation between years into account.

We conducted linear regression analyses to see if the number of fire salamanders sighted was influenced by temperature, humidity, observer-effect, or the time of year. Climatic data (mean, minimum and maximum daily temperature, total daily precipitation, and the mean daily relative atmospheric humidity) were obtained from the weather station of the Royal Netherlands Meteorological Institute (www.knmi.nl) in Maastricht (N 50°55′, E 5°47′). None of these predictor variables significantly influenced the number of sighted salamanders, and were therefore excluded from further analysis.

We chose to use data from the eight visits with the highest counts to compensate for the effect of more frequent monitoring on the average counts per year for 2010-2012. We could not define a clear rationale to choose four visits in these years comparable to the four in previous years. Using all visits of four randomly chosen visits would probably result in a lower average while using the four visits with highest counts could have the opposite effect. Using the eight visits with higher counts may have resulted in a too conservative population trend, but this was preferred above a Type I error which may have exaggerated the rate of decline.

Post-mortem examination was severely limited by the rapid autolysis of the animals, and post mortem studies were conducted on 5 specimens (Ghent University), including macroscopic examination and (q)PCRs for the detection of *Batrachochytrium dendrobatidis*, ranavirus, Chlamydiales, herpes viruses and circovirus (Mao, Hedrick and Chichar, 1997; Boyle et al., 2004; Halami, 2007; Martel et al., 2012). Due to severe autolysis, resulting in a lack of recognizable internal organs, routine histology, bacteriology and virology were not performed on these specimens. On a single specimen, found in 2010, histological, bacteriological and cytological tests were performed, in addition to macroscopic examination (Utrecht University).

Results

In the period 1997-2010, the maximum number of fire salamanders sighted annually over all areas fluctuated between 10 and 241 (table 1). In the largest population 'Bunderbos', annual maximum numbers fluctuated between 71 and 241 for that period, and in 2011 the total number dropped to four, despite intensive monitoring (26 visits that year). Taking the monitoring period as a whole (1997-2012) the species showed a very strong and significant decrease in all populations (fig. 1). Over this time frame, the total population decreased by 96%, illustrated by the trend, which is expressed as a slope of -0.2189 ± 0.018 ($P < 0.01$), indicating an annual decrease of 19.7%. In 'Vijlenerbos', only five individuals were seen in 2010; none were sighted in 2012 despite 57 visits to monitor the site that year. The population discovered in 'Putberg' in 1994 (Janssen and Huijgens, 2001) was visited infrequently the last years. Earlier visits saw the maximum number of adults fluctuating between 1 and 15 per year. The fire salamander (2) was last seen there in 2010.

The first reports of dead animals reached us in 2008. They were generally found without external signs of injury on footpaths in broad daylight (fig. 2). To date 25 have been found (2008: 3; 2009: 0; 2010: 16; 2011: 6), twenty

Table 1. Monitoring results per year, indicated as the maximum number of seen salamanders per year over all transects (second column), yearly indices and standard deviation (SD) of the index (calculated by TRIM), as well as the number of transects and the mean number of visits per transect per year.

Year	Sighted	Index	SD (index)	# Transects	Mean nr visits/transect
1997	167	100	0	8	3
1998	141	77.1	0.030	7	4
1999	241	165.5	0.060	6	4
2000	103	60.3	0.025	7	4
2001	183	89.8	0.038	8	3
2002	106	35.2	0.020	9	3
2003	115	49.2	0.025	9	3
2004	169	48.8	0.026	7	4
2005	90	43.6	0.035	9	3
2006	2	72.3*	0.033	1	5
2007	150	119.8	0.047	6	4
2008	10	109.5*	0.036	1	3
2009	140	70.1	0.042	8	7
2010	102	26.4	0.015	11	15
2011	4	1.4	0.001	10	16
2012	2	0.3	0.001	11	13

* Estimated values by TRIM (Trends & Indices for Monitoring data).

Figure 2. Deceased fire salamanders were generally encountered without external lesions (photo: M. van Mullekom). This figure is published in colour in the online version.

in ‘Bunderbos’, and five in ‘Putberg’. During the years 2009 and 2012 no dead or moribund specimens were encountered in the field.

A total of six fire salamanders was necropsied. All specimens were too decomposed for extensive pathological examination. However, the tests we carried out yielded no conclusive results about the cause of death; we did not detect *Batrachochytrium dendrobatidis*, ranavirus, Chlamydiales, herpes viruses or circovirus. In 2012, twenty-two visits searching outside the monitoring transects for animals for the ex-situ conservation programme, yielded a total of 39 individuals.

Discussion

Although the population of the fire salamander in the Netherlands has always been small, it was not considered to be at risk. However, the extent and speed of decline of the population is worrying and it has now shrunk to a size approaching extinction. Although the monitoring was not standardized optimally and the trend analyses did not fully account for the possible effect of pseudo-replication, the decline is to such an extent and the level of significance so strong, that it cannot be overlooked. Likewise, any bias due to variation in sighting probability would not lead to a different conclusion about the extent of the decline. To our knowledge, no similar decline in occurrence has been observed in the neighbouring regions of Belgium (pers. comm. R. Jooris, D. Verbelen, A. Laudelout) and western North Rhine-Westphalia (Germany) (pers. comm. M. Aletsee).

Although the cause has not yet been established, considering the sudden and steep nature of the decline and the number of dead individuals found, the most likely cause seems to be either an infectious agent or intoxication, possibly in combination with other causes. Amphibian population declines are caused by various abiotic and biotic factors acting together in a context-dependent fashion (Blaustein and Kiesecker, 2002). The decline we describe in this report strongly resembles the population crashes after entry and subsequent build-up of *B. dendrobatidis* infections in Australia, Central America, the Sierra Nevada (USA) and southern Europe (Berger et al., 1998; Bosch et al., 2001; Lips et al., 2006; Vredenburg et al., 2010). However, we did not find any trace of *B. dendrobatidis* in any of the fire salamanders sampled. Other possible causes such as climate change, habitat degradation, disturbed population demography, genetic erosion and the illegal capture of animals, or a combination of these, cannot be excluded at this moment. Indeed, habitat degradation has caused a strong range reduction of *S. salamandra* in the Netherlands in the past, but it is unlikely that this has caused the present collapse in the species' status. Although it is expected that climate change will cause streams to dry up (Goosen et al., 2010), this has not been seen to date. Habitat degradation can also be ruled out: the remaining habitat consists of a climax vegetation, the management of which has not changed since 1952. Genetic erosion or a demographic problem would result in a more gradual decline, and the occurrence of dead individuals cannot be explained by activities of illegal collectors. Emigration can more or less be ruled out as a possible cause of decline. This is supported by the absence of sightings of the species in any other areas, the lack of suitable habitat between the three source populations and fact that there have never been any records of road casualties in the surroundings.

However, it is known that high nitrate concentrations can seriously affect amphibian health (Rouse, Bishop and Struger, 1999; Camargo, Alonso and Salamanca, 2005; Ortiz-Santaliestra, Fernández-Benéitez and Marco, 2012). In the 'Bunderbos' the nitrate concentrations are very high in both the spring water and in the top layer of the forest soil. In the area where the fire salamander is found, mean nitrate concentrations of up to 2000 μmol l^{-1} were measured in the spring water in 2001 (Hendrix and Meinardi, 2004), which are lethal values for tadpoles (Baker and Waights, 1993; Camargo, Alonso and Salamanca, 2005). Exposure to deleterious substances can be an important cofactor suppressing the amphibian immune system which would facilitate outbreaks of infectious diseases and result in reduced adult fitness or mortality (Mann et al., 2009). However, a clear association between the high nitrate levels and the observed decline could not be made since the dead animals were in a severe state of postmortal decay, rendering proper histopathological examination impossible.

To date, 11 juvenile and 28 sub-adult and adult fire salamanders have been safely removed from ‘Bunderbos’; they were collected from outside the monitoring transects. They are being kept in captivity to safeguard them from possible threats on that site. These individuals will form the basis of a breeding programme for repopulating the sites once the cause or causes of the decline are clear and reversed (Stuart et al., 2004). These captive animals will be intensively examined for the presence of pathogens. Should a mortality occur, it could provide valuable material to help to elucidate the cause of the decline. Currently, a demographic study is being carried out, together with one on the genetic variation of the whole population. There is also an investigation being carried out into the possibility of pesticide residues in the brain of the dead individuals that have been found, and additionally monitoring in the field will be continued in 2013.

The present case illustrates how small populations believed to be stable can suddenly collapse and possibly go extinct. Stochastic variation greatly influences demographic rates in small populations. This was recognized at a relatively early stage due to monitoring, early enough, we hope, to prevent the complete loss of the fire salamander from the Netherlands.

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