Article

Drastic Population Size Change in Two Populations of the Golden-Striped Salamander over a Forty-Year Period—Are Eucalypt Plantations to Blame?

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Abstract: Over the last half century the Iberian peninsula has seen the large scale planting of exotic gum trees (*Eucalyptus* sp.) therewith reducing space for native wildlife. An additional effect of the gum tree plantations may be the lowering of the water table in adjacent streams, to which amphibian species with a larval niche in the running sections of small streams would be especially susceptible. In northwestern Iberia that niche is occupied by the Golden-striped salamander, *Chioglossa lusitanica*. I here report on the demographic trends of two *C. lusitanica* populations over a forty-year period, in two areas of one mountain range near Porto in northwestern Portugal. In both areas advantage was taken of the migration pattern of *C. lusitanica* to sites for aestivation and breeding in summer and fall. The area of the Silveirinhos brook was transformed in a plantation of gum trees shortly after the research started, while the area of Poço do Inferno remained virtually unaffected. At Silveirinhos the adult *C. lusitanica* population declined by one or two orders of magnitude, from *ca.* 1500 individuals to less than 50 at present. Demographic models that operate under a uniform larval mortality yielded population sizes that are compatible with field observations, including the late onset of the decline at 14 or more years after the planting of the gum trees and the near-extinction at year 32. An alternative reason for the relatively recent population collapse of *C. lusitanica* may have been disease, but no sick individuals or corpses have become available for clinical investigation. Conversely, the control population at Poço do Inferno increased in size by a factor of five or more. These data support the hypothesis that gum tree plantations have a strong negative effect on *C. lusitanica*. The population size increase at Poço do Inferno is probably attributable to the installation of wastewater treatment in the adjacent town of Valongo, with a discharge in the Simão river and closely connected to Poço do Inferno. This result suggests that the Simão river

contributes to the Poço do Inferno population and that medium-large streams may constitute prime *C. lusitanica* habitat. Larval mortalities estimated for stable and declining populations are remarkable close (0.69 and 0.73, respectively), but dissimilar to the larval mortality at *ca.* 0.51 of an increasing population such as at Poço do Inferno. Suggestions for further research include (i) the continued monitoring of the Silveirinhos population with inspections at 5–10 year intervals, and (ii) checking for the persistence of *C. lusitanica* populations in and around gum tree plantations where the species was reported decades ago.

1. Introduction

To document and understand natural population size fluctuations in long-lived animal species such as many vertebrates requires long-term data. To distinguish natural fluctuations from a pattern of change, such as may be expected from large-scale anthropogenic activities, requires even more data. Long-term data are, however, notoriously difficult to obtain. One dramatic change in the landscapes of southwestern Europe has been the planting of exotic gum trees (*Eucalyptus* sp.) over vast areas and one of the most drastic projects took effect in the last quarter of the 20th century, through the Portuguese Forestry Project with assistance of the World Bank [1,2]. In Portugal, the area with gum tree plantations—mostly the Tasmanian blue gum, *Eucalyptus globulus*—has increased eight-fold from the mid 1960s to a coverage of *ca.* 25% of today's forested area, with a substantial reduction in space for native wildlife as a consequence. An additional effect may be a reduced run-off causing watercourses to dry up early in the season, even if the streambed itself was not affected by the plantation. Such a drying effect would be most detrimental to species that live in the running section of streams [3,4]. I set out to study the possible effect of the large scale planting of gum trees in an area in the vicinity of Porto, Portugal, where shortly before I had studied the natural history of an enigmatic amphibian, the Golden-striped salamander, *Chioglossa lusitanica* (Figure 1). The study took place along two headwater streams at different slopes of one mountain range. One area remained unaffected, whereas in 1980 the other area was turned into a gum tree plantation. The planting involved the rip-plowing of the Serra de Santa Justa, except for a *ca.* 20 m wide buffer zone covering the Ribeira de Silveirinhos and its margins, as required by Portuguese law [5]. With data going back to 1976, the control population has increased in size, whereas the population at the gum tree plantation has decimated.
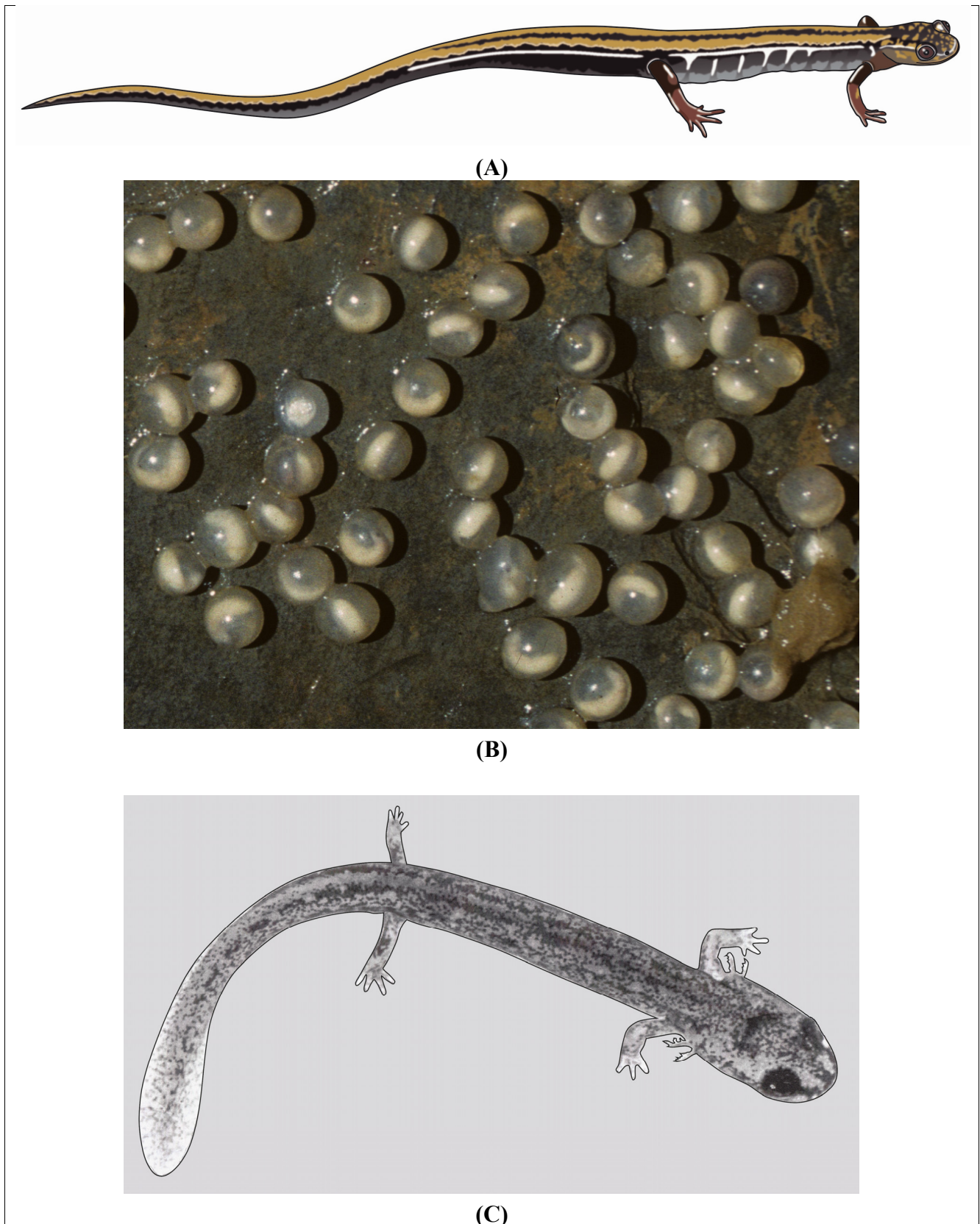
Figure 1. Golden-striped salamander, *Chioglossa lusitanica*: (**A**) adult, drawing and copyright Bas Blankevoort © Naturalis Biodiversity Center, reproduced from [6] with permission; (**B**) embryos in various stages of development, Photo M. Sparreboom; and (**C**) first-year larva, Photo JWA.

2. Materials and Methods

2.1. Study Areas and Fieldwork

The two study areas, called "Ribeira de Silveirinhos" and "Poço do Inferno", are located at the northern flanks of the Serra de Santa Justa, immediately south of the town Valongo at *ca*. 10 km east of Porto, Portugal. The Silveirinhos brook runs in southerly direction, has a length of 4 km and discharges in the Rio Ferreira. The Poço do Inferno brook runs in northeastern direction, has a length of *ca*. 600 m and discharges in the Simão river. The distance between the study plots is 1340 m "as the crow flies", while the "as the salmon swims" distance along the Ferreira and Simão rivers is *ca*. 10 km (Appendix 1 with Figure A1).

The Ribeira de Silveirinhos is a small semi-permanent mountain brook in slate and quartzite terrain. In a zone of up to 30 m wide the valley has a dense vegetation of *Alnus glutinosa*, *Eucalyptus globulus* and *Acacia* sp., with an undergrowth of brambles, *Digitalis purpurea*, *Pteridium aquilinum* and gum tree and *Acacia* shoots. The search plot is situated close to the Silveirinhos source and includes a dry stone, V-shaped built structure of *ca*. 720 m^3—presumably constructed from the debris of a local excavation— spanning the valley (41.17330°N, 8.50080°W). This "dam" of 28 m length, up to 7 m high and up to 22 m wide has a tunnel for the stream to run through (see Figure 1 in [7]). The age of the dam is unknown, but judging from the size of the trees growing on top of it, already in 1977, it must have been old. The tunnel, the dam wall, the brook and the vegetated zones at either side over the 200 m long downstream section of the brook were regularly surveyed by night for post-metamorphic *C. lusitanica*. The open maquis vegetation at further distance from the brook was initially inspected also, but no golden-striped salamanders (*Chioglossa*) were found and searching outside of the densely vegetated zone was abandoned. By daylight the selected stretch of the Silveirinhos was dip-netted for larvae of *C. lusitanica* and other amphibian species. In the 1979–1981 period the Serra de Santa Justa and adjacent mountain ranges were rip-plowed and transformed from semi-natural open maquis (see Figure 4 in [8]) into gum tree plantations almost entirely (details for the Valongo municipality are in Figure 8 in [9]), but the planting on newly created terraces stopped short of the densely vegetated zone at *ca.* 10 m from the Silveirinhos brook. The area upstream of the dam remained unchanged. The area is rather left alone and I have observed no management of the gum tree plantations other than the clear-cutting of sections.

The "Poço do Inferno" is a small impermanent mountain brook in schistose/quartzite terrain rich in iron oxide and with a vegetation consisting of *Pteridium aquilinum*, *Cytisus striatus*, *Calluna vulgaris*, *Erica* sp. and *Frangula azorica* and stands of gum and pine trees on its banks (see Figure 3 in [7]). Several narrow and more or less deep corridors with entrances close to the brook were built for gold-digging in Roman times (locally called "minas" [10]). However, bore holes from modern machinery witness a more recent human activity. The research was carried out in two mines, the "Upper mine" with the entrance at 41.18064°N and 8.48816°W at a distance of 260 m from the Simão, and the "Lower mine" with the entrance at 41.18185°N and 8.48677°W at 80 m from the Simão. The investigated area includes an 80 m section of the brook in between them, upstream from the dirt road crossing the brook.

Both mines consist of a main gallery and two side galleries, roughly laid out in the shape of a cross (Appendix 2 with Figure A2). Galleries are up to 2 m wide. The height allows straight up walking except

at several places deeper in the mines that are littered with heaps of rock debris. The Upper mine has shallow but permanent standing water for the deeper part of the main gallery and a water level of *ca*. 25 cm in one short side gallery. This mine has an additional gallery starting near the entrance. This stretch of *ca*. 25 m length is fairly dry, rarely visited by *C. lusitanica* and, with a few exceptions, was not included in the searches. The entrance of the Upper mine is located at a short distance from the Poço do Inferno brook with no observable discharge, except after periods of very heavy rain when the mine's water level rises markedly as to overflow the heap of soil at the entrance (see Figure 4 in [7]). In the Lower mine water drips down the walls and the ceilings at many points and a small stream runs through the mine permanently and at the mine entrance discharges in the Poço do Inferno brook. The main gallery is wetter than the two arms at either side. Both mines were prospected over their length of *ca*. 105 m (Upper mine) and 236 m (Lower mine). Adult salamanders and mated pairs were counted along with a rough count of the number of eggs (or, actually, embryos) (Figure 1). A further search by turning stones from the heaps of stone debris indicated the presence of many more adult and embryonic *C. lusitanica*, but this was not carried through, because of the danger of harming salamanders and the work load involved.

I explored for the presence of an underground connection from the Upper mine to the Poço do Inferno brook. In spring 2011 an aqueous solution containing 60 g Fluorescein sodium salt (Sigma F6377) was released in the standing water section deep in the Upper mine. Water samples of 50 ml were taken at the Poço do Inferno brook at 20 m upstream (negative control), aside and at 170 m downstream of the entrance of the Upper mine, at eight-hour intervals over a three day period. The pooled samples were measured for Fluorescein with a Perkin Elmer LS 45 Fluorescence Spectrometer under FL WinLab software and a detection limit of 0.013 µg Fluorescein/L.

2.2. Pattern of Annual Migration

The dam serves as an aestivation site for adult *Chioglossa* salamanders that in fall, winter and spring reside along the brook [8,11]. Both mines are places of *C. lusitanica* shelter, mating and egg-deposition. In both areas, observations on *C. lusitanica* were made on a regular basis in the spring, summer and autumn of 1977 and irregularly ever since. Most visits were during the autumnal period of reproduction. Egg numbers for 1976 were estimated in early spring 1977, by a count of molded embryos and the empty gelatinous shells left behind by hatched embryos. In the course of 1977 and in October 2010, adult salamanders in the dam were marked by toe-clipping. The adult population size was calculated with Begon's weighted mean method [12], based upon the recapture of marked salamanders. Additional data have become available from colleagues and from the literature.

2.3. Natural History of Chioglossa lusitanica and Unknowns

The Golden-striped salamander is an exceptionally slender and long-tailed salamander of the family *Salamandridae*, endemic to the northwestern corner of the Iberian Peninsula. While described 150 years ago [13], *C. lusitanica* remained virtually unstudied until a broad survey in 1957 [14] and my own ecological work in the late 1970s [15,16]. More has become known about the ecology of the species [4,17,18] and about important life history parameters such as demography and reproductive cycle [19,20]. The species distribution is by now well documented [21–23] and we have some knowledge on morphological

variation [24–26] and phylogeography [27–30]. By far the most prominent feature of *C. lusitanica* is its dependency on conditions of moisture, which is reflected by (i) a distribution confined to areas of high precipitation (>1000 mm annually); (ii) a drought-induced pattern of migration along the brooks, for which distances of >350 m overnight have been recorded; and (iii) a period of aestivation followed by mating and egg-laying in the rainy season [16]. Still, many questions regarding the life history of *C. lusitanica* are open, especially on the early stages of life, the use of underground habitats and dispersal.

2.4. Population Modeling

The population demography of *C. lusitanica* was modeled with Vortex 10 [31]. The variables required for modeling fell into one of four categories: (i) inferred from general amphibian knowledge (e.g., breeding system); (ii) known or inferred from the literature, including present paper (adult age distribution, sex-ratio, clutch size, adult mortality, population size at the start of the experiment, *etc.*); (iii) virtually unknown and modeled over a wide range of possible conditions (carrying capacity, K); and (iv) the parameter of interest as hypothesized to be affected by the planting of gum trees (larval mortality, q_l). Scenarios in which larval mortality was not uniform over time (e.g., "continuous change" or "broken stick" models) were not carried out because of the lack of data over the 1996–2009 period. The life table produced by Vortex indicates that the size of the breeding population is typically 1/5th–1/6th of the total population. Vortex was run for 1000 iterations per set of variable combinations. For details on how the demographic parameters were determined see Appendix 3 along with Figure A3. In the below Ň refers to population size estimates, Ń refers to census population size and Ñ refers to modeled population size and q_l and q_b are larval and breeding adult annual mortality rates, respectively.

3. Results

With data from the literature and colleagues included, estimates on the *C. lusitanica* adult population size (Ň_b) in the dam were made on seven annual occasions (Appendix 4). Larval counts (Ń_l) for the Silveirinhos brook were made on five annual occasions. Counts on the numbers of adults (Ń_b) and embryos (Ń_e) in the Poço do Inferno mine galleries were made on 12–16 annual occasions. The size of the adult *C. lusitanica* population in the Silveirinhos dam was estimated at Ň_b = 1324 with a standard error (SE) of 265 in 1977, at Ň_b = 1477 ± 207 in 1992 and at Ň_b = 113 ± 56 in 2010. In 1977, the number of larvae dip-netted in the adjacent 200 m long section of the Siveirinha brook was maximally 81 first-year larvae and eight second-year larvae. In 2015, these numbers were five and three, respectively. The maximum number of adults observed in the Upper mine was Ń_b = 78 in 1977 and 279 in 2014. In the Lower mine the numbers were 100 in 1977 and 336 in 2014. The embryos numbers counted in the mines rose gradually from Ń_e ~650 in 1976 to *ca.* 3550 in 2014. The most telling data points are probably those of 1977 and 1995 when accumulate counts of freshly deposited eggs were made over 16-25 visits spanning the entire reproductive period. These counts gave Ń_e ~ 550 in 1977 and *ca.* 5000 in 1995. In 1977, the ratio of juveniles to adults was Ń_j/Ń_b = 0.42 along the Silveirinhos brook (751 salamanders in 17 visits) and 0.55 along the Poço do Inferno brook (116 salamanders in 11 visits). No juveniles were observed inside the dam or in the mines. Over the 39 years of study, the pattern is that of a sharp population decline at the Silveirinhos and a substantial increase at the Poço do Inferno (Figure 2).
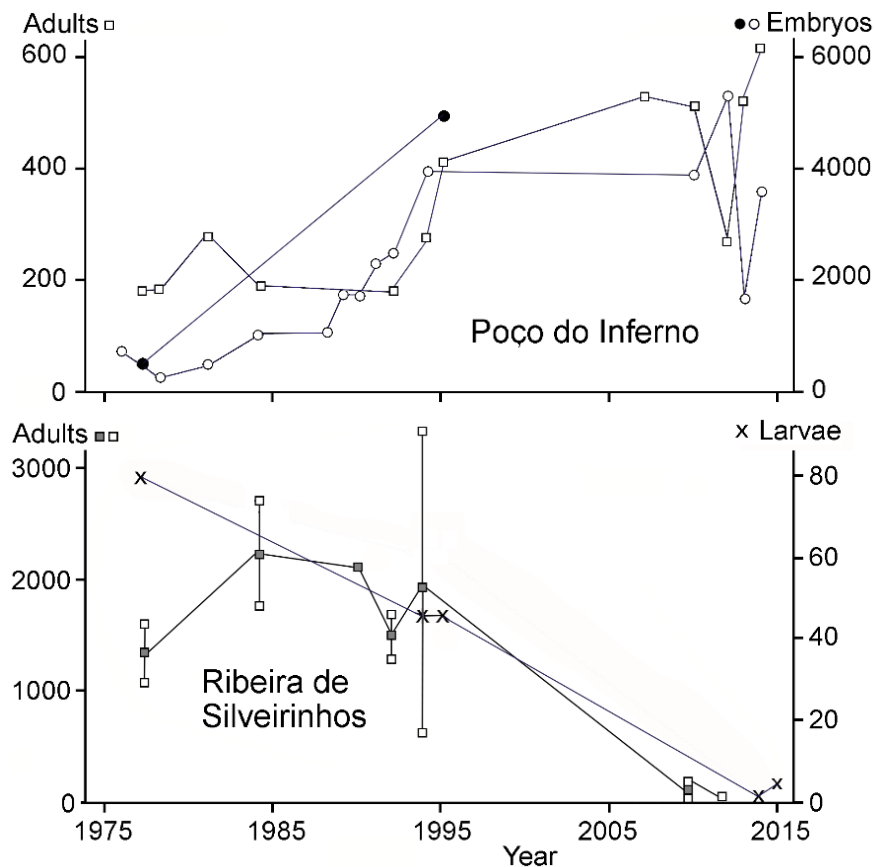
Figure 2. Numbers of *Chioglossa lusitanica* observed and population sizes estimated in the Serra de Santa Justa in northwestern Portugal over the 1976–2015 period. (**Top panel**) Embryo (round symbols) and adult numbers (squares) counted in two mine galleries at Poco do Inferno. The 1977 and 1995 cumulative embryo counts are shown separately with solid round symbols. (**Bottom panel**) First-year larvae counted in the Ribeira de Silveirinhos (crosses) and adult population size estimates from capture–mark–recapture in the "dam" (squares, $Ň_b$ solid symbols, $Ň_b \pm$ SE open symbols). For numerical detail see Appendix 4.

Following the application of Fluorescein in the Upper mine the concentrations in the Poço do Inferno brook were 0.26 μg Fluorescein/L adjacent to the mine entrance and 1.49 μg Fluorescein/L at 170 m downstream. The control sample (upstream of the Upper mine) was under the detection limit.

I let Vortex determine the values of q_l at which a hypothetical *Chioglossa* population would retain a 10,000 initial population size at year 100 with demographic parameter values as in Appendix 3. A stable population (rate of increase $r_{stoch} = 0$ [31]) is obtained at $q_l = 0.69$ when $q_b = 0.22$ and at $q_l = 0.62$ when $q_b = 0.28$ (Figure 3). A ten-fold population size increase, such as observed at Poço do Inferno, is associated with $q_l = 0.51$ when $q_b = 0.22$ and with $q_l = 0.38$ when $q_b = 0.28$. Demographic modeling further indicated that the recent low population size at Silveirinhos may be associated with a uniform larval mortality since the planting of gum trees in 1980 of $q_l = 0.76$–0.79 (2010, 30 years) (Figure 4). The figure also shows that K has little effect on such a declining population, in particular if $q_l > 0.70$.
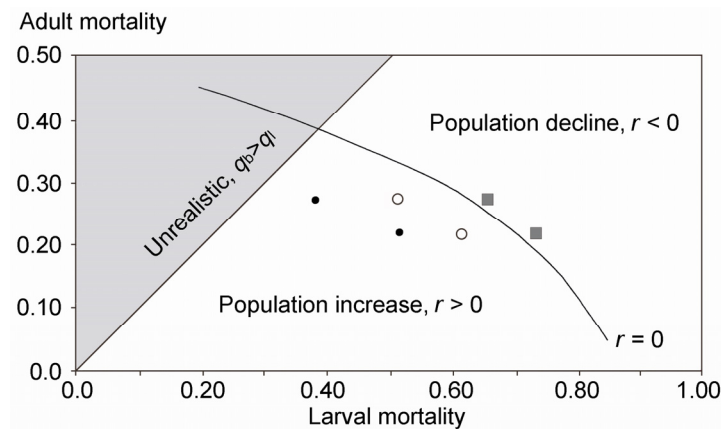
Figure 3. Population size development in a hypothetical population of *Chioglossa lusitanica* as a function of adult mortality (q_b, vertical axis) and larval mortality (q_l, horizontal axis) modeled with Vortex. The curved line indicates parameter combinations at which population size is stable ($r_{stoch} = r = 0$ [31]). Higher and lower q-values result in declining and increasing population sizes, respectively. The situation in which adult mortality is higher than larval mortality (parameter space shaded, $q_b > q_l$) is considered unrealistic. For details see text and Appendix 3. Round symbols refer to parameter combinations determined for Poço do Inferno where population size is increasing and square symbols refer to parameter combinations determined for the declining Ribeira de Silveirinhos population (symbols as in Figure 2).
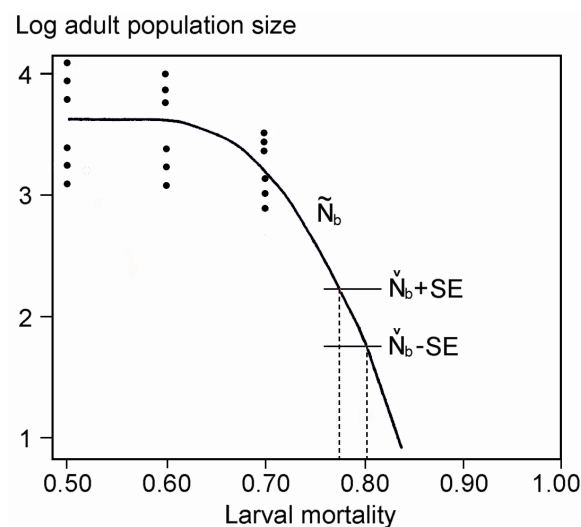
Figure 4. Adult population size of *Chioglossa lusitanica* at the Ribeira de Silveirinhos modeled with Vortex (log $\tilde{N}_b$ median value, vertical axis) as a function of larval mortality (q_l, horizontal axis) modeled with Vortex under a carrying capacity of K = 33,000. The year is 2010, *i.e.*, at 30 years after the widespread planting of gum trees. The dots drawn in for low q_l values represent the modeled variation in carrying capacity of, from top to bottom, 100,000, 70,000, 50,000, 20,000, 10,000 and 7000. Note that the size of the breeding population is about 1/5th–1/6th of the total population; for details see Figure A3 in Appendix 3. The horizontal lines represent the adult population size as estimated in 2010 (log$\check{N}_b \pm$ SE) and the interrupted vertical lines indicate the values of larval mortality at which modeled and observed data coincide, at around $q_l = 0.78$ ($0.77 < q_l < 0.80$).

More precise figures are derived from plotting the proportion of modeled population sizes ($\tilde{N}_b$) as falling inside or outside the $\check{N}_b \pm$ SE range, with q_l varying over the 0.5–1.0 range (Figure 5). The 1984 (4 year) and 1994 (14 years) population size estimates do not help to secure q_l estimates, presumably because the population had little opportunity to already decline (1984 [3]) or because the precision was poor (1994 [32]). The fairly precise 1992 estimate (12 years) is incompatible with $q_l < 0.58$ and $q_l > 0.83$ and the estimate of near-extinction in 2012 (32 years) is incompatible with $q_l < 0.70$. By its own the 2010 (30 years) $\check{N}_b$ estimate is the most informative with a range of $0.70 < q_l < 0.86$ and a best estimate of $q_l = 0.78$. Considering all data points, the best estimate for q_l is in the 0.70–0.83 range (Figure 5). Under a higher adult mortality (from $q_b = 0.22$ to $q_b = 0.28$) the q_l estimates decrease by *ca*. 8% points (e.g., from 0.73 to 0.65; Appendix 5 along with Figure A4). New and additional information on this project may be placed at http://www.repository.naturalis.nl/record/569285 under the sole responsibility of the author.
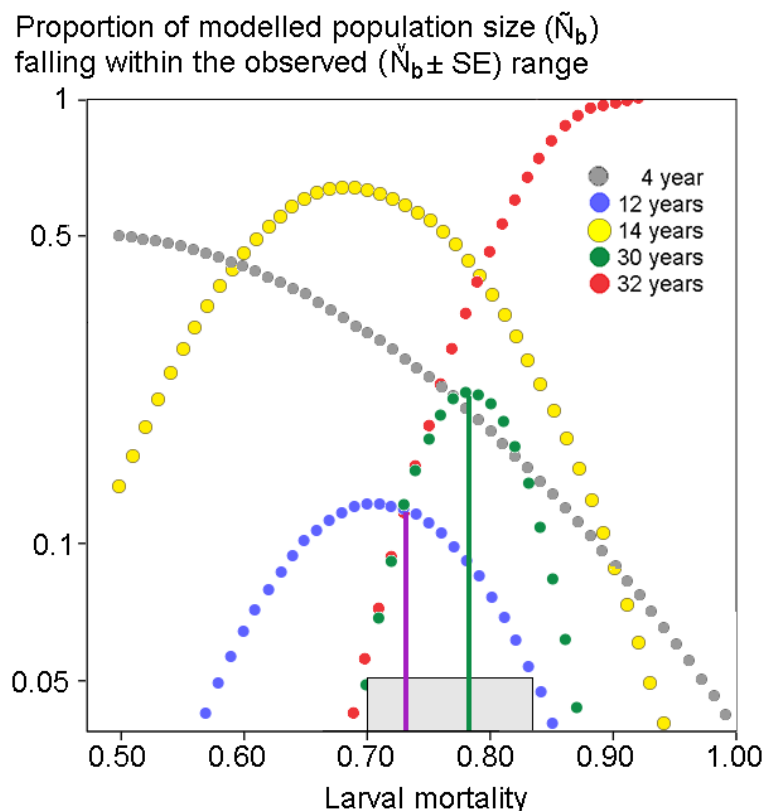
Figure 5. The probability of modeled population sizes of *Chioglossa lusitanica* at Ribeira de Silveirinhos falling within the population size range estimated in the field ($\check{N} \pm$ SE), as a function of larval mortality (q_l, horizontal axis). Populations were modeled with Vortex for 4–32 years following the widespread planting of gum trees in 1980. The results were fitted to a Gaussian distribution with Prism 6 software [33] ($r^2 > 0.95$) and then log-transformed. Support is maximal for $q_l = 0.73$ (purple bar, $p > 0.10$). The grey rectangle indicates the $0.70 < q_l < 0.83$ range over which none of the $\tilde{N}$ - $\check{N}$ combinations are implausible ($p > 0.05$). The green bar highlights the $q_l = 0.78$ value already retrieved for 2010 in Figure 4. Adult mortality q_b is set at 0.22; for results with $q_b = 0.28$ see Appendix 5.

4. Discussion

4.1. Population Development at Ribeira de Silveirinhos

The adult population size of *C. lusitanica* in the Silveirinhos dam was in the order of Ň = 1300–2200 in the 1977–1994 period and *ca.* 100 in 2010 (Figure 2). Observations at 11 monthly visits in 2012–2013 yielded ten sightings and just two salamanders were caught, so that a capture–mark–recapture estimate was impossible to make [34]. The data indicate a decline in *C. lusitanica* adult population size of one or two orders of magnitude. However, some assumptions are implicit to this conclusion and for interpreting the data it is important to understand the spatial dynamics of *C. lusitanica* at the Silveirinhos brook and dam system.

The schist and quartzite structure of the Serra de Santa Justa does not provide deep shelter and the thin layer of soil dries out quickly. The Silveirinhos dam serves as a summer hiding site for the adult population that at wetter periods of the year is distributed along the brook. The decision to migrate to the dam or stay put depends on the quality and depth of the shelter the individual salamander is in [11]. The total length of the Silveirinhos over which *Chioglossa* salamanders migrate to the dam is not known, but distances of >700 m have been recorded [7]. In summer 1994, the population size of the dam was estimated at $Ň_b$ = 1974 and the density of adult *C. lusitanica* in winter 1994–1995 was estimated at 11.4 ± 2.16 per m of brook [32]. Arntzen concluded that 60% of adult salamanders in the 200 m stretch downstream of the dam used the dam for aestivation, so that a length of *ca.* 330 m of the brook would account for the numbers estimated for the dam [8].

Is the decline real? One explanation for the low numbers of adult *C. lusitanica* in the dam in recent years is that migration for aestivation is no longer required, for example because the newly created gum tree terraces provide deep and sufficiently moist shelters year round. Another possibility is that low numbers were observed in exceptionally wet years in which migration for shelter from drought was not required. However, these explanations do not hold since the low number of adults in the dam is paralleled by low numbers of larvae in the brook (Figure 2, Appendix 4). In addition, adults were rare along the brook in winter 2014 and in spring 2015. I conclude that the current *C. lusitanica* population size at Silveirinhos is one or two orders of magnitude down relative to the 1977–1994 period. At this low size, the population may be especially prone to stochastic events, eventually leading to local extinction [31,35]. Is the population collapse at Silveirinhos to be attributed to (i) the gum tree plantations created in 1980? Other, not mutually exclusive, explanations are (ii) destruction of the sites that *C. lusitanica* uses for egg-deposition; (iii) increased competition or predation; and (iv) an outbreak of disease.

4.2. Potential Reasons for the Decline

Gum tree plantations: The streambed of the Silveirinhos, along with a vegetated zone on either side of the brook, stayed outside the plantation and were not structurally altered. The salamander decline is thought to operate through a reduced aquatic run-off causing the stream to fall dry earlier in the season and over a larger section of the valley. In rip-plowed plantations, gum tree transpiration rates remain high even when water supply from the soil would be dwindling [36]. This would in first instance affect the running sections of the brook, which is the larval niche of *C. lusitanica* [8,37]. Drought would

therewith affect *C. lusitanica* more than the other local amphibians. Considering a generation time of *ca*. 6 years ([19], Appendix 3), a gradual decline in *C. lusitanica* population size would be expected from 1980 onwards. While the precision of the dam population size estimates is low, especially for the crucial year 1994 (Figure 2), the larval count for that year confirms that the population size of *C. lusitanica* was yet substantial. A sharp decline in population size appears to have taken place only after 1994, which is 14 or more years after the creation of the gum tree plantation. For the population demographic modeling exercise to shed light on this issue, see below.

Destruction of egg-deposition sites: At the Ribeira de Silveirinhos, the places where *C. lusitanica* lays the eggs are unknown. At Poço do Inferno, oviposition takes place in the deeper parts of the mines—and even there, frequently hidden from sight. This suggests that normally the deepest available underground sections of the stream will be used or, at Silveirinhos, perhaps at wet and moist sites deep inside the dam. The gum trees taproots do normally not exceed a length of 3 m [38] but roots grow quickly towards aquatic resources and may then be reaching a depth of 14 m [39]. Gum trees are reported to exploit aquifers through which more water is consumed than is available from precipitation [40] and could therewith destruct *Chioglossa* egg-laying sites. I have, however, no information that would suggest the physical destruction or drying up of underground habitats to explain the Silveirinhos population collapse.

Increased competition or predation: Along with the gum tree plantation, the Silveirinhos has seen the building of a small causeway spanning the brook at the latitude of São Pedro da Cova, for use by trucks and other forestry equipment (41.15717°N, 8.49575°W, point C in Appendix 1). With this causeway, a pond was unintentionally created of size 17 m × 35 m. The exact date is not known, but pond creation was after 1995 (V. Lima, pers. comm.) and when I found it in spring 2011, the aquatic vegetation was well-developed, indicating an age of several years. Amphibian species, such as the urodeles *Lissotriton boscai*, *Salamandra salamandra*, *Triturus marmoratus*, and the toad *Alytes obstetricans*, that have larval stages in pond sections of the stream, are more common at Silveirinhos at the present than they were before [41]. This raises the question, has interspecific competition among larvae increased? However, the niche of *C. lusitanica* larvae is composed of the running and turbulent sections of a brook and only overlaps with the niche of *S. salamandra* larvae and not with the other species [8,37]. Adult toads (*Bufo spinosus*) and salamanders (*Salamandra salamandra*) are mentioned as potential predators [4] but their niches are largely outside the brook margins where *C. lusitanica* lives and, at Santa Justa, their density is not so high that they could extirpate the *C. lusitanica* population.

Outbreak of disease: In favor of this explanation is the sudden and massive decline of the *C. lusitanica* population after 1994, suggesting low *versus* high mortality rates before and after that date. In 1994, I observed a new arrival (the Green frog *Pelophylax perezi*) at the Silveirinhos and, in 2011, a small, unidentified species of carp. These vertebrates may have served as a disease vector. Drastic patterns of decline have been reported for a suite of amphibian species in central and northern Spain that were linked to infections with a Chytrid fungus and a Ranavirus, respectively [42–44]. The drastic decline of the marbled newts (*Triturus marmoratus*) in a pond in northern Portugal may have been caused by an Iridovirus carried by introduced Pumpkinseed sunfish (*Lepomis gubbosis*) [45,46]. Unfortunately, at Silveirinhos no surveys were carried out during the episode of population collapse and no corpses have become available for clinical investigation. However, given that the observed population development of the Golden-striped salamander (Figure 2) is not significantly at odds with demographic models that

operate under a uniform larval mortality rate there is, strictly speaking, no need to invoke an *ad hoc* explanation, such as disease (for a comparison, see, e.g. [47]).

4.3. Population Development at Poço do Inferno

At Poço do Inferno the data for the Upper mine and the Lower mine can be taken together because *C. lusitanica* migration from one mine to the other is frequent and may occur overnight ([7,48]. At just under one order of magnitude the local increase in *C. lusitanica* population size is unequivocal and can only to a minor degree be explained by, e.g., inter-observer variation, phenology, or weather conditions. To appreciate the healthy status of this population, it is important to note that the counts refer to visible eggs and visible adults—many more could have been found in the heaps of rock at the deeper sections of the mine galleries that though were left undisturbed. An increase in population size such as observed in the Poço do Inferno population is difficult to associate with a particular value of larval mortality, because of the dependency of q_l on carrying capacity (in expanding populations) and the availability of only count data.

Reproduction in the Lower mine can proceed unhampered. Matings are readily observed and embryos easy to find. Hatched larvae discharge in the stream running through the mine that will eventually assist transport to the outside. Downstream migration along the Ribeiro do Inferno over 80 m would bring the larvae in the Rio Simão. Upstream larval migration seems improbable due to a *ca.* 4 m high incline adjacent to the Lower mine entrance. The Upper mine also serves for mating and egg-deposition, over the season the larvae getting thinner and less numerous. In March 1977 and October 2010, I observed second-year larvae that may have been feeding on unfertilized eggs, dead embryos or larvae, since no other food sources than *C. lusitanica* itself appear to be available [49]. The water in this mine is stagnant with no visible connection to the outside, with the exception of periods of excessive rain. The water to overflow the heap of soil at the entrance of the mine has been observed on two occasions (March 1977 [50] and November 1994 [51]). The Fluorescein measured in the lower section of the Poço do Inferno brook indicates the presence of an underground aquatic connection with the Upper mine, but if the openings are large enough for *Chioglossa* larvae to pass it is not known. All in all, it is difficult to determine to what extent *Chioglossa* reproduction in the Upper mine contributes to the Ribeiro do Inferno population, but the mine's continued use for reproduction—quite possibly for 2000 years—suggests that it is unlikely to be negligible. Still, a simple and effective conservation measure would be the removal of the heap of soil at the mine entrance, so that larvae staying behind will be washed out of the mine following periods of rainfall.

At the Poço do Inferno, obvious environmental change has been absent. This edge of the Serra de Santa Justa remained free of large-scale gum tree plantation and the mines and their immediate surroundings are essentially unchanged. Minor changes in the area involve the remodeling of some of the dirt tracks, the local extraction of wood and the creation of fire break zones. Most pine trees fell victim to forest fires and were taken over by gum trees, but the area stayed free of terraced plantations such as at the Ribeira de Silveirinhos (see Figure 23 in [9]). One prominent change just outside the research area is the cleaning of the Simão stream through the installment of facilities for sewage treatment in Valongo in the early 1980s. Prior to this, the Simão was an opaque and smelling stream with plentiful remains of cloth washing and other household rubbish. The Simão valley saw further environmental improvement

through the project "Requalificação Paisagística das Margens do Rio Simão" and at present, the stream makes a clean and biologically healthy impression (http://www.valongoambiental.com). In the absence of other environmental change I attribute the drastic population size increase of *C. lusitanica* at Poço do Inferno to the improved health of the Simão River. The section of the Simão adjacent to the Poço do Inferno (in between Valongo and Campo) may be particularly amenable to *C. lusitanica* due to the lining of the river borders with dry stone walls and even the slate roofing over a *ca.* 190 m long section of the stream, presumably constructed with the stone debris from the Lower mine (Appendix 1).

4.4. Are Eucalypt Plantations to Blame? Suggestions for Future Investigations

Serious environmental problems have been attributed to gum tree plantations, foremost through a substantial reduction of total water yields when the trees are planted in semi-arid water catchment areas [52,53]. Toxins are released through evaporation, transferred to the soil by fog drip and leached out from leaf litter by rain [54]. More controversial is the impact the plantations would have on soil nutrients and erosion [1]. Gum tree plantations are typically associated with low biodiversity, including bird wildlife [55,56] and have a negative effect on ground arthropods and on the many species of vertebrates that depend on them as prey [57]. Others concluded that gum tree plantations have less diverse flora and fauna than indigenous forests [52]. In central Portugal it was reported that streams running through gum tree plantations had lower number and diversity of aquatic invertebrates than streams running through deciduous forests, but the effect is not consistent [58,59]. The effect of frequent logging through clear cutting may drastically affect the aquatic invertebrate and fish communities in adjacent riverine habitats ([59] and references therein). However, the supposed negative effects of gum trees on wildlife are frequently inconsistent or unrepeatable and alternative explanations usually cannot be ruled out. Moreover, effects may be different for different species [60]. As for *C. lusitanica*, negative effects of gum tree plantations are assumed to be indirect because the postmetamorphic salamanders live mostly in the buffer zone between the rip-plowed stands and the stream courses. The larval niche is likely to have become reduced, but regretfully no data on change in flow rates of the Silveirinhos are available.

The surveying of two *Chioglossa* populations over four decades has yielded some remarkable results. At the downside is a decline near to extinction of the Silveirinhos population that may be attributed to the monoculture of gum trees since 1980. The decline is late and sharp, but demographic models indicate that a pattern like this might be expected in *ca.* 12% of replicates. Nonetheless, the pattern of decline is so strong and abrupt as to be at odds with observations that *C. lusitanica* is regularly present in gum tree affected areas elsewhere [4]. Another example of an amphibian population possibly affected by an introduced plant species is Fowler's toad [61].

An outcome of the survey at the upside is the drastic and consistent population size increase of *C. lusitanica* at the Poço do Inferno. This may be attributed to the improved health of the adjacent Simão River. Moreover, this would suggest that *C. lusitanica* lives around larger streams than currently appreciated—for a description of stream size and types with and without the species see [4]. This hypothesis can be tested by the marking of adult salamanders along the Simão and their later retrieval in the mines. *Chioglossa lusitanica* living around large streams would also explain the observation of dense populations without local recruitment, such as at the tiny Salto brook adjacent to the Sousa River with

ca. 6 adults per m of the brook [14,15]. The main habitat of *C. lusitanica* in this area could be the adjacent Salto River. I advocate research to be undertaken to test for the regular presence and reproduction of *C. lusitanica* in high-order riverine habitats. However, large rivers of the size of the Douro are probably not a good habitat, as suggested by the barrier function of that river revealed by a substantial level of genetic differentiation across the river [28].

In search of a definitive answer on the effects of gum tree plantations on *C. lusitanica*, I advocate the resurveying of localities with and without newly created plantations that were prospected before, such as under the "EU-life" project in northern Portugal [62] and in Galicia [4]. I also recommend that the population size in the dam of the Silveirinhos be estimated at intervals of five to ten years. A population size with reasonable precision may be obtained with *ca.* one week of work, if carried out during and following rainy summer and autumn nights. The *C. lusitanica* population may rebound, either from the small number of local survivors, or from new arrivals that would colonize the area along the lower stretches of the Silveirinhos brook. A recovery to the level of the late past century would indicate the absence of strong effects from the gum tree plantations on *C. lusitanica*. Where *C. lusitanica* lays its eggs if there are no mines remains unclear. I suggest that the regular places are deep underground sections of smaller and larger streams. However, testing this proposition will probably have to wait for technical innovations to take place.
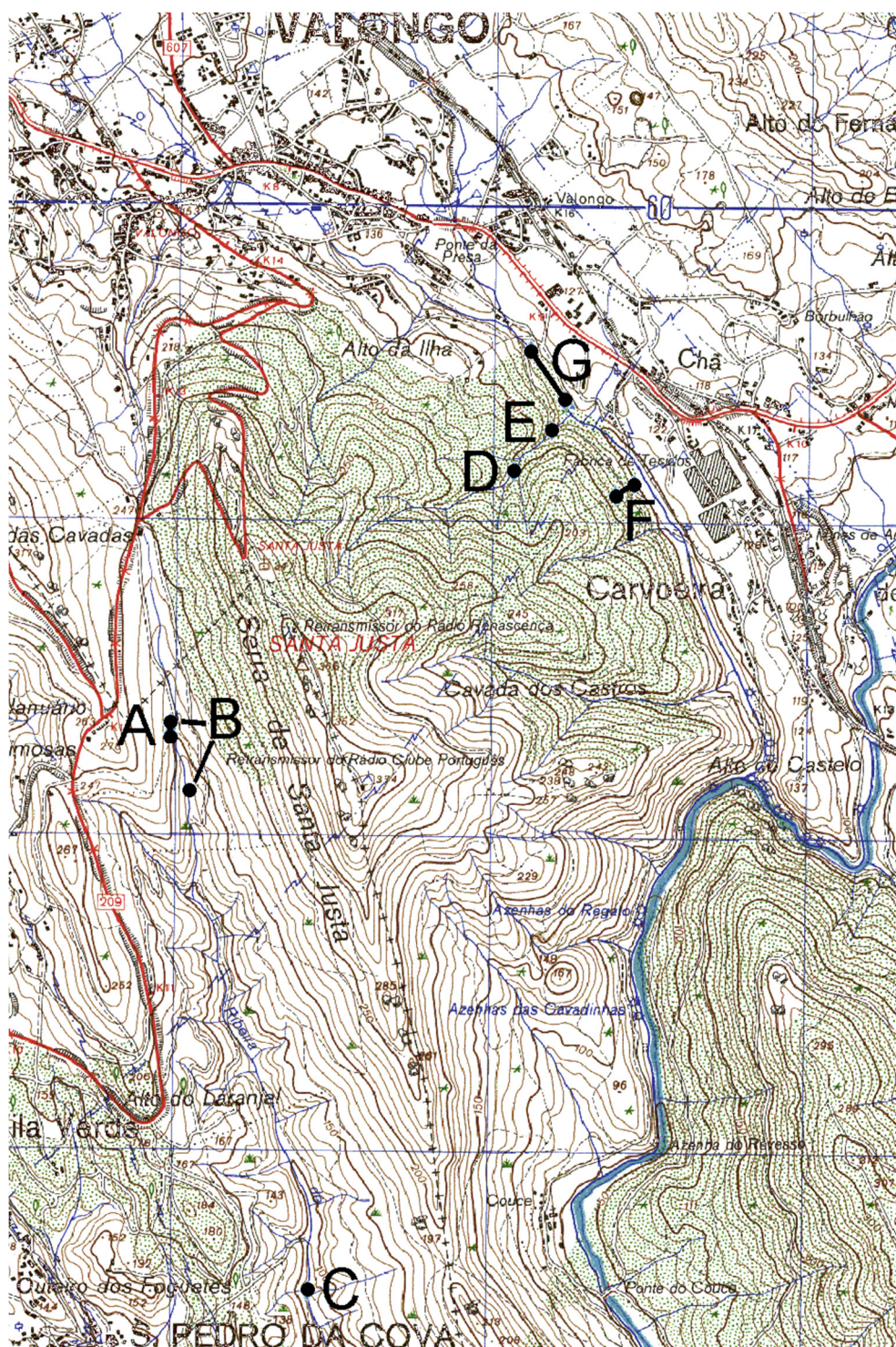
Conflicts of Interest

The author declares no conflict of interest.

Appendix 1

Figure A1. Map of the Valongo–São Pedro da Cova area with the Serra de Santa Justa [63]. The blue squares have a size of one km². Points of interest are: A: dam; B: research section of the Ribeira de Silveirinhos; C: newly created pond in the Silveirinhos streambed; D: entrance of Upper mine at Poço do Inferno; E: entrance of Lower mine; F: Simão mine (no data presented, inaccessible for man since 2010); and G: covered stretch of the Simão river. Note that the two study areas are in clockwise direction interconnected downstream through the Poço do Inferno-Simão-Ferreira river and upstream through the Silveirinhos.

Appendix 2
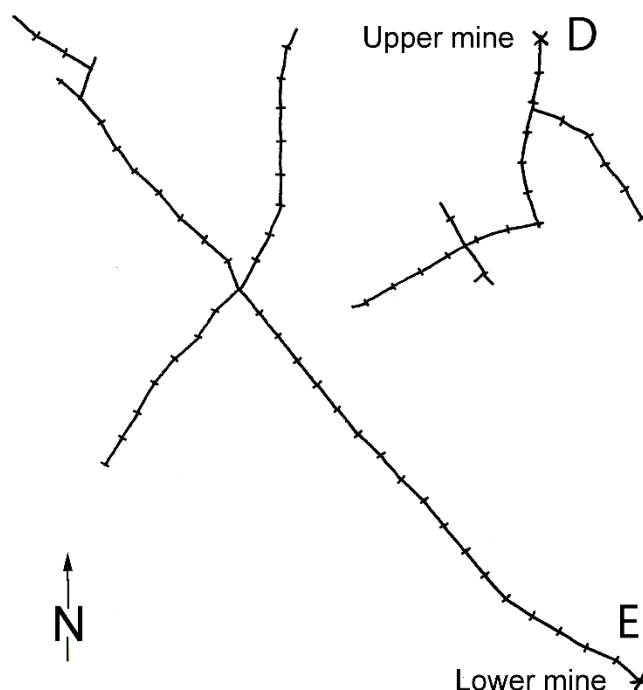
Figure A2. Layout of the Upper and Lower mine. Stretches are 5 m long. Entrances at the Poço do inferno brook are shown by "X" marks. For localities see Appendix 1.

Appendix 3. Parameter Settings for the Population Demographic Modelling with Vortex [31].

A3.1. Hypothetical Population of Chioglossa lusitanica

A3.1.1. Scenario Settings

Number of iterations: 1000 in population based analyses
Number of years: 100
Extinction definition: N < 50. This reflects the observed number of adults in the dam at the Ribeira de Silveirinhos ($\acute{N}_b$ = 10) at the most recent survey [34].
Number of populations: single
State variables: as defined under sensitivity testing, see larval mortality rates below.
Species description
Inbreeding depression: no
Concordance of the effect of environmental variation on reproduction and survival: no

A3.1.2. Reproductive System

Polygynous

    Age of first offspring: males 5 and females 6. This ignores a few males breeding at age 4 and a few females breeding at ages 4 and 5 (Figure 2 in [19], note that this figure shows post-metamorphic age). Breeding at age 5 age is also suggested by the pattern of 'lines of arrested growth' that, as in tree rings,

represent age and that show a tightening ("rapprochement") at age 5, indicating the switch in resource allocation from growth to reproduction (see also [64]).

With data for the sexes combined, the age distribution observed at the Ribeira de Silveirinhos indicates an annual mortality $q_b = 0.22$ (see [65]). However, the male age 5 class is relatively small and may be underrepresented, either through chance or because it is about to join the breeding population. Annual mortality determined from just the female age distribution gives $q_b = 0.28$, which parameter value was also analyzed (Appendix 5).

Maximum age of male and female reproduction: 10

Maximum lifespan: 10

Maximum number of progeny per brood (clutch size): 35

Sex ratio at birth: 50/50

Density dependent reproduction: does not apply

Reproductive Rates

Percentage adult females breeding: 100 with standard deviation (SD) due to environmental variation of 10%. Eggs are visible through the thin semi-transparent skin at ventral side of the females body and I observed the build-up of yolked oocytes over the spring and summer [15]. The first occurrence of mature sized females without eggs coincided with the onset of egg-deposition, from which I conclude that females skipping an annual breeding opportunity are absent or rare. Sequeira *et al.* observed that in summer almost all adults had mature sperm (males) and oocytes (females) [20]. However, part of the sample was drawn from the Lower mine, itself a breeding site, and may have been biased. Average clutch size is 18 ± 1, as in [20].

Mortality Rates

Mortality at age 0, *i.e.* embryo and larval mortality (q_l): The embryonic phase takes 12 weeks for hatching [66,67]. Most larvae metamorphose at *ca*. 9 months [8,19] while some stay in the water for a second year. In the modeling I equate the first year with the larval period. Under sensitivity testing larval mortality is analyzed over the 0.0 to 1.0 range with increments of 0.01.

Mortality at age 1: It here concerns recently metamorphosed salamanders ("efts") and a small proportion of the cohort as second-year larvae [8]. Efts live in the spatter-zone and in the immediate vicinity of the water courses [11].

Mortality at age 2: These are juveniles that generally live very close to the brook [11]. Mortality data for these cohorts are unavailable. Considering the habitat selection over an increasingly wide zone along the brook, annual mortality is scaled between larval mortality q_l and adult mortality q_b.

Mortality from age 4 onwards: $q_b = 0.22$, $q_b = 0.28$ (see above).

Mortality at age 3: These are young adults that are distinguished from full adults on the basis of body size and the development of secondary sexual characteristics [8]. Mortality set as for the age 4 year class.

SD due to environmental variation is set at $q/3$.

Initial population size: 10,000

Carrying capacity: The genuine value for K is virtually impossible to be determined and I analyze the effect over a large range (10,000–14,000–20,000–33,000–50,000–70,000–100,000).

Catastrophes: none

Mate monopolization: none

Harvest: none

Supplementation: none

Genetics: not investigated

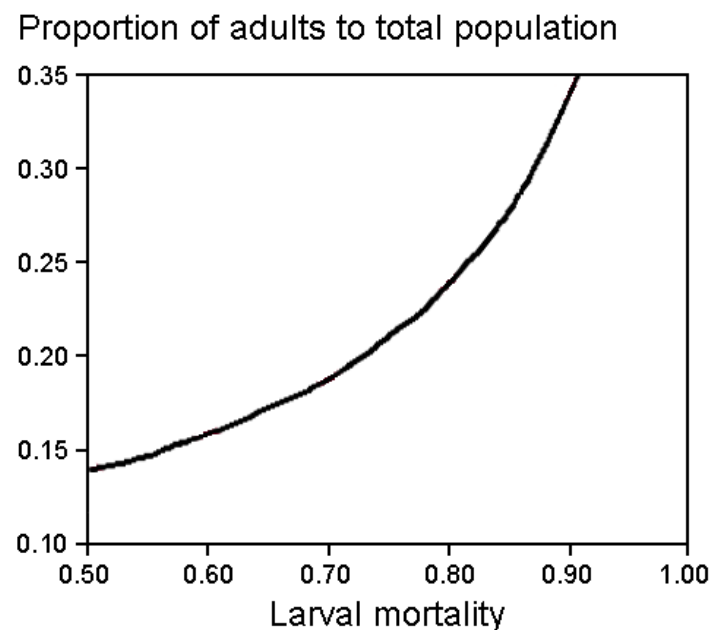*A3.2. Ribeira de Silveirinhos Population of Chioglossa lusitanica*

As under 1, with the following changes:

Population demographic development at the Ribeira de Silveirinhos is expressed as the number of adults in the breeding population (N_b), which is a proportion of the total population size N. The N_b/N ratio is dependent on the larval mortality rate and was determined from the age distribution with Vortex (Figure A3).

Number of years: 4, 12, 14, 30 and 32, corresponding to the adult population size estimates in 1984, 1992, 1994, 2010 and 2012.

Initial population size: N = 10,000 which roughly corresponds to N_b = 1324 + 2 × SE population size in 1977.

Carrying capacity: K = 33,000 which refers to the N_b = 1974 + 2SE adult population size in 1994. K was set at the high side in order not to force the model towards the observed population decline.

Figure A3. The contribution of adults to the total population of *Chioglossa lusitanica* (vertical axis, N/N_b) as a function of larval mortality (horizontal axis, q_l).

A3.3. Poço de Inferno Population of Chioglossa lusitanica

As under 1, with the following changes:

Number of years: 18, representing the 1977 to 1995 period in which the cumulative number of observed embryos increased by an order of magnitude (Appendix 4).

Initial population size: 10,000, representing the number of different adults observed in 1977 ($Ń_b$ = 1025, [15]).

Carrying capacity: K = 200,000, on the basis of population density relative to the Ribeira de Silveirinhos.

Appendix 4. Numerical Data on the Abundance of *Chioglossa lusitanica* in Two Study Areas of the Serra de Santa Justa, Portugal over the 1976–2015 Period

Table A1. Dam Ribeira de Silveirinhos.

Year	Adult Population Size ± Standard Error	Method	Source
1977	1324 ± 265	Weighted mean	[8,15]
1984	2234 ± 488	Weighted mean	[3]
1990	2117	Nil-recapture	[4]
1992	1477 ± 207	Weighted mean	[68]
1994	1974 ± 1352	Weighted mean	[32]
2010	113 ± 56	Weighted mean	
2012	10–50	Interpretation	[34]

Table A2. Silveirihos brook.

Year	Number of Larvae Observed in the Silveirinhos, Maximum (Date) *		Number of Visits	Source
	First Year Cohort	Second Year Cohort		
1977	81 (3/7)	8 (20/4)	5	[8,15]
1994	46 (December)	40 (December)	1	[32]
1995	46 (June)	18 (March)	4	[32]
2014	1 (26/12)	2 (26/12)	1	
2015	5 (15–16/4)	3 (15–16/4)	1	

Table A3. Poço do Inferno mine galleries.

Year	Largest Number of Adults Observed (Date)		Number of Visits	Source
	Upper Mine	Lower Mine		
1977	78 (19/9)	100 (20/9)	22, 16	[8,15]
1978	63 (13/10)	116 (22/10)	4, 3	[8,15]
1981	100 (13/10)	175 (13/10)	6, 6	
1984	56 (5/10)	134 (28/8)	6, 4	[3]
1992	37 (25/9)	141 (25/9)	1, 1	[68]
1994	117 (29/10)	153 (9/10)	6, 10	[51,66]
1995	209 (4/11)	199 (9/9)	25, 25	[51,66]
2007	311 (18/10)	216 (17/10)	1, 1	
2010	284 (3/11)	227 (28/10)	3, 2	
2012	146 (October)	121 (October)	1, 1	[34]
2013	264 (11/11)	253 (13/11)	2, 2	
2014	279 (30/9)	336 (30/9)	1, 1	

Table A3. *Cont.*

Year	Newly Deposited Eggs Totaled over Multiple Visits		Number of Visits	Source
	Upper Mine	**Lower Mine**		
1977	334	208	22, 16	[15]
1995	2975	2050	25, 25	[51]

Year	Maximum Number of Eggs and Embryos Observed		Number of Visits	Source
	Upper Mine	**Lower Mine**		
1976 **	487	169	1, 1	[15]
1978	130	78	4, 3	[8,15]
1981	219	233	6, 6	
1984	97	922	6, 4	[3]
1988	56	987	Not reported	[4]
1989	506	1200	Not reported	[4]
1990	150	1562	Not reported	[69] in [4]
1991	71	1778	2, 2	
1991	213	2043	Not reported	[4]
1992	46	2420	5, 5	[68]
1994	2536	1394	Not reported	[32]
2010	2503	1369	3, 2	
2012	2017	3283	3, 3	[34]
2013	970	720	2, 2	
2014	1520	2039	1, 1	

* over a 200 m long stretch of the brook. Data by V. Lima [32] were collected over 150 m; note that values in Figure 2 are adjusted accordingly.

** the numbers refer to empty egg shells and molded eggs recorded in early spring 1977.

Appendix 5
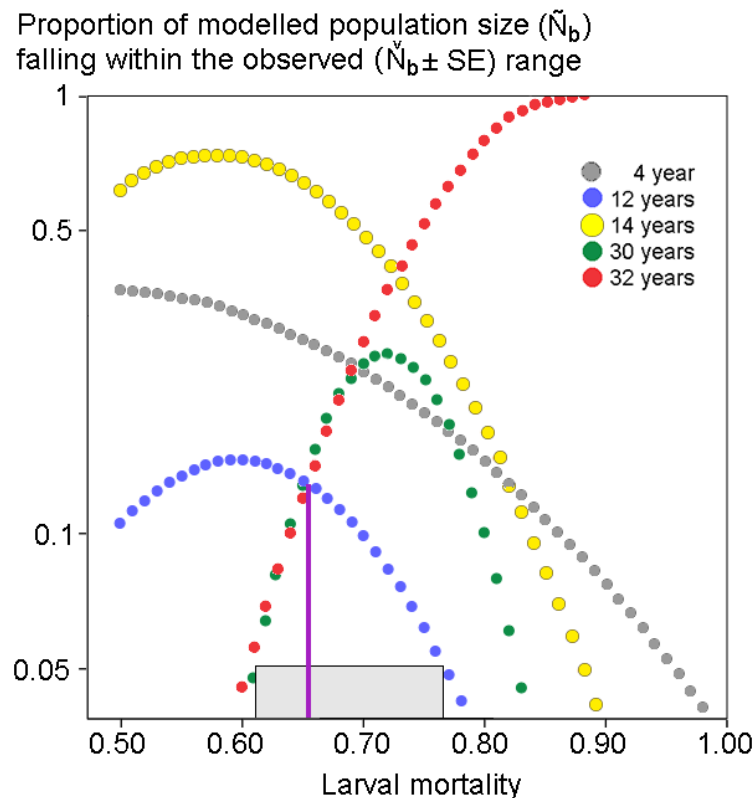
Figure A4. The probability of modeled population sizes of *Chioglossa lusitanica* at Ribeira de Silveirinhos falling within the population size range estimated in the field($\check{N} \pm SE$), as a function of larval mortality (q_l, horizontal axis). Adult mortality $q_b = 0.28$, otherwise as in Figure 4. Support is maximal for $q_l = 0.65$ (purple bar, $p > 0.10$). The grey rectangle indicates the $0.61 < q_l < 0.77$ range over which none of the $\tilde{N}$–$\check{N}$ combinations are implausible ($p > 0.05$).

References

1. Turnbill, J.W. Eucalypt plantations. *New For.* **1999**, *17*, 37–52.
2. Reboredo, F.; Pais, J. Evolution of forest cover in Portugal: A review of the 12th–20th centuries. *J. For. Res.* **2014**, *25*, 249–256.
3. Veenstra, G. Heeft de aanplant van *Eucalyptus gevolgen* voor de goudstreepsalamander, *Chioglossa lusitanica* ? *Lacerta* **1986**, *44*, 106–113. (In Dutch)
4. Vences, M. Habitat choice of the salamander *Chioglossa lusitanica*: The effects of eucalypt plantations. *Amphibia-Reptilia* **1993**, *14*, 201–212.
5. Ferreira, A.J.D.; Coelho, C.O.A.; Walsh, R.P.D.; Shakesby, R.A.; Ceballos, A.; Doerr, S.H. Hydrological implications of soil water-repellency in *Eucalyptus globulus* forests, north-central Portugal. *J. Hydrol.* **2000**, *231*, 165–177.
6. Arntzen J.W.; Beukema, W.; Galis, F.; Ivanović, A. Vertebral number is highly evolvable in salamanders and newts (family Salamandridae) and variably associated with climatic parameters. *Contrib. Zool.* **2015**, *84*, 85–113.

7. Arntzen, J.W. Speedy salamanders: Sedentariness and migration of *Chioglossa lusitanica*. *Rev. Esp. Herpetol.* **1994**, *8*, 81–86.

8. Arntzen, J.W. Ecological observations on *Chioglossa lusitanica* (Caudata, Salamandridae). *Amphibia-Reptilia* **1981**, *1*, 187–203.

9. Pinto Oliveira, C.I. Susceptibility to Forest Fire in the Municipality of Valongo. Implications for area Planning. Master's Thesis, Department of Geography, Faculty of Arts, University of Porto, Porto, Portugal, 2006.

10. Gonçalves, L. A reprodução de *Chioglossa lusitanica* Bocage. Algumas notas. *Naturalia* **1962**, *8*, 1–3. (In Portuguese)

11. Arntzen, J.W. Temporal and spatial distribution of the Golden-striped salamander (*Chioglossa lusitanica*) along two mountain brooks in northern Portugal. *Herpetol. J.* **1995**, *5*, 213–216.

12. Begon, M. *Investigating Animal Abundance*; Edward Arnold: London, UK, 1979.

13. Bocage, B. Note sur un nouveau batracien du Portugal, *Chioglossa lusitanica*, et sur une grenouille nouvelle de l'Afrique occidentale. *Rev. Mag. Zool. Pure Appl.* **1864**, *16*, 248–254. (In French)

14. Goux, L. Contribution a l'étude écologique, biologique et biogéographique *de Chioglossa lusitanica* Barb. (Urodela, Salamandridae). *Bull. Soc. Zool. Fr.* **1957**, *82*, 361–377. (In French)

15. Arntzen, J.W. De Levenswijze van de Goudstreepsalamander, *Chioglossa lusitanica* Bocage, 1864. Master's Thesis, Institute of Taxonomic Zoology, University of Amsterdam, Amsterdam, The Netherlands, 1979. (In Dutch)

16. Arntzen, J.W. *Chioglossa lusitanica* Bocage 1864—Der Goldstreifen-salamander. In *Handbuch der Reptilien und Amphibien Europas*; Grossenbacher, K., Thiesmeier, B., Eds.; Akademische Verlagsgesellschaft: Wiesbaden, Germany, 1999; pp. 301–321. (In German)

17. Vences, M. Untersuchungen zur Ökologie, Ethologie end geografischen Variation von *Chioglossa lusitanica* BOCAGE, 1864. *Salamandra* **1990**, *26*, 267–297. (In German)

18. Sequeira, F.; Gonçalves, H.; Faria, M.M.; Meneses, V.; Arntzen, J.W. Habitat-structural and meteorological parameters influencing the activity and local distribution of the Golden-striped salamander, *Chioglossa lusitanica*. *Herpetol. J.* **2001**, *11*, 85–90.

19. Lima, V.; Arntzen, J.W.; Ferrand, N.M. Age structure and growth pattern in two populations of the golden-striped salamander *Chioglossa lusitanica* (Caudata, Salamandridae). *Amphibia-Reptilia* **2000**, *22*, 55–68.

20. Sequeira, F.; Ferrand, N.; Crespo, E.G. Reproductive cycle of the golden-striped salamander *Chioglossa lusitanica* (Caudata, Salamandridae) in NW Portugal. *Amphibia-Reptilia* **2003**, *24*, 1–12.

21. Pleguezuelos, J.M.; Márquez, R.; Lizana, M. A*tlas y Libro Rojo de los Anfibios y Reptiles de España*; Ministerio de Medio Ambiante: Madrid, Spain, 2002. (In Spanish)

22. Arntzen, J.W.; Teixeira, J. History and new developments in the mapping and modelling of the distribution of the golden-striped salamander, *Chioglossa lusitanica*. *Z. Feldherpetol.* **2006**, *10*, 113–126.

23. Loureiro, A.; de Almeida, N.F.; Carretero, M.A.; Paulo, O.S. *Atlas dos Anfíbios e Répteis de Portugal*; Instituto da Conservação da Natureza e da Biodiversidade: Lisboa, Portugal, 2008. (In Portuguese)

24. Arntzen, J.W. Allometry and autotomy of the tail in the Golden-striped salamander, *Chioglossa lusitanica. Amphibia-Reptilia* **1994**, *15*, 267–274.

25. Alexandrino, J.; Arntzen, J.W. Morphological variation in two genetically distinct groups of the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). *Contrib. Zool.* **2005**, *74*, 213–222.

26. Arntzen, J.W.; Groenenberg, D.S.J.; Alexandrino, J.; Ferrand, N.; Sequeira, F. Geographical variation in the golden-striped salamander, *Chioglossa lusitanica* Bocage, 1864 and the description of a newly recognized subspecies. *J. Nat. Hist.* **2007**, *41*, 925–936.

27. Alexandrino, J.; Froufe, E.; Arntzen, J.W.; Ferrand, N. Genetic subdivision, glacial refugia and postglacial recolonization in the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). *Mol. Ecol.* **2000**, *9*, 771–781.

28. Alexandrino, J.; Arntzen, J.W.; Ferrand, N. Nested clade analysis and the genetic evidence for population expansion in the phylogeography of the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). *Heredity* **2002**, *88*, 66–74.

29. Arntzen, J.W.; Alexandrino, J. Ecological modelling of genetically differentiated forms of the Iberian endemic Golden-striped salamander, *Chioglossa lusitanica. Herpetol. J.* **2004**, *14*, 137–141.

30. Sequeira, F.; Alexandrino, J.; Rocha, R.; Arntzen, J.W.; Ferrand, N. Genetic exchange across a hybrid zone within the Iberian endemic golden-striped salamander, *Chioglossa lusitanica. Mol. Ecol.* **2005**, *14*, 245–254.

31. Lacy, R.C.; Pollak, J.P. *Vortex: A Stochastic Simulation of the Extinction Process. Version 10.0*; Chicago Zoological Society: Brookfield, IL, USA, 2014.

32. Lima, V. Estudo Comparativo de Alguns Aspectos da Biologia de *Chioglossa lusitanica* em Duas Populações do Noroeste de Portugal. Master's Thesis, Faculty of Sciences, University of Porto, Porto, Portugal**,** 1995. (In Portuguese)

33. Science Plus Group. *PRISM*, v. 6; Science Plus Group: Groningen, The Netherlands.

34. Silva Santos, J. Estudo Sobre o Tamanho de Três Populações de Salamandra Lusitânica (*Chioglossa lusitanica*) nas Serras de Valongo. Master's Thesis, Faculty of Sciences, University of Porto, Porto, Portugal, 2013. (In Portuguese)

35. Smith, K.F.; Acevedo-Whitehouse, K.; Pedersen, A.B. The role of infectious diseases in biological conservation. *Anim. Conserv.* **2009**, *12*, 1–12.

36. Pryor, L.D. *The Biology of Eucalypts*; Edward Arnold: London, UK, 1976.

37. Thiesmeier, B. Trophische Beziehungen und Habitatpräferenzen sympatrisch lebender *Salamandra salamandra* und *Chioglossa lusitanica*-Larven. *Abhandlungen und Berichte für Naturkunde*; Museum für Naturkunde Magdeburg: Magdeburg, Germany, **1994**, *17*, 119–126. (In German)

38. Hall, N.; Johnston, R.D.; Chippendale, G.M. *Forest Trees of Australia*; Australian Government Publishing Service: Canberra, Australia, 1970.

39. Sellers, C.H. *Eucalyptus, Its History, Growth and Utilization*; A.J. Johnston Co.: Sacramento, CA, USA, 1910.

40. Calder, I.R.; Rosier, P.T.W.; Prasanna, K.T.; Parameswarappa, S. *Eucalyptus* water use greater than rainfall input—A possible explanation from southern India. *Hydrol. Earth Syst. Sci.* **1997**, *1*, 249–256.

41. Arntzen, J.W. (Naturalis Biodiversity Center, Leiden, The Netherlands). Unpublished data, 2014, 2015.

42. Bosch, J.; Martínez-Solano, I.; García-París, M. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of Central Spain. *Biol. Conserv.* **2001**, *97*, 331–337.

43. Bosch, J.; Martínez-Solano, I. Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in the Peñalara Natural Park (Central Spain). *Oryx* **2006**, *40*, 84–89.

44. Price, S.J.; Garner, T.W.J.; Nichols, R.A.; Balloux, F.; Ayres, C.; de Alba, A.M.; Bosch, J. Collapse of amphibian communities due to an introduced ranavirus. *Curr. Biol.* **2014**, *24*, 2586–2591.

45. Froufe, E.; Arntzen, J.W.; Loureiro, A. Dead Newts in Peneda-Gerês. Available online https://www.amphibians.org/wp-content/uploads/2012/05/Froglog31.pdf (accessed on 21 July 2015).

46. Soares, C.; de Matos, A.A.; Arntzen, J.W.; Carretero, M.; Loureiro, A. Amphibian Mortality in a National Park in the North of Portugal. Available online: http://issuu.com/amphibiansdotorg/docs/froglog56/1 (accessed on 21 July 2015).

47. Prowse, T.A.A.; Johnson, C.N.; Lacy, R.C.; Bradshaw, C.J.A.; Pollak, J.P.; Watts, M.J.; Brook, B.W. No need for disease: Testing extinction hypotheses for the thylacine using multi-species metamodels. *J. Anim. Ecol.* **2013**, *82*, 355–364.

48. Arntzen, J.W. (Naturalis Biodiversity Center, Leiden, The Netherlands). Unpublished data, 1981.

49. Arntzen, J.W. (Naturalis Biodiversity Center, Leiden, The Netherlands). Unpublished data, 1977, 2010.

50. Arntzen, J.W. (Naturalis Biodiversity Center, Leiden, The Netherlands); Gerats, A.G.M. (University of Amsterdam, Amsterdam, The Netherlands). Unpublished data, 1977.

51. Faria, M.M. (R. Cooperativa 7 Bicas 130 3° E, Senhora da Hora, Portugal). Personal communication, 2015.

52. Poore, M.E.D.; Fries, C. *The Ecological Effects of Eucalyptus*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1985.

53. Florence, R.G. Cultural problems of *Eucalyptus* as exotics. *Commonw. For. Rev.* **1986**, *65*, 141–163.

54. Del Moral, R.; Muller, C.H. Fog drip: A mechanism of toxin transport from *Eucalyptus globulus*. *Bull. Torrey Bo. Club* **1969**, *96*, 467–475.

55. De la Hera, I.; Arizaga, J.; Galarza, A. Exotic tree plantations and avian conservation in northern Iberia: A view from a nest-box monitoring study. *Anim. Biodivers. Conserv.* **2013**, *36*, 153–163.

56. Proença, V.M.; Pereira, H.M.; Guilherme, J.; Vicente, L. Plant and bird diversity in natural forests and in native and exotic plantations in NW Portugal. *Acta Oecol.* **2010**, *36*, 219–226.

57. Zahn, A.; Rainho, A.; Rodrigues, I.; Palmeirim, J.M. Low macro-arthropod abundance in exotic *Eucalyptus* plantations in the Mediterranean. *Appl. Ecol. Environ. Res.* **2009**, *7*, 297–301.

58. Abelho, M.; Graça, M.A.S. Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. *Hydrobiologia* **1996**, *324*, 195–204.

59. Graça, M.A.S.; Pozo, J.; Canhoto, C.; Elosegi, A. Effects of *Eucalyptus* plantations on detritus, decomposers, and detritivores in streams. *Sci. World* **2002**, *2*, 1173–1185.

60. Larrañaga, A.; Basaguren, A.; Pozo, J. Impacts of *Eucalyptus globulus* plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. *Int. Rev. Hydrobiol.* **2009**, *94*, 497–511.

61. Greenberg, D.A.; Green, D.M. Effects of an invasive plant on population dynamics in toads. *Conserv. Biol.* **2013**, *27*, 1049–1057.

62. Sequeira, F.; Teixeira, J.; Alexandrino, J.; Lima, V.; Ferrand, N. Distribución de *Chioglossa lusitanica* en Portugal. *Bol. Aso. Herpetol. Esp.* **1996**, *7*, 7–8. (In Portuguese)

63. IGC—Instituto Geográfico e Cadastral. *Carta Corográfica de Portugal na Escala 1/50,000*; IGC: Lisboa, Portugal, 1977. (In Portuguese)

64. Francillon-Vieillot, H.; Arntzen, J.W.; Géraudie, J. Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela). A skeletochronological comparison. *J. Herpetol.* **1990**, *24*, 13–22.

65. Caughley, G. *Analysis of Vertebrate Populations*; John Wiley & Sons: London, UK, 1977.

66. Faria, M.M.; Gonçalves, H.; Meneses, C.; Sequeira, F. Reproductive habits of the golden-striped salamander, *Chioglossa lusitanica* in three mine galleries in Valongo (North of Portugal). In: Proceedings of the IV Congresso Luso-Espanhol de Herpetologia, Porto, Portugal, 5–8 December 1996; p. 82.

67. Ortiz-Santaliestra, M.E.; Fernández-Benéitez, M.J.; Lizana, M.; Marco, A. Influence of a combination of agricultural chemicals on embryos of the endangered Gold-striped salamander (*Chioglossa lusitanica*). *Arch. Environ. Contam. Toxicol.* **2011**, *60*, 672–680.

68. Belcher, J.; Cornish, C. Personal communication, 1992.

69. Arntzen, J.W. (Naturalis Biodiversity Center, Leiden, The Netherlands); Vences, M. (Technical University of Braunschweig, Braunschweig, Germany). Unpublished data, 1990.