

Heavy metacercariae infestation (*Parastrigea robusta*) promotes the decline of a smooth newt population (*Lissotriton vulgaris*)

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Abstract. A previously unexplained decline of a *Lissotriton vulgaris* population following breeding habitat management measure improving the pond's physical features is reported. We used CMR-analyses, skeletochronology, trematode load and fat body assessment to evaluate the response of newts to local stressors with respect to demographic life-history traits. We provide evidence that in *L. vulgaris* survival and consequently longevity are deeply affected by infestation with *Parastrigea robusta* metacercariae. Demographic responses as compared with those of unaffected populations include earlier sexual maturation and decreased longevity. In fact, infested newts survived rarely to the next-year breeding period probably due to the lack of metabolic reserves (undeveloped fat bodies, starvation-hypothesis). The local decline of smooth newts is most probably the result of an exceptional combination of the invasion of a neophyte (*Elodea*) and a neozoon snail (*Gyraulus parvus*) promoted by pond restoration establishing the complete developmental cycle of a newt parasite (*P. robusta*) in the breeding pond. Future studies are needed to evaluate whether or not the observed fatal impact of the trematode on newt survival is general and a new threat in habitats favorable to support high parasite densities.

Key words. Amphibia, age at maturity, longevity, parasite-mediated mortality, population size, skeletochronology, starvation-hypothesis, Trematoda.

Introduction

Declines in amphibian populations have been observed all over the world, in some cases leading to local or final extinction of species (HOULAHAN et al. 2000, WAKE & VREDENBURG 2008). The causes of declines include habitat loss, infectious disease, invasive species, and climate change, and may interact through complex mechanisms (BLAUSTEIN & KIESECKER 2002). For example, fungal pathogens such as *Batrachochytrium dendrobatidis* cope often better with the global warming than do their hosts intensifying the malign effect (BERGER et al. 1998, DASZAK et al. 1999, POUNDS et al. 2006). Host–parasite systems show a common pattern of disease-induced host population decline: the parasite invades a native host population producing a disease outbreak or epidemic, leading to mass mortality, population decline and, eventually, extirpation (VALENZUELA-SÁNCHEZ et al. 2017). Life-history theory predicts that increased extrinsic mortality can result in a wide range of life-history responses, including decreased lifespan, and rapid early development (STEARNS 1992). For example, age at maturity was consistently earlier in *B. dendrobatidis*-infected populations of *Litoria verreauxii alpina* than in uninfected populations increasing an in-

dividual's probability of survival to reproductive maturity (SCHEELE et al. 2017).

The role of helminth parasites in amphibian declines is less well understood (e.g. TINSLEY 1995, LORAS et al. 2011). The combined effects of the pesticide atrazine and larval trematodes contributed to the decline of leopard frogs *Rana pipiens* (ROHR et al. 2008). Trematode infections are known to cause amphibian malformations, but linking amphibian mortality and morbidity with trematode infection is often difficult due to the complex life cycle of the trematodes (BLAUSTEIN & JOHNSON 2003, GOREN et al. 2014). In many digenean species, the first intermediate host is a water snail, amphibians are second intermediate hosts and birds are the definitive hosts (e.g. *Strigea* spp., ODENING 1967), but only the adult worms have been described morphologically, whereas the identification of miracidia, cercariae and metacercariae require molecular tools (JOUSSON et al. 1999, LOCKE et al. 2011). Moreover, infestation of amphibian hosts is not externally visible (except for *Clinostomum complanatum* metacercariae, CAFFARA et al. 2014, BRUNI & ANGELINI 2016), and thus remains mostly undetected. Consequently, unexplained declines of amphibians may be related to undetected, endoparasitic helminths.

In this paper we report the marked decline of a large smooth newt population (*Lissotriton vulgaris*) inhabiting a pond which we monitor since 1992 in the former military training area Schmidtenhöhe near Koblenz (SINSCH et al. 1995). The decline to about 30% of initial population size came unexpected because following pond rehabilitation in autumn of 2008 (removal of silt and part of the reed vegetation), the syntopic crested newt population (*Triturus cristatus*, target species for conservation management) increased to almost double pre-restoration size and the alpine newt population (*Ichthyosaura alpestris*) remained unchanged in size (SINSCH, unpublished observations). Since *L. vulgaris* populations reproducing in nearby ponds (distance 200–2500 m) did not show similar declines, we started analyzing the local population dynamics and demographic life-history traits in 2013 to identify the pond-specific stressors affecting smooth newts, but not the syntopic crested and alpine newts. Specifically, we performed capture-mark-recapture (CMR) studies to follow the individual fate of adult smooth newts, assessed skeletochronologically the age structure of the breeding population, tested experimentally for a predatory impact of crested and alpine newts on smooth newts and finally measured the individual helminth parasite load. The helminth parasite community of local smooth newts comprised at least five species of trematodes and nematodes (SINSCH et al. 2018). Aim of this study is to reconstruct the scenario leading to the local population decline by evaluating the contribution of habitat change and biotic interactions on demographic life-history traits and population dynamics.

Materials and methods

Study area

Adult smooth newts *Lissotriton vulgaris* were collected from a permanent eutrophic pond (= pond 1; 50.347°N, 7.674°E, 333 m a.s.l.) and a temporarily water-filled ditch (= pond 2; 50.346°N, 7.644°E, 279 m a.s.l.) at the former military training area Schmidtenhöhe (area: ca. 700 ha) near Koblenz (Rhineland-Palatinate, Germany; SINSCH et al. 1995, SINSCH & BREUER 2018). The dynamics of a *Triturus cristatus* population reproducing in Pond 1 were regularly monitored since 1995 (SINSCH et al. 2003) and therefore, continuous data on the succession of the pond are available (Fig. 1). The pond originated from a former tank track and has a surface area of about 86 m² and about 0.9 m depth at maximum water level. Following the end of tank training in 1992, the vegetation belt at the shore region (mainly *Typha latifolia*, *Eleocharis palustris*, *Alisma plantago-aquatica*, *Lycopus europaeus*, *Juncus* sp.) and the submerse vegetation (*Chara globularis*) increased successively in extension reducing the open water surface area finally to about 4 m² in 2008. In October 2008, the pond was restored to former dimension and depth by removing submerse vegetation and all silt accumulated and about 80% of the shore vegetation. Following a two-years period of very scarce pond vegetation, the reed belt recovered and succes-

sion proceeded again. The present plant community differs from the pre-restoration community by the invasive neophyte *Elodea canadensis* establishing in 2014 and replacing the *Chara* algae. In 2016 and 2017, nearly the complete water body was filled with dense *Elodea* vegetation. Along with the rise of *Elodea* the invasive planorbid *Gyraulus parvus* became co-dominant with the native *Lymnaea stagnalis* in the snail community of the pond (SINSCH & BREUER 2018). The two species are well-known first intermediate hosts for several trematode parasite species.

Capture-Mark-Recapture surveys in pond 1

Smooth newts *L. vulgaris* were captured once a week by time-constrained dip-netting in pond 1 for five years: April 11 to July 25, 1995, April 20 to June 10, 2005, April 29 to July 1, 2013, April 17 to June 26, 2014, and April 13 to May 13, 2015. All individuals collected were sexed by examination of external secondary sexual characters (males have a high dorsal crest and a prominent cloaca; e.g. ALTUNİŞİK et al. 2014), the belly pigmentation pattern was recorded (1995 and 2005 by photocopies, 2013–2015 by digital photographs), and immediately released again in the pond. Individual snout-cloaca length (SCL, distance between snout tip and cloaca) was measured to the nearest 0.1 mm based on the photographs. The total number of belly pigmentation pattern recorded was 1109 in 1995, 911 in 2005, 259 in 2013, 540 in 2014, and 643 in 2015. Belly patterns were compared manually by US and JW to identify within-year and among-years recaptures of individuals (HAGSTRÖM 1977). The capture-recapture matrices were used to estimate the total size of the newt population reproducing in the pond by the CHAPMAN formula for low numbers of recaptures (CHAPMAN 1951) and the corresponding 95% confidence interval (standard error estimation according to SEBER 1970). Absence of overlap between the confidence intervals was considered as a significant difference between two estimates. We are aware that population size calculated by the described procedure tends to overestimate the real population size due to newt mortality during the monitoring period, but assume that the magnitude of error was similar in all years studied.

Predation trials

Since an adult *T. cristatus* male feeding on a *L. vulgaris* male was captured by chance in pond 1, we quantified predation of *T. cristatus* and *I. alpestris* on adult smooth newts and their larvae in replicated laboratory trials in temperature-controlled chamber at 15 ± 2°C and a light-dark cycle of 12:12 hours. Eight trial boxes (60 × 40 × 20 cm) were filled with tap water to 9 cm depth and a central large stone allowing the newts to leave water. Four boxes were additionally fitted with plants and more stones to provide shelter sites within the water. In the first trial series with two replicates each per predator species and shelter availability each



Figure 1. Varying states of pond 1 succession from 1995 to 2017. The 2008 photographs show the state before and after restoration. In 2017, *Elodea* pads reach up to water surface in most of the pond.

box was equipped with two *T. cristatus* or *I. alpestris* males and 10 adult *L. vulgaris* collected in pond 1 at June 15, 2015. Predator exposure lasted five days (June 15–19, 2015) and all surviving smooth newts were released again in situ. In the second and third trial series experimental design and predators were the same as in the first trial, but instead of adults 20 *L. vulgaris* larvae were placed in each box and experiments lasted two or three days (June 22–24, 24–25, 2015). At the end of all trials the surviving larvae and predators were released again in pond 1. The predation rate was calculated as number of prey items per predator and day. The effect of predator species and shelter availability on smooth newt survival was tested for by ANOVA.

Bone sampling, skeletochronological processing and age data analyses

In 2015, the 3rd digit of a hind limb was toe-clipped in 30 male newts and 24 females collected in pond 1 before release and stored in 4.5% formaldehyde. In 2016, another 28 males and 5 females were collected in pond 1 and 20 males in pond 2 for parasitological examination (see below). Following sacrifice by exposure to an overdose of the anesthetic MS-222 (buffered 1% solution for five minutes) the 3rd digit of a hind limb was removed from the carcasses. Skeletochronological age determination followed the standard laboratory protocols (SINSCH 2015). The samples were embedded in Histo-resin™ (JUNG) and stained with 0.5% cresylviolet (SINSCH et al. 2001). Diaphysis was cross-sectioned at 12 µm using a JUNG RM2055 rotation microtome. Cross sections were examined under a light microscope (OLYMPUS BX 50) for the presence of growth marks at magnifications of 400×. We distinguished strongly stained lines of arrested growth (LAGs) in the periosteal bone, separated by faintly stained broad growth zones (e.g. SINSCH et al. 2007). We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of periosteal bone at its maximum. The number of LAGs was assessed independently by all authors to estimate age. Demographic life-history traits quantified were (1) age at maturity (number of LAGs of the youngest reproductive individual), (2) median age, and (3) longevity (number of LAGs of the oldest reproductive individual). We pooled age data for males and females because previous studies (and this one) failed to detect significant sex-specific differences with respect to age (e.g. ALTUNIŞIK et al. 2014). Since age data deviated significantly from a normal distribution, we compared medians by the non-parametric Mann-Whitney-Wilcoxon W-Test and shape of distribution by the two-sided Kolmogorov-Smirnov-Test.

In a meta-analysis we compared the demographic data on the two study site populations with those published on another 11 populations throughout the geographical range of smooth newts (HAGSTRÖM 1977, DOLMEN 1982, VERRELL & FRANCILLON 1986, KALEZIC et al. 1996, NOBILI & ACCORDI 1997, MARNELL 1998, COGĂLNICEANU & MIAUD 2003, MALETZKY et al. 2004, ALTUNIŞIK et al. 2014). The

influence of latitudinal and altitudinal variation on age at maturity, median age and longevity was assessed using multiple regression models (procedure: backward selection). As pond 1 population seemed to differ from all other populations with respect to demographic life-history traits, we tested multiple regression models on the complete data set and a reduced data set excluding pond 1. We predict that the amount of variance explained by the model will increase considerably using the reduced data set as compared to the complete data set, if the demographic features of the pond 1 population were unrelated to its geographic position.

Assessment of helminth parasite load

In 2016, 33 newts were collected in pond 1 at June 14 and 20 newts in pond 2 at June 16. The pond 1 individuals were sacrificed immediately after collection ($n = 15$), or following being kept in captivity at room temperature and food ad libitum at July 15 ($n = 5$), August 8 ($n = 7$) and September 7 ($n = 6$). All pond 2 individuals were sacrificed upon capture at June 16. Each individual was photographed in life, sexed and SCL measured to the nearest 0.1 mm using a caliper, corresponding body mass following sacrifice to the nearest 1 mg. Body cavity, digestive tract, lungs, kidneys and bladder were examined subsequently macroscopically and microscopically for the presence of helminth parasites (for details see SINSCH et al. 2018). For the purpose of this study, we focus on the effects of infestation with *Parastrigea robusta* metacercariae (Trematoda, Strigeidae) because nematode infestation was similar in both population and at low frequency (SINSCH et al. 2018). Carcasses of newts were fixed in 10% buffered formalin, and deposited in the collection of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK 101147–101179, newts from pond 1; ZFMK 101180–101199, newts from pond 2).

Newt traits considered were (1) SCL [mm], (2) condition index as measure of nutrition state (studentized residuals of the SVL–mass relationship using the multiplicative model; READING 2007), (3) age [n LAGs], (4) size of fat body as a semi-quantitative measure of energy reserves (0 = very small, 1 = medium-sized, 2 = large-sized), and (5) metacercariae load [n cysts per individual]. Statistical analyses of size and mass-related traits were limited to data on males because of the sexual size dimorphism in *L. vulgaris* (e.g. MALMGREN & THOLLESON 1999) and the lack of data on females originating from pond 2. For univariate comparisons we used the non-parametric Mann-Whitney-Wilcoxon W-Test and shape of distribution by the two-sided Kolmogorov-Smirnov-Test. For multivariate comparisons of log₁₀-normalized parasite load and condition index as dependent variables we applied ANCOVAs with locality as fixed factor and age and size as continuous covariates. The significance level was set to $\alpha = 0.05$. All statistical analyses were performed using the program package STATGRAPHICS Centurion XVIII.

Table 1. CHAPMAN estimates of population size and numbers of individual *L. vulgaris* captured in pond 1. Estimates are given with the corresponding 95% confidence limit.

	1995	2005	2013	2014	2015
CHAPMAN estimate of reproductive adults	909 ± 95	824 ± 97	239 ± 89	249 ± 24	497 ± 57
Individuals actually collected	562 (266 males + 296 females)	522 (270 males + 252 females)	162 (77 males + 85 females)	231 (122 males + 109 females)	366 (194 males + 172 females)
Recaptured individuals marked in 2013			–	1 (0.6%)	0
Recaptured individuals marked in 2014			–	–	8 (3.5%)

Results

Population dynamics in pond 1

Estimates of the number of reproductive adults gathering in pond 1 during spring and early summer showed a significant among-year variation (Table 1). The number of individuals captured per reproductive season represented 62% and 93% of the corresponding CHAPMAN estimates and were within the 95% confidence interval in 2013 and 2014. Population size did not differ significantly between 1995 and 2005, but decreased from 1995–2005 to 2013–2015 to about 30–45% (confidence intervals did not overlap, $P < 0.05$).

Return rates of registered individuals to the study pond during the next-year breeding period ranged from 0.6–3.5% (Table 1). Since smooth newts of this pond showed a strong breeding site fidelity (SINSCH & KIRST 2016) and occasional surveys in neighboring ponds up to 500 m distant never yielded recaptures of registered individuals, the low return rates were considered mainly due to low survival during the post-reproductive period and hibernation prior the next-year reproductive period.

Predation trials

Independent of shelter availability or predator species in the trial boxes, all adult smooth newts survived the predation trials. In contrast, *L. vulgaris* larvae suffered from predation by crested newts at a rate of 0.8 larvae per day and predator in the shelter-rich boxes and of 1.3 larvae per day and predator in the shelter-free boxes (ANOVA, $F_{1,7} = 1.02$, $P = 0.3517$). The corresponding predation rates by alpine newts were 1.5 larvae per day and predator in the shelter-rich boxes and 1.0 larvae per day and predator in the shelter-free boxes (ANOVA, $F_{1,7} = 0.65$, $P = 0.4506$). Predation rates did not differ significantly between crested and alpine newts (ANOVA, $F_{1,15} = 0.21$, $P = 0.6549$).

Local age structure and meta-analysis

Skeletochronological age determination was successful in 55 (2015) and 31 (2016) adults collected in pond 1 and in 19 (2016) in pond 2 (Table 2). Between-years variation of

Table 2. Age structure of two *L. vulgaris* populations in the study area. In 2016, the infestation of individuals with *P. robusta* metacercariae was assessed.

Age class [n LAGs]	Frequency in pond 1 (2015)	Frequency in pond 1 (2016)	Frequency in pond 2 (2016)
1	13	4 (3 infested)	–
2	34	18 (14 infested)	1
3	6	7 (3 infested)	4
4	2	1 (1 infested)	6 (1 infested)
5	–	–	5
6	–	1	2 (1 infested)
7	–	–	1

age distribution at pond 1 was significant with respect to shape of distribution ($P < 0.0001$, two-sided Kolmogorov-Smirnov-Test), but not with respect to medians (2 LAGs; $W = 1025.0$, $P = 0.077$, Mann-Whitney-Wilcoxon W -Test). Between-ponds variation of age distribution (irrespective of parasite infestation) in 2016 was significant with respect to medians (2 LAGs [pond 1] vs. 4 LAGs [pond 2]; $W = 526.0$, $P < 0.0001$, Mann-Whitney-Wilcoxon W -Test) and to shape of distribution ($P < 0.0001$, two-sided Kolmogorov-Smirnov-Test). If the age-dependent annual survival rates of mature smooth newts were similar in the period of 2013–2016, the estimated annual survival rate of LAG 2 individuals was 29% on average in pond 1, i.e. only 29% of these age class survived to the age of LAG 3.

The meta-analyses of the effects of latitude and altitude on age at maturity, median age and longevity of the smooth newt populations revealed that only the impact of altitude was significant (multiple regression analysis, backward selection, $P < 0.05$; Fig. 2). Using the complete data set, altitude explained 34.9% of variation in age at maturity ($F_{1,112} = 5.91$, $P = 0.0334$), 69.1% of variation in median age ($F_{1,112} = 24.57$, $P = 0.0004$), and 54.6% of variation in longevity ($F_{1,112} = 13.26$, $P = 0.0039$). Excluding the pond 1 population from analysis, explained variation increased notably to 55.6% of variation in age at maturity ($F_{1,11} = 12.53$, $P = 0.0054$) and to 86.4% in median age ($F_{1,11} = 63.78$, $P < 0.0001$), but variation explained in longevity was very similar with 56.3% ($F_{1,11} = 12.89$, $P = 0.0049$).

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Table 3. Demographic life-history traits of adult *L. vulgaris* in the study area (ponds 1 and 2). Analogous data compiled from literature are given for several populations from a wide latitudinal and altitudinal range. Female and male age data are pooled.

Locality	Latitude [°N]	Altitude [m a.s.l.]	Median age [n LAGs]	Age at maturity [n LAGs]	Longevity [n LAGs]	Source
Trondheim, Norway	63.4	240	4	3	6	DOLMEN (1982)
Nygaard (Hunneberg), Sweden	58.3	96	5	3	10	HAGSTRÖM (1977)
Dublin, Ireland	53.3	70	4	3	7	MARNELL (1998)
Linford, Soulbury, England	51.9	87	4	2	6	VERRELL & FRANCILLON (1986)
Near Koblenz, Germany: Pond 1 (2015)	50.3	333	2	1	4	This study
Near Koblenz, Germany: Pond 1 (2016)	50.3	333	2	1	6	This study
Near Koblenz, Germany: Pond 2 (2016)	50.3	279	4	2	7	This study
Ameisensee, Österreich	47.5	1282	7.5	4	11	MALETZKY et al. (2004)
Fundu Marc, Ostrovu Popa, Romania	44.7/45.2	4-8	4	3	6	COGĂLNICEANU & MIAUD (2003)
Velika Osječenica, Bosnia	44.5	990	7	4	9	KALEZIC et al. (1996)
Settecamini, Italy	41.9	50	3	2	5	NOBILI & ACCORDI (1997)
Castelporziano, Italy	41.7	15	3	3	6	NOBILI & ACCORDI (1997)
Çanakkale, Turkey	40.1	32	3	2	5	ALTUNIŞIK et al. (2014)
Bozcaada Island, Turkey	39.7	3	3	2	4	ALTUNIŞIK et al. (2014)

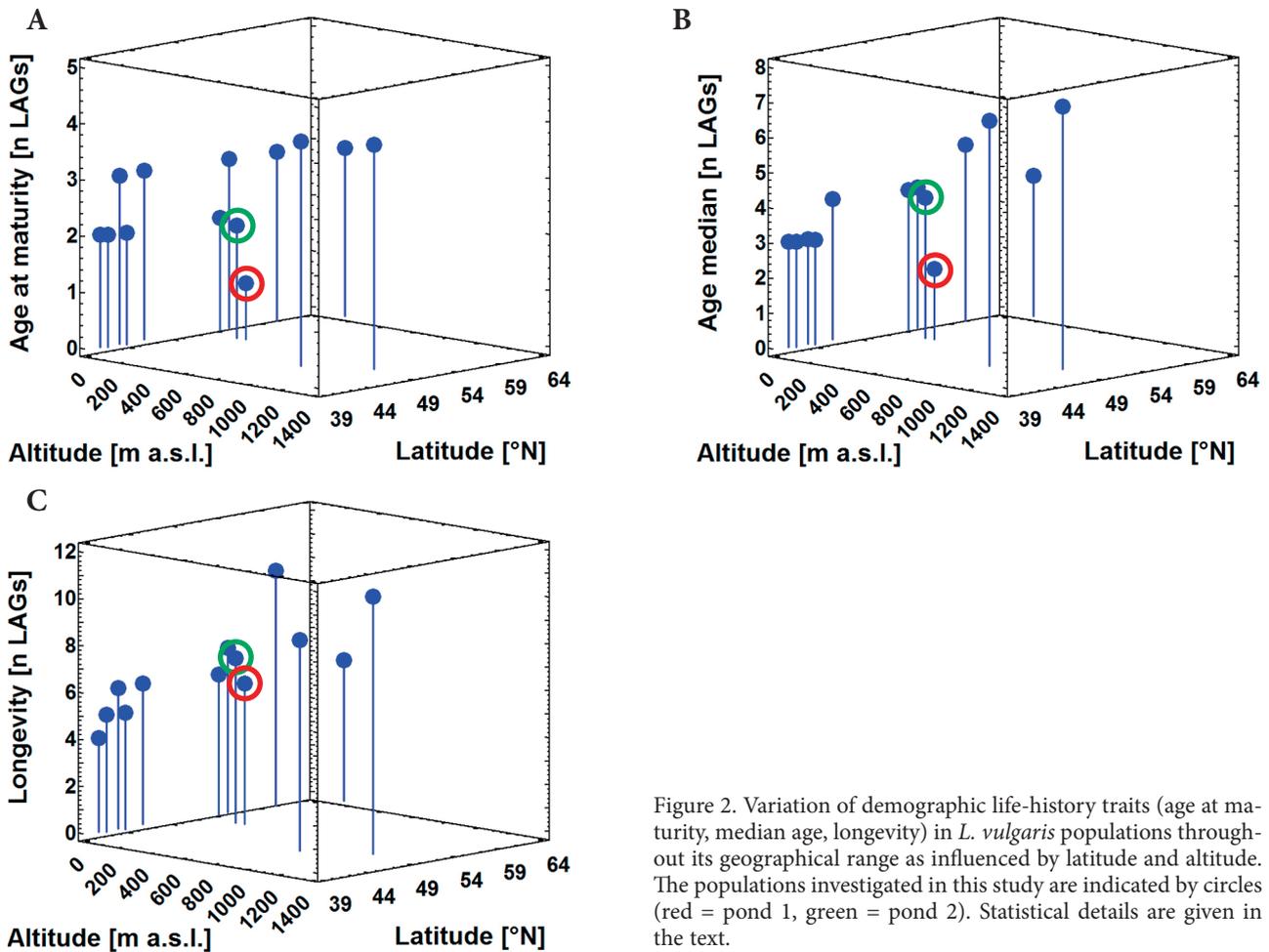


Figure 2. Variation of demographic life-history traits (age at maturity, median age, longevity) in *L. vulgaris* populations throughout its geographical range as influenced by latitude and altitude. The populations investigated in this study are indicated by circles (red = pond 1, green = pond 2). Statistical details are given in the text.

Effects of metacercariae load on newt hosts

Metacercariae load: The frequency of newts infested with *Parastrigea robusta* metacercariae was 73% in pond 1 and 10% in pond 2. Trematode cysts were attached exclusively to the wall of the body cavity, mostly at the dorsal part (Fig. 3; SINSCH et al. 2018). The number of metacercariae per infested newt ($n = 23$) varied between 1 and 23 (median: 4.5; Fig. 4). Log₁₀-normalized parasite load per newt did not differ significantly between localities (ANCOVA: $F_{1,22} = 3.18$, $P = 0.0904$) and the covariates age (ANCOVA: $F_{1,22} = 1.77$, $P = 0.1994$) and size (ANCOVA: $F_{1,22} = 0.26$, $P = 0.6172$) did not account for a significant amount of variance.

Size, body mass and condition index: Median SCL of pond 1 males was significantly smaller than that of pond 2 males (40.9 mm vs. 43.6 mm; $W = 426.5$, $P = 0.0022$, Mann-Whitney-Wilcoxon W-Test). In contrast, median body mass did not differ significantly between pond 1 and pond 2 males (1428 mg vs. 1368 mg; $W = 91.0$, $P = 0.7084$, Mann-Whitney-Wilcoxon W-Test). The condition index was therefore biased significantly towards pond 1 males (1.0 vs. -0.36; $W = 38.0$, $P = 0.0068$, Mann-Whitney-Wil-

coxon W-Test). When kept in terraria from June 14 to September 8, all newts survived captivity. The condition index decreased slowly, but significantly by -0.013 per day (linear regression model, $F_{1,27} = 6.87$, $P = 0.0145$; $R^2 = 20.9\%$). Comparing the time course of condition decrease between infested and parasite-free pond 1 males, neither slopes ($P = 0.3142$) nor intercepts ($P = 0.8503$) of regression models differed significantly. The condition index of infested males did not differ significantly between localities (ANCOVA: $F_{1,19} = 1.44$, $P = 0.2459$) and the covariate age (ANCOVA: $F_{1,19} = 0.02$, $P = 0.8964$) did not account for a significant amount of variance.

Size of fat body: With a few exceptions the fat body of pond 1 males was undeveloped (size class 0), but almost all pond 2 males had fully developed fat bodies (size class 2). Consequently, median size of fat body in pond 1 males was significantly smaller than that in pond 2 males (0 vs. 2; $W = 544$, $P < 0.0001$, Mann-Whitney-Wilcoxon W-Test). However, independent of the pond of collection 18 out of 21 infested individuals had fat bodies of size class 0 and only 3 of size class 1.

Age: Between-ponds variation of age distribution limited to newts infested with metacercariae was significant

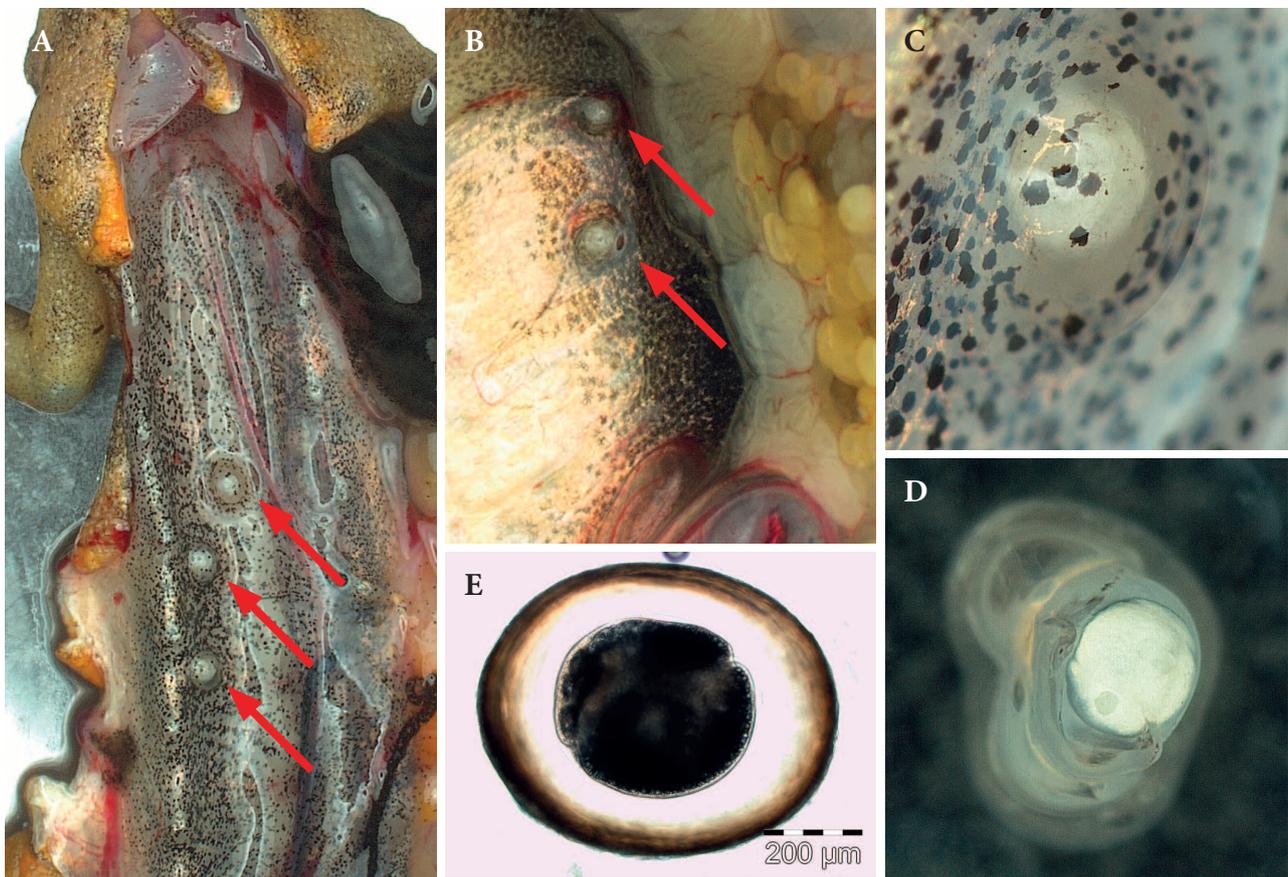


Figure 3. Infestation of (A) male and (B) female *L. vulgaris* with metacercariae cysts of *Parastrigea robusta* as indicated by red arrows. Details of metacercariae: (C) cyst *in situ* covered by the pigmented newt epithelium, (D) same cyst explanted (reflecting microscope view), (E) isolated cyst (transmission microscope view).

with respect to medians (2 LAGs (pond 1) vs. 5 LAGs (pond 2); $W = 35.5$, $P = 0.015$, Mann-Whitney-Wilcoxon W-Test). Age distributions of infected and uninfected newts differed significantly with respect to medians (2 LAGs vs. 3 LAGs; $W = 482.5$, $P = 0.0005$, Mann-Whitney-Wilcoxon W-Test) and to shape of distribution ($P < 0.0001$, two-sided Kolmogorov-Smirnov-Test; Fig. 5).

Discussion

Initially unexplained declines of amphibian populations have often led to the discovery of pathogens previously unknown or even undescribed for these specific hosts such as a ranavirus (BOLLINGER et al. 1999) and the fungi *Batrachochytrium dendrobatidis* (LONGCORE et al. 1999) and *B. salamandrivorans* (MARTEL et al. 2013). In the smooth newt population studied the agents of decline were the metacercariae of the trematode *Parastrigea robusta*, previously known to infest *Rana arvalis*, *R. temporaria*, *Triturus cristatus*, and *L. vulgaris* without known impact on the host populations in the wild (ODENING 1965, VOJTKOVÁ & VOJTEK 1972, VOJTEK 1972, 1989, SINSCH et al. 2018). An infection rate of 73% newts with 1–23 *P. robusta* metacercariae is unique for central European urodelans, as are the observed consequences for demographic life-history traits and population dynamics.

A long-known parasite becomes a new threat for smooth newt populations

The trematode *P. robusta* has been described by SZIDAT (1928) and its life cycle in the Berlin area analyzed in detail by ODENING (1965). Significant differences between the local life cycles in Berlin and the study pond are the first intermediate host (*Planorbis planorbis*/*Anisus vortex* vs. *Gyraulus parvus*) and the second intermediate host

(*R. temporaria*/*R. arvalis* tadpoles vs. *L. vulgaris* adults), whereas the common ground is the definitive host the mallard *Anas platyrhynchos* (SINSCH & BREUER 2018, SINSCH et al. 2018). Key to the up rise of the trematode as a significant newt threat is probably the sequence pond rehabilitation – invasion of the neophyte *Elodea canadensis* (maybe introduced by the mallards) – invasion of the neozoon *G. parvus*. The removal of the original *Chara* submerse vegetation opened space for the fast growing *Elodea* which in turn provided perfect habitat conditions for *Gyraulus*. With the presence of a suitable snail host and an acceptable amphibian host for metacercariae the life cycle of *P. robusta* was completed.

The infection pathway to brown frog hosts is the infection of tadpoles by cercariae according to ODENING (1965). Mortality of heavily infested *R. arvalis* tadpoles was high, in the surviving tadpoles metacercariae cysts developed externally visible in the tail fin and within the body. In contrast, we never observed any cyst in *L. vulgaris* larvae suggesting that infection does not occur at the larval stage, in agreement with VOJTEK (1972). Infestation rates of brown frog metamorphs in the Berlin area were 60–100%, with metacercariae cysts located visibly in the dorsal subcutis and within the body cavity (ODENING 1965, ZHIGILEVA & KIRINA 2015). It is unknown whether or not infected brown frog juveniles survive to adulthood. In contrast, smooth newts seem to become infected, when they return as adults to the pond for reproduction. Feeding on infected snails (we often observed *G. parvus* in the intestines of newts) is one possible pathway, the other one is direct penetration of skin by waterborne cercariae (VOJTKOVÁ & VOJTEK 1972). As we never observed subcutaneous cysts as in brown frogs, skin penetration by cercariae appears to be the less likely pathway.

Susceptibility of amphibian hosts to *P. robusta* infections seems to vary for unknown reasons. Post-metamorphic *R. arvalis* and *R. temporaria* from the Berlin region were heavily infested (60–100%; ODENING 1965), whereas only a

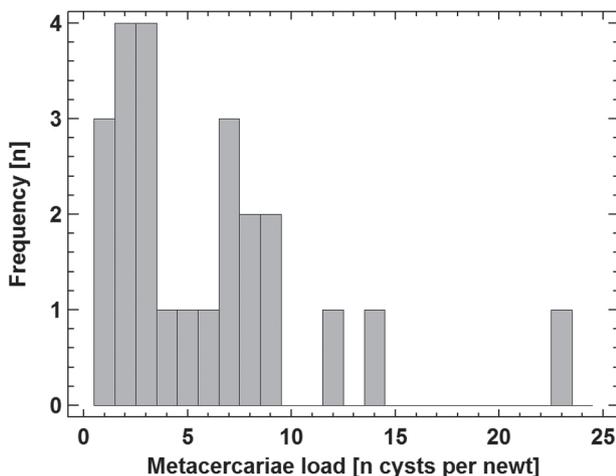


Figure 4. Frequency distribution of *P. strigea* cysts detected in 23 infested *L. vulgaris*.

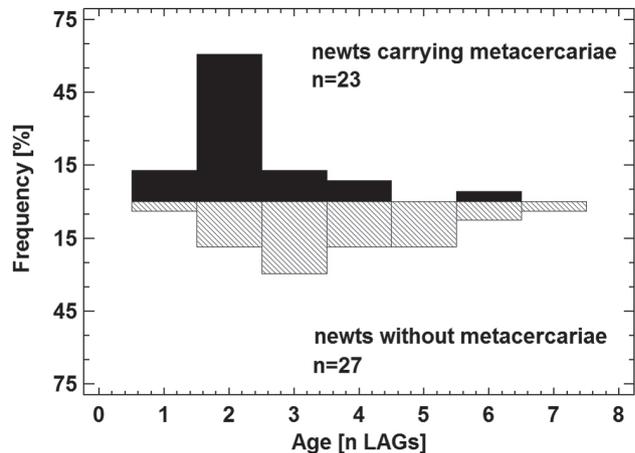


Figure 5. Age distributions of newts carrying metacercariae and of those without trematode infestation. Statistical details are given in the text.

single *R. arvalis* population out of eight studied in western Siberia was infested at 2.5% (ZHIGILEVA & KIRINA 2015), and frogs from the Brandenburg region near Berlin and from the Duisburg region were all *P. robusta*-free (SPIELER 1990, ANDREAS 2007). Brown frogs from southern Moravia and Slovakia were never infested in the field, but syntopic newts were (VOJTEK 1972, 1989). Experimental infection trials in the laboratory with anuran hosts remained unsuccessful as well (VOJTEK 1972). In contrast, *T. cristatus* and *L. vulgaris* adults from different localities in the Czech Republic and Slovakia were moderately infested (ca. 35%; VOJTKOVÁ & VOJTEK 1972), whereas at the study area *T. cristatus* were not infested at all, and infestation rates of syntopic *L. vulgaris* were either 10% or 73% (SINSCH et al. 2018). The low infestation rate at pond 2 was probably due to the fact that mallards visited rarely this ditch and that the *G. parvus* was not detected (SINSCH & BREUER 2018), i.e. the *P. strigea* life cycle could not be completed in this pond. We speculate that two of the 20 smooth newts captured in pond 2 had become infected elsewhere before reaching the breeding pond. The factors controlling epidemic spread of and immunity against *P. robusta* infestation within a population or species in amphibians remain to be studied, but a deep impact on demography and population dynamic as observed in pond 1 seems to be exceptional.

Impact on demographic life-history traits

As predicted by life-history theory in response to increased external mortality sources (STEARNS 1992), the heavily infested smooth newt population differed from all other *L. vulgaris* populations (including that of pond 2) analyzed so far by early sexual maturation and a significantly reduced median lifespan, whereas observed longevity was within the expected range (Fig. 2). This is consistent with the apparently low return rates of newts to pond 1 during the next-year breeding period which suggest low survival during the terrestrial phase following reproduction.

The unprecedented evidence that a substantial proportion (19.5%) of a smooth newt population mature and reproduce following the first hibernation (one year earlier than observed before) parallels the finding that *Bd*-infected *Litoria verreauxii alpina* populations from Australia reach maturity one year earlier than *Bd*-free populations (SCHEELE et al. 2017). These authors attribute the observed shift in age at maturity in *Bd*-challenged populations to chytridiomycosis as a substantial new selection pressure, increasing the probability to reproduce at least once. A comparable selection in response to the newts' metacercariae load does not appear plausible, given the relatively short period of *P. robusta* presence and an infection not before reaching the adult stage. Thus, the proximate cause of very early sexual maturation in this particular *L. vulgaris* population remains enigmatic at present.

Reduced median lifespan supports the inference of low survival of first breeders in the pond 1 population, but still one exceptional individual reached the expected longevi-

ty of six years. This individual was *P. robusta*-free suggesting that the infestation with metacercariae is the proximate cause of reduced longevity in the newt hosts. If we assume that most infested newts die within one year after infection, the fact that newts of any age were found carrying metacercariae may indicate that breeding newts have a low, but significant chance to avoid infestation during a single aquatic reproduction period. The rate of metacercariae-free newts during a given breeding period (27%) agrees well with the skeletochronologically estimated survival rate (29%) of the 2-years age class. Probabilistic estimation of infection chance during the following breeding periods suggests that about 98% of a cohort should have become infected (and subsequently died) before reaching the fifth year of life. This is in agreement with the very low number of the 4-years age class in pond 1, as compared with the sympatric pond 2 and other populations at similar altitudes.

The surprising result that feeding, infested newts survive at least until September, i.e. until a few weeks before the onset of hibernation, and the very low loss of condition index during this period suggest that there is no parasite-induced additive mortality component during the terrestrial activity period. However, the infested newts lacked almost completely metabolic reserves (fat body) enabling them to survive a prolonged hibernation without feeding (JØRGENSEN et al. 1979). Integrating the available features of infested newts, we propose the starvation-hypothesis (increased adult mortality of infested newts is due to starvation during hibernation) to explain the unusual low survival of smooth newts to be tested in future studies. Observational data as given below seem to support this hypothesis. If starvation during winter is the proximate cause for mortality, colder winter (= lower metabolic rates) are expected to promote higher survival rates than warmer ones. In fact, average minimum air temperatures (December–March) during the winter 2013/14 were 2.1°C higher than during 2014/15 (www.wetterkontor.de/de/wetter/deutschland/extremwerte.asp, [accessed January 8, 2018]), and in agreement with the starvation-hypothesis return rates of registered individuals were only 0.6% in spring 2014, but 3.5% in spring 2015 (Table 1).

Impact on population dynamics

The significant decline of smooth newt population size from fairly stable 800–1000 individuals to 200–500 at present is associated with pond restoration and the failure of recovery from that physical impact which was not observed in the syntopic crested and alpine newt populations recovering within four years to at least pre-restoration number (U. SINSCH, unpubl.). As the terrestrial habitat was not altered by pond restoration and breeding site fidelity is well-developed in this and other *L. vulgaris* populations throughout its range (e.g. BELL 1977, VERRELL 1987, SINSCH & KIRST 2016), the potential for recolonization and population growth was at least similar to that of the other newt species. A population size of about 1000 individuals is not

unusual in *L. vulgaris* breeding in ponds of comparable size (e.g. VERREL & HALLIDAY 1985, ORTMANN 2009), whereas 100–300 individuals are typical numbers of much smaller ponds (e.g. 4 m²; SCHATZ et al. 2012; 20 m²; KÜHNEL & SCHNEIDER 2013). Increased levels of predation on smooth newts by water birds such as the locally abundant herons (*Ardea cinerea*) and mallards (*Anas platyrhynchos*) as well as the occasionally visiting black stork (*Ciconia nigra*) are not probable because they do not discriminate among newt species. Recruitment of first breeders during the 2013–2015 study periods remained stable or doubled breeding population size from 2014 to 2015 indicating the impressive potential of this population to increase substantially.

The link between parasite load and observed and long-lasting decline of local population size following invasion of *E. canadensis* and the snail host *G. parvus* of *P. robusta* cercariae is supported by the fact that the failure of the *L. vulgaris* population is due to the unusual great adult mortality. The starvation-hypothesis proposes a comprehensible mechanism explaining adult mortality rates above all previously observed levels in smooth newts. Moreover, the decline restricted to a single breeding population emphasizes that the origin of the stressor is a local such as the presence of abundant *P. robusta* larval stages.

In conclusion, the local decline of smooth newts is most probably the result of an exceptional combination of the invasion of a neophyte (*Elodea*) and a neozoon (*G. parvus*) promoted by pond management measures establishing the successful developmental cycle of a newt parasite (*P. robusta*) in the breeding pond. Yet, future studies are needed to evaluate whether or not the observed fatal impact of the trematode on newt survival is general and a new threat in habitats favorable to support high parasite densities.

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