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No short term effect of *Clinostomum complanatum* (Trematoda: Digenea: Clinostomatidae) on survival of *Triturus carnifex* (Amphibia: Urodela: Salamandridae)

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Abstract. In this paper we evaluated the effects of the metacercariae of *C. complanatum* on body condition and survival probability of the Italian crested newt. We studied two populations of *T. carnifex*, with one of the two infected by the trematode, in central Italy, for a year. We found that: i) paedomorphic newts were more likely to be infected; ii) cysts were mostly located on the head and into the mouth; iii) the infection did not affect the body size of newts; iv) survival probabilities of infected and not infected newts from the same population did not differ, and it was similar to the survival of newts from the not-infected population. We conclude that, at least in the short term, the metacercariae of *C. complanatum* are not damaging for *T. carnifex*.

Keywords. Short term survival, *Triturus carnifex*, *Clinostomum complanatum*, host-pathogen interaction, capture-mark-recapture

The global amphibian decline rekindled the interest in amphibian diseases and the pathogens that cause them. In particular, the fungi *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, iridoviruses and bacterial infections have received much attention, since they caused mass mortalities worldwide (Daszak et al., 1999; Green et al., 2002; Wake and Vredenburg, 2008; Martel et al., 2014). Infections by other parasites got less attention, but it is known that a variety of organisms, such as Protozoans and Nematoda (Schmidt and Roberts, 2009), Maxillopoda (Marquardt et al., 2000) and Trematoda, can be amphibians' pathogens.

A remarkable number of species of Trematoda infecting amphibians is reported (e.g., http://www.colorado.edu/eeb/facultysites/pieter/Parasite_Database/Trematodes.html). Trematoda cause harmful infections in fish (Kim and Nagasawa, 1996; Lane and Morris, 2000) and reduced growth and poor body condition, limb malformation and even death in amphibians (Hsu et al., 2004; Johnson and McKenzie, 2008).

We have studied the infection of the digenetic trematode *Clinostomum complanatum* Rudolphi, 1814 at the expense of a population of *Triturus carnifex* (Laurenti, 1768). *Clinostomum* infect snails as first intermediate hosts and fish or amphibians as second intermediate ones. In amphibians the infection usually consists of dermal cysts (Sutherland, 2005) which contain metacercariae (yellow grub). The final host is represented by fish-eating birds where metacercariae mature in and eggs are released via bird feces or from their beak.

Clinostomum species have been found to have infected several species of amphibians (reviewed in Caffara et al., 2014) resulting in pathological changes (Miller et al., 2004). Reports of amphibians infected by *Clinostomum* were from North America only, until Caffara et al. (2014) found a site in Central Italy where *Lissotriton vul-*

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garis and *T. carnifex* were infected by *C. complanatum*. The present epidemiological study mainly focuses on the prevalence of the yellow grub infection and its effects on body condition and survival in this Italian Crested Newt population.

We studied two sites in Tuscany, Central Italy. The site where newts are infected by yellow grubs (already reported in Caffara et al., 2014; from this point forward it will be coded as SF) is an artificial pond built in 2008, 35 m a.s.l., surface 256 m^2 and maximum depth 2 m, inside the SCI-SPA IT51140011, in Sesto Fiorentino municipality. As a control site, we studied a natural pool (coded as AN) in a xerophytic wood, 655 m a.s.l., surface 53 m^2 and maximum depth 1 m, in Anghiari municipality. Both sites host *L. vulgaris* and *T. carnifex*; no potential predators like fish or crayfish have been found inside; water level remains almost constant all year round in both sites.

The Italian Crested Newts were sampled by dip-netting from March 2012 to March 2013 at SF (16 capture occasions, one every 26.7 days on average), and from July 2012 to May 2013 at AN (seven capture occasions, one every 53 days on average); capture sessions lasted about two hours. At SF two funnel traps were also placed in the pond for the whole night before the dip-netting session. Newts were marked by photographing their ventral spot pattern, they were sexed, and most of them were measured (snout-vent length, SVL henceforth; weight was not recorded since we supposed it would have varied during the study period); the life stage (paedomorphic or metamorphosed adult) has also been recorded. Paedomorphic individuals were discriminated from larvae on the basis of cloaca shape and by the presence of secondary sexual characteristics.

The metacercariae of *Clinostomum* sp. show a predilection for the skin (Sutherland, 2005), causing evident cysts (Gray et al., 2007), thus they are easy to be found by visual inspection. Cysts similar to *Clinostomum* ones may be caused by mesomycetozoan organisms, such as *Amphibiocystidium*, but they can be distinguished by a trained observer (see also Raffel et al., 2008). We also inspected the inner mouth by using a Kimura spatula. The number and location of cysts of each single newt have been recorded. Newts were immediately released after this in-field procedure.

The data we collected at SF has been used: i) to evaluate the association between infection (infected/not-infected newts), life stage (paedomorphic/metamorphosed) and sex (log-linear analysis, delta = 0.5, 100 maximum iterations, convergence criterion = 0.01); ii) to assess if the cysts were randomly distributed on the whole body or if they were clustered (chi-square test); iii) to evaluate if the infection per se affected the body size (for the ANCOVA the covariate was the interaction term between sex and life stage) or if the number of metacercariae did (Spearman correlation). Parametric statistics were applied when the data met normality assumption and when appropriate; the software STATISTICA 7 (StatSoft Inc.) has been used for statistical analyses.

Capture-mark-recapture (CMR) data were used to evaluate if the infection affected the individual survival. Estimates of survival probability were obtained via the program MARK (White and Burnham, 1999) under the Cormack-Jolly-Seber (CJS) model. For SF we considered 334 newts for an amount of 567 captures. Basing on this dataset, we built 24 models, considering the following parametrizations for survival: constant [phi(.)], time dependent [phi(t)], dependency on life stage [phi(.*l)], interaction between time and life stage [phi(t*l)], interaction between life stage and sex [phi(.*l*s)], interaction between time, life stage and sex [phi(t*l*s)]; and the following parametrizations for capture probability: constant [p(.)], time dependent [p(t)], dependency on life stage [p(.*l)], interaction between time and life stage [p(t*l)]; further, the same 24 models were run including the number of parasites as individual covariate for survival, thus obtaining an amount of 48 models at SF.

For the sake of comparison, a CMR study was also carried out at AN (141 newts were captured, 230 captures). No newts in this site showed visible lesions consistent with *C. complanatum*, and only 3 paedomorphic newts were captured (they have been removed from further analyses). Consequently, we ran eight CMR models under CJS model, considering survival as phi(.), phi(t), phi(.*s) or phi(t*s), and capture probability as p(.) or p(t).

Before the analyses, a closure test confirmed that the populations were open [Stanley and Burnham (1999) closure test: SF: chi-square = 420.04, d.f. = 25, $P < 0.001$; AN: chi-square = 205.57, d.f. = 13, $P < 0.001$]. Program U-CARE (Choquet et al., 2005) was used to evaluate the goodness of fit of the CJS model to both data-sets. CJS model fits the data collected at SF (chi-square = 115.43, d.f. = 134, P = 0.87), but not at AN (chi-square = 27.84, d.f. = 16, $P < 0.05$) due to trap-happiness of females. However, since the resulting c-hat was quite low (1.73), we only corrected for overdispersion at AN by adjusting the c-hat in the program MARK. Consequently, the evaluation of the best model is based on AICc for SF, and on QAICc for AN.

Out of 340 newts captured at SF, 98 were infected (28.8%; no individual out of 141 from AN was infected), with one to eight yellow grubs. Log-linear analysis returned as the model that best fits the data the association sex×life stage and life stage×infected/not-infected (chi-square = 0.68, d.f. = 2, P = 0.71). A significantly larger proportion of paedomorphic newts were infected (29.5%) in comparison with metamorphosed newts (23.5%) (chi-square = 7, d.f. = 1, P < 0.01).

Forty-one infected newts were captured more than once. The number of parasites changed in 22 newts without any apparent time pattern. Two individuals that became infected and four that lost their only parasite were removed from further analyses.

Cysts were found in the head (86 cysts in 57 newts), into the mouth (51 in 41 newts), in the trunk (7 in 7 newts), limbs (5 in 3 newts) and tail (10 in 8 newts) (chi-square = 163.46, d.f. = 4, P < 0.001). The average number of parasites per individuals (1.74 ± 0.12) was not correlated with SVL (R_S = -0.11, P = 0.3, n = 82).

Body size of infected newts did not differ from the size of not-infected newts ($F_{1,297}$ = 0.59, P = 0.44), even when taking into account life stage and sex (ANCOVA, $F_{1,294}$ = 1.92, P = 0.16; a two-way ANOVA revealed significant differences between life stages, sexes and their interaction).

Model fitting for survival analysis reported phi(t) p(.*l) as the best model for SF, with an AICc weight of 0.998. Consequently, none of the models including the number of parasites as covariate got support. Survival estimates from the best model are reported in Fig. 1. The best model for AN, phi(t)p(.), did not have a strong support, with a model weight equal to 0.59; the sixth ranked model had a ΔQAICc equal to 5.95 and model weight 0.03, the seventh model had weight < 0.001 and ΔQAICc equals to 13.52, thus estimates of survival at AN in Fig. 1 are based on model averaging (survival differences among sexes got a global weight of 0.13, meaning numerical difference at the third decimal number; for convenience, the figure reports only female survival, which was the lower one).

Our findings show that *Clinostomum* infection neither caused higher mortality rate nor affected body size of *T. carnifex*. In the infected SF population survival did not differ between infected and not-infected newts, and it was very similar to survival of the non-infected AN population (during the time period the two studies overlapped). Usually, infectious diseases are studied at individual level, while few studies evaluated the effects of an infection on population demography (Woodhams et al., 2011). We showed that during a short time period yellow grubs infection did not affect adult newts survival. SF population had been continuously monitored since 2008, and the first record of *C. complanatum* dates back to October 2010. *Clinostomum* metacercariae can survive in the host for about 4 years (Elliot and Russert, 1949), consequently any possible effect on survival could have been evident at the time of the study (March 2012-March 2013). Therefore, although the data were collected in a short time, our findings may be considered substantial.

The abundant presence of metacercariae on the head and into the mouth may be linked to the exposure of

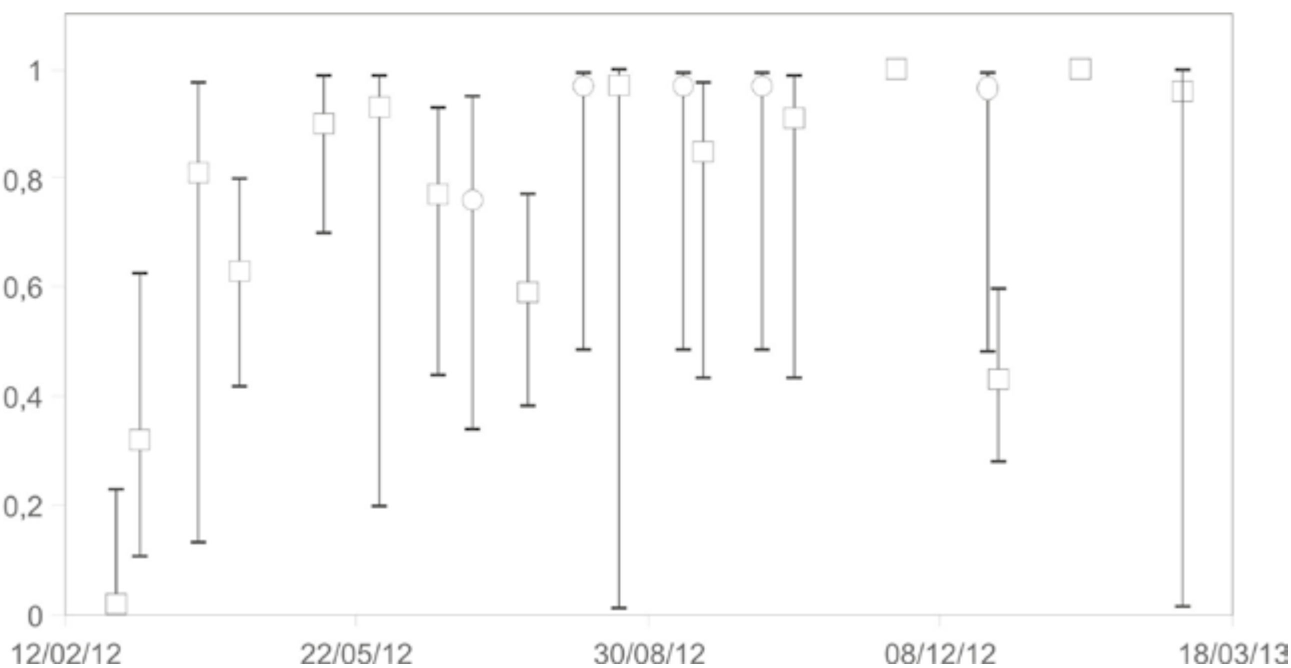

Fig. 1. Survival probabilities at SF (squares) and AN (circles) sites, with 95% C.I.

these regions while feeding. In fact the diet of *T. carnifex* includes freshwater molluscs (Fasola and Canova, 1992), that are intermediate host of *Clinostomum*. The cephalic region could also be selected by the parasite as the location with the main organs of the newts, where the parasite can cause damage and inhibit some vital functions (Miller et al., 2004), likely making it easier for the birds to prey on newts.

The success of the infestation by trematodes is strongly influenced by environmental and ecological characteristics, including abundance of intermediate hosts (e.g., Johnson and Chase, 2004) and the presence of seasonal definitive hosts. SF pond is surrounded by wetlands where bird species, like *Ardea cinerea*, *A. purpurea*, *A. alba*, *Ardeola ralloides*, *Nycticorax nycticorax* and *Egretta garzetta*, which can host adults of *C. complanatum* (Al-Salim and Ali, 2010; Shamsi et al., 2013) have been frequently observed. In the pond there are also various species of freshwater molluscs. Thus, all the hosts required for the life cycle of *C. complanatum* to be completed are present. On the contrary, AN is a small, isolated basin in a wooded area, where we did not observe any waterfowls. However, it is also important to take into account that occurrence of helmint infections in amphibians may indicate an environmental stress or a disequilibrium in the trophic web (Johnson and Lunde, 2005; Green et al., 2009). Environmental modifications, such as water pollution by pesticides and introduction of allochthonous species might foster the parasitosis emergence (Daszak et al., 2000) and modify the natural dynamics between host and parasite (Rohr et al., 2008; Poulin, 2010). Indeed, the area surrounding SF are heavily influenced by agriculture and infested by allochthonous species like *Procambarus clarkii*, *Gambusia holbrooki*, *Pseudorasbora parva*, *Trachemys scripta* and *Myocastor coypus*. On the contrary, the wooded area surrounding AN could preserve it from environmental modifications.

A larger proportion of paedomorphic newts were infected compared to metamorphic ones. This suggests that the probability of infection is related to the time spent in water by the newts. Metamorphic newts spent part of the year on land (Vanni et al., 2007), therefore they are less exposed to the parasite. On the contrary, the aquatic larval and paedomorphic stages are prone to be infected. During our surveys we did not search deliberately for larvae, and the few we captured apparently were not infected. Even if further monitoring is required to analyse the long term survival, basing on our findings adult survival does not seem lowered by yellow grubs. However, it is important to assess if *Clinostomum* can represent a threat for amphibians by acting on larvae, something that can be achieved by including the recruitment rate in the study of populations' dynamic.

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