

Annual spawn clump losses in a population of the agile frog *Rana dalmatina* in western France

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ABSTRACT – The agile frog *Rana dalmatina* is a common and frequently studied species in Europe including long-term studies of population change. Several have employed spawn clump counts to give estimates of annual presence of reproductive females. Spawn clumps are also subject to predation but little is known of the extent of losses but it could impact population densities significantly. The objective of the present study was to assess the extent of spawn clump losses due to predation in a population of *R. dalmatina* in western France using data from numbers of spawn clumps recorded over a four-year period (2019–2022). Spawn counts were greatest during 2019 but numbers declined with a zero count in 2022. Predation was greatest in 2021, the year total counts were lowest, but there were no statistical difference between annual losses, which varied from 22.9–41.6 %. Most spawn was deposited in a series of ditches rather than in the two available ponds but statistically predation levels were in agreement, with ditches ranging from 26.1–40 % versus ponds 28.6–40 %. Observed predators were ducks and alien crayfish *Procambarus clarkii*. The latter consumes all stages of frog development and hence likely represents the greater threat, especially since it has the capacity for population increase to very high numbers.

INTRODUCTION

Pond breeding amphibians face a series of decisions when selecting areas for oviposition, these include pond temperature, probability of ponds drying out, the presence of food for both their larvae and adults and for predator presence, the latter in both the aquatic and surrounding terrestrial environments. The agile frog *Rana dalmatina* (Fig. 1) breeds in ponds throughout Europe where it is listed as of Least Concern by the IUCN in Appendix II of the Bern Convention (Kaya et al., 2009). Various aspects of its ecology have been studied (e.g. Gollman et al., 2002; Hartel, 2003; 2004; Puky et al., 2006; Bartoń & Rafiński, 2006; Meek, 2012; 2018; Combes et al., 2018; Jovanović & Crnobrnja-Isailović, 2019). Among these is a long-term population study of *R. dalmatina* in Vendee, western France based on counts of spawn clumps and road mortalities. This showed that although numbers fluctuated widely they were, in general, stable over the long-term (Meek, 2018). Reproductive activity in *R. dalmatina* begins when the adults arrive at ponds in late winter, when females lay one clutch of around 500–2000 eggs (e.g. Gollman et al., 2002; Hartel, 2003; 2004; 2008; Bartoń & Rafiński, 2006; Puky et al., 2006; Meek, 2012). These are usually initially fixed to underwater twigs or plants that slowly float to the pond surface. The benefits of surface floating spawn is probably increased heat from warm sunshine and hence more rapid rates of larvae development, but the potential costs are increased risk of predation due to high visibility on water surfaces and also risk of the spawn being encased in ice during extreme winter conditions (Meek, 2012).

Survival of spawn masses is a critical aspect of pond breeding amphibian population dynamics influencing numbers of larvae and ultimately breeding adults. Therefore



Figure 1. Example of *Rana dalmatina* found in the study area

when spawn mass predation levels are high, and if they remain high especially over several years, there is a potential for population collapse. In this study numbers of missing spawn clumps, assumed due to predation, were recorded during the 4-year period from 2019 through to 2022 in Vendee, western France. Two main questions were addressed:-

1. Were there differences in annual proportions of spawn clumps lost as a proportion of the total numbers laid? This is important because survivorship of larvae is one critical aspect of long-term population stability.
2. Were there differences in spawn mass lost in ditches compared to ponds? This is important because differences in predation pressure between ponds and ditches could also impact on long-term population stability especially if there are major differences in numbers of spawn deposited.

METHODS

The study area is a fragmented landscape dominated by agriculture in Vendée, western France (46° 27' N; 1° 53' W). The first spawn clumps were usually sighted in early to mid February and counts were made by a single observer along the edges of three ditches and two ponds. Photographs of the spawn clumps were usually made alongside habitat features to enhance count accuracy, especially when concentrations were large.

Sampling periods were for 13 days between 10 February and 14 March (2019), 12 days between 2 to 23 March (2020) and 12 days from 23 February to 9 March 2021. During 2022, sampling began 1 February and continued into late April, due to the need to confirm a zero count. Figure 2 shows a map of the study area and Figure 3 photographic examples. Clutches of *R. dalmatina* were counted in ditches situated alongside a hedgerow abutted by farmland, alongside roads, a New Pond excavated in 2009 and a long established pond (Old Pond).

To compare missing spawn clumps as proportions of total numbers deposited, z-tests for two independent proportions were used. Comparisons were between years and between ponds and ditches. The null hypothesis in both data sets is that annual losses were in approximate agreement, $H_0: p_1 = p_2$ where p_1 is the proportion of the first sample and p_2 the proportion of the second sample. The resulting P-values are based on two-tailed tests. Sample precision tests for the amount of variation around the z-scores derived from sample sizes for each of the three-year data sets were ± 11.6 (2019), ± 16.6 (2020) and $\pm 20\%$ (2021).

RESULTS

Spawn mass numbers declined during the 4-year sampling period. In 2019 a total of 74 spawn clumps were counted with 17 of these recorded missing (22.9 %). For 2020 spawn count was 35, with 10 recorded missing (28.6 %). During 2021 total spawn mass was 24 with 10 recorded missing (41 %). However, the proportional losses were not significantly different between years; 2019 versus 2020, $z = 0.62$, $P = 0.53$; 2019 versus 2021 $z = 1.59$, $P = 0.11$ and 2020 versus 2021, $z = 0.96$, $P = 0.33$. Hence despite the differences in sample sizes the proportional losses were in statistical agreement (Fig. 4). The majority of spawn clumps were deposited in the three ditches. To improve sample sizes for comparisons the data from ponds and ditches were each pooled. Spawn counts in the three ditches formed 87.5 % ($n = 91$) of total spawn counts from 2019 to 2021, with the remainder deposited in the two ponds ($n = 13$; C & D in Fig. 3). Most spawn losses were recorded for rue de Bourneau and in the ditch alongside open fields (A in Fig. 2). The proportional losses for the pooled 3 year total counts between ponds and ditches were not significant $z = 0.32$, $P = 0.74$.

DISCUSSION

The results of this study have indicated that, irrespective of total annual counts, spawn mass losses in each of the surveyed areas were approximately similar (question 1) with



Figure 2. Map of the study areas showing sections sampled. Marked are **A** ditch running alongside agricultural fields, **B** and **C** roadside ditches along with the location of the two ponds. See Fig. 3 for photographs of these features.

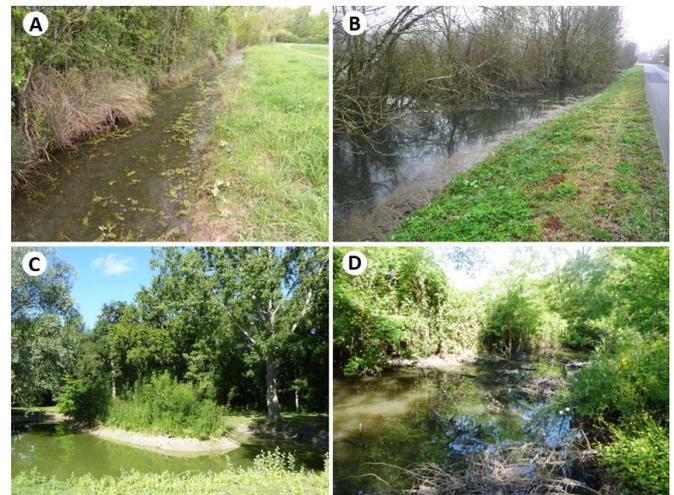


Figure 3. Water bodies in which *Rana dalmatina* spawn clumps were counted – **A.** & **B.** ditches, **C.** New Pond, and **D.** Old Pond

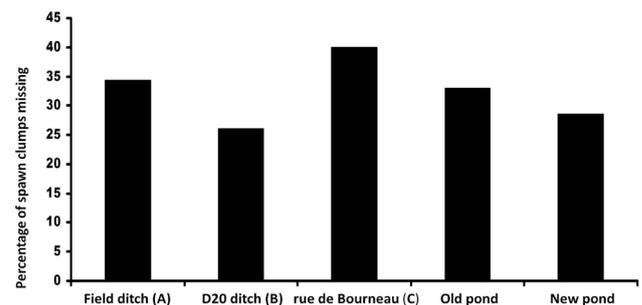


Figure 4. % of spawn clumps missing, assumed predated, during the study period. Data shown are pooled losses for the 3-year study period. Labeling on the x-axis refers to Fig. 2.

proportional losses also in agreement between ponds and ditches (question 2, and thus the null hypothesis is confirmed in both questions). This was perhaps an unexpected result given the habitat difference between pond and ditches.

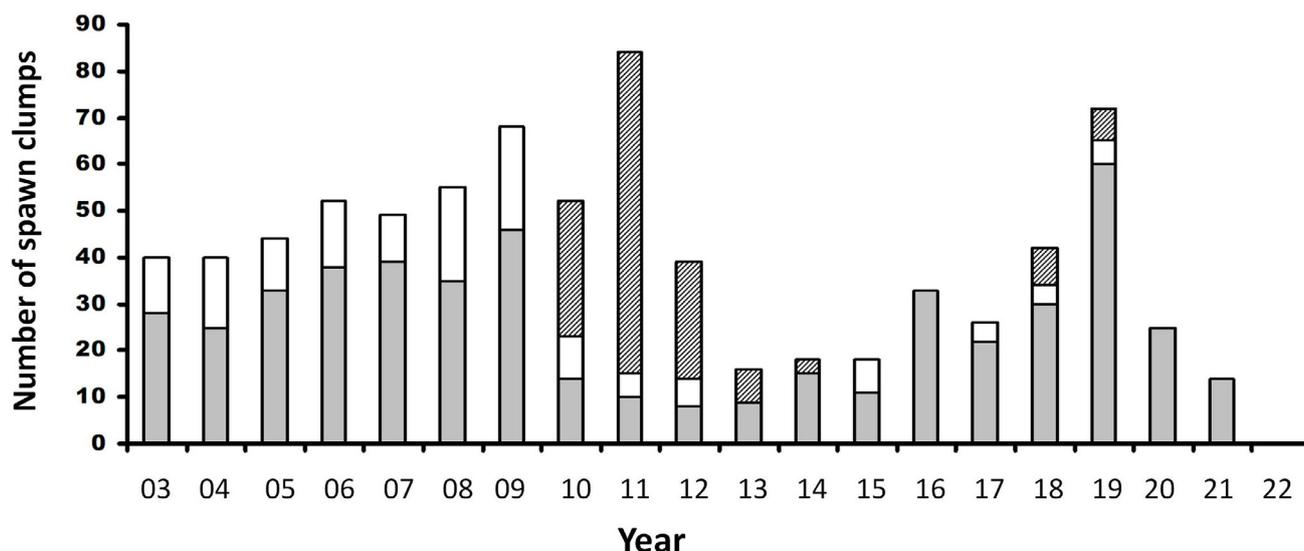


Figure 5. Spawn mass numbers deposited in the study area between 2003 and 2022. The histograms show final counts at the finish of the spawning period and hence do not include missing spawn counts. Grey bars represent pooled data for ditches; open bars Old Pond and crosshatched bars New Pond. Data from 2003 to 2018 are taken from Meek (2018), and 2019 to 2022, this study.

However, during the four-year study period spawn counts were mostly in ditches with smaller quantities in ponds and hence data comparison was potentially less reliable. The results suggest however that, despite total annual spawn mass counts, predation pressures were essentially similar, although further data are needed to support this finding.

The zero count recorded in 2022 was also unexpected but it should be kept in mind that spawn clumps represent only a proxy for numbers of reproductive females and hence does not necessarily indicate a local extinction, since males and smaller non-reproductive females may still be present in the locality. This followed a period of declining numbers beginning 2019 but was similar to the trend observed from 2011 when numbers declined from a 20 year high to low counts between 2013 and 2015 (Fig. 5). This trend was inversely mirrored by increases in numbers of alien crayfish *Procambarus clarkii* (Ficetola et al., 2011) a species well known to consume all stages of amphibian development, including adults. Crayfish numbers also fluctuate widely and in the study locality experience regular population crashes followed by increases. In the present study *P. clarkii* numbers began to increase from around 2018/19 and in 2021 were seen for the first time in areas B and C (Fig. 2). High numbers have been recently reported 6 km from the study area, for example in large garden ponds in the village of St Denis du Payre and in Deux-Sèvres region (e.g. Xavier Bonnet pers. com.). In addition to consuming spawn and larvae they effectively eliminate macrophyte cover, one of the key pond requirements for *R. dalmatina* breeding pond selection (Pavignano et al., 1990; Puky et al., 2006). Absence of macrophyte cover is normal in the New Pond when *P. clarkii* is present.

Several species of European amphibian are apparently able to detect *P. clarkii* presence. For example, Nunes et al.

(2013) found that five of nine European species of anurans changed their behaviour when *P. clarkii* was present, apparently chemical cues emitted from predated or injured conspecifics is the means of detection. High numbers of spawn were being deposited in the New Pond during 2010 and 2011 after it was created in 2009 but declined with the arrival of crayfish, becoming absent by 2015. *Rana dalmatina* forages widely across the landscape and appears not to be faithful to particular breeding sites. Consequently, if it is capable of detecting *P. clarkii* presence then it may simply migrate to other breeding areas (Blab, 1986; Puky et al., 2002; Gollmann et al., 2002). A second observed predator is ducks that feed on spawn although they will not necessarily consume a whole spawn mass leaving perhaps 10 % or so.

Previous amphibian research has shown that many, including European species, may occasionally miss breeding in certain years due to some ecological disturbance (Renoirt et al., 2021; Meek, 2021; 2022); pond drying is a good example (e.g. Lomas & Anderson, 2006). In the case of the green frog *Pelophylax lessonae* numbers in the study locality declined from high annual counts to a 4-year total absence followed by population recovery (Meek, 2021). A zero count was also recorded in a population of sympatric *Bufo spinosus* followed by a limited recovery (Meek, 2022). The recoveries were attributed to immigration from adjacent populations in both species. Absence of breeding female *B. spinosus* has been observed in other areas of western France (Renoirt et al., 2021). These species are classed as highly fecund pond-breeding amphibians with wide annual variations in population size. This renders them susceptible to population crashes along with capacity for recovery (Green, 2003). The present results have relevance in that they suggest predation pressure on *R. dalmatina* spawn clumps persist at an almost constant rate. If a major ecological disturbance occurs when

spawn mass numbers are at a lower bound during a period of population oscillation, for example, during high numbers of *P. clarkii*, the potential for a zero count increases. Continual monitoring of spawn mass counts is therefore needed to understand not only general long-term population trends and relationship with numbers of *P. clarkii* and other predators, but also the frequency of population zero counts.

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