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Low occurrence of the great crested newt *Triturus cristatus* at the limits of its range: an alarming preliminary study

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ABSTRACT - The lower Rhône valley constitutes the southern limit of the great crested newt's (*Triturus cristatus*) global distribution range. The initial objectives of this study were to confirm historical presence data, to identify potential new great crested newt populations and to characterise their habitats (with a Habitat Suitability Index) in order to assess their status in the lower Rhône valley. Despite the use of four different survey methods (eDNA, Ortmann traps, collapsible nylon traps, amphibian dip net), great crested newts were not detected except for one known population in Arles. HSI value results highlight the poor quality of wetlands in the studied area. Furthermore, the invasive crayfish *Procambarus clarkii* (detected in 60% of ponds) and the presence of fish (detected by sight in 50% of ponds) are known to have a negative impact on habitat quality and amphibian populations. These limiting factors might be one explanation for the critical conservation state of the *T. cristatus* Rhône populations.

INTRODUCTION

Amphibian populations are in decline worldwide (Collins & Storfer, 2003; Stuart et al., 2004). This has led to public concern, particularly because amphibians are indicator species of wetland quality (Welsh & Ollivier, 1998). The great crested newt, *Triturus cristatus*, is a large urodele species with a wide distribution across central and northern parts of Europe. Despite this wide distribution, southern populations have recently declined significantly in Europe (Gasc et al., 2004; Edgar & Bird, 2006; Denoël, 2012). The middle and lower Rhône valley constitutes the southern limit of its distribution range in France and in the World. Unfortunately during the 20th century, development of hydraulic infrastructure along the river reduced the area of associated functional wetlands. In this southern valley, the species is rare and only a few relict populations have been recorded in Bouches-du-Rhône (Brogard et al., 1996), in Gard (Brogard et al., 1996; Gendre & Rufray, 2005; Gendre et al., 2006; Geniez & Cheylan, 2012), in Vaucluse (Mourgues, unpublished data); in Ardèche (Parrain, 2005; Grossi, 2015) and in Drôme (Parrain, 2005 ; Parrain, 2010; Grossi, 2015). Among the recorded populations, some have been regularly monitored since the 1990's. For the others, the latest positive data are from the early 2000's or before. The objectives of this study were to confirm those historical presence data, to identify potential new great crested newt populations and to characterise their habitats in order to assess their status in the southernmost part of the Rhône valley.

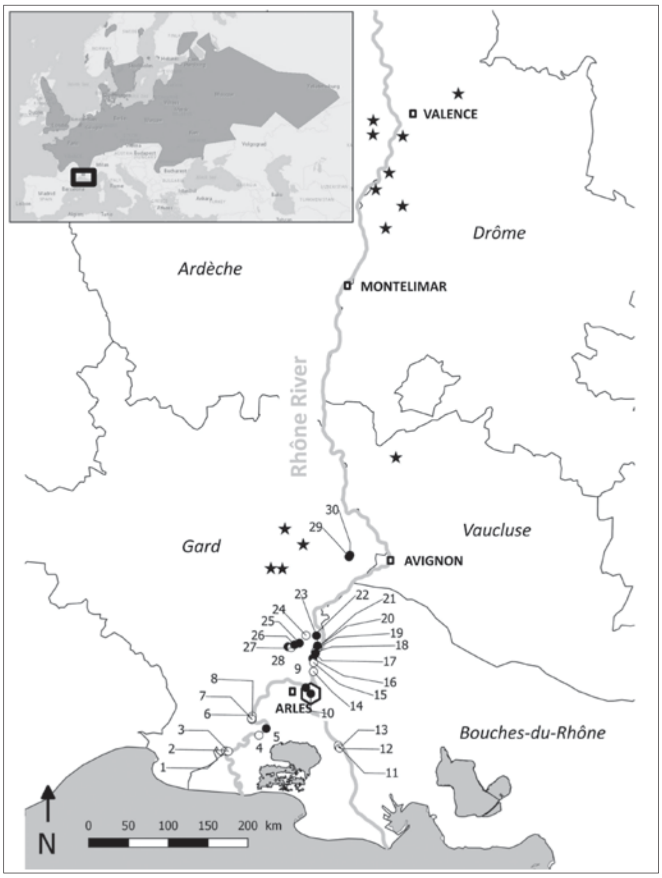

Figure 1: *Triturus cristatus* populations in the southern Rhône valley and location of studied sites; black dots: prospected historical sites; white dots: prospected non historical sites; diamond: presence of crested newts in 2017 in prospected sites; black stars: known populations outside of the studied area from Geniez & Cheylan (2012) and Grossi (2015). Box at top left: Worldwide distribution range of *T. cristatus* (Esri, USGS, NOAA | Sources: Esri, Garmin, USGS, NPS | IUCN)

Table 1. Results of field survey site by site; H.P.: Historical presence; L.D.: Last data; P.P.: Prospecting pressure (number of survey methods used); HSI score: Habitat Suitability Index score; P.S: Pond suitability (based on HSI score); P.A: Presence (1) – absence (0) of *T. cristatus*

Id	Latitude	Longitude	H.P.	L.D.	P.P.	HSI score	P.S.	P.A.
1	43°32'51.468" N	4°20'47.580" E	No	-	4	0.39	Poor	0
2	43°32'48.660" N	4°20'54.564" E	Yes	2006	4	0.37	Poor	0
3	43°32'47.364" N	4°21'17.892" E	No	-	2	0.44	Poor	0
4	43°34'57.252" N	4°26'58.848" E	No	-	4	0.63	Average	0
5	43°35'52.116" N	4°28'20.280" E	Yes	2002	1	0.54	Below average	0
6	43°37'4.6560" N	4°25'40.548" E	No	-	4	0.4	Poor	0
7	43°37'11.676" N	4°25'36.948" E	No	-	4	0.62	Average	0
8	43°37'30.864" N	4°25'43.608" E	No	-	4	0.49	Poor	0
9	43°41'21.768" N	4°35'40.920" E	Yes	2006	4	0.29	Poor	0
10	43°40'32.952" N	4°36'36.720" E	Yes	2016	2	0.37	Poor	1
11	43°33'34.200" N	4°41'42.360" E	No	-	1	0.44	Poor	0
12	43°33'26.424" N	4°41'42.936" E	No	-	2	0.53	Below average	0
13	43°33'14.148" N	4°41'58.272" E	No	-	3	0.63	Average	0
14	43°43'30.936" N	4°37'6.6360" E	No	-	2	0.33	Poor	0
15	43°44'43.188" N	4°37'9.1920" E	No	-	1	0.25	Poor	0
16	43°45'17.244" N	4°36'59.112" E	No	-	4	0.54	Below average	0
17	43°45'56.628" N	4°37'27.948" E	Yes	2002	4	0.57	Below average	0
18	43°46'7.6080" N	4°37'26.256" E	No	-	4	0.59	Below average	0
19	43°46'42.492" N	4°37'54.336" E	No	-	3	0.57	Below average	0
20	43°46'47.424" N	4°37'48.540" E	Yes	2001	4	0.42	Poor	0
21	43°46'46.992" N	4°37'56.676" E	No	-	3	0.41	Poor	0
22	43°46'59.232" N	4°37'50.664" E	Yes	2006	2	0.54	Below average	0
23	43°48'20.772" N	4°37'40.188" E	Yes	2002	4	0.6	Average	0
24	43°48'22.068" N	4°35'43.620" E	Non	-	2	0.39	Poor	0
25	43°47'20.148" N	4°34'30.360" E	Yes	2004	4	0.23	Poor	0
26	43°47'7.5840" N	4°33'34.452" E	Yes	1995	4	0.66	Average	0
27	43°46'45.120" N	4°32'58.632" E	No	-	3	0.61	Average	0
28	43°46'51.492" N	4°32'22.236" E	No	-	2	0.77	Good	0
29	43°58'53.976" N	4°43'42.636" E	Yes	1956/57	4	0.49	Poor	0
30	43°59'8.1240" N	4°43'53.076" E	Yes	1956/57	4	0.64	Average	0

MATERIALS AND METHODS

Studied sites were geo-referenced historic sites located in the lower floodplain of the Rhône River which have not been confirmed in over 10 years or more except for one pond (n°10), a known presence location, which was used in this study as a control site. Also, other peripheral wetlands potentially suitable for *T. cristatus* detected on ortho-photographs (photographs geometrically adjusted for perspective) or on exploratory field work were surveyed.

Field surveys started in March and ended in June 2017. In order to maximize and compare detection probability, four techniques were combined:

• Ortmann funnel traps, which can be applied in aquatic environments for sampling and monitoring amphibian species both at larval and adult stages (Drechsler et al., 2010).

• Collapsible nylon traps (45 x 22 x 22 cm), also used to detect adult or larval stages of amphibian species, particularly *T. cristatus* (Madden & Jehle, 2013). To provide constant access to air, the traps were placed in order to make sure that part of the net always emerged from the water. Both trapping systems were installed at the end of the day and recovered the following morning.

• Dip netting by day, was used to detect the larval stage during the emergence period (May and June).

• Environmental DNA (eDNA) monitoring, is an emerging detection method which uses nuclear or mitochondrial DNA released from an organism into the environment (Ficetola et al., 2008). This technique is used to detect mostly rare and discreet aquatic species and is very effective for *T. cristatus* detection even at low density (Rees et al., 2014; Rees et al., 2017). We used the VigiDNA SW1 kit from SPYGEN society. We used one kit (20 samples of 100 ml each) for each water body with a surface area < 1 ha.

In total, 16 sites were sampled with all four methods, four sites with three methods, seven sites with two and three sites with only one of the four methods.

To determine habitat suitability for *T. cristatus* we used the Habitat Suitability Index (HSI) (Oldham et al., 2000). The HSI is a geometric mean of ten suitability variables: location, pond area, pond drying rate, water quality, shade, fowl abundance indicator, fish abundance indicator, number of ponds, terrestrial habitat and macrophyte abundance indicator. HSI scores are between 0 and 1, where 1 indicates habitats that best meet the ecological requirements of the focal species (in our case, *T. cristatus*). This tool can therefore be used to predict likely presence or absence of *T. cristatus* (O'Brien et al., 2017). In addition of HSI, two other variables that may impact the presence of *T. cristatus* were collected: presence of *Procambarus clarkii*, a common invasive crayfish species in the southern Rhône valley, and water turbidity. The detection of other urodele species was possible with three of the four methods used: we noted these species. We surveyed a total of 30 wetlands (Fig. 1). Among these, 12 were historical presence sites for *T. cristatus*; the other 18 were peripheral ponds or stagnant branches of the Rhône River.

RESULTS

The crested newt *T. cristatus* was detected in only one pond (n°10) with both Ortmann funnel traps and collapsible nylon traps (eDNA was not monitored in this pond). Palmate newts *Lissotriton helveticus* were found in 16% of the surveyed ponds (n=5). *Procambarus clarkii* were detected in 60% (n =18). The turbidity evaluation revealed that water was clear in 33% of wetlands (n=10), cloudy in 30% (n=9) and opaque in 37% of them (n=11). Fish were detected by sight in 50% of the sites (n = 15).

The mean HSI was 0.49 which represents a "below average" mean habitat suitability according to Oldham's et al., (2000) index interpretation. In the only pond where *T. cristatus* was detected, the HSI was 0.37 ("poor"). Of the searched ponds, 73% had a "below average" or "poor" HSI score (< 0.50) (Table 1).

DISCUSSION

This study failed to confirm the presence of *T. cristatus* in the lower Rhône valley, except in an already known population (pond n°10) that had been monitored for several years (Renet & Olivier, 2012). As only the presence of a species can be unequivocally confirmed, its absence can only be inferred with a degree of probability (Kery, 2002). Therefore, it is necessary to remain cautious about these results, for example survey effort was not equal between all searched sites. Indeed, it was only possible to use all four detection methods in 54% (n=16) of the ponds due to extremely low water levels. However, a minimum of two detection methods were applied in 90% (n=27) of studied ponds and eDNA was monitored in all of them (except for pond n°10). This study failed to identify the populations recorded around 2000 (Callégari & Gendre, unpublished data) despite using multiple, effective detection methods at most sites. Therefore, it is thought very probable that all of those populations are now extinct.

The population of pond n°10 is the only known remaining population in the lower Rhône floodplain, and also the most southerly in the worldwide distribution range of *T. cristatus*. Currently, the pond is threatened by an urban project that increases the major conservation issues for this population.

Because only one extant population was detected, it was not possible to:

1 - Statistically compare detection methods

2 -Test the reliability of the HSI in a Mediterranean context.

The HSI results from this study suggest wetlands in the studied area are of poor quality for *T. cristatus*. This bad quality might be one explanation for the critical conservation state of the *T. cristatus* Rhône valley populations.

The present study also recorded fish presence on half of the sites but this could be an underestimate considering that there was no fish detection protocol, and that, in contrast to *P. clarkii*, fish cannot be detected with the traps used in the study. Generally, amphibians are less abundant, and sometimes absent, when predatory fish are present because of the high predation pressure they exert (Hecnar & M'Closkey, 1997; Denoël et al., 2005; Porej & Hetherington, 2005; Hartel et al., 2007). *Procambarus clarkii* was also present in the majority of surveyed ponds (63%) following its introduction to the lower Rhône valley in 1976 (Rosecchi & Poizat, 1997; Vigneux, 1997). This invasive crayfish is a polytrophic keystone species that can exert multiple pressures on ecosystems by modifying biotic factors. As an example, it has been demonstrated that the introduction of *P. clarkii* to ponds can reduce macrophyte biomass, and increase the turbidity (Rodríguez et al., 2003; Gherardi & Acquistapace, 2007; Rodríguez-Pérez et al., 2016; Souty-Grosset et al., 2016). The species is also suspected to directly prey upon amphibian larvae (Cruz et al., 2006; Cruz et al., 2008). Despite the likely disapearance of *T. cristatus* in the studied ponds, *L. helveticus* populations persisted in five ponds. Among these, *P. clarkii* was detected in only one. The deleterious actions of the crayfish are no doubt one of the reasons for this observation.

The low HSI scores of the Rhône wetlands highlight that they probably do not fulfil the ecological requirements of *T. cristatus*. The deterioration of those habitats might be only partly explained by the arrival of *P. clarkii* on the national territory. Indeed, long term environmental modifications induced by land-use policies probably affected the resilience of *T. cristatus* populations by isolating them and diminishing the number of shelter ponds within pond networks. With the arrival of exogenous species like *P. clarkii* the situation has deterioated. Moreover, the geographical location of these *T. cristatus* populations is disadvantageous: range limit populations are particularly sensitive to habitat loss and fragmentation (Sexton et al., 2009; Slatyer et al., 2013).

Nevertheless some *T. cristatus* populations have maintained in the middle and lower Rhône Valley outside of the study area. This might be explained by the isolation of those populations from the hydraulic network that has prevented fish or crayfish invasions and their negative consequences on the habitat. Survey efforts must be maintained in order to confirm the results of this one year study. The effort need to be focused firstly on historical sites and on peripheral ponds isolated from the hydraulic network, less likely to be impacted by *P. clarkii*. Subsequently, efforts should be extended to the surrounding area, especially further north where other small isolated populations are known.

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