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Long-time effect of an invasive fish on the Odonata assemblage in a Mediterranean lake and early response after rotenone treatment

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Abstract. Over a thirty year period from 1977 to 2007, 16 Odonata species were recorded in the Nature Reserve "Laguna de Zóñar", Cordova, Andalusia, Spain. Thermophilic dragonflies with wide distribution in the African continent were dominant in recent years. In 1985, Common carp *Cyprinus carpio* L., 1758 were introduced in an uncontrolled and illegal action, and aquatic macrophytes and benthonic macroinvertebrates of the lake practically disappeared within ten years. One of the most successful tools for controlling and eradicating invasive fish is the use of chemical compounds such as rotenone. This compound adversely affects aquatic organisms with gill respiration by inhibiting oxygen intake at the cell level. Here, we analyse the effect on the Odonata assemblage of this lake after treatment with rotenone intended to eradicate the carp population. During the first year after treatment, nine Odonata species were recorded, and at least six of them obviously had completed their life cycle in the lake. We also carried out the determination of the five last growth stadia in *Orthetrum cancellatum* larvae, and we propose that, in the southern Iberian Peninsula, this species has a univoltine life cycle with asynchronous emergence.

Further key words. Dragonfly, conservation, invasive species control, Mediterranean area, life cycle.

Introduction

Invasive species are considered severe threats to biodiversity and constitute a growing problem with progressive globalisation. At the world level, the number of introduced species has doubled compared to three decades ago (Williamson & Fitter 1996; Vitousek et al. 1997; Sala et al. 2000; Gozlan 2008). The introduction of an invasive species into a new ecosystem can prove fatal to the system (Gozlan & Newton 2009) or to some of its species due to diverse mechanisms, such as predation, competition, or hybridisation (Gozlan et al. 2010).

Globally, fish pose an urgent problem and 136 species of freshwater fish (26 families, 13 orders) have been introduced to the Mediterranean regions (Marr et al. 2013). The control of these species becomes necessary to maintain the high diversity that characterizes these ecosystems with respect to land systems (Sala et al. 2000).

The threat that the presence of Common carp *Cyprinus carpio* L., 1758 presents for larval Odonata populations and the deterioration of water quality in the lakes of the Mediterranean basin has been discussed by Samraoui et al. (1993). In 1985, Common carp was introduced to Zóñar Lake (Cordova province, southern Spain) in an uncontrolled and illegal action. The activity of the carp caused resuspension of the sediments, increased water turbidity and eutrophication, uprooting of the aquatic macrophytes, disappearance of the benthonic macroinvertebrates, progressive decrease in the occurrence of diving ducks, viz. *Oxyura leucocephala* (Scopoli, 1769) and *Aythya ferina* Boie, 1822, until their complete absence, and an increase in the presence of fish-eating birds, such as *Ardea cinerea* L., 1758 and *Phalacrocorax carbo* L., 1758 (Fernández-Delgado et al. 2004).

One of the most successful tools for controlling and eradicating invasive fish is the use of chemical compounds. This especially applies to rotenone, a crystalline isoflavone extracted from the roots of several tropical legume species originating from the jungles of South-East Asia and the Peruvian and Brazilian Amazon (Finlayson et al. 2000), which is used as a broad-spectrum insecticide and piscicide.

Rotenone can be lethal to aquatic organisms with gill respiration, as it inhibits the uptake of oxygen at the cell level (Lockett 1998). Consequently, during the treatment of a water body with this compound, damage to other aquatic fauna, such as other species of fish, amphibians in the larval phase, and aquatic invertebrates is inevitable. In the latter group, the effects have not been well studied as they vary depending on water temperature, application rate, and the taxa involved (Binns 1967; Cook & Moore 1969; Engstrom-Heg et al. 1978; Mangum & Madrigal 1999; Eriksen et al. 2009; Finlayson et al. 2010; Kjærstad & Arnekleiv 2011). Rotenone has been used in the USA (for the first time in 1934), several South American countries (e.g., the Galapagos Islands, Ecuador), Norway, and Oceania, but, as far as we know, never before in the Mediterranean area.

In the present work, the effect on the Odonata community after rotenone treatment used to eradicate the carp population in Zóñar Lake, a protected area in southern Spain, is analysed. This study increases the knowledge concerning the way, in which this tool for controlling invasive fish species affects macroinvertebrate communities in the lakes of the Mediterranean basin.

Material and methods

Study site

The Nature Reserve "Laguna de Zóñar" (Fig. 1) is situated near Aguilar de la Frontera, Cordova province, Andalusia, southern Spain (37°29'05"N, 04°41'27"W; 300 m a.s.l.). The water of Zóñar Lake has a surface area of almost 38 ha and reaches a depth of 16 m (Torres-Esquivias et al. 2009). This lake forms part of the Natural Reserve of the Wetlands of Southern Cordova, according to regional law (Ley andaluza 2/1989), and in 1987 it was designated ZEPA (Zona de Especial Protección de Aves; Directive 79/409/CEE) and was included on the list of the Ramsar Convention (Convention on Wetlands of International Importance) in 1989. In November 1977, Zóñar Lake was the only site in Western Europe where a continuous population of White-headed Duck *Oxyura leucocephala* existed, however, with only 22 individuals (Torres-Esquivias 1982).

Meta-analysis about Odonata assemblage

According to the available bibliography and the present study records, four damselfly and twelve dragonfly species had been recorded in Zóñar Lake over a period of thirty years. The first studies on Odonata from this lake date from November 1977 and from the period of February to October 1980; the results of both samplings were published by Ferreras-Romero (1983). The first sampling in November 1977 yielded larvae of *Erythromma lindenii* (Selys, 1840), *Ischnura graellsii* (Rambur, 1842), *Crocothemis erythraea* (Brullé, 1832), and *Selysiothemis nigra* (Vander Linden, 1825). In the course of eight samplings in 1980, besides these four species, *Sympecma fusca* (Vander Linden, 1820), *Enallagma cyathigerum* (Charpentier, 1840), *Anax* *ephippiger* (Burmeister, 1839), *A. imperator* Leach, 1815, *A. parthenope* Selys, 1839, *Diplacodes lefebvrii* (Rambur, 1842), *Orthetrum cancellatum* (L., 1758), *Sympetrum fonscolombii* (Selys, 1840), and *Trithemis annulata* (Palisot de Beauvois, 1807) were also recorded. In the same year, larvae and exuviae of *I. graellsii* and *A. parthenope*, plus larvae of *S. fonscolombii* were also collected.

Later, García-Rojas et al. (1986) reported the capture of larvae of *I. graellsii*, *A. imperator*, *A. parthenope*, *C. erythraea*, and *S. fonscolombii* from February to May 1981 and July to December 1984. In a compilation of unpublished records of Odonata from Spain (Jödicke 1996), adults from Zóñar Lake recorded in mid-April 1988 by Peter Jahn are listed: *Enallagma cyathigerum*, *I. graellsii*, *Aeshna isoceles* (Müller, 1767), *A. parthenope*, and *S. fonscolombii*. Of particular interest is the record of *A. isoceles*, which in Andalusia had previously been reported only from the area of the Coto

Figure 1. Aerial photograph of the Nature Reserve "Laguna de Zóñar" near Aguilar de la Frontera, Cordova province, Andalusia, southern Spain, facing north (vi-2013). Photo by Juan de la Cruz Merino

Table 1. Records of Odonata species at and in Zóñar Lake, Cordova, Andalusia, Spain, over a period of 30 years, from 1977 to 2007. Common carp was introduced in 1985 and a rotenone treatment was effected in July 2006. L – larvae; E – exuviae; A – adults. References: (1) Ferreras-Romero (1983); (2) García-Rojas et al. (1986); (3) Jödicke (1996); (4) Fernández-Delgado (1997); (5) this study.

Study period	1977–80 (1)	1981–84 (2)	1988 (3)	1993–94 (4)	2006–07 (5)
S. fusca	A				
E. cyathigerum	A		A		A
E. lindenii	L, A				
I. graellsii	L, E, A	L	A		A
P. genei					L, E, A
A. isoceles			A		
A. ephippiger	A				
A. imperator	A	L			
A. parthenope	L, E, A	L	A		L, A
B. impartita					A
C. erythraea	L, A	L			L
D. lefebvrii	A				
O. cancellatum	A				L, A
S. nigra	L, A				
S. fonscolombii	L, A	L	A		L, E, A
T. annulata	A				L, A

Doñana wetlands (Belle 1979) and for which records in the Iberian Peninsula are generally very scarce. As this was an isolated sampling in mid-spring, it may be that the Odonata community continued basically unaltered until the end of the 1980s (Table 1).

In 1985, Common carp was introduced to Zóñar Lake. General consequences on the fauna were analysed by Sánchez & Fernández-Delgado (1997), Fernández-Delgado (1997), and Fernández-Delgado et al. (2004).

After the introduction of carp, a macroinvertebrate study was carried out between May 1993 and February 1994, in which four seasonal sampling campaigns were conducted (one per season: spring, summer, autumn, and winter), at different depths (1, 2, and 7 m) (Fernández-Delgado 1997).

This study demonstrated beyond doubt that the situation of the macroinvertebrate community had drastically changed with respect to that of previous years: only few specimens of Diptera (Chironomidae), one specimen of Heteroptera (Corixidae), and an Oligochaeta worm were collected. In addition, Fernández-Delgado (1997) only lists empty shells of ostracod crustaceans and molluscs. Probably, these first years of the decade of the 1990s was the beginning of the period of the lowest faunistic richness in the history of Zóñar Lake.

Rotenone treatment

The treatment in Zóñar Lake consisted of pouring 11 600 litres of rotenone (commercial brand CFT Legumine®) into the water column by specialised workers of the Andalusian Environmental Agency using four boats. The application took place in two phases: the first (on 10–12-vii-2006) with greater concentration (90 ppb) than the second (*ca* 50 ppb), which was applied six days later (17–18-vii-2006). It was estimated that the rotenone concentration was lethal to fish for approximately 15 days (10–24-vii-2006). The mean persistence of the rotenone was estimated at some six days due to the weather and water conditions (pH 9.0, water temperature *ca* 28°C, maximum air temperature *ca* 40°C, 14 h of sunlight per day). As a result of the treatment, *ca* 13 000 kg of dead carp were collected during these days. The whole process was supervised by the Andalusian Environmental Regional Agency, which also bore the cost of approximately 600.000 €.

A year later, in June 2007, it was confirmed that the action had reduced the average percentage of the anoxic component of the water column to between 45 and 57 % compared to averages measured in April 1994. Transparency measured by Secchi disk had increased from 1.0 to 2.3 m in depth, and the density of submerged macrophytes (*Zannichellia palustris* L., 1758, *Chara* sp., *Najas marina* L., 1758) in shallow areas of the lake had increased as well (Fernández-Delgado et al. 2007).

Study on the early response of Odonata

Field collections were performed under optimal weather conditions for dragonflies, i.e., sunny days without clouds and with low wind velocity <10 km/h.

To cover all phenological groups, from early spring species to late summer and autumn species, and to record the 'Representative Spectrum of Odonata Species' (Schmidt 1985; Chovanec & Waringer 2001), seven samplings of adult Odonata were conducted in 2006 and 2007. Two samplings were carried out in autumn (06-x- and 09-xi-2006) and five more during spring and early summer (24-iv-, 10-v-, 28-v-, 12-vi-, and 28-vi-2007). Each sampling consisted in recording and collecting specimens by walking around the entire perimeter of the lake.

Likewise, to identify reproducing populations of odonate species in the lake, five samplings of larvae and exuviae were conducted in 2006 and 2007. Two samplings of larvae and exuviae were performed in autumn (03-x- and 09-xi-2006) and three more during winter and spring (15-ii-, 24-iv-, and 21--v-2007). Each sampling consisted of the screening of approximately 10 m^2 of sediment with hand nets at four points of the lake, near the shore in the North, South, East, and West.

In the laboratory, head width – i.e., the maximum distance between the lateral margins of the compound eyes – of each larva was measured to the nearest 0.1 mm using a Nikon SMZ800 binocular microscope with an eyepiece micrometre. The number of abdominal segments covered by the metathoracic (hind) wing sheaths, if present, was also recorded. On the basis of head width and number of abdominal segments covered by wing sheaths, each larva was either assigned to one of the last five stadia or designated as a "smaller larva". Here we follow common practice, designating the final, penultimate, and preceding stadia as F-0, F-1, F-2, etc. Because the number of stadia in Odonata varies, within a population and probably also within a hatching cohort, it is informative to assign exact serial designations to stadia only near the beginning and end of larval life (Corbet 1999). External signs of metamorphosis, such as an extension of the compound eyes progressing behind their posterior limits, wing sheaths swollen and with the costal vein of the adult wing visible and folded concertina-like within the wing sheaths, and the prementum of the labium void of muscular tissue, were recorded to determine the onset and temporary location of intra-stadial changes discernible in F-0 larvae (Ferreras-Romero & Corbet 1999). The criteria for assigning medium-sized or large larvae of *O. cancellatum* to the respective larval stadium are listed in Table 2.

Table 2. Determination of the growth stadium of *Orthetrum cancellatum* larvae collected during five samplings from October 2006 to May 2007 in Zóñar Lake, Cordova, Andalusia, Spain. Number of larvae used to establish the size ranges of the different stadia is given in parentheses (n). S – abdominal segment.

Stadium (n)	Head width [mm]	Relative length of hind wing sheaths
F-0 (10)	5.3–5.7	Reaching end of S5 or middle of S6
F-1 (9)	4.2–4.3	Reaching S4 or beginning of S5
F-2 (9)	3.0–3.5	Reaching end of S2 or middle of S3
F-3 (3)	2.5–2.8	Reaching end of S1 or middle of S2
F-4 (4)	2.0–2.2	Reaching beginning or middle of S1
Smaller (7)	1.6–1.7	Not reaching beyond thorax

Results

Adults

During the autumn of 2006, three Odonata species were collected: *Brachythemis impartita* (Burmeister, 1839) (1♂), *S. fonscolombii* (4♂, 4♀), and *T. annulata* (3♂, 2♀). Four teneral specimens of *S. fonscolombii* collected at the beginning of October obviously had emerged only a few hours earlier (Table 3).

In the first sampling of spring 2007, on 24 April, *O. cancellatum* (1♂, teneral) and *S. fonscolombii* (2♂, 2♀) were the only species captured. More *O. cancellatum* tenerals were collected in May and early June, indicating asynchronous emergence. Male and female *S. fonscolombii* were captured in all five samples taken in April, May, and June 2007, with tenerals occurring only in early June (2♂, 4♀).

Males and females belonging to *B. impartita* were collected again from late May, but specimens in teneral stage (3♂) were found only in the late-June sampling (presumably the second generation of the year). *Paragomphus genei* (Selys, 1841) was captured in the late-May sampling (1♂, teneral) and in the two June samplings (1♂ early June, 1♀ late June). *Anax parthenope* was captured both in late May as well as in early June (1♂ each time). In the early-June sampling, *T. annulata* (1♂, 1♀, both teneral) appeared.

Concerning Zygoptera, *E. cyathigerum* and *I. graellsii* adults were found consistently from early May onwards, with tenerals of the former species appearing only in June samples.

Table 3. Observations of odonate tenerals at Zóñar Lake, Cordova, Andalusia, Spain, during the sampling period of adults. The potential annual generation (gen.) is indicated in parentheses for bi- and multivoltine species.

Species	First or sole record	Second record	Last record
Sympetrum fonscolombii	Oct. 2006 (3rd gen.)	early June 2007 (2nd gen.)	early June 2007
Orthetrum cancellatum	April 2007	early May 2007	early June 2007
Paragomphus genei	late May 2007		
Trithemis annulata	early June 2007 (2nd gen.)		
Enallagma cyathigerum	early June 2007 (2nd gen.)	late June 2007 (2nd gen.)	late June 2007
Brachythemis impartita	late June 2007 (2nd gen.)		

Larvae

Six species were collected as larvae and/or exuviae, the most frequent and abundant being *O. cancellatum* and *S. fonscolombii* (Table 4). All of them were present during autumn 2006, after the rotenone treatment, and four species appeared again in the sampling six months later in May 2007. The collection of F-0 larvae, many of them with evident signs of metamorphosis, and exuviae proved that *O. cancellatum*, *T. annulata*, and *P. genei* were completing their life cycles in the lake (Table 4). *Orthetrum cancellatum* showed growth during winter and spring, as well as metamorphosis in F-0 larvae from the end of winter to late May (Table 4). Specimens in various growth stadia were collected in almost all of the sampling campaigns. According to these data from the southern Iberian Peninsula, the species developed in a univoltine cycle with asynchronous emergence and with adults beginning flight in April. In addition, numerous *S. fonscolombii* larvae were collected in many developmental stadia, especially in spring.

Discussion

The Odonata assemblage in Zóñar Lake in southern Spain has been the object of several studies since the second half of the 1970s. Apparently, the Odonata community remained without major alterations until the end of the

Table 4. Larvae and exuviae found from October 2006 to May 2007 in aquatic samples from Zóñar Lake, Cordova, Andalusia, Spain. Larval head width and presence or absence of external signs of metamorphosis in final stadium larvae is indicated. Number of larvae or exuviae (n); final, penultimate, and preceding stadia (F-0, F-1, F-2, F-3, and F-4) or smaller larvae (F-s) are differentiated.

Species	Sampling date (n)	Head width [mm]	Range of stadia
Paragomphus genei	03-x-2006 (1)	2.0	F-s
	09-xi-2006 (1)	5.0	F-0 (without metamorphosis signs)
	21-v-2007 (2)		Exuviae
Anax sp.	03-x-2006 (1)	3.4	F-s
Orthetrum cancellatum	03-x-2006 (13)	1.6–4.3	F-s–F-1
	09-xi-2006 (3)	2.8–4.2	F-3–F-1
	15-ii-2007 (10)	2.0–5.7	F-4–F-0 (with metamorph. signs)
	24-iv-2007 (14)	2.0–5.6	F-4–F-0 (with metamorph. signs)
	21-v-2007 (2)	5.5	F-0 (with metamorphosis signs)
Crocothemis erythraea	03-x-2006 (2)	2.5–3.0	F-3
	09-xi-2006 (1)	3.2	F-2
Sympetrum fonscolombii	03-x-2006 (14)	1.1–5.4	F-s–F-0 (without metamorph. signs)
	09-xi-2006 (13)	1.5–4.3	F-s–F-1
	21-v-2007 (73)	0.6–4.1	F-s–F-1
Trithemis annulata	03-x-2006 (3)	2.0–3.7	F-s–F-1
	09-xi-2006 (9)	1.6–2.8	F-s–F-2
	21-v-2007 (1)	4.8	F-0 (with metamorphosis signs)

1980s. Fourteen species were recorded and evidence was found that at least seven species bred in this lake (Ferreras-Romero 1983; García-Rojas et al. 1986; Jödicke 1996). The introduction of Common carp in 1985 had the consequence that the situation of the macroinvertebrate community drastically changed during the first years of the 1990s, including the disappearance of all breeding populations of both dragonflies and damselflies. According to Dalu et al. (2015) benthic Aeshnidae (*A. imperator*) demonstrate lower mortality rates after rotenone treatment than gill-respiring taxa, such as Ephemeroptera, Plecoptera, and Trichoptera. Likewise, gill-respiring aquatic insects, such as Coenagrionidae, and plastron-respiring aquatic insects, such as Corixidae, were found to be differentially susceptible to rotenone (Booth et al. 2015). Results of our study suggest that the rotenone treatment in Zoñar Lake did not immediately impede the larval growth of several species during autumn and before emergence in the following spring (*S. fonscolombii*).

During the autumn after the rotenone treatment, larvae of *P. genei*, *S. fonscolombii*, *O. cancellatum*, *C. erythraea*, *T. annulata*, and *Anax* sp. were collected. Of the first three species, some individuals were in an advanced stage of growth (F-0, F-1), presumably originating from eggs laid during the summer of 2006. Likewise, adults belonging to three thermophilic libellulid species that in the southern Iberian Peninsula produce several generations per year (Montes et al. 1982; Agüero-Pelegrín & Ferreras-Romero 1992; Corbet et al. 2006) were recorded: *Brachythemis impartita*, *S. fonscolombii*, and *T. annulata*.

There is evidence that in *P. genei*, *O. cancellatum*, and *T. annulata* larval growth continued from autumn to spring, followed by metamorphosis and subsequent emergence. Imagoes of *P. genei* emerged in spring 2007 as we collected exuviae and teneral adults. In Central European temperate climate zones, *O. cancellatum* is considered a late-spring species (Schmidt 1985), whereas in North Africa it is regarded as a summer species (Samraoui & Corbet 2000), however, with a long flight period spanning from April to the end of August. There are noteworthy coincidences, although not absolute, with the findings of Robert (1958) in terms of head width and extension of the metathoracic hind wing sheaths over the abdominal segments of the last five larval stadia in this species. However, while Robert estimated the existence of between 11 and 12 moults and at least three years to complete the cycle in Switzerland, Central Europe (»Cycle complet: 3 ans au moins? – 11–12 mues?«), our results indicate that in the southern Iberian Peninsula *O. cancellatum* is univoltine with asynchronous emergence from the second half of April to June, at least. The presumably extensive period of oviposition explains the wide range of larval sizes in our samples and the coexistence of at least five larval stadia from autumn to spring. A *T. annulata* larva of the last stage with signs of metamorphosis was found in the May sample. However, it was difficult to determine whether this specimen belonged to the first or second generation of the year (Agüero-Pelegrín & Ferreras-Romero 1994).

On the other hand, no evidence was found about whether the first generation of *S. fonscolombii* in 2007 emerged from Zóñar Lake: the first teneral adults were observed in June, a date rather indicating the second of the at least three generations the species produces each year (Corbet et al. 2006). The individuals of this species captured in April and May could have originated from other places more or less close to the lake. With the data compiled, it is only certain that the eggs that produced the second generation of the year had been deposited there by the adults during spring of the same year. A similar dynamic might occur with *B. impartita*: adults arriving at the lake in spring successfully produced the second generation of the year. Tenerals of both species were collected in June. The data gathered did not give evidence that *A. parthenope*, a resident species in the lake, completed its cycle after the rotenone treatment.

Finally, two Coenagrionidae species, *E. cyathigerum* and *I. graellsii*, were captured after winter 2007. Coenagrionidae oviposit endophytically, and consequently, they need submerged and floating aquatic vegetation to complete their biological cycle. Thus, the limiting factor explaining the absence of larvae and exuviae of these Zygoptera in the lake during the immediate months after the rotenone treatment, i.e., during autumn 2006, is probably the lack of aquatic vegetation during the years prior to treatment due to the high density of carp. This must have strongly affected the development of stable populations of *E. cyathigerum* and *I. graellsii*, which were common before the introduction of carp (Ferreras-Romero 1983; García-Rojas et al. 1986; Jödicke 1996). In the southern Iberian Peninsula, *I. graellsii* is multivoltine (Montes et al. 1982) and the most ubiquitous and tolerant of all Iberian Zygoptera (Ferreras-Romero et al. 2009). Therefore, *I. graellsii* will undoubtedly become established again in the lake with the recovery of aquatic vegetation. In the case of *E. cyathigerum*, together with the collection of teneral specimens in June, the presence of adults from May onwards indicates the establishment of a population of this species, which in Southern Europe is bivoltine (Corbet et al. 2006).

The data gathered in the present study with the collection of adults, larvae, and exuviae provides consistent and solid evidence that at least six species obviously completed their life cycles in Zóñar Lake within 12 months following the rotenone treatment: *Enallagma cyathigerum*, *O. cancellatum*, *P. genei*, *B. impartita*, *S. fonscolombii,* and *T. annulata*. The last four of these species are thermophilic and have a wide distribution across the African continent. *Paragomphus genei* and *B. impartita* had not been recorded in Zóñar Lake in the previous studies.

In summary, the rotenone treatment obviously did not harm populations of the Gomphidae or Libellulidae, which can be considered potential regular residents of Zóñar Lake. In addition, the elimination of Common carp has resulted in the regeneration and rapid extension of submerged aquatic vegetation, permitting the establishment of breeding populations of two species of Coenagrionidae cited from the lake in 20th century studies.

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