

## Trophic behaviour of the dragonfly *Cordulegaster boltoni* (Insecta: Odonata) in small creeks in NW Italy

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The order Odonata comprises many predaceous species that inhabit aquatic systems, and a few that are restricted to flowing waters. *Cordulegaster boltoni* is a widespread Odonata in Europe, which usually inhabits small lotic systems. In this study we analysed the gut contents of *C. boltoni* immature stages, collected in the Rocchetta Tanaro Natural Park (Italy, Piemonte). Two hundred and eleven individuals were collected, and their diet analyzed by dissection or clearing. Larvae appeared to be opportunistic predators, feeding on a variety of prey. Aquatic insects dominated their diet, while crustaceans, annelids, molluscs and terrestrial invertebrates were sporadically observed in the gut contents. An ontogenetic shift in the diet was detected, as small larvae consumed different prey than large ones. Our study suggests that *C. boltoni* is one of the dominant predators in the benthic communities of lowland small order streams of Piemonte, which, because of their environmental characteristics, are devoid of fish and stoneflies.

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### 1. Introduction

Aquatic insects have an important role in the trophic structure of lotic systems, and the number of studies focused on their feeding habits has been increasing (e.g. Monakov 2003, Bo *et al.* 2008, López-Rodríguez *et al.* 2009). Odonata inhabit a broad range of habitats, including ponds, lakes, swamps, marshes and streams. Few families,

such as Calopterygidae, Cordulegasteridae, and, to a lesser extent, Gomphidae, are restricted to flowing waters.

Despite their important trophic role, relatively few studies investigated feeding habits of Odonata, particularly in flowing water ecosystems. Odonata are predatory insects, both in the adult and nymphal stages. In particular, Odonata immatures are generally ambush predators that

detect prey using mechanical and visual stimuli. Rebola *et al.* (2004) tested the response of Anisoptera larvae to different kind of prey stimuli, and observed that chemical cues seem to have little or no contribution in the detection of prey. Blois (1990) investigated prey preferences of *Anax imperator* Leach, 1815 in an experimental study with two prey types (Chironomidae and Corixidae), and detected that larvae of this species generally feed on the most abundant prey. A recent study, conducted on large species such as *Anax imperator*, *Aeshna cyanea* (Müller, 1764) and *Libellula depressa* Linnaeus, 1758, concluded that larval diets changed with developmental stage (Blois 2006), with an increase of size and diversity of prey items correlated to increasing size of larvae. In the same study, prey preferences and selection mechanisms in these three species were observed. The trophic preferences of nymphs of *Enallagma* Charpentier, 1840 (Coenagrionidae) have been investigated in laboratory enclosures with different habitat complexity and using selected prey types. The results from this study indicate that prey vulnerability to *Enallagma* predation is species-specific rather than habitat-determined (Lombardo 1997). These and other studies are focused mainly on lentic species of Odonata, and mostly analysed feeding behaviour in laboratory assays (Bergelson 1985, Blois 1985, Blois & Cloarec 1985, Johnson 1991, Rebola *et al.* 2004).

The family Cordulegasteridae is represented in Europe by the sole genus *Cordulegaster* Leach, 1815 (Lohmann 1992, 1993), which has a Holarctic distribution (Askew 2004). Three species are present in Italy: *Cordulegaster bidentata* Sélys, 1843, *C. boltoni* (Donovan, 1807), and *C. picta* Sélys, 1854. *Cordulegaster trinacriae* Waterston, 1976, known from Sicily and mainland Italy, can be considered a subspecies of either *C. boltoni* or, according to other authors, *C. picta* (Askew 2004). *Cordulegaster boltoni*, commonly named Golden-ringed Dragonfly, occurs in Europe from southern Spain and Italy to southern Scandinavia, and is also reported from Tunisia, Algeria and Morocco (Askew 2004). This species inhabits small lotic systems, where larvae construct shallow depressions in fine sediments. *Cordulegaster boltoni* is a partivoltine taxon (Corbet *et al.* 1960, Corbet 1962), with a pro-

longed and relatively flexible pre-imaginal development. Different lengths of larval life has been observed in different areas: three or two years in southern Spain (Ferrerias-Romero & Corbet 1999) and Mediterranean France (Schütte 1997), four to five years in northeastern Germany (Donath 1987) and Yugoslavia (Kiauta 1964), and more than five years in the United Kingdom (Corbet *et al.* 1960).

The aim of our study was to characterise the diet of immature stages of a strictly lotic dragonfly, *C. boltoni*, in natural conditions. Furthermore, we tested whether shifts in prey selection occurred during larval ontogenesis.

## 2. Material and methods

The study sites are located in the Rocchetta Tanaro Natural Park, northwestern Italy (44°51'35"N, 8°20'48"E, 130 m a.s.l.). The landcover is composed largely of dense woodlands with a few, little villages. The environmental quality of lotic systems of the area is good (Bo *et al.* 2010). The study sites are classified as first class in the Extended Biotic Index system (Ghetti 1997), which corresponds to an environment with minimal human-induced impacts.

Two small creeks, Rio Rabengo and Rio Roninaggio, were sampled (four stations in each lotic system). The two creeks are very close (about 1.5 km) and have identical environmental characteristics. Rio Rabengo and Rio Roninaggio are 2<sup>nd</sup> order perennial lotic environments, characterized by gentle slope, low to moderate water current (ranging from 10 to 20 cm/s on average), fine substrata (80% sand – 20% fine gravel), and high amounts of coarse vegetal detritus. The mean water depth and width were 16.4 cm ( $\pm 7.6$  SD) and 91.8 cm ( $\pm 16.8$  SD), respectively. Riparian vegetation is mainly composed of *Alnus glutinosa*, *Corylus avellana*, *Salix alba*, *Populus alba*, and *Acer campestre*. The vegetation is very dense and causes significant shading of the stream channels.

Every month from April 2008 to May 2009, macroinvertebrates were collected using a kick-net (250  $\mu$ m mesh), and specimens were preserved in 90% ethanol. All organisms were counted and identified to the genus level, except

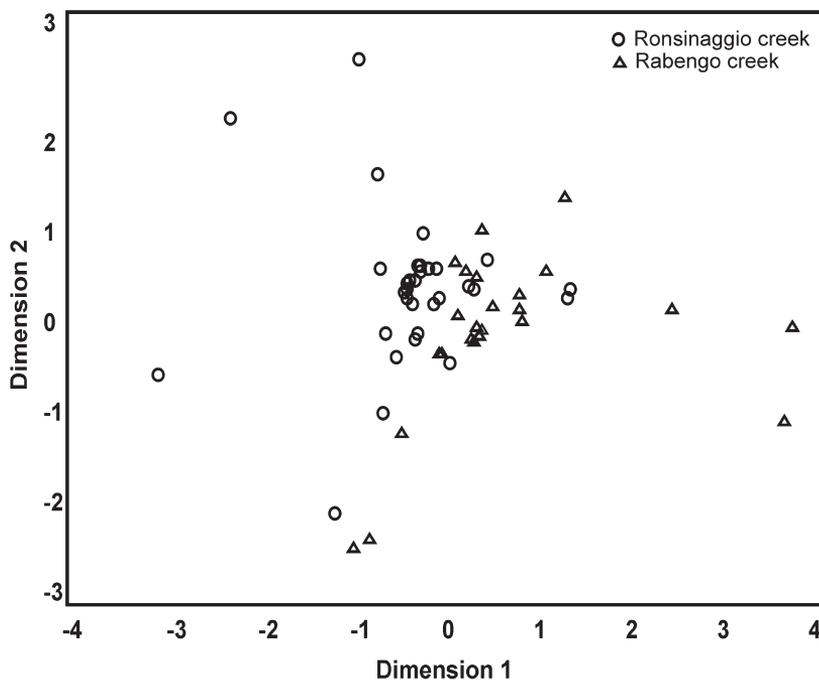


Fig. 1. A graph showing no clear grouping between two creeks, based on results of Multidimensional Scaling analysis of ingested prey items of *Cordulegaster boltoni* individual nymphs.

for a few groups, such as Oligochaeta and early instars of some Diptera and Trichoptera, which were identified to the family level.

The nymphs of *C. boltoni* were measured using a micrometer before their gut contents were analysed. Total length, pronotum width and right fore-wing pad length were measured (0.1 mm accuracy). Two different methods were used to analyse gut contents, depending on the size of the nymphs (as in López-Rodríguez *et al.* 2009). Larger nymphs were dissected, while smaller larvae were chemically cleared. Dissections were performed in the ventral right side of the thorax to remove the foregut. Gut contents were spread on a Petri dish and prey identified and counted. The quantity of other food items (detritus, hyphae, coarse particulate organic matter, mineral matter and animal matter) was recorded. For smaller nymphs, the method proposed by Bello and Cabrera (1999) was used to render individuals transparent, as previously done by our team in other macroinvertebrate feeding studies (Tierno de Figueroa *et al.* 2003, Cammarata *et al.* 2007, Fenoglio *et al.* 2008, López-Rodríguez *et al.* 2009). Each individual was placed in a vial with Hertwigs' liquid and heated in an oven at 65°C for approximately 120 hours. The specimens were

then mounted on slides for microscope analysis. Prey items were identified and counted from complete individuals or from sclerotized body parts, such as head capsules, mouthparts and legs. The count of sclerotized fragments can give a reasonably accurate estimate of prey consumed, as demonstrated for other macroinvertebrates (Stewart & Stark 2002). Individuals that did not contain any invertebrate prey were analysed with a compound microscope equipped with an ocular micrometer to identify non-animal gut contents. The absolute percentage of gut content (at 40× as percentage of total area occupied by the contents in the whole digestive tract) and the relative abundances of each food item (at 400× as percentage of area occupied by each component of the total gut contents) were recorded.

Statistica 8.0 was used for statistical analyses (StatSoft Inc. 2008). Normality of the data was assessed by means of the Kolmogorov-Smirnov test. Variables did not follow a normal distribution, so non-parametric tests were used. Correlations between total length, pronotum width and right fore-wing pad length, as well as the correlation between size and number of prey items, were calculated using a Kendall *Tau* test. A Gamma correlation test (Siegel & Castellan 1988) was

Table 1. Gut content of *Cordulegaster boltoni* nymphs (Mean, Min and Max of the number of the prey item in the gut,  $N = 110$  guts in all cases; No. = number of guts that contained the item).

Prey item	Mean	Min	Max	SD	No.	% presence
Ephemeroptera undet.	0.05	0	1	0.23	6	0.05
Baetidae	0.01	0	1	0.10	1	0.01
<i>Paraleptophlebia</i> sp.	0.01	0	1	0.10	1	0.01
<i>Ecdyonurus</i> sp.	0.05	0	1	0.23	6	0.05
Plecoptera undet.	0.05	0	1	0.21	5	0.05
<i>Nemoura</i> sp.	0.02	0	1	0.13	2	0.02
Odonata undet.	0.01	0	1	0.10	1	0.01
<i>Cordulegaster</i> sp.	0.01	0	1	0.10	1	0.01
<i>Calopteryx</i> sp.	0.02	0	1	0.13	2	0.02
Trichoptera undet.	0.05	0	1	0.23	7	0.06
Hydropsychidae	0.04	0	3	0.30	2	0.02
Polycentropodidae	0.02	0	2	0.19	1	0.01
Coleoptera undet.	0.01	0	1	0.10	1	0.01
Elmidae	0.01	0	1	0.10	1	0.01
Diptera undet.	0.01	0	1	0.10	1	0.01
Chironomidae	0.35	0	3	0.66	30	0.27
Tanypodinae	0.01	0	1	0.10	1	0.01
Stratiomyidae	0.01	0	1	0.10	1	0.01
Simuliidae	0.09	0	3	0.40	7	0.06
Dixidae	0.01	0	1	0.10	1	0.01
Gammaridae	0.57	0	22	2.33	25	0.23
Ostracoda	0.03	0	3	0.29	1	0.01
<i>Pisidium</i> spp.	0.01	0	1	0.10	1	0.01
Lumbricidae	0.01	0	1	0.10	1	0.01
Staphylinidae larvae	0.01	0	1	0.10	1	0.01
Terrestrial spiders	0.02	0	1	0.13	2	0.02

used to analyse the relationship between total length of nymphs and number of prey items in the gut contents.

To assess if individuals from both streams could be treated and analysed together, a Multidimensional Scaling analysis was performed using ingested prey items as variables (Quinn & Keough 2002).

### 3. Results

The three biometric measures of nymphal size were highly correlated to each other (total length vs. pronotum width: Kendall  $Tau = 0.90$ ; total length vs. fore-right wing pad length: Kendall  $Tau = 0.85$ ; pronotum width vs. fore-right wing pad length: Kendall  $Tau = 0.91$ ;  $p < 0.05$  and  $N = 211$  in all the cases). Thus we selected total length to represent nymphal size.

From both streams, 211 individuals were studied, 82 of which were dissected and 129 were cleared.

Guts of 30 nymphs were completely empty, and 71 contained only non-animal material. A Multidimensional Scaling analysis was performed using ingested prey items as variables to detect possible groupings in individuals by stream. The Stress value obtained with this analysis was equal to 0.014 (1.4%), indicating an almost perfect fit. As shown in Fig.1, individuals from both streams do not show a clear ordination or grouping, so all the individuals can be analysed together.

Non-animal material in gut contents was represented by detritus (mean  $\pm$  standard deviation of the total gut content =  $80.6 \pm 31.8\%$ ), coarse particulate organic matter ( $6.4 \pm 21.7\%$ ) and hyphae ( $0.17 \pm 0.78\%$ ). Twenty-four of these nymphs had ingested some mineral matter ( $11.2 \pm 22.8\%$ ).

The remaining 110 nymphs contained both animal and non-animal matter, the latter probably derived from the gut of their prey. These nymphs fed mainly on Chironomidae (30 guts), Gamma-

Table 2. Gamma correlation between total length (mm) and number of aquatic invertebrate prey type in guts of *Cordulegaster boltoni* nymphs. Values marked with an asterisk are significant at  $p < 0.05$ .

Prey item	Total length
Ephemeroptera	0.36*
Plecoptera	0.02
Odonata	0.77*
Trichoptera	0.44*
Coleoptera	0.58
Chironomidae	-0.52*
Simuliidae	-0.39*
Diptera	0.32
Gammaridae	0.62*
Ostracoda	0.70
<i>Pisidium</i> spp.	0.49
Lumbricidae	0.77

ridae (25 guts), Trichoptera (10 guts), Simuliidae (7 guts), Ephemeroptera (14 guts) and Plecoptera (7 guts) (Table 1).

There was a weak positive correlation between total length and number of prey ingested (Kendall  $Tau = 0.27$ ;  $p < 0.05$ ). Size of the nymphs was positively correlated to the number of Ephemeroptera, Odonata, Trichoptera and Gammaridae in the gut content, and negatively correlated to that of Chironomidae and Simuliidae (Table 2).

#### 4. Discussion

*Cordulegaster boltoni* is widespread in Europe, and represents one of the most typical Anisopteran dragonflies in running water systems. Our study provides the first record on the diet of a Mediterranean population of *C. boltoni*.

Larvae appeared to be opportunistic predators, taking a wide variety of prey, which were widely distributed. Aquatic insects dominated the diet of nymphs, but crustaceans, annelids and molluscs were also preyed upon. Furthermore, we found that terrestrial organisms that accidentally fall into the water can be captured and ingested by *C. boltoni* larvae. We detected an ontogenetic shift in the diet that supports previous studies (Ormerod *et al.* 1990). Small juvenile larvae appeared to feed particularly on small, less mobile and soft-bodied prey, such as Chironomi-

dae and Simuliidae, whereas larger individuals fed on larger, mobile and hard-bodied organisms, such as Gammaridae, Ephemeroptera, Odonata, and Trichoptera. Accordingly, it is likely that as the size of the dragonfly increases, its ability to capture larger, more robust and mobile prey also improves. Inter alia, the presence of different trophic spectra in different size classes may constitute an important advantage to minimize intra-specific competition, which has been reported as an important limiting factor in low order streams (Bo & Fenoglio 2005).

It has been observed in previous studies that Odonata larvae do not tend to actively seek for prey but rather to wait immobile and concealed in vegetation or (as is the case in *Cordulegaster* spp.) sediment, until a suitable food item comes within range of a forward rush and strike with the mouthparts (Askew 2004). Studies regarding trophic behaviour of this lotic species are until now scarce and conducted mostly in North Europe. For example, Jones (1950) analysed gut contents of 11 specimens of *C. boltoni* in the river Rheidol (Wales) and reported that Trichoptera larvae (Limnephilidae), Ephemeroptera nymphs (Siphonuridae, Baetidae), and Coleoptera (a large larva and one adult of Dytiscidae) were included in the diet. Moreover, a study in Upland Wales pointed out that the diet of *C. boltoni* included Oligochaeta, Plecoptera (Leuctridae and Perlodidae) and Ephemeroptera (Baetidae and Leptophlebiidae) nymphs, and Trichoptera (Rhyacophilidae, Polycentropodidae and Limnephilidae) and Diptera (Simuliidae, Chironomidae and Tipulidae) larvae (Ormerod *et al.* 1990). These authors also observed that larger larvae ate a wider variety of prey types than smaller larvae, as reported in our study. Moreover, they recorded that small individuals of *Cordulegaster* fed mostly on small chironomids, while larger specimens fed more frequently on simuliids, stonefly nymphs and polycentropodid caddisflies. In a more recent study, Woodward and Hildrew (2002) examined the impact of *C. boltoni* as a new top predator having recently invaded Broadstone Stream (England). Field enclosure/exclosure experiments were conducted to assess the impact of the invader on the benthic community. Depletion of prey varied seasonally and among taxa, and was highest when prey densities and en-

counter rates were high. The authors observed that mobile prey were most strongly captured and that depletion of prey was due solely to consumption (predators did not induce emigration as previously hypothesized for other top predators with stalking behaviour, such as large-sized Systellognatha stoneflies).

Our study suggests that *C. boltoni* is not exclusively an ambush predator, but it can explore actively the river bottom to capture a wide range of prey. This large-sized dragonfly can be considered one of the most representative carnivorous insects in lowland small order streams (which, because of their environmental characteristics, are often devoid of fish and stoneflies), where it acts as the top predator in the benthic trophic food webs.

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