ORIGINAL ARTICLE

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Are gobiid fish more susceptible to predation if parasitized by *Eustrongylides excisus*? An answer from robbed snakes

Abstract Aspects of the predator–prey relationship between dice snake, *Natrix tessellata* and gobiid fish infected with *Eustrongylides excisus* were studied in Lake Sinoe, Romania. A population of snakes residing here shows a high prevalence of subcutaneous larvae of this nematode. The hypothesis of the altered motility in infected fish leading to increased depredation by snakes was tested by comparing gobiids collected from dice snakes with gobiids caught via electrofishing. Out of a total of seven identified gobiid species, three were used for analysis: syrman goby *Neogobius syrman*, mushroom goby *Neogobius eurycephalus*, and round goby *Neogobius melanostomus*. No significant differences in prevalence and intensity of *E. excisus* infection were found between fish caught by snakes and those obtained by electrofishing. However, significantly higher abundance of *E. excisus* larvae in fish caught by snakes was reported. These findings suggest limited influence of the presence of *E. excisus* larvae in studied gobiids regarding their susceptibility to predation by dice snakes.

Introduction

Parasites alter the behavior of their hosts to increase the probability of parasite survival (e.g., Hurd 1990). Many parasitic organisms with complex lifecycles modify host behavior in a way that results in increased susceptibility of infected host to predation and thus, they enhance their transmission by influence of predator–prey interactions (Holmes and Bethel 1972; Lafferty and Morris 1996).

An international collaborative study aimed to understand the population biology of the dice snake, *Natrix tessellata* in the Dobrogea region of Romania was established in 2005, also including evaluation of parasitism and health status (Mihalca et al. 2007; Carlsson et al. 2009). The occurrence of skin nodules

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formed by nematode larvae was recorded; nematodes obtained from dissected snake carcasses were determined as L3 and L4 larvae of *Eustrongylides excisus* (Mihalca 2007) with prevalence exceeding 50% (Carlsson et al. 2009).

Nematodes of the genus *Eustrongylides* Jägerskiöld 1909 are heteroxenous in their life cycle, involving oligochaetes as first and fish as second intermediate hosts. Piscivorous birds, commonly cormorants, are the definitive hosts (Karmanova 1968; Anderson 2000). In some cases, vertebrates other than fish (e.g., piscivorous snakes) may enter the life cycle of *Eustrongylides* (Kirin 2002; Yildirimhan et al. 2007) and severe pathology is frequently observed in these hosts (Lichtenfels and Lavies 1976; Bursey 1986; Mihalca et al. 2007).

Natrix tessellata is a medium-sized snake that is abundant in aquatic habitats throughout Europe, showing ecological plasticity in niche utilization. In all populations, fish represent the major part of the diet (Luiselli and Rugiero 1991; Filippi et al. 1996). In fact, *N. tessellata* is the only European snake foraging in sea and/or brackish water and, therefore, the diet composition varies according to inhabited areas (Luiselli et al. 2007). In the Dobrogea region, Romania, *N. tessellata* forages in the brackish waters of Lake Sinoe and it feeds almost exclusively upon gobiid fish (Perciformes: Gobiidae) (Carlsson et al. 2009).

In fish, infection of *Eustrongylides* larvae has been recorded to increase the predation susceptibility (Coyner et al. 2001) when *Eustrongylides*-infected fish exhibited erratic movement compared to non-infected ones. A pilot study performed by the authors in 2005–2006 indicated a high prevalence of *E. excisus* larvae in gobiids (exceeding 20%, unpublished data) in *N. tessellata* habitats in Dobrogea region. Based on these findings, a possible explanation for the high occurrence of *E. excisus* larvae in dice snakes in the studied population is the effect of higher predation susceptibility of infected fish in accordance with Coyner et al. (2001).

Dice snakes usually return to the shoreline or into protective reeds to ingest their prey while preventing escape. This feeding behavior gave us the opportunity to "steal" the fish from snakes and to examine them for the presence/absence of *E. excisus* larvae. We here report on an investigation whether or not infected gobiids are more susceptible to predation from dice snakes by collecting ("robbing") recently caught fish–prey from captured snakes and comparing the results with the representative sample from the same fish population obtained by electrofishing.

Methods

Study site, timing

This study was conducted from 8 July 2007 to 26 July 2007 in Lake Sinoe, Histria, Constanta County, Romania (44.54°N, 28.93°E). Lake Sinoe is the southernmost lagoon lake of the Danube Delta system, a shallow lake, mostly less than 2 m deep, and about 170 km^2 in area, with reeds (*Phragmithes australis*) growing along almost the entire shoreline (Dorosencu et al. 2004). It is still connected with the Black Sea and thus contains brackish water. Salinity levels fluctuate and depend on freshwater from the Danube and varying influx of saline water from the Black Sea (Alexandrov et al. 2000).

Snake captured fish (SC samples)

A 100-m strip of shoreline was patrolled daily from 0700 to 1600 hours. Snakes were captured as they were swallowing their piscine prey at or near the shoreline, or as they were moving towards the shore (Fig. 1a). They were "robbed" either by taking the fish out of their mouth, or from their stomach by forced regurgitation if they had already swallowed the fish. For the shortest possible time prior to the processing (maximum 2 h), fish specimens were stored individually in plastic vials due to frequently observed active evasion of *E. excisus* larvae through the body wall, which might otherwise have biased the observed data (Fig. 1b).

Collection of fish by electrofishing (EC samples)

Electrofishing was performed on 12 July 2007 in the in the same area where the feeding snakes were collected. Scubla ELT60 electrofishing equipment was used, with a maximum output power of 940 V, pulsed with a frequency of 25–100 impulses/s or with waveform output power of maximum 500 V; stunned fish were collected with a net.

Fish examination, nematode collection

Alive fish were anaesthetized with clove oil and killed with a solution of tricaine methane sulfonate (TMS/MS222, 250 mg/l); total length was measured prior to dissection. The body cavity was opened with scissors; viscera were removed and examined for the presence of *E. excisus* larvae. In order to count the prevalence (% of infected fish in both EC and SC), abundance (total no. of larvae in EC and SC) and intensity (number of larvae in infected individuals in EC and SC), larvae were counted in each fish specimen. Following dissection, the fish were stored in 70% ethanol. Taxonomic determination of fish was performed according to keys given by Banarescu (1964) and Otel (2007).

Statistical analysis

Three fish species most abundant in both the EC and SC samples (*Neogobius eurycephalus*, *N. syrman*, and *N. melanostomus*) were used for analysis. To compare

Fig. 1 Poikilotherm hosts of *Eustrongylides excisus* in the study site. **a** *Natrix tessellata* coming to ingest the preyed gobiid fish to the shore of the Lake Sinoe, **b** active evasion of *E. excisus* larva (*arrow*) after euthanasia of the gobiid fish, **c** *E. excisus* larva, free in the body cavity of gobiid fish. *Scale bars* for *b* and *c* = 1 cm
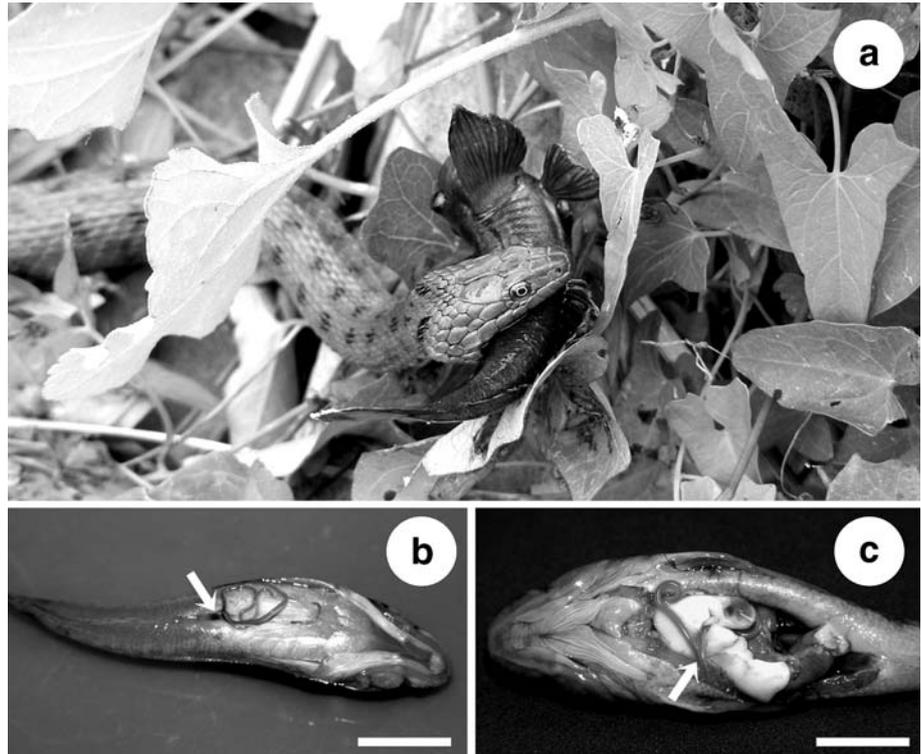
frequencies of fish positive for *E. excisus* between SC and EC samples we performed generalized linear model (GLZ) analysis of covariance (ANCOVA) with binomial distribution and species of fish as categorical factor. The length of fish was included as covariate to remove a possible effect of length of fish on parasite infection (Coyner et al. 2002). To examine possible differences in abundance and intensity of *E. excisus* infection between SC and EC samples we employed generalized linear model (GLZ) analysis of covariance (ANCOVA) with Poisson distribution and with length of fish as covariate and species of fish as a categorical factor. All statistical analyses were performed in STATISTICA (version 8.0, StatSoft, Inc. 2008).

Results

Gobiid fish obtained

Altogether, 149 gobiids were collected from a total of 96 snakes (SC samples) and a total number of 192 gobiid fish were caught by electrofishing (EC samples). Seven species were identified in SC, EC samples, or both: *Neogobius eurycephalus* (Kessler, 1874), *N. fluviatilis* (Pallas, 1814), *N. kessleri* (Günther, 1861), *N. syrman* (Nordmann, 1840), *N. melanostomus* (Pallas, 1814), *N. ratan* (Nordmann, 1840) and *Knipowitschia caucasica* (Berg, 1916). Data on mean values of prevalence, abundance and intensity of *Eustrongylides excisus* infection for the three gobiid species (*N. syrman*, *N. eurycephalus* and *N. melanostomus*) with sufficient

numbers from both SC and EC samples analyzed in this study, are presented in Table 1.

Prevalence, abundance, and intensity of *E. excisus* infection

No gross pathological changes were observed in infected fish during the dissections. Larvae were found either moving freely (Fig. 1c) or coiled up within capsules in the body cavity of fish.

We found no significant differences in prevalence of *E. excisus* between SC and EC (GLZ ANCOVA: length of fish: $\chi^2 = 0.39$, $p = 0.53$; origin: $\chi^2 = 2.00$, $p = 0.16$; species: $\chi^2 = 0.50$, $p = 0.78$; origin × species: $\chi^2 = 2.14$, $p = 0.34$). However, the abundance of *E. excisus* was higher in the SC samples than in the EC especially for *N. eurycephalus* and *N. melanostomus* (GLZ ANCOVA: length of fish: $\chi^2 = 0.12$, $p = 0.73$; origin: $\chi^2 = 3.99$, $p = 0.05$; species: $\chi^2 = 0.17$, $p = 0.92$; origin × species: $\chi^2 = 3.15$, $p = 0.21$, see Table 1), but the intensity of infection did not significantly differ between the EC and the SC samples (GLZ ANCOVA: length of fish: $\chi^2 = 0.02$, $p = 0.89$; origin: $\chi^2 = 0.17$, $p = 0.68$; species: $\chi^2 = 0.14$, $p = 0.93$; origin × species: $\chi^2 = 0.34$, $p = 0.84$).

Discussion

Behavioral changes of piscine hosts involved in the life cycle of parasitic helminths are well known (Moore

Table 1 Mean values of prevalence, abundance and intensity of *Eustrongylides excisus* infection in selected species of gobiids

Gobiid species	Prevalence of *E. excisus* (%)		Abundance of *E. excisus* (larvae)		Intensity of *E. excisus* infection (larvae/infected fish)	
	SC (no. of fish)	EC (no. of fish)	SC (no. of fish)	EC (no. of fish)	SC (no. of fish)	EC (no. of fish)
N. syrman	23.3 (n = 60)	21.5 (n = 121)	0.3 (n = 60)	0.3 (n = 121)	1.2 (n = 14)	1.2 (n = 26)
N. eurycephalus	29.6 (n = 27)	9.5 (n = 21)	0.4 (n = 27)	0.1 (n = 21)	1.5 (n = 8)	1.5 (n = 2)
N. melanostomus	25.0 (n = 4)	10.0 (n = 10)	0.5 (n = 4)	0.1 (n = 10)	2 (n = 1)	1 (n = 1)

SC fish obtained from snakes, *EC* fish obtained by electrofishing

2002). An example might be *Euhaplorchis californiensis*, trematode infecting California killifish (*Fundulus parvipinnis*). Cercariae of this parasite migrate to the brain, where they encyst, and by settling on the brain, this parasite has the potential to steer the fish towards a bird definitive host (Lafferty and Morris 1996). Another case worth mentioning might be *Schistocephalus solidus*, a cestode infecting the threespine stickleback, *Gasterosteus aculeatus*. It has been shown to be responsible for delayed/limited response to attacks by predators in infected fish–hosts (Ness and Fostrer 1999). The effect of *Eustrongylides* nematodes on the behavior of their piscine hosts was confirmed for *E. ignotus* where higher predation susceptibility occurs in infected fish (Coyner et al. 2001). All these parasitic helminths utilize two main strategies in increasing the likelihood of intermediate host predation as suggested by Holmes and Bethel (1972); (a) alteration of the body condition and movement of the intermediate host and (b) its disorientation.

In concordance with these findings, we anticipated that infected fish would be preyed upon by dice snakes to a higher extent than non-infected ones. However, we did not find significant differences in prevalence or intensity of *E. excisus* infection in fish caught by snakes compared to fish caught by electrofishing. Yet, higher abundance of *E. excisus* in fish obtained from snakes in comparison to abundance in electrofished specimens was recorded, especially for *N. eurycephalus* and *N. melanostomus*, although sample sizes were limited. To clarify if parasitized *N. eurycephalus* and *N. melanostomus* are really more susceptible to snake predation under natural conditions, increased sample sizes are needed.

The presence of eustrongylid larvae has been described in 17 orders of fish worldwide (Bangham 1939, 1940; Karmanova 1968; Spalding et al. 1993). After ingestion of infected oligochaetes by a fish, the eustrongylid larva migrates from the digestive tract to the body cavity or muscles of the body wall (Measures 1988). In the latter, it might cause hyperplasia of the tissue resulting in encystment of the larva in a capsule (Cooper et al. 1978), while in other cases larvae might remain free in the body cavity (Paperna 1974). A swelling of the body with a formation of a strongly melanized capsule has been described (Brugni and Viozzi 1999). Mortality of infected fish has also been documented in commercial fisheries (Bangham 1972). In our study, we observed both encysted and free larvae of *E. excisus* with no gross lesions, suggesting limited pathogenicity of these nematodes in examined gobiid fish.

Pelagic *Gambusia affinis* that were used to test the similar hypothesis were most likely altered in movement if infected with *Eustrongylides* larvae (Coyner et al. 2001). Such alteration caused their higher predation susceptibility to centrarchid fish, the sight predators that cue on movement of the prey. However, gobiid prey fish tested in our study are benthic, with most species preferring shelter under rocks (Hoese 1998). Moreover, the visibility in the eutrophic water of Sinoe Lake is minimal and the snakes hunt the fish under the stones, on the bottom. Then, the altered reaction times or motility of infected fish, if any, might not necessarily handicap them compared to the non-infected ones.

Ecological differences between the studied gobiids in our locality could explain the varying results obtained from the different species. *Neogobius fluviatilis* and *N. kessleri* seamed to be more available for EF. These species have a wider habitat preference and mobility (Banarescu 1964; Otel 2007), which might make them more difficult to catch for the snakes. In fact, *N. syrman* is the only really stagnophillic species among the dominant gobiid species from our samples. The high frequency of *N. syrman*, which prefers sandy substratum (Otel 2007) in our SC sample set suggests that the dice snakes hunt for fish in a variety of habitats and not only along the surveyed rocky shoreline. Lake Sinoe is shallow and mainly sandy bottomed, with some rocky shoreline areas. Electrofishing worked well in both sandy and rocky areas and the stunned fish usually floated to the surface, especially on sandy substrate. In rocky areas, fish commonly remained underneath the rocks where they had been hiding and had to be located and collected by hand. Hence, we attribute the large number of electrofished *N. syrman* compared to *N. eurycephalus* to methodological artifacts. The number of snake-caught *N. syrman* being more than twice the number of *N. eurycephalus*, is reflective of the abundance of the respective species in bottom habitats.

The reported prevalence of *Eustrongylides* larvae in fish other than gobiids is usually lower, e.g., 2.45% in *Gambusia holbrooki* (Frederick et al. 1996) or 0.78–8% in *Galaxias maculatus* (Brugni and Viozzi 1999) than those we recorded in gobiids in Lake Sinoe. High prevalence, abundance and intensity of *Eustrongylides* larvae in gobiid fish in comparison to other syntopic piscines are given by Sattari et al. (2005, 2008).

Considering the fact that, in Lake Sinoe, *N. tessellata* feeds almost exclusively on gobiids, the infection pressure of *E. excisus* is very high. This fact alone might explain the high infection rates in studied dice snakes in Lake Sinoe rather than anticipated manipulation of fish by *Eustrongylides* larvae.

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