SHORT NOTE

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The early catfish catches the worm: predation of *Corydoras aeneus* (Siluriformes, Callichthyidae) on freshwater nematodes

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Abstract – In this study, we measured the daily consumption of four different nematode species by a small freshwater catfish species, *Corydoras aeneus* (Gill, 1858). Consumption of nematodes by fishes was significant with a single *C. aeneus* individual being able to consume in 24 h between 40 581 and 75 849 adult nematodes depending on the nematode species offered. This represented the ingestion of up to 238 mg wet weight when considering the largest nematode species: *Panagrellus redivivus*. Our results strengthen the growing evidence of a significant trophic channel existing between meiobenthic invertebrates like nematodes and small bottom-feeding fishes like *C. aeneus*. We also discuss the relevance of using *P. redivivus* as live food for rearing *C. aeneus* which is a popular ornamental fish.

Keywords: Predator-prey interaction / aquaculture / benthic ecology / Caenorhabditis elegans / Panagrellus redivivus

1 Introduction

Nematodes are diverse and widely distributed and likely one of the most abundant groups of metazoans on Earth (Bongers and Ferris, 1999; Abebe *et al.*, 2008), presumably being only restricted by the presence of amenable substrates, of water, of oxygen and of enough microscopic resources to sustain population growth. Hence, free-living nematodes are important key organisms in freshwater food webs as they contribute to transfer microbial production to macroscopic animals like macro-invertebrates and fishes (Majdi and Traunspurger, 2015).

A massive consumption of meiofauna, and of nematodes in peculiar, by larvae and juveniles of bottom-feeding fishes has been only recently acknowledged through field and laboratory studies (Schlechtriem *et al.*, 2004, Spieth *et al.*, 2011, Weber and Traunspurger, 2014a; 2015; 2016; Tillner *et al.*, 2015). The fish-nematode trophic channel consists in a predator-prey body mass ratio over 5 orders of magnitude, which is in the upper bound of predator-prey body mass ratios commonly found in freshwater ecosystems for ectothermic vertebrates (Brose *et al.*, 2006). This trophic channel should be biologically interesting in terms of energy conservation as (1) it overrides trophic intermediaries (like in the whales-zooplankton channel), and (2) as effective strategies allow bottom-feeding fishes to consume enough nematodes at relatively low

energetic costs. It has been shown that juvenile bottomfeeding fishes were able to collect particles the size of nematodes out of bites of mud, sediment or biofilms. For example, in carp juveniles, Spieth *et al.* (2011) found a correspondence between the "mesh"-size of the branchial basket and the size-spectrum of the sediment-dwelling invertebrates that the carps were able to consume. However, it is becoming urgent to expand this field of research in order to better understand the ecological relevance of the fishnematode trophic channel. Especially as nematodes are one of the few benthic organism groups (with chironomids and oligochaetes), which can reach high densities in polluted rivers (Heininger *et al.*, 2007).

Corydoras aeneus (Gill, 1858) is a typical predator of benthic invertebrates in tropical streams (Aranha *et al.*, 1993; Lopes *et al.*, 2016). *C. aeneus* belongs to a fish family with species that were recorded to be tolerant to abrupt temperature changes, low-quality habitat and low dissolved oxygen concentrations in South America (Mol, 1993; De Araujo and Garutti, 2003). Thus, *C. aeneus* are commonly found in the most degraded urban streams (Casatti *et al.*, 2010), in addition *C. aeneus* are also heavily traded as ornamental fishes all over the world, making them of general interest and easily available for experiments.

In continuation of this endeavor, we seek (1) to examine the predation pressure of *C. aeneus* on different species of freeliving nematodes, and (2) to measure the amount of nematode biomass ingested daily.

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2 Material and methods

2.1 Rearing conditions and acclimation of fishes

C. aeneus were obtained from a local aquarium dealer (Hameln, Germany), acclimated, and maintained following Nickum et al. (2004). Two groups of 50 juveniles were accommodated in two 250-L aquaria $(100 \text{ cm} \times 50 \text{ cm} \times 50)$ cm) equipped with an aerating system (there were two groups in case a disease would break out, a reserve group would still be available for experiments; This was not the case, and individuals were randomly selected from the two stock tanks for the experiment). The 250-L tanks contained 5-cm of natural river sand (collected ~500-m downstream the spring of the Ems river, Germany). Java moss (Vesicularia sp.) and water trumpet (Cryptocoryne sp.) were used as refuges. The fishes were acclimatized to the experimental conditions for 8 weeks at 18-19 °C under a light:dark cycle of 12:12 h (58 W Osram fluorescent tubes). Fishes were fed TetraWafer Mix (TETRA, Melle, Germany) and frozen chironomid larvae (Amtra Aquaristik, Rodgau, Germany). The fishes were starved for 24 h before the feeding experiment.

2.2 Cultivation of nematodes species

Caenorhabditis elegans (Maupas, 1900) and Panagrellus redivivus (Linnaeus, 1767) were cultivated at 20 °C on 8-cm Agar (NG) plates (0.85% Agar, 0.125% peptone, 0.15% NaCl, 500 µl CaCl₂/L, 500 µl MgSO₄/L, 12.5 ml KH₂PO₄/L and 500 µl cholesterol/L), seeded with *Escherichia coli* OP50 (a uracil-requiring mutant of *E. coli*) (Sulston and Hodgkin, 1988). Panagrolaimus cf. thienemanni (Hirschmann, 1952), and Poikilolaimus sp., were cultured at 20 °C on nematode growth gelrite plates following the protocol of Muschiol and Traunspurger (2007).

2.3 Experimental set-up

Fine-grained sand (3–5 mm diameter) was obtained from the uppermost 5-cm of sediment collected from a local stream (Ems spring), washed several times to remove fine-grained particles and organisms, and then sterilized by two rounds of autoclaving (121 °C, 20 min) followed by drying for 48 h at 200 °C. No bacteria were added to the sediment, because nematodes can survive over 24 h without a food source (Donkin and Williams, 1995). The sediment was distributed to a depth of 2-cm in sixty four 12-L aquaria (30 cm × 20 cm × 20 cm, bottom: 600 cm²) equipped with an aeration system and under 12:12 light cycle (58W Osram fluorescent tubes). Six hours before fishes were placed in the aquaria, a homogeneous suspension of each of the four nematode species was spread out grid-like using a pipette near the sediment surface.

Starting densities of 300 000 nematodes per aquarium (500 nematodes cm⁻²) were estimated by counting (under a dissection microscope, $40 \times$ magnification) the aliquots of a homogeneous nematode suspension, which was used for inoculation of aquaria. The density achieved in sediment simulated a high, but realistic nematode abundance in the environment (see Traunspurger *et al.*, 2012). Every starting

suspension consisted only of adult nematodes. Adult nematodes were sorted out from juvenile stages in cultures by washing culture dishes through a cascade of mesh-sizes (500, 250, 100, 63 μ m). The body length of nematodes inoculated in control (no fish) and fish aquaria was inferred by counting the first 400 individuals in sub-samples from the starting nematode suspensions. For *Poikilolaimus* sp. and *Panagrolaimus cf. thienemanni*, individual biomass was estimated directly from length-wet mass regression after Muschiol and Traunspurger (2007). For *C. elegans* and *P. redivivus*, we first derived body-width from body-length after Muschiol and Traunspurger (2007), before using the regression of Andrássy (1956) to estimate individual wet weight (WW).

Two juvenile *C. aeneus* individuals with a total length of 25–30 mm, were added to 32 aquaria (8 replicate aquaria per nematode species) and 32 aquaria without fish served as controls (8 aquaria per nematode species). After 24 h of presence in aquaria, fishes were removed and released in the 250-L tank.

2.4 Sample processing

After removing the fishes, 20 sediment subsamples were collected randomly from each aquarium using an acrylic tube (0.71-cm intern. diam. × 2-cm-deep column of sediment). The 20 subsamples were pooled, and considered as one sample. Each sample was preserved immediately in formaldehyde (4% final concentration) and stained with rose Bengal (300 µg mL⁻¹). Nematodes were further extracted from sediment by density centrifugation (LUDOX TM 50[®] colloidal silica, adjusted to 1.14 g L^{-1} , using 10-µm mesh size to retain nematodes) according to the method of Pfannkuche and Thiel (1988). All nematodes present in samples were counted, and densities were further expressed as number of individuals per aquarium (*i.e.* per 600 cm²), or in terms of biomass as mg WW per aquarium.

2.5 Statistical analysis

For each nematode species, we used Student's *t*-test to compare mean nematode densities remaining in the aquaria after exposure to fish predation. Homoscedasticity of data was checked using Levene's test: data were not transformed. We used the mean individual biomass values measured from inoculums to infer the corresponding biomass of nematodes consumed by fishes during the experiment (24 h), assuming that no juveniles were produced and adult's individual biomass did presumably not vary much during the course of the experiment.

3 Results

After 24 h, the two *C. aeneus* individuals were able to dampen significantly the number of all nematode species dwelling the sediment (*t*-test, *Poikilolaimus*, *t*=-9.14, *p* < 0.001; *C. elegans*, *t*=-14.8, *p* < 0.001; *Panagrolaimus*, *t*=-11.5 *p* < 0.001; *Panagrellus*, *t*=-11.5 *p* < 0.001). The two *C. aeneus* removed, on a daily basis, on average 81162 *Poikilolaimus*, 103 285 *C. elegans*, 151 697 *Panagrolaimus*,



Fig. 1. Nematode densities after 24 h exposition in control aquaria without fishes (grey bars) or in aquaria with two *Corydoras aeneus* (25–30 mm) catfishes (black bars). Values are means \pm SD. Aquaria contained 1.2 L of sandy sediment spread on a surface of 600 cm². Nematode species offered: *Poikilolaimus* sp. (*Poikilo*), *Caenorhab-ditis elegans* (*Celegans*), *Panagrolaimus cf. thienemanni* (*Panagro*), and *Panagrellus redivivus* (*Panagre*).

and 136 200 *Panagrellus*, in comparison to controls (Fig. 1). Which represented for each *C. aeneus* individual, an average daily ingestion of 75, 137, 197, and 238 mg WW of adult nematodes of the species *Poikilolaimus* sp., *C. elegans*, *Panagrolaimus cf. thienemanni*, and *P. redivivus*, respectively (see Tab. 1 for nematode species individual body-mass).

4 Discussion

In the present study we showed that C. aeneus massively consumed adults of different free-living nematode species dwelling sandy sediment. Since Diatin et al. (2015) reported a wet weight of 440-510 mg for C. aeneus individuals measuring 22-23 mm, the predator-prey ratio in our study could be estimated at *ca*. 5 orders of magnitude, and the daily consumption of nematodes could be roughly estimated to represent between ca. 14 and 54% of C. aeneus individual biomass. We are aware that this estimation is probably overestimating the strength of the Corydoras-nematode trophic transfer occurring in the field, because it is based on optimal laboratory conditions (e.g. absence of alternative prey, relatively high density of size-calibrated nematodes, homogeneous structure of the substrate). However, our results clearly show that C. aeneus is able to feed massively on nematodes; in case those are the only prey available, strengthening growing evidence that this trophic channel can be commonly used by

many benthivorous fishes (Weber and Traunspurger 2014b, 2015; 2016; Abada *et al.*, 2017). Feeding on nematodes and other small-sized invertebrates by means of substrate ingestion/filtration reduces food chain length, presumably allowing field fish populations to sustain higher productivity rates than if preying on larger animals occupying higher trophic levels. Also as nematodes are abundant in nearly all benthic habitats, using them means less risk of food shortage, which might represent a considerable advantage, especially for the fitness of juvenile cohorts.

Indeed, this experiment does not reflect the true diet of *C. aeneus*, which might be much more diverse in the field as this species is opportunistic. For example, *C. aeneus* has long been considered as an efficient biological control of gnat due to its voracious feeding on mud-dwelling chironomid larvae, but they are also able to dampen populations of oligochaetes, chaoborids, and of small ostracods and cladocers swimming above the mud (Cook Jr., 1962). We recommend further laboratory investigations proposing mixtures of different categories (or sizes) of prey to better understand under which conditions nematodes might be used over other benthic invertebrate prey (see *e.g.* Dineen and Robertson, 2010; Weber and Traunspurger, 2014a).

Nematodes usually dominate in heavily polluted bottoms (*e.g.* Coull and Chandler, 1992; Heininger *et al.*, 2007), and contrary to chironomids, their life-cycle is fully benthic, which implies that the structure of nematode species assemblages can be a relevant indication of both short- and long-term contaminations (Höss *et al.*, 2011; Semprucci *et al.*, 2015; Haegerbaeumer *et al.*, 2017). Fish-nematode feeding studies can be performed under various specific abiotic and biotic conditions and provide coherent measures of feeding rates (see *e.g.* Weber and Traunspurger, 2014a). Thus, we believe that a better consideration of the fish-nematode trophic channel could bring valuable insights into the response of aquatic food webs to various environmental stressors, including realistic measures of trophic dynamics under heavy pollution scenarios.

C. aeneus is a popular ornamental fish, so conditions for its culture are of commercial interest (e.g. Diatin et al., 2015). Among the four species of nematodes offered, Panagrolaimus cf. thienemanni and Panagrellus redivivus were especially preyed upon. Panagrolaimus sp. has been proposed as a relevant substitute to rotifers as live food in fish larvae aquaculture, because it is easy to culture and yields better dry weight per individual than rotifers (Honnens and Ehlers, 2013). Also, P. *redivivus* alone or in mixture with other food items (*e.g.* algae). are known to represent a relevant live food for fish larvae (e.g. Biedenbach et al., 1989; Schlechtriem et al., 2004; Brüggemann, 2012). Knowing that methods exist to cheaply produce massive number of P. redivivus nematodes (e.g. Ricci et al., 2003), and knowing that the fatty acid content of P. redivivus can be improved by simple improvements of culture media (Rouse et al., 1992), we argue that P. redivivus could be a relevant live prey for stock cultures of fish juveniles and even for rearing adults of small bottom-feeding fish species like C. aeneus.

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Nematode species	Individual body mass (µg wet weight)				
	Mean	Median	Min.	Max.	Std. Dev.
Poikilolaimus sp.	1.84	1.97	0.34	2.71	0.55
Caenorhabditis elegans	2.66	2.78	0.05	3.18	0.58
Panagrolaimus cf. thienemanni	2.60	2.60	0.53	4.80	0.82
Panagrellus redivivus	3.50	3.33	0.82	17.92	1.51

Table 1. Individual body mass of nematode species inoculated in aquariums (n = 400 individuals measured).

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