

# RESEARCH NOTES

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## Infection by Anisakid Nematodes *Contracaecum* spp. in the Mayan Cichlid Fish '*Cichlasoma (Nandopsis)*' *urophthalmus* (Günther 1862)

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**ABSTRACT:** Larval nematodes that parasitize the Mayan cichlid fish '*Cichlasoma (Nandopsis)*' *urophthalmus* (Günther 1862) in southern Florida were identified as *Contracaecum* spp. (Nematoda: Anisakidae, Anisakinae). The objective of this study was to determine whether infection intensity and prevalence of these parasites differ between a brackish water and freshwater habitat or through ontogeny in the freshwater habitat only. The nematodes were removed from the abdominal cavity of the fishes and counted. Infection intensity was compared between habitats using analysis of covariance and evaluated through ontogeny using Spearman rank order correlation. Prevalence was compared between habitats and between adults and juveniles from the freshwater habitat using a  $z$ -test. Although infection intensity did not differ between habitats, infection prevalence was greater at the freshwater site (FWS). Both the prevalence and intensity of nematode infection increased through ontogeny at the FWS, and no nematode was found in fishes that were smaller than 93 mm standard length. Thus, the parasites appear to accumulate during the lifetime of the fishes.

Native to the Atlantic slope of tropical Central America (Miller, 1966), the Mayan cichlid '*Cichlasoma (Nandopsis)*' *urophthalmus* (Günther 1862) was first recorded in Florida in 1983 from the Everglades National Park (Loftus, 1987). It is referred to in this study as '*C. (Nandopsis)*' *urophthalmus* in accord with the convention for taxonomically undetermined cichlids (Stiassny, 1991). The means by which '*C. (Nandopsis)*' *urophthalmus* was introduced into Florida are unknown (Loftus, 1987), but it is likely that it was released by private aquarists. This species is euryhaline and can survive in a range of salinity from 0 to 40 ppt (Martinez-Palacios and Ross, 1992; Martinez-Palacios et al., 1993). '*Cichlasoma (Nandopsis)*' *urophthalmus* is susceptible to infection by parasites such as nematodes (roundworms), digenean trematodes, and poecilostomatoid copepods (Vidal-Martínez et al., 1994; Salgado-Maldonado and Kennedy, 1997). In the present study, '*C. (Nandopsis)*' *urophthalmus* was found to have larval *Contracaecum* spp. (Nematoda: Anisakidae, Anisakinae) (E. C. Greiner, pers. comm.) in the coelom. The species of *Contracaecum* infects '*C. (Nandopsis)*' *urophthalmus* in Mexico (Vidal-Martínez et al., 1994; Salgado-Maldonado and Kennedy, 1997). Lament (1999) found parasitic nematodes in the visceral cavity of '*C. (Nandopsis)*' *urophthalmus* collected in the Everglades region but did not identify them. The nematode described in Lament (1999) may also be *Contracaecum* spp.

*Contracaecum* spp. are widely distributed parasitic nematodes. Besides '*C. (Nandopsis)*' *urophthalmus*, they have also been recovered from other cichlids and many other fishes and aquatic birds (Deardorff and Overstreet, 1980; Banks and Ashley, 2000; Machado et al., 2000). Anisakids, such as *Contracaecum* spp., usually infect the alimentary tract of their definitive hosts, which are generally marine mammals. Marine crustaceans, cephalopod mollusks, or fishes often serve as intermediate or paratenic (transport) hosts. Anisakids are not found in the flesh of fishes, they infect the visceral organs instead. Thus, these parasites are not usually harmful to the fish they infect (Deardorff and Overstreet, 1980; Bush et al., 2001). In experimental infections of juvenile and adult chickens, mallard ducklings, mice, hamsters, and rats, *Contracaecum multipapillatum* was not harmful. However, in marine birds, *C. multipapillatum* can infect the proventriculus portion of the stomach and cause lesions. This parasite can also cause granuloma in dogs that eat fish. *Contracaecum multipapillatum* is also capable of infecting domestic cats (Deardorff and Overstreet, 1980; Vidal-Martínez et al., 1994). Anisakids are dangerous when ingested by humans, causing a condition known as anisakiasis, which causes violent abdominal pain, nausea, and vomiting (Bush et al., 2001; Plath et al., 2001).

The goal of this study was to determine whether the pattern of in-

fection by *Contracaecum* spp. in '*C. (Nandopsis)*' *urophthalmus* differs among habitats or through ontogeny. Because *Contracaecum* spp. parasitize both marine and freshwater fishes, it was hypothesized that neither prevalence nor infection intensity of the nematodes would differ significantly between adult '*C. (Nandopsis)*' *urophthalmus* from a brackish water site (BWS) and a freshwater site (FWS). Finally, on the basis of the notion that *Contracaecum* spp. might accumulate in '*C. (Nandopsis)*' *urophthalmus* with time, it was hypothesized that both prevalence and infection intensity would increase significantly through ontogeny at the FWS where juvenile fishes were collected.

The 2 sites chosen for this study, one of which is a brackish water habitat and the other a freshwater, are part of the canal system of southern Florida. The first site was located approximately at the intersection of US-41, the Tamiami Trail, and State Route 29. This is the site of intersection of the Tamiami Canal and the Main Barron River Canal in Carnestown, Collier County (25°54'41"N, 81°21'53"W), at the western border of the Big Cypress National Preserve. This region of the Tamiami Canal passes through brackish water and consists mostly of salt marsh and some mangrove stands and naturally experiences seasonal saltwater influx from Chokoloskee Bay. Data from a nearby water monitoring station on the Tamiami Canal (25°57'40"N, 81°30'60"W) are used in this study as indicators of the salinity fluctuations at this first collection site. In this region, salinity fluctuates seasonally from 0.00 to 34.30 ppt. Sampling at this site took place on 29 April 2000 and 27 May 2000. The second collection site was within the Big Cypress National Preserve in the canal along State Route 94 (Loop Road), east of Trail City, Monroe County (25°45'35"N, 81°0'9"W). This area is a freshwater cypress swamp, and sampling took place on a monthly basis from 24 June 2000 to 19 October 2000. Data from a nearby water monitoring station on the Tamiami Canal (25°50'35"N, 80°55'4"W) verify that this is a FWS, with salinity ranging from 0.00 to 0.29 ppt only.

Fishes were collected from the BWS and the FWS using seine and dip nets, hook and line angling, and minnow traps. Samples were taken on a monthly basis from 29 April 2000 to 19 October 2000 to minimize the confounding effect of changing seasons; 257 '*C. (Nandopsis)*' *urophthalmus* were collected for this study. Fifty-three adults were collected from the brackish water population. Sixty-eight adults and 136 juveniles were collected from the freshwater population. Only adult '*C. (Nandopsis)*' *urophthalmus* were considered when comparing the brackish and freshwater habitats. All individuals greater than 102 mm standard length (SL) were considered to be adults (Caso Chávez et al., 1986; Martinez-Palacios and Ross, 1992; Martinez-Palacios et al., 1993; Faunce and Lorenz, 2000). It was presumed that the 2 groups sampled are not subpopulations capable of intermixing because '*C. (Nandopsis)*' *urophthalmus* is philopatric or site tenacious (Caso Chávez et al., 1986; Salgado-Maldonado and Kennedy, 1997; Faunce and Lorenz, 2000).

Fishes were killed with an overdose of tricaine methanesulfonate (MS-222). They were then injected with 10% buffered formalin and placed on ice for preservation. On return to the laboratory, the fishes were cut from vent to pelvic girdle. All parasitic nematodes found in the coelomic cavity were removed and counted. The parasites were then fixed in 10% buffered formalin and stored in 70% ethanol. Twelve nematodes from both the brackish and freshwater populations were identified by E. C. Greiner, Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida. The fact that only 1 site was used for each habitat type means that there is no replication for each treatment. Therefore, statistical power is low, and the results of the analysis presented in this study must be interpreted with caution. Parasite infection was quantified using both prevalence (the proportion of the host population infected) and intensity (the number of parasites per host). Descriptive statistics were computed using

the arithmetic mean as the measure of central tendency and the standard deviation as the measure of data dispersion. Because nematodes are individual organisms, descriptive statistics are reported in this study using whole numbers. Prevalence was compared between sites and between adults and juveniles at FWS using a  $z$ -test with an alpha level of 0.05. Comparison of infection intensity between sites was accomplished using analysis of covariance (ANCOVA), with SL as covariate and an alpha value of 0.05. Both the number of nematodes and the SL were square-root transformed for analysis. Infection intensity was examined through ontogeny at FWS using Spearman rank order correlation on untransformed data (Zar, 1996). The statistical programs used were SigmaStat 2.03 and SYSTAT 10.0 (SPSS Science).

*Contracaecum* spp. found in the visceral cavity of '*C. (Nandopsis)*' *urophthalmus* ranged from approximately 5 to 20 mm in length. They were less than 1 mm in cross-sectional diameter, light pink in color, and generally found in a coiled position, although some were extended. These larvae were probably all in the L3 stage (B. G. Chitwood and M. B. Chitwood, 1974; E. R. Noble and G. A. Noble, 1982; Olsen, 1986), and only these larvae were considered in this analysis. Of the 56 fish collected from BWS, 22 contained *Contracaecum* spp. (none among the 3 juvenile specimens, and all 22 among the 53 adults). This is equivalent to 39.3% of all BWS fish sampled or 41.5% of the adults. Of the 204 fishes collected at FWS, 47 were infected with the nematodes (3 among the 136 juveniles and 44 among the 68 adults). This is equivalent to 23.0% of all FWS fishes or 2.2% of juveniles and 64.7% of adults. The  $z$ -tests revealed that prevalence of infection was significantly higher in FWS adults than in either BWS adults ( $z = 2.359$ ,  $P = 0.018$ ) or FWS juveniles ( $z = 9.850$ ,  $P < 0.001$ ).

Infection intensity per individual fish at BWS ranged from 1 to 16 nematodes, whereas it ranged from 1 to 43 nematodes at FWS. If the individual fish that contained 43 nematodes is discarded from the analysis, infection intensity at FWS ranged from 1 to 6 nematodes. Mean infection intensity among adults at BWS was  $2 \pm 4$  worms/fish. At FWS, mean infection intensity was  $2 \pm 5$  worms/fish for adults and  $0 \pm 0$  worms/fish for juveniles. ANCOVA indicates that infection intensity is not significantly different between sites ( $F = 2.621$ ,  $P = 0.108$ ).

Nematode infection showed an increase through ontogeny at FWS. Very small fish showed no evidence of infection. Ninety-three millimeters was the first SL at which infection was recorded. Beyond this length, there was no clear relationship between SL and infection intensity as the fish continued to grow. A Spearman rank order correlation was performed to evaluate *Contracaecum* spp. infection through ontogeny. Two separate tests were performed, 1 including the outlying data point of 43 nematodes in the coelom of 1 fish and 1 excluding that data point. If the outlier was included, there was a significant and positive correlation between number of nematodes in the coelom and SL ( $R^2 = 0.612$ ,  $P < 0.001$ ). If the outlier was excluded, a similar result was obtained ( $R^2 = 0.605$ ,  $P < 0.001$ ).

The broad ranges of *Contracaecum* spp. appear to be naturally occurring, probably because of migratory birds (Deardorff and Overstreet, 1980). It is presently unclear whether '*C. (Nandopsis)*' *urophthalmus* in Florida are infected by a *Contracaecum* spp. from Florida, from Central America, or by species from both locations (Hoffman and Schubert, 1984; Lament, 1999). This study shows that although infection intensity among adults did not differ significantly between sites, adults at FWS exhibited a significantly higher prevalence of infection by *Contracaecum* spp. than adults at BWS. The cause for this higher prevalence of infection at FWS could be attributed to a greater density of fishes at this site, but this remains to be determined. This study also shows that smaller '*C. (Nandopsis)*' *urophthalmus* harbors fewer parasites than do larger individuals. The smallest infected fish from BWS was 115 mm SL, and the smallest infected fish from FWS was 93 mm SL. However, there is no clear relationship between size and infection intensity beyond the minimum age of infection.

Larger individuals may harbor more parasites because they have had more time to accumulate them. '*Cichlasoma (Nandopsis)*' *urophthalmus* are carnivorous, feeding on decapod crustaceans such as crayfish *Procambarus* sp. and shrimp *Palaemonetes paludosus*. They also prey on fish such as mosquitofish *Gambusia holbrooki*, sailfin mollies *Poecilia latipinna*, and juvenile '*C. (Nandopsis)*' *urophthalmus* (Caso Chávez et al., 1986; Martínez-Palacios and Ross, 1988; Bergmann, 2002). Consuming these prey items could have led to postcyclic parasitic transmission (Scott, 1954; Smith and Wootten, 1975). However, '*C. (Nan-*

*dopsis)*' *urophthalmus* is also detritivorous (Caso Chávez et al., 1986; Martínez-Palacios and Ross, 1988; Bergmann, 2002). In the detritivorous striped mullet, *Mugil cephalus*, fish size is likewise positively correlated with the intensity of infection by *Contracaecum* spp. and other parasites combined (Valles-Rios et al., 2000). Thus, detritivory may lead to an increase in infection intensity through ontogeny, perhaps because of repeated exposure to the parasite. Further investigation would be necessary to determine whether predation, detritivory, or other factors are the primary sources of infection by *Contracaecum* spp. in '*C. (Nandopsis)*' *urophthalmus*.

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## Ex Vivo Anthelmintic Activity of Albendazole-Sulphoxide Enantiomers

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**ABSTRACT:** The antiparasitic activity of racemic albendazole-sulphoxide (Ricobendazole = *rac*RBZ) and its (+) and (–) enantiomers was tested in an ex vivo murine model for *Trichinella spiralis* infection. Larvae were isolated from the muscle of infected mice and exposed to concentrations between 0.01 and 1 µg/ml of the racemic mixture or to each of its enantiomers. The activity of each compound was then assayed by measuring the ability of the treated larvae to infect naive mice (larval viability). At a concentration of 0.5 µg/ml, all 3 compounds were highly effective in reducing the viability of the larvae, achieving reductions of 91.26% (*rac*RBZ), 96.7% (+), and 89.2% (–), when compared with untreated controls. At lower concentrations (0.1 µg/ml), only treatment with (+)RBZ rendered a significant reduction in larval viability in comparison with controls (84.3%;  $P < 0.01$ ), whereas at 0.01 µg/ml, none of the compounds altered larval viability ( $P > 0.05$ ).

Albendazole (ABZ), methyl (5-(propyl-thio)-1-*H*-benzimidazole-2-yl) carbamate, is a broad-spectrum drug that acts against the most important animal and human helminth parasite species (Horton, 2000). After administration, ABZ is rapidly transformed by 2 distinct hepatic microsomal enzyme systems: the flavin-containing monooxygenase system (Lanusse et al., 1993) and the cytochrome P-450 chain (Souhaili-El Amri et al., 1987). These modifications produce albendazole-sulphoxide (ABZSO) and albendazole-sulphone, the main metabolites that can be recovered from the plasma of sheep (Lanusse et al., 1995), cattle (Sanchez et al., 1997), mice (Rueda-Polo et al., 1998), and humans (Prochanzkova et al., 2000). Because of their affinity for the parasite's beta-tubulin, both ABZ and ABZSO exhibit antiparasitic activity (Lacey, 1988). However, ABZ can be further metabolized and is thus only found at low levels in plasma. Hence, the ABZSO product is thought to be the more active of the 2 in combating tissue-dwelling parasites (Mariner and Bogan, 1980). The chiral core in the sulfur atom of ABZSO permits 2 enantiomers to be generated, (+)ABZSO and (–)ABZSO. The pharmacokinetic disposition of ABZSO enantiomers after oral administration of ABZ in distinct animal species and humans (Delatour, Benoit et al., 1991; Delatour, Garnier et al., 1991; Garcia et al., 1999) reveals differences that possibly reflect the selective metabolism of ABZ and ABZSO enantiomers (Marques et al., 1999; Solana et al., 2000; Virkel et al., 2002). Indeed, even sex-related differences in the phar-

macokinetic behavior of ABZSO enantiomers have been documented in sheep (Capece et al., 2000). Furthermore, the selective uptake of ABZSO enantiomers and their selective binding to cytosolic proteins isolated from different helminth parasites has also been demonstrated (Alvarez et al., 2000; Solana et al., 2002). Thus, these factors must be taken into account because they may contribute significantly to the pharmacological properties of this chiral molecule.

However, despite the interest in these agents, the antiparasitic activity of the (+)ABZSO and (–)ABZSO enantiomers has never been directly tested. Using optimized chiral high-performance liquid chromatography (HPLC), we have been able to obtain very pure samples of (+)ABZSO and (–)ABZSO. This has enabled us to test the anthelmintic activity of these enantiomers in an ex vivo model system, using the well-established murine model of *Trichinella spiralis* infection.

To purify ricobendazole enantiomers from a racemic ABZSO mixture (Ricobendazole = RBZ), a modular liquid chromatography apparatus was used, equipped with: a Jasco PU-1580 isocratic pump (Tokyo, Japan); an automatic sampler (Gilson 231 XL, Villers le Bels, France) fitted to a 100-µl sampler loop (Rheodyne, Rohnert Park, California); a variable wavelength detector (UV-1575, Jasco); and a PC integrator (Borwin 1.5, JMBS developments [Jasco]). Before injection, the samples were filtered through a polyvinylidene fluoride Durapore® 0.45-µm filter (Millipore Co., Boston, Massachusetts). Subsequently, the racemic ricobendazole (*rac*RBZ) sample mix was injected into the HPLC system at a concentration of 0.1 mg/ml, and chiral HPLC separation was performed according to a modified version of the method described previously by Delatour et al. (1990) (Garcia et al., 1999). Briefly, a chiral- $\alpha$  1-acid glycoprotein column (100 × 4 mm, 5 µm) and a mobile phase containing sodium phosphate buffer (8 mM, pH 7.0), at a flow rate of 0.9 ml/min, were used. The samples were analyzed at 290 nm, and under these conditions, the retention times for (–) and (+)RBZ were 3.1 and 10.5 min, respectively. The liquid samples were collected in accordance with the retention times of the enantiomers. The samples were concentrated to dryness under vacuum at 70 °C using a Savant Speedvac® concentrator (Holbrook, New York). The samples recovered were quantified by the same HPLC method described above but included 2-propanol (1 ml/L) as an additive in the mobile phase. Under these conditions, the retention times after injection were 2.6 min for