

Infection patterns of *Myxobolus heterospora* in two tilapia species (Teleostei: Cichlidae) and its potential effects

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ABSTRACT: A *Myxobolus heterospora* (Baker, 1963) infection was found in 2 euryhaline tilapia species, *Sarotherodon melanotheron melanotheron* (Rüppel, 1853) and *Tilapia zillii* (Gervais, 1852), from a brackish water lake, Lake Nokoué (Benin, West Africa). The histology and ultrastructure of different levels of infection in intestinal connective tissues and wall tissues is described. A total of 391 *S. melanotheron melanotheron* and 222 *T. zillii* were examined from October 1987 to October 1989. *M. heterospora* was found throughout the study period, with a total prevalence of 42.19 and 26.57% for *S. melanotheron melanotheron* and *T. zillii* respectively. There was a statistically significant difference in occurrence as a function of season in *S. melanotheron melanotheron* but not in *T. zillii*, and there was a significant difference for size and sex in the former and for sex in the latter. *M. heterospora* induces total destruction of the intestine structure and probably leads to osmoregulatory disturbance. Further investigations of this myxosporean infection are necessary to determine its real effect on the host, since host survival and osmoregulatory rate have not yet been assessed.

KEY WORDS: Fish · Tilapia · Intestine · Myxosporea · Potential effect · Benin

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INTRODUCTION

Myxosporean parasites are known to be responsible for several forms of damage, including myoliquefaction of muscle tissues after the death of the host (Barja & Toranzo 1993, Pampoulie et al. 1999), destruction or infection of ovaries (Swearer & Robertson 1999, Gbankoto et al. 2001a) and reduction of respiratory capacity (Molnár & Székely 1999).

While studying tilapia populations in a brackish water lake, Lake Nokoué (Benin, West Africa), we detected the presence of an intestinal myxosporean, *Myxobolus heterospora* (Baker, 1963), in 2 species — *Sarotherodon melanotheron melanotheron* (Rüppel, 1853) and *Tilapia zillii* (Gervais, 1852). In West Africa, tilapia farms, aimed at augmenting low catches in the natural environment, are located in lagoons and estuaries where salinity varies from 0 to 90 g l⁻¹ (Payne

1983, Albaret 1987). Adaptation to salinity by euryhaline fishes is a complex process involving a suite of physiological and behavioural responses to the environment with different osmoregulatory requirements (Swanson 1998). It is mainly driven by their capacity to use specific osmoregulatory mechanisms (see Prunet & Bornancin 1990) involving such organs as the intestine (Ando 1975), gills (Foskett et al. 1981, Avella et al. 1993, Hiroi et al. 1999), urinary system (Cataldi et al. 1991) and skin (Marshall & Nishikoa 1980). When located in organs such as gills, intestine and skin, parasites such as myxosporeans can have an important negative impact on osmoregulatory mechanisms, and it is surprising that myxosporean infections are not well investigated in tilapias despite the worldwide intensification of tilapia culture (Landsberg 1985).

The aims of this study were: (1) to determine the pathogenic effect of an intestinal myxosporean para-

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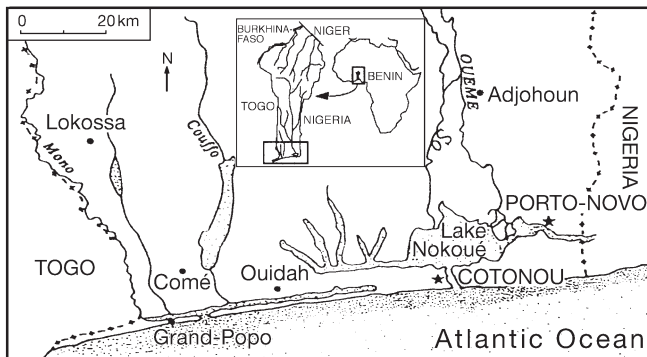


Fig. 1. Sampling site in Lake Nokoué (Benin, West Africa)

site *Myxobolus heterospora* (Baker, 1963) that occurs in the intestine of 2 euryhaline tilapia species, *Sarotherodon melanotheron melanotheron* (Rüppel, 1853) and *Tilapia zillii* (Gervais, 1852), in Lake Nokoué (Benin, West Africa); and (2) to investigate its prevalence and the main factors (season, size and sex of fish) that influence it.

MATERIALS AND METHODS

Environment. Monthly samples were collected over a period of 2 yr from October 1987 to October 1989 from Lake Nokoué in Benin, West Africa (Fig. 1). The maximum area of this lake is 160 km², and its water level varies from 0.5 to 3 m throughout the year. The climate is characterised by 2 dry and 2 wet seasons. The sampling months were grouped as S1 (long dry season from December to March), S2 (long wet season from April to July), S3 (short dry season from August to September) and S4 (short wet season from October to November). Thus, the long dry season of 1989 was designated S1-89 (see Gbankoto et al. 2001b).

The mean water temperature varied from 25.6 to 31.5°C (Fig. 2). Salinity varied greatly during the year (Fig. 2), with a minimum of 0.02 g l⁻¹ and a maximum of 29.77 g l⁻¹; it increased in the dry season with the influx of ocean water through the Cotonou channel (Fig. 1), and decreased during the wet season. Precipitation peaked from May to August (Fig. 2). pH varied between 6.20 and 7.75.

Population structure of fish hosts. We collected 119 male and 103 female *Tilapia zillii*, and 271 male and 113 female *Sarotherodon melanotheron melanotheron* in all. The sex of 7 *S. melanotheron melanotheron* could not be determined because

of their small size. The fish were sorted into size groups (50 to 100, 100 to 150, 150 to 200 and 200 to 250 mm). In *S. melanotheron melanotheron*, 1.28% of the individuals ranged between 50 and 100 mm, 53.45% between 100 and 150 mm, 43.74% between 150 and 200 mm and 1.53% between 200 and 250 mm. In *T. zillii*, 5.41% were between 50 and 100 mm, 68.47% between 100 and 150 mm, 25.22% between 150 and 200 mm and 0.90% between 200 and 250 mm.

Parasite infection. Approximately 30 fish per month were brought alive to the laboratory for species identification, sex determination, and total length (mm) and weight (mg) measurements. Size-frequency distributions were determined for both species. After dissection of the digestive tract, parasites were detected by the presence of white cysts on the external wall of the fish intestine (low-power stereomicroscope), and later by *in vivo* examination of the spores (phase-contrast light microscope). The dimensions of *Myxobolus heterospora* were determined on fresh tissues (n = 30) with an eyepiece micrometer (×1000).

Samples of infected intestine were fixed with 2.5 glutaraldehyde in 0.1 M sodium cacodylate buffer for 1 h and then with 2% osmium tetroxide in the same buffer for 1 h. After dehydration in ethanol, fragments were embedded in Spurr resin. The sections were cut on a Reichert OM U2 microtome, stained with uranyl acetate followed by lead citrate. Observations were made with a JEOL 1200-EX II transmission electron microscope (Central Electron Microscopy Laboratory, University of Montpellier II).

Data collection. Each fish was examined for parasite infection, determined as described above. Prevalence was calculated as the ratio of the number of individuals containing at least 1 visible white cyst to the total number of fish examined. The effect of size, sex and season on prevalence was tested using a χ^2 -test.

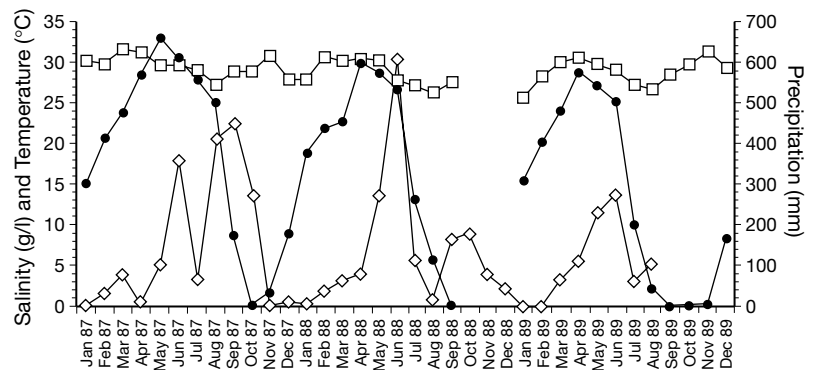


Fig. 2. Seasonal changes in salinity (●), temperature (□) and precipitation (◇) in Lake Nokoué

RESULTS

Parasite and infection description

In both host species, observations under a low-power stereomicroscope revealed cyst forms (50 to 100 × 100 to 250 μm) of *Myxobolus heterospora* (Baker, 1963) embedded in the walls of the intestine (Figs. 3 & 4). As described by Sakiti et al. (1991), the spores (Figs. 5 & 6) were ovoid or ellipsoidal (11.70 ± 1.30 × 8.20 ± 0.60 μm) or almost spherical (8.3 ± 0.8 × 6.98 ± 0.58 μm), with thin walls. The polar capsules were pear-shaped and of equal size (2.7 ± 0.3 × 2.8 ± 0.3 μm). The polar capsule filaments were coiled and formed 5 to 6 coils.

The histological and ultrastructural microscope observations revealed different levels of infection up to total destruction of the intestinal connective and muscular tissues. Invaginations of the intestinal epithelium villi were visible (Figs. 3 & 4). Spores were located between peripheral connective tissue fibres (Fig. 4) or all along the muscle tissue fibres (Fig. 5). In a final stage they destroyed these tissues, while penetrating progressively into the intestine walls. Accumulation of spores could be observed inside an envelope that increased in volume parallel to destruction of the peripheral fibres (Fig. 5). Fig. 6 shows tissue debris among spores that are floating in conjunctive and muscular fibres which are almost liquefied (Fig. 6).

Seasonal patterns of prevalence

The total prevalence of *Myxobolus heterospora* was 42.19% for *Sarotherodon melanotheron melanotheron* (165 infected out of 391) and 26.57% for *Tilapia zillii* (59 infected out of 222). *M. heterospora* was found in *S. melanotheron melanotheron* throughout the study period, with statistically significant seasonal variations ($\chi^2 = 32.01$, $df = 8$, $p < 0.001$; Fig. 7). *M. heterospora* was also found in *T. zillii* throughout the study period, but its occurrence did not vary significantly between seasons ($\chi^2 = 13.31$, $df = 8$, not significant; Fig. 7).

Prevalence as a function of host size and sex

Prevalence of *Myxobolus heterospora* differed significantly ($\chi^2 = 14.29$, $df = 3$, $p < 0.01$) in *Sarotherodon melanotheron melanotheron* as a function of fish size, but not in *Tilapia zillii* ($\chi^2 = 4.08$, $df = 3$, ns) (Fig. 8). Medium-sized fishes were the most parasitised in *S. melanotheron melanotheron*.

The prevalence of *Myxobolus heterospora* differed significantly between male (49.44%, 134 infected fish

out of 271 examined) and female (28.31%, 32 infected fish out of 113 examined) *Sarotherodon melanotheron melanotheron* ($\chi^2 = 14.48$, $df = 1$, $p < 0.001$), and between male (26.51%, 35 infected fishes out of 132 examined) and female (11.11%, 10 infected fishes out of 90 examined) *Tilapia zillii* ($\chi^2 = 7.84$, $df = 1$, $p < 0.001$).

DISCUSSION

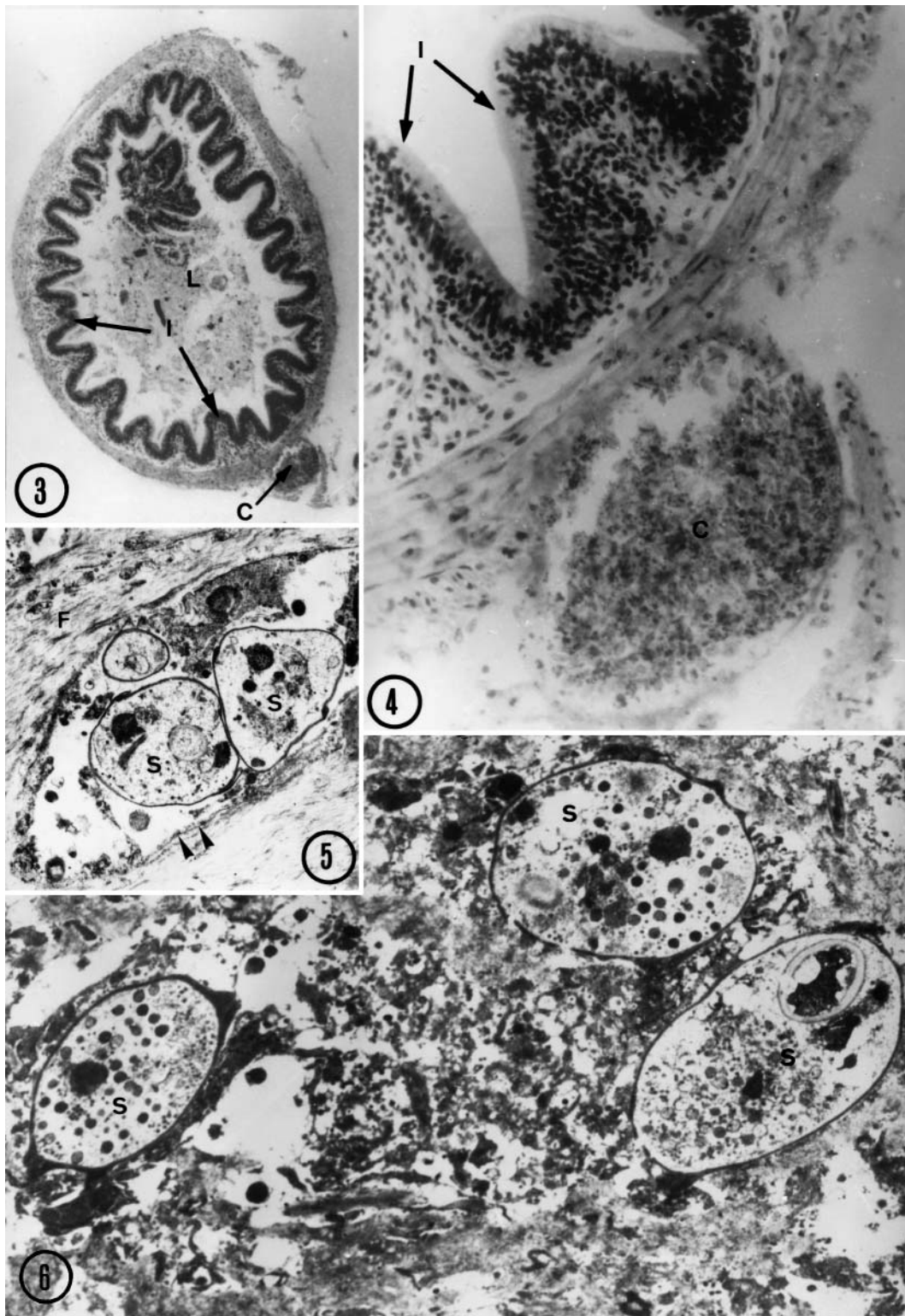
Parasite infection

Intestinal myxosporean parasites are often found in tissue locations similar to those observed in this study on *Myxobolus heterospora*. *Kudoa ciliatae* infects the smooth muscle layer of the intestine of sand whiting *Sillago ciliata* (Lom et al. 1992), *M. colossomatis* has been described inside serous membranes around the intestine of the tambaqui *Colossoma macropomum* (Molnár & Békési 1993), *Myxidium leei* in the intestinal wall of cultured sea bream *Sparus aurata* (Diamant et al. 1994), and *Henneguya sarotherodoni* in the intestine wall of the tilapia *Sarotherodon galilaeus* (Fall et al. 2000). Prevalence data are scarce, but Ghaffar et al. (1995) found that 14% ($n = 80$) of the African catfish *Clarias gariepinus* were infected by cysts of *H. branchialis* on the outer wall of the small intestine, and Landsberg (1983) reported cysts of *Myxidium giardi* in the intestine of 29 of 30 European eels *Anguilla anguilla*.

The high prevalence of *Myxobolus heterospora* (42.19 and 26.57% in *Sarotherodon melanotheron melanotheron* and *Tilapia zillii* respectively) indicates the potential impact of this parasite, and the necessity of further studies on this aspect, as suggested by Molnár & Békési (1993), who concluded that such intensive infections in cultured fingerlings point to the pathogenic nature of this myxosporean parasite.

Seasonal prevalence

Myxobolus heterospora was found throughout the study period but a seasonal pattern of statistically significant variation in prevalence was found only in *Sarotherodon melanotheron melanotheron*. A combination of fluctuations in salinity, temperature and pH between the dry and wet seasons might affect both the parasite life cycle and host behaviour, leading to modifications in prevalence. The sensitivity of the host to the parasite might thus fluctuate in accordance with fluctuations in the osmoregulatory cost arising from salinity changes (from 0 to 29.77 g l⁻¹). In similar cases, modifications of the contact zone between parasites



Figs. 3 to 6. Figs. 3 & 4. *Sarotherodon melanotheron melanotheron*. Light microscopy (Fig. 3. $\times 250$; Fig. 4. $\times 1000$) of a transverse section of intestine (Masson's trichrome stain), showing intestine lumen (L) and invaginations (I) of epithelial villi of fish adapted to salt water; note position of cysts (C) in the intestine wall. Fig. 5. Transmission electron microscopy ($\times 4000$) showing spores (S) between collagen fibres (F); spores are encased in a plasmic membrane (arrowheads). Fig. 6. Transmission electron microscopy ($\times 8000$) of spores (S) in contact with collagen fibres after destruction of the membrane

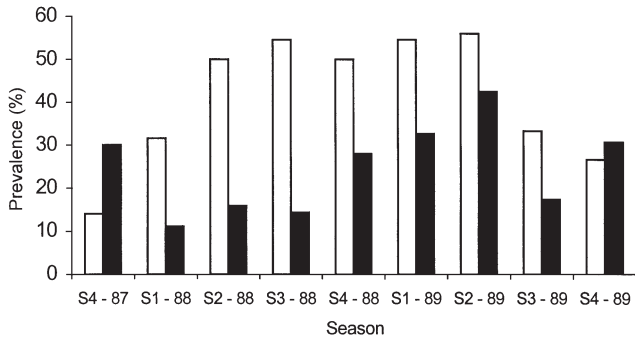


Fig. 7. Prevalence of *Myxobolus heterospora* in *Sarotherodon melanotheron melanotheron* (open bars) and *Tilapia zillii* (filled bars) from Lake Nokoué as a function of season from October 1987 to October 1989. S1: long dry season (December to March); S2: long wet season (April to July); S3: short dry season (August to September); S4: short wet season (October to November)

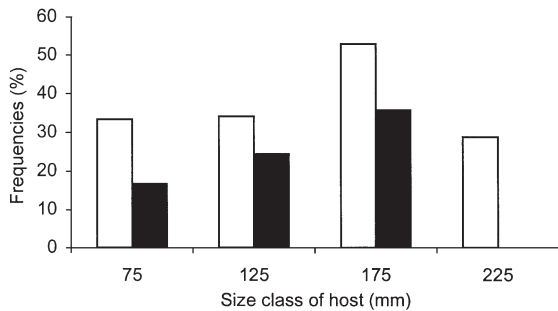


Fig. 8. Prevalence of *Myxobolus heterospora* in *Sarotherodon melanotheron melanotheron* (open bars) and *Tilapia zillii* (filled bars) from Lake Nokoué as a function of size

and their host, or synchronisation of the parasite cycle with the host cycle have been observed that resulted in a modification of the parasite's prevalence in relation to the host life cycle (Pampoulie et al. 2000).

Throughout our study, the temperature in southern Benin varied over a small range (from 25.6 to 31.5°C), so the seasonal pattern of *Myxobolus heterospora* cannot be explained by temperature variations, as was the case for *Sphaerospora renicola* in common carp *Cyprinus carpio* (Grupcheva et al. 1985) and for different species of the genus *Ceratomyxa* (Alvarez-Pellitero & Sitja-Bobadilla 1993). Host-parasite relationships in myxosporeans have been shown to be affected by the biochemical composition of the fish intestine. These parasites are adapted to only a narrow range of specific hosts; therefore in natural waters (including Lake Nokoué), high fish mortality may occur when the highly evolved host-parasite equilibrium is disturbed (Yunchis 1984). Thus seasonal environmental fluctuations may well affect the prevalence of these parasites.

Prevalence as a function of host size and sex

In this study, *Myxobolus heterospora* affected significantly more males than females of both *Sarotherodon melanotheron melanotheron* and *Tilapia zillii*. Male fishes are known to be more sensitive to myxosporean parasites than females (Gonzalez-Lanza & Alvarez-Pellitero 1985, Gbankoto et al. 2001b). This could be primarily due to the energetic cost of testosterone synthesis decreasing immune competency (Poulin 1996).

During our study, medium-sized fish were most parasitized in *Sarotherodon melanotheron melanotheron*. The lower prevalence in the large size class was probably due to mortality of highly infected individuals (which were thus absent from the collections), but could also be due to host adaptation to the parasite infection. Ventura & Paperna (1984) examined 233 intestines of the European eel *Anguilla anguilla* and established a tolerance in older eels to *Myxidium giardi* infection. However, in our study, *Myxobolus heterospora* infection led to complete destruction of the intestine which thus did not allow older fish to develop tolerance. In contrast, *M. heterospora* infection did not vary significantly as a function of fish size in *Tilapia zillii*, suggesting the absence of a lethal effect in this latter species. This difference may simply be due to interspecific differences in the resistance of host species to infection by this parasite. Mitchell (1989) also reported that the prevalence of various myxosporeans such as *M. muelleri* and *M. dujardini* (parasites of *Psychocheilus oregonensis*, *P. caurinus* and *Richardsonius blateatus*) was higher in the oldest fishes.

Potential pathogenic effect

In the present study, histological and ultrastructural examination revealed intestine degradation as a consequence of parasite infection. *In situ*, *Myxobolus heterospora* reproduces by asexual reproduction and progressively colonises the intestine wall. No lethal cases due to *M. heterospora* have yet been described, but they cannot be excluded when the infection is acute, with death probably resulting from intestinal degradation involving possible septicaemia, metabolite loss and osmoregulation dysfunction.

Osmoregulation is known to involve both gills (Potts et al. 1967) and the intestinal epithelium (Rawdon & Cornish 1973, Mainoya 1982). Consequently, we believe that one of the first effects of *Myxobolus heterospora* before total intestinal degradation could be dysfunction of the osmoregulation processes, prior to complete intestinal destruction. Moreover, there is evidence that in response to changes in environmental conditions such as salinity and changes in physiologi-

cal status, fishes use flexible strategies for the allocation of energy to osmoregulation, maintenance, activity and growth (Wieser & Megyesy 1991, Swanson 1998). Therefore, their capacity for resistance to infection could approach zero in the interconnected lagoon of Lake Nokoué, where salinity varies from 0.02 to 29.77 g l⁻¹ and, in turn, intestine infection could affect their osmoregulatory ability. Thus, infestation of the intestine by *M. heterospora* could negatively affect the survival of tilapia, which are considered to be the fishes most suitable for aquaculture in the brackish water lagoons of West Africa (Pauly 1976, Legendre 1983, Stickney 1986, Doudet 1991).

In conclusion, our results demonstrated the high occurrence of the intestine myxosporean *Myxobolus heterospora* in 2 tilapia species *Sarotherodon melanotheron melanotheron* and *Tilapia zillii*, and clearly showed the impact of this parasite on their intestinal structure and its potential effects on osmoregulatory mechanisms. Further investigations are required to detect the interactive effects of salinity and parasite infection on the physiology of economically important fishes.

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