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Intestinal trematodes *Prosorhynchoides ozakii* (Bucephalidae; Bucephalinae) in pond-cultured catfish *Pangasianodon hypophthalmus* in the Mekong Delta (Vietnam)

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Abstract

Catfish farming in Vietnam based on *Pangasianodon hypophthalmus* has expanded significantly and gained status as an important export business. As in other intensive fish farm enterprises some disease problems may be foreseen in the systems and disease surveys are needed to secure sustainable conditions and health control programmes for the fish. We have conducted a survey in catfish farms in two provinces of Southern Vietnam. Fish were collected in freshwater ponds from April to October and were thoroughly investigated for the presence of intestinal adult digenean trematodes. In the intestine of many catfish numerous adult bucephaline trematodes of the species *Prosorhynchoides ozakii* were found. All specimens were sexually mature and some were gorged with eggs. Overall prevalence was 19.7 % and the overall mean intensity was 9.6 (SD 4.6). Prevalences in individual samples varied from 12.5 to 69 % and intensities varied from 1-37 parasites per host. The variations of the parasite occurrence in relation to farm type, season, host age, host body length and habitat (including occurrence of intermediate hosts) were studied. A clear trend for increasing worm loads in larger fish was observed. In addition, farms with high hygienic measures (drying and disinfection of ponds, treatment of pond water from rivers) exhibited lower infection.

Introduction

The Mekong Delta of Vietnam is well known as a region rich in aquatic resources. Intensive fish pond culture in the Mekong Delta based on the species *Pangasianodon hypophthalmus* has proved successful and the species is today one of the most important pond cultured fish species with a high potential for export. Studies have indicated that it may be relevant to implement disease surveys in order to secure improved health condition of the fishes. Recent investigations have shown that catfish is host to specific monogeneans in various regions of South East Asia (Lim, 1996; Lim et al., 2001; Pariselle et al., 2002; 2006; Thuy & Buchmann, 2008) and the pond-cultured *P. hypophthalmus* in the Mekong Delta was recently found to host two monogenean species (Thuy & Buchmann, 2008). Thus, *Thaparocleidus siamensis* dominated the samples whereas *T. caecus* was extremely rare. Further, investigations for other parasites in *P. hypophthalmus* in Malaysia conducted by Molnar et al. (2006) demonstrated that also

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myxosporeans are frequent findings in cultured catfish. Due to the fact that digenean parasites also may cause morbidity among the production fishes (Wang et al., 2001; Ogawa et al., 2004) a thorough survey of digeneans in catfish in Vietnam is needed as well. The presence of intestinal adult trematodes parasitizing North Vietnamese fishes (but not *P. hypophthalmus*) was previously documented by Moravec & Sey (1989) but detailed data for prevalences and intensities were not provided. Further, recent investigations have pointed to the presence of different species of digenean metacercariae in Vietnamese fishes, including catfish from the Mekong Delta (Thien et al., 2007), from cyprinids in Japan (Ogawa et al., 2004) and from bullhead catfish from China (Wang et al., 2001) but we still lack detailed documentation on the infection of *P. hypophthalmus* with regard to adult intestinal trematodes. The present paper reports on an investigation on adult digenean trematodes in *P. hypophthalmus* collected in the Mekong Delta. Such a work could provide the basis for development of future control methods needed to support a sustainable aquacultural production with high quality products in the region.

Materials and methods

Farms

A total of 351 specimens of *P. hypophthalmus* were collected in pond culture systems with intensive and semi-intensive management plans in the two provinces Cantho and Vinhlong in Southern Vietnam. Sampling was conducted on 49 occasions from April 2006 to late October 2006 and each sample comprised 5-12 fish which were caught by hand-net on the farms. The pond management system and type of farm (cleaning of ponds between each cycle, pond size) were noted.

Examination of fish

The fish were transported to the laboratory (RIA2, Ho Chi Minh City) and fish age (days post-hatch) and full body length (cm) of the fish were recorded. Fish were killed and subsequently the intestines with contents were examined under a dissection microscope (7-40 x magnification). Live parasites were isolated, fixed in cold neutral formalin, stained with haematoxylin and mounted in glycerine jelly. Drawings were made by the use of a drawing tube and photographs taken on a Leica DMLB. Infection was described as prevalence (the percentage of the host population which is infected with a certain parasite) and mean intensity (mean number of parasites in the infected fish only) according to Bush et al. (1997). Differences of means in compared groups were evaluated by the use of the Mann-Whitney U-test. The Spearman rank correlation coefficient was calculated to illustrate associations between body length and intensity of infection. A 5 percent probability level was applied for all tests.

Results

Farm management types investigated

The fish production system called "Farmhouse system" was represented by 26 samples. These production units are characterized by smaller size, low investment and continous stocking of fish in ponds without drying, sludge removal and disinfection between production cycles. Feeding is less regular and not always based on commercial feed pellets. Water supply is direct from the river but no treatment of water is conducted on a regular basis.

The farm-type designated "Farmhousehold system" was represented by 23 samples. These systems are characterized by larger units and high investment. Hygienic measures including drying and disinfection of ponds between production cycles are always performed. Feeding is exclusively based on commercial feed pellets. The water supply from the river is treated weekly by disinfectants (formalin, copper sulphate).

Fish size

The investigated catfish expressed a clear size increase throughout the season from April to June. Samples in August, September and October comprised a mixture of older fish and smaller fish from stocking during the late season. The increase of size during the first months reflected age related growth. Young fish (less than 10 cm body length) stocked from April and harvested later in the season reached a body length of more than 30 cm by harvest.

Infection

A total of 351 fish were examined for infection with intestinal trematodes. The ages of hosts ranged from 4 to 230 days post-hatch. The body lengths varied from 3 to 34 cm. Of these were 69 fish infected with a total of 662 parasites corresponding to an over all prevalence of 19.7 % and a mean intensity of infection of 9.6 parasites per infected fish (SD 4.63). The minimum number of parasites in infected fish was 1 and the maximum number 37.

A total of 49 samples each comprising 5-12 fish were collected and investigated. It was found that 32 samples contained infected fish (65.3 %). There was found no significant seasonal variation of the infection level although the highest prevalence (60 %) was found in November samples. Likewise, a trend for more uninfected samples was seen in April/May. When the material was analysed for association with fish age only a slight and non-significant trend was seen for higher prevalence and intensity of infection in older fish (Table 1). However, a weak positive correlation between body length and intensity (only infected fish included, n=69) was found (Spearman rank correlation coefficient $r = 0.24$, $p<0.05$). This was also reflected by an increased number of parasites in hosts larger than 31 cm compared both to fish <10 cm and

Fish age (days)	**4-50**	**51-100**	**>100**
Number of samples	14	18	17
Prevalence % (mean, SD)	20.71 (20.97)	18.33 (18.7)	21.75 (18.38)
Intensity (mean, SD)	4.43 (6.43)	5.8 (6.08)	6.94 (7.66)

Table 1. Association between fish age (days) and infection level (prevalence and intensity).

Total body length (cm)	**1-10**	**11-20**	**21-30**	**31-40**
N	5	29	25	10
Mean intensity (SD)	6.6 (3.9)	9.6 (7.7)	7.4 (6.4)	16.1 (10.2)*

*: Significantly different from other size classes, p<0.05, Mann- Whitney U test.

Table 2. Association between fish body length and mean intensity (number of parasites per infected fish).

Farming system	Farmhouse	Farmhousehold
Number of samples	26	23
Prevalence % (mean, SD)	23.65 (20.13)	13.04 (16.5)
Intensity (mean, SD)	7.04 (8.43)	4.96 (6.05)

Table 3. Association between farming system and infection level expressed as prevalence and intensity.

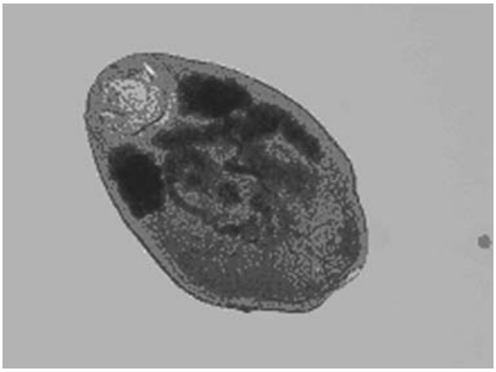

Figure 1. Whole mount of young *P. ozakii* recovered from intestine of *P. hypophthalmus.*

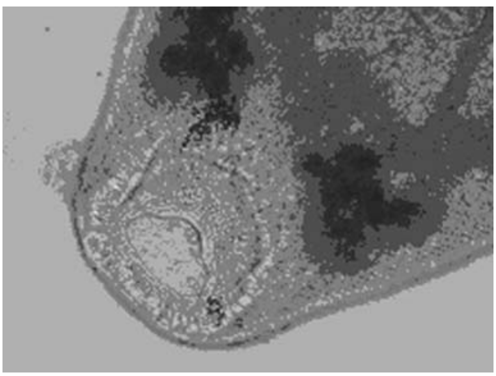

Figure 2. Rhynchus morphology.

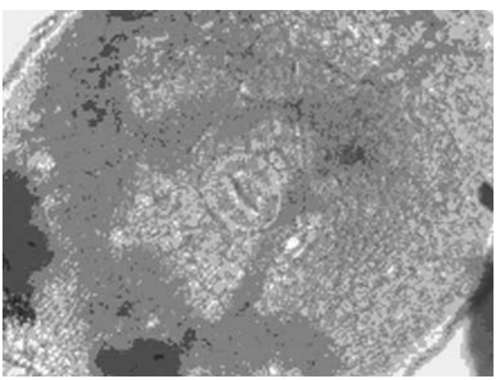

Figure 3. Mouth and pharynx.

compared to fish 21-30 cm (Table 2). Infection level showed no association with pond size. In addition, the geographic location had no influence on infection (data not shown). In contrast, the farm management type showed an association with the infection level. Thus, farmhousehold systems showed a lower prevalence and mean intensity compared to farmhouse systems (Table 3). In addition, more uninfected samples were obtained illustrated by six negative samples (comprising 48 fish) collected from two farmhousehold farms in April and May. Further, samples with prevalences of more than 30 % were mainly taken from farmhouse systems (9 out of 11). In contrast, samples with prevalences below 30 % were more often farmhousehold collections (10 out of 21).

Parasite identification

General morphology: One species of Digenea belonging to the family Bucephalidae and the subfamily Bucephalinae was found. It was diagnosed as *Prosorhynchoides ozakii* according to the description provided below. The early less mature stages (Figure 1) were slightly elongate. The rhynchus was simple without ornamentation (Figure 2). The mouth and pharynx was placed in the midpart of the body over the caecum (Figure 3). The tegument was spiny (Figure 4) and the vitellaria were characteristically located in the forebody just below the level of the rhynchus and the vitelline duct extended to the posterior of the body (Figure 5). The fully mature specimens were loaded with oval yellow eggs (Figure 6).

Morphometric description

Number of specimens measured: 10. Body length: 0.4-1.4 mm. Body width: 0.3-1.0 mm. Tegument structure: spiny in transverse rows. Rhynchus: (simple sucker without ornaments), Length: 0.13-0.32 mm, Width: 0.15-0.24 mm. Mouth: slightly anterior to midbody. Caecum: Saclike, small under pharynx. Testes: (round to elongate placed posteriorly to ovary), Length: 0.2-0.3 mm, Width: 0.1-0.2 mm. Ovary: pretesticular Length: 0.1-0.3 mm. Eggs yellow. Egg size: Length: 0.016-0.026 mm, Width: 0.010-0.015 mm. Cirrus sac placed posteriorly: L: 0.2-0.3 mm. Vitellaria: Characteristically placed in two groups in anterior part of the body, vitelline ducts extending to posterior part.

Discussion

The present investigation has shown that pond cultured catfish *P. hypophthalmus* in the Mekong Delta are infected with adult intestinal trematodes of the genus *Prosorhynchoides* belonging to the family Bucephalidae and the subfamily Bucephalinae (Overstreet & Curran, 2002). Bucephalids lack oral and ventral suckers but carry a number of specific characteristics including the apical holdfast, a rhynchus, a muscular pharynx and an oesophagus connected to a saclike caecum which never bifurcates. In addition, the posterior genital complex comprises a cirrus sac and a genital atrium opening to the exterior (Overstreet & Curran, 2002). The vitellaria are often characteristically grouped in the forepart of the worm. The species collected was designated *P. ozakii* despite the

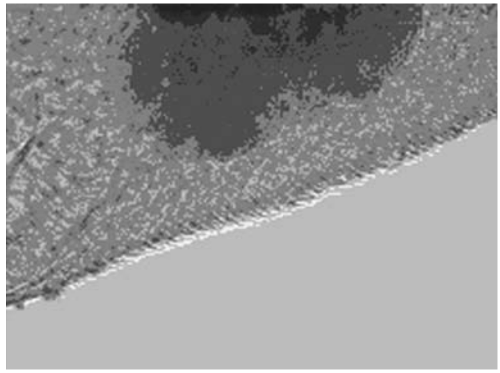

Figure 4. Spines in anterior tegument.

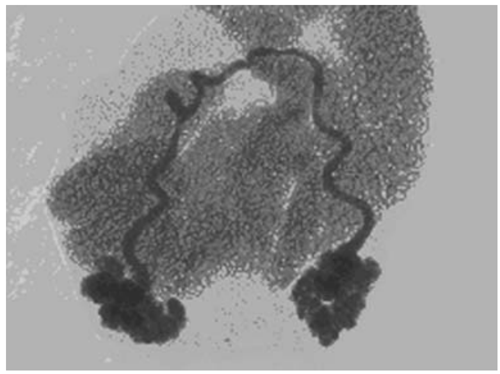

Figure 5. Characteristically located vitelline glands in mature specimen loaded with eggs.

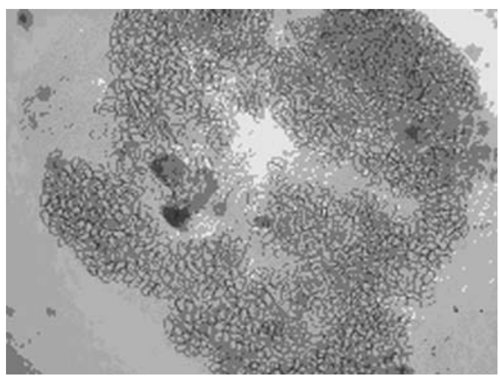

Figure 6. Eggs in fully mature specimen.

fact that minor differences in size of eggs and body length was noted compared to worms collected by Urabe et al. (2007). However, similar variations within the species occur as noted from other reports (Moravec & Sey, 1989). This could partly be due to differences in the fixation and compression techniques used (cold versus hot formalin). Further, the intestinal microhabitat in *P. hypophthalmus* could be different from host fishes (*Silurus biwaensis*) examined by Urabe et al. (2007) and thereby affect growth performance of parasites. Thus, the host factor could also account for the recorded slightly lower egg-size. On this basis it was decided not to erect and describe a new variant within the species. The examined catfish hosts (19.7 %) were found infected with up to 37 parasites and the complete dominance of *P. ozakii* in the pond cultures studied points to the fact that *P. hypophthalmus* is a satisfactory host for this species. The parasite has previously been recorded from a range of hosts including *Pelteobagrus (Pseudobagrus) vachelli*, *Saurogobius dobryi*, *Parasilurus asotus* (Moravec & Sey, 1989) and *Silurus biwaensis* (Urabe et al., 2007). The life cycle has not been fully elucidated but it is known that bucephalids generally use lamellibranch molluscs as first intermediate hosts, fish as secondary intermediate hosts (carrying metacercariae) and predatory fishes as final hosts (Overstreet and Curran, 2002). The related species *Parabucephalopsis parasiluri* uses the golden mussel *Limnoperna fortunei* as the first intermediate host and several fish species as intermediate hosts (Urabe et al., 2007). The first intermediate host of *P. ozakii* has not been identified yet but according to Urabe et al. (2007) various cyprinid, centrarchid and gobiid teleosts carry the metacercariae and serve as second intermediate hosts.

The infection levels may in some fish reach relatively high levels but the impact of these parasites on host health is not clear. There is reason to believe that they can lead to some morbidity in their metacercarial stage in fish intermediate hosts (Ogawa et al., 2004; Urabe et al., 2007) which calls for a deeper analysis of the infection dynamics.

It cannot be excluded that the catfish, in the present study, were infected by the time of stocking reflecting infection at an earlier stage in another location (hatchery and nursery ponds). However, the worm burden increases during growth of the fish which indicates that the life cycle is active in the farms and that fish can contract the infection during the pond period. In addition, the parasite seems to have satisfactory abiotic (including temperature) conditions in the ponds investigated. The temperature in the pond water during the first part of the season is near 28-29 °C whereas the temperature drops off to around 20 °C in October. The flooding period from August may increase the risk of immigration of infected intermediate hosts (Thien et al., 2007) and thereby contribute to the rise of infection level. Nonetheless, it is noteworthy that the farm type and management tended to influence the infection level. The farmhousehold systems experiencing lower infection use hygienic measures including drying and disinfection of ponds between production cycles and the water supply from the river is treated weekly by disinfectants. Thus, first intermediate hosts (probably lamellibranch molluscs) and various introduced second intermediate hosts (different species of smaller fish) are likely to have lower survival in these more intensive systems. Likewise, freely swimming cercariae liberated from lamellibranches may experience a lower survival in the treated intensive system. It is not yet known if catfish themselves can act as second intermediate hosts. If this is the case smaller catfish infected with *P. ozakii* metacercariae could be ingested by larger predatory catfish getting infected by cannibalism. Catfish from the Mekong Delta were in fact examined for metacercariae by Thien et al. (2007) and these authors detected quite a few un-identified metacercariae. Future studies should elucidate if metacercariae of *P. ozakii* also infect *P. hypophthalmus.* In this context it should be noted that various species of catfish carry metacercariae of related bucephalids (Wang et al., 2001; Ogawa et al., 2004; Urabe et al. 2007).

By including only infected fish in an analysis of fish size and intensity it became clear that larger fish carried higher worm burdens. This could probably be due to a longer exposure period (and ingestion of more intermediate fish hosts) combined with an extended longevity of the adult intestinal trematodes. To what extent the host immune system affect these worms are at present unknown but this biotic parameter may affect the parasite population as well.

Generally the worm burdens experienced should not be considered excessive and alarming. The maximum number of worms was 37 per host and the main part of the infected hosts carried between 2 and 15 worms. On the other hand the intestinal trematodes may in association with other pathogens including ectoparasites of the genus *Thaparocleidus* (Thuy & Buchmann, 2008) affect the general health and growth of the production fishes. Likewise, occurrence of myxosporean infections (Molnar et al., 2006) has been shown in some catfish systems. It could therefore be relevant to investigate possibilities for controlling these infections. In this context, the life cycle of the parasite should be elucidated. However, based on existing knowledge on the bucephalid biology it will be relevant to eradicate putative first intermediate hosts (lamellibranch molluscs) and metacercaria infected second intermediate hosts (smaller fish). Alternatively, mechanical filtration of water to remove infective parasite stages (cercariae) (Larsen et al., 2005) could be an option to avoid excessive use of chemicals in Vietnamese fish farming.

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