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Risk assessment of parasitic helminths on cultured Nile tilapia (*Oreochromis niloticus*, L.)

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ABSTRACT

Disease outbreaks constrain aquaculture development. Knowledge on the potential risks of pathogens to farmed fish can help in designing management strategies for increased aquaculture productivity and sustainability. This study compares the helminth infections in reservoirs and therein operated cages as well as earthen fish ponds and the feeding stream in order to assess the significance of life cycle style and water sources in parasite transmission. In addition field experiments were setup to determine loading time and transmission rate. From 650 fish examined, 8 helminth species were recorded (3 from caged- and all 8 from pond-raised fish). The parasite community was dominated by trophically-transmitted species in both culture systems indicating the importance of trophic pathway in helminth transmission. The occurrence of trophically-transmitted helminths in caged-fish was positively related to their prevalence in reservoir-dwelling hosts indicating the importance of water supply in spread of helminths. The prevalence in pond-raised fish was higher than in stream-dwelling ones suggesting the presence of local sources of infective stages within ponds. Risk assessment revealed that monogeneans are high-risk parasites while heteroxenous helminths pose low to negligible threats to farmed fish. Although, cages appeared safer to heteroxenous parasites than ponds, their location in the water body, especially the distance from shores and depth is critical.

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1. Introduction

In an attempt to increase fish production, various water bodies both natural and manmade are either utilized or being considered for aquaculture. However, ecological conditions in culture facilities may create favorable conditions for parasite proliferation (Lafferty and Kuris, 1999) thereby threatening aquaculture production. Outbreak of diseases in production facilities can cause significant financial losses to fish farmers (Brun et al., 2003; Faruk et al., 2004). Therefore, designing and implementation of appropriate contingency plans and management strategies, based on scientific information are required for disease prevention and control (Arthur et al., 2009; Bondad-Reantaso et al., 2008).

Risk analysis was adopted by World Trade Organization as a tool to minimize threats associated with the trade of live animals and/or their products (MacDiarmid, 1997; WTO, 1994). Recently, the tool was proved useful in minimizing disease cross-transmission between wild and farmed fish (Hutson et al., 2007; Nowak, 2004; Peeler et al., 2007). Risk analysis generates information on the likelihood of pathogen infections and their consequences, as such provide essential information for decision making (MacDiarmid, 1997). The essential step in risk analysis is risk assessment. It evaluates the routes of infection, establishment and effects of the pathogens. The procedure requires knowledge about the ecological framework that governs host interactions. This is the crucial link between epidemiology and ecology because parasite-related threats may vary under different aquatic habitats and weather conditions (Lafferty, 2008; Lafferty and Kuris, 1999; Marcogliese, 2001; Ondrackova et al., 2004).

This study evaluates the potential risk posed by helminths to cultured *Oreochromis niloticus* in cage and earthen pond systems in Uganda. The specific objectives were to (i) examine the importance of life cycle styles in helminth transmission to two culture systems, (ii) evaluate the significance of water sources to helminth infections by determining the relationship between their prevalence in cultured and wild fish, and (iii) assess the risks of helminth infestation to different aquaculture systems.

2. Materials and methods

2.1. Fish and parasite collection

Specimens of *O. niloticus* for parasitological examination were collected from the BOMOSA fish cage culture experimental systems

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(www.bomosa.org) in Ndolwa Dam, Lake Kyoga basin (1.21°N 33.10°E) and from ponds and their water source, the Kajjansi stream at Aquaculture Research and Development Centre (Kajjansi ADRC) within Lake Victoria basin (0.13°N 32.32°E; Fig. 1). Random samples of up to 30 fish were collected monthly between January and November 2008 from each site using gillnets, seine nets, hooks and scoop nets, except June, when no samples were collected from Kajjansi ADRC. The entire sample consisted of 650 fish with total lengths ranging from 2 to 34 cm (mean 13.2 cm) and wet weight ranging from 0.5 to 470 g (mean 58.4 g). The fish were transported alive in the original water at the prevailing temperature to the laboratory and examined within 2 h after arrival. Observed parasites were isolated, counted, and sent to the Department of Veterinary, Public Health and Animal Pathology, Faculty of Veterinary Medicine, University of Bologna, Italy for identification. The monogeneans were identified from the Institute of Aquaculture, University of Stirling, Scotland.

2.2. Importance of life cycle style in helminth transmission

The helminths were grouped according to the transmission pathway. FTP (free-living transmitted species) were those infecting fish via free living stages such as oncomiracidia (monogeneans) and cercariae (digeneans). TTP (trophically transmitted helminths) were those transmitted through food items e.g. procercoid (cestodes) and cystacanth (acanthocephala).

2.3. Importance of the water source in helminth transmission

To evaluate the significance of the water source to helminth infections, the relationship between the monthly prevalence of the dominant species (*Bolbophorus* sp., *Acanthogyrus* (*Acanthosentis*) *tilapiae* and *Amirthalingamia macracantha*) in wild hosts and culture systems was determined using Spearman's correlation coefficient. The parasitological terms of prevalence and mean intensity of each parasite species recorded from fish from the systems were used following Bush et al. (1997). The correlation between the prevalence of *Cichlidogyrus* spp. in the culture and wild systems was not computed because of their presence in the hosts prior to stocking.

2.4. Risk assessment

The four components of risk assessment: a) hazard identification, b) release assessment, c) exposure assessment and d) consequence assessment, were determined according to Murray and Peeler (2005), Bartley et al. (2006) and Arthur et al. (2009).

2.4.1. Hazard identification

The most prevalent parasite species recorded in/on *O. niloticus* examined during parasitological investigations (Akoll et al., 2012; Florio et al., 2009): *Cichlidogyrus* sp., *Bolbophorus* sp., *Acanthogyrus* (*A.*) *tilapiae* and *A. macracantha* were selected to represent monogeneans, digeneans, acanthocephalans and cestodes, respectively and considered as hazards.

2.4.2. Release assessment

Release assessment was classified according to colonization strategy and the known host range.

i. Colonization strategy, which is the parasites' dispersal range and the ability to colonize new environments, was used according to Esch et al. (1988) and hence parasites were categorized into two groups. The allogenic parasites mature in birds or mammals and thus, can be dispersed widely and have high colonization rate — hence have high chances of establishment and spread. In contrast, autogenic are those that mature in fish and would require anthropogenic translocation of the fish to colonize a wide area, as such, have low risk. Monogeneans are categorized as autogenic. However, because *Cichlidogyrus* spp. frequently occurred in fish for stocking (Akoll et al., in press) and have short-direct life cycle, the likelihood of spread and establishment was considered high.
ii. With regard to the host range (number of hosts the parasite species has been reported), parasites were categorized as specialist (strictly

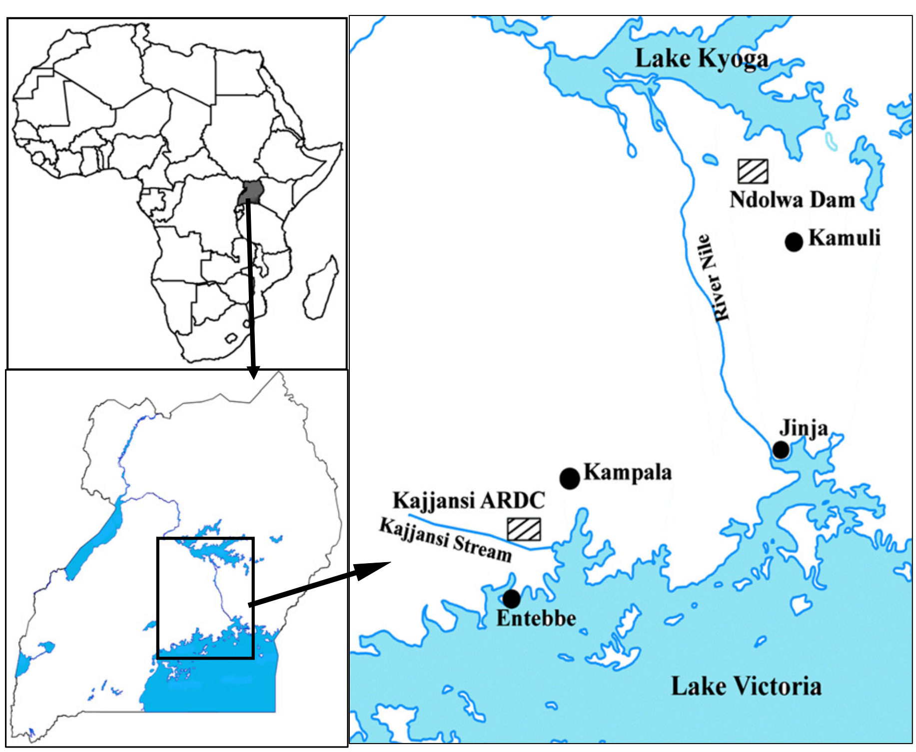

Fig. 1. Map of Uganda showing the sampling sites (▨): Ndolwa Dam — the reservoir (and cages therein) and Kajjansi.

one host) or generalist (several hosts) based on the available published information on the species. The risk was categorized as low (1 host), moderate (between 2 and 5 hosts) and high (more than 5).

2.4.3. Exposure assessment

Exposure assessment was determined using infection level and transmission rate, which is the rate at which parasite spreads among the hosts, after the initial appearance and loading time, the period at which the species is first observed on the fish after stocking.

i. The infection level was classified according to the prevalence and intensity of the parasites recorded across habitats during the present study for both wild and cultured *O. niloticus* populations. The risk from overall prevalence was categorized as negligible (0%), very low (between 0 and 10%); low (between 10 and 25%), moderate (between 25 and 50%) and high (>50%).
ii. Transmission rate and loading were identified by analyzing fish in two cages and one pond over a period of 6 months in parallel to the other study program. The transmission rates for *A.* (*A.*) *tilapiae* and *A. macracantha* were calculated as the change in prevalence of each parasite species during the study period (Eq. (1)).

$$R = \frac{P_1 - P_0}{P_0} \quad (1)$$

where, R — transmission rate, P_1 — prevalence in the new sample and P_0 — prevalence in the previous sample. Meanwhile, the transmission rate for the monogeneans was considered high because of the short and direct lifecycle (5–6 days). The loading time risk for all parasites was classified as high (between 0 and 2 months post-stocking), moderate (3 months), low (4 months), very low (5 months) and negligible (6 month and above).

2.4.4. Consequence assessment

Assessment of consequences was based on the potential economic losses accrued from parasite infestation from available literature. The losses was evaluated with regard to

i. fish mortalities,
ii. predation — possibility of increasing fish predation,
iii. pathology — assessed according to the severity of the parasite on the host tissues as: very low (no information available on the pathology and is likely to cause very minimal tissue alterations), low (limited or restricted pathology), moderate (mild pathology but there are chances of inducing severe tissue alteration), and high (significant or severe tissue changes that threaten fish health),
iv. public health concerns, and
v. the potential to reduce marketability of the fish.

2.4.5. Overall risk

The overall risk for each species was calculated as the product of likelihood (release plus exposure risks) and consequence using a likelihood and consequence matrix according to Arthur et al. (2009).

3. Results

3.1. Parasite occurrence in culture systems

Between January and November 2008, 650 fish were examined, of which, 165 fish were caught in the reservoirs, 213 from cages, 153 from the earthen ponds and 119 from the Kajjansi stream feeding the ponds. Eight helminth species were identified including two monogeneans: *Cichlidogyrus tilapiae* and *C. sclerosus*; four digeneans: *Clinostomum cutaneum*, *Tylodelphys* sp., *Diplostomum* sp. and *Bolbophorus* sp.; a cestode: *A. macracantha*; and an acanthocephalan: *Acanthogyrus* (*Acanthosentis*) *tilapiae*. All the eight helminth species were present in/on the pond-raised and stream-dwelling fish. The eye lens parasite: *Diplostomum* sp. was absent in the reservoir both in free-living and caged fish while *Tylodelphys* sp., and *C. cutaneum* were absent in caged-fish only. With exception of the monogeneans, all the other helminths were recorded on cultured fish between one and two months post-stocking. The parasite composition included six free-living transmitted parasites (FTP), namely, *C. tilapiae*, *C. sclerosus*, *C. cutaneum*, *Tylodelphys* sp., *Diplostomum* sp. and *Bolbophorus* sp. and two trophically transmitted species (TTP): *A. macracantha* and *A.* (*A*) *tilapiae*. The overall prevalence and intensity of helminths found during this study revealed that trophically transmitted helminths dominated heteroxenous species communities in both culture facilities.

3.2. Relationship of parasite prevalence between water supply and culture systems

The prevalence of TTP (*A. macracantha* and *A.* (*A.*) *tilapiae*) in cages was positively correlated with that recorded in reservoir-dwelling fish ($r = 0.32$, and 0.86 respectively, $p < 0.05$). No significant correlation was found between the prevalence of TTP in pond-raised and stream-dwelling hosts ($r = -0.07$, and -0.01, $p > 0.05$). The prevalence in the pond-raised fish was slightly higher than in the stream-dwelling hosts. With regard to TFP represented by *Bolbophorus* sp., the prevalence in fish from both culture facilities was not significantly correlated with the prevalence in the wild hosts ($r = 0.0002$ for cages, and $r = 0.005$ for ponds, $p > 0.05$).

3.3. Risk assessment

The results of the risk assessment revealed that the overall risk posed by the *Cichlidogyrus* spp. (monoxenous parasites) to both culture systems was high and for heteroxenous parasites (*A. macracantha*, *A.* (*A.*) *tilapiae* and *Bolbophorus* sp.) the risk ranged from negligible to very low for cages and very low to low for ponds (Table 1). Among the culture systems, the results showed that fish in cages appeared to be at a lesser risk to *A. macracantha* and *A.* (*A.*) *tilapiae* than in ponds but not to digeneans and monogeneans.

4. Discussion

Wild and cultured *O. niloticus* from Uganda harbor several parasites (Akoll et al., 2012; Florio et al., 2009). In culture facilities, these parasites can proliferate to devastating infections. As such, comprehensive management strategies should be put in place to ensure sustainable aquaculture development. Moreover, policy formulation, and designing and implementing appropriate disease management plans require science-based information (Arthur et al., 2009; Bondad-Reantaso et al., 2008). Pathogen risk-related information can be obtained through risk assessment, which determines the likelihood of spreading, establishing and the potential consequences of pathogens, all important in fish health management.

The present parasitological investigation yielded eight helminth species. With exception of monogeneans, the TTP dominated the helminth communities in both culture systems. This highlights the importance of trophic pathway in enhancing dispersal capacity of the parasites (Marcogliese, 1995). In contrast, free-living stages rely on their energy reserves and swimming abilities to seek and penetrate suitable fish. The transmission ability of these free-living stages is also significantly reduced by fluctuations in environmental conditions; predation and alternative hosts (Combes et al., 2002; Esch and Fernandez, 1993; Pietrock and Marcogliese, 2003; Thieltges et al., 2008). Overall, the life cycle strategy contributes significantly to enhanced transmission success of parasites.

The positive relationship found between trophically transmitted helminths in cages indicates the importance of water in spreading

Table 1
The risk assessment scheme for helminths in cultured *O. niloticus* from Uganda.

Parameter	*Amirthalingamia macracantha*		*Acanthogyrus (A) tilapiae*		*Bolbophorus* sp.		*Cichlidogyrus* spp.	
	Cage	Pond	Cage	Pond	Cage	Pond	Cage	Pond
1. Release assessment								
Colonization strategy	High (allogenic)		Low (autogenic)		High (allogenic)		High (autogenic)	
Host range	Moderate (2 hosts[a])		High (>30 host[b])		High (several hosts[c])		High (17 hosts[d])	
Sum of release risk	Moderate	Moderate	Low	Low	High	High	High	High
2. Exposure assessment								
Infection level in wild[e]	Moderate	High	Low	Moderate	Low	Low	Moderate	Moderate
Infection level in aquaculture[e]	Very low	High	Low	Moderate	Very low	Very low	Moderate	Moderate
Loading time[f]	Moderate (3)	High (2)	High (2)	High (2)	–	–	High (0)	High (0)
Transmission rate[f]	Moderate (1.2)	High (2.3)	High (1.3)	High (1.2)	Moderate	High	High	
Sum of exposure risk	Very low	High	Low	Moderate	Very low	Very low	High	High
Likelihood (sum of release and exposure risks)	Very low	Moderate	Very low	Low	Very low	Very low	High	High
3. Potential consequences								
Predation	High		Low		High		High	
Mortalities	Low		Low		High[g]		High[h]	
Pathology[i]	Moderate		High		High		High	
Public health	Negligible		Negligible		Negligible		Negligible	
Marketability[j]	Low		Low		High		Low	
Sum of consequence	Moderate	Low	Low	Low	High	High	High	High
4. Overall risk (sum of likelihood and consequence)	Very low	Low	Negligible	Low	Very low	Very low	High	High

[a] Aloo (2002); Scholz et al. (2004).
[b] Amin et al. (2008).
[c] Paperna (1996).
[d] Pariselle and Euzet (2009); le Roux and Avenant-Oldewage (2010).
[e] Infection level: assessed from the data on prevalence and mean intensity.
[f] Loading time: given in months post-stocking in parenthesis and transmission rate is provided in parenthesis.
[g] Paperna (1996) reported mortalities associated with *Bolbophorus* sp.
[h] Kabata (1985), reported mortalities due to *C. sclerosus*.
[i] Paperna (1996) and Florio et al. (2009) have documented pathologies of these parasites.
[j] Kabunda and Sommerville (1984) reported rejection of tilapia.

the parasites from wild populations to caged fish. The present finding supports the previous evidence on the role of water supply as a source and dispersion pathway of parasites to cultured fish (Hutson et al., 2007; Murray and Peeler, 2005; Rückert et al., 2009; Sepulveda et al., 2004). This relationship affirms the fact that the presence of high population densities of infective stages within the reservoir is an important strategy for parasites to increase transmission success (Combes et al., 2002). In contrast, the high prevalence recorded in pond-raised fish compared to the stream-dwelling hosts demonstrates the importance of local sources of parasite infective stages within the pond. Indeed, the shallow water depth, occurrence of snails and zooplankton as well as the likelihood of access by birds to ponds provide an optimum platform for the interaction between the hosts. This enables completion of the parasites' indirect lifecycle within the pond, which may explain the high prevalence in ponds compared to the feeding stream.

In the present study, the overall risk was high for monoxenous helminths and ranged from low to negligible for heteroxenous species. The high risk for monoxenous parasites could be attributed to their short and direct lifecycles which result in rapid proliferation to adverse levels (e.g. Kabata, 1985; Ogawa, 2002; Thoney and Hargis, 1991). Indeed, *Cichlidogyrus* spp. eggs hatch within 2–6 days and larvae mature within 4–6 days at 20 °C–28 °C (Paperna, 1996). Besides, *Cichlidogyrus sclerosus* and *C. tilapiae* infect 16 and 24 fish species respectively in Africa (le Roux and Avenant-Oldewage, 2010; Pariselle and Euzet, 2009). This broad spectrum of hosts can enhance their establishment in new water bodies. In contrast, heteroxenous parasites have complex life cycles requiring the presence and close interaction of several hosts. The degree of interactions is governed by the prevailing ecological conditions and habitat characteristics (Lafferty, 2008; Lafferty and Kuris, 1999; Marcogliese, 2001; Ondrackova et al., 2004). As such, the positioning of cages in reservoirs and the quality of pond husbandry practices such as screening, clearing the dykes and routine dredging may lower the likelihood of establishment and transmission of heteroxenous parasites. The differences in the risk among the heteroxenous species was largely attributed to the colonization strategy. Owing to the migration of birds and/or mammals (definitive hosts), allogenic species (*A. macracantha* and *Bolbophorus* sp.) would spread widely (Bush, 2001; Esch et al., 1988) as compared to autogenic species (*A. (A) tilapiae*) which matures within the fish host. Although acanthocephalans can inflict significant damage on the hosts (Amin and Heckmann, 1992; Florio et al., 2009; Martins et al., 2001; Paperna, 1996; Sanil et al., 2011; Taraschewski, 1988), there is no information concerning fish mortalities.

5. Conclusion

This study highlights the importance of risk assessment in provision of scientific information for aquatic health decision making. However, appropriate selection of the risk assessment parameters is crucial. In aquatic health management perspective, the study confirms the economic importance of monogeneans in aquaculture. Therefore, management options should consider risk factors which aggravate monogenean infections especially, high stocking density, low water exchange and poor bottom hygiene. For cages, the distance from the shore and the water bed can influence considerably the transmission of oncomiracidia (Akoll et al., in press). Thus, location of cages should emphasize water depth and distance from the shores. Besides, biosecurity measures such as screening fish for monogeneans and application of appropriate treatment, in case of infections, prior to stocking are essential. Although heteroxenous parasites especially trophically transmitted species are frequent in farmed fish, they pose lower threats than monoxenous. Nonetheless, key risk factors necessary for the establishment and proliferation of these species must be considered in management options. Importantly, reduced access of birds to culture systems and clearing vegetation along littoral zones to eliminate the optimum conditions for the occurrence and interaction of snails, various invertebrates, fish and bird is necessary to control parasitic infection. Overall, we recommend surveying potential water sources for presence of parasite prior to establishing fish farming facilities and implement systematic monitoring to guide in the preparation of contingency plans and general disease management options.

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