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STUDY ON PARASITIC HELMINTHS INFECTING THREE FISH SPECIES FROM KOKA RESERVOIR, ETHIOPIA

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ABSTRACT: A cross-sectional study was conducted in three species of fish from Koka reservoir from November 2008 - April 2009 to determine the prevalence of helminth parasites and identify the most common genera infecting fish. A total of 315 fish comprising of 97 (30.8%) *Barbus intermedius*, 145 (46.0%) African catfish (*Clarias gariepinus*), and 73 (23.2%) Nile tilapia (*Oreochromis niloticus*) were caught by gill nets of variable sizes and long line hook. All the sampled fish were examined externally and internally for the presence of helminth parasites. Of all fish examined, 209 (66.3%) were found to harbour either single or multiple species of helminth parasites. A significantly higher ($P<0.05$) infection was found in *C. gariepinus* than other fish species. There was no significant variation ($P>0.05$) between *B. intermedius* and *O. niloticus.* There was no significant ($P>0.05$) relationship between infections and size (body length) categories. The study demonstrated that *Contracecum* species from mesentery and pericardial cavity was most prevalent nematode (24.8%) in *C. gariepinus* followed by 16.5% in *B. intermedius* and 5.4% in *O. niloticus*. *Gyrodactylus* species was the only monogenean detected from the fins of *B. intermedius*. *Clinostomum* species from branchial and pericardial cavities was the dominant digenean with high prevalence in *O. niloticus* (27.4%). The highest prevalence (77.9%) of *Diplostomum* species was recorded in *C. gariepinus* from cranial cavity followed by unidentified cestodes (35.9%) detected from the mesentery and body cavity. *Proteocephalus* species was detected in 11.34% of *B. intermedius* from intestine and body cavity. This work revealed that helminth parasites are widespread in fish from Koka reservoir and *C. gariepinus* was particularly the most highly infected fish than *B. intermedius* and *O. niloticus*. The study cautions the development efforts when stocking of community aquaculture facilities and artificial reservoirs as part of the food security programme.

Keywords/phrases: ***Barbus intermedius, Clarias gariepinus*, fish, helminths, *Oreochromis niloticus***

INTRODUCTION

Fish are the most diverse groups of vertebrates occupying a variety of marine and freshwater habitat (DACA, 2006). Fish represent a very important food source for low-income populations for whom it may be the only source of protein (Tombi and Bilong, 1971; Tomas, 1999). Moreover, about a third of the world population, for example several million people in Africa, depend on fish for their livelihood from fishing, processing, transporting, and retailing (Tombi and Bilong, 1971; World Book, 2001).

In Ethiopia, commercial fishing has not developed to any significance that in turn has not encouraged the government to promote systematic studies on the local fish fauna (Tudorancea *et al*., 1999). The commercially most valuable fish species in Ethiopia include the tilapia *Oreochromis niloticus,* and several species of the genera *Clarias, Lates, Barbus* and *Bagrus* (Shibru Tedla, 1973; Tudorancea *et al*., 1999). In order to create new fisheries in some lakes or to improve the existing ones, transfers of indigenous or exotic fish species were carried out in the country (Balarin, 1986). For instance, in the late 1950s, Koka reservoir was built for the purpose of hydro-electric power generation and it could also be used more efficiently for commercial fishery (Tudorancea *et al*., 1999).

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Currently, *Oreochromis niloticus, Clarias gariepinus, Cyprinus carpio* and *Barbus intermedius* are the commercially important fish species from this reservoir (Kasahun Asmare, 2005).

Presently, only a few reservoirs are found in Ethiopia and these are not adequately studied for fishery purposes. As such, there is lack of sufficient knowledge on these water bodies compared to natural lakes (Melaku Mesfin *et al.*, 1988). To support the livelihood of the increasing population of Ethiopia, which is periodically confronted with severe drought, an organized fishing program was initiated in the late 1970s and early 1980s in Lake Tana and some of the rift valley lakes. There is an increased need for animal protein in Ethiopia. The country has a relatively large freshwater network with a huge fishery development potential that need to be supported with basic studies on the local fish fauna (Tudorancea *et al.*, 1999).

The occurrence of a wide variety of fish diseases remains a major constraint to successful economic development (Tombi and Bilong, 1971). Diseases of fish are known to cause productivity losses from high mortality, both in aquaculture and extensive inland fisheries aside from being causes of human diseases in many areas of the world (Eshetu Yimer and Mulualem Eneyew, 2003). Fish parasites are among the major pathogens, which cause fish diseases and spoil the appearance of fish thus resulting in consumer rejection. Several groups of parasites belonging to helminths, arthropods, protozoans and other groups of miscellaneous taxa are known to infect fish and produce harmful effects on their hosts (Paperna, 1996). However, only few studies were carried out to estimate status of disease causing pathogens (mainly parasites) in fish of Ethiopian water bodies and fish culture. Therefore, this study was designed to study the prevalence and intensity of parasitic infection among three commercially important fish species of Koka reservoir.

MATERIALS AND METHODS

Study area

The study was conducted in Koka dam (8°26′ N, 39°10′ E; altitude 1,660 m) (Tudorancea *et al.*, 1999; Gashaw Tesfaye, 2006), constructed on the Awash River in the Ethiopian rift valley in the late 1950s for the purpose of generating hydro-electric power. The dam which has an area of 255 km^2 and mean depth of 9 m, is located about 80–90 kms southeast of Addis Ababa. The Awash River is one of the longest perennial rivers in Ethiopia. Besides the Awash River, the dam is also fed by Modjo River.

The Ethiopian rift valley is characterized by a four month dry season (November-February) and an eight month of rainy season (March-October). December and July are the coldest months (Daniel Gamatchu, 1977). The reservoir has a large area of flood plain (Gashaw Tesfaye, 2006). In the northern part of the rift valley, which includes Koka reservoir and associated Awash River flood plains, the main rainy season is between July and September (Daniel Gamatchu, 1977). Because of the evaporation that exceeds the rainfall in the rift valley area, the closed drainage basin of Koka reservoir may suffer seasonal or annual water deficit (Kasahun Asmare, 2005).

Study methodology

Fish were collected early in the morning and kept in polythene bags filled with oxygenated water. All collected fish specimens were examined within a day while still in the field. Records of the total length (cm) were taken for each fish as it was thought to show the size reflective of age category. Then the location, date of sampling, organ of fish examined, types of parasite detected, and number of parasites per organ were recorded for each fish. All sampling procedures followed that of Marcogliese (2001).

Fish were examined externally and internally, keeping them wet throughout the procedure. Before dissection, an external examination was carried out. Mucus sample was taken from the body surface, especially from pectoral and ventral fins, and a direct smear was made on a slide and examined using compound microscope at 100× and 400× magnification. External parasites found on skin, fin, opercula and gill were identified. Skin, fins, opercula and gills were examined for parasites in a petri dish, containing water, and using a stereomicroscope. Gill rakers were forced apart by a needle and inspected for parasites. The eye balls were taken out using scissors and forceps, then crushed and examined under microscope. The abdominal wall was opened up to the mouth along the ventral midline by inserting a sharp end of scissors through the anus. Then another incision was made from the anus up to the lateral line and further along the lateral line up to the gill cover. Then the detached part of the abdominal wall was removed and the internal organs exposed. Body cavity, pericardial cavity, heart, and mesentery were examined for parasites that were identified using naked eye, a stereomicroscope and a compound microscope. The intestine was taken out, separated from adipose tissue, dissected, and a portion placed in petri dish containing 0.75% saline solution for examination. Sliced portions of liver and kidney were visually examined and put on slide for further microscopic examination. The skin was removed and the exposed muscle was then examined with naked eye. The brain of *C. gariepinus* was dissected longitudinally and the cranial cavity washed away into petri dish using water dropper and checked for parasites especially *Diplostomum* spp. metacercariae.

All parasites collected were fixed and preserved in 70% ethanol (Marcogliese, 2001). Identification of most parasites was made immediately following standard keys in literature (Paperna, 1996; Roberts, 2001; Klinger and Francis-floyd, 2002; Roy, 2002; Amlacher, 2005; Pouder *et al.*, 2005). Most of the cestodes were difficult to characterize. Cestode samples collected from *B. intermedius* and *O. niloticus* were sent to the Natural History Museum of Vienna (Austria) and were identified.

Data analysis

Data were captured into electronic data sheet. Records of the lengths (cm) of the fish were plotted on a scatter to categorize sizes as small, medium and large. This size category was used to point out the effect of age of fish on the occurrence of infection. The corresponding results of infection were analyzed by STATA version 9.2 (STATA for windows special edition) taking into account different risk factors, which could have effect on prevalence of helminths. Mean intensity of infection, abundance and prevalence were calculated for all the recovered parasites as previously described (Bush *et al.*, 1997). Mean intensity (Mi) was calculated as the number of parasites of a given taxonomic group per number of infected hosts. Likewise, abundance (Ab) was calculated as the number of parasites of a given taxonomic group per total number of hosts examined. A p-value less than 0.05 at 95% confidence interval was considered significant.

RESULTS

Fish species sampled

A total of 315 fish, including 145 (46.03%) African catfish (*C. gariepinus*), 97(30.8%) *B. intermedius* and 73 (23.17%) Nile tilapia (*O. niloticus*), were caught during the period from November 2008 to April 2009 and examined (Table 1). Gill nets with various mesh size (40, 60, 80,100, 120 mm) and long line hooks were used for catching fish from different sites of the reservoir by using motorized boat.

Overall infection rates

From a total of 315 fish examined, 209 (66.3%) had helminth infection harbouring at least one helminth parasite and 99 (31.4%) had multiple infections (Table 2). All single and multiple infections within each fish host revealed a significantly higher prevalence ($P<0.05$) in *C. gariepinus* than in both *B. intermedius* and *O. niloticus*. Prevalence in the latter two species were similar ($P>0.05$).

Table 1. Size distribution of fish examined at Koka reservoir, Nov. – Apr. 2009.

Species of fish	N	Range of size (cm)	Mean size	95 % CI
B. intermedius	97	18–39	28.52	27.52–29.53
C. gariepinus	145	23–117	47.37	44.78–49.95
O. niloticus	73	8–35	24.96	23.35–26.56

CI: confidence interval

Occurrence of helminth infections in different size categories of the three fish species

Prevalence of helminth infections considering different size categories of three fish species at Koka reservoir in Ethiopia is shown in Table 3. There is no significant ($P>0.05$) association between prevalence of helminth infection and fish size in the three host species. However, there is an increasing trend in the proportion of infection with simultaneous increase in fish size from small to large in *C. gariepinus*.

Table 2. Percentage of fish of species parasitized by helminths examined at Koka reservoir, Nov. – Apr. 2009.

Species of fish	N	Range of size (cm)			
		Total Infection	95 % CI	Multiple infection	95 % CI
Barbus intermedius	97	41 (42.27)	32.26–52.27	13 (13.4)	06.50–20.30
Clarias gariepinus	145	137 (94.48)	90.72–98.24	80 (55.17)	46.98–63.36
Oreochromis niloticus	73	31 (42.47)	30.85–54.07	06 (8.22)	01.76–14.67
Total	315	209 (66.3)	61.10–71.60	99 (31.4)	26.3–36.6

CI: confidence interval

Table 3. Prevalence of helminth infections in fish species of different size from Koka reservoir, Nov – Apr. 2009.

Species of fish	Size category	N	Range of size (cm)			
			Total Infection	95 % CI	Multiple infection	95 % CI
Barbus intermedius	Small	29	12 (41.37)	22.31–60.44	2 (6.9)	–2.9–16.7
	Medium	47	17 (36.17)	21.90–50.43	6 (12.77)	2.86–22.66
	Large	21	12 (57.14)	34.06–80.22	5 (23.81)	3.94–43.67
	Total	97	41(42.27)	32.26–52.27	13 (13.4)	6.5–20.3
Clarias gariepinus	Small	49	44 (89.79)	81–98.58	20 (40.08)	26.55–55.07
	Medium	77	74 (96.10)	91.68–100	46 (59.74)	48.53–70.94
	Large	19	19(100)	–	14 (73.68)	51.87–95.49
	Total	145	137(94.48)	90.72–98.24	80 (55.18)	46.98–63.63
Oreochromis niloticus	Small	12	3 (25)	–3.74–53.74	0	–
	Medium	24	11 (45.83)	24.34–67.3	3 (12.5)	–1.77–26.77
	Large	37	17 (45.94)	29.1–62.79	3 (8.1)	–1.11–7.33
	Total	73	31(42.47)	30.85–54.07	6 (8.22)	1.77–14.67

Helminth infections in B. intermedius

Forty-one of 97 *B. intermedius* (42.27%) were infected, 13 (13.4%) of which harboured multiple infections with helminths belonging to different genera (Table 4). *Contracaecum* sp., *Proteocephalus* sp., and *Clinostomum* sp. appear to be most prevalent in *B. intermedius*.

Helminth infections in C. gariepinus

Hundred and thirty seven of 145 *C. gariepinus* (94.48%) were infected with one or more helminth species; 80 (55.17%) of them had multiple infections with helminths belonging to different genera and some unidentified cestodes and cysts. Metacercariae of *Diplostomum* sp. were the most prevalent and abundant followed by unidentified cestodes (Table 5).

Helminth infections in O. niloticus

Out of 73 *O. niloticus*, 31 (42.47%) were infected; of which 6 (8.22%) harboured multiple helminths belonging to the several genera (Table 6).

Table 4. Prevalence (P), mean intensity (Mi), and mean abundance (Ab) of infection by different helminths in *B. intermedius* (n=97) at Koka reservoir, Nov. – Apr. 2009.

Helminth parasites	Fish organs infected	P (%)	Mi	Ab
Nematodes				
Contracecum sp.	Mesentery, Pericardial cavity	16.49	3.38	0.56
Capillaria sp.	Mesentery	2.06	2.5	0.05
Camallanus sp.	Mesentery	6.18	2.67	0.16
Monogeneans				
Gyrodactylus sp.	Pectoral fins	4.1	1.5	0.06
Digeneans				
Diplostomum sp.	Eyes	1.03	1	1.03
Digenean metacercariae	Intestines	1.03	20	0.21
Euclinostomum sp.	Kidneys	2.06	12.5	0.26
Clinostomum sp.	Branchial/pericardial cavities	7.21	1.71	0.12
Cestodes				
Bothriocephalus sp.	Intestines	3.09	12.33	0.38
Ligula intestinalis	Body cavity	3.09	1.33	0.04
Proteocephalus sp.	Intestines	11.34	11.81	1.34

Table 5. Prevalence (P), mean intensity (Mi), and mean abundance (Ab) of infection by different helminths in *C. gariepinus* (n=145) at Koka reservoir, Nov. – Apr. 2009.

Types of parasites	Fish organs infected	P (%)	Mi	Ab
Nematodes				
Contracecum sp.	Mesentery, Pericardial cavity	24.82	6.08	0.22
Capillaria sp.	Mesentery	6.20	2	0.12
Camallanus sp.	Mesentery	4.82	2.86	0.14
Monogeneans				
Gyrodactylus sp.	Fins	0.60	1.00	1.00
Digeneans				
Diplostomum sp.	Cranial cavity	77.93	10.61	8.26
Clinostomum sp.	Pericardial cavity, Branchial cavity and muscle	6.90	3.20	0.22
Cestodes				
Unidentified cestodes	Mesentery, Body cavity	35.86	4.75	1.89
Encysted Cestode larvae	Muscles	14.48	4.43	0.64

Table 6. Prevalence (P), mean intensity (Mi), and mean abundance (Ab) of infection by different helminths in *O. niloticus* (n=73) at Koka reservoir, Nov–Apr. 2009.

Types of parasites	Fish organs infected	P (%)	Mi	Ab
Nematodes				
Contracecum sp.	Mesentery	5.48	3.75	0.21
Camallanus sp.	Mesentery	1.36	3	0.41
Digeneans				
Tylodelphis sp.	Eye	4.1	1.33	0.05
Euclinostomum sp.	Kidney	2.73	10	0.27
Clinostomum sp.	Pericardial and Branchial cavity	27.39	2.55	0.7
Digenea metacercariae	Intestine	1.36	12	0.27
Cestodes				
Amirthalingamia macracantha	Liver	2.73	4.5	0.12
Unidentified cestode	Intestine	2.73	4.5	0.12
Acanthocephala sp.	Intestine	2.73	3.66	0.15

DISCUSSION

The present results revealed different external and internal helminth parasites in freshwater fish from Koka reservoir. The total prevalence of infection in all examined fish hosts was 66.3%; this value is close to that reported by Olofintoy (2006) with 62.6% prevalence from some freshwater fish species in Nigeria. The mean intensity of all helminths remained very low and this could be partly associated to the environmental and host-parasite relationship existing in the areas during the sampling periods and to water current velocity. Ergens (1983) argued that the degree of infection depends on the conditions of host density. Moravec *et al.* (1997) also stated that parasite fauna diversity could be affected by seasonal environmental changes.

Among the three fish species examined, *C. gariepinus* had higher prevalence of total infection (94.5%) and multiple infections (55.2%) than *B. intermedius* and *O. niloticus*. This could be linked to the high occurrence of *Diplostomum* species in *C. gariepinus*, prevalence of 77.9% and mean abundance of 8.3 worms/host. Recently, Birhanu Tadesse (2009) reported a 100% prevalence of *Diplostomum* metacercariae from cranial cavity of *C. gariepinus* sampled from Lake Hawassa. Likewise, Mukama (2008) indicated that *Diplostomum* sp. was recovered from the cranial cavity of the same fish species with a similar higher prevalence of 83.3% in Tanzania. Such a consistently higher prevalence being found in *C. gariepinus* could be attributed to the less selective or omnivorous behaviour of this fish species (World Book, 2001; Peter, 2005). Ogawa (1996) also stated that problems of parasitic infections are associated with feeding behaviour of fish. This might have exposed the species for easy transmission of parasites from invertebrate intermediate hosts and fish intermediate hosts.

This study also demonstrated that *C. gariepinus* had high prevalence of multiple infections of 40.1%, 59.7% and 73.7% in small, medium and large size groups, respectively. This suggests that the average total helminth load tend to increase with the host size, an indication of a rise in infection rate in older hosts. This was also reported by Tombi and Bilong (1971) confirming that the prevalence of intestinal helminth infections in fish species increases with their standard length. The likely reason for the rise in the prevalence of infection between the juvenile and adult fish is related to their standard length or due to change in diet from weeds, seeds, phytoplankton and zooplanktons to insect larvae, snails, crustaceans, worms and fish (Reed *et al.*, 2002).

Of all the nematodes identified from mesentery and pericardial cavity, *Contracecum* sp. was the dominant in all species of fish examined. The occurrence of *Contracecum* sp. in Ethiopia was also reported from Lakes Chamo, Hawassa, Ziway and Tana (Amare Tadesse, 1986; Eshetu Yimer, 2000; Eshetu Yimer and Mulualem Eneyew, 2003) and Lake Babogaya (Yohanes Tafere, 2008; Birhanu Tadesse, 2009). Amare Tadesse (1986) reported a 10.6% prevalence of *Contracecum* sp. in *O. niloticus* from Lakes Hawassa and Chamo while Tefera Wondim (1990) reported a 68.7% prevalence in *O. niloticus* from Lake Tana. These findings prove that the occurrence of parasitism varied from one habitat to other that could be due to host-parasite relationship and biotic factor (Anderson, 1992). It might also be associated to environmental and host factors in the areas during the sampling periods. However, the finding confirms that *Contracaecum* sp. is widespread in most fish species examined. Other nematodes like *Camallanus* and *Capillaria* species were also detected from the mesentery of *B. intermedius* and *C. gariepinus* in the current work. This could suggest the adaptive behaviour of nematodes in relation to host specificity as reported by Olofintoy (2006).

Gyrodactylus sp. Found on the fins of *B. intermedius* (with 4.1% prevalence) and least prevalent in *C. Gariepinus* (0.6%) was the only monogenea recorded in this study. Yohanes Tafere (2008) reported a prevalence of 2.8% and 2.0% in *O. niloticus* from Babogaya and Hora lakes, respectively. Water currents might have a double effect on monogeneans; it brings their infective larvae on to the hosts, or gets rid of them (infective larva and mature individual) (Gerassev and Staravoiter, 1988).

Clinostomum sp. was found in all fish species with high prevalence in *O. niloticus*; this parasite showed a 66.2% prevalence in the same host from Lake Tana (Eshetu Yimer and Mulualem Eneyew, 2003). Birhanu Tadesse (2009) also reported 15% prevalence in *C. gariepinus* from Lake Hawassa. Such reports demonstrate that these parasites are widespread in fish of Ethiopian lakes. Fish infection by metacercariae of *Clinostomum* and *Euclinostomum* spp. are common in Africa (Paperna, 1996). However, the disparity of results from different sites could be attributed to the effect of environment and host factors.

Euclinostomum sp. showed a prevalence of 2.7% in *O. niloticus*. Prevalence of 4.6% was detected in the same host (*O. niloticus)* from Lake Tana (Eshetu Yimer and Mulualem Eneyew, 2003). Previously, a 3.3% prevalence of *Tylodelphis* and *Diplostomum* spp. was reported in eyes of *O. niloticus* (Birhanu Tadesse, 2009). *Diplostomum* spp. were found in *O. niloticus* from different localities of the Ethiopian water bodies (Eshetu Yimer *et al*., 2001).

This study recorded a high proportion of some unidentified cestodes from mesentery and body cavity of *C. gariepinus* with prevalence of 35.86%. Birhanu Tadesse (2009) also reported 15% and 26.3% prevalence of unidentified cestodes from Lakes Hawassa and Babogaya respectively. *Proteocephalus* species, identified from intestine and body cavity, were the second prevalent helminth with prevalence of 11.34% and mean intensity of 11.81 in *B. intermedius*. There is a report of 0.8% prevalence of *Proteocephalus* species in fish of Lake Tana (Eshetu Yimer and Mulualem, Eneyew, 2003). The prevalence of *Bothriocephalus* species in the intestine of *B. intermedius* was about 3.09% in the current work. This cestode was reported by Zhokov *et al*., (2007) in *B. intermedius* from Lake Tana. *L. intestinalis* was found in 3.09% of *B. intermedius* examined. Eshetu Yimer and Mulualem Eneyew (2003) reported a 12.4% prevalence of *L. intestinalis in* body cavity of *B. intermedius* sampled from Lake Tana. Amare Tadesse (1986) reported only one *L. intestinalis* plerocercoid in body cavity of *B. intermedius* from Lakes Hawassa and Chamo. Fish infected with *L. intestinalis* showed poor body condition, inhibited gonadal maturation and spawning migration (Lester, 1988). This indicated that *L. intestinalis* is an important pathogen. *Amirthalingamia macracantha* was recorded in 2.7% of liver samples of *O. niloticus*. Birhanu Tadesse (2009) detected this cestode with a prevalence of 3.3% in the liver of *O. niloticus* from Wonji ponds. Dick and Choudhury (1999) stated that intensities and prevalence of most cestodes have seasonal peaks. Cestodes are atypical parasites of fish in lakes or reservoir and outbreaks occur where copepod or *Tubificid* *oligochaetes* are often abundant (Dick and Choudhury, 1999; Roberts, 2001).

Acanthocephalans have been reported in different African fish families (Paperna, 1996). In the present study, Acanthocephalans were detected only in the intestine of *O. niloticus* with 2.73% prevalence. Despite their fearsome proboscis with their rows of hooks, Acanthocephalans have not generally been observed as serious pathogens of fish. This is surprising in view of the sometimes-severe local damages to the intestine of fish caused by proboscis (Roberts, 2001).

The present findings confirm that helminth parasites are widespread in fish from Koka reservoir. The presence of these helminths in fish of the reservoir may be the result of poor water quality, crowding and other problems that give suitable habitats for those parasites and intermediate hosts. The greater susceptibility of *C. gariepinus* to helminth infections may require further investigation. Further, a general survey of all parasites and other pathogens need to be conducted by considering additional risk factors with full coverage to all seasons. The government should promote systematic studies on the local fish fauna so as to encourage the expansion of commercial fishing industry. We have also found unidentified cestodes in this survey, and future studies should address the biology and zoonotic potential of these. The finding of *Clinostomum* and *Contracaecum* species is also indicative of the existing risks of public health, as these species are known to infect humans from consumption of raw or inadequately cooked fish (Hirai *et al.*, 1987; Shamsi and Butcher, 2011). Therefore, people living around the reservoir and who consume raw or inadequately cooked fish need to be aware of the potential risk of acquiring zoonotic parasites and be advised to avoid these habits. Moreover, the presence of an array of parasites in this water body demands extreme caution during stocking of fingerlings and parents to the emerging aquaculture as an effort towards food security of the government extension program. Hence, information pertaining to basic fish culture, pond management, water quality and related issues should be available for those interested in the activities of fish culture.

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