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Original paper

Spatio-temporal and host-dependent variations in prevalence and intensity of heterophyid (Digenea: Heterophyidae) metacercariae infection in brackishwater and freshwater fishes: a comparative study

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ABSTRACT. Studies on distribution of parasites are important to reveal the ecology of host-parasites interactions. The objective of the study was to compare the variations in heterophyid encysted metacercariae (EMC) infection in freshwater and brackishwater fish hosts and variation in parasitological descriptors of metacercariae infection during different seasons. The status of infection with heterophyid EMCs in the second intermediate host fishes from brackish- and freshwater resources was investigated. Seasonal changes in the occurrence of EMCs in different fish hosts were monitored in a longitudinal field survey lasting 12 months from June 2018 to May 2019. Distribution of heterophyid EMCs was found varied in freshwater and brackishwater fishes with higher prevalence in brackishwater fish. There was a distinct seasonal trend in the prevalence of infection for all heterophyid EMCs in the brackishwater fish examined with high burden during summer. Thus variations in parasitic infection among hosts underpin the importance of parasites as an evolutionary or ecological force.

Keywords: Heterophyidae, intermediate hosts, fishes, brackishwater, freshwater

Introduction

Trematodes of the family Heterophyidae are common intestinal parasites of fish-eating birds and mammals [1–4] with snails as first intermediate host and a broad spectrum of second intermediate fish hosts [5,6]. Heterophyidae includes a set of over 50 genera [7] and the heterophyid encysted metacercariae (EMC) can infect different organs of fishes [8,9]. Fish-borne zoonotic trematodes comprising heterophyids are of common in East and Southeast Asia [10–12]. Geographic areas, where raw fish is a favorite meal, people are often get parasitized by small flukes, particularly of the family Heterophyidae [13,14]. Despite this fact, little attention has been paid to these trematodes, most probably because of their smaller size and difficulty in taxonomic designation. Although heterophyid EMCs from fishes of India were documented by many authors [15–23] little is known about their epidemiology and ecology in their natural hosts.

The risk of parasitism is often structured both spatially and temporally because of spatial aggregation of infected individuals including intermediate hosts [24,25] and seasonal changes in the release of infective stages of parasites [26]. Consequently, the impact of parasites can also vary among host populations [27,28]. Dobson et al. [29] and Khan [30] suggested that temperature, habitat etc. are the important environmental variables that determine the levels of parasite abundance. Seasonal variation of metacercarial infection in fish has been reported for several species of heterophyids [31,32]. Thien et al. [14] carried out a seasonal investigation on the occurrence of fish-borne zoonotic trematodes, especially of heterophyids in economically important monocultured hybrid catfish and giant gourami in Vietnam. Elsheika and Elshazly [4] studied the seasonal prevalence of heterophyid EMC in brackishwater fishes of Egypt. Similarly Krailas et al. [33] explored the seasonal prevalence of heterophyids of various freshwater fishes in Thailand. Elsheika and Elshazly [34] made preliminary observations on infection of brackish- and freshwater fishes by heterophyid EMC in Egypt.

The pattern of seasonal variation and effect of biotic factors on heterophyid EMC could be of considerable importance in planning for parasite control [35]. Many researchers focused their studies on the seasonality and the environmental factors that influence the prevalence and intensity of fish parasites generally. Factors affecting the occurrence of heterophyid infection, the seasonal variation and the possible interaction among these factors are poorly studied [35,36]. An attempt was made, through this study, to reveal the seasonal dynamics and habitat dependent variation in heterophyid occurrence in order to broaden the current knowledge of host-parasite interactions of these digeneans in their second intermediate hosts.

Materials and Methods

Fish sample collection

The fishes were collected from Kannur Kozhikode, Kasaragod and Wayanad districts, Kerala, India during June 2018 to May 2019. Live specimens of 18 species of brackishwater fishes, *Mugil cephalus*, *Leiognathus equulus*, *Gerres filamentosus*, *Gerres oyena*, *Scatophagus argus*, *Etroplus suratensis*, *Etroplus maculatus*, *Caranx ignobilis*, *Lutjanus argentimaculatus*, *Thryssa malabaricus*, *Thryssa mystax*, *Rastrelliger kanagurta*, *Terapon jarbua*, *Nematolosa nasus*, *Sillago sihama, Ambassis gymnocephalus, Arius subrostratus* and *Drepane punctata*, were collected from water bodies of Kannur, Kozhikode and Kasaragod districts and 15 species of freshwater fishes, *Aplocheilus lineatus*, *Haludaria fasciata*, *Pseudosphromenus cupanus*, *Danio rerio*, *Barilius gatensis*, *Channa striata*, *Pethia punctata*, *Lepidocephalichthys thermalis*, *Rasbora dandia*, *Puntius conchonius*, *Puntius bimaculatus*, *Puntius filamentosus*, *Garra mullya*, *Poecilia reticulata* and *Gambusia affinis*, collected from water bodies of Wayanad were brought to the laboratory and examined for heterophyid EMC.

Isolation and identification of metacercariae

The sacrificed fishes were examined for parasites and the heterophyid EMC collected from the scales, gills, liver, muscles and mesenteries were transferred to physiological saline (0.75% NaCl solution). Cysts of each type were observed under a Nikon ECLIPSE Ni-U phase contrast research microscope. Larvae were excysted either by rupturing the cyst wall with fine needles or by mounting them under cover glass and applying gentle pressure over it by a fine needle. The metacercariae were observed under Nikon ECLIPSE Ni-U phase contrast research microscope with or without vital staining. For permanent preparation, metacercariae fixed in 5% formalin were stained with acetocarmine, following Cantwell [37]. Photographs were taken with the Nikon Y-TV55 camera, with the Nikon NIS Elements Imaging Software, attached to the Nikon ECLIPSE Ni-U phase contrast research microscope. Parasitological descriptors of infection were measured following Bush et al. [38].

Seasonal variation

The seasonal variation of heterophyid EMC's infection in *Mugil cephalus* and *Haludaria fasciata*

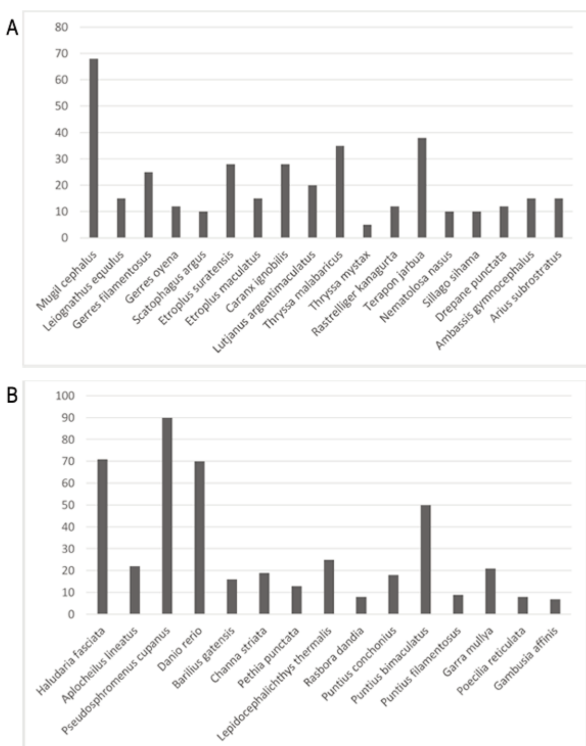

Figure 1. Fishes examined for heterophyid EMCs. A. Brackishwater fishes, B. Freshwater fishes

Table 1. Prevalence, intensity, abundance and site of heterophyid EMCs infection in *Mugil cephalus*

Heterophyid EMCs	Site of infection	Prevalence (%)	Mean intensity	Mean abundance
Centrocestus formosanus	Gills	1.47	9	0.13
Haplorchoides sp.	Mesenteries	14.70	1.50	0.22
Haplorchis sp.	Liver	4.40	2	0.08
Heterophyes sp.	Muscles	2.94	4.50	0.13

were measured. Infection in *Barilius gatensis* was excluded due to small sample size. The parasitological descriptors (prevalence, intensity and mean abundance) of infection during different seasons, monsoon (June–November), winter (December–February) and summer (March–May) were measured following Bush et al. [38].

Results

Spatial distribution of heterophyid EMC

A total of 18 species of brackishwater and 15 species of freshwater fishes were explored for heterophyid EMCs (Fig. 1). Among the fishes studied, *Mugil cephalus*, *Haludaria fasciata* and *Barilius gatensis* were infected with metacercariae. Pattern of distribution of heterophyid EMC varied in freshwater and brackishwater fishes and presence of five morphologically distinct metacercariae were recorded. The heterophyids studied were identified as *Centrocestus formosanus*, *Haplorchoides mehrai*, *Haplorchoides* sp., *Haplorchis* sp. and *Heterophyes* sp. (Fig. 1 A–E). Among the five heterophyid EMC recovered in the present study, only *C. fomosanus* was common to both brackish- and freshwater systems.

The brackishwater fish, *M. cephalus* was infected with *C. formosanus*, *Haplorchoides* sp., *Haplorchis* sp. and *Heterophyes* sp. Out of the 68 *M. cephalus* examined 1, 10, 3 and 2 fishes were found infected with *C. formosanus*, *Haplorchoides* sp., *Haplorchis* sp. and *Heterophyes* sp., respectively. The prevalence, mean intensity, mean abundance and sites of infection of different heterophyid EMCs varied with host (Tab. 1). *Haplorchoides* sp. being the most prevalent followed by *Haplorchis* sp., *Heterophyes* sp. and *C. formosanus*. The EMCs of *C. formosanus* and *H. mehrai* were recovered from the freshwater fish, *H. fasciata* and *C. formosanus* from *B. gatensis*. Out of 71 specimens of *H. fasciata* examined, one was infected with *H. mehrai* under the scales and three with *C. formosanus* on the gills.

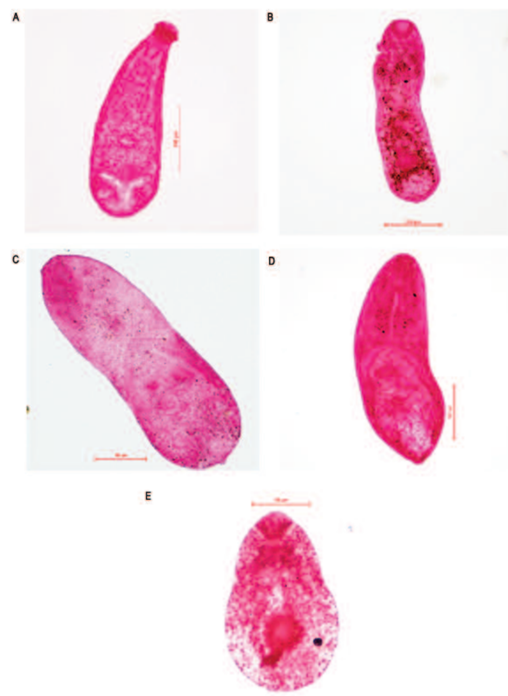

Figure 2. Photomicrographs of heterophyid EMCs recovered. A. *Centrocestus formosanus*, B. *Haplorchoides mehrai*, C. *Haplorchoides* sp., D. *Haplorchis* sp., E. *Heterophyes* sp.

The prevalence, mean intensity and mean abundance of *H. mehrai* infection were 1.41, 11 and 0.15, respectively. These parasitological descriptors for *C. formosanus* infection in *H. fasciata* were 4.23, 85 and 3.59, respectively. Out of 16 *B. gatensis* examined, one was infected with *C. formosanus* in the gills and prevalence, mean intensity and mean abundance of infection were 6.25, 97 and 6.06, respectively.

Temporal variation of heterophyid EMC infection in fishes

The heterophyid EMCs infection in the brackishwater fish, *M. cephalus* and freshwater fish, *H. fasciata* showed temporal variations (Fig. 3–5). There was a distinct seasonal trend in the prevalence of infection for all heterophyid EMCs in the

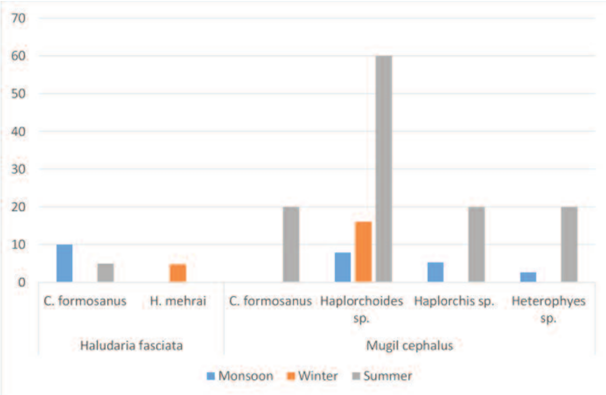

Figure 3. Variation in prevalence of heterophyid EMCs infection in *H. fasciata* and *M. cephalus* during June 2018–May 2019

brackishwater fish examined with high burden during summer. On the other hand, the prevalence of infection in freshwater fish with two heterophyid species varied with seasons (Fig. 3).

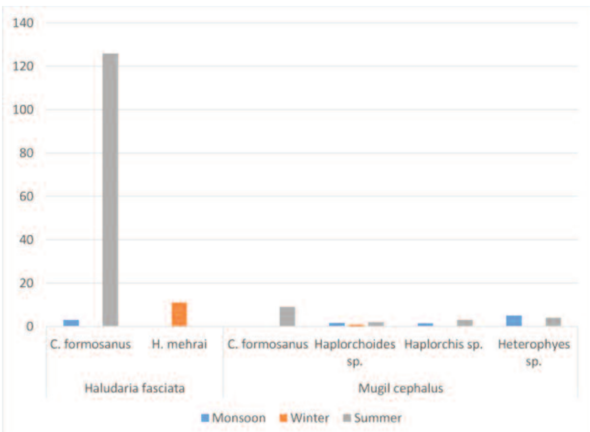

Figure 4. Variation in mean intensity of heterophyid EMCs infection in *H. fasciata* and *M. cephalus* during June 2018–May 2019

In *C. formosanus* of brackishwater fish, infection was noted only during summer, while in freshwater fish infections were observed during all season with the highest prevalence during monsoon. The highest values of intensity and mean abundance of *C. formosanus* infection in *H. fasciata* were recorded during summer with 252 metacercariae in two fishes. Infection of *Haplorchoides* sp. was recorded in all seasons and the highest value of prevalence and intensity of infection were noted during summer. While the highest mean abundance of infection recorded was during winter and the lowest was recorded during monsoon. On the other hand, *H. mehrai* was recorded only in the winter season. In summary, the infection of *Haplorchoides* metacercariae varied with seasons.

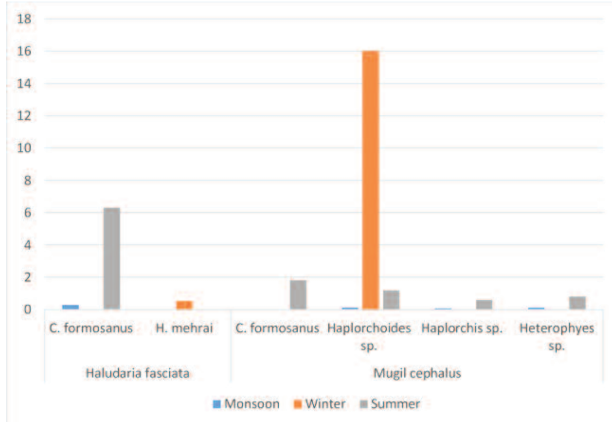

Figure 5. Variation in mean abundance of heterophyid EMCs infection in *H. fasciata* and *M. cephalus* during June 2018–May 2019

The infection of EMCs of *Haplorchis* sp. and *Heterophyes* sp. in *M. cephalus* were recorded during monsoon and summer periods only. In the case of *Heterophyes* sp. the highest values of prevalence and mean abundance of infection were noted during summer. While the highest mean intensity of infection was recorded during monsoon. The highest values of these parasitological descriptors for *Haplorchis* sp. infection was recorded during summer season.

Discussion

Spatio-temporal variation in parasitism is a common feature of most host-parasite interactions, including parasitic infections in freshwater [28] and brackishwater [39] fishes. According to Marcogliese [40] diversity of parasites within a host reflects the presence of diverse intermediate and definitive hosts of the parasites in that ecosystem. On the basis of the differences in morphological characters a total of five species of heterophyid EMCs were recovered during the present study, *C. formosanus*, *H. mehrai*, *Haplorchoides* sp., *Haplorchis* sp. and *Heterophyes* sp. The heterophyid EMC common to both habitats was *C. formosanus*. Four species of heterophyid EMCs were obtained from the brackishwater fish *M. cephalus*, whereas, two species from the freshwater fish *H. fasciata*. This indicates that the type of habitat seems to play an important role in the infection of fish by different types of heterophyid parasites [4,30]. According to Rekharani and Madhavi [20] the conditions in the brackish water environment including the presence of massive populations of cerithiid snails which act as vectors for many species of heterophyids are congenial for the completion of many heterophyid life cycles [12,20].

Temporal variation in parasite population is associated with host biology [41,42] and environmental characteristics [43], especially those associated with annual or seasonal changes [44]. There was a distinct seasonal trend in the prevalence of EMC infection for all the heterophyid species in brackishwater fishes examined, with high burdens in summer. Whereas, infection with EMCs were recorded throughout the year in freshwater fishes. Sukontason et al. [45] and Noikong et al. [31] recorded heterophyid infections in cyprinoid fishes of Northern Thailand throughout the year with greater prevalence during winter season than rainy and hot seasons. Similarly, in the present study the heterophyid infections in freshwater fish *H. fasciata* were found throughout the year. *Haplorchoides mehrai* was recorded during winter, whereas *C. formosanus* during both monsoon and summer seasons with higher prevalence during summer. However, in the brackishwater fish *M. cephalus* the highest prevalence was recorded during summer for all heterophyid EMCs recovered. This result agrees with the studies of Kang et al. [46], El-Naffar and El-Shahawi [47] and Raef [48], who reported that heterophyid infection is more abundant during summer. Elsheikha and Elshazly [4] observed that the transmission of encysted metacercariae of heterophyids to the fish host was highly temperature dependent. They pointed out that increased prevalence and intensity of metacercarial infection during summer and spring was the result of massive release of cercariae during the hot seasons and the decreased level during the winter season was due to the death of cercariae. Similarly, in the present study the highest prevalence and intensity of heterophyid EMCs infection in *M. cephalus* were recorded during summer months with the lowest or no infection during winter. The high prevalence of infection in summer can be attributed to the coincidence of infestation with the time of emergence of cercaria from snail vectors, which is usually supported by the increased temperature of water [49]. This coincides with the time of maturation of the snail host that flourishes better in warm water when the surrounding environmental conditions become favorable [50].

Moreover, the interest in parasite site selection stems from its pivotal role in many aspects of host parasite interactions including parasite transmission dynamics and parasite-induced host pathology [51]. According to Ibrahim and Soliman [52] site preference of each species of heterophyid EMC is influenced by the differences in host species, the environmental conditions, the geographical distribution and the genetic variations of metacercariae. In freshwater fishes, the metacercariae were found as ectoparasites i.e. on scales and gills, whereas in brackishwater fishes they were found in the internal organs. This may be treated as an adaptive strategy adopted by the parasite to survive the salinity fluctuations in the brackishwater system.

Spatial and temporal variations in trematode parasite populations are associated with natural changes in the environmental conditions and the interspecific associations prevailing in every ecosystem [44] as the developing stages of the parasites are directly exposed to the environment at some stage of their lives [53]. In the present study, there was a clear spatial and temporal variation in the heterophyid EMCs infection in freshwater and brackish water fishes, with more infection and clear seasonality in brackish water fish, *M. cephalus*. According to previous studies the heterophyids were well established in the brackishwater system with host specificity, mullets act as an important host for variety of heterophyid EMCs [4,20] and seasonality, with the highest infection during summer season [54].

Determinants of parasite prevalence in host communities influence important ecological and evolutionary processes of parasites [25]. According to Luque and Poulin [55] some lineages of fish harbor more larval helminths (more species and/or more individuals) than others merely because of historical reasons (ancient associations between certain parasite taxa and fish taxa) and not really because of their present ecological characteristics. Poulin and Valtonen [56] suggested that assemblages of larval helminth parasites in fishes are not random collections of locally available species, but rather structured packets of larval parasites that travel together along common transmission routes. Further studies are required to establish the factors that are supposed to influence the high prevalence of heterophyid EMCs in the brackish water fishes. Parasites may be expected to become locally adapted to their hosts. Variations in levels of parasitism among hosts underpin the importance of parasites as an evolutionary or ecological force [57]. There are results which confirmed the importance of the phylogeny of hosts as a confounding factor in any analysis on the influence of host features on parasite species richness and abundance [56,58].

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