Haya: The Saudi Journal of Life Sciences (SJLS)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-623X (Print)
ISSN 2415-6221 (Online)

Prevalence of Sympatric Parasites in the Flathead Grey Mullet *Mugil cephalus* (Linnaeus, 1758) - Arabian Gulf - Saudi Arabia

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Original Research Article

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Article History
Received: 10.04.2018
Accepted: 20.04.2018
Published: 30.04.2018

DOI:
10.21276/haya.2018.3.4.8

Abstract: A total number of 1042 flathead mullet *Mugil cephalus* were searched for parasites during summer & autumn 2015 and winter & spring 2016. Six different parasite taxa belonging to Microsporidia, Myxozoa, Ciliophora, Anisakidae and Gnathostomatidae were identified. The total number of infested mullets along the study period with one or more parasites was 396 representing a percentage of 38.0%. Mullets were infested with *Microsporum velveticum* through ingesting the intermediate host. This parasite was observed in the intestinal epithelia, adipose tissue, liver, stomach epithelia, muscles and air bladder. *Myxidium depressum* was observed in the liver and gall bladder of infested mullets in a plasmid stage that had undergone sporogony forming multicellular myxospores. *Trichodina murmanica* was observed on the gill surface, the operculum and respiratory epithelium where superficial to deep ulcerative lesions were visible. Third Larval stages of *Anisakis simplex*, *Raphidascaris acus* and *Gnathostoma spinigerum* were observed free in the duodenum and ileim or encapsulated in the stomach epithelia, liver, and muscles. The infra-community of protists was highest in winter and fall while for parasitic nematodes was during spring and summer. Seasonal fluctuation of the parasite community was demonstrated by using standard calculations.
Keywords: *Mugil cephalus*; Microsporidia; Myxozoa; Ciliophora; Anisakidae; Gnathostomatidae.

INTRODUCTION

The biodiversity of parasites in marine ecosystem has been investigated by several authors. Sympatric parasites infest hosts that exist within their same habitat more precisely. Parasites consumed in raw seafood can cause human serious aliments [1]. It is highly probable that almost every organism, at least temporarily, is parasitized within its life span [2]. Among parasites in the marine ecosystem are Microsporidia, Myxozoa, Ciliophora, Anisakidae and Gnathostomatidae. The number of parasites in the marine ecosystem is currently estimated to be 20,000 to 100,000 species [3]. Some endoparasites infect their host through skin penetration or by being accidentally ingested [4]. The pathogenic parasites may shed free-living stages that migrate through the host's tissue into the sea water, where they actively search for other hosts [5]. Heteroxenous endoparasites require many hosts to complete their life cycles and depend on stable ecological interactions to get from one host to another. So, the parasites in an ecosystem reflect the "health" of that system. Homoxenous endoparasites have direct life-cycles, involving one host species. Fishes may obtain parasites from their food, or are directly infected by free-living parasitic stages in the sea water [3].

Microsporidia live inside the cells of their hosts and build small unicelled spores [6]. Alternation between cyclic merogony and sporogony within host tissues take place [6]. The trophic stage has a single nucleus or maximally two and is enclosed in a sporophorous vesicle or present free in the host cell cytoplasm [4]. Many species infect fishes and infestation is always in epithelial tissues causing focal lesions. Microsporidia infect marine crustacean, fishes and mammals including man [6]. The complete life cycles of Microsporidia for most species remains under study. Transmission of, microspodians from one host to another is common by the fecal oral method similar to *Nucleospora salmonis*, *Glugea* spp. and *Loma* spp [6, 4], or indirectly transmitted through an intermediate host.

Myxozoa are predominantly parasites of fish [7]. They are virtually identical with cnidarian nematocysts. The life cycle requires a vertebrate and an

invertebrate host [8]. The genus *Trichodina* is common inhabitant of some freshwater and marine invertebrates and fishes [9]. They are found as ecto-commensals on some fish species [10]. On the other hand, this species causes severe tissue damage in *Sepia* sp [11].

Roundworms are endoparasites that infest the intestinal mucosa of the host and cause severe tissue damage and sometimes penetrate the intestine. The herring worm *Anisakis simplex* and *Pseudoterranova decipiens* are parasites of the GI tracts of marine vertebrates [12, 13]. They have a fecal oral method of distribution as eggs are taken in by crustaceans where they mature into an infective stage [14]. Some hosts, such as fishes, are infected when they ingest the infected crustaceans where the juveniles burrow into the tissues and if a definitive host eats this fish, it begins its vitality [15]. *Anisakis simplex* can complete its life cycle when crustaceans, small fish, and marine animals live simultaneously in the vicinity [16]. Ascaroids have developed number of adaptions to overcome the internal responses and physiology of hosts [17].

Fish-eating birds are always migratory and are potential reservoir hosts for parasites which seriously influence human public health [18, 17]. The world health organization (WHO) reported that 18 million of citizens were infected with parasites through fishes and citizens endangered worldwide exceeded half a billion [19]. Before being placed on the market, fish should be subjected to a visual examination process and should not be 'obviously contaminated' by parasites. Water temperatures and salinity fluctuation in marine ecosystem determine the ability of parasites to complete their life-cycles [20].

This study was intended to provide a detailed insight into the parasites of commercially mariculture mullet *Mugil cephalus* living in the Arabian Gulf in Saudi Arabia, and to analyze the seasonal estimation of each parasite in the different months of the year.

MATERIALS AND METHODS

Animal collection and identification

Adults of the flathead grey mullet *Mugil cephalus* [21] were collected (boat and fishers) from Ad-dammam estuarine beach of the Arabian Gulf, Saudi Arabia in summer & autumn 2015, and Winter & Spring 2016. Surface water samples were collected from the Ad-dammam estuarine beach of the Arabian Gulf (Saudi Arabia) using polyethylene bottles (2-litres capacity). A total number of 1042 mullet were collected in the study and transported to the laboratory in ice tanks. Temperature (°C) of surface water was measured by dipping a mercury thermometer and recorded during sampling period (Fig. 1A). Measurement of salinity was done using WTW 320 and Sentix 41 probe with range 0-14 pH scale and temperature sensor conductivity meter (Fig. 1B). Measurement of pH was done using Orion 420 A pH meter (Fig-1C). Identification of this fish species was carried out according to Menezes *et al*., [22]. Ethical approval was obtained from the Deanship of Scientific Research, Imam Abdulrahman bin Faisal University ethics committee.

Parasite collection

The viscera of each mullet were incised and thoroughly tested for parasites. Nematodes collected from the GI tracts were placed in Eppendorf vials containing 10% neutral formalin as fixative, distilled water for 24 hrs, and dehydrated through ascending series of ethyl alcohol then a series of tertiary butyl alcohol. Eherlich haematoxylin and eosin [23] were applied. These preparations were then photomicrographed to ensure their identification. An Olympus IMT-2 phase Contrast microscope with an automatic camera was used following the method of Nomarski. For scanning electron microscopy (SEM) nematodes were fixed in 2.5% Glutaraldehyde at 1200 mOsm (pH 7.5) and dehydrated in a graded ethanol series. The dehydrated parasites were critical point dried, mounted on specimen holders, and subsequently sputter-coated with gold. Specimens were examined and photographed using a FEI Quanta 200 SEM at 15 kV.

To search for microscopic parasites, fresh specimens of mullets were dissected, and their GI tracts and the other viscera were isolated and placed in saline solution. Supernatants were fixed in 2.5% Glutaraldehyde and prepared for micrography and/or SEM [24].

Parts of the GI tracts and the other viscera were isolated and fixed in 2.5% Glutaraldehyde in 0.05 M PBS containing 0.33 M NaCl (1h, 4°C). The fixative was removed by washing samples several times with PBS. Post-fixation was carried out using 2% Osmium tetroxide in PBS for 30-60 min at 4°C. These preparations were subsequently washed with PBS, dehydrated in a graded ethanol series, and propylene oxide and embedded in araldite resin. Semithin and ultrathin sections (60-70nm) were obtained using the Leica UC6 microtome equipped with diamond knives. Ultrathin sections were picked with formvar-coated single-slot copper grids, stained automatically with uranyl acetate and lead citrate in a Nanofilm TEM STAINER, and examined on a Phillips CM 120 transmission electron microscope at 60 kV. Semithin sections were placed on glass slides and stained with toluidine blue (1% toluidine, 1% Na2B4O7, 20% sucrose) for 1 min at 60°C.

The number of infested mullets with one or more parasites was calculated in all months of the year and the number of each parasites was subjected to two-way analysis of variance. Tukeys post-hoc test was used to identify the significant difference between the different parasites. Significance difference was considered when $p<0.05$.

RESULTS

Parasitic protists

Parasitic protists belonging to Microsporidia, Myxozoa and Ciliata can change their life style from an active feeding trophozoite in the host to an inactive resistant cyst which infect another host. They are always opportunistic and ubiquitous in marine ecosystem. Protist parasites cause the most potentially lethal diseases in fishes, tortoises, shrimps, and other marine organisms. They transmit from one host to another through oral- fecal method of infested foods, water or through sexual reproduction. Our study identified three different parasitic protists in the viscera of mullets.

Microsporum velveticum [25]

M. velveticum is ameboid and multinucleated in the adult stage and spores are produced during the life of the trophozoite. Each spore is produced from several cells that contain polar capsule(s) from which long filaments can protrude.

Mullets were infested with microspores likely through ingesting food contaminated with infective spores that were found in feces of other infested fishes. This parasite was observed in the intestinal epithelia, adipose tissue, liver, stomach epithelia, muscles, and air bladder of infested mullets. It was unicellular and exhibited intracellular parasitism (Fig-2A). It was isolated from cysts which were embedded in the infested tissues of the mullet (Fig-2B-D). Pyriform spores had a thick wall containing the toxic sporoplasms which is important in the invasion process (length 4.5 µm; width 2.3 µm) (Fig-2E). They had a coiled polar tube that was extricable to inject the sporoplasms into the mullet tissues. Each spore had a posterior large vacuole and limited with a spore's pansporoblast membrane (Fig-2E). Identification of this parasite was done according to Lom, and Dyková, I. [26].

Myxidium depressum [27]

This parasite was found in the gall bladder and liver of infested mullets in a plasmid stage that had undergone sporogony forming multicellular myxospores (Fig-3A). They were elongated, semilunar shaped and encapsulated in two valves, length 10.7 µm; width 8.7 µm; average thickness 6.1µm. Each myxospore bore an irreversible polar filament which served as a holdfast organ. It had valvogenic, spore shell valves, amoeboid infective sporoplasms (sporoplasmic cells) and capsulogenic cells forming a polar capsule (Fig-3B). This parasite was observed in the GI lumen and embedded, as plasmid stage, in the gastric epithelia of the mullet. In the hepatic cells of infested mullet, stages of sporogonic plasmodia could be detected where myxospores formed (Fig-3C). Identification of this parasite was done according to MacKenzie *et al*., [7].

Trichodina murmanica [26]

The agametic longitudinal fission and gametic conjugation stage of the parasite was observed exhibiting a direct life style from one mullet to another. It was found in urinogenital system of infested mullets. It was observed on the gill surface, operculum, and epithelium (Fig-4A) where superficial to deep ulcerative lesions were visible. On the branches of gills of infested mullets nearly circular cell structures reached a total diameter of 42.7 µm which were representatives of the family Trichodinidae (Fig-4B). Living cells have a cylindrical or hemispherical shape. It had an internal tooth like skeletal ring. The basal adhesive disk was well-developed, consisting of skeletal ring of radially arranged denticles composed of broad outer blade, a central cone, and an inner ray. An adoral region of cilia aligned in a spiral that makes one or two turns was present on the disks. The Adoral disc was situated opposite the Aboral one facing the underlay. The diameter of the adhesive plate was 23.6 µm with three concentrically arranged rings containing radially arranged hooks interlocked (3.0 µm) (Fig-4C) [9].

Nemathelminths

These round worms pass through six stages in the marine habitat, including an egg, first larval stage, second-, third- and fourth larval juvenile and finally adult. Molts separate each juvenile stage from the preceding stage.

Anisakis simplex [28]

Third Larval stages of *Anisakis simplex* [28] were observed in the small intestine or encysted on the liver, stomach wall, and muscles of infested mullets. The examined mullets were asymptomatic. Total length ranged from 13.5-20.5mm and maximal width 0.12-0.39 mm. The body was broad posteriorly and tapered anteriorly (Fig-5A, B). Cuticle striations were transversely visible throughout the worm and irregularly wrinkled posteriorly (Fig-5C, D). The foregut length was 1.20-2.2 mm. Three lips were visible, one dorsal and two ventro-lateral with a prominent boring tooth between the two ventro-lateral lips (Fig-5E). An excretory opening was present anteriorly below the mouth ventrally (Fig-5F). A terminal spine was found on the posterior broad part of the worm (Fig-5C). Ventricular appendix and intestinal cecum were absent [14].

Raphidascaris acus [29]

Third larval stage of *Raphidascaris acus* [30] was observed in the intestine. The cuticle was smooth, without striations (Fig-6A). Lateral alae extended along the body. The lips were poorly developed. A ventral boring tooth was present (Fig-6B). The ventriculus was broader and a ventricular appendix was present (Fig-6C). Absence of intestinal cecum. An excretory opening was present under the nerve ring. Rectal glands

were present. The posterior part was pointed and transversally striated (Fig-6D). Male body length was 17.12 mm and maximal width 0.720 mm. Female body length was 25.23 mm, maximal width 0.67-0.82 mm [31-33, 12]. Third-stage larvae of *Raphidascaris* sp. are previously described in the work of Barson & Marshall [18] & Mattiucci *et al*., [17].

***Gnathostoma spinigerum* [34]**

G. spinigerum require two intermediate hosts, the larvae developing first in a *Cyclops*, continuing in a fish or a vertebrate, and reaching sexual maturity in a mammal. Third-stage larva of *G. spinigerum* was 2-15 mm in length and had a terminal mouth and an anus (Fig-7A). The mouth was surrounded with two lips (Fig-7B). The GI tract was simple and composed of an esophagus and an intestine. It had four rows of hooklets extruding from the cephalic bulb and used to fix larvae into the tissues of infested mullets (Fig-7B). The body was covered with fine spines (Fig-7C). A cervical papilla extended in the body and two labial papillae were found on the cephalic bulb. The cephalic bulb had four openings. Concerning spawning of adult worm, ova were oval shaped with a mucus plug at one end. These ova were released into the mullet GI tract, from which they will be excreted with the feces to infest other mullets. The worm was also detected in the stomach and ovary of the mullet [32].

Enumeration of parasites during the study period

Along the period of this study, different parasites were searched in 1042 mullets during summer & autumn 2015 and winter & spring 2016. The total number of infested mullets along the study period with one or more parasites was 396. Infestation with Microsporidia, Myxozoa and Ciliophora was intense during September to February representing 27% - 44% of the total number of mullets searched for parasitic protists. Infestation with round worms was intense during March till August representing 40.5% - 63.4% of the total number of mullets searched for parasites in this period.

Infestation with *Microsporum velveticum* was intense during September to February representing 7-10% of the total number of mullets searched for *Microsporum velveticum* in this period (Fig-8A). Infestation with *Myxidium depressum* was intense during October to February representing 4-6% of the total number of mullets searched for *Myxidium depressum* in this period (Fig-8A). Infestation with *Trichodina murmanica* was intense during October to March representing 5-6% of the total number of mullets searched for *Trichodina murmanica* in this period (Fig-8A). Infestation with *Anisakis simplex* was intense during March - August representing 10-19 % of the total number of mullets searched for *Anisakis simplex* in this period (Fig-8B). Infestation with *Raphidascaris acus* was intense during March - August representing 7-12 % of the total number of mullets searched for *Raphidascaris acus* in this period (Fig-8B). Infestation with *Gnathostoma spinigerum* was intense during April- July representing 10-17% % of the total number of mullets searched for *Gnathostoma spinigerum* in this period (Fig-8B). The percentage of infestation with roundworms was more intense than infestation with Microsporidia, Myxozoa and Ciliophora.

The total number of mullets infested with the parasites studied were subjected to two-way analysis of variance (ANOVA). This statistical analysis showed a significant difference in the means among the parasitic protists and roundworms. Roundworms were prevalent in the warmer months ($p<0.0001$), while protists dominated in the cooler months ($p<0.05$).

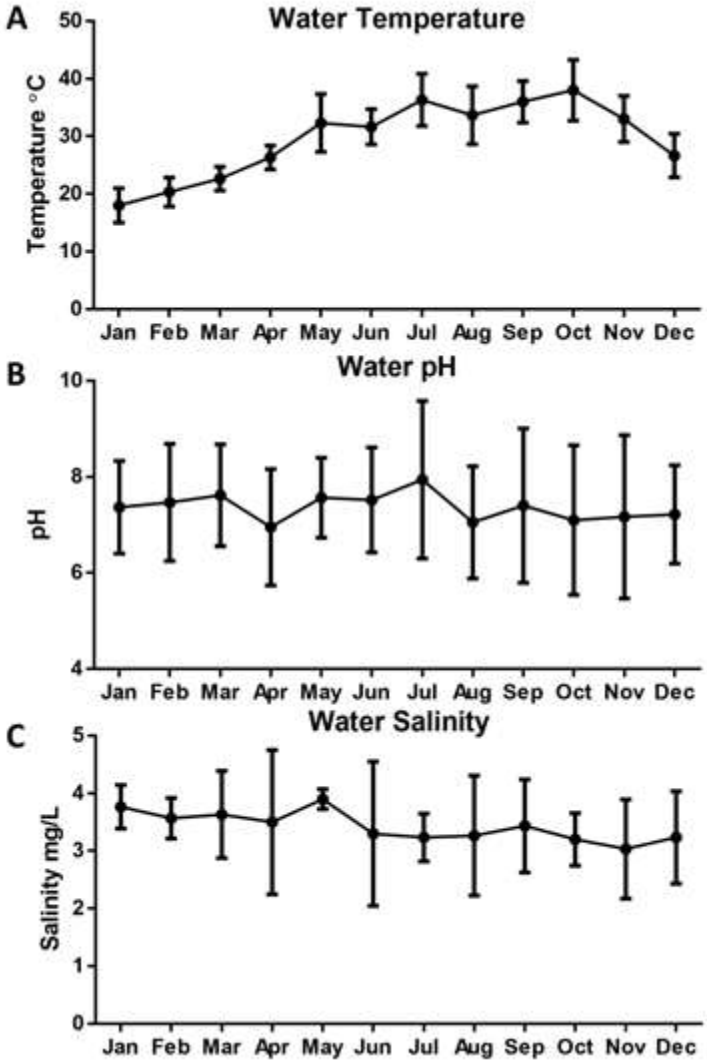

Fig-1: Water temperature, salinity, and pH measurements from the study site. A. Temperature was measured during collection of samples. B, C. For measurement of salinity and pH water samples were collected and analysed in the lab.

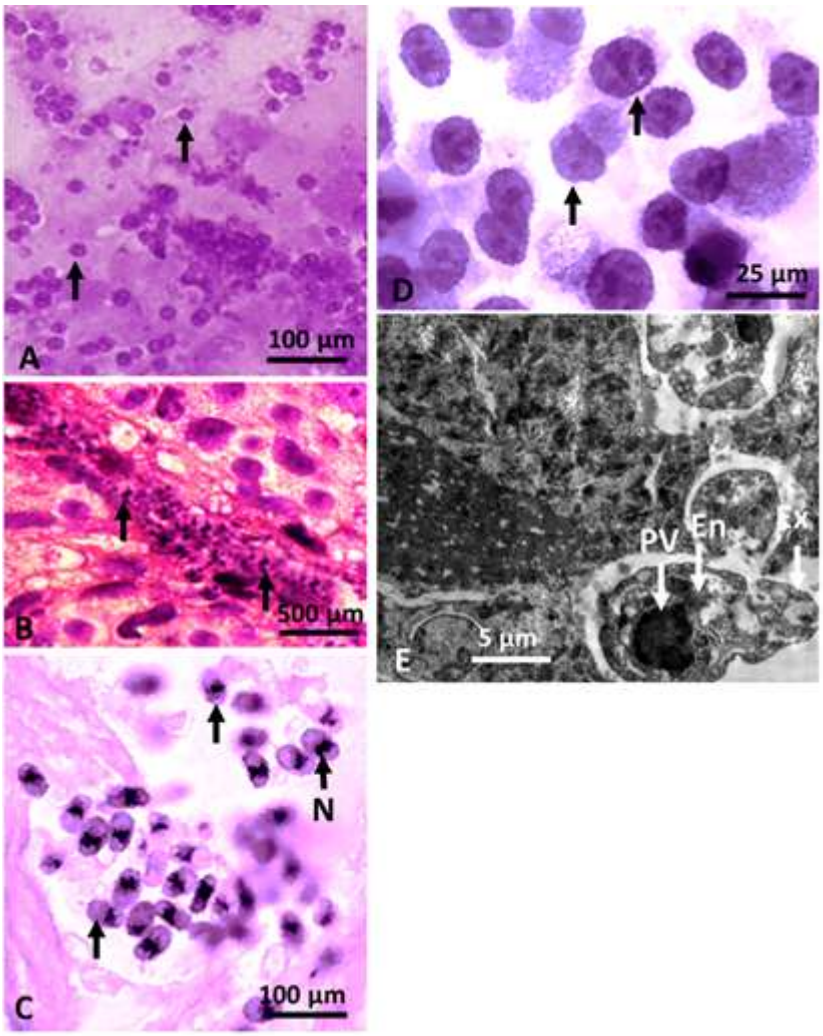

Fig-2: A. Photomicrograph of *Microsporum velveticum* spores indicated by arrows. These spores were Pyriform and had thick wall containing the toxic sporoplasms which was important in invasion process (average length 4.5 µm; average width 2.3 µm). B, C, D. Photomicrograph of *Microsporum velveticum* cysts indicated by arrows. This parasite was observed in the intestinal epithelia, liver, and air bladder of infested mullets. It was unicellular and exhibited intracellular parasitism. N, Nucleus. E. TEM of *Microsporum velveticum* spores. They had a coiled polar tube that was extricable to inject the sporoplasms into the mullet tissues. Each spore had a posterior large vacuole and limited with a spore's pansporoblast membrane. PV, Posterior Vacuole; En, Endospore; Ex, Exospore.

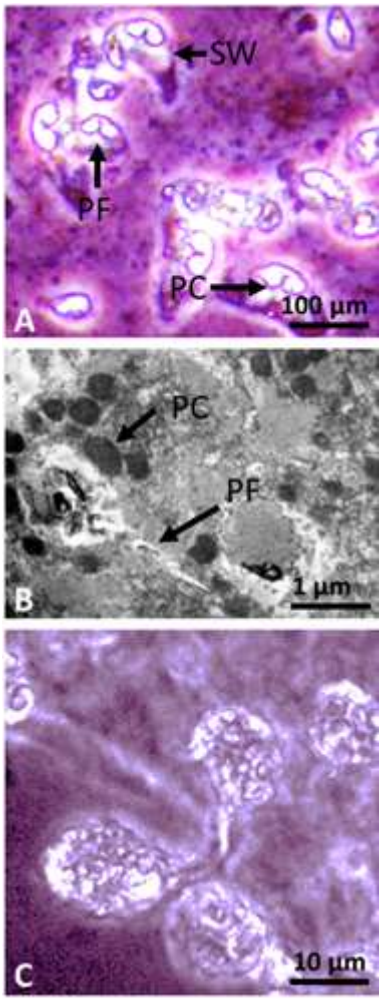

Fig-3: A. Photomicrograph of *Myxidium depressum* [27], indicated by arrows. This parasite was found in the liver and gall bladder of infested mullets in a plasmid stage that had undergone sporogony forming multicellular myxospores. B. SEM of *Myxidium depressum* [27]. It appeared as spore shell valves, amoeboid infective sporoplasms (sporoplasmic cells) and capsulogenic cells forming a polar capsule. SW, Spore Wall; PC, Polar Capsule; PF, Polar Filament. C. Photomicrograph of *Myxidium depressum* [27]. This parasite was observed in GI lumen and embedded in the gastric epithelia as plasmid stage of the mullet.

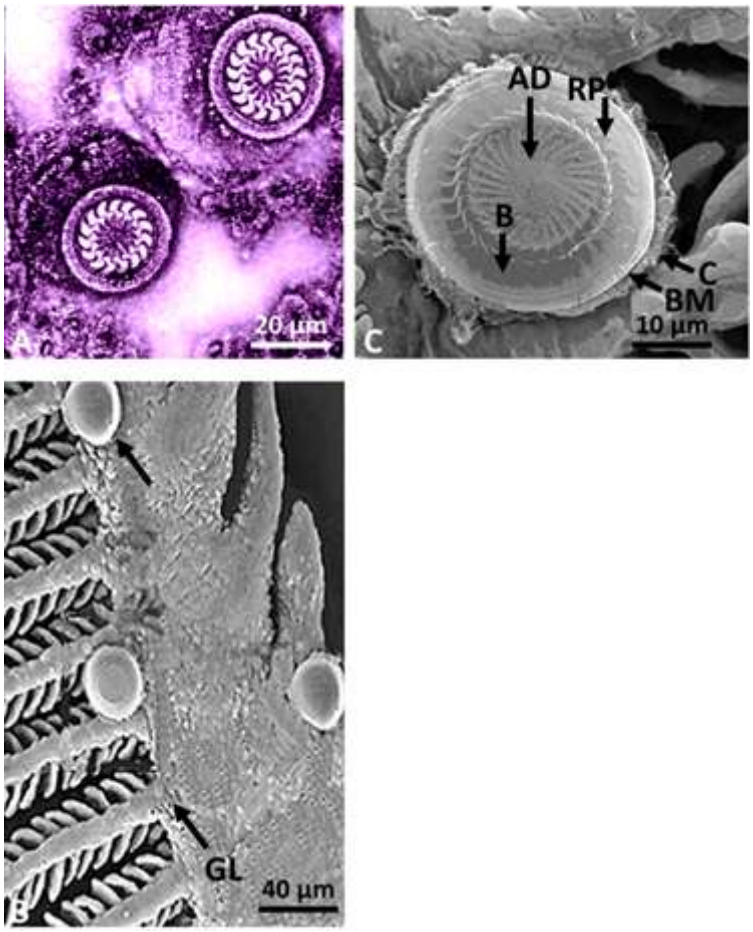

Fig-4: A. Photomicrograph of *Trichodina murmanica* [26] on the epithelial cells covering the gill surface. B, C. SEM of *Trichodina murmanica* [26] observed on the epithelial cells covering the gill surface and on the branches of gills of infested mullets nearly circular cell structures reached a total diameter of 42.7 µm were representatives of the Family Trichodinidae. AD, Anchoring Disc; B, Blade; BM, Border Membrane; C, Cilia; RP, Radial Pin; GL, Gill Lamillae.

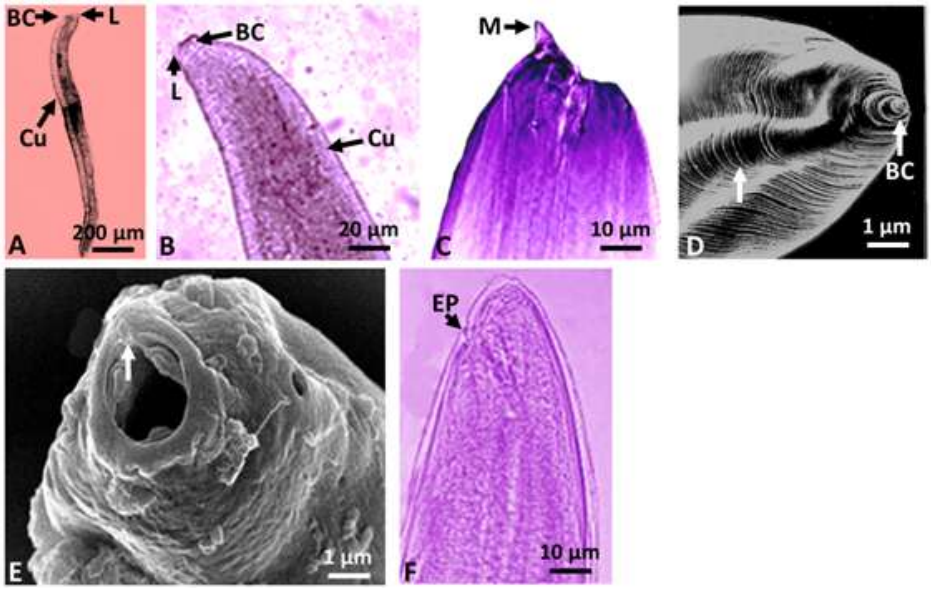

Fig-5: A. Photomicrograph of the third larval stages of *Anisakis simplex* [28]. Total length ranged from 13.5-20.5mm and maximal width 0.12-0.39 mm. B. The body was tapered anteriorly. BC, Buccal Capsule; L, Lip; Cu, Cuticle. C. The posterior was broad with a terminal mucron (M). D. SEM of the third larval stages of *Anisakis simplex* [28]. Cuticle striations were transversely, irregularly wrinkled posteriorly as indicated by an arrow. E. TEM of the third larval stages of *Anisakis simplex* [28]. There were one dorsal and two poorly developed ventro-lateral lips with a prominent boring tooth below the oral opening and between the two ventro-lateral lips indicated by an arrow. E. Anterior showing the Excretory Pore (EP).

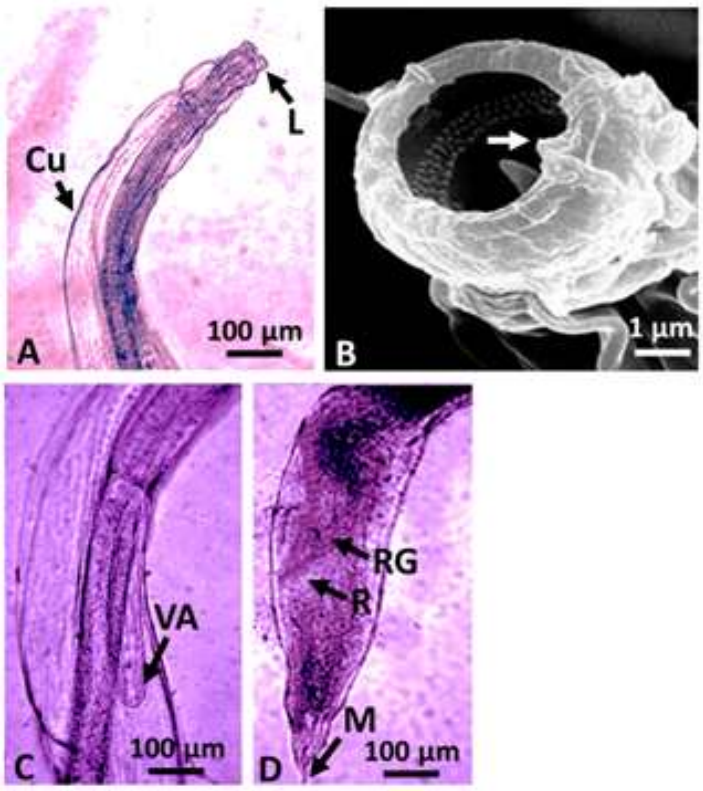

Fig-6: A. Photomicrograph of the third Larval stages of *Raphidascaris acus* [30]. It was observed in the intestine. The cuticle was smooth, without striations. A lateral alae extended along the body. Poor developed lips were present. L, Lip; Cu, Cuticle. B. SEM of the third Larval stages of *R. acus*. A ventral boring tooth was present as indicated by an arrow. C. Photomicrograph of the third Larval stages of *R. acus* showing a broad ventriculus. A ventricular appendix (VA) is present. D. Photomicrograph showing the posterior end of third larval stage. M, mucron; R, Rectum; RG, Rectal Gland.

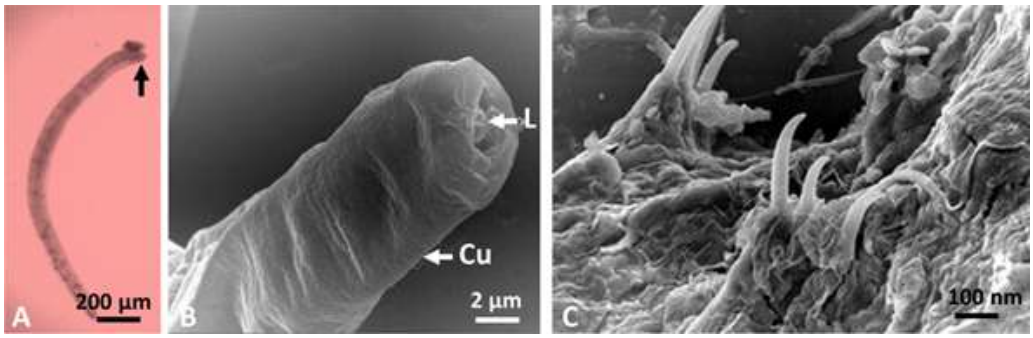

Fig-7: A. Photomicrograph of the third-stage larva of *Gnathostoma spinigerum.* It was 2-15 mm in length, had a terminal mouth (indicated by the arrow) and an anus. B. SEM of the third-stage larva of *Gnathostoma spinigerum* showing the anterior. The mouth was surrounded with two lips. It had four rows of hooklets extruding from the cephalic bulb and used to fix larvae into the tissues of infested mullets. L, Lip; Cu, Cuticle. E. SEM of the posterior showing the body covered with fine spines.

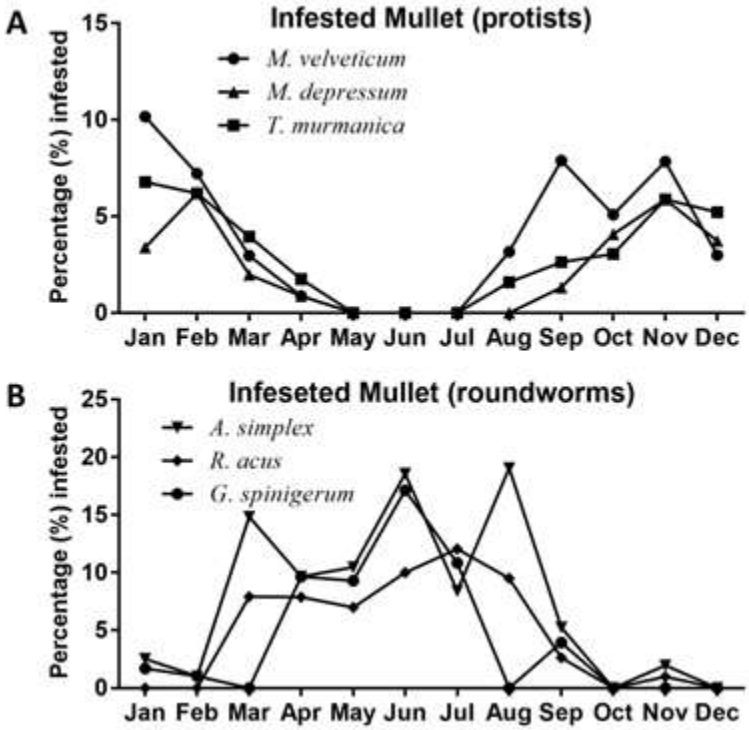

Fig-8: Infestation of mullets with the different parasites during the study period. A. Percentage of mullet infested with parasites belonging to the protist kingdom. Increased infestation was observed during the colder months of the year. B. Percentage of mullet infested with roundworms. Infestation increased during the hotter months of the year.

DISCUSSION

In marine ecosystems organisms live by eating each other. Almost all marine organisms are susceptible to parasites. Parasites can be dangerous to their fish hosts, causing reproduction inability, hinder development, weakness and increased mortalities [35, 15]. Parasites, live in or on hosts, exert mechanical destruction of cells, tissues and organs or deprive their hosts of nutrients and provide intoxication [36]. Moreover, parasites may cause bacterial secondary infections and are considered as vectors for pathogens or other parasites [36]. On the other hand, parasites can provide important information about their hosts as well as their habitats. There are studies from a wide range of areas for the use of parasites as bio-indicators or already successfully used as such [20, 37]. Marcogliese *et al.*, [38] investigated the effects of abiotic factors on marine communities including parasites. Parasites exert a dangerous impact in aquaculture as fishes become reservoirs for protozoans and helminthes [4, 38]. Myxosporeans are divesting parasites in cultured fishes the websites of Fisheries and Oceans Canada, 2003, 2004) [39]. In this study, pathogens in mullets were tested in relation to climatic changes. It was concluded that the intensity of parasites was affected by water temperature, while salinity and pH did not exert any

effect on parasite density. It is known that there are host parasite and parasite host categories specific to abiotic and biotic factors of marine ecosystems. This was evidenced by numerous other studies on water temperature, salinity water depth food and the occurrance of intermediate hosts that act directly or indirectly on the marine food structure [40-42, 3, 9].

The microsporidian *Loma branchialis* has been found in the cod fish *Melanogrammus aeglefinus* [42]. This parasite infects the gill filaments, pseudobranchs, the heart and liver [42]. In gill lamellae it causes clubbing which leads to respiratory problems [43]. The present study found that Mullets were infested with microspores of *Microsporum velveticum* likely through ingesting food contaminated with infective spores that were found in feces of other infested fishes. This parasite was observed in the intestinal epithelia, adipose tissue, liver, gastric epithelia, muscles, and air bladder of infested mullets.

Marine myxozoans require an alternative invertebrate host to complete their life cycle [44]. Myxosporeans are considered as potential parasites in aquaculture [45, 46]. The genera *Kudoa*, *Sphaerospora*, *Ceratomyxa*, and *Myxidium* have caused considerable problems in North Atlantic mariculture. The flat fishes of Hokkaido were more susceptible to the infection of the myxosporidian parasites than the allied forms in the North Sea [11]. Our study showed that *Myxidium depressum* infested the liver and gall bladder of mullets in a plasmid stage that had undergone sporogony forming multicellular myxospores.

We observed *Trichodina murmanica* in the urinogenital system of infested mullets. It was also found on the gill epithelia, operculum, and body surface where superficial to deep ulcerative lesions were visible. *Trichodina murmanica* and *T. cooperi* were found by Mitra *et al*., [47] on cod fishes held in captivity. Heavy *Trichodina* infection in flatfish was also found in farmed plaice and in turbot cultivation [13, 48].

Parasitic nematodes cause enormous losses in aquaculture and many species are responsible for serious human diseases. Despite the large variety of the species, their structure is monotonous. A typical life cycle includes four larvae and one adult stage. Parasitic species have either direct or indirect life cycles. Larval stages of *Anisakis simplex* and *Raphidascaris acus* inhabit in the systems and muscles of fishes [39]. Our findings showed that *Anisakis simplex* and *Raphidascaris acus* were present in various tissues of mullet. Anisakid nematodes inhabiting the tissues of edible fishes causes the most economical damage in marine fisheries [26, 49, 50]. Infection of fishes with seal-worm *Pseudoterranova* sp. is primarily harmless while the whale-worm *Anisakis* sp. Exerts danger to human health. Anisakids are not host specific as they have a wide range of different fish host species which may result in a higher probability of dispersal [51]. Anisakiasis, in humans is acquired by eating smoked fish or raw fish [15, 16].

Worms of *Gnathostoma* inhabit the stomach or esophageal epithelia of definitive mammalian hosts [52]. When eggs are deposited in marine water, free-swimming larvae are liberated and ingested by *Cyclops* in which 3rd-stage larvae (L3) form. They develop into advanced L3 in the second intermediate hosts such as fishes. They are passed to a wide range of paratenic hosts including birds, and mammals including humans. The principal mode of human infection by *Gnathostoma* is consumption of raw or undercooked flesh of second intermediate hosts containing 3rd-stage larvae [53]. In infested mullets, the GI tract showed concentric long stenosis, resulting in circumscribed wall thickening. Mucosal lesions were not present. Gnathostomiasis is common in Southeast Asia, where people have the habit of ingesting uncooked or partially cooked food. Furthermore, influx of Southeast Asian people to various parts of the world increases the chance of spreading the disease to other regions since there are now more reported cases in nonendemic areas [54].

Along the period of this study, different parasites were found in mullets during summer & autumn 2015 and winter & spring 2016. Statistical studies on parasites, including protists and metazoans have been done in the perch *Bairdiella chrysura* collected along the coast of Florida [55]. Fluctuations in parasites intensities in the different months of the year were recorded in seals living in Gulf of St. Lawrence [56, 57]. In these studies, it was concluded that variation of parasitic population in seals was dependent upon feeding behaviour on capelin or on cod. Our analysis found that parasites were extremely sensitive to climatic change. Microsporidia, Myxozoa, Ciliophora predominated during cold months while Anisakidae and Gnathostomatidae were predominate during the warm months. Therefore, infections with roundworms with complex and indirect life cycles tend to decrease in cold months when the intermediate host have a non-reproductive state and are few in number. In warmer months the intermediate host increases in number and would be eaten by mullets. Infections with ectoparasites like *Trichodina* with direct single-host life cycles and the endoparasites *Microsporum velveticum* and *Myxidium depressum* tend to increase with increasing number of mullets in cold months.

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