

Interactions between Monogenean Parasites and Fish

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Abstract: Monogeneans are ubiquitous, less understood parasite in context of aquaculture practices which generate a potentially stressful environment to the cultured animals, with the possible suppression of the immune system, rendering the fish more susceptible to different infectious diseases. Among the different parasites, monogenean ectoparasites are known to parasitize a large number of fish. Fish hosts susceptible to a certain parasite show an ability to mount a protective response after infection. The innate host factors like complement, lectins, acute phase reactants, macrophages are reported to bind monogeneans and elicit severe damage to the parasites by targeting the monogenean tegument, gastro-dermis and glands. Increase in the host's production of adaptive and non-adaptive factors following monogenean infections of certain duration explains the acquired response. However, clever skill of the monogenean to avoid and even exploit the wide array of immunological elements of the host poses an interesting event in the dynamic interactions between host and monogenean. Understanding of diverse monogenean morphology in context of its life-cycle and host interaction may give clue to avoid negative impact of this parasite.

Keywords: Monogenean, Aquaculture, Morphology, Immune response.

1. INTRODUCTION

Aquaculture is the fastest growing and emerging food production sector in the world as well as in India. With the gradually declining of capture fishery resources, the aquaculture industry worldwide is making significant progress in the development of technology to increase production. These aquaculture practices generate a potentially stressful environment to the cultured animals, with the possible suppression of the immune system, rendering the fish more susceptible to different infectious diseases (Hoffman, 1967; Villamil *et al.*, 2003). Among various infectious diseases, parasite diseases in aquaculture have been gaining increasing economic importance now-a-days (Alvarez-Pellitero, 2008; Dash *et al.*, 2014). Among different parasites, monogenean ectoparasites are known to parasitize a large number of fish (Dove and Ernst, 1998; Del Rio-Zaragoza *et al.*, 2010). Species belong to the helminth group with a site preference of gill is also known as gill fluke and commonly infect cyprinid fish. The infected fish shows pale gill with excessive mucus secretion, enhanced respiratory rate, loss of appetite and gill hemorrhages which often leads to mortality (Dash *et al.*, 2014). Indian major carps have been reported to be infected by gill monogeneans with high prevalence and intensity (Ramudu and Dash, 2013; Dash *et al.*, 2015). Monogenean infections, thus, can be accounted for production loss in many ways and control of such ectoparasites has now become an urgent need for the fish farming industry. Control of this parasite would not be possible without the knowledge of host-parasite interaction from immunological point of view. It is very much important to understand which cells, molecules or pathways of the immune system get activated during an infection that would assist in developing suitable immunoprophylactic measure against the pathogen (Dash *et al.*, 2014).

2. SYSTEMATIC CLASSIFICATION

See table no. 1 classification of monogenean parasites

Table 1 classification of monogenean parasites

kingdom	animalia
phylum	platyhelminthes
class	monogenea
sub class (1)	monopisthocotyleas
order	dactylogyridae
	gyrodactylidae

	capsilidae
	diclidophoridae
sub class (2)	polyopisthocotyleas
order	chimaericolidea
	diclybothriidea
	mazocraeidea
	polystomatidea

3. MORPHOLOGY OF ADULT MONOGENEAN

See figure no. 1 the morphology of adult Monogenea.

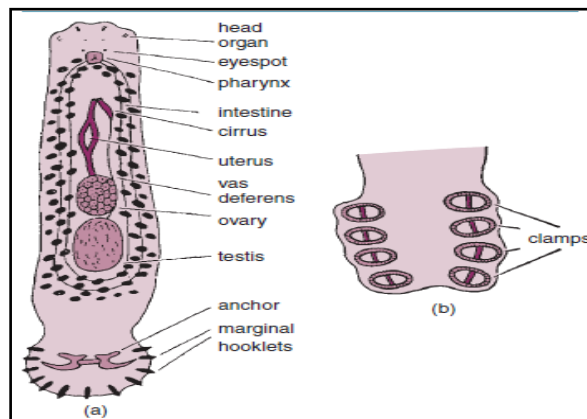


Figure 1: The morphology of adult Monogenea. (a) Monopisthocotylean. Note the undivided posterior haptor. (b) Haptor of polyopisthocotylean. Note the subdivision of the haptor into clamps (source: Roberts, 2012)

3.1. Opisthaptor

An important adhesion apparatus in the posterior part which is equipped with sclerotized structures (large hooks, anchors or hamuli). Some opisthohapter penetrate, some serve non penetrating spring function on the epithelium surface of host. Marginal hooklets assist attachment.

3.2. Tegument

The outer lining composed of outer anucleated layer and basal nucleated layer. Outer tegumental plasma membrane is covered by glycocalyx, which shows positive reactions for carbohydrates. The tegumental layer equipped with mitochondria, secretory bodies and microvilli (especially in the adhesive region of parasite). Tegument serves as osmoregulatory and excretory function.

3.3. Anterior adhesive pads

It is a fore part-prohaptor (attachment) which play crucial role in feeding, movement and reproduction. The anterior adhesive pads produce sticky secretion. The secretions are proteinaceous and involved in the parasite attachment. Sticky secretions allow parasite to attach the host and additional secretions released can allow the worm to detach gland cells have crucial role in attachment and communication with host.

3.4. Digestive gland cells

An enzyme produced includes alkaline and acid phosphatase, esterases and proteases. These enzymes are released into host tissue, initiating extracorporeal digestion. They also act as important antigens in host immune response.

3.5. Sensory system

Respond actively to both mechanical and chemical stimuli. Various Chemoreceptors, rheoreceptors, tangoreceptor, photoreceptor function in monogeneans. Compound receptors (group of multicellular receptors) on anterior part of worm are function as chemo and tango sensors. Papillae equipped with and penetrated by nerves, which may serve a function in attachment to the host.

3.6. Nervous system

The monogenean parasites are mobile and move actively on the surface of host body. These parasites possess orthogonal central nervous system and 3 pairs of longitudinal nerve trunks (ventral, dorsal, lateral) connected by transverse commissures. They have rudimentary brain composed of cerebral ganglionic collections of nerve cells

connected by commissures. The esterases (acetylcholinesterases) found in both Central nervous system and Peripheral nervous system. Addition to cholinergic nerve, aminergic nerves are also found in parasites.

3.7. Muscular system

The feeding, movement, reproduction based on the coordinated muscular actions of parasite body. The muscles are arranged as inner longitudinal and outer transverse fibre. The Opisthaptor, pharynx, male copulatory organs are richly supplied with muscles. Muscles are non-striated and rich with mitochondria allows the typical coordinated movement composed of stretching, shortening and bending.

3.8. Alimentary canal

The digestive system consist of mouth, pharynx and intestinal caeca which is a range of enzymes are released by gland cells. The gastrodermis serves for absorption of nutrients and developed according to food type eaten by parasites. For polyopisthocotyleans it is adapted for absorption of haemoglobin and for monopisthocotylean it is for the digestion of epithelial cells and mucus.

3.9. Excretory system

The basic unit is flame cells in excretory system. The waste released through two anteriolateral opening leading to collecting duct which again connected to a protonephrdial system consisting of flame cells, capillaries and ducts. The structure of flame bulbs and capillaries differs among groups which serve as osmoregulatory in addition to excretory function.

3.10. Reproductive system

Most of the plathyhelminth parasites are hermaphroditic flat worms with testis and ovary. They possess vitellaria, an ootype and seminal receptacle. Sclerotized structures found are cirrus, accessory cirrus and vagina. Polyopisthocotyleans have genito-intestinal canal and have a different organization of spermatozoan microtubules.

4. REPRODUCTION AND LIFE CYCLE

The most are oviparous with simple life cycle and Hermaphrodite in nature. Gyrodactylus are viviparous and have mature embryo inside the uterus. The egg morphology differ considerably dactylogyrous and pseudodactylogyrous produce small oval egg with sticky stalk allowing egg for attachment, some have short or long extensions. Generally have ciliated larvae on oncomiracidium and exception-non ciliated larvae such as Acanthocotyle greeni. Within 24 hrs larvae attaches to host. These are influenced by temperature including oviposition rate, embryonation, hatching time, the free living phase, the post larval development and adult life span of parasites.

5. DIFFERENCE BETWEEN MONOPISTHOCOTYLEAS AND POLYOPISTHOCOTYLEAS

Table 2 Difference between Monopisthocotyleas and Polyopisthocotyleas

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Monopisthocotyleas	Polyopisthocotyleas
Opisthohaptor: Single unit comprising several , large centrally located sclerotized anchors	Battery of small, muscular adhesive suckers or clamps supported by cuticular sclerites
Use of anchors or hooks for attachment tend to pierce the tissue	Clamps have opposing sections that grasp the host tissue between them
Feeds on the superficial layer of skin and gills	Feeds on blood
Extensive hyperplasia & Tissue reactions	Anaemia ,associated with lethargy , anorexia , dark skin colour
Gastro dermis adapted for digestion of epithelial cell & mucus	Gastro dermis adapted for absorption of hemoglobin
Larvae have 2 pair of eyes with lens	Larvae have 1 pair of eyes without lens
eg: family: dactylogyridae	eg: family: dicilidophoridae

6. DIFFERENCE BETWEEN FAMILY DACTYLOGYRIDAE AND FAMILY GYRODACTYLIDAE

Table 3 Difference between family dactylogyridae and family gyrodaetylidae

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DACTYLOGYRIDAE	GYRODACTYLIDAE
Microhabitat : gills	Microhabitat : skin& gills
Oviparous (usually upto 2mm length)	Viviparous (usually .3 – 1 mm length) ,have uterus with developing embryo
Anterior part of body Contains pair of pigmented light receptors	Absent
Two cephalic lobes containing secretions of adhesive gland cells	Cephalic lobes present
Muscular pharynx, tubular posteriorly confluent intestine	Muscular pharynx, intestinal caecae simple and end blindly
Haptor contains 2 pairs of hamuli and 14 marginal hooks	Haptor contains 1 pair of ventrally located hamuli and 16 marginal hooklets
Transmission by attachment of eggs	Transmission from fish to fish
eg: <i>Dactylogyrous vastator</i> (infects common carp)	eg: <i>Gyrodactylus salaris</i> (infect salmon) *** OIE LISTED PATHOGEN

7. LIFE CYCLE OF MONOGENEAN

See figure no. 2 Life cycle of Monogenean

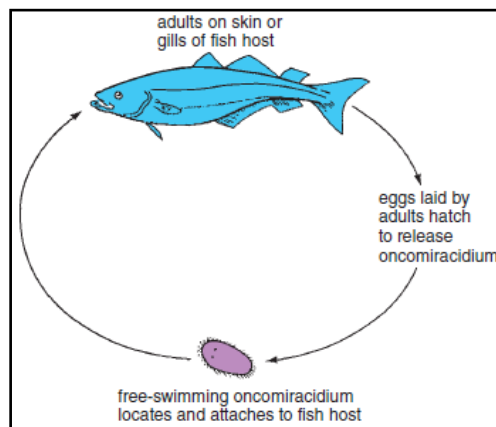


Figure2: Life cycle of Monogenean (source: Roberts, 2012)

8. HOST – PARASITE RELATIONSHIP

8.1. Egg hatching

Both abiotic and host factors can affect the early stage of parasite. The changes of light intensities initiate hatching in certain species: *Entobdella sereola* - hatching is stimulated by illumination after a period of darkness; in contrast *Discicotyle sagittata*- egg hatching is induced by darkness. The mucus extract from fish host has been observed as an efficient hatching stimulus. When fully-embryonated egg is subjected to mucus extracts the oncomiracidium will initiate active movements and subsequently escape through the opercular opening.

8.2. Host finding

Monogenean ability to detect differences between different fish species by its sensory structures may be based on both chemical and mechanical stimuli on the host surface. The selection of the correct host and the mechanism behind the choice is insufficiently understood. The high host-specificity of monogeneans is due to some chemo-attraction of the infective oncomiracidia (*Entobdella soleae*). The isolated epithelium-covered by scales from various fish species when exposed to larvae showed clear selection of sole scales.

The dissected epithelia from cornea in fish when presented to the parasite did not show similar attraction. Some oncomiracidia also attracted agar plugs with sole mucus Substances. The *Gyrodactylus salaris* infects salmon, (see figure no. 4) *Salmo salar*, when it has the choice between rainbow trout, carp and salmon. Similarly *Gyrodactylus derjavini* preferentially infect rainbow trout when offered the same three possibilities.

This ability was also cross-checked with in vitro studies using fish scales covered with live host cells. In vitro studies on migration of isolated gyrodactylids offered mucus or in agar plugs in petri-dishes did not show any specific selection. This indicates that some volatile host substances from intact cells only could be involved in host selection. pH of mucus also plays an important role in host identification.(see Figure no. 3)

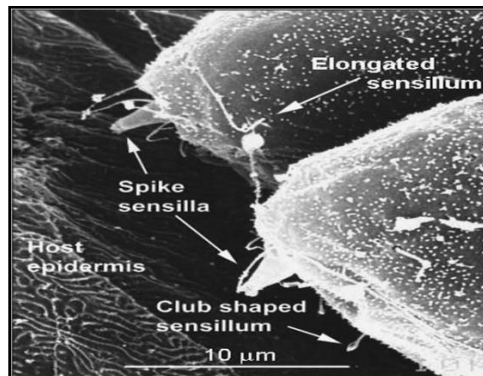


Figure3: Scanning electron micrograph of the sensory apparatus at the anterior lobes of *Gyrodactylus salaris* actively searching the skin surface of an Atlantic salmon parr, anterior lateral view. (Source: Bakke *et al.*, 2004)

9. HOW DOES THE HOST RECOGNIZE THE MONOGENEAN PARASITE?

9.1. Initiation of the immune response

Pathogen recognizing receptors (PRRs) (Toll-like receptors, TLRs and other PRRs)

Two types of host-defence mechanisms have been traditionally accepted, innate and adaptive (also known as acquired). The main distinction between them is the receptor types used to recognize pathogens (Medzhitov, 2007). Innate immune recognition relies on a growing number of receptors (PRRs), with a broad specificity, which are germline encoded and have evolved to recognize pathogen-associated molecular patterns (PAMPs) (Janeway and Medzhitov, 2002). One unifying feature of PRRs is their highly conserved structures, which are steady among given class of microorganisms. In contrast, adaptive immune recognition is mediated by antigen (Ag) receptors, with random but narrow specificities. The different PRRs are involved in performing specific tasks, including opsonization, activation of complement cascade, phagocytosis, etc. (Pasare and Medzhitov, 2004). There are several functionally distinct classes of PRRs, but the best characterized are Toll-like receptors (TLRs), type-I transmembrane proteins with extracellular leucine-rich repeat (LRR) motifs and intracellular Toll/interleukin-1 receptor domain. TLR family members contribute both to cell-cell interaction and to signalling. The study of TLR genes in several teleost species (*Danio rerio*, *Takifugu rubripes*, *Carassius auratus*, *Oncorhynchus mykiss*, *Panaeolus olivaceus*) has demonstrated the evolutionary conservation of the key components of TLR signalling in vertebrates (reviewed in Roach *et al.*, 2005; Purcell *et al.*, 2006). Following these reviews, new data on TLR-signalling molecules has been obtained for some of the above cited species (Takano *et al.*, 2007; Chen *et al.*, 2008), and also for gilthead sea bream *Sparus aurata* (Franch *et al.*, 2006), Atlantic salmon *Salmo salar* (Tsoi *et al.*, 2006), *Ictalurus punctatus* (Baoprasertkul *et al.*, 2007) and five sea breams (Chen *et al.*, 2008).

Creagh and O'Neill (2006) reported two additional families of innate receptors have been described that join the TLRs as key pathogen sensors *viz.*, intracellular receptors NOD-like receptors (NLRs) and RIG-I (retinoic acid-inducible gene I)-like proteins (RLRs). All NLRs contain a nucleotide-binding oligomerization domain (NOD) followed by a LRR at the carboxy terminus. Another important group of PRRs is represented by C-type lectins, which are best known for their ability to recognise specific pathogen-associated carbohydrate structures. These are characterized by C-type lectin receptors (CLRs), proteins that contain carbohydrate recognition domains (CRDs) (McGreal *et al.*, 2004). Some CLRs are produced as transmembrane proteins present on dendritic cells and macrophages (MFs) (Medzhitov, 2007; Cambi and Figdor, 2005), or are secreted as soluble proteins. The involvement of a mannose receptor in phagocytosis has been suggested for gilthead sea bream (Rodriguez *et al.*, 2003). Amongst soluble PRRs, the mannan-binding lectins (MBLs) and ficolins bind N-acetyl-glucosamine and mannose structures common among microbes (Fujita *et al.*, 2004)

The immune mechanisms and their pathways against fish parasites are concerned; substantial progress has recently been made. In teleost, cellular and humoral factors of both innate and adaptive immunity are involved in immunity against monogeneans infecting gill tissue with superficial capillaries and several effector mechanisms has been suggested for that (Buchmann, 1999).

Apart from different systemic immunity components, several other cellular and humoral factors of the mucosa such as mucosal cells, leucocytes, complement, C-reactive protein, lysozyme, anti-microbial peptides etc. is thought to play important role in mediating immunity against monogeneans (Buchmann 1999). The immune responses of Indian major carps against monogeneans have poorly been demonstrated. The immune-relevant gene expression in gill and head kidney have been reported in one of the Indian major carps, *Labeo rohita*, parasitized by the monogenean ecto-parasite *Dactylogyrus catlaeus* (Dash *et al.*, 2014).

9.2. Non-specific cellular responses

Inflammation and Phagocytosis reactive oxygen and nitrogen species: Activation of the fish skin epidermis, which mainly causes the production of cytokines leading to decrease of the ectoparasite population (Lu *et al.*, 2013). Non-specific cellular responses, injury or parasitic invasion of the fish epidermis is known to elicit inflammatory reactions involving several cell types, including neutrophils, macrophages, eosinophils and basophils (Buchman and Lindenstrøm, 2002). Infiltrated leucocytes secrete cytokines and other immunoactive compounds, which have several effects induction of cellular migration to the inflammatory site; regulation of mucus production in goblet cells; and induction of macrophage respiratory burst activity, among others (Secombes, 1996). Gene encoding interleukin-I beta and tumour necrosis factor alpha can be detected. It leads to inflammatory response, leading to the production of reactive nitrogen species. Cyclooxygenase responsible for prostaglandin production regulates the inflammatory process.

9.2.1. Eosinophilic and mast cells

Eosinophils are frequently involved in the response to parasite diseases. The term eosinophilic granule cells (EGCs) was introduced by Roberts *et al.* (1972) to designate mononuclear eosinophilic granule-containing cells distributed in the connective tissues of various teleosts. EGCs have been suggested to be mast cell analogous or equivalent (Reite, 1998), similar to intestinal Panneth cells (Sveinbjornsson *et al.*, 1996) or representing a cell type originating from the evolutionary precursors of both Panneth cells and mast cells (Paulsen *et al.*, 2001). Their heterogeneity and staining diversity have been stressed (Sveinbjornsson *et al.*, 1996; Rocha and Chiarini-Garcia, 2007), and some of them produce anti-microbial peptides or lysozyme. Recently, Mulero *et al.* (2007) found also histamine in mast cells of several perciform fish. Important differences can also occur between fish species, and thus, the characterization of these cells using specific markers is needed. Changes in EGC abundance and distribution can occur in response to parasites. Adams and Nowak (2003) reported EGCs adjacent to lesions produced by amoebas in *Salmo salar* with AGD. Two apparently different types of EGCs involved in the response of *Diplodus puntazzo* to the myxozoan *Enteromyxum leei*; EGC1 (very similar to the EGC of salmonids), degranulated in this infection and their number decreased with the progression of the infection, in parallel with an increase in EGC2-type cells (Alvarez-Pellitero *et al.*, 2008). The releasing of bioactive granules from eosinophils, causing the expulsion of most metacercaria, was also reported in *Carassius auratus* infected by the digenean *Ribeiroia marini* (Huizinga and Nadakavukaren, 1997). Degranulating eosinophils were also involved in the cellular response elicited by *Sepiella inermis* in *Common carpio* (Richards *et al.*, 1996).

9.3. Non-specific humoral defences

9.3.1. Lysozyme

Lysozyme is an important defence molecule of the innate immune system which play role in mediating protection against microbial invasion. It is a lytic enzyme formed by leucocytes, especially monocytes, MFs (macrophages) and neutrophils. Fish lysozyme possesses lytic activity against bacteria and can activate complement and phagocytes. Saurab and Sahoo (2008) mentions fish lysozyme in mucus, lymphoid tissue, plasma and other fluids and reported to be expressed in a wide variety of tissues. Parasite infections changes serum lysozyme levels in fish. An increase was reported in rainbow trout following immunisation with live theronts of *Ichthyophthirius multifiliis* (Alishahi and Buchmann, 2006), in *Dicentrarchus labrax* immunized with *Sphaerospora dicentrarchi* spores and in fish infected by *Ceratomyxa shasta* (Foot *et al.*, 2004).

9.3.2. Complement

The complement components, present in skin or in serum, of various fish has been reported to be involved in killing mechanisms against various parasites including monogeneans (Buchmann *et al.*, 1998; Harris *et al.*, 1998; Leiro *et al.*, 2008). Many species, including *Gyrodactylus derjavini* and *G. salaris*, are extraordinarily sensitive to host complement.

9.3.3. Acute-phase proteins

As reviewed in Bayne and Gerwick (2001), the acute phase response (APR) is a pervasive physiological response of the body to injury, trauma or infection. In its broadest context, the APR involves changes in at least the hepatic, neuroendocrine, hematopoietic, musculo-skeletal and immune systems, and is induced by pro-inflammatory cytokines such as IL-1, IL-6 and TNF α . Among the humoral components increasing their concentrations in APR in mammals are included complement system, clotting system, anti-proteases, metal-binding proteins, lectins, lysozymes, antimicrobial peptides and opsonins.

9.3.4. Lectins

Turner (2003) and Klein (2005) reported the role of C-type lectins as PRRs. Among soluble PRRs, MBL (Mannose-binding lectin) is well known for its role as initiator of the primary immune response and its participation in the lectin complement pathway. MBL homologous have also been demonstrated in teleosts (Russell and Lumsden, 2005). The pentraxins CRP and serum amyloid P (SAP), as well as transferrin and thrombin have been identified in teleosts (reviewed in Bayne and Gerwick, 2001). However, information on changes in relation to parasite infections is scarce. CRP is known to inhibit egg production in *Bothriocephalus scorpii* cultured *in vitro*, via phosphorylcholine/complement (Fletcher *et al.*, 1980).

9.3.5. Anti-proteases:

Host protease inhibitors modulate protease activities and control a variety of critical protease-mediated processes, including the resistance to invasion by infectious agents. Variation in the Macroglobulin family antiproteases, mainly α -2-macroglobulin which functions as clearance of active proteases from the tissue fluids have been observed in several parasite infections by Armstrong and Quigley (1999) which is also shown to interact with innate and adaptive mechanisms by Dalmo *et al.* (1997). In fish resistant to cryptobiosis, α -2-macroglobulin can neutralise the metalloprotease involved in *Cryptobia salmositica* virulence.

9.3.6. Mucosal epithelia

The mucosal epithelia play a significant role in fish immunology. Medzhitov (2007) reported the receptors involved in the response of mucosal epithelia are TLRs and NODs. Fritz *et al.* (2007) acknowledged that the specialised Ag presenting cells (APCs) and epithelial cells which constitute the primary cellular barrier recognize the pathogen. Thus, both types of cells can activate the corresponding signals leading to the different mechanisms and pathways (phagocytosis, complement, acute-phase reaction, cytokine activation), so that the epithelial response is integrated into the whole immune response. Shao *et al.* (2001) reported that the epithelial cell/T cell relationship plays an important role in the immune regulation of the gut. In ectoparasitic monogeneans the role of mucus in limiting the parasite load has been demonstrated (Buchmann and Lindenstrøm, 2002)

9.4. Specific cellular defences

9.4.1. Major Histocompatibility Complex (MHC):

The MH receptors are immunoglobulin superfamily member proteins that interact with T-cells through a specific T-cell receptor (TCR) in order to initiate immune responses. Dixon and Stet (2001) has demonstrated their role in the integration of innate and adaptive responses., Chistiakov *et al.* (2007) mentions that in tetrapods and sharks, MHC genes linked to a complex on a single chromosome, whereas in teleosts MH genes are not linked and are even located in different chromosomes. MH receptors, class I and class II receptors, are present in teleosts, and their function is to display foreign peptides (Ags) to T cells (usually class I for intracellular pathogens and class II for extracellular pathogens). Class I and II MH genes are highly polymorphic, particularly in the peptide-binding-encoded region, and such polymorphism has been found in some fish species (Chistiakov *et al.*, 2007). Cuesta *et al.* (2006) reported granulocytes of *Sparus aurata* express MHC II genes, suggesting a role as APCs. Morrison *et al.* (2006) acknowledged the highly regulated expression of MH II in *Salmo salar* inoculated with *Neoparamoeba* sp. by the numerous positive cells detected within amoebic gill disease lesions, and thus such cells could contribute to Ag presentation. Dendritic-like cells were described in the gills of Chinook salmon *Oncorhynchus tshawytscha* infected by *Loma salmonae* (Lovy *et al.*, 2006).

9.4.2. Cytokines:

Interactions between immune cells are mediated by direct cell to cell contact and through the release of soluble factors (cytokines). Fish cells release several cytokines analogous to mammalian cytokines (Manning and Nakanishi, 1996; Bird *et al.*, 2006) and like higher vertebrates, different immune responses elicited by pathogen infection are activated through differential synthesis of cytokines by activated subset of cells. Usually, Th1 cytokines interleukin 2 (IL-2), interferon gamma (IFN- γ) and tumor necrosis factors alpha (TNF- α) and beta (TNF- β) induce defences against intracellular pathogens by activating macrophages, enhancing antigen presentation and inducing T cell differentiation (Janeway *et al.*, 2005). In contrast, Th2 cytokines like IL-4, IL-5, IL-10 and IL-13 activate B cells and thus coordinate immunity against extracellular pathogens through antibody production. Recently, Th1 (Type 1 T helper cells) responses

mediated by IFN- γ have been shown functionally in rainbow trout *O. mykiss* (Zou *et al.*, 2005). Infection by gyrodactylids induces the localised expression of the proinflammatory cytokine IL-1, which initiates and drives subsequent protective responses (Buchmann, 1999). Indeed, IL-1 β is expressed in rainbow trout skin a few days following infection by *G. derjavini* (Lindenstrøm *et al.*, 2003). IL-1 β induces mucus secretion, but this response is arrested in resistant fish within a few days through the expression of the IL-1 β decoy receptor (Lindenstrøm *et al.*, 2003); susceptible fish continue to overexpress IL-1 β . Once the initial phase of protection abated, the expression of a set of cytokines, such as TNF- α 1, TNF- α 2 and transforming growth factor-beta (TGF- β) elicits the subsequent –as of yet undescribed— responses (Lindenstrøm *et al.*, 2004). Similarly, it has been shown to invoke IL-1 β expression in Atlantic salmon *Salmo salar* after attachment of *G. salaris*; and that susceptible fish express this cytokine for a longer period than resistant fish (Lindenstrøm *et al.*, 2006). Thus, effective responses against gyrodactylids seem to be of a cellular, Th1 type. No data are available on specific cellular responses against polyopisthocotyleans, but it is possible that these occur.

9.5. Specific humoral defences

It involves the production of antibodies, which can be released into the blood, the gut mucosa or the skin mucus. Antibodies have been detected in natural infections of blood-feeding monogeneans such as *Heterobothrium okamotoi* (Wang *et al.*, 1997), *Pseudodactylogyrus* (Monni and Cognetti-Varriale, 2002). An antibody as a communication tool and protection measure has been reported in various fishes by various authors' Wang *et al.* (1997).

10. PATHOGENICITY

10.1. Polyopisthocotyleas Feeding Activity

Direct blood feeding by polyopisthocotyleas can result in anaemia. Higher the infection intensity, the higher the loss of blood. Erythrocyte counts, haematocrit values, haemoglobin content and mean corpuscular measures of blood from parasitized fish were lower than in normal fish. The condition is associated with lethargy, anorexia, dark skin colour and paleness of muscle, gill, kidney and liver of infected fish. Lower levels of protein in serum and reduced enzyme activity (lactate dehydrogenase, alkaline phosphatase *etc.*). Higher levels of urea and creatinine. Pathogenicity of sanguivorous *Neobenedenia hirame* on olive flounder is associated with anaemia. However, mortality is further increased by concomitant infection with marine form of VHS (Viral hemorrhagic septicemia). The viral infection alone did not produce significant mortality but the effect of monogenean infection made the host increasingly susceptible to these viral pathogens.

10.2. Monopisthocotyleas Feeding Activity

Browsing of epithelia by monopisthocotyleas elicit another type of pathological disturbance. This depends on the size and number of monogeneans. High densities of *G. salaris* and *G. derjavini* severely injure epithelia on fins. However, large capsalids may produce large feeding wounds with heavy erosion of epidermal layers on yellowtail. The tissue ingested by the monopisthocotyleas comprises epithelial cells, mucus and blood (if haemorrhages occur). This results in severe injury to the host, besides other pathological reactions. (See figure no. 5) Extensive hyperplasia and tissue reactions at the site of attachment are also associated with smaller monogeneans. These host reactions will impair the normal physiological function of the organ. Gill tissues develop into club-shaped filaments with fused lamellae or extensive outgrowths, partly embedding the invading parasite. This decreases in the respiratory surface of gills will lead to mortality due to hypoxia.

10.3. Effect of gland secretion

The enzymes, such as proteases, phosphatases and others in the pharynx, oesophagus and intestinal caeca, associated with digestion may affect the host epithelia and provoke reactions. Gland secretion from cephalic glands involved in attachment of the prohaptor and gland secretions from opisthaptor glands may all have effects on host tissue. Although monogeneans inflict extensive pathogenic effects on fish, the host reactions to eliminate them are only partly expressed. This indicates that the parasites are able to produce immune-evading or immuno-suppressive substances.

10.4. Immune evasion strategies

The parasites have complex life cycles and many undergo extensive migrations within or outside of the host body – both of which also act to subvert or lessen the toxic effects of immune responses to invasion. Immune evasion strategies used by fish monogeneans like invasion of immunologically privileged sites; encystment; adsorption of host proteins on the parasite surface, high surface membrane turnover. The parasite can survive essentially undamaged throughout the life of the fish (Hoole and Arme, 1982). This may be a function of the leucocytotoxic and proteinase inhibitory activities on lymphocyte proliferation identified (Taylor and Hoole, 1994).

10.5. Prevention and Control

According to Noga (2011) in culture: oxidizers (hydrogen peroxide, sodium per carbonate) they destroy worm surface. Organophosphate inhibit acetyl cholinestrases in nervous system leading to paralysis (dichlorovos -1 ppm). Benzimidazoles bind to tubulin monomer and prevent microtubule assembly in worm cells. Egg and oncomiracidia-filter by nylon mesh with pore size less than 80 micro meters.

Biological control Using cleaner fish – (control of *Macroglyrodactylus polypteri*, infecting African lung fish using tilapia: control of *psuedodactylogyrous* using cyclopoid copepods). Chemical control: copper sulphate, sodium chloride, hydrogen peroxide. Use of anthelmintics: benzimidazole, praziquantal, Vaccination.

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