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6 Fish host-monogenean parasite interactions, with special reference to Polyopisthocotylea

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Abstract

Teleost fishes possess immune systems capable of mounting humoral and cellular responses against pathogens, both specific and non-specific. Monogenea (Platyhelminthes) are common ectoparasites of fish, and can have significant pathogenic effects on hosts, particularly confined animals. There are two types of monogeneans, Monopisthocotylea and Polyopisthocotylea, which differ in their biology and effects on the host. Several reviews have summarised the information available on the interaction between fish hosts and monopisthocotylean monogenean parasites; this review focuses on case studies involving polyopisthocotyleans

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(species of the genera Discocotyle, Heterobothrium and Neoheterobothrium) known to cause pathology/mortality in wild and/or farmed fishes, for which laboratory-based studies on host-parasite interactions are available. Known immune responses (humoral and cellular; innate and acquired) elicited against polyopisthocotyleans are reviewed; and contrasted to defence mechanisms against monopisthocotyleans.

Introduction

The fishes are the most numerous and diverse of the major vertebrate groups; a recent compilation of fish species lists 23'250 species with valid descriptions (1). The total number of described fish helminths exceeds far more than 30'000 species (2). Platyhelminths include trematodes, cestodes and monogeneans, all of which possess a dorsoventrally flattened body. Worms of the class Monogenea are important and numerous ectoparasites of fish which exhibit a relatively high degree of host specificity, with most fish species being infected by one or more specific parasites (2). This would lead to the prediction that there are well over 23'250 monogenean species; however, less than 4'000 species have been described (3). Monogeneans have been responsible for important epizootics with serious consequences, particularly in farmed fishes (4, 5).

Monogenea

Monogenea (Platyhelminthes) are mostly skin and gill parasites of marine and freshwater fishes. A few parasitize cephalopods, amphibians, chelonians and one species, *Oculotrema hippopotami*, is found on the eyes of the hippo. Their life cycle involves only one host and they mostly spread by way of eggs and free-swimming infective larvae (oncomiracidia). As opposed to most monogeneans, worms of the genus *Gyrodactylus* are viviparous. Thus, gyrodactylid transmission primarily relies on host to host contact (6), although parasites may also invade new hosts by drifting with water currents or clinging to the surface of the water (7), and can remain viable and infective on dead hosts for some days (8). One consequence of this simple, one-host life cycle is that monogeneans multiply readily in man-made environments, like aquaria and fish farms, sometimes overwhelming and killing their hosts (5, 9). Most monogeneans are small, ranging in size from about 0.3 to 20 mm (10). The anterior end of the body, called the prohaptor, has various feeding and adhesive structures. In some species, the prohaptor has a series of glands that secrete adhesive compounds (11). The mouth is linked to a muscular pharynx and is located in the ventral, cephalic portion of the body. All monogeneans possess a posterior attachment organ, called the opisthaptor or haptor. This organ is equipped with varying numbers and types of accessory hooks or anchors

Figure 1. Ventral view of the haptor of the monopisthocotylean monogenean *Capsala* sp. from the gills of *Euthynnus affinis*. Micrograph kindly provided by Ian Whittington.

Figure 2. Ventral view of the haptor of the monopisthocotylean monogenean *Gyrodactylus* sp. Micrograph kindly provided by Kurt Buchmann and José Bresciani.

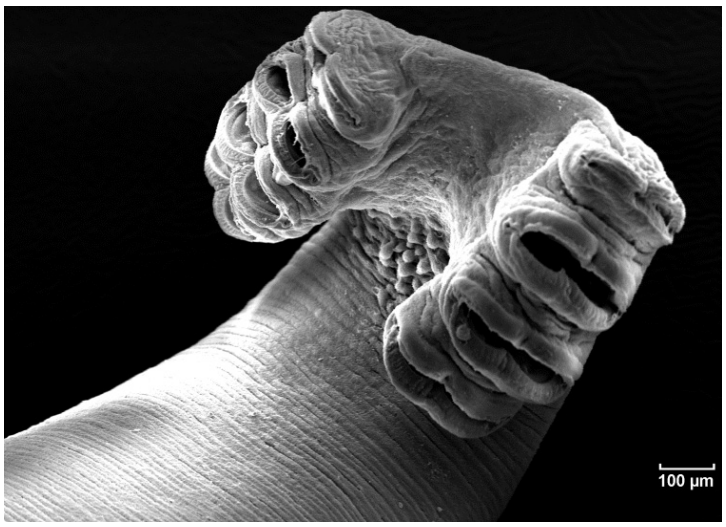

Figure 3. Ventral view of the haptor of the polyopisthocotylean monogenean *Discocotyle sagittata*. Micrograph by the author in collaboration with Robert Porter.

(hamuli), hooklets, and suckers or clamps. The structure of the haptor has been used for the subdivision of the Monogenea into two main clades (3): the Monopisthocotylea and the Polyopisthocotylea (with few exceptions, these groups correspond, respectively, to Polyonchoinea and Oligonchoinea, a further classification). Figures 1-3 show representative haptors of two monopisthocotylean monogeneans (*Capsala*; *Gyrodactylus*) and of a polyopisthocotylean monogenean (*Discocotyle sagittata*).

Monopisthocotylea and Polyopisthocotylea differ in their biology. Monopisthocotyleans primarily feed by grazing on host epithelia (skin, fins and gills) and mucus, and are mobile over the host's surface; worms move in a leech-like manner, securing attachment through the hooks and adhesive secretions of the prohaptor while the opisthaptor is repositioned. Polyopisthocotyleans are blood-feeders and are much less motile, generally infecting the gills, and the branchial and buccal cavities. Both monopisthocotyleans and polyopisthocotyleans exhibit preference for attachment to particular sites on the host (12), which indicates recognition by the parasite of variation in microhabitat conditions. For instance, most oncomiracidia of the monopisthocotylean *Entobdella soleae* invade the upper surface of the common sole *Solea solea*, but adults live on the lower surface (13). For polyopisthocotyleans, there is comprehensive documentation of species-specific differences in distribution on the host respiratory apparatus with respect to gill arch, primary and secondary gill lamellae, etc. Examples include *Diclidophora merlangi*, occuring most frequently on the second gill arch of whiting *Gadus merlangus*, *D. luscae* primarily present on the second and third gill arches of pouting *G. luscus* (14); *Diplozoon paradoxum*, favouring the first 2 gill arches of the roach *Rutilus*

rutilus (15); and several *Demidospermus* spp. and *Scleroductus yuncesi* commonly found on the second gill arch of catfish *Pimelodus maculatus* (16). Occurrence on particular gill regions has been interpreted in relation to differences in the force of water passing over the gill apparatus (14, 17) or in the surface of different gill arches (17) although it may also result from movement of parasites to species-specific mating areas ("rendez-vous") facilitating cross-fertilisation (18, 19). In the case of polyopisthocotyleans with very complex attachment apparatus, it has often been assumed (especially in the older literature based primarily on "snapshots" of parasite distribution on wild fish) that these monogeneans are relatively immobile on the gills. However, studies on cohorts of the polyopisthocotylean *Discocotyle sagittata* examined at intervals during development on experimentally-infected rainbow trout *Oncorhynchus mykiss* show that site specificity may change during the course of an infection, and that site selection is achieved by post-invasion migration (20).

The following sections briefly review host-parasite interactions involving monopisthocotyleans from the best-studied genera (*Gyrodactylus* and *Neobenedenia*), and those involving polyopisthocotyleans from the genera *Discocotyle, Heterobothrium,* and *Neoheterobothrium*.

Host pathology / mortality

The differences between monopisthocotyleans and polyopisthocotyleans are reflected in the type of pathological damage inflicted on their hosts. In many cases, host mortality may not be directly caused by the parasite, but by secondary infections associated with or facilitated by monogeneans; this is particularly the case for Monopisthocotylea, whose feeding on host epidermis and mucus erodes protective mechanisms (4, 21, 22); sometimes the epithelium can be eaten away to the bone (2). The feeding activity of skin-dwelling monogeneans like *Gyrodactylus* spp. causes disseminated thinning of the epidermis, vacuolar degeneration and infiltration of mononuclear cells; when *Gyrodactylus* invades the gills, hypertrophy and fusion of secondary lamellae of gill filaments results (23). Further damage occurs by the insertion of marginal hooklets into the epithelium, which leave numerous minute holes (21, 22). These perforations facilitate bacterial and other opportunistic infections (2) and can result in kidney damage, probably subsequent to systemic osmoregulatory problems due to the breached epidermis (23).

Within the Monopisthocotylea, members of three superfamilies have been implicated in causing disease and mortality of fishes (4): the superfamilies Gyrodactyloidea, Dactylogyroidea, and Capsaloidea. These parasites all have a wide geographical range and are pathogenic to a wide phylogenetic range of hosts. Perhaps the most infamous gyrodactylid is *Gyrodactylus salaris*, responsible for major epizootics on wild Atlantic salmon *Salmo salar* in Norway

since 1975, following introduction from the Baltic Sea. Despite the extreme measures taken to control this pathogen, it continues to devastate Norwegian *S. salar* populations (8). In general, Norwegian salmon stocks lack a *G. salaris*-regulating response, while it is not considered a pathogen in the Baltic Sea where fish stocks are resistant. However, there is a growing awareness that not all Baltic salmon may be resistant to Norwegian *G. salaris*, and that Norwegian and Baltic *G. salaris* strains may differ in virulence (24, 25).

Pathogenic species within the superfamily Dactylogyroidea belong to four families: the Dactylogyridae, the Tetraonchidea, the Ancyrocephalidae and the Diplectanidae. For instance, several species of *Dactylogyrus* and *Cichlidogyrus* have been reported as pathogenic to carp and tilapia in Africa (26); and eels in European farms are highly susceptible to both *Pseudodactylogyrus anguillae* and *P. bini* (27).

Members of the superfamily Capsaloidea affecting cultured marine fishes include species from the genera *Benedenia* and *Neobenedenia*: these parasites are a threat to mariculture because of their low host specificity, wide distribution and ability to cause mortality due to heavy infection (28). Examples include *B. seriolae* infecting several *Seriola* species, such as yellowtail *S. quinqueradiata*, amberjack *S. dumerili*, and kingfish *S. lalandi* (29); and *N. girellae* infecting amberjack, Japanese flounder *Paralichthys olivaceus*, tiger puffer *Takifugu rubripes* (28), tilapia *Oreochromis mossambicus* (30), barramundi *Lates calcarifer* (31), and cobia *Rachycentron canadum* (32).

Most polyopisthocotyleans are not overtly pathogenic. This might to some extent be accounted for by the fact that, in contrast to monopisthocotyleans that rely partially on anchors or marginal hooks for attachment and tend to pierce host tissues, polyopisthocotyleans have clamps that actually grasp the host tissue. However, due to their sanguinivorous feeding habit, polyopisthocotyleans can induce anaemia and potentially host mortality. Documented examples of pathology caused by Polyopisthocotylea include *Axine heterocerca* killing sea cage-cultured *Siganus* sp. (33), *Heteraxine heterocerca* affecting *Siganus quinqueradiata* (4), *Microcotyle sebastis* damaging the rockfishes *Sebastes melanops* (4) and *S. schlegeli* (34), and *Zeuxapta seriolae* killing amberjacks *Seriola dumerili* (35). In a few cases, the probable involvement of the parasite in inducing host anaemia has been shown. Thus, significant negative correlations have been found between worm burdens and host haematocrit in *Siganus* sp. infected with *Allobivagina* sp. (36), Japanese flounder *Paralichthys olivaceus* infected with *Neoheterobothrium hirame* (37), and rainbow trout *O. mykiss* infected with *D. sagittata* (38). Moreover, negative correlations between parasite burdens and host body condition were found in *D. sagittata* (39) and *N. hirame* infections (40). In addition to anaemia, *D. sagittata* and other polyopisthocotyleans have been reported to induce serious gill damage (33). The host response in infected gills includes increased mucus production, epithelial hyperplasia, loss of

lamellar structure, clubbing or fusion of gill filaments, haemorrhage, aneurysms and secondary invasion by bacteria or fungi; these pathological changes lead to a reduction or total cessation of gas exchange (2).

Fish host immunity

Teleost fishes have relatively well-developed immune systems, which have been described in great detail (41-43). Piscine immune defences have several similarities with the more characterised defence systems of higher vertebrates (44). Thus, fish immunity comprises innate and acquired responses, which involve cellular and humoral effectors. Non-specific cellular responses involve leucocytes, which are not limited to inflammation and phagocytosis but also produce reactive oxygen and nitrogen species, and release reactive mediators upon antigenic stimulation. Non-specific humoral defences include lysozyme, complement, interferon, C-reactive protein, transferrin, lectin and a series of other substances. Specific cellular defences are mediated by lymphocytes bearing major histocompatibility complex (MHC) antigens, which communicate by means of cytokines. Specific humoral defences involve the production of antibodies, which can be released into the blood, the gut mucosa or the skin mucus.

Several studies have shown that teleosts are capable of activating immune defences to prevent infection or to combat established pathogens (45-48); these include monogeneans, particularly monopisthocotyleans. Early work demonstrated that extensive proliferation and hyperplasia of gill epithelia reduced parasite burdens following infection by *Dactylogyrus vastator* in carp (49) and by *D. macracanthus* in tench (50); and that several marine fishes mounted protective responses against *Neobenedenia (Epibdella) melleni* (51, 52). As recently reviewed by Buchmann *et al*. (9, 53), later work has confirmed the ability of various fishes to respond immunologically to monopisthocotyleans: examples include common carp combating infections by *Dactylogyrus vastator* (54), *D. minutus* and *D. extensus* (55); European eel *Anguilla anguilla* reacting against *Pseudodactylogyrus* spp. (27); and Japanese flounder against *Neobenedenia girellae* (56). Several host species mount effective responses against gyrodactylids, and some of the immune mechanisms and effectors mediating protection have been well documented (9, 21, 45, 53). Therefore, this review will summarize the available information on host responses against polyopisthocotyleans; and draw comparisons to known immune mechanisms against monopisthocotyleans, establishing commonalities and differences.

Innate immune responses

Complement

The probable involvement of innate humoral components present in fish mucus (complement, lysozyme, lectins, C-reactive protein and haemolysins (41)) in

controlling monogenean infections was recognised early on: the monopisthocotylean *N. melleni* survived longer *in vitro* in mucus from susceptible hosts or in sea water than in mucus from immunized susceptible hosts or non-susceptible fish species (57). Similarly, oncomiracidia of the polyopisthocotylean *D. sagittata* survived longer when incubated in naïve plasma and immune sera from rainbow trout, *O. mykiss* than from brown trout, *Salmo trutta* (58). The killing of infective stages was mediated via the alternative pathway of complement. Oncomiracidia exposed to brown trout plasma exhibited more extensive structural damage than

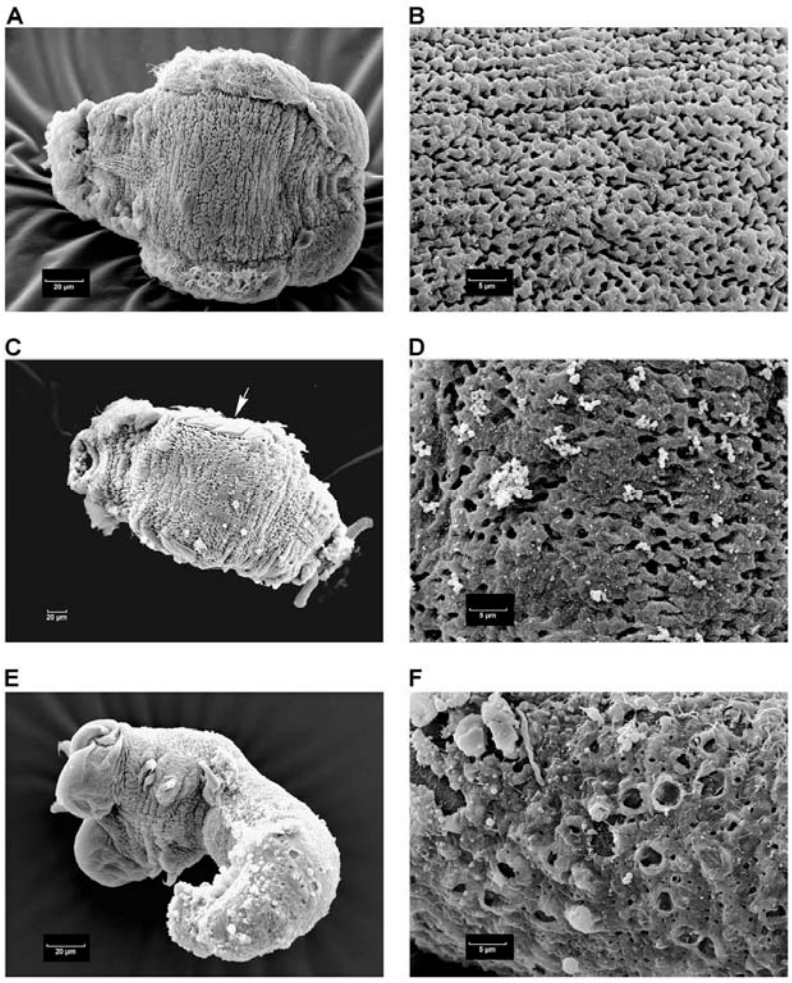

Figure 4. Scanning electron micrographs of *Discocotyle sagittata* oncomiracidia incubated for 1 h at 4°C in the presence or absence of fish plasma at 1:25 dilution. (A) Control oncomiracidium incubated in PBS only, and (B) regular aspect of its tegument at higher magnification; (C) oncomiracidium incubated in rainbow trout (*Oncorhynchus mykiss*) plasma (note arrow pointing to cells which have shed their cilia), and (D) higher magnification showing tegument irregularities; (E) oncomiracidium incubated in brown trout (*Salmo trutta*) plasma, and (F) close-up showing tegument disruption. Micrograph reproduced with permission, Fish & Shellfish Immunology.

parasites incubated in rainbow trout plasma (FIGURE 4). The different killing ability of complement from the two salmonid species may partially account for the observation in UK farms that native brown trout consistently harbour lower parasite burdens than introduced rainbow trout (39). Controlled infection of rainbow trout and brown trout demonstrated that the former is more susceptible to infection by *D. sagittata* (59). The contrasting susceptibility of these 2 salmonid species to *D. sagittata* may illustrate different levels of coevolution of hosts with the parasites encountered in their environment.

The *in vitro* ability of fish serum to lyse parasites via the alternative pathway of complement has been demonstrated in a number of systems: examples include monopisthocotylean monogeneans of the genus *Gyrodactylus* (60, 61), digeneans (62), kinetoplastids (63), ciliates (64), and myxozoans (65). Fish resistant to *Gyrodactylus* spp. have been shown to have higher complement titres than susceptible fish (6). Moreover, *G. derjavini* infection of rainbow trout has been shown to induce the expression of the pro-inflammatory cytokine interleukin-1 beta (IL-1β) in the skin of rainbow trout (66). IL-1β is a mucus secretagogue and gyrodactylid infection is known to induce increased mucus production (4, 21). Similarly, increased mucus production was observed in the gills of pompano *Trachinotus marginatus* infected by the polyopisthocotylean *Bicotylophora trachinoti* (67). The protective role of mucus production is suggested by an association between reduced mucus cell discharge and reduced host resistance to *Gyrodactylus* infection (68), as well as by the migration of parasites to body parts with lower density of mucus cells as the infection progresses (69) (FIGURE 5). The fact

Figure 5. Two *Gyrodactylus derjavini* on the cornea of a rainbow trout (*Oncorhynchus mykiss*). Micrograph kindly provided by Kurt Buchmann and José Bresciani.

that skin monogeneans move away from areas of local immune responses raises the possibility that gill flukes may also migrate during the course of infection in response to developing local reactions or to habitat damage.

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 (53). Infiltrated leucocytes secrete cytokines and other immunoactive compounds, which have several effects, such as induction of cellular migration to the inflammatory site; regulation of mucus production in goblet cells; and induction of macrophage respiratory burst activity, among others (70). Infection by monogeneans can induce an inflammatory reaction, as is the case for the polyopisthocotylean *H. okamotoi,* which first infects the gill arches of the tiger puffer *T. rubripes,* then matures in the wall of the branchial cavity (71); no apparent host response occurs in the gills, but the posterior body of the parasite is covered by inflammatory tissue in the branchial cavity wall (72). Similarly, adult *N. hirame* attach to the branchial cavity wall and induce a strong inflammatory response that encapsulates the haptor (73). It has been proposed that haptor encapsulation is the first step in the process of parasite elimination (74). This might be the case for tiger puffer persistently infected with *H. okamotoi*, which can develop partial immunity following the inflammatory process in the branchial cavity (75). However, a few monogeneans have turned the host inflammatory response to their advantage. Provided that encapsulation does not lead to elimination nor interferes with reproduction, it reduces the energetic expenditure of the parasite by providing secure attachment at no expense to the worm. Thus, at an advanced stage of attachment, *N. hirame* no longer grasp host tissue with their clamps, but are held in place by inflammatory and partially necrotic host tissues (73). Over evolutionary time, this energy-saving mode of attachment has been proposed to lead to a reduction in the size of the haptor and its armature, as seen in the monopisthocotylean *Ancylodiscoides parasiluri* and the polyopisthocotyleans *Callorhynchicola multitesticulatus* and *Heterobothrium elongatum,* all of which are fastened by host inflammatory tissue (76).

A further example of host non-specific responses that are utilized by monogenean parasites is provided by *Gyrodactylus* infecting salmonids. Resistant fish initially respond to parasite invasion by discharging the contents of mucus cells and producing IL-1β, a proinflammatory cytokine and mucus secretagogue (77, 78). However, IL-1β production decreases a few days post-invasion, which moderates mucus cell proliferation and creates a microhabitat poor in goblet cells. In contrast, in highly susceptible fish, persistent overexpression of IL-1β and goblet cell hyperplasia have been described.

Lindenstrøm *et al*. (78) suggested that the continually-produced and secreted mucus facilitates gyrodactylid proliferation, as it serves as chemoattractant and food source. Thus, effective immunity to *Gyrodactylus* is characterized by a biphasic response: the initial inflammatory response is quenched and gives way to other, as of yet uncharacterised, protective mechanisms. A similar biphasic response has been described in pompano *Trachinotus marginatus* infected with the polyopisthocotylean *Bicotylophora trachinoti* (67). In resistant fish, parasite abundance and phagocytosis by spleen and head-kidney cells increased early after infestation, but later phagocytosis became depressed and parasite burdens decreased.

Specific humoral responses

Detectable levels of specific antibodies have been documented following natural infection with some Monogenea. Examples involving Monopisthocotylea include carp producing immunoglobulin against *D. vastator* and *D. extensus* (79), and eels against *P. bini* and *P. anguillae* (27, 80). Humoral responses against Polyopisthocotylea include tiger puffer producing antibodies against *H. okamotoi* (81) and rainbow trout against *D. sagittata* (82). It could be argued that the sanguinivorous habit of polyopisthocotyleans would favour the development of systemic defences, because parasites are in direct contact with the host's blood. However, no antibodies could be found in spot croacker *Leiostomus xanthurus* infected with the polyopisthocotylean *Heteraxinoides xanthophilis* (83); and it is noteworthy that in tiger puffer antibody production against *H. okamotoi* only starts following the inflammatory processes elicited by mature worms established in the branchial cavity, but not while the parasites are attached to and feeding from the gills (75). Similarly, no antibodies were detected in fish harbouring monopisthocotyleans, like Japanese flounder infested with *N. girellae* (56), or rainbow trout infected with *G. derjavini* (60).

There is some evidence that immunoglobulin can mediate partial protection against polyopisthocotyleans. For instance, immunization of rainbow trout against *D. sagittata* resulted in reduced burdens following controlled infection; and a significant negative correlation was found in vaccinated fish between antibody titres and parasite intensity (84). A second instance is that of tiger puffer persistently infected with *H. okamotoi* (75). Fish with infections lasting >1 year remained infected but apparently controlled parasite burdens at low levels despite continuous exposure to infective stages. In contrast, naïve fish held in the same tanks developed significantly heavier infestations. Persistently-infected fish had significantly higher antibody titres than naïve fish throughout the period studied (70 days). In both cases, it would be interesting to determine what parasite structure(s) the antibodies mediating protection bind to; a possibility would be that immunoglobulin contained in a blood meal attached to

components of the digestive system and interfered with food uptake. Even if antibody titres were not determined, it is probable that the effective immunization of rockfish *Sebastes schlegeli* against the polyopisthocotylean *Microcotyle sebastis* induced the production of specific immunoglobulin (85).

Although antibodies might mediate protection against monogeneans, effective immunity apparently results from a combination of factors. This would account for the observation that vaccination of fishes has so far only induced partial immunity; for the persistence of infection in fish with high antiparasite immunoglobulin titres, as found for rainbow trout *O. mykiss* and brown trout *S. trutta* naturally-infected with *D. sagittata* (82); and also for the fact that partial immunity can develop independently of antibodies following primary infection. Examples include Japanese flounder acquiring partial protection to *N. girellae* (56) and rainbow trout to *D. sagittata* (38).

Specific cellular responses

Even though no studies have demonstrated the occurrence of specific cell-mediated immune responses in fish against monogeneans, it is probable that these occur. Typical cell-mediated responses have been documented in fish, like graft rejection, specific cytotoxicity, lymphocyte proliferation in response to mitogenic stimulation, and delayed hypersensitivity (86). Interestingly, delayed hypersensitivity has been demonstrated against the protozoan parasites *Cryptobia salmositica* (87) and *Ichthyophthirius multifiliis* (88), and it is conceivable that such a reaction could be induced by monogeneans.

Interactions between immune cells are mediated not only by direct cell-to-cell contact, but also through the release of soluble factors (cytokines). Fish cells release several cytokines analogous to mammalian cytokines (86, 89), and as is the case for higher vertebrates, the different types of immune responses elicited by pathogen infection are activated through differential synthesis of cytokines by activated cell subsets. Generally, 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 (90). In contrast, Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) activate B cells and thus coordinate immunity against extracellular pathogens through antibody production. Recently, Th1 responses mediated by IFN-γ have been shown functionally in rainbow trout *O. mykiss* (91). Buchmann (21) proposed that infection by gyrodactylids induces the localised expression of the pro-inflammatory cytokine IL-1, which initiates and drives subsequent protective responses. Indeed, IL-1β is expressed in rainbow trout skin a few days following infection by *G. derjavini* (66). IL-1β induces mucus secretion, but this response is arrested in resistant fish within a few days through the expression of the IL-1β

decoy receptor (66); susceptible fish continue to overexpress IL-1β. Once the initial phase of protection has subsided, the expression of a suite of cytokines, such as TNF-α1, TNF-α2 and transforming growth factor-beta (TGF-β) elicits the subsequent –as of yet undescribed— responses (92). Similarly, it was shown that attachment of *G. salaris* induces IL-1β expression in Atlantic salmon *S. salar;* and that susceptible fish express this cytokine for a longer period than resistant fish (78). 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.

Conclusion

Teleost fishes have been shown to respond immunologically to infection by monogenean parasites. Responses involve a series of immune effectors, which most likely act in combination. Although monogenean infections may result in host mortality, particularly in farmed and/or confined fish (4), immunity seems to play an important protective role. Several lines of evidence support this. First, differences in susceptibility to infection have been significantly associated to immune parameters, at both individual host and species levels; e.g., specific antibody levels of vaccinated rainbow trout negatively correlated to *D. sagittata* burdens (84); and differences in the susceptibility of 4 salmonids (rainbow trout, brown trout, Conon salmon, Iijoki salmon) to infection with *G. derjavini* were partially related to mucus cell density (93). Second, the importance of immunity in controlling monogeneans is illustrated by the increase in susceptibility to infection following immunosuppressive treatments or stress. For instance, gyrodactylid infection was facilitated by the treatment of fish with steroids (68, 94, 95); and the burdens of *D. sagittata* increased markedly in naturally-resistant farmed brown trout subjected to stress (39), a factor known to affect immune capabilities of fish (96). Finally, comprehensive field data on polystomatids, polyopisthocotylean monogeneans that parasitize amphibians, suggest that host immunity exerts a very strong influence on the parasite populations of wild hosts. Despite very high transmission rates, the prevalence and intensity of adult polystomatids are generally very low (97, 98). Host factors may be involved in the marked attrition of prereproductive worms, as suggested by long-term laboratory experiments demonstrating that primary infection of the clawed toad *Xenopus laevis* with *Protopolystoma xenopodis* can elicit strong, long-term protective immunity against re-infection (99). While primary burdens of *P. xenopodis* showed high prevalence of adult worms, challenge infections resulted in only a few infected hosts harbouring small burdens of parasites exhibiting reduced egg production. The occurrence of long-lasting immunity post-infection would provide an explanation for the low burdens

observed in wild hosts. As opposed to the traditional view of high parasite fecundity as a compensation for losses during transmission, Tinsley (100) has suggested that monogenean reproductive adaptations have their most significant role in countering the additional losses that occur post invasion due to host immunity. Monogenea may additionally have evolved immunoevasive mechanisms to avoid host defences; their sheer specific abundance is a testament of their capacity to cope with host immunity. Further study of host-parasite interactions between hosts and monogeneans will provide more insight into the mechanisms resulting from their co-evolution.

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