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Biology and Mucosal Immunity to Myxozoans

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Abstract

Myxozoans are among the most abundant parasites in nature. Their life cycles involve two hosts: an invertebrate, usually an annelid, and a vertebrate, usually a fish. They affect fish species in their natural habitats but also constitute a menace for fish aquaculture. Using different strategies they are able to parasitize and cause damage in multiple organs, including mucosal tissues, which they use also as portals of entry. In fish, the main mucosal sites include the intestine, skin and gills. Recently the finding of a specific mucosal immunoglobulin in teleost (IgT), analogous to mammalian IgA, and the capacity of fish to develop a specific mucosal immune response against different pathogens, has highlighted the importance of studying immune responses at mucosal sites. In this review, we describe the major biological characteristics of myxozoan parasites and present the data available regarding immune responses for species that infect mucosal sites. As models for mucosal immunity we review the responses to *Enteromyxum spp.* and *Ceratomyxa shasta*, both of which parasitize the intestine. The immune response at the skin and gills is also described, as these mucosal tissues are used by myxozoans as attaching surfaces and portal of entry, and some species also parasitize these sites. Finally, the development of immunoprophylactic strategies is discussed.

Keywords

Myxozoan; fish; intestine; gill; skin; mucosal immune response

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1. Introduction

The phylum Myxozoa is a highly diverse taxon of microscopic endoparasites. While most myxozoans are not highly pathogenic for their fish hosts, some cause severe disease and many others cause economic losses in aquaculture as a result of decreased fish growth, fecundity or flesh quality. The pathogenic potential of many species is unknown, as they infect species that are not currently of economic value and hence receive little attention. Thus, as new fish species are brought into culture we can expect to see new myxozoan diseases emerge. While this review focuses on species that infect mucosal tissues, we include a broader review as most myxozoans use mucosal tissues as portals of entry.

With over 2,000 myxozoan species described (Lom and Dykova, 2006), those affecting fish in freshwater aquaculture are the most studied. Because of the economic importance, long history as cultured species, and now nearly global distribution of salmon and trout, their parasites have received a great deal of attention. Certainly, the best known myxozoan is *Myxobolus cerebralis*, the cause of whirling disease (Hofer, 1903). This parasite invades cartilage tissue in young rainbow trout (*Oncorhynchus mykiss*) causing skeletal deformities and a striking whirling behavior. It has been reported across four continents as a result of the translocation of salmonids, and although no longer a severe problem in culture as a result of improved culture practices, concerns about *M. cerebralis* resurged following its detection in wild rainbow trout populations in North America in the 1980's (Hedrick, 1998).

Tetracapsuloides bryosalmonae, the cause of proliferative kidney disease, also has a broad distribution, infecting wild and cultured salmonids in Europe and North America (Henderson and Okamura, 2004). In contrast, *Ceratomyxa shasta* (Noble, 1950) has remained geographically restricted to the Pacific Northwest of North America despite the movements of its salmonid hosts. This parasite infects the intestinal tissues, and although it can cause problems in culture facilities with unprotected water supplies, it is largely a problem of free-ranging salmonids (Hallet and Bartholomew, 2012). In North America, catfish (*Ictalurus punctatus*) are the most intensively cultured freshwater fish, and infections by *Henneguya ictaluri* (Pote et al., 2000) cause a condition known as “hamburger gill”, as a result of the numerous cysts found in the gills. This myxozoan causes outbreaks with mortality rates exceeding 50% (Pote et al., 2012). Worldwide, carp culture is also plagued by serious myxozoan pathogens, including *Myxobolus koi*, which infects the gills and causes respiratory dysfunction in juvenile koi (*Cyprinus carpio*) (Yokoyama et al., 1997) and *Sphaerospora dykova* (= *S. renicola*), the cause of a serious swimbladder infection in cultured carp (*C. carpio*) (Dykova and Lom, 1988).

Infections in saltwater aquaculture are equally common, but more difficult to control because of the open water nature of most culture facilities, and even less is known about the impact of myxozoans on wild fish populations in oceans. In Atlantic salmon (*Salmo salar*) cultured in Norway, *Parvicapsula pseudobranchiola* infects the pseudobranch, resulting in low-grade to significant mortalities (Karlsbakk et al., 2002). *Kudoa thyrsites* infects the muscle of a variety of marine fishes and causes a post-mortem condition known as “soft flesh” that decreases market value, although during the life of the fish it appears to have little effect. *Enteromyxum leei* and *E. scopthalmi* infect intestinal tissues of a broad range of host species and have caused losses in important Mediterranean aquaculture species. *E.*

leei parasitizes gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and Mediterranean sharpnose sea bream (*Diplodus puntazzo*); while *E. scopthalmi* parasites turbot (*Psetta maxima* or *Scophthalmus maximus*). Infections by these species may result in direct mortality, weight loss, poor food conversion rates, delayed growth and decreased marketability of the fish (Sitjà-Bobadilla and Palenzuela, 2013).

In summary, there are many myxozoan species parasitizing different tissues in a wide variety of fish species. Understanding the host immune responses against these parasites may provide insight into new avenues for developing effective treatments against these myxozoan parasites.

2. Biology of myxozoans

2.1. Classification

First discovered in the early 1800's, the first 150 years of myxozoan research focused on classical descriptions and pathology studies. A seminal advance in our understanding of this group occurred with the elucidation of the first myxozoan life cycle in the early 1980's (Markiw and Wolf, 1983), and with that discovery, the recognition that the two sister classes, Myxosporea and Actinosporea, were actually alternate life stages of single organisms. Thus began major taxonomic revisions of the group, which despite their multicellular nature, continued to be classified as Protozoa. The class Actinosporea was suppressed (Kent et al., 1994), and with the advent of molecular approaches, the metazoan affinity of myxozoans was confirmed (Smothers et al., 1994). Subsequent analyses have linked this group to cnidarians (Jiménez-Guri et al., 2007), with Medusozoa being their closest relative (Nesnidal et al., 2013). As it stands, the phylum is comprised of two classes: the Myxosporea (Bütschli, 1882) being the larger, more diverse group containing over 2000 species, and the Malacosporea (Canning et al., 2000), with only four described species that are assigned to the genera *Buddenbrockia* and *Tetracapsuloides*. Although the majority of myxozoans utilize fish as their vertebrate host, myxosporeans also infect amphibians, reptiles, birds and mammals (moles and shrews) (Reviewed by Lom and Dykova, 2006; Yokoyama et al., 2012). Malacosporeans infect byozoans, with one species, *T. bryosalmonae*, also infecting fish.

2.2. Life cycle and development

Although 30 years have passed since the first life cycle description, very few life cycles have been resolved (Yokoyama et al., 2012). Of these, most require an invertebrate host: annelids (primarily oligochaetes and polychaetes) for Myxosporea; bryozoans for Malacosporea. Myxozoans develop as two distinct spore phenotypes in their different hosts (see review by Lom and Dykova, 2006). Myxosporea develop in their invertebrate hosts into delicate, tri-radially symmetric actinospores, that are highly variable in size (5-500 µm) and number of infectious cells (2 to >100); in vertebrates they develop into bilaterally symmetric myxospores with hard shell valves, and are relatively smaller (5-20 µm) with 1 to 2 infectious sporoplasms. Both stages have the characteristic cnidarian polar capsules, the number of which differs between life stages, with actinospores having 1 or 3 (usually 3), and myxospores 1-10 (usually 2). Fewer malacosporean life cycles have been described, but at

least one species, *T. bryosalmonae*, has a small ($12 \times 7 \mu\text{m}$), myxospore-like “malacospore” stage in the fish host, and 2 polar capsules. In the bryozoan host, however, the parasite exists as polysporous sac-like or vermiform stages (Lom and Dykova, 2006), and spores are similar in size to the fish malacospores but have 4 polar capsules. Direct transmission between fish has been reported for a few marine species of the genus *Enteromyxum* (Diamant, 1997; Redondo et al., 2002; Yasuda et al., 2002), but the possibility that they additionally utilize an invertebrate host has not been dismissed.

2.2.1. Invasion of the fish host—The life cycle of *M. cerebralis* was the first to be established in the laboratory and has served as a model myxosporean life cycle (Figure 1). As the actinospore is released from the annelid host its caudal appendages inflate, facilitating flotation and encounter with the fish host. Actinospores attach to the fish by firing their polar filaments, allowing the vegetative sporoplasm to penetrate (El-Matbouli et al., 1995). Portals of entry for *M. cerebralis* include the skin, fins, buccal cavity, digestive tract and gills (Antonio et al., 1998; El-Matbouli et al., 1999; Kallert et al., 2009; Markiw, 1989). Mucosal tissues also represent a primary invasion route for other myxozoans (Table 1). Invasion by multiple routes, including skin, buccal cavity, stomach and intestine, has been observed for *H. ictaluri* (Belem and Pote, 2001) and *T. bryosalmonae* can invade through mucus cells in the skin (Longshaw et al., 2002) and gills (Grabner and El-Matbouli, 2010; Morris et al., 2000). The gills are portals for *C. shasta* (Bjork and Bartholomew, 2010) and *Sphaerospora truttae* (Holzer et al., 2003). The intestinal myxozoan *E. scopthalmi* can reach the intestine directly by ingestion, a route facilitated by intensive culture systems, but has been also detected in the blood, which suggests entry by similar routes as other myxozoans (Redondo et al., 2004). Thus, it is likely that myxozoans take advantage of multiple epithelial invasion routes.

2.2.2. A model for the infection process—Attachment of *M. cerebralis* to the fish occurs when a combination of chemical (primarily mucus-derived nucleosides) and mechanical cues signal the discharge of polar filaments of the infectious actinospore (triacinomyxon). During the invasion process, the three polar filaments anchor the parasite to the fish, facilitating the emergence of the ameboid sporoplasm from between the valve shells and its entry into the fish through the secretion opening of a mucus pore (El-Matbouli et al., 1999; Kallert et al., 2007). These initial steps are not host specific, and may occur on contact with any fish species (Kallert et al., 2009), but successful infection only occurs in salmonids. Once in the fish, the sporoplasm begins an asexual replicative phase (presporogonic phase) and at the same time begins to migrate through the epidermis and peripheral nerves to the cartilage (El-Matbouli et al., 1995; El-Matbouli et al., 1999). At the final infection site the parasite engulfs and digests chondrocytes, destroying the structural framework and resulting in skeletal deformities. The parasite forms plasmodia, and begins spore development. Invasion processes of other myxozoans have also been examined, but in less detail: *T. bryosalmonae* was detected in the skin of rainbow trout 1 min post-exposure (Longshaw et al., 2002) and *C. shasta* actinospores attach to the fish gill and the sporoplasm penetrates between epithelial cells (Bjork and Bartholomew, 2010).

2.2.3. Migration and tissue localization in the fish host—Myxozoans have evolved to take advantage of nearly every niche in their hosts, and consequently have a variety of migration paths and development pathways. Some species remain localized at their site of entry in the skin or scales; others migrate and become coelozoic (inhabiting body cavities) or histozoic (occupying tissues). Migration pathways in the host have been described for only a few species, but the migration of *M. cerebralis* along the nerves to the vertebral cartilage (El-Matbouli et al., 1995) suggests a route for species that end up in immunoprivileged sites like the brain and nervous tissue. Blood stages are recognized for a number of species that infect gills and visceral organs, for example *T. bryosalmonae* (Kent and Hedrick, 1985), *S. truttae* (McGeorge et al., 1996), *H. ictaluri* (Belem and Pote, 2001) and *C. shasta* (Bjork and Bartholomew, 2010) (Figure 2A), which suggests that the circulatory system is a primary migration route.

Developmental strategies are also varied. The sporogonic stage of most species develops extracellularly, either as large plasmodia, with numerous spores, or as pseudoplasmodia, which form mono or di-sporoblasts (Lom and Dykova, 2006). Extrasporogonic development often occurs at sites other than the tissue where spore formation occurs. *M. cerebralis* develops intraepithelially in the dermis before migrating along nerve cells to the cartilage (El-Matbouli et al., 1995). *C. shasta* remains sequestered in the gill blood vessel endothelium, where it replicates and releases presporogonic stages into the circulatory system, delivering the parasite to the intestine (Bjork and Bartholomew, 2010), where sporogenesis occurs in small disporic pseudoplasmodia (Bartholomew et al., 1989; Yamamoto and Sanders, 1979) (Figure 2B). Plasmodia that develop within tissues may occur as cysts or, in the case of *H. ictaluri*, as pseudocysts in the gill (Molnar, 2002).

2.2.4. Transmission to the invertebrate host—Release of spore stages from the fish depends on the infection site. For example, species inhabiting kidney tubules are excreted in urine and those in scale pockets may be sloughed from the skin during the life of the fish. However, spores that form in the brain, cartilage, muscle and visceral organs are typically released into the environment after the death of their host. Independent of the mode spores are released, they sink to encounter their benthic invertebrate hosts, or are filtered out of the water column by filter-feeding invertebrate hosts. After ingestion, the myxospores open and release the infective sporoplasm, which then penetrates the intestinal epithelium. Development within invertebrates has fewer permutations because of the simple architecture of the host. Some species continue their development in the intestinal epithelium and are shed with feces. Others migrate from the intestine to begin their replicative phase in the body cavity or body wall, and are released from the host through mucus pores or on death of the annelid (Bartholomew et al., 1997). Developmental phases in the invertebrate host are more uniform, with pansporocysts resulting in development of 4 or 8 actinospores.

2.3. Laboratory models

Experimental transmission models have been difficult to establish for these parasites and this has hampered research on myxozoans. Some life cycles have been inferred from DNA matches of myxospore and actinospore stages without demonstrating transmission and development of the parasite in the subsequent host. This is due to difficulties in obtaining

pathogen-free stocks of both the fish and worm host and because of challenges in maintaining invertebrate hosts under laboratory conditions. Laboratory infection models of only a few species have been established, and most of these are for myxobolid-type myxozoans with tubificid oligochaete invertebrate hosts (e.g. *M. cerebralis* (Wolf and Markiw, 1984), *M. parviformis* (Kallert et al., 2005), and *M. rotundus* (Szekely et al., 2009). Exceptions to this include *C. shasta* with its polychaete host (Bjork and Bartholomew, 2009) and *T. bryosalmonae* with its bryozoan host (Feist et al., 2001; Morris and Adams, 2006). Establishment of these cultures, and of direct transmission models for *E. leei* and *E. scophthalmi* (Diamant, 1997; Redondo et al., 2002), has permitted controlled experiments to determine the effects of parasite dose, timing and temperature, as well as to examine the process of invasion and the host response to that process.

2.4. Pathogenic mechanisms

Invasion mechanisms and virulence factors in myxozoans are poorly understood compared with well-studied bacterial and viral pathogens. Host-parasite interactions are complex and vary depending on myxozoan species, host infection site and environmental factors such as temperature. Many myxozoans, particularly those infecting coelozoic spaces of the gall and urinary bladders, do not elicit a host response. Other species avoid the immune system by infecting immunoprivileged sites, such as the central nervous system (e.g. *M. cerebralis*, *M. fryeri*; Ferguson et al., 2008), eye (e.g. *M. clariae*; Hemananda et al., 2009) and gonads (e.g. *Sphaerospora testicularis*; Sitjà-Bobadilla and Alvarez-Pellitero, 1990). Some species, particularly those infecting muscle, develop intracellularly (Lom and Dykova, 2006). Some cyst-forming species, like *M. insidiosus*, which infects the striated muscle of salmonids, appear to elicit little host reaction (Amandi et al., 1985), while others, like *M. squamalis*, which inhabits the scale pockets of salmonids, cause modification of the epithelium (Iversen, 1954). In general, histozoic species are more pathogenic than coelozoic species.

Examination of factors associated with invasion and pathogenesis has focused primarily on proteases, and myxozoan cysteine and serine protease genes have been identified from several species of *Kudoa* (Funk et al., 2008; Martone et al., 1999) and from *M. cerebralis* (Dörfler and El-Matbouli, 2007; Kelley et al., 2003; Kelley et al., 2004). In other parasites, proteases play a variety of roles, including degradation or activation of host immune molecules, and they may also serve as targets for chemotherapeutants or vaccines. However, studies that would address these functional questions in fish have not yet been conducted. The following sections review known pathogenic mechanisms for myxozoans according to tissue specificity.

2.4.1. Species that infect the intestine—Although a number of myxozoans infect the intestine, only a few preferentially infect this tissue. *E. scophthalmi*, *E. leei* and *C. shasta* are the most pathogenic, resulting in chronic enteritis, which impairs nutrient absorption leading to anorexia, cachexia and eventually to death. However, clinical manifestations of disease vary depending on the host species and susceptibility. Rates of parasite replication and disease progression are temperature-dependent: in infections of *C. shasta*, Chinook salmon held at 13°C have lower and later mortality (~30 days post-exposure (dpe)) compared with fish held at 21°C, which had higher and earlier mortality (within 16 dpe) (Ray et al., 2012).

Despite the importance of these pathogens, there is a paucity of information on their virulence factors. Invasion of *E. scophthalmi* in the intestine of turbot has been shown to involve lectin-carbohydrate interactions, as the process was inhibited by pre-treatments with certain lectins (Redondo and Alvarez-Pellitero, 2010). Proteases are likely a major component of both invasion and proliferation, but challenges in purifying proliferative parasite stages from host tissues make parasite-derived factors difficult to characterize. Indirect evidence of parasite protease involvement during infection was presented in a study that used molecular profiling to examine the response of gilthead sea bream to infection by *E. leei* (Davey et al., 2011). They interpreted the up-regulation of protease inhibitors as an attempt to inhibit parasite-derived proteases, or host proteases released from infected tissues.

2.4.2. Species that infect the skin—Species that infect the skin are generally not serious pathogens, but most myxozoans must cross this barrier (Table 1). There has been some research on pathogenic mechanisms, particularly for *M. cerebralis*, which invades using this route. In two studies that examined the involvement of proteases in the pathology of whirling disease, genes that encoded for a cathepsin Z-like cysteine protease gene (MyxCP-1) and a serine protease (MyxSP-1) were isolated. High levels of transcription of these genes in the dorsal fin and gills of fish at 5 min and 2 h post infection suggest an enzymatic role in host tissue invasion (Kelley et al., 2003; Kelley et al., 2004). Subsequently, an additional serine protease gene (MyxSubtSP) was identified from this parasite (Dörfler and El-Matbouli, 2007).

2.4.3. Species that infect the gills—Parasites infecting the gills can compromise respiration if in sufficient numbers, or may cause damage by inducing a proliferative cellular response or cellular hypertrophy that result in clubbing of the gills and fusion of the lamellae. This becomes so severe in *H. ictaluri* infections that it produces characteristic breaks in the gill cartilage. In contrast to the rapid development of the *C. shasta* myxospore, mature spores of *H. ictaluri* were not seen until 70 dpe, long after the resolution of the inflammatory response (Pote et al., 2012).

2.5. Resistance to myxozoan infections (natural or acquired)

Myxozoans can be generalists in their infection strategies, or highly host specific. Thus resistance can encompass non-permissive hosts, behavioral avoidance (lack of overlap with parasite), as well as evolutionary adaptation. Among species with relatively narrow host ranges, interspecific differences in resistance among salmonids have been well documented for *M. cerebralis* (MacConnell and Vincent, 2002), *C. shasta* (reviewed by Bartholomew, 1998) and *T. bryosalmonae* (Arkush and Hedrick, 1990). Parasites with a broad host range cause a variety of pathologies depending on the host. For *E. leei*, some marine species have been shown refractory to infection (Padrós et al., 2001), while others become infected but do not die or show clinical disease signs (European sea bass) (Sitjà-Bobadilla et al., 2007a). Disease severity and rate of progression differ among those susceptible hosts: Mediterranean sharpnose sea bream (*D. puntazzo*) (Alvarez-Pellitero et al., 2008; Golomazou et al., 2006a), tiger puffer (*Takifugu rubripes*) (Yanagida et al., 2006) and gilthead sea bream (*S. aurata*) disease progresses more slowly (Sitjà-Bobadilla et al., 2006a). Similar differences in disease severity and progression have been demonstrated for

E. scophthalmi, which is highly pathogenic for turbot *S. maximus*, but less so for sole *Solea senegalensis* (Palenzuela et al., 2007).

Of most interest are the intraspecific differences in resistance to infection that point to genetic adaptation of the host. Natural evolution of this adaptation is evident in the relative resistance of salmonids sympatric with *C. shasta* (Bartholomew, 1998). “Human-assisted” evolution of this trait is evidenced by a strain of rainbow trout that has become highly resistant to *M. cerebralis* as a result of prolonged rearing in a hatchery water supply where the parasite was established (Hedrick et al., 2003). Interspecific differences in susceptibility have also been reported among brown trout to infection by *T. bryosalmonae* (Quigley and McArdle, 1998), carp to *Thelohanellus nikolskii* (Molnar, 2002), gilthead sea bream to *E. leei* (Sitjà-Bobadilla et al., 2007b) and turbot to *E. scophthalmi* (Quiroga et al., 2006; Sitjà-Bobadilla et al., 2006b). These intraspecific differences have clear applications for aquaculture, as well as for furthering our understanding of the genetic and immunological mechanisms that contribute to disease resistance.

Mechanisms for genetic resistance to myxozoan infections are only beginning to be examined. Genetic analysis of resistance to *C. shasta* using molecular markers shows the trait is linked to multiple loci, which supports the hypothesis that resistance in this species is polygenic (Nichols et al., 2003). In contrast, a single QTL region explained a large percentage of the phenotypic variation in resistance to *M. cerebralis* (Baerwald et al., 2010). Studies like these are beginning to provide insight into potential pathways that may be differentially regulated in resistant and susceptible fish.

3. Immune responses to myxozoans parasitizing mucosal tissues of fish

Myxozoan parasites can invade different tissues and cavities of fish, including immunoprivileged sites, such as the central nervous system, eye and gonads (Ferguson et al., 2008; Hemananda et al., 2009; Sitjà-Bobadilla and Alvarez-Pellitero, 1990). In these latter cases, if the parasite is not controlled by the immune system at the beginning of the infection process, it arrives at the immunoprivileged site where it becomes inaccessible to the immune system surveillance until the infection has advanced to a stage where it breaks through these barriers. For example, in the case of *M. cerebralis*, the immune response appears to be effective early in the infection, as parasites that do not reach the nerves are eliminated (Hedrick, 1998). Once in the nerves they are protected until they reach the cartilage. As the parasite consumes cartilage there is an inflammatory response (Hedrick, 1998; MacConnell and Vincent, 2002) and development of an antibody responses can take up to 12 weeks after exposure (Ryce, 2003).

Immune responses against these parasites vary depending on the myxozoan species, the target tissue, and the host species. Differences occur also among individuals of the same species, depending on its natural resistance or susceptibility to the parasite. For example, a transcriptome analysis of the responses of gilthead sea bream to *E. leei* showed differences between control, exposed-parasitized, and exposed-non-parasitized fish, as well as between intestine and head kidney tissues. This study also demonstrated a complex interplay between parasite derived elements and host defense elements in the exposed fish (Davey et al., 2011).

Although, innate and adaptive immune responses against myxozoans have been described (reviewed by Alvarez-Pellitero, 2008; Sitjà-Bobadilla, 2008b), mucosal responses to myxozoans have only been reviewed in relation to immunoglobulin and antibody production (Salinas et al., 2011). In the next sections, immune responses related to myxozoan infections of the three major mucosal areas in fish (intestine, gills and skin) are reviewed and compared.

3.1. Immune responses to intestinal parasites

The intestine is the most studied mucosal tissue with respect to immune responses in mammals and also in fish. There are several myxozoan parasites that infect the gut, with some *Enteromyxum spp.* and *C. shasta* being the most studied (Table 2).

3.1.1. *Enteromyxum* spp—*Enteromyxum* infections tend to produce damage to the epithelial tissue which can be correlated with the development of the parasite and the severity and progression of the disease. This can be evidenced, for example, as necrosis, detachment, vacuolization and sloughing of the epithelial intestinal cells (Alvarez-Pellitero et al., 2008). The first immunological barriers: mucus protein composition, epithelial cells and goblet cells of the intestine, are important components in the immune responses of fish against *Enteromyxum spp.* It has been shown that certain terminal carbohydrate residues play a role during the initial contact between parasite and host. (Redondo et al., 2008). The carbohydrate residues glucose-mannose, N-acetyl-glucosamine, N-acetyl-galactosamine and alpha-D-galactose were shown to be the dominant structures in the membrane of *E. scophthalmi* as well as at the interface with its host, turbot. In correlation with the latter, mannose-glucose and N-acetyl-glucosamine residues were detected on the epithelial brush border, rodlet cells and mucus of turbot intestine (Redondo et al., 2008). Similarly, for *E. leei* parasitizing gilthead sea bream, terminal carbohydrates residues containing glucose-mannose and fucose were dominant on the parasite membrane, and at the host-parasite interface (Redondo and Alvarez-Pellitero, 2009). This suggests that the composition of the parasite surface may be very important for initial recognition by the host and for subsequent host-parasite interactions. Related to this, down-regulation in expression of mannose binding lectin genes was evident in infected fish of the same species (Davey et al., 2011), which could indicate that *E. leei* is able to inhibit the recognition mechanism in its host as a strategy for a successful infection. Mucins of gilthead sea bream parasitized with *E. leei* showed lower glycoprotein content and decreased glycosylation in the anterior and middle intestine, while both glycoprotein content and glycosylation, were higher in the posterior intestine compared with control fish. These highly glycosylated mucins resulted in lower adhesion of bacterial pathogens compared with mucins from control fish (Estensoro et al., 2013), demonstrating that the structure and abundance of mucosal proteins change in response to pathogenic infections, perhaps as a strategy to prevent the adhesion of the pathogen to the intestinal surface. Additionally, histological examinations of naturally infected fish showed that the number of goblet cells was significantly decreased in gilthead sea bream heavily infected with *E. leei* presenting severe enteritis (Fleurance et al., 2008), suggesting that mucus production may be affected by the presence of the parasite in the intestinal epithelium, thereby facilitating its establishment on this surface.

Several studies have examined the regulation of innate components like enzymatic activity, complement and cytokines, following *Enteromyxum* spp. infection. For enzymatic activity, the release of nitrogen species, produced by the inducible nitric oxide synthase (iNOS), was evaluated in turbot infected by *E. scophthalmi*. This enzyme is found primarily in phagocytes, but also in structural cells from the intestinal mucosa. Immunohistochemistry studies revealed the presence of iNOS in the apical border of enterocytes and some goblet cells in control, and more clearly in infected fish. Moreover, moderate numbers of iNOS⁺ cells were also located in the lamina propria-submucosa of infected fish presenting moderate and severe inflammatory infiltrates (Losada et al., 2012). These results are in agreement with those obtained for *D. puntazzo* exposed to *E. leei*, as serum nitric oxide levels were gradually increased after infection with the parasite, compared to levels in control fish (Golomazou et al., 2006a). Similarly, respiratory burst, serum lysozyme and antiprotease activity vary between infected and uninfected hosts, showing a similar pattern of response in several studies for diverse host-parasites interactions. In that regard, respiratory burst increased in parasitized fish, as evidenced in Mediterranean sharpsnout and gilthead sea bream exposed to *E. leei* (Alvarez-Pellitero et al., 2008; Sitjà-Bobadilla et al., 2008). Serum lysozyme decreased significantly in gilthead sea bream exposed to *E. leei* (Sitjà-Bobadilla et al., 2008) and in turbot exposed to *E. scophthalmi* (Sitjà-Bobadilla et al., 2006b), while it could not be detected in serum of *D. puntazzo* exposed to *E. leei* (Golomazou et al., 2006a). An increase in total serum antiprotease levels post-infection was registered in turbot exposed to *E. scophthalmi* (Sitjà-Bobadilla et al., 2006b) and in *D. puntazzo* exposed to *E. leei* (Muñoz et al., 2007), while serum antitumor activity was slightly lower in *D. puntazzo* fish exposed to *E. leei* (Muñoz et al., 2007). The only parameter that gave contradictory results was peroxidase activity, which decreased significantly in serum (Sitjà-Bobadilla et al., 2008) and head kidney (Cuesta et al., 2006a) of gilthead sea bream exposed to *E. leei*, yet was higher in serum of infected *D. puntazzo* exposed also to *E. leei* (Muñoz et al., 2007). These results suggest that there is an active innate immunological response against *Enteromyxum* spp., with some components of the response being universal while others varying from one host species to the other.

With regards to complement, it has been shown that alternative complement activity increased in the serum of gilthead sea bream exposed to *E. leei* (Cuesta et al., 2006a), and it was slightly, but not significantly, increased in serum of parasitized fish (Sitjà-Bobadilla et al., 2008). Contrary to this, the expression of complement-related genes was down-regulated in the intestine of exposed fish (Calduch-Giner et al., 2012; Davey et al., 2011). In infections of turbot with *E. scophthalmi*, a drop in serum complement activity was registered at 40 dpe (Sitjà-Bobadilla et al., 2006b). Thus, complement expression and activity may vary between tissues (systemic versus mucosal) and depending on the time after parasite exposure.

Patterns of cytokine expression also change depending on the tissue and time analyzed after infection with *Enteromyxum* spp. Expression of the pro-inflammatory cytokine IL-1 was increased at 10 dpe while expression of TNF decreased at later times post-infection in the head kidney of gilthead sea bream infected by *E. leei* (Cuesta et al., 2006a). Meanwhile in the intestine of the same species, parasitized fish exhibited a down-regulation of IL-1 and TNF at 113 dpe (Sitjà-Bobadilla et al., 2008).

The cellular immune responses to *Enteromyxum spp.* infecting the intestine of fish have also been examined histologically. Analysis of *E. leei* intestinal infections in the Mediterranean sharpnose sea bream showed that initially the host fights the pathogen through an increase in leukocyte numbers, respiratory burst activity, haematopoietic activity and melanomacrophage centers (MMC) (Alvarez-Pellitero et al., 2008). Interestingly, two types of eosinophilic granular cells (EGC) behaved differently in the intestinal epithelium and the lamina propria: EGC1 decreased with the progression of the infection, while EGC2 increased, mainly in the lamina propria. This suggests that diminishing these cellular responses could contribute to the high virulence of *E. leei* in this fish species (Alvarez-Pellitero et al., 2008). Similarly, extensive epithelial vacuolization with accumulation of EGCs and lymphocytic infiltration was detected in the intestinal lamina propria and epithelium of European sea bass, but not of gilthead sea bream infected with *E. leei*, thus demonstrating a different cellular reaction between fish species against the same pathogen (Sitjà-Bobadilla et al., 2007a). Histological examination in sea bream naturally infected by *E. leei* showed an inflammatory cell infiltration and sclerosis of the intestinal lamina propria (Fleurance et al., 2008). A regenerative epithelium was detected in the intestines of some survivor fish that showed severe chronic enteritis but no parasites, suggesting that development of an acquired immune response may help in the elimination of parasites (Fleurance et al., 2008). Similarly, leukocyte infiltration in the intestine of turbot was seen after 20 dpe to *E. scophthalmi*. In these same fish an increase in circulating granulocytes, haematopoietic depletion in head kidney, and an increase in the number of apoptotic cells and MMC was observed. In contrast, a decrease in the percentage of lymphocytes in the head kidney and spleen occurred in the same fish (Sitjà-Bobadilla et al., 2006b). In addition, cellular immune responses to *E. leei* in gilthead sea bream have been studied in head kidney leukocytes, showing that the percentage of acidophilic granulocytes, leukocyte peroxidase content and phagocytic activity decreased significantly at different sampling times in infected fish, whereas cytotoxic activity increased. This suggests that cytotoxic activity could play an important role in the defense against this myxozoan parasite, as it is stimulated after infection (Cuesta et al., 2006b). These results demonstrate that fish respond with a strong cellular response to *Enteromyxum spp.* parasitizing their intestinal mucosal surface, by recruiting leukocytes to the affected mucosal tissue, which is also reflected in a reduction in the number of these cell types in hematopoietic sites such as the head kidney and spleen.

Genes related to the major histocompatibility complex (MHC) also appear to be regulated after exposure to *Enteromyxum spp.* For example, alpha-2 microglobulin (related to MHC-I) was over-expressed in the intestine of turbot parasitized with *E. scophthalmi* (Sitjà-Bobadilla et al., 2006b) and of gilthead sea bream parasitized with *E. leei* (Sitjà-Bobadilla et al., 2008). Similarly, interferon-stimulated and MHC class II genes were up-regulated in the intestine of gilthead sea bream exposed but non-infected with *E. leei*, possibly indicating that an active immune response at the local level is important to avoid infection with the parasite or to restrict its subsequent proliferation (Davey et al., 2011).

Finally, an adaptive immune response to *Enteromyxum spp.* has been detected in the intestine of parasitized fish. In gilthead sea bream exposed to *E. leei* a significant increase in expression levels of IgM and in the number of IgM⁺ B cells in the posterior intestine was detected in parasitized fish later than 3 months after exposure. This response was stimulated

by a plant protein-based diet containing 66% vegetable oil (Estensoro et al., 2012). Similarly, the number of IgM⁺ B cells significantly increased in the intestine of turbot exposed to *E. scophthalmi*, whereas in spleen and kidney the same cells decreased (Bermudez et al., 2006). Immunoassays developed for the detection of specific IgM antibodies against *E. scophthalmi* in turbot (Sitjà-Bobadilla et al., 2004), did not detect specific IgM antibodies in serum from exposed fish collected up to 43 dpe (Sitjà-Bobadilla et al., 2006b). Nevertheless, in an experimental challenge of three groups of turbot that had survived outbreaks of *E. scophthalmi*, a progressive and statistically significant increase in the mean antibody production up to 152 and 350 dpe was detected in previously infected challenged fish, but not in naive challenged fish, and this, correlated to differences in mortality after challenge (Sitjà-Bobadilla et al., 2007b). These studies indicate that while there is a development of an adaptive immune response against *Enteromyxum* parasites, this could occur at very late times after infection. The possibility exists that if specific intestinal mucosal immunoglobulins were analyzed during *Enteromyxum* infections, an immune response might be detected at the mucosal surface, similar to the IgT responses recently described for *C. shasta* infections in rainbow trout (Zhang et al., 2010). Whether early IgT responses may occur during infection is an interesting question that will have to wait for future studies.

3.1.2. Ceratomyxa Shasta—A variety of mechanisms have been proposed to explain the resistance of certain strains of fish to infection by *C. shasta*, including exclusion of the parasite at the site of invasion and/or preventing establishment in the intestine. However, a recent study comparing infections in a susceptible strain of rainbow trout and a resistant strain of Chinook salmon (*Oncorhynchus tshawytscha*) could not support these hypotheses (Bjork and Bartholomew, 2010).

C. shasta infections in susceptible strains of salmon and trout result in an acute inflammatory response in the intestine similar to the enteronecrosis described for *Enteromyxum* spp. In infections caused by this parasite, infiltration of polymorphonuclear leukocytes, fibroblasts and macrophages can be observed in the gut. In severe infections, the epithelial lining necrotizes and is replaced by fibrous connective tissue containing host cells and parasites at different stages (Bartholomew et al., 1989; Bjork and Bartholomew, 2010). Despite the severity of the response, specific antibodies against the parasite could not be detected with an anti-IgM antibody (Bartholomew et al., 1989). There is some evidence that resistant fish strains are able to clear the parasite from the blood within 2 weeks, before infection is fully established in the intestine (Bjork and Bartholomew, 2010), suggesting a role for innate immune defenses at the beginning of the infection process. In another study, infections with *C. shasta* in relatively resistant production stocks of *O. mykiss* and *Oncorhynchus clarki* that received prolonged exposure to the parasite were characterized by the presence of high numbers of parasites on the surface of the intestinal epithelia, without associated inflammation. However, when fish were later released as yearlings, the prevalence of infection increased and many fish developed an extensive inflammatory response, evidenced as severe granulomatous enteritis, with tissue damage and necrosis. This pathology was also associated with a decreased fitness of the fish (Bartholomew et al., 2004). In a more recent study of *C. shasta* infections in rainbow trout, a large accumulation of IgT⁺, but not IgM⁺ B

cells, mainly in the intestinal lamina propria, was demonstrated in survivor fish after 3 months post-infection (Figure 3). This increase in IgT⁺ B cells was concomitant with an increase in the overall concentration of IgT and the production of specific IgT antibodies against the parasite, primarily in the intestinal mucus (Zhang et al., 2010). Conversely, specific IgM responses against the parasite could not be detected in the gut mucus, although they were present in the serum. The aforementioned findings showed for the first time that a specialized mucosal immunoglobulin (IgT), functionally analogous to mammalian IgA, was present in teleosts. In these same infections with *C. shasta*, the transcription of CCR7 was dramatically increased in both IgM⁺ and IgT⁺ B cells from the gut. CCR7 is the chemokine receptor for the CCL19 and CCL21 chemokines in mammals, which have a major role in the recruitment of lymphocytes to lymph nodes and Peyer's patches in the intestinal mucosa, especially naive T lymphocytes. This suggests that CCR7 plays an important role in lymphocyte trafficking during infection in rainbow trout, possibly in the recruitment of B lymphocytes into the gut (Ordas et al., 2012). However, since IgT⁺ B cells were the only B cell subset increasing in the gut of fish having survived *C. shasta* infections (Zhang et al., 2010), there likely exist additional chemokines or mechanisms modulating the trafficking of B cells in the fish gut.

3.2. Immune responses to gill parasites

The gills represent an important portal of entry for many myxozoan parasites (Table 1) but also the site of infection and establishment for others (Table 2). For example, *Myxobolus pendula* develops in the gill arch of creek chub (*Semotilus atromaculatus*). The plasmodia develop within complex cysts, which become encapsulated by host immune cells that form a granuloma in the epithelium. The granuloma area produces high levels of alkaline phosphatase activity which does not inhibit the development of the spores, although it may prevent the spread of the parasite to surrounding tissues (Koehler et al., 2004).

Some of the most studied myxozoans that parasitize gills are from the genus *Henneguya*. For most of them an inflammatory immune response from the host has been described. *H. multiplasmodialis*, located in the gill arch and filaments of *Pseudoplatystoma corruscans* and *P. reticulatum*, induced an inflammatory infiltrate in the tissue surrounding the plasmodium and in the internal septa. This plasmodium was surrounded with stratified epithelium and numerous club cells were observed, indicating that modifications of the epithelia occur also as a response to myxozoan parasites in this mucosal tissue (Adriano et al., 2012). Infections of *H. ictaluri* in catfish are also characterized by a significant inflammatory response. In areas adjacent to the lesions, the gill lamellae fuse and contain an inflammatory infiltrate which includes granulocytes-like and Langerhans-like cells. In areas of disrupted cartilage, the inflammatory infiltrate is composed of a mixed population of granulocytes-like cells including neutrophils and cells that resemble eosinophils, the latter presenting evidence of degranulation (Lovv et al., 2011). In a similar manner, infections by *Henneguya* spp., together with other parasites in *Piaractus mesopotamicus* raised in Brazil, produced mild to moderate multifocal necrosis and lympho-plasmocytic inflammation with hypertrophy and fusion of secondary gill lamellae (de Sant'Ana et al., 2012). These examples suggest that destruction of the gill epithelia is unavoidable in the presence of parasites growing in the tissue, but a variety of innate cellular components are attracted to the site in host defense.

Different responses have been described for *H. pseudoplatystoma* which infects the connective tissue of the gill filaments and lamella in farmed pintado (a hybrid between *Pseudoplatystoma fasciatum* and *P. corruscans*). This parasite caused reduction in gill function, but no inflammation (Naldoni et al., 2009).

Whether adaptive immune responses are also involved in controlling myxozoan parasites in gill is an important point that remains to be addressed. Nevertheless, studies of gill infections by other parasite species like *Ichthyophthirius multifiliis* suggest that B and T cells accumulate in the gill of infected fish after exposure (Olsen et al., 2011). Along the same lines, it has been demonstrated that parasites like *C. shasta* which enter through the gills and use the blood stream as a migration route, elicit a significant IgM immune response in the serum of rainbow trout, while provoking a strong IgT mucosal immune response at the final infection site, the intestine (Zhang et al., 2010), as previously described in section 3.2.1. Additionally, the existence of a gill associated lymphoid tissue (GIALT) has been described in teleosts (Salinas et al., 2011) and thus, it would be expected that an adaptive mucosal immune response could be generated at this portal of entry. Nevertheless, as *C. shasta* does not remain at the gills, it is unlikely that fish will generate an adaptive immune response in this tissue. However, this remains to be demonstrated.

3.3. Immune responses to skin parasites

Although there are few myxozoan parasites that use the skin of fish as a preferred site for development (Table 2), the skin represents a portal of entry and a site for attachment of several myxozoan spores as reviewed early in this manuscript (Section 2.2.2 and Table 3).

In addition to the diverse array of innate immune components (i.e., lysozyme, AMPs) present in skin mucus, the cell surface of the skin also contains lectins. These lectins allow the parasite's spores to attach and adhere to the host cells thereby allowing them to migrate to target tissues (Table 1). In this regard, spores of *Kudoa clupeiidae* have been found in the skin muscle of Atlantic menhaden producing ulcerative lesions associated with chronic inflammatory infiltrates (Reimschuessel et al., 2003), indicating that this parasites can cross the mucosal barrier of the epidermis. The epidermal mucus of rainbow trout has been shown to stimulate *M. cerebralis* actinospores to attach to their hosts via extrusion of filaments from the polar capsules (Kallert et al., 2010). Similarly, the skin mucus of goldfish (*Carassius auratus*) can stimulate actinospores of *Myxobolus cultus*, suggesting that the spores recognize fish hosts by detecting low-molecular-weight mucin components in skin mucus, which triggers the parasite to use the outer surface of the fish as a portal of entry (Yokoyama et al., 1995). In another study, surface-associated glycans were detected in the developmental stages of *M. cerebralis* infecting rainbow trout and the oligochaete host *Tubifex tubifex*. Mannose-containing glycans were detected in all the developmental stages of the parasite and at the host tissues, while galactose-specific lectins were reactive in the host tissues but were not found in the parasite (Kaltner et al., 2007). These parasite-specific carbohydrates may help in the adhesion process to the fish epidermis, and simultaneously protect the host by activating the complement system or stimulating an adaptive immune response by serving as antigens.

The only myxozoan parasites which infect skin specifically and have been studied are *Henneguya spp.* Nevertheless, two studies from the same research group, described different immunological reactions in two catfish species in response to different *Henneguya spp.* The first study reported channel catfish infected by a new species of myxozoan, *H. sutherlandi*, which was confined to the dermis and elicited no inflammatory reaction from the fish (Griffin et al., 2008). The second study described infections of *H. pellis* producing cyst-like lesions in the skin and body wall of the peritoneal cavity in blue catfish (*Ictalurus ictalurus*). In the skin it affected mainly the dermis, presenting mild mononuclear inflammatory infiltrates in the epidermis and occasionally in the hypodermis, while in the peritoneal cavity there was an intense granulomatous inflammatory infiltrate (Griffin et al., 2009). These contradictory results for similar myxozoan species parasitizing phylogenetically related fish species demonstrate that the capacity to elicit an immune response depends on the specific interaction between the parasite and its particular host.

Further studies will be needed to determine the immune responses generated by myxozoan parasites in the skin. However, other skin parasites, like the protozoan *I. multifiliis*, are able to produce strong specific mucosal IgT and IgT⁺ B cells responses (Xu et al., 2013), similar to those described previously for *C. shasta* in the gut (Zhang et al., 2010). The aforementioned skin study with *I. multifiliis* represents the first example showing that IgT is the prevalent immunoglobulin responding to infection in the fish skin. We hypothesize that other parasites, such as myxozoans, may also elicit strong IgT responses in the skin, although this hypothesis remains to be demonstrated.

4. Immunoprophylactic strategies to control myxozoan parasites

As reviewed here (section 3) and in previous reviews related to immune responses against myxozoans (Alvarez-Pellitero, 2008; Sitjà-Bobadilla, 2008a, b), fish have all the elements to mount an adaptive immune response and acquire resistance to myxozoan parasites. The existence of immunological memory has been demonstrated for *E. leei* (Sitjà-Bobadilla et al., 2007b) and *T. bryosalmonae* (Foott and Hedrick, 1987), as fish are able to mount a more effective response in a second encounter with these parasites. Nevertheless, at present there are no vaccines available against any myxozoan parasite. This is, in part, because of the inability to culture myxozoans *in vitro*, providing a source of antigen for experimentation and vaccination. Moreover, there is a lack of information regarding the interactions between parasite and their hosts; more specifically about the parasite antigens that trigger the host immune response and which could potentially be used in the design of a vaccine. In addition, until recently it was not known that IgT was the prevalent mucosal immunoglobulin in the fish skin and gut (Xu et al., 2013; Zhang et al., 2010), which in turn prevented the evaluation of IgT-mediated immune responses in mucosal sites in response to vaccination.

For myxozoans infecting mucosal surfaces, immersion vaccines that prevent infections in skin and gills, and oral vaccines that protect against intestinal parasites, would appear as an excellent alternative to the traditional injectable vaccines currently in use (Plant and LaPatra, 2011). Additionally, the use of probiotics added to the water or in the diet may help to create a more effective microbiota at the mucosal sites that could improve natural immunity at

these surfaces and prevent future infections. Some probiotics are currently applied in aquaculture and they have been shown effective in improving systemic and mucosal immunity (Martínez Cruz et al., 2012). Nevertheless, their effects are temporary, as it has been shown that the intestinal microbiota of fish become established early during development and therefore the single addition of a probiotic will change the microbiota composition only temporarily, returning to its original composition after a few days, unless the probiotic is continuously administered (Verschuere et al., 2000).

As an alternative to vaccination, exploitation of non-specific immune factors has received some consideration, primarily by the use of immunostimulants now included in many commercial feeds. However, their efficacy against myxozoan infections has been low or difficult to interpret. For instance, prophylactically feeding *O. mykiss* with a diet containing an immunomodulatory compound including glucan from yeast and an antiparasitic drug from *Aspergillus fumigatus* (fumagilin) decreased the number of fish infected with *C. shasta*, but didn't decrease the overall survival rate (Whipple et al., 2002). In contrast, diets containing fumagilin or a combination of the anticoccidial drugs Amprolium and Salinomycin (AS), were effective in treating sharpnose seabream infected with *E. leei* as they reduced the development of myxozoan spores and the prevalence, intensity and mortality of the disease, especially with the AS treatment (Golomazou et al., 2006b). Diets with AS were also effective in reducing the prevalence of *Myxobolus sp.* infections in *Puntazzo puntazzo* (Athanassopoulou et al., 2004). Nevertheless when AS were tested in diets for channel catfish to control *H. ictaluri*, the diet presented poor palatability and its efficacy in controlling the parasite could not be demonstrated (Manning et al., 2013).

Currently, treatment of the water and bathing of fish with chemicals are the most commonly used methods for preventing and treating myxozoan infection outbreaks. Traditional treatments have included the use of chemicals like malachite green (Ibarra et al., 1990), triazine derivatives (Schmahl et al., 1993) and hydrogen peroxide (Angelucci et al., 2008), especially because of their low cost. Nevertheless, more effective and less toxic treatments are available. For example, exposure of *E. leei* to low salinity (below 8 parts per thousand) was effective in controlling infections of anemonefish with the parasite (Yokoyama and Shirakashi, 2007). UV irradiation and ozonation, were effective in preventing *Kudoa neurophila* infections in post-larvae and juveniles striped trumpeter (*Latris lineata*) (Cobcroft and Battaglene, 2013). All of these treatments likely affected the survival or infectivity of the fragile actinospore stage of the parasite. More interestingly, for *M. cerebralis*, UV irradiation of the infective triactinomyxozoan prevented or significantly inhibited development of the parasite life cycle in juvenile *O. mykiss*. While this treatment did not prevent the initial attachment and penetration of the parasite in the skin, it prevented the posterior development, and the fish exposed to irradiated parasites developed a protective response to subsequent challenges with fully infective parasites (Hedrick et al., 2012). The treatment of water systems with UV irradiation is an effective means of preventing infection, and may provide the additional benefit of enhancing immunity to future contact with the parasite.

In conclusion, there are no effective immunoprophylactic agents against myxozoans and current treatments are not effective, leaving avoidance of infection as the primary means of controlling myxozoan disease.

5. Concluding remarks and future directions

Myxozoan parasites represent threats to natural fish populations and a great menace to aquaculture, but little attention has been paid to developing preventive treatments. As described here, myxozoan parasites are able to trigger strong immune responses in their hosts. Specifically, at mucosal surfaces it has been demonstrated that innate and adaptive responses are generated, and that the development of immunological memory results in resistance to subsequent encounters with the parasite. Nevertheless, the parasites have also the capacity to evade the immune response by hiding in immunoprivileged sites or by migrating to different tissues.

Therefore, since the infection strategies developed by different myxozoans are variable, it will be important to better understand these parasites, including their life cycles, routes of infection, portals of entry and tissues affected. Additionally, it will be important to develop systems to culture the parasite *in vitro* with the goal to establish experimental infections as well as to develop a source of pathogen antigens for the future development of vaccines. Most importantly, it will be pivotal to understand the interactions between pathogens and their hosts, to learn the strategies used by the parasite to evade the host's immune system and to investigate the antigens recognized by the host to mount an effective immune response against the parasite. In this regard it is important to consider that specific mucosal immune responses can be generated in teleosts, and therefore, future studies need to consider the evaluation of IgT responses elicited by parasite infections and vaccine or immunostimulation treatments. This knowledge will permit us to better understand the biology of host-myxozoan interactions and will facilitate the design of more effective and novel immunotherapeutic tools, including vaccines.

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Highlights

- Myxozoans are among the most abundant parasites in nature.
- Many species infect both wild and farmed fish.
- Here we highlight the biology of some of the most important myxozoans infecting mucosal surfaces of fish.
- We describe the current knowledge on immune responses elicited by Myxozoans in fish mucosal sites.
- The development of immunoprophylactic strategies is also discussed in this review.

Fig. 1.

Life cycle of *Myxobolus cerebralis* showing salmonid and tubificid hosts, with myxospore and triactinomyxon stages. Graphics by Stephen Atkinson, Oregon State University. Reprinted from the American Fisheries Society Fish Health Section Blue Book (MacConnell and Bartholomew, 2010). Copyright American Fisheries Society. Used with permission.

Fig. 2.

In situ hybridized sections of *Ceratomyxa shasta*-infected tissues. (A) Parasite stages in the blood vessel of the gill filaments 1 day after exposure. (B) Parasite proliferation in the intestine with resulting inflammation and tissue destruction. Parasites are labeled by dark purple stain. Scale bars 10 μm.

Fig. 3. Accumulation of IgT⁺ B cells in the gut of rainbow trout surviving an infection with the parasite *Ceratomyxa shasta*. *Upper panel:* Immunofluorescence staining of a gut cryosection from rainbow trout three months post-infection with *C. shasta*. Cryosection was stained for trout IgM (red), IgT (green) and *C. shasta* (magenta); nuclei are stained with DAPI (blue). *Lower panel:* Differential interference contrast image of the same section shown in upper panel. A parasite is localized in the gut lumen.

Table 1

Described portals of entry for myxozoans.

Myxozoan	Final site of infection	Portal of entry	Reference
<i>Myxobolus cerebralis</i>	Cartilage	Skin, fins, buccal cavity, digestive tract.	Markiw (1989)
		Skin, fins.	El-Matbouli et al. (1995)
		Fins, gills.	Antonio et al. (1998)
		Skin, fins, respiratory epithelium, buccal cavity.	El-Matbouli et al. (1999)
		Skin, gills, fins.	Kallert et al. (2009)
<i>Henneguya ictaluri</i>	Gills	Stomach, skin, buccal cavity, gills.	Belem and Pote (2001)
<i>Sphaerospora truttae</i>	Kidney	Gills (most frequent), skin, fine	Holzer et al. (2003)
<i>Tetracapsuloides bryosalmonae</i>	Kidney	Skin	Longshaw et al. (2002)
		Gills	Morris et al. (2000)
			Grabner and El-Matbouli (2010)
<i>Ceratomyxa shasta</i>	Intestine	Gills (most frequent).	Bjork and Bartholomew (2010)
<i>Enteromyxum scopthalmi</i>	Intestine	Intestine ^a	Redondo et al. (2004)

^aInfections detected in intestine following ingestion of infected material.

Table 2

Immunological studies for myxozoans infecting mucosal tissues of fish.

Tissue infected	Myxozoan	Host	Immune response analyzed	Reference
Intestine	<i>Enteromyxum leei</i>	Gilthead sea bream (<i>Sparus aurata</i>)	Intestine mucus composition (glycoproteins). Transcriptome analysis of immune genes in posterior intestine. IgM expression and IgM ⁺ cells in posterior intestine.	Estensoro et al. (2013) Calduch-Giner et al. (2012) Estensoro et al. (2012)
			Transcriptome analysis of immune genes in intestine and head kidney. Histopathology of intestine inflammatory cell infiltration and goblet cells. Genetic analysis of redox and immune relevant genes in head kidney and intestine.	Davey et al. (2011) Fleurance et al. (2008) Sitià-Bobadilla et al. (2008)
			Analysis of innate immune response in head kidney. Measurement of cell-mediated cytotoxicity in head kidney.	Cuesta et al. (2006a) Cuesta et al. (2006b)
Intestine	<i>Enteromyxum leei</i>	Gilthead sea bream (<i>Sparus aurata</i>) & European sea bass (<i>Dicentrarchus labrax</i>)	Histopathology analysis of intestine.	Sitià-Bobadilla et al. (2007a)
Intestine	<i>Enteromyxum leei</i>	Mediterranean sharpshout sea bream (<i>Diplodus puntazzo</i>)	Analysis of cellular immune response in intestine. Measurement of humoral innate immune parameters in serum.	Alvarez-Pellitero et al. (2008) Muñoz et al. (2007)
Intestine	<i>Enteromyxum scopthalmi</i>	Turbot (<i>Psetta maxima</i>)	Expression of iNOS in intestine, head kidney and spleen. Detection of specific antibodies in serum. Histology analysis of carbohydrate terminals at the host-parasite interface in intestine. Innate and adaptive response in serum and different tissues.	Losada et al. (2012) Sitià-Bobadilla et al. (2007b) Redondo et al. (2008) Sitià-Bobadilla et al. (2006b)
Intestine	<i>Enteromyxum scopthalmi</i>	Turbot (<i>Scophthalmus maximus</i>)	Histology analysis of Ig ⁺ cells in intestine, spleen and kidney. Detection of specific IgM antibodies in	Bermudez et al. (2006) Sitià-Bobadilla et al. (2004)

Tissue infected	Myxozoan	Host	Immune response analyzed	Reference
Intestine	<i>Ceratomyxa shasta</i>	Rainbow trout (<i>Oncorhynchus mykiss</i>)	serum. Expression of chemokine receptor CCR7 by B cells in intestine.	Ordas et al. (2012)
Intestine	<i>Ceratomyxa shasta</i>	Rainbow trout (<i>Oncorhynchus mykiss</i>) & cutthroat trout (<i>O. clarki</i>)	Analysis of IgT and IgM immunoglobulin and B cells responses in serum and intestine.	Zhang et al. (2010)
Intestine	<i>Ceratomyxa shasta</i>	Steelhead trout (<i>Salmo gairdneri</i>)	Histopathology analysis of intestine inflammatory response.	Bartholomew et al. (2004)
Gill	<i>Henneguya multiplasmidialis</i>	<i>Pseudoplattystoma</i> spp.	Histopathology analysis of intestine inflammatory response. Detection of specific antibodies.	Bartholomew et al. (1989)
Gill	<i>Henneguya pseudoplattystoma</i>	Farmed pintado (South american catfish)	Histopathology analysis of gill inflammatory response.	Adriano et al. (2012)
Gill	<i>Henneguya</i> spp.	Channel catfish (<i>Ictalurus punctatus</i>)	Histopathology analysis of gill inflammatory response.	Naldoni et al. (2009)
Gill	<i>Henneguya</i> spp.	<i>Piaractus mesopotamicus</i>	Histopathology analysis of gill inflammatory response.	Lovy et al. (2011)
Gill	<i>Myxobolus pendula</i>	Creek chub (<i>Semotilus atromaculatus</i>)	Histopathology analysis of gill inflammatory response. Measurement of alkaline phosphatase activity.	de Sant' Ana et al. (2012)
Skin and gills	<i>Kudoa clupei</i> sp.	Atlantic menhaden (<i>Brevoortia tyrannus</i>)	Histopathology analysis of gill and skin inflammatory response.	Kochler et al. (2004)
Skin	<i>Henneguya sutherlandi</i> .	Channel catfish (<i>Ictalurus punctatus</i>)	Histopathology analysis of skin.	Reimschuessel et al. (2003)
Skin (portal of entry)	<i>Myxobolus cultus</i>	Goldfish (<i>Carassius auratus</i>)	Skin mucus activity.	Griffin et al. (2008)
				Yokoyama et al. (1995)

Table 3

Myxozoans found in the skin mucosal tissues of fish.

Myxozoan	Host	Immune response	Reference
<i>Enteromyxum scophthalmi</i>	Turbot (<i>Scophthalmus maximus</i>)	N/D	Redondo et al. (2004)
<i>Henneguya ictaluri</i>	Channel catfish (<i>Ictalurus punctatus</i>)	N/D	Belem and Pote (2001)
<i>Henneguya laterocapsulata</i>	Hybrid African catfish	N/D	Obiekezie and Schmahl (1993)
<i>Henneguya pellis</i>	Blue catfish (<i>Ictalurus Ictalurus</i>)	N/D	Griffin et al. (2009)
<i>Henneguya sutherlandi</i>	Channel catfish (<i>Ictalurus punctatus</i>)	Confined to dermis. No inflammatory reaction.	Griffin et al. (2008)
<i>Kudoa clupei</i>	Atlantic menhaden (<i>Brevoortia tyrann</i>)	Chronic inflammatory infiltrates.	Reimschuessel et al. (2003)
<i>Myxobolus cerebrales</i>	Common carp and Rainbow trout	N/D	Kallert et al. (2009); Kaltner et al. (2007)
<i>Myxobolus charii</i> <i>Myxobolus mbaitaoi</i>	<i>Citharus citharus</i>	N/D	Fomena et al. (2004)
<i>Myxobolus cordeiroi</i>	Jau catfish (<i>Zungaro jahu</i>)	N/D	Adriano et al. (2009)
<i>Myxobolus cultus</i>	Goldfish (<i>Carassius auratus</i>)	Mucus activity.	Yokoyama et al. (1995)
<i>Myxobolus episquamalis</i>	Sea mullet (<i>Mugil cephalus</i>)	N/D	Rothwell et al. (1997)
<i>Myxobolus hakyi</i>	Sutchi catfish (<i>Pangasianodon hypophthalmus</i>)	N/D	Baska et al. (2009)
<i>Myxobolus hyborhynchi</i>	Fathead minnow (<i>Pimephales promelas</i>)	Mononuclear inflammatory cells in perichondral and periosteal connective tissue.	Cone and Frasca (2002)
<i>Myxobolus rotundus</i>	Crucian carp (<i>Carassius auratus</i>)	N/D	Lu et al. (2003)
<i>Myxobolus spp.</i>	<i>Capoeta sp.</i> and <i>Alburnus sp.</i>	N/D	Masoumian et al. (2007)
<i>Myxobolus</i> , <i>Henneguya</i> & <i>Thelohanellus</i>	Cyprinid fishes and eels	N/D	Molnar (2002)
<i>Sphaerospora molnari</i>	Common carp (<i>Cyprinus carpio</i>)	N/D	Eszterbauer et al. (2013)
<i>Thelohanellus hovorkai</i>	<i>Cyprinus carpio</i>	N/D	Liyanage et al. (1998)
<i>Unicauda clavicauda</i>	<i>Notropis hudsonius</i> - Cyprinidae	N/D	Cone and Melendy (2000)

N/D: Not determined.