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Transmission Biology of the Myxozoa

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1. Introduction

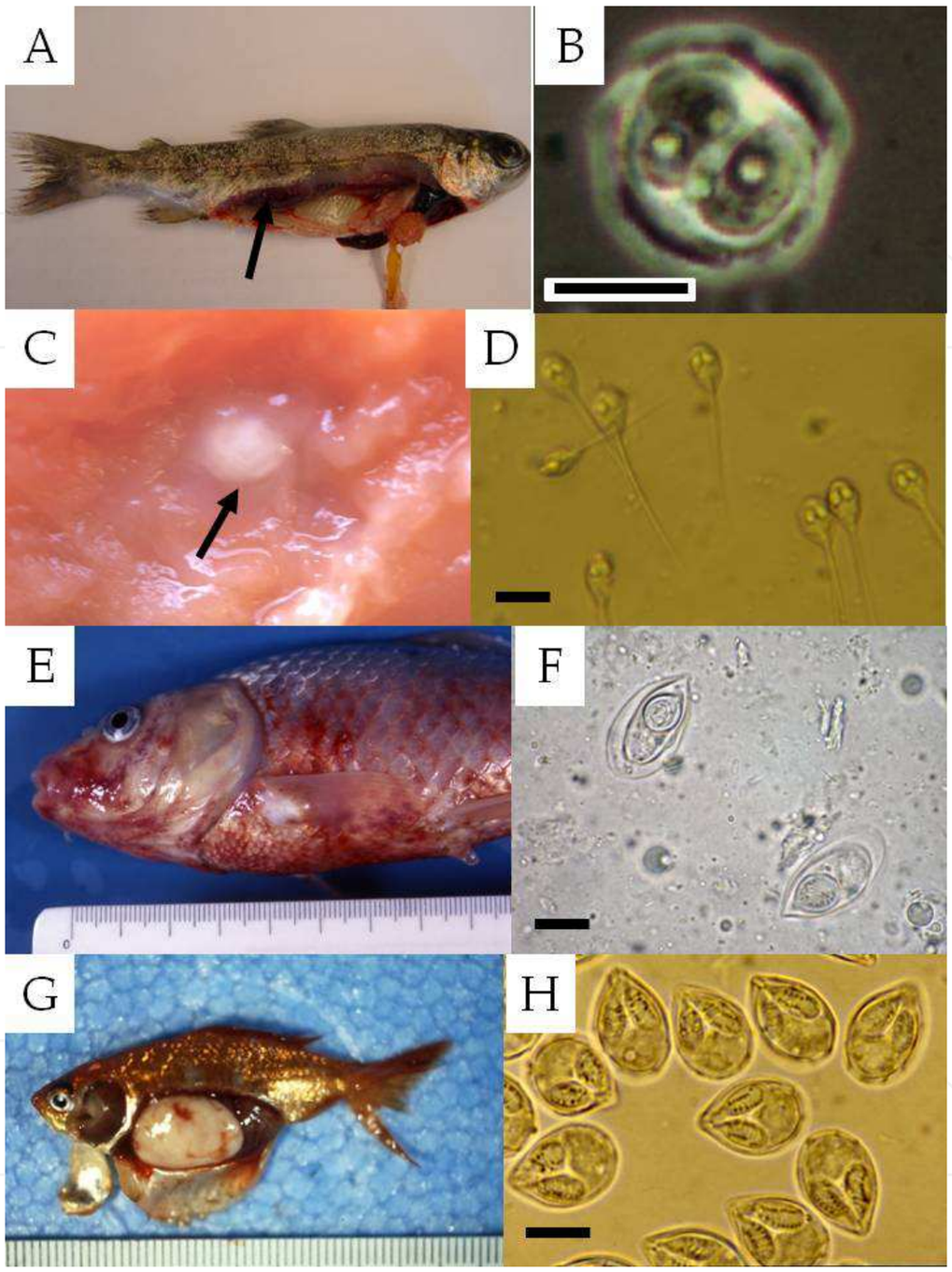
Myxozoans are spore-forming parasites of both freshwater and marine fishes (Lom & Dyková, 1992, Kent et al., 2001; Feist & Longshaw, 2006). The Myxozoa were previously classified as protozoans, although the multicellular state and functional specialization of the cells composing spores were considered to exceed protozoan level (Lom & Dyková, 1992). Indeed, molecular studies demonstrated that myxozoans are metazoans (Smothers et al., 1994, Siddal et al., 1995). However, there were two conflicting views concerning the phylogenetic origin of myxozoans; the Bilateria (Smothers et al., 1994, Schlegel et al., 1996, Anderson et al., 1998, Okamura et al., 2002) vs. the Cnidaria (Siddal et al., 1995). More recently, the Cnidaria-hypothesis has been strongly supported by phylogenetic analyses of protein-coding genes of myxozoans (Jimenez-Guri et al., 2007, Holland et al., 2010). The phylum Myxozoa, of which more than 2100 species in 58 genera are described to date, is divided into two classes, Myxosporea and Malacosporea (Lom & Dyková, 2006). Most of myxozoans are not harmful to host fish, however, some species cause diseases in cultured and wild fish which are problems for aquaculture and fishery industries worldwide. Generally, freshwater myxosporeans appear to be specific at the family or the genus level of the host, while some marine myxosporeans have a low host-specificity. Some examples are mentioned below.

For freshwater species, myxozoans infecting salmonids have been relatively well studied. For example *Myxobolus cerebralis*, the causative agent of whirling disease, *Tetracapsuloides bryosalmonae*, the cause of proliferative kidney disease (= PKD), and *Ceratomyxa shasta*, causing ceratomyxosis, have fatal effects on farmed salmonid fish (Table 1). Salmonid ceratomyxosis is a local disease which is restricted only to North America (Bartholomew et al., 1997), while whirling disease and PKD are widely distributed in the world (Hedrick et al., 1993, 1998). *M. cerebralis* infects cartilage tissue and causes a whirling behaviour (tail-chasing swimming), a black tail, and skeletal deformities of affected fish. Whirling disease was previously known as a hatchery disease, but recently, it has been recognized as one of the causes for the decline of natural rainbow trout populations in several western states of the USA (Hedrick et al, 1998). Symptoms of PKD in salmonid fish are a swollen kidney (Fig. 1A) and anemic gills, evoked by chronic inflammation of the kidney interstitium. The

Myxozoans	Disease names or typical signs	Fish	References
Ceratomyxa shasta	Ceratomyxosis	Salmonids	Bartholomew et al. (1997)
Chloromyxum truttae	Hypertrophy of gall bllader	Salmonids	Lom & Dyková (1992)
Henneguya ictaluri	Proliferative gill disease (PGD)	*Ictalurus punctatus*	Pote et al. (2000)
Henneguya salminicola	Milky condition	Salmonids	Awakura & Kimura (1977)
Hoferellus carassii	Kidney enlargement disease (KED)	*Carassisus auratus*	Yokoyama et al. (1990)
Myxidium giardi	Systemic infection	*Anguilla* spp.	Ventura & Paperna (1984)
Myxobolus artus	Muscular myxobolosis	*Cyprinus carpio*	Yokoyama et al. (1996)
Myxobolus cerebralis	Whirling disease	Salmonids	Hedrick et al. (1998)
Myxobolus cyprini	Malignant anemia	*Cyprinus carpio*	Molnár & Kovács-Gayer (1985)
Myxobolus koi	Gill myxobolosis	*Cyprinus carpio*	Yokoyama et al. (1997a)
Myxobolus murakamii	Myxosporean sleeping disease	*Oncorhynchus masou*	Urawa et al. (2009)
Myxobolus wulii	Cysts in gill or hepatopancreas	*Carassius auratus*	Zhang et al. (2010b)
Parvicapsula pseudobranchicola	Inflammation and necrosis of filaments	*Salmo salar*	Karlsbakk et al. (2002)
Sphaerospora dykovae	Swimbladder inflammation (SBI)	Cyprinids	Dyková & Lom (1988)
Tetracapsuloides bryosalmonae	Proliferative kidney disease (PKD)	Salmonids	Hedrick et al. (1993)
Thelohanellus hovorkai	Hemorrhagic thelohanellosis	*Cyprinus carpio*	Yokoyama et al. (1998)

Table 1. Economically important freshwater myxosporeans.

causative agent of PKD has not been identified for a long time, and thus the organism was previously called PKX (Hedrick et al., 1993). It was assigned to the Myxozoa in 1999 and initially called *Tetracapsula bryosalmonae* (Canning et al., 1999). Canning et al. (2000) erected the new class Malacosporea in the Myxozoa, and later, in the course of nomenclature changes by Canning *et al.* (2002) *Tetracapsula bryosalmonae* was renamed to *Tetracapsuloides bryosalmonae* (Fig. 1B). Salmonids suffering from ceratomyxosis show abdominal distension and exophthamia, possibly caused by osmotic imbalance due to *C. shasta* infection in the internal organs (Bartholomew et al., 1997). *Henneguya salminicola* produces cysts in the musculature of anadromous salmonid fish (Fig. 1C, D). This parasite does not cause a health



A & B: Proliferative kidney disease of rainbow trout (*Oncorhynchus mykiss*). Note the swollen kidney (arrow). Malacospore of *Tetracapsuloides bryosalmonae* from bryozoans host (B). C & D: Milky condition of pink salmon (*Oncorhynchus gorbuscha*). White exudate (arrow) filled with spores of *Henneguya salminicola* (D). Photos of courtesy by Dr. T. Awakura. E & F: Hemorrhagic thelohanellosis of common carp (*Cyprinus carpio*). Note extensive haemorrhages in mouth and abdomen caused by *Thelohanellus hovorkai* (F) in the subcutaneous tissue. G & H: Creamy appearance of enlarged hepatopancreas of goldfish (*Carassius auratus*) infected with *Myxobolus wulii* (H). Scale bars for B, D, F and H are 10μm.

Fig. 1. Myxozoan diseases of freshwater fish and the causative myxozoan parasites.

problem of the host, but renders the infected fish unmarketable due to the milky condition of the flesh (Awakura & Kimura, 1977). Myxosporean sleeping disease is caused by *Myxobolus murakamii* infecting the peripheral nerve of masu salmon (*Oncorhynchus masou*). This disease has been known only in Hiroshima Prefecture, in south-western Japan, although *M. murakamii* occurs also in Hokkaido, the northernmost area of Japan. It remains to be clarified why the sleeping disease does not occur in Hokkaido (Urawa et al., 2009). *Chloromyxum truttae* infects the gallbladder of brood stock of rainbow trout (*Oncorhynchus mykiss*), while it infects the yearlings of Atlantic salmon (*Salmo salar*). Affected fish showed loss of appetite, yellow colouration of body, and hypertrophic gall bladder (Lom & Dyková, 1992). Pseudobranch infection with P*arvicapsula pseudobranchicola* has been reported in Atlantic salmon in Norway, showing lethargy, disorganized swimming, exophthalmia and low-grade to significant mortalities (Karlsbakk et al., 2002). Affected fish exhibited eye bleeding and cataracts, possibly due to obstruction of the blood supply to the choroid bodies of the eyes.

Myxobolus koi, *Thelohanellus hovorkai*, and *Sphaerospora dykovae* (= *S. renicola*) are well-known pathogens in cultured common carp (*Cyprinus carpio*) in Europe and Asia (Dyková & Lom, 1988, Yokoyama et al., 1997a, 1998). *M. koi* infects the gills and causes a respiratory disfunction of carp juveniles. Yokoyama et al. (1997a) reported that there are two types of *M. koi* infections; the one forms large-type (pathogenic) cysts in the gill filaments, while the other forms small-type (non-pathogenic) cysts in the gill lamellae. *T. hovorkai* infecting the connective tissue is the causative agent of the hemorrhagic thelohanellosis of common carp (Yokoyama et al., 1998). Spore dispersion of *T. hovorkai* in subcutaneous connective tissue causes extensive hemorrhages and edema, finally resulting in death of affected fish (Fig. 1E, F). *S. dykovae*, the cause of swimbladder inflammation (SBI) was previously known as *S. renicola*, but has recently been renamed as *S. dykovae* in association with revised taxonomy of the genus *Leptotheca* (Gunter & Adlard, 2010). The target organ (spore forming site) for *S. dykovae* is the kidney, but the extrasporogonic stage of *S. dykovae* proliferates in the swimmbladder, which causes SBI of carp (Dyková & Lom, 1988). *Myxobolus artus* produced rice bean-like cysts in the musculature of common carp. Adult carp (over 1-year old) do not die of the disease but lose their commercial value. In contrast, juvenile carp (0-year old) heavily infected with *M. artus* exhibit hemorrhagic anemia and increased mortality rate. After degeneration of *M. artus* cysts in the musculature, spores engulfed by macrophages are transferred into gills, where numerous spores accumulate and pack within the lamellae. As a result, the gill epithelia are exfoliated, causing the hemorrhagic anemia (Yokoyama et al., 1996). *Myxobolus cyprini* infecting the skeletal muscle of common carp was also reported to cause the malignant anemia (Molnár & Kovács-Gayer, 1985), but it is unknown whether the disease mechanisms are the same as *M. artus*. *Thelohanellus kitauei* forms large cysts in the intestinal mucosa of common carp so that the intestine was occluded to emaciate the infected fish.

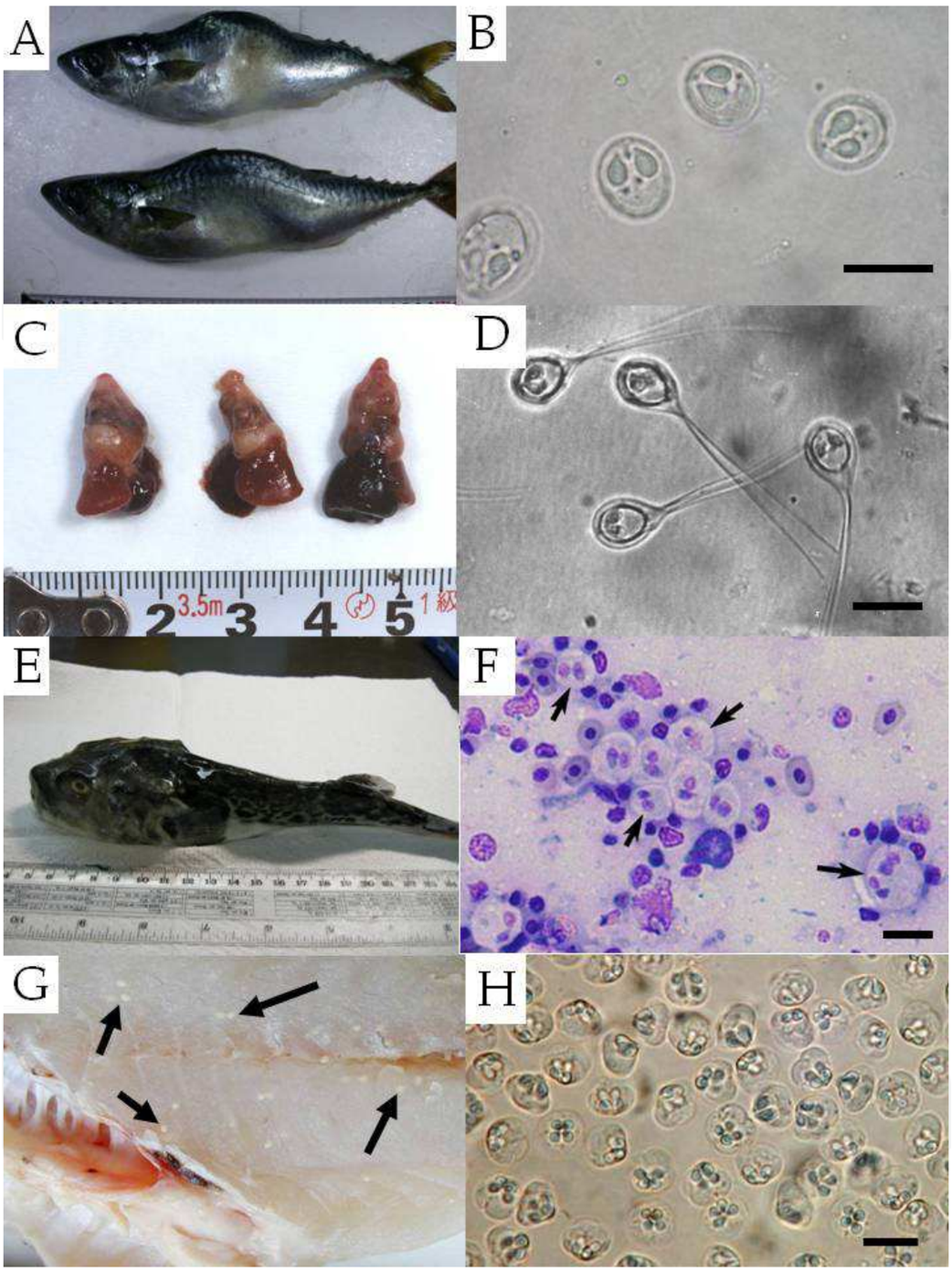
Hoferellus carassii infecting the kidney of goldfish (*Carassius auratus*) is the causative agent of kidney enlargement disease (KED). This parasite does not cause a high mortality of affected fish, but a low marketability as an ornamental fish (Yokoyama et al., 1990). *Myxobolus wulii* forms numerous cysts in the gills of goldfish in some cases, whereas large cysts are formed in the hepatopancreas in other cases (Fig. 1G, H). In both cases, infection of fish results in high mortality (Zhang et al., 2010b). Gill infections with *Henneguya ictaluri* and *H. exilis* are typical

myxosporean diseases in catfish culture. *H. ictaluri* causes proliferative gill disease of catfish (*Ictalurus punctatus*) (Pote et al. 2000). *Myxidium giardi* infects multiple organs including gills and kidney of several eel species, *Anguilla anguilla*, *A. rostorata*, and *A. japonica*. Infected elvers exhibit dropsy, ascites, and swollen kidney (Ventura & Paperna, 1984).

Compared to freshwater myxosporeans, many marine species have a broad host range, such as *Kudoa thyrsites*, *K. yasunagai* and *Enteromyxum leei* (Table 2). *K. thyrsites* lowers the

Myxozoans	Disease names or typical signs	Fish	References
Enteromyxum leei	Enteromyxosis or myxosporean emaciation disease	*Diplodus puntazzo*, *Sparus aurata*, *Paralichthys olivaceus*, *Pagrus major*, *Takifugu rubripes*	Diamant (1997) Yasuda et al. (2002)
Enteromyxum scophthalmi	Enteromyxosis		Palenzuela et al. (2002)
Henneguya lateolabracis	Cardiac henneguyosis	*Lateolabrax* sp.	Yokoyama et al. (2003)
Henneguya pagri	Cardiac henneguyosis	*Pagrus major*	Yokoyama et al. (2005a)
Kudoa amamiensis	Kudoosis amami	*Seriola quinqueradiata*	Yokoyama et al. (2000)
Kudoa iwatai	Cysts in multiple organs	*Dicentrarchus labrax*, *Lateolabrax japonicus*, *Mugil cephalus*, *Sparus aurata*, *Pagrus major*, *Oplegnatus punctatus*	Diamant et al. (2005)
Kudoa lateolabracis	Post-mortem myoliquefaction	*Lateolabrax* sp., *Paralichthys olivaceus*	Yokoyama et al. (2004)
Kudoa lutjanus	Systemic infection	*Lutjanus erythropterus*	Wang et al. (2005)
Kudoa neurophila	Meningoencephalomyelitis	*Latris lineata*	Grossel et al. (2003)
Kudoa shiomitsui	Cysts in the heart	*Takifugu rubripes*, *Thunnus orientalis*	Zhang et al. (2010)
Kudoa thyrsites	Post-mortem myoliquefaction	*Salmo salar*, *Paralichtys olivaceus*, *Coryphaena hyppurus*	Moran et al. (1999a)
Kudoa yasunagai	Abnormal swimming	*Lateolabrax japonicus*, *Oplegnathus fasciatus*, *Seriola quinqueradiata*, *Takifugu rubripes*, *Thunnus orientalis*, *Plotosus lineatus*	Zhang et al. (2010a)
Myxobolus acanthogobii	Myxosporean scoliosis or skeletal deformity	*Seriola quinqueradiata*, *Scomber japonicus*	Yokoyama et al. (2005b)
Sphaerospora epinepheli	Disorientation, hemorrhage	*Epinephelus malabaricus*	Supamattaya et al. (1991)
Sphaerospora fugu (= *Leptotheca fugu)*	Myxosporean emaciation disease	*Takifugu rubripes*	Tin Tun et al. (2000)

Table 2. Economically important marine myxosporeans (see also Fig. 2).



A & B: Skeletal deformity (A) of Japanese mackerel (*Scomber japonicus*) infected with *Myxobolus acanthogobii* (B) in the brain. C & D: Enlarged bulbus arteriosus (C) of Chinease seabass (*Lateolabrax* sp.) infected with *Henneguya lateolabracis* (D) in the heart. E & F: Myxosporean emaciation disease (E) of tiger puffer (*Takifugu rubripes*) infected with developmental stages (arrows) of *Enteromyxum leei* (F) in the intestine. Diff-Quik stain (F). G & H: Cysts (arrows) in the skeletal muscle (G) of red sea bream (*Pagrus major*). Cysts are packed with spores of *Kudoa iwatai* (H). Scale bars for B, D, F and H are 10 μm.

Fig. 2. Myxosporean diseases of marine fish and the causative myxozoan parasites.

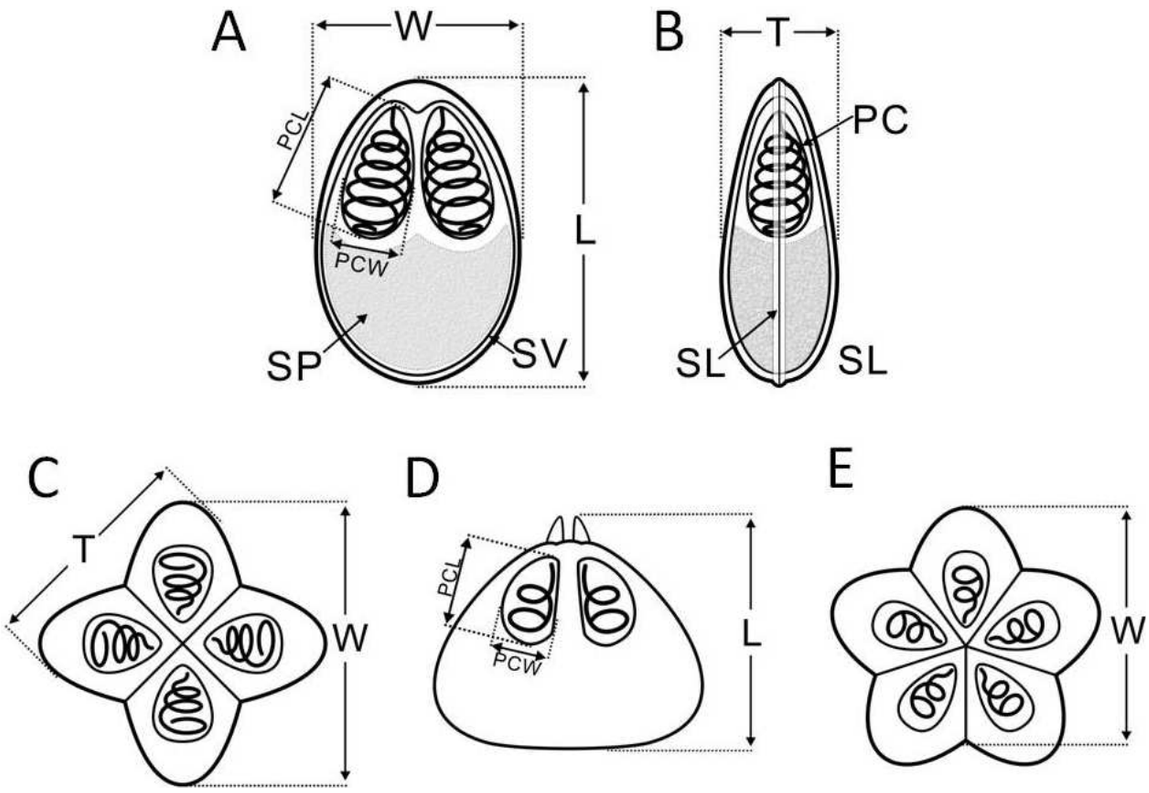
commercial value of various cultured marine fish species, particularly Atlantic salmon (*Salmo salar*) in North America, by causing post-mortem myoliquefaction (Moran et al., 1999a). *K. yasunagai* forms numerous cysts in the brain, probably causing disorder of swimming performance of many fish species (Zhang et al., 2010a). Recently, enteromyxosis or myxosporean emaciation disease, caused by *E. leei*, has emerged as a new threat in various cultured marine fish, e.g. gilthead sea bream (*Sparus aurata*) in Mediterranean countries and tiger puffer (*Takifugu rubripes*) in Japan (Diamant, 1997, Yasuda et al., 2002). In contrast, *Enteromyxum scophthalmi* and *Sphaerospora fugu* (= *Leptotheca fugu*) have been found only in the intestine of turbot (*Psetta maxima*) and tiger puffer (*Takifugu rubripes*), respectively, although the signs of the disease appear to be similar to *E. leei* infection (Tin Tun et al., 2000, Palenzuela et al., 2002). Heart infections have been documented such as *Henneguya lateolabracis*, *H. pagri*, and *Kudoa shiomitsui*. The former two species are highly pathogenic to Chinese sea bass (*Lateolabrax* sp.) and red sea bream (*Pagrus major*), respectively (Yokoyama et al., 2003, 2005a), whereas the pathogenic effects of *K. shiomitsui* are not clear (Zhang et al., 2010a). Many *Kudoa* infections in skeletal muscle may render the infected fish unmarketable by producing cysts (e.g., *K. amamiensis* and *K. iwatai*) or causing myoliquefaction (e.g., *K. lateolabracis* and *K. neothunni)*. *K. neurophila* has become an impediment to the juvenile production of striped trumpeter (*Latris lineata*) in Tasmania, due to meningoencephalomyelitis of hatched larvae (Grossel et al., 2003). *Myxobolus acanthogobii* infects the brain and causes the myxosporean scoliosis in yellowtail (*Seriola quinqueradiata*), while infected Japanese mackerel (*Scomber japonicus*) exhibits the lordosis (dorso-ventral deformity) and infected goby (*Acanthogobius flavimanus*) is subclinical (Yokoyama et al., 2005b). *Sphaerospora epinepheli* infects the kidney of *Epinephelus malabaricus*, which shows disorientation of the body and hemorrhages (Supamattaya et al., 1991).

2. Myxosporeans

The class Myxosporea is comprised of the two orders, Bivalvulida and Multivalvulida. Bivalvulids include 52 genera with more than 2100 species described from freshwater and marine fishes, while multivalvulids contain 5 genera with more than 60 species predominantly from marine fish (Lom & Dyková, 2006). Morphology, life cycle, phylogeny, and biology of myxosporeans are summarized below.

2.1 Morphology of myxosporean

Myxosporean spores are composed of shell valves, sporoplasms, and polar capsules containing coiled polar filaments (Fig. 3). Number of valves and polar capsules, arrangement of the polar capsules, and ornamentation of spores allow the genus-level diagnosis of myxosporeans. Identification at the species-level is based on spore dimensions. Species description of myxospores should follow the guidelines of Lom & Arthur (1989). For bivalvulids, spore length and spore width in frontal view, spore thickness in side view, length and width of polar capsules are measured (Fig. 3). If ornamentations such as the caudal appendages for *Henneguya* are present, the length is also measured. For multivalvulids, spore length (including the apical projections, if present) in side view, spore width and spore thickness in top view, length and width of polar capsules are determined. Care must be taken to avoid confusion of thickness and width of spores, because multivalvulids are radially symmetrical.

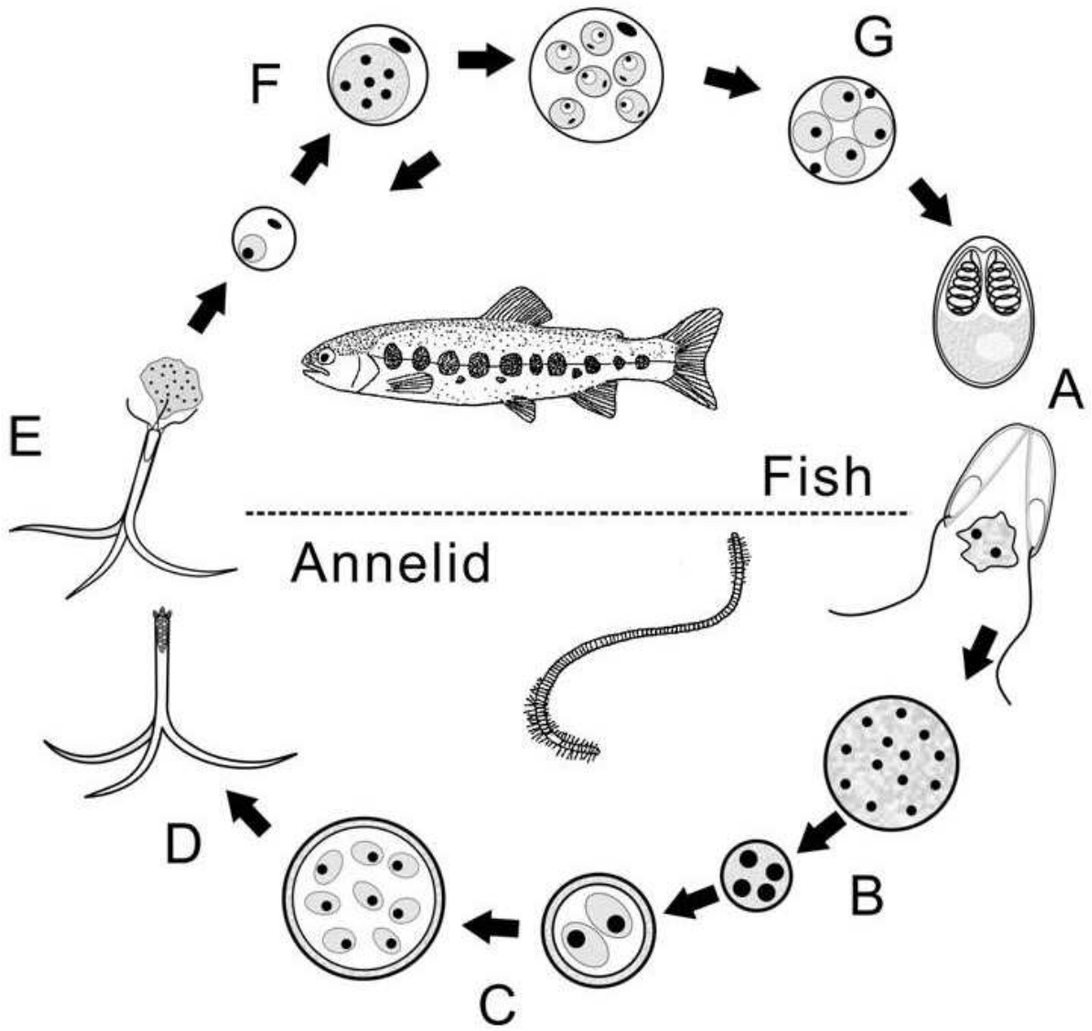


PC: polar capsule, SP: sporoplasm, SV: shell valve, SL: sutural line, L: spore length, W: spore width, T: spore thickness, PCL: polar capsule length, PCW: polar capsule width.

Fig. 3. Diagrams of bivalvulid (A: frontal view, B: side view) and multivalvulid (C & E, top view, D: side view) myxosporean spores.

2.2 Life cycle of myxosporeans

The first myxozoan life cycle was discovered for *M. cerebralis* by Wolf & Markiw in 1984 and was later confirmed by many other researchers, who reported similar life cycles for more than 30 myxosporean species. These life cycles involve an annelid invertebrate (mainly oligochaetes for freshwater species and polychaetes for marine species) and a vertebrate host which is typically a fish (Fig. 4). In the latter, myxosporean spore stages (= myxospores) develop. Myxospores are ingested by annelids, in which the polar filaments extrude to anchor the spore to the gut epithelium. Opening of the shell valves allows the sporoplasms to penetrate into the epithelium. Subsequently, the parasite undergoes reproduction and development in the gut tissue, and finally produces usually eight actinosporean spore stages (= actinospores) within a pansporocyst. After mature actinospores are released from their hosts they float in the water column (El-Matbouli & Hoffmann, 1998). Upon contact with skin or gills of fish, sporoplasms penetrate through the epithelium, followed by development of the myxosporean stage. Myxosporean trophozoites are characterized by cell-in-cell state, where the daughter (secondary) cells develop in the mother (primary) cells. The presporogonic stages multiply, migrate via nervous or circulatory systems, and develop into sporogonic stages. At the final site of infection, they produce mature spores within mono- or disporic pseudoplasmodia, or polysporic plasmodia (El-Matobouli & Hoffmann, 1995).

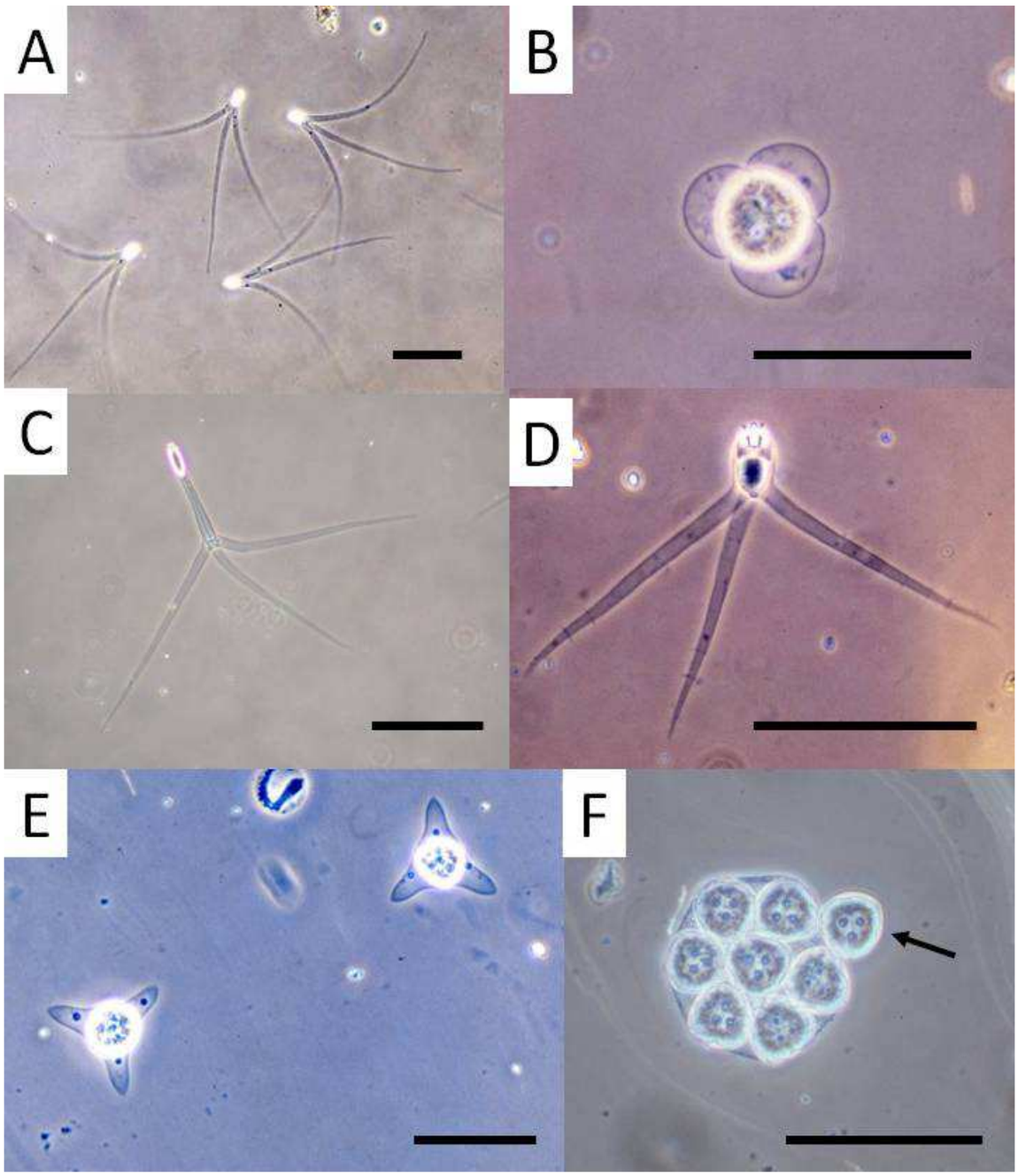


A: The polar filaments are extruded to anchor the spore to the gut epithelium, followed by opening of shell valves of myxospore. B: Gametogony. C: Sporogony of actinosporean phase. D: Mature actinospore stages develop in a pansporocyst, and actinospores are released into the water. E: Upon contact of actinospores with the skin or gills of the fish host, polar filaments extrude to anchor the spore to the skin or gills, facilitating invasion of the sporoplasms into the fish. F: Presporogonic multiplication in a cell-in-cell state. G: Sporogony of myxosporean phase.

Fig. 4. Diagram of the life cycle of myxosporean alternating fish and annelid hosts.

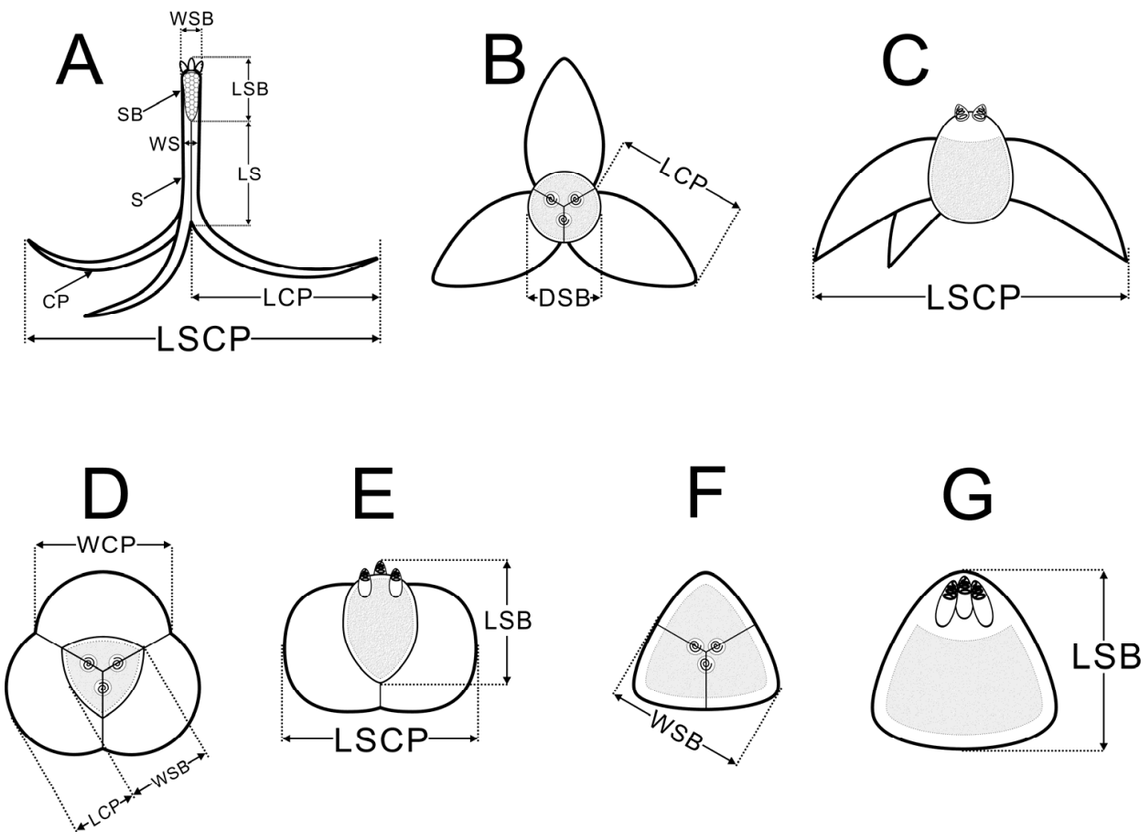
2.3 Morphology of actinospores

Actinospores that are formed in the invertebrate hosts have a triradiate form with exclusively 3 polar capsules and mostly 3 caudal processes (Figs. 5 & 6). To characterize actinosporean stages, researchers should follow the guidelines of Lom et al. (1997); shape of the caudal processes (straight, curved or branched), presence of the style (small stalk below the spore body) and formation of spore nets (pattern of connection between several spores), number of daughter cells in the spore body, and measurements of the spore body, style, polar capsules and processes (Fig. 6).



A: Raabeia-type actinospores of *Myxobolus cultus* from oligochaete *Branchiura sowerbyi*, B: Neoactinomyxum-type actinospore from *B. sowerbyi*. C: Triactinomyxon-type actinospore of *M. arcticus* from oligochaete *Lumbriculus variegatus*, D: Echinactinomyxon-type actinospore from *B. sowerbyi*, E: Aurantiactinomyxon-type actinospore of *Thelohanellus hovorkai* from *B. sowerbyi*, F: Sphaeractinomyxon-type actinospores from unidentified marine oligochaete, which was collected in May 1990, on the coast of Mie Prefecture, the middle part of Japan. Arrow shows an actinospore released from a pansporocyst which develops 8 actinospores. Scale bars for A, C and D are 100 μm, and those for B, E and F are 50 μm.

Fig. 5. Several morphotypes of actinosporean spores.



A: Triactinomyxon, B & C: Aurantiactinomyxon, D & E: Neoactinomyxum, F & G: Tetractinomyxon.B, D & F: top views , C, E & G: side view. SB: spore body, LSB: length of spore body; WSB: width of spore body; S: style; LS: length of style; WS: width of style; CP: caudal process; LCP: length of caudal process (regardless of curvature). LSCP: largest span of between the tips of the caudal processes; PC: polar capsule; DSB: diameter of spherical spore body

Fig. 6. Diagram of actinosporean spores.

There have been 18 collective groups described thus far (Lom & Dyková, 2006, Rangel et al., 2011). Based on the total length of spore (or interconnected spore mass), they are distinctly divided into two morphotypes; the small-type ranges from 15 to 40 µm, e.g., Endocapsa, Sphaeractinomyxon, Tetraspora, Tetractinomyxon, Aurantiactinomyxon, Neoactinomyxum and Guyenotia, while the large-type ranges from approximately 100 to 400 µm, e.g., Echinactinomyxon, Raabeia, Triactinomyxon, Pseudotriactinomyxon, Hexactinomyxon, Ormieractinomyxon, Siedleckiella, Synactinomyxon, Antoactinomyxon, Hungactinomyxon and Unicapsulactinomyxon. From the practical point of view, the large-type actinospores are more likely to be removed by filtration systems than the small-type actinospores. Thus it is important to determine the type of the corresponding actinospore, not only for parasitology, but also for disease management in aquaculture.

Practical key for determination of actinospore-types:

1. a. Processes are absent.....2
   b. Processes are present.....4

2. a. Spores are tetrahedral with a single binucleate sporoplasm ....**Tetractinomyxon**
   b. Spores are subspherical with polar capsules embedded beneath the spore surface.....3
3. a. Eight spores, rounded in side view, are developed within a pansporocyst…….. .....**Sphaeractinomyxon**
   b. Four spores, flattened in side view, are developed within a pansporocyst..... .....**Tetraspora**
4. a. Spores do not connect each other.....5
   b. Spores connect each other at the end of the processes, forming a net structure.....7
5. a. Processes are reduced to bulge-like swellings.....6
   b. Spores with curved leaf-like processes resemble an orange with partly opened peel .....**Aurantiactinomyxon**
   c. Spores have a subspherical spore body with 3 finger-like processes.....**Guyenotia**
   d. Spores have an ovoid spore body with 3 straight spine-like processes..... .....**Echinactinomyxon**
   e. Spores have an elipsoidal spore body with 3 curved, and sharp-tipped processes... .....**Raabeia**
   f. Spores have an elongated spore body with a style and 3 anchor-like processes..... .....**Triactinomyxon**
   g. Spores are similar to triactinomyxon, but the processes have longitudinal sutures, which remain fused over all their length.....**Pseudotriactinomyxon**
   h. Spores have an elongated spore body with a style and 3 diverged (in total 6) processes.....**Hexactinomyxon**
   i. Spores have a single and large polar capsule in an elliptical spore body..... .....**Unicapsulactinomyxon**
6. a. Flattened in side view, and polar capsules are embedded below the spore surface .....**Endocapsa**
   b. Rounded triangular in top view, and polar capsules protrude at the spore apex..... .....**Neoactinomyxum**
7. a. Spore units are echinactinomyxon whose 4 processes of different spores form the junction.....**Antoactinomyxon**
   b. Spores units are triactinomyxon whose 3 processes of different spores form the junction.....**Siedleckiella**
   c. Spore units are echinactinomyxon whose 8 processes have anchor-like hooks at the end, adhering together.....**Ormieractinomyxon**
   d. Spores have two wing-like and one short, conical process, forming a star-like structure.....**Synactinomyxon**
   e. Four spores form a cube-like net interlaced with another cube made of 4 spores..... .....**Hungactinomyxon**

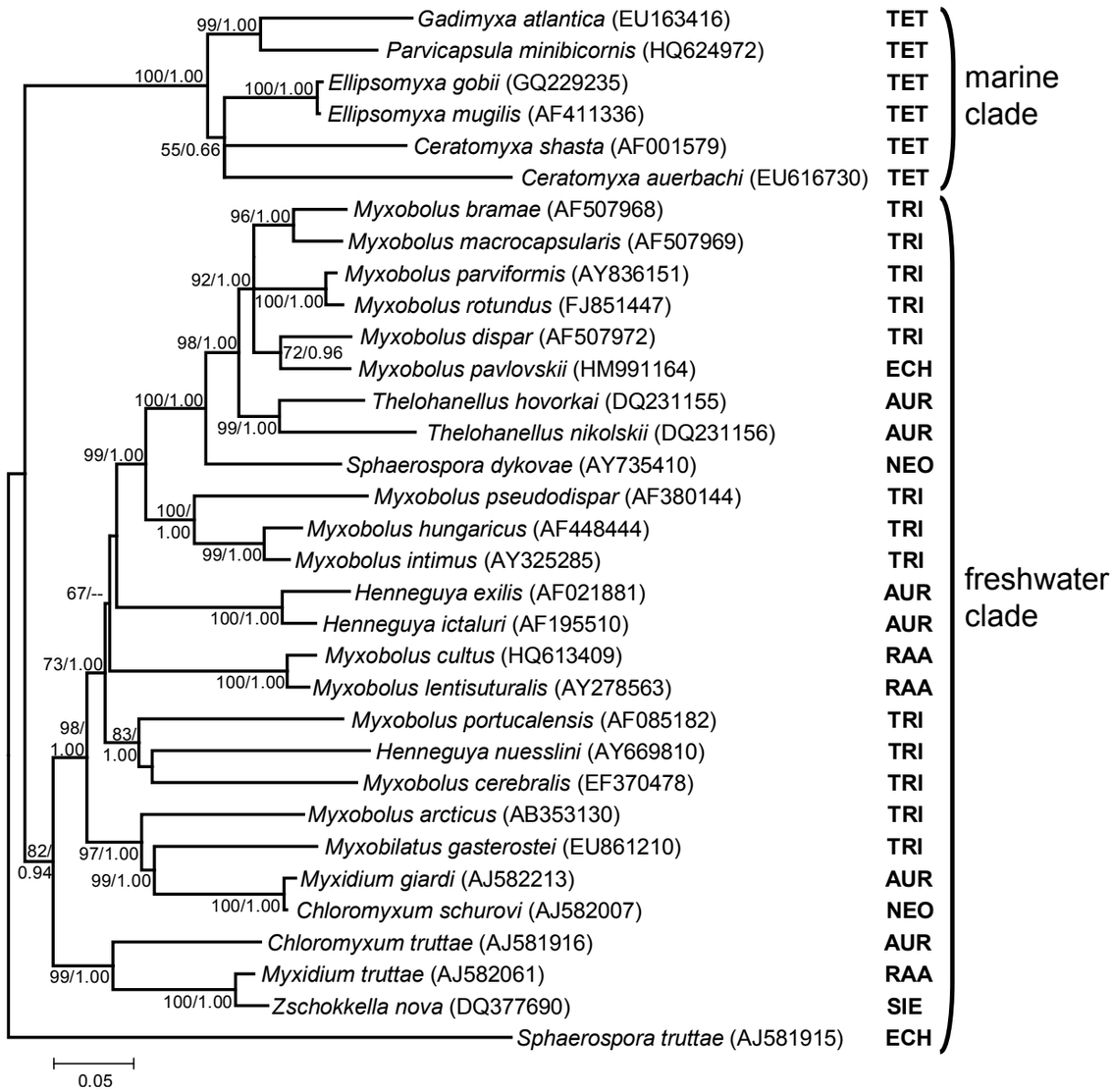
2.4 Phylogeny of myxosporeans and actinospore-types

As far as we know, the corresponding actinospore stages have been identified for 39 myxosporean species. Among them, 18S rDNA sequences of 33 species were registered in GenBank for either myxospore or actinospore stages, or both (Table 3). Cladistic analysis of myxosporean and actinospore-types revealed a lack of taxonomic congruity between the

Myxosporean species	GenBank No.	Actinospore type	GenBank No.
Ceratomyxa shasta	AF001579	Tetractinomyxon	nr
Ceratomyxa auerbachi	EU616730	Tetractinomyxon	EU616733
Chloromyxum schurovi	AJ581917	Neoactinomyxum	AJ582007
Chloromyxum truttae	AJ581916	Aurantiactinomyxon	AJ582006
Ellipsomyxa gobii	GQ229235	Tetractinomyxon	AY505127
Ellipsomyxa mugilis	AF411336	Tetractinomyxon	EU867770
Gadimyxa atlantica	EU163416	Tetractinomyxon	EU163412
Henneguya exilis	AF021881	Aurantiactinomyxon	nr
Henneguya ictaluri	AF195510	Aurantiactinomyxon	nr
Henneguya nuesslini	AY669810	Triactinomyxon	nr
Myxidium giardi	AJ582213	Aurantiactinomyxon	nr
Myxidium truttae	AJ582061	Raabeia	AJ5820009
Myxobilatus gasterostei	EU861210	Triactinomyxon	EU861209
Myxobolus arcticus	AB353130	Triactinomyxon	AB353128
Myxobolus bramae	AF507968	Triactinomyxon	nr
Myxobolus cerebralis	EF370478	Triactinomyxon	MCU96492
Myxobolus cultus	HQ613409	Raabeia	AB121146
Myxobolus dispar	AF507972	Raabeia	nr
Myxobolus hungaricus	AF448444	Triactinomyxon	nr
Myxobolus intimus	AY325285	Triactinomyxon	nr
Myxobolus lentisuturalis	AY278563	Raabeia	nr
Myxobolus macrocapsularis	AF507969	Triactinomyxon	nr
Myxobolus parviformis	AY836151	Triactinomyxon	AY495704
Myxobolus pavlovskii	HM991164	Echinactinomyxon	nr
Myxobolus portucalensis	AF085182	Triactinomyxon	nr
Myxobolus pseudodispar	AF380144	Triactinomyxon	EF466088
Myxobolus rotundus	EU710583	Triactinomyxon	FJ851447
Parvicapsula minibicornis	HQ624972	Tetractinomyxon	DQ231038
Sphaerospora dykovae (= *S. renicola*)	AY735410	Neoactinomyxum	nr
Sphaerospora truttae	AJ581915	Echinactinomyxon (?)	nr
Thelohanellus hovorkai	DQ231155	Aurantiactinomyxon	DQ231155
Thelohanellus nikolskii	DQ231156	Aurantiactinomyxon	nr
Zschokkella nova	DQ377690	Siedleckiella	nr

Table 3. List of myxosporean species and the corresponding actinosporean types registered in GenBank. nr: not registered.

two stages (Xiao & Desser, 2000a). Different phenotypes may be subject to environmental factors. Since the first study of molecular relationship between myxosporean and actinospore-types based on the 18S rDNA by Holzer et al. (2004), some more life cycles of marine myxosporeans have been discovered. Thus we update the phylogenetic analysis of species, where both life stages are described using the data available in GenBank. It is widely accepted that freshwater and marine myxosporeans are separated into two major branches (Kent et al., 2001, Fiala, 2006), and the phylogenetic tree in the present study also supports this (Fig. 7). Further, the close relationship between the marine clade myxosporeans and the



TET: tetractinomyxon, TRI: triactinomyxon, ECH: echinactinomyxon, AUR: aurantiactinomyxon, NEO: neoactinomyxum, RAA: raabeia, SIE: siedleckiella.

Fig. 7. Phylogram of myxosporeans based on 18S rDNA. Bayesian and maximum likelihood analyses. Myxosporean species names were followed by GenBank accession numbers in parenthesis and the corresponding actinospore-types.

tetractinomyxon-type actinospores has been strongly supported, whereas no obvious pattern was observed for actinospore morphology for the freshwater clade myxozoans.

To date, the information on myxozoan life cycles is still limited. Therefore, the species used for this phylogenetic analysis only covers a small portion of the wide myxozoan diversity. This leads to instability in some parts of the tree. The marine "tetractinomyxon-clade" was well defined, only the position of *C. auerbachi* and the rather aberrant *C. shasta* was not resolved properly. Considering that the hosts for *C. shasta* are anadromous salmonids and a polychaete which is typically marine, *C. shasta* may be an originally marine parasite, which migrated secondarily to the freshwater environment. The inclusion of further *Ceratomyxa*-species would probably help to stabilize this placement, but unfortunately *C. shasta* and *C. auerbachi* are the only species of the genus where both actinospore and myxospore are known. The exact positions of *M. cerebralis*, *H. exilis* + *H. ictaluri*, *H. nuesslini*, *M. portucalenis* and *M. cultus* + *M. lentisuturalis* at the base of the "*Myxobolus*-clade" were not clarified. Again, the inclusion of more species might stabilize their branches. The placement of *M. rotundus* with *M. parviformis* is quite different compared to the analysis of Fiala (2006), but the sequence of the actinospore was used in the present study, because it exhibits a higher quality at the 3′ end compared to the available myxospore-sequence of this species.

According to the hypothesis of Fiala & Bartošová (2010), who stated that the common ancestor of myxozoans was a freshwater species, the congruence of the marine clade actinospore-type (tetractinomyxon) might reflect the divergence of freshwater and marine myxozoans. When colonizing polychaetes as hosts, the tetractinomyxon type of spore developed or was already present in the freshwater ancestor of marine myxozoans. This actinospore-type persisted at least in most myxozoans parasitizing marine polychaetes that we know to date. Knowledge of more life cycles of marine myxozoans is necessary to provide information on marine actinosporean diversity. At present, there are 13 marine actinosporeans for which the myxosporean stage of the life cycle is still unknown (Table 4); 2 types of endocapsa from oligochaetes, 3 types of sphaeractinomyxon from polychaetes, 4 tetractinomyxon from polychaetes and sipunculids, 2 tetraspora from oligochaetes, 1 triactinomyxon from oligochaete, and 1 unicapsulactinomyxon from polychaete. Among them, most of the oligochaetes are benthic living in beach sediments whereas most of the polychaetes are sedentary tube worms (fan worm) attaching on the rocks or shells in coastal areas. The sipunculid (peanut worm) lives in shallow waters, either in burrows or in discarded shells.

2.5 Biology of actinosporeans

Since the discovery of the life cycle of *M. cerebralis* by Wolf & Markiw (1984), many scientists have focused on biological studies of actinosporeans, such as emergence from annelid hosts, waterborne stage, invasion mechanisms, and the portals of entry into fish host. Invasion process has been also investigated in relation to the mechanisms in the host specificity of the parasites. The current knowledge on the aforementioned points is summarized below.

2.5.1 Methodology for actinosporean biology

To obtain materials for research on actinosporeans, it is desirable to maintain the life cycle of the model-myxosporean in the laboratory. Released actinospores can be harvested by

Actinospore type	Invertebrate host	Corresponding myxosporean
Endocapsa rosulata (Hallett et al., 1999)	*Heterodrilus* cf. *keenani* (Oligochaeta)	nd
Endocapsa stepheni (Hallett et al., 1999)	*Heterodrilus* cf. *keenani* (Oligochaeta)	nd
Sphaeractinomyxon stolci (Caullery & Mesnil, 1904)	*Clitellio, Peloscolex, Tubifex* (Oligochaeta)	nd
Sphaeractinomyxon ersei (Hallett et al., 1998)	*Doliodrilus diverticulatus* (Oligochaeta)	nd
Sphaeractinomyxon leptocapsula (Hallett et al., 1999)	*Heronidrilus* sp. (Oligochaeta)	nd
Tetractinomyxon (Køie et al., 2008)	*Chone infunduliformis* (Sabellidae: Polychaeta)	*Ceratomyxa auberbachi*
Tetractinomyxon (Køie et al., 2004)	*Nereis diversicolor* and *N. succinea* (Nereididae: Polychaeta)	*Ellipsomyxa gobii*
Tetractinomyxon (Køie et al., 2007)	*Spirorbis* sp.(Spirorbidae: Polychaeta)	*Gadimyxa atlantica*
Tetractinomyxon (Køie, 2002)	*Hydroides norvegica* (Polychaeta)	nd
Tetractinomyxon (Køie, 2005)	Unidentified spionid (Polychaeta)	nd
Tetractinomyxon intermedium (Ikeda, 1912)	*Nephasoma minuta* (Sipunculidae: Sipuncula)	nd
Tetractinomyxon irregulare (Ikeda, 1912)	*Nephasoma minuta* (Sipunculidae: Sipuncula)	nd
Tetraspora discoidea(Hallett & Lester, 1999)	*Doliodrilus diverticulatus* (Oligochaeta)	nd
Tetraspora rotundum (Hallett & Lester, 1999)	Tibificidae spp. (Oligochaeta)	nd
Triactinomyxon (Roubal et al., 1997)	*Duridrilus* sp. (Oligochaeta)	nd
Unicapsulactinomyxn (Rangel et al., 2011)	*Diopatra neapolitana* (Polychaeta)	nd

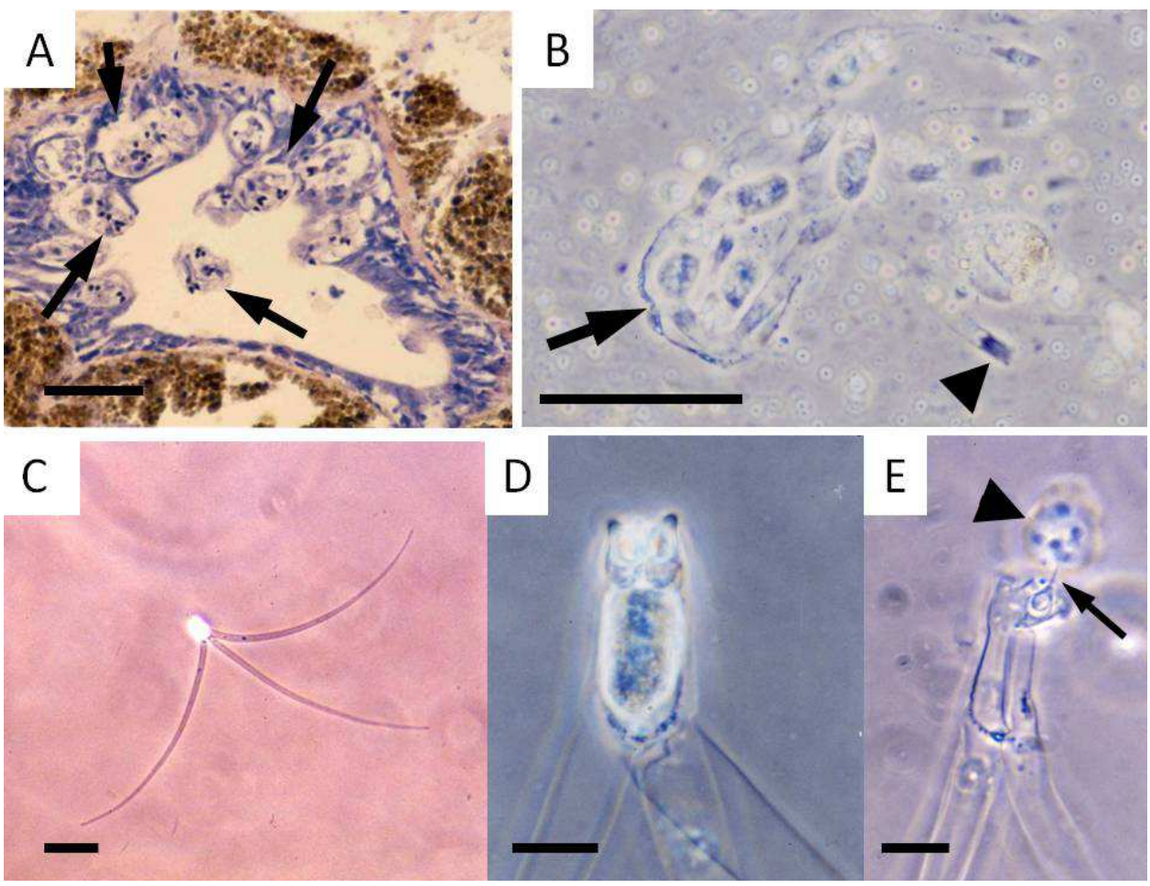
Table 4. Marine actinosporeans from annelids or sipunculids. nd: not determined.

filtering of the aquarium water through mesh screens (El-Matbouli et al., 1995). If a laboratory system is not available, study materials are obtained from naturally infected wild invertebrate worms. Yokoyama et al. (1991) developed a multi-well plate method to collect actinospores of a single myxozoan species. Oligochaetes are placed individually in wells filled with dechlorinated tapwater. One of the advantages of this method is that even small-size actinospores which are hard to trap by filtration can be collected easily from wells. However, it may be difficult to apply this method to fragile or large-size worms. Also, if actinospores are released after host death, the well plate method will be inapplicable

(Rangel et al., 2009). In that case, worms may be crushed on a glass slide with gentle pressure. However, Rangel et al. (2011) successfully obtained marine actinosporeans of *Zschokkela mugilis* from the coelomic fluid of the polychaete host with a hypodermic needle and syringe. To determine the viability of actinospores, presence or absence of the sporoplasms in the spore body has been used as an indicator (Yokoyama et al., 1993, Xiao & Desser, 2000b), because aged actinospores spontaneously release sporoplasms so that spores become empty. Alternatively, a vital staining technique with fluorescein diacetate (FDA) and propidium iodide (PI) can be applied (Markiw, 1992, Yokoyama et al., 1997b, Wagner et al., 2003, Kallert et al., 2005).

2.5.2 Emergence pattern

Most of freshwater actinosporeans infect the intestinal epithelium of oligochaetes and emerge into the environment by defecation (Fig. 8A, B), whereas *C. shasta* actinosporeans



A: Pansporocysts (arrows) develop in the intestinal epithelium of *Branchiura sowerbyi*. B: Pansporocyst is excreted from *B. sowerbyi*. As the pansporocyst membrane (arrow) is ruptured, actinospores are released. Tip of the caudal process is still folded (arrowhead). C: Free actinospore. Note completely unfolded processes. D & E: Chemical response of actinospore to fish mucus. D: Intact spore. E: Empty spore releasing sporoplasm (arrowhead) immediately after contact with mucus. Polar filaments (arrow) are discharged. Scale bars for A, B and C are 50 µm, and those for D and E are 10 µm.

Fig. 8. Process of emergence, floating and invasion of *Myxobolus cultus* actinospores.

develop in the epidermis of the polychaete *Manayunkia speciosa* and actinospores are released directly from the epidermis into the water column (Meaders & Hendrickson, 2009). In many myxosporean species, actinospores are shed from the annelid hosts between spring and summer (Yokoyama et al., 1993, El-Mansy et al., 1998a, b, Özer & Wootten, 2002), which may be an adaptation to synchronize with hatching and growing seasons of larval fish. However, in some species, actinospores are released throughout the year. Prevalence of infection in the invertebrate hosts has been reported to be relatively low, 0.1-4% (Yokoyama et al., 1993, Özer & Wootten, 2002), but in some cases, it reached extremely high value of over 90% (El-Mansy et al., 1998a). Actinospore release may persist for the natural life-span of oligochaete hosts, at least for 2 years in case of *Tubifex tubifex* infected with *M. cerebralis* (Gilbert & Granath, 2001). Actinospore emergence follows a circadian rhythm with a significant peak in the middle of the night or early morning (Yokoyama et al., 1993, Özer & Wootten, 2001). It is unclear if this daily pattern in spore release is due to the rhythm of the oligochaete itself or of the actinosporean, and the ecological significance of this phenomenon for transmission to the next host remains to be investigated. Alteration of the photoperiods affected the release pattern of actinospores (Yokoyama et al., 1993), and thus artificial control of lighting condition may have some effects on myxosporean transmissions in the field.

2.5.3 Waterborne stage

Actinosporeans with long processes are buoyant (Fig. 8C) and can remain suspended in the water column for more than 24 hours (Kerans & Zale, 2002). Longevity of actinospores in the water ranges from 4 to 25 days, depending on temperature and species (Markiw, 1992, Yokoyama et al., 1993, Xiao & Desser, 2000b). Life-span decreases with increasing temperature (Yokoyama et al., 1993, Özer & Wootten, 2002). At ambient temperature (20 ^{o}C), viability of raabeia actinospores persisted for 10 days, while echinactinomyxon spores survived for 21 days (Yokoyama et al., 1993). In contrast, Özer & Wootten (2002) reported that raabeia and synactinomyxon spores remain viable only for 2-3 days at 22 ^{o}C. Markiw (1992) showed that the infectivity of actinospores of *M. cerebralis* persisted for 3-4 days at 12.5 ^{o}C, whereas El-Matbouli et al. (1999a) indicated that *M. cerebralis* actinospores survived and maintained their infectivity for 15 days at 15 ^{o}C. Using morphological characteristics and vital staining technique, Kallert & El-Matbouli (2008) showed that actinospores of the myxosporean species survive longer at lower temperature (4 °C vs. 12 °C). *M. cerebralis* actinospores were most sensitive and showed a significant decrease of viability already after 1 d at 12 °C, while *M. pseudodispar* and *Henneguya nuesslini* survived longer, even at 12 °C. Water flow has been recognized as an environmental factor which have some effects on myxsporean infections (Hallett & Bartholomew, 2008, Bjork & Bartholomew, 2009). Higher water velocity resulted in lower infection prevalence of *C. shasta* in polychaete and decreased infection severity in fish (Bjork & Bartholomew, 2009). During the planktonic phase of actinospores, high flow velocity may cause mechanical damages and dilution effects on actinospores. Also, high flow rates may limit the time for actinospores to encounter and attach to the fish host (Hallett & Bartholomew, 2008).

2.5.4 Invasion mechanisms

Polar filament discharge and sporoplasm release of actinospores are induced by chemical responses to fish mucus (Fig. 8D & E), suggesting the role of chemoreception in the host

attachment of actinospores (Yokoyama et al., 1993, 1995, Uspenskaya, 1995, McGeorge et al., 1997, Xiao & Desser, 2000b). However, the percentage of actinospores reacting to the mucus varied among fish and parasite species (Yokoyama et al., 1993, Özer & Wootten, 2002). Thus, it is not clearly understood whether the chemical stimulation with fish mucus reflects the host specificity of myxosporeans. Actinospores of *M. cultus* reacted not only to the skin mucus from natural host but also to the mucus from abnormal host (Yokoyama et al., 1993) and even to mucin from bovine submaxillary gland (Yokoyama et al., 1995). Further, purification of the reactants from fish mucus by gel filtration and ultrafiltration revealed that they were low-molecular-weight (<6000 MW) substances (Yokoyama et al., 1995). Yokoyama et al. (2006) indicated that *M. arcticus* actinospores reacted to the mucus of the susceptible host, masu salmon (*Oncorhynchus masou*) as well as non-susceptible hosts, sockeye salmon (*O. nerka*) and goldfish (*Carassius auratus*), whereas *T. hovorkai* actinospores reacted only to the susceptible host, common carp (*Cyprinus carpio*). In contrast, actinospores of *Myxobolus cerebralis* did not react to fish mucus alone (El-Matbouli et al., 1999b) and required both mechanical and chemical stimuli (Kallert et al., 2005). Nevertheless, *M. cerebralis* actinospores were unable to specifically detect susceptible fish (salmonids), but also penetrated gills of carp at the same rate as gills of trout (Kallert et al., 2009). Further, Kallert et al (2007) revealed the process of host invasion of *M. cerebralis* actinospores in detail; immediately after filament discharge of actinospores, contraction of the filaments brings the actinospore apex to contact with the host surface. Then, opening of the apical valves is followed by penetration of the sporoplasms through the epithelium. The active fraction inducing the polar filament discharge of *M. cerebralis* actinospores was small molecular, amphiphilic to slightly hydrophobic organic substances (Kallert et al., 2010). More recently, several nucleosides derived from surface mucus of fish, inosine, 2'-deoxyinosine and guanosine have been determined by HPLC method as 'chemical cues' triggering host recognition for *M. cerebralis* actinospores (Kallert et al., 2011).

2.5.5 Portals of entry into fish

Entry of myxozoans into the fish host via the skin, fins and buccal cavity was first demonstrated in rainbow trout experimentally exposed to actinospores of *Myxobolus cerebralis* by Markiw (1989). Within 5-10 min of exposure, aggregates of sporoplasms were observed in the epithelia of exposed fish (Markiw, 1989, El-Matbouli et al., 1995). Further, El-Matbouli et al. (1999b) revealed by scanning electron microscopy that *M. cerebralis* actinospores penetrate into the secretory openings of the mucous cells of the epidermis. Belem & Pote (2001) showed by indirect fluorescent antibody test that *Henneguya ictaluri* has the multiple entry sites; the gut mucosa, skin and buccal cavity of the channel catfish (*Ictalurus punctatus*). Some actinospores may be able to enter the fish through different portals of entry. *Sphaerospora truttae* and *Ceratomyxa shasta* utilize predominantly the gills as entry site (Holzer et al., 2003, Bjork & Bartholomew, 2010). Yokoyama & Urawa (1997) suggested that small actinospore (aurantiactinomyxon) invade the fish through the gills, whereas large actinospores (triactinomyxon and raabeia) penetrate mainly through the fin and skin.

2.5.6 Other biological characteristics

Effects of physical and chemical treatments on viability of actinosporeans were investigated, although the information is available only for *Myxobolus cerebralis* and *Myxobolus cultus*. For

M. cerebralis, drying at room temperature for 15 min, freezing at -20ºC for 1 hour, temperatures above 75 ºC for 5 min and sonication (47 kHz, 130 W) for 10-13 min were effective in killing actinospores, but pressure of 6.2 x 10^7 Pa (9000 psi) was not (Wagner et al., 2003). To inactivate actinospores of *M. cerebralis* chemically, chlorine of 13 ppm for 10 min, hydrogen peroxide of 10% for 10 min, and povidone-iodine of 50% solution (5000 ppm active iodine) for 60 min were effective (Wagner et al., 2003). Electricity with a pulse length of 99 µsec at 3 kV induced polar filament discharge of *M. cerebralis* actinospores, suggesting a potential use of direct current as a means of disinfection (Wagner et al., 2002). For *M. cultus*, drying at 5 ºC for 1 day and ultraviolet irradiation at 600 mW s cm^{-2} were highly effective in killing actinospores, whereas sodium chloride of 0.5% had a moderate effect (Yokoyama et al., 1997b). However, even high concentrations of malachite green (10 ppm), metrophonate (5 ppm) and formalin (1000 ppm) did not affect the treated spores (Yokoyama et al., 1997b).

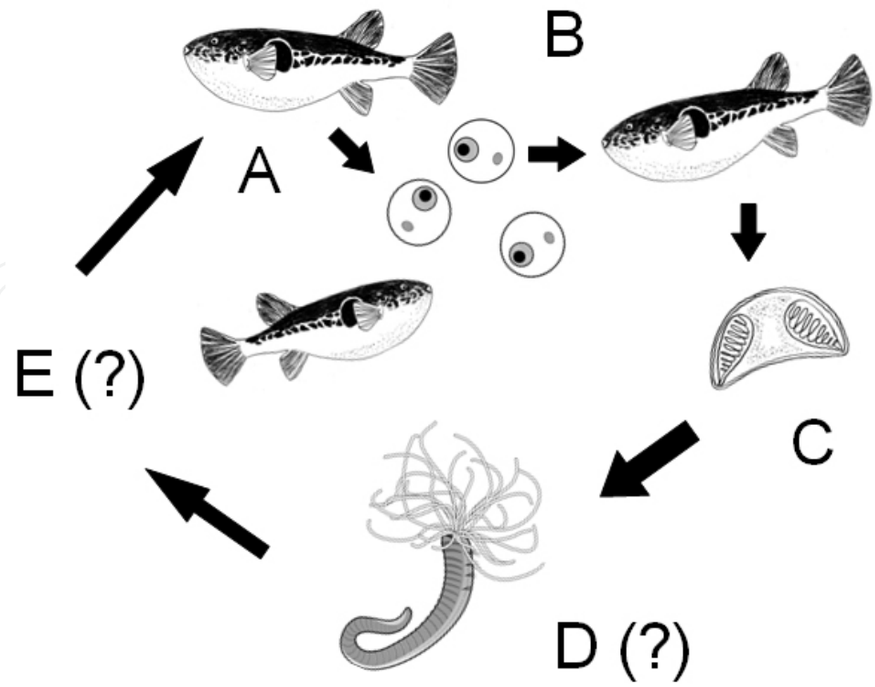
Actinosporean infections are also influenced by various biological and ecological factors, such as host (annelid) susceptibility, water temperature, and sediment type. Susceptibility to *M. cerebralis* varied among different genetic strains of *T. tubifex* (Beauchamp et al., 2002). Development and release of *M. cerebralis* actinospores from *T. tubifex* were temperature-dependent; High temperatures above 20 ºC were lethal for the parasite, whereas low temperatures between 5 and 10 ºC delayed development, and moderate temperatures between 15 and 20 ºC accelerated development, and increased the number of spores released (El-Matbouli et al., 1999a). Blazer et al. (2003) also reported a similar pattern of temperature effects on development of *M. cerebralis* actinospores in *T. tubifex*. Environmental factors like substratum and water quality may influence the actinosporean production. Blazer et al. (2003) indicated that the mud substrate produced the highest total number of *M. cerebralis* actinospores in *T. tubifex*, whereas the leaf litter was the least productive substratum in number of actinospores released. Aquatic oligochaetes have habitat preferences which are closely associated with some environmental parameters, such as substrate type, texture, nutritional potentials, and anaerobic conditions (Koprivnikar et al., 2002, Liyanage et al., 2003). Actinospore production of *M. cerebralis* is also affected by environmental pollutants (Shirakashi & El-Matbouli, 2010).

3. Fish-to-fish transmission of marine myxosporeans

Enteromyxum leei develops within the gut epithelium of marine fish, and the developmental stages are excreted to the water (Fig. 9). Released stages are orally ingested by other fish, resulting in establishment of horizontal infection (Diamant, 1997, Yasuda et al., 2002, 2005, Sitja-Bobadilla et al., 2007). This route of transmission may occur only in intensive culture systems, where it facilitates rapid spread of the parasite. Broad host range of *E. leei* also appears to assist the parasite's dispersion (Diamant et al., 2006). Indeed, an episode of enteromyxosis in 25 different fish species in an exhibition aquarium was reported (Padrós et al., 2001). *E. scophthalmi* and *E. fugu* also transmit from fish to fish directly, but their host ranges are narrow.

3.1 Infective developmental stages of *Enteromyxum* spp. in water column

Although actinosporean stages for *Enteromyxum* spp. have not been discovered, some biological characteristics of infective developmental stages have been investigated. Viability



A: *E. leei* develops in the intestine, followed by excretion through the vent. B: Developmental stages are horizontally transmitted to other fish by oral ingestion. C: Mature myxospores are released into water column. D: Myxospore possibly infects marine annelids. E: Actinospore is possibly released from annelids, followed by infection to fish.

Fig. 9. Diagram of fish-to-fish transmission and putative life cycle of *Enteromyxum leei*

of *Enteromyxum* spp. stages was determined *in vitro* by dye-exclusion assays (Redondo et al., 2003, Yokoyama & Shirakashi, 2007), tetrazolium-based cell-proliferation assay (Redondo et al., 2003), and vital staining with fluorescent dyes, Hoechst 33342 and propidium iodide (Yokoyama et al., 2009). Longevities of *E. leei* and *E. scophthalmi* were estimated to be at most 1 day in seawater (Redondo et al., 2003, Yokoyama et al., 2009). However, intestinal mucosal remnants covering the parasites may protect them from osmotic shock, resulting in retaining their viability in seawater (Redondo et al., 2002, Yokoyama et al., 2009). Survivability of developmental stages of *E. leei* decreased significantly in low salinity of less than 8‰ (Yokoyama & Shirakashi, 2007). Also, fish size and parasite dose likely affect the success of fish-to-fish transmission (Sitja-Bobadilla et al., 2007, Yokoyama & Shirakashi, 2007).

3.2 Invasion and development of *Enteromyxum* spp. in fish host

Following ingestion of the infective stages, the first barrier is the intestinal mucosa of the fish. A role of lectin/carbohydrate interaction in the turbot-*E. scophtahlmi* relationship was suggested (Redondo & Alvarez-Pellitero, 2009). Further, attachment and invasion of *E. scophthalmi* to the turbot intestinal epithelium were inhibited by pre-treatments of parasites by some lectins, Con A and SBA, suggesting the involvement of N-acetyl-galactosamine and galactose residues and also of mannose/glucose residues (Redondo & Alvarez-Pellitero, 2010). After penetration of the developmental stages into the intestinal epithelium, several factors are involved in the progression of the disease (Quiroga et al., 2006). One of the most

important factor is water temperature.Yanagida et al. (2006) showed that temperatures below 15 ºC suppressed the development of *E. leei* and onset of the disease, but a temperature increase to 20 ºC promoted *E. leei* development. Similarly, infection with *E. scophthalmi* was established earlier at higher temperature (Redondo et al., 2002).

4. Malacosporeans

The class Malacosporea is a recently discovered group, and only three species belonging to two genera have been described to date (Canning & Okamura, 2004; Canning et al., 2007). All of them are known to be parasites of freshwater bryozoans, but the life cycle is described only for *T. bryosalmonae*. Besides the bryozoan stage, it involves the infection of salmonid fish (Saulnier et al., 1999) where the parasite causes the Proliferative Kidney Disease (PKD). According to recent findings, species of the second malacosporean genus *Buddenbrockia* might also require a fish host in their life cycles (Grabner & El-Matbouli, 2010a).

4.1 Morphology of malacosporeans in bryozoan hosts (bryozoa-spores)

Malacosporean spores developing in the bryozoan host (bryozoa-spores) are small (15 – 20 µm), approximately spherical without appendices (Fig. 10). They consist of two haploid sporoplasms including one secondary sporoplasm cell each, four capsulogenic cells and eight valve cells (Canning et al., 2000, McGurk et al., 2005). Only minimal morphological differences have been recorded between spores of different malacosporean species. Morris et al. (2002) documented ornamented spores with a mean diameter of 19.0 µm in the bryozoan *Plumatella repens* infected with worm-like malacosporean stages. The spores observed by McGurk et al. (2006a), also released by a worm-shaped malacosporean in *P. repens*, were spherical and 17.7 µm in diameter.

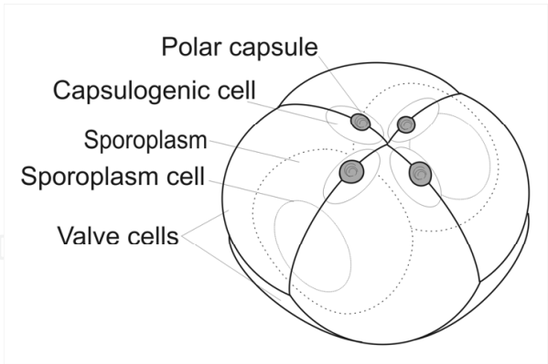


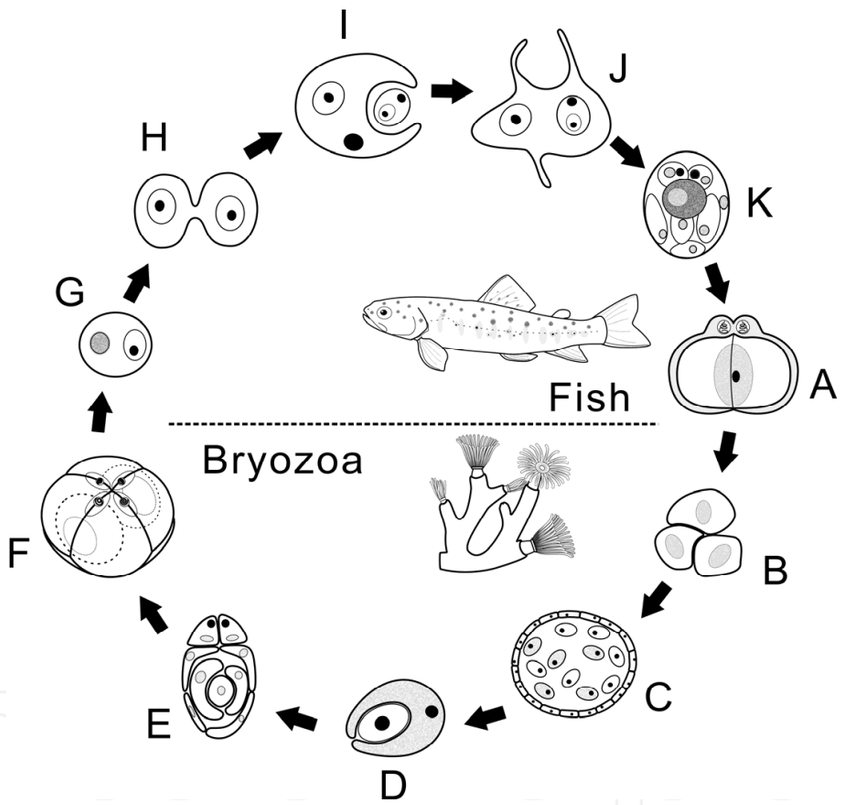
Fig. 10. Diagram of malacosporeans in bryozoans (bryozoa-spores).

4.2 Morphology of malacosporean in fish hosts (fishmalacospores)

To date, fresh and mature fishmalacospores were only described for *T. bryosalmonae*. They are about 12 × 7 µm in size and bear two polar capsules with 4 to 6 turns of their polar filament, one sporoplasm and four valve cells (Kent et al., 2000, Hedrick et al., 2004, Morris & Adams, 2008). Apparently, only few fishmalacospores are released at a time by *T. bryosalmonae*-infected fish, because only small numbers of spores were found in urine samples from infected fish over a prolonged period of time (Hedrick et al., 2004).

4.3 Life cycle of malacosporeans

Life cycles of malacosporeans still remain mysterious, but recent studies have revealed most parts of the development and transmission of *T. bryosalmonae* (Canning et al., 1999, Kent et al., 2000, Morris & Adams, 2006a). This parasite develops as sac like stages in the body cavity of freshwater bryozoans, followed by release of malacospores to the surrounding water. When the spores come in contact to the skin or gills of a fish host (salmonid), the sporoplasm penetrates the epithelium and is transported by the blood into the kidney interstitium, causing PKD. Sporogony commences after migration to the kidney tubules and mature spores are released with the urine to the water, where they are infective for bryozoans (Fig. 11). The whole development, beginning with the penetration into the fish, to the presence of mature spores in the kidney tubules takes about 9 weeks in brown trout (Morris & Adams 2006a).



A: Fishmalacospore infects freshwater bryozoans. B: Presaccular cell aggregates in coelomic cavity of bryzoans. C: Early spore sac floating in bryozoan coelomic fluid. It contains stellate and sporogenic cells. D: Sporogenic cell becomes enclosed by stellate cells. E: Maturing spore with casulogenic cells, valve cell and forming sporoplasms. F: Mature bryozoa-spore infects fish. G: Proliferative stage (cell doublet with primary cell and secondary cell inside) in kidney interstitium. These stages are in close contact to host phagocytes (not shown). H: Division of cell doublet resulting in 2 cell doublets. I: Engulfment of one cell doublet by another resulting in a S-T-doublet (primary cell enclosing one secondary and one secondary with tertiary cell). J: S-T-doublet in kidney tubule. Note that contact to the host phagocyte is lost during migration through the tubule epithelium. K: Sporogony inside of primary cell (pseudoplasmodium).

Fig. 11. Diagram of the life cycle of *T. bryosalmonae* (Malacosporea), alternating between fish and bryozoan host.

T. bryosalmonae can infect a wide variety of salmonid fish. Most affected are species of the genera *Salmo* and *Oncorhynchus*, but also *Salvelinus* species (Hedrick et al., 1993, El-Matbouli & Hoffmann, 1994). Severe outbreaks of the disease were also noted in grayling (*Thymallus thymallus*) (Hoffmann & Dangschat, 1981). Northern Pike (*Esox lucius*) is the only non-salmonid fish species, in which extrasporogonic stages similar to those of *T. bryosalmonae* were found (Seagrave et al., 1981, Morris et al., 2000a). It was observed that fish become resistant against reinfection with *T. bryosalmonae* after surviving the disease (Ferguson, 1981, Foott & Hedrick, 1987). However, in some fish species sporogonic stages seem to persist after clinical infection and possibly continue to form spores chronically (Kent et al., 1998, Kent et al., 2000). Recently it was shown by transmission experiments conducted with European parasite lineages that brown trout (Morris & Adams, 2006a) and brook trout (Grabner & El-Matbouli, 2008) can transmit the parasite to bryozoans. In contrast, rainbow trout and grayling became infected, but no infection appeared in bryozoans cohabitated with these fish. But as mature fishmalacospores were reported from rainbow trout infected with *T. bryosalmonae* in North America, it seems likely that there is a regional difference in host specificity (Morris & Adams, 2006a). Additionally, infection experiments have shown that common carp (*Cyprinus carpio*) and minnow (*Phoxinus phoxinus*) can become infected by *Buddenbrockia* species, but the proof for the completion of the life cycle is still missing (Grabner & El-Matbouli, 2010a). Additionally, intra-bryozoan cycles without involvement of a fish host might be possible for some malacosporeans (Hill & Okamura 2007).

4.4 Biology of malacosporeans

Knowledge on the biology of malacosporeans is still limited. Most information exists for *T. bryosalmonae*, while the understanding of life cycles of other malacosporeans is still in its infancy. The information concerning the transmission of malacosporeans will be summarized below.

4.4.1 Emergence pattern

Occurrence of PKD is seasonal and occurs from spring till autumn. This can be explained by the higher abundance of the bryozoan host in warmer months and therefore higher spore load in the water, but also by increase in severity of infection in fish at higher temperatures (Foot & Hedrick, 1987, Hedrick et al., 1993). It has to be noted, that in most cases infections with *T. bryosalmonae* become apparent only in trout farms. Mortalities or diseased fish in the wild are not found in most cases. Therefore, the dynamics of natural life cycles are difficult to investigate (Okamura et al., 2011).

4.4.2 Waterborne stage

Malacosporean bryozoa-spores do not possess hard valves for protection against external damage. Therefore, they are very short-lived and lose their infectivity after about 24h (de Kinkelin et al., 2002). Hedrick et al. (2004) described that fishmalacospores degrade already within minutes on a microscope slide. The floating characteristics of malacosporean spores are not investigated, but the lack of processes that might prevent sinking down in the water column and short life-span suggest that contact to the host must occur soon after release of spores.

4.4.3 Portals of entry into fish

The *T. bryosalmonae* spores released from parasitized Bryozoa most likely enter the fish through the gills (Morris et al., 2000b, Holzer et al., 2006, Grabner & El-Matbouli, 2010b) or the mucus cells of the skin (Longshaw et al., 2002), while the blood stream was considered to be the most probable route to the target organs (Morris et al., 2000b, Holzer et al., 2006). The infection seems to be very effective that one single spore is sufficient to infect a fish and to cause clinical symptoms of PKD (McGurk et al., 2006b).

4.4.4 Other biological characteristics

Transfer of malacosporeans to new habitats can also occur by fragmentation and reattachment of bryozoan colonies, which was found to be common for *Fredericella sultana*-colonies (Morris & Adams, 2006b). Another way for propagation of malacosporeans without a fish host might be the infection of durable stages (statoblasts) of bryozoans (Hill & Okamura, 2007). Water quality, especially increase of organic material, seems to influence disease outbreaks, most likely by fostering growth of bryozoan colonies and thereby increasing numbers of infective stages in the water (El-Matbouli & Hoffmann, 2002).

5. Control strategies of myxozoans

To date, there are no commercially available chemotherapeutants and vaccines to treat myxozoan infections. Thus, the current disease control strategies can only be based on the biology of myxozoans. Compared to myxospores, waterborne actinospores are generally short-lived and highly susceptible to several treatments. The actinospore stage can be considered as 'weak point' in the life cycle of myxozoans and should be targeted for the control strategy. This paragraph deals with possible control strategies of myxozoan diseases with emphasis on prevention of transmission to fish hosts.

5.1 Eradication of invertebrate hosts

In case of most myxozoans with indirect life cycle, transmission success largely depends on the size of population of invertebrate hosts. The most effective way for prevention of myxozoan transmission is to eradicate the invertebrate hosts in the aquaculture environment. Habitat manipulation may be an effective means to remove oligochaetes for example by dredging mud from the pond bottom or by conversion of earthen ponds to concrete raceways. Replacing the muddy substrate with coarse sand reduced the number of *Branchiura sowerbyi* which is the alternate oligochaete host for *Thelohanellus hovorkai*, mitigating the hemorrhagic thelohanellosis of carp (Liyanage et al., 2003). This was explained by a delicate body surface of *B. sowerbyi* was damaged by rugged-edged sand particles. Removing the vegetation upstream of the water inlet to a fish farm with PKD problems is considered as a possibility for prevention of PKD-outbreaks because it reduces habitats for bryozoan and spore load in the water, but this measure is not be feasible in most cases (de Kinkelin et al., 2002). Besides the substrate amendment for eliminating the habitat of invertebrate hosts, use of a benthos-eating fishes as a biological control of oligochaete abundance is worth considering in fish farms (Yokoyama et al., 2002).

5.2 Avoidance of infective period

If actinospore emergence occurs only in a certain time of the year, rearing fish outside the infective period may be useful. To reduce PKD-related losses, it is recommended to delay the transfer of young fish to the endemic water until autumn when water temperature is decreasing. As bryozoan populations decline under low temperature conditions in autumn, the number of *T. bryosalmonae* spores in the water becomes significantly low at this time. Additionally, low water temperature prevents the clinical outbreak of the disease and the fish usually become resistant against this pathogen in the subsequent years (Foott & Hedrick, 1987).

5.3 Removal of actinospores

Sand filtration is highly effective in removing actinospores of *M. cerebralis* (Arndt & Wagner, 2003, Nehring et al., 2003). However, it may be applied only for large-type actinospores. The sand filtration of water supply was also suggested to prevent enteromyxosis caused by *E. scophthalmi* in turbot farms, though the actinospores of this species have not been determined (Quiroga et al., 2006). In contrast, the infective stage of *K. thyrsites* was not removed by filtration of seawater (Moran et al., 1999b). Ozone and ultraviolet treatments of water supply are also effective for disinfection of *M. cerebralis* and *C. shasta* (Sanders et al., 1972, Tipping, 1988, Hedrick et al., 2000). Increase of water velocity may dilute the density of actinospores and reduce infection severity in culture facilities or rivers where water release is managed e.g. by dams (Hallett & Barthomew, 2008, Bjork & Bartholomew, 2009). Chemical treatments with toxic compounds may be effective but not be environmentally acceptable. Biological filtration of floating actinospores using a planktonic copepod (*Cyclops* spp.) may be practical in fish farms (Rácz et al., 2006). Murakami (1983) reported based on his empirical observations that rearing of rainbow trout, which is non-susceptible to *M. murakamii,* upstream of the masu salmon farm reduced the myxosporean sleeping disease, suggesting that rainbow trout plays a role of biological filter of the waterborne infective stage of *M. murakamii*. This may be explained by the nonspecific response of actinospore to fish mucus. The same was shown experimentally for *M. cerebralis* by preincubation of carp with actinospores of the parasite. Thereby, infection rate in susceptible rainow trout was reduced significantly (Kallert et al., 2009).

5.4 Interception of fish-to-fish transmission of *Enteromyxum* spp.

Fish-to-fish transmission of *Enteromyxum* spp. should be considered as an exceptional case. Because of their direct life cycle, epidemics of *Enteromyxum* frequently occurs in closed aquaculture systems. Effective control may be achieved with the integrated management strategies. The foremost strategy is to prevent infected fish from entering the culture system. Early diagnosis using a highly sensitive PCR assay greatly reduces the potential for dispersal of *E. leei* via infected juveniles (Yanagida et al., 2005). Fish farmers should minimize their risk of pathogen introduction by cultured fish from uncertified sources. Fish cages with different age classes should not be set up in a close proximity, because infection rate may increase with fish age, and older fish could spread the pathogen to younger fish. Infected fish must be removed as soon as the disease becomes apparent. Fallowing is also effective in intercepting the transmission cycle of *Enteromyxum* spp., if all fish farmers in the area cooperate in this practice at the same time. However, wild fish living in the farming

area may act as carrier or reservoir of the parasite. In this case, the fallowing practice becomes useless, though *Enteromyxum* infection has never been detected from wild fish so far. Increase of water flow possibly lowers the chance of ingestion of infective parasites by dilution effect. Evacuation of sea cages to offshore area with faster water flow may reduce further transmission of *E. leei*. In case of land-based culture farms, increasing water exchange rates of rearing water would help to flush the waterborne parasite out of fish tank (Yokoyama et al., 2009). Development of *E. leei* is strongly influenced by water temperature. Rearing of Malabar grouper (*Epinephelus malabaricus*) at 30 ºC had both for preventive and curative effects on *E. leei* infection, although a similar treatment was not effective in tiger puffer. Hyposalinity treatment below 1/4 seawater (8‰) was effective in killing developmental stages of *E. leei* in *in vitro* (Yokoyama & Shirakashi, 2007), but *in vivo* trials where tiger puffer were reared in low salinity seawater were unsuccessful for prevention of the disease. Further studies are required to clarify these inconsistent results.

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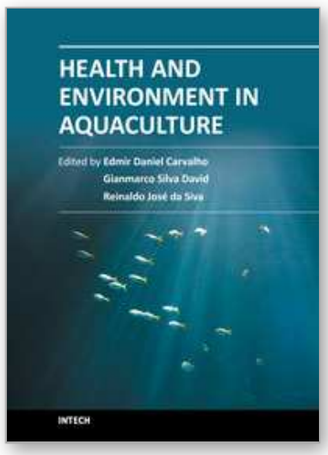
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Aquaculture has been expanding in a fast rate, and further development should rely on the assimilation of scientific knowledge of diverse areas such as molecular and cellular biology, and ecology. Understanding the relation between farmed species and their pathogens and parasites, and this relation to environment is a great challenge. Scientific community is involved in building a model for aquaculture that does not harm ecosystems and provides a reliable source of healthy seafood. This book features contributions from renowned international authors, presenting high quality scientific chapters addressing key issues for effective health management of cultured aquatic animals. Available for open internet access, this book is an effort to reach the broadest diffusion of knowledge useful for both academic and productive sector.

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