

Hematodinium/Ichthyodinium

(protist: flagellate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). One protistan supergroup known as SAR comprises the Stramenopiles (with heterokont flagella), Alveolata (with cortical alveoli) and Rhizaria (with fine pseudopodia). Three major alveolate groups are recognized: ciliates, apicomplexans and dinoflagellates. Flagellates are protists that swim using one or more flagella (undulipodia); each arising from a small centriole (basal body, kinetosome) and having a microtubular axoneme core (2+9 configuration). Phytoflagellates possess chloroplasts, which require sunlight to function, so most species are free-living aquatic organisms. Dinoflagellates have a distinctive haploid nucleus (dinokaryon) where the chromosomes remain condensed during interphase and are evident as beaded strands due to low levels of histones and associated proteins (condition termed 'mesokaryotic'). Many dinoflagellates form characteristic free-swimming dinospores (thecate and/or pigmented) and various species have been associated with coloured tides/blooms, bioluminescence and even neurotoxicity in mammals (paralytic shellfish poisoning). Some species, however, are heterotrophic and parasitic in marine animals, including the syndinids which form multinucleated plasmodial trophonts (without chloroplasts) in the tissues of crustacea and fish.

Classification:

Domain: Eukaryota (membrane-bound nucleus)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria)
Group: Alveolata (with cortical alveoli)
Phylum: Dinoflagellata (with unique mesokaryotic nuclei (lacking histones), autotrophs and heterotrophs)
Class: Syndiniophyceae (multinucleate plasmodial trophonts)
Order: Syndiniales (parasitic in copepods, appendicularians, crabs, radiolaria and fish eggs)
Family: Syndiniaceae (multinucleate trophonts without chloroplasts)
Genus: *Hematodinium* (parasitic in tissues of decapod crustaceans)
Genus: *Ichthyodinium* (parasitic in eggs of fish)
Species: various species cause lesions in fish and shellfish

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Flagellated species have one or more flagella with an internal microtubular core (in a characteristic 2+9 configuration comprising 2 single central microtubules and 9 peripheral doublets) anchored to a submembranous protein structure (known variously as a centriole, basal body, kinetosome or blepharoplast). Many types of flagellated cells have been described and recent phylogenetic studies have classified them into several disparate groups: including the metamonads (amitochondriate flagellates), heteroloboseans (amoeboid flagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). While most flagellated protists are free-living organisms swimming and feeding in aquatic environments, representatives of several groups have developed symbiotic relationships with various hosts; some being endoparasitic in vertebrates (notably anaerobic metamonads in tubular organs, and heterotrophic euglenozoans occurring in blood or tissues), and some being parasitic in invertebrates (alveolates in crustacean tissues) (representatives tabulated below).

Higher taxonomy	Class or order	Family	Genera	Hosts (tissues)	Transmission*
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups unified by molecular studies)					
Group: Alveolata (with cortical alveoli)					
Phylum: Dinoflagellata (with unique mesokaryotic nuclei)	Order: Blastodinales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	<i>Amyloodinium</i> <i>Crepidodinium</i> <i>Piscinoodinium</i>	fish (skin)	direct (w)
	Order: Syndiniales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	<i>Haematodinium</i> <i>Ichthyodinium</i>	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	<i>Perkinsus</i>	gastropods, bivalves (tissues)	direct (w)

Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: Metamonad (amitochondriate flagellates with karyomastigonts)					
Phylum: Fornicata (diplomonads)	Order: Diplomonadida (1-2 karyomastigonts)	Hexamitidae (2 karyomastigonts with binary axial symmetry)	<i>Giardia</i>	vertebrates (gut)	direct (f-o)
			<i>Hexamita</i> <i>Spiroucleus</i>	vertebrates (tissues)	direct (f-o, w)
Phylum: Parabasalia (with parabasal body)	Order: Trichomonadida (3-5 anterior flagella plus recurrent flagellum)	Monocercomonadidae (costa absent, most without undulating membrane)	<i>Histomonas</i>	birds (gut, liver)	direct (f-o)
			<i>Dientamoeba</i>	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	<i>Trichomonas</i>	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	<i>Cochlosoma</i>	birds (gut)	direct (f-o)
Group: Discoba (diverse group supported robustly by molecular studies)					
Phylum: Euglenozoa (flagella inserted in anterior pocket, heterotrophs, autotrophs)	Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)	Ichthyobodonidae (flagellar pocket continues as groove)	<i>Ichthyobodo</i> (= <i>Costia</i>)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	<i>Cryptobia</i>	fish (gills, skin)	direct (w)
			<i>Trypanoplasma</i>	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	<i>Trypanosoma</i>	vertebrates (blood, tissues)	indirect (v-b)
		<i>Leishmania</i>	vertebrates (blood, tissues)	indirect (v-b)	

*f-o = faecal-oral transmission; v-b = vector-borne transmission, w = water-borne transmission; v = venereal transmission

The group Alveolata in the supergroup SAR is characterized by the possession of cortical membranous alveoli (vesicles or sacs) underlying and supporting the cell wall, and includes many otherwise disparate groups comprising 3 large assemblages (Apicomplexa, Ciliophora, Dinoflagellata) and 6 smaller taxa (Acavomonidia, Chromerida, Colpodellida, Colponemidia, Perkinsozoa and Voromonadida). The phylum Dinoflagellata comprises a diverse group of aquatic organisms, with many free-living species being important in food chains either as producers (autotrophs) or consumers (heterotrophs), some being endosymbiotic in invertebrates (e.g. in photosynthetic corals) and some being parasitic mainly in invertebrates but also a few vertebrates (fish). Dinoflagellates have a distinctive haploid nucleus (dinokaryon) where the chromosomes remain condensed during interphase and are evident as beaded strands due to low levels of histones and associated proteins (condition termed 'mesokaryotic'). Many dinoflagellates contain chloroplasts so early classification systems have used either botanical or zoological nomenclature, whereas recent molecular phylogenetic studies on eukaryotes have recognized and characterized cognate groups and provided appropriate names. Trophonts (or dinospores) of most species have an unequal heterodynamic pair of flagella; one ribbon-like and lying in equatorial furrow (girdle or cingulum) and the other directed posteriorly and often lying in a longitudinal ventral furrow (sulcus). Many species have thecate dinospores encased in armour composed of cellulosic plates; the anterior section is termed the epitheca (epicone in unarmoured species) and the posterior section is called the hypotheca (hypocone). Most species have unique membranous cytoplasmic organelles called pusules which are thought to be associated with osmoregulatory or excretory function. Many species have coloured chloroplasts and/or coloured pigments, some even exhibiting bioluminescence when disturbed by wind, wave or boat wakes. Several pigmented species bloom under suitable environmental conditions and cause 'red tides' and some pigments are neurotoxic to mammals when concentrated in the tissues of fish or filter-feeding shellfish (causing PSP 'paralytic shellfish poisoning'). Exemplars of toxic dinoflagellates are tabulated below (for interest only):

Toxic dinoflagellates	Toxin	Transvector	Effect
<i>Prorocentrum</i>	venerupin	shellfish	human hepatotoxicity
<i>Dinophysis</i>	okadaic acid	shellfish	human enterotoxicity
<i>Pyrodinium</i>	saxitoxin	shellfish	human neurotoxicity (PSP)
<i>Protogonyaulax</i>	saxitoxin	shellfish	human neurotoxicity (PSP)
<i>Gymnodinium</i>	saxitoxin	shellfish	human neurotoxicity (PSP) & fish kills
<i>Ptychodiscus</i>	brevetoxin	shellfish	human neurotoxicity & fish kills
<i>Peridinium</i>	glenodinine	-	fish kills
<i>Gambierdiscus</i>	ciguatoxin	fish	ciguatera

Over 4,000 dinoflagellate species are recognized in 550 genera: the majority being free-living autotrophs in pelagic or neritic surface waters. However, some 140 species from 5 families are heterotrophic and parasitic in aquatic hosts: including Oodiniidae (e.g. *Amyloodinium*, *Piscinoodinium* and *Crepidoodinium* on skin and gills of fish); Syndiniidae (e.g. *Ichthyodinium* and *Hematodinium* in tissues of fish and crabs); Chytriodiniidae (e.g. *Chytriodinium* on eggs of copepods and shrimps); Paradinidae (e.g. *Paradinium* in body cavities of copepods); and Ellobiopsidae (e.g. *Ellobiopsis* and *Thalassomyces* on exoskeletons of crustacea). Genera parasitic on fish and in shellfish that are covered in this document are tabulated below.

Taxon	Genus	Hosts	Site	Transmission
Class: Blastodiniophyceae [†] (uninucleate trophonts, often with chloroplasts)				
Order: Blastodinales [†] (ectoparasitic on zooplankton, algae, crustacea and fish)				
Oodiniidae (trophonts with rhizoid-like holdfast organelles, gymnosporos)	<i>Amyloodinium</i>	fish	skin	direct (water)
	<i>Crepidoodinium</i>	fish	skin	direct (water)
	<i>Piscinoodinium</i>	fish	skin	direct (water)
Class: Syndiniophyceae (multinucleate trophonts, without chloroplasts)				
Order: Syndiniales (endoparasitic in copepods, appendicularians, crabs, radiolaria and fish eggs)				
Syndiniidae (plasmoidal trophonts, dinospores not of <i>Gymnodinium</i> type)	<i>Hematodinium</i>	decapods	tissues	direct (water)
	<i>Ichthyodinium</i>	fish	tissues	direct (water)

[†]In recent classifications, these names are no longer valid, and the genera are considered *incertae sedis* in the Dinoflagellata.

Molecular biological studies on environmental and clinical isolates have shown that endobiotic parasites belonging to the Syndiniales include at least 5 major marine alveolate groups (MALV I-V), sometimes given interchangeably as Syndiniales I-V): namely, MALV I infecting ciliates and fish (including *Ichthyodinium*); MALV II infecting dinoflagellates and radiolarians; MALV III recovered from the environment; MALV IV infecting metazoans (including *Hematodinium* infecting crabs); and MALV V recovered from the environment. Endoparasitic syndinid dinoflagellates form multinucleate plasmoidal trophonts (without chloroplasts) in the tissues of their hosts, which then produce free-swimming biflagellated dinospores (not of the *Gymnodinium* type). *Ichthyodinium* parasitizes embryos and larvae of marine fin fish living and reproducing in the ichthyoplankton primarily in tropical and warm-temperate oceanic ecosystems. There have been several suggestions to transfer *Ichthyodinium* to Perkinsoidea on the basis of some similarities in parasite morphology and biology (nuclei without the typical dinoflagellate appearance, presence of rhoptry-like structures, possible pseudo-conoid, biflagellated spores, and schizogony), but they have not been supported by subsequent molecular analyses of small and large subunit ribosomal RNA genes. *Hematodinium* parasitizes the haemolymph and haemocoels of marine decapod crustaceans (crabs, lobsters, and some shrimp) around the world, with heavy infestations imparting a stringent taste to the flesh (termed bitter crab syndrome). The parasites form a series of dividing stages in internal organs, including amoeboid, filamentous and arachnoidal trophonts (schizonts), gorgonlocks and clump colonies, and finally arachnoidal sporonts which produce free-swimming dinospores. Molecular characterization studies have detected some genetic differences between isolates from different crustacean species and from different oceanic regions, but further studies are required to determine whether they are intra- or inter-specific differences.

Parasite species	Hosts	Location (disease)	Dinospores	Distribution
Genus <i>Ichthyodinium</i> (amoeboid trophonts growing to multinucleate plasmodia)				
<i>I. chabelardi</i>	marine fish: Anguilliformes: anguillid (European eel); Carangiformes: carangid (Atlantic horse mackerel); Clupeiformes: alosid (European pilchard), engraulid (European anchovy); Gadiformes: gadid (Atlantic cod, blue whiting); Perciformes: serranid (leopard coral grouper), sparid (gilthead bream, bogue); Pleuronectiformes: scophthalmid (turbot); Scombriformes: scombrid (Atlantic mackerel, yellowfin tuna, Pacific bluefin tuna); Stomiiformes: sternoptychid (bristlemouth)	vitelline sacs in eggs, larvae	8-12 µm	Eurasia
Genus <i>Hematodinium</i> (parasitic in tissues of decapodid crustaceans)				
<i>H. australis</i>	Decapoda: portunid (blue swimmer crab, sand crab, mud crab), trapeziid (coral/guard crab)	haemolymph, haemal spaces		Australia
<i>H. perezii</i>	Decapoda: carcinid (European green crab, Pennant's swimming crab), cancrinid (Jonah crab, Atlantic rock crab, brown crab), ovalipid (lady crab), polybiid (harbour crab, velvet crab), portunid (Atlantic blue crab, Asian blue crab), varunid (mud-flat crab)	haemal sinuses, haemocytes, soft tissues (bitter crab disease)	macrospores 12-14 µm microspores 7-9 µm	Atlantic, Pacific, North Sea

<i>H. spp.</i> (unidentified isolates, including novel? genotypes)	Decapoda: cancrinid (brown crab, Jonah crab, Atlantic rock crab), epialtid (common spider crab, longnose spider crab), lithodid (scarlet king crab, red king crab, blue king crab), menippid (Florida stone crab), munidid (rugose squat lobster), nephropid (Norway lobster), oregoniid (inshore tanner crab, triangle tanner crab, grooved tanner crab, snow crab, great spider crab, contracted crab), ovalipid (lady crab), pagurid (common hermit crab, Prideaux's hermit crab), palaemonid (ridgeway prawn), panopeid (flatback mud crab, saltmarsh mud crab, Say mud crab), penaeid (giant tiger prawn), polybiid (velvet crab), portunid (lesser blue crab, mud crab), trapeziid (coral crab, guard crab)	haemal sinuses, haemocytes		Atlantic, Pacific, North Sea
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Parasite morphology: These endoparasitic dinoflagellates form at least 4 different types of stages during their development: uninucleate trophonts (amoeboid and/or filamentous); multinucleate trophonts (including stages described as arachnoid trophonts, plasmodia, schizonts, and sometimes gorgonlocks and clump colonies); sporonts (arachnoid) with sporoblasts forming prespores; and free-swimming biflagellated zoospores (dinospores). *Ichthyodinium* first appears as uninucleate amoeboid trophonts 8-20 µm long with condensed chromatin in the nucleus (dinokaryon-like) and cytoplasmic trichocysts (thought to be involved in attachment). The trophonts are vegetative and undergo division (plasmotomy) involving at most 3 schizont generations forming multinucleate plasmodia. Primordial schizonts are formed when trophonts divide by multiple mitotic divisions without subsequent cytoplasmic division to form large multinucleated stages 100-140 µm. The schizont cytoplasm then forms cylindrical projections around each nucleus, with the resultant cells budding from the rest. These cells were initially cylindrical or racquet-shaped measuring around 20 µm in length. In some host species, they formed secondary schizonts by undergoing successive longitudinal bipartitions, but the posterior poles of the daughter cells remained attached, so they assumed a rosette shape. The secondary schizonts then formed long cords (up to 1-2 mm) of successive groups of 8 cells before releasing cylindrical-oblong cells (note that some studies have failed to find secondary schizonts, rosettes or cords in several host species). The products of the previous schizont generations then formed the last schizont generation where the cells became spherical after a series of divisions producing numerous (sometimes thousands) small (20-24 µm in diameter) uninucleate zoosporangia. When released into water, the sporangia underwent 1-2 divisions producing free-swimming dinospores. Note that in some studies, zoosporangia were not observed as the spherical cells transformed directly into dinospores. The dinospores did not have a *Gymnodinium*-like configuration but had a wide shallow slanted cingulum (not deep and equatorial) and a shallow ventral sulcus (not pronounced). The dinospores ranged in size from 8-12 x 4-5 µm and possessed 2 smooth flagella which were spherical in cross-section (typical dinoflagellates have a ribbon-like transverse flagellum with a flattened cross-section due to the possession of an axoneme along the outer edge and a contractile fiber (often referred to as a striated strand) along the inner edge). The dinospores possessed alveolate pellicles and contained trichocysts and some apical complex organelles (rhoptries and microtubules). *Hematodinium* also formed uninucleate trophonts (initially filamentous then rounded amoeboid stages) that developed into large amorphous multinucleate trophonts (known as arachnoid trophonts or schizonts) which then formed arachnoid sporonts producing sporoblasts, prespores and finally dinospores. The trophonts were small rounded stages measuring 6-18 µm in diameter with a single dinokaryon nucleus with chromatin condensed as small beads. They formed large amorphous multinucleate plasmodia 20-48 µm which were vermiform with 2-20 nuclei in *H. perezii* or ovoid with 2-5 nuclei in *H. australis*. The sporonts were found to produce 2 types of naked (athecate) gymnodinoid dinospores, macro-spores measuring 12-14 µm long and micro-spores measuring 7-9 µm long. *In vitro* culture studies also revealed the presence of several intermediate stages, with arachnoid trophonts giving rise to smaller schizonts which transform into polypodial (tentacular) 'gorgonlocks' colonies which then round up to form clump colonies giving rise to further trophonts.

Site of infection: Endoparasitic stages of *Ichthyodinium* are found within the vitelline (yolk) sacs in eggs and larvae of fin fish while those of *Hematodinium* are found within the haemolymph or haemocoels of internal organs (hepatopancreas, heart, gills, muscle) of marine decapods (crabs, lobster, and some shrimp). Infections by *Ichthyodinium* have been recorded in the eggs and/or larvae of some 14 species of fin fish belonging to 10 different families from the Mediterranean Sea and the Atlantic and Pacific Oceans. Infections by *Haematodinium* spp. have been found in some 38 crustacean species around the world, including economically-important species such as edible crabs from Europe, tanner and snow crabs from the northern Pacific and Atlantic Oceans, blue crabs from the Atlantic, lobsters from Norway, and swimming and mud crabs from Asia and Russia.

Pathogenesis: Both genera of endoparasitic dinoflagellates may cause morbidity and mortality in their respective hosts. *Ichthyodinium* stages in the yolk sacs of fish eggs and larvae have been associated with their subsequent destruction (the condition sometimes referred to as parasitic castration, even though infections do not destroy the gonads). Instead, heavy infections cause discolouration (opacities) and distention of the yolk sacs leading to their rupture before or after hatching resulting in the death of the eggs or larvae. Epizootics have been recorded in various fishes, both wild and farmed species, often accompanied by drastic reductions in their reproduction success (low egg hatching rates, poor larval survival). The highest rates of infection have been

observed in the winter and early spring in cold-water fish species but in the warmer months in temperate and tropical fish species. In either case, infections are intimately linked to the breeding cycles of the resident fish species as parasites only infect eggs shortly after spawning (allegedly through weaknesses in the eggshell, possibly sperm tracts). It has been suggested that global climate change leading to rising ocean temperatures may facilitate expansions in the host and geographic ranges of the parasites, affecting commercial fishing industries or preventing the establishment of stable seed stocks for aquaculture. *Hematodinium* undergoing massive proliferation in the haemolymph and tissues of marine decapods cause a series of physiological and pathological changes resulting in functional deficits contributing to mortality. The haemolymph becomes opalescent or milky-white in colour, slow to clot and exhibits dramatic reductions in haemocytes (sometimes absent). Infections have been associated with milky blood disease in cultured swimming crabs, yellow water disease in cultured mud crabs, and milky shrimp disease in prawn aquaculture. Infected animals undergo significant detrimental biochemical changes, involving nutrient depletion and reduced levels of haemolymph proteins (including haemocyanin and glycogen) with lowered oxygen-carrying capacity of the haemolymph but elevated rates of oxygen consumption within tissues. Nearly all major organs and tissues exhibit cellular damage and necrosis, with dilation of haemal sinuses, pressure necrosis in spongy connective tissues, lysis of hepatopancreatic tubules, muscular degeneration, discoloured shells and respiratory dysfunction due to loss of gill epithelial cells, lamellar distention, fusion and clubbing (swelling), loss of trabecular cells and haemocyte infiltrations. Infected crustaceans become lethargic with drooping limbs and mouthparts, lose weight and have short survival times in captivity. The main cause of death is attributed to disruption of gas transport and tissue anoxia caused by massive proliferation of the parasites, and some crabs are also thought to die from metabolic exhaustion. A key feature of many infections is the condition known as bitter crab disease, which occurs when cooked muscles take on a chalky texture and an astringent after-taste that makes them unmarketable. The bitter taste of cooked crabs may be due to the dinoflagellates themselves or their metabolites. Small numbers of diseased crabs have been shown to render entire batches of crabs unpalatable if they are processed together. Infections have also been associated with immunosuppression as evidenced by alterations in gene expression and regulation, with pronounced haemocytopenia hindering normal immune responses (clotting, phagocytosis, encapsulation of foreign material, production of antibiotic factors) and facilitating secondary bacterial infections resulting in septicæmia (commonly associated with stress in crustaceans). Epidemics have been recorded in commercially-fished decapod species resulting in significant economic losses, particularly around bays and fjords in northern America.

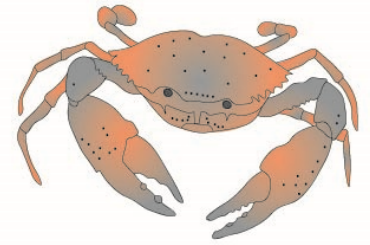
Developmental cycle and mode of transmission: Few complete life-cycles have been determined but they involve dinospore attachment or ingestion, the development of endoparasitic vegetative trophonts forming large amorphous plasmodia that give rise to free-swimming spores as the main transmissive stages. The invasive stages of *Ichthyodinium* form small amoeboid mononuclear cells in the yolk sacs of fish eggs (evident after gastrulation). The trophonts grow and undergo several cycles of schizogony (dividing by plasmotomy) to form multinucleate plasmodia (schizonts) that eventually release cells that either form zoosporangia or directly divide to form biflagellated dinospores. Molecular biological studies have detected parasite DNA in fertilized fish eggs, fish larvae, and rearing water, but not in the gonads of the parent fish nor in smaller fish used as feed. Infections have been detected in fish eggs as early as 2-8 hours after spawning, and free-swimming dinospores have been detected several days later in lysed fish eggs or larvae. Dinospores have been observed as early as 2 hours after yolk sac rupture and they are actively free-swimming for several days but die unless they invade new fish eggs. Epidemiological studies initially suggested that *Ichthyodinium* infected adult fish from benthic prey and then spread vertically through gametes, but there is now overwhelming evidence to refute this. Transmission occurs horizontally when free-swimming dinospores infect fish eggs soon after spawning. Freshly spawned eggs transferred to containers of sterilized seawater do not become infected, but if the eggs are left in rearing water for at least one hour they become infected. *Hematodinium* also undergoes a complex developmental cycle whereby dinospores invade the haemolymph in their decapod hosts and form filamentous and then amoeboid trophonts which proliferate through arachnoid trophonts (interconnected plasmodial colonies) that eventually form syncytial arachnoid sporonts which release sporoblasts and/or pansporoblasts that develop to prespores then motile dinospores. Some isolates have also been observed to undergo an alternative developmental pathway when arachnoid sporonts in the late stages of development release pansporoblasts (or multinucleated schizonts) that formed gorgonlocks (filamentous) colonies that underwent segmentation and formed clump colonies which developed directly to arachnoid trophonts. Studies on lobsters revealed cycles involving fusion of macro- and micro-spores to form filamentous trophonts which developed directly to gorgonlocks and then clump colonies to produce more arachnoid trophonts. In all cases, transmission between hosts was achieved by water-borne free-swimming dinospores, although some studies demonstrated that the experimental transfer of trophonts and plasmodia may lead to infections in recipient hosts although transmission between crabs by feeding (scavenging, predation or cannibalism) were not highly successful. Epidemiological studies revealed that most *Hematodinium* infections were seasonal, although the patterns differed according to host species, type of fisheries and geographic location. Infection peaks were observed in the summer and autumn in boreal regions, but in winter in temperate regions. Parasite development occurred over a wide temperature range (best between 8-15°C) and outbreaks were often associated with high salinity waters (above 18 parts per thousand (ppt)) with some evidence to suggest dinospores lost viability promptly in low salinity water (< 15 ppt). Infections generally took 9-12 months to develop and there were strong associations with moulting cycles (most prevalent post-moult). Outbreaks have been associated with rising water temperatures which stimulate moulting activity. Most outbreaks have been confined to sites with unique hydrologic features (such as fjords or poorly draining estuaries with shallow sills), areas with salinity > 11 ppt and stressful conditions such as seasonal hypoxia or predation pressure. However, some outbreaks have also occurred in more open ocean regions in crabs from depths > 250 meters.

Differential diagnosis: Clinical infections may be indicated by unique discolourations of host tissues which may then be confirmed by the direct detection of endoparasitic developmental stages by microscopic examination or by molecular probes. *Ichthyodinium* developmental stages cause dark opacities in the yolk sacs of fish eggs or larvae which can be observed by visual examination. Microscopic examination of excised samples (aspirates, smears, squashes, sections) reveals the presence of numerous ovoid trophonts, schizonts and/or dinospores depending on the stage of infection. Molecular biological techniques have also been used to detect parasites following the polymerase chain reaction (PCR) amplification of nuclear gene sequences, notably small subunit (SSU) ribosomal RNA (rRNA) and internal transcribed spacer (ITS) regions). Phylogenetic studies have suggested there may be 2 species of *Ichthyodinium*: with *I. chabelardi* found in European fish populations; and a slightly different genotype found in some Asian fish populations (leopard coral grouper and yellowfin tuna). However, further studies are required to characterize these and additional isolates. *Hematodinium* infections may cause darker red colouration of the carapace and pale pink colouration of the appendage joints of infected decapods (cooked appearance) and the haemolymph appears cloudy and opaque ('milky'). Microscopic examination of wet mounts or smears of haemolymph or histological sections of haemal spaces in tissues reveals the presence of various parasitic stages (uninucleate trophonts, multinucleate plasmodia, sporonts and developing spores depending on the stage of infection). Parasites stain relatively well with Jenner-Giemsa or other blood stains, and often have distinctive dinokaryon nuclei (with condensed chromatin) and multinucleate stages tend to have a 'frothy' cytoplasm. Researchers have developed immunological tools (fluorescent-antibody tests) to detect infections in Norway lobsters, which appear to be quite sensitive, but little is known about their specificity. Molecular biological techniques have been used to detect parasites in clinical and environmental samples by PCR amplification of nuclear gene sequences (SSU rRNA, ITS). Phylogenetic studies have confirmed the differentiation of *H. australis* from *H. perezii*, but have also suggested that 3 genotypes of *H. perezii* may occur varying in host range and geographic location: including a generalist clade containing isolates from tanner crabs, king crabs, green crabs, contracted crabs, hermit crabs and lobsters from the North Atlantic and North Pacific Oceans; a small clade containing isolates from snow crabs from Canada, lobsters from Norway, brown crabs and hermit crabs from Europe; and another small clade containing isolates from harbour crabs from Europe, Atlantic blue crabs from eastern North America, and Asian blue crabs and mud crabs from China. Further studies are required to determine the extent of the genetic variation between isolates.

Treatment and control: Infections by endoparasitic dinoflagellates are difficult to control as there are no effective chemotherapeutic agents available and preventive strategies based on hygiene can only be implemented in some aquaculture enterprises and not in wild fisheries. *Ichthyodinium* has been detected sporadically in wild fish (especially in sardines and mackerel) but has proven to be a pest species on some fish farms. Infections in fish eggs and larvae are prevalent during spawning seasons, so it is imperative that broodstock be regularly screened for infections to prevent outbreaks. Studies have shown that maintaining good water quality and incubating fertilized eggs and rearing broodstock in disinfected seawater (oxidant-treated (ozone) or UV-sterilized) prevented the horizontal transfer of infections. *Hematodinium* infections have been found in a variety of marine decapods, both in wild and cultured stocks. Several studies have found that chemotherapy with broad-spectrum antibiotics helped crabs survive infections, but the most effective measures for controlling infections in mariculture involved regular screening (of both cultured and co-habiting sentinel species), quarantine (isolation of infected stocks), limiting translocations of live animals, avoiding polyculture pond systems (infections can occur in co-cultured shrimp as well as wild sympatric crabs and prawns), harvesting animals in winter (when fewer crabs are infected and meat is more marketable), and culling or removing dead animals (preferentially disposing of them through onshore fertilizer processing plants, and not using infected crabs for bait). Following outbreaks, some success has also been reported rebuilding populations from lightly infected stock (but not from heavily infected animals).

Haematodinium

syndinid dinoflagellates
with parasitic stages
in aquatic hosts

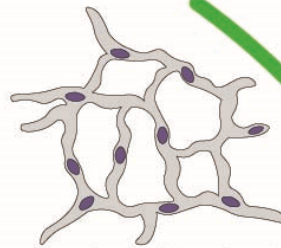


Hosts
(marine decapods)

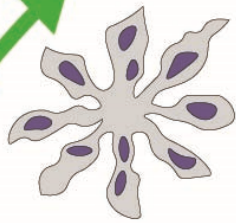

haemolymph
(‘milky blood’,
bitter crab disease)



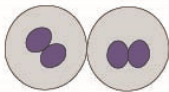
clump colony



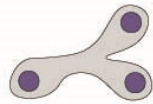
arachnoid trophont



gorgonlocks colony

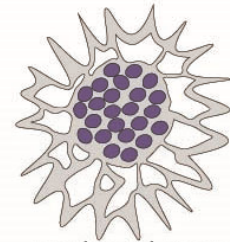


amoeboid trophont



filamentous trophont

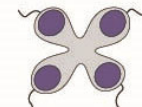
multinucleated
‘plasmodial’ stages
(cells 6-18 μm ,
colonies < 200 μm)



arachnoid sporont

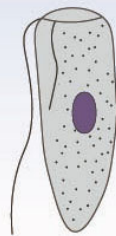


sporoblasts



pre-spores
released

dinospores
invade host



macrospores
(12-14 μm)

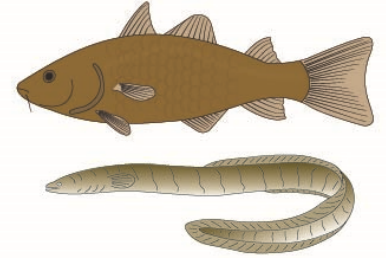


microspores
(7-9 μm)

direct transmission
via free-swimming dinospores

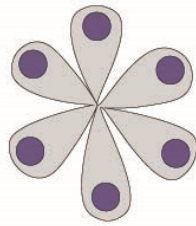
Ichthyodinium

syndinid dinoflagellates
with parasitic stages
in aquatic hosts

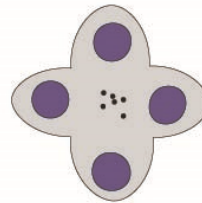


Vertebrate Hosts
(marine fish)

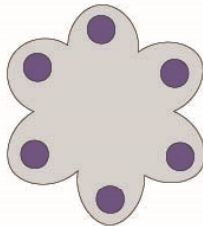
fish eggs/larvae
(yolk sac discolouration,
distention, rupture,
'parasitic castration')



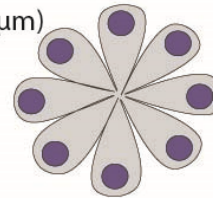
primordial schizont
(100-140 μm)



secondary schizont
(20-40 μm)



multinucleated
'plasmodial' stages
divide by plasmotomy



tertiary schizont
(20-24 μm)



zoosporangia



amoeboid
trophont
(8-20 μm)

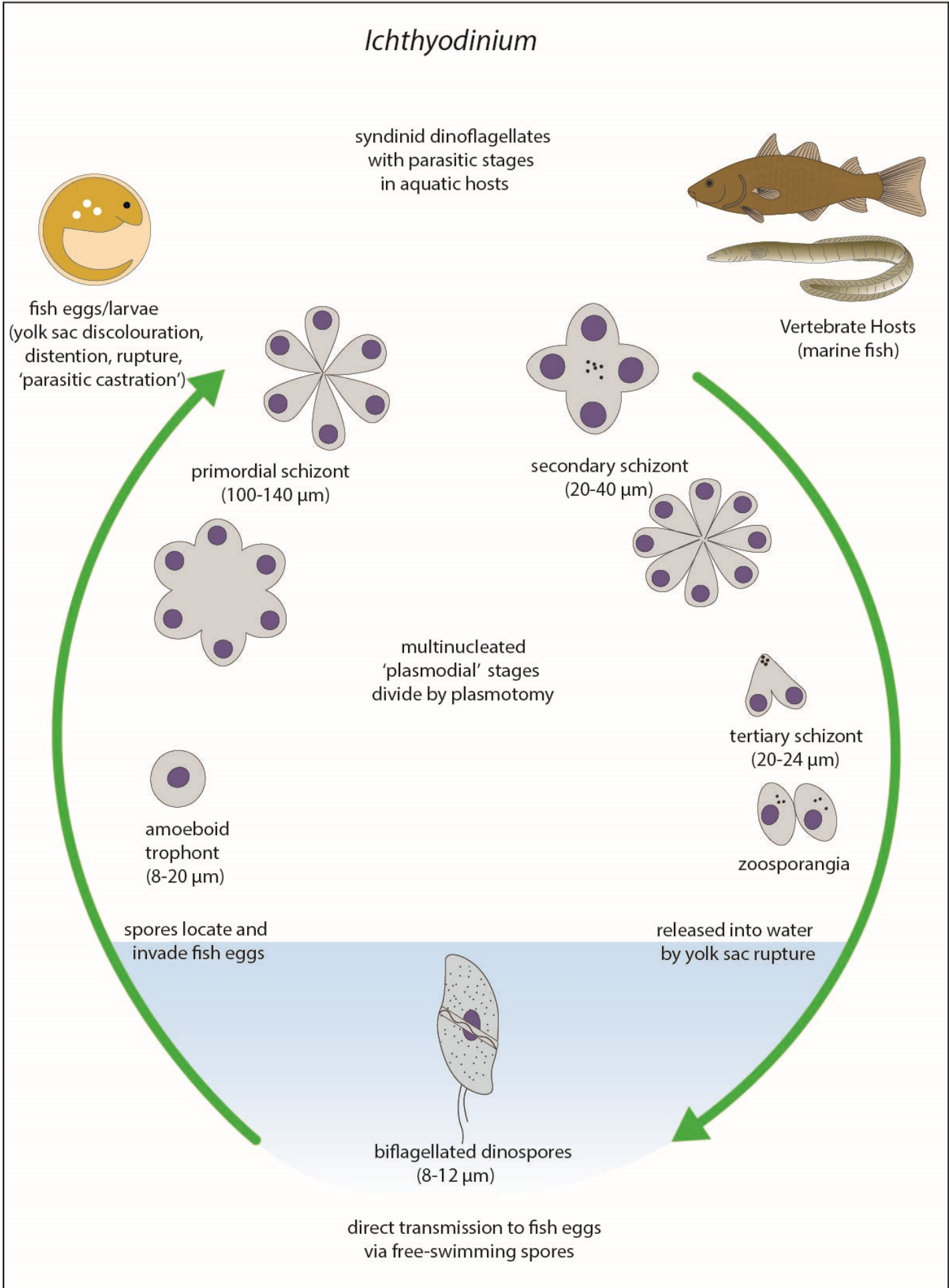
spores locate and
invade fish eggs

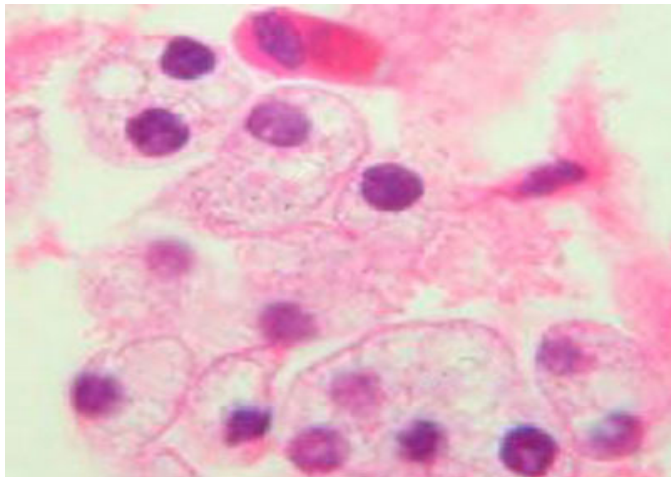
released into water
by yolk sac rupture



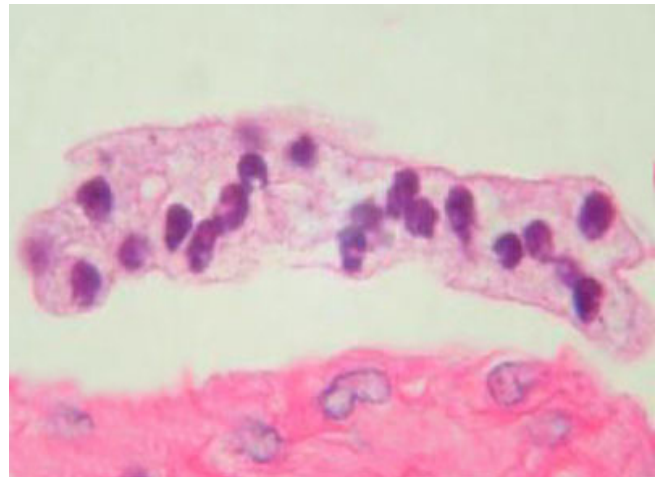
biflagellated dinospores
(8-12 μm)

direct transmission to fish eggs
via free-swimming spores

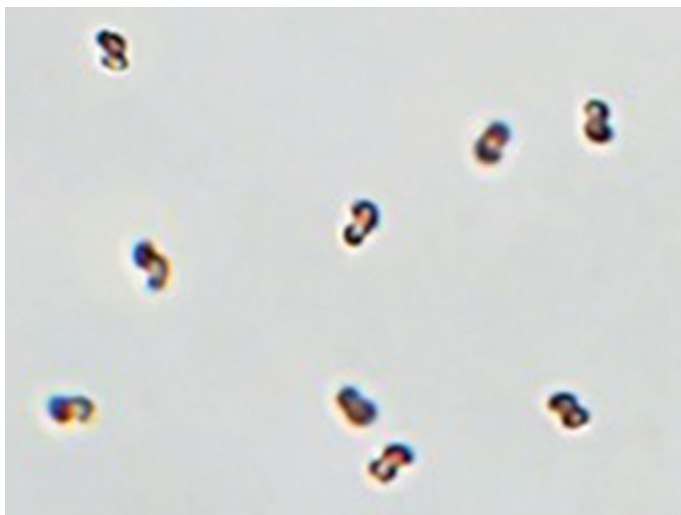




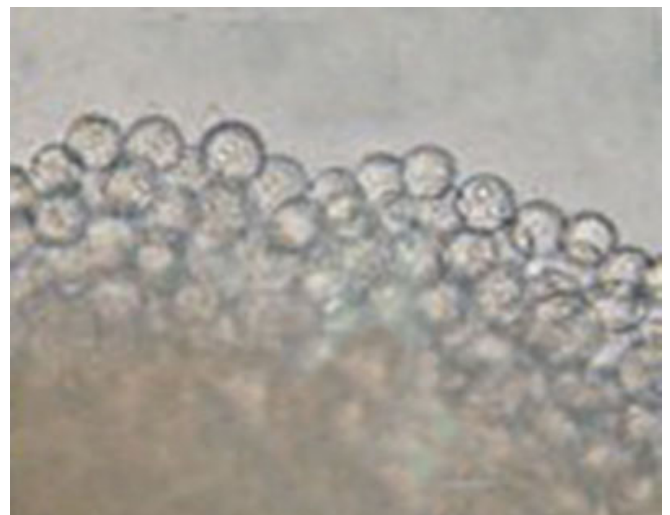
Haematodinium trophonts in haemolymph of crab



Haematodinium colony in crab tissues



Haematodinium dinospores



Ichthyodinium trophonts in fish egg