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6 Infectious Diseases of Warmwater Fish in Fresh Water

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6.1 Introduction

Eurythermal fish species are those that can tolerate a wide range of temperatures (i.e. 2–32°C) (Molnár *et al*., 2019). There is, however, no consensus on the definition of a warmwater species. Pope *et al*. (2009) consider these as species preferring temperatures >15°C, while Howard (2019) defines them as species preferring water temperatures around 27°C. In 2020, freshwater aquaculture accounted for 53,494,889 tonnes (i.e. 43.64% of total aquaculture production); a map of global finfish aquaculture is provided in Fig. 6.1. Finfish accounted for 90.04% of freshwater production in 2020 with 197 species or categories of fish (FishStatJ, 2022) farmed across 179 territories. Freshwater finfish production is increasing at 2.95% year on year (2016–2020), against an annual growth of 3.48% for the entire freshwater aquaculture sector.

Global freshwater cultured finfish production in 2020 is provided in Table 6.1. The cyprinids have major representation, while the top ten species, not including the general category 'freshwater fishes', account for 71.89% of the total tonnage. A summary of country-level cage and pen culture is presented in Tables 6.2 and 6.3.

For some species, cage culture is growing (e.g. *Siniperca* spp. in China) (Gui *et al*., 2018), while elsewhere new policies linked to environmental protection have resulted in cage culture being discouraged (Nogueira *et al*., 2020) or banned (Hu and Lv, 2016). In China, the demolition of cages used for rearing *Ictalurus punctatus* led to a 10.64% decrease in production (i.e. down 236,786 tonnes in 2016) and a consequential change to farming practices. At the same time, the Ministry of Fisheries and Aquaculture, Brazil has explored the creation of 100 aquaculture parks within 11 reservoirs for cage culture that could result in annual production of 226,934 tonnes of fish. The assessment and development of additional aquaculture parks could result in Brazil emerging as one of the largest producers of cage aquaculture in the world (Bueno *et al*., 2015).

While there is a wealth of information on diseases of feral and cultured fish in warm fresh water (Lio-Po and Lim, 2014; Paladini *et al*., 2017; Jahangiri *et al*., 2021; Shinn *et al*., 2022), there is a comparative scarcity of information on freshwater, cage culture-reared stocks. For many disease reports, the culture system is not clearly specified. Infections appear to occur

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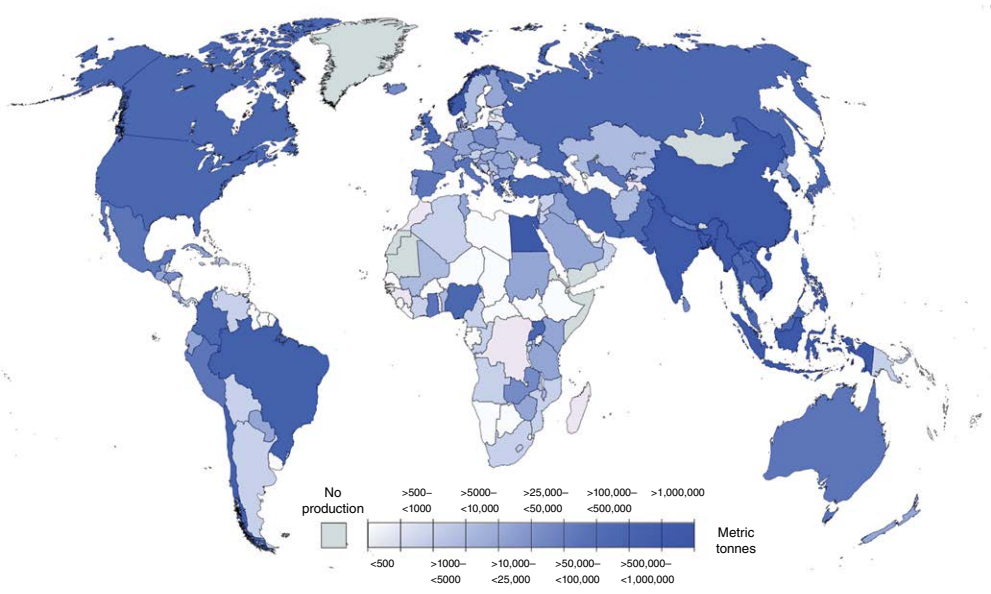


Fig. 6.1. Global finfish aquaculture by production (metric tonnes) in 2019. (Data drawn from FishStatJ, 2022.)

more frequently in cages than in ponds, where the captive-held stocks are exposed to pathogens of feral fish and to a greater number of intermediate hosts. Disease reports of cage culture in river systems and in urban waters exemplify this, where the biosecurity threats are greater and more challenging to control. There are also commensurate health risks to humans from stocks reared in unsanitary waters, polluted waters and in areas where fish-borne zoonotic diseases are prevalent. Diseases afflicting pond-reared and cage-cultured fish are in many cases similar; those that are significant in pond aquaculture are treated as potential problems of finfish reared in net enclosures and are the focus of this chapter. The potential impacts of climate change on the frequency and severity of infections of finfish reared in cage culture are also commented upon.

6.2 Viral Infections

Viral infections often cause mass mortality among fry, fingerlings and juveniles, although older fish may either develop pathological signs and die or may appear unaffected but are carriers. Most viral infections in fish occur at cool water temperatures of 15–25°C (Dishon *et al.*, 2007). Stress from handling, poor water quality, water temperature, age of fish, high stocking density and poor nutrition are factors that facilitate the development of viral diseases (Plumb, 1999). Viral infections of freshwater fish in tropical Asia are summarized in Table 6.4.

6.2.1 Tilapia lake virus (TiLV) disease

Tilapia lake virus disease (TiLVD), or syncytial hepatitis of tilapia (SHT), is an important viral disease of tilapia. Although first recorded in Israel and Ecuador from 2011, suspicious mortality events predating 2009 were known (Ferguson *et al.*, 2014). Reports from 16 countries rapidly followed, suggesting that the translocation of infected fish prior to the discovery of the pathogen may have contributed to its transboundary spread (Dong *et al.*, 2017a; Jansen *et al.*, 2019; Tang *et al.*, 2021).

The causative agent was initially termed tilapia lake virus (TiLV) upon the first discovery in Israel (Eyngor *et al.*, 2014). Further genome characterization suggested that TiLV is a novel

Table 6.1. The top freshwater finfish species/species groups with tonnages over 50,000 tonnes landed from aquaculture activities in 2020 with details regarding their distribution and production compared with tonnages landed from capture fisheries. (As categorized by FishStatJ, 2022; additional data are drawn from Froese and Pauly, 2022.)

Species (ranked by tonnage)	Latin name	Temperature range (°C)	No. of countries/islands reported from			Tonnage from aquaculture	No. of aquaculture producing countries	Tonnage from capture fisheries (no. of countries)	
			Native/endemic	Introduced	Not established			Native/endemic	Introduced
Grass carp (= white Amur)	*Ctenopharyngodon idella* (Valenciennes, 1844)	0–35	2	68	23	5,791,541	42	215 (1)	24,681 (17)
Silver carp	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	6–28	4	58	19	4,896,612	35	1,016 (1)	33,182 (15)
Common carp	*Cyprinus carpio* (L., 1758)	3–35	17	116	6	4,236,326	80	29,048 (193)	100,421 (34)
Nile tilapia	*Oreochromis niloticus* (L., 1758)	14–33	22	77	6	3,561,316	74	208,307 (6)	73,337 (7)
Catla	*Gibelion catla* (Hamilton, 1822)	25–32	6	7 (8)[a]	4	3,540,312	8	nr	nr
Bighead carp	*Hypophthalmichthys nobilis* (Richardson, 1845)	4–26	1	44	23	3,187,236	20	nr	2,762 (8)
Carassius spp.	e.g. *Carassius carassius* (L., 1758)	2–22	42	15 (16)[a]	3	2,753,342	14	–	–
Freshwater fishes nei	Not specified					2,567,220	69	–	–
Striped catfish	*Pangasianodon hypophthalmus* (Sauvage, 1878)	22–26	4	4 (5)[a]	1	2,520,422	11	nr	nr
Roho labeo	*Labeo rohita* (Hamilton, 1822)	22–31	6	9	4	2,484,817	11	nr	nr
Torpedo-shaped catfishes nei	e.g. *Clarias batrachus* (L., 1758)	8–35	1	7	12[b]	1,249,012	10	–	–
	e.g. *Clarias gariepinus* (Burchell, 1822)		40	23 (24)[a]	5				

Tilapias nei	Various *Coptodon*, *Oreochromis*, *Sarotherodon*, tilapia spp.	8–36				1,146,316	69	–	–
	e.g. *Coptodon rendalli* (Boulenger, 1897)		11	26 (29)[a]	0				
	e.g. *Coptodon zillii* (Gervais, 1848)		28	23 (26)[a]	3				
	e.g. *Oreochromis aureus* (Steindachner, 1864)		10	35	4				
	e.g. *Oreochromis mossambicus* (Peters, 1852)	17–35	7 (9)[a]	93 (95)[a]	8 (9)[b]		10	0	21,450 (2)
	e.g. *Sarotherodon galilaeus* (L., 1758)		29	3 (4)[a]	1				
Wuchang bream	*Megalobrama amblycephala* (Yih, 1955)	10–20	1	2	0	781,737	1	nr	nr
Rainbow trout	*Oncorhynchus mykiss* (Walbaum, 1792)	12–21	6	84	14	733,999	75	140 (3)	1,216 (13)
Black carp	*Mylopharyngodon piceus* (Richardson, 1846)	0–40	4	17 (18)[a]	7	695,541	3	nr	nr
Snakeheads									
e.g. Indonesian snakehead	*Channa micropeltes* (Cuvier, 1831)	23–27	6	1 (2)[a]	1	648,363	10	15,335 (1)	0
e.g. striped snakehead	*Channa striata* (Bloch, 1793)		14	10	0		6	55,280 (2)	8,439 (2)
Largemouth black bass	*Micropterus salmoides* (Lacepède, 1802)	10–32	3	61 (63)[a]	11	621,327	8	863 (1)	180 (1)

Continued

Table 6.1. Continued

Species (ranked by tonnage)	Latin name	Temperature range (°C)	No. of countries/islands reported from			Tonnage from aquaculture	No. of aquaculture producing countries	Tonnage from capture fisheries (no. of countries)	
			Native/endemic	Introduced	Not established			Native/endemic	Introduced
Cyprinids nei	Various *Abramis*, *Chanodichthys*, *Cirrhinus*, *Rutilus*, *Tinca* spp.	4–26	–	–	–	578,041	26	–	–
Mrigal carp	*Cirrhinus cirrhosus* (Bloch, 1795)	14–31	4	8 (9)[a]	2	576,513	9	nr	nr
Yellow catfish	*Tachysurus fulvidraco* (Richardson, 1846)	16–25	5	0	0	565,477	1	nr	nr
Pangas catfishes nei	Various *Pangasius* spp.	26–28				462,216	4	–	–
	e.g. *Pangasius bocourti* (Sauvage, 1880)		4	0	0				
	e.g. *Pangasius pangasius* (Hamilton, 1822)		5	2 (4)[a]	2				
Channel catfish	*Ictalurus punctatus* (Rafinesque, 1818)	10–32	3	23	7	453,986	6	966 (1)	0
Blue-Nile tilapia, hybrid	*Oreochromis aureus* × *O. niloticus*	8–33	na	na	na	414,042	2	–	–
Mandarin fish	*Siniperca chuatsi* (Basilewsky, 1855)	4–22	3	1	0	376,986	1	nr	nr
Pond loach	*Misgurnus anguillicaudatus* (Cantor, 1842)	5–25	13	9	0	368,406	3	nr	nr
Amur catfish	*Silurus asotus* (L., 1758)	5–25	6	0 (1)[a]	0	351,468	3	nr	nr

Silver barb	*Barbonymus gonionotus* (Bleeker, 1849)	22–28	5	9	0	335,863	7	36,350 (2)	0
Asian swamp eel	*Monopterus albus* (Zuiew, 1793)	25–28	15	2	0	307,333	2	800 (1)	0
Japanese eel	*Anguilla japonica* (Temminck et Schlegel, 1846)	4–27	9	2 (3)[a]	2	279,011	4	121 (3)	0
North African catfish	*C. gariepinus* (Burchell, 1822)	8–35	39 (40)[a]	24	5	222,996	47	74,573 (7)	10 (1)
Giant gourami	*Osphronemus goramy* (Lacepède, 1801)	20–30	8	12	1	149,216	3	6,635 (1)	0
Sturgeons nei	Various *Acipenser*, *Huso* spp.	5–22	na	na	na	123,451	38	–	–
Pirapatinga (= piaractus)	*Myletes brachypomus* (G. Cuvier, 1817)	23–28	6 (7)[a]	2	3	122,021	10	2,772 (3)	0
Milkfish	*Chanos chanos* (Forsskål, 1775)	15–43	88	2	0	116,151	5	6,472 (1)	0
Cachama (= pacu or tambaqui)	*Colossoma macropomum* (G. Cuvier, 1818)	22–28	4	12	3	104,915	9	4,104 (3)	0
Africa–bighead catfish hybrid	*C. gariepinus* (Burchell, 1822) × *Clarias macrocephalus* (Günther, 1864)	8–35	na	na	na	99,344	1	–	–
Silver, bighead carps nei	*Hypophthalmichthys harmandi* (Sauvage, 1884), etc.	0–43	na	na	na	68,974	4	–	–
Climbing perch	*Anabas testudineus* (Bloch, 1792)	22–30	17	3	1	59,914	6	33,216 (2)	1,500 (1)
Orangefin labeo	*Labeo calbasu* (F. Hamilton, 1822)	19–33	7	1	0	52,808	2	–	–

nei, not included elsewhere; na, not applicable; nr, not reported.

[a] Includes unconfirmed/questionable reports.

[b] Reports are based on misidentifications.

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Table 6.2. A summary of freshwater cage and pen aquaculture production in selected countries ('est.' indicates an estimate).

Country	Cage/pen details	No.	Hectares	No. of culturists	Tonnage	Reference
Bangladesh	Cage		17.9		4,590	Department of Fisheries, Bangladesh (2021)
	Pen culture		7,263.0		13,337	
Brazil		100 parks	1,920.0		est. 284,778	Roubach *et al*. (2015)
China	Cage culture (given for 2020)		1,350.9		320,905	Ministry of Agriculture, Rural Affairs
	Cage culture (given for 2015)		14,737.6		1,379,086	Fishery Administration (2021)
India	Cages/pens in reservoirs	15,454	138.7			Department of Fisheries, India (2020)
Indonesia	Floating net cages (Karamba)				183,509	Indonesian Ministry of Marine Affairs and Fisheries (2020)
	Floating net (Jaring Apung Tawar)				432,698	
	Jaring Tancap Tawar				19,287	
Malaysia	Freshwater cages	30,416	55.0	1,963	15,736	Department of Fisheries Malaysia (2020)
	Pen culture	317	8.0	155	678	
Philippines					80,182	Philippine Statistics Authority (2022)
Thailand	Cage sites	7,471	220.3		52,335	Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand (2020); Shinn *et al*. (2022)
Total			25,711.4		2,502,343	

orthomyxo-like virus (Bacharach *et al*., 2016). The virus was then officially named *Tilapinevirus tilapiae* (previously known as *Tilapia tilapinevirus*), a member of the family Amnoonviridae. TiLV is an enveloped single-stranded RNA (ssRNA) virus with a diameter of 55–100 nm.

TiLV affects several species in the family Cichlidae, including *Oreochromis niloticus*; a list of natural and susceptible hosts is summarized elsewhere (Surachetpong *et al*., 2020; Tang *et al*., 2021).

TiLV infects all stages of tilapia but is more severe in young fish (<50 g) than in older fish. Disease outbreaks usually occur in the first few weeks after stocking and result in a mortality of 20–90% leading to significant economic loss (Eyngor *et al*., 2014; Ferguson *et al*., 2014).

Table 6.3. Finfish species farmed in net and cage aquaculture. For each, where available, the production is provided in metric tonnes ('est.' indicates an estimate). Figures in brackets are production figures specifically from pen culture and are in addition to the figures provided. (Data sources: a = Department of Fisheries, Bangladesh, 2021; b = FAO, 2022; c = Lio-Po and Lim, 2014; d = Gui *et al*., 2018; e = Radhakrishnan *et al*., 2019; f = Indonesian Ministry of Marine Affairs and Fisheries, 2020; g = LARReC, 2001; h = Department of Fisheries, Malaysia, 2020; i = Griffiths *et al*., 2019; j = Gurung *et al*., 2010; k = Philippine Statistics Authority, 2022; l = Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand, 2020.)

Common name	Latin name	Bangladesh[a]	Brazil[b]	Cambodia[c]	China[d]	India[e]	Indonesia[f]	Lao PDR[g]	Malaysia[h]	Myanmar[i]	Nepal[j]	Philippines[k]	Thailand[l]
Basa	*Pangasius bocourti*							yes					
Bata	*Labeo bata*	(319)											
Bighead carp	*Hypophthalmichthys nobilis*				yes						yes		
Black carp	*Mylopharyngodon piceus*				yes								
Black ear catfish	*Pangasius larnaudii*							yes					157
Blunt-snout bream	*Megalobrama amblycephala*				yes								
Boggut labeo ('ghonia')	*Labeo boggut*	(177)											
Carp spp.	Not known								47.7			419.9	
Catla	*Gibelion catla*	(1,288)		yes (*Labeo* spp.)		yes				yes			
Channel catfish ('baung')	*Hemibagrus nemurus*								792.6				
Channel catfish	*Ictalurus punctatus*				yes								
Common carp	*Cyprinus carpio*	(292)					148,542.0	yes	0.2	yes	yes		
Common climbing perch ('koi')	*Anabas testudineus*	(18)											10

Continued

Table 6.3. Continued.

Common name	Latin name	Bangladesh[a]	Brazil[b]	Cambodia[c]	China[d]	India[e]	Indonesia[f]	Lao PDR[g]	Malaysia[h]	Myanmar[i]	Nepal[j]	Philippines[k]	Thailand[l]
Common silver barb/ Java barb	*Barbonymus gonionotus*	(1,440)					289.1	yes	53	yes			30
Crucian carp	*Carassius carassius*				yes								
Freshwater catfish ('keli')	*Clarias* sp.			yes			18,754.6		1,093.6 (101.3)	yes			769
Giant gourami	*Osphronemus goramy*						529.2	yes	0.1				96
Giant/ Indonesian snakehead	*Channa micropeltes*			yes									
Grass carp/ Chinese carp	*Ctenopharyngodon idella*	(327)			yes			yes		yes	yes		
Kalibaus	*Labeo calbasu*	(146)				yes							
Kuria labeo	*Labeo gonius*					yes							
Mandarin fish	*Siniperca* spp.				yes								
Milkfish	*Chanos chanos*											8,265.20	
Mozambique tilapia	*Oreochromis mossambicus*						2,145.2		7.3				2
Mrigal	*Cirrhinus cirrhosus*	(1,179)				yes							
Mud carp	*Cirrhinus molitorella*							yes					
Nile tilapia (incl. GIFT and red)	*Oreochromis niloticus*	4,590 (3,261)	est. >186,448		yes	yes	318,515.8	yes	6,108.5 (0.8)	yes		71,488.40	50,079
Pacu	*Piaractus mesopotamicus*		est. 10,112										
Pirapitinga	*Piaractus brachypomus*		est. 8,518										
Pomfret	Various pomfret species						25,044.7						

River carp ('lampam sungai')	*Barbonymus schwanenfeldii*								13.6			
River carp ('jelawut')	*Leptobarbus hoevenii*			yes			1,508.1		80.1			
Rohu	*Labeo rohita*	(1,849)				yes						
Sand/marble goby ('ketutu')	*Oxyeleotris marmoratus*			yes (spp.)			143.6		0.8			1
Silver carp	*Hypophthalmichthys molitrix*	(1,003)			yes			yes		yes		
Silver shark minnow	*Osteochilus vittatus*						63.5					
Small scale mud carp	*Cirrhinus microlepis*			yes								
Snakehead, various and hybrids	*Channa* spp.	(47)	yes		yes		42,637.3	yes				4
South American catfish/ jundiá	*Rhamdia quelen*		yes									
Streaked prochilod/ curimbatá	*Prochilodus lineatus*											
Striped catfish	*Pangasianodon hypophthalmus*	(345)		yes (spp.)		yes	72,437.6	yes	7,525.1 (576)			66
Sturgeons	Various species				yes							
Swamp eels	*Monopterus* and *Ophichthys* spp.	(13)			yes							
Tambaqui	*Colossoma macropomum*		est. 21,856									
Tambaqui × pacu hybrids	Various hybrids		yes									

Continued

Table 6.3. Continued.

Common name	Latin name	Bangladesh[a]	Brazil[b]	Cambodia[c]	China[d]	India[e]	Indonesia[f]	Lao PDR[g]	Malaysia[h]	Myanmar[i]	Nepal[j]	Philippines[k]	Thailand[l]
Walking catfish	*Clarias batrachus*	(9)											
Wallago catfish ('boal')	*Wallago attu*	(51)											
Yellow mystus	*Mystus nemurus*				yes		4,867.9						
Other freshwater fish	Other freshwater fish	(1,573)					15		13.8			8.5	835
Total		17,927	226,934				635,493.60		16,414.50			80,182	52,049

Table 6.4. Taxonomic classification, viral properties, susceptible host(s) and geographical distribution of major viruses causing diseases in tropical finfish commonly cultured in fresh waters.

Disease	Virus agent	Genus/Family	Nucleic acid/ Genome	Morphology/Size (nm)	Enveloped	Susceptible host(s)	Geographical distribution
Tilapia lake virus (TiLV) disease or syncytial hepatitis of tilapia (SHT)	*Tilapinevirus tilapiae*	*Tilapinevirus* Amnoonviridae	ssRNA 10.323 kb 10 RNA segments	Icosahedron 55–100	yes	Tilapia, giant gourami, African cichlids	17 countries in Asia, Africa and American continents
Tilapia parvovirus (TiPV) disease	Tilapia parvovirus (TiPV)	*Parvovirus Parvoviridae*	ssDNA >4269 bp	Spherical ~30	no	Tilapia	China, Thailand
Carp edema virus disease (CEVD) or koi sleepy disease (KSD)	*Cyprinid herpesvirus 1*	*Poxvirus Chordopoxvirinae*	dsDNA	Spherical to pleomorphic shape ~416–450	yes	Carps	Europe, USA, Japan, China, India, Korea, Thailand
Spring viraemia of carp (SVC)	Spring viraemia of carp virus (SVCV)	*Vesiculovirus* Rhabdoviridae	ssRNA	Bullet shape 60–90 wide by 90–180 long	yes	Carps	Europe, Asia
Koi herpesvirus disease (KHVD)	*Cyprinid herpesvirus 3* (CyHV-3) or koi herpesvirus (KHV)	*Herpesvirus* Alloherpesviridae	dsDNA	Icosahedron ~100–110	yes	Carps, tilapia	Worldwide
Grass carp reovirus disease (GCRVD)	Grass carp reovirus (GCRV) or Chinese grass carp reovirus (CGRV) or golden shiner virus (GSV)	*Aquareovirus C* Reoviridae	dsRNA	Icosahedron ~60–70	no	Carps	Asia
Channel catfish virus disease (CCVD)	Channel catfish virus (CCV) or *Ictalurid herpesvirus 1*	*Ictalurivirus* Alloherpesviridae	dsDNA	Icosahedron ~90–100	yes	Channel catfish, blue catfish, striped catfish	USA, Malaysia
Epizootic ulcerative syndrome (EUS)	EUS rhabdovirus	*Vesiculovirus* Rhabdoviridae	ssRNA	Bullet shape ~65–175	yes	Snakehead, catfish, goby, gourami, climbing perch, barbs	South-East Asia, East Asia and Africa

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Diagnosis

Affected fish may present lethargy, loss of appetite, abnormal swimming, cessation of schooling, skin erosion, scale protrusion, gill pallor, abdominal distension, anaemia and exophthalmia (Ferguson *et al.*, 2014; Dong *et al.*, 2017b). In diseased hybrid red tilapia, a pale body and reddish opercula are usually observed, while diseased Nile tilapia usually present a darkened body (Fig. 6.2A). Internally, abnormalities include pale, necrotic and watery livers, with bile diffusion giving it a dark or an unusual green colour. An enlarged spleen and gall bladder, and fluid accumulation in the intestine and abdominal cavity, may also be observed (Dinh-Hung *et al.*, 2021; Tang *et al.*, 2021).

Pathognomonic lesions for the histopathological diagnosis are the presence of syncytial cells containing multiple nuclei in the liver (syncytial hepatitis) (Ferguson *et al.*, 2014; Del-Pozo *et al.*, 2017; Dong *et al.*, 2017b) (Fig. 6.2B). Stereotypical histopathological changes were also reported in the liver including hepatic vacuolization, focal necrosis, losing storage fats, cell dissociation, and a foamy cytoplasm to hepatocytes containing lipoprotein-like droplets or eosinophilic inclusions. Pancreatic degeneration and infiltration of inflammatory cells were also described (Jansen *et al.*, 2019). Other histopathological alterations in the spleen, kidney, brain, gills, gastrointestinal tract and reproductive organs are not pathognomonic.

Molecular methods for the early screening of TiLV from asymptomatic fish or as a confirmatory diagnosis of clinically sick fish include conventional reverse transcription polymerase chain reaction (RT-PCR), nested RT-PCR, semi-nested RT-PCR, SYBR Green- and TaqMan probe-based quantitative RT-PCR (RT-qPCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) methods for pond-side diagnosis of TiLV (see Taengphu *et al.*, 2022). Taengphu *et al.* (2022) published a validated RT-qPCR method that can detect and quantify TiLV from both fish and environmental samples. Delamare-Debouteville *et al.* (2021) integrated rapid amplicon sequencing, called Oxford Nanopore Technologies (ONT), with a PCR assay that serves as a promising platform for backpack, pond-side diagnosis and genotyping of TiLV in a single day. Other molecular methods for the localization of TiLV from infected tissues include *in situ* hybridization (ISH) using TiLV-specific probes (Bacharach *et al.*, 2016; Dong *et al.*, 2017b; Dinh-Hung *et al.*, 2021) and immunohistochemistry (IHC) using TiLV-binding antibodies (Piewbang *et al.*, 2021).

Several cell lines are reported to propagate TiLV including E-11, a clone of the cell line SSN-1 derived from *Channa striata*, tilapia primary brain cells OmB and TmB derived from *Oreochromis mossambicus*, CFF derived from *Pristolepis fasciatus*, OnlB and OnlL derived from *O. niloticus* liver and brain, TiB cell line established from tilapia brain, and CAMB originated from hybrid snakehead brain (see Wang *et al.*, 2020). Cytopathic effects (CPEs) typically appear 3–12 days after inoculating cells at 25°C with filtered homogenate containing TiLV virions.

Transmission

TiLV can be transmitted both horizontally and vertically. Cohabitation of infected and naïve fish induced disease and resulted in 56–80% mortality in a couple of weeks (Eyngor *et al.*, 2014; Liamnimitr *et al.*, 2018). Using environmental RNA (eRNA) to quantify viral loads in water, Taengphu *et al.* (2022) found ~10^3–10^5 copies per litre of pond water during disease outbreaks, confirming its waterborne transmission. Vertical transmission of TiLV from fertilized eggs and fry was confirmed through experimental infection (Yamkasem *et al.*, 2019; Dong *et al.*, 2020).

Control and prevention

Biosecurity, selective breeding and vaccination are effective in managing viral diseases in aquaculture. Using TiLV-free broodstock for seed production, early screening for TiLV-negative stocks by PCR before stocking, reducing stress and improving fish immunity in the early days of stocking are recommended to reduce the risk of disease outbreaks (Jansen *et al.*, 2019; Tang *et al.*, 2021).

Disinfection of the pond, water and equipment could prevent contamination. Jaemwimol *et al.* (2019) reported that 2.5 ppm iodine, 10 ppm sodium hypochlorite, 300 ppm hydrogen peroxide, 80 ppm formalin and 0.5% Virkon™ can inhibit the *in vitro* replication of TiLV; while Soto *et al.* (2019) found that 50 ppm buffered

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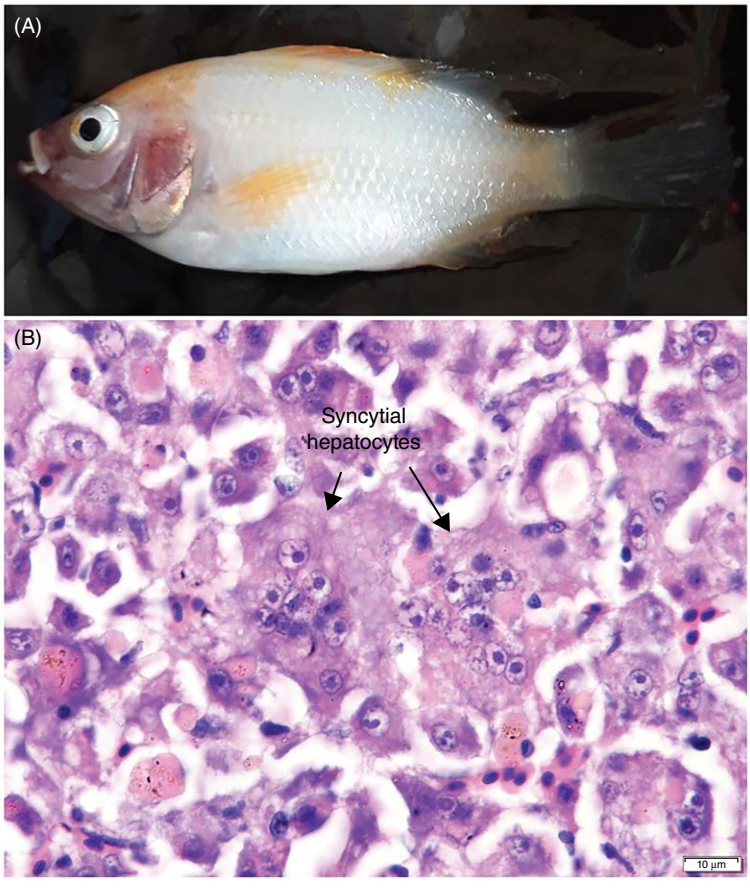


Fig. 6.2. (A) Juvenile hybrid red tilapia naturally infected with TiLV showing pale colour, scale protrusion, reddish colour around the mouth and on the operculum. (B) Pathognomonic lesions (syncytial hepatocytes) found in the fish liver are useful for histopathological diagnosis of TiLV infection. (Images by H.T. Dong.)

povidone iodine and household bleach (20 ppm free chlorine) can inactivate TiLV in 30 min.

Several studies report vaccination efficacy in preventing TiLVD. A live attenuated vaccine developed in Israel had a relative percentage survival (RPS) of 56–58% (Bacharach and Eldar, 2016). In China, a β-propiolactone-inactivated vaccine using the adjuvant Montanide IMS 1312 gave protection with RPS of 85.7% (Zeng *et al.*, 2021). Segment 8 (VP20)-based DNA and recombinant protein vaccines have also been investigated. DNA vaccine prime-recombinant VP20 (rVP20) boost, resulted in 72.5% protection (Zeng *et al.*, 2021). In Thailand, Mai *et al.* (2021) developed heat- and formalin-killed vaccines which gave good protection in tilapia juveniles with RPS of 71.3 and 79.6 %, respectively. These vaccines stimulated both branches of the adaptive immune systems (B-cell and T-cell responses) and elicited both systemic and mucosal anti-TiLV immunoglobulin M (IgM). Interestingly, the protective antibodies raised in vaccinated broodstock can be transferred maternally to the fertilized eggs and these persist in 1- to 3-day-old larvae. Thus, vaccination of tilapia broodstock was suggested to be a promising strategy for preventing TiLV infection in tilapia fertilized eggs and larvae (Mai *et al.*, 2022). Up-scaling TiLV vaccine production at an affordable cost for tilapia farmers remains a bottleneck for the tilapia industry.

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6.2.2 Tilapia parvovirus (TiPV) disease

The first mass mortality report of adult *O. niloticus* was from China in 2020. The causative agent was identified as a novel parvovirus, called tilapia parvovirus (TiPV), a new member of the genus *Chapparvovirus* (family Parvoviridae) (Liu *et al.*, 2020). TiPV has been detected in tilapia samples collected from multiple provinces in China during 2015–2019 with relatively high prevalence (23.1 to 64.6%) (Du *et al.*, 2019; Liu *et al.*, 2020). Recently, a co-infection of TiLV and TiPV resulted in mortalities of 50–70% in juvenile hybrid red tilapia (*Oreochromis* sp.) in Thailand (Yamkasem *et al.*, 2021; Piewbang *et al.*, 2022). Co-infection of TiPV and *Streptococcus agalactiae* in adult Nile tilapia was also reported most recently in Thailand where <1% of the affected fish population showed signs of illness, and only minor mortalities were recorded because of handling stress (Dong *et al.*, 2022).

TiPV is a non-enveloped, spherical-shaped virus that is ~30 nm in diameter. Its genome is linear single-stranded DNA (ssDNA).

Relatively high mortality (60–70%) was estimated in adult Nile tilapia during disease events in China. Experimental infection using tissue homogenates from diseased fish resulted in 90% mortality within a couple of weeks post-infection (Liu *et al.*, 2020).

Diagnosis

Currently, no pathognomonic gross signs are proposed for the diagnosis of TiPV disease. Sick adult *O. niloticus* displayed lethargy, anorexia, abnormal corkscrew swimming movements, skin haemorrhages, exophthalmia and pronounced ocular lesions (Liu *et al.*, 2020) (Fig. 6.3A). TiLV and TiPV co-infected hybrid red tilapia displayed lethargy, haemorrhages and focal ulceration on the body surface, exophthalmos and abnormal swimming (Yamkasem *et al.*, 2021; Piewbang *et al.*, 2022). On the other hand, TiPV and *S. agalactiae* co-infected fish showed skin erosions, cloudy eye lenses and corneal injury. Most of the internal organs appeared normal, except for a few cases of microhepatica, intestinal pallor and necrotic areas in the ovary (Dong *et al.*, 2022).

Cowdry type A inclusion bodies (CAIB) within pancreatic acinar cells were proposed as the pathognomonic lesions that are diagnostic of TiPV infection (Dong *et al.*, 2022) (Fig. 6.3B).

Conventional PCR and SYBR Green-based quantitative PCR (qPCR) methods for the diagnosis of TiPV were published by Liu *et al.* (2020).

Liu *et al.* (2020) successfully isolated TiPV from infected fish using the tilapia brain cell line (TiB) originating from Nile tilapia. After inoculation with tissue homogenate, CPEs were initially observed at 3 days post-infection (dpi) and were clearly observed at 6 dpi, with completely detached cells at 8 dpi. Observed CPEs were cell shrinkage, rounding, and cell fusion with cytoplasmic vacuolization.

Transmission

The TiPV transmission route is still unknown. TiPV, like other aquatic viruses, is likely to spread vertically and horizontally. Dong *et al.* (2022) found TiPV within the oocytes of infected Nile tilapia.

Control and prevention

TiPV is currently reported in China and Thailand; wider distribution of the virus is likely due to the long history of active movement of live fish for aquaculture. Most disease cases were co-infections of TiPV and other pathogens, suggesting that further investigation to assess the true impact of TiPV is required. Apart from disease surveillance, increasing awareness and biosecurity measures are recommended to prevent the transboundary spread of this virus.

6.2.3 Carp edema virus (CEV) disease

Carp edema virus disease (CEVD) is caused by carp edema virus (CEV), a pox-like virus belonging to the Poxviridae (Adamek *et al.*, 2017a). As infected fish become lethargic and unresponsive at the bottom of their culture system, the disease is therefore also known as 'koi sleeping disease' (KSD). It was first described in juvenile koi carp in Japan in the 1970s with a mortality rate of up to 80–100%. Later, it was reported sporadically in adult koi and common carp (Oyamatsu *et al.*, 1997a; Miyazaki *et al.*, 2005). Due to the popularity of koi and common carp and their international trade, the virus has been introduced to

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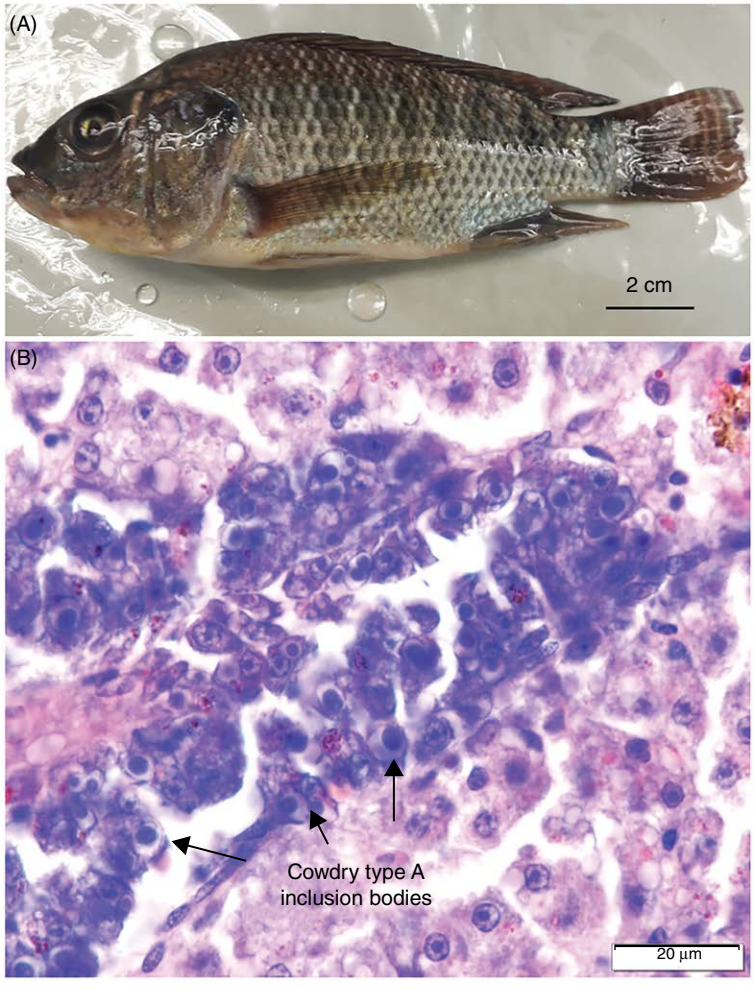


Fig. 6.3. (A) Adult Nile tilapia naturally infected with TiPV showing ocular damage. (B) Haematoxylin and eosin-stained section revealing pathognomonic lesions (Cowdry type A inclusion bodies) in the pancreatic tissue. (Images by H.T. Dong.)

many other nations (Marsella *et al.*, 2021). These reports serve as a warning of a new threat to the carp industry, which constitutes about 8.3% (in 2018) of the aquaculture food production sector globally (FAO, 2020).

The virus is described as enveloped with a 'mulberry-like' morphology. Two types of virions are described: immature ones with a rounded shape ranging from 416 to 450 nm in diameter, and mature ovoid virions measuring 300–400 nm × 250–400 nm. Mature virions have capsomeres arranged around a kidney-shaped nucleoid (Miyazaki *et al.*, 2005). The genome of CEV consists of double-stranded linear DNA; its complete genome has been published recently (Mekata *et al.*, 2021). Based on the P4a nucleotide sequence encoding the P4a core protein, at least three genogroups have been confirmed (Soliman *et al.*, 2019).

Diagnosis

In addition to the 'sleeping' behaviours of fish, signs of infection include gill necrosis, swelling of the primary filaments, sunken eyes, an over-secretion of mucus, and haemorrhages at

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the mouth and the bases of fins (Miyazaki *et al.*, 2005; Way *et al.*, 2017). The virus mainly attacks the gills, causing congestion, interfering with respiration and rendering fish lethargic (Oyamatsu *et al.*, 1997a).

Over 80% of the virus was found in the gills (Oyamatsu *et al.*, 1997a), making it a suitable tissue for diagnosis. Histopathology of the gills may show vacuolization in the primary gill lamellae; clubbing and fusion of the secondary gill lamellae; blockage of the interlamellar spaces by cellular debris; and epithelial cell enlargement (Adamek *et al.*, 2017a).

Molecular detection is based on the gene that encodes the P4a core protein. The first PCR protocols were developed in Japan using single and nested PCR (Oyamatsu *et al.*, 1997b), thereafter nested PCR and qPCR methods (Adamek *et al.*, 2016; Matras *et al.*, 2017) were developed. More recently, a novel droplet digital PCR (ddPCR) assay was developed that can detect about 2.2 ± 0.26 copies of CEV DNA per reaction and serves as a valuable tool for the diagnosis of this emerging disease (Wang *et al.*, 2021).

To date, no susceptible cell line has been reported for CEV, making it difficult to isolate the virus *in vitro* as part of disease diagnostics.

Transmission

Outbreaks occur more frequently in low temperatures (15–25°C) (Miyazaki *et al.*, 2005) and lower (6–15°C) (Way and Stone, 2013; Soliman *et al.*, 2019). Although temperature is the key factor, stressors such as poor water conditions and transportation may lead to manifestation of clinical signs (Lewisch *et al.*, 2015). Following lethargy, mortality occurs within 4–5 days, with a peak of mortality around 2 weeks when 75–100% of fish die (Miyazaki *et al.*, 2005). Co-infections of CEV with other pathogens such as *Cyprinid herpesvirus 3* (CyHV-3) (Adamek *et al.*, 2016; Kim *et al.*, 2020), flavobacteria (Adamek *et al.*, 2018) and *Aeromonas* spp. (Jung-Schroers *et al.*, 2015; Lewisch *et al.*, 2015) have been casually reported. The findings suggest that CEV acts as the primary agent that injures the gill structure or as an immunosuppressant paving the way for secondary infection (Jung-Schroers *et al.*, 2015; Way *et al.*, 2017).

To date, there is no solid evidence regarding the mechanism of transmission, including effective vectors of CEV. Water, however, is suggested to be the main cause of contagion by carrying epithelial cells that have been shed from the gill filaments of infected fish (Hedrick *et al.*, 1997).

Control and prevention

Currently there is no effective treatment for CEVD, hence preventive measures are the best resort for this disease. Translocation of fish remains a high-risk factor that can introduce the pathogen, so an effective diagnosis regime is imperative. As temperature constitutes a factor in virus proliferation, the harvesting and handling of fish in low temperatures of 15–25°C should be avoided. In Japan, keeping fish in 0.5% saline water prior to stressful events such as translocation, grading and handling is a common practice that helps delay the growth of CEV and helps rejuvenate the physiological condition of the host. It should be noted though that bathing fish in saline does not eradicate the virus. CEVD appears to cause more serious clinical signs in some strains of carp than in others, so in production it is judicious to select varieties that can resist the virus such as wild Amur carp (Adamek *et al.*, 2017b).

6.2.4 Koi herpesvirus (KHV) disease

Outbreaks of koi herpesvirus disease (KHVD), also known as carp nephritis and gill necrosis virus (CNGV) disease (Pikarsky *et al.*, 2004), have been reported extensively in Europe and North America. In Asia, epizootic levels were reported in Israel, Japan, Indonesia, Taiwan, Thailand, Singapore and Korea (Lio-Po and Lim, 2014). A three-year surveillance in 2004 to 2006 did not detect koi herpesvirus (KHV) in Cambodia, Lao PDR, Myanmar, the Philippines and Vietnam (Lio-Po *et al.*, 2009). Economic losses can be significant (Sunarto *et al.*, 2005; Yuasa and Sano, 2009).

KHV or CyHV-3 belongs to the family Herpesviridae (see Waltzek *et al.*, 2005). This double-stranded DNA (dsDNA) virus has an icosahedral nucleocapsid measuring 101.9 ± 10.3 nm and a total genome length of approximately 295 kbp (Aoki *et al.*, 2007). The KHV strains found in the USA, Israel and the Netherlands were closely related and of genotype E, while those found in Japan, Indonesia,

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Taiwan and China were also closely related and of genotype A (Kurita *et al*., 2009).

KHV infects common carp, koi carp and hybrid ghost carp although there are conflicting reports on the susceptibility of other fish species to KHV.

Natural and experimental transmission of the infection from common carp to *Carassius auratus*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *O. niloticus*, *Pangasianodon hypophthalmus*, *Plecoglossus altivelis*, etc. were unsuccessful (Perelberg *et al*., 2003; Ito *et al*., 2007; Yuasa and Sano, 2009). Hedrick *et al*. (2006), however, reported that goldfish × common carp hybrids were moderately sensitive to KHV.

Natural infections of susceptible fish result in 80–90% mortality within a week after the onset of clinical signs (Lio-Po and Lim, 2014). The virus can be transmitted from infected fish to naïve koi or common carp via intraperitoneal injection, by bath or by cohabitation.

Experimental exposure of healthy common carp to KHV at 22°C can cause up to 82% mortality within 15 days (Ronen *et al*., 2003). The incubation period of the disease is 5–7 days, characterized by onset of clinical signs, and spread to koi and common carp was rapid when water temperatures were 15–25°C (Gilad *et al*., 2003; Ronen *et al*., 2003).

Diagnosis

Infected fish typically have white, necrotic patches on the gill filaments, sunken eyes, haemorrhages on the body surface, and excessive mucus production with rough pale patches on the skin. Affected fish often swim at the surface and exhibit respiratory distress.

The gills develop lamellar epithelial degeneration, focal areas of necrosis and exfoliation. There is hyperplasia and fusion of the secondary gill lamellae (Tu *et al*., 2004). Intranuclear inclusion bodies (Fig. 6.4), a pathognomonic lesion for histopathological diagnosis of KHVD, can be observed from multiple organs of the KHV-infected fish such as the branchial epithelium, liver, kidney and spleen. The virus can be visualized by transmission electron microscopy (TEM). Necrotic tissues are also seen in the liver, spleen, and kidney parenchymal cells (Hedrick *et al*., 2000). Non-specific secondary infections may be associated with KHV infections (e.g. *Flavobacterium columnare* and *Aeromonas* spp.) (Sunarto *et al*., 2005).

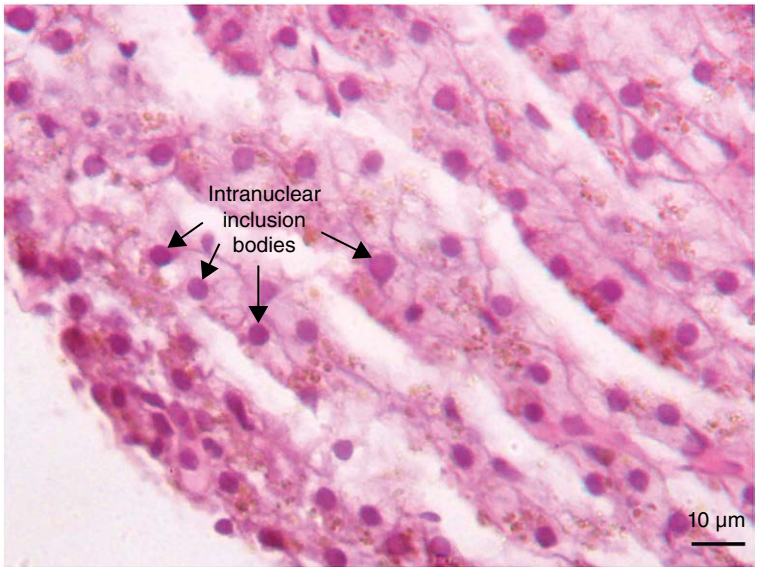


Fig. 6.4. Naturally KHV-diseased koi carp showed pathognomonic lesions (intranuclear inclusion bodies) in the liver (haematoxylin and eosin stain). (Image by H.T. Dong.)

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To date, PCR is one of the most sensitive, specific and rapid tests for KHV detection (Gray *et al.*, 2002; Gilad *et al.*, 2004; Bercovier *et al.*, 2005; El-Matbouli *et al.*, 2007). A ring trial confirmed that the Bercovier-TK (1) and modified Gray SpH (2) primer sets were the most robust for detection of KHV DNA (Way *et al.*, 2008). A real-time PCR for the quantitative estimation of the KHV virus has also been developed using TaqMan real-time PCR (Gilad *et al.*, 2004).

Despite high sensitivity, the detection of latent and persistent stages, or during the onset of the disease when viral loads are low, may result in false negative results (Gilad *et al.*, 2003; Bergmann *et al.*, 2010). Subsequently, Monaghan *et al.* (2015a) assessed the sensitivity of seven PCRs for the early detection of KHV and found all PCRs could detect higher positivity from mucus samples (gill, skin, fin base swabs) compared with fish organs (gill, spleen, kidney, gut) in early infection (<4 days post-challenge). Viral DNA may be more concentrated in the mucus during the first days of transmission. The World Organisation for Animal Health (Office International des Epizooties, OIE), however, recommends diagnosis from internal organs of late-stage infected fish (OIE, 2019) as the virus colonizes these organs in abundance.

The development of a rapid LAMP test proved equally sensitive in its detection (Yoshino *et al.*, 2006). The analysis of mucus serves as an appropriate non-destructive sampling of suspected fish, particularly given the value of koi specimens. The recently developed fluorescence real-time LAMP assays to simultaneously detect CyHV-3 and CEV genogroup II with high sensitivity serve as an appropriate tool for the screening of transboundary fish (Cano *et al.*, 2021).

Other RT-PCR methods targeting the mRNA terminase (Yuasa *et al.*, 2012) and ISH (Haenen *et al.*, 2004) can also detect KHV in paraffin-embedded tissues from infected common carp. Strong ISH-positive responses were detected within epithelial cells of the secondary gill lamellae and in mucus cells of the basal area (Lee *et al.*, 2012). ISH demonstrated its utility in detecting viral DNA in gill and gut sections at 1 h post-infection (Monaghan *et al.*, 2015b).

Enzyme-linked immunosorbent assay (ELISA) allows for the detection of antibodies to KHV in the serum of fish previously exposed to the virus (St-Hilaire *et al.*, 2009). This method confirms the presence of anti-KHV antibodies found in the sera of survivors from KHV outbreaks that become carriers of the virus. The combination of ELISA and qPCR is recommended in the diagnosis of KHV-suspected carrier fish (Soto *et al.*, 2020).

The virus may be isolated in susceptible fish cell lines such as koi fin (KF-1), koi fin (KFC), koi tail (KT-2), common carp brain (CCB), koi snout (KS) or head kidney of common carp (KoK) (Eckart *et al.*, 2019). The KF-1 cells inoculated with KHV exhibit typical vacuolations at 20–25°C in 7–14 days. In contrast, survival of KHV in CCB cells was maintained for 30 days at 30°C (Dishon *et al.*, 2007). After 3 dpi, CPE is characterized by giant syncytia. Current recommended cell lines may not be highly sensitive to KHV (OIE, 2019a); newer cell lines such as KoK are expected to improve detection of KHV (Eckart *et al.*, 2019).

Transmission

Temperature is a critical factor in the pathogenesis of KHV infection. Cell cultures inoculated with KHV and incubated at optimum temperature develop typical vacuolation. However, the vacuolated cells may revert to normal, and plaques may disappear when the cells are shifted to non-permissive temperatures and can reappear after transfer to permissive temperatures (Dishon *et al.*, 2007). *In vivo*, the virus induces infection/mortalities at 18–25°C; if the fish are held at 13 or 30°C, no infection or mortality develops (Goodwin, 2005).

Some studies reported that virulence and pathogenesis of KHV are strain dependent. The virus can remain latent in the host for long periods without manifesting clinical signs of KHVD, becoming active only at permissive temperatures (Lio-Po and Lim, 2014).

Other species may be potential vectors for KHV transmission. In an experimental set-up, Matras *et al.* (2019) confirmed several potential vectors with the detection of viral DNA at 49 dpi. Fabian *et al.* (2013) found that with naturally occurring infections, many wild cyprinid and non-cyprinid species cohabitating with carp can become carriers of the virus, regardless of season, temperature variation, age and infection status of the carp stock. In addition, Minamoto *et al.* (2011) found that several rotifers from natural lakes tested positive for KHV DNA.

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Prevention and control

A live attenuated KHV commercial vaccine, developed in Israel, is available for the prevention of KHV (Ronen *et al.*, 2003; Perelberg *et al.*, 2005). Vaccinated carp develop high antibody titres resulting in RPS of 80–95%. Protective immunity after vaccination lasts for at least 8 months. Using two formalin-inactivated vaccines entrapped within the liposomal membrane experimentally sprayed on to dry pellets and fed to common carp, Yasumoto *et al.* (2006) produced RPS scores of 74.4 and 65% when challenged with its homologous virus 22 days after vaccination. By an immersion method, the recombinant plasmid of the ORF149 gene of KHV can be delivered by single-walled carbon nanotubes (SWCNTs) proved to confer 56% protection (Hu *et al.*, 2021). Other recombinant DNA vaccines using partial viral genes (such as ORF25) are in research.

The virus is persistent, and this is related to a cycle of persistent infection and reactivation in hosts given that the virus was found to be present not only in water and pond sediment (Honjo *et al.*, 2012), but also in tank standpipes up to 6 days after removing fish (Tolo *et al.*, 2021). Effective disinfection is crucial to reduce the spread of the disease. The virus can be killed by iodophor (200 mg/l), benzalkonium chloride (60 mg/l) or 30% ethyl alcohol for 20 min (Kasai *et al.*, 2005). Other disinfectants such as peracetic acid (0.1% v/v), quicklime (0.015 M) and a protease, Neutrase® (8 mU/ml), were able to reduce the viral load of KHV and viral haemorrhagic septicaemia virus (VHSV) by at least four orders of magnitude within 24 h (Amtmann *et al.*, 2020).

The transboundary introduction of KHV into non-endemic countries should be closely monitored. Somga *et al.* (2010) intercepted an illegal importation of koi carp found positive for KHV in Manila. Thus, adequate biosecurity measures on importation of koi and common carp should be in place. Sensitive and quick detection of KHV prior to translocation undoubtedly is necessary.

6.2.5 Grass carp reovirus (GCRV) disease

Grass carp reovirus (GCRV) disease was previously known as haemorrhage of grass carp (Nie and Pan, 1985), haemorrhagic disease of grass carp (Jiang, 2009) and Chinese grass carp reovirus (CGRV) disease in China. This is the most serious viral infection reported in grass carp in China, causing ~80% mortalities (Jiang, 2009). Annual outbreaks with losses in central, southern and eastern China and northern Vietnam occurred at water temperatures of 24–30°C (Jiang, 2009). In the USA, golden shiner virus (GSV) disease, a similar disease, was first detected in cultured *Notemigonus crysoleucas* in 1977 (Plumb *et al.*, 1979). Outbreaks of a haemorrhagic GCRV-like disease also occurs in *I. punctatus* called channel catfish reovirus (CCRV) disease. Fish were introduced into China in 1984, and infection at 28°C results in mortalities of up to 60% in cultured fingerlings (Xu, J. *et al.*, 2013).

The *Aquareovirus* causing GCRV disease is a 60–80 nm virus belonging to the family Reoviridae. The virus is characterized by a non-enveloped icosahedron with a 5:3:2 three-dimensional symmetry. Its genetic material consists of 11 segmented double-stranded RNAs (dsRNAs) encoding seven structural proteins (VP1–VP7), the remainder are non-structural proteins. Proteins VP1–VP4 and VP6 build the core layer, while VP5 and VP7 form the outer capsid composed of 200 trimers (Cheng *et al.*, 2008; Fan *et al.*, 2010). Two serotypes with distinct antigenicities were reported in China, namely GV-87/3 from Hunan Province and GV-90/14 from Hubei Province (Jiang, 2009). CCRV-730 from *I. punctatus* measures 60–70 nm in diameter and has 99–100% nucleotide sequence similarity to the GCRV-873 strain. Strains are based on structural proteins V4, V6 and V7; among GCRV strains, GCRV-873, GCRV-HZ08 and Hubei GCRV are the three representative genotypes (Rao and Su, 2015).

In China, epizootics occur in grass carp *C. idella*, *Mylopharyngodon piceus*, *Pseudorasbora parva* and *Gobiocypris rarus* (see Wang *et al.*, 1994); however, the virus can replicate subclinically in *H. molitrix* and *Hemiculter bleekeri* (see Ding *et al.*, 1991). Other cyprinids such as bighead carp, common carp and golden carp can be asymptomatic carriers (Jiang, 2009).

In the USA, GSV infects golden shiner and grass carp (McEntire *et al.*, 2003); the virus induces low-grade mortality (Plumb *et al.*, 1979) but under crowded conditions and at high water temperatures, acute epizootics result in 50–75% mortality (Schwedler and Plumb, 1982).

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Diagnosis

Signs of infection in grass carp include exophthalmia, dark body coloration, haemorrhages in the mouth cavity, and haemorrhagic or pale gills, fin bases and opercula. Affected fish are listless, swimming near the surface, have petechial haemorrhages in the cornea and dorsal musculature (reddish back), petechiae on the ventral body surfaces, in visceral fat and in the intestinal mucosa. Internally, haemorrhages develop in the musculature, intestine, liver, spleen and kidney. Degeneration and necrosis of the liver cells, hyperaemia and haemorrhagic vessels of the liver and spleen occur (Guo and Jiang, 1993). The virus replicates rapidly in the head kidney of infected fish causing an acute infection and significant mortalities of up to 80% among fingerlings and sometimes yearlings.

CCRV-infected channel catfish exhibit pale gills, localized haemorrhages in the liver, a dark and enlarged spleen, an empty gut, and haemorrhages on the intestinal wall with a clear yellow to blood-tinged fluid in the abdomen (Xu, J. *et al.*, 2013). Experimental infection yielded initial deaths at 2 dpi, reaching up to 50% mortalities at 7 dpi; clinical signs were like those observed in natural infections. Diseased channel catfish fingerlings become lethargic, float at the surface, display spiral swimming and die within 24 h manifesting severe haemorrhages of the operculum, lower jaw and fin bases, body colour depigmentation, bilateral exophthalmia and abdominal distension.

A neutralization test, ELISA or RT-PCR is used for virus detection (Li *et al.*, 1997). Seng *et al.* (2004) developed an RT-PCR for the detection of GCRV and other aquareoviruses. Later, a multiplex PCR was designed to detect GCRV and to distinguish between the three genotypes (Zeng *et al.*, 2013).

Isolation of GCRV is possible in cell cultures of grass carp kidney (CK or CIK), swim bladder cells of grass carp (GSB) and proboscis snout into fibres (PSF) cells of grass carp at 25°C (Li *et al.*, 2016). CPEs in CCRV-exposed channel catfish kidney (CCK) cells occurs 48 h post-inoculation (Xu, J. *et al.*, 2013).

Transmission

Experimental transmission of the virus by immersion in water containing GCRV resulted in disease and mortality within 1–2 weeks at 25–28°C. The serotype GV-90/14 showed increased virulence, inducing 100% mortality in 3–5 days in experimentally exposed grass carp fingerlings, while only 38% mortalities in 5–10 days were attributed to the serotype GV-87/3 (Jiang, 2009).

Prevention and control

In China, vaccination of naïve fingerlings with formalin-inactivated GCRV containing lipopolysaccharide induced immunity of up to 90%, a week after vaccination, when held at >20°C (Jiang *et al.*, 1991). An inactivated vaccine prepared from infected fish was effective when given to fish from the same region but less effective when given to fish in another region (Yang *et al.*, 1989). Vaccine administration by immersion yielded lower protection rate; however, the rate of protection can be increased using hyperosmotic infiltration (Ye *et al.*, 1992). An attenuated vaccine was developed but gave poor protection (Jiang, 1995). The vaccination of grass carp fingerlings in China has reduced mortality from >80 to <30% (Jiang, 2009). Subunit vaccines focusing on partial structural proteins show promise. The V35 subunit vaccine resulted in a 60% survival rate post-challenge (Gao *et al.*, 2018), and the VP56 subunit vaccine gave 71–75% protection (Pei *et al.*, 2019). Laboratory-scale trials, using DNA vaccines based on S6 and S10, gave moderate (59.9%) to low (23.1%) protection against GCRV-II (Chen *et al.*, 2018).

6.2.6 Channel catfish virus (CCV) disease

Channel catfish virus (CCV) is a viral pathogen of fry and fingerling channel catfish that can result in significant losses in commercial production. The first outbreak of channel catfish viral disease (CCVD) occurred in the USA in 1968 and it now occurs wherever channel catfish are reared (Plumb, 1999). *Ictalurus furcatus* and *P. hypophthalmus* are naturally susceptible to CCV, while common carp, koi carp and *Carassius carassius* are experimentally susceptible to CCV; other catfish species such as *Clarias gariepinus*, *Clarias batrachus* and *Silurus glanis* were resistant to an experimental challenge with CCV. CCV or *Ictalurid herpesvirus 1*, measuring 90–100 nm, is

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a double-stranded DNA virus belonging to the family Alloherpesviridae.

Diagnosis

Clinical signs are abdominal distension, exophthalmia, pale or haemorrhagic gills, and petechial haemorrhages at fin bases and the ventrum. In 20–50% of epizootics, affected fish swim in an upright or hanging position. Weak fish sink to the bottom and respire weakly, which increases prior to death (Plumb, 1999).

Acute infection occurs primarily in <10 g fish following waterborne exposure to CCV (Plumb, 1999). Infection is a haemorrhagic viraemia, initially replicating in the kidney and then in the spleen. Thereafter, the virus is transported to the intestine, liver, heart and brain. After experimental immersion, CCV was detected at 2–8 dpi in the skin and blood, at 10 dpi in the posterior kidney and gills (Kancharia and Hanson, 1996). Necrosis of the renal haematopoietic tissue and tubules; oedema, necrosis and congestion of the liver; intestinal oedema; and congestion and haemorrhage in the spleen are characteristic histopathological findings (Wolf, 1988). In experimentally infected fish, skeletal muscle haemorrhages are seen. Severe mortality approaching 100% occurs in <1-year-old fish in water temperatures of >25°C within 7–10 days. CCV causes moderate mortalities at 21–24°C and almost no mortalities at <18°C.

A rapid, sensitive and specific test for CCV detection is by PCR, which can detect the virus in brain, blood, intestine, kidney and liver of acutely infected fish (Gray *et al*., 1999). Likewise, a nested PCR assay can detect <10 copies of CCV DNA in 10^8 times as much DNA of asymptomatic broodstock carriers (Baek and Boyle, 1996).

CCV replicates in channel catfish ovary (CCO) and in brown bullhead (BB) cells at 25–33°C (Wolf and Darlington, 1971; Bowser and Plumb, 1980). The virus can also be detected by TEM, a serum neutralization test and an indirect fluorescent antibody technique (IFAT). In addition, ELISA can detect antibodies against CCV in the serum of previously CCV-exposed fish (Crawford *et al*., 1999).

Transmission

Transmission is horizontal from fish to fish or through infected water. Experimental infection is possible by injection (intramuscular or intraperitoneal), incorporation of the virus into the feed, swabbing the gills with a virus suspension and by immersion in virus-inoculated water. Juveniles (<10 g) can be infected by CCV via waterborne transmission while injection is needed for larger fish. Infection of 1-year-old fish results in low mortality which is usually associated with transportation or handling stress (Plumb, 1999).

Fingerlings surviving a primary epizootic become carriers of the virus. Survivors were 33% shorter and 85% lighter than their naïve control counterparts (McGlamery and Gratzek, 1974).

The portal of virus entry is via the gills and the gut. Naturally exposed fry die within 3 dpi, while experimentally infected fry die within 7–10 days following the death of the first fish used for the challenge. The virus also persists in apparently healthy adult broodfish in a latent state. CCV can occur in conjunction with a secondary bacterial infection with *F. columnare* that prolongs mortality (Plumb, 1999).

Prevention and control

Experimental studies have shown that attenuated CCV can induce protective immunity to channel catfish after injection or bath exposures (Walczak *et al*., 1981). Passive transfer of adult channel catfish sera with anti-CCV neutralizing antibody activity also protected juvenile channel catfish from challenge with CCV (Hedrick and McDowell, 1987). Subsequent reports, likewise, indicated that vaccination of channel catfish with a combination vaccine pair (ORF59 and ORF6) provided strong protection against CCV challenge (Nusbaum *et al*., 2002). These results showed that virus neutralizing antibodies were elicited with an anamnestic response upon viral challenge.

6.3 Bacterial Diseases

Intensive culture with high stocking density of fish leads to increased feed ration and waste with concomitant increases in ammonia and nitrite toxicity (Mitchell, 1997). This increases the organic load of the rearing water and permits rapid bacterial multiplication. Stress and trauma from handling are also predisposing factors.

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In addition, most bacterial pathogens release enzymes that facilitate their entry/invasion into the fish host tissues. Although they may cause primary infection, they may also act as secondary disease agents to a primary virus or parasite. The major bacterial infections among warm freshwater fish are motile *Aeromonas* septicaemia, *Pseudomonas* septicaemia, edwardsiellosis, francisellosis, enteric septicaemia of catfish, columnaris disease and streptococcal septicaemia/meningoencephalitis (Table 6.5).

6.3.1 Motile *Aeromonas* septicaemia (MAS)

Motile *Aeromonas* septicaemia (MAS) is the most common bacterial infection in fish, also known as haemorrhagic septicaemia, infectious dropsy, infectious abdominal dropsy, red pest, red disease or red sore. MAS affects tropical freshwater fish worldwide and occasionally brackish-water fish. MAS infections are frequently reported in farmed milkfish, common carp, grass carp, Nile tilapia, hybrid red tilapia, snakehead fish, giant gourami, channel catfish and striped catfish (Lio-Po and Lim, 2014; Dong *et al*., 2017c; Hoai *et al*., 2019; Ngo *et al*., 2022). Co-infection of motile *Aeromonas* and other pathogens is common during disease outbreaks (Dong *et al*., 2015a).

Aeromonas hydrophila is known as the aetiological agent of MAS. Recent studies, however, revealed an increasing number of pathogenic motile *Aeromonas* which can also be attributed to outbreaks of MAS including *Aeromonas veronii*, *Aeromonas jandaei*, *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas schubertii* and *Aeromonas dhakensis* (Dong *et al*., 2017c; Hoai *et al*., 2019; Azzam-Sayuti *et al*., 2021; Mursalim *et al*., 2022). A recent investigation on the prevalence of *Aeromonas* isolated from cultured freshwater fish in Malaysia revealed that *A. dhakensis* was the predominant species (43%), followed by *A. veronii* (22%), *A. hydrophila* (20%), *A. caviae* (8%) and *A. jandaei* (7%) (Azzam-Sayuti *et al*., 2021). In Thailand, *A. veronii* was the most dominant (72.1%) motile *Aeromonas* recovered from disease outbreaks from freshwater fish, while *A. hydrophila* was occasionally found (2.3%) (Mursalim *et al*., 2022). *Aeromonas* spp. are a free-living, mesophilic bacteria found in the soil, freshwater lakes, ponds, streams, bottom mud, domestic tap water and sewage. They are often associated with the normal flora of fish and have been isolated from both healthy and diseased fish (Lio-Po and Lim, 2014). Motile *Aeromonas* species cause infections not only in aquatic animals but also in avian hosts, cows and sometimes humans.

Immersion of scarified milkfish fingerlings in *A. hydrophila* resulted in up to 100% mortality at 2 dpi but not in fish with intact skin, while intraperitoneal injection of milkfish with the bacterium caused mortalities within 12 h of injection (Lio-Po and Duremdez-Fernandez, 1986). In walking catfish and snakeheads, *A. hydrophila* induced dermal lesions after intramuscular injection of at least 10^5 cells per fish that eventually ulcerated (Lio-Po *et al*., 1992). Likewise, immersion of Nile tilapia fingerlings yielded an LD_{50} of 1.5×10^6 colony-forming units (cfu)/ml, with 100% mortality at 10^8 cfu/ml and no mortality at 10^3 cfu/ml (Yambot, 1997). In channel catfish with mechanically abraded skin, *A. hydrophila* experimentally induced systemic infection in 80% of exposed fish while cutaneous lesions developed in the remaining fish (Matsche and Grizzle, 1999). Challenge of *P. hypophthalmus* with *A. hydrophila* induced external signs of haemorrhagic infection (Ly *et al*., 2009).

Experimental infections with *A. veronii* or *A. jandaei* resulted in 100% mortality in Nile tilapia within 24 h when high doses ($3.7–8.9 \times 10^6$ cfu/fish) were administered intraperitoneally; a tenfold reduction in dose resulted in 50–70% mortality (Dong *et al*., 2017c). An *A. veronii* dose of 10^5 cfu/fish given to *I. punctatus* induced 100% mortality in 10 days (Hoai *et al*., 2019). In another study, experimental infection of hybrid red tilapia with *A. dhakensis* (1.86×10^5 cfu/g fish) resulted in over 80% mortality in 24 h (Soto-Rodriguez *et al*., 2018).

Diagnosis

Infected fish lose appetite, become lethargic and swim near the surface. External signs are typical of bacterial septicaemia, namely exophthalmia, distended abdomen, fin and tail erosion, skin haemorrhage, ulceration and loss of scales. Septicaemia in acute MAS can be fatal with no obvious clinical signs. Internally, infected fish present ascites, swollen internal organs and

Table 6.5. Important bacterial diseases, causative agents, susceptible host(s), distribution and zoonotic potential.

Bacterial pathogen	Disease name	Susceptible host(s)	Geographical distribution	Zoonotic potential
Aeromonas hydrophila *Aeromonas veronii* *Aeromonas jandaei* *Aeromonas caviae* *Aeromonas sobria* *Aeromonas dhakensis* *Aeromonas schubertii*	Motile *Aeromonas* septicaemia (MAS) Aeromonasis	Freshwater fish, e.g., carp, tilapia, catfish, snakehead fish, etc.	Wide distribution in tropical countries	Yes (*A. hydrophila*, *A. veronii*, *A. jandaei*, *A. dhakensis*)
Pseudomonas fluorescens *Pseudomonas anguilliseptica* *Pseudomonas aeruginosa* *Pseudomonas alcaligenes* *Pseudomonas otitidis* *Pseudomonas plecoglossicida*	*Pseudomonas* septicaemia	Eel, tilapia	Wide distribution in tropical countries	No
Francisella orientalis	Francisellosis	Tilapia	Wide distribution in tropical countries	No
Edwardsiella ictaluri	Enteric septicaemia of catfish (ESC) Bacillary necrosis of pangasius (BNP) Edwardsiellosis of tilapia (EOT)	44 fish species including channel catfish, striped catfish and tilapia	Wide distribution in the USA, Japan, South-East Asia	No
Edwardsiella tarda *Edwardsiella anguillarum* *Edwardsiella piscicida*	Edwardsiellosis	Tilapia, catfish	Wide distribution in tropical countries	Yes (*E. tarda*)
Flavobacterium columnare *Flavobacterium covae* *Flavobacterium davisii* *Flavobacterium oreochromis*	Columnaris (or saddleback disease)	Carp, tilapia, catfish and various freshwater fish	Worldwide	No
Streptococcus agalactiae *Streptococcus iniae* *Streptococcus dysgalactiae* *Streptococcus ictaluri* *Streptococcus suis*	Streptococcal septicaemia (= meningoencephalitis or streptococcosis)	Freshwater fish, e.g. tilapia, striped bass, channel catfish, rainbow trout, barramundi, snakeskin gourami, etc.	Wide distribution in tropical countries	Yes (*S. agalactiae* ST283)

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haemorrhages, and an accumulation of yellowish liquid and gas in the intestine (Dong *et al.*, 2017c; Hoai *et al.*, 2019). Tilapia infected with *A. schubertii* exhibited visceral white spots in the liver, spleen and kidney (Liu *et al.*, 2018).

The infection elicits an intense inflammatory response, with massive infiltration of monocytic and granulocytic cells into infected tissues. Infected goldfish are anaemic and there is a shift in the differential counts of lymphocytes to a predominance of neutrophils. Nile tilapia infected with *A. veronii* or *A. jandaei* present severe blood congestion, tissue degeneration with hemosiderin accumulation in the liver, hyperaemia and haemorrhages within the spleen, and there is severe necrosis of microvilli and sloughing of epithelial cells within the intestine (Dong *et al.*, 2017c). In tilapia infected with *A. schubertii*, histopathological manifestation includes vacuolization in the liver, splenic haemorrhages and swelling of capillaries in the brain. Necrotic lesions in the spleen, liver and kidney filled with many short, rod-shaped bacteria are seen (Liu *et al.*, 2018; Ren *et al.*, 2019).

Motile *Aeromonas* spp. are flagellated, Gram-negative, short rods. The bacteria grow at between 18 and 39°C. In tryptic soy agar (TSA) or in brain heart infusion agar (BHIA) at 25–30°C incubation for 24–48 h, *Aeromonas* spp. produce white to creamy, convex, moist colonies. In Rimler–Shotts' medium, the bacterium forms orange-yellow colonies at 35°C (Lio-Po and Lim, 2014). Identification of motile *Aeromonas*-complex is typically a combination of biochemical testing together with sequencing of 16S rRNA and/or *gyrB* for phylogenetic analysis. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been used as a rapid diagnostic tool for *Aeromonas* species (Dong *et al.*, 2017c; Hoai *et al.*, 2019; Mursalim *et al.*, 2022; Ngo *et al.*, 2022). A multiplex PCR assay has also been developed for the detection of multiple *Aeromonas* species (Persson *et al.*, 2015).

Transmission

Motile aeromonads are mostly isolated from mucosal surfaces and intestine of healthy fish (Harikrishnan and Balasundaram, 2005). Outbreaks of these opportunistic pathogens in farmed freshwater fish are usually related to transport and handling stress, adverse environmental conditions of low oxygen concentration, low pH, and increased levels of ammonia and carbon dioxide (Dong *et al.*, 2017c). These predisposing conditions are possibly immunodepressive and, along with the virulence of the *Aeromonas* strain, are important factors in the development of MAS epizootics (Thune *et al.*, 1993). Dong *et al.* (2015a) reported concurrent infections involving three to five pathogens in each individual tilapia where *A. veronii* was found in all diseased fish. Horizontal transmission through waterborne cohabitation is confirmed but vertical transmission is not yet elucidated.

Prevention and control

Prevention of stressful conditions in cultured fish will minimize MAS outbreaks. Inactivated vaccines for injection and immersion have been developed and tested in various freshwater fish species, but the focus has been on *A. hydrophila* (see Lio-Po and Lim, 2014). There have been a limited number of vaccine studies for other *Aeromonas* species (e.g. *A. veronii*, *A. jandaei* and *A. dhakensis*).

Using antibiotics (novobiocin and rifampicin) to induce mutation in pathogenic *A. hydrophila* strains, several live attenuated vaccines were obtained that showed impressive protection against pathogenic strains in tilapia and channel catfish (Pridgeon *et al.*, 2013, 2014). Bivalent live or inactivated vaccines were effective in laboratory trials (Pridgeon *et al.*, 2013; Pasaribu *et al.*, 2018). Nevertheless, further effort would be required to produce cost-affordable vaccines on a large scale.

Prophylactic bath treatments with 1–3% NaCl can help reduce post-handling infections. Likewise, bath treatments with potassium permanganate 2–4 mg/l are also effective for external lesions. Medicated feed with 2–4 g oxytetracycline/kg feed (50–100 mg/kg fish) for 14 days is recommended (Plumb, 1994); however, drug-resistant strains of *A. hydrophila* may evolve (Aoki, 1999). Administration of *A. hydrophila*-specific bacteriophages *per os* or by immersion was reportedly effective in protecting tilapia and striped catfish from *A. hydrophila* challenge (Dang *et al.*, 2021; Dien *et al.*, 2021a). A recent report describing ozone nanobubble technology as a non-chemical

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method for reducing the pathogenic bacterial load in water, thereby enhancing the survivability of Nile tilapia when challenged with a multidrug-resistant *A. hydrophila*, suggests this may serve as a promising technology for modern aquaculture (Dien *et al.*, 2021b).

6.3.2 *Pseudomonas* septicaemia

Pseudomonas (order Pseudomonadales) are Gram-negative, aerobic, motile, rod-shaped bacteria with one to three polar flagella that are found in diverse aquatic and terrestrial environments. Most *Pseudomonas* species are non-pathogenic, but a few cause fish diseases. In freshwater culture systems, *Pseudomonas* septicaemia has been associated with epizootic outbreaks in Nile tilapia, grass, silver and bighead carp, walking catfish, and Japanese and European eels (Thune *et al.*, 1993; Haenen and Davidse, 2001). *Pseudomonas fluorescens*, *Pseudomonas anguilliseptica* and *Pseudomonas aeruginosa* are the aetiological agents of the disease. Other species including *Pseudomonas alcaligenes* and *Pseudomonas otitidis* are usually associated with unusual problems of tilapia eggs and fry in the Philippines and Thailand (Duremdez and Lio-Po, 1985; S. Senapin, Thailand, 2022, personal communication). Most recently, *Pseudomonas plecoglossicida* was reported as an emerging pathogen causing visceral white nodule disease in cultured *Larimichthys crocea* in China (Li *et al.*, 2020).

Diagnosis

The clinical signs in fish affected with *Pseudomonas* septicaemia are very similar to those of MAS. Gross signs include ascites, exophthalmia, septicaemia and ulcers. The infection may be acute or chronic with the latter commonly associated with skin lesions. The pathogen has been associated with fin rot. Histopathological findings in Nile tilapia include focal necrosis, abscesses and granulomas in the eyes, gills, liver, swim bladder, kidney and spleen (Miyashita *et al.*, 1984).

Isolation and identification of the pathogen are required, and it can be cultured on nutrient agar, *Pseudomonas* F agar and blood agar (Austin and Austin, 2007). For strains pathogenic to fish, the optimum growth temperature is 20–25°C. These secrete oxidase, catalase and gelatinase, but not amylase, galactosidase, urease or hydrogen sulfide. They are citrate-positive, oxidative for glucose and produce a fluorescent pigment (Plumb, 1994). Molecular identification of *Pseudomonas* species is usually successful with amplification and sequencing of the 16S rRNA gene.

Prevention and control

Stress from low dissolved oxygen, high stocking density, physical trauma and poor nutrition are predisposing factors in the development of *Pseudomonas* septicaemia (Post, 1983). Bath treatments during the early stage of the disease include 1–2 mg benzalkonium chloride/l for 1 h and 0.5–1 mg furanace/l for 5–10 min (Austin and Austin, 2007). Swain *et al.* (2007) showed that vaccination using formalin-killed whole-cell antigens of *P. fluorescens* singly or as a polyvalent vaccine with *A. hydrophila*, *E. tarda* and *P. fluorescens* provided protection to *Labeo rohita*, yielding RPS of 80 and 70% in single and polyvalent vaccines, respectively.

6.3.3 Enteric septicaemia of catfish (ESC)

Enteric septicaemia of catfish (ESC) caused by *Edwardsiella ictaluri* is also known as bacillary necrosis of pangasius (BNP) in striped catfish, as red head disease in yellow catfish and as edwardsiellosis of tilapia (EOT) in tilapia (Machimbirike *et al.*, 2022). In American channel catfish culture, infection accounts for significant losses when temperatures are between 22 and 28°C (Peterman *et al.*, 2019). Infections are reported from Asia, Australia, the Caribbean, Europe and North America; to date, 44 fish species are reported as being susceptible to *E. ictaluri* infection (31 naturally susceptible and 13 experimentally susceptible) (Machimbirike *et al.*, 2022).

The causative agent *E. ictaluri* is a Gram-negative, pleomorphic, rod-shaped bacterium belonging to the family *Enterobacteriaceae*. The genomic sizes of *E. ictaluri* isolates range between 3.6 and 3.9 Mbp. Only 11 *E. ictaluri* genomes are currently available in GenBank (Machimbirike *et al.*, 2022).

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E. ictaluri is pathogenic to channel catfish but only slightly pathogenic to *I. furcatus*. Recently, outbreaks in tilapia resulting in 30–65% mortality are considered an emerging problem for the Vietnamese tilapia industry and possibly wider. The causative agent is a highly pathogenic, multidrug-resistant *E. ictaluri* strain (Dong *et al.*, 2019; Nhinh *et al.*, 2022). The origin of this isolate remains unclear; however, most disease outbreaks originated from imported stocks.

Diagnosis

In acute infections, ingested bacteria enter the bloodstream through the intestine and colonize various organs, causing necrosis and ulceration (Noga, 2010). There is haemorrhaging, liver necrosis, splenic and renal hypertrophy; the peritoneal cavity secretes a bloody or clear fluid. Fish exhibit abdominal distension, exophthalmia and pale gills. With chronic infections, bacteria invade the olfactory organ through the nasal openings and spread from the meninges to the skull and skin, forming a hole-in-the-head lesion (Shotts *et al.*, 1986).

Infected striped catfish and tilapia are pale, having a swollen abdomen, a protruding bloodshot anus and cloudy eyes. Internally, ascites and numerous white nodules in the spleen, kidney, liver, gills, and rarely in the intestine, are associated with infection. Histopathologically, moderate to severe multifocal necrosis and pyogranulomatous lesions are observed in in the spleen (Fig. 6.5), kidney, liver and other organs, with the presence of white nodules. Other changes include an infiltration of inflammatory cells, cell pyknosis and karyorrhexis, and the presence of basophilic rod-shaped bacterial clusters in the necrotic tissues (Ferguson *et al.*, 2001; Crumlish *et al.*, 2002; Soto *et al.*, 2013a; Dong *et al.*, 2015b, 2019; Nhinh *et al.*, 2022).

E. ictaluri produces pinpoint to small-sized, off-white, translucent, irregular-surfaced colonies (0.14 ± 0.13 mm). After growth for 48 h on trypticase soy agar (TSA) at 28°C, the biochemical profile can be determined using conventional biochemical tests, the API 20E kit, combined with genus- or species-specific PCR and sequencing of the 16S rRNA. qPCR can be used to detect *E. ictaluri* in the brain, gill, kidney and liver of channel catfish (Shoemaker *et al.*, 2012).

Transmission

Horizontal transmission of *E. ictaluri* from infected channel catfish to naïve fish was confirmed though cohabitation experiments. *E. ictaluri* shed from dead fish, or cannibalism of them, are recognized mechanisms of horizontal transmission in channel catfish (Klesius, 1994). Infected channel catfish that recover from the disease can be asymptomatic carriers and may serve as a reservoir for disease transmission (Klesius, 1992). Detection of bacteria in the gonads indicates the possibility of vertical transmission (Mqolomba and Plumb, 1992).

Fish exposed to *E. ictaluri* at 25°C had the highest death rate, while those grown at 20 and 30°C had moderate death rates; infected groups kept at 15 and 35°C showed no or minor mortality (Baxa-Antonio *et al.*, 2011). Soto *et al.* (2013a) suggested the cutaneous and oral routes as the main portals of entry of the bacteria with the spleen and head kidney as major targets of infection.

Co-infection with either *Ichthyophthirius multifiliis* or *F. columnare* increased the disease severity, resulting in higher rates of mortality when compared with single infections (Shoemaker *et al.*, 2012; Dong *et al.*, 2015b).

Prevention and control

Current approaches to disease prevention and control are systematically reviewed by Machimbirike *et al.* (2022). Only a few commercial vaccines are available, including a live attenuated vaccine (AQUAVAC-ESC) for channel catfish in the USA and two oil-based inactivated vaccines (Alpha Ject Panga 1, Alpha Ject Panga 2) for striped catfish in Vietnam (Machimbirike *et al.*, 2022). In the USA, immersion vaccination of catfish against ESC is recommended at the fry stage (Bebak and Wagner, 2012). In Vietnam, the vaccines are injectable for juvenile striped catfish (>15 g). In Asia, the use of antibiotics for disease control in the catfish and tilapia industries has led to an alarming rise in the prevalence of multidrug-resistant strains (Machimbirike *et al.*, 2022; Nhinh *et al.*, 2022). Barnes *et al.* (2022) stated that autogenous vaccination in aquaculture is a locally feasible solution to tackle the global antimicrobial resistance crisis. Selective breeding for selection of *E. ictaluri*-resistant strains is

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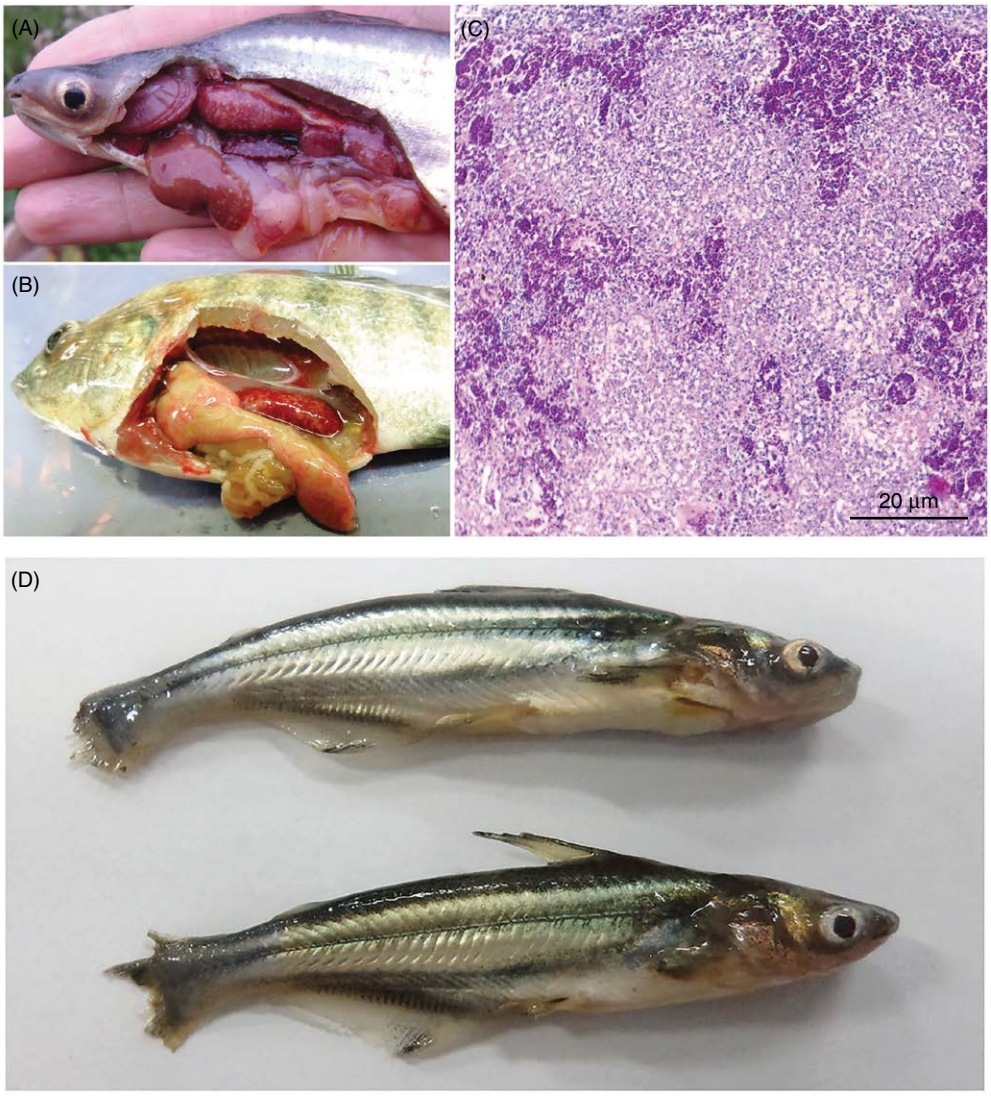


Fig. 6.5. (A) Striped catfish infected with *Edwardsiella ictaluri* showing numerous white spots in the head kidney, spleen and liver. (B) Nile tilapia infected with *E. ictaluri* exhibiting white spots in the swollen spleen, pale liver and ascites. (C) Histopathology of *E. ictaluri*-infected Nile tilapia spleen showing multifocal necrosis with multiple pyogranulomas. (D) Clinical signs of striped catfish naturally infected with *Flavobacterium columnare* showing severe fin erosion and skin necrosis. (Images by H.T. Dong.)

being developed for both channel catfish and striped catfish. In another study, macrophage chemotaxis in channel catfish in response to an *E. ictaluri* exoantigen was significantly low among unfed fish vis-à-vis the fish group that were fed daily. Moreover, mortality from ESC was significantly lower for fish fed daily or every other day than for fish that were not fed before and after challenge (Lim and Klesius, 2003).

6.3.4 Edwardsiellosis

The causative bacteria of edwardsiellosis are *Edwardsiella tarda*, *Edwardsiella piscicida* and *Edwardsiella anguillarum*, members of the family *Enterobacteriaceae* (Katharios *et al.*, 2019; Leung *et al.*, 2019; Oh *et al.*, 2020). *E. ictaluri* is considered separately (see Section 6.3.3 above). Infections are reported worldwide (Austin and

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Austin, 2007), affecting eels, mullet, channel catfish, tilapia, carp, striped bass and olive flounder (see Han *et al.*, 2006; Lio-Po and Lim, 2014; Leung *et al.*, 2019; Oh *et al.*, 2020).

Diagnosis

Infected fish may exhibit haemorrhagic areas on the body and malodorous lesions on the flank or caudal peduncle, and a swollen abdomen. Larger fish (>40 cm) are commonly infected. Internally, white nodules may develop in the liver. Histopathology of edwardsiellosis in tilapia consists of liquefactive necrosis of infected tissues with bacterial infiltration of macrophages engorging bacterial cells that eventually leads to granuloma formation in the liver, spleen and kidney (Miyazaki and Kaige, 1985). Virulence factors of *E. tarda* include haemolysin, dermatotoxin, siderophore, superoxide dismutase and catalase (Kokubo *et al.*, 1990; Han *et al.*, 2006). Infection is usually prevalent in channel catfish at 30°C. *E. tarda* is considered a zoonotic pathogen.

Edwardsiella species can be diagnosed based on typical clinical signs and isolation/identification of the pathogen *in vitro*. This Gram-negative, motile bacillus can be isolated on common bacterial culture media. On TSA at 25–30°C, *Edwardsiella* produces small, grey, circular transparent colonies. Species identification is based on either conventional biochemical tests or determined using the API 20E kit combined with molecular analysis, particularly sequencing of *16S rRNA* and DNA gyrase subunit B (*gyrB*) for phylogenetic analysis (Oh *et al.*, 2020).

Prevention and control

Vaccination by hyperosmotic infiltration of *O. niloticus* did not provide protection against *E. tarda* infection (Lio-Po and Wakabayashi, 1986). In contrast, vaccination by intraperitoneal injection using formalin-killed whole-cell antigens of *E. tarda* singly or as a polyvalent vaccine consisting of *A. hydrophila*, *E. tarda* and *P. fluorescens* provided protection to *L. rohita*. An RPS of 80% in either single or polyvalent vaccine was observed (Swain *et al.*, 2007). Castro *et al.* (2008) developed an *E. tarda* adjuvanted vaccine for cultured turbot which showed RPS values of >90% at least 6 months post-vaccination. Vaccination with the outer membrane recombinant protein OmpA was effective against *E. anguillarum* in Japanese eels, *Anguilla japonica*, with RPS of 77.7% (LiHua *et al.*, 2019).

6.3.5 Francisellosis

Francisellosis caused by *Francisella orientalis* (formerly known as *Francisella noatunensis* subsp. *orientalis*, *Rickettsia*-like or *Piscirickettsia*-like organism) is a significant bacterial disease of farmed *Oreochromis* spp. Francisellosis infections are reported worldwide and are summarized in Nguyen *et al.* (2016) and Ramirez-Paredes *et al.* (2020).

Francisellosis outbreaks usually occur in water temperatures of 23–26°C resulting in a 50–60% mortality in cultured tilapia (Nguyen *et al.*, 2016). A co-infection of *F. orientalis* and *I. multifiliis* resulted in higher mortality and more severe clinical signs of infection (Nguyen *et al.*, 2020).

The causative agent, *F. orientalis*, is a Gram-negative, non-motile coccobacillus to pleomorphic spherical bacterium measuring 0.1−1.5 µm in size (Colquhoun *et al.*, 2014). Intracellular, strictly aerobic and fastidious, *Francisella* requires specific media for its cultivation (Colquhoun *et al.*, 2014; Nguyen *et al.*, 2016).

Diagnosis

Presumptive diagnosis of francisellosis can be made through observation of gross signs. Clinically sick fish typically present whitish nodules (granulomas) on the gills, spleen, head kidney and sometimes in other organs (i.e. liver, intestine, muscle). Other non-specific signs such as a pale body, lethargy and anaemia are also seen during disease outbreaks (Soto *et al.*, 2009; Nguyen *et al.*, 2016).

Rapid tissue stamp smears of the head kidney or spleen stained with Giemsa can be used to visualize numerous coccobacilli, intracellular bacteria in the macrophages under a light microscope. Histopathologically, infected fish exhibit typical granulomas in multiple organs, notably the spleen and head kidney. Isolation of *F. orientalis* from infected fish can be achieved using enriched blood agar plates supplemented with 0.1% cysteine and 1% glucose, cysteine

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heart agar (CHA) with 5% sheep's blood, CHA with 1% haemoglobin (CHAH) or Thayer–Martin medium (Colquhoun *et al.*, 2014). Supplement of polymyxin B (100 units/ml) with or without ampicillin (50 µg/ml) added to the culture medium increases the chance of success; the optimal temperature for isolation is 28–30°C (Soto *et al.*, 2009).

Molecular diagnostic methods for the detection and quantification of *F. orientalis* include conventional PCR (Nguyen *et al.*, 2016), real-time qPCR (Rodrigues *et al.*, 2018), recombinase polymerase amplification (RPA) (Shahin *et al.*, 2018), LAMP (Pradeep *et al.*, 2017) and ISH (Dong *et al.*, 2016). Amplification of *16S rRNA* combined with sequencing and phylogenetic analysis is a common approach for the identification of *Francisella* from new geographical locations.

Transmission

The waterborne transmission of *F. orientalis* in *O. niloticus* fingerlings has been confirmed by laboratory trials (Soto *et al.*, 2013c). Vertical transmission of *F. orientalis* is also possible; asymptomatic tilapia broodstock can transmit *F. orientalis* to their reproductive organs, fertilized eggs and larvae (Pradeep *et al.*, 2017; Nguyen *et al.*, 2019).

Prevention and control

Using tilapia broodstock that test negative for *F. orientalis* is recommended to prevent vertical transmission and to produce specific pathogen-free seed. The onset of francisellosis in tilapia is significantly influenced by temperature, but not by salinity. Infected fish cultured at a high temperature (i.e. 30°C) displayed suppressed development of clinical signs and mortality while at lower temperatures (i.e. 25°C) the fish developed francisellosis with high mortality. Vaccination is effective in disease prevention. Pulpipat *et al.* (2020) reported that an injectable formalin-killed vaccine administered to cultured tilapia via intraperitoneal injection elicited protective antibodies and protection with an RPS of 71–76%. The presence of *F. orientalis*-specific IgM antibodies in the serum and mucus of tilapia that received an oral vaccine dose suggests that oral immunization has the potential to protect tilapia from francisellosis (Hoare *et al.*, 2021). Effective treatment using antibiotics incorporated with feed is also reported (Soto *et al.*, 2013c). Removing infected fish and disinfecting culture facilities is recommended to inactivate planktonic and biofilm forms of *F. orientalis* (Soto *et al.*, 2015).

6.3.6 Columnaris disease

Columnaris disease is a common infection of freshwater fish worldwide and can result in significant economic loss (Bernardet and Bowman, 2006). Natural outbreaks in tilapia hatcheries and grow-out farms result in a 10–70% mortality (Dong *et al.*, 2015c).

F. columnare, formerly known as *Flexibacter columnaris*, is the causative agent. It is a slender, Gram-negative, non-flagellated rod (about 0.5 µm × 4–12 µm) with gliding motility that forms 'haystacks' or columns. Recently, three new species were split out from *F. columnare*, namely *Flavobacterium covae*, *Flavobacterium davisii* and *Flavobacterium oreochromis* (see LaFrentz *et al.*, 2022).

Diagnosis

Columnaris disease is easy to recognize based on external clinical signs. Diseased fish usually show fin erosion with greyish to white margins; depigmented, necrotic skin lesions with yellowish or pale margins which can develop into shallow ulcers; yellowish mucoid material at the mouth; and light to dark brown gill discoloration. Infection primarily begins at the mouth, fins and gills. Gills or dermal/muscular capillaries of infected fish become congested and degenerated (Declercq *et al.*, 2013; Dong *et al.*, 2015c). Figure 6.5D shows an example of striped catfish with typical clinical signs of columnaris disease.

Gill lesions initiate at the distal end of the filaments and extend to the base. Epithelial vacuolation, necrosis, congestion, oedema, fusion and degeneration of the secondary lamellae subsequently follow. Colonization of filamentous bacteria on the necrotic areas of the gills and skin can be observed in both wet-mount and histological sections (Declercq *et al.*, 2013). Acute mortality is usually associated with gill lesions. Internal pathology or host inflammatory

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response may occur, and the pathogen may be isolated from internal tissues (Shotts and Starliper, 1999).

Primary isolation of the pathogen can be achieved on selective Anacker and Ordal's medium (or Cytophaga agar), modified Shield agar (MSA) or tryptone yeast extract salts (TYES) agar supplemented with neomycin at 5 µg/ml and polymyxin B at 200 IU/ml (Bernardet and Bowman, 2006). Colonies are yellow to orange, and rhizoid. This aerobic organism cannot tolerate more than 0.5% NaCl and grows between 4 and 36°C, producing gelatinase, caseinase, catalase, oxidase and chondroitin sulfatase (Bernardet and Bowman, 2006; Declercq *et al.*, 2013; Dong *et al.*, 2015c).

Rapid diagnosis is by specific PCR (Mabrok *et al.* 2020), LAMP (Suebsing *et al.*, 2015) and monoclonal antibodies (Ponpukdee *et al.* 2021). Amplification and sequencing of *16S rRNA* and multilocus sequence analysis (MLSA) are commonly used for identifying and characterizing this bacterium (Dong *et al.*, 2015a; LaFrentz *et al.*, 2018).

Transmission

Bacterial transmission is via water. The disease is most associated with stress from high temperatures, elevated organic loads, high stocking density, low dissolved oxygen and trauma from excessive handling. In channel catfish, it occurs more often at 25–32°C with significant mortality. Young fish are more susceptible than older fish. It may occur as a primary infection or as a mixed infection with another bacterium, *E. ictaluri* or *A. hydrophila*, or in association with a parasite, such as *Henneguya* sp. or *Ichthyobodo* sp. (Duarte *et al.*, 1993; Plumb, 1994). Columnaris disease appears to follow outbreaks of other diseases (Duarte *et al.*, 1993).

Survivors of columnaris disease release the pathogen into the water at rates of up to 5×10^3 cells/ml per h (Fujihara and Nakatani, 1971), and surviving fish may release the bacterium up to 140 dpi. The severity of the lesion depends on the virulence of the strain and the ability of the pathogen to elaborate proteolytic enzymes (Lio-Po and Lim, 2014).

The bacterium can survive for up to 16 days at 25°C in hard, alkaline water with high organic load; survival decreases at pH 7 or less and in waters with <50 mg $CaCO_3$/l and with low organic matter. In sterile mud at 25°C, the organism survives for 16 days (Becker and Fujihara, 1978).

F. columnare was found in the reproductive organs of apparently healthy tilapia broodstock, fertilized eggs and newly hatched fry, implying possible maternal transmission (Suebsing *et al.*, 2015).

Prevention and control

Maintenance of fish under optimal environmental conditions, proper handling of fish, prophylactic treatment and good health management practices are recommended for disease prevention (Declercq *et al.*, 2013). Fish exposed to *F. columnare* and maintained at 3–9 ppt salinity did not develop clinical signs or mortality, whereas fish cultured at lower salinity or in fresh water died with clear clinical symptoms of columnaris disease (Altinok and Grizzle, 2001).

Daily oral vaccination with heat-killed *F. columnare* for 4 weeks reportedly reduced mortality of rainbow trout from 48 to 8%, with protection correlated with antibody levels (Fujihara and Nakatani, 1971). Similarly, tilapia also mounts a significant humoral response in plasma and cutaneous mucus to *F. columnare* after intraperitoneal immunization with formalin-killed sonicated cells of *F. columnare* in Freund's complete adjuvant within 2 weeks. The mean titre remained significantly elevated above controls even at 10 weeks post-immunization (Grabowski *et al.*, 2004). The development of mucoadhesive immersion nanovaccines, based on chitosan, for the prevention of columnaris in tilapia resulted in an RPS of 58–81% (Kitiyodom *et al.*, 2019a,b).

Experimental application of FCP1 phage therapy via the intramuscular route in walking catfish, *C. batrachus*, provided protection against *F. columnare* infection. After treatment, gross signs disappeared and all experimental fish survived (Prasad *et al.*, 2011).

Therapeutic agents such as potassium permanganate at 5 mg/l (depending on the organic load of the rearing water) in combination with oxytetracycline added to feed at 50 mg/kg fish per day for 10 days is effective in controlling outbreaks in cages (Duarte *et al.*, 1993).

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6.3.7 Streptococcal septicaemia/ meningoencephalitis

Streptococcosis caused by *S. agalactiae* is the most important bacterial disease of farmed tilapia worldwide; infections result in significant economic loss (Shinn *et al*., 2018). Species that are pathogenic to freshwater fish include *S. agalactiae* (syn. *Streptococcus difficile*), *Streptococcus iniae* (syn. *Streptococcus shiloi*), *Streptococcus dysgalactiae*, *Streptococcus ictaluri* and *Streptococcus suis*. *Streptococcus* spp. are facultative, anaerobic, Gram-positive bacteria that are non-motile and non-spore-forming, exhibiting varying degrees of haemolysis according to species and strain (Buller, 2004; Noga, 2010). To date, *S. agalactiae* is the most dominant and significant of the reported species causing disease in freshwater fish. *S. agalactiae* (GBS) can be divided into ten serotypes (Ia, Ib, II–IX) based on capsular polysaccharides. Five serotypes have been identified in aquatic animals, namely serotypes Ia, Ib, II, III and IX. GBS sequence type 283 (GBS ST283) is a reported zoonotic type which is widely distributed in South-East Asia (Barkham *et al*., 2019).

Diagnosis

Clinical signs include unilateral and bilateral exophthalmia with or without conjunctival haemorrhaging and corneal opacities. Petechiae occur beneath the opercula, perinal, on the caudal and pectoral fins, and around the mouth. There may be nodular or abscess formation, darkening and discoloration of the body. Abdominal swelling with ascites is common. Affected fish are anorexic, swim sluggishly in a circle, turn laterally and eventually die (Zamri-Saad *et al*., 2010).

Internal signs include petechiae and haemorrhaging of the intestinal tract, liver and pyloric caeca. Systemic infection with evidence of bacterial dissemination in the heart, liver, kidney, stomach, small intestine, brain, eyes and musculature has been recorded. Multiple necrosis with granulomas, increasing melanomacrophage centres with an overload of melanophores, occur in the hepatic parenchyma. The spleen develops hyperplasia of the reticuloendothelial cells with necrotic foci. Degenerative changes in the renal tubules, catarrhal enteritis in the small intestine and stomach, bacterial meningitis and abscess formation in the muscle have been recorded (Buller, 2004; Rodkhum *et al*., 2011).

Microscopic examination of Gram- or Giemsa-stained tissue smears from head kidney of infected fish is useful for a rapid presumptive diagnosis of streptococcosis. Diseased fish usually show numerous extra- and intracellular Gram-positive cocci. *Streptococcus* spp. can be isolated from the brain, kidney, heart, spleen, blood and exophthalmic eyes using nutrient agar (NA) supplemented with sheep's or goat's blood, BHIA or TSA. After incubation for 24–48 h at 28–30°C, *Streptococcus* spp. usually form pinpoint colonies on culture media. Modified Hucker's Gram staining showing small Gram-positive cocci, approximately 0.3–0.5 µm in diameter, most often occurring in chains, is a presumptive diagnosis (Kitao *et al*., 1981; Buller, 2004). These organisms are non-motile and encapsulated. The pathogen does not grow in 40% bile, 6.5% saline, 0.1% methylene blue milk, or at 10 and 45°C (Kusuda and Salati, 1999; Buller, 2004). Details on the classification of *Streptococcus* spp. based on biochemical and serological tests are provided in Kitao *et al*. (1981) and Plumb (1994). Isolates from freshwater fish are usually β-haemolytic (Buller, 2004). Molecular methods for identification include specific duplex PCR for *S. agalactiae* and *S. iniae* (see Rodkhum *et al*., 2012), qPCR for *S. agalactiae* (see Leigh *et al*., 2020), and LAMP for *S. agalactiae* and *S. iniae* (see Suebsing *et al*., 2013). Amplification of *16S rRNA* for sequencing is usually used for identification of *Streptococcus* species recovered from diseased fish.

Transmission

Experimental transmission occurs by immersion, injection, via the oral route or cohabitation and is enhanced by injury to the skin or stressful environment. Infection via the nares is a potential route of infection in Nile tilapia and hybrid striped bass (Evans *et al*., 2000). Sources of infection are water, mud, contaminated feed or carrier fish (Plumb, 1994). Hatchery-to-grow-out farm transmission of *S. agalactiae* has also been reported (Amal *et al*., 2013). Streptococcosis has been shown to be transmitted from parents to offspring (Pradeep *et al*., 2017). *Streptococcus* is also

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more pathogenic to Nile tilapia than to channel catfish (Chang and Plumb, 1996).

Streptococcal outbreaks are triggered by several environmental factors, one of which is high temperature during the summer. Several studies revealed that tilapia infected with *S. agalactiae* and maintained at 33–35°C exhibited significantly higher rates of mortality compared with those maintained at 25–28°C (Rodkhum *et al.*, 2012; Kayansamruaj *et al.*, 2014).

Prevention and control

Avoidance of stress due to adverse or poor water quality, rough handling, high stocking density or overfeeding should be followed, and infected or dead fish removed.

Formalin-killed *S. difficile* vaccine injected intraperitoneally protects tilapia (Eldar *et al.*, 1995). In addition, Klesius *et al.* (2000) showed that intramuscular injection of a combined vaccine prepared from two strains of *S. iniae* obtained from Nile tilapia provided RPS of 63.1 and 87.3% when challenged with its homologous pathogens. Anti-*S. iniae* whole sera provided immunity to *O. niloticus* against *S. iniae* (see Shelby *et al.*, 2002). Subsequent studies showed that intraperitoneal injection of *O. niloticus* with *S. iniae* bacterin vaccine yielded RPS of 79–100% upon challenge with heterologous *S. iniae* derived from diverse geographical locations (Shoemaker *et al.*, 2010). In Malaysia, Ismail *et al.* (2016) developed a formalin-killed *S. agalactiae* feed-based vaccination regimen for prevention of streptococcosis in hybrid red tilapia with 45–70% survival after bacterial challenge. In the USA, novel attenuated *S. iniae* and *S. agalactiae* vaccines developed through selection for antibiotic resistance offered relatively good protection in tilapia (Pridgeon and Klesius, 2011; Liu *et al.*, 2019). Recently, Linh *et al.* (2022) reported that the pre-treatment of Nile tilapia with ozone nanobubbles for 10 min enhanced uptake of immersion heat-killed *S. agalactiae* vaccine and resulted in better protection compared with control without nanobubble treatment.

Selective breeding for *S. agalactiae*-resistance has gained promising results for Nile tilapia in Thailand. Selection response revealed that the risk of death decreased to 54% and survival rate increased to 21%, suggesting possible genetic improvements for the fish population (Suebsong *et al.*, 2019).

6.4 Diseases Caused by Oomycetes

Commonly known as water moulds, oomycetes are fungus-like eukaryotes which have cell walls that do not contain chitin but instead possess glycans and small amounts of cellulose (Yanong, 2003; Walker and van West, 2007). Most oomycetes are aquatic and produce characteristic heterokont types of zoospores with two flagella, one whiplash and one tinsel type. Most oomycetes that are pathogenic to fish belong to the subclass Saprolegniomycetidae and to the orders Saprolegniales (e.g. *Achlya*, *Branchiomyces*, *Dictyuchus*, *Saprolegnia*, etc.), Leptomitales (e.g. *Aphanomyces*) and Pythiales (e.g. *Pythium*) (Phillips *et al.*, 2008) (Fig. 6.6).

Diagnosis

Accurate identification is based on morphology; the sexual reproductive stages are typically required, although their general lack can make speciation difficult. Direct histochemical staining and/or fluorescent microscopy can assist in visualizing the hyphae and in determining cell-wall composition (see Paladini *et al.*, 2017). Molecular-based methods are crucial for identification (e.g. Diéguez-Uribeondo *et al.*, 2009).

6.4.1 Saprolegniosis

Most species in the *Saprolegniaceae* family are relatively intolerant of brackish water but thrive in fresh water, affecting a wide range of warm-water fish species. Some species of *Achlya* and *Saprolegnia* are important parasites of fish and their eggs (Panchai *et al.*, 2007). *Achlya* do not possess motile primary zoospores; instead they encyst as a hollow ball at the mouth of the zoosporangium. The infective secondary zoospores then emerge from the zoosporangium. *Achlya* spp. are reported from cage-reared *O. niloticus* (see Shinn *et al.*, 2022) and from *Gibelion catla* and *C. idella* from Pakistan (Iqbal *et al.*, 2012). The primary zoospores of *Aphanomyces* also encyst in a ball at the mouth of the sporangium, while in *Dictyuchus* the zoospores encyst inside the sporangium. *Saprolegnia* releases motile primary zoospores that possess two apical flagella. After a brief period of swimming, they encyst then release another motile secondary zoospore

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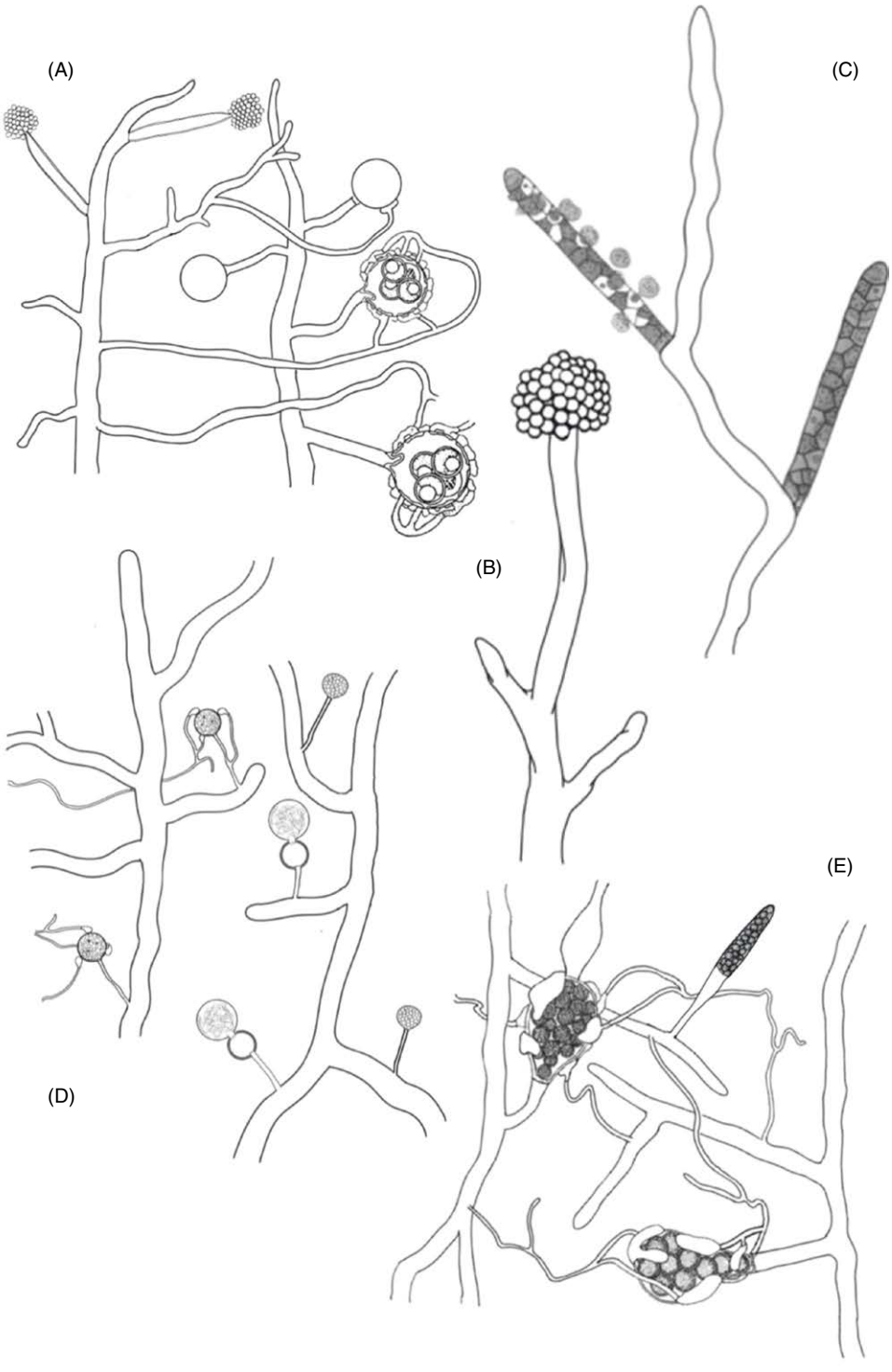


Fig. 6.6. Commonly encountered oomycetes. (A) *Achyla bisexualis* (modified from Willoughby, 1994; Markovskaja, 2004). (B) *Aphanomyces invadans* (modified from Noga, 2010). (C) *Dictyuchus sterilis* (modified from Webster, 1986). (D) *Pythium debaryanum* (modified from Webster, 1986; Trigiano *et al*., 2004). (E) *Saprolegnia parasitica* (modified from Coker, 1923; Willoughby, 1994).

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with two flagella. These secondary zoospores encyst; the cyst is covered by hooked hairs facilitating their attachment. Infections of *Saprolegnia parasitica* have resulted in significant mortalities of *P. hypophthalmus* in farm sites in India (Ravindra *et al.*, 2022). Infections are also reported from *G. catla*, *Cirrhinus mrigala* and *L. rohita* in India (Chauhan *et al.*, 2012); *G. catla* and *C. idella* in Pakistan (Iqbal *et al.*, 2012); *Channa punctata* from India (Mastan, 2015); and *Cyprinus carpio* from India (Magray *et al.*, 2021). Lio-Po and Lim (2014) and Chauhan (2014) provide a summary of oomycete infections various catfish, cyprinids, oreochromids and other fish species.

Pathology

Following their release from zoosporangia, the zoospores exhibit positive chemotactic responses to amino acids released from the tissues of susceptible hosts (El-Feki *et al.*, 2003; Sarowar *et al.*, 2014). Host contact triggers encystment of the zoospore; germination and host infection follow. Subsequent secretion of effector proteins by the pathogen can modulate or inhibit the host's immune responses, facilitating infection (Pradhan *et al.*, 2020). With the progression of infection there may be swelling, erosion and ulceration which can result in impaired osmoregulation, haemodilution, respiratory failure in the case of infections within the gills, and mortality. With *Aphanomyces invadans*, the hyphae invade the fish skin and skeletal muscles causing ulceration, commonly resulting in death (Lilley *et al.*, 1998). Infections of *Aphanomyces*, *Achlya*, *Allomyces* and *Saprolegnia* spp. are also reported in association with epizootic ulcerative syndrome in snakeheads (see Lio-Po and Lim, 2014). Experimental infection with *Aphanomyces* has been shown to induce lesions in naive snakeheads (Lilley and Roberts, 1997).

6.4.2 Branchiomycosis

Branchiomycosis or gill rot is due to infection either by *Branchiomyces demigrans* resulting in the penetration of gill tissue or by *Branchiomyces sanguinis* in the blood vessels of the gills. Infections result in respiratory distress and are associated with high rates of mortality, notably in waters >20°C. While infections are commonly reported in cyprinids (Post, 1983), significant losses are also documented for various oreochromids (Shinn *et al.*, 2022).

Pathology

Infected fish may be lethargic and present gills that are ragged, corroded or have evident areas of infarctive necrosis due to the intravascular growth of *Branchiomyces*. Infection can result in hyperplasia, fusion of the gill lamellae, reduction in blood supply to the gills, thrombosis, vascular necrosis and tissue sloughing (Post, 1983; Roberts, 2012). Spores are released from the necrotic gills; secondary bacterial invasion of the gill filament edges follows. Under favourable conditions, the disease may develop in 2–4 days; *in vitro* culture of the pathogen produced spores on day 14 of culture (Post, 1983). The hyphae of *B. sanguinis* are branched, thin-walled (0.2 µm) and non-septate, measuring 8–30 µm in diameter, with spores measuring 5–9 µm, that infect the gill filaments and lamellar capillaries. By comparison, *B. demigrans* has a thicker hyphal wall (0.5–0.7 µm), spores that are 12–17 µm in diameter and hyphae that infect the gill parenchyma (Post, 1983).

Prevention and control

The presence of organic matter, algal blooms, low pH <6.5, low dissolved oxygen, high stocking density and temperatures between 25 and 32°C are considered predisposing factors. Affected fish should be burned and/or buried. Survivors of an epizootic are carriers of the pathogen and should not be cultured with naïve fish or transported into *Branchiomyces*-free geographical areas.

6.4.3 Epizootic ulcerative syndrome (EUS) attributed to the EUS rhabdovirus, *Aeromonas hydrophila* and *Aphanomyces*

The epizootic ulcerative syndrome (EUS) is characterized by severe, ulcerative, dermal necrosis with extensive erosion/sloughing of the underlying musculature. Externally, fish may have red spots and/or large red or grey, shallow ulcers which are subject to secondary infections. Lesions on snakehead can be extensive leading to

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complete erosion of the posterior; necrosis to both the soft and hard tissues of the cranium can result in the brain being exposed.

The EUS caused serious economic losses in a wide range of wild and cultured fish, such as snakeheads (*Ophicephalus striatus*; *Channa* spp.), catfish (*Clarias* spp.), climbing perch (*Anabas testudineus*), barbs (*Puntius* spp.), goby (*Oxyeleotris marmoratus*, *Glossogobius giuris*), gourami (*Trichogaster pectoralis*, *Trichogaster trichopterus*, *Trichopsis vittatus*), Siamese fighting fish (*Betta splendens*) and eels (*Fluta alba*, *Mastacembelus armatus*, *Monopterus albus*) (Lilley *et al*., 1998; Lio-Po, 1998). In the Philippines, cage-cultured snakeheads are very susceptible to the disease while carps (*C. carpio*), tilapia (*O. niloticus*) and milkfish (*Chanos chanos*) are considered naturally resistant. In Laguna de Bay, Philippines, the EUS morbidity rate among snakeheads was approximately 59% in January 1986 (Lio-Po and Lim, 2014).

By and large, EUS outbreaks show a seasonal pattern occurring from September to March when fish-rearing water temperatures in the region are at their lowest range of below 25°C. First outbreaks were reported in Vietnam in 1973, Singapore in 1977, Malaysia in 1979, Indonesia and Thailand in 1980, Cambodia, Myanmar and Lao PDR in 1984, the Philippines in 1985, Sri Lanka in 1987, Bangladesh and India in 1988, and Bhutan and Nepal in 1989. This spreading pattern of EUS outbreaks in South-East and East Asia strongly indicates the infectious nature of the aetiological agent/s. EUS was also reported in *C. gariepinus* in South Africa and Zambia (Huchzermeyer *et al*., 2018; Malherbe *et al*., 2019). To date, EUS rhabdovirus, *A. hydrophila* and *A. invadans* have been associated with EUS-affected fish (Frerichs *et al*., 1986; Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Kasornchandra *et al*., 1992; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Chinabut *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Lilley and Roberts, 1997; Lilley *et al*., 1998; Lio-Po, 1998; Kanchanakhan *et al*., 1999; Lio-Po *et al*., 2000).

The EUS rhabdovirus was isolated from EUS-affected fishes in Thailand, Sri Lanka, Myanmar and the Philippines (Frerichs *et al*., 1986; Kasornchandra *et al*., 1992; Kanchanakhan *et al*., 1999; Lio-Po *et al*., 2000). Positive isolations in catfish spleen (CFS) and snakehead spleen (SHS) cell cultures were predominantly obtained from slightly lesioned fishes (Lio-Po, 1998). CPEs were also induced in monolayer cells of blue gill fry (BF2), snakehead (SSN-1) and CCO, resulting in a virus titre of 10^6 $TCID_{50}$/ml at 25°C in 2–3 days with optimum replication at 15°C (Lilley and Frerichs, 1994; Frerichs *et al*., 1989; Kasornchandra *et al*., 1992; Kanchanakhan *et al*., 1999; Lio-Po *et al*., 1999, 2000). Electron micrographs of the virus showed its bullet shape, typical of the family Rhabdoviridae, with an estimated size of 65 nm × 175 nm (Lio-Po *et al*., 2000) (Table 6.4). Characterization and serological comparison of the virus with other fish rhabdoviruses associated with EUS-affected fish in Thailand showed that the Philippine virus isolate is morphologically similar and slightly antigenically related to the ulcerative dermal rhabdovirus (UDRV) isolated in Thailand.

The bacterium, *A. hydrophila*, has been consistently isolated from lesions of EUS-affected fish (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Costa and Wejeyaratne, 1989; Pal and Pradhan, 1990; Torres, 1990; Lio-Po *et al*., 1992; Pathiratne *et al*., 1994; Angka *et al*., 1995; Karunasagar *et al*., 1995; Thanpuran *et al*., 1995; Rahman *et al*., 1999). Related studies experimentally demonstrated the dermonecrotic effects of this pathogen to naïve fish.

Mycotic isolations and identification of *Aphanomyces* were reported in EUS-affected snakeheads (Roberts *et al*., 1993; Willoughby *et al*., 1995). In Africa, only *Aphanomyces* was tested in sampled EUS-affected fish (Huchzermeyer *et al*., 2018; Malherbe *et al*., 2019). Fungal hyphae invade the fish skin and skeletal muscles causing ulceration, commonly resulting in death (Lilley *et al*., 1998). Experimental infection with *Aphanomyces* induced lesions in naïve snakeheads and sand whiting (Roberts *et al*., 1993; Chinabut *et al*., 1995; Lilley and Roberts, 1997; Catap and Munday, 1998). The pseudofungi grow invasively through the fish skin and skeletal muscles causing severe myonecrosis and ulceration, commonly resulting in death (Callinan *et al*., 1995; Chinabut *et al*., 1995; Lilley and Roberts, 1997; Lilley *et al*., 1998). However, granuloma development was observed at 26°C or above, while at lower temperatures only acute inflammation developed (Chinabut *et al*., 1995). In addition, Catap and Munday (1998) observed that sand whiting

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injected with zoospores of *Aphanomyces* at 26°C developed highly inflamed, haemorrhagic external lesions while similarly treated fish held at 17°C had slightly inflamed injection sites. Temperature-related growth rate of this pathogen correlates with the findings that *Aphanomyces* from EUS-affected fish thrives better at 26–30°C than at lower temperatures (Lilley and Roberts, 1997).

Temperature is a significant factor in the pathogenesis of EUS. Replication of the EUS virus was observed at 15–25°C and the virus induced dermal lesions in fish reared at 20–22.5°C but not at 28–32°C (Lio-Po *et al.*, 2001). On the other hand, *A. hydrophila* and *Aphanomyces* thrive at 18–39°C and 26–30°C, respectively, with the latter pathogen producing 1.3–8 mm larger colonies at the high temperature. As such, among these three EUS-associated pathogens, only the EUS virus is temperature sensitive in terms of replication and dermal lesion induction in EUS-susceptible naïve fish viz-à-viz the EUS-season prevailing temperature of below 25°C. Moreover, studies on the interaction of these three pathogens in inducing EUS-like lesions indicate that the EUS virus is the primary pathogen and superimposed by a change to secondary/tertiary infections with either *A. hydrophila* and/or *Aphanomyces* (Lio-Po, 1998).

Diagnosis

The EUS-associated virus can be isolated from organ tissues of slightly lesioned catfish and snakeheads in cell monolayers of CFS, SHS, snakehead liver (SHL), CCO, BF2 and SSN-1 (Frerichs *et al.*, 1989; Kasornchandra *et al.*, 1992; Kanchanakhan *et al.*, 1999; Lio-Po *et al.*, 1999, 2000). Tissue filtrates derived from the visceral organs of EUS-affected fish induce CPEs when inoculated on to susceptible cells.

Bacterial isolation and identification of *A. hydrophila* from EUS-affected fish are detailed in Section 6.3.1 of this chapter on MAS. Bacterial colonies can also be histologically demonstrated in EUS-affected snakeheads (Cruz-Lacierda, 1995).

The pseudofungus, *Aphanomyces*, is isolated from ulcerated muscles of EUS-affected fish in Czapek Dox medium (Callinan *et al.*, 1995; Willoughby *et al.*, 1995). The hyphae of *Aphanomyces* are aseptate, 12–25 µm in diameter. Histological sections of muscular lesions of EUS-affected fish show the development of a necrotic granulomatous mycosis that may invade the abdominal viscera (Lilley *et al.*, 1998). Moreover, *Aphanomyces* can also be detected by PCR (Phadee *et al.*, 2004; Vandersea *et al.*, 2006; OIE, 2019b).

Transmission

Intramuscular injection of cell-cultured EUS rhabdovirus induced dermal lesion development and mortality of naïve snakehead (*Ophicephalus striatus*) juveniles reared at 20–22.5°C but not at 28–32°C (Lio-Po *et al.*, 2001). Slight dermal lesions developed 3 to 10 days following intramuscular inoculation which progressed to moderate lesions at 10–12 dpi but not deep ulcers in 5 to 12 days. Moreover, naïve snakehead fry and fingerlings exposed to cell-cultured EUS rhabdovirus by bath manifested significant mortalities ($P < 0.01$) of 100% at 5 and 9 dpi, respectively, with no apparent lesions (Lio-Po *et al.*, 2001; Kanchanakhan *et al.*, 2002). The lower temperature throughout the infection experiments simulated the water temperature during natural EUS outbreaks in EUS-affected countries. Moreover, cohabitation of naïve snakeheads with EUS fish or with apparently healthy snakeheads in lake water led to EUS-like lesion development attaining 100% morbidity by day 12 and 14, respectively, at 23–26°C (Lio-Po *et al.*, 2003), while naïve snakeheads stocked with EUS fish in aquifer water did not manifest EUS-like lesions. That study demonstrated the horizontal transmission of the virus from EUS fish and apparently healthy snakeheads held in endemic areas to naïve fish.

A. hydrophila has been associated with EUS-affected *C. striata* (syn. *O. striatus*) and *C. batrachus* in the wild as well as in ponds and cages (Lio-Po and Lim, 2014). Intramuscular injection of pure cultures of *A. hydrophila* induced dermonecrotic lesions in healthy catfish and snakeheads (Lio-Po *et al.*, 1992, 1996, 1998; Pathiratne *et al.*, 1994; Angka *et al.*, 1995; Karunasagar *et al.*, 1995). This bacterium thrives at 18–39°C and secretes a dermonecrotic factor at 10 and 30°C (Olivier *et al.*, 1981). Moreover, extracellular proteins such as protease are secreted by *A. hydrophila* associated with EUS-affected fish (Yadav *et al.*, 1992; Leaño *et al.*, 1996; Uddin *et al.*, 1997).

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The hyphae of *Aphanomyces* develop primary zoospores within the sporangium which are then released to the tip where they form a spore cluster (Fig. 6.6B). Here the primary zoospores quickly transform into reniform, laterally biflagellated, motile secondary zoospores. On attaching to the fish skin, the spore germinates, and the hyphae invade the skin, musculature and internal organs resulting in the development of mycotic granulomas. Refer to Section 6.4.1 on saprolegniosis for further details.

Prevention and control

Quarantine and restricted movement of EUS-susceptible fish from endemic areas to non-endemic sites should be practised. Outbreaks are common when water temperatures drop to <25°C and the immunity of the fish is at its lowest (Catap and Munday, 1998). The transmission of the EUS virus/*A. hydrophila*/*A. invadans* is horizontal, with the presence/release of the virus, bacteria and zoospores from infected fish or through infected water supplies (Lio-Po *et al.*, 2003). Raising the salinity of the water to >2 ppt can help stop the spread of the pathogen/s, but on detection of infection it is advised that water exchanges are stopped, stock movements are restricted, and ponds are limed (Lilley *et al.*, 1998). Proper sun-drying and liming of ponds, coupled with thorough disinfection of all farm materials used for cage production, can destroy the pathogen. Currently, there is no vaccine available and there are no effective chemotherapeutant treatments. Dietary inclusion (2 g/kg) of the immunostimulant Salar-Bec, fed to satiation for 14 days prior to injection with *A. invadans* zoospores, resulted in a 59.2% higher survival in the treatment group at 40 days post-challenge (Miles *et al.*, 2001). Prophylactic treatment with 5 ppm Coptrol (a chelated copper compound) was reported to prevent induction of EUS lesions, while a proprietary mixture, CIFAX, may be curative (Lilley *et al.*, 1998).

6.5 Parasitic Diseases

While there is a wealth of information on the parasite fauna and diseases of warm, freshwater finfish aquaculture (Lio-Po and Lim, 2014; Paladini *et al.*, 2017; Jahangiri *et al.*, 2021; Shinn *et al.*, 2022), there is comparatively little information dealing with parasitic diseases in cage-pen culture systems. In many instances, the specific identity of the parasites is infrequently provided and at best only the genera are recorded (Paperna, 1991). Rarely are details regarding patterns of infection, pathology and prevailing factors predisposing fish to disease provided.

6.5.1 Diseases caused by protistans

Protistan parasites of importance include those belonging to the Ciliophora, Myxozoa, Microspora, Sarcomastigophora and Apicomplexa (Canning and Lom, 1986; Woo, 1995). The pathogenic protistans most reported include the myxosporeans, trichodinids and the dinoflagellates. The general under-reporting of protistan infections may result from infections going undetected, undiagnosed, not recorded at the farm level, or ignored because of their common occurrence. The translocation of fish stocks for aquaculture and fisheries has contributed to the worldwide distribution of many parasites, notably parasitic protistans (Shinn *et al.*, 2022). The earliest recorded translocations of tilapia, for example, were to South-East Asia in the late 1930s (Atz, 1954). Shinn *et al.* (2022) summarizes >820 translocations of tilapia worldwide, many of which occurred prior to mandatory health certification as part of national strategies in health management and aquaculture biosecurity.

6.5.1.i Diseases caused by ciliates

The ciliates (phylum Ciliophora) are common ectoparasites of fish, especially in hatcheries and on young fish in grow-out ponds. The vast majority of protistan problems in aquaculture are due to poor husbandry. Under normal culture conditions, *Apiosoma* spp., *Epistylis* spp. and *Trichodina* spp. are commonly encountered, as harmless ectocommmensals. However, if culture conditions deteriorate (e.g. due to overstocking, increased organic loading of the water due to overfeeding or poor waste management, low water exchange) then under these favourable, organic-rich conditions, the size of the parasite populations can increase quickly. Without the

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need for an intermediate host and reproducing by binary fission, parasite infections can spread rapidly, leading to morbidity and mortality. While notable pathogenic ciliates such as *I. multifiliis*, *Chilodonella* spp. and *Trichodina* spp. are readily identifiable, the specific identities of most ciliate infections in tropical aquaculture remain undetermined. For infections of *Chilodonella* spp., refer to the works of Lom and Dyková (1992), Basson and Van As (2006) and Bastos Gomes *et al.* (2016).

6.5.1.ii Trichodinid diseases

Pathogenic trichodinids include members of the genera *Trichodina*, *Tripartiella* and *Trichodinella*. Many trichodinids are associated with *C. idella*, *C. carpio*, *H. molitrix* and *H. nobilis*, and these were introduced into Israel and South-East Asia from China (Chen, 1955; Paperna, 1991). Various trichodinids from African cichlids have also been introduced into South-East Asia (Albaladejo and Arthur, 1989; Shinn *et al.*, 2022). Trichodinids commonly cause mortality in hatcheries, and these may continue to be a problem after fish are transferred to cage-culture systems. Trichodinids are prevalent on young clariid hybrids of *C. gariepinus* and *Clarias* sp. in cages. They are also found on *C. idella*, *H. molitrix* and *H. nobilis* in hatcheries in China and Vietnam, as well as on pangasiids and *Catla* spp. in cage culture. Although there are many species of trichodinids, only a few are known to be pathogenic (Lom, 1995). For a review of trichodinids infecting commercial fish species in South America, see the review of Maciel *et al.* (2018). For a review of infections on commercially important species of tilapia, see Shinn *et al.* (2022).

Pathology

Pathological effects are dependent on the host's response, the intensity of infections and environmental conditions, since stressful conditions can compromise the host's ability to counteract infections (Paperna, 1996). Infections on the fish's exterior may be at preferred sites (e.g. gill or fin bases). Parasite activity results in abrasion, cell damage, erosion and desquamation of the epidermis. The host response is through increased mucus secretion and epithelial hyperplasia, cellular destruction and inflammation. Infections may lead to respiratory distress, emaciation and mortalities, if left untreated (Paperna, 1996). It is important to emphasize that ciliate infections frequently facilitate the establishment of secondary microbial species; a review of their interactions and control is provided in Jahangiri *et al.* (2021) and is the subject of several case studies that investigate various bacterial and ciliate pathogens (Xu, D.H. *et al.*, 2013; Xu *et al.*, 2019).

Diagnosis

Trichodinids are easily observed microscopically from skin and gill scrapings (Paperna, 1996). Taxonomy of the trichodinids is based on the structure of the buccal ciliature, the morphology of the adhesive disc, and the number and size of its components (Lom, 1995). Trichodinids are essentially flat discs, with somatic ciliature consisting of three or four ciliary wreaths around the aboral surface of the body which is transformed into an adhesive disc. The disc is a proteinaceous skeleton, composed of a ring of hollow conical denticles. The denticles consist of blades (centrifugal flat projections) and horns (rod-like centripetal projections), connected to each other by radial pins (Fig. 6.7D). *Trichodina* is characterized by denticles with massive central conical parts, flat semi-circular blades, straight thorns and a diameter of 50–100 µm. Comprehensive reviews are provided by Van As and Basson (1989), Lom and Dyková (1992) and Basson and Van As (2006).

Prevention and control

In most cases an outbreak of *Trichodina* results from deteriorating environmental conditions, which are common in intensive culture systems. If *Trichodina* numbers rise, then fouled nets should be changed to ensure good water exchange through the net pen, pond aerators repositioned if necessary to facilitate water flow through the cages, feed management reviewed to ensure the fish are not being overfed, stocking density reduced by splitting stock out into clean cages, and waste management issues reviewed and addressed. To eliminate trichodinids, a saline solution (0.1–0.2% as a dip for 1–2 days) or formalin (150–250 ppm as a dip for 30–60 min)

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can be applied (Lom, 1995). The efficacy of formalin in controlling trichodinids depends on water quality (pH, salinity and ambient temperature) and the species of fish to be treated.

6.5.1.iii Ich or white spot disease

I. multifiliis, ich or white spot, is a pathogenic ciliate infecting freshwater fish and causing ichthyophthiriosis. This pathogen was first reported from China (Dickerson and Dawe, 1995), but it is a cosmopolitan pathogen commonly encountered in temperate and tropical warmwater fish in temperatures typically 2–27°C (Matthews, 1994). Development is temperature dependent, making them a potential danger to cage-culture systems in tropical warm waters. This parasite is maintained within the fish as a low subclinical (enzootic) infection and as encysted tomonts. It persists in the environment, becoming epizootic clinical infections when fish are stressed because of poor management practices (e.g. poor feed, overcrowding and poor sanitation). The pathogen is not host specific and recovery from the disease confers resistance to reinfection (Paperna, 1996).

Pathology

The parasitic trophont stage located within the host's epidermis feeds on the basal layer of the epidermis. Aggregations of trophonts can arise within galleries because of multiple entry at single sites, from parasite tunnelling and/or possibly from reproduction (Matthews, 1994) (see Fig. 6.7C). Trophonts need to reach a critical size before they can exit their hosts and their departure is an active process which may involve the discharge of contractile vacuoles (Ewing and Kocan, 1987). The exiting of mature trophonts damages the epidermis, causing detachment. The synchronized exiting of numerous trophonts, notably in small fish or from the gills, can be traumatic, resulting in osmotic shock, haemodilution and high rates of mortality.

Diagnosis

Clinical signs include anorexia, lethargy and respiratory distress; infections are characterized by white spots on the skin and gills (Dickerson and Dawe, 1995). Skin and gill scrapes examined under a compound microscope reveal ciliates (up to 1 mm in diameter) with a small cytostome. *Ichthyophthirius* fixed and stained with Giemsa or haematoxylin reveals a large crescent-shaped macronucleus and a small micronucleus.

Prevention and control

The management and control of *I. multifiliis* lies in having a comprehensive understanding of its biology, life cycle, and the factors contributing to its establishment and pathogenicity. In warm waters, the life cycle can complete in *c.*8 days (i.e. 7 days for development of the parasitic trophont; 1 day for the external phase leading to the release of infective theronts). Infection dynamics can be affected by several factors including the speed of water moving through cages, stocking density, cage/system design, water depth, aeration and cleaning frequency. The chemotherapeutants that have been assessed *in vivo* have typically been administered as a short (i.e. <60 min) high-dose application, delivered either as a single dose or as a regimen of daily treatments over a period of *c.*7 days (Picón-Camacho *et al.*, 2012a,b). Most treatments have had limited efficacy because the chemical has been unable to treat the parasite situated beneath the epithelium. The narrow treatment window serves only to treat the external stages of the parasite in the water column at the time of the treatment. By increasing the window of chemical exposure, protomonts can be treated as they are continuously released from the host and before they encyst, and tomites/theronts treated as they are released and before they infect. There are, however, practical challenges and welfare considerations in delivering long-duration bath treatments and these need to be carefully factored into any treatment. The efficacy of potassium permanganate, widely used throughout South-East Asia, is affected by the organic load of the water; it can lower the dissolved oxygen concentration of the water and its toxicity increases in waters with a high pH. The long duration of 1–5 ppt NaCl as a permanent bath can reduce the number of trophonts establishing on fish and may prove beneficial, helping the host recover the osmotic imbalance and loss of salts created by exiting trophonts.

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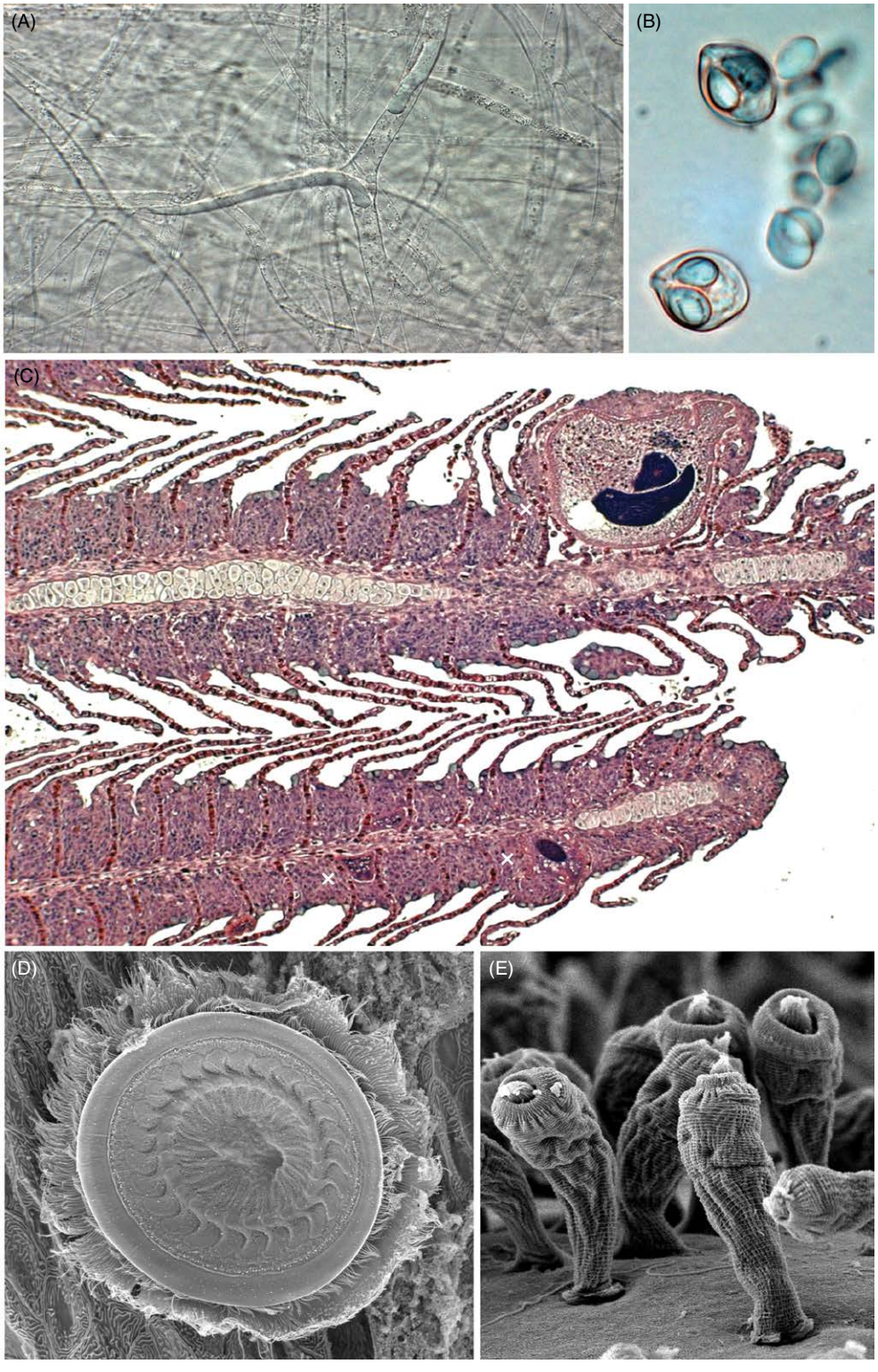


Fig. 6.7. Some commonly encountered parasites of warm freshwater cage culture-reared fish. (A) *Saprolegnia* sp. (image by A. Shinn). (B) *Myxobolus* sp. from *Pangasianodon hypophthalmus* from Vietnam (image by A. Shinn). (C) Developing trophonts (indicated as ×) of *Ichthyophthirius multifiliis* in the

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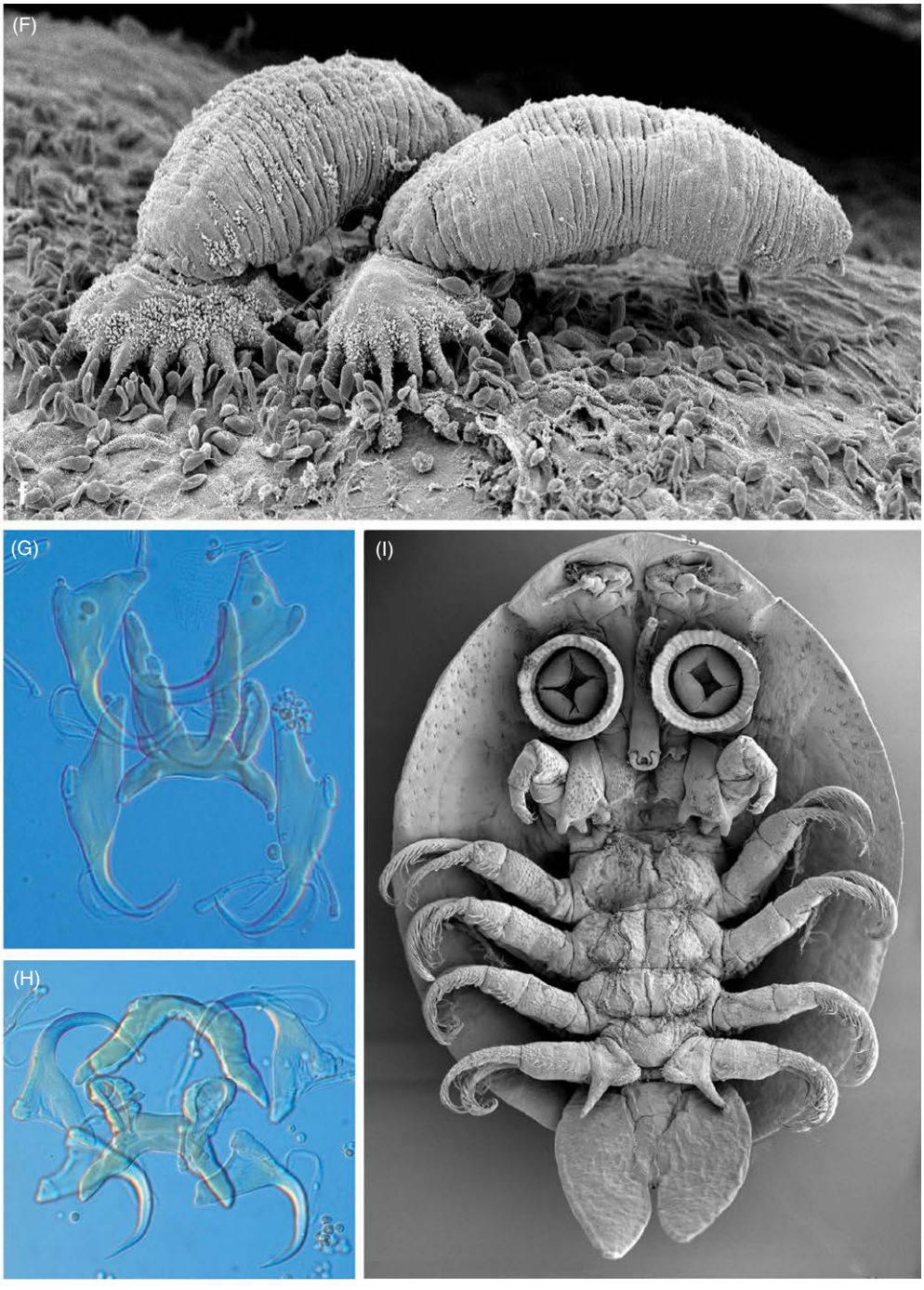


Fig 6.7. Continued.
gills of a rainbow trout, *Oncorhynchus mykiss* (image by A. Shinn). (D) *Trichodina* sp. from the skin of Nile tilapia, *Oreochromis niloticus* (image courtesy of G. Hanako Rosas Saito and A. García-Vásquez). (E) *Apiosoma* sp. (image courtesy of G. Paladini). (F) *Gyrodactylus* sp. and *Ichthyobodo necator* (image courtesy of G. Paladini). (G) *Cichlidogyrus tilapiae* from *O. niloticus* (image courtesy of A. García-Vásquez and A. Shinn). (H) *Cichlidogyrus sclerosus* from *O. niloticus* (image courtesy of A. García-Vásquez and A. Shinn). (I) *Argulus* sp. from *O. niloticus* reared in Mymensingh, Bangladesh (image by A. Shinn).

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At present there are no commercial vaccines for *I. multifiliis*. However, fish can acquire a protective immunity against *I. multifiliis* (see Jørgensen *et al.*, 2008). Different vaccines using live, killed, parasite homogenate and DNA have been assessed for the protection they confer against *I. multifiliis* (see review of Shivam *et al.*, 2021). Studies by Cross and Matthews (1992) and Gurunathan *et al.* (2000) demonstrated that non-lethal infections or intraperitoneal injections of live theronts can confer immunity (Matthews, 2005). A DNA vaccine encoding immobilization antigens administered as an intramuscular injection in *I. punctatus* offered a lower level of protection than that of a live vaccine (Xu *et al.*, 2019).

6.5.2 Diseases caused by dinoflagellates

There are five genera of parasitic oodinid dinoflagellates on fish: *Amyloodinium*, *Piscinoodinium*, *Crepidoodinium*, *Ochthyodinium* and *Oodinioides* (Noga and Levy, 2006). The ichthyotoxins produced by dinoflagellates cause massive mortality in cultured and feral fish (Steindinger and Baden, 1984). In fresh water, the most important pathogenic dinoflagellate in fish is *Piscinoodinium*, which is closely related to the marine dinoflagellate pathogen, *Amyloodinium*. *Piscinoodinium* is not host specific and has been reported on feral, aquarium and cultured food-fish species from diverse families in warm waters (see Lio-Po and Lim, 2014).

6.5.2.i Velvet or rust disease

Fish with excessive mucus covering the body together with a rust-coloured appearance of the skin are infected with *Piscinoodinium pillulare*, the causative agent for velvet rust diseases, gold dust disease, pillularis disease and freshwater *Oodinium* disease (see Lio-Po and Lim, 2014 and references therein). *Piscinoodinium*, like its marine relative *Amyloodinium*, is found on a wide range of host species and is known to cause mortality in warmwater fish. Infections of *P. pillulare* in Malaysia have been reported to cause mortality by inducing hyperplastic gills and obstruction of the respiratory surface. Outbreaks with up to 100% mortality of farmed *Piaractus mesopotamicus* have been reported from Brazil (Sant'Ana *et al.*, 2012). A 30% mortality of farmed juvenile *C. macropomum* is reported from Peru (Arbildo-Ortiz *et al.*, 2020). Infections from farmed populations of tambacu hybrids, *P. mesopotamicus*, *C. macropomum*, *Leporinus macrocephalus*, *O. niloticus* and *Prochilodus lineatus* are reported from Brazil (Martins *et al.*, 2001). There is also a record of 8-year-old *Piaractus brachypomus* at a semi-intensive farm dying during the dry season in Brazil when temperatures fluctuate between 11 and 35°C (Ramos *et al.*, 2020). Infections of *Piscinoodinium* sp. on farmed *O. niloticus* are summarized in Shinn *et al.* (2022).

Pathology

Histopathological changes to gill structures occur with a massive proliferation of the gill epithelium, fusion of adjacent lamellae and separation of the gill respiratory epithelium resulting in a severe hyperplasia of the entire gill filament. The trophonts of *P. pillulare* penetrate the host cells by nail-like extensions resulting in degeneration and collapse of the cells, leading to focal erosion and proliferation of the epithelium and obliteration of the gill lamellae. The inner strata of the epithelium become spongious and may undergo complete lysis.

Diagnosis

Initial diagnosis can be based on clinical signs and confirmed by microscopic examination of the trophont stage (Gomes *et al.*, 2018). *Piscinoodinium* infects skin and gills with clinical signs like amyloodiniosis. Infected fish have a yellow- to rust-coloured (velvety) skin, a dense covering of mucus resulting in darkening of the skin, dyspnoea, anorexia and skin ulcers. All oodinids have a parasitic trophont stage and a sessile, stalked, sac-like trophozoite stage which feeds on the skin and gill epithelia. The trophont has a prominent stalk, which anchors the parasite to the host. It probably uses the stalk to absorb nutrients. After feeding, the trophont detaches, withdraws the stalk and forms an encysted tomont (reproductive cysts). The tomont divides asexually forming dinospores, the mobile infective stages. The trophonts and tomonts are important for definitive diagnosis, and

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microscopic identification of these stages is necessary. Trophonts are oval with smooth walls, usually visible to the naked eye as white spots (80–100 μm) and turn dark blue in Lugol's iodine. *Piscinoodinium* is distinguished from other oodinid dinoflagellates based on the morphology of the trophont, especially the type of host attachment and mode of nutrition. Fish should be examined live or immediately after death, and biopsies of the gills can be removed from live or recently dead fish and examined. Trophonts are removed by brushing the fish gently in a dish of water and the sediment is examined under the microscope. The trophont of *Piscinoodinium* is a yellow-green, pyriform or sac-like cell, almost round, 12 μm × 29 μm, with a rudimentary sulcus, and having a short stalk with an attachment disc extending from its base and thin holdfasts (rhizocysts) radiating from the stalk. Head parts of the rhizocysts are inverted in separate compartments (rhizothecas) in the sole of the disc, while their shafts are firmly embedded in the host cell cytoplasm. The theca covers the entire cell except for the area of the attachment disc.

Prevention and control

Outbreaks of oodinid infections result from stress due to poor environmental conditions. Hence, environmental manipulation is probably a viable approach to control outbreaks of *Piscinoodinium*. Long-duration treatments may result in better efficacies and should cover a period that allows for the emergence of dinospores from trophonts and the period over which dinospores remain infective (i.e. *c*.2 days). Salt (1.5 g/l) remains the safest method as a permanent bath; however, for-life threatening infections, Noga (2010) suggests that high concentrations of salt (e.g. up to 35 ppt) for 1–3 min dislodges trophonts. The latter treatment though will require establishing a treatment station where there is no possibility of the dislodged dinospores finding their way back into the main culture system.

6.5.3 Myxosporean diseases

Myxozoans are specialized cnidarian parasites which typically use a vertebrate and invertebrate host as part of their life cycle. Myxosporean cysts can be found infecting the skin and subcutaneous layer, muscle, gills, central nervous system and visceral organs, causing extensive lesions as cysts break and mortality (Lom and Dyková, 1995). Myxozoans are traditionally classified using morphological features of the spores (number and arrangement of polar capsules, features of spore walls, etc), morphometrics and tissue tropism. Notable problematic species in warmwater fish include *Myxobolus lentisuturalis* in *C. gibelio* (see Dyková *et al*., 2002) and *Thelohanellus hovorkai* in *C. carpio* (see Yokoyama *et al*., 1998). High rates of myxosporean-related mortality have been reported in *Colossoma macropomum* fry (see Videira *et al*., 2016) and in cage-cultured *P. hypophthalmus* (see Molnár *et al*., 2006).

Pathology

Myxozoan infections can result in a range of pathologies. *Myxobolus drjagini* infecting the skin of the head, the olfactory and oculomotor nerves in the cranial cavity and the intrafilamental epithelium of the gills results in destruction of the nerves and a condition known as 'silver carp twist disease' with consequential mass mortalities (Wu *et al*., 1975; Chen and Ma, 1998). Infections are widely reported in *H. molitrix* and *Hypophthalmichthys nobilis*. There is, however, uncertainty regarding the identity of the myxosporeans recovered from different tissues; those from the gills may represent another morphologically similar species, *Myxobolus paratypicus* (see Xi *et al*., 2019). Heavy infections of *Myxobolus pavlovskii* in the interlamellar epithelium and secondary lamellae in fingerling *H. molitrix* and *H. nobilis* results in impaired respiratory function and impacts survival (Molnár, 1979). Infection of *Myxobolus koi* in the gills of *C. carpio* and *C. auratus* results in numerous cysts forming within the gills (Paperna, 1991; Egusa, 1992). The formation of large cysts (*c*.5 mm in diameter) enveloped by vacularized host connective tissue within the gills restricts opercula movements; increased mucus production and epithelial proliferation leads to congestion and impacts on respiratory function (Hoshina, 1952). Spores of *M. koi* were also observed in the heart, liver, kidney and intestine. Heavy infections of *Myxobolus dispar* in *C. carpio* also result in impaired respiration (Lom and Dyková,

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1992). *Myxobolus dahomeyensis* infection in the ovaries of *O. niloticus*, which results in a suppurating thick liquid replacing mature oocytes, can result in sterilization (Okaeme *et al.*, 1988; Gbankoto *et al.*, 2001). *O. niloticus* with ocular infections of *Myxobolus sarigi* present exophthalmos (Okaeme *et al.*, 1988). The elongated plasmodia of *Myxobolus encephalicus* in the brain blood vessels of *C. carpio* are in contact with the vessel walls and are coated with a layer of endothelial cells to prevent blood coagulation, which results in impaired blood flow, dilation of blood vessels and localized oedema. In the brain, mature spores released from plasmodia may provoke a heavy granulomatous inflammation. Heavily infected fry and fingerlings are emaciated, have shrunken eyes, have a loss of balance and swim in a circular motion (Lom and Dyková, 1992). The mass infection of *Sphaerospora molnari* in the gills of *C. carpio* results in heavy losses. Infections can elicit epithelial hyperplasia, with parasites replacing tissue consequentially leading to muscular dystrophy, circulatory disorder, and the necrotic destruction of the tissue with the release of spores into the water (Lom and Dyková, 1992). The plasmodia of *Thelohanellus nikolskii* in close association with the fin cartilage of *C. carpio* results in cartilage fragmentation and fin rays breaking off, impairing swimming performance (Molnár, 1982). In Indonesia, *Thelohanellus pyriformis* forms large plasmodia in the subcutaneous tissue and muscle of cyprinids causing fatal epizootics (Lom and Dyková, 1995). A co-infection of *Zschokkella auratis* together with various bacterial pathogens and *I. multifiliis* resulted in the complete loss of *C. striata* stock at a farm unit in India (Paul *et al.*, 2020). For a summary of myxozoans infecting oreochromids, where typically infections result in hyperplasia or hypertrophy of host tissues, see the recent review of Shinn *et al.* (2022).

Diagnosis

The gross signs of infection may be varied, but histozoic (i.e. those within tissues; cf. coelozoic, which are those occupying cavities) species frequently form cysts of various colours (i.e. usually white, or yellow), shapes and sizes (i.e. round, oval or branched). Heavy gill infections may hamper opercular movement and impair respiratory function, while infections of the brain, nervous system and fins may result in abnormal swimming behaviours and performance. Identification is assisted by spore morphology and based upon: the general spore shape; the number of polar capsules; the orientation of the polar filament within the polar capsule and the number of coils; the number of shell valves and whether these are smooth, ridged or possess protrusions; details of the infective sporoplasm (i.e. one binucleate or two uninucleated cells) with or without an iodophilic vacuole; use of morphometric data collected from key structures; and by the presence/absence of a mucus envelope. To identify the spores, cysts can be carefully excised, transferred to a glass slide and then the cyst broken to release the spores. For helpful guides to the Myxozoa, refer to the works of Dyková and Lom (1988), Lom and Dyková (1992, 1995, 2006) and Feist and Longshaw (2006).

Prevention and control

Currently, there are no effective treatments for myxosporeans and the best method is to remove and destroy heavily infected fish from cages. Management and control lie in the practice of good biosecurity to minimize the likelihood and potential impact of infections. New stocks should be held under quarantine while the relevant health checks are conducted to ensure their disease-free status prior to stocking, and the health of stock fish should be assessed regularly throughout production. For stocks reared in earth ponds, ponds should be drained, the sediment removed on a regular basis, the pond thoroughly dried between production cycles, and hydrated lime applied to ensure that any intermediate hosts (e.g. oligochaetes) are killed. Removing pond sediment reduces the amount of detritus available to oligochaetes. Using high-density polyethylene to completely line culture ponds may help in sediment management and its effective removal between production cycles, but the same disinfection practices of comprehensive cleaning of the liner between cycles is recommended. Where possible, the incoming water should be filtered and treated with ozone or by ultraviolet irradiation to minimize the likelihood of actinosporean stages and infected oligochaetes entering culture systems. Producers should be aware of the risks of feeding live oligochaetes

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which can harbour infective myxospores (Hallett *et al.*, 2005). Using specific pathogen-free oligochaetes sourced from a reputable supplier is therefore recommended.

6.5.4 Diseases caused by monogeneans

Monogeneans are among the most reported parasitic agents of fish (Scholz *et al.*, 2018; Shinn *et al.*, 2022). They are mainly ectoparasitic on the gills, buccal cavity, body surface and fins of freshwater fish, although some are endoparasitic (e.g. Gussev and Fernando, 1973; Euzet and Combes, 1998; Mendoza-Franco *et al.*, 2018). Monogeneans are oviparous except for the viviparous gyrodactylids. Although there are some species that cause epizootics in wild populations of fish (Bakke *et al.*, 2007; Paladini *et al.*, 2021), they are important pathogens in intensive fish culture (Ogawa *et al.*, 1995; Lopez *et al.*, 2002; García-Vásquez *et al.*, 2007; Grano-Maldonado *et al.*, 2011). Their direct life cycle results in rapid and continuous recruitment, especially in warm waters; this makes monogeneans especially dangerous in intensive culture (Deveney *et al.*, 2001). Disease caused by monogeneans is normally more debilitating than fatal, and subsequent mortality is usually attributed to viral or bacterial infection. Monogeneans stress the fish hosts by destroying the epidermal integrity of the fish, thus predisposing their hosts to other pathogens as mechanical vectors of bacterial and viral diseases (Xu *et al.*, 2007; Abdel-Latif and Khafaga, 2020).

In intensive culture systems, where the intensity of infection can be high on the gills, monogeneans can cause death directly by inhibiting respiration through physical damage to the gills that destroys the gill architecture. Fish mortality from monogenean infections may result from damage to gill tissues and skin caused by attachment organs, and by feeding on the integument, which stimulates cell proliferation and secretion of copious amounts of mucus (Paperna, 1991). Cage culture in tropical areas is usually conducive to the perpetuation of parasitic diseases with high stocking density. The nets trap eggs, infective larvae and food debris around the cages, which attract carrier/reservoir feral fish. Most monogenean genera are specific to a group of related host species; for example, *Dactylogyrus* is found on cyprinids, *Cichlidogyrus* on cichlids, while catfish harbour *Thaparocleidus* (see Shaharom, 1985). Although at the species level most monogeneans are specific to a particular host, some, like *Thaparocleidus caecus*, are found on several pangasiids (Lim *et al.*, 2001; Šimková *et al.*, 2013).

Many of the monogenean species on warm freshwater-cultured fish have not been identified or are incorrectly classified. For example, *Dactylogyrus* spp. have also been incorrectly implicated as being pathogenic to snakeheads, tilapia and clariids cultured in South-East Asia (Kabata, 1985). *Trianchoratus* is found on snakeheads other than giant snakehead, which is infected by *Sundanochus* spp., while *Cichlidogyrus* spp. infect the tilapias and *Quadriacanthus* spp. and *Bychowskyella* spp. infect the South-East Asian clariids. The gyrodactylids, on the other hand, are ubiquitous, although at species level they might be host specific. The most reported monogeneans on warm freshwater-cultured fish are the *Dactylogyrus* spp. on carp; *Cichlidogyrus* spp., *Enterogyrus* spp. and *Scutogyrus* spp. on cichlids; *Bychowskyella* spp. and *Quadriacanthus* spp. on clariids; *Trianchoratus* spp. on snakehead; *Pseudodactylogyroides* spp. on *Oxyeleotris marmorata*; *Thaparocleidus* spp. on catfish other than clariids; and the *Gyrodactylus* spp. (see Section 6.5.4.iv below) (Fig. 6.8).

In many cases, the specific identity of the pathogenic monogeneans, signs and pathology of the infection, disease mechanism, and control and preventive measures have not been specifically elucidated and documented. For instance, it is known that *Thaparocleidus siamensis* occurs in greater intensity than *T. caecus* on cultured *P. hypophthalmus* in Peninsular Malaysia and Thailand (Lim, 1990, 1996; Thuy and Buchmann, 2008), but it is not known which of the two species is pathogenic. The translocation of monogeneans, along with their hosts, has been well documented for the various *Dactylogyrus* spp. on imported Chinese carp, for *Cichlidogyrus* spp. on tilapia (Shinn *et al.*, 2022) and for *Quadriacanthus clariadis* on the *C. gariepinus* imported into Thailand (Paperna, 1991; Lerssutthichawal, 1999). There is information on the signs, pathology and control measures for some species of *Dactylogyrus*, *Gyrodactylus* and *Cichlidogyrus*, but not for other monogenean pathogens. In most cases, the information is derived from

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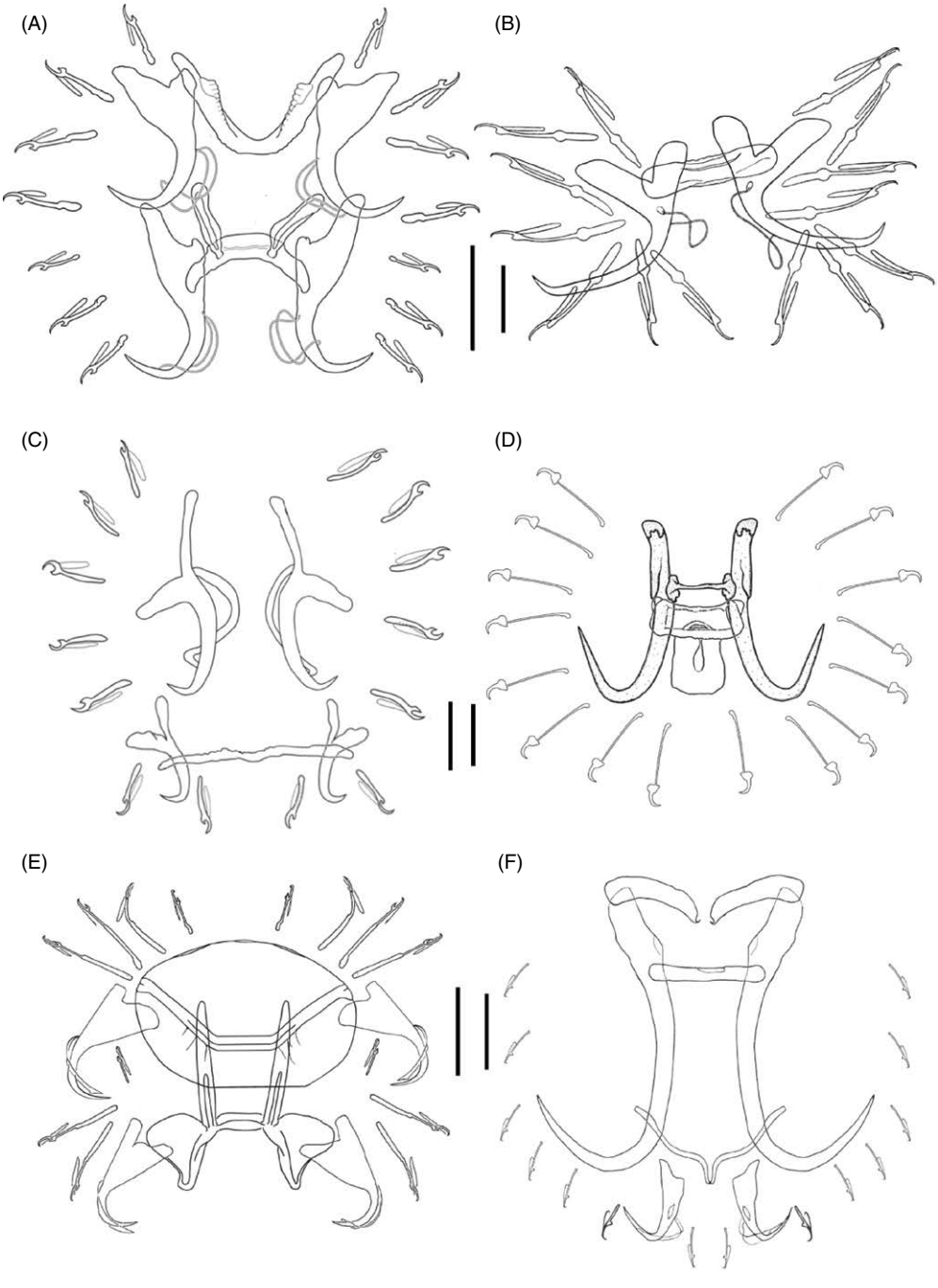


Fig. 6.8. Arrangement of the attachment hooks in six key species of monogenean. (A) *Cichlidogyrus tilapiae* Paperna, 1960 (modified from Šimková *et al*., 2019). (B) *Dactylogyrus vastator* Nybelin, 1924 (modified from Gussev, 1985). (C) *Enterogyrus malmbergi* Bilong Bilong, 1988 (modified from Zhang *et al*., 2019). (D) *Gyrodactylus cichlidarum* (adapted from García-Vásquez *et al*., 2007). (E) *Scutogyrus longicornis* (adapted from Pariselle and Euzet, 1995). (F) *Tharoparocleidus* gen. sp. (adapted from Pariselle *et al*., 2006). Scale bars: (A–D) 20 µm; (E, F) 30 µm.

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pond-culture systems and not from cage-culture systems. It should be also noted that habitat can affect parasitic infections, as indicated by the infection of tilapia by *Neobenedenia* spp. instead of *Cichlidogyrus* spp. when farmed in cages in estuarine waters (see Shinn *et al.*, 2022).

6.5.4.i Diseases caused by *Dactylogyrus* species

Dactylogyrus species are specific to the Cyprinidae although they are also found on Hemiramphidae and one species on a catfish (Gussev, 1976). This genus is frequently listed as a disease-causing agent since cyprinids are the most cultured fish group. The genus *Dactylogyrus* includes over 900 species (Gibson *et al.*, 1996) and approximately 50 species are known from the seven top cultured species of cyprinid listed in Table 6.1. The four important species of *Dactylogyrus* that cause disease in cultured common carp in Israel are *Dactylogyrus anchoratus*, *Dactylogyrus extensus*, *Dactylogyrus minutus* and *Dactylogyrus vastator* (see Paperna, 1991). These have different temperature preferences: for example, *D. extensus* flourishes at low water temperatures (optimum temperatures of 16–17°C), while *D. vastator* prefers warmer waters (20–24°C). *Dactylogyrus* has also been shown to cause mass mortality of fry, small fish and broodfish (Paperna, 1991; Kritsky and Heckmann, 2002).

Pathology

The pathology caused by *Dactylogyrus* spp. on exotic carp has been reported in studies done in Europe, but not for the species infecting the indigenous cyprinids of South-East Asia. Feeding on epithelial cells and anchorage (attachment) by the monogeneans cause severe destruction of the gills, resulting in haemorrhage and metaplasia of the gill tissue. Secondary bacterial infections usually occur and result in death of the fish. The pathologies caused by *D. vastator* and *Dactylogyrus lamellatus* are similar (Molnár, 1972; Paperna, 1991). *D. vastator* infections cause severe hyperplasia of the epithelium of gill filaments. Extensive proliferation of the respiratory epithelium of the gills interferes with respiratory functions and may be a direct cause of death. The sites of proliferation are dependent on the preferred sites of the monogenean species. *D. vastator* prefers the tips of the gill filaments and causes mass mortality in young fish but seldom on fish greater than 32–35 mm since the functions of the remaining gill filaments are not affected. Massive infections of *D. extensus* can cause mortality in 4–7 kg broodfish (Paperna, 1991).

Diagnosis

Fish infected with *Dactylogyrus* spp. are lethargic and are seen swimming at the water surface. Fish heavily infected with *Dactylogyrus* have pale to greyish gills, swollen at the edges, and the opercula appear to open wider than normal and secrete an excessive amount of mucus. Heavily infected fish may be anorexic and seen gasping for air and exhibiting abnormal behaviour such as jumping out of the water. *Dactylogyrus* spp. are usually found on the gills, although in massive infections they can also be found on the buccal cavity. *Dactylogyrus* spp. can kill directly by damaging gill structures and affecting respiration. In warm eutrophic waters with low oxygen concentrations, this becomes serious (Molnár, 1994). *Dactylogyrus* infections usually result in secondary bacterial infections with subsequent mortality.

At present, *Dactylogyrus* infections are confirmed by examination of the gills and infected fins for presence of the monogeneans. The signs and pathology of monogenean infections are neither generic nor species specific. Hence, the diagnosis of a monogenean infection is based on the identification of the pathogen itself. Correct diagnosis of monogeneans requires proper preparation of the parasite specimens. Gills can either be completely removed or gill biopsies can be taken from the infected fish. Each parasite is removed carefully from the gills under a dissecting microscope, placed on a slide and covered with a coverslip. Excess water is removed, and the corners of the coverslip sealed with nail polish to prevent it from moving. Ammonium picrate is added underneath the coverslip to clear and fix the specimens, which are examined using a phase contrast microscope. Monogenean species are usually identified based on the hard reproductive and haptoral armaments (i.e. attachment hooks) on the cleared and flattened

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specimens. *Dactylogyrus* are oviparous monogeneans with or without four eye spots, 14 marginal hooks, two anchors, one or two connective bars and two needle-like structures, and spindle-shaped dactylogyrid-type seminal vesicles (Fig. 6.8B). For identification keys to the relevant species, see the works of Mizelle and Price (1964), Paperna (1991), Chinabut and Lim (1993), Wu *et al.* (2000), Pandey and Agrawal (2008) and Galli *et al.* (2010).

6.5.4.ii Diseases caused by *Cichlidogyrus* species

Cichlids are cultured in cages in warm fresh water as well as warm estuarine water. Tilapias cultured in fresh waters are affected by *Cichlidogyrus* spp., while in marine waters they are infected by the marine monogeneans, *Neobenedenia girellae* and *Neobenedenia melleni* (see Shinn *et al.*, 2022). In 2019, 11 commercially important species of tilapia (not including hybrids) were cultured in 124 countries worldwide resulting in an aquaculture production of >6.2 million tonnes (Shinn *et al.*, 2022). Cichlids are hosts to species of *Cichlidogyrus*, *Onchobdella* and *Enterogyrus* (an endoparasitic monogenean that lives within the stomach of its host). Shinn *et al.* (2022) documents >820 translocations of tilapia and comments on the parasites that have been moved with them. The most notable species that have been translocated to several countries include *Cichlidogyrus halli*, *Cichlidogyrus longicornis*, *Cichlidogyrus sclerosus*, *Cichlidogyrus thurstonae*, *Cichlidogyrus tilapiae* and *Scutogyrus longicornis* (see Zhang *et al.*, 2019; Shinn *et al.*, 2022). No accounts of mortality due to *Cichlidogyrus* spp. have been recorded, but *C. sclerosus* is reported to cause severe gill damage in tilapias cultured in the Philippines (Kabata, 1985) as does *Cichlidogyrus philander* on *Pseudocrenilabrus philander* in South Africa (Igeh and Avenant-Oldewage, 2020).

Diagnosis

The behaviour of the fish can indicate the presence of parasites, and this is like infections of *Dactylogyrus*. An accurate diagnosis requires removing the gills or taking gill biopsies; the monogeneans are collected and prepared as stated for *Dactylogyrus* (see Section 6.5.4.i above). *Cichlidogyrus* can be distinguished from other monogeneans by having a haptor with four anchors, with two bars, one of which is V-shaped and the other made up of three parts (see Fig. 6.8A).

6.5.4.iii Diseases caused by *Thaparocleidus* species

Species belonging to this monogenean genus are found on cultured pangasiids and bagrids in South-East Asia (see Pariselle *et al.*, 2002; Thuy and Buchmann, 2008; Šimková *et al.*, 2013; Lio-Po and Lim, 2014) (Fig. 6.8F). As with *Pseudodactylogyroides*, little is known about the pathology caused by this group of monogeneans, or the level of pathogenicity.

Diagnosis

Monogenean species from pangasiids are collected and prepared as for *Dactylogyrus* infection (see Section 6.5.4.i above). *Thaparocleidus* spp. (Fig. 6.8F) have four anchors, two connective bars, one of which may be whole or separated into two, 14 marginal hooks and a sac-like seminal vesicle (Lim, 1996).

6.5.4.iv Diseases caused by *Gyrodactylus* species

The gyrodactylids can be readily differentiated from the dactylogyrids – they are colourless, lack eye spots, viviparous with most specimens carrying a developing embryo within their uterus, epithelial grazers rather than blood feeders, and possess 16 rather than 14 marginal hooks. Mothers give birth to live young which immediately attach to the surface of their host. Following the first birth, gyrodactylids may increase their activity on their hosts, moving to the fin edges where, on close contact with another suitable host, they can loop across. The research findings of Soleng *et al.* (1999) show that gyrodactylids that become detached from their hosts and are drifting in the water column can reattach on contacting a new host. Detached specimens may also attach to the substrate and wait

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until another host passes over making immediate contact. Of the *Gyrodactylus* species infecting warm freshwater fish, some have wide host specificity and can cause fish mortalities in juvenile populations of fish (García-Vásquez *et al.*, 2007, 2011). For example, *Gyrodactylus cichlidarum* is reported from six species of commercially farmed cichlids and from at least 19 countries (Shinn *et al.*, 2022). *Gyrodactylus* and *Macrogyrodactylus* infections are also common on *Clarias* spp. with at least seven species of *Gyrodactylus* and three species of *Macrogyrodactylus* described from *C. gariepinus* (see Paperna, 1973; Přikrylová *et al.*, 2012; Maduenyane *et al.*, 2021).

Pathology

Gyrodactylus spp. are usually found on the skin, fins and gills and commonly in conjunction with protozoan and bacterial infections (Xu *et al.*, 2007; Abdel-Latif and Khafaga, 2020). Mucus secretion is increased during heavy infections, fins become frayed, skin ulcerated, swimming performance is impaired, and gills may be damaged by the feeding and attachment processes of the worm. Fish infected with *Gyrodactylus* exhibit abnormal behaviour, namely rubbing against the net, anorexia, hyperproduction of skin mucus, haemorrhagic ulcers on the body sides, fin rot (mainly anal and caudal fins), and thickening and opacity of the eye cornea. Infections can result in the mass mortality of juvenile fish (García-Vásquez *et al.*, 2007, 2011; Abdel-Latif and Khafaga, 2020).

Diagnosis

Initial diagnosis can be based on clinical signs with confirmation by examination of the parasites. The monogeneans are collected from skin and gill scrapings and prepared as for *Dactylogyrus* spp. (see Section 6.5.4.i above). Two sets of head glands are used as a means of temporary attachment during locomotion. Its haptor is armed with 16 marginal hooks, two anchors and two connective bars, one of which is a simple dorsal bar, the other is a triangular-shaped ventral bar (Fig. 6.8D). There are >400 species of *Gyrodactylus* (see Harris *et al.*, 2004); morphologically, species are separated on subtle differences in the shape of the attachment hooks, particularly the sickles of the marginal hooks (Malmberg, 1970; Gussev, 1985; Shinn *et al.*, 2001; Galli *et al.*, 2010). *Gyrodactylus* spp. in the tropical regions are poorly studied and more investigations are required.

6.5.4.v Other important monogenean infections of cage-culture species

For information regarding infections of *Pseudodactylogyroides marmorata* found on cage-cultured *O. marmorata*, *Sundanonchus* spp. primarily found on *Channa micropeltes* and *Trianchoratus* spp. commonly found on other channids and anabantids, refer to the review of Lio-Po and Lim (2014).

Prevention and control of monogenean infections

The main method for the control of small monogenean species is by a salt or formalin treatment. The recommended doses and concentrations to be used will vary according to host, size, stocking density, existing condition of the gills and parasite species, as well as the physicochemical properties of the water, notably the temperature (Shinn and Bron, 2012). Tancredo *et al.* (2019) recommend a short bath (1 h) using formalin at 75 mg/l, and not exceeding 100 mg/l, for the treatment of *C. carpio* fingerlings (average weight 1.7 g; 4.7 cm total length) infected with *D. minutus* in 28°C water. In all situations where a treatment may be required, it is advisable to seek veterinary advice and support. Eradication of feral reservoir fish from ponds is possible but not when the cages are in rivers or large lakes. The best alternative management strategy includes good husbandry based on knowledge of the reproductive biology and ecological requirements of the parasites such as temperature dependency. Using healthy fish fry from reliable hatcheries, limiting stocking density of fish, providing good-quality feed and sanitation of nets will help to keep infection at a low level.

6.5.5 Diseases caused by other helminths

While many digeneans, nematodes, cestodes and acanthocephalans have been recorded from

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tropical aquaculture species (Paperna, 1996), details regarding the pathogenic species causing disease in cage-culture systems are limited (Lio-Po and Lim, 2014). Important species include infections of the blood fluke *Sanguinicola* which has been recorded on exotic cultured grass carp and bighead carp; the nematodes *Anguillicola crassus* and *Philometroides cyprini* in common carp; the adult Asian tapeworm, *Schizocotyle* (syn. *Bothriocephalus*) *acheilognathi*, which causes mortality in heavily infected grass carp in Europe; and the cestodes *Lytocestus* spp. found in cultured and wild *C. batrachus* and *Senga* spp. found in cultured and wild snakeheads, *Channa* spp. Fish infected with intestinal (adult) cestodes have retarded growth, erratic swimming behaviour and distended abdomen, become emaciated, cease to feed, develop a haemorrhagic enteritis caused by destruction of the intestinal epithelium, and heavily infected fish have varying degrees of aseptic dropsy (Paperna, 1996). A cyclopoid copepod is the intermediate host, and the cestodes could be an important pathogen in cage-culture systems since the fish are in intimate contact with the environment. The helminths infecting a range of farmed tilapia species are provided in the review of Shinn *et al.* (2022).

6.5.6 Diseases caused by parasitic arthropods

Lernaea and *Ergasilus* spp. (Copepoda), *Argulus* (Branchiura) and *Alitropus* (Isopoda) have been recorded on a wide range of cultured fish species (Kabata, 1985; Shariff and Sommerville, 1986). For further details regarding infections on warmwater fish species, pathology and diagnostics, readers are signposted to the reviews provided by Lester and Roubal (1995) and Lio-Po and Lim (2014).

Prevention and control

Many of the treatments that were once commonly used for the treatment of parasitic copepods are no longer appropriate or permitted for use. For cages in rivers or large lake systems, however, the use of chemicals is ineffective and dipping fish in chemicals was not efficient in removing all the copepodid stages (Lester and Roubal, 1995). Raja *et al.* (2022) used emamectin benzoate (EB) given as a dose of 50 µg EB/kg per day for ten consecutive days to control infections of *Lernaea cyprinacea* on caged *Lates calcarifer* and on pond-reared *C. auratus* and *Cyprinus rubrofuscus*.

6.5.6.i *Argulus* infections

Most branchiurans are freshwater parasites with a few estuarine and marine species (Kabata, 1985). *Argulus* or the fish louse (Fig. 6.7I) is macroscopic and easily observable on the fish's exterior or in the oral cavity. These ectoparasitic crustaceans feed on the mucus layer, flesh and blood. The prolonged feeding and strong attachment of *Argulus* by its suckers on to the host result in direct mechanical damage to the skin and disruption of epithelial structure, resulting in lesions and subsequent invasions by opportunistic pathogens. The life cycle of the parasite is direct. Argulid eggs (ovoid, average size 0.2 mm × 0.3 mm) are covered by a gelatinous material and are laid in rows, typically on hard structures with rough rather than smooth surfaces. Development of the eggs is temperature dependent and is *c.*17 days at 23°C (Rahman, 1995). The eggs hatch into free-swimming larvae which must find a host within 2–3 days. Species such as *Argulus japonicus*, *Argulus foliaceus*, *Argulus indicus* and *Argulus siamensis* are economically important parasites of fish in warm freshwater aquaculture (see reviews of Lio-Po and Lim, 2014; Shinn *et al.*, 2022).

Pathology

This parasite is not host specific and is found on a wide range of fish species from cyprinids to siluriforms and perciforms, and infections can result in significant losses (Kumari and Nomani, 2021). Heavily infected fish are lethargic, listless, cease to feed and rub themselves on the substrate to dislodge attached parasites. The parasite is also common in India, affecting the major Indian carp, especially rohu species. *Argulus* feed via a mouth tube, a proboscis, that encloses heavily armed mandibles and two syphons for the release of digestive enzymes. There is also a slender, partially sheathed pre-oral stylet which repeatedly punctures the skin to induce blood

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flow to the feeding site (Kabata, 1970). Products released through the stylet and mouth tube result in the breakdown of host epithelial cells, facilitating blood feeding, which creates a localized inflammatory response with subcutaneous haemorrhaging (Walker *et al.*, 2011). AmbuAli *et al.* (2020) identified 27 proteins within the secretory products that play a role in digestion and immunomodulation. Haemorrhagic responses from adult feeding were observed within 24–48 h post-infection (Walker *et al.*, 2011). The lesion or wound made by the feeding *Argulus* may be restricted to the epidermis or may penetrate through to the stratum spongiosum of the dermis and even the stratum compactum, turning the dermis oedematous (Lester and Roubal, 1995). The area may become necrotic with secondary bacterial and fungal infections. Mortality may be associated with changes in the ionic and osmotic homeostasis, anorexia and secondary infections. *Argulus* feeding on blood causes fish to become anaemic and its piercing proboscis stylet causes haemorrhagic spots on the epidermis. The spots are formed by epidermal hyperplasia. Bacterial infections occur around the site of infection. *Argulus* may also serve as a vector of viral infections, such as spring viraemia of carp (SVC) (Pfeil-Putzien, 1977; Ahne, 1985) and carp pox (carp papilloma) which occurred in conjunction with an infection of *A. japonicus* infection in Israel (Sarig, 1971). *Argulus* also acts as an intermediate host for various skrjabillanid and daniconematid nematodes (Moravec, 1998) and argulids have been implicated in facilitating the establishment of several bacterial infections, for example *A. hydrophila* and *F. columnare* (see Bandilla *et al.*, 2006; Shameena *et al.*, 2021).

Diagnosis

The parasite is oval to round, dorsoventrally flattened (about 4–8 mm in diameter), with a pair of modified sucker-like first maxillae. Its proboscis or feeding organ is for inserting into the epidermis and the underlying tissue of the fish hosts to feed on blood (Fig. 6.7I).

Prevention and control

Historically, a range of noxious chemicals have been recommended for the control of *Argulus* infections; all are now either banned or undesirable (Kabata, 1985; Egusa, 1992). Current chemotherapeutic methods for the control of *Argulus* include the use of EB. Hakalahti *et al.* (2004) used a dose of 50 µg EB/kg per day for seven consecutive days to control infections of *Argulus coregoni* on rainbow trout. Raja *et al.* (2022) applied the same dose for seven to ten consecutive days to control *A. siamensis* on Indian major carps and *L. rohita*, and in removing *Argulus* spp. on caged *P. hypophthalmus* and on pond-reared Indian major carps. The fallowing of sites – that is, the drying of ponds or not stocking ponds until the larval stages have died – is an effective approach in the control of crustacean pathogens. Other non-chemical strategies include the use of removable substrates on to which *Argulus* lay their eggs, the substrates are then periodically removed, rotated, sun-dried or cleaned, and redeployed (Gault *et al.*, 2002).

6.6 Impacts from Climate Change

Since 2000, sea levels have risen by 61.74 mm (Church and White, 2006; Sweet *et al.*, 2022) and Arctic polar ice is decreasing at a rate of 3.5–4.1% per decade (IPCC, 2014). Ocean acidification has seen a mean global pH drop from 8.11 in 1989 to 8.05 in 2020 (Our World in Data, 2022a). Global atmospheric carbon dioxide has risen from 339.6 ppm in 1980 to 418.28 ppm in 2020 (Our World in Data, 2022b); atmospheric methane levels have risen from 1625.9 ppb in 1983 to 1906.8 ppb in 2022 (Our World in Data, 2022c). Global temperatures have risen by 0.354°C over the period 1970–2021; sea temperatures by 0.286°C over the same time frame (Morice *et al.*, 2021). Eckstein *et al.* (2021) predict that the frequency of severe tropical storm events will increase with every 0.1°C rise in global temperature. Changing climatic conditions resulting in flood events, altered patterns of precipitation, salt intrusion, siltation, oxygen depletion, heatwaves, drought, changes to water flow and velocity, can all exert effects with direct impacts on aquaculture production, systems, stocks, and the frequency of disease events. Damage to aquaculture structures can result in the loss of stock. Escaped fish may acquire new infections and, as

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reservoirs of infections, their activity around caged stocks can result in the transfer of infections to caged stock or in extending the window of infection as the treatment of fish falls outside the health management of cages. Rising water temperatures though may have the greatest impact on the frequency and severity of disease events, favouring pathogens like *Streptococcus* spp. which thrive in temperatures above 30°C. Without management interventions, warmer waters lower dissolved oxygen concentrations imposing greater degrees of stress on fish species, some of which may already be farmed at the upper extreme of their temperature tolerance (see Table 6.1). Warmer waters may also result in a general acceleration in parasite development times or may permit certain pathogens/parasites and/or their intermediate hosts to extend their geographic range.

The temporal, decadal change in global surface temperature is presented in Fig. 6.9. When the average climate risk index for each country is considered (i.e. countries most exposed and vulnerable to extreme weather events; Fig. 6.10A) and combined with current aquaculture production (Fig. 6.10B), then analysis reveals that many aquaculture industries within the tropical zone are at most risk (i.e. Myanmar, Haiti, Philippines, Mozambique, Bangladesh, Pakistan, Thailand, Nepal, Madagascar and Vietnam). Safeguarding those aquaculture industries at most risk from climate change will require national coordinated strategic plans of area management to protect water resources and the aquaculture industries within them. At the farm level, this will require a move away from abstracting and discharging waters from common water sources to closed farm systems, with on-site disinfection and recycling of water, as a biosecurity measure, to minimize the likelihood of waterborne pathogens entering farm sites. For cage-culture operations, protecting stocks from climate change and disease threats may lie in genetic breeding programmes selecting for strains that are specific-pathogen resistant (SPR) or specific-pathogen tolerant (SPT), or can tolerate increases in various environmental factors (salt, extremes in temperature, toxic nitrogenous compounds, etc.), or have faster growth reducing the 'in pond' culture time. There also need to be parallel programmes of vaccine development and vaccination against those pathogens where there is currently no protection or for new emerging diseases in warmwater aquaculture. The last decade has also seen radical changes to the chemotherapy arsenal – many of the products that

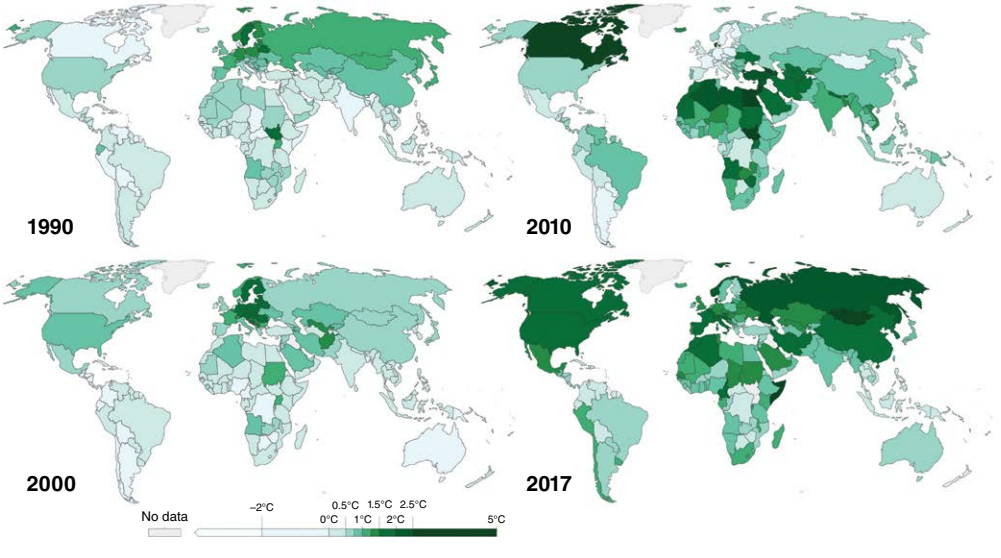


Fig. 6.9. Global surface temperature change, measured in °C, for the last four decades. (Data are based on HadCRUT analysis from the Climatic Research Unit of the University of East Anglia, together with that produced by the Hadley Centre, UK Meteorological Office.)

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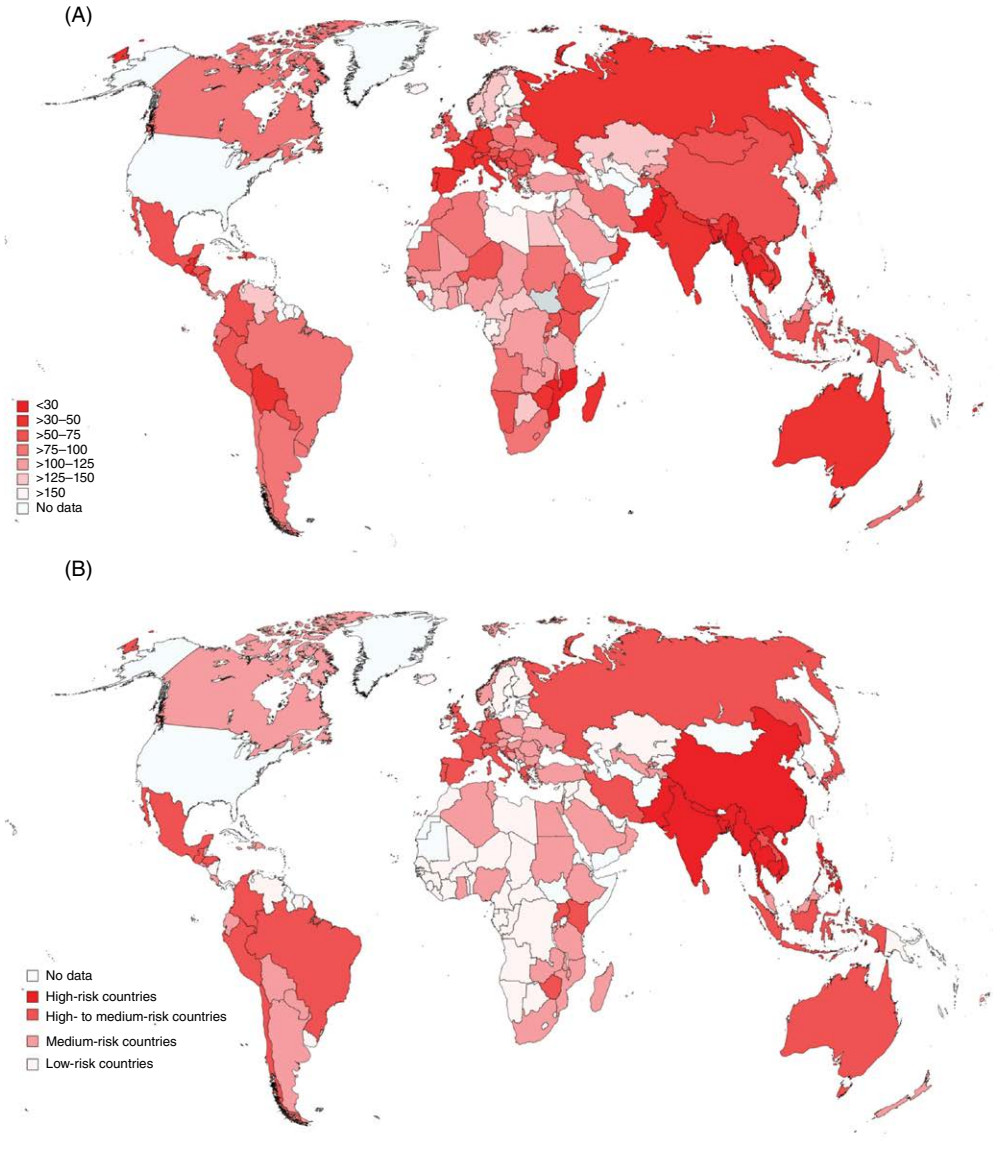


Fig. 6.10. (A) Average climate risk index (CRI) for 2000–2019 using scores calculated by Eckstein *et al*. (2021). Countries are colour-coded according to their exposure and vulnerability to extreme weather events and their capacity to cope with these – countries at the greatest risk are those with low CRI scores. The map indicates that the top ten countries currently at the greatest risk of changes to climatic conditions are Myanmar (most affected), Haiti, Philippines, Mozambique, Bangladesh, Pakistan, Thailand, Nepal, Madagascar and Vietnam. Please note that data for some countries, such as the USA, were not scored by the Eckstein *et al*. (2021) report. (B) Aquaculture-producing countries at most risk from the impacts of climate change. Countries are ranked by their average CRI score (2000–2019) and by the size of national finfish production. (Data on CRI scores are drawn from Eckstein *et al*., 2021; while finfish production data are taken from FishStatJ, 2022.)

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were once commonly used in tropical aquaculture as parasite treatments are no longer permitted or suitable for the treatment of cages and large water bodies. The limited availability of licensed efficacious treatments, therefore, remains a barrier to effective parasite control in aquaculture. While new and alternative regimens are researched, good aquaculture biosecurity and husbandry practices (e.g. stocking of disease-free stocks, recognizing the early signs of disease, conducting regular health surveillance, training in treatment administration) and access to good veterinary/health support can help minimize the frequency and magnitude of disease events.

6.7 Conclusions and Recommendations for Future Research

Microorganisms and parasites are part of a fish's normal flora and fauna and under normal conditions, many of these do not induce disease in their host. Changing environmental conditions from pollution, overstocking and/or climate change may be stressful to fish. Bacterial multiplication, for instance, is enhanced with increasing organic matter from uneaten feeds. Stress predisposes fish to invasion by opportunistic pathogens, with subsequent morbidity and mortality. Stress is also associated with handling, stocking, grading and shipping of fish. Often fish mortality can be attributed to several factors (e.g. fish condition, pathogens, poor husbandry, environment), and frequently it is difficult to determine the significance of any one of these factors (Mitchell, 1997). Some parasites may not result in direct mortality, but they debilitate fish, predisposing them to other pathogens. Parasite activity can create portals of entry for viral, bacterial and oomycete pathogens of fish. Other parasites may serve as reservoirs of viral pathogens. Despite the long history of aquaculture in the tropics and the importance of disease in aquaculture, large knowledge gaps in the health of cage-reared aquaculture species remain. This may be due to lack of trained manpower and institutional support. The usual approach to disease management is to use chemicals (usually indiscriminately) or, when this fails, to discard the fish and start afresh. In many countries there is no mandatory requirement to report fish deaths, and until recently fish were usually imported and stocked without quarantine. With the cross-boundary movement of fish there are risks of pathogen transfer (Hedrick, 1996) and of fish escapes. For commercial species of tilapia, Shinn *et al.* (2022) summarizes >820 translocations and comments on how some have facilitated the spread of parasites into new regions. In this regard, the provisions of the OIE International Aquatic Animal Health Code should be adhered to (OIE, 2021). The lack of institutional support in many countries results in reduced research on pathogens and consequently an inability to control and prevent diseases. There is also a lack of trained personnel in disease management and little reliable information on the specific identity of pathogens. A related issue is the lack of legislation and guidelines pertaining to the use of drugs and chemicals in aquaculture. In many instances, control agents are unregulated, used indiscriminately (usually the aetiological agents are not identified) and without a specific withdrawal period prior to the sale of the fish. Trained competent fish disease managers, who can diagnose pathogens and are capable of dispensing proper prevention and control measures, are important to sustain aquaculture.

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