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Aquatic Invertebrates

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Porifera (sponges)

Coelenterates:

- Cnidarians (Hydrozoa, Scyphozoa/jellyfish, Anthozoa/anemones and corals)
- Mollusca
  - Bivalves (oysters, clams, mussels, scallops, cockles)
  - Cephalopod (Nautiloidea, Coleiodea; Decapodiformes/cuttlefish and squid, Octopodiformes/octopus)

  Gastropods:
  - Prosobranchia (limpets, abalone, cowries, conch, winkles, snails, whelks)
  - Opistobranchia (sea hares, sea slugs)
  - Pulmonata (snails)
- Crustaceans:
  - Shrimp
  - Lobsters
  - Crabs
  - Crayfish
- Echinoderms:
  - Crinoidea (crinoids, sea lilies, feather stars)
  - Asteroidea (sea stars/starfish)
  - Ophiuroidea (brittle stars, basket stars)
  - Echinoidea (sea urchins, sand dollars)
  - Holothuroidea (sea cucumbers)
  - Concentrocycloidea
- Urochordates:
  - Ascidians (sea squirts)
  - Thalaceans (salps, doliolids)
  - Larvacians

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Porifera (Sponges)

Overview

The phylum Porifera is a diverse group of ancient and anatomically undifferentiated animals commonly referred to as sponges. Until the middle of the 18th century, sponges were classified as plants (Ruppert et al., 2004). Sponges occur in the fossil record back to the Precambrian era (over 600 million years ago) and were the most important contributors to reefs during the Paleozoic and Mesozoic eras (Hooper and Van Soest, 2002). All members lack defined organs, with differentiated connective tissue cells performing necessary biologic functions. A unique system of water canals facilitates transport of food, gametes, and waste products. Nearly all species are sessile, and most are marine. Of the approximately 8300 species belonging to over 680 genera, only about 3% occur in freshwater (Hooper and Van Soest, 2002; Ruppert et al., 2004). Sponges are normally found on firm substrates in shallow water, although some occur on soft bottoms, or at great depth.

Despite their being termed "primitive" or "simple" in form and function, Srivastava et al. (2010) discovered that at least one species shares over 70% of its DNA with humans. This finding may lead to further use of sponges in biomedical research, where they are already making important contributions in the areas of embryology, pharmacology, and toxicology. Sponges are highly sensitive to water quality and flow changes, including suspended sediments. Zinc and copper may be toxic to some species. Infectious diseases are not well characterized. Bacterial and algal microbiota, and occasionally protozoa have been implicated.

Clinical Signs

- Sudden death – toxicity, trauma, extreme sedimentation.
- Poor growth – water pollution, sedimentation, inadequate food supply, loss of symbiotic bacteria.

A listing of outbreaks of the major disease syndromes of sponges is given in Table 1.1. A list of the etiology of the major disease syndromes of sponges can be found in Appendix 1.1.

Coelenterates

Taxonomic Overview

This large phylum is made up of the cnidarians, including scyphozoans (jellyfishes), anthozoans (stony corals, soft corals, sea anemones) and hydrozoans (hydras, fire coral, Portuguese Man-o-War) and the ctenophores (comb jellies). This is an economically important group for research, environmental monitoring, public and private display, and tourism. Jellyfish exhibits are now some of the most popular displays in public aquariums and upscale restaurants throughout the world. Coral reefs collectively are one of the most beautiful, diverse, and fragile ecosystems on the planet. Investigations on the diseases of corals are some of the most active areas of research for any aquatic animal group.

Key Points

- Some diseases of hard and soft corals have been well documented, but etiologies are often an enigma. The nomenclature for many of the infectious diseases is in the process of standardization.

Table 1.1 Miscellaneous disease outbreaks in invertebrates – Porifera (sponges).

Outbreak	Species	Ecological description	Microbiological description
Indian Ocean, 1884	*Ircinia*	Unknown	Fungal filaments destroy sponge body leaving a crust of spongin fibers
Florida, 1895	*Commercials*	Loss of commercial sponges	Internal tissue completely eroded
Tunisia, 1906	*Hippospongia equina*	Attributed to shallow water and low water exchange	Dermal cortex covered in white, gray or green liquid
Caribbean, 1938	*Hippospongia*	Heavy mortality	Fungi always present
Florida Keys, 1939	Commercials	70–95% mortality	Unidentified fungal filaments in diseased tissue
Cuba, 1939	Commercials	70–95% mortality	Unidentified fungal filaments in diseased tissue
British Honduras, 1939	Commercials	Unseasonably high temperature and salinity	Unbranched fungal filaments between live and dead tissue
Panama, 1984–1995	*Iotrochota, Amphimedon compressa, Aplysina fulva, Callyspongia vaginalis, Niphates, Xestospongia, Verongula rigida, Ircinia*	Disease symptoms evident over multiple census periods. Cross species contact infections unsuccessful. Excision of diseased tissue often successful as a treatment	Spreading lesions. *I. birotulata*: dulling of surface tissue, tissue loss and white discoloration. *A. compressa*: glossing of surface and advancing narrow brown band. *Ap. fulva*: glossing of surface tissue and a narrow brown band progression, exposure of skeletal fibers
Belize, 1985	*Geodia papyracea*	Mutualism changed to disease in high water temperature	Extensive tissue decay caused by proliferation of cyanobacterial symbiont
Mediterranean, 1986–1990	*Hippospongia, Spongia*	Devastated commercial sponge populations and more prevalent in shallow, warm water. Spread rapidly	Rapidly spreading white spots, brittle skeletons, putrefied tissue, white veil (Oscillatoria?). Unidentified bacteria tunnel through skeleton
Sicily and Ligurian coast, 1986–1995	*Spongia* and *Hippospongia* spp., *Petrosia ficiformis, Ircinia variabilis, Anchinoe paupertas*	Disappearance of *Spongia officinalis* in Marsala Lagoon; 60% of commercial specimens affected in 1987, 5% in 1988 and 20% in 1989. Recolonization by 1995	White patches observed on the surface of affected sponges. Ovoid bacteria filled the canaliculi of exposed skeletal fibers
Libya, 1987	Commercial	Pollution, environmental conditions, and neglect of fishery	Unknown

(*Continued*)

Table 1.1 (Continued)

Outbreak	Species	Ecological description	Microbiological description
North Wales, 1988–1989	*Haliclona oculate, Halichondria panicea*	A number of individuals affected in 1988–1989 but no disease detected since	Brown decaying patches in *H. oculata* (possibly algal overgrowth) and decayed patches in *H. panicea* covered in white bacterial film
Mediterranean, 1994–1996	*Ircinia spinosula, Ircinia*	Massive mortality in 1994 Decay of spongin fibers	No excavation of spongin fibers. Putative agent unknown
Belize, 1996	*Xestospongia muta*	No data	Not performed
Great Britain, 1998	*Rhopaloeides odorabile*	Soft and fragile tissue with portions of pinacoderm eroded away to reveal the skeletal fibers	Bacteria observed burrowing through the spongin fibers. Causative agent identified as an alpha-proteobacterium
Mediterranean, 1994–1996	*Ircinia spinosula, Ircinia*	Massive mortality in 1994 Decay of spongin fibers	No excavation of spongin fibers. Putative agent unknown
Papua New Guinea, 1996–2000	*Ianthella basta*. Diseased specimens of *Jaspis* and *Xestospongia*	Decline in health and abundance of *I. basta*. Brown lesions with rotted tissue, large holes and brown biofilm	Five bacterial strains within the *Bacillus* and *Pseudomonas* genera were strongly correlated with disease
Curacao, 2000	*Xestospongia muta*	White holes with brittle dead tissue around perimeter of lesions	Not performed
Greece, 2001	*Aplysina aerophoba*	Massive necrosis associated with temperature increase. Suspected bacterial pathogen	Not performed
Antarctica, 2002	*Cinachyra antarctica, Inflatella belli, Isodictya setifera*	Discolored and fragile tissue in *I. belli* and *Is. setifera*. Putrefaction of tissue in *C. antarctica*	Not performed
Bahamas, 2004	*Aplysina cauliformis*	*Aplysina* red band syndrome. Rust -colored leading edge, necrosis	Cyanobacteria responsible for coloration but etiological agent uncertain
Florida Keys, 2005	*Xestospongia muta*	Orange band between healthy and bleached tissue	No environmental trigger identified; putative agent unknown
Great Britain, 2006	*Coscinoderma, Rhopaloeides odorabile*	Pinacoderm eroded away to reveal skeletal fibers	Not performed
Mexico, 2004–2005	*Xestospongia muta, Geodia, Ircinia, Xestospongia gigantea, Callyspongia plicifera*	100% of *X. muta* populations affected by disease. White with brittle dead tissue around perimeter of lesions	Not performed
Mediterranean, 2006	*Spongia officinalis* and others	Significant mortality and necrosis	Not performed
Lake Baikal, 2017	11 different	Decreased thriftiness	Not performed

- Many coelenterates, especially the sessile hard and soft corals, are important indicators of ecosystem health and can be sentinels for environmental changes and problems.
- Trauma and "eversion syndrome" are major concerns when keeping captive jellyfish.
- A variety of therapeutic compounds have been used in this phylum, almost exclusively on an empirical basis, and with mixed results.
- Our overall knowledge of coelenterate medicine and surgery is growing steadily.
- Fragging is a term used to describe "surgical propagation" of hard and soft corals using a variety of instruments, adhesives, and substrates. This technique is important for propagation of corals for conservation, scientific study, and captive display.

Clinical Signs

- Jellyfish (scyphozoans): a listing of the etiology of the major disease syndromes of jellyfish and anemones (Actinaria) is found in Appendix 1.1.

Clinical Signs: Corals (Anthozoans)

- Sudden death – toxicity, trauma, predation, extreme water-quality parameters. White film, excessive mucus, when draped over the coral, appears like a white film. White film can be a response to chemical irritation, rough handling, or areas of anaerobic decay.
- Poor growth – inadequate nutritional quality, inadequate food supply, loss of symbiotic bacteria, water pollution, interspecific competition.

Clinical signs for the major disease syndromes of corals are listed in Appendix 1.1.

Diseases of Coral in Cultivation and Aquaria

- Clinical signs – as with naturally occurring reefs; specific lesions due to parasites.
  Major diseases:
  - Trauma – induced by parasitic copepods, flatworms, coral eating fish, fragging, extreme water current. Healing and regeneration are possible if water conditions are stabilized.
  - Toxicity – Heavy metals like copper are known to be toxic to coral (Patel and Bielmyer-Fraser, 2015). Some species are much more susceptible to toxicity, such as *Acropora*. Coral is very sensitive to ammonia. Fluctuations in calcium and alkalinity will kill many stony coral species.
  - Inadequate environment – different species require different amount of current and lighting for optimal health. Generally, soft corals are the most tolerant and small polyp stony corals are the more exigent.

Diseases of Small Polyp Stony Coral

Acropora-Eating Flatworm

Species:	*Amakusaplana acroporae*
Host:	*Acropora* spp.
Diagnostics:	Visual inspection of eggs and feeding mark (the actual flatworm is difficult to see)
Eggs:	Orange-reddish brown
Adult:	Brown, clear brown spots (difficult to see)
Feeding marks:	Small, diffuse, circular, lighter in color

Treatment 1: Flatworm eXit™ (Salifert) Read the treatment sheet for the dosage. There are reports of both success and failure with this product; use at your own risk. Other species of *Acropora*-eating flatworm (AEFW) should be treated the same way.

Treatment 2: Egg Removal The eggs are resistant to treatment and are best manually removed (Figure 1.1). If possible, remove the infected colony from the display and place in a separate container. Generally (AEFW) lay their eggs at the base of or underneath the coral (Figure 1.2). Many egg clusters can be found at the junction of live tissue and coral skeleton. The eggs laid on dead coral skeleton adjacent to live tissue, so once the larva hatch their first meal is nearby (Figures 1.3, 1.4 and 1.5).

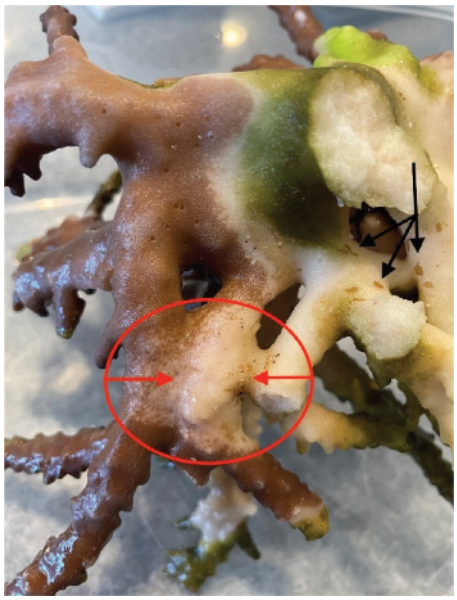


Figure 1.1 Egg clusters (black arrows) and larval migration (red circle) of *Amakusaplana acroporae*.

Figure 1.2 Large quantities of *Amakusaplana acroporae* egg clusters under the branches of a dead *Acropora* species.

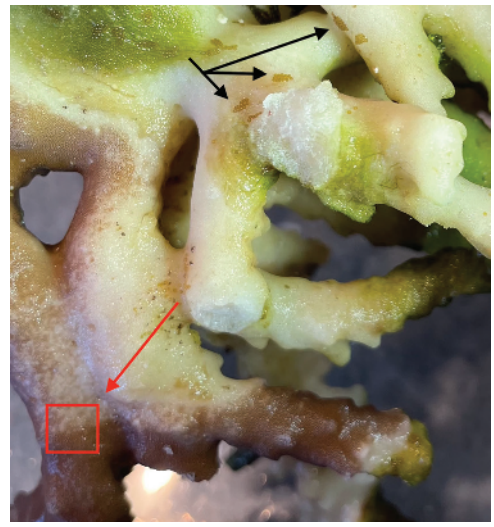


Figure 1.3 Egg clusters (black arrows) of *Amakusaplana acroporae*. Eggs hatch and then the larva migrate to the junction where the dead and alive tissues meet, where they begin to feed (red arrow to box).

Figure 1.4 Circular feeding marks induced by *Acropora*-eating flatworms (circle). It is often easier to see these marks than the adults themselves.

Figure 1.5 *Acropora*-eating flatworms. Arrows: 1 – Adult. 2: Feeding marks. Circle – egg clusters.

Using a toothbrush thoroughly brush the base of the skeleton. Try to remove as many egg capsules as possible. Be careful at the junction between the live tissue and bare skeleton. Make sure this area is thoroughly cleaned. Be gentle, as the toothbrush bristles can irritate the already stressed tissue.

Treatment 3: Biological Control Some wrasses will tend to eat these flatworms like sixline and melanurus wrasses. However, these fish do not eat the eggs; they only eat adult AEFWs. This may be a means to control a chronic AEFW infestation but will never be curative. As a result, the worms may continue to spread with time. There are also reports of blue velvet nudibranch eating these flatworms.

Treatment 4: Dip Treatment New corals should always be dipped before being added to a reef, especially *Acropora*. There are various dips that can be used, such as ReVive Coral Cleaner™ (Two Little Fishies) and iodine, but no firm data on efficacy have been established. These dips are not guaranteed to remove or kill these worms and the coral should always be carefully inspected before addition into the tank.

Parasitic Copepods/Amphipods

Species:	Tegastes acropranus ("red bug") is one of the most common parasites infesting *Acropora* in the home aquarium, as fragments of infected coral can easily be passed from one aquarist to another (Figures 1.6 and 1.7).
Host:	*Acropora* spp.
Diagnostics:	Visual inspection, low magnification
Treatment:	milbemycin oxime (0.1% otic solution)
Dosage:	0.167 µg/l
Duration:	Treat twice weekly for three weeks.
Notes:	0.25-ml tubes can be applied at a rate of 1 tube/1500 l During treatment, carbon and other filtration modalities should be turned off for at least 6 hours. Perform a 20% water change after each treatment.

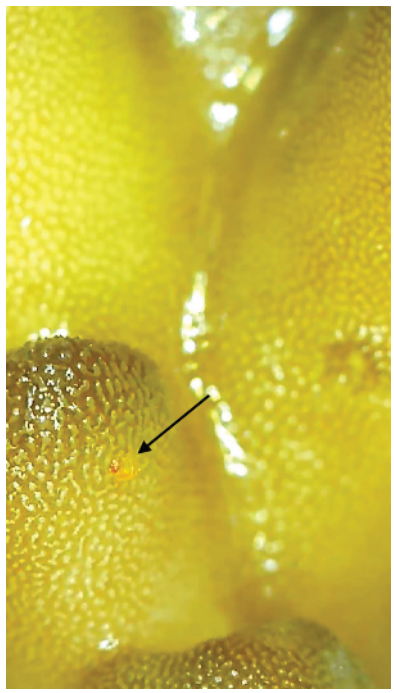


Figure 1.6 *Tegastes acroporanus* on the surface of an Acropora species.

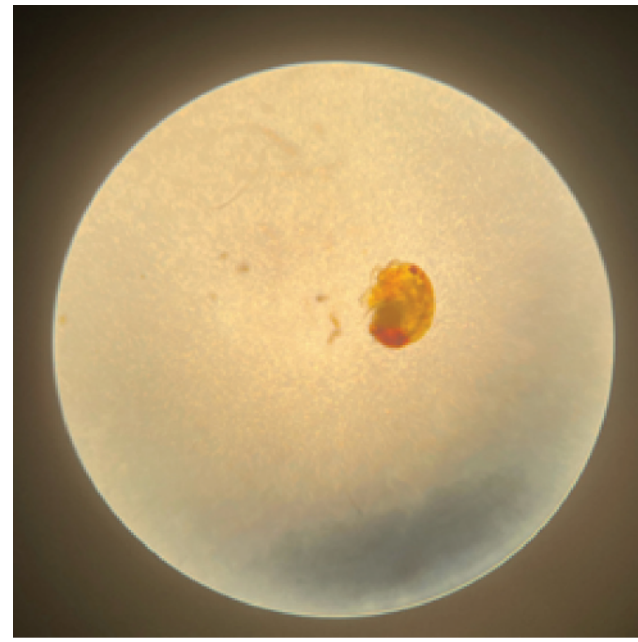


Figure 1.7 *Tegastes acroporanus* (40× magnification).

Milbemycin oxime is toxic to some invertebrates. Certain species of crabs, shrimp, and copepods are extremely susceptible. If the infestation is caught early enough, it is recommended to remove the infected colonies and treat via dip.

For other species of parasitic copepods/amphipod the dose may vary slightly. Published milbemycin oxime dose range is 0.167 μg/l to 0.625mg/l (Christie and Raines, 2016).

Ciliates

Species:	*Heterotrich* (Figures 1.8 and 1.9), *Acropora* ciliates.
Host:	*Acropora* spp.
Diagnostics:	Visual inspection, low magnification
Treatment:	No known treatment.
Notes:	*Halofolliculina corallasia*, a heterotrich ciliate, has been identified as the probable causal agent of skeletal eroding band syndrome (Figure 1.10).

Metronidazole has been reported to kill similar protozoa; however, the coral succumbs to secondary bacterial infection (Sweet et al., 2014). Infected branches should be immediately removed from the tank.

Montipora-Eating Nudibranch

Species:	*Phestilla* (Figure 1.11)
Host:	*Montipora* spp.
Diagnostics:	Visual identification, light microscopy

Treatment 1: Manual Removal The eggs are resistant to treatment and are best manually removed. If possible, remove the infected colony from the display and place in a separate container. Generally, *Phestilla* lay their eggs at the base of or underneath the coral. The eggs laid on dead coral skeleton adjacent to live tissue, so once the larva hatch, they begin feeding nearby.

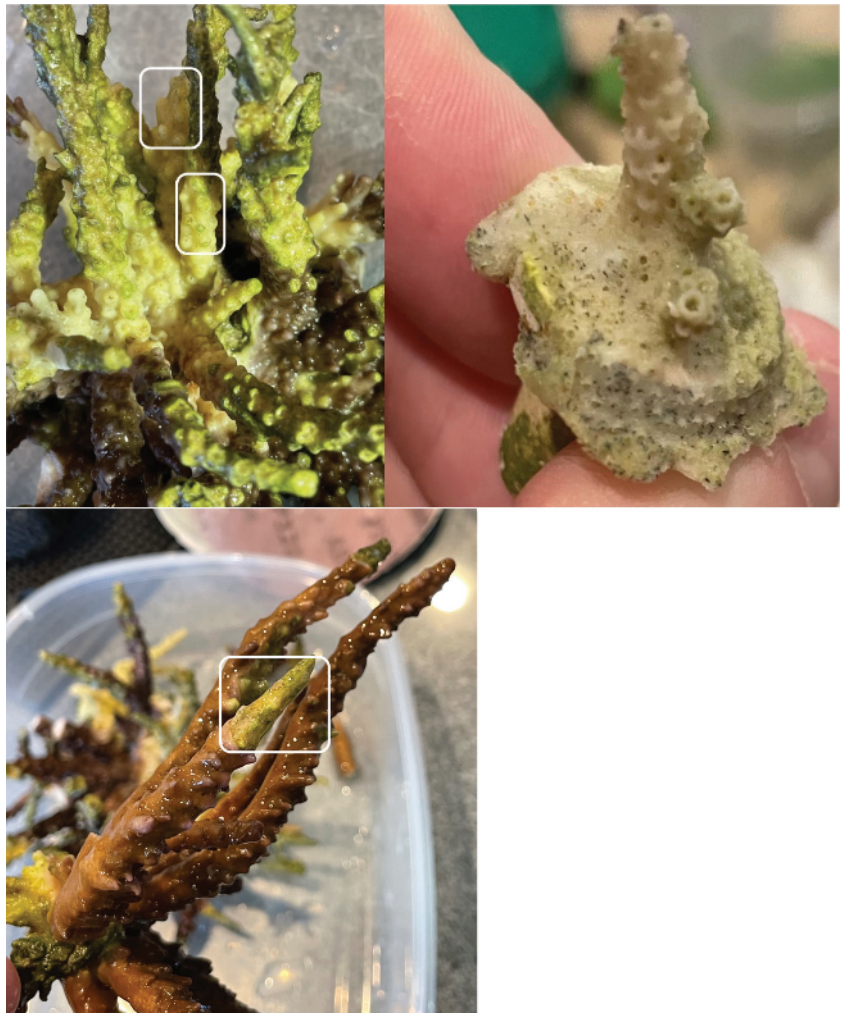


Figure 1.8 Skeletal eroding band syndrome. Note the black/green protozoa embedded in the coral skeleton.

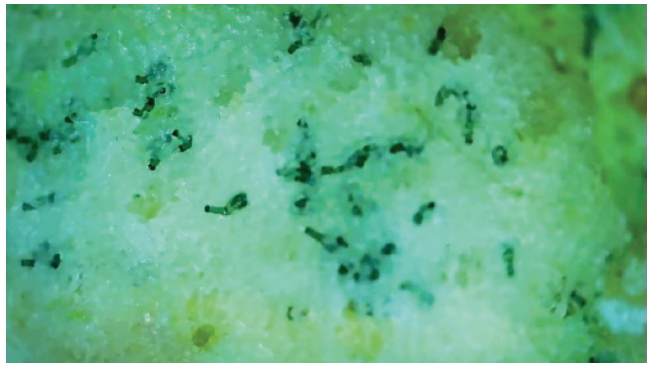


Figure 1.9 *Heterotrich* spp. (2.5× magnification).

If possible, remove the colony and remove all visible adults. If removal is not an option, use a powerhead or turkey baster to blow the adults off to be eaten by predatory wrasses. Consistently removing the adults may help to limit the infestation, but the coral may still struggle to thrive. Chemical treatment is risky as more stress is put on the dying coral.

Treatment 2: Iodine Iodine dips for one hour will kill most adults (but not the eggs).

Treatment 3: Potassium Permanganate Remove the colony and treat in an external tank.

Dosage: 50 mg/l for 30–90 minutes (Borneman, 2007)

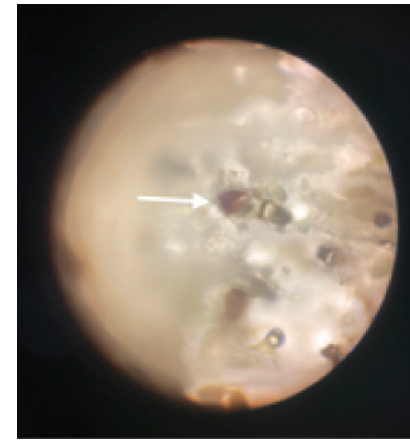


Figure 1.10 *Heterotrich* spp. (100× magnification).

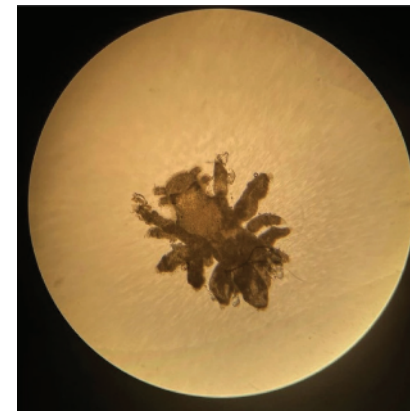


Figure 1.11 *Phestilla* spp. (10× magnification).

Diseases of Large Polyp Stony Corals

Euphyilla Flatworms

Species:	*Polyclad* (Figure 1.12)
Host:	*Euphyilla spp*. (Figure 1.13)
Diagnostics:	Visual inspection, microscopy.
Note:	Once a polyp is infected, it will close with the worms inside, making it nearly impossible to identify the disease while the infected coral is still in the tank. Remove a closed but not a dead polyp, and leave in the open air for around five minutes; the flatworms should begin to come to the surface (Figure 1.14).

Treatment Polyclad species are notoriously hard to treat and the risk of collateral damage is high when treating chemically. While in the tank, the flatworms are resistant to chemical treatment since they are protected inside the closed polyp. If the colony cannot be removed from the tank, using a turkey baster, apply current directly into the center of the closed polyp to dislodge the flatworms. By doing this multiple times a day, a few hours apart, it may be possible to mitigate damage. This method of dislodgement should be applied in combination with all attempted chemical treatments.

Chemical Treatment

- Treatment 1: Several over the counter treatments are available. Efficacy is still debated, and dose unestablished. Treatment may cause stress to small polyp stony species.
- Treatment 2: Levamisole; the optimal dose is unestablished. This treatment should only be used in a separate tank if the coral can be removed from the display.

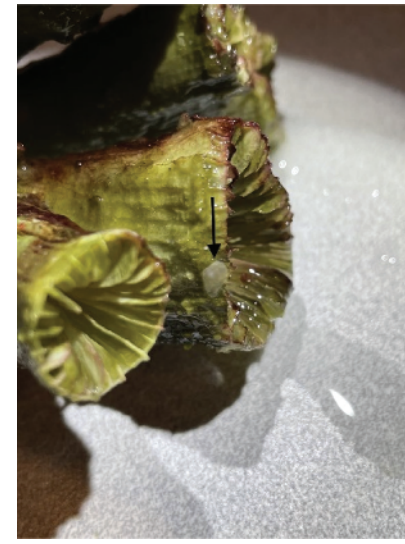


Figure 1.12 *Polyclad* species remaining on a dead *Euphyilla* polyp.

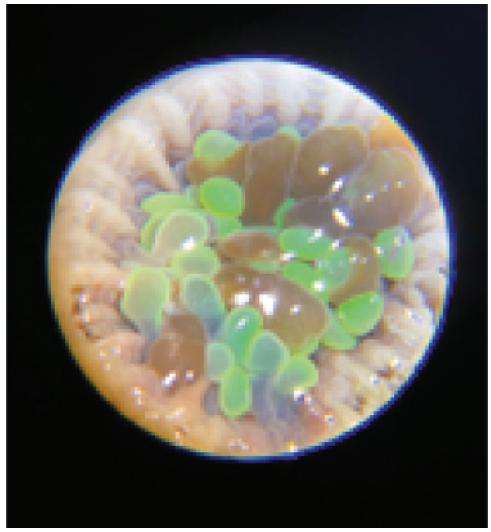


Figure 1.13 Euphilia flatworms under low-level magnification (< 2.5×).

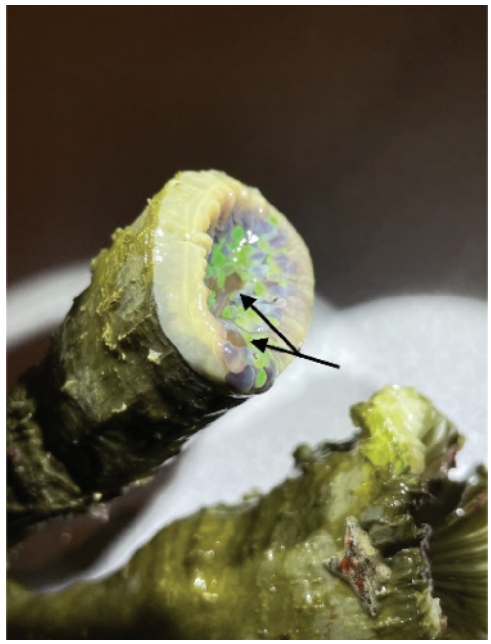


Figure 1.14 *Polyclad* species emerging from an infected polyp after being removed from the water (arrows).

- Treatment 3: Iodine immersion. Place the infected colony in a small bucket of saltwater, apply iodine until a "dark tea" is achieved. Leave the colony submerged for 10–15 minutes. After the bath, in a new bucket containing saltwater only, vigorously shake the colony under the water to dislodge the remaining flatworms.

Dendrophilia-Eating Nudibranch

Species:	*Phestilla melanobrachia*
Host:	*Dendrophylliidae*
Diagnostics:	Visual inspection. These nudibranchs look extremely similar to the coral polyp they consume! Look for horizontal movement of the mimicked "polyp."
Treatment:	Manual removal. They are not very prolific, so it is possible to clear the infection.

Diseases of Zoanthids

Zoanthid-Eating Nudibranch

Species: Unknown
Host: *Zoanthid* spp.
Diagnostics: Visual inspection. Zoanthid-eating nudibranch (Figure 1.15) will glow under blue light, typically green, but other colors are possible.
Treatment: Manual removal of the eggs is necessary as they are resistant to chemical treatment. Some predatory wrasses may consume adults. Use a moderate current created by a power head and systematically blow out the colony to dislodge adults, making them easier for fish to eat.

Zoanthid Sea Spider

Species: *Pycnogonida* (Figure 1.16)
Host: *Zoanthid* spp.
Diagnostics: Visual inspection; look for the legs hanging out from the closed polyp.
Treatment: They must be manually removed with a forceps as they are protected from chemical treatment while they are inside the polyp.

Other Parasites

Red Planaria (*Convolutriloba retrogemma*) (Figure 1.17)

Diagnostics: Visual inspection, low magnification

Pathogenicity The pathogenicity of these worms comes from their ability to proliferate and an internal toxin. These flatworms can proliferate out of control if unregulated, resulting in two problems. The worms do not eat coral but they do irritate the coral tissues, resulting in less polyp

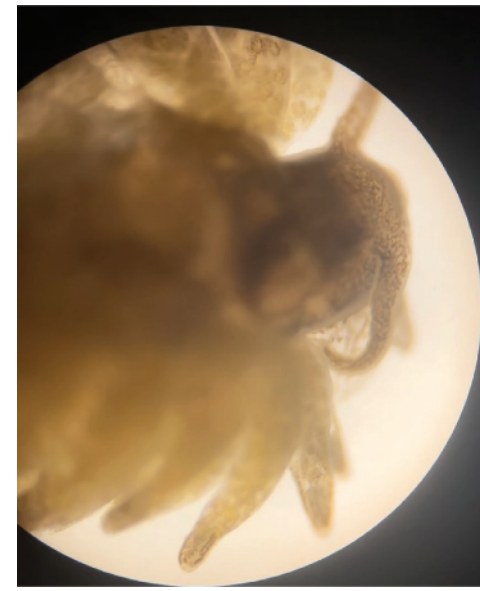


Figure 1.15 Zoanthid-eating nudibranch (10× magnification).

Figure 1.16 Pycnogonida species (10× magnification).

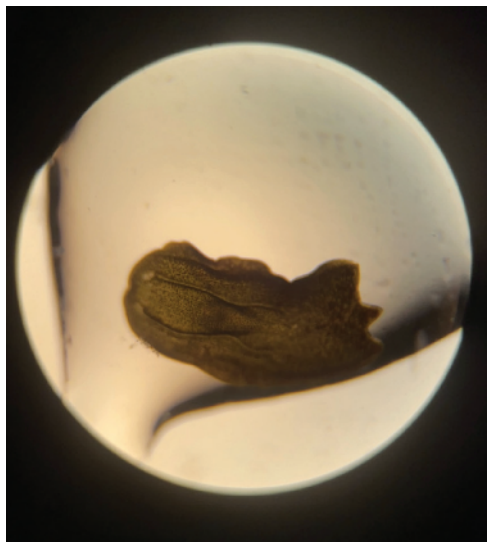


Figure 1.17 *Convolutriloba retrogemma* (10× magnification).

extension and unnecessary stress. If uninterrupted, these flatworms will proliferate until the population is too large for the environment to support them (Figure 1.18). This can result in a mass death of the flatworms and the release of their internal toxin. This toxin in a high enough concentration can cause a tank "crash." To prevent this toxic crash event, if you have red planaria it is imperative to be running fresh activated carbon, which has the ability to absorb the toxin that may be released into the water.

Treatment 1: Manual Removal Manual removal will be one of the biggest components of red planaria management. These flatworms proliferate at a tremendous rate. For the other treatment

Figure 1.18 There is a large amount of planaria around the base of this *Euphyilla*.

modalities to be effective, they must be accompanied by manual removal of the worms. Methods of removal include:

1) Vacuuming the sand bed. These flatworms tend to stay towards the lower portions of the tank and the sand bed. Vacuuming the sand bed can remove a massive quantity of them.
2) Airline syphon. These flatworms have few adhesive properties and are easily knocked off their substrate. A gentle syphon with an airline manually moved over the rocks will easily suck the flatworms out of the tank.
3) Refugium cleaning. These worms can accumulate in massive quantities in a tanks refugium. Freshwater washes of hardy macroalgae can be very effective at flatworm removal.

Treatment 2: Biological Control These flatworms contain an unappetizing toxin, which deters natural predators. Thus, some of the usual flatworm consuming fish tend to ignore this species. Melanarus wrasses (*Halichoeres melanurus*) and sixline wrasses (*Pseudocheilinus hexataenia*) may be able to control minor infestations of red planaria. Alternatively, blue velvet nudibranch (*Chelidonura varians*) have a diet consisting of flatworms of various species.

Treatment 3: Chemical Control (Risky) Treatment of these worms chemically without collateral damage has proven to be challenging. There are several over the counter products labeled to treat these flatworms with uncertain efficacy.

Conclusion: As always, prevention and good biosecurity are always more effective than treatment.

Vermetidae Infestation

Species: *Vermetidae* spp.
Host: Any solid surface
Diagnostics: Visual inspection. Sometimes they are easier to spot after feeding as their mucus nets will be full of particulate.

These snails can grow on any hard surface, generally on the rock or near glass, near but not directly on growing corals (Figure 1.19). These worms release a mucus net to catch food. However, this mucus is extremely irritating to most species of coral, causing prolonged closure. Since these

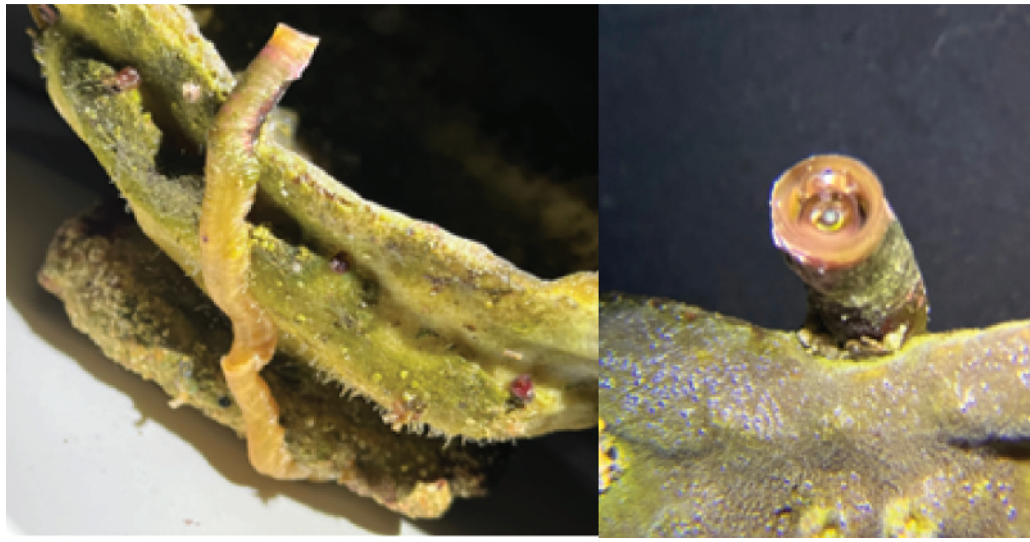


Figure 1.19 Vermentid snails can attach to any hard substrate. In this example, the sessile snail is growing off a Turbinaria species. Make sure to remove the bottom of the snail, as they will survive if only the top is crushed.

worms do not move their location, this persistent mucus will eventually kill nearby corals. Use bone cutters to crush the shell and remove the snail from its substrate.

Class Hydrozoa

Hydrozoans are the hydras, colonial hydroids, and colonial pelagic jellies like *Physalia physalis* (the Portuguese Man-of-War). Little is known about their pathology and diseases.

- Phylum Ctenophora: These are the comb jellies. Little is known about their pathology and diseases.
- Subphylum Urochordata: These are the tunicates (sea squirts) and a few other less well-known taxa (e.g. Salps). There are some economically important diseases that affect this group.

Introduction

The urochordates or tunicates make up an evolutionarily important group. They bridge invertebrates and vertebrates, making them interesting and valuable to researchers in a variety of disciplines. Many research facilities maintain cultures of urochordates or their cell lines. There is also some demand for these animals as human food or display in aquaria. There is relatively little information available on the diseases or treatments of these animals, but a few important disease problems are reviewed here.

Infectious Diseases

Secondary invasion of bacteria occurs as a result of injury or decomposition of dead epibiota. The latter is more common among aquarium animals, a function of deteriorating tissue remaining on the host as opposed to removal by scavengers in nature (Monniot, 1990).

Urochordate large body cavities with extensive water circulation make them good parasitic and commensal hosts, but there are few reports of pathogen-induced morbidity (Monniot, 1990; Moiseeva et al., 2004). The line between organisms being parasitic or commensal is often not clear

in the literature. Metazoan commensals and parasites of urochordates are numerous and varied. Amphipods, annelids, barnacles, bivalves, bryozoans, cephalopods, cnidarians, copepods, ctenophores, decapods, fish, gastropods, isopods, nemerteans, and turbellarians have also been widely identified (Monniot, 1990). Appendix 1.1 lists the major disease syndromes of Urochordata.

Mollusks (Bivalves)

Mollusks represent a large phylum of invertebrates abundant in aquatic environments. Organisms within this grouping are highly diverse, including but not limited to bivalves (hinge-shelled mollusks such as mussels, oysters, scallops, and clams), cephalopods (squids, octopus, cuttlefish) and gastropods (slugs, snails, limpets). Discussion of the structure, life strategies, and diseases of all mollusks is out of the scope of this text. However, an effort has been made to present the basic assessment and differential diagnoses of a number of important aquacultured species from across the world, focused on bivalves.

Wild fisheries of bivalves farm such species as abalone, razor clams, scallops, and geoducks. Also harvested are gastropods such as winkles and whelks, sea urchins, and the cephalopods. In addition to fisheries, bivalves represent important aquaculture species in many countries, and are the focus of much research into their health for optimized production. Although many species are cultured for aquaria or display, this chapter focuses on two commonly cultured mollusks for human consumption: oysters and mussels. Cultured mussel species include blue or edible mussels (*Mytilus edulis*), as well as many other *Mytilus* and *Perna* species. Cultured oysters include the American oyster (*Crassostrea virginica*), European flat oyster (*Ostrea edulis*), and Pacific oysters (*Crassostrea gigas*). Other commercially important bivalves such as blood clams and Palourde clams are not covered here.

Bivalves vary greatly in their anatomy and life strategies. Sessile bivalves like mussels and oysters attach themselves to hard substrate using cement or byssal threads, forming large colonies in tidal or deeper water environments. The life cycles of bivalves vary between species, but a "standard" model involves external fertilization of released eggs, development to free-swimming larvae, and growth to spat and juvenile stages with shell development. Stage of settlement varies with species, but both mussels and oysters require a substrate for growth. Aquaculture of these organisms is then performed in different ways. Mussels produced using aquaculture can be cultured in various systems, including on longline ropes, rafts, or posts (Bouchot), whereas oysters are often grown in container systems. Both freshwater and marine mussel production occurs, although the majority are cultured in the marine environment.

Anatomy and Clinical Features

Shared anatomy of bivalves includes complete enclosure of organisms within the two valves of a shell, hinged by a ligament of elastic protein and held closed by adductor muscles. Food and oxygen are obtained from the aquatic environment when the shell is 'open' via filter feeding across bivalve gills, with inhaled and exhaled products often passed via siphons. Starvation and water quality are therefore important initial avenues of exploration in investigation of apparent pathology in bivalves. As with fish, uniform mortalities are suggestive of an environmental or husbandry issue, whereas less uniform patterns of impact suggest an infectious etiology.

Bivalves are problematic to assess clinically in open water. This is due partially to the presence of some organisms beneath substrate, or in non-tidal zones, but also due to their limited clinical signs. It is possible to assess larval feeding and behavior, however, and the behavior of gaping or

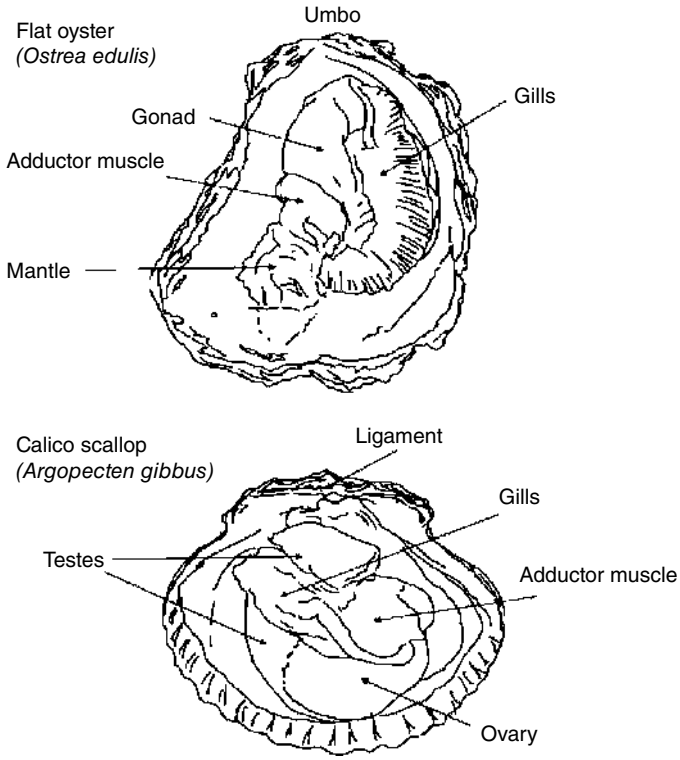


Figure 1.20 Internal anatomy of *Ostrea edulis* and *Argopecten gibbus* immediately on removal of one side shell valve.

delayed closer in adults is a useful indicator of impaired health. Further behavioral indices such as mobility can be assessed for a number of groups; scallops swim, clams burrow, and abalone graze. Signs are unfortunately mainly non-specific, and further investigation is often required.

When assessing the gross anatomy of bivalves, assessment should begin externally with the shell. The shell should then be opened carefully to avoid damaging soft internal structures (Figure 1.20). Visceral organs within can be described as firm/fat, medium, or of poor condition. Poor condition bivalves have limp, watery flesh that sinks away from shell edges and is often translucent in appearance. Mantle retraction is considered a common sign of poor health. Condition can also be quantitively measured using a tissue condition index (dry tissue/shell weight) or shell condition index (dry tissue weigh × 100/shell length). Overall, it should be noted that condition varies seasonally with events such as spawning, but poor conditions can also be a useful indicator of infectious pathology within animals.

Diagnostic interventions in the treatment of bivalves are similar to those available in the veterinary care of fish, including collection of samples for histology, molecular workup, and hemolymph (the bivalve equivalent to blood) for smears. Hemolymph is often obtained from the adductor muscle of bivalves. This can be done destructively or in live animals. Live sampling is performed through insertion of a needle between the shell valve opening, simply performed in some species such as *Mya arenaria* where valves do not tightly oppose. Other bivalves require a small notch to be made in the shell to allow access. Squash or smear preparations of tissue are useful initial diagnostic techniques too, particularly for protozoan organisms. A number of protozoans within

bivalves are identified as notifiable diseases by the World Organisation for Animal Health (WOAH). Histology and molecular diagnostics thus often represent confirmatory diagnosis. When submitting samples for histology or molecular diagnostics, guidance is available from the Food and Agriculture Organization regarding totally sample number requirements to ensure detection of pathogens of different prevalence within a population. Davidson's fixative is recommended for bivalve tissue sections for histology.

For hobbyists and commercial producers, water quality is paramount for optimized bivalve health. Flow dynamics are also crucial for these organisms, in both environmental and recirculating settings. Nitrogenous waste buildup and availability of food are also important points to cover when taking a history for aquaria-reared bivalves. In addition to the infectious and production diseases that can impact bivalves, their method of filter feeding can lead to bioaccumulation of contaminants, algal toxins, and pathogens such as Norovirus or bacteria. If contamination is suspected, specialised sampling must be conducted in line with regional guidelines and legislation to ensure food safety.

Although contaminants such as algal toxins can induce altered gene expression within tissue, clinical indicators of exposure are often not observable. Specialised testing is therefore essential. Infectious diseases of concern for bivalve production largely differ from those of concern from a public health or zoonotic viewpoint. Some indicators used in public health surveillance can be informative though in exploring pathology observed within a population, such as assessment of levels of metallothionein as an indicator of level of heavy metal exposure, or mixed function oxygenase as indicator of organic xenobiotic compound (hydrocarbons, pesticides) exposure.

Clinical Differentials

Clinical presentation is hard to discern for living bivalves beyond determining if the organism is alive, responsive or motile (where relevant). A healthy mussel, for example, will be either closed or the shell partially opened with the siphon extended for feeding. Gently touching an open mussel should lead to the shell closing. A "gaping" shell is suggestive of death, and a delayed closure response might be considered lethargy. The remainder of mollusk anatomy is not externally visible. Assessment of the shell can though provide useful insight into bivalve health. In addition to any visible shell lesions or fouling, appraisal of the shell can give an indication of previous stresses. Expected growth rate of mussels varies between species and with environmental parameters (with selectively bred aquaculture mussels tending to grow faster than their wild counterparts, and slower growth occurring in winter months). With a good understanding of the life history of the bivalve in question, however, growth rings on the shell can be a valuable indicator of patterns of stress or periods of reduced growth. Shell is deposited sequentially throughout the life of bivalves, so a closer pattern of ridges indicates periods of slower growth. A disrupted pattern might indicate a stress event or pathological condition that impaired growth or calcium metabolism. Calcareous shell tissue is also laid down internally to wall off damage or the attempted ingress of parasites to the viscera. These shell nodules are often described as "blisters" and can be mud filled. Generally, however, holes in the shell are of little consequence unless full-thickness perforation has occurred – in which case this can lead to further pathogen ingress. Biofouling on the inner shell can also indicate general weakness of the bivalve.

In addition to the causative agents of clinical signs described below, incidental findings might be reported from diagnostic workups. Ciliates, parasitic copepods, and lipofuscin pigment accumulation (associated with pollution exposure and other stresses, but a common finding in older mussels) are usually of little consequence in health screens. A strong smell when handling freshly obtained bivalves can indicate a soft tissue pathology, is also suggestive of less than fresh samples.

Gaping or moribund bivalves should be avoided in sample collection where possible. One shocking but occasional finding can be the expulsion of red liquid on shucking of a bivalve. This is usually due to release of pigmented algae in seawater from a closed shell. Algal presence is of little concern unless associated with toxic algal blooms.

Specific Diseases

Of the major economically significant diseases in oysters and mussels, many are WOAH notifiable. Detailed description of the diagnosis and control of these conditions and the other notifiable diseases of mollusks are available in the WOAH *Manual of Aquatic Animal Disease*. The conditions of concern for other aquaculture mollusks including diseases of razor clams, scallops, limpets, and other clams are not covered in this chapter, but do present essential reading in understanding the breadth of infectious disease in shellfish.

Reported diseases of importance in abalone include abalone viral ganglioneuritis (abalone herpes virus) and the notifiable condition withering syndrome (*Xenophaliotis californiensis*). Brief details of some of the major pathologies of concern in oysters and mussels are given here; however, this list is not exhaustive, and further reading of texts listed in the bibliography are recommended.

Ostreid Herpes Virus

Brief overview:	Ostreid herpes virus (OsHV-1) is a viral disease with a number of variants identified within a different mollusk species.
Host range and distribution:	It is documented as causing mortalities primarily in the larvae and juvenile life states of *Crassostrea gigas* and *Crassostrea angulate*. Other variants can have different host species.
Transmission:	Transmission is considered to be host to host by shedding from adjacent infected individuals. Latently infected individuals, as well as actively diseased stock, are thought to transmit viral particles.
Signs/diagnosis:	High mortality (up to 100%) is the main indicator of active infection within a population, often occurring during the summer (temperatures exceeding 16°C). A reduction in swimming and feeding behavior of larvae can be seen, whereas adults present as gaping and slow to close when stimulated to do so, however none of these signs are pathognomonic. Definitive diagnosis can only be reached using molecular techniques such as polymerase chain reaction and sequencing.
Differentials:	Diseases that present similarly include other viral pathologies, as well as systemic bacterial infections, and the major protozoan infections of bivalves. Samples should be collected for molecular as a priority, as well as histopathology and electron microscopy.
Treatment:	No treatment is currently available for OsHV-1.
Management:	Biosecurity is paramount. Quarantine procedures and hygiene such as filtration and ultraviolet treatment of water are recommended.

Bonamia

Brief overview:	*Bonamia ostrea* and *Bonamia exitosa* are protozoan parasites within the Haplosporidian phylum that cause lethal disease in bivalve oysters (Figure 1.21).

Host range and distribution: Transmission: *B. ostrea* is known to infect Australian mud oyster (*O. angasi*), Chilean flat oyster (*Ostrea chilensis*), Olympia flat oyster (*Ostrea conchaphila*), Asiatic oyster (*Ostrea denselammellosa*), European flat oyster (*O. edulis*) and Argentinian oysters (*Ostrea puelchana*). *B. exitosa* infects *Ostrea stenina*, European flat oysters, Australian mud oysters, and Chilean flat oysters. Transmission occurs both vertically and horizontally through released infective particles, hypothesized to be via the gills. These are ingested by oysters and infective particles are phagocytosed by hemocytes where they are infective. Older individuals appear more susceptible to disease. *B. ostreae*, *C. gigas*, and *Mytilus edulis* appear resistant to infection.

Signs/diagnosis: Clinical indicators are non-specific. Mass mortalities within a population can be observed, with infection late autumn/winter and deaths peaking in spring of *B. ostrea*. Infected individuals can also be clinically normal. Previously reported gross changes include yellow to black discoloration, lesions such as ulcers in the connective tissues of the gills, mantle, and digestive gland (Comps et al., 1980). These gross signs are not pathognomonic for infection with *B. ostrea* and most infected oysters appear normal. Gill imprints and histology are both useful in diagnosing infection with this parasite (Figure 1.21a). Transmission electron microscopy can help differentiate *Bonamia* organisms. Specific PCR represents an option for confirmatory information, as well as diagnosis from larvae.

Differentials: High mortalities can occur due to a number of infectious and non-infectious challenges to oysters. Tissue ulceration when present is not unique to *B. ostrea* infection, and discoloration can be caused by other protozoal infections, as well as viral, bacterial and fungal infections. Gill erosions can occur as well with OsHV-1 infections.

Treatment: There is currently no treatment available for *B. ostrea* or *B. exitosa* infection in oysters.

Management: General husbandry practices should be implemented to avoid stressing a population.

Perkinsus

Brief overview: *Perkinsus* species (previously referred to as *Dermocystidium*) infect mollusks globally. *Perkinsus olensi marinus* is the causative agent of 'dermo' disease in oysters, and although *P. olensi* infects primarily clams, it is also a concern in oysters.

Host range and distribution: *P. marinus* infects *Crassostrea virginica* (the most susceptible species), *Crassostrea gigas*, *Crassostrea ariakensis*, *Crassostrea rhizophorae*, and *Crassostrea corteziensis* oysters, as well as the mollusks *Mya arenaria* and *Macoma balthica*. Infection can be lethal or may persist throughout life. *P. olseni* is primarily a pathogen of venerid clams, although it does also infect *C. virginica*.

Transmission: All *Perkinsus* life stages are infective and are acquired through horizontal transmission in host feces or disrupted diseased tissue, with uptake by feeding.

Signs/diagnosis: Infection is often eventually lethal. Oysters can be infected for one to two years prior to mortality, with deaths coinciding with high water temperatures. Infection presents as a chronic wasting condition, with *P. martinus* infection leading to pale, poor-condition tissue. Macroscopic nodules in tissue are also reported. Multifocal lesions containing *Perkinsus* cells and infiltrated haemocytes can be observed in histopathology sections, with definitive diagnosis achieved using molecular techniques.

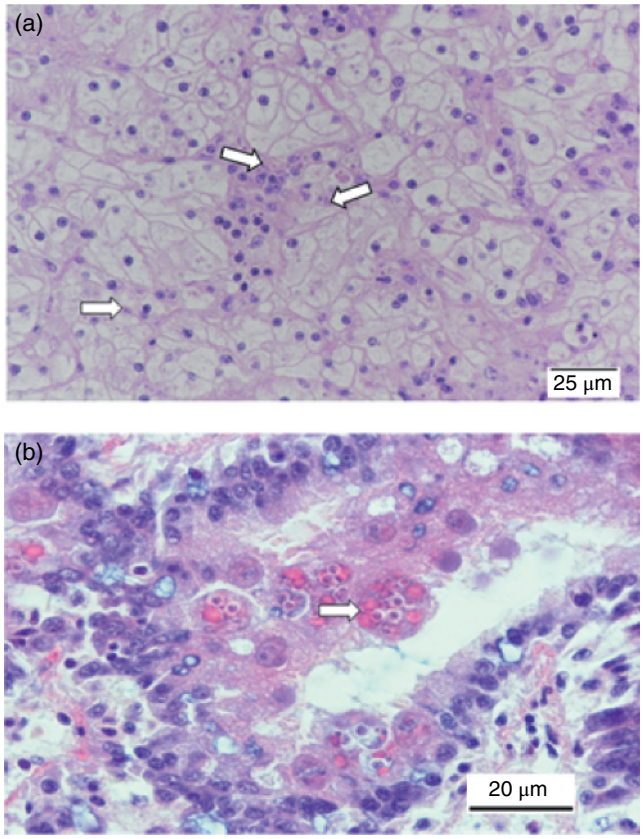


Figure 1.21 *Bonamia ostrea* and *Marteilia refringens* in oyster tissues. Scale bar 25µm. Hematoxylin and eosin stain. a) *B. ostrea* parasites (arrows) within connective tissue cells of European flat oyster *Ostrea edulis*. Scale bar 20µm. Hematoxylin and eosin stain. b) *M. refrigens* parasites within the digestive gland of European flat oyster, *O. edulis*. Note the cell within cell arrangement of the cells (arrow). Scale bar = 20 micrometers/Hematoylin and eosin stain.

Differentials: Similar wasting conditions are reported with viral infections such as hemocyte infection virus, or even seasonal changes associated with spawning or over stocking. Prolonged *Marteilia* infection presents similarly with poor condition and mantle retractions.

Treatment: Bacitracin, cycloheximide and freshwater treatments have been demonstrated to reduce *P. marinus* load in hosts. N-halamine compounds kill *P. marinus* cells without impacting larvae, indicating a use in disinfection of contaminated eggs and larvae.

Marteilia

Brief overview: *Marteilia* parasites are thought to have an indirect life cycle with suggested intermediate hosts including nematodes, copepods and cnidaria. *Marteilia refringens* infects both mussels and oysters as the final host, although geographical distribution is thus far limited mainly to Europe and Northern Africa.

Host range and distribution: Oyster and mussel species in which *Marteilia* have been observed include *Ostrea edulis*, *Crassostrea virginica*, *Mytilus edulis*, *Mytilus galloprovincialis*, *Xenostrobus secures*, *Ostrea chilensis*, *Ostrea puelchana*, *Ostrea angasi*, *Ostrea denselamellosa*, and *Ostrea stentina*. *M. refringens* also impacts the clams *Solen marginatus*, *Chamelea gallina*, *Ruditapes decussatus*, *Ruditapes philippinarum*, *Tapes rhomboides*, *Tapes pullastra*, *Ensis minor*, *Ensis siliqua*, and cockles (*Cerastoderma edule*).

Transmission: Juveniles and adults can become infected by horizontal transmission of shed propagules in feces. The impact on oysters is much greater from this infection, which can last over a lifetime.

Signs/diagnosis: Wild populations of *O. edulis* and mussels can present without obvious clinical impact, but naïve populations can also see very high mortalities. Infection in oysters can also be lethal. Deaths coincide with parasite sporulation in late summer/autumn (water temperature 17°C). Gross changes include pale, poor-condition flesh with watery viscera and mantle retraction. Reduced growth rate is reported in oysters and mussels from impaired uptake ability of nutrition and storage, with impaired gonad development also reported for infected mussels.

Histopathology is considered diagnostic in infected juveniles and adults (Figure 1.21b). Tissue imprints can also provide initial diagnosis, with confirmatory diagnosis as ever achieved using molecular techniques such as PCR and sequencing.

Differentials: Chronic wasting in this condition presents similarly to *Perkinsus* infection, as well as viral conditions and starvation.

Mikrocytos mackini

Brief overview: *Mikrocytos mackini* infection (sometimes referred to as Denman Island disease) is a condition that appears ubiquitous to some regions of Western North America, but is not found and therefore of great importance if isolated elsewhere.

Host range and distribution: *Crassostrea gigas*, *Crassostrea virginica*, *Ostrea edulis* and *Ostrea lurida* oysters are all susceptible to infection.

Transmission: Transmission appears to be horizontal open death of the initial host, and through feeding uptake by the next.

Signs/diagnosis: Infection at cooler temperatures (10°C for 3–4 months) is considered essential for disease development. Mortalities occur therefore in the spring, and infections appear otherwise to be subclinical. Moribund individuals gape and are slow to close. Foci of pustules (up to 5 mm in diameter) are seen in the body wall, adductor or on the mantle and labial palps due to hemocyte accumulation. Pustules typical of this disease are usually green but can range from yellow–brown to colorless.

Differentials: Other conditions can cause high mortality in the spring. Pustules appear similar to conditions such as systemic bacterial disease, *Bonamia* infection and Pacific oyster nocardiosis (*Nocardia crassostreae*).

Treatment: There are no currently available treatments, vaccinations, or decontamination protocols for this pathogen.

Management: New stock should not be seeded at low tide or prior to June. Mature stock should be harvested within three years and before February.

Haplosporidia

Overview:	Haplosporidia are organisms of importance as major marine epizootics not only in bivalves but also crustaceans. They are a concern for both cultured and wild populations, with consequences for aquaculture, food security, and ecosystem health.
Host range:	Research is continuing to characterise the diversity of these organisms. *Haplosporidium nelson* and *Haplosporidium costale* infect Pacific oysters such as *Crassostrea virginica* and *Crassostrea gigas*. Recently reclassified, *Haplosporidium amoricana* infects mussels such as *Ostrea edulis* as well as crab species, and recently described *Haplosporidium pinnae* is linked with disease in *Pinna nobilis* (fan mussel). Parasite abundance is linked with seasonal changes, including warm temperatures and high salinity.
Transmission:	It is suspected that an intermediate host acts in transmission.
Signs/diagnosis:	Infection of *H. nelson* and *H. costale* can manifest as slow growth or deformities within a population, or with altered environmental conditions, mass mortalities can occur. Brown discoloration to the gill and mantle have been previously described for all species. Diagnosis is achieved by histology and molecular techniques.
Differentials:	Brown discoloration presents similarly to changes with bacteria disease, and phaeohyphomycosis. The majority of notifiable pathologies can cause high mortalities, and deformities can be induced by any number of stressed.
Treatment:	There are currently no treatment options available for these organisms.
Management:	Filtration and ultraviolet radiation of influent water in hatcheries seems to be effective in prevention.

Other Pathologies

Although not covered here, a number of important pathologies impact other commercially important bivalves. Resources are provided at the end of the section to assist in understanding the health of these additional species.

In addition to pathologies caused by viruses, bacteria, fungi, and parasites, a number of additional factors can impact bivalve health. Infectious neoplastic conditions of unknown etiology appear important in large-scale mortality events of various oyster and mussel species for example, and bacterial disease are frequently reported, either as systemic infections or due to endotoxic effects. Of major consideration are environmental and non-infectious impacts on health. Temperature, pH, and salinity are important to bivalve growth and survival, as are presence of toxic algae, pollutants, and suspended solids. High inorganic suspended solids can impair feeding in bivalves, and bioaccumulation of aquatic contaminants has been linked to many instances of reduced production and even failure of stocks. Die-off can occur from as simple a reason as altered water parameters, so environmental factors must always be considered when exploring suspected disease in bivalves.

Zoonoses

Handling shellfish often leads to cuts or mild abrasions of the skin because of their sharp shells. Systemic infection leading to sepsis is rare but not unheard of. An important consideration for bivalves and public health specifically is their adaptation for filter feeding, and the potential to

bioaccumulate toxic products of phytoplankton. Shellfish toxins and infectious agents like Norovirus and various bacterial pathogens from bivalves are a food safety concern, causing poisoning in humans. Even with proper cooking, toxic algae and viral pathologies can cause severe disease, and in some extreme cases, death. Care should therefore be taken in harvesting bivalves during seasonal peaks of algal blooms. Commonly held knowledge is that bivalves are unsafe to eat when gaping (which is true); however, those that do not gape can also pose a risk to public health.

Abalone

Abalone are herbivorous marine gastropods. Common species of abalone are listed; those with asterisks are commercially grown in aquaculture: brownlip abalone (*Haliotis conicopora*), greenlip abalone (*Haliotis laevigata*), Roe's abalone (*Haliotis roei*), blacklip abalone (*Haliotis rubra*), "tiger" abalone (hybrid of *H. laevigata* and *H. rubra*), diversicolor or jiukong abalone (*Haliotis diversicolor*). The anatomy and function of abalone are illustrated in Figures 1.22 and 1.23).

Culture Methods

- Land-based raceway: Long, shallow ponds are situated adjacent to the sea for access to seawater. Hides to provide habitats and parallel ridges along the base of tanks are positioned to allow periodic flushing of detritus down the channels. Abalone are fed a pelleted diet.
- Ranch: Habitats made of large concrete blocks anchored to a sandy seabed are placed in the ocean. Abalone stay in their habitats and do not escape since they dislike travelling across the sand substrate. Ocean currents deliver detached seaweed to the hides.

Breeding

Broodstock may be harvested from the wild or are line bred. Spawning is by adding peroxide to the water to simulate stormy weather that creates free radicals in the water. Larvae are grown on plastic sheets with a lawn of algae.

Anesthetics

Various anesthetics have been used for handling, grading, or transferring animals, and all have depressive effects on growth rates. However, benzocaine has the fewest adverse effects.

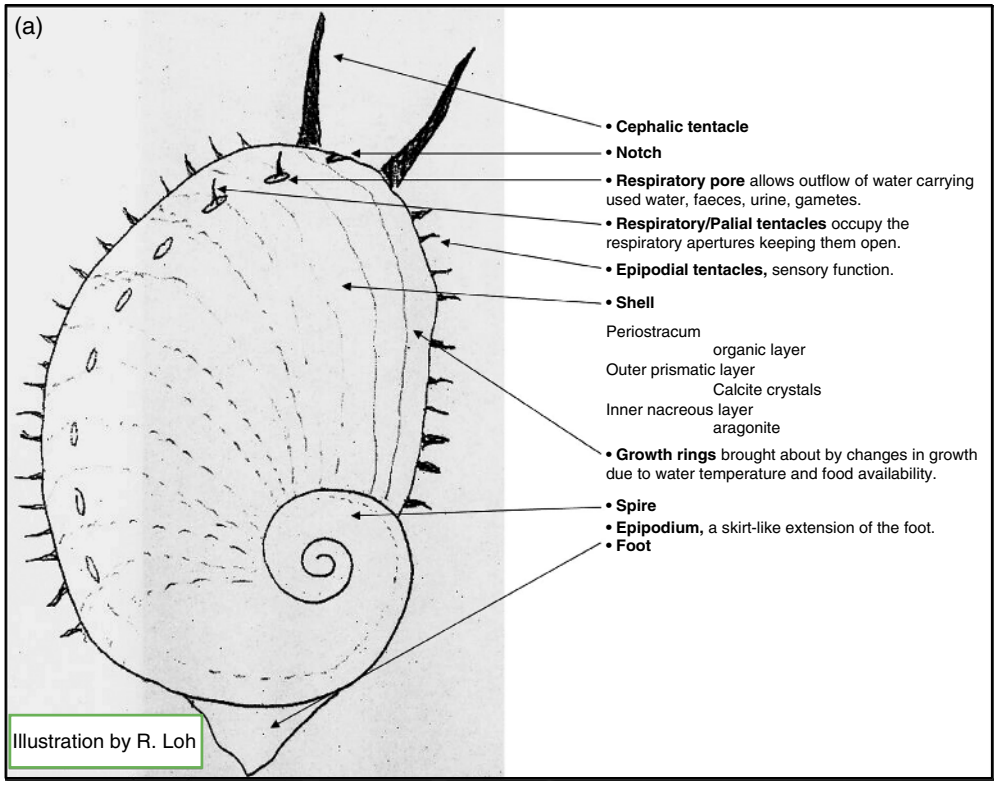
Environmental Requirements

Temperature:

- Greenlip: growth 12–22°C (prefer 18°C)
- Blacklip: growth 10–22°C (prefer 16–18°C)
- Roe's: growth 14–26°C
- Donkey ear: growth 20–32°C (prefer 28°C)
- Diurnal variations may also impact growth rates.

pH:

- Greenlip: 7.78-8.77
- Blacklip: 7.93-8.46
- Hazardous if < 7.16 or > 9.01.



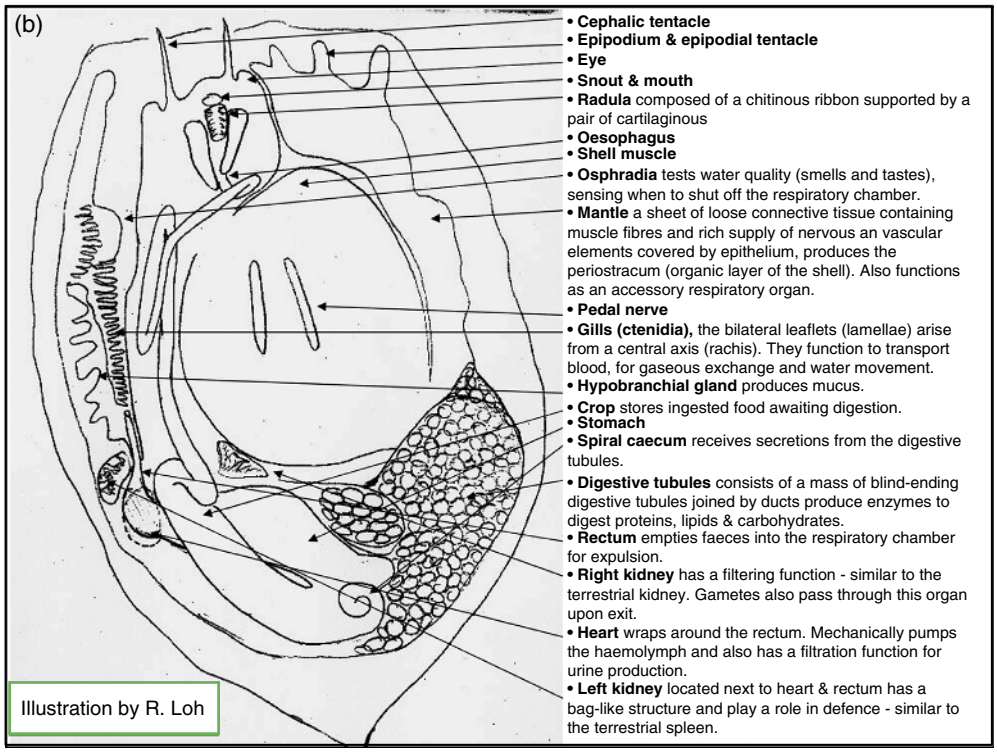


Figure 1.22 Anatomy and function of abalone. a) Dorsal view. b) Internal structure. (Illustrations by R. Loh).

Salinity:

- Optimal: 35 ppt
- Greenlip: 25–40 ppt
- Blacklip: 25–40 ppt
- Abalone do not tolerate freshwater and will develop skin blisters if exposed to raindrops.

Stocking density:

- No more than 40kg/m^3
- Average commercially 20kg/m^3

Ammonia:

- Chronic exposure is detrimental, and its effect depends on the interaction with dissolved oxygen.

Refuge:

- Abalone prefer dim to dark areas with increased activity, including feeding at night. Provide refuges and house in sheds or under thick shade cloth.

Infectious and Parasitic Diseases

Viral Abalone viral ganglioneuritis is a herpesvirus that causes intense hemocytic inflammation of the nervous tissue, causes weakness and paralysis with a rapid and high cumulative mortality of up to 90%. Radula may protrude, but usually, affected abalone are found dead. In wild populations, there may be large numbers of clean (empty) shells on the substrate due to predation or scavenging by other animals of moribund or dead abalone.

Bacterial Commonly *Vibrio* species cause "abscesses" or septicemia. Primary bacterial diseases are associated with *Vibrio splendidus*, *Vibrio alginolyticus, Vibrio parahaemolyticus* and *Vibrio harveyi*. Other bacterial infections tend to be secondary to stress, mainly due to heat stress, handling, and poor water quality.

Withering syndrome is a fatal disease caused by an intracellular *Rickettsia*-like bacterium *Xenohaliotis californiensis*, with upwards of 95% mortalities. It is a WOAH-listed disease. Other kinds of *Rickettsia*-like organisms are considered incidental. Superficial epithelial erosion is associated with *Flavobacterium*-like bacteria.

Fungal *Atkinsiella awabi* causes tubercle disease, with animals dying from rapidly progressing focal foot lesions with extensive inflammation. Histologically, fungal hyphae are poorly staining and are of variable thickness. Shell mycosis creates blisters of conchiolin and nacre on the interior rather than the shell's exterior. This results in loss of value of the shell and can be fatal if it affects the foot muscle attachment.

Protistans *Perkinsus olseni* is the most pathogenic. They are found systemically, incite intense hemocytic reaction and can cause vessel blockage (infarction) and gill necrosis. Other *Perkinsus* species are considered incidental. Detectable by histology, molecular tests, or incubating fresh gill tissues in Ray's thioglycollate broth for detecting Perkinsus infection.

The thraustochytrid *Labyrinthuloides haliotidis* only infects very young abalone when their cellular and humoral defenses are immature. Older animals (over one year) are refractory to becoming parasitized.

Incidental Findings

- Coccidian-like infection of the oesophageal pouch and intestinal mucosa.
- Cryptosporidian-like parasites have been observed in the intestinal epithelium.
- Holotrichous ciliates are found free in the lumen or adherent to the mucosa of the digestive gland, including the oesophageal pouch, in gills, and on the surface of the foot, with no associated host reaction.
- Mantoscyphidian-like ciliates (peritrichous ciliates) may be found attached to the gills, mantle cavity, and esophageal pouch epithelium, or attached to or cover without obvious host response or pathology.
- Intracellular parasites of the digestive gland.
- *Pseudoklossia*-type coccidia in the left kidney.
- *Nematopsis*-like gregarines are found in the interstitial tissue.

Helminths

Spionid Mudworms The polychetes *Boccardia knoxi*, *Boccardia chilensis*, *Polydora hoplura*, *Polydora*, *Polydora websterii*, and *Polydora armata* cause damage to the shells as they bore into the shell of abalone. The internal shell surfaces will have "mud blisters" covered by nacre. This diverts energy demand towards walling off the polychetes and potentially contaminates meat at processing or are unsightly for live trade. Small "chimneys" made by the worms may be observed on the external shell surfaces. The polychetes can be extracted for speciation from abalone shells by placing shells in a 50% alcohol and 50% seawater mix. This method causes the worms to either escape their burrows completely and then die where they can be easily collected, or at least expose themselves out of their burrows where they could be pulled out manually. An interactive key has been constructed (Wilson and McDiarmid 2004) and was used in conjunction with a review of the spionids present in Southern Australia by Blake and Kudenov (1978) to identify the key features of these taxa.

The sabellid polychete *Terebrasabella heterouncinata* causes poor productivity in abalone, and affected animals develop deformed capped-shaped shells. The sabellid larvae only settle on live gastropod shells as they rely on the host reaction to cover the parasite to form their burrows. Burrows appear as pinpoint lesions on the exterior surface from which the brachial crown of the worm protrudes during feeding.

Nematodes *Echinocephalus pseudouncinatus* causes foot muscle weakness and decreases the efficacy of this structure as a hold-fast organ making infected abalone easy to remove from the rock substrate. It is zoonotic, where consumption of live worms can lead to larval migrans. Abalone and sea urchins act as intermediate hosts, with adult worms maturing in elasmobranchs as definitive hosts. Other nematodes are found incidentally reported in the connective tissue near the mouth/esophagus.

Cestodes Plerocercids (the second intermediate host of the cestode) have been reported as incidental findings, with birds likely as the final host. They possibly belong to the genus *Polypocephalus* or *Tylocephalum* in the Family Lecanicephalidea.

Trematodes Metacercarial infections have been reported with little effect on survival. The abalone's gonad appeared to be the initial site of infection, and heavy infections in the gonad may occasionally

cause castration. Imparts an orange discoloration if large numbers are encysted within the gills. Metacercariae may also be found in the right kidney and the esophagus's lamina propria.

Non-Infectious Diseases

Heat stress Abalone from temperate environments are prone to heat stress. The author's laboratory findings for heat-stressed greenlip abalone are summarized below:

- Intravascular glassy hypereosinophilic material (presumed fibrin) in the interstitium between digestive tubules.
- Left kidney fimbrial sinuses were expanded by moderate amounts of eosinophilic fluid (interpreted as hemolymph pooling), and others as hypereosinophilic glassy material (presumed fibrin).
- Gonads were consistent with the end of spawning or the beginning of a new cycle.
- Hemocytosis of the epipodium and mantle were seen in some.
- Reduced mucus cells at the gill tips (C. Hooper, personal communication, 2020).

Miscellaneous Findings

Wild-caught animals carry a range of fauna on their shells, including algae, sponges such as *Cliona* spp., cunjevoi (sea squirt), barnacles, oysters, other mollusks, and other harmless worms (spirorbids and *Pomatoceros* spp.). In the gills of wild abalone, there may be foreign-body type, hemocytic granulomatous reactions related to *Bacillariophyta* diatom algae or sponge spicules.

Sampling for Laboratory Testing

Necropsy A video detailing abalone anatomy and how to safely shuck (remove the abalone from its shell), draw hemolymph (abalone blood) for hemocyte analysis and bacteriology, and sampling organs for histopathology, *Perkinsus* testing and molecular analysis in a necropsy/autopsy by Dr. Loh the Fish Vet is available on YouTube (Biology lesson: Abalone autopsy/necropsy dissection of abalone, preparation for laboratory testing, 2016. https://youtu.be/vnms4gXOKik).

Hemocyte Counts A Neubauer hemocytometer is filled with undiluted hemolymph, and hemocyte counts are carried out immediately on five 0.2 mm squares. The average count is multiplied by a factor of 250 to derive the total chamber count for 1 cubic mm. The mean hemocyte count in greenlip abalone is 7317 cells/mm^3. Abalone infected with hemocyte parasites have lower counts, averaging 5680 cells/mm^3. The author has conducted hemocytes counts in heat-stressed sick animals, finding considerably lower counts at 450–2700 cells/mm^3.

Hemolymph Biochemistry It is thought that marine invertebrates are iso-osmotic with their environment. To investigate this preconception, the author has conducted some biochemistry testing by comparing heat-stressed ($n = 12$), recovered ($n = 15$) and healthy ($n = 10$) greenlip abalone. It is admittedly a small sample size; however, some patterns emerged. Healthy abalone appear able to achieve homeostasis for total protein ($\bar{x} = 4.7$ mmol/l), ammonia ($\bar{x} =$ 71–91 mmol/l), and creatinine ($\bar{x} =$ 108–126 mmol/l). The levels in sick abalone were unchanged for total protein ($\bar{x} = 5.0$ mmol/l) but were elevated for ammonia ($\bar{x}$=130 mmol/l), and reduced for creatinine ($\bar{x}$=97 mmol/l). All abalone appear to maintain above seawater levels for Ca ($\bar{x} =$ 12–13 mmol/l), Mg ($\bar{x} =$ 56–59 mmol/l), Na ($\bar{x} =$ 495–513 mmol/l), Cl ($\bar{x} =$

549–582 mmol/l), and K ($\bar{x}$ = 12.0–13.5 mmol/l). The results for these parameters in sick animals had a downward trend toward seawater levels. The iron level was higher in the recovering animals ($\bar{x}$ = 8.9 mmol/l) compared to the sick ($\bar{x}$ = 5.4 mmol/l) and healthy ($\bar{x}$ = 4.0 mmol/l) animals. Other biochemical parameters tested in the panel, with futile results, included creatine kinase, alanine transaminase, gamma-glutamyl transferase, glutamate dehydrogenase, total bilirubin, conjugated bilirubin, urea, phosphate, β-hydroxybutyric acid, cholesterol, albumin, and albumin/globulin.

Histology Assessment Examine for and quantify epithelial loss from foot, dilation of hemolymph channels, protein deposits in soft tissues or intravascular, pigmentation in the kidney or digestive gland, hemocytic response, necrosis, and infectious agents. A nutritional or general health assessment can be based on the digestive tubule spacing scoring system developed by Dr. Anna Mouton. Any separation of spaces between the digestive tubules is regarded as abnormal and indicates suboptimal conditions. Dilated right kidney tubules indicate poor water quality, overstocking, insufficient water flow, and poor tank hygiene, and also occur with returning abalone to water after handling and transport.

Cephalopods

A listing of clinical signs with differentials of cephalopods can be found in Appendix 1.1.

Gastropods

It is beyond the scope of this text to address all the current knowledge of this group. Some publications on gastropod diseases are included in the bibliography.

Crustacea

A listing of clinical signs with differentials of *Crustacea* is provided in Appendix 1.1.

Overview

The crustaceans are a group of invertebrate organisms containing many commercially important farmed and wild fisheries species. A great number of different aquaculture industries exist, but marine species Pacific white shrimp (*Penaeus vannamei*) and tiger shrimp (*Penaeus monodon*) are the most commonly farmed species, with the remainder of aquaculture crustaceans largely farmed in fresh water. The majority of this aquaculture occurs in Asia, but is also found in Central and South America. Crustacea of note in North America and Europe are largely obtained from fisheries, particularly focused on crabs and lobsters, although an industry for crayfish production does exist.

Each species and method of production has its own set of challenges and common disorders, some beyond the scope of this chapter to describe. Insight into the disease of both cultured and wild fisheries is, however, of importance to aquatic veterinary professionals, and an overview is provided here, with further reading recommended for greater detail. In addition to fisheries and aquaculture for food production, crustaceans are relatively popular in small aquaria, where multiple shrimp species, hermit crabs, and other small crabs are common ornamental additions.

Biology

Crustacea are a diverse subphylum within Arthropoda that includes copepods such as parasitic sea lice and even woodlice. The crustaceans identified as particularly of interest to aquatic veterinary specialists in this section are the decapods: crabs, lobsters, prawns, shrimp, and crayfish. The complex development, varied morphology, and divergent physiology of crustaceans must be appreciated to fully understand the approach to diagnosis and treatment of disease. This section provides a very brief overview of crustacean biology, with additional reading highly recommended. Not all diseases listed are documented as impacting all species within defined groups, so care must also be taken to avoid presumptive diagnosis without further reading.

A large amount of anatomical and physiological features separate crustaceans from fish (Figures 1.23, 1.24 and 1.25). Respiration is still achieved using gills, with similar important functions in osmoregulation and nitrogenous waste excretion. Unlike fish, however, a number of Crustacea species are able to breath atmospheric oxygen. Indeed, species such as hermit crabs are obligate air breathers and can drown. For many other crustaceans, though, provided that the gills are kept moist, respiration can be achieved both above and under water. Instead of blood, crustacean organs are bathed in oxygen-carrying fluid as part of an open circulatory system. The decapods addressed in this chapter use hemocyanin [KB(1)] as their oxygen-carrying pigment, a copper-based protein that is blue when oxygenated. Oxygen is supplied in this manner by the hemolymph plasma, rather than contained within erythrocyte cells. The hemocyanin gives a blue color appearance to the blood analog hemolymph, a color that can be seen externally in some thin-shelled species. When challenged by infectious agents, the repertoire of crustacean immune defenses is divergent from that of vertebrates. Crustaceans are not capable of building adaptive immunity and rely solely on innate responses. These responses are based mainly on the phagocytic

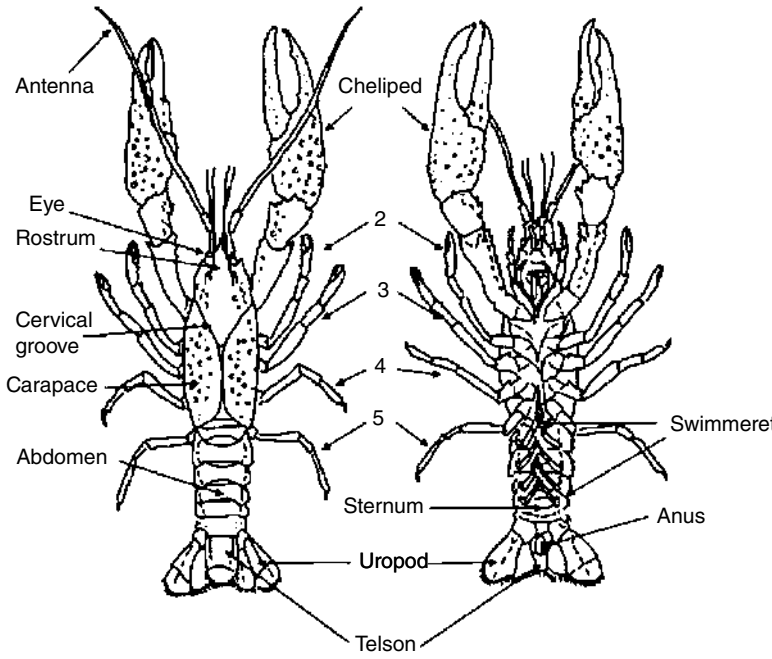


Figure 1.23 General anatomy of a lobster (clawed); dorsal and central views.

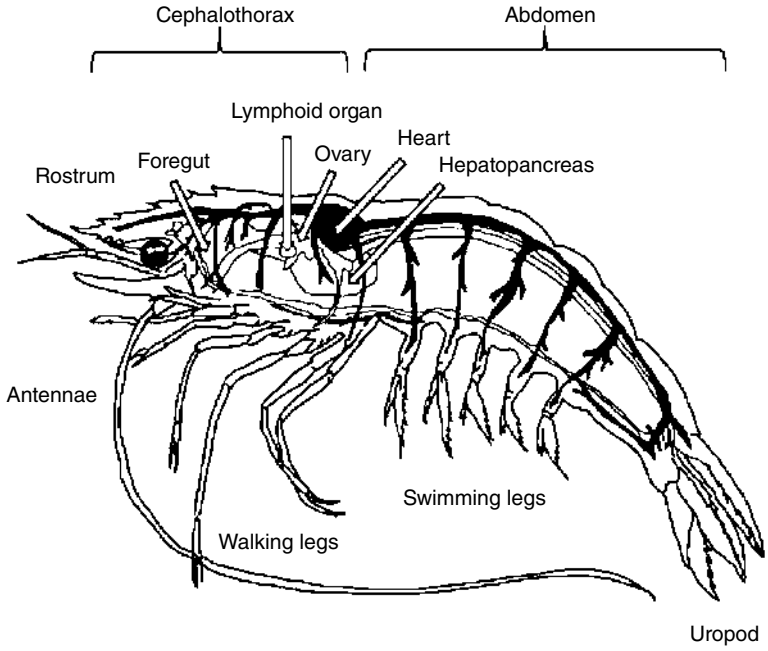


Figure 1.24 The internal anatomy of shrimp and prawns can, to an extent, be observed during the clinical examination. The majority of organs (anterior ganglion, brain, heart, gonads, foregut, and hepatopancreas) are located in the cephalothorax.

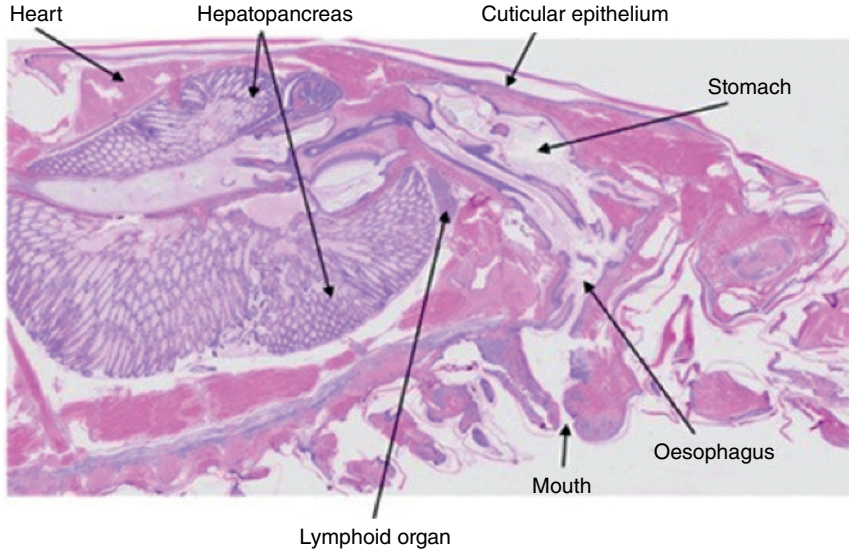


Figure 1.25 Penaeid shrimp cross-sectional anatomy.

and reactive oxygen species production capacity of immunologically active hemocytes as part of their alternative to blood, hemolymph, as well as antimicrobial peptides. Encapsulation and melanization of localized insults as "nodules" is considered an important aspect of host defense.

Individuals are usually of separate sexes, producing eggs that are either laid into the water column or remain attached to the female until they are ready to hatch. Larval development

progresses through various stages of molting until adult morphology is achieved. Adult decapods are protected by an exoskeleton, which might include a carapace on the cranial aspect, the site of much externally observable pathology. The exoskeleton arrangement depends on the form and segmentation of the crustacean in question, with variation in antennae and appendage numbers.

Crustaceans shed their shells in a process called molting, which allows an increase in body size. This process occurs most frequently in larvae, but even adults molt [KB(2)], albeit less frequently. A new exoskeleton is laid down under the old, and regeneration of any lost limbs begins. The appearance and structure of various structures alters during this process, and this must be considered by clinicians when assessing animals for disease. The outer shell demineralizes and blood is withdrawn from appendages, which shrivel, grow soft, and take on a pale, almost blue appearance. Other color and externally apparent changes are documented and vary with the species in question. Many crustaceans cannot take on water for respiration during the metabolically expensive process of molting, and so if unnaturally prolonged, the individual can die. Immediately after molting, the flesh of crustacean is of lower quality and altered in appearance. Most fishery regulators do not allow the harvest of 'soft shelled' (post-molt) crustaceans such as crabs and lobsters. This is a stage of particular susceptibility to trauma and disease, and also easily confused with infectious pathology.

Reproduction occurs in crustaceans generally as an annual cycle. Crustaceans feed on a broad range of substrates, including small vertebrates, plankton, mollusks, other crustaceans, vegetation, and detritus. Females will, however, often not feed during the brooding period, depleting their body reserves. This impacts brown and white meat quality. Male and female individuals can be differentiated in different ways. For shrimp, males have two petasma on their first pleopods; small appendages for attaching onto females. Female shrimp do not have these. Differentiating using this method is similar in crayfish and lobsters, although species differences account for a lot of variation. There is a lot of information online regarding differentiating sex using other means, but this presence of a calcified appendage extension is the most reliable in these animals. Crab sex is differentiated by the shape of their apron (Figure 1.26).

Aquaculture and Economic Significance

Of the many varied aquacultured crustaceans, shrimp and prawns represent the largest combined industries. Although the major aquacultured shrimp and prawn species are tiger shrimp and Pacific white shrimp, also of note are other *Penaeus* species, such as the banana prawn

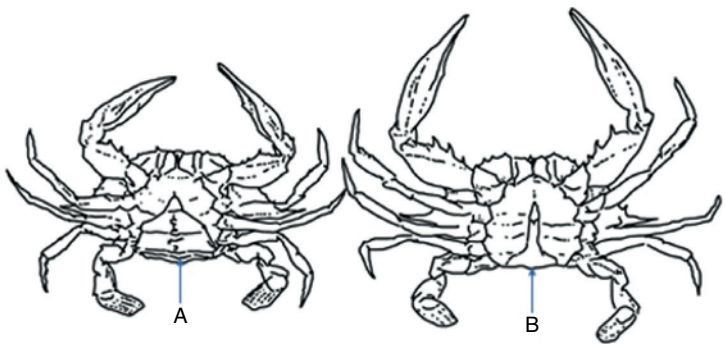


Figure 1.26 Male and female crabs can be differentiated by their external anatomy. In some species, the claws are a different color between males and females. Apron shape is also different between the sexes. Mature females (sooks) have a rounded apron (A) and males (jimmies) have a long, pointy apron (B). The apron of immature females can be more triangular.

(*Fenneropenaeus indicus*), kuruma prawn (*Marsupenaeus japonicus*) and fleshy shrimp (*Fenneropenaeus chinensis*). The majority of shrimp and prawn aquaculture occurs in Asia, with tiger shrimp and Pacific white shrimp being marine species. Aquaculture production of freshwater species is primarily of species such as the giant river prawn (*Macrobrachium rosenbergii*), crayfish, and freshwater crabs. The terms shrimp and prawn are often used interchangeably, despite taxonomic and morphological differences. A simple rule of thumb is that prawns have a "bend" to their body and release their eggs, whereas shrimp have a more linear form, and brood eggs on their underside.

Production of shrimp and prawns generally occurs in earthen pond systems, with a fresh or saline environmental water source depending on species adaptations. Although production times are short and profits can be very high, these organisms can be highly sensitive to environmental changes and stress. Infectious outbreaks can also be devastating, and a number of notifiable diseases with impact on production are detailed at the end of this section. Increasing use of recirculating aquaculture systems reduces the risk of disease outbreaks, although these come with their own suite of challenges. Large-scale wild fisheries of shrimp and prawns can have issues of sustainability owing to the focus on the use of trawling for capture, and although aquaculture removes some of the issues of seasonality in production and exploited fisheries, aquaculture has a number of issues too. Challenges such as sustainability, pollution and biosecurity all remain to the fully addressed.

The farming of a number of crab species such as *Eriochier sinensis* (Chinese mitten crab) and *Scylla* species occurs in Asia, but many others are harvested as part of fisheries. Commercially important crabs to Pacific fisheries include the horse crab (*Portunus trituberculatus*), flower crab (*Portunus pelagicus*), blue crab (*Callinectes sapidus*), mangrove mud crab (*Scylla serrata*) and *Charybdis* species. The edible or brown crab (*Cancer pagurus*) is an important catch species in the Atlantic, and several additional genera are important to northern fisheries, including genus *Chionoecetes* (snow crabs), *Metacarcinus magister* (Dungeness crab) and *Paralithodes camtschaticus* (king crab), [KB(3)].

Lobsters are a high value crustacean group, with varied taxonomy of members. Edible lobsters include the spiny lobster species (clawless species of genus *Panulirus* with robust antennae) and a group referred to as clawed lobsters that includes commercially important *Homarus* species. Spiny lobsters represent the only major aquacultured lobster species, farmed primarily in Vietnam, where wild-caught juveniles are raised to harvest. Culture efforts are currently being pursued, although a protracted larval phase limits entirely aquacultured individuals. European lobsters (*Homarus gammarus*) are generally dark blue in color (turning red only with cooking). American lobster (*Homarus americanus*) are generally brown/orange in color and can grow very large. Both species are found in the North Atlantic ocean. Closely related *Metanephrops*, also clawed, are commonly known as scampi.

Crayfish are an important aquacultured species in the Americas and Oceania and Asia. Red clawed crayfish (*Cherax quadricarinatus*) are farmed in Central America and Australia, whereas procambarid crayfishes, also known as 'crawdads', such as *Procambarus clarkia* (red swamp cray) and *Procambarus zonangulus* (white river crayfish), are commercially important to the Southern United States. Certain species of crayfish can be particularly invasive, and although desirable for harvest in their native regions, are considered pests elsewhere. Aquaculture production methodologies vary between species, but in general are less intensive than shrimp and prawn production [KB(4)]. *C. quadricarinatus* production can occur directly in ponds, without hatcheries, and omnivorous crayfish graze at night from the pond bottom without the requirement for high rate feeding of other aquaculture-farmed crustaceans. Important wild fisheries include those in Scandinavia and China.

Aquaria

Crustaceans such as shrimp make attractive editions to both freshwater and marine aquaria, and thus are popular amongst hobbyists. A huge variety of species are cultured for display, including *Neocaridina* and *Palaemonetes* commonly seen in freshwater tanks, Mantis shrimp (order Stomatopoda) in marine displays, and even microscopic daphnia. Different species have different environmental requirements that cannot be covered within the scope of this section. However, many clinical symptoms are shared across species, and similar principles can be applied in assessment of both aquacultured and home aquaria crustaceans. Further reading is required for specific species.

Disease and Diagnostics

Many infectious and environmental challenges exist to aquaculture production of crustaceans. Conditions impacting wild individuals are of importance to the aquatic health professional. For example, crustaceans obtained from fisheries are often transported live, and risk the spread of any pathogens they host not only to naïve wild populations, but also the geographical area of important aquacultured stock. Infections are potentially devastating in both commercially important wild populations and cultured stocks [KB(5)]. Many of the pathologies associated with high mortalities in aquaculture production require sophisticated diagnostics for definitive diagnosis. The clinical changes of crustaceans, although more varied than that of bivalves, are still fairly limited in their specificity to any given condition. Cageside diagnostic options include assessment of the clotting reaction of hemolymph, consistently delayed or even absent in many infections of shrimp, and non-destructive sampling for assessment of tissue squash preparations. Small sections of gill or pleopod connective tissue can allow observation of various indicators of disease, including presence of parasitic organisms, cellular changes such as hypertrophy of hemocytes or refractive aggregates of specific pathogens.

An appreciation of normal presentation for specific species is essential for performing accurate clinical assessments. For example, observation of reddening is a common feature of many pathological conditions. This color change occurs due to chromatophore expansion with carotenoid pigments (distributed throughout the body by hemolymph from the hepatopancreas) as part of necrosis. But reddening can also be a normal color change in lobsters due to seasonality, and in shrimp spawning, where broodstock can often be redder in color than expected (thought to be linked to a carotenoid-rich diet). Female lobsters can also take on a green coloration as part of reproductive cycling, where egg development gives the tail a green appearance. Diet can also give individuals a green appearance. This color can be striking, and easily mistaken for pathology.

Clinical assessment of crustaceans should proceed similarly to other aquatic organisms. An initial external assessment might include the notation of damage or loss to the eyes, antennae, pleopods (legs), and europod (tail), as well as appraisal of the gill tissue beneath the operculum. Identification of shell legions, testing for exoskeleton consistency and any discoloration can also be assessed. Owing to the more translucent nature of shrimp and prawn shells, the external examination of these animals can include assessment of internal body features. Stomach and intestinal fill, muscular coloration, and the appearance of the hepatopancreas can all be assessed. Noting deformities or deviation within a population provides essential initial information in building a clinical picture. Molt stage, sex, and carapace length can all be important information, and should be recorded.

Many of the pathologies associated with high mortalities in aquaculture production can be identified using sophisticated diagnostics. However, there is also a varied suite of initial investigations that can be explored. The clinical changes of crustaceans, although fairly limited in their

specificity to any given condition, can be highly informative. Cageside diagnostic options include assessment of the clotting reaction of hemolymph, consistently delayed or even absent in many infections, and non-destructive sampling for assessment of tissue squash preparations. Appraisal of small sections of gill or pleopod connective tissue can allow observation of various indicators of disease, including presence of parasitic organisms, cellular changes such as hypertrophy of hemocytes, or refractive aggregates of specific pathogens. Microscopic assessment of pleopods involves removing a pleopod for examination with a compound microscope. This can be used to diagnose and even stage *Hematodinium* infections. Hemolymph smears stained with neutral red provides an easy method for observing pathogens and can always be photographed to share with colleagues if a clinician lacks experience in pathogen identification (Figure 1.27). Hemolymph smears, either fresh or stained, can demonstrate parasites and bacteria. If examination cannot be performed immediately, clinicians should avoid air drying smears to reduce artefacts. Even without microscopy however, assessment of the opacity or clotting ability of hemolymph can be informative.

The preferred method of euthanasia of lobsters and crabs [KB(6)] is through electrical stunning. Many other methods have been described (including boiling, freezing, and pithing), however electrical stunning is the only method that results in rapid loss of consciousness due to the decentralized nervous system of crustaceans. A number of water-soluble anesthetics have also been demonstrated as effective in multiple crustaceans, and might be used for sedation and euthanasia.

When performing disease diagnostics, moribund individuals should be selected for sampling, particularly for bacteriology and histopathology. In some suspected viral pathologies, however, hemolymph should be collected from clinically normal individuals cohabiting with moribund hosts, as hemocytes are depleted in later-stage infections. For the majority of conditions, juveniles and subadults provide the best samples for surveillance, although exceptions apply. Greater detail regarding sampling technique is available within WOAH disease guides.

Sampling should be performed as soon as possible following live transport of animals to avoid physiological stress. To avoid cannibalism for species such as crabs, holding individuals of varied size is not recommended. Small tissue sections of recently euthanized crustacean gill and hepatopancreas may be used in squash preparations and impression smears. The use of Davidson's fixative is recommended for routine histological studies of crustaceans. The formulation of the fixative should depend on the species to be studied, and the environment it resides in (freshwater or marine). A typical formulation of Davidson's fixative is 33% ethyl alcohol [95%], 22% formalin [approximately 37% formaldehyde], 11.5% glacial acetic acid and 33.5% distilled or tap water. Small individuals can be fixed whole following impregnation with fixative; approximately 5–10% of the body weight of the individual should be injected directly to the hepatopancreas and adjacent regions of the cephalothorax and abdomen. The cuticle should then be opened just lateral to the dorsal midline from the sixth

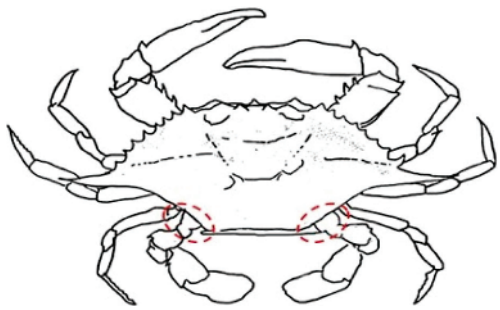


Figure 1.27 Hemolymph can be obtained via arthrodial membranes of the legs or the junction of the carapace and abdomen. Recommended sampling sites vary between species, but a common prebranchial site is from the base of the fifth leg. An appropriate gauge needle might be 27 gauge in crustaceans with a carapace greater than 15 mm, with small gauges for smaller individuals.

segment to rostrum base, to enhance penetration. Larger crustaceans such as lobsters, crabs and crayfish should be dissected, with tissues placed inside cassette(s) for fixation. Fixation should not exceed 48 hours, after which transfer to 70% ethyl alcohol for storage is necessary.

Clinical Signs

The research of pathogens of crustaceans is an emerging field, in which great progress has been made in recent years, particularly for those species of importance to aquaculture. There exists, however, a multitude of potentially pathogenic agents recently described, for which the gross clinical signs of infection are not yet fully understood.

Behavior Behavior can be assessed for animals within the pond, but by removing a subset of organisms to a bucket or bowl, observation becomes easier, particularly for smaller animals. Lethargy and altered feeding are particularly important clinical signs for most of the major diseases. Shrimp and prawns will also aggregate at the pond side in respiratory distress with many conditions, a behavioral change that might be first noticed due to the increased presence of predators that this attracts. Reduction or loss of the "tail flick" escape response can be an indication of continuing pathology and lethargy in some crustaceans. Poor coordination and collisions can also be helpful in diagnosis, often suggestive of blindness. Trying to escape, thrashing, spasms, and autotomy (limb shedding) can all be considered signs of distress in crabs and lobsters, sometimes due to as simple a reason as handling. Healthy lobsters can even be seen to avoid others within a cohort infected with disease (such as in the case of *Panulirus argus* virus 1, PaV1, in spiny lobsters).

Environmental Parameters Environmental changes can be an important cause of reported pathology within the *Crustacea*, and readings or assessment of the environment should be factored into any investigative approach. Algae, temperature, salinity, oxygen, turbidity, pH, and predator activity are just a few of the many changes that might be linked either directly or indirectly to apparent pathology in crustaceans. Recent run-off from nearby fields can be an important element of case history in pond-reared crustaceans, whereas fungal infections are considered more likely in recirculating systems, with high temperatures and high levels of nutrients within the water.

Shell Changes Shell changes are the most obvious clinical change that can be observed in crustaceans. Although the thin, semi-opaque shells of prawns and shrimp can allow visualization of organs within, shells are made of a very hard substance called chitin. As the exoskeleton is quite hard, many shell changes of thicker shelled crustaceans, such as lobsters and crabs, can be non-lethal. These changes can lead to rejection for sale of individuals on aesthetic grounds.

Environmental conditions are important factors in shell disease; ocean acidification, rough handling, high temperatures and poor water quality are considered predisposing to shell weakness across the decapod group. Crustacea are considered to be most vulnerable to shell lesions immediately after molting, when the new larger shell is potentially thinner and weaker due to relative mineral deficiency (calcium, phosphate and magnesium are all important dietary minerals for shell health). Shrimp and prawns have generally softer, thinner shells, and so many of the color changes observed in shrimp and prawns are not color changes to the carapace itself, rather internal changes visible through the shell.

Aggression and traumatic damage can induce shell lesions, but environmental stressors such as pollutants or temperature are also considered important factors in shell pathologies. Specific infectious agents of most shell disorders are not yet fully understood. Although bacteria are frequently

isolated from lesions and researchers have seen limitation of lesion progression through experimental treatment with malachite green and formalin, these may represent secondary agents. Outcomes appear to depend on frequency of shell molts, meaning adults are most commonly observed as impacted due to their longer period between molts.

Incidental Findings Findings can at times be incidental, and of little significance to crustacean health. Some shell lesions are considered this way, determined to be merely melanized spots from previous trauma that do not extend full thickness through the carapace. Other findings without apparent clinical impact include intestinal parasites such as *Porospora nephropis* and *Stichocotyle nephropis* in lobsters, seen in squash or histological preparations of the gut as cyst like structures. *Histriobdella homari* worms can be found in the egg mass or gills of crustaceans, often without apparent ill effect, and documented findings of copepod and isopod infections, although rare within the literature, seem rarely of concern. Digenea infestations, often localized to the antennal gland in crayfish, are another incidental finding, and certain ciliates do not appear to be associated with direct pathology. Many apparently incidental pathogens can, however, cause disease given the correct set of circumstances. As with fish, the outcome of disease is predicated by a complex suite of contributory factors. Stress, nutritional status, age, stage of molt and a host of other factors influence disease outcome in crustaceans. Ultimately, lobster health status determines the outcome of survival in animals with disease such as shell lesions. General practice is to remove impacted individuals from an aquaculture population, whatever the causative agent. In many infectious outbreaks, destocking is required.

Specific Diseases

Similar to fish, crustaceans suffer a number of bacterial, viral, and parasitic infectious disorders, on top of non-infectious challenges such as water quality and temperature changes. Many clinical considerations are the same, such as the complexities of differentiating incidental findings from causative agents of observed clinical changes. Owing to the varied nature of host Crustacea and pathologies, only a limited number can be discussed in this chapter. The diseases presented here are majority WOAH notifiable conditions, of consequence not only to impacted farms, but regional production. Many of the listed diseases are associated with rapid high mortalities in shrimp and prawns. It is important to remember, however, that viral infections can be accompanied by secondary bacterial and epicommensal infestations, which may play a role in the ultimate cause of death. Many additional diseases can impact crustaceans beyond just those notifiable to the WOAH. Further reading is recommended from the expanding field of literature for a more complete overview of the topic.

Notifiable Diseases

White Spot Disease

Overview: White spot disease is highly infectious WOAH notifiable viral infection caused by white spot syndrome virus (WSSV), of which there are a number of genetic variants reported.

Etiology: WSSV has been assigned as the only member of the *Whispovirus* genus in the Nimaviridae family. It can be found in fresh, brackish, and marine environments and appears to be unrelated to other known viruses. Geographical isolates with genotypic variability have been identified, but they remain classified as a single species. The virus may remain viable in seawater for around 30 days at 30°C and 3–4 days in freshwater. Simulation of pond bottoms suggest sunlight will inactive it in a dried pond bed after 21 days or around 40 days in a pond with a still muddy bed. All life stages are susceptible, from eggs to broodstock.

Host range and distribution: WSSV infects crustaceans and all decapods (order Decapoda) including crabs and lobsters are considered susceptible. Indeed, to date, no decapod has shown any signs of being refractory to infection, although morbidity and mortality as a result of infection are variable and some species can carry high levels of virus without clinical signs. WSSV has been found in crustaceans in China, Japan, South Korea, South-East Asia, South Asia, the Indian Continent, the Mediterranean, the Middle East, and the Americas. Present within these regions are zones and compartments free from infection.

Signs/diagnosis: WSSV infections can be subclinical or clinical in nature. Affected shrimp are lethargic and exhibit abnormal swimming patterns such as swimming on their side, near the surface, or near pond edges. Feed consumption decreases or ceases. High morbidity and mortality are observed within days of the onset of signs, and some may die without showing any clinical signs. Non-penaeid species (e.g. crab, lobster) generally have subclinical infections under natural conditions.

WSSV is best detected following exposure to stressors such as eye-stalk ablation, spawning, molting, salinity fluctuation, temperature or pH changes, and during plankton blooms. The virus targets ectodermal and mesodermal tissues, especially the cuticular epithelium and subcuticular connective tissues and while it does infect connective tissues in the hepatopancreas and midgut, it does not infect the endodermis-derived tubular epithelial cells in either organ. Multifocal to coalescing white spots, seen within the exoskeleton, are the most commonly observed clinical sign and can range from barely discernible up to 3 mm in diameter. White spots are not a reliable diagnostic sign of infection, though, since environmental stress factors, such as high alkalinity or bacterial disease, can also cause white spots on the carapace of shrimp, and some WSSV-infected shrimp may have few, if any, white spots. Red to pink discoloration can also be seen in diseased populations.

The carapace can be loosely attached to underlying cuticular epithelium in clinically affected individuals and it can easily be removed (Figure 1.28); anorexia results in empty gastrointestinal tracts on inspection while debilitation results in excessive fouling of the gills and exoskeleton. Infected shrimp show delayed, or even absent clotting of hemolymph.

Figure 1.28 The carapace of a shrimp infected with white spot disease is loose and when removed, large smooth-edged white spots can be seen, often with smaller white spots also evident on the abdominal segments. Note that clinically affected shrimp may appear grossly normal. *Source:* Reproduced with permission B. Diggles/Department of Agriculture, Fisheries and Forestry/ CC BY 4.0.

Hypertrophied nuclei can be seen in squash preparations of the gills and cuticular epithelium, which can be stained or unstained (Figure 1.29). Otherwise, affected tissues can be identified in histopathology, where virus-infected cells exhibit hypertrophied nuclei with marginated chromatin, eosinophilic to pale basophilic (on hematoxylin and eosin, H&E) stained intranuclear viral inclusions within hypertrophied nuclei and multifocal necrosis associated with pyknotic and karyorrhectic nuclei in affected tissues of ectodermal and mesodermal origin. For details of polymerase chain reaction protocols, see the WOAH Manual of Diagnostic Tests for Aquatic Animals. Otherwise, diagnosis can be confirmed with in-situ hybridization, immunohistochemistry, and electron microscopy.

Differentials: Infection with infectious hypodermal and hematopoietic necrosis virus produce similar inclusions that need to be differentiated from those of WSSV as well as possibly white mottling of the shell. Environmental stress factors, such as high alkalinity, or bacterial disease can also cause white spots on the carapace of shrimp. Other viral conditions such as acute hepatopancreatic necrosis disease, infection with *Hepatobacter penaei*, infection with shrimp hemocyte iridescent virus, infection with Taura syndrome virus and infection with yellowhead virus genotype 1 (YHV1) should also be considered.

Treatment: There is no known treatment.

Management: Restocking with resistant species is not an option, although producers are attempting to breed improved resistant strains. Avoiding stocking in the cold season, use of specific pathogen-free or PCR-negative seed stocks, use of biosecure water and culture systems, and even polyculture of shrimp and fish have all apparently been attempted. Disinfecting eggs may help avoid trans-ovum transmission. Details on inactivation of the virus are available in the WOAH *Manual of Diagnostic Tests for Aquatic Animals*.

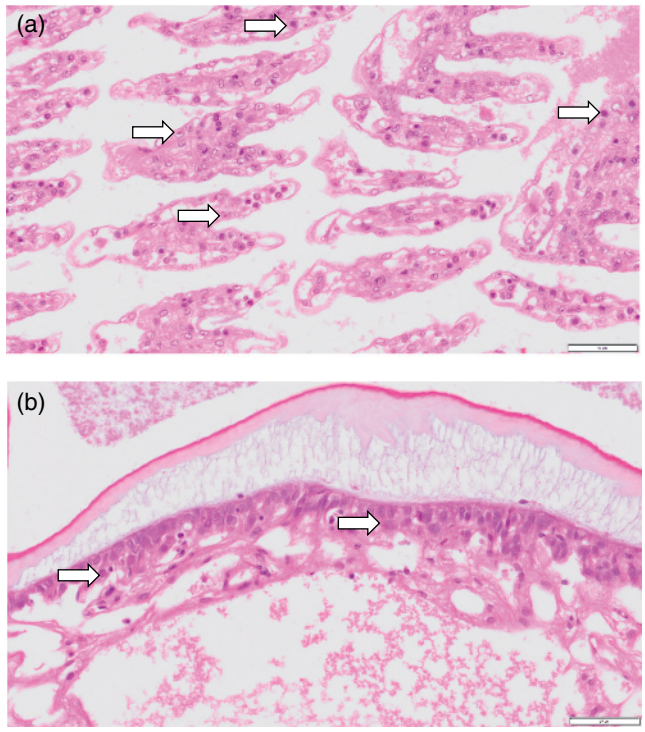


Figure 1.29 White spot syndrome virus infection in whiteleg shrimp (*Penaeus vannamei*). a) Virus infecting the nuclei of the circular epithelial cell lining in the gills. Infected nuclei appear hypertrophied with marginalized chromatin and containing an eosinophilic inclusion body (arrows). Hematoxylin and eosin stain. Scale bar = 50 μm. b) Virus infecting the nuclei of the cuticular epithelial cells lining the gut. Infected nuclei appear hypertrophied with marginalized chromatin and containing an eosinophilic inclusion body (arrows). Hematoxylin and eosin stain. Scale bar = 50 μm.

Taura Syndrome

Overview: Taura syndrome is a WOAH notifiable viral disease caused by infection with Taura syndrome virus (TSV), a small picorna-like RNA virus that belongs to the genus *Aparavirus* in the family Dicistroviridae. TSV replicates in the cytoplasm of host cells and has caused severe losses in shrimp farming regions of the Americas and Southeast Asia although impacts are declining due to improvements in good biosecurity practices and increasing use of tolerant stocks.

Etiology: TSV appears to remain infectious for up to 48 hours in the feces of sea gulls that have ingested infected shrimp carcasses, which may implicate birds as an important route of transmission of the virus within affected farms or farming regions. In addition, aquatic insects feeding on prawn carcasses, such as the water boatman (*Trichcorixa* spp.) may also act as vectors.

Host range and distribution: TSV causes disease in a wide range of penaeids and is known to be carried by a wide range of non-penaeids. *Penaeus monodon* and *Penaeus* (*Marsupenaeus*) *japonicus* susceptibility to TSV is unclear, but the whiteleg shrimp (*Litopenaeus vannamei*) and the western blue shrimp (*Litopenaeus stylirostris*) are probably the most susceptible species. The first known occurrence of a TSV outbreak was in 1992 when the virus impacted the shrimp population near the Taura river in Ecuador. The disease quickly spread to other countries in the Americas. By 1999, TSV was introduced into Southeast Asia through infected broodstock and post-larvae of *L. vannamei* intended for aquaculture and subsequently spread throughout much of the region.

Signs/diagnosis: Taura syndrome is seen mainly in the nursery phase of *Penaeus* (*Litopenaeus*) *vannamei*. It usually occurs within 14–40 days of stocking post-larvae into grow-out ponds or tanks. Mortalities are usually in the range of 40–90% in cultured populations of post-larval, juvenile, and subadult shrimp. Lesions usually appear during the acute phase of the disease and are present in specific target tissues, particularly the cuticular epithelium (hypodermis). Producers may notice lethargic prawns and cessation of feeding initially. Affected animals gather at the pond edge when moribund resulting in a sudden increase in seabird activity fishing in ponds. Producers will notice sudden onset of high mortalities in late post-larvae, juvenile, or subadult prawns.

The virus replicates mainly in the hypodermis but also in the gut, gills, and appendages. Gross pathology of the acute phase includes empty stomachs and pale red body surfaces and appendages, and notably, the uropods and pleopods may appear red due to the expansion of red chromatophores (Figure 1.30). The tail fan edges may also appear somewhat roughened by focal epithelial necrosis. Acutely affected shrimp may also exhibit soft shells and multiple, irregularly shaped, and randomly distributed melanized (dark) cuticular lesions in the transition phase. This will be accompanied by increased mortalities, usually at molting. There are few grossly obvious pathological signs of disease in the chronic phase.

Histopathology reveals necrosis of the cuticular epithelium of appendages, multiple melanized foci in the cuticular epithelium (transition phase), and abundant pyknotic and karyorrhectic nuclei, which have been described as giving Taura syndrome lesions a "peppered appearance" (Figure 1.31). In the chronic form of the disease, lymphoid organ spheroids may be apparent. Definitive diagnosis is based on consistent histopathology, positive in-situ hybridization in target tissues or reverse transcription PCR (rtPCR) followed by sequencing, or real-time PCR (Figures 1.32 and 1.33).

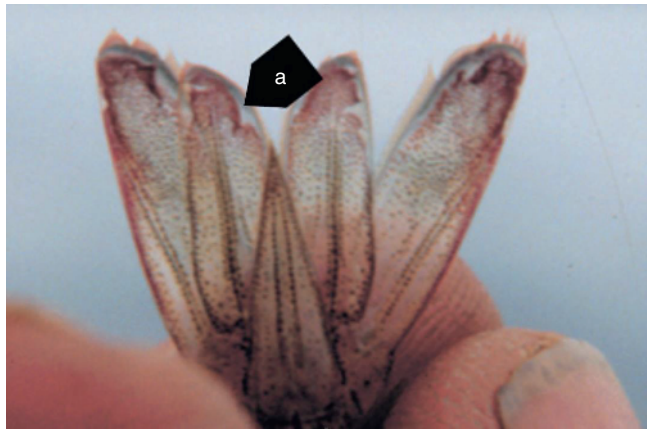


Figure 1.30 Reddened uropods with irregular edges suggesting focal necrosis caused by Taura syndrome virus. *Source:* Reproduced with permission from DV Lightner/ Australia Department of Agriculture, Fisheries and Forestry/CC BY 4.0.

Figure 1.31 Multifocal melanization during a transitional phase infection with Taura syndrome virus. *Source:* Reproduced with permission from DV Lightner/Australia Department of Agriculture, Fisheries and Forestry/ CC BY 4.0.

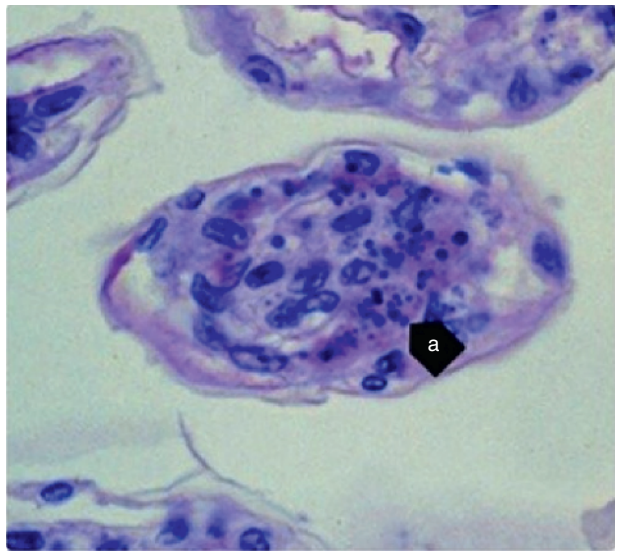


Figure 1.32 *Pennaeus vannamei* gill lesions exhibiting pyknosis, karyorrhexis, and a large number of cytoplasmic inclusion bodies (a). with Taura syndrome virus. *Source:* Reproduced with permission from DV Lightner/Australian Department of Agriculture, Fisheries and Forestry/CC BY 4.0.

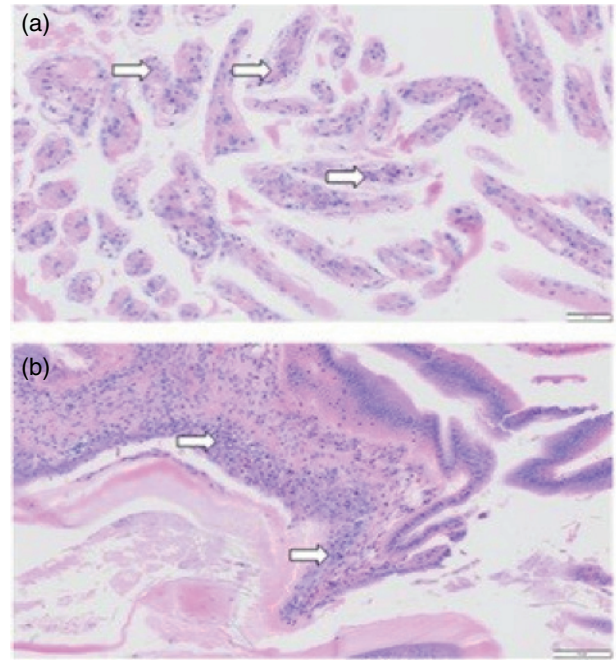


Figure 1.33 Taura syndrome virus and yellowhead virus 1 in whiteleg shrimp (*Pennaeus vannamei*). a) Infected cells within the cuticular epithelium of the gut are rounded with densely stained pyknotic nuclei (arrows). Hematoxylin and eosin stain. Scale bar = 100 µm. b) Infected cells within the cuticular epithelium of the gut are rounded with densely stained pyknotic nuclei (arrows). Hematoxylin and eosin stain. Scale bar = 100 µm.

Differentials: Affected prawn may show signs similar to yellowhead virus and WSSV.

Treatment: No confirmed effective treatments are in use, although blocking agents like injected short random double-stranded RNAi have been used with some improved resistance in trials. Some lines of TSV-resistant *P. vannamei* are available commercially.

Management: Outbreaks are reportedly more frequent when salinity falls below 30 ppt. Eradication methods for TSV depend on total depopulation of infected stocks, disinfection of the culture facility, avoiding the reintroduction of the virus from other nearby culture facilities, wild shrimp and fomites, and restocking with Taura syndrome-free post-larvae that have been produced from Taura syndrome-free broodstock. This approach has been successfully applied to certain aquaculture situations. It is good hygiene practice to disinfect eggs and larvae to reduce the potential of TSV contamination of spawned eggs and larvae produced from them.

Yellowhead Disease

Overview: Yellowhead disease is a WOAH notifiable concern in black tiger prawn aquaculture globally, with the majority of outbreaks reported in Southeast Asia. A number of viruses are included in the yellowhead complex: yellowhead virus genotype 1 (YHV1) is associated with yellowhead disease, while genotypes 2–6 are frequently isolated with apparently healthy individuals, and are rarely associated with disease, although genotype 2 is implicated with a gill-associated disease.

Host range and distribution: Yellowhead disease is infective in a wide array of prawn, shrimp, and krill species, both naturally and experimentally. Outbreaks have thus far been isolated however to *P. monodon* (black tiger prawn) and *P. vannamei* (white Pacific shrimp).

Transmission: Infective to post-larval organisms, this disease can be transmitted horizontally by cohabitation with live infected organisms, ingestion of infected material from deceased individuals, and through environmental water (where the virus can remain viable up to 72 hours). Infected organisms can remain lifelong carriers through viral persistence and chronic infections. Vertical transmission has been demonstrated for closely related genotype 2 yellowhead virus, but not yet for yellowhead disease.

Signs/diagnosis: Yellowhead disease is associated with rapid epidemics with high mortality, particularly in juvenile organisms. Behavioral changes common to many infectious pathologies are noted, including anorexia and congregation at pond edges. Producers report abnormally high feeding behavior prior to cessation and onset of clinical signs (two to four days). Total farm loss can occur within days of onset of clinical disease. The syndrome is named for the pallid, sometimes yellow, coloration of the dorsal cephalothorax area (due to enlargement and discoloration of the hepatopancreas). General appearance of an infected organism is a pallid/bleached presentation, with altered gill appearance, and a softened hepatopancreas. Presumptive diagnosis can be achieved using histopathology or Davidson-fixed, H&E-stained wet mounts with xylene alongside results of hemolymph smears (Figure 1.34).

Differentials: Cessation of feeding, congregation at pond edges, and lethargy of stock are shared features with many other pathologies in shrimp and prawns. In addition to infectious causes, yellow discoloration of individuals may occur due to stress or nutritional variation for altered metabolism and pigment deposition, as well as environmental toxicity (copper).

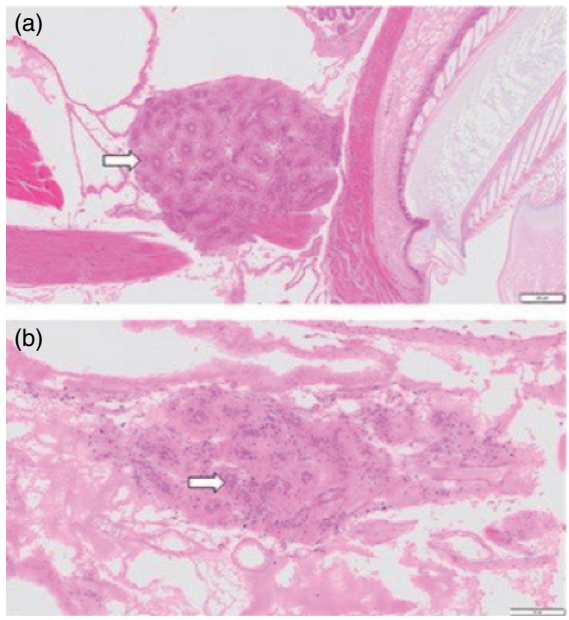


Figure 1.34 Taura syndrome virus (TSV) and yellowhead virus 1 in whiteleg shrimp (*Pennaeus vannamei*). a) TSV does not infect the lymphoid organ tissues; tubule structure is unaffected (arrow) and nuclei appear normal. Scale bar = 200 μm. b) Yellowhead virus infects the lymphoid organ tissues; loss of tubule structure is evident and nuclei rounded with densely stained pyknotic cells.

Treatment: No chemotherapy or preventative vaccinations are available for this pathogen. Specific disease-free animals are available for stocking. Following outbreaks, sites must be drained and treated to remove yellowhead virus particles. Yellowhead virus can be inactivated by heating and appears susceptible to chlorine treatment. Nested rtPCR is suggested as part of screening protocols for stock.

Infectious Hypodermal and Hematopoietic Necrosis Virus

Overview: The WOAH defines infection with infectious hypodermal and hematopoietic necrosis virus as infection with the non-enveloped and pathogenic DNA agent IHHNV of the Family Parvoviridae, an infection reportable to the WOAH and many state authorities. It is formally known as decapod Penstyldensovirus 1 but can also be known as *P. stylirostris* densovirus (PstDNV). In regions where the virus is endemic in wild stocks, the prevalence of IHHNV has been found in various surveys to range from 0% to 100%. Some reported mean values for IHHNV prevalence in wild stocks are 26% and 46%.

Host range and distribution: At least two distinct genotypes of IHHNV infectious to *P. vannamei* and *P. monodon* have been identified, with type 1 from the Americas and East Asia (principally the Philippines) and type 2 from South-East Asia (World Organisation for Animal Health, 2022a). Two sequences homologous to part of the IHHNV genome are found embedded in the genome of penaeids. These were initially described as type 3A from East Africa, India, and Australia, and type 3B from the western Indo-Pacific region including Madagascar, Mauritius, and Tanzania (World Organisation for Animal Health, 2022a; Tang and Lightner, 2006). In East Africa, Australia, and the western Indo-Pacific region, IHHNV-homologous sequences have been found in the *P. monodon* genome and are not infectious to the susceptible host species *P. vannamei* and *P. monodon*. Other susceptible species include yellowleg shrimp (*P. californiensis*), northern white shrimp (*Penaeus setiferus*), and blue shrimp (*P. stylirostris*). The northern brown shrimp (*Penaeus aztecus*) may be susceptible, and PCR positives (not active infection) have been reported from a number of other species: giant river prawn (*Macrobrachium rosenbergii*), kuruma prawn (*Penaeus japonicus*), green tiger prawn (*Penaeus semisulcatus*), Argentine stiletto shrimp (*Artemesia longinaruis*), Cuata swimcrab (*Callinectes arcuatus*), and some fish: Mazatlan sole (*Archirus mazatlanus*), yellowfin mojarra (*Gerres cinereus*), tilapias (*Oreochromis* sp.), Pacific piquitinga (*Lile stolifera*) and blackfin snook (*Centropomus medius*) (World Organisation for Animal Health, 2022a). IHHNV appears to be distributed worldwide in both wild and cultured penaeid shrimp (Owens et al., 1992). Infection has also been reported in farmed penaeids from Pacific islands including the Hawaiian Islands, French Polynesia, Guam, and New Caledonia. In the Indo-Pacific region, the virus has been reported from cultured and wild penaeid shrimp in East Asia, South-East Asia, and the Middle East (Bondad-Reantaso et al., 2001). An IHHN-like virus sequence was reported from Australia (Owens et al., 1992), and the presence of infection with IHHNV in farmed prawns in Australia was reported to the World Organisation for Animal Health in 2008.

Signs/diagnosis: IHHNV has been detected in all life stages (eggs, larvae, post-larvae, juveniles, and adults) of *P. vannamei*. When broodstocks are sourced from endemic areas, the hatching success of eggs is reduced, survival and culture performance of the larval and post-larval stages are lowered, and any nauplii produced from infected broodstock have a high prevalence of infection with IHHNV (Motte et al., 2003). Infection with IHHNV in this species is often acute, with very high mortalities occurring in the juvenile life stages of this species. Vertically infected larvae and early post-larvae do not become diseased, but in approximately 35-day-old or older juveniles, gross signs of the disease may be observed, followed by mass mortality. In horizontally infected juveniles, the incubation period and severity of the disease is somewhat size and/or age dependent, with young juveniles always being the most severely affected. Infected adults seldom show signs of the disease or mortalities. Gross signs are not specific, but juvenile *P. stylirostris* with acute infection with IHHNV refuse food, followed by changes in behavior and appearance. *P. stylirostris* have been observed in tanks to rise slowly to the surface, where they become motionless and then roll over and slowly sink (ventral side up) to the tank bottom. Shrimp exhibiting this behavior may repeat the process for several hours until they become too weak to continue, or until they are attacked and cannibalized by their healthier siblings. At this stage of infection, *P. stylirostris* often have white or buff-colored spots (which differ in appearance and location from the white spots that sometimes occur in shrimp with WSSV infections) in the cuticular epidermis, especially at the junction of the tergal plates of the abdomen, giving such shrimp a mottled appearance. This mottling later fades in moribund shrimp as such individuals become more bluish. In *P. stylirostris* and *P. monodon* with terminal-phase IHHNV infections, moribund shrimp are often distinctly bluish in color, with opaque abdominal musculature. Some surviving *P. stylirostris* and *P. vannamei* may carry the virus for life and may pass the virus to their progeny and other populations by vertical and horizontal transmission. Infections can be either acute or chronic depending on shrimp species and population. Some populations of *P. stylirostris* can suffer acute, usually catastrophic disease, with mortalities approaching 100% as described above. In contrast, infection in some populations of *P. vannamei*, *P. stylirostris*, and *P. monodon* results in a more subtle, chronic form of IHHVV disease, runt-deformity syndrome (RDS), in which high mortalities are unusual but where growth suppression and deformities of the cuticle are common (Kalagayan et al., 1991). The syndrome results in poor and highly disparate growth. RDS is a more commonly seen in *P. vannamei* where juvenile shrimp may display a bent (45–90-degree bend to left or right) or otherwise deformed rostrum, a deformed sixth abdominal segment, wrinkled antennal flagella, cuticular roughness, "bubble heads," and other cuticular deformities (Australia Department of Agriculture, Water and the Environment, 2020b). In *P. vannamei* and *P. stylirostris* with RDS, a deformed rostrum bent to the left or right, is said to be pathognomonic for infection with IHHNV. Affected shrimp show a wide distribution of sizes and the coefficient of variation, which should be between

10% and 30%, can approach 90% (World Organisation for Animal Health, 2022a). The principal target tissues for IHHNV include connective tissue cells, the gills, hematopoietic nodules and hemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells (World Organisation for Animal Health, 2022a). Hence, whole shrimp (e.g. larvae or post-larvae) or tissue samples containing the target tissues are suitable for most tests using molecular methods. In general, spawned eggs and larvae are not suitable samples for detection or certification (World Organisation for Animal Health, 2022a). Hemolymph or excised pleopods may be collected and used for testing (usually for PCR or dot-blot hybridization with specific probes) when non-lethal testing of valuable broodstock is necessary. IHHNV is a systemic virus, and it does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of IHHNV. Using Davidson's fixative, only live, moribund, or compromised shrimp should be selected for fixation and histological examination – dead shrimp should not be used. Selected shrimp are killed by injection of fixative directly into the hepatopancreas; the cuticle over the cephalothorax and abdomen just lateral to the dorsal midline is opened with fine-pointed surgical scissors to enhance fixative penetration (the abdomen may be removed and discarded), the whole shrimp (or cephalothorax less the abdomen) is immersed in fixative for 24–48 hours, and then transferred to 70% ethyl alcohol for storage. After transfer to 70% ethyl alcohol, fixed specimens may be transported (via post or courier to the diagnostic laboratory) by wrapping in cloth or a paper towel saturated with 70% ethyl alcohol and packed in leak-proof plastic bags. Acute (but not chronic) infections in *P. stylirostris* can be readily diagnosed using routine H&E-stained sections. For diagnosis of chronic infections, the use of molecular methods are recommended for IHHNV detection (e.g. by PCR or application of IHHNV-specific DNA probes to dot-blot hybridization tests or in-situ hybridization of histological sections). Histological demonstration of prominent intranuclear, Cowdry type A inclusion bodies provide a provisional diagnosis of infection with IHHNV (Australia Department of Agriculture, Water and the Environment, 2020b). These characteristic IHHNV inclusion bodies are eosinophilic and often haloed (with H&E stains of tissues preserved with fixatives that contain acetic acid, such as Davidson's AFA and Bouin's solution), intranuclear inclusion bodies within chromatin-marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of fore- and hindgut, nerve cord, and nerve ganglia) and mesodermal origin (hematopoietic organs, antennal gland, gonads, lymphoid organ, and connective tissue). Intranuclear inclusion bodies caused by and often haloed (with H&E stains of tissues preserved with fixatives that contain acetic acid, such as Davidson's AFA and Bouin's solution), intranuclear inclusion bodies within chromatin-marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of fore- and hindgut, nerve cord, and nerve ganglia) and mesodermal origin (hematopoietic organs, antennal gland, gonads, lymphoid organ, and connective tissue). Intranuclear

inclusion bodies caused by infection with IHHNV may be easily confused with developing intranuclear inclusion bodies caused by WSSV infection. In-situ hybridization assay of such sections with a specific DNA probe to IHHNV provides a definitive diagnosis of infection with IHHNV. Direct detection methods using DNA probes specific for IHHNV are available in dot-blot and in-situ hybridization formats. PCR tests for IHHNV have been developed and a number of methods and commercial PCR detection kits are readily available (World Organisation for Animal Health, 2022a).

Infection with IHHNV is suspected if at least one of the following criteria are met: i) clinical signs indicative of infection with IHHNV; ii) histopathology indicative of infection with IHHNV; or iii) positive result by PCR. Infection is confirmed if two of the following criteria are met: i) positive result by in-situ hybridization; ii) positive result by PCR (always genotype specific); iii) and sequence analysis to confirm IHHNV nucleic acid sequence (World Organisation for Animal Health, 2022a).

Differentials: Clinical presentation and histological changes can be difficult to differentiate from white spot syndrome and abdominal segment deformity disease. In addition to infectious etiology, inappropriate nutrition or temperature for growth and development, as well as genetic defects, must be considered.

Treatment: No success with vaccination, chemotherapy, or immunostimulation has been described.

Management: The replication rate of IHHNV at high water temperatures appears to be significantly reduced. In one study with *P. vannamei*, after a suitable incubation period, shrimp held at 32°C had approximately 102 times lower viral load than shrimp held at 24°C (Montgomery-Brock et al., 2007). PCR prescreening of wild or pond-reared broodstock or their spawned eggs/nauplii and discarding those that test positive for the virus, as well as the development of specific pathogen-free shrimp stocks of *P. vannamei* and *P. stylirostris* have proven to help control the virus, with the latter being the most successful husbandry practice for the prevention and control of IHHN (Lightner, 2005). Selected stocks of *P. stylirostris* that are resistant to infection with IHHNV have been developed, and these have had some successful application in shrimp farms but they do not have increased resistance to other diseases, such as WSSV, so their use has been limited. A genetic basis for IHHN susceptibility in *P. vannamei* has been reported in some stocks. The relative resistance of these shrimp to infection with IHHNV is considered to be one of the main reasons that led to *P. vannamei* being the principal shrimp species farmed in the Western hemisphere, and since 2004, globally (Lightner, 2009).

Necrotising Hepatopancreatitis (Acute Hepatopancreatic Necrosis Disease)

Overview: Often observed as a protracted bacterial infection, this condition impacts postlarvae and juvenile giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*Penaeus annamei*) following stocking.

Host range: Reported in susceptible organisms in China, Vietnam, Thailand, Malaysia, Mexico, and the Philippines. High salinity is considered a risk factor to infection, as is hot dry weather between April and July. It is unknown whether persistent carriers might transmit bacteria, but the causative agent *Vibrio parahaemolyticus* is common in environmental water; demonstrated as capable of surviving for more than two weeks. Vector species are suspected but not yet demonstrated.

Transmission: Transmission between infected individuals occurs via oral routes or cohabitation. Onset of infection often coincides with an external stressor such as poor water quality or algal blooms.

Signs/diagnosis: Onset of clinical signs and mortality occurs post-stocking. Changes include a pale, atrophied hepatopancreas, soft shells, and empty guts. Melanized spots and hardening can also occur within the hepatopancreas due to melanin deposition, hemocytes, and increase fibrous connective tissue content. Presumptive diagnosis by histopathology should be confirmed using PCR.

Differentials: General presentation is similar to numerous other viral and systemic bacterial infections.

Treatment: There are no recommended treatments for this infection, nor are there available resistant strains or disease-free strains.

Management: Strict feeding rate control and appropriate stocking density are considered important husbandry practices to reduce disease impact. Screening stock, sanitation, and strict biosecurity can reduce the likelihood of an outbreak. The bacterium is known to be sensitive to freezing, disinfection and heating, important considerations as it is associated with food poisoning in humans.

Acute Hepatopancreatic Necrosis Disease, Vibrio parahaemolyticus

Overview: Acute hepatopancreatic necrosis disease (AHPND) was formally known as early mortality syndrome and refers to infection with strains of *Vibrio parahaemolyticus* (Vp AHPND), a Gram-negative rod-shaped bacterium that contains an approximate 70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) binary toxins, PirA and PirB (World Organisation for Animal Health, 2022b) and is capable of causing rapid death of infected shrimp. *Photorhabdus* is a genus of bioluminescent, gram-negative bacilli known to be pathogenic to a wide range of insects and has been used as biopesticide in agriculture. Although other *Vibrio* species have been isolated from clinical cases of AHPND, only Vp AHPND has been demonstrated to cause AHPND. Removal (or "curing") of the plasmid pVA1 abolishes the AHPND-causing ability of Vp AHPND strains. It is important to recognise from a diagnostic perspective that the Pirvp operon may be deleted naturally due to the instability of repeat sequences that flank the Pir toxin operon. Any affected Vp AHPND strain will lose its ability to cause AHPND. If the Pir toxin sequence is used as a target for detection, a false negative may occur if the colony isolated has this deletion even though the colony was derived from an isolate of AHPND-causing Vp AHPND. The pVA1 plasmid also carries genes related to conjugation, meaning this plasmid is potentially transferable to other bacteria.

Host range and distribution: The giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*Penaeus vannamei,* also known as king prawn or Pacific white shrimp) are currently listed by the WOAH as fulfilling all the criteria for susceptible species; there are possibly more, including the fleshy prawn (*Penaeus chinensis*) that partially fulfils the criteria, or the kuruma prawn (*Penaeus japonicus*) that has demonstrated a PCR-positive product in the absence of clinical signs. The disease has been reported from China, Vietnam, Malaysia, Thailand, Mexico, and the Philippines.

Signs/diagnosis: In regions where the bacterium is endemic in farmed shrimp, evidence suggests a prevalence approaching 100% (Tran et al., 2013a). AHPND is characterised by sudden, mass mortalities (up to 100%) usually within 30–35 days of stocking grow-out ponds with post-larvae or juveniles (Food and Agriculture Organization, 2013), although older juveniles may also be affected (de la Peña et al., 2015). The onset of clinical signs and mortality can start as early as 10 days post-stocking, with moribund prawns sinking to the bottom of the pond.

Clinical signs include hepatopancreatic pallor and atrophy, soft shells, intestines with discontinuous or no contents, black foci or linear streaks visible within the hepatopancreas (due to melanized tubules). In addition, the hepatopancreas does not squash easily between the thumb and forefinger (possibly due to increased fibrous connective tissue and hemocytes) (Network of Aquaculture Centres in Asia-Pacific, 2014). The disease has been described as having two distinct phases: an acute phase characterized by a massive and progressive degeneration of the hepatopancreatic tubules from proximal to distal, with significant rounding and sloughing of tubule epithelial cells into the tubules, collecting ducts and posterior stomach in the absence of bacterial cells, and a terminal phase characterised by marked intratubular hemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells (Food and Agriculture Organization, 2013; Tran et al., 2013a).

Wet mounts and smears are not appropriate, nor are fixed sections for in-situ hybridization or electron microscopy. Vp AHPND can be isolated on standard media used for isolation of bacteria from diseased shrimp and species identification may be carried out using 16S rRNA PCR or toxR-targeted PCR and sequencing. AHPND-specific PCR methods, protocols and primers that target the Vp AHPND toxin genes are described in the *Manual of Diagnostic Tests for Aquatic Animals* (World Organisation for Animal Health, 2017b).

Differentials: Infection with *Enterocytozoon hepatopenaei*, *Hepatobacter penaei* or shrimp hemocyte iridescent virus (Australia Department of Agriculture, Water and the Environment. 2020a).

Treatment: There are no known treatments, vaccinations, immunostimulants or resistance-breeding successes to-date (Food and Agriculture Organization, 2013).

Management: *Vibrio* spp. are ubiquitous in the marine environment, and the possibility that there are vector species, particularly various genera of polychete worms that are thought to be naturally susceptible (World Organisation for Animal Health, 2022a), can be expected, although to date none has been conclusively identified. The bacteria are thought to be naturally transmitted through horizontal oral transmission and cohabitation. Low salinity (< 20 ppt) seems to reduce

the incidence of the disease and peak occurrence appears during the hot and dry season from April to July. Overfeeding, poor seed, water, and feed quality, algal blooms or crashes are also factors that may lead to occurrences of AHPND in endemic areas (Food and Agriculture Organization, 2013). As with other infectious diseases of shrimp, establishing good sanitary and biosecurity practices, such as improvement of hatchery sanitary conditions and post-larval screening are likely to be beneficial; good broodstock management, use of high-quality post-larvae and good shrimp farm management (strict feeding rate control, appropriate stocking density) are all well-established practices that reduce the impact of disease, including AHPND (Network of Aquaculture Centres in Asia-Pacific, 2012).

Vp AHPND is expected to possess similar properties to other strains of *V. parahaemolyticus* found in seafood that have been shown to survive up to 9 days in filtered estuarine water and 18 days in filtered seawater at an ambient temperature of 28°C ± 2°C (Karunasagar et al., 1987). Experimental studies have shown that Vp AHPND could not be transmitted via frozen infected shrimp (Tran et al., 2013a). Other strains of *V. parahaemolyticus* are known to be sensitive to freezing, refrigeration, heating, and common disinfectants.

Shrimp White Tail Disease The clinical disease based on appearance of "white tail" has been associated with several etiological agents in both shrimp and freshwater prawns. These are:

- infectious myonecrosis virus (IMNV)
- *Macrobrachium rosenbergii* nodavirus (MrNV)
- extra-small virus-like particle (XSV).

Infectious Myonecrosis Virus

Overview: IMNV is similar to the Totiviridae and is related to the *Giardia lamblia* virus. IMNV infects penaeid shrimp, including the most commonly farmed species, causing mortalities that can reach to 70% in farmed *Penaeus vannamei*. Losses due to IMNV from 2002 through 2011 were estimated to be more than US $ 1 billion (Lightner, 2012). In regions where the infection is endemic in farmed stocks of *P. vannamei*, its prevalence may reach 100%.

Host range and distribution: Anecdotal information on the introduction of *P. vannamei* stocks from Brazil to Indonesia in 2006 was confirmed when investigations of stock in both countries revealed a shared 99.6% similar genome sequence identity. The virus appeared to be contained in Indonesia until 2008, when it subsequently spread to multiple provinces including East Java, Bali, Lampung, Central Java, West Kalimantan, and West Nusa Tenggara. Susceptible species include the brown tiger prawn (*Penaeus esculentus*), banana prawn (*Penaeus merguiensis*), and whiteleg shrimp (*P. vannamei*) (World Organisation for Animal Health, 2022a), while it is thought that the giant tiger prawn (*Penaeus monodon*) and blue shrimp (*Penaeus stylirostris*) may be susceptible, and the southern brown shrimp (*Penaeus subtilis*) has yielded a PCR-positive only.

Transmission: IMNV has been demonstrated to be transmitted horizontally by cannibalism, and although vertical transmission is suspected from anecdotal evidence, it is not known whether this occurs transovarially or by surface contamination of newly spawned eggs. Transmission via water probably occurs. Real Time PCR for screening pond-reared broodstock and/or their spawned eggs/nauplii and discarding those that test PCR-positive, fallowing and restocking of affected farms or entire culture regions with IMNV-free stocks of *P. vannamei*, and the development of specific pathogen-free shrimp stocks of *P. vannamei* most suited to local culture conditions have proven to be the most successful husbandry practice for preventing and controlling other virus diseases of shrimp, and should be applicable to control and prevent infection with IMNV (Tang et al., 2019).

Signs/diagnosis: Juveniles and subadults of *P. vannamei*, farmed in marine, brackish, and low salinity brackish water, appear to be most severely affected by infection with IMNV (Lightner, 2011). The virus targets the striated muscles (skeletal and less often cardiac), connective tissues, hemocytes, and the lymphoid organ parenchymal cells (Figure 1.35). Simple dissection will allow the hypertrophied (three to four times their normal size) paired lymphoid organs to be seen. On histopathology, lesions in striated muscles and lymphoid organs are not pathognomonic for infection with IMNV since white tail disease of penaeid shrimp caused by *P. vannamei* nodavirus (PvNV) can mimic infection with IMNV. Hence, diagnostic information from other sources (e.g. history, gross signs, morbidity, mortality, or rtPCR findings) may be required to confirm a diagnosis of infection with IMNV (Tang et al., 2019). Routine H&E-stained paraffin sections of acute-phase infections with IMNV show myonecrosis with characteristic coagulative necrosis of striated (skeletal) muscle fibers, often with marked edema among affected muscle fibers. Some shrimp may present a mix of acute and older lesions. In this shrimp, affected muscle fibers appear to progress from coagulative necrosis to liquefactive necrosis, accompanied by moderate infiltration and accumulation of hemocytes (Tang et al., 2019). In the most advanced lesions, hemocytes and inflamed muscle fibers are replaced by a loose matrix of fibrocytes and connective tissue fibers that are interspersed with hemocytes and presumed foci of regenerating

Figure 1.35 Opaque muscle in the abdominal sections and an erythematous tail caused by infectious myonecrosis virus. *Source:* Reproduced with permission from DV Lightner/Australia Department of Agriculture, Fisheries and Forestry/CC BY 4.0.

muscle fibers (Poulos et al., 2006). Significant hypertrophy of the lymphoid organ caused by accumulations of lymphoid organ spheroids (LOS) is a highly consistent lesion in shrimp with acute or chronic-phase infection with IMNV lesions. Often, many ectopic LOS are found in other tissues not near the main body of the lymphoid organ. Common locations for ectopic LOS include the hemocoelom in the gills, heart, near the antennal gland tubules, and ventral nerve cord (Poulos et al., 2006). Stained or unstained tissue squashes of skeletal muscle when examined with phase or reduced light microscopy may show loss of the normal striations. Fragmentation of muscle fibers may also be apparent. Squashes of the lymphoid organ may show the presence of significant accumulations of spherical masses of cells (LOS) among normal lymphoid organ tubules. See the *Manual of Diagnostic Tests for Aquatic Animals* for further details on sampling and diagnosis based on antibody and molecular techniques (Tang-Nelson F-J. 2017a).

In endemic areas, following stressful events such as capture by cast netting, feeding, and sudden changes in water salinity or temperature, there may be outbreaks of infection associated with sudden high mortalities in early juvenile, juvenile, or adult *P. vannamei*. In the acute phase, IMNV presents as focally extensive areas of white necrosis in striated (skeletal) muscles, especially in the distal abdominal segments, uropods and telson, which can become reddened and necrotic in some shrimp. Severely affected shrimp become moribund with high mortalities following a "stress" event that may continue for several days. Only shrimp with the acute phase of disease exhibit behavioral changes. Typically, severely affected shrimp become lethargic during or soon after stressful events. Feed conversion ratios of affected populations can increase from a normal value of approximately 1.5 up towards 4.0 or higher. Some *P. vannamei* that survive IMNV infections or epidemics may carry the virus.

Differentials: Whitish, necrotic tail muscle is observed in dead and moribund shrimp, especially in samples from Brazil, Indonesia, India, and Malaysia. IMNV should be included in the list of diseases for diagnosis. However, this clinical sign can also be caused by a number of factors other than IMNV infection (e.g. hypoxia, crowding, sudden changes in temperature or salinity, PvNV, and MrNV; Tang, 2019).

Management: The virus is non-enveloped and it is likely that IMNV will remain infectious in the gut and feces of birds that feed on dead or moribund shrimp at farms with continuing infection, and spread within and among farms by feces or regurgitated shrimp carcasses. Anecdotally, IMNV appears to be more difficult to inactivate with typical pond disinfection procedures (e.g. sun drying, chlorination, etc.) than other penaeid shrimp viruses like WSSV, YHV1, TSV, and IHHNV; however, disinfection of eggs and larvae is a good management practice recommended to reduce the transmission potential of a number of penaeid shrimp diseases from female spawners to their eggs or larvae, and the practice may reduce IMNV contamination of spawned eggs and larvae produced from them. Reservoir hosts are suspected, but none have been documented consistently. There are no specific data on vectors.

Macrobrachium rosenbergii Nodavirus

Overview: The MrNV virus is infective to brown tiger prawn (*Penaeus esculentus*), banana prawn (*Penaeus merguiensis*), and whiteleg shrimp (*Penaeus vannamei*) and is identified with no clinical signs in southern brown shrimp (*Penaeus subtilis*). Juveniles and subadults appear most severely impacted.

Transmission: Transmission occurs from infected tissue, including possibly within the gastrointestinal tract of predatory birds, through feces or regurgitate. Horizontal transmission is demonstrated, and vertical suspected, however this might be through contamination of eggs after laying.

Signs/diagnosis: High mortalities following stressful events such as handling or sudden alterations to environmental conditions. This disease impacts the striated muscles (skeletal and cardiac) and lymphoid organ parenchymal cells, causing white necrotic areas within the musculature. Reddening is seen in some shrimp in the distal abdominal segments and tail fan. Animals become moribund and high mortalities (40–70%) can be seen for a number of days. Confirmatory diagnosis can be achieved through histopathology and a careful clinical history, or molecular techniques such as PCR or in-situ hybridization.

Differentials: Differential diagnoses include other white tail disease agents. Necrotic muscle tissue can also occur due to stresses such as low dissolved oxygen or altered temperature.

Management: There is currently no treatment or verified prevention strategy for this disease, although research does support the existence of more resistant lines of *P. vannamei* (a species that, in general, appears more susceptible to this disease than others). Screening broodstock is recommended to prevent outbreaks, and specific pathogen-free lines of *P. vannamei* seem the most successful prevention strategy. This virus appears particularly resistant to traditional disinfection protocols.

Enterocytozoon hepatopenaei

Overview: *Enterocytozoon hepatopenaei* (EHP) is a microsporidian parasite of emerging concern in aquaculture production. Although infection does not appear to be associated with mortalities, it has been linked to notable reduction in growth performance.

Host range and transmission: EHP is known to infect *Penaeus monodon*, *Penaeus vannamei* and *Penaeus stylirostris* and is potentially endemic to the Australasian Pacific region. The full life cycle remains to be characterized, although infection is known to occur via infective spores, considered to be acquired through excretion in the feces or through cannibalism of infected tissue.

Signs/diagnosis: Clinical signs appear to be exclusively in a retardation of growth of infected individuals. Diagnosis is best achieved via molecular means due to the small spore size of this organisms.

Differentials: A general lack of clinical signs typifies this infection. Generalized poor growth can occur due to a multitude of different conditions in shrimp (poor nutrition, altered temperature as well as viral and bacterial pathologies) and is therefore not pathognomonic.

Treatment: No treatment is currently available for this condition.

Management: PCR screening can be used to detect infection within a population and screen juveniles prior to stocking. Various trials have been aimed at killing spores, including temperature and chemical water treatments.

Freshwater Infections

Prawn "White Tail" Disease

Overview: White tail disease is a viral infection of brackish and freshwater environments that can present entirely without clinical signs for variable mortality in non-adult freshwater prawns.

Transmission: Infects giant freshwater prawn (*Macrobrachium rosenbergii*) by both vertical from broodstock and horizontal (via infected individuals and contaminated water) transmission. Larvae, post-larvae, and early juveniles are impacted, with variable mortality up to 95%.

Signs/diagnosis: Infection impacts striated muscle tissue and intratubular connective tissue of the hepatopancreas. Externally visible discoloration begins at the tail and extends cranially to cephalothorax, with extensive necrosis of muscle fibers, followed by death. Presumptive diagnosis can be reached by observation of post-larval stock with milky, opaque tails, and high mortality. Confirmatory diagnosis of MrNV and/or XSV is via rtPCR and loop-mediated isothermal amplification.

Treatment: No vaccination protocols or treatments are currently available for this infection.

Management: Broodstock screening with rtPCR is highly recommended.

Necrotizing Hepatopancreatitis

Overview: The WOAH defines infection with *Hepatobacter penaei* as infection with the pathogenic agent *Candidatus Hepatobacter penaei*, an obligate intracellular bacterium of the Order α-Proteobacteria that targets all hepatopancreatic cell types. The disease is commonly known as necrotizing hepatopancreatitis (World Organisation for Animal Health, 2022a). In *Penaeus vannamei*, it can result in an acute, usually catastrophic disease, with mortalities approaching 100%. Infection with *H. penaei* has been demonstrated in juveniles, adults, and broodstock of *P. vannamei* but other penaeids are susceptible.

Host range and distribution: Prevalence of *H. penaei* in wild stocks has been estimated between 5% and 17% in *Penaeus duorarum* and *Penaeus aztecus* in Mexico. Reported values for *H. penaei* prevalence in shrimp farms are between 0.6% and 1.3% in *P. vannamei* from shrimp farms in Belize, Brazil, Guatemala, Honduras, Mexico, Nicaragua, and Venezuela. *H. penaei* appears to have a Western hemisphere distribution in both wild and cultured penaeid shrimp and in addition to those territories above, is also found in Colombia, Costa Rica, Ecuador, El Salvador, Honduras, Panama, Peru, and the United States. On-farm infection may increase during long periods of high temperatures (> 29°C) and high salinity (20–38 ppt). In Mexico, the bacterium has a prevalence of less than 7% in shrimp farms between April and August; however, in September and October when temperatures are high during the day and low at night, a high prevalence and mortality (> 20%) are observed (Morales-Covarrubias, 2010).

Signs/diagnosis: No clinical signs are pathognomonic. Infection with *H. penaei* often causes an acute disease with very high mortalities in young juveniles, adult, and broodstock. In horizontally infected young juveniles, adult, and broodstock, the incubation period and severity of the disease is often size and/or age dependent. Gross signs are not specific, but acute infection with *H. penaei* shows a marked reduction in food consumption, followed by changes in behavior and appearance. These include lethargy, reduced food intake, atrophied hepatopancreas, anorexia, and empty guts, noticeably reduced growth and poor length to weight ratios ("thin tails"), soft shells and flaccid bodies, black or darkened gills, heavy surface fouling by epicommensal organisms, bacterial shell disease, including ulcerative cuticle lesions or melanized appendage erosion, and expanded chromatophores resulting in the appearance of darkened edges in uropods and pleopods. The principal target tissue for *H. penaei* is hepatopancreas but it infects most enteric tissues meaning feces may be collected and used for testing (usually by PCR, or dot-blot hybridization with specific probes) when non-lethal testing of valuable broodstock is necessary (Morales-Covarrubias et al., 2012). *H. penaei* do not replicate in the midgut, caeca, connective tissue cells, the gills, hematopoietic nodules, and hemocytes, ventral nerve cord and ganglia, antennal gland tubule epithelial cells, and lymphoid organ parenchymal cells, and these tissues are not appropriate for sampling (World Organisation for Animal Health, 2022a, chapter 2.2.3).

Differentials: Infection with EHP, infection with Vp AHPND and infection with (SHIV) (World Organisation for Animal Health, 2022a).

Transmission: Surviving prawns may be able to carry the intracellular bacterium for life and act as a source of infection by horizontal transmission, which is typically via cannibalism or contaminated water (Aranguren et al., 2006; Morales-Covarrubias, 2010). Feces shed into water has also been suggested as a source of transmission.

Management: Prevention includes early detection of the initial phase of clinical infection because of the potential for cannibalism to amplify and transmit the disease; sufficient feeding since starvation and cannibalism of infected shrimps with positive conditions for *H. penaei* multiplication are important factors for spread of the bacterium; using hydrated lime to treat pond bottoms during pond preparation before stocking can help reduce infection with *H. penaei*; preventive measures including raking, tilling, and removing sediments from the bottom of the ponds, fallowing ponds with prolonged drying (through exposure to sunlight) of ponds and water distribution canals for several weeks, disinfection of fishing gear and other farm equipment using calcium hypochlorite, and extensive liming of ponds between batches; employing best practice and using specific pathogen-free broodstock where possible (World Organisation for Animal Health, 2022a).

In the face of an outbreak, the best treatment currently available is antibiotic treatment with oxytetracycline and florfenicol in medicated feeds every 8 hours for 10 days, particularly if infection with *H. penaei* is detected in the initial phase (Morales-Covarrubias et al., 2012). There are no vaccinations or confirmed successes with immunostimulants, breeding for resistance, restocking with resistant strains or blocking agents. Disinfection of eggs and larvae is good management practice (Lee & O'Bryen, 2003) and is recommended for its potential to reduce *H. penaei* contamination of spawned eggs and larvae (and is good hygiene practice to prevent contamination by other infectious agents). The prevalence

and severity of infection with *H. penaei* may be increased by rearing shrimp in relatively crowded or stressful conditions. Some husbandry practices have been successfully applied to the prevention of infection with *H. penaei* including the application of PCR to prescreen wild or pond-reared broodstock.

Non-Notifiable Diseases

Epizootic Shell Disease

Overview: A number of shell conditions are described in lobster, *Homarus americanus*, including impoundment shell disease (ISD), burn-spot or rust-spot shell disease, epizootic shell disease (ESD), and the enzootic form of ESD. ESD is a form of shell disease that presents differently to "classical" shell pathologies in that it develops rapidly with high prevalence in a population.

Host range and transmission: ESD impacts clawed lobsters. Important factors in onset appear to be water temperature, as well as environmental contaminants. Shell pathologies are considered to be predisposed by conditions that reduce the integrity of the carapace, including altered environmental pH or direct trauma, but the full causality of epizootic shell disease appears complex, involving multiple anthropogenic factors. Various bacteria can be cultured from lesions, including *Aquimarina homari* and *Thalassobius* species (also observed in ISD). These mixed bacterial communities appear to be associated with subsequent necrosis of tissues.

Signs/diagnosis: Shell erosions (moderate to deep) occur in the carapace along the dorsum of the cephalothorax and abdomen. Lesions are generally thin and deep, with pillars of unpigmented tissue seen deeper within the erosion. Diagnosis is based on clinical presentation and a case history of high prevalence of shell disease within a population.

Differentials: Several forms of shell disease can present clinically similarly, and most shell disease syndromes linked to bacterial infection are documented as presenting with irregular "cigarette burn marks" that progress and overlap. They can be distinguished by their characteristic clinical signs and prevalence. Chitinolytic bacterial infections can impact all lobster species, as can diet-induced shell disease. Additional shell diseases, including traumatic damage from substrate or aggression and "rust spot" disease (linked to aquatic pollution) cause brown necrotic pits across the shell surface, both often localized to claws and appendages. Impoundment shell disease, linked with overcrowding, poor water quality or inadequate diets, is seen bilaterally on the dorsal carapace, centered on setal pores, but also progresses to coalescing lesions. Fusarium fungal infection can also cause black discoloration to the carapace. Blackspot (auto-oxidation and polymerization of polyphenol oxidase oxidizing diphenols to form dark pigments during traumatic events such as molting and rough handling) is cosmetic only.

Treatment: As causation appears to be multifactorial, prevention must also be multifactorial. At this stage, treatments are only used in the study of this condition.

Gaffkemia

Overview: Gaffkemia (also known as red-tail) is an extremely virulent infectious disease of lobsters caused by the bacterium *Aerococcus viridans*.

Host range and distribution: Gaffkemia is thought to be a risk in all species of lobster but appears particularly virulent in European lobsters.

Signs/diagnosis: Dark orange to pink discoloration to the ventral surface of infected individuals occurs due to stress-associated pigmentation of hemolymph with astaxanthin, seen through thin ventral membranes. Lethargy and anorexia are also common findings, with infection progressing to moribund individuals that may lie on their sides or lose appendages. Rate of progression is impacted by temperature but can occur within a few days of infection in warm conditions. Diagnosis can be achieved by culture, PCR or fluorescent antibody techniques.

Differentials: Lethargy and inappetence are common findings as with most diseases. Reddening of the tail is a finding also seen in generalized stress syndromes, as well as with *Hematodinium* (parasitic) and *Haliphthoros* (fungal) infections.

Treatment: Antibiotics can be successful in the treatment of this condition, although legislation varies globally on their use.

Management: Good hygiene and low stress environments are key to prevention. Low stocking density and prevention of traumatic injury that might allow bacterial ingress are thought to be key.

Hematodinium

Overview: *Hematodinium* are parasitic dinoflagellates documented in a number of crustacean species globally and one of the most economically significant pathogens of decapod crustaceans (Figure 1.36).

Host range and distribution: *Haematodinium* infections are documented in many crustacean hosts, including the Atlantic blue crab (*Callinectes sapidus*), Norwegian lobster (*Nephrops norvegicus*), mud crabs (*Scylla serrata*), snow crab (*Chionoecetes opilio*), tanner crab (*Chionoecetes bairdi*), velvet swimming crab (*Necora puber*) and ridgetail white prawns (*Exopalaemon carinicauda*). European lobsters do not appear to be susceptible. The currently described species are *Haematodinium perezi* and *Haematodinium australis*, and they appear to have varied host prevalence.

Figure 1.36 *Hematodinium* infection of *Nephrops norvegicus* pleopod. Pleopod assessment is used for diagnosis and staging severity. Cells similar in size to the hemocytes are seen as darkened areas due to dense aggregations using compound light microscopy. Intensely red pigmentation is due to carotenoid pigment released from the hepatopancreas during disease. This reddening can be observed macroscopically but is considered a less reliable indicator of disease. *Source:* Image kindly provided by Irene Molto-Martin, Institute of Aquaculture, Stirling and Cefas, Weymouth.

Transmission: Transmission is thought to occur horizontally through release of spores from infected animals.

Signs/diagnosis: Sometimes described as "bitter crab disease" due to the impact infection imparts on the taste of these animals, this disease causes high mortalities, with seasonal mortalities or even near eradication of stock occurring in wild crustaceans. Other clinical signs include altered carapace coloration, as well as paling and necrosis of muscle tissues. Hemolymph is cloudy, and animals can display altered swimming behavior and lethargy.

Dead animals sink to the bottom and are not observed unless washed upon the shoreline in wild populations. Infections can therefore go unnoticed in juveniles and females not targeted by fisheries. Diagnosis can be achieved using wet smears, histology, and molecular detection techniques. Neutral red stains *Hematodinium* well for wet mounts, although histology can allow observation of parasites in multiple tissue types.

Differentials: Pallid muscle due to glycogen depletion can occur in many chronic conditions, as well as naturally with seasonal variation. Pallid shell appears similar to recently molted individuals. Clouded hemolymph in crabs can be due to a number of systemic diseases, including *Paramoeba* and bacterial infections.

Treatment: Currently, there are no practical treatments or means of prevention.

Paramoeba perniciosa

Overview: *Paramoeba perniciosa* causes paramoebiasis (also known as "gray crab disease") in multiple *Crustacea* and is of concern to fisheries globally.

Host range and distribution: Infection occurs in crabs (*Callinectes sapidus* and *Cancer irroratus*) and lobsters (*Homarus americanus*).

Transmisison: Transmission appears to be horizontal through contact.

Signs/diagnosis: Infection causes pallid or a grayish discoloration of the shell, often accompanied by altered behavior ranging from reduced activity to flaccid paralysis. Reduced or absent clotting of the hemolymph can be indicative of disease presence, and high mortalities can be observed following stressful events. Parasites can be observed in squash preparations, hemolymph smears, and histology, as well as being diagnosed by PCR.

Differentials: Diseased species may provide an indication of which condition to expect; however, cloudy hemolymph and altered shell coloration are both symptoms seen in paramoebiasis and *Hematodinium* infections in crabs. Systemic infections of bacteria or viral diseases can also cause mass mortalities such as can be seen with paramoebiasis.

Treatment: There are currently no methods of treatment or control for this organism.

Zoonoses

Handling shellfish often leads to cuts or mild abrasions of the skin because of their tough shells and ability to pinch. Similar to mycobacterial infection from tropical fish, care must be taken to avoid infection of the skin with *Erysipelothrix rhusiopathiae*, particularly from lobsters. If infection does occur, a red-purple circular lesion will develop, progressing to a blister. Systemic infection leading to sepsis is rare but possible.

Like many aquatic organisms, crustaceans can also pose a risk to human health if improperly cooked or prepared. Bacterial infections such as *Vibrio* can be transmitted from crustaceans to human hosts to cause gastritis, and digenea of the *Paragonimus* species are considered zoonotic from uncooked crayfish. Crayfish-related rhabdomyolysis (Haff disease) is a condition reported in humans following consumption of crayfish but the toxicity is currently unclear. Similarly, to bivalves, toxic algae may be a concern (although less so than in filter-feeding mollusks) and *Paragonimiasis* (lung fluke) can also be contracted by eating uncooked crayfish or crabs.

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Appendix 1.1

Etiology and clinical signs: coelenterates

Condition/body part affected	Etiology/clinical signs
Cnidarians	
Scyphozoa (jellyfish)	
Sudden death	Toxicity, trauma, extreme water quality parameters
Poor growth	Inadequate nutritional quality, inadequate food supply, loss of symbiotic bacteria, water pollution
Major diseases:	
Trauma	Nearly any species is susceptible. Healing and even regeneration is possible. The term "symmetrization" has been used to describe injured jellyfish healing and regaining their radial symmetry
Toxicity	Heavy metals like copper are known to be toxic to medusae and polyps
Aging	In some cases, it is possible that animals are merely undergoing senescence, although, it is very easy to make this "diagnosis" despite the fact that husbandry-related problems could be in play
Infectious diseases	Numerous species of bacteria have been cultured from jellyfishes, but in many cases these may represent normal flora. *Bacillus* spp. have been associated with moribund sea nettles (Doores and Cook, 1976). No viral or fungal diseases have been reported in jellyfish
Parasitic diseases:	
Nematodes	Larval nematodes of the genus *Thynnascaris* have been found associated with several species of jellyfish but successful treatment has not been reported
Digenean trematodes	Jellyfish can serve as an intermediate host for digenean trematodes, with the first host being a mollusk. In most cases the definitive host is a vertebrate such as a fish. It is not clear what clinical affect these infections have on the jellyfish
Cestodes	Cestode plerocercoids have been widely reported in jellyfish but their clinical impact is not well understood. It is likely that fish are the definitive hosts for many of these infections
Amphipods	The parasite of most concern in captive situations are the hyperiid amphipods. It appears the amphipods use the jellyfish as a haven for seasonal reproductive efforts (Crossley et al., 2009; Boonstra et al., 2015; Newton and Smolowitz, 2020). Treatment includes manual removal and chitin synthesis inhibitors like diflubenzuron (Crossley et al., 2009). Milbemycin oxime (0.66 mg/l) has been used to successfully treat *Hyperia medusarum* in the crystal jellyfish (*Aequorea victoria*) (Boonstra, 2015)
Isopods	Isopods have been reported in jellyfish and include the infestation of *Catostylus mosaicus* with *Cymodoce gaimardii* (Browne, 2017)
Crustaceans	Barnacles and decapod crustaceans are commonly found "hitch-hiking" in medusae of many jellyfish species. While some of these relationships may be deleterious to the host, others may be neutral, or even symbiotic (Yusa et al., 2015)

Condition/body part affected	Etiology/clinical signs
Anthozoa (sea anemones)	
Sudden death	Toxicity, trauma, extreme water quality parameters
Poor growth	Inadequate nutrition, inadequate lighting, inadequate water flow/motion, water pollution, loss of symbiotic bacteria
Trauma	All species are susceptible. Healing and regeneration occurs in many species
Major diseases:	
Toxicity	Heavy metals like copper are known to be toxic to anemones (Patel et al., 2014)
Aging	In some cases, it is possible that animals are merely undergoing senescence, although, its very easy to make this "diagnosis," despite the fact that husbandry-related problems could be in play
Inadequate environment	Anemones require at least some water current to respire, feed, and expel waste
Infectious diseases	No viral or fungal diseases have been reported in true anemones. Hydra adenovirus causes disease in the form of decreased growth in the hydrozoan *Hydra vulgaris* (Van Etten et al., 1982). There is no known treatment. It appears that anemones are not susceptible to at least some of the bacteria (*Vibrio* sp.) that affect their coral cousins based on in vivo studies (Zargoza et al., 2014). However, one laboratory study found that *Serratia marcescens* results in clinical disease in *Aiptasia pallida* (Krediet et al., 2014)
Parasitic diseases	Protozoal parasites have the potential to impact anemones. *Helicostoma nonatum*, a marine ciliate, has been implicated in "brown jelly infection" of corallimorph anemones (Sprung and Delbeek, 1997). It is possible, if not likely, that this is an opportunistic infection secondary to trauma
Anthozoa (corals)	
Sudden death	Toxicity, trauma, predation, extreme water quality parameters
Poor growth	Inadequate nutritional quality, inadequate food supply, loss of symbiotic bacteria, water pollution, interspecific competition
Brown jelly presence	A brown gelatinous material appears suddenly on the surface of a coral or soft coral. The material is the product of necrosing polyp tissues, and bare skeletal material will become visible as the condition progresses
Coral bleaching (loss of symbiotic zooxanthellae)	Increased temperature, pH, carbon dioxide; decreased light, wave action/water current; other water quality aberrations, bacterial infection, fungal infections
Goniopora slow wasting	The lower marginal polyps stop expanding and then die. The condition can progress vertically until all polyps are gone
Gorgonian sloughing	This spontaneous condition starts at the base, the tissue becomes soft and spongy, and then separates from the dark core
Large polyp recession	Apparently healthy large polyp corals begin to lose tissue at the polyp margins. Algal growth or brown jelly reactions may follow
Leather coral collapse	Distinct from normal shedding of leather corals. The polyps stop expanding, darken, after several weeks the tissue loses integrity, and degenerates. Rotting tissue ulcers appear on the capitulum or stalks and increase in size

(*Continued*)

Condition/body part affected	Etiology/clinical signs
Mushroom shrink	Corallimorphs shrink, cannot fully expand, and appear mottled
Polyp coagulative necrosis	Polyps becomes soft, display an external white friable deposit, and usually progress to death
Polyp extrusion	This is similar to bleaching, but small spherical structures (intact polyps) float out from the coral's skeleton. This is common and normal for pocilloprids but can also be a response to disease or stress
Polyp shutdown	Colonial polyps do not open for prolonged periods even after water changes
Soft coral collapse	Loss of turgor; the coral may wilt or even collapse
Stony coral tissue loss	Known as stony coral tissue loss disease, this disease syndrome has caused major stony coral mortality in Florida since 2014 (Aeby et al., 2019). To date, an etiological agent(s) has not been identified
White film	Excessive mucus, when draped over the coral, appears like a white film. White film can be a response to chemical irritation, rough handling, or areas of anaerobic decay
White paste	More extreme than white film. This aggressive response to irritants results in a thick white pasty substance engulfing stony corals
Xeniid melt	Healthy soft corals, particularly in the family Xeniidae, melt into a pile of small fragments and stumps within 24 hours
Trauma	Nearly all species are susceptible. Healing and regeneration is possible and probably common if the environmental conditions are good
Toxicity	Heavy metals like copper and zinc are known to be toxic to coral polyps of many species (Bilemyer-Fraser et al., 2018; Deschaseaux et al., 2018). Other heavy metals, such as iron, nickel, and vanadium are also toxic to at least some reef-building corals (Jafarabadi et al., 2018)
Infectious diseases	This is a very complicated area but one of much active research. Rarely have Koch's postulates been fulfilled for any soft or hard coral infectious disease. Mohamed and Sweet (2019) provide a comprehensive review that includes a very helpful and fully referenced table. Bacterial organisms that have been associated with specific disease are *Aurantimonas* spp. and *Sphingomonas* spp. (white plague types II and III), *Geitlerinema* spp., *Leptolyngbya* spp., *Oscillatoria* spp., *Pseudoscillatoria* spp. (black band disease), Serratia marcescens. (white pox disease), *Vibrio* sp. (yellow band disease), *Vibrio harveyi* and *Vibrio alginolyticus* (rapid tissue necrosis), *Vibrio coralliilyticus* and *Vibrio shiloi* (bacterial bleaching), *Oscillatoria* spp. (red band disease), *Vibrio* spp. (ulcerative white spot disease), *Vibrio charcharia* (white band disease type II), *Vibrio* spp. (porites white patch syndrome). A number of fungal organisms have been associated with coral disease. These include *Aspergillus sidowii* (aspergillosis), *Rhystisma* spp., (dark spots syndrome), *Curvularia l*unata (pink line syndrome – also includes *Phormidium valderianum*, a cyanobacterium)
Parasitic diseases	Protozoans are known to cause disease in corals. *Philaster* sp., a ciliate, has been implicated in brown band disease and brown jelly syndrome. *Halofolliculina* spp. has been linked to Caribbean ciliate infection. *Halofolliculina corallasia* is the causative agent for skeleton eroding band of *Acropora* and *Pocillopora* spp. *Amakusaplana acroporae*, *Acropora*-eating flatworm. *Tegastes acropranus*, red bug. *Heterotrich* spp. (ciliate). *Phestilla* sp., *Montipora*-eating nudibranch, *Dendrophilia*-eating nudibranch. *Polyclad* spp., *Euphyilla* flatworms. Zoanthid-eating nudibranch. *Pycnogonida* sp., zoanthid sea spider. *Convolutriloba retrogemma*, red planaria. *Vermetidae* spp. vermentid snail

Condition/body part affected	Etiology/clinical signs
Hydrozoa	
Hydras, colonial hydroids, colonial pelagic jellies (Portuguese Man-o-War)	Little is known about the pathology and diseases of this phylum
Ctenophora	
Comb jellies	Little is known about the pathology and diseases of this phylum
Urochordata	
Sea squirts, salps	
Infectious diseases	Secondary invasion of bacteria from injury or decomposition of dead epibiota
Cup cell diseases	Parasitic disease caused by a haplosporidian. Affects Botryllus schlosseri
Soft tunic syndrome	Thinning, softening of the body wall (tunic) of cultured *Halocynthia roretzi*. Causative agent is the kinetoplastid *Azumiobodo hoyamushi*
Mollusca	
Bivalves (oysters, mussels, clams, abalone)	
Sudden death	Parasites (*Haplosporidia*/*Minchinia*, *Bonamia*, *Hexamita*), transmissible hemocytic neoplasia, *Vibrio*, *Oyster herpesvirus*, acute viral necrosis of scallops, iridoviruses, malacoherpesvirus
Poor growth	*Perkinsus*, insufficient food
Larval death	Velar virus disease (iridovirus), vibriosis
Biofouling	Generalized sign of disease
Ulcers/pustules	Mikrocytos, *Nocardia*, *Bonamia*, foreign-body haemocytic granulomas (metacercariae, nematodes, *Crustacea*, algae)
Emaciation/gaping	*Perkinsus*, *Haplosporidia*/*Minchinia*, *Marteilia*, hinge erosion (bacteria), Oyster herpes virus, *Mikrocytos*
Gills:	
Ulcers	*Bonamia* (parasite)
Nodules	*Marteila* (parasite)
Inflammation	*Trubellaria*, *Trichodina*
Brown discoloration	*Haplosporidia*
Shell:	
Deformity/poor growth	Juvenile oyster disease, shell disease (mudworm, boring mussels – *Lithophaga*, boring sponges – clina, pine, possibly fungus), *Haplosporidia*, *Mikrocytos*, thickening or marked extension of shells in oysters caused by disturbance of calcium metabolism
Mantle:	Rickettsia-like organisms (aka prokaryote inclusion bodies, *Chlamydia*-like organisms, intracellular microcolonies of bacteria)
Brown/yellow digestive gland	Necrosis (*Marteila*)
Gametes	Viral gametocytic hypertrophy, *Marteilioides chungmuensis*, metacercariae, nematodes, transmissible hemocytic neoplasia, hermaphrodites

(*Continued*)

Condition/body part affected	Etiology/clinical signs
Digestive gland	*Ancistrocoma* (incidental)
Unknown etiology	Akoya oyster disease, oyster edema disease
Cephalopods	
Sudden death	Bacterial septicemia (*Vibrio*), water quality issues (ammonia, oxygen, hydrogen sulfide), toxicosis (heavy metal-copper, herbicide, pesticide), parasites, trauma, gas supersaturation, handling stress
Poor growth, emaciation	Anorexia, insufficient caloric intake, nutritional deficiency, stress/water quality issues, heavy parasitism
Uncoordinated swimming/ nervousness	Generalized infectious disease (bacteria,fungal, protist infection), electrocution, copper toxicosis, central nervous system disease, water quality issues, gas bubble disease, parasitism, low strontium
Asphyxia	Gill disease (parasites), anemia (parasites), low dissolved oxygen, toxin exposure/ammonia, organic/inorganic contaminants
Skin/mantle:	
Petechiae	Bacterial septicemia
Increased mucus	Water quality issues, parasitism
Hemorrhage/ulcers/ necrosis	Trauma (handling, tankmate aggression/contact), improper pH, bacterial infection (*Vibrio*, *Lactococcus*, *Photobacterium*, *Pseudomonas*, *Aeromonas*, *Staphylococcus*), labyrinthulomycetes (thraustochytrid) infection, fungus (*Cladosporidium*, *Fusarium*) infection, irritant exposure, cuttlebone fracture
Parasites	Isopods, copepods, branchiurans
Nodules/lumps	Granulomatous disease (bacteria, fungi), parasites (coccidians – Aggregata), virus (iridovirus-like), foreign body
Pale spots	Acute skin injury, neurologic injury, scarring, deep infection
Edema	Trauma, skin ulcers, fatal idiopathic condition
Gills:	
Hemorrhage/ulcers	Bacterial infection (*Vibrio*), parasites, toxins
Pallor	Anemia, mild hyperplasia
Parasites	Copepods, cestodes, trematodes, coccidians
Hyperplasia/fusion	Water quality issues, chronic inflammation, parasitism (ciliates – Ancistromidae)
Gas bubbles	Supersaturation
Necrosis	High/low pH, chlorine, toxins
Nodules	Encysted trematodes, coccidians, cysts, neoplasia, bacteria (*Vibrio*, *Rickettsia*-like organisms) fungus, labyrinthulomycetes (thraustochytrid) infection, fibrosis, spermatophores (other cephalopods)
Hemorrhage/ulcers	Bacterial infection (*Vibrio*), parasites, toxins
Pallor	Anemia, mild hyperplasia
Increased mucus	Water quality issues, bacterial infection, parasites
Eyes:	
Exophthalmia/ buphthalmia	Bacterial septicemia, trauma, gas bubble disease/supersaturation, idiopathic

Condition/body part affected	Etiology/clinical signs
Ulcers/hemorrhage	Fungus, bacterial septicemia, viral infection, trauma
Corneal edema	Poor water quality, sepsis, fungus, labyrinthulomycetes (thraustochytrid) infection
Ophthalmitis	Trauma, bacteria (*Lactococcus*, *Photobacterium*), hematogenous spread of pathogens
Cataract	Rapid osmolar fluctuations, gas bubble disease
Gas bubbles	Supersaturation, bacterial infection, corneal perforation
Nodules	Granulomas (bacteria, fungi, parasite
Enucleation	Trauma, severe ophthalmitis
Heart:	
Inflammation/pericarditis	Bacterial sepsis (*Vibrio*)
Nodules	Granulomas (bacteria, fungus)
Skeleton:	
Cuttlebone fracture	Trauma
Deformity	Trauma, hereditary, nutritional deficiency, adverse environment conditions, toxicant exposure, healing cuttlebone fracture
Loss of limb	Autophagy (self-destruction)
Small cuttlebone	Hypercapnia
Stomach	Ulcers
Digestive tract:	
Nodules	Coccidians, encysted trematodes, encysted cestodes
Connective tissues	Coccidians, encysted cestodes, encysted nematodes, fibrosis, neoplasia
Crustacea (shrimps, lobsters, crabs, crayfish)	
Sudden death/ lethargy	Viruses (many), bacteria (*Vibrio*, *Micrococcus*, *Aerococcus*, *Rickettsia*), water quality, *Aphanomyces*, aflatoxin, pesticides, herbicides, ultraviolet light
Floating	Gas supersaturation
Milky hemolymph	Bacteria (*Rickettsia*), microsporidians, dinoflagellates, scuticociliates, amoeba
Dwarfism/deformity/poor growth	Viruses (IHHNV, hepatopancreatic parvovirus, monodon slow growth syndrome), vibriosis, diet
Cramped tail	Nutritional imbalance
Muscle opacity	Viruses (iridovirus, totivirus, nodavirus), microsporidians (Ameson), muscle necrosis (exertion/stress), vibriosis, trematodes, asbestos fibers, selenium deficiency, *Aphanomyces*
Cuticle:	
Black spots	Melanin production (inflammation: bacterial sepsis – *Vibrio*, especially secondary to viral disease), fungus, *Lagenidium*, virus (Taura syndrome), parasite (microsporidians), Aphanomyces, water quality); ultraviolet light
Yellow spots	Viruses, bacteria

(*Continued*)

Condition/body part affected	Etiology/clinical signs
White spots	white spot virus, mineral deficiency (water quality), bacteria (Bacillus), phosphatidylcholine deficiency
Pink coloration	Chromatophore expansion, especially of telson, due to stress, sepsis
Mucus	Bacteria
Ulcers	Bacteria (*Vibrio*, *Mycobacterium*), fungi, neoplasia, ultraviolet light, *Aphanomyces*
Biofouling of gills, cuticle	Poor water quality, generalized debility from disease
Dysecdysis	Multiple causes, especially viruses, phosphatidylcholine deficiency
Nodules	Granulomas, neoplasia (rare)
Soft shell	Nutritional imbalance, rancid feeds, pesticides
Luminescence	Luminescent vibriosis
Gills:	
Biofouling	Poor water quality, generalized debility from disease
Black spots	Inflammation (bacteria, fungi, protozoans), sediment, heavy metals (iron, cadmium, chromium, copper), oxidants (chlorine, potassium permanganate), ultraviolet light, gas supersaturation, ammonia, acids, crude oil, ascorbic acid imbalances
Parasites	Isopods
Hepatopancreas:	
Nodules	Granulomas (bacterial, parasite – cestode, trematode, fungus)
Yellow/pale	Can be normal, necrosis (yellowhead virus), microsporidians
Atrophy	Emaciation, viral infection, bacterial infection (*Vibrio*, spirochetes)
Dark	Emaciation/atrophy, lack of lipid
Speckles (various colors)	Granulomas – bacteria, microsporidians, fungus
Gut:	
Darkened	Vibriosis, parasites
Red feces	Virus (suspected parvovirus)
Parasites	Gregarines, cestodes, nematodes
Antennal gland nodules	Granulomas (melamine)
Gonads: black spermatophore (penaeids)	Unknown etiology
Eye: Corneal plaques	Unknown etiology