

Cleaner fish in aquaculture: review on diseases and vaccination

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Abstract

Combating and controlling sea lice causes large economic costs for the farmers, with estimated values of more than 305 million euros (€) per year. Increased resistance against traditional chemotherapeutants due to evolutionary drivers in the sea lice combined with the lack of an effective vaccine and few other chemical treatments available are expected to cause these costs to increase. Several possible methods for managing sea lice infestations have been investigated, but only cleaner fish has proven to have an effect on lice levels. Cleaning activity is well known in marine fish and has been observed in the wild as a form of symbiosis between two species: one species, the ‘client’ fish, seek out the other species, the ‘cleaner’ fish, to have ectoparasites and dead tissue cleared from its body. The Atlantic lumpfish is a relatively new aquaculture species, and wild-caught mature fish are used as brood stock for farmed production. This poses a biosecurity risk, as wild fish can carry pathogens, and the use of quarantine and health screening is recommended. Vaccine development is unfortunately lagging far behind relatively to the wide spread and high utilisation of the fish. This review contains description of the main pathogens and diseases that affect cleaner fish.

Key words: Atlantic lumpfish, cleaner fish, diseases, health management, vaccination, wrasse.

Introduction

Aquaculture is currently one of the fastest growing food sectors in the world, with the majority being finfish production. The total world fish production is expected to reach 196 million tons (Mt) by the year 2025, where aquaculture is estimated to surpass the total production of capture fisheries. The majority of growth will take place in developing countries, where freshwater species is expected to become more important. However, the capture sector is expected to remain dominant for a number of fish species and still be vital for supplying seafood both locally and globally (Organisation for Economic Co-operation and Development/Food and Agriculture Organization of the United Nations, OECD/FAO (2016)).

In 2018, the global production of farmed salmonids exceeded 2.36 million tons, while the total catch volume of wild salmonids was a bit more than 1/3 of that size. Atlantic salmon (*Salmo salar* L.) is produced in high amounts and is used for smoked, fresh, sushi and ready-made meals. Farmed Atlantic salmon is produced in Norway, Chile, UK,

North America, Faroe Islands, Ireland, New Zealand and Tasmania (MOWI, 2019). These are the countries which produce most of the salmon. Several fish health-related issues inhibit continued industry growth, however the main challenge being ectoparasitic infestation by the copepod (small crustaceans) sea lice (Jones *et al.*, 2015; Treasurer, 2018b). Several species exist, but the majority of disease outbreaks in the Atlantic Ocean are caused by *Lepeophtheirus salmonis* (specific for salmonids) and *Caligus elongatus* (generalist; less host-specific) (Boxaspen, 2006). Other species have been described on salmonids in the Pacific Ocean, such as *Caligus rogercresseyi* in Chile (Boxs hall & Bravo, 2000).

When attached to a host (Figs 1 and 2), the parasite use rasping mouthparts to feed on mucus, skin, blood and underlying tissue (Costello, 2006; Thorstad *et al.*, 2015). This leads to tissue damage/loss, bleedings and increased mucus discharge from the host's skin, eventually causing the host to suffer from reduced growth, loss of bodily fluids, stress, reduced osmoregulatory and respiratory ability, impaired body defences, risk of secondary infections and,

ultimately, death (Whelan, 2010; Thorstad *et al.*, 2015). Combating and controlling sea lice causes large economic costs for the farmers, with estimated values of more than 305 million euros (€) per year (Costello, 2009). Increased resistance against traditional chemotherapeutants due to evolutionary drivers in the sea lice (Denholm *et al.*, 2002; Aaen *et al.*, 2015; Helgesen *et al.*, 2018), combined with the lack of an effective vaccine and few other chemical treatments available, is expected to cause these costs to increase (Powell *et al.*, 2018; Brooker *et al.*, 2018). For the Norwegian salmon farming industry alone during 2016, the total costs for controlling, preventing and treating sea lice were close to 5 billion NOK (about 500 million €) (Iversen *et al.*, 2017). Only two years later, in 2018, that number had risen to 5, 2 billion NOK, which was a five-fold increase since 2011 (Berghlin, 2019b). Also considering the threat the parasite poses for wild stocks of salmonid fish (Forseth *et al.*, 2017; Thorstad & Finstad, 2018; Nekouei *et al.*, 2018; Kristoffersen *et al.*, 2018), it becomes clear how sea lice currently is one of the major challenges for the aquaculture industry to overcome.

Integrated pest management was introduced to salmon farming in 2002 (Mordue & Pike, 2002), an ecosystem approach that was already used for healthy crop productions in agriculture. It integrates different management strategies and practices to suppress and keep pest populations below the crop's economically sustainable limits, while keeping the use of pesticides and other interventions to levels that minimise risks to humans and the environment. It also encourages the use of natural control mechanisms. The same principles are important for combating sea lice, and several non-medicinal methods have been developed as alternatives to chemical removal. This includes barriers between the sea



Figure 1 Atlantic salmon infested with sea lice at different developmental stages. Adult female (fat arrow), and different chalmus stages can be observed (↑). Photograph: Mattias B. Lind (Norway Royal Salmon Ltd.).



Figure 2 Adult female sea lice bearing egg strings. Photograph: Tore Seternes.

lice and the salmon (e.g. skirts, snorkel cages, bubble curtains), anti-sea lice diets (that strengthen the fish natural defence system or affects the lice), traps, lasers, thermal treatment and different forms of mechanical removal of the lice (e.g. water flushers). Other methods under development/investigation include ultrasound and freshwater treatments (Global Salmon Initiative; Aaen *et al.*, 2015; Holan *et al.*, 2017). In Norwegian salmon farming, the number of such non-medicinal approaches has increased in recent years, the majority being from use of thermic delousing (Helgesen *et al.*, 2018). However, such methods are not without issues of its own, which affect the health and welfare of the treated salmon (Poppe *et al.*, 2018).

Another strategy is to apply biological pest control by natural enemies. This is the utilisation of other living organisms, such as parasites, pathogens and predators, for controlling pests, by using their beneficial actions (parasitism, infections and predation) to manage pest invasions (Treasurer, 2002). Several possible methods for managing sea lice infestations have been investigated, but only cleaner fish has proven to have a deleterious effect on lice levels (Treasurer, 2002). Cleaning activity is well known in marine fish and has been observed in the wild as a form of symbiosis between two species: one species, the 'client' fish, seek out the other species, the 'cleaner' fish, to have ectoparasites and dead tissue cleared from its body. This mutually beneficial association apparently results in the client fish having its parasite burdens removed while the cleaner fish receives a source of food (Hobson, 1969; Grutter, 2001; Arnal *et al.*, 2001; Leung & Poulin, 2008). In salmonid aquaculture, different species of European wrasse (Labridae), such as ballan (*Labrus bergylta* Ascanius), goldsinny (*Ctenolabrus rupestris* L.), corkwing- (*Symphodus melops* L.), wrasses and Atlantic lumpfish (*Cyclopterus lumpus* L.) (Fig. 3), are used for this specific purpose (Treasurer, 2002; Erkinharju, 2012; Powell *et al.*, 2018; Imsland *et al.*, 2018a). In 2018, a total of near 49 million cleaner fish were put to sea together with salmon and rainbow trout in

Norway, of which near 31 million were lumpfish. The majority of these originate from commercial lumpfish production (approximately 93%), while the rest are wild-caught (Norwegian Directorate of Fisheries 2019).

The lumpfish is a relatively new aquaculture species, and wild-caught mature fish are used as brood stock for farmed production. This poses a biosecurity risk, as wild fish can carry pathogens, and the use of quarantine and health screening is recommended (Powell *et al.*, 2018; Brooker *et al.*, 2018; Scholz *et al.*, 2018a). Once hatched, the lumpfish can display rapid growth rates (up to 3.65% per day) under optimal conditions (Nytrø *et al.*, 2014). This makes it possible to produce lumpfish of deployment size within a few months (about 5–7 months for 20 g fish), which is significantly shorter than farming ballan wrasse (about 18 months for 40–50 g fish) (Powell *et al.*, 2018; Brooker *et al.*, 2018). Ballan wrasse, however, are considered to be extremely efficient in delousing salmon (Skiftesvik *et al.*, 2013). Lumpfish have in studies shown to lower sea lice infestation levels on affected salmon (Imsland *et al.*, 2014a; Imsland *et al.*, 2018a), but the species feeding behaviour is strongly opportunistic, and as such can vary with whatever food items are presentable (Imsland *et al.*, 2014c; Eliassen *et al.*, 2018). Sea lice foraging behaviour might be possible to improve using different implementations strategies (Imsland *et al.*, 2016a; Imsland *et al.*, 2016b; Imsland *et al.*, 2019a). Recently, the current knowledge base of lice removal by cleaner fish has come under scrutiny for being far too limited and not reflecting the conditions within sea cages on commercially scaled levels (Overton *et al.*, 2020). More species-targeted, detailed and replicable experiments performed at larger scaled levels under various environmental conditions and farm management procedures are among the recommendations for additional studies needed

to address the concerns and justify the continued use of cleaner fish in aquaculture (Overton *et al.*, 2020).

Lumpfish is preferably deployed in sea pens when the water temperatures are low, as the fish continue to actively feed at temperatures close to 4°C (Nytrø *et al.*, 2014; Eliassen *et al.*, 2018), and it has been suggested that higher temperatures (>10°C) make them more susceptible to diseases (Nordstrand *et al.*, 2017; Ronneseth *et al.*, 2017; Brooker *et al.*, 2018). Interestingly, a recent study observed that keeping mature lumpfish at a temperature level of 14°C resulted in notable reductions to their reproductive performances and that an optimum holding temperature for spawning would be within the >6°C and <14°C thermal window (Pountney *et al.*, 2020). Wrasse are also affected by temperature, showing reduced activity at lower levels and eventually entering torpor, a state of reduced physiological activity, at temperatures below 5–7°C (Sayer & Reader, 1996; Espeland *et al.*, 2010). As such, lumpfish have been suggested as a cold-water cleaner fish, better suited to remove sea lice when deployed at the northernmost salmon farms and during the autumn/winter season (Imsland *et al.*, 2014a). However, the infestation pressure or dispersion of sea lice in northernmost coastal areas, with low sea water temperature, may be lower; this may limit the use of cleaner fish in salmon aquaculture (Myksvoll *et al.*, 2018).

In order to ensure healthy and well-kept cleaner fish, the aquaculture sector has established their own guidelines for good farming practices (available at Lusedata, n.d.). Important factors for lumpfish in sea pens include shelters, feeding, clean nets, stocking density and health checks. Shelters are essential for providing refuge and a place to rest for the lumpfish when it is not actively searching for feed or feeding (Imsland *et al.*, 2014c). Artificial substrates appear to function just as good as natural variants, as long as they

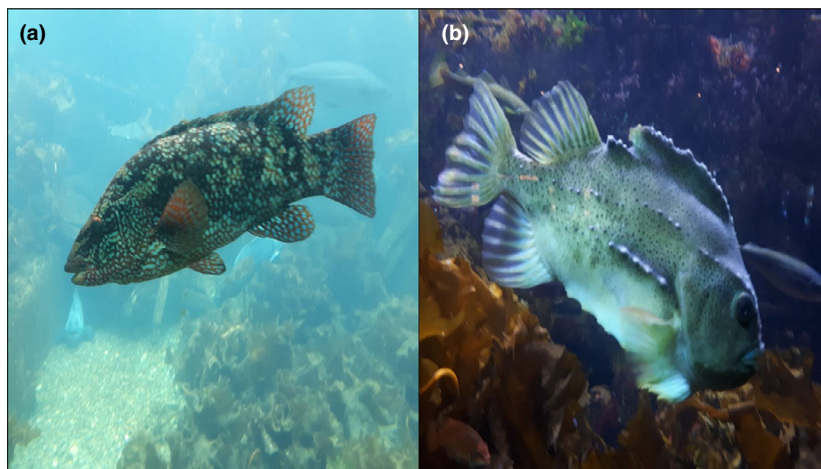


Figure 3 Two commonly used fish species for cleaner fish management in salmonid farming. Ballan wrasse (a) and Atlantic lumpfish (b). Photographs taken at Bergen Aquarium, Norway. Photograph: Toni Erkinharju.

have smooth, vertical surfaces for the lumpfish to attach to (Imstrand *et al.*, 2015). Supplementary feeding is necessary during periods with low lice counts, to ensure that the cleaner fish stay healthy and fit (Skiftesvik *et al.*, 2013; Imstrand *et al.*, 2019b). Anecdotal evidence from the field also indicates that lumpfish will search for alternative food sources, or nibble on the fins and skin of salmon, if not fed properly (Lusedata, n.d.). Regular cleaning of nets is also recommended; otherwise, the cleaner fish might eat fouling organisms on the net instead of sea lice (Skiftesvik *et al.*, 2013). There are no clear recommendations for optimal stocking densities of lumpfish together with salmon, and ratios vary from 2–5% (Brooker *et al.*, 2018). Imstrand *et al.* (2014b) reported that differences in cleaner fish size, rather than stocking ratios, had negative effects on feed conversion ratio, specific growth rates and sea lice infection levels for cohabitated Atlantic salmon. In this study, cleaner fish size of 360 g (average) duocultured with large salmon (ca. 2.4 kg) resulted in higher feed conversion rate and lower specific growth rate – compared to results where smaller salmon (average weight of 619 g) was reared with smaller lumpfish (54 g). However, any size recommendation for cleaner fish to be cultured along the salmon has not been provided. The current practise is culling lumpfish after only one salmon production cycle. This has received harsh criticism by animal welfare organisations (Powell *et al.*, 2018).

Finally, monitoring of health and welfare through routine checks and investigations of the cleaner fish are important to keep the prevalence of diseases as low as possible and ensure the natural behaviour of the fish are maintained (Brooker *et al.*, 2018, Lusedata). Establishing proper indicator levels for each cleaner fish species are as such helpful. Lumpfish for instance does not show a clear stress response compared to salmonids, which is possibly due to evolutionary adaptations in the species (Hvas *et al.*, 2018; Espmark *et al.*, 2019). A recent study suggested using liver colouration as a welfare indicator of lumpfish, with increased incidence of dark reddish-brown livers being a sign of reduced lipid reserves and nutritional status, while pale livers could be an indication of a struggling immune system, such as caused by disease (Eliassen *et al.*, 2020). In addition, both lumpfish and wrasses swim poorly in fast currents compared to salmon, which might be challenging at farming localities with strong water currents (Hvas *et al.*, 2018; Hvas *et al.*, 2019).

Atlantic lumpfish

The Atlantic lumpfish, also called lumpsucker (*Cyclopterus lumpus*, Linnaeus 1758), is a marine teleost belonging to the order Scorpaeniformes, family Cyclopteridae (Davenport, 1985; Nelson *et al.*, 2016). It is morphologically

distinct from other fish. A compact, near spherical body shape with vivid skin colouration is observed in sexually mature specimens; males typically display red, orange or purple colour, while females are usually grey or blue-green. On the dorsal side of the body, the anterior fin is covered by thick skin, which forms a long and high crest with compressed, bony tubercles that increases in height with age, giving the back a more humped appearance in older fish. Compressed, pointed tubercles are adorning the lateral sides of the fish in three longitudinal rows (upper, middle and lower) along its body length. No lateral line is present. On the ventral side of the body, between the pectoral fins, is a suction disc that is a modification of the pelvic fins, which the fish use to adhere to the substrate in its environment. Lumpfish have a peculiar skin structure, scale less, with a high proportion of subcutaneous gelatinous tissue, giving it a rubbery texture. The majority of the skeleton, except for the skull, is ossified but has been perceived to be of a cartilaginous nature, which initially led the species to be classified together with the elasmobranch fish (Hase, 1911; Davenport, 1985; Budney & Hall, 2010; Nelson *et al.*, 2016). Specific features to note internally (Fig. 4) include a long gastro-intestinal tract with numerous pyloric caeca, a bi-lobed anterior kidney, lack of a swim bladder and a large urinary bladder, especially in adult females (Davenport, 1985; Budney & Hall, 2010; Nelson *et al.*, 2016; Treasurer, 2018a).

The lumpfish is typically found in colder regions of the northern hemisphere. Adults are semi-pelagic and spend most of the year freely swimming out in the open ocean, often over abyssal depths and across distances of several kilometres (km) per day. They return to the shoreline during springtime for spawning, which occurs in shallow



Figure 4 Internal organs of juvenile lumpfish with the left operculum and abdominal wall removed. Left gill (G), the heart (H), the liver (L), bands of pyloric caeca (P), posterior kidney (K), part of the stomach (St), parts of the intestine (I), layers of skeletal musculature (Mu) and parts of the skin (Sk) are visible. External features, such as the mouth (Mo), left eye (E), the dorsal hump (DH), several bony tubercles (Tu), the tail (Ta) and some fins (F) are also noticeable. Photograph taken at Tromsø Aquaculture Research Station. Photograph: Toni Erkinharju.

coastal waters. Females lay their eggs as sizeable masses on to the surface of a nest, while males start fertilising the eggs immediately. During incubation, the eggs receive paternal care and guardianship from the male fish. Upon hatching, the larvae disperse rapidly with the water currents within a short time. They remain in shallow, warmer water until they are fully developed adults (Davenport, 1985; Kennedy *et al.*, 2015; Bakketeig *et al.*, 2017; Treasurer, 2018a). During this time, hatchlings mainly feed on surface plankton and then switch to larger invertebrate fauna upon reaching juvenile life stages. They are opportunistic as juveniles and adults and display a variable diet, including jellyfish, small crustaceans, insects, young fish and seagrass – and also copepods. Some have even shown preference for the salmon feed pellets, when kept in sea cages together with salmon (Davenport, 1985; Davenport & Rees, 1993; Ingólfsson & Kristjánsson, 2002; Vandendriessche *et al.*, 2007; Rusyaev & Orlov, 2014; Imsland *et al.*, 2014c; Treasurer, 2018a).

Wild lumpfish populations are regarded as abundant, distributed across both sides of the North Atlantic Ocean in coastal areas with 32 000 km of coastline (Fig. 5) (Powell *et al.*, 2018; Treasurer, 2018a). The Western distribution: as far north as the island of Disko off the west coast of Greenland; from there southwards along most of eastern Canada, down to the Chesapeake Bay area in eastern USA. On the Eastern side: in northern parts of Europe, including Jan Mayen, the Svalbard archipelago and east across the Barents Sea to Novaya Zemlya in northern Russia; from there southwards along countries bordering the North Sea (especially Iceland, Norway, the Faroes, the UK, Ireland and France), down to the Iberian peninsula, just off the Algarve coast in southern Portugal (Davenport, 1985; Holst, 1993; Vasconcelos *et al.*, 2004; Eriksen *et al.*, 2014; Treasurer, 2018a). There have also been records of lumpfish observed in the Mediterranean Sea, near the coast of Croatia; however, this has been reported as a probable case of a vagrant specimen (Dulcic & Golani, 2006; Katsanevakis *et al.*, 2020). In Norway, the largest natural stocks are considered to be just off the coast of Nordland, Troms and Finnmark county, although the fish can be found along the entire Norwegian coastline (Bakketeig *et al.*, 2017). Despite the species having a wide geographic distribution, and being of considerable economic value, there is limited knowledge available on the genetic structure of lumpfish populations from different origins. One study identified three distinct genetic groups: Maine-Canada-Greenland, Iceland-Norway and Baltic Sea, with limited detections of gene flow between them (Pampoulie *et al.*, 2014). However, there are still several knowledge gaps for other areas of the Atlantic Ocean (Powell *et al.*, 2018).

Lumpfish had little commercial value up until the late 20th century, when several countries in the North Atlantic

began large-scale targeting of female fish for their roe, starting with Iceland and Norway in the 1940s-1950s, Canada in the 1970s and Greenland in the 1990s (Davenport, 1985; Kennedy *et al.*, 2019). The eggs are marketed as either whole roe or separated from the ovaries and further processed into lumpfish caviar, with Iceland, Canada and Norway as major producers (Johannesson, 2006). Apart from the roe, the fish carcass has low economic value and is usually discarded at sea. The fishery is seasonal, and timing can vary between different areas. Participation has been declining in all countries except Iceland in recent years, with a price drop on lumpfish roe compared to other fish species suggested as the main reason (Kennedy *et al.*, 2019). The increased use of lumpfish as cleaner fish in the salmonid aquaculture industry has in some places led to a demand for ripe females and fertilised eggs. This has also generated some concern, however, as increased lumpfish capture leads to depletion of wild stocks (Halvorsen *et al.*, 2017; Treasurer, 2018a; Kennedy *et al.*, 2019). Currently, lumpfish is listed as near threatened (NT) on the IUCN Red List (Lorance *et al.*, 2015). In order to prevent disease transmission, lumpfish deployed as cleaner fish are culled after the production cycle ends, not reused or put to alternative uses. This leads to demand for additional cleaner fish, to replenish the fish that were removed, when the next salmon production cycle begins (Erkinharju *et al.*, 2013; Anonymous, 2013; Brooker *et al.*, 2018). Some proposals for alternative use to reduce wastage include human or animal feed, reconditioning into brood stock for captive breeding or a source for extraction of valuable marine components, such as proteins, lipids, minerals and pigments (Davenport, 1985; Mudge & Davenport, 1986; Nøstvold *et al.*, 2016; Clark *et al.*, 2016; Powell *et al.*, 2018; Brooker *et al.*, 2018; Treasurer, 2018a; Dave *et al.*, 2019). However, despite the large numbers of cleaner fish used, there are large knowledge gaps concerning several factors affecting the lumpfish health and welfare under farmed conditions, such as possibility for expressing natural behaviour, optimal morphological and physiological status, the impacts of stressful situations, injuries and environmental conditions, and dietary/feeding requirements. Combined with the potential negative effects from these different factors, in addition to those from diseases and parasites, the overall welfare of cleaner fish in sea cages has been considered poor for both lumpfish and wrasse (Grefsrud *et al.*, 2019).

Wrasse (Labridae)

Wrasses (order Labriformes, family Labridae) are a family of marine fish commonly found on rocky reefs along the coastlines of the Atlantic, Indian and Pacific oceans. The pharyngeal region of wrasse and closely related species is particularly designed for food processing, with thick,

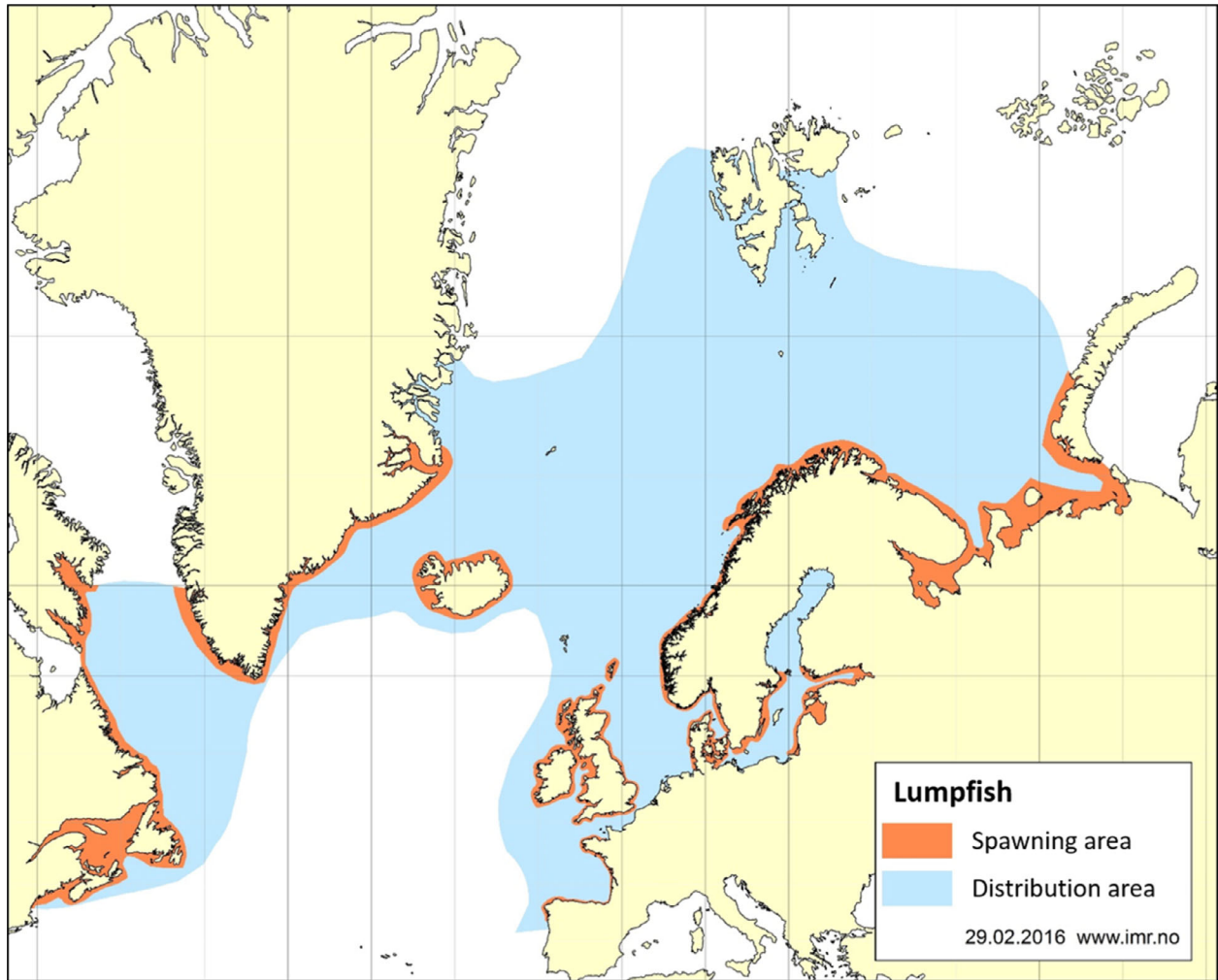


Figure 5 Spawning and distribution area of wild lumpfish stocks in the Atlantic Ocean, the North Sea, the Baltic Sea and the Barents Sea. Adapted from the Norwegian Institute of Marine Research's topic pages on Atlantic lumpfish (available at <https://www.hi.no/hi/temasider/arter/rognkjeks-rognkall>) (01.03.2020). Used with permission.

protruding lips, strong teeth and protractile mouths, suited for preying on different invertebrates, such as hard-shelled crustaceans. Protruding from the upper body is the dorsal fin with 8–21 spines and 6–21 soft rays, while the anal fin with 2–6 spines and 7–18 soft rays is located on the lower body. They possess cycloid, overlapping scales and a continuous or interrupted lateral line system (Erkinharju, 2012; Nelson *et al.*, 2016). Features to note internally include an agastric digestive system and a closed swim bladder. Most wrasse species are protogynous hermaphrodites, capable of changing sex with female-to-male change being most common under natural conditions. This can result in complex mating systems. Many species are brightly coloured, with diverse variations in colour patterns depending on the sex and size of the fish (Fig. 6). In addition to cleaner fish utilisation, several wrasse species are

commonly used as display fish in public aquaria (Erkinharju, 2012; Nelson *et al.*, 2016; Brooker *et al.*, 2018). Interestingly, the cleaner wrasse, *Labroides dimidiatus*, has recently been reported as the first fish to seemingly pass the mirror mark test, a behavioural technique used to measure and determine whether an animal possess self-awareness (Kohda *et al.*, 2019). The study has received some criticism, however, as the mirror mark tests is viewed as being too limited and simplistic, and a more gradualist model is needed to determine how different animal species, including fish, construe and position the self in the world around them (de Waal, 2019).

Initial laboratory trials for wrasse as cleaner fish began near the end of the 1980s, which later commenced with field experiments in sea cages, before commercial fishing for wrasse as lice cleaners increased, as sea lice in several



Figure 6 Cuckoo wrasse (*Labrus mixtus*) with highly colourful skin patterns. Photograph taken at Bergen Aquarium, Norway. Photograph: Toni Erkinharju.

regions developed resistance to chemical treatment (Erkinharju, 2012; Skiftesvik *et al.*, 2013). Of the three most commonly used wrasse species in Norway, ballan wrasse is highly prized for its size, hardiness and grazing efficiency. However, ballan wrasse also has the lowest natural abundance, compared to goldsinny and corkwing wrasse (Skiftesvik *et al.*, 2013; Skiftesvik *et al.*, 2014; VKM *et al.*, 2019a). Rock cook- (*Centrolabrus exoletus* L.), cuckoo- (*Labrus mixtus* L.) and scale-rayed wrasse (*Acantholabrus palloni* L.) are either less suited for captivity or are rarely caught in commercial fisheries and are as such not commonly used as cleaner fish (VKM *et al.*, 2019a). Currently, commercial farming of wrasse is still in its infancy and ballan wrasse is the species predominantly used in northern Atlantic countries (Erkinharju, 2012; Brooker *et al.*, 2018; Treasurer, 2018a).

Diseases of lumpfish and wrasse

Bacteria

Bacterial diseases are one of the major health and welfare challenges in aquaculture and the most significant cause of mortality in cleaner fish. Some are primary pathogens, while others are opportunistic, and do not cause disease in healthy individuals with an uncompromised body defence system (Nilsen *et al.*, 2014; Scholz *et al.*, 2018a). Fish pathogenic bacteria isolated from cleaner fish disease outbreaks in Norwegian aquaculture, include atypical *Aeromonas salmonicida*, different species of *Vibrio*, *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Tenacibaculum* spp. and *Moritella viscosa* (Hjeltnes *et al.*, 2019). It is expected that the number of recorded bacterial pathogens will no doubt increase (Table 1), as the use of cleaner fish in salmonid aquaculture continue to grow (Powell *et al.*, 2018).

Aeromonas salmonicida is a well-known bacterium isolated from a wide range of different fish species worldwide and the cause of typical furunculosis as *A. salmonicida* also causes atypical furunculosis (Wiklund & Dalsgaard, 1998; Cipriano & Bullock, 2001). It is considered a primary pathogen of fish, with infection often resulting in formation of skin ulcers and large boils (furuncles), and granulomas and haemorrhages in internal organs. Mortality can be quite high (Cipriano & Bullock, 2001; Roberts, 2012). A presumptive diagnosis is often made on observation of classical furuncle type lesions and/or internal granulomas, and on histological observation of characteristic bacterial microcolonies within organs of susceptible fish (Fig. 7). A definitive diagnosis requires verification of the pathogen, through bacterial isolation and/or molecular analysis (Whitman, 2004; Cain & Polinski, 2014). As for *A. salmonicida*, agglutination tests are commonly used for species confirmation but does not discriminate between subtypes (Wiklund & Dalsgaard, 1998). The species consists of several subspecies that are grouped into typical and atypical divisions. Typical *A. salmonicida*, or *A. salmonicida* ssp. *salmonicida* (ASS), is the cause of the disease furunculosis in salmonids (Hirvelakoski *et al.*, 1994; Menanteau-Ledouble *et al.*, 2016). Typical furunculosis caused mortality of lumpfish (while the cohabitated salmon appeared unaffected most probably due to vaccination against this disease) in a sea farm in mid-Norway in 2015. The cause was traced back to a local strain of ASS affecting wild salmonid populations in the area (Johansen *et al.*, 2016a). Another outbreak was reported the following year, but ASS is otherwise rarely seen in lumpfish (Hjeltnes *et al.*, 2019). Mortalities from typical furunculosis have been reported from wrasse (Treasurer & Cox, 1991; Treasurer, 2012). Atypical *A. salmonicida*, however, is often described as the largest disease challenge of both lumpfish and wrasse in many areas (Scholz *et al.*, 2018a). Recently, a new method of characterising *A. salmonicida* has emerged based on sequence variation in a region of the virulence array protein gene (*vapA*), which encodes the outer membrane protein known as the 'A-layer'. Bacterial isolates were grouped into 14 different clusters or A-layer types, with the A-layer type V and VI almost exclusively consisting of isolates from cleaner fish (Gulla *et al.*, 2016). Type VI was most prominent in lumpfish. The A-layer protein appears to be related to the pathogenicity of the bacteria, as strains lacking the protein only cause mortality of cleaner fish when other stressors are present (Ronnseth *et al.*, 2017).

Vibrio anguillarum (synonym *Listonella anguillarum*) causes the disease classical vibriosis and is a significant bacterial pathogen of marine fish (Haastein & Holt, 1972; Myhr *et al.*, 1991; Frans *et al.*, 2011). Infection initially starts with haemorrhages in the skin and musculature, which then proceeds to a generalised septicæmia (Fig. 8).

Table 1 Summary of bacterial pathogens reported from wrasse (Labridae), lumpfish (*C. lumpus*) and Atlantic salmon (*Salmo salar*) with relevant references

Bacterial pathogens	Wrasse	Lumpfish	Salmon	Comments	References
<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i> (ASS)	X	X	X	Furunculosis vaccination provide protection for salmon. Disease outbreaks are rare in all species. Transmission from salmon to cleaner fish in sea cages has been suggested.	Hjeltnes <i>et al.</i> (2019), Treasurer and Cox (1991), Treasurer (2012), Menanteau-Ledouble <i>et al.</i> (2016), Hjeltnes <i>et al.</i> (1995), Hirvelä-Koski <i>et al.</i> (1994)
Atypical <i>Aeromonas salmonicida</i>	X	X	X	Vaccination for furunculosis might provide cross-protection. Salmon appear less susceptible to atypical variants. Vaccination of cleaner fish might reduce infection pressure.	Hjeltnes <i>et al.</i> (2019), Wiklund and Dalsgaard (1998), Laidler <i>et al.</i> (1999), Frerichs <i>et al.</i> (1992), Treasurer (2012)
<i>Vibrio anguillarum</i> serotype O1	X	X	X	Vaccination provide protection for salmon. Disease outbreaks are rare in all species. Primarily serotype O1 and O2 are considered pathogenic for fish. Vaccination of cleaner fish might reduce infection pressure.	Hjeltnes <i>et al.</i> (2019), Marcos-López <i>et al.</i> (2013), Biering <i>et al.</i> (2016), Frans <i>et al.</i> (2011), Haastein and Holt (1972), Ronneseth <i>et al.</i> (2014), Myhr <i>et al.</i> (1991)
<i>Vibrio anguillarum</i> serotype O2 and O3	X	X	X	Vibriosis vaccination (<i>V. anguillarum</i> serotype O2a) provide protection for salmon. Primarily serotype O1 and O2 are considered pathogenic for fish. Vibriosis outbreaks in salmon is rare.	Hjeltnes <i>et al.</i> (2019), Johansen <i>et al.</i> (2016), Sørensen and Larsen (1986), Frans <i>et al.</i> (2011), Myhr <i>et al.</i> (1991)
<i>Vibrio ordalii</i> (formerly <i>Vibrio anguillarum</i> biotype 2)	X	X	X	Selective breeding of <i>V. ordalii</i> disease-resistant lumpfish stock might be possible. The bacterium has caused severe disease of farmed salmon in Chile and other parts of the world. Genetic differences between North Atlantic and Pacific bacterial strains have been reported. Severe pathogen for lumpfish but disease outbreaks are rare.	Hjeltnes <i>et al.</i> (2019), Johansen <i>et al.</i> (2016), Colquhoun <i>et al.</i> (2004), Poppe <i>et al.</i> (2012), Bornø <i>et al.</i> (2016), Breiland <i>et al.</i> (2015), VKM <i>et al.</i> (2017), Steinum <i>et al.</i> (2016)
Misc. <i>Vibrio</i> spp.	X	X	X	Salmon appear unaffected by certain species. Some species, <i>V. splendidus</i> , <i>V. ichthyenteri</i> and <i>V. pacinii</i> , have been found in the digestive tract of wrasse. <i>V. tapetis</i> causes disease in bivalves. <i>V. splendidus</i> commonly isolated from ballan wrasse with fin erosions.	Hjeltnes <i>et al.</i> (2019), Bergh and Samuelsen (2007), Myhr <i>et al.</i> (1991), Jensen <i>et al.</i> (2003), Birckbeck and Treasurer (2014), Gulla <i>et al.</i> (2015), Gulla <i>et al.</i> (2017), Paillard <i>et al.</i> (2006)
<i>Pasteurella</i> sp.	-	X	X	<i>Pasteurella</i> pathogen in lumpfish (not yet characterised) and <i>Pasteurella</i> pathogens in salmon (<i>P. skyensis</i> and <i>P. varracalbmi</i>) are considered genetically distinct. Recently, <i>Pasteurella</i> isolates of same genotype were found in diseased salmon and lumpfish kept at the same farming locality. Considered a pathogen of special concern regarding importation of lumpfish.	Hjeltnes <i>et al.</i> (2019), Poppe <i>et al.</i> (2013), Birckbeck <i>et al.</i> (2002), Alarcon <i>et al.</i> (2016a), Valheim <i>et al.</i> (2000), Reid and Birckbeck (2015), VKM <i>et al.</i> (2019b)
<i>Pseudomonas anguilliseptica</i>	X	X	X	Considered opportunistic. Suggested as a primary lumpfish pathogen. Isolated from several different fish species. Considerable variability in disease susceptibility indicate some degree of host specificity. Important emerging fish disease. Reported from one locality with wrasse in Norway in 2017.	Hjeltnes <i>et al.</i> (2019), Wiklund and Bylund (1990), Poppe <i>et al.</i> (2012), Treasurer and Birckbeck (2018), Scholz <i>et al.</i> (2018a), Wakabayashi and Egusa (1972), Mjølnerød (2019)
<i>Tenacibaculum</i> spp.	X	X	X	Several different variants exist, showing little degree of host specificity. Cross-species transmission have been suggested. <i>Tenacibaculum</i> spp. often detected in diseased cleaner fish. <i>T. maritimum</i> (formerly <i>Flexibacter maritimus</i>) can cause disease in both lumpfish and salmon. Appear opportunistic. <i>T. finnmarkense</i> and <i>T. dicentrarchi</i> reported from lumpfish, and <i>T. dicentrarchi</i> and <i>T. solae</i> reported	Hjeltnes <i>et al.</i> (2019), Johansen <i>et al.</i> (2016), Habib <i>et al.</i> (2014), Avendaño-Herrera <i>et al.</i> (2006), Olsen <i>et al.</i> (2017), Nilsen <i>et al.</i> (2014), Småge <i>et al.</i> (2016), Småge <i>et al.</i> (2018), Avendaño-Herrera <i>et al.</i> (2016), Klakegg <i>et al.</i> (2019),

Table 1 (continued)

Bacterial pathogens	Wrasse	Lumpfish	Salmon	Comments	References
				from wrasse. <i>T. dicentrarchi</i> is an emerging salmonid bacterial pathogen in Chile. Associated with 'crater disease' in lumpfish.	Ostland <i>et al.</i> (1999), Johansen and Poppe (2017), Frisch <i>et al.</i> (2018)
<i>Moritella viscosa</i> (formerly <i>Vibrio viscosus</i>)	X	X	X	Primarily a disease problem in salmonid aquaculture. Vaccination does not appear to offer full protection. Two genetic divisions exist, a 'type' strain and a 'variant' strain. Only the variant strain has been detected in lumpfish. Type strain is common in salmon and can cause severe problems. Variant strain includes other fish species and has caused disease problems for salmon on a few occasions. Indications that salmon are susceptible to lumpfish <i>M. viscosa</i> isolates. Could become a problem if lumpfish is used more often during the winter season.	Hjeltnes <i>et al.</i> (2019), Lunder <i>et al.</i> (1995), Gudmundsdottir <i>et al.</i> (2007), Benediktsdottir <i>et al.</i> (2000), Johansen <i>et al.</i> (2016), Grove <i>et al.</i> 2010, Karlsen <i>et al.</i> 2014, Einarsdottir <i>et al.</i> (2018), VKM <i>et al.</i> (2017)
<i>Piscirickettsia salmonis</i>	-	X	X	Only reported on one occasion from lumpfish in Ireland. SRS has been challenging in salmon aquaculture in Chile. Appear to be differences in virulence between European and Chilean strains. A pathogen of special concern regarding import of cleaner fish.	Hjeltnes <i>et al.</i> (2019), Marcos-Lopez <i>et al.</i> (2017), Rozas and Enriques (2014), Fryer <i>et al.</i> (1992), House <i>et al.</i> (1999), Olsen <i>et al.</i> (1997), Reid <i>et al.</i> 2004, Rosas-Serri <i>et al.</i> 2017, VKM <i>et al.</i> 2019b
<i>Photobacterium damselae</i> subsp. <i>damselae</i> (formerly <i>Vibrio damselae</i>)	X	-	X	Reported from a single wild-caught ballan wrasse with systemic infection. Not reported from lumpfish. Closely related bacteria, <i>P. damsela</i> subsp. <i>piscicida</i> , cause disease in Mediterranean countries. Reported from diseased rainbow trout during warm summer periods. Demonstrated pathogenicity to Atlantic salmon during experimental settings. Usually a disease issue for warm water marine fish. <i>P. damselae</i> infection might potentially become problematic with increasing ocean temperatures. Virulence properties tied with increasing water temperatures. Also reported as a human pathogen.	VKM <i>et al.</i> (2019b), McMurtrie <i>et al.</i> (2019), Austin <i>et al.</i> (1997), Pedersen <i>et al.</i> (1997), Colorni and Diamant (2014), Matanza and Osorio (2018), Rivas <i>et al.</i> (2013)
<i>Lactococcus garvieae</i>	X	-	X	Reported from wild Red sea wrasse (<i>Coris aygula</i>). Not detected in Norway, or from any wrasse species currently used as cleaner fish. Pathogenicity connected to increased water temperatures. Rainbow trout is very susceptible to disease. Atlantic salmon appears less susceptible than rainbow trout. Carrier state of live bacteria might occur. Might potentially become problematic with increasing ocean temperatures. Considered an emerging fish pathogen worldwide. May potentially be a zoonotic pathogen. Associated with disease conditions in humans, such as bacterial endocarditis.	VKM <i>et al.</i> (2019b), Colorni <i>et al.</i> 2003, Algöet <i>et al.</i> (2009), Colorni and Diamant (2014), Vendrell <i>et al.</i> (2006), Fefer <i>et al.</i> (1998), Elliot <i>et al.</i> (1991)
<i>Mycobacterium</i> spp.	-	-	X	Might potentially be a zoonotic pathogen. Associated with disease conditions in humans, such as bacterial endocarditis. Over 120 bacterial species have been documented, and at least 167 fish species have been reported as susceptible to mycobacterial infection and disease. <i>M. salmoniphilum</i> and <i>M. marinum</i> are among those present in Norwegian waters	Hjeltnes <i>et al.</i> (2019), Zerihun <i>et al.</i> (2019), VKM <i>et al.</i> (2019b), Jakobs <i>et al.</i> (2009), Hashish <i>et al.</i> (2018), Aro <i>et al.</i> (2014), Brocklebank <i>et al.</i> (2003), Colorni and Diamant (2014)

Table 1 (continued)

Bacterial pathogens	Wrasse	Lumpfish	Salmon	Comments	References
Epitheliocystis bacteria (phylum <i>Chlamydiae</i>)	X	X	X	Intracellular bacterial disease, sometimes observed in varying numbers within secondary gill lamellae. The novel species <i>Candidatus sp. Similichlamydia labri. nov.</i> have been reported from ballan wrasse. None of the species detected in wrasse have been found in farmed salmonid fish. As such, they do not likely represent a disease problem with salmonid and cleaner fish polyculture. New species on other wrasse fish have recently been suggested. Reported from lumpfish, but no species have been characterised. A few species have been reported from Atlantic salmon. Associated with complex gill disease in Atlantic salmon.	Nilsen <i>et al.</i> (2014), Brooker <i>et al.</i> (2018), Steigen <i>et al.</i> (2015), Steigen <i>et al.</i> (2018), Colorni and Diamant (2014), Gjessing <i>et al.</i> (2019)

It can be quite severe, with high and rapid mortalities in susceptible fish (Frans *et al.*, 2011; Roberts, 2012). Both lumpfish and wrasse have demonstrated disease and mortality after challenge with *V. anguillarum* (Rønneseth *et al.*, 2014; Biering *et al.*, 2016). Diagnosis is often based on the clinical history, combined with histopathology and bacterial isolation with biochemical characterisation and serotyping (Whitman, 2004; Jansson & Vennerström, 2014). Serotyping is based on variability of the bacterium's O-antigens, and a total of 23 different serotypes (O1–O23) have been described from fish (Pedersen *et al.*, 1999). Among these, serotype O1 and several subtypes of O2 (O2a, O2a-biotype II and O2b) are frequently isolated from Norwegian cleaner fish, with serotype O1 being the most prominent in lumpfish (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019). Serotype O1 and O2 infections have also been reported in salmonids (Sørensen &

Larsen, 1986; Myhr *et al.*, 1991). Classical vibriosis are often associated with increasing temperatures (Frans *et al.*, 2011); however, disease outbreaks in lumpfish have occurred at temperature levels as low as 6°C in Scottish aquaculture (Marcos-Lopez *et al.*, 2013).

Vibrio ordalii (formerly *Vibrio anguillarum* biotype 2) is a different bacterial species, known for causing severe haemorrhagic septicaemia in salmonids in Europe, South America, Oceania and parts of Asia (Colquhoun *et al.*, 2004; Frans *et al.*, 2011; Jansson & Vennerström, 2014). The bacteria was isolated from lumpfish in Norway in 2011 (Poppe *et al.*, 2012) and can potentially lead to high mortalities and recurring disease outbreaks (Fig. 9) (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019). However, the susceptibility to infection can vary widely between different

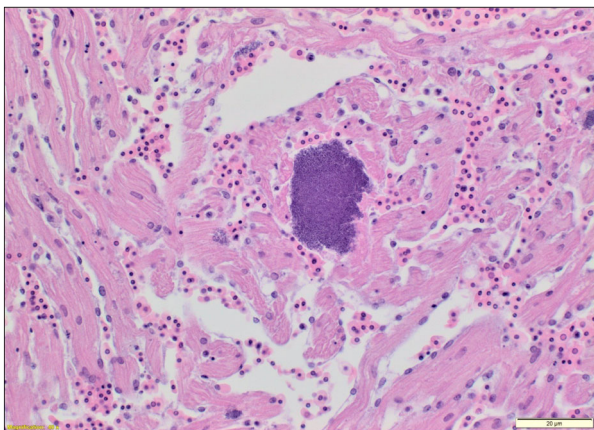


Figure 7 Bacterial microcolony in the heart of a lumpfish with suspected atypical furunculosis disease caused by infection with atypical *Aeromonas salmonicida*. Haematoxylin and eosin (HE) stain. Scalebar: 20 µm. Photograph: Toni Erkinharju.

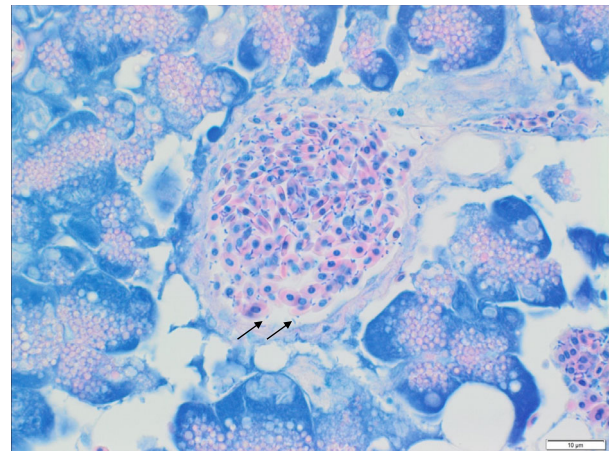


Figure 8 Peripancreatic blood vessel with several curved, rod-shaped bacteria (arrows) in a lumpfish affected by generalised infection with *Vibrio anguillarum* serotype O1. May–Grünwald–Giemsa (MGG) stain. Scalebar: 10 µm. Photograph: Toni Erkinharju.

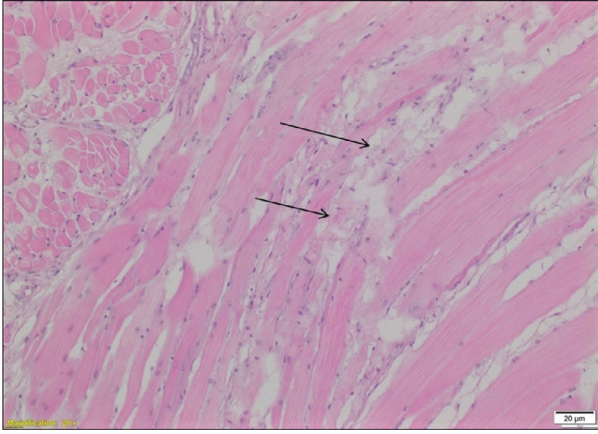


Figure 9 Lumpfish skeletal muscle with liquefactive necrosis of muscle tissue (arrows). *Vibrio ordalii* infections show predilection for muscle and skin with resulting necrosis and haemorrhage. HE stain. Scalebar: 20 μ m. Photograph: Stefanie C. Wüstner.

lumpfish families (Breiland *et al.*, 2015). The bacteria have also been associated with lesions of the eyes in infected lumpfish (Bornø *et al.*, 2016). Several other *Vibrio* species commonly found in the marine environment have also been isolated from cleaner fish, such as *V. splendidus*, *V. logei*, *V. wodanis* and *V. tapetis*, but their significance as disease-causing pathogens is largely unclear (Scholz *et al.*, 2018a; Hjeltnes *et al.*, 2019). *V. splendidus* for instance is often isolated from farmed ballan wrasse with fin erosions, sometimes in mixed culture with *Tenacibaculum* spp. bacteria (Scholz *et al.*, 2018a; Hjeltnes *et al.*, 2019). *V. splendidus* and *V. tapetis* have been reported as pathogenic for certain wrasse species (Sigmund *et al.*, 2003; Bergh & Samuelsen, 2007; Johansen *et al.*, 2016a), although recent studies have suggested they might represent opportunistic pathogens rather than causing primary infections (Gulla *et al.*, 2015; Gulla *et al.*, 2017). *V. tapetis* is also the cause of brown ring disease in the bivalve *Ruditapes philippinarum* (Paillard *et al.*, 2006). Some species, *V. splendidus*, *V. ichthyenteri* and *V. pacinii*, have been found in the digestive tract of different larval wrasse species (Birkbeck & Treasurer, 2014).

Pasteurellosis is a severe bacterial disease that was first detected in lumpfish in Norway in 2012 and is occasionally observed in aquaculture (Poppe *et al.*, 2013; Gu & Skjelstad, 2018a; Scholz *et al.*, 2018a). The bacterium *Pasteurella* sp. has yet not been characterised to species level, but show close relation to *P. skyensis* and *P. varracalbmi*, two *Pasteurella* bacteria affecting salmon (Valheim *et al.*, 2000; Poppe *et al.*, 2013; Alarcon *et al.*, 2016a). Infection leads to bacterial septicemia and mortality may be as high as 100%. Macroscopically visible lesions in moribund fish include tail rot, bleeding gills, fin haemorrhages, ascites and skin ulcers

appearing as white nodules, especially on the head. Internally, affected fish display multiple granulomas and haemorrhages in visceral organs (Alarcon *et al.*, 2016a; Gu & Skjelstad, 2018; Hjeltnes *et al.*, 2019; Ellul *et al.*, 2019b). Aggregates of bacteria, often with variable tissue reactions (necrosis, inflammation, haemorrhages or none at all), are observed histologically, which can easily be mistaken for atypical furunculosis (Fig. 10) (Poppe *et al.*, 2013; Alarcon *et al.*, 2016a; Gu & Skjelstad, 2018). Both infections can also occur together (Gu & Skjelstad, 2018). Definitive diagnosis should therefore only be made on proper identification of the bacterial agent. *Pasteurella* sp. is considered a pathogen of special concern regarding importation of lumpfish (VKM *et al.*, 2019a).

Pseudomonas anguilliseptica is an opportunistic pathogen causing disease in a wide variety of fish species in freshwater, seawater and brackish water. Originally, it was a serious problem in eel farming being the cause of red spot disease (Wakabayashi & Egusa, 1972; Wiklund & Bylund, 1990; Scholz *et al.*, 2018a). It manifests as a haemorrhagic septicemia, causing external skin haemorrhages on the ventral side of the body, and petechial haemorrhaging in the peritoneum, liver and adipose tissue. Some individuals also develop lesions in the eyes. Mortality can vary greatly depending on the species affected (Roberts, 2012; Scholz *et al.*, 2018a). Bacterial colonies are observed histologically, both within blood vessels and in internal organs (Fig. 11; Roberts, 2012). Diagnosis can be challenging, as the bacterium grows slowly on culture media, and is as such easily overgrown by more rapidly dividing bacterial species (Roberts, 2012; Scholz *et al.*, 2018a). *P. anguilliseptica* was first isolated from lumpfish in Norway in 2011 and has since then been diagnosed on several occasions (Poppe *et al.*, 2012; Johansen *et al.*, 2016a; Treasurer & Birkbeck, 2018). The bacteria has been reported from a single farming locality with wrasse in Norway in recent years (Hjeltnes *et al.*, 2019), but has otherwise not been described from wrasse (Scholz *et al.*, 2018a). It has recently been indicated as a primary pathogen of lumpfish (Mjølnerød, 2019). Infected fish present similar clinical signs to other susceptible fish species, in addition to haemorrhages within the brain and the operculum, which have not been previously described from other species (Poppe *et al.*, 2012; Hjeltnes *et al.*, 2019; Mjølnerød, 2019).

Tenacibaculosis is an ulcerative disease affecting marine fish, caused by species of *Tenacibaculum* bacteria, such as *T. maritimum* (previously *Flexibacter maritimus*) (Averdano-Herrera *et al.*, 2006). It causes deep ulcers with erosion and necrosis of the skin, especially on the fins, tail and parts of the head. Histologically, *Tenacibaculum* spp. can be observed as mats of abundant long, thin and rod-shaped bacteria associated with epithelial ulcers, which can lead to a presumptive diagnosis (Roberts, 2012; Jansson & Vennerström, 2014). Bacterial isolation can be challenging,

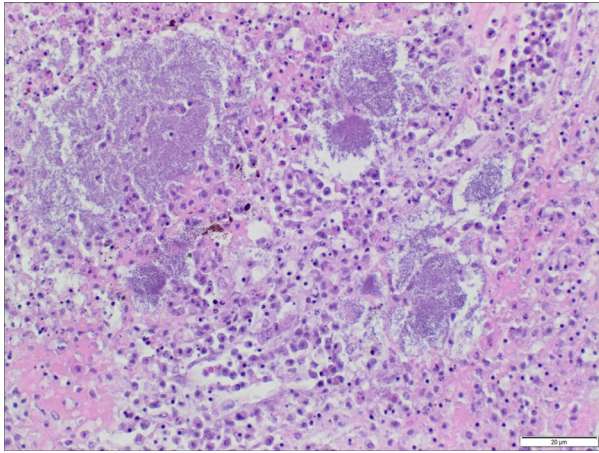


Figure 10 Lumpfish skin infected by several bacterial microcolonies of *Pasteurella* sp., with surrounding inflammation and necrosis. HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

however, as *T. maritimum* grows slowly on agar media, and colonies can be overgrown or inhibited by other bacterial species in mixed infections (Avendano-Herrera *et al.*, 2006; Scholz *et al.*, 2018a). It has been suggested to be an opportunistic pathogen, since the bacteria are found within the mucus layer of fish skin, and as such appear to be a natural part of the skin microbial population (Avendano-Herrera *et al.*, 2006). *T. maritimum* was first isolated from diseased lumpfish in Norway in 2015. Affected fish were characterised by increased mucus production and white necrotic lesions in the skin on the head, around the eyes and over the bony tubercles (Småge *et al.*, 2016). Although *T. maritimum* infection can be systemic, descriptions of

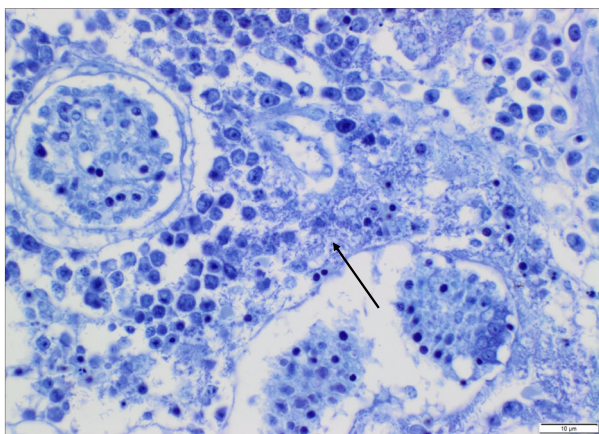


Figure 11 Kidney of lumpfish with suspected bacterial septicemia caused by *Pseudomonas anguilliseptica*. The renal tissue appears necrotic (arrow), and several thin, rod-shaped bacteria are observable. Giemsa stain. Scalebar: 10 μ m. Photograph: Toni Erkinharju.

associated pathologic changes in internal organs have not been reported from lumpfish (Scholz *et al.*, 2018a). *Tenacibaculum* spp. are regularly detected in diseased lumpfish (Fig. 12), in both pure and mixed culture (Hjeltnes *et al.*, 2019). Other species reported from lumpfish are *T. finnmarkense* and *T. dicentrarchi* in Norway, Scotland, Ireland and the Faroes (Olsen *et al.*, 2017; Scholz *et al.*, 2018a). *T. dicentrarchi* and *T. soleae* have been reported from wrasse (Olsen *et al.*, 2017). *T. dicentrarchi* is an emerging disease problem in Chilean aquaculture (Avendano-Herrera *et al.*, 2016), but its significance for cleaner fish is still unknown. *Tenacibaculum* spp. have also been associated with ‘crater disease’ in lumpfish, which forms circular crater-like depressions in skin, especially near the bony tubercles (Johansen & Poppe, 2017).

Winter ulcer disease is caused by infection with the bacterium *Moritella viscosa* (formerly *Vibrio viscosus*), which forms large open ulcers in the flanks of affected fish during the winter season (Lunder *et al.*, 1995; Roberts, 2012; Jansson & Vennerström, 2014). This can lead to severe economic loss, as the ulcers can lead to high mortality during the production cycle, and in addition, cause downgrading or rejection of affected salmon at time of slaughter (Tørud & Håstein, 2008; Jansson & Vennerström, 2014). Diagnosis of winter ulcer disease in salmon is generally made on the basis of clinical history and bacterial culture (Whitman, 2004; Gudmundsdottir & Bjornsdottir, 2007). The bacterium is regularly isolated from skin lesions of cleaner fish, sometimes in mixed infections (Fig. 13) (Hjeltnes *et al.*, 2019). *M. viscosa* in lumpfish was initially isolated as a low-

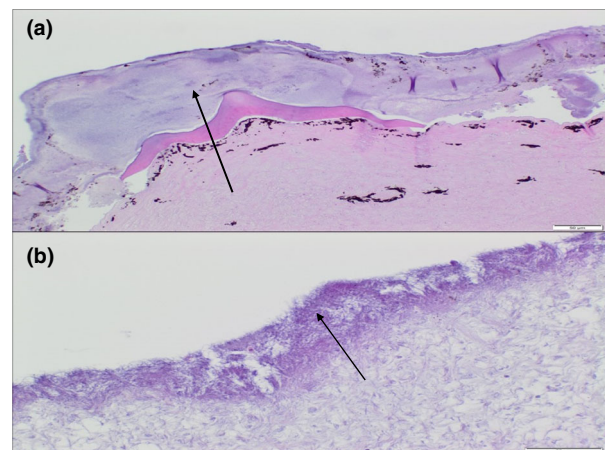


Figure 12 A: Infected dermal ulcer of lumpfish, covered by a large matrix of mixed bacterial types (arrow) (long, filamentous and shorter, rod-shaped). The epidermal layer is missing. The fish presented clinical symptoms characteristic for ‘crater disease’. B: Skin erosion with several long, filamentous bacteria (dark pink; arrow) (*Tenacibaculum* spp.). HE stain. Scalebar 50 μ m (a) and 20 μ m (b). Photograph: Toni Erkinharju.

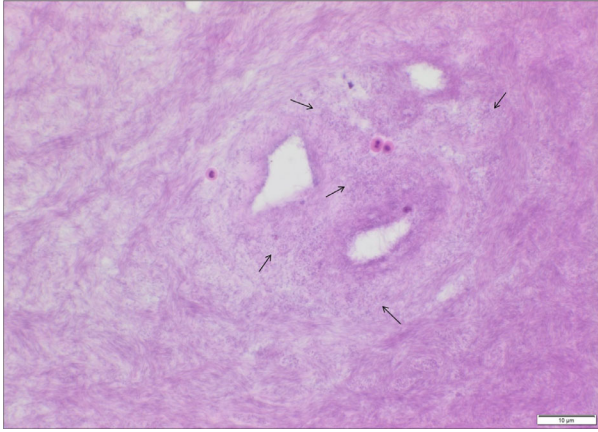


Figure 13 Mixed bacterial infection in skin ulcer of lumpfish. Several short bacterial rods (arrows), of which some might be *Moritella viscosa*, can be seen around the centre of the image. HE stain. Scalebar 10 μm . Photograph: Toni Erkinharju.

non-virulent strain from the gills of one healthy individual (Benediktsdottir *et al.*, 2000), but have recently been isolated from skin lesions on fish at several occasions (Hjeltnes *et al.*, 2019). It has been reported that the bacterium has been the cause of mortalities of lumpfish in Norway (Scholz *et al.*, 2018a). Among the known *M. viscosa* isolates, there appears to be two different clusters based on phenotypic (Western blotting, plasmid profile, pulsed field electrophoresis) and genetic analysis (16S rRNA and *GyrB* sequencing), a 'type strain' consisting of almost exclusively isolates from Atlantic salmon, and a 'variant strain' consisting of several different fish species, including lumpfish (Grove *et al.*, 2010). To date, very few cleaner fish *Moritella* isolates have been studied and more information is needed to better understand the significance of *M. viscosa* in lumpfish (Johansen *et al.*, 2016a; Scholz *et al.*, 2018a). However, in a study by Einarsdottir *et al.* (2018) it was shown that salmon was susceptible to *M. viscosa* isolated from affected lumpfish, but not the other way around. Vaccination of salmon is possible, but does not appear to offer full protection from the disease (Gudmundsdottir & Bjornsdottir, 2007; Scholz *et al.*, 2018a).

Salmonid rickettsial septicaemia, or piscirickettsiosis, is a disease affecting fish in marine and brackish waters. It is caused by an intracellular bacterium called *Piscirickettsia salmonis* and was initially observed in salmonid fish in Chilean aquaculture (Fryer *et al.*, 1992; Rozas & Enriquez, 2014). Mortality can be quite high and is difficult to treat with antibiotics, possibly due to the bacteria's intracellular life cycle (Jansson & Vennerström, 2014). Typical signs of infection are white, sometimes haemorrhagic, circular nodules in the liver, which occasionally rupture and forms crater-like lesions (Roberts, 2012; Rozas & Enriquez, 2014). A presumptive diagnosis is usually made on clinical history

and histological demonstration of pleomorphic bacteria, associated with necrotic and inflammatory lesions in affected organs. Confirmation of the pathogen requires additional analysis by either immunohistochemistry, isolation with cell culture or specific bacteriological media, serological or molecular methods (Jansson & Vennerström, 2014; Rozas & Enriquez, 2014; Scholz *et al.*, 2018a). *P. salmonis* has never been detected in Norwegian cleaner fish (Hjeltnes *et al.*, 2019), but was isolated from a lumpfish hatchery in Ireland in 2015 (Marcos-Lopez *et al.*, 2017). Infected fish displayed distended abdomen with marked ascites, pale organs, enlarged kidney and green-coloured liver with numerous cream-coloured patches. Although the reported mortality and morbidity rates were very low, there is a risk of subclinical infection resulting in the infection going unnoticed, and thus potentially spreading the bacteria when moving lumpfish to new areas (Marcos-Lopez *et al.*, 2017). *P. salmonis* has caused disease in Atlantic salmon in Norway (Olsen *et al.*, 1997).

Systemic infection with *Photobacterium damsela* subsp. *damsela* (Pdd) (formerly *Vibrio damsela*) was recently reported from a single wild-caught ballan wrasse from the British south-west coast during the warmest average water temperatures that year (McMurtrie *et al.*, 2019). Photobacteriosis is a devastating fish disease in warm and temperate aquaculture locations, where a closely related subspecies, *Photobacterium damsela* subsp. *piscicida*, has been described from several marine fish species in Mediterranean countries, in the United States and in Japan (Colorni & Diamant, 2014). Fish photobacteriosis might potentially become a problem with increasing water temperatures (VKM *et al.*, 2019a). Regulation of physiological and virulence-related properties of Pdd have been suggested to be associated with changes in the water temperature (Matanza & Osorio, 2018). Pdd is also an opportunistic human pathogen, potentially causing severe necrotising fasciitis in affected individuals (Rivas *et al.*, 2013).

As for other bacterial species, there are only limited information available. *Lactococcus garviae* has been isolated from wild Red sea wrasse *Coris aygula* (Colorni *et al.*, 2003). Lactococcosis in fish manifests as a hyperacute and haemorrhagic septicaemia and is regarded as an emerging pathology, and as with Pdd, the pathogenicity is closely tied to increasing water temperatures (Vendrell *et al.*, 2006; Colorni & Diamant, 2014). Rainbow trout is very susceptible to disease, while other salmonids, such as Atlantic salmon and brown trout (*Salmo trutta*), appear less susceptible (Algöet *et al.*, 2009). Fish surviving infection might potentially become carriers, and thus contributing to further spread of the disease if it is undetected (Vendrell *et al.*, 2006; Algöet *et al.*, 2009). It has been associated with rare cases of bacterial endocarditis in humans (Elliott *et al.*, 1991; Fefer *et al.*, 1998). Its relevance as a fish pathogen in

northern parts of Europe might change with increasing water temperatures (Algöet *et al.*, 2009; VKM *et al.*, 2019a). It has not been detected in Norway or in any wrasse species used as cleaner fish to date (VKM *et al.*, 2019a).

Mycobacterium spp. causes chronic disease in fish with formation of granulomatous lesions within internal organs in severe cases (Colorni & Diamant, 2014). Numerous bacterial species have been recognised, and at least 167 fish species, both cultured and wild, have been reported as susceptible to infection and development of disease (Jacobs *et al.*, 2009). *M. salmoniphilum* and *M. marinum* are among those species considered present in Norwegian waters (VKM *et al.*, 2019a). *M. salmoniphilum* has been diagnosed as an increasing pathogen in Norwegian Atlantic salmon farms lately (Zerihun *et al.*, 2019; Hjeltnes *et al.*, 2019). Similar cases of fish mycobacteriosis in Atlantic salmon have also been reported from other parts of the world, such as *M. salmoniphilum* in Chile and *M. chelonae* in Canada (Brocklebank *et al.*, 2003; Aro *et al.*, 2014). There is some degree of zoonotic potential associated with these bacteria, and especially *M. marinum* infection is considered a possible occupational hazard when handling aquarium fish (Colorni & Diamant, 2014; Hashish *et al.*, 2018). As far as the authors are aware, there have been no reports of *Mycobacterium* spp. in fish species used as cleaner fish.

Epitheliocystis bacteria (phylum *Chlamydiae*) are several intracellular bacterial species, capable of causing gill disease with formation of spherical or ellipsoid 'cysts' within the gill tissue (Colorni & Diamant, 2014). A novel species, *Candidatus* sp. *Similichlamydia labri. nov.*, was recently discovered in ballan wrasse in Norway (Steigen *et al.*, 2015; Brooker *et al.*, 2018). Subsequently, two putative new species of *Chlamydiae* were then reported from wrasse collected on the west coast of Norway (Steigen *et al.*, 2018). Epitheliocystis has been reported from lumpfish (Fig. 14) (Nilsen *et al.*, 2014), but no species have been identified. A few species that have been reported from Atlantic salmon, *Ca. Piscichlamydia salmonis* and *Ca. Branchiomonas cysticola*, may be associated with the multifactorial disease condition known as complex gill disease (Gjessing *et al.*, 2019). The newly described species in wrasse have not been found in farmed salmonids and are as such not considered a relevant disease problem for aquatic polyculture (Steigen *et al.*, 2018).

Fungi

Fungal infections are common in lumpfish aquaculture and can cause significant mortalities, especially in hatcheries. Different species are probably involved, but *Exophiala* spp. (melanised fungi, also known as black yeast) are considered the most common, and have also been found in wild-caught lumpfish (Table 2) (Powell *et al.*, 2018; Brooker *et al.*, 2018). *Exophiala* infections have also been reported from numerous

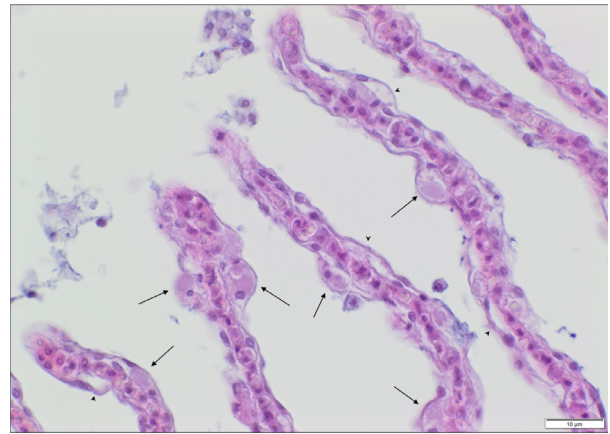


Figure 14 Gill tissue from lumpfish, with several epitheliocysts located within the secondary lamellae (arrows). Slight lifting of the respiratory epithelium is also noticeable (arrowheads). HE stain. Scalebar 10 μ m. Photograph: Toni Erkinharju.

fish species, such as Atlantic salmon (Langdon & McDonald, 1987; de Hoog *et al.*, 2011; Jansson & Vennerström, 2014). Generally, the pathogen causes systemic infection which manifests as multiple granulomas in internal organs, and occasionally as dark dermal nodules on the body surface. Histologically, septated fungal hyphae are observed in affected organs, such as the kidney and liver (Fig. 15) (Gjessing *et al.*, 2011; Roberts, 2012). Different special stains (such as periodic acid-Schiff (PAS) and Grocott's silver stain) may aid in the interpretation of pathologic changes (Jansson & Vennerström, 2014). Culturing usually requires special growth media, and molecular analysis is required for species identification (de Hoog *et al.*, 2011; Scholz *et al.*, 2018a).

Currently, five species have been identified in lumpfish; these are *E. angulospora*, *E. psychrophile*, *E. salmonis*, *E. aquamarina* and *Cyphellophora* sp (de Hoog *et al.*, 2011; Johnson *et al.*, 2018; Scholz *et al.*, 2018b). *E. angulospora* have previously been isolated from a lumpfish hatchery in Scotland (Saraiva *et al.*, 2019). Both *E. angulospora* and *E. psychrophila* were recently detected in a lumpfish broodstock facility, in addition to *E. salmonis* and another fungus, *Chyphellophora* sp., from lumpfish at sea, in Ireland and Iceland (Scholz *et al.*, 2018b). Affected fish had several dark-pigmented ulcerative lesions in the skin, especially around bony tubercles, and multifocal black discoloration or nodules on internal organs. Diffuse swelling was also observed, especially in the kidneys (Johnson *et al.*, 2018; Scholz *et al.*, 2018b; Saraiva *et al.*, 2019). Finally, the species *E. aquamarina* has also been reported from lumpfish, in addition to several species of fish commonly kept in aquariums (de Hoog *et al.*, 2011). It is currently unknown to what degree *Exophiala* spp., or other pathogenic fungus, might be present in farmed lumpfish in Norway. Systemic

Table 2 Summary of fungal pathogens reported from wrasse (Labridae), lumpfish (*C. lumpus*) and Atlantic salmon (*S. salar*) with relevant references

Fungal agent	Wrasse	Lumpfish	Salmon	Comments	References
<i>Exophiala</i> spp.	-	X	X	<i>E. salmonis</i> , <i>E. psychrophila</i> and <i>E. pisciphila</i> reported from Atlantic salmon. <i>E. salmonis</i> , <i>E. psychrophila</i> , <i>E. angulospora</i> and <i>E. aquamarina</i> reported from lumpfish. Reported from several fish species. Fish appear generally susceptible to disease. There is a general lack of information regarding fungal pathogens in cleaner fish. May potentially cause opportunistic zoonotic infections in humans.	Powell <i>et al.</i> (2018), Scholz <i>et al.</i> (2018a), de Hoog <i>et al.</i> (2011), Pedersen and Langvad (1989), Langdon and McDonald (1987), Johnson <i>et al.</i> (2018), Scholz <i>et al.</i> (2018b), Saraiva <i>et al.</i> (2019), Bornø <i>et al.</i> (2016), Jansson and Vännerström (2014), Gjessing <i>et al.</i> (2011), Kebbe and Mador (2016), VKM <i>et al.</i> (2019b)

mycoses are occasionally reported (Hjeltnes *et al.*, 2019), but the species is not always identified. It has also been suggested that some species, such as *E. pisciphila* and *E. salmonis*, might have potential as zoonotic pathogens (Revankar & Sutton, 2010; Yoon *et al.*, 2012; Kebbe & Mador, 2016). The Table 2 sums up the occurrence of known fungal pathogens in the fish species.

Parasites

Several different parasites have been reported from both wild-caught and farmed lumpfish and wrasse (Table 3). Some are specific to their host species, while others require intermediate hosts as part of their life cycle. A few can potentially infect cohabitated salmon, and some are a concern due to their zoonotic potential. Most of the time, parasites are not detrimental to their host, but may become

harmful under stressful conditions. (Karlsbakk *et al.*, 2014; Johansen *et al.*, 2016a; Powell *et al.*, 2018; Scholz *et al.*, 2018a). Important parasites in lumpfish include the protozoans *Paramoeba perurans* (Oldham *et al.*, 2016), *Nucleospora cyclopteri* (Freeman *et al.*, 2013), *Trichodina* sp. (*T. cyclopteri*, *T. galaye*) (Karlsbakk *et al.*, 2014), *Ichtyobodo* sp. (Karlsbakk *et al.*, 2014), coccidians (*Eimeria* sp.) (Kristmundsson *et al.* 2018), and the metazoans *Kudoa islandica* (Kristmundsson & Freeman, 2014), *Gyrodactylus* sp., *Gyrodactylus cyclopteri* (Karlsbakk *et al.*, 2014; Pietrak & Rosser, 2020), nematodes (*H. aduncum*, *A. simplex*) (Rolbiecki & Rokicki, 2018) and *Caligus elongatus* (Davenport, 1985; Heuch *et al.*, 2007).

Paramoeba perurans (synonym *Neoparamoeba perurans*) is the cause of amoebic gill disease (AGD) affecting many different fish species all over the world (Mitchell & Rodger, 2011; Jansson & Vennerström, 2014). The main tissue affected are the gills, which become hyperplastic and display increased mucus production upon colonisation with the amoeba. The thickening of gill filaments then causes impairment in the diffusion of respiratory gases across the gill surface, potentially resulting in respiratory distress, cardiac changes, disturbances in the acid-base homeostasis and, ultimately, death (Roberts, 2012). Diagnosis of the disease is made on observation of the parasite on affected gills with hyperplastic respiratory epithelium and fused filaments, while confirmation of the species require identification by additional analysis, such as *in situ* hybridisation (ISH) or PCR (Mitchell & Rodger, 2011; Jansson & Vennerström, 2014). AGD can have high impacts on aquaculture production due to the increased mortalities and reduced growth of affected fish, caused either by the parasite alone, or from concurrent infection with other gill pathogens. Another important factor is the increased production costs from labour-intensive and time-demanding parasite treatments, which does not completely eliminate all of the infective amoeba from the fish (Mitchell & Rodger, 2011; Jansson & Vennerström, 2014; Johansen *et al.*,

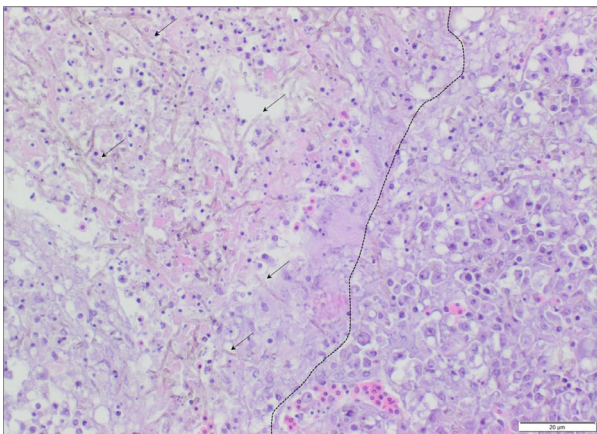


Figure 15 Necrotic liver of lumpfish with systemic mycosis. Several light brown septated fungal hyphae can be seen (arrows), suspected of being *Exophiala* sp. A demarcation between severely affected and less affected liver tissue can be noted (dotted line, ---). HE stain, Scalebar 20 μ m. Photograph: Toni Erkinharju.

Table 3 Summary of parasitic agents reported from wrasse (Labridae), lumpfish (*C. lumpus*) and Atlantic salmon (*S. salar*) with relevant references

Parasitic agents	Wrasse	Lumpfish	Salmon	Comments	References
<i>Paramoeba perurans</i> (syn. <i>Neoparamoeba perurans</i>)	X	X	X	Experimental transmission confirmed between lumpfish and salmon. Cleaner fish a possible reservoir. Salmon is very susceptible to infection. Low host specificity. The amoeba is considered common along the Norwegian coastline as far north as Nordland county. Treatment is difficult and costly.	Hjeltnes <i>et al.</i> (2019), Mitchell and Rodger (2011), Young <i>et al.</i> (2007), Young <i>et al.</i> (2008), Steinum <i>et al.</i> (2008), Karlsbakk <i>et al.</i> (2013), Karlsbakk <i>et al.</i> (2014), Oldham <i>et al.</i> (2016), Haugland <i>et al.</i> (2017), Steigen <i>et al.</i> (2018), Scholz <i>et al.</i> (2018a)
<i>Nucleospora cyclopteri</i>	-	X	-	<i>N. cyclopteri</i> only described from lumpfish. Closely related to <i>N. salmonis</i> in salmon. Direct transmission has been shown for certain microsporidian parasites in fish, such as <i>N. salmonis</i> . The parasite is common in wild lumpfish along the Norwegian coast.	Hjeltnes <i>et al.</i> (2019), Mullins <i>et al.</i> (1994), Freeman <i>et al.</i> (2013), Freeman and Kristmundsson (2013), Karlsbakk <i>et al.</i> (2014), Alarcon <i>et al.</i> (2016b), Scholz <i>et al.</i> (2018a), VKM <i>et al.</i> (2017), Aloui <i>et al.</i> (2006), Lom and Nilsen (2003), Johansen <i>et al.</i> (2016a)
<i>Tetramicra brevisfolium</i>	-	X	-	Only described from lumpfish in Ireland. Not reported from cleaner fish in Norway. Direct transmission has not been proven. Potential problem in turbot farming in Southern Europe. Transmission to salmon has not been investigated, but considered unlikely.	Hjeltnes <i>et al.</i> (2019), Scholz <i>et al.</i> (2017), Lom and Nilsen (2003), Figueras <i>et al.</i> (1992), Scholz <i>et al.</i> (2018a), Estevez <i>et al.</i> (1992)
Other microsporidia	X	-	X	<i>Loma</i> sp. and <i>Desmozoon lepeoptherii</i> (syn. <i>Paranucleospora theridion</i>) reported from wrasse. The parasites have also been reported from salmon. Cross-infection between fish are unknown. Direct transmission has been shown for <i>Loma salmonis</i> . Sea lice are considered vector for <i>D. lepeoptherii</i> .	Hjeltnes <i>et al.</i> (2019), Scholz <i>et al.</i> (2018a), Mitchell and Rodger (2011), Ramsay <i>et al.</i> (2002), Nylund <i>et al.</i> (2010), Kent <i>et al.</i> (1989), Steigen <i>et al.</i> (2018), Lom and Nilsen (2003)
<i>Ichthyophonus</i> sp.	X	X	X	<i>I. hoferi</i> -like organisms reported from both wrasse and lumpfish. Can potentially infect 'all' fish. Opportunistic generalist. Transmission directly through water or potentially from salmon consuming infected fish, such as cleaner fish, or zooplankton.	Hjeltnes <i>et al.</i> (2019), Mo and Poppe (2018), Hansen and Nilsen (2018), Gozlan <i>et al.</i> (2014), Zubchenko and Karaseva (2002), Teffer <i>et al.</i> (2020), Rahimian (1998), Kochan (2019)
<i>Trichodina</i> sp.	X	X	X	Two species identified on lumpfish, <i>T. cyclopteri</i> and <i>T. galaye</i> . Commonly found on cleaner fish. Considered host-specific. Makes transmission between cleaner fish and salmon less likely. Usually not associated with severe pathology. Might be present among other gill pathogens.	Hjeltnes <i>et al.</i> (2019), Johansen <i>et al.</i> (2016), Karlsbakk <i>et al.</i> (2014), VKM <i>et al.</i> (2017), VKM <i>et al.</i> (2019b), Treasurer (1997), Steigen <i>et al.</i> (2018)
Scuticociliates	X	X	X	Widespread in the marine environment. Free-living organisms, considered secondary pathogens. Organisms occasionally reported from lumpfish, resembling <i>Uronema marinum</i> . Reported from several fish. Scuticociliates also reported from wrasse and salmon. Not considered a significant problem for healthy salmon.	Scholz <i>et al.</i> (2018a), Østevik <i>et al.</i> (2018), McMurtrie <i>et al.</i> (2019), Erkinharju <i>et al.</i> (2019), Bermingham and Mulcahy (2007), Ferguson <i>et al.</i> (1987), Dyková <i>et al.</i> (2010), Piazzon <i>et al.</i> (2013), Colorni and Burgess (1997)
<i>Ichthyobodo</i> sp. (also known as Costia)	X	X	X	The flagellates, <i>I. salmonis</i> and <i>I. necator</i> have been identified in salmon. Considered species-specific. Lumpfish and wrasse most likely have their own, novel species. Not	Hjeltnes <i>et al.</i> (2019), Karlsbakk <i>et al.</i> (2014), Isaksen <i>et al.</i> (2011), Johansen <i>et al.</i> (2016), Mitchell and Rodger (2011), VKM <i>et al.</i> (2019b),

Table 3 (continued)

Parasitic agents	Wrasse	Lumpfish	Salmon	Comments	References
<i>Cryptobia dahlia</i>	-	X	-	considered a severe pathogen. Often found on wrasse. Specific species for fish. Salmon has the well-known hemoflagellate <i>Cryptobia</i> (<i>Trypanoplasma</i>) <i>salmositica</i> . Considered harmless for the lumpfish host, due to lack of apparent damage to the gastric tissue. Commensals. <i>Spironucleus</i> sp. also reported from intestinal canal of lumpfish.	Scholz <i>et al.</i> (2018a), Steigen <i>et al.</i> (2018), Treasurer (1997) Karlsbakk <i>et al.</i> (2014), Erkinharju <i>et al.</i> (2019), Freeman and Kristmundsson (2018), Treasurer (1997), VKM <i>et al.</i> (2019b), Woo (2003)
<i>Cycloptericola marina</i>	-	X	-	Similar to <i>C. dahlia</i> , considered harmless for the lumpfish host. May cover parts of the gastric tissue.	Karlsbakk <i>et al.</i> (2014), Erkinharju <i>et al.</i> (2019)
Coccidea (resembling <i>Eimeria</i> sp.)	X	X	X	<i>Eimeria</i> -like coccidians reported from both lumpfish and wrasse recently. Coccidian parasites have been reported from the gastro-intestinal tract of wild-caught salmon fry at Trøndelag county. <i>Eimeria</i> sp. in fish show very high host specificity. Obligate parasites with a direct life cycle. Highly prevalent in wild cleaner fish. Common parasite within the fish intestine. Usually minimal severe pathology described from marine fish. Might affect the cleaner fish appetite, thus affecting grazing of sea lice.	Hjeltnes <i>et al.</i> (2019), Kristmundsson <i>et al.</i> (2018), VKM <i>et al.</i> (2019b), McMurtrie <i>et al.</i> (2019), Erkinharju <i>et al.</i> (2019), Åkesson <i>et al.</i> (2020), Sweco (2017), Molnar <i>et al.</i> (2012), Xavier <i>et al.</i> (2018), Gjerde (2011b)
<i>Kudoa islandica</i>	-	X	-	Isolated from both lumpfish and wolffish. <i>Kudoa thyrssites</i> occur in Atlantic salmon. <i>Kudoa</i> -like myxozoa are incidental findings in wrasse. Typically, low or no mortality. <i>Kudoa</i> is not strictly host-specific; possibility for transmission might exist.	Hjeltnes <i>et al.</i> (2019), Kristmundsson and Freeman (2014), Alarcon <i>et al.</i> (2016b), Lom and Dyková (2006), Henning <i>et al.</i> (2013), Karlsbakk <i>et al.</i> (2014), St-Hilaire <i>et al.</i> (1997), Johansen <i>et al.</i> (2016), Swearer and Robertson (1999), Scholz <i>et al.</i> (2018a)
<i>Myxobolus aeglefini</i>	-	X	X	Intermediate hosts often required for myxozoan parasites, such as tubifex worms. Benthic organisms found on the sea bottom. Most are considered host-specific. Salmon could potentially be infected from cod kept in the same cage. Resemble <i>Myxobolus cerebralis</i> infection. <i>M. aeglefini</i> has not been reported from lumpfish in Norway.	Cavin <i>et al.</i> (2012), Karlsbakk <i>et al.</i> (2014), Scholz <i>et al.</i> (2018a), Mo <i>et al.</i> (1992), Gilbert and Granath (2001), Erkinharju <i>et al.</i> (2019), Blazer <i>et al.</i> (2004)
<i>Gyrodactylus</i> sp.	X	X	X	Specific host species. Could potentially cause problems, not fully surveyed.	Karlsbakk <i>et al.</i> (2014), Johansen <i>et al.</i> (2016), Hjeltnes <i>et al.</i> (2019), VKM <i>et al.</i> (2019b), Alarcon <i>et al.</i> (2016b), Pietrak and Rosser (2020), VKM <i>et al.</i> (2017), Erkinharju <i>et al.</i> (2019)
Nematodes	X	X	X	Some species, such as <i>Hysterothylacium aduncum</i> , has a wide range of hosts. Several are zoonotic. Low to no prevalence in surveys of farms. <i>H. aduncum</i> , <i>Anisakis simplex</i> , <i>Contracecum oscalatum</i> and <i>Pseudoterranova</i> sp. have all been reported from lumpfish. <i>H. aduncum</i> , <i>A. simplex</i> , <i>Pseudoterranova</i> sp. and <i>Contracecum oscalatum</i> found in wrasse. <i>H. aduncum</i> ,	Rolbiecki and Rockiki (2008), Karlsbakk <i>et al.</i> (2014), McMurtrie <i>et al.</i> (2019), Hjeltnes <i>et al.</i> (2019), VKM <i>et al.</i> (2019b), Mo and Poppe (2018), Deardoff and Kent (1989), Lunestad, 2003, Mo <i>et al.</i> (2010), Levsen and Maage (2016), Adams <i>et al.</i> (1997), Johansen <i>et al.</i> (2016), Treasurer (1997)

Table 3 (continued)

Parasitic agents	Wrasse	Lumpfish	Salmon	Comments	References
Flukes	X	X	X	<i>A. simplex</i> and <i>Pseudoterranova</i> sp. found in salmon. Salmon usually eat feed pellets, but might eat cleaner fish. If the cleaner fish is infected, it could potentially transmit to salmon. <i>Microcotyle donavini</i> , <i>Macvicaria alacris</i> and <i>Gaevskayatrema perezii</i> are special concerns regarding import of wrasse. <i>Cryptocotyle lingua</i> found on lumpfish and wrasse. Considered relatively harmless and also found in salmon and cod.	(Karlsbakk <i>et al.</i> (2014), Treasurer (1997), Erkinharju <i>et al.</i> (2019), VKM <i>et al.</i> (2019b), Alarcon <i>et al.</i> (2016b), McMurtrie <i>et al.</i> (2019), Heuch <i>et al.</i> (2011)
Cestodes	X	X	X	<i>Eubothrium</i> sp. been reported from both salmon and lumpfish. Cestodes also occur in wrasse. Might use copepods as intermediate hosts. Cestode larvae may be found in the intestine of lumpfish.	McMurtrie <i>et al.</i> (2019), Hjeltnes <i>et al.</i> (2019), Rolbiecki and Rokicki (2008), Karlsbakk <i>et al.</i> (2014), Johansen <i>et al.</i> (2016)
<i>Caligus elongatus</i>	X	X	X	Lumpfish important host for genotype I. Commonly observed on wild fish. Generalist.	Hjeltnes <i>et al.</i> (2019), Treasurer (1997), VKM <i>et al.</i> (2019b), Johansen <i>et al.</i> (2016), Davenport (1985), Karlsbakk <i>et al.</i> (2014), Powell <i>et al.</i> (2018), Øines <i>et al.</i> (2006), VKM <i>et al.</i> (2017), Todd (2006)
Other copepods	X	X	X	<i>C. centrodoni</i> and <i>Hatschekia</i> sp. reported from wrasse. Lumpfish is intermediate host for <i>Lernaeocera branchialis</i> , while cod is end host. <i>L. salmonis</i> has not been observed in cleaner fish.	Treasurer (1997), McMurtrie <i>et al.</i> (2019), Steigen <i>et al.</i> (2018), Karlsbakk <i>et al.</i> (2014), Khan <i>et al.</i> (1990), Powell <i>et al.</i> (2018), Brooker <i>et al.</i> (2007), Davenport (1985)

2016a; Scholz *et al.*, 2018a). *P. perurans* has been identified from lumpfish in both Scotland and Norway (Karlsbakk, 2015; Oldham *et al.*, 2016), and AGD are occasionally reported in lumpfish (Fig. 16), both in land-based facilities

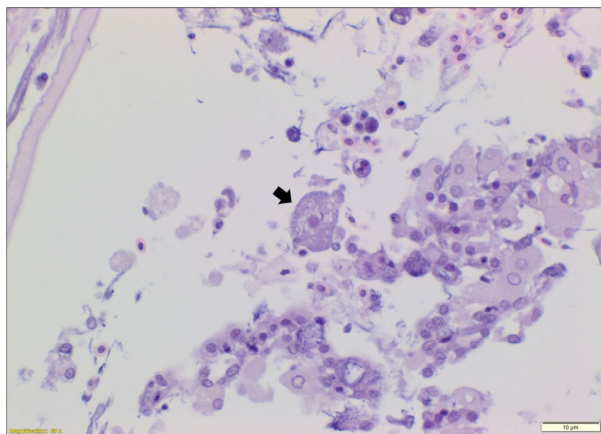


Figure 16 Amoeba-like organism (thick arrow) found among cellular debris located in-between gill filaments of lumpfish affected by amoebic gill disease (AGD). HE stain. Scalebar 10 µm. Photograph: Toni Erkinharju.

and at sea together with salmon (Hjeltnes *et al.*, 2019). Infected lumpfish can develop similar gill lesions as other fish species, but they appear to be more resistant, as progression is slower, mortality lower and fewer fish affected,

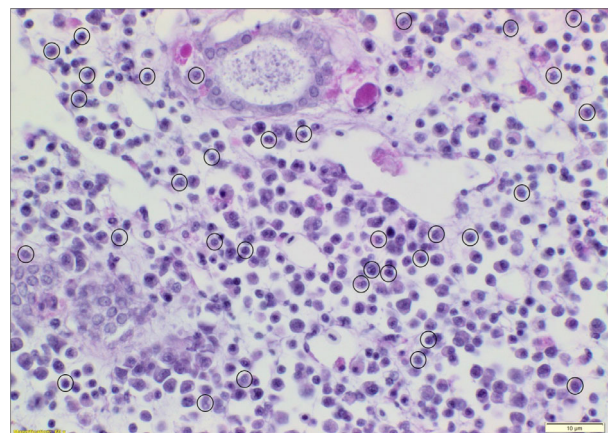


Figure 17 Kidney from lumpfish with suspicion of infection with microsporidian parasites. Several cells display intranuclear oval-shaped structures (some indicated by black circles). PAS stain. Scalebar 10 µm. Photograph: Toni Erkinharju.

when compared to infection in Atlantic salmon (Haugland *et al.*, 2017). *P. perurans* is currently the only pathogen shown experimentally to be transmitted from lumpfish to cohabitated salmon, indicating that the lumpfish could function as a reservoir for the amoeba, unless it is successfully removed from both species (Johansen *et al.*, 2016a; Haugland *et al.*, 2017; VKM *et al.*, 2017).

Nucleospora cyclopteri is a microsporidian parasite only reported from lumpfish. It was initially described from captive lumpfish in eastern Canada in the middle of the 1990s (Mullins *et al.*, 1994) but was first characterised from Icelandic lumpfish in 2013 (Freeman *et al.*, 2013; Freeman & Kristmundsson, 2013). Recently, the parasite has also been reported in both wild and farmed lumpfish in Norway (Karlsbakk *et al.*, 2014; Alarcon *et al.*, 2016b), and in broodfish from the UK (Scholz *et al.*, 2018a). It is closely related to *N. salmonis* (Freeman *et al.*, 2013), which is known to cause high mortality in salmonids (El Alaoui *et al.*, 2006; Cain & Polinski, 2014). It is uncertain whether the parasite in lumpfish use intermediate hosts/vectors as part of its life cycle (Scholz *et al.*, 2018a), but direct transmission has been shown for certain microsporidian parasites in fish, such as *N. salmonis* (Lom & Nilsen, 2003). *N. cyclopteri* has been proven a significant lumpfish pathogen, infecting the nucleus of the fish's leucocytes, mainly lymphocytes and lymphocyte precursor cells (Fig. 17). Pathology and mortality can be severe in affected fish. The most prominent clinical signs are in the kidneys, which can be presented as several white nodules on the surface, or as swollen, enlarged and pale organs due to extensive proliferation of parasite-infested leucocytes within the tissue (Freeman *et al.*, 2013; Karlsbakk *et al.*, 2014; Alarcon *et al.*, 2016b). The parasite has also been detected in high densities in the spleen, heart, gills and other organs, suggesting that the infection may be systemic (Freeman *et al.*, 2013; Warland, ; Hansen *et al.*, 2019). It is also possible that the parasitic infection of leucocytes will lead to impairment of the fish's immune system (Scholz *et al.*, 2018a). Definitive diagnosis is made on histological observation of the microsporidian in the nuclei of lymphocytes (usually as oval-shaped spores), supported by special staining techniques (such as Gram-Twort, Calco-Fluor-White or *in situ* hybridisation), and by PCR analysis (Freeman *et al.*, 2013; Hansen *et al.*, 2019).

Another microsporidian parasite is *Tetramicra brevifilum* that has been reported causing disease in farmed turbot in southern Europe (Figueras *et al.*, 1992; Estevez *et al.*, 1992). It was recently isolated from lumpfish brood stock in Ireland (Scholz *et al.*, 2017). Infected fish presented severe bloating, exophthalmos, white corneal patches and numerous blister-like nodules on the body surface. Internally, numerous cysts and white nodules were noted on all organs, and a mucoid-like fluid were observed in the abdominal cavity. The livers

were the most heavily affected organ, some pale yellow and mottled with cysts on the surface. Xenomas ('xenoparasitic complexes') are located inside the cysts, which may form multiple large space occupying complexes within affected organs. Inflammation is usually not observed, unless the xenomas rupture and free microsporidians enter the surrounding tissue (Scholz *et al.*, 2017). A presumptive diagnosis is made on clinical history and pathological findings. Confirmation of the species through molecular analysis is advised (Scholz *et al.*, 2018a). *T. brevifilum* is often found in lumpfish at sea on the coast of Ireland (Scholz *et al.*, 2018a). It has not been reported from lumpfish in Norwegian aquaculture (Hjeltnes *et al.*, 2019).

Other reported microsporidian parasites are *Loma*-like parasites (species unknown) and *Desmozoon lepeoptheirii* (syn. *Paranucleospora theridion*) from gills of wrasse with minimal or no associated pathology (Steigen *et al.*, 2018; Scholz *et al.*, 2018a). *D. lepeoptheirii* and *Loma salmonis* are significant gill pathogens for salmonid fish (Kent *et al.*, 1989; Ramsay *et al.*, 2002; NYLUND *et al.*, 2010; Mitchell & Rodger, 2011). Atlantic salmon appear relatively resistant to infection with *L. salmonis* (Mitchell & Rodger, 2011), while both Atlantic salmon and salmon louse (*Lepeophtheirus salmonis*) may function as natural reservoirs for *D. lepeoptheirii* (NYLUND *et al.*, 2010; Mitchell & Rodger, 2011). It is unknown whether wrasse could potentially transmit such microsporidian parasites to cohabitated salmon (Steigen *et al.*, 2018). Direct transmission has been demonstrated for *L. salmonis* (Lom & Nilsen, 2003).

The protist *Ichthyophonus hoferi* is a fungal-like parasite that can potentially affect all species of fish and cause severe mortality (Jansson & Vennerström, 2014). Infected fish are typically emaciated with numerous cysts located beneath

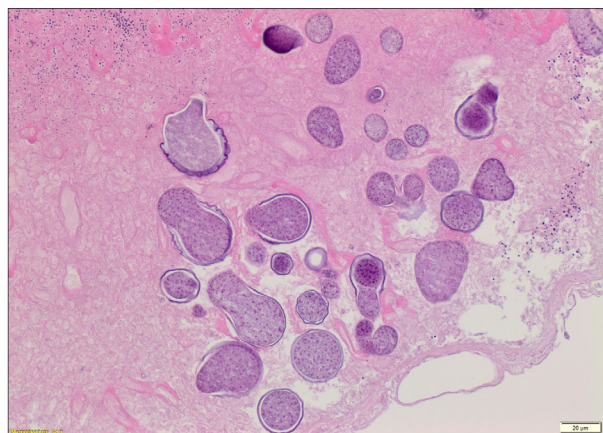


Figure 18 Several budding spores in the spleen of lumpfish systemically infected by *Ichthyophonus* sp. HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

the skin. Some may also display central nervous symptoms if the parasite has spread to the brain. Internally, several white nodules formed by granulomatous inflammation can be observed in the heart, liver, kidney, spleen and in the intestinal wall (Rahimian, 1998; Roberts, 2012). The disease is diagnosed by histologically observing granulomas with large club-shaped spores, surrounded by a thick double contoured wall (Fig. 18) (Rahimian, 1998; Bruno *et al.*, 2006). The spores also stain positive with periodic acid-Schiff (PAS) stain, which can aid in reaching a diagnosis. *I. hoferi*-like organisms have previously been reported in infected lumpfish in Norway, causing high mortality and characteristic lesions (Hansen & Nilsen, 2018; Hjeltnes *et al.*, 2019). It is often detected in heart and skeletal muscle tissue, due to a preference for active musculature (Kocan *et al.*, 2006; Hansen & Nilsen, 2018). There is a concern that infected lumpfish could potentially transmit the parasite to cohabitated salmon at sea (Mo & Poppe, 2018). It has recently been reported from ballan wrasse suffering from increased mortality (Hjeltnes *et al.*, 2019).

Trichodinids are mobile ciliates that can be found on the skin and gills of several species of fish (Khan, 1991; Gaze & Wootten, 1998; Lio-Po & Lim, 2014). Two species of *Trichodina*, *T. cyclopteri* and *T. galaye*, are host-specific and commonly found on the gills of lumpfish (Fig. 19a) (Karlsbakk *et al.*, 2014; Johansen *et al.*, 2016a). Trichodinids are

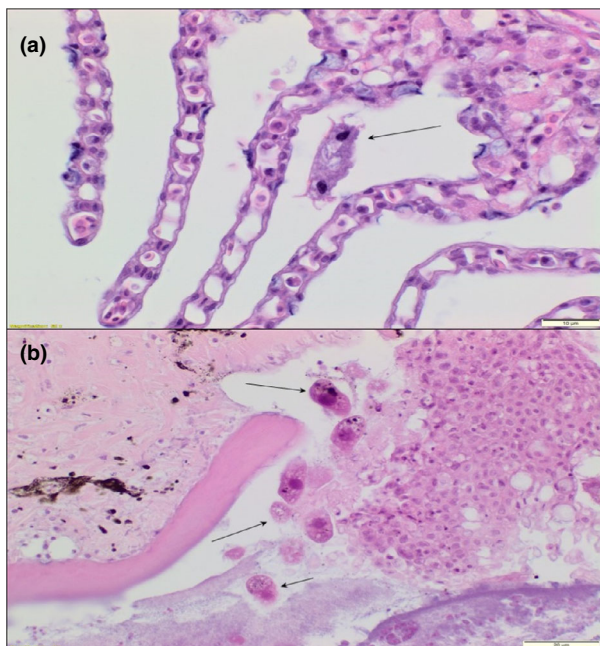


Figure 19 Ciliates infesting lumpfish. (a) *Trichodina* sp. (arrow) on gills of lumpfish. HE stain. Scalebar 10 μ m. (b) Scuticociliatosis of lumpfish skin. Multiple scuticociliates (arrows) can be seen infesting the underlying skin tissue. HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

usually a problem for juvenile fish in stressful and overcrowded habitats, where massive infestations can cause severe damage to the epidermal tissue and gills, resulting in respiratory distress and mortality. They are easily observed microscopically in gill and skin samples (Roberts, 2012; Lio-Po & Lim, 2014; Scholz *et al.*, 2018a). Heavy infestations of juvenile lumpfish with *Trichodina* sp. have been reported (Powell *et al.*, 2018; Scholz *et al.*, 2018a). The parasites are a commonly reported finding in Norwegian aquaculture, but are not usually related to major health problems (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019). Trichodinids have also been found in varying abundance on the gills of wild-caught wrasse, usually without any associated pathology (Steigen *et al.*, 2018; Scholz *et al.*, 2018a).

Scuticociliates are free-living, fast-moving marine parasites widely distributed in the oceans (Roberts, 2012; Piazzon *et al.*, 2013). They are considered secondary pathogens of lumpfish and are often observed associated with skin lesions of affected fish in Ireland. It was very recently reported from Norwegian farmed lumpfish (Østevik *et al.*, 2018; Scholz *et al.*, 2018a). Infected fish usually present deep, white ulcers on the skin (Fig. 19b); however, the parasite can also infest the gills, and sometimes the infection progresses systemically, which can cause necrosis of the brain tissue. The parasite can be observed microscopically, sometimes filled with phagocytised red blood cells (Roberts, 2012; Østevik *et al.*, 2018; Scholz *et al.*, 2018a). The species has of yet not been identified, but have been reported to resemble *Uronema marinum*, which have been observed in several marine fish species (Piazzon *et al.*, 2013; Scholz *et al.*, 2018a). Other ciliates have been reported from the gills of wrasse without any apparent damage to the tissue, such as the scuticociliate *Pseudocohnilembus persalinus*, and *Cryptocaryon*-like ciliates (McMurtrie *et al.*, 2019). The

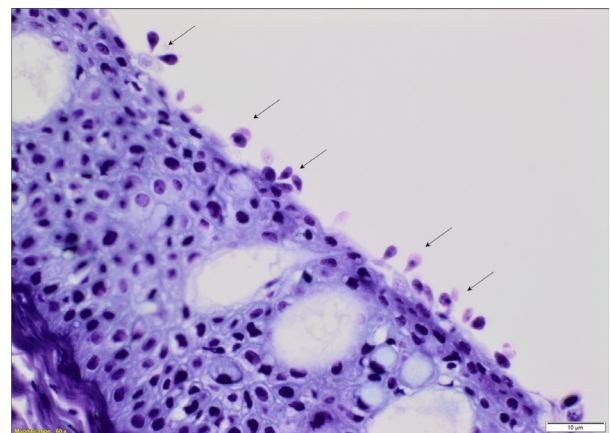


Figure 20 Skin section from lumpfish with several *Ichtyobodo* sp. ('Costia') parasites (arrows) located on the surface of epidermis. Giemsa stain. Scalebar 10 μ m. Photograph: Toni Erkinharju.

species *C. irritans* is known as the cause of ‘white spot’ disease in marine fish (Colorni & Burgess, 1997). Similarly, protozoan ciliates have been found on Atlantic salmon (Ferguson *et al.*, 1987; Bermingham & Mulcahy 2007; Dyková *et al.*, 2010), but scuticociliates have not been observed causing disease in healthy salmon (Scholz *et al.*, 2018a).

Ichthyobodo (formerly *Costia*) are flagellates that infect gill or skin tissue of both freshwater and marine fish (Isaksen *et al.*, 2011; Mitchell & Rodger, 2011; Cain & Polinski, 2014). It consists of a number of species, two of which have been identified from farmed salmonids. *I. salmonis* is described as eurohaline, and usually found on sea-based salmon, while *I. necator* appears to be specific for freshwater (Mitchell & Rodger, 2011; Cain & Polinski, 2014). Both species can cause ichthyobodosis, potentially leading to mortality if infections become severe. Typical clinical signs are greyish coating on the body surface, caused by epithelial hyperplasia in the skin, and ‘flashing’ behaviour (fish trying to scratch itself by rubbing against objects or the bottom) (Roberts, 2012; Scholz *et al.*, 2018a). *Ichthyobodo* parasites are easily identified microscopically on affected skin or gills (Isaksen *et al.*, 2011; Cain & Polinski, 2014). *Ichthyobodo* spp. have been found on skin and gills of lumpfish (Fig. 20) and gills of wrasse, and appear both to be novel species (Karlsbakk *et al.*, 2014; Steigen *et al.*, 2018). They are not considered to be a severe pathogens in lumpfish and wrasse (Johansen *et al.*, 2016a; Scholz *et al.*, 2018a).

Some organisms are commensals rather than true parasites. For example, *Cryptobia dahli* is a flagellate commonly found within the stomach of wild-caught lumpfish (Karlsbakk *et al.*, 2014). It is regarded as harmless for the lumpfish, since it does not cause any apparent damage to the gastric tissue, even when present in extremely high numbers. As such, it is not considered to become a problem for lumpfish aquaculture in the future (Freeman & Kristmundsson, 2018). On the other hand, the hemoflagellate *Cryptobia* (Trypanoplasma) *salmositica* is well known for causing disease in salmonids (Woo, 2003). Uncharacterised *Cryptobia* sp. organisms have been observed in some species of wrasse (VKM *et al.*, 2019a). *C. dahli* is often spotted together with *Cycloptericola marina*, a fungal-like organism sometimes covering parts of the stomach mucosal wall (Fig. 21), which is also considered harmless for the lumpfish host (Karlsbakk *et al.*, 2014; Erkinharju *et al.*, 2019). Finally, a flagellate described as *Spiroucleus* sp. has been observed within the intestinal tract of lumpfish (Karlsbakk *et al.*, 2014).

Piscine apicomplexans are obligate parasites affecting both marine and freshwater fish, where the majority belong to the taxonomic class Coccidea (Colorni & Diamant, 2014; Xavier *et al.*, 2018). They infest a wide range of different organs, including the intestine, swim bladder, liver,

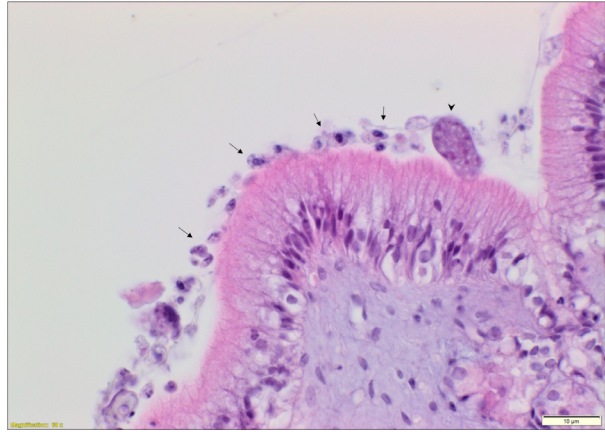


Figure 21 *Cryptobia* sp. (arrows) and *Cycloptericola* sp. (arrowhead) on the mucosa surface of stomach from lumpfish. HE stain. Scalebar 10 µm. Photograph: Toni Erkinharju.

spleen, testes, kidney, gills, blood cells. Fish coccidian infections have been the cause of large losses in freshwater aquaculture (Roberts, 2012; Colorni & Diamant, 2014). However, little is known of the impact this group pose on marine aquaculture, and subclinical infections might go unnoticed. In addition, most of the reported disease outbreaks are in different species of warm water fish (Colorni & Diamant, 2014). Diagnosis is usually made on microscopical identification of different coccidian life stages (Roberts, 2012). An eimerid apicomplexan has recently been reported from infected lumpfish juveniles in Norway and wild-caught broodfish in Iceland. Affected fish displayed a period of high mortality, but few clinical signs, except for thinning of parts of the intestinal tract, were

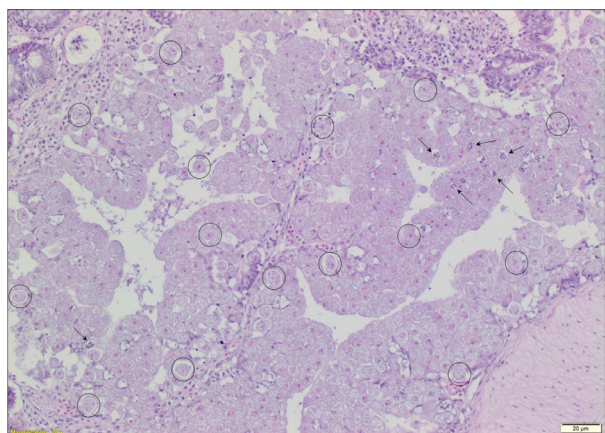


Figure 22 Parts of the intestine of lumpfish with developing coccidian parasites inside hypertrophic epithelial cells. Numerous macrogamonts (some indicated by black circles) and several microgamonts (black arrows) are noticeable. HE stain. Scalebar 20 µm. Photograph: Toni Erkinharju.

observed. Parasites were found inside hypertrophic epithelial cells in the anterior part of the intestines and in the pyloric caeca on histology (Fig. 22). Large parts of the intestine epithelial layer were sloughed off, caused by bursting of the parasite infected cells (Kristmundsson *et al.*, 2018). Intestinal coccidian infestations (*Eimeria* sp.) in varying abundance are sporadically reported from lumpfish in Norwegian aquaculture (Hjeltnes *et al.*, 2019) and are highly prevalent in wild-caught lumpfish. *Eimeria*-like intestinal coccidians have also recently been reported from wrasse in Norway and the UK (McMurtrie *et al.*, 2019; Åkesson *et al.*, 2020). Coccidian parasites are also found in salmonids, such as in wild-caught juvenile fry in inland waters of Norway (Sweco, 2017). Although *Eimeria* sp. is commonly found in many fish species, it displays a high degree of host specificity and is as such not considered a significant pathogen, regarding cohabitated cleaner fish and salmon (Molnár *et al.*, 2012; Åkesson *et al.*, 2020). Coccidiosis in terrestrial animals might cause reduced intestinal function, which then may lead to reduced growth and appetite (Gjerde, 2011b). As such, it has been suggested that severe coccidian infections might negatively affect the cleaner fish's appetite, thus affecting its efficiency as lice eaters (Erkinharju *et al.*, 2019).

Kudoa are a group of myxozoan parasites comprising numerous species (Eiras *et al.*, 2014). Most species infect the skeletal muscle of fish, and some cause post-mortem histolysis (known as 'soft flesh disease') where the musculature become very soft and liquefied (Roberts, 2012; Henning *et al.*, 2013). For example, *Kudoa thyrmites* has been reported from muscle tissues of Atlantic salmon (St-Hilaire *et al.*, 1997; Jansson & Vennerström, 2014). The muscle fillet becomes unsuitable for human consumption (Henning *et al.*, 2013; Scholz *et al.*, 2018a). In recent years, some species, mainly *K. septempunctata* and *K. hexapunctata*, have been suggested as possible causes of food poisoning after ingestion of raw fish products (Kawai *et al.*, 2012; Suzuki *et al.*, 2015). The parasite is easily identified by microscopically observing its characteristic spores (with four polar capsules) within infected muscle tissue (Fig. 23) (Roberts, 2012; Jansson & Vennerström, 2014). *Kudoa islandica* has been reported from wild-caught lumpfish and two species of wolffish in Iceland, where numerous parasite plasmodia (white, tubular structures) had substituted considerable parts of the muscle fibres and heavily infected fish showed extensive liquefactive necrosis of muscle tissue (Kristmundsson & Freeman, 2014). The parasite has also been recently observed in farmed lumpfish in Norway (Alarcon *et al.*, 2016b), is prevalent in wild-caught lumpfish and is sporadically detected in skin/muscle samples submitted for histology (Hjeltnes *et al.*, 2019). No species have been described from wrasse used as cleaner fish, but *Kudoa*-like myxozoan organisms are observed regularly within the

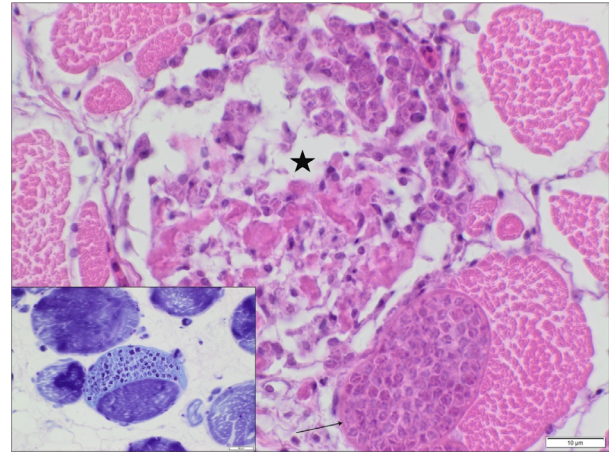


Figure 23 Necrosis of infected muscle fibre (star) and plasmodium with myxozoan spores (*Kudoa* sp.) (arrow) within skeletal musculature of lumpfish. Insert shows parasite spores with their characteristic four polar capsules visible. HE stain and Giemsa stain (insert). Scalebar 10 μ m. Photograph: Toni Erkinharju.

skeletal musculature (Scholz *et al.*, 2018a). *Kudoa ovivora* n. sp. has been found in the ovaries of wild-caught Caribbean labroid fish (Swearer & Robertson, 1999). Among the known *Kudoa* species, the host specificity can range from rather strict to more wide (Lom & Dyková, 2006). As *K. islandica* is capable of infecting other fish than lumpfish, it may be able to infect cohabitated salmon in sea cages (Kristmundsson & Freeman, 2014; Scholz *et al.*, 2018a).

Myxobolus aeglefini (synonym *M. albi*) is a myxozoan parasite known to infect cartilaginous tissue of fish. The parasite has a wide host species preference (Yokoyama & Wakabayashi, 2000; Molnar *et al.*, 2008; Karlsbakk *et al.*, 2017). It has been observed in wild-caught lumpfish in Maine, the USA (Cavin *et al.*, 2012). The most common clinical signs were uni- or bilateral exophthalmos and white to tan coloured nodules in the sclera of the eye. The parasites were also observed in cartilaginous tissue other places in the body, such as in the skull, tongue, gill arches, vertebrae, fins and pectoral girdle. Large and coalescing myxozoan cysts were formed within the cartilage resulting in degeneration and necrosis of cartilage, extending into adjacent bone and surrounding tissue (Cavin *et al.*, 2012). Some of the external symptoms also resemble the typical clinical signs associated with *M. cerebralis* infection, the cause of whirling disease in salmonids (Blazer *et al.*, 2004; Roberts, 2012; Cain & Polinski, 2014; Powell *et al.*, 2018). It is speculated that this lumpfish disease is underdiagnosed, as subclinical infections might occur and go unnoticed (Scholz *et al.*, 2018a). It has not been reported from lumpfish in Norway (Erkinharju *et al.*, 2019). *Myxobolus* species has not been reported from wrasse. *M. aeglefini* has been

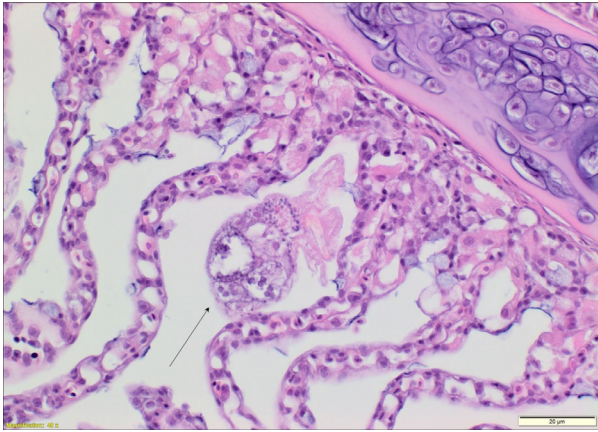


Figure 24 Monogenean parasite (*Gyrodactylus* sp.) located between two filaments (arrow) in lumpfish gills. HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

reported from Atlantic salmon kept in close proximity to infected Atlantic cod (*Gadus morhua*) in sea cages; however, it was not discovered how the salmon had become infected with the parasite (Mo *et al.*, 1992). Myxozoan parasites typically has a two-host life cycle, such as *M. cerebralis* alternating between the invertebrate *Tubifex tubifex* and numerous salmonid species (Gilbert & Granath, 2001). However, tubifex worms are benthic organisms found on the sea bed, typically far below the depth of sea cages,

making it unlikely that salmon would become infected from consuming these (Mo *et al.*, 1992).

Gyrodactylus are well-known monogenean ectoparasites (flatworms) of many fish species (Cain & Polinski, 2014). Especially, *G. salaris* is a major challenge, as it causes high mortality in both wild and farmed salmonids and can easily be transmitted to new areas (Bakke *et al.*, 1992). The parasite has made huge impacts on Atlantic salmon in Norway, decimating the natural populations by nearly 90% in many river areas (Johnsen *et al.*, 1999; Cain & Polinski, 2014). In lumpfish, large numbers of *G. cyclopteri* can be found attached to the gills and skin (Fig. 24) (Karlsbakk *et al.*, 2014; Alarcon *et al.*, 2016b; Pietrak & Rosser, 2020). This parasite could potentially cause problems in aquaculture. However, the occurrence of *Gyrodactylus* and associated gill lesions in Norwegian lumpfish has not been fully surveyed (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019; Erkinharju *et al.*, 2019). *Gyrodactylus* sp. has been observed on wrasse (VKM *et al.*, 2019a).

Known parasitic helminths of fish are different species of nematodes (roundworms), flukes and cestodes (tapeworms) (e.g. see Bristow (1993), Jyrwa *et al.* (2016), Eiras (2016), Rauque *et al.* (2018)). These are larger internal parasites, of which several can be observed with the naked eye in infected fish (Jansson & Vennerström, 2014). They are relatively common in wild-caught fish and several different species have been observed in cleaner fish (Fig. 25). For example, both *Eubothrium crassum*, a cestode, and *Cryptocotyle* sp., a trematode, have been reported from lumpfish (Rolbiecki & Rokicki,

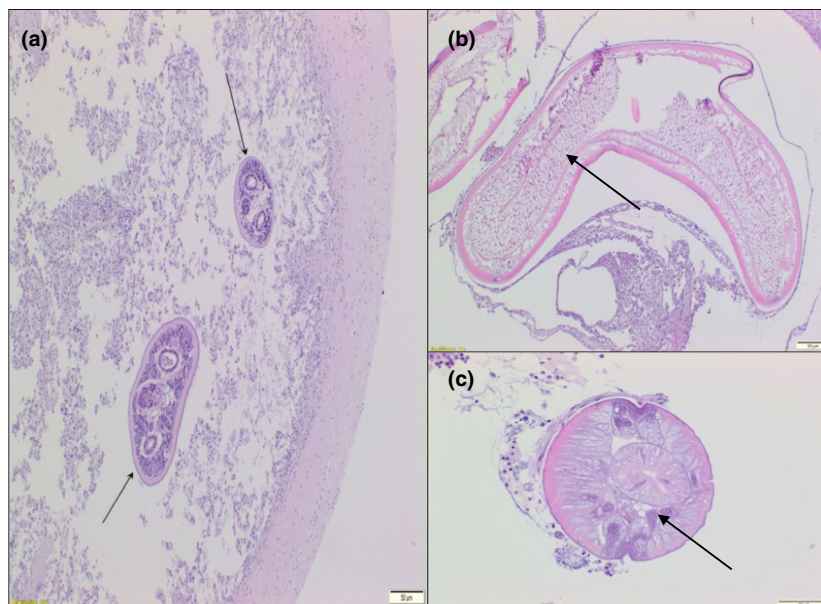


Figure 25 Helminths in lumpfish. (a) Two digenean trematodes (arrows) of unknown species within the lumen of pyloric caeca in lumpfish. Surrounding tissue is heavily autolysed. (b) Nematode (arrow) (unknown species) in abdominal cavity, longitudinal section. (c) Nematode (arrow) (unknown species) in abdominal cavity, cross section. HE stain. Scalebar 50 μ m (a, b) and 20 μ m (c). Photograph: Toni Erkinharju.

2008; Alarcon *et al.*, 2016b). Cestodes might be found within the intestinal tract of lumpfish and wrasse, but do not appear to be major health concerns (Karlsbakk *et al.*, 2014; McMurtrie *et al.*, 2019). In recent years, there has been a reported increase on the occurrence of cestodes, *Eubothrium* sp., within the intestine of Atlantic salmon kept in sea cages in Norway (Hjeltnes *et al.*, 2019). *Cryptocotyle lingua*, which is not considered a particularly problematic parasite in regards to fish health, can be found on wrasse and other fish, such as cod and salmon (Treasurer, 1997; Heuch *et al.*, 2011; VKM *et al.*, 2019a). On the other hand, three selected species, *Microcotyle donavini*, *Macvicaria alacris* and *Gaevskayatrema perezi*, are of special concerns regarding import of wrasse to Norway (VKM *et al.*, 2019a).

Two species of nematodes in particular, *Hysterothylacium aduncum* and *Anisakis simplex*, are considered common in wild-caught lumpfish, and encapsulated larvae can be found within internal organs of affected fish (Rolbiecki & Rokicki, 2008; Karlsbakk *et al.*, 2014; Mo & Poppe, 2018; Scholz *et al.*, 2018a). Other known species include *Contratecum oscalatum* and *Pseudoterranova* sp. (Rolbiecki & Rokicki, 2008; Karlsbakk *et al.*, 2014). Same species has also been reported from wrasse and salmon (Treasurer, 1997; Mo *et al.*, 2010; Johansen *et al.*, 2016b; Scholz *et al.*, 2018a; VKM *et al.*, 2019a). *A. simplex* is a zoonotic pathogen; it is one of two species frequently associated with human anisakiasis, which can result from ingestion of raw or undercooked fish meat (Adams *et al.*, 1997; Roberts, 2012; Jansson & Vennerström, 2014). This has raised some concern, as predation of cleaner fish by cohabitated salmon have been observed, which indicate that cleaner fish infected with *A. simplex* could potentially transmit the parasite to salmon meant for human consumption (Mo & Poppe, 2018). However, exactly how common this nematode is in farmed Norwegian cleaner fish, in addition to other helminths, is still unknown (Karlsbakk *et al.*, 2014; Mo & Poppe, 2018). For farmed Atlantic salmon, the amounts of different nematodes have ranged from none-to-low in previous reports (Deardorff & Kent, 1989; Lunestad, 2003; Mo *et al.*, 2014).

Sea lice are marine and brackish water crustaceans commonly found on farmed fish. The copepods *Lepeophtheirus salmonis* and different species of *Caligus* are widely known as the most important parasites in salmonid aquaculture (Boxaspen, 2006; Jansson & Vennerström, 2014). The parasite attaches to the fish body surface and causes severe skin erosions in heavily infested individuals (Costello, 2006; Roberts, 2012). Eroded skin lesions make the fish susceptible to secondary infections and causes osmoregulatory difficulties, which may ultimately result in death. Diagnosis is normally made by observing the parasite on infected fish. (Jansson & Vennerström, 2014). Wild-caught lumpfish appear to regularly suffer from infestation by *Caligus elongatus* (Fig. 26),

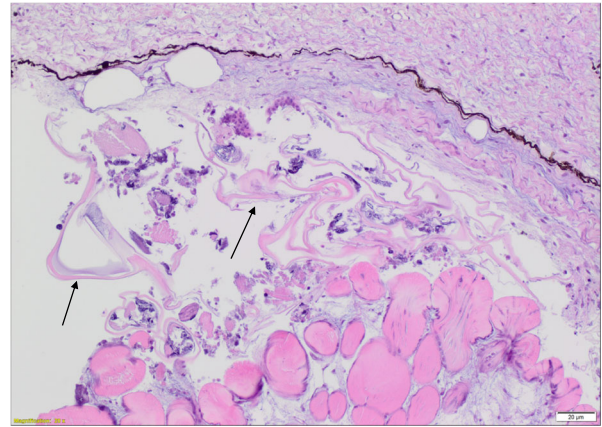


Figure 26 Skin section of lumpfish with suspected cuticle-like remnants from a parasitic copepod (arrows). HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

often with several parasites attached around the bony tubercles (Davenport, 1985; Heuch *et al.*, 2007; Karlsbakk *et al.*, 2014; Powell *et al.*, 2018). The crustacean is also considered a problem for farmed lumpfish on several sites in Troms and Finnmark county in northern Norway (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019). *C. elongatus* consists of two genotypic variants, genotype 1 and genotype 2, where lumpfish appear to be the main reservoir of genotype 1 (genotype 2 is mostly associated with farmed salmon) (Oines *et al.*, 2006; Oines & Heuch, 2007). As *C. elongatus* is considered a generalist and show low host specificity, infected lumpfish could potentially transmit the parasite to other fish species (Powell *et al.*, 2018; Hjeltnes *et al.*, 2019). The copepod has also been found on different species of wrasse (Treasurer, 1997; VKM *et al.*, 2019a) and Atlantic salmon (Todd, 2006). Other parasitic copepods have also been observed in lumpfish, such as *Lernaocera branchialis*, a sea louse sometimes found in Atlantic cod, whose early life stages can cause quite severe infections in the gills of affected lumpfish (Karlsbakk *et al.*, 2014). Lumpfish is an intermediate host for this parasite (Khan *et al.*, 1990; Brooker *et al.*, 2007). There are no known records of *L. salmonis* infections in lumpfish (Powell *et al.*, 2018). Other species, such as *Caligus centrodoni* and *Hatschekia* sp., have been reported from wild-caught wrasse (Treasurer, 1997; Steigen *et al.*, 2018; McMurtrie *et al.*, 2019; VKM *et al.*, 2019a).

Viruses

Viral infections can cause major disease outbreaks in aquaculture settings. Very few viral diseases have been reported from lumpfish or wrasse, and novel viruses are usually identified through investigation of diseased fish (Treasurer, 2012; Karlsbakk *et al.*, 2014; Scholz *et al.*, 2018a). Some are considered to be specific for the cleaner fish, while others

Table 4 Summary of viral pathogens reported from wrasse (Labridae), lumpfish (*C. lumpus*) and Atlantic salmon (*S. salar*) with relevant references

Viral agent	Wrasse	Lumpfish	Salmon	Comments	References
Viral haemorrhagic septicaemia virus (VHSV)	X	X	X	Lumpfish isolate novel subgroup in genotype IV. Wrasse isolates is of genotype III. Rainbow trout is susceptible to genotype III, Atlantic salmon appears less susceptible. Virus is present in wild marine fish. Low virulent strains might mutate into highly virulent strains. Special concerns regarding import of cleaner fish.	Guðmundsdóttir <i>et al.</i> (2019), Garver <i>et al.</i> (2013), King <i>et al.</i> (2001), Munro <i>et al.</i> (2015), Matejusova <i>et al.</i> (2016), Sandlund <i>et al.</i> (2014), Dale <i>et al.</i> (2009), Hjeltnes <i>et al.</i> (2019), Wallace <i>et al.</i> (2015), VKM <i>et al.</i> (2017), Johansen <i>et al.</i> (2016), Ito <i>et al.</i> (2016), VKM <i>et al.</i> (2019b)
Ranavirus (proposed European North Atlantic Ranavirus)	-	X	-	Closely related to EHN. EHN appear to be less pathogenic to salmon. Viral agent of special concern regarding import of lumpfish. Ranaviruses are common in many poikilothermic animals. Does not appear to be a primary pathogen for lumpfish.	Scholz <i>et al.</i> (2018a), Ariel <i>et al.</i> (2016), Whittington <i>et al.</i> (2010), Stagg <i>et al.</i> (2020)
Cyclopterus lumpus virus (CLuV)/Lumpfish flavivirus (LFV)	-	X	-	Initial trial failed to transmit virus from lumpfish to salmon, but still uncertain whether transmission is possible. Several reported cases of disease in lumpfish recently, at all stages of production.	Skoge <i>et al.</i> (2018), Vestvik <i>et al.</i> (2017), Hjeltnes <i>et al.</i> (2019), VKM <i>et al.</i> (2017)
Nodavirus (Nervous necrosis virus – NNV)	X	X	X	Wide range of marine fish species affected. Found in wild fish. Nodavirus-like particles detected in Atlantic salmon with CMS. Experimental injection of salmon has shown pathology. Special concern regarding import of wrasse. Lumpfish have recently shown susceptibility to nodavirus.	Toffan <i>et al.</i> (2019), Korsnes <i>et al.</i> (2017), Grotmol <i>et al.</i> (1997), Munday <i>et al.</i> (2002), VKM <i>et al.</i> (2017), Korsnes <i>et al.</i> (2005), VKM <i>et al.</i> (2019b)
Cyclopterus lumpus Coronavirus (CLuCV)	-	X	-	Only reported from lumpfish as of date. Little is known about the virus. Coronavirus are known for causing diarrhoea. Pathogen tests are needed for lumpfish. Closely related to viruses from other fish (such as Atlantic/Chinook salmon bafini virus)	NFO (2019), Johansen <i>et al.</i> (2019),
Cyclopterus lumpus Totivirus (CLuTV)	-	X	-	Only reported from lumpfish as of date. Little is known about the virus. Appear closely related to PMCV, causing disease in salmon. Need pathogen tests.	NFO (2019), Johansen <i>et al.</i> (2019)
Infectious pancreatic necrosis virus (IPNV)	X	X	X	Not detected in Norwegian cleaner fish aquaculture. Lumpfish have shown experimental susceptibility, and could potentially be a reservoir. The virus has a broad host range and reported from several fish species. Use of IPN-resistant salmon and trout might reduce the viral threat.	Hjeltnes <i>et al.</i> (2019), Breiland <i>et al.</i> (2015), Scholz <i>et al.</i> (2018a), Johansen <i>et al.</i> (2016), VKM <i>et al.</i> (2017), Gomez-Casado <i>et al.</i> (2011), Roberts and Pearson (2005), Treasurer (2012)
Salmonid alphavirus (SAV)	X	-	X	Not reported from lumpfish. Reported from wrasse (SAV subtype 6). Wrasse have not shown disease signs. No evidence of viral transfer to salmon. Vector/vehicle transmission considered possible. Horizontal transmission important for spread. Moderate if wrasse originate from SAV-endemic zone, low if outside of SAV-endemic zones. Disease from SAV is specific to salmon and trout. Considered special concern regarding import of wrasse.	Hjeltnes <i>et al.</i> (2019), Gomez-Casado <i>et al.</i> (2011), McLoughlin and Graham (2007), Ruane <i>et al.</i> (2018), Treasurer (2012), Røsaæg <i>et al.</i> (2017), Olsen <i>et al.</i> (2011), Kristoffersen <i>et al.</i> (2009), VKM <i>et al.</i> (2017), Johansen <i>et al.</i> (2016), Scholz <i>et al.</i> (2018a), VKM <i>et al.</i> (2019b), McCleary <i>et al.</i> (2014), Snow <i>et al.</i> (2010), Deperasińska <i>et al.</i> (2018)

Table 4 (continued)

Viral agent	Wrasse	Lumpfish	Salmon	Comments	References
				Isolated from wrasse in locality with diseased salmon. Sample contamination could not be excluded. Been detected in wild fish close to salmon farms.	
Infectious salmon anaemia virus (ISAV)	X	-	X	Reported from salmon cage with wrasse, although wrasse showed no pathology and no connection could be established. Cross-contamination possible. Reported from wild fish. Avirulent variant (ISAV-HPRO) is present in Norwegian wild salmon.	Hjeltnes <i>et al.</i> (2019), Treasurer (2012), Gomez-Casado <i>et al.</i> (2011), VKM <i>et al.</i> (2017), Nylund <i>et al.</i> (2002), Rimstad and Mjaaland (2002), Plarre <i>et al.</i> (2005), Madhun <i>et al.</i> (2019)
Piscine myocarditis virus (PMCV)	X	-	X	Reported causing disease in wrasse. Could potentially transmit virus to salmon. Contamination considered highly unlikely. CMS has been increasing in Atlantic salmon in Norway lately.	Scholz <i>et al.</i> (2018c), Hjeltnes <i>et al.</i> (2019), VKM <i>et al.</i> (2017), Garseth <i>et al.</i> (2018a), Rodger <i>et al.</i> (2014)
Piscine orthoreovirus (PRV)	X	-	X	Prevalent in wild fish. Described from wrasse without pathology. Cross-contamination from diseased salmon could not be excluded.	Hjeltnes <i>et al.</i> (2019), Wiik-Nielsen <i>et al.</i> (2012), Palacios <i>et al.</i> (2010), Wessel <i>et al.</i> (2017), Johansen <i>et al.</i> (2016), VKM <i>et al.</i> (2017)
Lymphocystis disease	X	-	-	Wide host range, reported from at least 140 fish species. Considered benevolent disease, causing skin lesions. Reported from ballan wrasse and also from bluestreak cleaner wrasse <i>Labroides dimidiatus</i> . Not known whether salmon could become affected.	Schonheit <i>et al.</i> (2017), Scholz <i>et al.</i> (2018a), Essbauer and Ahne (2001), VKM <i>et al.</i> (2017), VKM <i>et al.</i> (2019b), Whittington <i>et al.</i> (2010), Borrego <i>et al.</i> (2017)
Piscine orthoreovirus <i>Oncorhynchus mykiss</i> (PRVom; formerly 'Virus Y')	-	-	X	Isolated from rainbow trout (genotype PRV-3). Can cause experimental disease in Atlantic salmon. Not reported from cleaner fish.	Johansen <i>et al.</i> (2016), Hjeltnes <i>et al.</i> (2019), Vendramin <i>et al.</i> (2019), Olsen <i>et al.</i> (2015), Hauge <i>et al.</i> (2017)
Salmon gill pox virus (SGPV)	-	-	X	Isolated from gills of diseased salmon. Not reported from cleaner fish. Present in wild salmonids.	Johansen <i>et al.</i> (2016), Hjeltnes <i>et al.</i> (2019), Gjessing <i>et al.</i> (2015), Gjessing <i>et al.</i> (2017), Garseth <i>et al.</i> (2018b)
Infectious hematopoietic necrosis virus (IHNV)	-	-	X	Not detected in Norway. Never reported from cleaner fish.	Johansen <i>et al.</i> (2016), Saksida (2006), Hjeltnes <i>et al.</i> (2019), Dixon <i>et al.</i> (2016)

are also found in different teleosts, such as salmonids. Regarding salmonid viruses, it is not fully known, whether such viruses could be transmitted from cleaner fish to cohabitated salmon, and vice versa (Karlsbakk *et al.*, 2014; Scholz *et al.*, 2018a). Reported viruses from cleaner fish studies include viral haemorrhagic septicaemia virus (VHSV), an unspecified ranavirus, cyclopterus lumpus virus (CLuV) or lumpfish flavivirus (LFV), lymphocystis virus, nervous necrosis virus (NNV), a new totivirus, a new coronavirus, infectious pancreatic necrosis virus (IPNV), salmonid alphavirus (SAV), infectious salmon anaemia virus (ISAV), piscine myocarditis virus (PMCV), and piscine orthoreovirus (PRV) (Table 4) (Treasurer, 2012;

Johansen *et al.*, 2016a; Brooker *et al.*, 2018; Scholz *et al.*, 2018a; Hjeltnes *et al.*, 2019; 2019).

Viral haemorrhagic septicaemia (VHS) is a severe rhabdovirus disease reported from at least 80 different fish species in several parts of the world. It is classified as a notifiable disease by the World Organisation for Animal Health (OIE) (Skall *et al.*, 2005; Scholz *et al.*, 2018a). The virus has been grouped in four genotypes (GI-IV) with extended subgroups for genotype I and IV (Skall *et al.*, 2005; Cain & Polinski, 2014). Mortality can be quite severe, and characteristic clinical signs are widespread haemorrhaging on body surfaces, within the eyes, in internal organs and occasionally in the musculature. In severe cases,

the fish also appears anaemic. Occasionally, the central nervous system might also be affected, leading to abnormal fish behaviour (Al-Hussinee *et al.*, 2011; Roberts, 2012). Isolation of the virus with cell culture and identification by immunological or molecular analysis is recommended for diagnosis (Cain & Polinski, 2014; Office International des Epizooties, 2019). Recently, a novel VHS virus in genotype IV was found in wild-caught lumpfish meant for broodfish stock in Iceland. Intraperitoneal challenge with this virus isolate resulted in high mortality of lumpfish (3–10% survival) and by cohabitation with viral shedders (43–50% survival). However, the relative per cent survival after immersion challenge was at average 90% (performed in triplicate tanks) (Guðmundsdóttir *et al.*, 2019). By comparison, cohabitation of naïve Atlantic salmon with lumpfish shedders resulted in some mortality (80–92% survival), but the virus was not detected or re-isolated in any samples from the salmon (Guðmundsdóttir *et al.*, 2019). Viral isolates of VHSV genotype III have been reported from multiple wrasse species in Scotland (Munro *et al.*, 2015). Wild marine fish has been suggested as a possible origin of the viral outbreak (Wallace *et al.*, 2015). VHSV has so far not been detected in Norwegian cleaner fish (Karlsbakk *et al.*, 2014; Hjeltnes *et al.*, 2019).

As for other viruses, a novel ranavirus (genus iridovirus; proposed European North Atlantic Ranavirus) has been detected in lumpfish from the Faroes, Iceland, Scotland and Ireland. It is closely related to the epizootic hematopoietic necrosis virus (EHNV), but does not appear to be a primary pathogen in lumpfish (Scholz *et al.*, 2018a; Stagg *et al.*, 2020). Ranaviruses have also been reported from other marine fish, such as turbot and Atlantic cod, although their virulence remains unclear (Ariel *et al.*, 2016). Lumpfish flavivirus (LFV/CLuV) is another virus, discovered in 2015 from aquaculture facilities all over Norway and in Scotland. The virus is present in lumpfish at all stages of production; broodfish, hatchery, grow-out phase and when put to sea with the salmon. Infection has been associated with high mortality, but the virus has also been found in lumpfish without any clinical signs of disease. Pathologic changes are primarily observed in the liver (Fig. 27), which presents as necrosis and inflammation of the hepatic tissue during acute infection, which then turns more fibrotic as the infection progresses to chronic stages. Diagnosis is made by combining histological evaluation with molecular analysis, as the virus has proven incapable of isolation by cell culture (Vestvik *et al.*, 2017; Skoge *et al.*, 2018). CLuV has been frequently reported from farmed fish recently, especially in Hordaland, Troms and Finnmark county (Hjeltnes *et al.*, 2019). Transmission from infected lumpfish to salmon have been attempted, but not demonstrated (Vestvik *et al.*, 2017).

Nodavirus is the cause of the disease viral encephalopathy and retinopathy (VER) and viral nervous necrosis (VNN) in teleost fish (Munday *et al.*, 2002; Jansson & Venerström, 2014; Yong *et al.*, 2017). The susceptibility of lumpfish to the disease was recently tested with three different nodaviruses, which showed reduced survival, viral replication in the brain and typical vacuole formations in brain and retina of infected fish (Toffan *et al.*, 2019). The virus has been detected in Norwegian lumpfish, but actual prevalence has not been reported.

Early 2018 two more additions were added to the list of possible lumpfish viral pathogens, when two previously unknown viruses were discovered in material from lumpfish juveniles experiencing increased mortality. One was suggestively named *Cyclopterus lumpus* Totivirus (CLuTV) and appear to be closely related to PMCV. The virus has been detected in both dead and moribund lumpfish; however, its relevance is currently unknown ((NFO), 2019, Johansen *et al.*, 2019). The other virus was named *C. lumpus* Coronavirus (CLuCV), closely related to coronaviruses in other fish species, such as Chinook/Atlantic salmon bafini virus. Coronavirus typically leads to diarrhoea in mammals, but it is uncertain whether the same is true for lumpfish infected with CLuCV ((NFO), 2019, Johansen *et al.*, 2019). Functional pathogen tests are needed in order to better understand the significance of these novel viruses in lumpfish (Johansen *et al.*, 2019).

The occurrence and virulence of some common salmonid viruses in wild and farmed cleaner fish has also been investigated, especially IPNV, SAV, ISAV, PMCV and PRV (Johansen *et al.*, 2016a; Scholz *et al.*, 2018a). IPNV is the

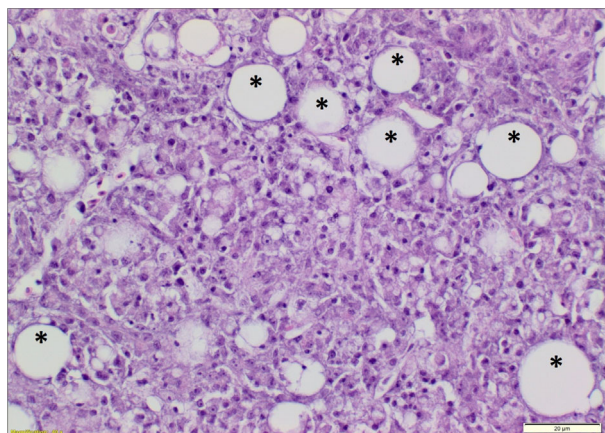


Figure 27 Necrosis in liver of lumpfish infected with flavivirus (LFV/CLuV). Multiple liver vacuoles are also present (*). HE stain. Scalebar 20 μ m. Photograph: Toni Erkinharju.

cause of infectious pancreatic necrosis (IPN), a disease capable of high mortalities in young salmonids, especially hatched fry (Roberts & Pearson, 2005; Gomez-Casado *et al.*, 2011; Jansson & Vennerström, 2014). The virus primarily affects the intestinal mucosa and pancreas of infected fish, by causing catarrhal enteritis in the pyloric caeca and necrosis of exocrine pancreatic cells (Roberts, 2012). It has been isolated from many different fish species and also from invertebrates (molluscs and crustaceans) (Roberts, 2012; Jansson & Vennerström, 2014). Recent experiments have shown lumpfish capable of being a reservoir of IPNV, indicating that the virus replicates within the fish and infected lumpfish can shed the virus into the environment (Breiland & Johansen, 2015). Viral susceptibility has been demonstrated in challenged goldsinny wrasse, without transmission to cohabitated salmon (Treasurer, 2012). IPNV has not been detected in cleaner fish used in Norwegian aquaculture (Hjeltnes *et al.*, 2019).

SAV, ISAV, PMCV and PRV are the aetiological causes of the salmonid diseases pancreas disease (PD), infectious salmon anaemia (ISA), cardiomyopathy syndrome (CMS) and heart and skeletal inflammation (HSMI), respectively (Rimstad & Mjaaland, 2002; Palacios *et al.*, 2010; Gomez-Casado *et al.*, 2011; Jansson & Vennerström, 2014; Rodger *et al.*, 2014; Wessel *et al.*, 2017; Deperasinska *et al.*, 2018; Garseth *et al.*, 2018a). The viruses have been isolated from wrasse stocked together with infected salmon at sea, but it is still unknown to what degree cleaner fish are susceptible to development of disease and/or capable of transmitting the viruses to other fish (Johansen *et al.*, 2016a; Scholz *et al.*, 2018a; Hjeltnes *et al.*, 2019). The viruses have not

been reported from lumpfish (Johansen *et al.*, 2016a; Hjeltnes *et al.*, 2019).

Non-infectious diseases

Diseases of non-infectious nature are a very broad topic, and only certain issues have been described from cleaner fish culture (Scholz *et al.*, 2018a). They are considered less economically devastating than the infectious diseases, but can potentially lead to a weakened defence system of affected fish, making them more susceptible to infection by different pathogens. Common problems of this nature in aquaculture settings (Table 5) are often related to either production management, environmental conditions, nutrition, vaccination and treatment, physical factors, hereditary factors or neoplasia (Schmidt-Posthaus & Marcos-López, 2014).

Nutrition and feeding of cleaner fish during production have been a challenge for the culturing of wrasse, but not so much for lumpfish as they are more easily weaned from live feed on to dry artificial diets (Powell *et al.*, 2018; Scholz *et al.*, 2018a), possibly due to a well-developed digestive system shortly after hatching (Marthinsen 2018). However, eye cataracts have been a common finding among growing lumpfish and broodfish stock (Bornø *et al.*, 2016; Powell *et al.*, 2018). The disorder leads to formation of opacities within the eye lens (Fig. 28), which causes disturbances in the dispersion of light passing through, resulting in reduced sight or, ultimately, blindness of affected fish (Paradis *et al.*, 2019). As lumpfish rely on sight to feed, this condition could negatively affect their efficiency as sea lice cleaners

Table 5 Summary of non-infectious diseases reported from wrasse (Labridae), lumpfish (*C. lumpus*) and Atlantic salmon (*S. salar*) with relevant references

Condition	Wrasse	Lumpfish	Salmon	Comments	References
Cataract	X	X	X	Cataract observed on lumpfish on several occasions. Reported from wrasse under experimental settings. Also reported from Atlantic salmon.	Powell <i>et al.</i> (2018), Bornø <i>et al.</i> (2016), Paradis <i>et al.</i> (2019), Jonassen <i>et al.</i> (2017), Imsland <i>et al.</i> (2019), Espmark <i>et al.</i> (2019), Bjerkås <i>et al.</i> (2004)
Deformities	X	X	X	Can cause welfare problems. Deformities in tail, fins, head, operculum, suction disc (for lumpfish) and spinal region. Cultured wrasse was recently reported having higher incidences of spinal deformities than cultured lumpfish.	Espmark <i>et al.</i> (2019), Scholz <i>et al.</i> (2018a), Brooker <i>et al.</i> (2018), Fjelldal <i>et al.</i> (2020), Rusyaev <i>et al.</i> (2019), Fjelldal <i>et al.</i> (2012), Treasurer (1994)
Other	X	X	X	Nephrocalcinosis has been reported from all species. Behaviour problems, such as tail biting and fin nipping, have been observed on both lumpfish and wrasse. Swim bladder over-inflation have occurred in wrasse. Lumpfish lack swim bladder, although clinical condition of gas supersaturation has been reported.	Scholz <i>et al.</i> (2018a), Powell <i>et al.</i> (2018), Fivelstad <i>et al.</i> (2018), Fivelstad <i>et al.</i> (2018), Weitkamp and Katz (1980), Poppe <i>et al.</i> (1997)

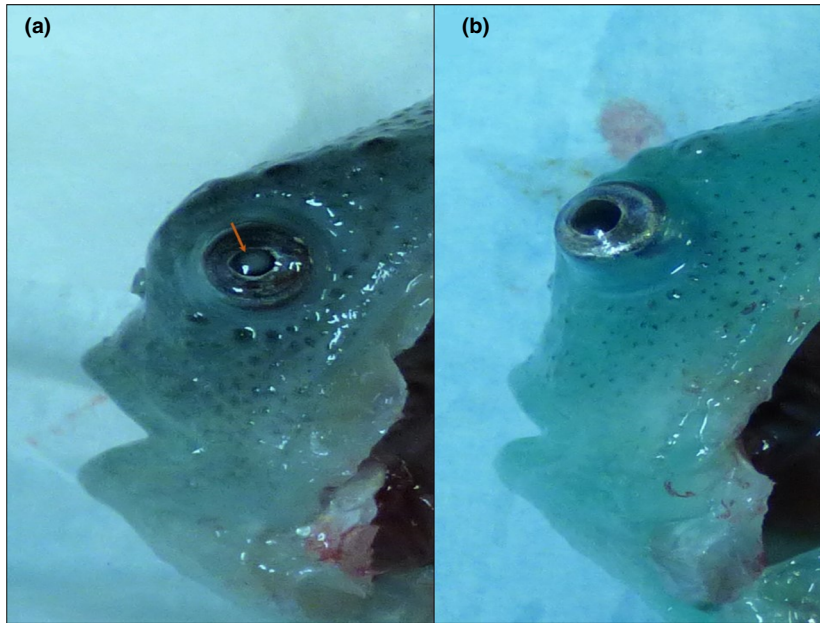


Figure 28 (a) Lumpfish with cataract formation (greyish lens) in the left eye (arrow). (b) Lumpfish with cataract-free left eye for comparison. Photographs taken at Tromsø Aquaculture Research Station. Photograph: Toni Erkinharju.

(Scholz *et al.*, 2018a; Paradis *et al.*, 2019). Cataract has been linked to nutritional deficiencies in other fish species (Waagbo *et al.*, 2003; Williams, 2006; Schmidt-Posthaus & Marcos-López, 2014). A recent study concluded that cataract formation in farmed lumpfish could be related to disturbed nutrient metabolism or malnutrition influencing the composition of specific amino acids in the tissue, causing osmotic imbalances and development of cataract (Jonassen *et al.*, 2017). Other studies have also found nutritional factors, such as feed composition and feeding frequencies, associated with cataract development in lumpfish (Imsland *et al.*, 2018b; Imsland *et al.*, 2019b). However, other factors such as environmental parameters, clinical conditions and physical injuries, could also be related (Jonassen *et al.*, 2017; Powell *et al.*, 2018; Imsland *et al.*, 2018b). Multifactorial causes have also been suggested for cataractogenesis in eye lenses of Atlantic salmon (Bjerk *et al.*, 2004). Development of cataract has been observed in ballan wrasse kept under experimental conditions (Espmark *et al.*, 2019).

Some lumpfish juveniles can develop deformities in the suction disc, leading to problems for attaching themselves to substrates during resting. These types of fish are more easily stressed and exhausted, especially during high current conditions at sea, and could potentially lead to increased mortality. The condition appears to be hereditary. The affected fish are usually found and culled from the production cycle and are as such not considered a major problem in lumpfish culture (Scholz *et al.*, 2018a; Espmark *et al.*,

2019). Jaw and spinal deformities have also been observed but not sufficiently documented (Brooker *et al.*, 2018; Scholz *et al.*, 2018a). A recent study observed high occurrences of vertebra deformities in cultured wrasse, while, surprisingly, the observed deformity rate was lower in cultured lumpfish (Fjellidal *et al.*, 2020). Spinal deformities have also been reported from farmed Atlantic salmon, with several environmental and nutritional conditions being potential risk factors (Fjellidal *et al.*, 2012). Developmental anomalies of the tail region have been reported from wild-caught lumpfish (Rusyaev *et al.*, 2019). An abnormal skull formation, termed ‘pugheadedness’, has been observed in wild-caught goldsinny wrasse (Treasurer, 1994).

Other non-infectious clinical conditions that have been reported are gas supersaturation and nephrocalcinosis from lumpfish (Fig. 29), and nephrocalcinosis and swim bladder over-inflation in wrasse (Scholz *et al.*, 2018a). Similar conditions have also been described from Atlantic salmon (Weitkamp & Katz, 1980; Poppe *et al.*, 1997; Fivelstad *et al.*, 2018). Behavioural problems, such as tail biting and fin nipping, which are likely related to different farming conditions, have also been observed on both lumpfish and wrasse during cultivation (Scholz *et al.*, 2018a; Espmark *et al.*, 2019). Finally, a persistent challenge is increased mortality and fish welfare issues, especially associated with ‘rough’ handling and non-medicinal lice treatments (warm water treatment (thermolicer), freshwater bathing (hydrolicer), high pressure water flushing, brushing), which makes the cleaner fish easily stressed, more prone to injuries and

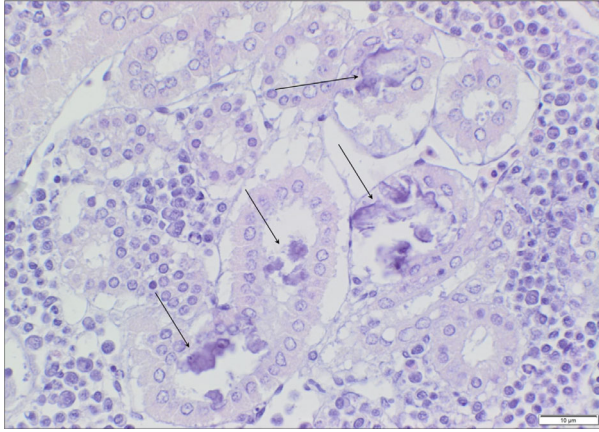


Figure 29 Lumpfish kidney with nephrocalcinosis. Several tubules with amorphous, basophilic and calcified material within the lumen can be noted (arrows). HE stain. Scalebar 10 μm . Photograph: Toni Erkinharju.

might amplify the severity of pathogen-related issues (Hjeltnes *et al.*, 2019; Espmark *et al.*, 2019).

Risk of disease transmission from cleaner fish to salmon

Cohabitation of cleaner fish at sea pens together with salmon poses the risk of possible disease transmission between the different species (Treasurer, 2012; Brooker *et al.*, 2018; Scholz *et al.*, 2018a). This is especially a concern when using wild-caught cleaner fish, as they are more likely to be carriers of disease agents than cleaner fish farmed in closed systems (Treasurer, 2012). In addition, lumpfish and wrasse farmed in production facilities are usually screened and cleared for possible pathogens before being deployed as cleaner fish at sea (Brooker *et al.*, 2018). However, wild-caught lumpfish are widely used as source for brood stock, as lumpfish production is still at an early age, and more knowledge on the species' reproductive biology, markers for preferred genetic traits and efficient fertilisation techniques under captive conditions are needed in order to establish fully closed breeding cycles (Davenport, 1985; Brooker *et al.*, 2018; Powell *et al.*, 2018). Proper quarantine, pathogen screening and parasite treatments are therefore important, before the fish is used for production purposes (Powell *et al.*, 2018). In addition, the demand for cleaner fish in Norwegian salmon farms has rapidly increased beyond what is attainable from national fishery and aquaculture production, making it necessary to import cleaner fish from other countries. Such transportation of live animals also comes with a risk of transferring disease-causing agents from one geographically distant area to the next (VKM *et al.*, 2019b). Regarding the possibility of cleaner

fish transmitting disease to cohabitated salmon, the issue has previously been addressed in two reported risk assessments, one by the research institute Nofima (Johansen *et al.*, 2016a) and one by the Norwegian Scientific Committee for Food and Environment (VKM *et al.*, 2017).

Bacteria and fungi

The potential risk of infected lumpfish transmitting bacterial disease to salmon is considered generally low for most of the known bacterial agents (Johansen *et al.*, 2016a; VKM *et al.*, 2017; Brooker *et al.*, 2018). Vaccinated salmon have so far shown protection against typical *Aeromonas salmonicida*, *Vibrio salmonicida* and *Vibrio anguillarum* serotype O1 and O2a (Johansen *et al.*, 2016a; VKM *et al.*, 2017). Interestingly, it has been suggested that salmon infected with typical *A. salmonicida* could potentially transmit the bacteria to wrasse, although under normal farming conditions this incidence would probably be rare (Treasurer & Cox, 1991; Hjeltnes *et al.*, 1995; Treasurer, 2012). The probability of transmitting atypical *A. salmonicida* is considered low, as the furunculosis vaccine might provide some degree of cross-protection (Johansen *et al.*, 2016a) and challenged salmon appear less susceptible to the cleaner fish variants (Frerichs *et al.*, 1992; Laidler *et al.*, 1999; Scholz *et al.*, 2018a). Several farmed lumpfish are also vaccinated against atypical *A. salmonicida* and *V. anguillarum*, which may reduce the number of infected individuals in the net pens (Brooker *et al.*, 2018; Hjeltnes *et al.*, 2019). However, there is a possibility of introducing, as of yet, undescribed strains of *A. salmonicida* existing in areas relevant for importation of cleaner fish (VKM *et al.*, 2019b). The probability of disease transmission with *V. ordalii* is considered low (Johansen *et al.*, 2016a; VKM *et al.*, 2017). While *V. ordalii* infections have caused mortality in cage-farmed Atlantic salmon in Chile (Colquhoun *et al.*, 2004), genetic differences between North Atlantic and Pacific strains have been reported (Steinum *et al.*, 2016; Johansen *et al.*, 2016b). The pathogenic potential of other species of *Vibrio* in cleaner fish is largely unknown, and more information is surely needed (Scholz *et al.*, 2018a). Salmon appear unaffected by *V. tapetis* and *V. splendidus* isolates from wrasse (Treasurer, 2012; Scholz *et al.*, 2018a).

Both *Pseudomonas anguilliseptica* and *Pasteurella* sp. have caused disease in Atlantic salmon (Wiklund & Bylund, 1990; Birkbeck *et al.*, 2002), but the probability of cross-infection from diseased lumpfish is considered low for these bacteria as well (Johansen *et al.*, 2016a; VKM *et al.*, 2017; Scholz *et al.*, 2018a). Different fish species display considerable variability in susceptibility for *P. anguilliseptica* infection (Wiklund & Bylund, 1990), and as such, some degree of host specificity might be ascertained (Johansen *et al.*, 2016a). *Pasteurella* isolates from infected lumpfish and

salmon have shown to be genetically distinct and appear to be from separate serotypes (Reid & Birkbeck, 2015; Alarcon *et al.*, 2016a; VKM *et al.*, 2017). Surprisingly, in 2018, the same genotype (usually associated with salmon) was identified from both diseased lumpfish and salmon kept within the same farming locality in Norway (Hjeltnes *et al.*, 2019). *Pasteurella* sp. also occur among lumpfish in the British Isles, but it is uncertain if they are of the same or of different strains than the Norwegian isolates (Scholz *et al.*, 2018a; VKM *et al.*, 2019b).

The isolate of *M. viscosa* from lumpfish have shown to be an atypical variant strain (Grove *et al.*, 2010), different from the typical strain associated with winter ulcer disease in salmon (Karlsen *et al.*, 2014; Johansen *et al.*, 2016a). Atypical strains have caused disease in Atlantic salmon, but only in a few exceptional cases in Norway (Grove *et al.*, 2010). Cleaner fish are also used to a far less degree during wintertime, when the bacteria is most active and causing disease in salmon (Johansen *et al.*, 2016a). As such, the probability of disease transmission is considered low for this bacterial pathogen (Johansen *et al.*, 2016a; VKM *et al.*, 2017), but could potentially become a problem (Brooker *et al.*, 2018; Scholz *et al.*, 2018a), especially if lumpfish is used more frequently throughout the winter season (VKM *et al.*, 2017). Following this, it has been shown that lumpfish isolate can be able to cause *M. viscosa* infection in salmon, as shown by Einarsdottir *et al.* (2018)

Piscirickettsia salmonis has generated special concern, due to its potential of being imported with lumpfish from other geographical regions (VKM *et al.*, 2019b). Even though *P. salmonis* has never been identified in Norwegian cleaner fish (Hjeltnes *et al.*, 2019), it has been considered low probability of transmission from cleaner fish to salmon (VKM *et al.*, 2017). *P. salmonis* can cause severe disease in infected Atlantic salmon (Rozas-Serri *et al.*, 2017), but there have appear to be differences in virulence between geographic regions, with European isolates being less severe than Chilean isolates (House *et al.*, 1999; VKM *et al.*, 2017).

There are several uncertainties regarding *Tenacibaculum* spp., due to data indicating that there are probably multiple different strains involved during ulcerative disease infections in affected fish (Johansen *et al.*, 2016a; Olsen *et al.*, 2017). *T. maritimum* is the most common species observed in marine fish (VKM *et al.*, 2017) and is capable of causing disease in Atlantic salmon (Ostland *et al.*, 1999; Frisch *et al.*, 2018). Several teleost host species exist (Avendano-Herrera *et al.*, 2006), and cross-species transmission has been suggested as a possibility in aquatic polyculture (Habib *et al.*, 2014). However, the species is considered more severe for juveniles than for adult fish (Toranzo *et al.*, 2005). The species *T. finnmarkense*, often found in Northern Norwegian salmon farms, is also capable of causing disease in salmon, although direct fish-to-fish transmission

was poor and possible vectors or reservoirs were not identified (Småge *et al.*, 2018). Isolates from disease outbreaks in Norwegian salmon farms have also shown similarity to *T. dicentrarchi* (Habib *et al.*, 2014), which was recently the cause of an acute disease outbreak in Atlantic salmon (Klaekgg *et al.*, 2019). *Tenacibaculum* spp. is often isolated in both mixed and pure culture from affected cleaner fish (Hjeltnes *et al.*, 2019). The probability of disease transmission from cleaner fish to salmon has as such been considered to be of moderate risk (VKM *et al.*, 2017), but there are several knowledge gaps that needs solving (Johansen *et al.*, 2016a; Scholz *et al.*, 2018a).

The potential disease transmission risks regarding other cleaner fish bacterial pathogens are currently not known. *Photobacterium damsela* subsp. *damsela* has been reported from diseased rainbow trout during warm summer periods in Denmark (Pedersen *et al.*, 1997), and Atlantic salmon have demonstrated susceptibility to disease under experimental conditions (Austin *et al.*, 1997). Further work is needed however, to investigate its potential risks at cooler northern European climate conditions (McMurtrie *et al.*, 2019).

Not much is known regarding the potential of lumpfish fungal diseases transferring to cohabitated salmon (Scholz *et al.*, 2018a), but there is some concern regarding species of the genus *Exophiala* (Johnson *et al.*, 2018; Scholz *et al.*, 2018b). Animals with moist skin, such as those with sweat glands or those being waterborne (i.e. fish), are more susceptible to *Exophiala* spp. infection (de Hoog *et al.*, 2011), and black yeast infections have been reported from Atlantic salmon (Otis *et al.*, 1985; Pedersen & Langvad, 1989). However, due to lack of available documentation, fungal pathogens have not been included in reported risk assessments of cleaner fish aquaculture (Johansen *et al.*, 2016a; VKM *et al.*, 2017; VKM *et al.*, 2019b).

Viruses

Our current knowledge of viral diseases in lumpfish are limited, and new ones are sure to emerge with time (Johansen *et al.*, 2016a). One characteristic feature of viruses is their capacity to adapt to new host species, the influenza virus being a well-known example. Rearing cleaner fish together with salmon in an enclosed space, such as in net pens at sea, involves several opportunities for direct contact between the species, often at high population densities. These are important factors, which could potentially promote adaptive changes in different viruses, causing them to switch from one fish species (the 'original' host) to another species (the 'new' host) (Parrish *et al.*, 2008).

Both wrasse and lumpfish have shown susceptibility to infection by the VHS virus (Munro *et al.*, 2015; Matejusova *et al.*, 2016; Guðmundsdóttir *et al.*, 2019). In addition,

VHSV genotype Ib has been detected in several wild marine fish along the Norwegian coastal line and could potentially be transferred to farmed fish (Sandlund *et al.*, 2014). As such, there is a possibility that cleaner fish could be carriers of VHSV. Atlantic salmon has not demonstrated significant susceptibility to VHSV (King *et al.*, 2001; Garver *et al.*, 2013) but Norwegian rainbow trout have developed disease after infection with VHSV genotype III (Dale *et al.*, 2009), which was of the same genotype isolated from infected wrasse in Scotland (Munro *et al.*, 2015). The VHS virus isolated from infected wild-caught lumpfish in Iceland, however, was of genotype IV and did not transmit to cohabitated salmon (Guðmundsdóttir *et al.*, 2019). It has been shown however, that low virulent viral strains might mutate into highly virulent strains when provided with the right opportunities (Ito *et al.*, 2016). Based on this information, an introduction of VHSV into a farming facility stocked with cleaner fish is considered a possible high probability of transmitting the virus from infected wrasse or lumpfish to cohabitated salmonids, especially rainbow trout (Johansen *et al.*, 2016a; VKM *et al.*, 2017). VHSV is considered a viral pathogen of special concern regarding importation of both lumpfish and wrasse (VKM *et al.*, 2019a).

For IPNV, both wrasse and lumpfish have shown infection in experimental settings and are considered possible reservoirs for the virus (Gibson *et al.*, 1998; Breiland & Johansen, 2015). The virus also has a broad host range (Scholz *et al.*, 2018a). As such, there is a moderate probability of transmitting the disease to cohabitated salmon (VKM *et al.*, 2017). Screening of susceptible stocks is advisable wherever IPNV is an issue (Scholz *et al.*, 2018a). However, the virus is already highly prevalent in salmonid aquaculture in Norway, and the production of IPN-resistant salmon through genetic selection, in addition to increased use in rainbow trout production, indicate that the possible threats from transmission is probably close to negligible (Johansen *et al.*, 2016a; VKM *et al.*, 2017).

Wild wrasse populations along the Norwegian and Swedish coast were recently screened for nervous necrosis virus (NNV) (Betanodaviridae), and an overall presence of 6.7% was detected in brain tissue from sampled fish (Korsnes *et al.*, 2017). It is considered a viral pathogen of special concern regarding importation of wrasse to Norway (VKM *et al.*, 2019a). The overall presence of nodavirus in lumpfish is currently not known, but the species has shown susceptibility for infection (Toffan *et al.*, 2019). Viral infections have primarily been described from marine fish (Munday *et al.*, 2002; Jansson & Vennerström, 2014), including farmed Atlantic cod (*Gadus morhua* L.) (Patel *et al.*, 2007), Atlantic halibut (*Hippoglossus hippoglossus* L.) (Johansen *et al.*, 2002) and turbot (*Scophthalmus maximus* L.) (Johansen *et al.*, 2004) in Norway. Nodavirus-like particles have

been detected in heart tissue of Atlantic salmon affected by CMS (Grotmol *et al.*, 1997), and the species has developed pathology from viral challenge with nodavirus under experimental conditions (Korsnes *et al.*, 2005). However, nodaviral infections are not commonly observed in salmonids and the probability of disease transmission from cohabitated cleaner fish is therefore regarded as low (Johansen *et al.*, 2016a; VKM *et al.*, 2017).

Salmonid alphaviruses (SAV) are currently among the most important pathogens of salmonid aquaculture, being the cause of pancreas disease (PD) (SAV subtype 1 and 3) in salmon and rainbow trout, and sleeping disease (SAV subtype 2) in rainbow trout (McLoughlin & Graham, 2007; Hjeltnes *et al.*, 2019). The virus has not been detected in lumpfish. SAV has been reported from both wild-caught wrasse and wrasse in sea cages during an outbreak of PD in the cohabitated salmon, but the fish did not show any signs of pathology associated with the disease (Ruane *et al.*, 2018; Scholz *et al.*, 2018a; Hjeltnes *et al.*, 2019). Different challenge experiments have also given similar results, where challenged wrasse did not develop disease signs (Treasurer, 2012; Røsæg *et al.*, 2017). However, there is a risk that cleaner fish might serve as non-affected mechanical vectors/vehicles for transmitting the virus to susceptible species, such as the salmon (Olsen *et al.*, 2011; VKM *et al.*, 2017). Horizontal transmission, such as fish-to-fish, is regarded as important for spreading PD to new areas (Kristoffersen *et al.*, 2009). SAV viruses have also been detected in non-salmonid wild marine fish species close to salmon farms (Snow *et al.*, 2010; McCleary *et al.*, 2014). Therefore, cleaner fish has been estimated a moderate risk of transmitting the SAV virus to cohabitated salmon, when using wild-caught cleaner fish originating from SAV-endemic areas (VKM *et al.*, 2017). Outside of the endemic zones, however, the probability is considered low (Johansen *et al.*, 2016a; VKM *et al.*, 2017). SAV is considered a viral pathogen of special concern regarding importation of wrasse (VKM *et al.*, 2019a).

For other salmonid viral diseases, the risk of disease transmission is generally considered negligible to low, such as for ISAV, PRV, salmon gill pox virus (SGPV), infectious hematopoietic necrosis virus (IHNV) and piscine orthoreovirus *Oncorhynchus mykiss* (PRVom, formerly 'Virus Y') (Treasurer, 2012; Johansen *et al.*, 2016a; VKM *et al.*, 2017). ISAV and PRV have been reported from wrasse and salmon kept at the same farming localities, but the wrasse displayed no clinical disease and cross-contamination of samples could not be excluded (Johansen *et al.*, 2016b; Scholz *et al.*, 2018a; VKM *et al.*, 2019a). Both ISAV and PRV have been reported from wild fish carriers, however, suggesting the possibility of natural viral reservoirs (Nylund *et al.*, 2002; Plarre *et al.*, 2005; Wiik-Nielsen *et al.*, 2012; Madhun *et al.*, 2019). PMCV is an exception, however, as the virus has

been detected in wrasse with heart lesions, which is therefore considered a potential disease reservoir for the pathogen (Scholz *et al.*, 2018c; VKM *et al.*, 2019a). The occurrence of CMS has been increasing in Norwegian aquaculture (Hjeltnes *et al.*, 2019), and infected cleaner fish could essentially become a biosecurity risk by transmitting the virus to salmon (Brooker *et al.*, 2018; Scholz *et al.*, 2018c). Based on the available information, it is considered a moderate probability when using cleaner fish (VKM *et al.*, 2017). PRVom (genotype PRV-3) causes disease in rainbow trout (Olsen *et al.*, 2015; Vendramin *et al.*, 2019), and experimental transmission of the virus has been demonstrated in Atlantic salmon, albeit with slower viral replication and minor pathology (Hauge *et al.*, 2017). SGPV is associated with gill disease in farmed Atlantic salmon (Gjessing *et al.*, 2015; Gjessing *et al.*, 2017) and has also been reported from wild anadromous salmon (Garseth *et al.*, 2018b). IHNV causes severe disease in salmonids, including Atlantic salmon and rainbow trout (Saksida, 2006; Dixon *et al.*, 2016), but the virus has never been detected in fish from Norway (Hjeltnes *et al.*, 2019). None of these salmonid viruses (PRVom, SGPV or IHNV) has been reported from lumpfish or wrasse (Johansen *et al.*, 2016b).

Several new viral diseases of cleaner fish have been reported, such as LFV/CLuV, CLuTV, CLuCV and ranavirus for lumpfish (Skoge *et al.*, 2018; NFO (2019) Stagg *et al.*, 2020), and lymphocystis for wrasse (Schonheit *et al.*, 2017). Due to their recent discovery, not much is known of their ability to infect and cause disease in cohabitated salmon (Scholz *et al.*, 2018a). However, some information has been reported regarding lumpfish ranavirus and lymphocystis in wrasse (both belonging to *Iridoviridae*). Experimental challenge of salmon with lumpfish ranavirus did not cause significant pathology after immersion or injection (Scholz *et al.*, 2018a). It is considered a viral agent of special concern regarding importation of lumpfish (VKM *et al.*, 2019a). Atlantic salmon does not appear to be very susceptible to infection with the iridovirus EHN (Whittington *et al.*, 2010). Regarding lymphocystis disease viruses, viral detection from more than 140 fish species has been shown (Essbauer & Ahne, 2001). It has been reported from wild-caught ballan wrasse in Norway (Schonheit *et al.*, 2017), and from bluestreak cleaner wrasse, *Labroides dimidiatus*, located at warmer waters (VKM *et al.*, 2019a). Lymphocystis is generally considered a benign, self-limiting disease in fish, causing primarily superficial lesions (Whittington *et al.*, 2010; Schonheit *et al.*, 2017), and the virus is regarded low probability of disease transmission in salmonid aquaculture (VKM *et al.*, 2017). However, infected fish could display lower growth rates and become more susceptible

to secondary infections, which could potentially lead to economic losses if large parts of the population is affected (Borrego *et al.*, 2017).

Parasites

Wild-caught cleaner fish usually harbour several different parasites, as this is a common occurrence in fish under natural conditions (Scholz *et al.*, 2018a). A great amount of species have been found in wrasse (Treasurer, 1997; Treasurer, 2012; VKM *et al.*, 2019b), and some have been reported from lumpfish (Rolbiecki & Rokicki, 2008; Karlsbakk *et al.*, 2014; Erkinharju *et al.*, 2019). Several of these species show a high degree of host specificity and some even have complicated life cycles involving different animal species/types as intermediate hosts and are as such not directly transmitted from fish-to-fish (Treasurer, 2012; Karlsbakk *et al.*, 2014; Johansen *et al.*, 2016a; Scholz *et al.*, 2018a). However, some species are a concern, partly due to their probability of spreading from infected cleaner fish to cohabitated salmon, but also from potentially being zoonotic pathogens (Mo & Poppe, 2018; Scholz *et al.*, 2018a). Interestingly, the likelihood of introducing novel parasites with importation of cleaner fish to Norway is regarded as lower with lumpfish than for wrasse (VKM *et al.*, 2019a).

Among the currently known parasitological organisms, the gill amoeba *Paramoeba perurans* is considered the most significant threat in sea cages stocked with cleaner fish and salmon (Karlsbakk *et al.*, 2014; Johansen *et al.*, 2016a). The parasite demonstrates a low degree of host specificity and can infect several different fish species (Johansen *et al.*, 2016a), including salmonids, wrasses and lumpfish (Young *et al.*, 2007; Young *et al.*, 2008; Steinum *et al.*, 2008; Karlsbakk *et al.*, 2013; Haugland *et al.*, 2017). In Norway, the amoeba can be found along the coastline as far north as Nordland county (Hjeltnes *et al.*, 2019). Experimental transmission of *P. perurans* from infected lumpfish to Atlantic salmon was also recently reported (Haugland *et al.*, 2017). As such, it is considered a high probability of being spread from infected cleaner fish to cohabitated salmon (VKM *et al.*, 2017).

Several helminths are regarded as foodborne pathogen concerns, especially nematode species in the genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Phocascaris* and *Hysterothylacium* (Family: Anisakidae). Encapsulated larvae of these nematodes can occur in internal organs and musculature of marine fish, potentially causing disease in humans (anisakiasis, commonly known as 'kveis' in Norwegian) ingesting raw or undercooked fish meat possessing these parasites (Karl *et al.*, 2011; Gjerde, 2011a; Jansson & Vernerström, 2014; Scholz *et al.*, 2018a). Infection with the species *Anisakis simplex* is especially a problem, as the invasive parasite can penetrate the gastro-intestinal tissue wall,

migrate to other parts of the body and potentially lead to immune hypersensitivity reactions in some individuals (Audicana & Kennedy, 2008; Jansson & Vennerström, 2014). Anisakiasis is considered an important emerging disease of global scale (Bao *et al.*, 2017).

For lumpfish, there are records of *A. simplex*, *Pseudoterranova* spp., *H. aduncum* and *C. osculatum* occurring in wild-caught fish (Rolbiecki & Rokicki, 2008; Karlsbakk *et al.*, 2014), and lumpfish kept in open sea cages can become infected by ingesting intermediate hosts (such as marine copepods) used by the nematodes (Gjerde, 2011a; Johansen *et al.*, 2016a). *A. simplex*, *H. aduncum* and *C. osculatum* also occur in wild wrasse (Treasurer, 1997; VKM *et al.*, 2019a). As a result, the cohabitated salmon can potentially become infected by predated on the cleaner fish (Johansen *et al.*, 2016a; Mo & Poppe, 2018). In Norwegian aquaculture, a national survey on the prevalence of Anisakis in farmed salmon only registered the nematodes in a few runts and concluded that the probability of the parasites occurring in fish meant for human consumption is very low (Levsen & Maage, 2016). Similar observations were also reported by others (Mo *et al.*, 2014). In addition, due to Atlantic salmon being fed artificial feed it is argued that they are unlikely to become infested with parasitic helminths (Levsen & Maage, 2016). However, although the probability of nematodes spreading from infected cleaner fish to salmon appear to be negligible, careful consideration and risk analysis is advised, as such transmission can lead to dire consequences (Johansen *et al.*, 2016a; Mo & Poppe, 2018).

The sea lice *Caligus elongatus* are frequently reported from lumpfish stocked in net pens at sea (Powell *et al.*, 2018; Hjeltnes *et al.*, 2019) and are commonly observed on wild-caught lumpfish (Heuch *et al.*, 2007; Karlsbakk *et al.*, 2014; Scholz *et al.*, 2018a). As motile *C. elongatus* are able to move between different fish hosts (Oines *et al.*, 2006; Jansson & Vennerström, 2014; Brooker *et al.*, 2018) and lumpfish are regarded as an important host species for genotype I of the marine copepod (Oines *et al.*, 2006; Oines & Heuch, 2007), there is a risk of cohabitated salmon becoming infected with the parasite (Johansen *et al.*, 2016a). Based on current knowledge, it is considered moderate probability in aquaculture settings, but there are uncertainties due to only limited information available (VKM *et al.*, 2017).

Historically, *Ichthyophonus hoferi* has been reported from numerous different fish species, including salmonids (Møllergaard & Spanggaard, 1997; Rahimian, 1998; Zubchenko & Karaseva, 2002; Kocan *et al.*, 2004; Tierney & Farrell, 2004; Jansson & Vennerström, 2014; Gozlan *et al.*, 2014; Teffer *et al.*, 2020). Fungal and fungal-like pathogens of fish typically have a generalistic nature and can infect a wide range of different hosts (Gozlan *et al.*, 2014). For

Ichthyophonus, the main route of transmission is via consumption of infected fish and (possibly) pelagic zooplankton, in addition to waterborne transmission from fish-to-fish (Jones & Dawe, 2002; Jansson & Vennerström, 2014; Kocan, 2019). Fungal infections in cultured fish are considered a secondary phenomenon (Gozlan *et al.*, 2014); disease outbreaks of *I. hoferi* are largely controlled by using processed and sterilised fish feed (Jansson & Vennerström, 2014), and waterborne infections have only been documented in a few fish species in the marine environment (Gregg *et al.*, 2012; Kocan, 2019). However, *Ichthyophonus* sp. has been reported from Norwegian cleaner fish (Hansen & Nilsen, 2018; Hjeltnes *et al.*, 2019), and as such, there is a risk that salmon can become affected by predated on cohabitated wrasse or lumpfish infected with the pathogen (Mo & Poppe, 2018).

The microsporidian parasite *Nucleospora cyclopteri* is capable of causing severe disease in infected lumpfish (Freeman *et al.*, 2013; Karlsbakk *et al.*, 2014; Alarcon *et al.*, 2016b). It has been observed in wild-caught lumpfish along the entire Norwegian coast (Karlsbakk *et al.*, 2014), meaning it is very probable that lumpfish used as cleaner fish will be affected by the parasite when put to sea (Scholz *et al.*, 2018a). However, most microsporidians in fish are generally species-specific and this seem to include *N. cyclopteri* (Cain & Polinski, 2014; Johansen *et al.*, 2016a; Scholz *et al.*, 2018a). Therefore, it is considered a low probability for cross-infection between infected lumpfish and cohabitated salmon in sea pens with this parasite (Johansen *et al.*, 2016a; VKM *et al.*, 2017). The possibility of transmitting other microsporidian parasites, such as *T. brevifilum* in lumpfish and *Loma* sp. in wrasse, is considered equally unlikely, although it has not been investigated (Scholz *et al.*, 2017; Steigen *et al.*, 2018).

For other lumpfish parasites, such as flagellates *Ichtyobodo* sp., ciliates *Trichodina* sp., *Scuticociliate* species, myxozoans *Kudoa islandica* and *Myxobolus aeglefini*, and monogeneans *Gyrodactylus* sp., any possible disease transmission from cleaner fish to salmon is regarded as either unlikely, as several species show high degree of host specificity, or unknown due to lack of available information (Johansen *et al.*, 2016a; VKM *et al.*, 2017; Scholz *et al.*, 2018a).

Commercial and experimental vaccines for cleaner fish

According to recent documentations by the Norwegian Food Safety Authority (Mattilsynet), there is on average a cleaner fish mortality above 40% in Norwegian aquaculture. However, there is uncertainty in regards to this number, since not all of the cleaner fish that are lost are registered (Mattilsynet, 2020). Others argue that near all of

the cleaner fish put in sea cages together with salmon die throughout the production cycle, which account for a daily mortality of about 150 000 fish within a year (Hjeltnes *et al.*, 2019; Berghlin, 2019a). Relevant causes include handling, such as non-medicinal lice treatments; also skin wounds, fin erosions and mechanical injuries (some of which might be directly or indirectly related to different handling procedures), in addition to poor fish quality, environmental conditions, aggression/predation and disease, especially bacterial infections (Nilsen *et al.*, 2014; Bornø *et al.*, 2016; Hjeltnes *et al.*, 2019; Mattilsynet, 2020). There is generally a lack of effective treatment protocols (Scholz *et al.*, 2018a), although there are some recommendations for medicinal treatment of infectious diseases in cleaner fish (Gu & Skjelstad, ; Treasurer & Birkbeck, 2018; Pietrak & Backman, 2018; Powell *et al.*, 2018; Scholz *et al.*, 2018a; Kverme *et al.*, 2019; Haugland *et al.*, 2019). In addition, antimicrobial treatment is not necessarily effective against all types of pathogens, such as *P. salmonis* in salmonids (Rozas & Enriquez, 2014). Currently, cleaner fish is the fish category with the largest number of prescribed antibiotic treatments (91 total for lumpfish in 2018) for bacterial infections in Norwegian aquaculture (Hjeltnes *et al.*, 2019). It is preferable to keep the antimicrobial usage in aquaculture as low as possible, in order to prevent the formation of antimicrobial-resistant (AMR) bacterial populations (Santos & Ramos, 2018). As such, vaccination is regarded as an essential option for preventing development of bacterial diseases and improving cleaner fish health and welfare (Nilsen *et al.*, 2014; Brooker *et al.*, 2018; Scholz *et al.*, 2018a).

There are a few commercial vaccines developed for cleaner fish – mainly targeted to combat *A. salmonicida* and/or *Vibrio* sp. infection (Pharmaq (Zoetis), Elanco, Vaxxinova Norway Ltd). A few experimental vaccines have been tested for use in cleaner fish. One of this is Vibrogen-2 (Elanco). This vaccine containing inactivated *V. anguillarum* O1 and O2, plus *V. ordalii* did not induce protection against *V. anguillarum* (Chakraborty *et al.*, 2019). In another study, lumpfish were immunised using an oil-in-water emulsion of two isolates of *A. salmonicida* (Alpha marine micro 3, Pharmaq), where the bacteria originally were isolated from Atlantic cod. The vaccine consisting of a high virulent strain yielded 73% relative survival (Rønneseth *et al.*, 2017). The protection induced by two experimental *Pasteurella* sp. vaccines (formalin inactivated bacteria in water-in-oil formulations) was assessed by Ellul *et al.* (2019a). This study concluded that there was a slight protection of immunised lumpfish, followed by bath challenge with autologous pathogen, over control fish – albeit non-significant (Ellul *et al.*, 2019a). The number of vaccine trials conducted in cleaner fish is remarkably low given that hundreds of millions are being used as lice eaters in

aquaculture. To the best of our knowledge, there are currently no vaccines available for fungal, viral or parasitic diseases in cleaner fish.

Conclusion

The cleaner fish are susceptible towards a high range of different pathogens that may cause diseases and mortalities. The animal welfare of cleaner fish, with regard to immune prophylactic measures, must be taken care of by developing efficient vaccination strategies. Vaccine development aiming to induce protection against well-known pathogens is by far lagging behind the current situation of the main aquacultured species. Whether cleaner fish are host of pathogens likely to be transmitted to salmon, impacting animal welfare, must thorough fully be evaluated in future studies. A better focus on cleaner fish welfare together with vaccine prophylactic measures would reduce the risk of transmitting infectious pathogens.

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