This article has been accepted for publication in Veterinary Record following peer review. The definitive copyedited, typeset version Brooker, AJ., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., Migaud, H. (2018) Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges Veterinary Record 183, 383 is available online at: https://doi.org/10.1136/vr.104966

Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges

Adam J. Brooker[1], Athina Papadopoulou[1], Carolina Gutiérrez[2], Sonia Rey[1], Andrew Davie[1] and Hervé Migaud[1]*

[1]Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK.

[2]Marine Harvest Scotland Ltd., Stob Ban House, Glen Nevis Business Park, Fort William, PH33 6RX, Scotland, UK.

*Corresponding author

E-mail: herve.migaud@stir.ac.uk

Telephone: +44 (0)1786 467886

Address: Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, Scotland, UK.

Abstract

Currently, cleaner fish are one of the most widely used sea lice control strategies in Atlantic salmon aquaculture. Two species are currently being farmed in North Atlantic countries, ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*), and the sector in most countries is rapidly expanding towards self-sufficiency. The species are very different both in terms of their biology and life histories and, consequently, production and husbandry methods must be tailored to each species. There are numerous health challenges currently experienced in both species, with bacterial and parasitic diseases being the most prevalent, and cohabitation with salmon may increase the risk of disease. Good husbandry and routine health monitoring are essential, although treatment is often required when disease outbreaks occur. Ballan wrasse and lumpfish are both proven to be effective salmon delousers, although delousing efficacy can be variable in farmed fish; the provision of suitable habitat and acclimation to net-pen conditions may encourage natural behaviours, including delousing, and the use of operational welfare indicators can highlight potential welfare issues. Cleaner fish research is progressing rapidly, although much of the basic knowledge regarding the species' biology remains unknown. The simultaneous domestication of two new marine aquaculture species is a significant challenge demanding sustained effort and funding over a prolonged period of time. Research must focus on enhancing the robustness of the farmed stocks and increasing hatchery outputs to meet the urgent demands from the salmon sector and protect wild stocks from overfishing.

1. Introduction

The greatest disease challenge currently limiting production within the global Atlantic salmon industry is infection by caligid sea lice. Along with causing significant physical and biochemical damage, including skin lesions, loss of protective skin function leading to risk of secondary infections, osmoregulatory imbalance, immunosuppression and increased stress[1,2], there are significant economic impacts due to production losses and treatment costs. The global economic impact of sea lice was estimated at £700 million in 2015, with costs likely to have continued to rise since then.[3] In terms of the production losses, Abolofia et al.[4] estimated that sea lice infection typically results in up to a 16% reduction in production biomass, which translates approximately to a 9% loss in farm revenues. Driven by environmental and welfare concerns, and economic pressures, many new innovative strategies for pest control in salmon farming have been developed over recent years, including lice removal technologies using brushes or water jets (e.g. hydrolicer), novel bath treatments using warm water (thermolicer) or fresh water, physical barrier technologies (e.g. snorkel cages and lice skirts), light regimes to manipulate salmon swimming behaviour and passive lice control using laser technology (Stingray, Stingray Marine Solutions) (reviewed in Holan et al.[5]). Other methods are in development, including new chemotherapeutants, vaccines, feed additives and selective breeding. Since 2010, one of the most widely adopted alternative pest control strategies is the use of cleaner fish as a biological control. While not a novel method with the first proof-of-principle reported in the early 1990s[6, 7], the expansion of farmed cleaner fish production has led to the emergence of a new sector with new production challenges, including the health and welfare management of these new aquaculture species.

Prior to *circa* 2011, all cleaner fish deployed in salmon sea cages were wild caught wrasse species from the family Labridae, including cuckoo (*Labrus mixtus*), ballan (*Labrus bergylta*), corkwing (*Symphodus melops*), goldsinny (*Ctenolabrus rupestris*) and rockcook (*Centrolabrus* *exoletus*). Based on farm experience, there was a preference towards the deployment of ballan, goldsinny and corkwing wrasse, driven primarily by stock availability[8] and enhanced delousing performance.[9,10] The level of exploitation in Norway (where the longest record of catch and deployment exists) provides an insight into the scale of application, where the use of wild wrasse increased from 1.7 million fish in 2008 to 20 million in 2016.[11] However, such increasing demands for cleaner fish due to the recent industry expansion together with increasing sea lice pressures and biosecurity concerns, has led to the farming of cleaner fish to control the quality and health of deployed animals and ensure the environmental sustainability of this pest management strategy. To this end, two species are currently being farmed in North Atlantic countries (UK, Ireland, Norway, Iceland and Faroe Islands)[12], ballan wrasse (reviewed in Davie et al.[13] & Lekva & Grotan[14]) and lumpfish (*Cyclopterus lumpus*) (reviewed in Powell et al.[15]). Despite farming being in its infancy with the first farmed ballan wrasse deployed in salmon pens in 2013 in Scotland[16], the sector in most countries is rapidly expanding towards self-sufficiency. In Norway, 17.2 million (46%) of the 37.4 million cleaner fish deployed in 2016 were of farmed origin, comprising 15.9 million lumpfish and 1.3 million ballan wrasse.[11] In comparison, UK farmed cleaner fish production in 2016 was 1.9 million lumpfish and 118,000 ballan wrasse, which was 68% of the total cleaner fish deployed.[17] Annually, these numbers are increasing significantly, primarily driven by lumpfish production as they are proving to be the least challenging in terms of hatchery production.

This paper provides a comparative overview of the scientific knowledge and industry practices regarding the biology and deployment of cleaner fish with an emphasis on the health challenges and welfare of both species.

1. Overview of cleaner fish production

Although being used for a common purpose, ballan wrasse and lumpfish are quite distinct, both in terms of their biology, ecology and life histories (Table 1). This can be beneficial as each species can be used for sea lice control under different conditions, with each having its own biological niche in the net-pen environment, but it also means that production and husbandry methods must be tailored to each species.

Ballan wrasse are protogynous hermaphrodites[18,19] with a complex hierarchy and a highly skewed sex ratio, which makes broodstock management challenging.[20] The spawning window is naturally in April–June[21] but this can be extended in captivity using environmental manipulation.[13] Due to their long generation time (reaching puberty at ~6 years for females and 12 years for males[19]), current hatchery production is exclusively from wild-caught broodstock, although F1 stocks are now being retained by commercial hatcheries to act as future potential broodstock. Ballan wrasse typically require 18 months to reach their deployment size of 40–50g[22], which includes a two-month live feeds period with weaning to formulated feeds being completed by about 70–90 days post hatch depending on hatchery protocols (Fig. 1). Research is ongoing to optimise the species' growth potential primarily through environmental and nutritional manipulations with the objective being to shorten the production period to reduce costs, optimise the use of hatchery and nursery facilities and increase overall productivity. The primary focus is on improving understanding of nutritional requirements and digestive physiology, especially given their agastric digestive system.[23] As in most other non-domesticated marine fish species, larviculture can be challenging with high mortalities during the early larval stages, primarily during first feeding and weaning. There are also anecdotal reports of deformities in juveniles, primarily jaw and spinal pathologies, which could reduce their delousing efficacy. However, there remains no clear data, nor has the aetiology for such conditions been identified, though, as with other marine species, it is most likely multifactorial.

Farmed lumpfish are currently produced from wild-caught broodstock, which are culled and the gametes stripped for artificial fertilisation.[24] Due to the increased biosecurity risk associated with wild broodstock, a post-mortem health screening is recommended, and only egg masses from clean parents should be used for production. Natural spawning in captivity is possible[25], although quality is generally poorer than in egg masses produced using artificial fertilisation[15]. Given the well-established supply chains for mature adults from caviar fisheries[26], wild fish remain the favoured source of broodstock. The production cycle for lumpfish is nearly 60% shorter (5–7 months to a deployment size of around 20g) than in ballan wrasse (Fig. 1), which is one of the main drivers for the increased focus on the production of this species in Ireland, UK, Norway, Faroes, Iceland & Canada. The rapid growth rate of lumpfish (SGR 1.5–3.5% per day[27]) brings with it challenges in production management, primarily due to a conflict between ensuring effective vaccination strategies while maintaining size grades desired for deployment. However, it is anticipated that closed-life-cycle management will be viable given the species' short generation time[15], which should enable the selective improvement of traits of interest (e.g. slower growth, enhanced delousing and disease resistance).

Table 1. Natural range, population dynamics and reproductive traits in wild ballan wrasse and lumpfish.

	Ballan wrasse	**Lumpfish**
Natural range	East-Atlantic coasts (Morocco – Trondheim and Iceland).[18]	Widely distributed in North Atlantic (Hudson Bay – Bermuda, Greenland, Iceland – Iberian Peninsula).[28]
Natural habitat	Rocky reefs.[18]	Bentho-pelagic.[29]
Home range	High site fidelity.[30,31]	Extensive migrations between feeding and breeding grounds.[32,33]
Status of wild stocks	Least concern on IUCN Red List[34], although not assessed since 2010.	Near threatened on the IUCN Red List[35]. Decrease in some spawning stocks in recent decades, may be overexploited.[36]
Population genetics	Two predominant genetic clades (Scandinavian & Celtic) based on mitochondrial haplotypes.[37]	Three distinct populations based on microsatellite markers: Maine-Canada-Greenland, Iceland-Norway and Baltic Sea.[36]
Gender system	Protogynous hermaphroditic resulting in highly skewed sex ratio.[19,38]	Gonochoristic.[39]
Natural diet and digestive system	Omnivorous, primarily hard-shelled crustaceans e.g. decapods, isopods and molluscs[19]; substrate grazers.[9] Agastric digestive system.[23]	Larger planktonic organisms (harpaticoids, amphipods, isopods). Sea lice regularly ingested.[29,40,41] Stomach present.
Fecundity	105–154 eggs/g per season in 650–950 g fish[21]; ~100 eggs/g per spawning in 500 g fish.[42]	Wild Greenland lumpfish potential fecundity of 49–60 eggs/g for 2 kg fish, increasing with latitude[43], and mean of 61 eggs/g for 2 kg fish from Gulf of St Lawrence.[44]

IUCN, International Union for Conservation of Nature.

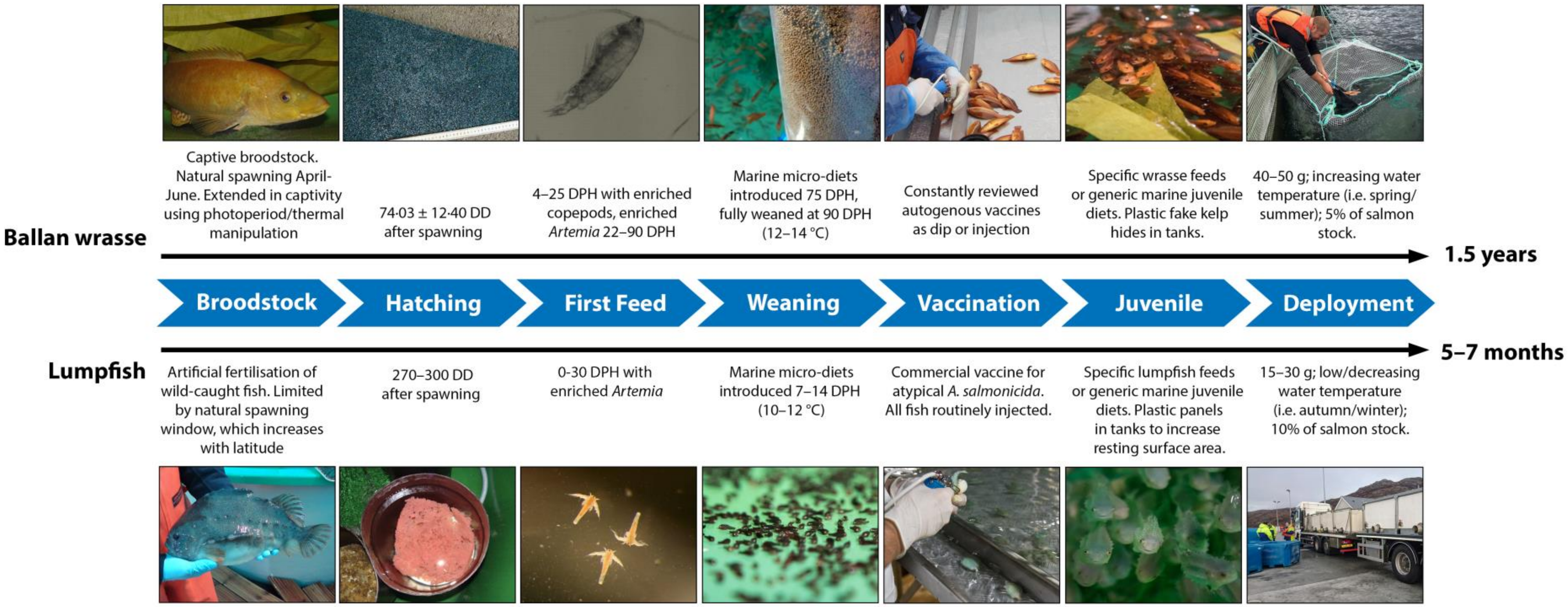

Figure 1. Typical commercial production timelines for ballan wrasse and lumpfish. DD = degree days, DPH = days post-hatch. Image credits: A. Brooker, C, Gutiérrez, A. Chalaris, T. Cavrois, J. C. Navarro.

2. Cage management: cleaner fish behaviour, welfare and re-use

2.1 Behaviour and delousing efficiency

Ballan wrasse and lumpfish are omnivorous, opportunistic feeders, and they are both proven to be effective salmon delousers.[45,46,47] However, it remains unknown whether they predate sea lice on salmon or other hosts in the wild. The natural diet of the two species is quite distinct and reflective of their different environmental preferences (Table 1). Cleaner fish mutualism is widely recognised in tropical-reef-dwelling wrasse[48,49], so cleaning behaviour may be innate, in the Labridae at least. In tank studies, farmed ballan wrasse naïve to both salmon and sea lice reduced sea lice prevalence from 12 to less than 0.5 adult lice per salmon after 60 h even when supplementary food (crushed mussels) was available.[45] Furthermore, Skiftesvik et al.[50] found that farmed wrasse were as effective as wild wrasse at delousing in cage studies. Imsland et al.[51] reported that lumpfish in experimental net pens maintained sea lice levels significantly lower than in controls, although there was evidence of variable performance that may be related to genetics or animal size. All studies have found that both species preferentially predate larger, motile lice stages, although chalimus stages can also be predated.[52]

Lumpfish are deployed at a smaller size than ballan wrasse (15–30g vs. 40–50g) as their broader cross-section prevents their escape from net pens at these sizes (Table 2). Small lumpfish (20–30g) are thought to be more effective delousers than larger lumpfish (>75g)[53], but more research is required to confirm this. Conversely, delousing was found to be more rapid in larger ballan wrasse (~75g) than smaller fish (~23g), although there is an increased risk of aggressive behaviour leading to salmon injuries when deploying larger wrasse during the first year of seawater production.[45]

Stocking rates are generally higher for lumpfish than for ballan wrasse. Treasurer[54], Skiftesvik et al.[50] and Leclercq et al.[45] recommended a stocking ratio of 5% wrasse:salmon, whereas Imsland et al.[47,51,55] used stocking ratios of 10% and 15% for lumpfish. Anecdotal evidence from the Scottish and Norwegian salmon industries indicate that stocking ratios of approximately 5% for ballan wrasse and 8–10% for lumpfish are widely used.

Strict biofouling control and salmon mortality removal is important when using ballan wrasse, as these alternative food sources can preclude delousing.[9,50] These practices are considered less important for lumpfish, which may benefit from the alternative food sources[46], and indeed, the Faroese aquaculture industry promotes net biofouling to reduce the effects of strong coastal currents in the net pens.[56]

Water temperature is an important consideration for both ballan wrasse and lumpfish as it dictates their deployment windows. The ballan wrasse is a temperate species and tends to have slower swimming and foraging activity at temperatures below 10°C[57], and below 6°C they enter into a state of torpor[30] (Table 2). In contrast, lumpfish continue to feed at 4°C[27], but industry reports suggest that they prefer lower temperatures and are more prone to disease at higher temperatures (>10°C). Consequently, wrasse are best deployed in the spring/summer when water temperatures are rising, while lumpfish are best deployed in the autumn/winter when water temperatures are dropping (Table 2).

Ballan wrasse and lumpfish are diurnal species; they are active during the day, when they are likely to exhibit delousing behavior, and rest at night.[30,58,59] Lumpfish tend to swim at shallower depths than ballan wrasse, which adjusted their swimming depth according to the time of day in commercial net-pen trials[59] (Table 2). The species' differences in temperature preferences and behaviour suggest that a combined wrasse/lumpfish deployment strategy may prove to be more effective than a single-species approach.

2.2 Husbandry practices

Good health and welfare can be promoted through good husbandry practices, and while many improvements in cleaner-fish husbandry have already been made, including transportation, acclimation, supplementary feeding, hides and substrates, many more will come as new knowledge becomes available.[60] In the wild, ballan wrasse inhabit coastal reefs, preferring the cover of rocks or kelp[19], and tend to be territorial with relatively small home ranges and limited migrations.[31] They are diurnally active and nocturnally quiescent, sheltering overnight in rocky crevices.[30,31,61] Wild lumpfish have an offshore, semi-pelagic lifestyle and are often associated with floating seaweed.[32,40,62]

Despite their different lifestyles, the habitat requirements of both ballan wrasse and lumpfish can be met through the use of artificial substrates, or hides (Table 2). These provide shelter for ballan wrasse, particularly during the night, although the net-pen corners and sides are preferred locations for ballan wrasse at any time of the day or night.[59] Imsland et al.[55] found that while lumpfish spent much of the daytime foraging, they were usually found resting within or under floating weed when not feeding, and at night they tended to aggregate on smooth substrates using their abdominal suckers.[58] Various hide configurations are produced commercially, but they are typically made from strips of plastic attached to ropes to form strands of artificial kelp with the addition of rigid plastic substrates for lumpfish to adhere to.[58] Continued research into hide types, colours and locations in the net pen may yield further enhancements.

Sea lice levels in commercial salmon net pens are maintained as low as possible. In the UK, for example, the Aquaculture and Fisheries (Scotland) Act 2007 sets a treatment threshold of 0.5–1 adult female lice per fish. These low lice levels are inadequate to sustain a population of cleaner fish, and although both wrasse and lumpfish are known to graze on biofouling in net pens[9,46,47], supplementary feeding is essential to maintain the condition and welfare of the cleaner fish.[50,63] Several feed manufacturers now produce pelleted diets for ballan wrasse and lumpfish, and these can be delivered by hand or automatic feeders into the hides or near the pen edges, which allows feeding behaviour to be monitored. Typical feeding rates are 2% of the fish biomass every other day.[60] As ballan wrasse are predominantly substrate grazers, their condition can be better maintained using agar-based feed blocks placed within small feeder shelters away from the main hides.[63] The use of this water-stable feed is becoming more widespread and has also being trialled for lumpfish.[64]

The acclimation of hatchery reared cleaner fish to the net-pen conditions is likely to be beneficial in reducing stress and encouraging natural behaviours (including delousing). For example, retaining ballan wrasse in a small conditioning pen containing hides and agar feed within the main net pen for several weeks before release has been reported to improve deployment success.[60]

2.3 Welfare

As cleaner fish are produced for their delousing behaviour as a pest management strategy rather than any physical characteristics, good welfare is essential to promote their natural behaviours. For any new species in aquaculture, it is important to develop indicators to define and monitor welfare.

The development and standardisation of best management practices (*e.g.* RSPCA cleaner fish welfare standards[65]) and routine health checks are essential to minimise disease and maintain a good welfare status. To monitor health and welfare both in hatcheries and following deployment at sea, operational welfare indicators (OWI) must be defined for each cleaner fish species, and these should be based on preferred environmental conditions, physical and physiological status or behaviour.[66] Mortalities are a definitive indicator of poor health and welfare, and they should be recorded along with condition and growth rates. Fulton's condition index[67,68] can be used for both species to indicate general animal condition. However, it should be noted that given their rotund body form, the typical ranges recorded for lumpfish (e.g. 4–4.5[55]) are much higher than in most other teleosts. Nonetheless, datasets have confirmed that the species follows an isometric growth pattern so the method is valid[69] (A. Davie, unpublished data).

In many fish species, fin damage can be a result of aggression and a sign of stress[70], and these injuries can be a portal for bacterial and fungal infections. Fin damage indices have been developed for both wrasse[71] and lumpfish (S. Rey, unpublished data) and could easily be implemented as a physical OWI. Elevated blood glucose and lactate is a sign of stress and can be measured using handheld meters, and this method has been validated for ballan wrasse.[72] Other physiological parameters could be used (e.g. hepatosomatic index[73] or liver-colour scoring index[56]), although they require sacrificial sampling. Behaviour can also be used to assess animal welfare.[74,75] Environmental, dietary and social preferences can be determined by choice tests or place preference tests, and routine monitoring of behaviour at salmon farms may be achieved by visually observing and recording behaviour at the surface or underwater using video cameras, or more quantitative techniques could involve sonar or acoustic tagging of sentinel cleaner fish.[59]

2.4 Re-use and end use

Cleaner fish trained in salmon delousing are a valuable resource, and once a production cycle is finished, the capture and redeployment or breeding of these fish could be considered to be an efficient use of this resource. However, biosecurity issues may prevent their reuse, and this practice may not be permitted in some countries. While reuse may be an option for wrasse, the rapid growth rates of lumpfish and their tendency to be poor delousers and aggressive when mature[53] precludes their reuse.

For cleaner fish that can no longer be used as delousers there are several possibilities for their end use if they are harvested appropriately. As availability increases, there is increasing interest from the retail sector for both species, including the use of ballan wrasse for sashimi (Cornwall

Good Seafood Guide[76]); there is an emerging market for whole and filleted lumpfish, especially in Asia, and exports to China bring in more than €18 million per year to the Icelandic economy.[77] However, further research is required to develop this market, and public perception may be an issue due to its unusual appearance.[78] A further market opportunity for lumpfish could be the production of lumpfish roe from mature captive females as a sustainable alternative to wild fisheries[26], although this would require the development of additional rearing facilities to ongrow the fish once they had exceeded their effective delousing size. Finally, biliverdin, a compound responsible for the blue coloration of ballan wrasse and lumpfish[79] has several potential applications in research, medicine and biotechnology, including fluorescence microscopy and as a storage medium for transplant organs. While large quantities could potentially be extracted from cultured ballan wrasse blood[80], the cost of extraction compared to other sources must be further studied.

Table 2. Comparison of deployment and husbandry practices for farmed ballan wrasse and lumpfish in salmon sea pens (with a focus on UK production).

	Ballan wrasse	Lumpfish
Deployment window	Spring/summer with increasing water temperature.	Late autumn/winter with decreasing water temperature.
Transportation	Fish starved 24 h prior transport via road in tanks with hides present, then secondary transport via boat to net-pen site.	Fish starved 24 h before transport via road in tanks with hides present, then secondary transport via boat to net-pen site. High-stress periods are loading, handling, secondary transport.[81]
Deployment size	At least 40–50g to prevent escape through net mesh.[54] Larger wrasse may be more effective delousers.[45]	Typically 15–30 g[81] (R. Hawkins, Marine Harvest Scotland, pers. comm., 2017). Ineffective delousers when mature (400–500g, 14–16 months[55]).
Stocking rate	5% of salmon number.[45,50,54]	10% of salmon number.[47,51,55]
Use of hides and substrates	Plastic fake kelp; various configurations available commercially, e.g. curtain, lantern, float frame.	Plastic fake kelp and smooth, flat surfaces for resting; various configurations available commercially.
Feeding behaviour	Will not feed below 6°C, winter dormancy.[57,82,83]	Will feed as low as 4°C.[27]
Swimming Activity	Slower than lumpfish, prefers edges and corners.[59]	Higher activity rates than ballan wrasse. Covers whole pen area.[59] Active foraging during day, aggregate on smooth surfaces at night.[58]
Buoyancy	Physoclistic; rapid pressure changes should be avoided.[84,85] Observations of swim bladder over-inflation in hatcheries and net pens.	No swim bladder, but near-neutral buoyancy due to cartilaginous skeleton, extensive sub-cutaneous jelly and loose-fibred muscles.[86]
Recapture	Un-baited creels are commonly used to sample or recapture.	Hides or habituation to feeding sites are preferred.

3. Health challenges, prevalence and management

3.1 Primary diseases during production

There are numerous health challenges currently experienced in both farmed ballan wrasse and lumpfish, and this is a top priority area for research.

3.1.1 Bacterial diseases

Bacterial diseases are currently the primary challenge in both species. Secondary bacterial infections by opportunistic pathogens may be triggered by poor husbandry or water quality in the hatchery/nursery, handling during vaccination, nutritional imbalance, stress or cannibalism (common in early stage lumpfish in hatcheries).[40]

Atypical strains of the bacterium *Aeromonas salmonicida* are the aetiological agent of atypical furunculosis, affecting both ballan wrasse and lumpfish when water temperatures exceed 13°C.[87] It is the most frequent cause of bacterial disease outbreaks in both species resulting in considerable economic losses as a commercial vaccine is not currently available. The bacterium is classified into subtypes (A-layer types) by the virulence array protein gene, vapA. Ballan wrasse are more susceptible to subtype V in Scotland and both V and VI in Norway, while lumpfish appear to be susceptible only to subtype VI.[88,89] Outbreaks have occurred in hatcheries and at cage sites, although asymptomatic fish can also be positive. Disease progression is chronic, and high mortalities have been recorded.[87] Affected fish show external ulcers on the skin and fins, granulomas in the internal organs and fluid accumulation in the abdominal region (Fig. 2a–d).

Vibriosis in cleaner fish is caused by *Vibrio anguillarum*, *V. ordalii* and *V. splendidus*. Pathology is similar for both species with external lesions (ulcers, oedema and haemorrhages), enlargement of the caudal peduncle due to fluid retention and necrosis of internal organs (Fig. 2e). While lumpfish are susceptible to both species, only *Vibrio anguillarum* has been isolated from wild ballan wrasse to date, with up to 60% mortality in 50g fish injected with the bacterium.[90,91] Other *Vibrio* spp. have been isolated from cleaner fish but their pathogenicity is unclear.[89] Birkbeck and Treasurer[92] demonstrated that *V. splendidus* and *V. ichthyoenteri* are part of the natural microbiota of wrasse, and hence, *Vibrio* spp. may be opportunistic, causing disease only if the immune system is suppressed.

Pasteurella sp. and other pathogens, such as *Pseudomonas anguilliseptica*, *Tenacibaculum maritimum*, *Moritella viscosa* and *Piscirickettsia salmonis*, have been reported as primary pathogens causing Pasteurellosis in lumpfish.[93,94,95,96,97,98,99,100,101] Symptoms of *Pasteurella* infections commonly include skin lesions as white patches around the eyes, tail rot and bleeding in the gills, the base of the fins and tail[99] (Fig. 2f), although similar symptoms have been observed in other bacterial infections, e.g. skin ulcers and tail rot in *Tenacibaculum maritimum* infections in lumpfish in Norway.[100]

In Norway, epitheliocystis has also been observed in ballan wrasse, which is an intracellular bacterial disease caused by *Candidatus sp.* Similichlamydia labri. nov. and affects the secondary lamellae of the gills.[102]

3.1.2 Parasitic diseases

The ubiquitous *Neoparamoeba perurans*, the causative agent of Amoebic Gill Disease (AGD), has been reported as a natural infection in both ballan wrasse and lumpfish, and experimental infection has been successful in lumpfish cohabiting with infected salmon.[103] Primary histopathology shows pale patches at the bases of gill filaments, hyperplasia of epithelial cells and fusion of gill lamellae[103,104] (Fig. 2g,h). Low-to-moderate mortalities in hatcheries and cage sites have been reported due to AGD.

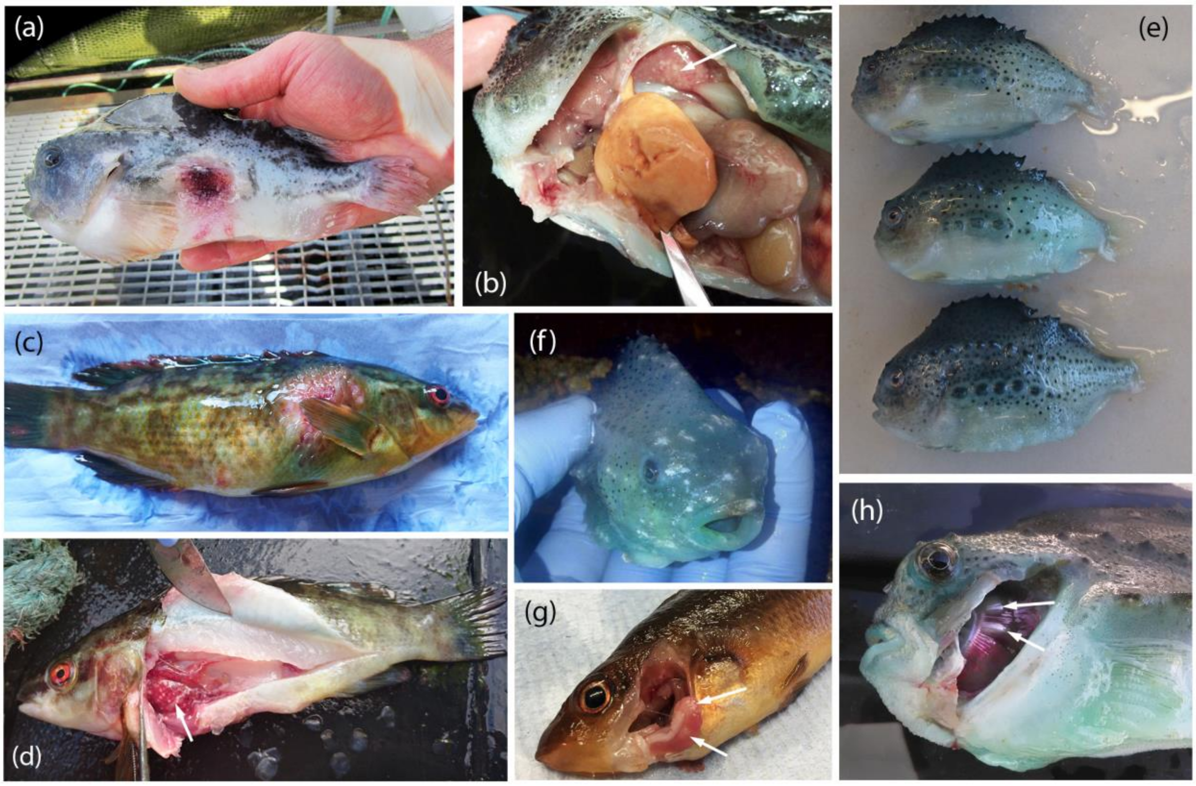

Figure 2. Clinical signs of bacterial and parasitic diseases in lumpfish and ballan wrasse: (a) external ulcer indicative of atypical *Aeromonas salmonicida* in lumpfish; (b) multifocal granulomas in lumpfish kidney characteristic of atypical *A. salmonicida*; (c) external ulcer indicative of atypical *A. salmonicida* in ballan wrasse; (d) multifocal granulomas in ballan wrasse liver characteristic of atypical *A. salmonicida*; (e) decolouration and enlargement of the caudal peduncle symptomatic of *Vibrio* spp. in lumpfish.; (f) skin lesions as white patches around the eyes symptomatic of *Pasteurella* sp. in lumpfish.; (h) amoebic gill disease in ballan wrasse resulting in pale plaques on the gills; and (g) amoebic gill disease in lumpfish resulting in pale plaques on the gills. Image credits: C. Gutiérrez, L. Sheriff, G. Ramírez-Paredes.

The microsporidian *Nucleospora cyclopteri* has been reported in wild lumpfish with 25% of fish showing chronic clinical signs, such as pale and uniformly enlarged kidneys (renomegaly), exophthalmia and skin lesions.[105] Horizontal transmission is confirmed, although vertical transmission may also occur due to the close association of spores with eggs. All wild lumpfish used as broodstock for commercial production must be tested for *N. cyclopteri* as there is no effective treatment available. Reports on farmed Norwegian lumpfish, have found co-infections of *N. cyclopteri* and *Kudoa* islandica.[106] The microsporidian *Tetramicra brevifilum* has recently been reported in lumpfish broodstock causing lethargy, anorexia, exophthalmia, severe bloating like ascites, vacuolisation and white nodules in most of the internal organs, while skeletal muscle liquefaction and microsporian xenomas were observed in the skin, internal organs, gills and eyes.[107]

Other ciliates, such as *Trichodina* sp. and *Uronema-like* species, have been reported as incidental findings on the skin or gills of lumpfish. Heavy infections may lead to mortalities if unattended.[15]

3.1.3 Viral diseases

Viral haemorrhagic septicaemia (VHS) is a notifiable disease in Scotland and has been reported in wild-caught cleaner fish from Shetland.[108,109,110] It has not been found in farmed ballan wrasse, but was isolated in Icelandic lumpfish for the first time in 2015.[111] Interestingly, lumpfish infected with VHS were more susceptible to the Infectious Pancreatic Necrosis (IPN) virus (another notifiable disease) during experimental infections.[94]

In a recent cardiomyopathy syndrome (CMS) event at a salmon farm in Ireland, ballan wrasse tested positive for low levels of piscine myocarditis virus (PMCV).[112] Only small numbers of fish were tested and the histopathology was inconclusive. However, ballan wrasse are known to be susceptible to the virus and can be potential carriers.

A recently discovered lumpfish flavivirus (fam. *Flavoviridae*) has been associated with moderate-to-high mortality in farmed lumpfish in Norway with the virus present in most tissues but elevated in liver and kidney, although it has also been detected in lumpfish with no clinical signs.[113] Clinical signs are anaemia, pale gills, liver inflammation and necrosis and inflammation of the abdomen, and it is recommended that all wild-caught lumpfish broodstock are tested for the pathogen using RT-PCR, with monitoring and regular screening during the production cycle.[113]

3.1.4 Fungal diseases

Systemic fungal infections in adult lumpfish by *Exophiala spp*. have been reported from marine hatcheries and seawater sites causing dark lesions in the skin, gills and internal organs, such as liver, kidney and musculature.[15] However, the source of the infection has not been identified, and treatments with bronopol, formalin and even itraconazole have not been 100% successful.

3.2 Prevalence and management

3.2.1 Prevalence

Bacterial diseases may lead to mass mortality, especially in the lumpfish. A survey conducted in Norway in 2013 reported 48% mortality among the stocked cleaner fish population, with 75% caused by bacterial infections.[90] Similar findings were reported in autumn/late summer 2015, when bacterial agents were confirmed in nearly 80% of case materials, and atypical furunculosis and vibriosis were the most common causes of mortality in cleaner fish.[114]

AGD is prevalent in hatcheries where water quality is poor and sand-filtered water is not used (flow-through hatcheries) or when recirculation systems are topped up. Some evidence suggests that UV irradiation or ozonisation is inadequate to kill free-living amoeba, and in some cases amoeba-forming pseudocysts can pass through the system and be re-activated.[115]

3.2.2 Management

Following deployment, the survival of cleaner fish is often poor in net pens cohabiting with Atlantic salmon, and there are concerns regarding the welfare of wild and farmed cleaner fish.[116] Good husbandry, such as the provision of hides, clean nets and supplementary feeding[63], and routine health monitoring are essential.

AGD can be controlled using hydrogen peroxide, which significantly reduces the numbers of amoebae present in the gills (C. Gutiérrez, personal communication). In Atlantic salmon, freshwater bathing significantly reduces the presence of amoebae and mucoid patches on gills[117]; the treatment is effective for lumpfish and is used where possible[118], but it is not used for ballan wrasse due to their low freshwater tolerance. Freshwater is preferred to hydrogen peroxide as clearance rates are higher, and hydrogen peroxide can cause mortalities if gills are compromised (CG, pers. obs.). In hatchery trials, 15 ppt brackish water over three days achieved 100% clearance of AGD in ballan wrasse (P. Featherstone, Marine Harvest Scotland, pers. comm., 2017), although further investigation of this treatment for wrasse in sea pens is required.

Authorised antimicrobial treatments (e.g. oxytetracycline or florfenicol) are used in net pens to control clinical outbreaks when required. While broad-spectrum antibiotics are effective against most bacteria, significantly improving survival rates, some bacterial diseases, such as pasteurellosis, are often recurrent requiring longer and more frequent treatments, which highlights the importance of disease prevention through good welfare and nutrition, the reduction of stress and the use of effective vaccines.

3.2.3 Vaccination

As mortality events in cleaner fish are often associated with bacterial diseases, vaccination is key to improve their health and welfare, improve survival and reduce the use of antimicrobials. Further development of vaccines for cleaner fish and improved vaccination strategies are required, and rapid progress has recently been made in this area.

Due to the lack of commercial (broad spectrum) vaccines available for ballan wrasse, the use of autogenous vaccines has increased as new pathogens are regularly isolated from clinical outbreaks. Autogenous vaccines currently available in Scotland (Ridgeway Biologicals Ltd.) and Norway are aqueous-based dip vaccines and oil-based injection vaccines, which are regularly reviewed based on emergent diseases in cleaner fish to meet the needs of the industry. Under experimental conditions, for instance, polyvalent autogenous vaccines against atypical *Aeromonas salmonicida* using a homologous strain were found to be protective for ballan wrasse with a relative percent survival (RPS) of 79% and 91% at LD_{50} and LD_{60}.[119] Several vaccines are available for lumpfish including an injection vaccine against atypical *Aeromonas salmonicida* (A-layer type VI) and *Listonella anguillarum* (syn. *Vibrio anguillarum*) serotype O1 and O2a antigens supplied by Pharmaq (Zoetis) and an autogenous vaccine against *Aeromonas salmonicida* and *Vibrio salmonicida* supplied by Vaxxinova Norway AS. In a recent trial, fish vaccinated against atypical *Aeromonas salmonicida* showed high levels of specific antibodies, providing 73% and 60% RPS in monovalent and trivalent vaccines, respectively, providing strong evidence that the optimisation of vaccines will improve the immunity of cleaner fish to specific diseases.[120]

3.3 Cohabitation

As both wild-caught and farmed cleaner fish are used for the control of sea lice in salmon net pens, the culture intensification of these species may lead to the emergence of novel diseases.

Farmed cleaner fish are usually tested for atypical *A. salmonicida*, *Vibrio*, *Pasteurella* (lumpfish) and AGD before they are transferred to sea pens, but this is not always the case for wild-caught wrasse, and their introduction to net pens should always be risk-assessed.

Farmed salmon are fully vaccinated and protected against the majority of significant bacterial pathogens and viruses, including typical *Aeromonas salmonicida*, *Vibrio salmonicida* and some viruses, such as IPN and PD.[121,122,123] However, cleaner fish may act as reservoirs/carriers for other potential pathogens, e.g. *Moritella viscosa*, *Piscirickettsia salmonis*, *Tenacibaculum maritimum* or possibly notifiable diseases, such as VHS.

AGD affects salmon[124], ballan wrasse[104] and lumpfish[103], and cohabitation is likely to increase the risk of disease outbreaks as histopathological changes are consistent in all three species. Furthermore, it is believed that cleaner fish may act as asymptomatic carriers, which poses a threat to cohabiting salmon.[103] Although 3-day brackish water treatments have proven to be effective in wrasse, it is logistically impossible to carry out these treatments in commercial net pens. Commercial trials have shown that cleaner fish (particularly lumpfish, but also ballan wrasse) can carry high numbers of amoebae compared to salmon despite being asymptomatic, and cleaner fish are often positive for AGD using molecular methods long after salmon are negative following freshwater bath/hydrogen peroxide treatments (CG, unpublished data). As AGD appears to develop more slowly in cleaner fish than salmon, they should be screened using RT-PCR (not scored) for AGD before they are deployed with salmon.[103]

While *Lepeophtheirus salmonis* only infects salmonids, both salmonids and cleaner fish are susceptible to infection by *Caligus elongatus*, and motile stages of *C. elongatus* can move between farmed salmonids and wild fish, especially when water temperatures are high.[1]

VHS has caused high mortalities in both wild wrasse[109] and lumpfish.[111] Therefore, more research on how cleaner fish can act as reservoirs of notifiable pathogens is required to mitigate the risks of cohabiting with salmon. Ballan wrasse (and corkwing wrasse, *Symphodus melops*) are susceptible to PMCV, and although they may not develop clinical CMS, they can pose a significant biosecurity risk to salmon, especially if re-used or moved between sites or pens.[112] Lumpfish are carriers of *Piscirickettsia salmonis*, and it is present at most sites where they are deployed[101], which may increase infection pressure on Atlantic salmon, so it is important that both species are treated synchronously.

4. Knowledge gaps and challenges

Cleaner fish farming is still in its infancy, and while research is progressing rapidly with strong scientific communities in the UK and Norway collaborating together, much of the basic knowledge regarding the species' biology, their environmental and nutritional requirements, their social and delousing behaviour, and their immune functions remains unknown or poorly described. The simultaneous domestication of two new marine aquaculture species is a significant challenge that demands sustained effort and funding over a prolonged period of time. Research must focus on enhancing the robustness of the farmed stocks (better survival in the hatchery, reduced prevalence of malformations and a disease-free status) and increasing hatchery outputs to meet the urgent demands from the salmon sector and to protect wild stocks from overfishing. As there are no selective breeding programmes for cleaner fish to date, current research is focusing on improved larval and juvenile performance through better microbial management, tailored environmental conditions and husbandry, and optimised diets (including live feeds, enrichment, weaning and grower diets that meet the species' nutritional requirements, optimise growth potential in ballan wrasse and limit growth in lumpfish). Health management is a critical priority as disease outbreaks throughout all life stages are responsible for significant losses in both species. Research is required to characterise immune function in these species, and then develop polyvalent vaccines against the most virulent bacteria (e.g. atypical furunculosis, *Vibrio*, *Pasteurella*) while monitoring for the emergence of new diseases.

Obviously, promoting welfare and delousing behaviour post-deployment are essential. This requires a better understanding of cleaner fish behaviour in captivity including cohabitation of different cleaner fish species and stocks, the development of acclimation and/or conditioning protocols for farmed stocks to cope quickly and reliably with the transfer from sheltered land-based hatchery systems to dynamic, open sea pens, and the development of indicators to monitor their welfare. Last but not least, there should be a major focus on domesticating the species by closing the life cycles, establishing breeding programs and identifying genomic markers for relevant traits (especially gender, growth, delousing behaviour and disease resistance/robustness) that can be actively selected, and studying population genetics in wild cleaner fish stocks across the North Atlantic region and the potential implications of translocation.

While many scientific knowledge gaps and production bottlenecks remain, impressive progress has been made over the past decade. The success of this innovative and unique pest management strategy will require the fast-tracking of the domestication process over the next few years to ensure its sustainability and reliability and support ambitions for the expansion of the global salmon industry.

Acknowledgements

The authors thank the following for contributing information to this review: Thomas Cavrois, Antonios Chalaris, William Clark, Sarah-Louise Counter Selly, Paul Featherstone, Ronnie Hawkins, Juan Carlos Navarro, Samuel Pountney, Gustavo Ramírez-Paredes and Lindsay Sheriff.

5. References

1. WOOTTEN, R., SMITH, J. W. & NEEDHAM, E. A. (1982) Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences 81, 185–197
2. GRIMNES, A. & JAKOBSEN, P. J. (1996) The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. Journal of Fish Biology 48, 1179–1194
3. BROOKER, A. J., SKERN-MAURITZEN, R. & BRON, J. E. (2018) Production, mortality and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling. ICES Journal of Marine Science 75, 1214–1234
4. ABOLOFIA, J., ASCHE, F. & WILEN, J. E. (2017) The cost of lice: quantifying the impacts of parasitic sea lice on farmed salmon. Marine Resource Economics 32, 329–349
5. HOLAN, A. B., ROTH, B., BREILAND, M. S. W., KOLAREVIC, J., HANSEN, Ø. J., HERMANSEN, Ø., GJERDE, B., HATLEN, B., MORTENSEN, A., LEIN, I., NOBLE, C., GISMERVIK, K., ESPMARK, M. (2017) Beste Praksis for Medikamentfrie Metoder for Lakseluskontroll (MEDFRI). (Best Practices for Drug-Free Methods for Salmon Lice Control (MEDFRI)). Nofima Report Series, No. 10/2017. Nofima. 108 pp [in Norwegian]
6. BJORDAL, A. (1991) Wrasse as cleaner fish of farmed salmon. Progress in Underwater Science 16, 17–29
7. TREASURER, J. (1994) Prey selection and daily food consumption by a cleaner fish, *Ctenolabrus rupestris* (L.), on farmed Atlantic salmon, *Salmo salar* L. Aquaculture 122, 269–277
8. SKIFTESVIK, A. B., BLOM, G., AGNALT, A. L., DURIF, C. M., BROWMAN, H. I., BJELLAND, R. M., HARKESTAD, L. S., FARESTVEIT, E., PAULSEN, O. I., FAUSKE,

M. & HAVELIN, T. (2014) Wrasse (Labridae) as cleaner fish in salmonid aquaculture–the Hardangerfjord as a case study. Marine Biology Research 10, 289–300

9. DEADY, S., VARIAN, S. J. & FIVES, J. M. (1995) The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. Aquaculture 131, 73–90

10. GONZALEZ, E. B. & DE BOER, F. (2017) The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fisheries Science 83 661–670

11. NORWEGIAN DIRECTORATE OF FISHERIES (2017) Sale of Farmed Cleaner Fish 2012–2016. www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse. Accessed November 23, 2017

12. BOLTON-WARBERG, M. (2017) An overview of cleaner fish use in Ireland. Journal of Fish Diseases 1–5

13. DAVIE, A., GRANT, B., CLARK, W. & MIGAUD, H. (2018) The Ballan wrasse (*Labrus bergylta*) reproductive physiology, broodstock management and spawning behaviour. In Cleaner Fish Biology and Aquaculture Applications. Ed J. Treasurer. 5m Publishing. pp 34–45

14. LEKVA, A. & GROTAN, E. (2018) Rearing of ballan wrasse. In Cleanerfish Biology and Aquaculture Applications. Ed J. Treasurer. pp 34–45

15. POWELL, A., TREASURER, W. J., POOLEY, L. C., KEAY, J. A., LLOYD, R., IMSLAND, K. A. & GARCIA DE LEANIZ, C. (2017) Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture 1–20

16. TREASURER, J. (2018) An introduction to sea lice and the rise of cleaner fish. In Cleaner Fish Biology and Aquaculture Applications. Ed J. Treasurer. 5m Publishing, pp 3–34

17. MUNRO, L. A. & WALLACE, I. S. (2017) Scottish Fish Farm Production Survey 2016. Marine Scotland Science. Edinburgh. 56 pp

18. QUIGNARD, J. P., PRAS, A. (1986) Labridae. In Fishes of the North-Eastern Atlantic and the Mediterranean, Vol. II. Eds P. J. P. Whitehead, M., -L., Bauchor, J. –C., Hureau, J. Nielsen, E. Tortonese. UNESCO. pp 919–942

19. DIPPER, F., BRIDGES, C. & MENZ, A. (1977) Age, growth and feeding in the ballan wrasse *Labrus bergylta* Ascanius 1767. Journal of Fish Biology 11, 105–120

20. LECLERCQ, E., GRANT, B., DAVIE, A. & MIGAUD, H. (2014) Gender distribution, sexual size dimorphism and morphometric sexing in ballan wrasse *Labrus bergylta*. Journal of Fish Biology 84, 1842–1862

21. GRANT, B., DAVIE, A., TAGGART, J. B., SELLY, S. L. C., PICCHI, N., BRADLEY, C., PRODOHL, P., LECLERCQ, E. & MIGAUD, H. (2016) Seasonal changes in broodstock spawning performance and egg quality in ballan wrasse (*Labrus bergylta*). Aquaculture 464, 505–514

22. HELLAND, S., DAHLE, S. W., HOUGH, C. & BORTHEN, J. (2014) Production of Ballan Wrasse (*Labrus bergylta*). Science and Practice. The Norwegian Seafood Research Fund (FHF). 136 pp

23. LIE, K. K., TØRRESEN, O. K., SOLBAKKEN, M. H., RØNNESTAD, I., TOOMING-KLUNDERUD, A., NEDERBRAGT, A. J., JENTOFT, S. AND SÆLE, Ø. (2018) Loss of stomach, loss of appetite? Sequencing of the ballan wrasse (*Labrus bergylta*) genome and intestinal transcriptomic profiling illuminate the evolution of loss of stomach function in fish. BMC genomics 19, 186

24. JONASSEN, T. M., LEIN, I. & MYTRO (2018) Hatchery management of lumpfish. In Cleanerfish Biology and Aquaculture Applications. Ed J. Treasurer. 5m Publishing. pp. 122–147

25. WITTWER, C. & TREASURER, J. W. (2018) Egg collection by hand-stripping and natural spawning in lumpfish *Cyclopterus lumpus* broodstock. In Cleaner Fish Biology and Aquaculture Applications. Ed J. Treasurer. 5m Publishing. pp. 147–160

26. JOHANNESSON, J. (2006) Lumpfish caviar: from vessel to consumer. FAO Fisheries Technical Paper. No. 485. Food and Agriculture Organization of the United Nations. Rome. 60 pp

27. NYTRØ, A. V., VIKINGSTAD, E., FOSS, A., HANGSTAD, T. A., REYNOLDS, P., ELIASSEN, G., ELVEGÅRD, T. A., FALK-PETERSEN, I. B. & IMSLAND, A. K. (2014) The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). Aquaculture 434, 296–302

28. STEIN, D. L. (1986) Cyclopteridae. In Fishes of the North-Eastern Atlantic and the Mediterranean, Vol. III. Eds P. J. P. Whitehead, M. -L. Bauchot, J. -C. Hureau, J. G. Nielsen, & E. Tortonese. UNESCO. pp 1269–1274

29. DABORN, G. R. & GREGORY, R. S. (1983) Occurrence, distribution, and feeding habits of juvenile lumpfish, *Cyclopterus lumpus* L. in the Bay of Fundy. Canadian Journal of Zoology 61, 797–801

30. MOREL, G. M., SHRIVES, J., BOSSY, S. F. & MEYER, C. G. (2013) Residency and behavioural rhythmicity of ballan wrasse (*Labrus bergylta*) and rays (*Raja* spp.) captured in Portelet Bay, Jersey: implications for Marine Protected Area design. Journal of the Marine Biological Association of the United Kingdom 93, 1407–1414

31. VILLEGAS-RÍOS, D., ALÓS, J., MARCH, D., PALMER, M., MUCIENTES, G. & SABORIDO-REY, F. (2013) Home range and diel behavior of the ballan wrasse, *Labrus bergylta* , determined by acoustic telemetry. Journal of Sea Research 80, 61–71

32. DAVENPORT, J. (1985) Synopsis of biological data on the lumpsucker, *Cyclopterus lumpus* (Linnaeus, 1758). FAO Fisheries Synopsis No. 147. 34 pp

33. KENNEDY, J., JÓNSSON, S. P., KASPER, J. M. & ÓLAFSSON, H. G. (2015) Movements of female lumpfish (*Cyclopterus lumpus*) around Iceland. ICES Journal of Marine Science 72, 880–889

34. POLLARD, D. (2010) IUCN Red List of Threatened Species 2017-3: *Labrus bergylta*. www.iucnredlist.org/details/full/187398/0. Accessed September 15, 2017.

35. LORANCE, P., COOK, R., HERRERA, J., DE SOLA, L., FLORIN, A. & PAPACONSTANTINOU, C. (2015) The IUCN Red List of Threatened Species 2017-3: *Cyclopterus lumpus*. www.iucnredlist.org/details/full/18237406/1. Accessed September 15, 2017

36. PAMPOULIE, C., SKIRNISDOTTIR, S., OLAFSDOTTIR, G., HELYAR, S. J., THORSTEINSSON, V., JÓNSSON, S. Þ., FRÉCHET, A., DURIF, C. M., F., SHERMAN, S., LAMPART-KAŁUŻNIACKA, M., HEDEHOLM, R. ÓLAFSSON, H., DANÍELSDÓTTIR, A. K. & KASPER, J. M (2014) Genetic structure of the lumpfish *Cyclopterus lumpus* across the North Atlantic. ICES Journal of Marine Science 71, 2390–2397

37. D'ARCY, J., MIRIMIN, L. & FITZGERALD, R. (2013) Phylogeographic structure of a protogynous hermaphrodite species, the ballan wrasse *Labrus bergylta*, in Ireland, Scotland, and Norway, using mitochondrial DNA sequence data. ICES Journal of Marine Science 70, 685–693

38. QUIGNARD, J. P. (1966). Recherches sur les Labridae (poissons téleostéens perciformes) des côtes européennes: systématique et biologie (Research on Labridae (teleost perciform fish) on European coasts: systematics and biology). Naturalia Monspeliensis Sér Zoology 5, 1–247. [in French]

39. HOENIG, J. M. & HEWITT, D. A. (2005) What Can We Learn about Mortality from Sex Ratio Data? A Look at Lumpfish in Newfoundland. Transactions of the American Fisheries Society 134, 754–761

40. INGÓLFSSON, A. & KRISTJÁNSSON, B. K. (2002) Diet of juvenile lumpsucker *Cyclopterus lumpus* (Cyclopteridae) in floating seaweed: effects of ontogeny and prey availability. Copeia 2, 472–476

41. VANDENDRIESSCHE, S., MESSIAEN, M., O'FLYNN, S., VINCX, M. & DEGRAER, S. (2007) Hiding and feeding in floating seaweed: floating seaweed clumps as possible refuges or feeding grounds for fishes. Estuarine, Coastal and Shelf Science 71, 691–703

42. CHALARIS, A. E. (2011) Preliminary study of fecundity, sperm characteristics, behaviour and hormonal induction in native wrasse species. MSc thesis. University of Stirling.

43. HEDEHOLM, R. B., POST, S. & GRØNKJÆR, P. (2017) Life history trait variation of Greenland lumpfish (*Cyclopterus lumpus*) along a 1600 km latitudinal gradient. Polar Biology 40, 1–10

44. GAUTHIER, J., GRÉGOIRE, F. & NOZÈRES, C. (2017) Assessment of lumpfish (*Cyclopterus lumpus*) in the Gulf of St. Lawrence (3Pn, 4RST) in 2015. DFO Canadian Science Advisory Secretariat Research Document, 2017/051. 47 pp

45. LECLERCQ, E., DAVIE, A. & MIGAUD, H. (2014) Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo salar*) post-smolts. Pest Management Science 70, 1274–1282

46. IMSLAND, A. K., REYNOLDS, P., ELIASSEN, G., HANGSTAD, T. A., NYTRØ, A. V., FOSS, A., VIKINGSTAD, E. & ELVEGÅRD, T. A. (2014b) Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. Journal of Ethology 32, 117–122

47. IMSLAND, A. K., REYNOLDS, P., ELIASSEN, G., HANGSTAD, T. A., NYTRØ, A. V., FOSS, A., VIKINGSTAD, E. & ELVEGÅRD, T. A. (2015b) Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.). Aquaculture 436, 47–51

48. BARBU, L., GUINAND, C., BERGMÜLLER, R., ALVAREZ, N. & BSHARY, R. (2011) Cleaning wrasse species vary with respect to dependency on the mutualism and behavioural adaptations in interactions. Animal Behaviour 82, 1067–1074

49. BSHARY, R. & OLIVEIRA, R. F. (2015) Cooperation in animals: Toward a game theory within the framework of social competence. Current Opinion in Behavioral Sciences 3, 31–37

50. SKIFTESVIK, A. B., BJELLAND, R. M., DURIF, C. M. F., JOHANSEN, I. S. & BROWMAN, H. I. (2013) Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402–403, 113–118

51. IMSLAND, A. K., REYNOLDS, P., ELIASSEN, G., HANGSTAD, T. A., FOSS, A., VIKINGSTAD, E. & ELVEGÅRD, T. A. (2014) The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 424–425, 18–23

52. ELIASEN, K., DANIELSEN, E., JOHANNESEN, Á., JOENSEN, L. L. & PATURSSON, E. J. (2018) The cleaning efficacy of lumpfish (*Cyclopterus lumpus* L.) in Faroese salmon (*Salmo salar* L.) farming pens in relation to lumpfish size and seasonality. Aquaculture 488, 61–65

53. IMSLAND, A. K., REYNOLDS, P., NYTRØ, A. V, ELIASSEN, G., ARNE, T., JÓNSDÓTTIR, Ó. D. B., EMAUS, P. A., ANDERS, T., LEMMENS, S. C. A., RYDLAND, R. & MAGNE, T. (2016). Effects of lump fish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. Aquaculture 465, 19–27

54. TREASURER, J. W. (1996) Wrasse (Labridae) as cleaner-fish of sea-lice on farmed Atlantic salmon in west Scotland. In Wrasse: Biology and Use in Aquaculture Eds M. D. J. Sayer, J. W. Treasurer & M. J. Costello. Fishing News Books Ltd. pp 185–203

55. IMSLAND, A. K., REYNOLDS, P., ELIASSEN, G., HANGSTAD, T. A., NYTRØ, A. V., FOSS, A., VIKINGSTAD, E. & ELVEGÅRD, T. A. (2014a) Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433: 137–142

56. ELIASEN, K. (2017) International perspectives: the Faroe Islands. International Cleaner Fish Summit, Glasgow, Scotland, May 8 to 10, 2017

57. TREASURER, J. W. (2013) Use of wrasse in sea lice control. SARF068. Scottish Aquaculture Research Forum. 31 p

58. IMSLAND, A. K., REYNOLDS, P., ELIASSEN, G., HANGSTAD, T. A., NYTRØ, A. V., FOSS, A., VIKINGSTAD, E. & ELVEGÅRD, T. A. (2015a) Assessment of suitable substrates for lumpfish in sea pens. Aquaculture International 23, 639–645

59. LECLERCQ, E., ZERAFA, B., BROOKER, A. J., DAVIE, A. & MIGAUD, H. (2018) Application of passive-acoustic telemetry to explore the behaviour of ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) in commercial Scottish salmon sea-pens. Aquaculture 495, 1–12

60. RABADAN, C. G. (2018) Improving the use of wrasse (Labridae) stocked in commercial sea cages with Atlantic salmon (*Salmo salar*) for sea lice control: Key features for a successful deployment. In Cleaner Fish Biology and Aquaculture Applications. Ed J. Treasurer. 5m Publishing. pp. 62–77

61. COSTELLO, M. J. (1991) Review of the biology of wrasse (Labridae: Pisces) in Northern Europe. Progress in Underwater Science 16, 29–51

62. KENNEDY, J., JÓNSSON, S. Þ., ÓLAFSSON, H. G. & KASPER, J. M. (2016) Observations of vertical movements and depth distribution of migrating female lumpfish (*Cyclopterus lumpus*) in Iceland from data storage tags and trawl surveys. ICES Journal of Marine Science 73, 1160–1169

63. LECLERCQ, E., GRAHAM, P. & MIGAUD, H. (2015) Development of a water-stable agar-based diet for the supplementary feeding of cleaner fish ballan wrasse (*Labrus bergylta*) deployed within commercial Atlantic salmon (*Salmon salar*) net-pens. Animal Feed Science and Technology 208, 98–106

64. IMSLAND, A. K., REYNOLDS, P., HANGSTAD, T. A., JÓNSDÓTTIR, Ó. D. B., NOBLE, T., WILSON, M., MACKIE, J. A., ELVEGÅRD, T. A., URSKOG, T. C. & MIKALSEN, B. (2018) Feeding behaviour and growth of lumpfish (*Cyclopterus lumpus* L.) fed with feed blocks. Aquaculture Research 00, 1–7

65. RODGERS, E. (2017) The development of welfare standards for cleaner fish. International Cleaner Fish Summit. Glasgow, UK, May 8 to 10, 2017

66. MARTINS, C. I. M., GALHARDO, L., NOBLE, C., DAMSGÅRD, B., SPEDICATO, M. T., ZUPA, W., BEAUCHAUD, M., KULCZYKOWSKA, E., MASSABUAU, J. C., CARTER, T., PLANELLAS, S. R. & KRISTIANSEN, T. (2012) Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry 38, 17–41

67. FULTON, T. W. (1904) The Rate of Growth of Fishes. 22nd Annual Report of the Fisheries Board of Scotland. pp 141–241

68. NASH, R. D. M., VALENCIA, A. H. & GEFFEN, A. J. (2006) The origin of Fulton's condition factor – setting the record straight. Fisheries 31, 236–238

69. COULL, K. A., JERMYN, A. S., NEWTON, A. W., HENDERSON, G. I. & HALL, W. B. (1989) Length/weight relationships for 88 species of fish encountered in the North East Atlantic. Department of Agriculture and Fisheries for Scotland. Aberdeen. p. 81

70. TURNBULL, J. F., RICHARDS, R. H. & ROBERTSON, D. A. (1996) Gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon, *Salmo salar* L., parr. Journal of Fish Diseases 19, 415–427

71. RODRIGUEZ, N. & GIFFORD, K. (2012) Development of Ballan wrasse (*Labrus bergylta*) fin key. Aquaculture Development note, NAFC Marine Centre. 3 pp

72. LECLERCQ, E., DAVIE, A. & MIGAUD, H. (2014c) The physiological response of farmed ballan wrasse (*Labrus bergylta*) exposed to an acute stressor. Aquaculture 434, 1–4

73. BOLGER, T. & CONNOLLY, P. (1989) The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology 34, 171–182

74. DAWKINS, M. S. (2008) The science of animal suffering. Ethology 114, 937–945

75. BÉGOUT, M. L., KADRI, S., HUNTINGFORD, F. & DAMSGARD, B. (2012) Tools for studying the behaviour of farmed fish. In Aquaculture and Behaviour. Ed F. Huntingford, M. Jobling & S. Kadri. Wiley-Blackwell. pp 65–86

76. CORNWALL GOOD SEAFOOD GUIDE (2018) Ballan Wrasse www.cornwallgoodseafoodguide.org.uk/fish-guide/ballan-wrasse.php. Accessed March 06, 2018

77. THORDARSON, G. (2013) Export value of lumpfish products from Iceland increase around 300 million IKR. http://www.coastalfisheries.net/wp-content/uploads/2013/06/Export-value-of-lumpfish-products-from-Iceland-increase-around-300-million-IKR.pdf. Accessed March 12, 2018

78. NØSTVOLD, B. (2017) Post-use. International Cleaner Fish Summit. Glasgow, UK, 8-10 May 2017

79. MUDGE, S. M. & DAVENPORT, J. (1986) Serum pigmentation in *Cyclopterus lumpus* L. Journal of Fish Biology 29, 737–745.

80. CLARK, W., LECLERCQ, E., MIGAUD, H., NAIRN, J. & DAVIE, A. (2016) Isolation, identification and characterisation of ballan wrasse *Labrus bergylta* plasma pigment. Journal of Fish Biology 89, 2070–2084.

81. JONASSEN, T. M., REMEN, M., HOFOSSETER, S. A. & KUNICKIENE, E. (2017) Live transport of lumpfish – what is best practice? International Cleaner Fish Summit. Glasgow, UK, 8-10 May 2017

82. KELLY, N. I., ALZAID, A., NASH, G. W., GAMPERL, A. K. (2014) Metabolic depression in cunner (*Tautogolabrus adspersus*) is influenced by ontogeny, and enhances thermal tolerance. PLoS ONE 9: e114765

83. TREASURER, J. W. (2002) A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Management Science 58, 546–558

84. RUMMER, J. L., BENNETT, W. A. (2005) Physiological effects of swim bladder overexpansion and catastrophic decompression on red snapper. Transactions of the American Fisheries Society 134, 1457–1470

85. CARLSON, T. J. (2012) Barotrauma in fish and barotrauma metrics. The Effects of Noise on Aquatic Life. Eds A. N. Popper & A. Hawkins. Springer. pp 229–234

86. DAVENPORT, J. & KJØRSVIK, E. (1986). Buoyancy in the lumpsucker, *Cyclopterus lumpus*. Journal of the Marine Biological Association of the UK 66, 159–174

87. BIERING, E., VAAGNES, Ø., KROSSØY, B., GULLA, S. & COLQUHOUN, J. D. (2016) Challenge models for atypical *Aeromonas salmonicida* and *Vibrio anguillarum* in farmed Ballan wrasse (*Labrus bergylta*) and preliminary testing of a trial vaccine against atypical *Aeromonas salmonicida*. Journal of Fish Diseases 39, 1257–1261

88. GULLA, S., LUND, V., KRISTOFFERSEN, A. B., SØRUM, H. & COLQUHOUN, J. D. (2015a) vapA (A-layer) typing differentiates *Aeromonas salmonicida* subspecies and

identifies a number of previously undescribed subtypes. Journal of Fish Diseases 39, 329–342

89. BORNØ, G. & GULLA, S. (2017) The Health Situation in Norwegian Aquaculture 2016. The Norwegian Veterinary Institute. 117 pp

90. NILSEN, A., VILJUGREIM, H., RØSAÆG, M. V. & COLQUHOUN, D. (2014) Rensefiskhelse – Kartlegging av Dødelighet og Dødelighetsårsaker (Cleaner Fish Health - A Survey of Mortalities and Causes of Death). Norwegian Veterinary Institute. 84 pp [in Norwegian]

91. GULLA, S., SØRUM, H., VÅGNES, Ø. & COLQUHOUN, J. D. (2015b) Phylogenetic analysis and serotyping of *Vibrio splendidus*-related bacteria isolated from salmon farm cleaner fish. Diseases of Aquatic Organisms 117, 121–131

92. BIRKBECK, T. H. & TREASURER, J. W. (2014) *Vibrio splendidus*, *Vibrio ichthyoenteri* and *Vibrio pacinii* isolated from the digestive tract microflora of larval ballan wrasse, *Labrus bergylta* Ascanius, and goldsinny wrasse, *Ctenolabrus rupestris* (L.). Journal of Fish Diseases 37, 69–74

93. MARCOS-LÓPEZ, M., DONALD, K., STAGG, H. & MCCARTHY, U. (2013) Clinical *Vibrio anguillarum* infection in lumpsucker *Cyclopterus lumpus* in Scotland. The Veterinary Record 173, 319

94. BREILAND, M. S. W., MIKALSEN, H. & JOHANSEN, L. -H. (2014) Susceptibility of Lumpfish (*Cyclopterus lumpus*) to *Vibrio ordalii* and infectious pancreatic necrosis virus (IPNV). Aquaculture Europe 2014, Donostia–San Sebastián, Spain, October 14 to 17, 2014.

95. BREILAND, M. S. W., JOHANSEN, L. –H., MIKALSEN, H., HANSEN, Ø., DRANGSHOLT, T. & MORTENSEN, A. (2015) Susceptibility of different lumpfish (*Cyclopterus lumpus*) families to *Vibrio ordalii* infection. 17th International Conference on

Diseases of Fish and Shellfish, Las Palmas de Gran Canaria, Spain, September 7 to 11, 2015. P 111

96. EINARSDOTTIR, T., BENEDIKTSDOTTIR, E., RAU, J. & HJARTARDOTTIR, S. (2015) *Moritella viscosa* in Icelandic lumpfish. 17th International Conference on Diseases of Fish and Shellfish, Las Palmas de Gran Canaria, Spain, September 7 to 11, 2015. p 69

97. HJELTNES, B., WALDE, C., BANG JENSEN, B. & HAUKAAS, A. (2016) Fish Health Report 2015. The Norwegian Veterinary Institute. pp 1–76

98. GULLA, S., DUODU, S., NILSEN, A., FOSSEN, I. & COLQUHOUN, J. D. (2016) *Aeromonas salmonicida* Infection levels in pre- and post-stocked cleaner fish assessed by culture and an amended qPCR Assay. Journal of Fish Diseases 39, 867–77

99. ALARCÓN, M., GULLA, S., RØSAEG, V. M., RØNNESETH, A., WERGELAND, H., POPPE, T., NILSEN, H. & COLQUHOUN, J. D. (2016a) Pasteurellosis in Lumpsucker *Cyclopterus lumpus*, Farmed in Norway. Journal of Fish Diseases 39, 489–95

100. SMÅGE, B. S., FRISCH, K., BREVIK, J. Ø., WATANABE, K. & NYLUND, A. (2016) First isolation, identification and characterisation of *Tenacibaculum maritimum* in Norway, isolated from diseased farmed sea lice cleaner fish *Cyclopterus Lumpus* L. Aquaculture 464, 178–184

101. MARCOS-LÓPEZ, M., RUANE, M. N., SCHOLZ, F., BOLTON-WARBERG, M., O'MITCHELL, S., O'SULLIVAN, S. M., MOORE, I. A. & RODGER, H. D. (2017) *Piscirickettsia salmonis* infection in cultured lumpfish (*Cyclopterus lumpus* L.). Journal of Fish Diseases 40, 1625–1634

102. STEIGEN, A., KARLSBAKK, E. & PLARRE, H. (2015) A new intracellular bacterium, *Candidatus similichlamydia* labri sp. nov. (Chlamydiaceae) producing epitheliocysts in ballan wrasse, *Labrus bergylta* (Pisces, Labridae). Archive of Microbiology 197, 311–318

103. HAUGLAND, T. G., OLSEN, B. A., RØNNESETH, A. & ANDERSEN, L. (2017) Lumpfish (*Cyclopterus lumpus* L.) develop amoebic gill disease (AGD) after experimental challenge with *Paramoeba perurans* and can transfer amoebae to Atlantic salmon (*Salmo salar* L.). Aquaculture 478, 48–55

104. KARLSBAKK, E., OLSEN, B. A., EINEN, B. C. A., MO, A. T., FIKSDAL, U. I., AASE, H., KALGRAFF, C., SKÅR, Å. S. & HANSEN, H. (2013) Amoebic Gill Disease due to *Paramoeba perurans* in Ballan wrasse (*Labrus bergylta*). Aquaculture 412–413, 41–44

105. FREEMAN, M., KASPER, J. M. & KRISTMUNDSSON, Á. (2013) *Nucleospora cyclopteri* n. sp., an intranuclear microsporidian infecting wild lumpfish, *Cyclopterus lumpus* L., in Icelandic waters. Parasites & Vectors 6, 49

106. ALARCÓN, M., THOEN, T. E., POPPE, T., BORNØ, G., MOHAMMAD, N. S. & HANSEN, H. (2016b) Co-Infection of *Nucleospora cyclopteri* (Microsporidia) and *Kudoa islandica* (Myxozoa) in Farmed Lumpfish, *Cyclopterus lumpus* L., in Norway: A case report. Journal of Fish Diseases 39, 411–18

107. SCHOLZ, F., FRINGUELLI, E., BOLTON-WARBERG, M., MARCOS-LÓPEZ, M., MITCHELL, S., PRODHOL, P., MOFFET, D., SAVAGE, P., O'SULLIVAN, S. M., O'CONNOR, I., MCCARTHY, E. & RODGER, D. H. (2017) First record of *Tetramicra brevifilum* in Lumpfish (*Cyclopterus lumpus, L*.). Journal of Fish Diseases 40, 757–771

108. HALL, M. L., SMITH, J. R., MUNRO, S. E., MATEJUSOVA, I., ALLAN, E. T. C., MURRAY, G. A., DUGUID, J. S., SALAMA, G. K., MCBEATH, A. J. A., WALLACE, S. I., BAIN, N., MARCOS-LÓPEZ, M. & RAYNARD, S. R. (2013) Epidemiology and control of an outbreak of viral haemorrhagic septicaemia in wrasse around Shetland commencing 2012. Scottish Marine and Freshwater Science 4, 1–45

109. MUNRO, E. S., MCINTOSH, E. R., WEIR, J. S., NOGUERA, A. P., SANDILANDS, M. J., MATEJUSOVA, I., MAYES, S. A. & SMITH, R. (2015) A mortality event in wrasse

species (Labridae) associated with the presence of viral haemorrhagic septicaemia virus. Journal of Fish Diseases 38, 335–341

110. WALLACE, I., DONALD, K. S., MUNRO, A. L., MURRAY, W., PERT, C. C., STAGG, H., HALL, M. & BAIN, N. (2015) A survey of wild marine fish identifies a potential origin of an outbreak of viral haemorrhagic septicaemia in wrasse, Labridae, used as cleaner fish on marine Atlantic salmon, *Salmo salar* L., farms. Journal of Fish Diseases 38, 515–521

111. TOWERS, L. (2015) Viral Hemorrhagic septicemia reported for first time in Icelandic fish. https://thefishsite.com/articles/viral-hemorrhagic-septicemia-reported-for-first-time-in-icelandic-fish. Accessed September 15, 2017

112. SCHOLZ, F., RUANE, M. N., MORRISSEY, T., MARCOS-LÓPEZ, M., MITCHELL, S., O'CONNOR, I, MIRIMIN, L., MACCARTHY, E. & RODGER, D. H. (2018) Piscine myocarditis virus detected in corkwing wrasse (*Symphodus melops*) and ballan wrasse (*Labrus bergylta*). Journal of Fish Diseases 41, 147–152

112. FREDRIK, N., JOHANSEN, R., BRUDAL, E. & NYLUND, S. (2017) Lumpfish Flavivirus – Kunnskap og Utfordringer (Lumpfish Flavivirus – Knowledge and Challenges). http://www.intrafish.no/fou/1331923/lumpfish-flavivirus-kunnskap-og-utfordringer. Accessed September 15, 2017 [in Norwegian]

114. BORNØ, G., ALARCÓN, M., LINAKER, L. M., COLQUHOUN, J. D., NILSEN, H., GU, J., GJERSET, B., HANSEN, H., THOEN, E., GULLA, S. & JENSEN, B. B. (2016) Akutt Dødelighet Hos Rognkjeks (*Cyclopterus lumpus*) (Acute mortality in lumpfish) I 2015. Report 2. Norwegian Veterinary Institute. 45 pp [in Norwegian]

115. WENNBERG, A. C. & POWELL, M. D. (2015) Disinfection of *Paramoebae perurans* with UV and ozone. In situ dose-response testing. NIVA-rapport: 6909. Norwegian Institute for Water Research. 18 pp

116. TREASURER, J. & FELEDI, T. (2014) The Physical Condition and Welfare of Five Species of Wild-Caught Wrasse Stocked under Aquaculture Conditions and When Stocked in Atlantic Salmon, *Salmo salar*, Production Cages. Journal of the World Aquaculture Society 45, 213–19

117. PARSONS, H., NOWAK, B., FISK, D. & POWELL, M. (2001) Effectiveness of commercial freshwater bathing as a treatment against amoebic gill disease in Atlantic Salmon. Aquaculture 195, 205–10

118. PERRY, B. & TREASURER, J. W. (2015) An overview of cleanerfish production at Ardtoe. Proceedings of the Cleanerfish Workshop. Inverness, December 1, 2015

119. RAMÍREZ-PAREDES, J. G., VERNER-JEFFREYS, D. W., RIMMER, G., SMITH, L., WALLIS, S. T., COCKERILL, D., PAPADOPOULOU, A. & ADAMS, A. (2017) Efficacy testing of a polyvalent autogenous vaccine against atypical furunculosis and vibriosis in Scottish ballan wrasse (*Labrus bergylta*). 18th International Conference on Diseases of Fish and Shellfish. Belfast, UK, September 4 to 8, 2017

120. RØNNESETH, A., HAUGLAND, T. G., COLQUHOUN, J. D., WERGELAND, I. H. & BRUDAL, E. (2017) Protection and antibody reactivity following vaccination of Lumpfish (*Cyclopterus lumpus* L.) against atypical *Aeromonas salmonicida*. Fish and Shellfish Immunology 64, 383–91

121. SOMMERSET, I., KROSSØY, B., BIERING, E. & FROST, P. (2005) Vaccines for fish in aquaculture. Expert Review of Vaccines 4, 89–101

122. BERIT, A., JORUN, L., MIDTLYNG, P., GRAVNINGEN, K., EMILSEN, V. & EVENSEN, Ø. (2014) Comparision of a serological potency assay for furunculosis vaccines (*Aeromonas salmonicida* subsp. *salmonicida*) to intraperitoneal challenge in Atlantic salmon (*Salmo salar* L.). Biologicals 42, 86–90

123. EVENSEN, Ø. (2016) Development of fish vaccines: Focusing on methods. In Fish Vaccines. Ed A. Adams. Springer. pp 55–56

124. OLDHAM, T., RODGER, H. & NOWAK, F. B. (2016) Incidence and distribution of amoebic gill disease (AGD) - an epidemiological review. Aquaculture 457, 35–42