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

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## **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<sup>1,2</sup>, 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.<sup>3</sup> In terms of the production losses, Abolofia et al.<sup>4</sup> 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.<sup>5</sup>). 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<sup>6, 7</sup>, 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<sup>8</sup> and enhanced delousing performance.<sup>9,10</sup> 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.<sup>11</sup> 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)<sup>12</sup>, ballan wrasse (reviewed in Davie et al.<sup>13</sup> & Lekva & Grotan<sup>14</sup>) and lumpfish (*Cyclopterus lumpus*) (reviewed in Powell et al.<sup>15</sup>). Despite farming being in its infancy with the first farmed ballan wrasse deployed in salmon pens in 2013 in Scotland<sup>16</sup>, 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.<sup>11</sup> 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.<sup>17</sup> 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.

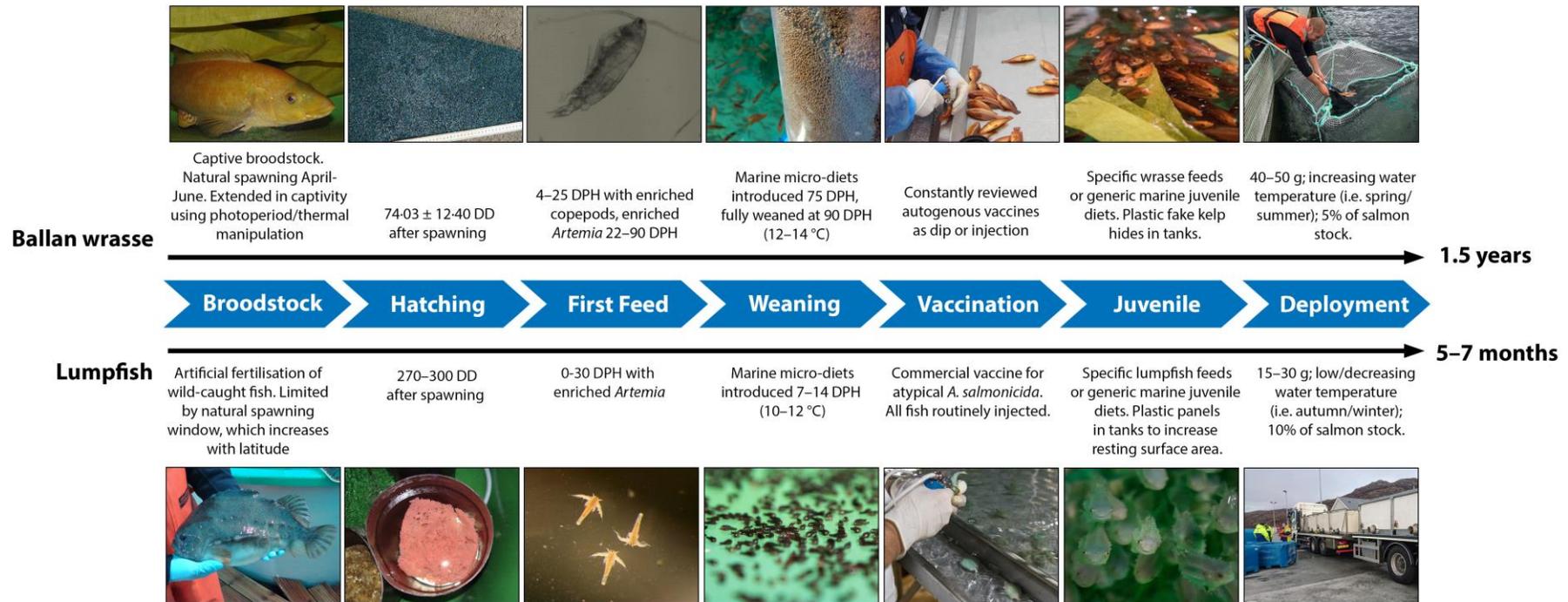
Ballan wrasse are protogynous hermaphrodites<sup>18,19</sup> with a complex hierarchy and a highly skewed sex ratio, which makes broodstock management challenging.<sup>20</sup> The spawning window is naturally in April–June<sup>21</sup> but this can be extended in captivity using environmental manipulation.<sup>13</sup> Due to their long generation time (reaching puberty at ~6 years for females and 12 years for males<sup>19</sup>), 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<sup>22</sup>, 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.<sup>23</sup> 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.<sup>24</sup> 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<sup>25</sup>, although quality is generally poorer than in egg masses produced using artificial fertilisation<sup>15</sup>. Given the well-established supply chains for mature adults from caviar fisheries<sup>26</sup>, 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<sup>27</sup>) 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<sup>15</sup>, 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.

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

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.<sup>45,46,47</sup> 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<sup>48,49</sup>, 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.<sup>45</sup> Furthermore, Skiftesvik et al.<sup>50</sup> found that farmed wrasse were as effective as wild wrasse at delousing in cage studies. Imsland et al.<sup>51</sup> 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.<sup>52</sup>

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)<sup>53</sup>, 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.<sup>45</sup>

Stocking rates are generally higher for lumpfish than for ballan wrasse. Treasurer<sup>54</sup>, Skiftesvik et al.<sup>50</sup> and Leclercq et al.<sup>45</sup> recommended a stocking ratio of 5% wrasse:salmon, whereas Imsland et al.<sup>47,51,55</sup> 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.<sup>9,50</sup> These practices are considered less important for lumpfish, which may benefit from the alternative food sources<sup>46</sup>, and indeed, the Faroese aquaculture industry promotes net biofouling to reduce the effects of strong coastal currents in the net pens.<sup>56</sup>

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<sup>57</sup>, and below 6°C they enter into a state of torpor<sup>30</sup> (Table 2). In contrast, lumpfish continue to feed at 4°C<sup>27</sup>, 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.<sup>30,58,59</sup> 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<sup>59</sup> (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.<sup>60</sup> In the wild, ballan wrasse inhabit coastal reefs, preferring the cover of rocks or kelp<sup>19</sup>, and tend to be territorial with relatively small home ranges and limited migrations.<sup>31</sup> They are diurnally active and nocturnally quiescent, sheltering overnight in rocky crevices.<sup>30,31,61</sup> Wild lumpfish have an offshore, semi-pelagic lifestyle and are often associated with floating seaweed.<sup>32,40,62</sup>

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.<sup>59</sup> Imsland et al.<sup>55</sup> 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.<sup>58</sup> 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.<sup>58</sup> 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<sup>9,46,47</sup>, supplementary feeding is essential to maintain the condition and welfare of the cleaner fish.<sup>50,63</sup> 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.<sup>60</sup> 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.<sup>63</sup> The use of this water-stable feed is becoming more widespread and has also been trialled for lumpfish.<sup>64</sup>

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.<sup>60</sup>

### 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<sup>65</sup>) 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.<sup>66</sup> 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<sup>67,68</sup> 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<sup>55</sup>) 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<sup>69</sup> (A. Davie, unpublished data).

In many fish species, fin damage can be a result of aggression and a sign of stress<sup>70</sup>, and these injuries can be a portal for bacterial and fungal infections. Fin damage indices have been developed for both wrasse<sup>71</sup> 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.<sup>72</sup> Other physiological parameters could be used (e.g. hepatosomatic index<sup>73</sup> or liver-colour scoring index<sup>56</sup>), although they require sacrificial sampling. Behaviour can also be used to assess animal welfare.<sup>74,75</sup> 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.<sup>59</sup>

#### *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<sup>53</sup> 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<sup>76</sup>); 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.<sup>77</sup> However, further research is required to develop this market, and public perception may be an issue due to its unusual appearance.<sup>78</sup> A further market opportunity for lumpfish could be the production of lumpfish roe from mature captive females as a sustainable alternative to wild fisheries<sup>26</sup>, 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<sup>79</sup> 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<sup>80</sup>, 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).

	<b>Ballan wrasse</b>	<b>Lumpfish</b>
<b>Deployment window</b>	Spring/summer with increasing water temperature.	Late autumn/winter with decreasing water temperature.
<b>Transportation</b>	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. <sup>81</sup>
<b>Deployment size</b>	At least 40–50g to prevent escape through net mesh. <sup>54</sup> Larger wrasse may be more effective delousers. <sup>45</sup>	Typically 15–30 g <sup>81</sup> (R. Hawkins, Marine Harvest Scotland, pers. comm., 2017). Ineffective delousers when mature (400–500g, 14–16 months <sup>55</sup> ).
<b>Stocking rate</b>	5% of salmon number. <sup>45,50,54</sup>	10% of salmon number. <sup>47,51,55</sup>
<b>Use of hides and substrates</b>	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.
<b>Feeding behaviour</b>	Will not feed below 6°C, winter dormancy. <sup>57,82,83</sup>	Will feed as low as 4°C. <sup>27</sup>
<b>Swimming Activity</b>	Slower than lumpfish, prefers edges and corners. <sup>59</sup>	Higher activity rates than ballan wrasse. Covers whole pen area. <sup>59</sup> Active foraging during day, aggregate on smooth surfaces at night. <sup>58</sup>
<b>Buoyancy</b>	Physoclistic; rapid pressure changes should be avoided. <sup>84,85</sup> 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. <sup>86</sup>
<b>Recapture</b>	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).<sup>40</sup>

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.<sup>87</sup> 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.<sup>88,89</sup> 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.<sup>87</sup> 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

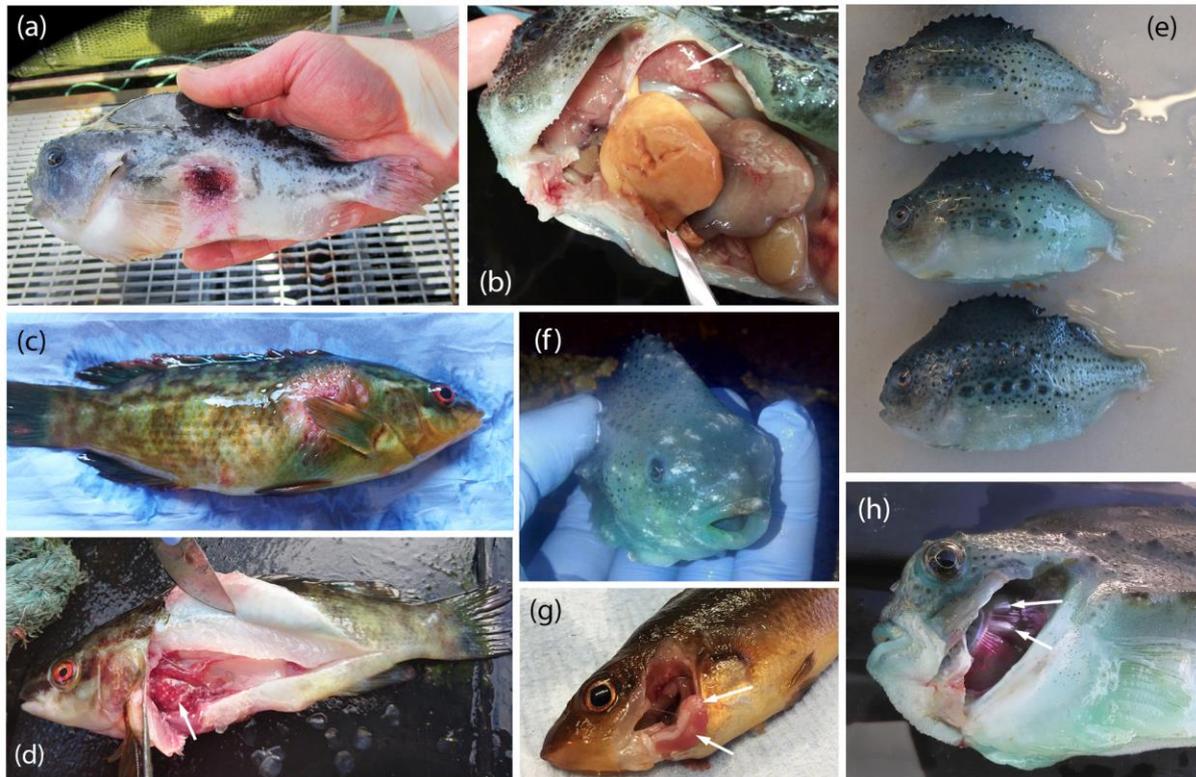
from wild ballan wrasse to date, with up to 60% mortality in 50g fish injected with the bacterium.<sup>90,91</sup> Other *Vibrio* spp. have been isolated from cleaner fish but their pathogenicity is unclear.<sup>89</sup> Birkbeck and Treasurer<sup>92</sup> demonstrated that *V. splendidus* and *V. ichthyenteri* 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.<sup>93,94,95,96,97,98,99,100,101</sup> 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<sup>99</sup> (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.<sup>100</sup>

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.<sup>102</sup>

### 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.<sup>103</sup> Primary histopathology shows pale patches at the bases of gill filaments, hyperplasia of epithelial cells and fusion of gill lamellae<sup>103,104</sup> (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.<sup>105</sup> 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*.<sup>106</sup> 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 microsporidian xenomas were observed in the skin, internal organs, gills and eyes.<sup>107</sup>

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.<sup>15</sup>

### 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.<sup>108,109,110</sup> It has not been found in farmed ballan wrasse, but was isolated in Icelandic lumpfish for the first time in 2015.<sup>111</sup> Interestingly, lumpfish infected with VHS were more susceptible to the Infectious Pancreatic Necrosis (IPN) virus (another notifiable disease) during experimental infections.<sup>94</sup>

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).<sup>112</sup> 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.<sup>113</sup> 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.<sup>113</sup>

#### *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.<sup>15</sup> 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.<sup>90</sup> 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.<sup>114</sup>

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.<sup>115</sup>

### *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.<sup>116</sup> Good husbandry, such as the provision of hides, clean nets and supplementary feeding<sup>63</sup>, 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<sup>117</sup>; the treatment is effective for lumpfish and is used where possible<sup>118</sup>, 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<sub>50</sub> and LD<sub>60</sub>.<sup>119</sup> 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.<sup>120</sup>

### 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.<sup>121,122,123</sup> 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<sup>124</sup>, ballan wrasse<sup>104</sup> and lumpfish<sup>103</sup>, 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.<sup>103</sup> 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.<sup>103</sup>

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.<sup>1</sup>

VHS has caused high mortalities in both wild wrasse<sup>109</sup> and lumpfish.<sup>111</sup> 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.<sup>112</sup> Lumpfish are carriers of *Piscirickettsia salmonis*, and it is present at most sites where they are deployed<sup>101</sup>, 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.

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