

RENSVEL OWI FACT SHEET SERIES:
AN INTRODUCTION TO OPERATIONAL AND
LABORATORY-BASED WELFARE INDICATORS
FOR LUMPFISH (*Cyclopterus lumpus* L.)



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INTRODUCTION

Fish welfare, defined by Stien et al., (2013) as the “*quality of life as perceived by the animals themselves*” requires various tools to assess and evaluate it (see e.g. Noble et al., 2018). These tools are termed Welfare Indicators (WIs) and can be classified as either animal or environment based (e.g. Nilsson, Stien, Iversen et al., 2018):

- Animal-based (Direct) indicators – an indicator that is on, or from the fish, applied at either the individual or group level.
- Environment-based (Indirect) indicators – an indicator that is based upon the rearing environment e.g. water quality, various management processes or the farm infrastructure.

WIs that can be used out on the farm are termed Operational Welfare Indicators, or OWIs (e.g. Noble et al., 2012). WIs that are sampled at the farm (either from the animal or the environment it is subjected to) but are then sent to a lab for further analysis are termed Laboratory-based Welfare Indicators, or LABWIs (Nilsson, Stien, Iversen et al., 2018).

Health and welfare are key to the successful rearing and deployment of cleaner fish out on the farms. However, knowledge on their welfare is relatively scarce in relation to other more established farmed species, although attempts are being made to correlate what is available and give an overview of the potential welfare challenges the fish face (e.g. Treasurer et al., 2018).

This fact sheet series will give a brief overview of some of the latest science-based findings and some practical experience with regard to a suite of life-stage and species-specific OWIs and LABWIs for lumpfish (*Cyclopterus lumpus* L.). For each OWI/LABWI we will i) briefly outline the indicator, ii) give a science-based overview of the information we have about it, such as potential risk factors and mitigation strategies that can be linked to it, and either iii) give an overview of some practical knowledge related to the OWI, or iv) address the methods for measuring the OWI/LABWI.

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OUTLINE OF THE OWIs AND LABWIs COVERED IN THIS FACT SHEET SERIES

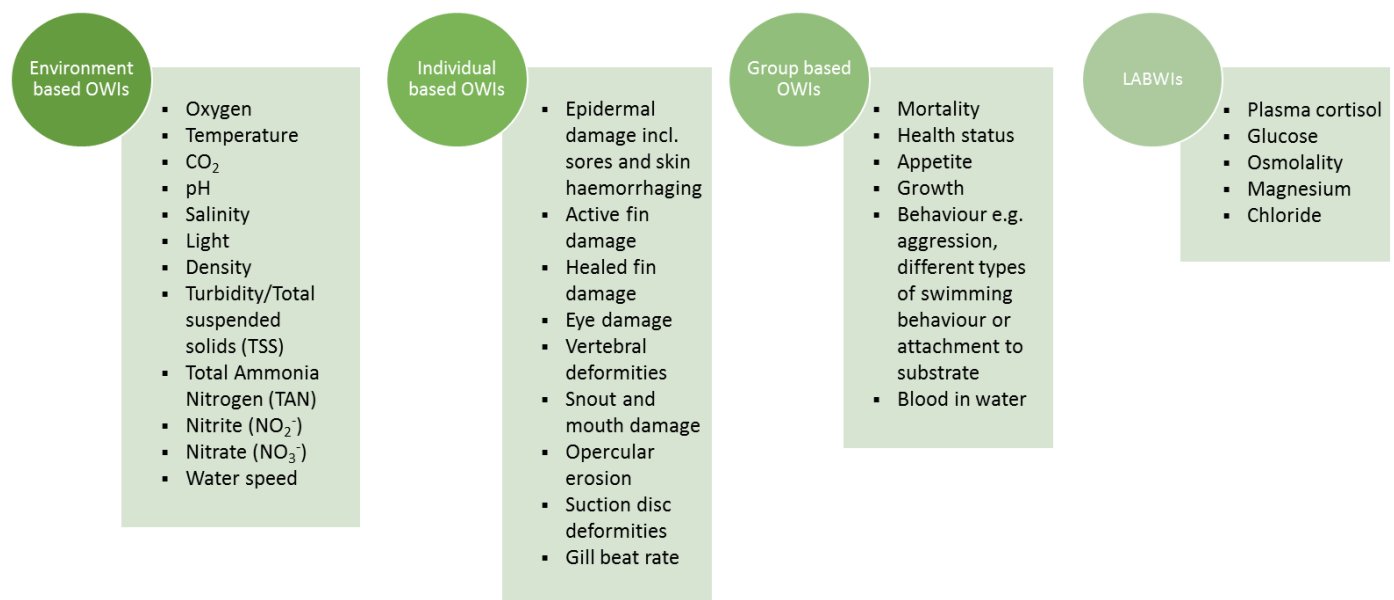


Figure 1: Overview of the OWIs and LABWIs addressed in the RENSVEL OWIs for lumpfish fact sheet series (Figure: Chris Noble, Nofima. Adapted from figures in “Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare 351pp.” with permission.

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ENVIRONMENT BASED OWIs

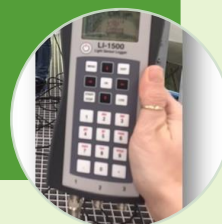


Environment based OWIs are well established indirect welfare indicators for numerous farmed species (Noble et al., 2018; RSPCA 2018a, b; Stien et al., 2013) including cleaner fish (Treasurer et al., 2018). However, there is little published information on environment based OWIs for lumpfish (Jørgensen et al., 2017; Treasurer et al., 2018).

Key Environment based OWIs covered in these Factsheets:

- Oxygen
- Temperature
- CO₂
- pH
- Salinity
- Light
- Density
- Turbidity/Total suspended solids (TSS)
- Total Ammonia Nitrogen (TAN)
- Nitrite (NO₂⁻)
- Nitrate(NO₃⁻)
- Water speed

Environment
based OWIs



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Oxygen



Oxygen is a critical water quality indicator and levels that are too low can cause welfare problems in numerous fish species including lumpfish (e.g. Jørgensen et al., 2017). Different life stages can also have differing oxygen requirements.

Science based knowledge

- In an experiment where juvenile lumpfish were subjected to Dissolved Oxygen (DO) saturations of 55%, 69%, 81% and 96%, growth was negatively affected at all DO saturations \leq 81% in comparison to fish held at 96% DO (Jørgensen et al., 2017).
 - DO levels of 55% and 69% also had a detrimental impact upon plasma cortisol levels in comparison to the higher DO saturations.
 - Appetite was also reduced in fish subjected to DO saturations of 55% and 69%.
 - If fish are exposed to hypoxic conditions they can respond by hyperventilating (Perry et al., 2009). Fish exposed to 55% and 69% DO increased their gill beat rate/min by 71% and 60% in comparison to fish held at 96% DO after 1 month exposure to those oxygen levels.
- DO saturation levels and recommendations for differing life stages:
 - Juveniles:
 - $>80\%$ DO saturation (Jørgensen et al., 2017).
 - 80-90% DO saturation (Treasurer et al., 2018).
 - > 7 mg/l (RSPCA, 2018a).
 - Adults and broodstock:
 - 80-90% DO saturation (Treasurer et al., 2018).
 - > 7 mg/l (RSPCA, 2018a).
- DO saturation levels should be kept at ca. 100% during transport (Jonassen et al., 2018a).
- Increasing DO levels from 100% to 150% during simulated transport studies (8 h transports) did not affect the stress response of the lumpfish (Remen and Jonassen, 2017).

Practical based knowledge

Practical DO saturation levels and recommendations:

- $>90\%$ DO saturation levels during start feeding in RAS (Johannesen et al., 2018a).
- 90-100% saturation during transport (Jonassen et al., 2018a).

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Temperature

Temperature is a key environmental parameter and affects poikilothermic fish in numerous ways (e.g. Jobling, 1997; EFSA, 2008). Temperature preferences and thresholds vary with life stage in lumpfish.



Science based knowledge

Lumpfish are a eurythermal fish that can be found across a wide temperature range, tolerating very low temperatures 0-20 degs (Powell et al., 2018a).

- Juveniles exhibit high ontogenetic variability in their optimal temperature (Jonassen et al., 2018b) and optimum temperatures for marine fish usually decrease as the fish get bigger (e.g. Jobling, 1997).
- Rapid growth during the juvenile phase (which may in part be due to higher temperatures) may be a risk factor for increased prevalence of cataracts (Jonassen et al., 2018b).
- Hvas et al., (2018) also reported qualitative evidence that adult lumpfish held at > 15 °C had more cataracts.
- Both low temperatures (< 4 °C, Imsland et al., 2018a) and high temperatures (18 °C, Hvas et al., 2018) can also increase mortalities.

Life stage	Temperature range	Reference
Juvenile	Optimal temperature = ca. 16 °C in 11-40 g fish 9 – 16 °C	Nytrø et al., (2014) Jonassen et al., (2018b)
Adult	Optimal temperature = ca. 13 °C in 100-110 g fish and ca. 9 °C for 120-200 g fish 6 – 10 °C < 15 °C	Nytrø et al., (2014) Treasurer et al., (2018) Hvas et al., (2018)
Broodstock	8 – 10 °C	Treasurer (2018b)

Practical based knowledge

- Practical based experience on water temperature requirements in relation to life stage:
 - Juvenile fish: < 14 °C (Treasurer et al., 2018).
 - Adult lumpfish can feed down to 4 °C and can be more susceptible to disease when temperatures are greater than 10 °C (Brooker et al., 2018).
- When transferring fish from the hatchery to sea cages, transferring fish from medium (8 °C) to lower temperatures (3 °C) led to more problems than transferring fish from medium (8 °C) to higher temperatures (15 °C) (Jonassen et al., 2018a).

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CO₂/pH

Elevated levels of water-borne CO₂ can be a welfare challenge for numerous fish species (see e.g. Nilsson, Stien, Iversen et al., 2018) as can pH levels that are either too low or high. However, as far as the authors are aware, optimal or critical levels for CO₂ have not been reported (see also Jørgensen et al., 2017) and this is also the same for pH (e.g. Powell et al., 2018a).



Science based knowledge

As stated above, the authors have not found any peer-reviewed published information on the optimal or critical levels for CO₂ and pH in lumpfish (see also Jørgensen et al., 2017; Powell et al., 2018a).

- Simulated transport conditions using either 8 h or 20 h transports, DO levels either 100% to 150%, temperatures either 8 °C or 12 °C, 30 or 60 g fish, 30 kg/m³ or 60 kg/m³ led to CO₂ values in the range of ca. 5 - 8.5 mg/l with no obvious negative effects for 30 g lumpfish (Remen and Jonassen, 2017).

Practical based knowledge

- With regard to CO₂, it has been suggested that the CO₂ tolerances of lumpfish are potentially comparable to other marine species (Treasurer et al., 2018).
- With regard to pH, it has also been suggested that the pH tolerances of lumpfish are potentially comparable to other marine species, and production data has reported pH values in the region 7.3-8.2 (Treasurer et al., 2018).
- Practical experience states that some lumpfish hatcheries maintain pH at ca. 8.1 during start feeding in RAS (Johannesen et al., 2018a).

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Salinity

Lumpfish are generally not found in low salinities in the wild, but certain populations are found in less saline waters (Davenport, 1985; Powell et al., 2018a) and lumpfish can tolerate freshwater exposure (Treasurer and Turnbull, 2019).



Science based knowledge

With regard to freshwater exposure:

- Treasurer and Turnbull (2019) have reported that lumpfish of up to 70 g can tolerate 5 h exposure to freshwater (0 ppt) and a 10 day exposure to brackish water (15 ppt) without any discernable negative effects on their welfare, although fish were less active at 0 ppt in some trials.

Practical based knowledge

- Most hatcheries operate with salinities in the range of 30-35 ppt (Jonassen et al., 2018b). There are some experience based observations that salinities below 32 ppt may increase the risk of cataracts (Jonassen et al., 2016).
- Salinity can be held at ca. 33 ppt during start feeding in RAS (Johannesen et al., 2018a).

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Light

The manipulation of daylength and changes in daylength is a widely used technique for e.g. controlling reproduction and initiating maturation in numerous farmed fish species including lumpfish (Imsland et al., 2018b). However, with regard to cleaner fish, the field is still in its infancy. As far as the authors are aware, there have been little in the way of scientific studies on the effects of light intensity or wavelength upon lumpfish welfare and performance. The limited information that is available will be reported here.



Science based knowledge

- As stated above, as far as the authors are aware, there is little in the way of published information regarding optimal light intensity or wavelength for lumpfish in relation to their welfare.
- Skiftesvik et al., (2017) reported that lumpfish lack eye pigments for red light and do not see it, colliding with tank walls and each other. They were very active under green light and swam more calmly under blue light.
- Results from RENSVEL suggest that in general, it seems that the wavelength of light has a greater effect on welfare than light intensity (Espmark et al., 2019). Results suggest that green light (520 nm wavelength) is more stressful (significantly increases plasma cortisol levels) for lumpfish than blue light (420 nm wavelength) and also increases the frequency of fish with cataracts.
- Cleaner fish detect lice by sight and short days or low light intensities during winter may reduce their lice detection abilities and effectiveness (Skiftesvik et al., 2017).
- Photoperiod manipulation has recently been successfully used to control the timing of maturation and spawning in lumpfish (Imsland et al., 2018b; Imsland et al., 2019a).

Practical based knowledge

- During egg incubation the eggs are usually subjected to low intensity 24 h light as practical experience suggests high light intensity has a negative effect upon larvae quality (Jonassen et al., 2018b).
- It has also been suggested that reduced light intensity can reduce aggression in the tank rearing juvenile phase (Jonassen et al., 2018b).
- There is no clear standardised approach to specific photoperiod lengths during the tank rearing phase. Some farmers utilise a 24:0 LD regime, others may utilise an 18:6 LD regime, with others preferring a 12:12 LD regime (Jonassen et al., 2018b).

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Density

Stocking density is a common OWI but its use can be somewhat problematic as its influence depends on other factors such as water quality, life stage, behavioural interactions, feed management etc., (e.g. Turnbull et al., 2008). Its potential welfare effects must therefore be considered in partnership with other OWIs (see Turnbull et al., 2005).



Science based knowledge

- As stated above, it is a challenge to define optimal or undesirable stocking densities, even in well established aquaculture species due to the number of factors that can influence its ultimate effect. However, it is clear that inappropriate densities (e.g. too high) can be detrimental to fish welfare, especially if they have a negative impact upon e.g. water quality, behavioural interactions or feed access.
- Another aspect to consider is that lumpfish can either be sedentary and sit and rest on the substrate or under shelters (Johannesen et al., 2018a) or can also be quite active swimmers, (Davenport, 1985). Suitable substrates for resting on or around should therefore be provided.
- Treasurer et al., (2018) have suggested a life stage specific “surface area index” for ensuring lumpfish have enough area for settlement would be very valuable.
- Stocking juvenile lumpfish at a density of 60 kg/m³ for eight hours during simulated transport did not appear to have a detrimental impact upon their welfare. Increasing density levels from 30 kg/m³ to 60 kg/m³ did increase plasma cortisol after 4 hours but not after 8 hours (Remen and Jonassen, 2017).
- Other stocking density recommendations:
 - < 80 kg/m³ during transport (RSPCA, 2018a).
- RENSVEL results (Espmark et al., 2019) suggest densities of 15, 30 and 60 kg/m³ did not affect the survival, growth or welfare of juvenile lumpfish. However, plasma cortisol levels were lower at low density and caudal fin damage was more prevalent at low density in relatively small fish (20 g).

Practical based knowledge

- Broodstock: during maturation, stocking density should be < 20 kg/m³ without enrichment and > 20 kg/m³ with enrichment (Puvanendran, personal observations reported in Treasurer et al., 2018).
- Boyce et al., (2018) also suggested that density should be 23-43 kg/m³ after fish reach 100 g in body weight.

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Turbidity/TSS

Kolarevic, Stien et al., (2018) state “*Turbidity refers to the clarity of the water and TSS refers to the suspended material in the water and while these two parameters are related they are not always highly correlated*”. Turbidity can be influenced by dissolved and suspended solids and the nature of these solids is critical for determining their effect on the fish. High concentrations of suspended solids can lead to reduced dissolved oxygen saturations due to microbial activity and smaller particles can also potentially affect gill function (Timmons and Ebeling, 2007). However, as far as the authors are aware, optimal or critical levels for turbidity or TSS have not been reported for lumpfish.



Science based knowledge

- As stated above, **the authors have not found any peer-reviewed published information on optimal or critical levels for Turbidity and TSS in relation to lumpfish welfare.**
- *Until this information becomes available, we draw the readers attention to the recommendations for TSS drawn up for Atlantic salmon (Thorarensen and Farrell, 2011):*
 - *The upper limit of TSS for Atlantic salmon salmon has been suggested to be ≤ 15 mg/l (Thorarensen and Farrell, 2011).*
- *TSS values for other aquaculture species have been reported as being between 10 and 80 mg/l and are species specific (Timmons and Ebeling, 2007).*

Practical based knowledge

- Practical experience regarding turbidity/TSS suggest increased turbidity and organic matter in RAS can cause an increase in temperature and a reduction in dissolved oxygen and should be monitored.

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Total ammonia nitrogen (TAN), Nitrite (NO_2^-) and Nitrate (NO_3^-)

Ammonia (NH_3) is a poisonous end product of protein catabolism and referred to as Unionised Ammonia, UIA (Thorarensen and Farrell, 2011) that reacts with water to form ionised ammonium (NH_4^+). The total quantity of NH_3 and NH_4^+ is termed Total Ammonia Nitrogen (TAN). Nitrite (NO_2^-) and nitrate (NO_3^-) can accumulate in water, particularly in cases where water is reused (RAS and/or transport). Nitrite can be toxic for fish, but mainly in freshwater where it competes with chloride uptake in the gills. In seawater the presence of chloride in the water alleviates the adverse effects of nitrite toxicity making it less harmful. Nitrate has been known to be less toxic for some species (for example Atlantic salmon) compared to nitrite. As far as the authors are aware, optimal or critical levels for TAN, nitrite or nitrate have not been reported in cleaner fish.



Science based knowledge

- As stated above, **the authors did not find any peer-reviewed published information on the optimal or critical levels for TAN, nitrite or nitrate in relation to lumpfish welfare.**
- *Until this information becomes available, we draw the readers attention to the conservative recommendations for UIA, nitrite or nitrate that have been drawn up for Atlantic salmon (as reported in Noble et al., 2018):*
 - *Recommendations regarding UIA in Atlantic salmon:*
 - *Short-term exposure (4 hours): ≤ 0.1 mg/l (Wedemeyer, 1996); long-term exposure: ≤ 0.012 mg/l (Fivelstad et al., 1995).*
 - *Recommendations regarding nitrite in Atlantic salmon:*
 - *≤ 0.1 mg/l (Wedemeyer, 1996; Thorarensen and Farrell, 2011).*
 - *Recommendations regarding nitrate in Atlantic salmon:*
 - *< 100 mg/l (Bregnballe, 2010).*

Practical based knowledge

- TAN: 0.004 ± 0.001 mg/l during start feeding of lumpfish in RAS (Johannesen et al., 2018a).
- Nitrite: ca. 0.2 ± 0.1 mg/l during start feeding of lumpfish in RAS (Johannesen et al., 2018a).
- Nitrate: 0.79 ± 0.12 mg/l during start feeding of lumpfish in RAS (Johannesen et al., 2018a).

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Water speed

Water speed is a well established indirect OWI (e.g. Kolarevic, Stien et al., 2018). It affects the swimming behaviour of the fish (Nilsson, Stien, Iversen et al., 2018) and its effects can be beneficial e.g. by exercising the fish (e.g. Kolarevic, Stien et al., 2018). However, currents that are too high can exhaust the fish, and force lumpfish up against the wall of the rearing system (see e.g. Powell et al., 2018a).



Science based knowledge

- Lumpfish can be sedentary and sit and rest on the substrate or under shelters (Johannesen et al., 2018a) but they can also be pelagic, covering long distances in the wild (Davenport, 1985). However, swimming is rather restricted in lumpfish irrespective of life stage (Treasurer et al., 2018) and lumpfish have been described by Hvas et al., (2018) as a typical benthic species.
- Lumpfish possess a ventral cartilaginous sucker/disc that is ca. 20% of the body length and is formed by modified pelvic fins. This disc helps the lumpfish attach themselves to substrates and reduces the risk of them being dislodged in e.g. high current conditions (see Powell et al., 2018a and references therein). Lumpfish that use the suction discs to attach to the substrate can tolerate higher water speeds than fish that are swimming (Hvas et al., 2018).
- Lumpfish have a somewhat limited aerobic scope in comparison to e.g. salmon and their critical swimming speeds can range from 1.3 - 1.7 body lengths/second (bl/s) in 300 g fish (Hvas et al., 2018).
 - According to Hvas et al., (2018):
 - 75 g fish had problems remaining attached to surfaces when water current speeds were 80 – 110 cm/s.
 - 300 g fish had problems remaining attached to surfaces when water current speeds were > 70 cm/s.
 - This suggests larger fish had more problems staying attached to the substrate than smaller fish and Hvas et al., (2018) suggested this was associated with drag.

Practical based knowledge

- Lumpfish have been observed to become exhausted and pushed up against the net in high energy farming conditions (Malthe Hvas, personal communication).

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INDIVIDUAL BASED OWIs

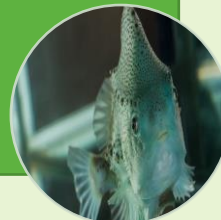
Individual based OWIs including morphological injuries are well established OWIs for numerous farmed species (Noble et al., 2018; RSPCA 2018a, b; Stien et al., 2013) including cleaner fish (Treasurer and Feledi, 2014; Treasurer et al., 2018) and are also applicable at differing lifestages (Treasurer et al., 2018). However, there is very little published information relating to individual based OWIs in lumpfish (Treasurer et al., 2018).



Key Individual based OWIs covered in these Factsheets:

- Sores
- Skin haemorrhaging
- Active fin damage
- Healed fin damage
- Eye damage
- Vertebral deformities
- Snout and mouth damage
- Opercular damage
- Suction disc deformities
- Gill beat rate

Individual
based OWIs



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Epidermal damage

Addresses the damage or loss of epidermal tissue, including sores/ulcers and haemorrhaging. The epidermis is a barrier to infection and contains nociceptors. Any skin damage is a well established risk for fish welfare (Noble et al., 2012).



Science based knowledge

Risk factors for sores and skin haemorrhaging:

- Sores/Ulcers:
 - Health related risk factors: *Moritella viscosa*, *Tenacibaculum* spp. (EURL, 2016; Bornø and Gulla, 2017); *Pseudomonas anguilliseptica* (Rimstad et al., 2017); *Gyrodactylus* sp. (Rimstad et al., 2017); Atypical *Aeromonas salmonicida* (Bornø and Gulla, 2017); *Tetramicra brevifilum* (Scholz et al., 2018); *Pasteurella* spp. (Scholz et al., 2018); *Scuticociliate* spp. (Scholz et al., 2018); *Trichodina* spp. (Scholz et al., 2018); *Nucleospora cyclopteri* (Scholz et al., 2018); *Exophilia* spp. (Scholz et al., 2018); VHSv (Scholz et al., 2018).
- Skin haemorrhaging:
 - Health related risk factors: *Aeromonas salmonicida* (Scholz et al., 2018); *Pseudomonas anguilliseptica* (Scholz et al., 2018); *Moritella viscosa* (Scholz et al., 2018); VHSv (Scholz et al., 2018); *Pasteurella* spp. (Scholz et al., 2018; Ellul et al., 2019); *Vibrio anguillarum* (Scholz et al., 2018).
- Skin discolouration:
 - Health related risk factors: *Aeromonas salmonicida* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); *Trichodina* spp. (Scholz et al., 2018); VHSv (Scholz et al., 2018).
- Epidermal damage has also been observed after transport (Jonassen et al., 2018a) and may be due to physical contact with sharp/hard/rough surfaces (Jonassen et al., 2018a).
- Knotless nets should be used for handling (e.g. Jonassen et al., 2018a).
- Epidermal damage may also be due to net cleaning or other operations (Rimstad et al., 2017).

Practical based knowledge

- Further potential risk factors for sores can be handling at low (< 7 °C) water temperatures (MarinHelse, 2018).

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Active and healed fin damage

Fin damage is a well established welfare issue in lumpfish (Treasurer et al., 2018) as it is damage to live tissue (Ellis et al., 2008) and active fin damage may also be a welfare and health risk due to infection risk via opportunistic pathogens (e.g. Scholz et al., 2018).



Science based knowledge

- Fins susceptible to fin damage include:
 - Caudal, Dorsal, Pectoral, with the caudal fin especially at risk (Treasurer et al., 2018). (Pelvic fin damage is covered in the later suction disc deformity section).
- The drivers for fin damage in lumpfish are unclear (Transport and Handling guidelines, <http://lusedata.no/wp-content/uploads/2012/05/Veileder-for-h%C3%A5ndtering-og-transport-av-rognkjeks-oppdatert-v%C3%A5r-2017.pdf>). However, we do have some clear indication that overt aggression (tail nipping) leads to caudal fin erosion in juvenile fish (Treasurer et al., 2018).
- Caudal fin damage may also emerge as a problem during maturation (Treasurer et al., 2018).
- Density may also be a risk factor for fin damage, as a consequence of potential increased territoriality amongst conspecifics (e.g. suggested in Jonassen et al., 2018b).
- Grading can be a good mitigation strategy for preventing fin damage in juvenile tank rearing before the fish are transferred to sea (Pattillo, undated).
- Active fin damage is also a potential route for opportunistic pathogens such as *Tenacibaculum* spp. (e.g. Jonassen et al., 2018b).
- With regard to health related issues, fin damage can be caused by *Pseudomonas anguilliseptica* (Scholz et al., 2018); *Gyrodactylus* sp. (Rimstad et al., 2017); Atypical *Aeromonas salmonicida* (Bornø and Gulla, 2017); *Pasteurella* spp. (Scholz et al., 2018; Ellul et al., 2019); *Scuticociliate* spp. (Scholz et al., 2018); *Trichodina* spp. (Scholz et al., 2018); *Tenacibaculum* spp. (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); VHSv (Scholz et al., 2018).

Risk factor	Juvenile	Adult	Broodstock	Reference
Aggression	✓	✓		Treasurer et al., 2018
Diseases, stress etc.	✓	✓	✓	Scholz et al., 2018; Sällebrant, 2018
Density	✓		✓	Treasurer et al., 2018; Espmark et al., 2019

Mitigation strategy	Juvenile	Adult	Broodstock	Reference
Environmental enrichment (EE)	✓	✓	✓	Treasurer et al., 2018
Continuous feeding (to reduce aggression)	✓	?	?	Treasurer et al., 2018

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Active and healed fin damage continued

Practical based knowledge

- Further potential risk factors for fin damage: feeding strategy and feed withdrawal?
- Fin damage has also been observed after transport (Jonassen et al., 2018a).
- Potential mitigation factors for fin damage: improved feed management.

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Eye damage including cataracts

Eye damage is also a well established welfare threat in fish and can be due to trauma from e.g. handling (see Nilsson, Stien, Iversen et al., 2018). Cataracts (clouding of the lens) can also be a problem in lumpfish (Jonassen et al., 2017; Treasurer et al., 2018).



Science based knowledge

- It has been reported that cataracts can be somewhat unusual in the larvae and juvenile life-stages and more common in the later life-stages (Powell et al., 2018b).
- Potential risk factors and mitigation strategies for eye damage:
 - Risk factor: Poor nutrition, especially in bilateral cataracts (Jonassen et al., 2017).
 - Risk factors: Handling, density, infectious diseases in unilateral cataract formation (suggested by Jonassen et al., 2017).
 - Risk factor: High feeding frequency (4 or 7 days/week) significantly increased the prevalence of cataracts in comparison to 3 days/week (fish fed 2% bw/day on feeding days). A feeding regime that reduced the number of feeding days was used to reduce weekly ration – which was greater in fish fed daily > 4 days > 3 days/week, leading to 35% larger fish in groups fed daily vs 3 days/week (Imsland et al., 2019b).
 - Mitigation strategy: reduce the number of feeding days? (Imsland et al., 2019b).
 - Risk factors for exophthalmos: *Scuticociliate* spp. (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); VHSv (Scholz et al., 2018); *Tetramicra brevifilum* (Scholz et al., 2018).
 - *Pasteurella* spp. can also be a risk factor for cataracts (Dawit, 2015).
 - Handling can be implicated in other forms of eye damage (e.g. Jonassen et al., 2017; Imsland et al., 2019b and references therein).
- *Risk factors in other species: environmental and nutritional factors are key to cataract formation.*

Risk factors	Juvenile	Adult	Broodstock	Reference
Cataracts: Rapid growth?	✓	✓	?	Imsland et al., (2019b)
Cataracts: Feeding regime?	✓	✓	?	Imsland et al., (2019b)
Cataracts: nutritional factors?	✓	✓	✓	Suggested by Jonassen et al., (2017)

Practical based knowledge

- Further potential risk factors for eye damage: abrasion or dessication during handling.
- Potential mitigation factors: gentle handling, knotless nets, limited air exposure, reducing the risk of potential drying of the eye.

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Vertebral deformities

Vertebral deformities are a well established welfare indicator in fish and can impact upon welfare by e.g. reducing the effectiveness of foraging or swimming behaviour (e.g. Noble et al., 2018 and references therein). However, as far as the authors are aware, there is limited information on vertebral deformities in cleaner fish, e.g. in terms of their aetiology, driving factors, prevalence and severity.



Science based knowledge

Risk factors for vertebral deformities:

- Constant 10 °C egg incubation temperature is a clear risk for increasing the frequency of vertebral deformities in newly hatched larvae (Imsland et al.,2019c).

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Snout and mouth damage

Snout damage can be due to e.g. handling and contact with the sharp edges, hard surfaces or abrasion with the net (see e.g. Gismervik et al., 2018). Mouth damage is also a clear welfare threat as it may hinder feeding and the ingestion of food items (Branson and Turnbull, 2008).



Science based knowledge

Risk factors for snout damage and mouth haemorrhaging:

- Diseases etc: *Tenacibaculum spp.* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018).
- Jaw damage and lesions around the mouth area have also been observed in both juvenile and broodstock lumpfish (Rey and Treasurer, personal communications cited in Treasurer et al., 2018).
- *In other species, it can be due to physical contact with the net, hard surfaces or sharp edges (e.g. Noble et al., 2012).*

Practical based knowledge

- Practical based experience suggests risk factors for snout and mouth damage include startle behaviours leading to collisions with e.g. the net or tank wall.

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Opercular damage

The opercula have a role in, and contribute to, the buccal pump mechanism and can improve the effectiveness of respiration in fish. Any damage can reduce this effectiveness and may become a welfare challenge, especially if the fish e.g. have gill health problems or are subjected to hypoxic conditions (Ferguson and Speare, 2006).



Science based knowledge

- Opercular damage is a widely used welfare indicator in other species e.g. Atlantic salmon (Noble et al., 2018; RSPCA, 2018a) and is also used as an OWI in lumpfish (e.g. Pooley et al., 2019). However, as far as the authors are aware, there is limited information available on the risk factors that can contribute to opercular damage in lumpfish, or its severity.
- Risk factors for opercular damage in lumpfish:
 - Health related risk factors: *Vibrio anguillarum* can lead to haemorrhaging around the operculum in lumpfish (Marcos-López et al., 2013).
- Risk factors for opercular damage in other species:
 - Opercular damage may also be due to physical damage, poor nutrition or poor rearing conditions (e.g. Eriksen et al., 2007; Nilsson, Stien, Iversen, et al., 2018).

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Suction disc deformities



Lumpfish possess a ventral cartilaginous sucker/disc that is ca. 20% of the body length and is formed by modified pelvic fins. This disc helps the lumpfish attach themselves to substrates and reduces the risk of them being dislodged in e.g. high current conditions (see Powell et al., 2018a and references therein).

Science based knowledge

- Suction disc deformities can prevent or limit how well the fish attach themselves to the substrate.
- This may be problematic for lumpfish in high energy tidal or exposed farming conditions (Treasurer, 2018a).
- Suction disc deformities can be passively diagnosed as present/absent if the fish are having problems attaching themselves to the substrate (Treasurer et al., 2018).
- The severity of the deformities can be diagnosed during manual inspection of the fish - with a deformed suction disc having a gap or break in the margin of the disc (Treasurer et al., 2018).
- **As far as the authors are aware there is no concrete information on drivers or risk factors for suction disc deformity (see also Treasurer et al., 2018).**
- **The effects of suction disc deformities on e.g. survival are also not established (Treasurer, 2018a).**

Practical based knowledge

- It has been observed that small juveniles in poor nutritional condition can develop suction disc deformities (Kousoulaki et al., 2018).

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Gill beat rate



An elevated gill beat rate or ventilation frequency may not always be due to stress or indicate a welfare risk. However, it can indicate low dissolved oxygen saturations or gill problems (Nilsson, Stien, Iversen et al., 2018).

Science based knowledge

- Potentially valuable OWI in slow moving or static fish such as lumpfish (Treasurer et al., 2018).
- Can be a good indicator of hypoxia - If fish are exposed to hypoxic conditions they can respond by hyperventilating (Perry et al., 2009).
- Jørgensen et al., (2017) reported increased gill beat rate at a dissolved oxygen (DO) saturation of $\leq 80\%$.
 - Fish exposed to 55% and 69% DO increased their gill beat rate/min by 71% and 60% in comparison to fish held at 96% DO after 1 month exposure. Gill beat rate was also 11% and 20% greater in fish held at 55% and 69% DO in comparison to those held at 81%.
- Treasurer and Turnbull (2019) have reported that lumpfish of up to 70 g can tolerate 5 h exposure to freshwater (0 ppt) and a 10 day exposure to brackish water (15 ppt) without any discernable negative effects on their welfare, including gill beat rate.

Practical based knowledge

- Another potential risk factor for increased gill beat rate is AGD (Treasurer personal observation, reported in Treasurer et al., 2018).

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Emaciation state



Emaciation is a robust indicator of a welfare challenge in lumpfish (Nilsen et al., 2014; MarinHelse, 2018) and in other species.

Science based knowledge

- Emaciation in lumpfish can be caused by poor nutrition e.g. Kousoulaki et al., (2018) or health related risk factors such as *Pseudomonas anguilliseptica* (Scholz et al., 2018) and *Nucleospora cyclopteri* (Scholz et al., 2018).

Practical based knowledge

- A lumpfish in good condition is normally wider in the ventral buccal cavity region than the head. Emaciated fish become progressively thinner and the head becomes the widest part of the body (MarinHelse, 2018).
- Potential additional risk factors for emaciation can be i) underfeeding/poor feed management in the cage and/or ii) fish being worn out by being deployed on sea localities that are too exposed (MarinHelse, 2018).
- Dominance hierarchies can also lead to poorer competitors being excluded from feeding which can lead to emaciation (MarinHelse, 2018).

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GROUP BASED OWIs



Group based OWIs are also well established OWIs for numerous farmed species (Noble et al., 2018; RSPCA 2018a, b; Stien et al., 2013) including cleaner fish (Treasurer et al., 2018) and are also applicable at differing lifestages (Treasurer et al., 2018).

Key Group based OWIs covered in these Factsheets:

- Mortality
- Health status
- Appetite
- Growth
- Behaviour e.g. aggression, different types of swimming behaviour or attachment to substrate
- Blood in the water

Group based
OWIs



RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Mortality rate

Mortality rate (especially changes in mortality rate) is a very widely used OWI. If mortality is high or begins to rise it is a clear indicator of a welfare problem. On the other hand, low mortalities do not automatically mean that welfare is good – fish can experience welfare problems that do not lead to mortality (e.g. Nilsson, Stien, Iversen et al., 2018).



Science based knowledge

- Mortality can be used as both a long and short-term OWI in numerous farmed species (e.g. Nilsson, Stien, Iversen et al., 2018; Treasurer et al., 2018).
 - Long term: The total accumulated mortality during a production cycle
 - Short term: Daily, weekly, monthly
- Short-term mortality such as an active increase in mortality rate can be used to identify ongoing welfare risks or potential problems on the farm. Long-term mortality can be used to retrospectively assess potential welfare or health problems in the rearing unit, farm, company or region (see e.g. Nilsson, Stien, Iversen et al., 2018).
- Mortality is benchmarked in a number of other farmed species e.g. Atlantic salmon (Soares et al., 2011; Stien et al., 2016) and this benchmarking, if carried out actively or in real time, can also be used to actively or retrospectively assess or evaluate potential welfare problems.
- **However, there is anecdotal evidence that the carcasses of lumpfish can rapidly decompose in the rearing unit (e.g. Nilsen et al., 2014) which is problematic for obtaining precise data on mortality rates and identifying the causes of mortality (Treasurer et al., 2018).**
- It has also been suggested that dead cleaner fish in sea cages may also be eaten by wild fish as they lay on the net floor (Nilsen et al., 2014).
- We need more information and knowledge on why the cleaner fish die. The cause of death is often multifactorial: e.g. environmental issues/stress, nutritional issues, suboptimal feeding strategies, infections and lack of protective vaccines etc. Another challenge with lumpfish is that multiple (bacterial) pathogens may be found in the same fish.
- Dead fish should be collected as fast as possible. If possible, potential causes of mortality should also be identified and stated and these can be classified according to e.g. the recently published *Veileder i Dødfiskkategorisering (Guidance on classifying mortalities) handbook* produced by MarinHelse AS (2018).

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Mortality rate continued

Science based knowledge

Risk factors for mortalities:

- Nilsen et al., (2014) carried out a mortality mapping study in 2013 that reported lumpfish mortalities in sea cages were due to: bacterial infections (75%), emaciation (9%), sores/fin erosion (1%), with 14% due to other causes (2% of mortalities were also old/rotten).
- Specific health related risk factors: *Aeromonas salmonicida* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); *Vibrio ordalli* (MarinHelse, 2018); *Pasteuralla* spp (Scholz et al., 2018; MarinHelse, 2018); *Neoparamoeba perurans* (Scholz et al., 2018); Scuticociliate spp. (Scholz et al., 2018); *Nucleospora cyclopteri* (Scholz et al., 2018); *Exophiala angulospora* (Saraiva et al., 2019); *Flaviviridae* spp. (Skoge et al., 2019).
- Both low temperatures (< 4 °C, Imsland et al., 2018a) and high temperatures (18 °C, Hvas et al., 2018) can also increase mortalities.

Practical based knowledge

- High mortalities after cage deployment can also be related to handling or due to chemical or mechanical de-licing practices (e.g. Kousoulaki et al., 2018).
- Mortalities may also be due to net cleaning, delicing baths or other operations (Rimstad et al., 2017; MarinHelse, 2018).

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Health status

Health has a major impact upon fish welfare and health status is a commonly used welfare indicator. In certain situations health problems can be diagnosed on the farm (OWIs) and other require samples from the fish to be sent to a laboratory for further diagnostics (LABWIs), see Nilsson, Stien, Iversen et al., (2018).



Science based knowledge

- The health status of lumpfish is regularly monitored by the farmer and internal or external fish health personnel. If a potential health problem is noticed and identified, the response time to the challenge can be much more rapid. A rapid response time is key as it has been previously reported that the majority of lumpfish mortalities can be related to health problems (Nilsen et al., 2014).
- Nilsson, Stien and Iversen et al., (2018) suggest detailed health plans are a good resource based WI. They also state *“While frequent treatments may indicate poor disease control and a welfare problem they can also indicate an effective monitoring and response to disease problems they therefore have to be considered in context”*.
- The impacts of infectious diseases on certain OWIs such as epidermal damage, eye damage, behaviour, appetite and mortality are addressed in each relevant OWI factsheet in this series.
- Numerous reviews and risk assessments also give a good overview of the overall health impacts (and welfare risks) posed by cleaner fish pathogens (e.g. Rimstad et al., 2017; Scholz et al., 2018) and will not be covered in these factsheets.

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Appetite and growth rate



A drop or sudden loss of appetite, or a poor feeding response can be due to stress (Huntingford and Kadri, 2014). Good monitoring of appetite and the feeding response of lumpfish can help the farmer identify any potential welfare problems as soon as possible (Treasurer et al., 2018). The same applies to growth rate: if farmers notice a sudden change or drop in growth rate, it may be due to a welfare problem (e.g. Nilsson, Stien, Iversen et al., 2018). On the other hand, rapid growth is not necessarily an indicator for good welfare in lumpfish as feed with a high fat content can not only lead to rapid growth but can lead to more fat in the liver, which may have consequences for fish health (Gerd M. Berge, pers. comm).

Science based knowledge

Appetite:

- Well established OWI in numerous fish species (e.g. Huntingford and Kadri, 2014; Noble et al., 2018; Treasurer et al., 2018). Usually used as a qualitative OWI as quantifying appetite can be difficult due to variability in appetite and the feeding response on a potentially hourly or daily basis (Treasurer et al., 2018).
- However, a drop in or lack of appetite may not just be due to a potential problem. For example, it may be because fish have just eaten and are satiated. It may also be related to environmental factors, such as water temperatures that are too low (Treasurer et al., 2018) or the life stage of the fish e.g. appetite suppression as the fish mature (Davenport, 1985).
- A drop in appetite can be an indicator for potential health problems including *Aeromonas salmonicida* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); *Tenacibaculum* spp. (Scholz et al., 2018); *Tetramicra brevilum* (Scholz et al., 2018).
- Long term appetite problems lead to emaciation (see earlier section of this factsheet).
- The presence of feed in the intestine is an indicator that the fish have recently eaten (although this is dependent on fish size and temperature). This check can be carried out during auditing of recently euthanised fish (e.g. Kolarevic, Stien et al., 2018) and its presence/absence is already monitored in vaccine control protocols (e.g. by PHARMAQ, as outlined in Haugland et al., 2018).
- Jørgensen et al., (2017) have reported reduced appetite in fish subjected to DO saturations $\leq 80\%$.

Growth and growth rate:

- Growth and growth rate are fundamentally connected with feeding, appetite and the nutritional status of the fish (Nilsson, Stien, Iversen et al., 2018) and can be a reflection of problems associated with these factors.
- Has been used as an OWI for lumpfish (Jørgensen et al., 2017).
- Requires good growth monitoring practices in order to be a robust, quantifiable OWI.
- As with appetite, poor growth may not just be linked to poor welfare *per se*, as longer term changes in growth rate may be linked to e.g. season or the life stage of the fish (Treasurer et al., 2018). To help identify whether poor growth is due to a welfare challenge, it should be coupled with other OWIs (Ellis et al., 2002)
- Size variation with the group may reflect feed access and can also be used as an OWI for lumpfish (Treasurer et al., 2018).

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Behaviour

Fish behaviour is a central OWI tool in the OWI toolbox (see Nilsson, Stien, Iversen et al., 2018). Potential behavioural OWIs suitable for lumpfish include aggression, different types of swimming behaviour and also tank distribution or attachment to the substrate (Treasurer et al., 2018).



Science based knowledge

General information regarding lumpfish swimming behaviour and attachment to the substrate:

- Lumpfish can be sedentary and sit and rest on the substrate or under shelters (Johannesen et al., 2018a) but they can also be pelagic, covering long distances in the wild (Davenport, 1985).
- It has been suggested that lumpfish can be classified as proactive or reactive (Johannesen et al., 2018a)
 - Proactive fish exhibit escape behaviours when exposed to a potentially threatening stimuli, reactive fish do not respond or freeze (Johannesen et al., 2018a).
- Lumpfish can also switch between 'sit and wait' or more active foraging in relation to feed abundance. For example, when food is plentiful they spend more time attached to the substrate but exhibit more active foraging behaviour when food is sparse (Killen et al., 2007).
- Swimming is rather restricted in lumpfish irrespective of life stage (Treasurer et al., 2018).
- Temperatures near or above their thermal threshold can lead to somewhat chaotic swimming, elevated swimming speeds, and increased breaking of the water surface with their head during swimming (Hvas et al., 2018).
- Lumpfish can be active foragers during start feeding (Brown et al., 1992).
- Juveniles have a somewhat restricted aerobic capacity and do need sufficient surface area to attach to substrates. If this surface area is not available, fish can resort to aggression. Juveniles become more pelagic as they grow, and can swim individually, in loose groups or school (Jonassen et al., 2018a).
- Treasurer et al., (2018) have suggested that a life stage specific "surface area index" for ensuring lumpfish have enough area for settlement would be very valuable.

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Behaviour continued

Science based knowledge cont.

Aggression in the tank rearing phases:

- Aggression is common in juvenile lumpfish especially during the first 3 months after hatching (Treasurer et al., 2018) and cannibalism has also been reported (Brooker et al., 2018).
- Lumpfish can be territorial and can form feeding hierarchies. This may lead to the exclusion of poorer competitors to the tank walls or substrate. Potential lost feeding opportunities both for poorer competitors as well as more dominant fish (due to increased energy expenditure during dominant behaviours and increased swimming activity) can exacerbate potential size differences between individuals (Jonassen et al., 2018a).
- Aggression due to the formation of size hierarchies can be reduced by size grading (Jonassen et al., 2018a) and also improved feed management such as continuous feeding (Treasurer et al., 2018). However continuous feeding combined with environmental enrichment can have a potentially negative effect upon welfare if the feeding method and deployment of the shelter are not given due consideration (Johannesen et al., 2018b).
- Aggression has also been observed in broodstock during maturation (Treasurer et al., 2018).
- It has also been suggested that reduced light intensity can reduce aggression (Jonassen et al., 2018a).
- Aggression can be mitigated against using environmental enrichment (Treasurer et al., 2018).

Health impacts upon behaviour:

- Lethargy can be due to *Aeromonas salmonicida* (Scholz et al., 2018); *Tenacibaculum spp.* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); *Tetramicra brevifilum* (Scholz et al., 2018); *Trichodina spp.* (Scholz et al., 2018).
- Erratic behaviour near the surface can be due to *Nucleospora cyclopteri* (Scholz et al., 2018).

Behaviour during sea cage deployment:

- The duoculture of lumpfish with salmon can make the lumpfish more active, spending less time resting (Imsland et al., 2014; Powell et al., 2018b).
- Little overt hostile behaviour between the species has been observed (Imsland et al., 2014).
- Imsland and Reynolds, (2018) suggest cage held lumpfish can exhibit the following behaviours: i) resting, ii) hovering, iii) swimming along the net, iv) swimming amongst the salmon, v) salmon inspection behaviours, vi) salmon cleaning behaviours and vii) shoaling, amongst others.
- Active daytime foragers (can spend >50% of their time searching for food, Imsland et al., 2014) and are more restful at night (Imsland and Reynolds, 2018; Powell et al., 2018b).
- However, lumpfish generally spend a lot of time in shallower waters (can spend 80% of their time at depths < 10 m, Leclerq et al., 2018), which may be problematic in terms of their potential lice eating efficacy if the salmon swim deeper (e.g. Skiftesvik et al., 2018).

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Behaviour continued

Practical based knowledge

- Juvenile tank reared lumpfish like shade and will attach to any available substrate in shaded areas. Staff should be careful to reduce the risk of overly dense 'clumping' of the fish (e.g. Jonassen et al., 2018b).
- It has been suggested that bright tank colours may reduce lumpfish settlement and attachment. Juvenile lumpfish may avoid attaching to white tank bottoms which may in turn aid tank cleaning (see Jonassen et al., 2018b).
- Lumpfish subjected to feed withdrawal may become more aggressive. This can be mitigated against by keeping the fish in reduced light/total darkness during fasting before transport (e.g. Jonassen et al., 2018a).

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Blood in the water



Blood in the water has been suggested as a potential OWI for Atlantic salmon by Nilsson, Stien, Iversen et al., (2018) and may also be an OWI for lumpfish. It is usually due to damage to the skin or gills and “red water” is evidence that the fish is injured.

Science based knowledge

- This OWI is manually and qualitatively diagnosed.
- Can be clearly diagnosed if the fish are in light coloured closed containers (Nilsson, Stien, Iversen et al., 2018) or if the water outflow from a pipe or tank is monitored.
- Is a rapid indicator of an acute or ongoing welfare problem, but its cause or severity cannot be diagnosed without a more detailed investigation of the problem (Nilsson, Stien, Iversen et al., 2018).

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PHYSIOLOGICAL LABWIs and OWIs



Physiological LABWIs and OWIs are often used to document stress-related loads and challenges in numerous species (e.g. Noble et al., 2018) including cleaner fish (Treasurer et al., 2018).

Key Physiological LABWIs and OWIs covered in these Factsheets:

- Plasma cortisol
- Glucose
- Osmolality
- Magnesium
- Chloride

Physiological
LABWIs and
OWIs



RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Plasma cortisol



Plasma cortisol is widely used as an indicator of the primary stress response in fish (Barton and Iwama, 1991). Elevated levels of plasma cortisol are widely accepted to be linked to negative experiences in fish, although its association with positive situations should not be overlooked (Ellis et al., 2012).

Science based knowledge

- Plasma cortisol levels can be assessed using i) enzyme-linked immunoassay (ELISA) or ii) radioimmunoassay (RIA) methods (Sopinka et al., 2016) and is therefore classified as a LABWI.
- The acute stress profile of lumpfish has a peak that is 4-6 times lower than the associated stress profile of ballan wrasse (Treasurer et al., 2018; Espmark et al., 2019) and other species such as salmonids (Hvas et al., 2018).
- Resting basal plasma cortisol levels in an unstressed lumpfish are < 20 nmol/l, whilst chronically stressed lumpfish often have cortisol levels well above 30 nmol/l (Sällebrant, 2018; Espmark et al., 2019).
- Jørgensen et al., (2017) suggest plasma cortisol levels in unstressed fish are < 10 ng/mL and levels in stressed fish are > 20 ng/mL. DO saturations of 55% and 69% led to plasma cortisol levels > 20 ng/mL, whilst DO saturation 81% and above were around 10 ng/mL and below.
- Small lumpfish (< 20g) seem to handle stress better than larger lumpfish (> 100g) (Sällebrant, 2018; Espmark et al., 2019)
- Exposing lumpfish to a daily stressor (lowering the water level, 264 kg/m³) will after 14 - 21 days lead to a chronic stress load that will adversely affect welfare (Sällebrant, 2018; Espmark et al., 2019).

Strengths and weaknesses of the LABWI

- If plasma cortisol levels are sampled before, during and after a routine or operation, they can give the user good, robust information on how that routine affects the fish (Barton, 2002).
- Can be difficult to interpret the samples (especially if limited or single samples are taken) and one should not interpret high levels of plasma cortisol as an indicator of reduced welfare without further information (Nilsson, Stien and Iversen et al., 2018).
- Analysis can be somewhat time consuming (taking at least 1-2 days).

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Glucose



Increased concentrations of plasma cortisol promote glycogenolysis, where tissue stores of glycogen are converted into glucose and discharged into the blood (Barton and Iwama, 1991).

Science based knowledge

- Elevated glucose levels have been widely used as a stress indicator in fish (Barton and Iwama, 1991) but as they can be impacted upon by various other factors such as feed ingredients, satiation levels etc., glucose levels not be benchmarked against a “standard level”, but measured e.g. before, during and after an operation (Nilsson, Stien, Iversen et al., 2018).
- As for cortisol, lumpfish do not show high levels of glucose in the blood during and after exposure to a stressor as compared to e.g. salmon (Treasurer et al., 2018).
- Neither acute nor chronic stress seem to affect glucose levels in lumpfish (Hvas et al., 2018; Sällebrant, 2018; Espmark et al., 2019).

Strengths and weaknesses of the OWI

- Easily accessible hand-held instruments are a validated and inexpensive tool for monitoring plasma glucose levels (Sopinka et al., 2016) and are both robust and easy to use out on the farm. Glucose is therefore classified as an OWI.
- It is difficult to interpret the samples (especially if limited or single samples are taken) and one should not interpret high levels of plasma glucose as an indicator of reduced welfare without further information, as levels can be affected by numerous other factors (Nilsson, Stien and Iversen et al., 2018).

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Osmolality

Deviations in plasma osmolality are elements of the the secondary stress response in fish (Veiseth et al., 2006). Nilsson, Stien, Iversen et al., (2018) defined osmolality as “*the number of dissolved particles in liquid, and salinity represents the amount of dissolved salt in water. Freshwater has a salinity of 0 ‰ and an osmolality of 0-10 mOsm kg⁻¹, whilst seawater has a salinity of 33-35 ‰ and an osmolality of 1000 mOsm kg⁻¹.*”



Science based knowledge

- According to the results of the RENSVEL project unstressed lumpfish have an osmolality that is around 350-360 mOsm / kg (Sällebrant, 2018; Espmark et al., 2019).
- Osmolality also appears to increase during both acute and chronic stress exposure and is most pronounced in small lumpfish (< 20g) (Sällebrant, 2018; Espmark et al., 2019).
- However, Hvas et al., (2018) reported no significant effect of acture stressor exposure on osmolality levels in 300 g lumpfish (unstressed lumpfish had an osmolality level of 374 ± 5, stressed fish exposed to an 8 minute chase protocol had levels of 369 ± 1 and 1-3 hours after stressor exposure levels were around 360 mOsm / kg, mean ± SEM).

Strengths and weaknesses of the LABWI

- Fluctuations in osmolality are a good way to assess the acute stress response (Sopinka et al., 2016).
- It is difficult to interpret the samples in relation chronic stress exposure without further information as osmolality levels can be influenced by numerous other factors (Sopinka et al., 2016).

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Magnesium



Secondary stress responses cause changes to plasma and tissue ions, such as magnesium and chloride (Treasurer et al., 2018).

Science based knowledge

- Results from RENSVEL suggest an unstressed lumpfish has a plasma magnesium level < 1.5 mmol/L (Sällebrant, 2018; Espmark et al., 2019).
- Plasma magnesium levels also appear to increase during both acute and chronic stress exposure and are a good indicator of disturbed ion balance under stress. There is also a good correlation between levels of plasma cortisol and magnesium (Sällebrant, 2018; Espmark et al., 2019).

Strengths and weaknesses of the LABWI

- Fluctuations in plasma magnesium levels are a good way to assess the acute stress response (Sopinka et al., 2016; Espmark et al., 2019).
- However, it is difficult to interpret the samples in relation to chronic stress exposure without further information as plasma magnesium levels can be influenced by numerous other factors (Sopinka et al., 2016).

RENSVEL OWI FACTSHEET SERIES: LUMPFISH

Chloride



Secondary stress responses cause changes to plasma and tissue ions, such as magnesium and chloride (Treasurer et al., 2018).

Science based knowledge

- Results from RENSVEL suggest an unstressed lumpfish has a plasma chloride level that is ca. 150 mmol/L (Sällebrant, 2018).
- Plasma chloride levels also appear to increase during both acute and chronic stress exposure and are a good indicator of disturbed ion balance under stress (Sällebrant, 2018; Espmark et al., 2019).
- However, Hvas et al., (2018) reported plasma chloride levels significantly decreased in 300g lumpfish during and after exposure to a stressor. Unstressed lumpfish had a chloride level of 161 ± 2 , stressed fish exposed to an 8 minute chase protocol had significantly reduced levels of 155 ± 1 and 1-3 hours after stressor exposure chloride levels were again significantly reduced around 148 ± 1 , mmol/l (mean \pm SEM). The authors suggested this decrease was due to active acid-base compensations (Evans et al., 2005).

Strengths and weaknesses of the LABWI

- Fluctuations in plasma chloride levels are a good way to assess the acute stress response (Sopinka et al., 2016; Espmark et al., 2019).
- However, it is difficult to interpret the samples in relation to chronic stress exposure without further information as plasma chloride levels can be influenced by numerous other factors (Sopinka et al., 2016).

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