

RENSVEL OWI FACT SHEET SERIES:
AN INTRODUCTION TO OPERATIONAL AND
LABORATORY-BASED WELFARE INDICATORS
FOR BALLAN WRASSE (*Labrus bergylta*)



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Suggested citation: Noble, C., Iversen, M. H., Lein, I., Kolarevic, J., Johansen, L. –H., Burgerhout, E., Puvanendran, V., Kousoulaki, K., Aas, G. H., Stene, A. & Espmark, Å. M. (2019). RENSVEL OWI FACT SHEET SERIES: An introduction to Operational and Laboratory-based Welfare Indicators for ballan wrasse (*Labrus bergylta*). 43 pp.

ISBN 978-82-8296-590-3

An output of the FHF financed project 901136 «RENSVEL: Velferd hos rensefisk – operative indikatorer»

April 2019

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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 ballan wrasse (*Labrus bergylta*). For each OWI/LABWI we will i) briefly outline the indicator, ii) give a science-based overview of the information we currently 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.

This fact sheet series is an output of the FHF financed project 901136 «RENSVEL: Velferd hos rensefisk – operative indikatorer», led by Nofima and is written in partnership with researchers from Nord University and NTNU. The authors would especially like to thank the steering group of the project (Olav Breck, Mowi ASA; Espen Lie Dahl, SalMar ASA; Kjetil Heggen, Lerøy Seafood Group ASA; Halvard Hovland, Havlandet Marin Yngel AS; Lars Jørgen Ulvan, Nordland leppefisk AS) for all of their guidance and inputs throughout the project.

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

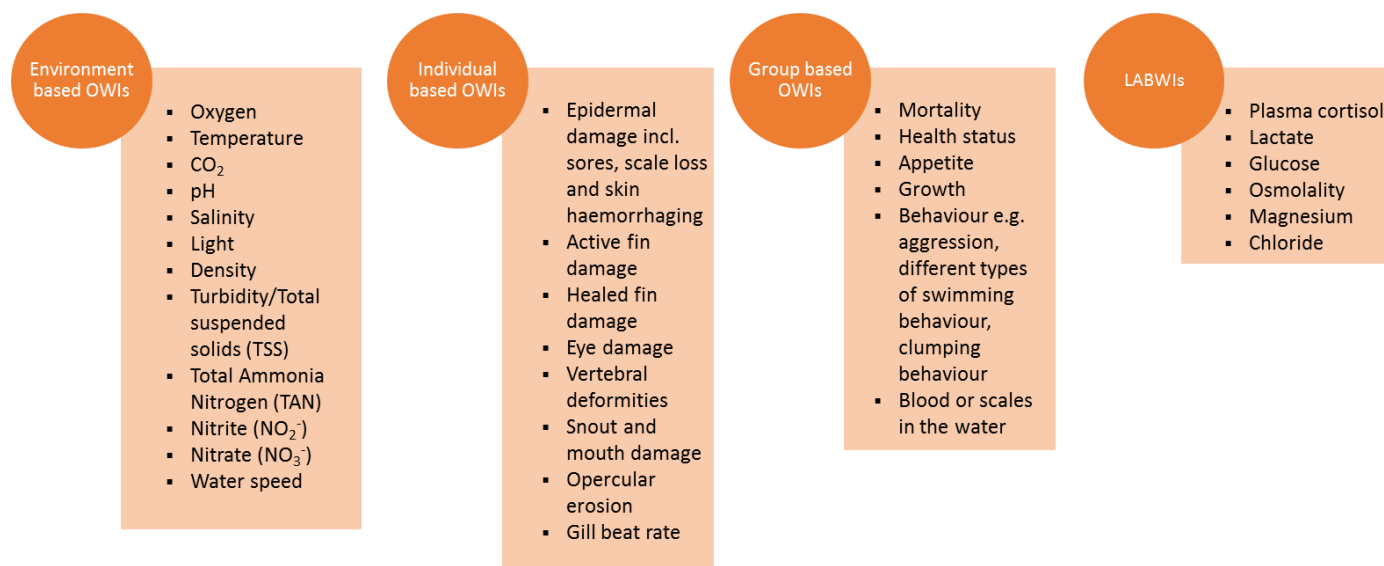


Figure 1: Overview of the OWIs and LABWIs addressed in the RENSVEL OWIs and LABWIs for ballan wrasse fact sheet series (Figure: Chris Noble, Nofima. Adapted from figures in “Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators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

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ENVIRONMENT BASED OWIs

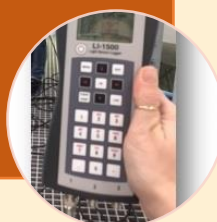
Environment based OWIs are well established indirect welfare indicators for numerous farmed species (Noble et al., 2018; RSPCA 2018a, b; Stien et al., 2013). However, there is little published information on environment based OWIs for ballan wrasse (Treasurer et al., 2018).



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 or high can cause welfare problems in numerous fish species including ballan wrasse (e.g. Treasurer et al., 2018). Different life stages can also have differing oxygen requirements.



Science based knowledge

- Dissolved Oxygen (DO) saturation levels and recommendations for differing life stages:
 - Juveniles:
 - > 7 mg/l in the hatchery (RSPCA, 2018a).
 - 80-90% DO saturation (Treasurer et al., 2018).
 - RENSVEL results suggest 100% DO saturation is optimal in juvenile fish weighing 20-30g (Gaffney et al., in prep).
 - Adults and broodstock:
 - As far as the authors are aware, there are no recommendations for DO saturation levels in adult/broodstock ballan wrasse but studies have reported using > 90% DO saturation for rearing broodstock (e.g. Grant et al., 2016) or recording average DO levels of > 11 mg/l in a study on cage held ballan wrasse (> 70g mean weight, Leclercq et al., 2018).

Practical based knowledge

Practical DO saturation levels and recommendations:

- > 7 mg/l when wrasse held in RAS (Treasurer et al., 2018).
- 80-100% saturation during transport and not greater than 125% (Transportation of wrasse guidelines <http://lusedata.no/wp-content/uploads/2012/05/05062015-Veileder-til-transport-av-leppefisk.pdf>).
- However, another recommendation is that oxygen levels should not exceed 110% during transport https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_lea_flet7_Farm_Application_of_Wrasse_web_version.pdf
- DO saturations of 80-90% have been used for rearing adult ballan wrasse with good results (I. Lein, pers. comm.).

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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 ballan wrasse.



Science based knowledge

- It has been suggested that wrasse can tolerate temperatures 4 – 20 °C (Treasurer et al., 2018) but become less active at < 10 °C (Brooker et al., 2018) and inactive at < 6 °C (Treasurer, 2002).

Life stage	Temperature range	Reference
Juvenile	Begin at 12 °C and then gradually increase to 16 °C 8 – 16 °C	Treasurer (2018) RSPCA (2018a)
Adult	Optimal temperature = ca. 16 °C in 20 g and >50 g fish Become less active at < 10 °C Become inactive at < 6 °C	Treasurer (2018) Brooker et al., (2018) Treasurer (2002)
Broodstock	11 °C	Treasurer (2018)

Practical based knowledge

- Practical based experience on water temperature requirements in relation to life stage:
 - Juvenile fish: 14 – 16 °C (Treasurer et al., 2018).
 - Brooker et al., (2018) suggests the best time to deploy wrasse is from spring onwards when the water temperature is increasing.
- It has been suggested that water temperature during transport should be 8 – 18 °C (Transportation of wrasse guidelines <http://lusedata.no/wp-content/uploads/2012/05/05062015-Veileder-til-transport-av-leppefisk.pdf>).
- Temperature during transport should not increase by more than 2 °C during the transport process. https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_leafile7_Farm_Application_of_Wrasse_web_version.pdf

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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 for ballan wrasse and this is also the same for pH (e.g. Treasurer et al., 2018).



Science based knowledge

As stated above, the authors have not found any peer-reviewed published information for optimal or critical levels for CO₂ and pH (see also Treasurer et al., 2018).

- However, the RSPCA welfare standards for Atlantic salmon suggest wrasse in hatcheries must be reared to a pH of 7.4 – 8.2 (RSPCA, 2018a).

Practical based knowledge

- With regard to CO₂, it has been suggested that CO₂ tolerances in ballan wrasse are potentially comparable to other marine species and should be < 15 mg/l (Treasurer et al., 2018).
- With regard to pH, it has also been suggested that pH tolerances for wrasse are potentially comparable to other marine species, and production data has reported pH values in the region 7.2 – 8.0 (Treasurer et al., 2018).
- CO₂ levels during transport should be < 15 mg/l and pH should be > 7.0 (Transportation of wrasse guidelines <http://lusedata.no/wp-content/uploads/2012/05/05062015-Veileder-til-transport-av-leppefisk.pdf>).

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Salinity

Ballan wrasse are a marine fish that reportedly has a low tolerance of freshwater (Bolton-Warberg, 2018; Brooker et al., 2018), unlike lumpfish (Treasurer and Turnbull, 2019).



Science based knowledge

With regard to freshwater exposure:

- Both Bolton-Warberg, (2018) and Brooker et al., (2018) have reported that ballan wrasse have a low tolerance to freshwater. This means they must be separated from the salmon before e.g. freshwater bathing.
- Skiftesvik et al., (2018) also state ballan wrasse do not withstand being directly transferred to freshwater, but can tolerate 2 hours of freshwater exposure if salinity is gradually decreased.

Practical based knowledge

- Salinity levels during transport should be > 25 ppt (Transportation of wrasse guidelines <http://lusedata.no/wp-content/uploads/2012/05/05062015-Veileder-til-transport-av-leppefisk.pdf>).
- Salinities during tank rearing should be ca. 34 ppt. https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_1_eaflet7_Farm_Application_of_Wrasse_web_version.pdf
- However, it has been suggested that wrasse can survive short-term exposure to salinities that drop as low as 14 ppt and can also survive for 5 – 7 days in salinities that are as low as 21 ppt. The same authors also state that low salinities are stressful for the wrasse and can lead to mortalities. See https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_1_eaflet7_Farm_Application_of_Wrasse_web_version.pdf

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Light

The manipulation of daylength and changes in daylength are widely used techniques for e.g. controlling reproduction and initiating maturation in numerous farmed fish species. However, with regard to cleaner fish, the field is still in its infancy. As far as the authors are aware, there have been few scientific studies on the effects of light intensity or wavelength upon cleaner fish welfare and performance. The information that we have found will be reported here.



Science based knowledge

- As stated above, as far as the authors are aware, there is little published information regarding optimal light intensity or wavelength for ballan wrasse in relation to their welfare.
- RENSVEL results suggest that neither wavelength (green light: 520 nm, blue light: 450 nm, or broadspectrum white) or intensity (1, 5, 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) affect survival or growth (Espmark et al., 2019).
- However, Espmark et al., (2019) suggest fish under green light (520 nm) had a higher proportion of cataracts than those under blue light (450 nm). Light intensity also affected the incidence of cataracts, with the highest proportion of fish with cataracts at 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Fish under green light (520 nm) also had higher activity levels, glucose levels and more epidermal damage than those under blue light (450 nm).
- Skiftesvik et al., (2017) have reported that wavelength can affect the behaviour of wild caught ballan wrasse, with fish held under green light being more active than those under blue, and fish held under red light being more aggressive towards conspecifics in comparison to fish held under other wavelengths.
- Helland et al., (2014) recommended that wrasse should not be abruptly transferred from a 24 h light regime to a 16:8 LD regime in the tank rearing phase and suggested a more gradual transition should be tested.
- Skiftesvik et al., (2014) reported that unexpected shadowing over the tank or sudden changes in light can trigger 'clumping' behaviour in wrasse larvae and juveniles. They suggested keeping light diffuse and uniform to reduce the risk of clumping.
- 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).

Practical based knowledge

- Blue light may be perceived as unpleasant for humans. A solution to this may be to use wide or full-spectrum white light which also contains the blue wavelength (450 nm).

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Density

Stocking density is a common OWI for fish 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.
- Suitable shelters should be provided for ballan wrasse to rest in at night and also to reduce the risk of potential predation by either salmon or trout (Rimstad et al., 2017) or by predatory seabirds around the pen https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_leaflet7_Farm_Application_of_Wrasse_web_version.pdf
- Other stocking density recommendations:
 - Wrasse > 10 grams in weight must not be subjected to stocking densities over 30 kg/m³ in hatcheries (RSPCA, 2018a).
 - Wrasse 20 g or > 50g should be subjected to densities of 15 – 25 kg/m³ during ongrowing (Treasurer, 2018).
 - Stocking density should be < 110 kg/m³ during transport (RSPCA, 2018a).

Practical based knowledge

- It has also been recommended that densities during tank rearing should not be > 5 kg/m³. https://www.northernperiphery.eu/files/archive/Downloads/Project_Publications/18/ECOFISH_leaflet7_Farm_Application_of_Wrasse_web_version.pdf
- Densities of 7-10 kg/m³ have been used for rearing ballan wrasse broodstock (I. Lein, pers. comm.)

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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 ballan wrasse.



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 ballan wrasse 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 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 product of protein catabolism and is 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). Ammonia concentrations in the water depend upon TAN, pH and temperature. Low DO levels and variable concentrations of NH_3 lead to increased ammonia toxicity whilst increased salinity and sodium levels reduce NH_3 toxicity (Colt, 2006). 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 ballan wrasse.



Science based knowledge

- As stated above, **the authors have not found any peer-reviewed published information on optimal or critical levels for TAN, nitrite or nitrate in relation to ballan wrasse 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).*

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Water speed

Water speed is a well established indirect OWI in fish (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 lead to exhaustion.



Science based knowledge

- In a study by Leclercq et al., (2018), wrasse distribution and swimming activity did not appear to be affected by the tidal cycle, although the farm site was considered to be sheltered with a mean water flow of ca. 6 cm/s.
- Results from RENSVEL suggest the critical swimming speeds of juvenile ballan wrasse (mean weight = 32g) can range from 1.7 – 2.5 body lengths/second (bl/s), dependent upon DO saturation (Gaffney et al., in prep).
 - DO saturations of 75% significantly reduced RU_{crit} to 1.7 bl/s.
 - DO saturations of 85% led to an RU_{crit} of 2.1 bl/s.
 - DO saturations of 100% led to an RU_{crit} of 2.5 bl/s.
 - DO saturations of 125% led to an RU_{crit} of 2.0 bl/s.
- When exposed to water velocities in a swim tunnel, 12.5 – 18.5 % of the fish do not swim at all (Gaffney et al., in prep.; Espmark et al., 2019).

Practical based knowledge

- When wrasse are held in cages over winter they become less active and should be protected from e.g. strong currents (Treasurer et al., 2018).

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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 still very little published information relating to individual based OWIs in ballan wrasse (Treasurer et al., 2018).



Key Individual based OWIs covered in these Factsheets:

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

Individual
based OWIs



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Epidermal damage

Addresses the damage or loss of epidermal tissue, including sores/ulcers, scale loss and haemorrhaging. The epidermis is a barrier to infection and contains nociceptors. Any damage to the epidermis is a well established risk for fish welfare (Noble et al., 2012) and has been used as an OWI in ballan wrasse (Treasurer and Feledi, 2014).



Science based knowledge

Risk factors for sores and skin haemorrhaging in ballan wrasse:

- Sores/Ulcers:
 - Health related risk factors: infections with *Moritella viscosa* (Rimstad et al., 2017; Scholz et al., 2018); *Tenacibaculum* spp. (Rimstad et al., 2017); Atypical *Aeromonas salmonicida* (Rimstad et al., 2017); Viral hemorrhagic septicemia (VHS) virus (Scholz et al., 2018).
- Skin haemorrhaging:
 - Has been used as an OWI in ballan wrasse by Treasurer and Feledi (2014) but no haemorrhaging was observed in their cage deployment study.
- Scale loss:
 - Scale loss has been widely used as an OWI in other species e.g. Atlantic salmon (Noble et al., 2018) and we suggest it can be used as a potential OWI for ballan wrasse.
 - Aggression has been reported in wrasse male broodstock during spawning and this can lead to scale loss and mortalities during the spawning season (Treasurer, pers. obs. reported in Treasurer et al., 2018).

Practical based knowledge

- Epidermal damage in wrasse has also been observed in cages in relation to handling and care should be taken when carry out any handling procedures (Rabadan, 2018). For example, knotless nets should be used for handling.
- Epidermal damage may also be due to net cleaning or other operations (Rimstad et al., 2017).
- 'Clumping' or 'balling' behaviour may lead to fin or epidermal injuries when the fish attempt to swim into the dense mass of fish and get injured.

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Active and healed fin damage

Fin damage is a well established welfare issue in cleaner fish (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). Treasurer and Feledi (2014) have suggested fin damage is one of the most common forms of physical injury in ballan wrasse.



Science based knowledge

- Fins susceptible to fin damage include:
 - Caudal, Dorsal, Pectoral, Anal fin with the caudal fin especially at risk (Treasurer and Feledi, 2014).
- Active fin damage is also a potential route for opportunistic pathogens such as *Tenacibaculum* spp. (e.g. Scholz et al., 2018).
- With regard to health related issues, fin damage can also be exacerbated by *Tenacibaculum* spp. (Scholz et al., 2018).
- Stress may also induce/exacerbate fin damage, especially in juvenile ballan wrasse (Virtanen, 2018; Espmark et al., 2019).

Practical based knowledge

- Further potential risk factors for fin damage may be the choice of feeding strategy and feed withdrawal.
- Potential mitigation factors for fin damage can be improved feed management and minimising exposure to stressors.
- 'Clumping' or 'balling' behaviour may lead to fin or epidermal injuries when the fish attempt to swim into the dense mass of fish and get injured.

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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 wrasse.



Science based knowledge

- Potential risk factors for eye damage:
 - Light (Green light: 520 nm) (Espmark et al., 2019).
 - RENSVEL results (Espmark et al., 2019) suggest light intensity also affected the incidence of cataracts, with the highest proportion of fish with cataracts at $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in comparison to either 1 or $20 \mu\text{mol m}^{-2} \text{s}^{-1}$.
 - Malnutrition is also a risk factor for eye damage and cataracts (FHF CleanFeed project 901131).
- *Risk factors in other species: environmental and nutritional factors are key to cataract formation.*

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 or prevalence and severity.



Science based knowledge

Risk factors for vertebral deformities:

- Subjecting the early life stages of ballan wrasse to inadequate nutrition, in particular due to the use of feed crumbles from hard, hot extruded diets which may be consumed by the stomachless larvae-juveniles may not be utilized enough, resulting in reduced growth, significantly lower levels of minerals in the body (such as phosphorus, calcium and magnesium) and multiple skeletal anomalies (Kousoulaki et al., 2018).
- Also poor quality live feed for larvae (Kjørsvik et al., 2014; Høyland, 2015).
- High incubation temperatures (the temperature optima lies between 10-15 °C, Shchepak, 2011).

Practical based knowledge

- Practical based experience suggests risk factors for vertebral deformities include high water current speed in the early tank rearing phase, leading to lordosis (Lekva and Grøtan, 2018).
- As stated above, vertebral deformities may also be due to nutritional problems or inappropriate rearing temperatures (Lekva and Grøtan, 2018).

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Snout and mouth damage

Snout damage can be due to e.g. handling and contact with 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). It is an established OWI in ballan wrasse (Treasurer and Feledi, 2014).



Science based knowledge

Risk factors and snout damage and mouth haemorrhaging:

- Pathogens such as *Tenacibaculum* spp. (Scholz et al., 2018).
- Inappropriate weaning diets can lead to head/jaw deformities in wrasse (Kousoulaki 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).*

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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 fish species e.g. Atlantic salmon (Noble et al., 2018; RSPCA, 2018a). However, as far as the authors are aware, there is limited information available on the risk factors that can contribute to opercular damage in ballan wrasse, or its severity.
- *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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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 (DO) saturations or gill problems (Nilsson, Stien, Iversen et al., 2018). It has been suggested as an OWI for ballan wrasse by Treasurer et al., (2018).



Science based knowledge

- Potentially valuable OWI if the fish are slow moving or the observer has good visibility (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).

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 incl. condition factor

Emaciation is a robust indicator of a welfare challenge in cleaner fish (Nilsen et al., 2014). Condition factor has also been used as an OWI for ballan wrasse (Treasurer and Feledi, 2014).



Science based knowledge

- Emaciation in ballan wrasse can be caused by poor nutrition and wrasse do need supplementary feeding during cage deployment to maintain their body condition as they may not get enough nutrition from eating lice alone (e.g. Skiftesvik et al., 2013) .
- With regard to certain weaning protocols (e.g. those involving Artemia and weaning onto dry feeds) the majority of fish starve if the weaning diet feed contains significant amounts of fish meal, in particular fish hydrolysates stabilized with the synthetic antioxidant ethoxyquin (Bogevik et al., 2015). This problem may be also be observed during later life stages if the fish meal used in the feed is of poor quality e.g. oxidized. The feed needs to contain significant amounts of high quality shrimp or krill meal.
- A reduction in condition factor has previously been used as an OWI in ballan wrasse (e.g. Skiftesvik et al., 2013).

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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, clumping behaviour
- Blood or scales in the water

Group based
OWIs



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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 such as 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 cleanerfish 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).**
- Dead fish should be collected as fast as possible to circumvent this. If possible the potential causes of mortality should also be identified.

Risk factors for mortalities:

- Nilsen et al., (2014) carried out a mortality mapping study in 2013 that reported ballan wrasse mortalities in sea cages were due to: sores/fin erosion (53%) or mechanical injuries (3%), with 37% due to other causes (8% of mortalities were also old/rotten). No mortalities were recorded as due to bacterial infections (unlike lumpfish).
- Specific health related risk factors for mortalities include: Atypical *Aeromonas salmonicida* (Rimstad et al., 2017; Scholz et al., 2018); *Vibrio anguillarum* (Rimstad et al., 2017; Scholz et al., 2018); VHSv (Rimstad et al., 2017); *Moritella viscosa* (Scholz et al., 2018); AGD (Scholz et al., 2018).
- Small wrasse may also be predated upon by salmon or trout. A high risk period for predation can be when the salmon or trout are fasted. This risk may be reduced by providing the wrasse with shelters (Rimstad et al., 2017).

RENSVEL OWI FACTSHEET SERIES: BALLAN WRASSE

Mortality rate continued

Science based knowledge continued

Risk factors for mortalities:

- Wrasse have a closed swim bladder, so any fish that are acclimated to deeper water in the net cage can die if they are brought to the surface too rapidly e.g. if the nets or the dead fish collector are lifted too fast (Treasurer and Feledi, 2014; Rimstad et al., 2017).
- Aggression has been reported in wrasse male broodstock during spawning, which can lead to scale loss and mortalities during the spawning season (Treasurer, pers. obs. reported in Treasurer et al., 2018).

Practical based knowledge

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

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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 others require samples from the fish to be sent to a laboratory for further analysis (LABWIs), see Nilsson, Stien, Iversen et al., (2018).



Science based knowledge

- The health status of ballan wrasse 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 wrasse mortalities can be related to health problems such as sores or fin rot (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 an 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: BALLAN WRASSE

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 ballan wrasse 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).



Science based knowledge

Appetite:

- A 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.
- A drop in appetite can be an indicator for potential health problems in wrasse including infections with *Aeromonas salmonicida* (Scholz et al., 2018); *Vibrio anguillarum* (Scholz et al., 2018); *Tenacibaculum* spp. (Scholz et al., 2018).
- Long term appetite problems lead to emaciation (see earlier section of these factsheets).
- 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). An assessor can carry out this check 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).

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.
- 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 cleaner fish (Treasurer et al., 2018).

RENSVEL OWI FACTSHEET SERIES: BALLAN WRASSE

Behaviour

Fish behaviour is a central tool in the OWI toolbox (see Nilsson, Stien, Iversen et al., 2018). Potential behavioural OWIs suitable for ballan wrasse include aggression, different types of swimming behaviour and clumping behaviour (Treasurer et al., 2018).



Science based knowledge

General information regarding their behaviour:

- Wrasse can become adapted to and tolerate exposed, energetic water conditions and habitats (Rimstad et al., 2017).
- Ballan wrasse are diurnal (Brooker et al., 2018).
- Wrasse > 10 grams must be provided with environmental enrichment in hatchery tanks (RSPCA, 2018a).
- Ballan wrasse can express escape /avoidance behaviours by actively swimming away from the perceived threat (e.g. Iversen et al., 2015). They can also express “clumping” behaviour where the fish form very tight high density groups.

Behaviour in the tank rearing phases:

- Water flow must not have a negative impact upon wrasse behaviour and the fish should express normal behaviour (RSPCA, 2018a).
- RENSVEL results suggest juvenile ballan wrasse (20-30 g) have a U_{crit} of 1.7 – 2.5 bl/s dependent upon DO saturation (Gaffney et al., in prep).
- Wrasse can exhibit clumping behaviour from around 40-50 days post hatch and this clumping behaviour can persist through the tank rearing phase (Lekva and Grøtan, 2018). It is thought to be a stress response in the absence of shelter (Lekva and Grøtan, 2018). Treasurer et al., (2018) have also suggested it is due to elevated stress levels and it has been suggested as a potential OWI by the same authors, especially if it happens regularly.
- Skiftesvik et al., (2014) reported that disturbances such as noise, unexpected shadowing over the tank, sudden changes in light and also feeding can trigger clumping behaviour in wrasse larvae and juveniles. They suggested reducing the fish’s exposure to disturbances, or keeping light diffuse and uniform.
- Aggression is not considered a problem in larval or juvenile rearing but has been reported in wrasse male broodstock during spawning, which can lead to scale loss and mortalities during the spawning season (Treasurer, pers. obs. reported in Treasurer et al., 2018).

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Behaviour continued

Science based knowledge cont.

Health impacts upon behaviour:

- Lethargy in wrasse can be due to infections with atypical *Aeromonas salmonicida* (Scholz et al., 2018); *Tenacibaculum spp.* (Scholz et al., 2018) or *Vibrio anguillarum* (Scholz et al., 2018).

Behaviour during sea cage deployment:

- The RSPCA (2018a) report that wrasse will often exhibit tight clumping or shoaling type behaviours when under duress.
- Small wrasse may also be predated upon by salmon or trout. A high risk period for predation can be when the salmon or trout are fasted. This risk may be reduced by providing the wrasse with shelters (EURL, 2016; Rimstad et al., 2017).
- Ballan wrasse prefer the sides of the net cage or the corners, irrespective of whether it's day nor night, and were also observed resting on or near the bottom of the cage (Leclercq et al., 2018).
- Wrasse adjust their swimming depths throughout the day, spending ca. 60% of their daytime activity at depths below 15 m and swimming shallower at night (Leclercq et al., 2018).
- In a study by Leclercq et al., (2018), wrasse distribution and swimming activity did not appear to be affected by the tidal cycle, although the farm site was considered to be shelter with a mean water flow of ca. 6 cm/s.

Practical based knowledge

- It has been reported that juvenile tank reared ballan wrasse will react to handling, grading or netting by resting immobile on the bottom of the tank for several minutes after the procedure (Leclercq et al., 2014).
- Practical experience suggests wrasse do need shelter for rest for periods and prefer artificial kelp type shelters (artificial kelp).
- 'Clumping' or 'balling' behaviour may lead to fin or epidermal injuries when the fish attempt to swim into the dense mass of other fish and get injured. This type of behaviour may lead to anoxic conditions within the clump of fish.

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Blood and scales in the water

Blood or scales in the water have been suggested as potential OWIs for Atlantic salmon by Nilsson, Stien, Iversen et al., (2018). Blood in the water is usually due to damage to the skin or gills and “red water” is evidence that the fish is injured. Scales floating in the water are an indicator of physical damage to the epidermis.



Science based knowledge

- These OWIs are 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 their cause or severity cannot be diagnosed without a more detailed investigation of the problem (Nilsson, Stien, Iversen et al., 2018).

RENSVEL OWI FACTSHEET SERIES: BALLAN WRASSE

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
- Lactate
- Glucose
- Osmolality
- Magnesium
- Chloride

Physiological
LABWIs and
OWIs



RENSVEL OWI FACTSHEET SERIES: BALLAN WRASSE

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 it is therefore classified as a LABWI.
- The acute stress profile of ballan wrasse is similar to Atlantic salmon (Treasurer et al., 2018; Espmark et al., 2019).
- In ~32g wrasse, swimming to exhaustion increased plasma cortisol levels 5-fold from 236 ± 25 nmol/l to 1233 ± 115 nmol/l (Gaffney et al., in prep; Espmark et al., 2019).
- Leclercq et al., (2014) also reported that when juvenile ballan wrasse were subjected to air exposure for 1 minute, plasma cortisol levels increased from 60.8 ± 5.5 ng/ml to 284.3 ± 26.7 ng/ml 30 minutes after exposure to the acute stressor. Levels were still significantly higher than the original pre-stressor levels for 1 – 2 hours post-stressor.
- Small wrasse (< 25g) seem to handle stress better than larger ballan wrasse (> 40g) (Virtanen, 2018; Espmark et al., 2019).
- Exposing wrasse to a daily stressor (lowering the water level, 264 kg/m^3) after 7-14 days will lead to a chronic stress load that will adversely affect welfare (Virtanen, 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).
- It 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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Lactate

Nilsson, Stien and Iversen et al., (2018) state “*Lactate is the product of anaerobic ATP production (glycolysis) in the cells, which occurs when oxygen is not available in sufficient amounts for the cells to utilise aerobic metabolism*”. However, it can also form under aerobic conditions (see Brooks, 2018). Lactate production can be driven by intensive exercise (e.g. Leclercq et al., 2014 and references therein).



Science based knowledge

- Elevated lactate levels have been widely used as a stress indicator in fish (Barton and Iwama, 1991) but they can be impacted upon by various other factors and as with glucose, they should not be benchmarked against a “standard level”, but measured e.g. before, during and after an operation (Nilsson, Stien, Iversen et al., 2018).
- Lactate is primarily an indicator of elevated levels of muscle activity (which can be due to stress). It can take a few hours to appear in the blood after the fish has been subjected to stress (see Nilsson, Stien, Iversen et al., 2018).
- Resting basal levels of lactate in an unstressed ballan wrasse are 1 – 2 mmol/l (Espmark et al., 2019).
- In ~32g wrasse, swimming to exhaustion increased plasma lactate levels 2.2-fold from 1.31 ± 0.1 mmol/l to 2.85 ± 0.4 mmol/l (Gaffney et al., in prep; Espmark et al., 2019).
- Exposing wrasse to a daily stressor (lowering the water level, 264 kg/m³) led to lactate levels of 4 – 10.5 mmol/l (Virtanen, 2018; Espmark et al., 2019).
- Leclercq et al., (2014) reported that when juvenile ballan wrasse were subjected to air exposure for 1 minute, lactate levels significantly peaked 30 mins post-stressor.
- Lactate appears to be a good indicator for measuring acute stress in ballan wrasse, and may also be a potential indicator for chronic stress in “large” ballan wrasse (> 40g) but this needs further study (Virtanen, 2018; Espmark et al., 2019).

Strengths and weaknesses of the OWI

- Easily accessible hand-held instruments are a validated and inexpensive tool for monitoring lactate levels (Sopinka et al., 2016) and are both robust and easy to use out on the farm. Lactate 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 lactate 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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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 they can be impacted upon by various other factors such as feed ingredients, satiation levels etc. Glucose levels should not be benchmarked against a “standard level”, but measured e.g. before, during and after an operation (Nilsson, Stien, Iversen et al., 2018).
- In ~32g wrasse, stress from swimming to exhaustion increased glucose levels increased from 2.4-fold from 1.46 ± 0.1 mmol/l to 3.44 ± 0.3 mmol/l (Gaffney et al., in prep; Espmark et al., 2019).
- Leclercq et al., (2014) reported that when juvenile ballan wrasse were subjected to air exposure for 1 minute, glucose levels increased nearly 90% from basal levels to 2.2 ± 0.1 mmol/l 30 minutes after exposure to the stressor. Levels returned to pre-stressor levels within 1 hour.

Strengths and weaknesses of the OWI

- Easily accessible hand-held instruments are a validated and inexpensive tool for monitoring 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 glucose as an indicator of reduced welfare without further information, as levels can be influenced by numerous other factors (Nilsson, Stien and Iversen et al., 2018).

RENSVEL OWI FACTSHEET SERIES: BALLAN WRASSE

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 ballan wrasse have an osmolality that is around 350-360 mOsm / kg (Virtanen, 2018; Espmark et al., 2019).
- Osmolality also appears to increase during both acute and chronic stress exposure and is most pronounced in “large” ballan wrasse (> 40g) (Virtanen, 2018; Espmark et al., 2019).

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: BALLAN WRASSE

Magnesium

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



Science based knowledge

- Results from RENSVEL suggest an unstressed ballan wrasse has a plasma magnesium level < 2 mmol/L (Virtanen, 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 (Virtanen, 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 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: BALLAN WRASSE

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 ballan wrasse has a plasma chloride level that is ca. 140 – 145 mmol/L (Virtanen, 2018; Espmark et al., 2019).
- Plasma chloride levels also appear to increase during both acute and chronic stress exposure and this response is most pronounced in “large” ballan wrasse (> 40g), suggesting chloride levels are a good indicator of disturbed ion balance under stress (Virtanen, 2018; Espmark et al., 2019).

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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