FEED X ANIMAL WELFARE

A report prepared
by FAI Farms Ltd.

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EXECUTIVE SUMMARY

FEED X aim to enable 10% of the global feed industry to transition to sustainably sourced oil and protein ingredients. As salmon and shrimp feed typically contains both animal byproducts and soy such as fishmeal, fish oil and soybean, this transition is liable to influence the contents of salmon and shrimp feed.

This report provides a practical framework in which to measure the welfare of salmon and shrimp in the context of novel feeds. A welfare outcome measure (WOM) framework was identified as the most objective way to directly assess animal welfare. Ethical scopes of the salmon and shrimp supply chains were carried out to identify where the key welfare risks lie and suggested WOM risk assessment frameworks for salmon and shrimp were described. The WOMs in these frameworks can be used to measure welfare in feed trial or commercial settings and provide a holistic view of animal welfare.

In commercial settings, WOM data should be collected from an entire lifecycle of standard production to establish a baseline level for each measure. Alternatively, in trial settings, groups fed standard and novel feeds can be grown alongside each other to provide truly comparable results. The environments of the tanks and care of the fish should be identical apart from the variable of interest (feed type). Nevertheless, environmental measures (e.g. water quality, temperature, salinity) should be recorded from each tank on a regular basis so that any deviations are known. The treatment of the fish should be as similar to that which would be expected in a commercial environment as possible so the results are commercially relevant.

The frameworks provided here do not take into account the welfare of other animals in the salmon and shrimp supply chain. Switching to novel proteins may mean increasing insect farming and soybean production which may pose welfare risks as well as benefits. These influences on animal welfare should also be considered alongside the direct risks to salmon and shrimp.

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1. AIMS

1.1. FEED X AIMS

The focus of FEED X is to source, test, finance and scale alternative feed ingredients into the global feed industry, focusing on salmon and shrimp feed. FEED X aims to enable 10% of the global feed industry to transition to sustainably sourced oil and protein ingredients. As salmon and shrimp feed typically contains fishmeal, fish oil and soybean, this transition is liable to influence the contents of salmon and shrimp feed.

1.2. REPORT AIMS AND STRUCTURE

The aim of this report is to provide a framework in which to measure the welfare of salmon and shrimp in the context of novel feed ingredients. The report will take into account key welfare risks throughout the salmon and shrimp supply chain alongside welfare risks specific to novel feed ingredients. FEED X identified three categories to be de-risked. An ethical risk matrix identifies the key potential animal welfare risks and benefits associated with each category (Table 1). Section 2 (Method) describes the background to our 'Welfare Outcome Measure' (WOM) framework that can be used in a broad sense to measure animal welfare in salmon and shrimp. Sections 3 and 4 (Risk assessment frameworks) outlines our WOM framework for salmon and shrimp, including species-specific measures to enable holistic welfare assessment. Section 5 (Discussion) includes a discussion of how to collect and analyse the data gained from the risk assessment frameworks outlined in section 3 along with a discussion of input measures, further analysis and the animal welfare impact of sustainable feeds throughout the broader supply chain. Section 6 (Conclusions) concludes the report and provides recommendations for innovators using a WOM framework to measure salmon and shrimp welfare.

1.3. ETHICAL RISK MATRIX

Table 1: The potential animal welfare risks and benefits associated with the three categories to be de-risked

Categories to be de-risked	**Solution description**	**Priority solutions**	**Animal welfare risks**	**Animal welfare benefits**
Feed ingredient categories	Nutritional solutions creating net positive environmental effects	Protein from: food by-products (e.g. land animals, cassava), insects, fermentation, energy production	Welfare risks associated with insect farming Ensuring alternative protein sources contain the correct nutritional profile and digestibility as fishmeal so as to not compromise salmon and shrimp growth and health	Reduction in fish production and/or trawling for fishmeal leading to decreased welfare risks associated with fish farming and trawling (including risks to non-target organisms)
	Nutritional solutions creating health effects equal to or greater than fatty acids	Oil from: microbes, seaweed, other plants	Ensuring alternative oil sources contain the correct nutritional profile and digestibility as fish oil so as to not compromise salmon and shrimp growth and health Risks to non-target animals associated with increased seaweed production	Reduction in fish production and/or trawling for fish oil leading to decreased welfare risks associated with fish farming and trawling (including risks to non-target organisms) Benefits to non-target animals associated with increased seaweed production
	Nutritional solutions using inputs that create environmentally restorative effects	Protein from seaweeds or insects fed on seaweeds	Welfare risks associated with insect farming Ensuring alternative protein sources contain the correct nutritional profile and digestibility as fishmeal so as to not compromise salmon and shrimp growth	Reduction in fish production and/or trawling for fishmeal leading to decreased welfare risks associated with fish farming and trawling (including risks to non-target organisms) Benefits to non-target animals associated with

			and health Risks to non-target animals associated with increased seaweed production	increased seaweed production
Feed production categories	Technology solutions creating net positive effects	Solar and wave power, packaging waste, energy waste, food waste	N/A	Renewable energy sources can decrease the loss of wild animal habitats associated with climate change. However, depending upon their location they can have an adverse (wind farms affect raptors, migratory birds and bats), or positive affect (new marine habitats as trawler free zones).
Feed performance categories	Technology solutions increasing the health, survival and growth performance of the fish/shrimp	Integrated technologies incorporating digital monitoring to increase the health, survival and growth performance of the fish/shrimp	New technologies must be introduced alongside traditional methods of monitoring (i.e. human assessment) so that health and welfare issues are not overlooked	New technologies have the potential to enable quicker detection of health and welfare issues leading to more rapid treatment
	Integrated information systems solutions increasing feed waste efficiencies	Systems (digital or otherwise) that use co-products including sludge water from pens or ponds	N/A	N/A
	Innovations moving the whole farm production foot print off land	Unknown innovations	If a disease challenge enters a recirculating aquaculture system, it may be more difficult to abolish	Decreased risk of disease/sea lice transfer from farmed to wild fish Easier control of the environment in recirculating aquaculture systems may lead to improved welfare via better water quality and disease control

2. METHOD

2.1. SUMMARY

Traditionally, ‘input’ measures have represented the main way in which farmers provide for good welfare. ‘Input’ measures include anything that is put into a farming system e.g. feed type, stocking density, stockmanship, animal breed etc. Although these measures are important to protect animals from practices which are widely recognised as leading to poor welfare (e.g. battery cages for laying hens), they do not directly measure the experience of the individual animal and often fail to capture the full effect of a system upon the animal’s welfare. Welfare outcome measures (WOMs) provide an objective tool to measure welfare, regardless of the production system, breed, climate, and so on. The data can be used to benchmark across farming operations, locate best practice and identify areas that can be improved within supply. WOM assessment can provide a scientific basis to support the adoption of certain input measures rather than relying on our anthropomorphic judgement of what we believe to be best for animals. WOM assessment provides valuable feedback for farmers to improve the welfare and profitability of their animals.

2.2. BACKGROUND

The importance of using WOMs to assess farm animal welfare has been highlighted by researchers (Hewson 2003; Webster 2005; Main 2009; Grandin 2010; Grandin 2015) and is recognised at international level by organisations such as the World Organisation for Animal Health (OIE) (World Organisation for Animal Health, OIE 2018). One of the first animal-based scoring systems for evaluating the welfare of cattle and pigs at slaughter was described by Grandin (1998). Grandin (1998) developed a protocol to assess animal welfare during handling, stunning and slaughter. This protocol was subsequently adapted and rolled out by McDonald’s Corporation when they started auditing U.S. beef and pork slaughter plants in 1999. Over the next five years improvements were seen in WOMs during handling and stunning in audited plants (Grandin 2006).

Key WOMs at slaughter and on-farm have been defined by the European Union (EU)-funded Welfare Quality® project (hereafter, Welfare Quality®). Welfare Quality® began in 2004 and comprised a partnership of 40 institutions in Europe and, after 2006, four in South America (Blokhuis et al. 2010). The project was funded for five years and, aimed to develop a standardised system for the assessment of animal welfare, focusing on pigs, cattle and chickens. The project realised that resource- or management-based measures (input measures, e.g. stocking density, housing system, feeding strategy) provide only partial information about animal welfare (Blokhuis et al. 2010). It was also noted that WOMs are sensitive to variations in farm management and specific system-animal interactions, meaning that WOMs can provide more detail than input measures. After expert consultation and discussion with members of the public, stakeholders and external experts, Welfare Quality® came up with four principles for good welfare with twelve associated criteria to form the basis of assessment protocols (Table 2). Welfare Quality® researchers developed standardised, primarily animal-based measures to check compliance of farms or slaughterhouses with the 12 welfare criteria. Where no WOM was available to check a specific aspect, or if it was not sufficiently sensitive or reliable, measures of the resources or management would be used to determine as much as possible whether or not a given welfare requirement was being met. The measures had to be both valid for determining something about the animals’ welfare and practical to collect on-farm or slaughter. Welfare Quality® outputted a welfare assessment protocol for pigs, cattle and chickens. Each protocol combines 30-50 input measures and WOMs to holistically assess animal welfare and a method to standardise scoring to produce an overall assessment of animal welfare on that particular unit (Botreau et al. 2009).

Table 2: The principles and criteria that are the basis for Welfare Quality ® assessment protocols

Welfare principles	**Welfare criteria**	
Good feeding	1	Absence of prolonged hunger
	2	Absence of prolonged thirst
Good housing	3	Comfort around resting
	4	Thermal comfort
	5	Ease of movement
Good health	6	Absence of injuries
	7	Absence of disease
	8	Absence of pain induced by management procedures
Appropriate behaviour	9	Expression of social behaviours
	10	Expression of other behaviours
	11	Good human-animal relationship
	12	Positive emotional state

In 2012, the European Food Safety Authority (EFSA 2012) provided an independent review of the use of WOMs to assess the welfare of dairy cows, pigs and broiler chickens. The report concluded by describing WOMs as 'the most appropriate indicators of animal welfare' and highlights the importance of the systematic collection of standardised field data on animal-based measures and subsequent availability in well-defined databases. This should enable researchers to select the most appropriate measures, or combinations of measures, from the 'toolbox' of available measures to answer the specific welfare question being asked.

Using Welfare Quality® as a reference, a system for farm animal welfare outcome assessment has been developed for major farm animals in the UK (Assurewel 2015; Assurewel 2018). WOM assessment is now used in farm assurance schemes (Main et al. 2012a; Main et al. 2012b) and has resulted in demonstrable improvements to animal welfare, including a reduction of feather loss in the laying hen industry (Mullan et al. 2016).

It has been acknowledged that the amount of time required to carry out a WOM assessment such as Welfare Quality® as part of a farm assurance audit limits feasibility (Knierim & Winckler 2009; de Vries et al. 2013). However, some WOMs can be collected at slaughter. The collection of WOMs at slaughter is beneficial because abattoirs process a large number of animals in a relatively short timeframe and scoring can be done in a central location by a few trained individuals, or even via automated machinery. Slaughter WOMs can be valid measures of welfare on the farm of origin, transport, lairage or at the time of slaughter (Llonch et al. 2015). There are six main criteria to consider when deciding whether a WOM is appropriate to be included in a welfare assessment (Table 3).

Table 3: Criteria for inclusion of welfare outcome measures (WOMs) in an animal welfare assessment (adapted from EFSA 2012)

Criteria	**Explanation**
Validity	WOMs should accurately measure (an aspect of) the welfare state of an animal. The relationship between a WOM and the actual welfare state of the animal should be based on published science (or at least expert opinion).
Practicality	WOMs should be practical and feasible to collect i.e. they should not involve too much time and should not be too costly.
Robustness	WOMs should not be affected by external factors that are not related to the welfare of the animal e.g. time of year, weather (unless these are linked to the welfare of the animal).

High intra- and inter-observer reliability	WOMs show low variability when repeatedly measured by the same observer over time or by different observers. Observers should be trained to the “gold standard” for the measure and training should be repeated at regular intervals so that observers are “recalibrated”.
Representative within animals	Where the measures vary over time, e.g. time of day or interval since a particular event, then the measures should be based on a representative time sample.
Representative between animals	Where WOMs are taken from only a sample of animals in the unit, WOMs should be taken from an unbiased, representative sample (in terms of sex, body size, age etc.) The way in which the sample should be chosen should be specified.

2.3. WOMS IN AQUACULTURE

As with terrestrial animals, animal-based WOMs can inform us about the welfare state of aquaculture. Stein et al. (2013) outlined potential WOMs for caged Atlantic salmon which were selected using a semantic modelling approach (first introduced by Bracke et al (1999a,b,c)) whereby the authors came up with a list of welfare needs for salmon based on available scientific literature and then linked these needs to input measures and welfare indicators, including animal-based WOMs and input measures such as the salinity and oxygen levels of the water. The outputs of the welfare indicators indicate whether the welfare needs are catered for. This formed the Salmon Welfare Index Model (SWIM 1.0), which was purposed to be a tool primarily for fish farmers to assess welfare in sea cages. The model also suggests weightings for each indicator discussed according to their predicted welfare impact on the fish. The welfare of the fish is calculated as an aggregated score from 0 (worst) to 1 (best).

A recent report (“FISHWELL”) indicates WOMs for salmon that are suitable for use on farms (termed operational welfare indicators) and WOMs that require access to a laboratory or other analytical facilities to provide useful information (termed laboratory-based welfare indicators) (Noble et al. 2018). Noble et al. (2018) suggest 13 welfare needs for salmon based on four categories (Table 4). To provide a holistic view of salmon welfare, WOMs should be able to inform us as to whether all of the 13 welfare needs are/have been catered for. Noble et al. (2018) describe a range of WOMs that could be used to measure the welfare of salmon, some of which can be carried out at the group level so do not require handling or disturbance of the fish. Some measures are taken at the individual fish level which, in most cases, involve handling and examination of individuals. Individual measures would be more suitable to be taken at the time of slaughter.

Table 4: Welfare needs of farmed salmon (adapted from Noble et al. 2018)

Category	**Welfare Needs**
Resources	Feeding and nutrition
Environment	Respiration
	Osmotic balance
	Thermal regulation
	Good water quality
Health	Body care
	Hygiene
	Safety and protection
Behaviour	Behavioural control
	Social contact
	Rest
	Exploration
	Sexual behaviour

2.4. FAI WOM TEMPLATE

A framework for WOM collection has been developed by FAI Farms (Bright et al., in prep) and is currently used to collect WOMs from a variety of farmed animals in a commercial setting (Table 5). This framework is to be viewed as a guide to drawing up WOM lists ensuring the main categories are covered and is relevant for **all species**. There will be a relatively wide variation in the types of welfare issues being measured. For example, specific measures directly related to a welfare issue such as the presence of sea lice infestation, and 'iceberg indicators' such as percentage mortality, which can reflect a variety of underlying welfare issues (EFSA 2012).

Because the WOM framework aims to take a holistic view of animal needs, this framework is suitable for measuring overall animal welfare. By using a WOM framework to assess salmon and shrimp welfare linked to novel feed ingredients, the overall welfare of the animals can be taken into account. Another advantage of using a WOM framework is that it can be utilized in both a commercial and trial setting.

The WOMs chosen will be dependent on the setting in which the research is carried out. In a trial environment with dedicated researchers, it is generally possible to include a longer list of WOMs than would be possible commercially. Commercially, due to time and personnel restraints, it is important to consider the practicality of the measures collected. The best way to do this is to work with producers/processors from the outset when deciding on the WOM list. Some measures may not be practical for collection commercially, especially those that take more time or are more difficult to collect. If producers and/or processors are being asked to collect novel WOMs, they will need to be trained on how to collect the measures and this training should be repeated if they are collected over a significant time period (see Table 3). In a commercial setting, WOM data would normally be collected for 6-12 months to establish a baseline level for each WOM and a basis from which to identify welfare issues within a supply. Twelve months of data is important when a WOM may be influenced by seasonality. In the case of animals with a long, variant life-cycle such as salmon, differences in life stages should also be taken into consideration. For example, some measures may be affected by the change in environment when the fish are moved from freshwater to sea cages. Such variation is not a problem for WOM assessment as long as it is taken into account, and 'like stages' are compared with 'like stages' or a whole cycle including both fresh- and salt-water stages is averaged and compared with another average cycle

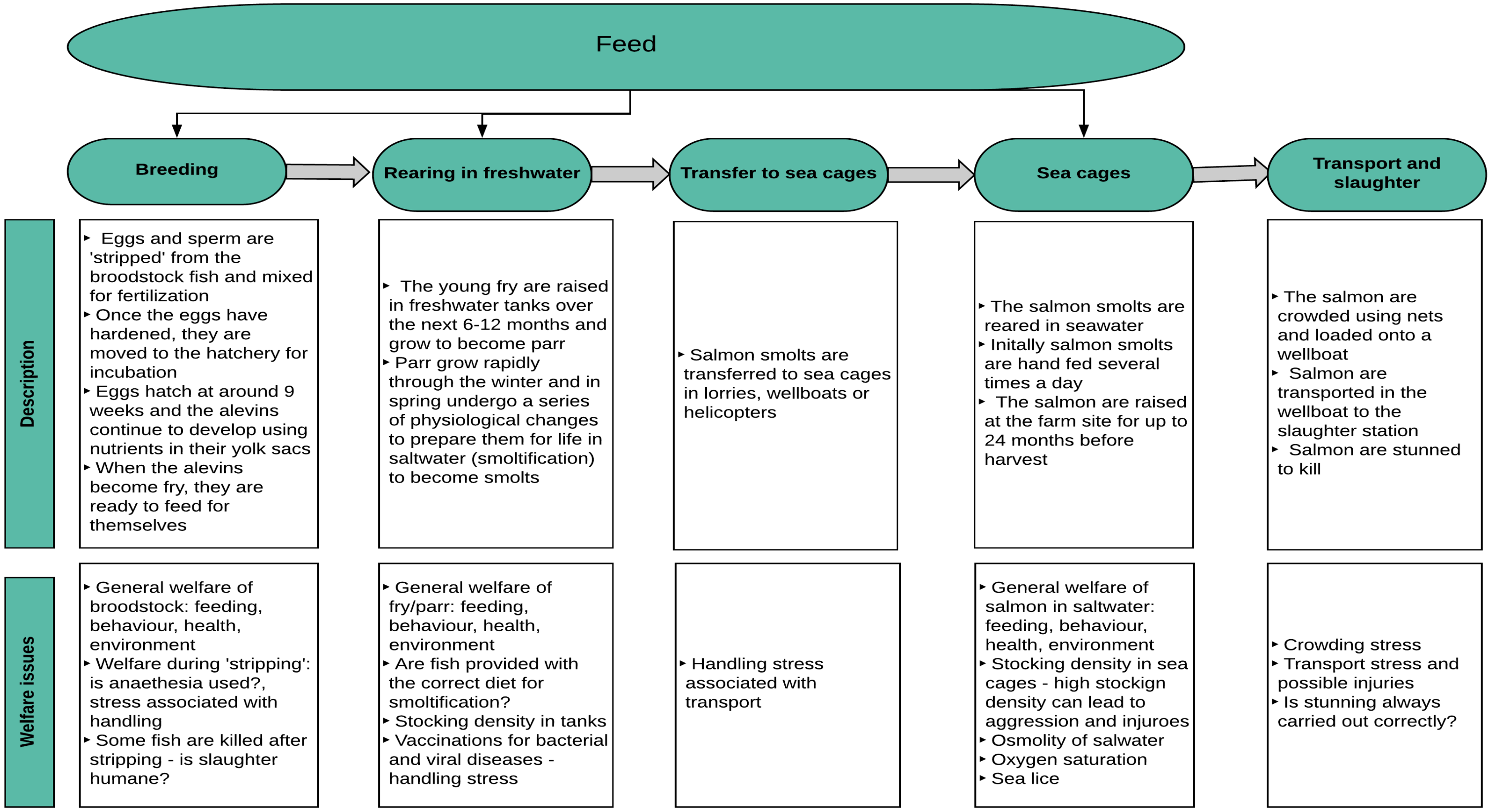
Table 5: WOM framework developed for all species (taken from Bright et al., in prep). See Table 6 and Table 7 below for more detail regarding how these are manifested in Salmon and Shrimp farming context.

Category	Example Measures
Liveability	Percent mortality (including culls)
	Percent dead on arrival
Disease	Percent PMI rejects / condemnations
	Antimicrobial use
	Notifiable disease status
Injury	Percent of animals with wounds
	Percent of animals with breaks/bruising
	Percent of animals with bruising
	Percent with mutilations
Mobility	Mobility score / Leg culls
Behaviour	Behaviour/activity measure (no current practical measure for continuous data collection)

3. RISK ASSESSMENT FRAMEWORK: SALMON

3.1. SCOPING PHASE

An ethical scope of the salmon supply chain was carried out in order to map where the key welfare risks lie (Figure 1).



	Breeding	Rearing in freshwater	Transfer to sea cages	Sea cages	Transport and slaughter
Description	‣ Eggs and sperm are 'stripped' from the broodstock fish and mixed for fertilization ‣ Once the eggs have hardened, they are moved to the hatchery for incubation ‣ Eggs hatch at around 9 weeks and the alevins continue to develop using nutrients in their yolk sacs ‣ When the alevins become fry, they are ready to feed for themselves	‣ The young fry are raised in freshwater tanks over the next 6-12 months and grow to become parr ‣ Parr grow rapidly through the winter and in spring undergo a series of physiological changes to prepare them for life in saltwater (smoltification) to become smolts	‣ Salmon smolts are transferred to sea cages in lorries, wellboats or helicopters	‣ The salmon smolts are reared in seawater ‣ Initally salmon smolts are hand fed several times a day ‣ The salmon are raised at the farm site for up to 24 months before harvest	‣ The salmon are crowded using nets and loaded onto a wellboat ‣ Salmon are transported in the wellboat to the slaughter station ‣ Salmon are stunned to kill
Welfare issues	‣ General welfare of broodstock: feeding, behaviour, health, environment ‣ Welfare during 'stripping': is anaethesia used?, stress associated with handling ‣ Some fish are killed after stripping - is slaughter humane?	‣ General welfare of fry/parr: feeding, behaviour, health, environment ‣ Are fish provided with the correct diet for smoltification? ‣ Stocking density in tanks ‣ Vaccinations for bacterial and viral diseases - handling stress	‣ Handling stress associated with transport	‣ General welfare of salmon in saltwater: feeding, behaviour, health, environment ‣ Stocking density in sea cages - high stockign density can lead to aggression and injuroes ‣ Osmolity of salwater ‣ Oxygen saturation ‣ Sea lice	‣ Crowding stress ‣ Transport stress and possible injuries ‣ Is stunning always carried out correctly?

Figure 1: Ethical scope of the salmon supply chain from breeding to slaughter. The upper boxes provide a brief description of what happens at each state of the salmon life cycle. At all stages, the welfare categories outlined in Table 5 will be influential, however specific welfare issues at each stage of the salmon life cycle are discussed in the lower boxes.

3.2. WOM IMPLEMENTATION

Table 6 outlines an example WOM list for salmon. The WOMs are described in detail below. In blue highlight, are measures considered significant welfare risks in the development of novel protein feeds.

Table 6: Example WOM list for salmon

Category	Measure	Unit of measurement	Measurement details	Inclusion
Liveability	**Mortality**	%	% of fish that died (e.g.at end of seawater production/harvest)	All
	Ineffective stun	%	% of fish with ineffective first stun	All
Disease	**Sea lice count**	Average sea lice count	Average (adult female) sea lice count per fish	Sample
	Medicine use	No. treatment courses	Total number of medicinal treatments per cage	All
	Non-medicinal treatments	No. treatments	Total number of non-medicinal treatments per cage	All
	Condition Factor	Average Condition Factor	Average Condition Factor per cage	Sample
	Gill score	Average gill score	Average gill score per cage (0-5)	Sample
	Gut morphology	Score	Six-parameter score	Sample
Injuries	**Skin condition**	%	% fish with a skin condition (descaling, wounds)	Sample
	Fin condition	%	% fish with a fin condition (dorsal, pectoral, pelvic, tail)	Sample
	Vertebral deformities	%	% fish with deformed vertebrae	Sample
	Eye condition	%	% fish with an eye condition (damage, loss)	Sample
	Snout condition	%	% fish with a snout condition (damage, injury)	Sample
Mobility & Behaviour	**Stocking density at site**	Kg/m^3	Max kg/m^3 at peak production	All
	Crowding incidents	Number	Total number of crowds during production	All

LIVEABILITY

Mortality rate is perhaps the most commonly used WOM (Noble et al. 2018). High mortality rates indicate that there is a welfare problem. However, it is not possible to discern what has caused the increase in mortality rate without collecting data on other measures. A low mortality rate does not necessarily mean good welfare as many issues such as disease, injuries and behavioural disturbances may decrease welfare without causing death.

Ineffective stun means that an animal has not sufficiently lost consciousness to be insensible to pain. Salmon may be stunned via an electric current or a percussive blow to the head. The general principle of electric stunning is to pass enough current through the brain to cause an epileptic-like fit. A percussive blow to the head may be administered manually or by an automated stunning machine and disrupts the normal electrical activity of the brain causing dysfunction and/or destruction and impaired blood circulation. Stunning results in immediate unconsciousness and insensibility to pain. Unconsciousness is tested via methods such as eye roll reflex, breathing and equilibrium test (Humane Slaughter Association 2016).

DISEASE

Sea lice count is an important measure of welfare as salmon lice infestations lead to tissue damage, a stress response, reduced appetite, changes to the gills and skin, a delayed healing response, osmotic problems and death (Bowers et al. 2000; Finstad et al. 2000; Costello et al. 2006). Infections with larger numbers of sea lice negatively affects swimming performance at high current velocities (Bui et al., 2016) and can be lethal in juveniles.

Medicine use should be recorded alongside other WOMs as using excessive medicine can 'cover up' a poor system. However, using excessive medicine, especially antibiotics, is not a sustainable option. It is therefore important that medicine use is considered alongside other WOMs to ensure that the fish are not showing WOM values indicative of good welfare because medicine use is excessive.

Non-medicinal treatments include sea lice skirts, thermal treatments, snorkels and cleaner fish. These are treatments used to control sea lice. The use of non-medicinal treatments is not necessarily bad for welfare but the use of these should be noted in conjunction with sea lice count and medicine use. NB if cleaner fish are used as a non-medicinal treatment, the WOM for salmon need to be applied to cleaner fish.

Condition factor (RISK FACTOR) or 'K' (Equation 1) is a measure of the nutritional status of the fish. The K value takes into account the mass and fork length of the fish. For adult salmonids, K values usually fall in the range 0.8 to 2.0 with 0.8 representing a poor fish and 2.0 representing a very large fish. A poor condition factor may represent poor welfare for a number of reasons such as; poor appetite, poor nutritional quality of feed, diseases and stress (Stein et al. 2013). Therefore, it is important that condition factor is considered in conjunction with other WOMs to clarify the reason for a poor condition factor. Generally, K decreases during winter and spring, and increases during summer and autumn (Stein et al. 2013). Therefore, it is important that comparisons are made between fish measured at the same time of year.

$$K = \frac{\text{mass}\ \ (\text{g}) \times 100}{\text{fork length}\ \ (\text{cm})^3}$$

Equation 1: Condition Factor (K) for salmonids

Gill score is a measure of amoebic gill disease (AGD), which is an important disease affecting farmed salmon caused by attachment of marine amoeba, *Paramoeba perurans* to the gill. AGD is scored on a 0-5 scale (Taylor et al. 2016). AGD can lead to high mortalities and is related to temperature and salinity stress (Taylor et al. 2016). Clinical signs attributed to AGD include anorexia, respiratory distress, flared opercula and lethargy (Mitchell & Rodger 2011).

Gut morphology (RISK FACTOR) is altered by intestinal disorders in salmon. The inclusion of soybean meal (SBM) in the diet of salmon can induce an inflammatory response of the distal intestinal mucosa, known as SBM-induced enteritis (Uran 2008). This disorder can impact feed digestibility and intestinal immunity (Nayak

2010). There are species-specific differences with Chinook salmon being more susceptible than Atlantic salmon, and pink salmon showing no signs of SBM-induced enteritis at 20% SBM inclusion (Booman et al. 2018). Inclusion of high (>45%) levels of faba bean protein concentrate have also been shown to cause gut inflammation in Atlantic salmon. Mixed plant-protein diets (including SBM and faba bean protein concentrate) have been shown to induce less extensive changes in the gut transcriptome than single-protein diets (Krol et al 2016). Enteritis can lead to impaired growth and increased risk of secondary diseases (Uran 2008). These secondary impacts of intestinal disorders should be identifiable within the on-farm/slaughter welfare outcome measures if it is not possible to collect data on gut morphology. However, gut morphology is included as a suggested additional lab-based measure due to its importance to fish health in the context of novel feeds. A six-parameter scoring system has been developed to score the intensity of enteritis based on gut morphology (Uran 2008).

INJURIES

Skin condition is an important measure of welfare because the integrity of the skin-scale complex provides a relatively impermeable barrier to water and electrolytes (Stein et al. 2013). Epidermal damage such as scale loss, wounds and ulcers can therefore result in a loss of body water and altered ion balance, which produces osmotic stress that may be life threatening (Bouck & Smith 1979). Factors associated with a high risk for mechanical damage to the skin include transport, sorting, vaccination, pumping, strong currents and high densities of fish, jelly fish burns, parasites, attack from other fish and predators (Noble et al. 2012). Poor skin condition can lead to decreased growth and increased disease susceptibility (Noga 2000), therefore it is important to consider skin condition alongside other WOMs. Also, poor skin condition can be a sign of an underlying viral or bacterial infection so may indicate decreased immunity.

Fin condition (RISK FACTOR) is measured as a factor in salmon quality grading as it is believed to reflect the general physiological condition or health of the fish. Fin damage can be painful, present a route of infection and may negatively affect swimming ability (Turnbull et al. 1996; Noble et al. 2012). Poor fin condition is correlated with a high stocking density, poor water quality, decreased condition factor and increased plasma glucose and cortisol levels (Turnbull et al. 2005; Adams et al. 2007). There are numerous risk factors for poor fin condition including husbandry practices such as pumping, construction material used in tanks, light regime and stocking density (Noble et al. 2012). Ration size may affect fin condition due to competition and increased levels of aggression, as can underfeeding (Moutou et al. 1998). Numerous studies have also reported that diet formulation, such as the choice of fishmeal versus krill as a protein or lipid source, can increase fin damage (Lellis and Barrows 1997).

Vertebral deformities (RISK FACTOR) are commonly associated with farmed salmonids (Noble et al. 2018). There is an array of potential risk factors for vertebral deformities including various nutritional factors (Dabrowski et al. 1990; Cahu et al. 2003; Gorman and Breden 2007). If a feed is lacking certain minerals, it is possible that vertebral deformity will ensue making vertebral deformities an important WOM to measure in feed trials. However, vertebral deformities are also associated with other factors such as infectious disease, the temperature eggs are harvested as, water quality and environmental pollution (Noble et al. 2018), therefore it is important to consider this WOM alongside other welfare and environmental measures to establish causation.

Eye condition (RISK FACTOR) such as cataracts have been linked to nutritional deficiencies as well as osmotic balances and water temperature fluctuations, parasitic infections in the eye, toxic factors, ultraviolet radiation, oxidative stress to the lens fibre, genetic predisposition with rapid growth and rapid change in water salinity (reviewed in Bjerkås and Sveier 2004). Specifically, cataract prevalence in farmed Atlantic salmon has been related to histidine deficiency in salmon feed (Breck et al. 2003, 2005; Waagbø et al. 2010) associated with the removal of blood and bone meal from the feed and also using more vegetable oil in salmon feed (Waagbø et al. 2003; Bjerkås and Sveier 2004). Mechanical injuries are also associated with eye damage (Noble et al. 2018).

Opercula (snout) deformities (RISK FACTOR) have been linked to dietary deficiencies (Baeverfjord et al. 1998) as well as traumatic injuries during highly competitive feeding (Noble et al. 2018). However, opercula deformities can also occur due to excessive cartilage deposition. Damage to the opercula is associated with increased mortality rates, susceptibility to diseases and therefore reduced animal welfare (Eriksen et al. 2007).

MOBILITY AND BEHAVIOUR

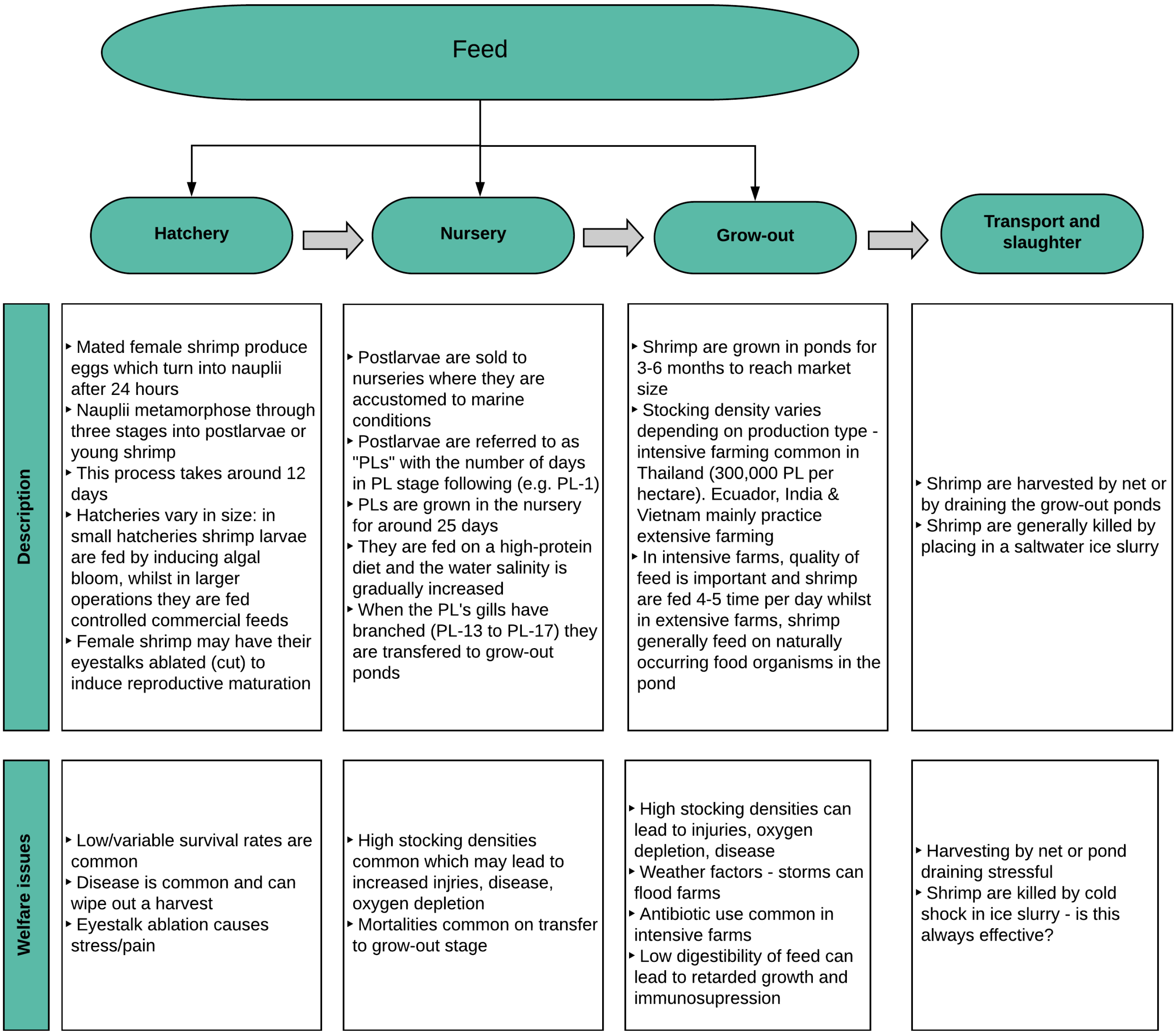
Stocking density is measured as the maximum stocking density at peak production and is expressed in kg/m^3. Although technically an input measure, stocking density is often measured as a proxy for a behavioural measure when collecting WOM data on farms. This is because at a very high stocking density, it is highly unlikely that salmon would be free to express natural behaviours and move around their environment unhindered and high stocking densities are associated with low welfare scores in salmon (based on body condition, fin condition and plasma concentrations of glucose and cortisol) (Turnbull et al. 2005). However, there exists some conflicting data that suggests that salmon welfare is compromised when stocking density is both too low and too high (Adams et al. 2007). At a high stocking density, welfare may be compromised due to a number of factors such as reduced water quality and increased feeding competition. Therefore, during feed trials, the maximum stocking density should be recorded to control for any influence of stocking density on fish welfare.

Crowding incidents such as when fish are crowded into a corner before grading or slaughter have been shown to cause stress, as measured by increased plasma cortisol concentration (Veiseth et al. 2006). Therefore, the number of crowding incidents should be recorded as this may influence WOMs.

4. RISK ASSESSMENT FRAMEWORK: SHRIMP

4.1. SCOPING PHASE

An ethical scope of the shrimp supply chain was carried out in order to map where the key welfare risks lie. Information on risks was informed by a Seafood Market Opportunity report by PwC (2018) (Figure 2).



	Hatchery	Nursery	Grow-out	Transport and slaughter
Description	Mated female shrimp produce eggs which turn into nauplii after 24 hours Nauplii metamorphose through three stages into postlarvae or young shrimp This process takes around 12 days Hatcheries vary in size: in small hatcheries shrimp larvae are fed by inducing algal bloom, whilst in larger operations they are fed controlled commercial feeds Female shrimp may have their eyestalks ablated (cut) to induce reproductive maturation	Postlarvae are sold to nurseries where they are accustomed to marine conditions Postlarvae are referred to as "PLs" with the number of days in PL stage following (e.g. PL-1) PLs are grown in the nursery for around 25 days They are fed on a high-protein diet and the water salinity is gradually increased When the PL's gills have branched (PL-13 to PL-17) they are transfered to grow-out ponds	Shrimp are grown in ponds for 3-6 months to reach market size Stocking density varies depending on production type - intensive farming common in Thailand (300,000 PL per hectare). Ecuador, India & Vietnam mainly practice extensive farming In intensive farms, quality of feed is important and shrimp are fed 4-5 time per day whilst in extensive farms, shrimp generally feed on naturally occurring food organisms in the pond	Shrimp are harvested by net or by draining the grow-out ponds Shrimp are generally killed by placing in a saltwater ice slurry
Welfare issues	Low/variable survival rates are common Disease is common and can wipe out a harvest Eyestalk ablation causes stress/pain	High stocking densities common which may lead to increased injries, disease, oxygen depletion Mortalities common on transfer to grow-out stage	High stocking densities can lead to injuries, oxygen depletion, disease Weather factors - storms can flood farms Antibiotic use common in intensive farms Low digestibility of feed can lead to retarded growth and immunosupression	Harvesting by net or pond draining stressful Shrimp are killed by cold shock in ice slurry - is this always effective?

Figure 2: Ethical scope of the shrimp supply chain from breeding to slaughter. The upper boxes provide a brief description of what happens at each state of the shrimp life cycle. At all stages, the welfare categories outlined in Table 5 will be influential, however specific welfare issues at each stage of the shrimp life cycle are discussed in the lower boxes

4.2. WOM IMPLEMENTATION

Table 7 outlines an example WOM list for shrimp. The WOMs are further described in detail below. In blue highlight, are measures considered significant welfare risks in the development of novel protein feeds.

Table 7: Example WOM list for shrimp

Category	Measure	Unit of measurement	Measurement details	Inclusion
Liveability	**Mortality**	%	Annual number of shrimp harvested, divided by the total number of shrimp stocked (%)	All
Disease	**Flaccid body**	%	% of shrimp with a flaccid body	Sample
	Empty gut	%	% of shrimp with an empty gut	Sample
	Muscle necrosis	%	% of shrimp with muscle necrosis	Sample
	Red colouration	%	% of shrimp with red colouration	Sample
	Dark gills or pleopoda	%	% of shrimp with dark gills or pleopoda	Sample
	Muscle cramp	%	% of shrimp with muscle cramp	Sample
	White colouration	%	% of shrimp with white colouration	Sample
	Medicine use	Mg/kg	Total mg/kg medicinal treatments used per pond	All
Injuries	**Lacerations, wounds, broken antennae**	%	% pf shrimp with lacerations, wounds, broken antennae per cage	Sample
	Post larvae stocked/year sourced from non-ablated broodstock	%	Annual % of shrimp sourced from non-ablated broodstock	All
Mobility/behaviour	**Stocking density**	Kg/m3	Stocking density at peak production	All

LIVEABILITY

High **mortality** rates indicate that there is a welfare problem. However, it is not possible to discern what has caused the increase in mortality rate without collecting data on other measures. A low mortality rate does not necessarily mean good welfare as many issues such as disease, injuries and behavioural disturbances may decrease welfare without causing death.

DISEASE

A **flaccid body** is a sign of diseases such as 'loose shell syndrome' (LSS) (Nunan et al. 2007) or necrotizing hepatopancreatitis (NHP-B) (Lightner 1996). The occurrence of NHP-B is related to specific environmental conditions such as high temperature and high salinity (Lightner 1996; Vincent et al. 2007). LSS results in growth rate and body weight reduction and poor survival and is likely due to chronic bacterial infections and toxic pond bottom conditions (Alavandi et al. 2007).

An **empty gut (RISK FACTOR)** is a sign of general poor health in shrimp as it suggests that they are not eating. This could be related to disease (e.g. vibrio: Soto-Rodriguez et al. 2015; Mastan & Begum 2016; white spot syndrome virus (WSSV): Escobedo-Bonilla et al. 2006). An empty gut may also be related to a lack of food/inedible feed (Lavilla-Pitogo et al. 2000) so is important to measure in a feed trial context.

Muscle necrosis can occur when shrimp are exposed to stressful condition such as low oxygen, high temperatures (Lakshmi et al. 1978) or overcrowding (Lavilla-Pitogo et al. 2000). The muscles lose their normal transparency and become blotched with white until the entire tail area takes on a whitish appearance.

Red coloration (RISK FACTOR) can occur due to the ingestion of rancid feeds (from poor storage), presence of the aflatoxin B1 fungus in feed, infection with *Vibrio* spp. (vibriosis) (Soto-Rodriguez 2010) or poor water conditions (Lavilla-Pitogo et al. 2000). Body and appendages of affected shrimp become yellowish, then yellowish-pink and eventually red. Affected shrimp will also show slow growth and reduced resistance to stress and disease.

Dark gills/pleopoda (RISK FACTOR) otherwise known as black gill disease is a result of a number of disease syndromes including ascorbic acid deficiency (Magarelli et al. 1979). Black gill disease can lead to destruction and dysfunction of gill processes and increased likelihood of secondary infections. Black gills can also arise from accumulation of organic matter that occurs in highly turbid water, indicating poor water quality.

Muscle cramp or 'cramped muscle syndrome' leads to shrimp with rigid dorsal flexure of the abdomen which cannot be straightened. These shrimps lie at the bottom of the pond and are susceptible to cannibalism. The exact cause of muscle cramp is not known but it has been associated with extreme temperatures, high salinities and handling stress (Erazo-Pagador 2001).

White coloration is a general sign of stress and/or disease in shrimp. It may be caused by cotton shrimp disease or microsporidiosis, which is a disease caused by small parasites called microsporidia. Microsporidia invade and replace the shrimp's host tissues making the product unmarketable. It may also cause low level mortalities (Bower et al. 1994). Shrimp with cotton shrimp disease appear cooked or 'milky white'. White coloration may also be caused by bacterial infection with *Vibrio* spp. or 'white gut disease'. A white body coloration should be distinguished from white eyes, which are most likely a sign that a shrimp is about to moult (shed their exoskeleton). Total moult cycle duration is around 5 and 6.5 days for 2g *P. vannamei* and *P. monodon* and 11 and 12 days for 15g *P. vannamei* and *P. monodon*, respectively (Corteel et al. 2012), so it should be taken into account that a number of shrimp will be ready to moult during sampling.

Medicine use should be recorded alongside other WOMs as using excessive medicine can 'cover up' a poor system by treating diseases. However, using excessive medicine, especially antibiotics, is not a sustainable option. It is therefore important that medicine use is considered alongside other WOMs to ensure that shrimp are not showing WOM values indicative of good welfare because medicine use is excessive.

INJURIES

Lacerations, wounds and broken antennae (RISK FACTOR) are generally related to high stocking densities or an inability to moult properly. Shrimp that do not have sufficient minerals, such as calcium or insufficient feed will fail to properly moult and may show signs of incomplete moulting which may look like lacerations or wounds (Inve Aquaculture, personal communication).

Eyestalk ablation is carried out in shrimp broodstock to induce reproductive maturation. Eyestalk ablation is associated with signs of stress and pain in shrimp, which are curtailed by use of an anaesthetic (Taylor et al. 2004). Eyestalk ablation also jeopardizes growth, shortens the moulting cycle, increases energetic demands and results in high mortality and loss in egg quality (Uawisetwathana et al. 2011). Therefore, a higher percentage of shrimp sourced from non-ablated broodstock relates to a higher level of overall welfare within the supply chain.

MOBILITY/BEHAVIOUR

Stocking density is measured as the maximum stocking density at peak production and is expressed in kg/m^3. Although technically an input measure, stocking density is often measured as a proxy for a behavioural measure when collecting WOM data on farms. This is because at a very high stocking density, it is highly unlikely that animals would be free to express natural behaviours and move around their environment unhindered. At high stocking densities, shrimp (*Litopenaeus vannamei*) move around more (da Costa et al. 2016) and a high frequency of swimming behaviour in *L. vannamei* has been shown to be associated with stress (Taylor et al. 2004) suggesting that high stocking densities may be stressful. A high stocking density can also lead to reduced growth performance (Arnold et al. 2005; Liu et al. 2017), poor immune status (Liu et al. 2017) and increased mortality rate (Arnold et al. 2005; da Costa et al. 2016).

5. DISCUSSION

5.1. DATA COLLECTION AND PRESENTATION

The WOMs outlined in Tables 6 and 7 represent a broad range of WOMs that could be collected to provide information on the welfare of salmon and shrimp. These WOMs are a good starting point for considering salmon and shrimp welfare; they do not represent a complete list of possible WOMs (see section 5.3), nor a minimum requirement for measuring welfare. An advantage of the WOM approach is that it can be applied in a trial or commercial setting. In a trial environment with dedicated researchers, it is generally possible to include a longer list of WOMs than would be possible commercially. Commercially, due to time and personnel restraints, it is important to consider the practicality of the measures collected (see section 2.4).

All WOMs should be considered alongside one another to build a holistic picture of animal welfare. For example, mortality should never be considered without taking into account medicinal treatments in order to ensure that animals are being treated when required and diseases are not being masked by excessive medicine use. Producing a welfare index or aggregated score from the WOM and/or weighting measures may be possible (e.g. Stein et al. 2013) but requires in-depth understanding of the data, results in a loss of detail, and also presents problems when presenting data to less knowledgeable stakeholders (Heath et al. 2014, Grandin 2015c).

WOM assessment generates a relatively large amount of data that is best handled in a centralised database in which stakeholders can upload, store and view data. WOM data can be trended and benchmarked in a number of ways. Presenting data graphically enables fast, accessible interpretation of trends and comparison across a variety of conditions. Where there is a specific research question (i.e. the effect of novel feed ingredients on welfare), there will be two datasets to compare: one in which animals are fed a standard feed and one in which a novel feed is provided. All parameters apart from the feed should be match controlled in the two conditions. The influence of novel feed should be measured at all stages of the lifecycle so as to control for the potential effect of novel feed at an earlier stage influencing welfare at a later stage (for example, inhibiting growth rate or affecting the immune response in later life). This will be particularly important for long lived species such as salmon.

5.2. INPUT MEASURES

As discussed in section 2, the use of welfare outcome measures provides a more objective overview of animal welfare than measuring inputs alone. However, input measures such as water quality, temperature and salinity should be recorded on a regular basis so that any deviations are known. For example, if one tank experiences a large increase in temperature, this may lead to poor welfare as measured by WOMs. However, if the increase in temperature has not been recorded and this tank is being provided with a novel feed, we may erroneously interpret this poor welfare as being a result of the novel feed. Ideally, environmental conditions in both groups would be identical and such circumstances would not occur however, because of factors such as human error or equipment malfunctions it is impossible to rule such circumstances out. Therefore, it is important to collect data on key input measures alongside WOMs.

5.3. FURTHER ANALYSIS

The WOMs outlined in tables 6 and 7 provide a practical framework for measuring the welfare of salmon and shrimp. However, they do not represent a completely holistic picture and there are further measures of welfare that are less well-understood.

Although stocking density and crowding incidents can be used as proxy measures for behaviour, there are no welfare indicators that directly measure behaviour. In salmon, behavioural welfare indicators include foraging behaviour, aggressive encounters, ventilatory activity, swimming behaviour and abnormal or stereotypic behaviours (Martins et al. 2012). By using underwater cameras, it is possible to monitor salmon behaviour, however an automated measure of salmon behaviour is not yet available and the time required to monitor behaviour from videos can be lengthy. Feed intake and condition factor are proxy measures for foraging behaviour, as they can inform us about how much the fish are eating. Injuries provide a proxy measure for aggressive interactions because a high level of aggression will result in an increase in injuries. Swimming behaviour may be altered under unfavourable conditions and abnormal swimming behaviour has been suggested as a sign of poor welfare in farmed fish (Huntingford et al. 2006). In salmon, decreased swimming speed has been linked to changes in disease status (Tierney & Farrel, 2004) and parasite load (Wagner et al, 2003), suggesting that swimming speed may indicate welfare state. However, more work on this is required before swimming speed can be utilised as a WOM in a feed trial or commercial environment.

In shrimp, higher stocking densities are associated with increased movement which may be an indicator of stress (da Costa et al. 2016). Because stocking density is more practical and feasible to measure than movement, stocking density can act as a proxy for movement. To directly measure behaviour, highly sensitive underwater video systems would be required due to the turbid, low-light water characteristics of shrimp ponds (see Hung et al. 2016).

5.4. ANIMAL WELFARE RISKS THROUGHOUT THE SUPPLY CHAIN

FEED X aim to enable 10% of the global feed industry to transition to sustainably sourced oil and protein ingredients. Currently, the salmon and shrimp feed industry rely on fishmeal and fish oil, alongside increasingly more vegetable products such as soybean. It is well-established that production of fishmeal, fish oil and soybean have negative environmental consequences. However, there are also animal welfare risks associated with their use.

Around 25-35% of fishmeal is produced from fish byproducts and this figure is expected to grow (FAO 2018). However, the majority of fishmeal is produced from whole fish which are either wild-caught or produced in fisheries, therefore their use is associated with animal welfare issues related to the catching and killing methods used and environmental conditions used in fisheries. Secondary animal welfare risks are related to the unintended catch of non-target species and damage to ocean ecosystems that occurs due to practices such as bottom-trawling (Victorero et al. 2018). Soybean may seem like a more welfare friendly option, however mass deforestation in the Amazon and Cerrado in Brazil due to soybean production has led to the destruction of habitats of many wild animals, threatening biodiversity and negatively influencing animal welfare (WWF 2014).

5.4.1. ANIMAL WELFARE RISKS ASSOCIATED WITH LAND USE CHANGE

As indicated in Table 1, there are risks associated with land use change to produce novel feeds. An increase in seaweed growth, insect production and other plant-based solutions may require land use change. Although land use change is generally considered a biodiversity rather than animal welfare issue, there are obvious potential risks to wild animal welfare. We cannot measure the welfare outcomes of wild species; however, it is vital that any impacts on wild species are considered, tracked and minimized.

There are many farming practices that can be instigated to make spce for wildlife, such as keeping or planting hedgerows, land sharing or sparing, intercropping and decreasing pesticide use using integrated pest management. A wildlife monitoring programme could be established to track the impacts on wild species.

The impacts of land-use change need to be considered on a case by case basis and may bring about both positive and negative impacts. In the case of seaweed, there may well be positive impacts on wild species of increasing production. For example, one study found that planting sugar kelp increased shelter, feeding and nursery areas for a very high diversity of associated organisms in these ecosystems such as; other seaweeds, invertebrates, crustaceans and echinoderms, and a variety of fish species (Hasselstrom et al. 2018).

5.4.2. ANIMAL WELFARE RISKS ASSOCIATED WITH INSECT FARMING

Insects represent a potentially more sustainable source of protein than fishmeal and soy (Dobermann et al. 2017) and therefore production could be scaled-up to meet protein requirements for aquaculture feed. However, there may be animal welfare implications of this change. Humans often treat insects in a manner that indicates we believe they do not experience negative mental states (eg pain, distress and suffering), or that they have a reduced capacity for these compared with vertebrates (Sherwin 2001). However, research challenges this position. Insects show short and long-term memory and can learn to avoid negative stimuli. For example, *Drosophila* learn to restrict their movements to one half of a heat box when the other half heats to a noxious temperature when entered (Wustmann et al. 1996), they can retain this memory for up to two hours and learn to transfer this behaviour to a different heat box (Putz & Heisenberg 2002). Studies of 'cognitive bias' whereby animals in a negative affective state show pessimistic decision-making and those in a positive affective state show optimistic decision-making have been cited as evidence of emotion-like states in a range of species. This evidence now extends to bees (Bateson et al. 2011; Schlüns et al. 2016; Perry et al. 2016) and fruit flies (Deakin et al. 2018). However, as with all non-human animals, whether these behaviours are accompanied by subjective conscious experiences cannot be ascertained (Mendl et al. 2011). Many insects continue to walk on injured limbs without limping, locusts will continue to feed by being eaten themselves and male mantids continue to mate as they are eaten by their partners (Eisemann et al. 1984). However, the observation that insect behaviour is different from human behaviour is not evidence that they do not have pain-like experiences. Being able to experience the emotional component of pain may not be an all-or-none phenomenon and insects could have some aspects of an emotional experience but still lack the full subjective experience (Adamo 2016). When it comes to animal emotions, an absence of proof does not imply proof of absence and therefore an ethical approach is encouraged.

Currently, there is no structured knowledge, based on scientific research, on how insects should be reared in conditions that are in accordance with their welfare (Erens et al. 2012). Unlike farmed mammals, birds and fish, EU law concerning the protection of animals kept for farming purposes does not apply to insects or other invertebrates. Therefore, it is down to individual breeders and farmers to uphold insect welfare. With an expansion of insect farming for animal feed, insect welfare should be accounted for. Erens et al. (2012) suggest that legislation on insect-rearing should be put into place and identify welfare at slaughter as an important area for concern. The WOM framework described in this report covering the categories of liveability, disease, injury, mobility and behaviour need to also be applied to insect welfare, if the expansion of insect farming and their utilisation as novel feed and food sources continues.

6. CONCLUSIONS

If salmon and shrimp feed producers are to transition to sustainably sourced oil and protein ingredients, the impact of these novel proteins on animal welfare must be measured. This report provides a practical, holistic framework in which to measure salmon and shrimp welfare in the context of novel feeds. While all WOMs described here should be considered alongside one another to build a holistic picture of animal welfare - specific welfare risks related to feed, have been identified and need to be carefully monitored during the development and commercial testing of novel proteins. In salmon these specific welfare risks are; **fin condition, vertebral deformities, eye condition, opercular deformities, condition factor** and **gut morphology**. In shrimp the specific risks are; **red colouration, dark gills/pleopoda, empty gut, lacerations/wounds/broken antennae**.

The WOM framework for salmon and shrimp can be utilised in a commercial or trial environment to provide an objective overview of animal welfare. It is recommended that innovators use this framework to compare animal welfare when a novel protein diet is provided to that under a standard diet. In commercial settings, WOM data should be collected from an entire lifecycle of standard production to establish a baseline level for each measure. Alternatively, in trial settings, groups fed standard and novel feeds can be grown alongside each other to provide truly comparable results.

Finally, the WOM framework utilised in this report covers the categories of liveability, disease, injury, mobility and behaviour, which can be applied to **any farmed species** as a means of assessing their welfare. As the species used in alternative protein production for feed and food expands, it is important that we use comprehensive frameworks and robust data collection to ensure that animal welfare is safeguarded. This is especially true for 'lower sentient' beings such as amphibians and molluscs as well as animals that are used as by-products such as chicken for pet feed, which are often excluded from welfare legislation.

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