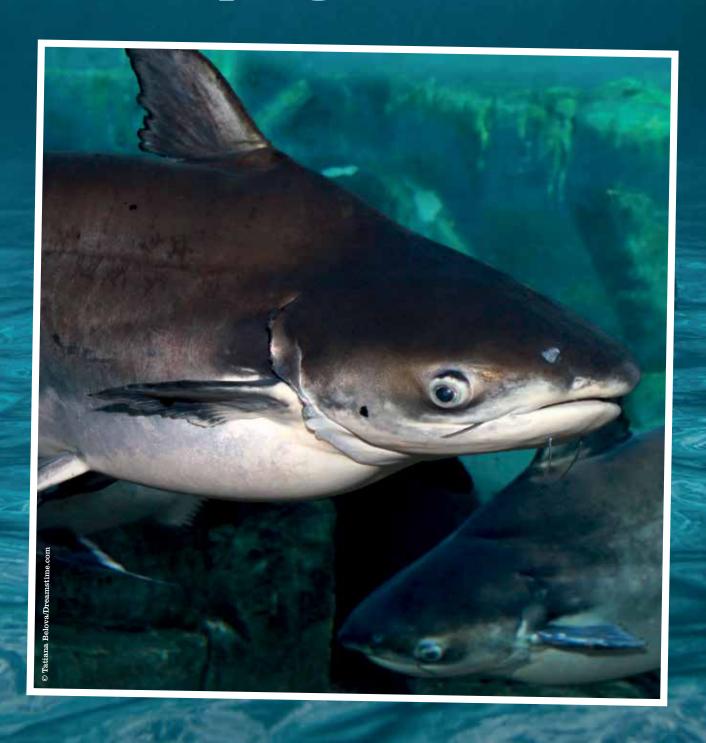
Improving the welfare of farmed pangasius



COMPASSION Food Business
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Foreword

Pangasius are sentient beings and must be provided with a good quality of life in a farmed environment. This document summarises research relevant to the grow-out phase of pangasius as a basis for our recommendations to improve fish welfare through the provision of good housing, good environment, good feeding, good health and opportunities to express appropriate behavior. This is in line with the adapted Five Freedoms model of Welfare Quality. When referring to pangasius in this document, we refer to two species, *Pangasianodon hypophthalmus* and *Pangasius bocourti*. Both of these species are referred to with numerous names in the literature and in the aquaculture industry (see Annex 1 for details).

GOOD ENVIRONMENT

When considering pangasius welfare in terms of good housing/environment, two types of growout systems exist which affect factors such as stocking density and water quality. Grow-out of pangasius occurs in earthen ponds or in cages and nets in natural water bodies (Hung et al., 2003). Ponds are the most common culturing method and are commonly intensive (60-80 fish/m² but can be as high as 120 fish/m²) (Phuong & Oanh, 2010; Poulsen et al., 2008). Earthen ponds are filled with water from nearby rivers and water is replaced regularly via tidal flow or pumping (Griffiths et al., 2010; Ngoc et al., 2016; Phan et al., 2009; WWF, 2010). Between water changes the water is stagnant and the water quality can degrade rapidly; thereby providing pangasius with a low-quality environment (Lefevre, Huong, Ha, et al., 2011). Floating nets or cages are maintained in rivers where the water flows through the nets or cages and reduces the risk of water degradation. Cage or net culture is more intensive than pond culture and the stocking density is generally 100-150 fish/m³ (Poulsen et al., 2008). Regardless of the system used, pangasius are sometimes exposed to polluted water including heavy metals (Hoang et al., 2010), insecticides, herbicides and antibiotics (Murk et al., 2018).

Stocking density

The concept of a minimum rearing space for fish is more complex than for terrestrial species as fish utilize a three dimensional medium (Conte, 2004; Ellis *et al.*, 2002). Additionally, stocking density will increase as fish grow, therefore it is hard to measure precisely at any one time in the farm environment.

Stocking density is an important management tool for optimizing farmed fish welfare but is strongly influenced by both environmental factors and fish behavior, some of which are still poorly understood. Environmental parameters such as dissolved oxygen and toxic ammonia levels can fluctuate within a pond, net or cage and have a significant effect on welfare by creating areas of poor water quality (Lefevre, Huong, Ha, et al., 2011). In ponds, research has shown that individual pangasius respond to these changing conditions by congregating in areas of better water quality (Lefevre, Huong, Ha, et al., 2011). The majority of ponds have depths between 3.5 to 4.5 m (Phan et al., 2009), however, the spatial variability of water quality parameters restricts the space the fish can occupy. Pangasius congregate at the top of the pond (between 0 to 1 m depth) at densities 3 to 4 times higher than the calculated stocking densities (the total biomass divided by pond volume) which assume pangasius are spread uniformly across the ponds/nets/cages (Lefevre, Huong, Ha, et al., 2011). This congregation is a result of avoidance behavior to low quality water (Lefevre, Huong, Ha, et al., 2011) restricting their space to live and move, thereby reducing their overall welfare. Reducing the stocking density and ensuring high water quality will alleviate this.

As of yet, there is no published research on water quality in net and cage farms. Regardless of the rearing environment, water quality must be monitored regularly to ensure water quality is within a suitable range for the fish at all times (see next subsection for more information).

Pangasius are commonly reared at exceedingly high stocking densities (Griffiths et al., 2010; Poulsen et al., 2008). The FAO states that governmental recommendations for stocking density in ponds are 20-40 fish/m² (Griffiths et al., 2010) and the Philippine government recommends a stocking density of 3-5 fish/m³ in ponds (Department of Agriculture, 2012) however average stocking densities are 60-80 fish/m² and densities as high as 120 fish/m² (max 120 kg/m²) have been reported (Poulsen et al., 2008). Nets and cages are typically stocked at 100-150 $fish/m^3$ (max 100-150 kg/m³) (Griffiths et al., 2010; Poulsen et al., 2008) (see Text box 1) yet the Philippine government recommends stocking densities of 5-10 fish/m³ in nets/cages (Department of Agriculture, 2012). Pangasius are stocked at these rates from entry to the grow-out ponds or cages/nets at the fingerling stage (1-3 g) until harvest (~0.8-1 kg); stocking density increases, therefore, as the fish grow from 0.1-0.45 kg/m³ at the start of grow-out and can reach 100-150 kg/m³ at harvest. Welfare outcome measures are rarely reported at these high stocking densities. Regardless of water quality, research has shown that rearing pangasius at these stocking densities present welfare challenges. High stocking density (>44 fish/m2) has been found to lower survival rates (Belton et al., 2011; Malik et al., 2014); lower weight gain and increase feed conversion rate (Azimuddin et al., 1999; Rahman et al., 2006); as well as increase the frequency of disease outbreaks (Poulsen et al., 2008) (see Disease section below) (WWF, 2010). The Aquaculture Stewardship Council (2019) maximum stocking density is 38 kg/m² for ponds and 80 kg/m³ for cages and sets a threshold of 20% for real mortality from stocking to harvest (((number of stocked fish - number of harvested fish)/number of stocked fish)x100) for disease detection and prevention (Aquaculture Stewardship Council, 2019a; WWF, 2010). This mortality threshold is high when compared to other species (Table 1). Both Naturland (2019) and Soil Association (2017) operate at a maximum stocking density of 10 kg/m³ and Naturland (2019) states that "in no case shall the animals display any injuries (e.g. of the fins) indicating too high stocking densities". Therefore, Compassion recommends a maximum stocking density of 10 kg/m³ in order to prevent high mortality, to aid lower levels of disease and to provide pangasius with space to live.

Stocking density in pangasius.

Stocking density in farmed fish is commonly reported as kg of fish per cubic meter as fish occupy a three-dimensional space. Pangasius stocking density is commonly reported as number of fish per square metre. Ponds, nets and cages are of variable depths which is not always reported, as a result, the stocking density per cubic metre cannot always be calculated and it can only be reported based on the surface area of the pond, net or cage. Pond depth varies between 3.5-4.5 m on average but shallow ponds with 2.5 m depth and deep ponds with 6 m depth have been reported (Phan *et al.*, 2009). With such variable depths the three-dimensional space available for pangasius is unknown and will not be consistent across ponds, nets or cages for identical stocking densities when they are reported in square meter.

Pangasius stocking density is reported as number of fish and not as kg of fish as is commonly done for farmed fish as pangasius are not graded between the time they enter the grow-out pond/net/cage and harvest. Therefore, the stocking density increases with age as the fish grow.

Table 1. Mortality comparison table

Species	Standard	Notification to vet	Reported mortality
Pangasius	20% total mortality (Aquaculture Stewardship Council, 2019a; WWF, 2010)	All mortality events with daily mortality above the average daily mortality on the farm (Aquaculture Stewardship Council, 2019a; WWF, 2010)	Cumulative: 30% during early grow-out, 10% during late grow-out phase (Phan et al., 2009), 20-25% at 44 fish/m² and 10% at 6 fish/m² (Belton et al., 2011)
Salmon	<0.5% per week <5% cumulative (RSPCA, 2018a)	>0.5% per week >1-1.5% per week must be reported to RSPCA (RSPCA, 2018a)	Monthly: 0.54-2.79% (Scottish Salmon Producers Organisation, 2019) Cumulative: 20-30% (Tett et al., 2018; The Humane Society of the United States, 208 C.E.)
Trout	<0.5% per week <5% cumulative (RSPCA, 2018b)	>0.5% per week (RSPCA, 2018b)	Cumulative: 5-10% (Isaac, 2017)
Sea bass/ sea bream	<0.5% per day (Aquaculture Stewardship Council, 2019b)	≥0.5% per day (Aquaculture Stewardship Council, 2019b)	Cumulative: 10-25% (Algers et al., 2008)

Based on existing science and knowledge from industry practice, Compassion recommends that pangasius are kept at a stocking density of 10 kg/m³ during grow-out. This along with monitoring and management of water quality will reduce the high localised stocking density currently observed. Ideally, at each site, environmental factors should be regularly monitored every 0.5-1m for the entirety of selected pond/net/cage depth and these factors taken into consideration along with the prevailing conditions and fish behavior when deciding whether stocking density is appropriate. Poor welfare can occur at any given stocking density and stocking density should be reviewed after every production cycle (Oppedal et al., 2011). There is a lack of scientific evidence for the optimal stocking density in pangasius, therefore more research is needed to give more specific recommendations.

Water quality

Water quality has a fundamental role in the health and welfare of farmed pangasius (Waycott, 2015c). Indeed, one of the principal concerns regarding high stocking density is that it can lead to a deterioration in water quality. Oxygen, temperature, turbidity and toxic ammonia are all important parameters (Lefevre, Huong, Ha, et al., 2011; Phuong et al., 2010). Water circulation also plays a vital role in disposing of waste products and allowing oxygenated water to circulate (Phuong et al., 2010). Some of these factors can be controlled by farm management practices while others are related to the environmental characteristics of the site and should be assessed prior to starting farming (Phuong et al., 2010).

Pangasius are facultative air-breathers, meaning they can withstand dissolved oxygen (DO) as low as 0.05-0.10 mg/l, high turbidity and highly polluted water (chemical oxygen demand = 25) (Graham, 1997; Waycott, 2015c). Research has shown that in ponds with a stocking density of 14 fish/m³ (~ 4.9-14 kg/m³) pangasius are exposed to chronically low levels of DO and the fish spend the majority of their time near the surface (<1m) where DO is higher (Lefevre, Huong, Ha, et al., 2011). The stocking density used in this study was 1.4-2.8 times lower than the governmental recommendations for stocking density and 4.2-5.7 times lower than the average stocking densities used by the industry. Even at these shallow depths, pangasius stocked at 14 fish/m³ were exposed to hypoxic conditions for 50% of the time when the fish are medium-sized (200-500 g) and 87% of the time when the fish are ready for harvest (~1000g) (Lefevre, Huong, Ha, et al., 2011). Pangasius display infrequent air-breathing in normoxic conditions (Lefevre et al., 2013) however air-breathing behaviour increases in frequency as DO decreases (Lefevre, Huong, Wang, et al., 2011). Frequent airbreathing is an indicator of inadequate environment (Lefevre, Huong, Wang, et al., 2011) and has been found to be energetically costly for pangasius (Lefevre et al., 2013). In addition, when carbon dioxide is elevated, the oxygen carrying capacity of the blood decreases as a result of nitrate uptake (Hvas et al., 2016). This is likely to increase the need for costly air-breathing. The ability to survive in environments poor in DO does not mean such conditions do not cause stress and suffering. When denied access to air in water high in DO, pangasius display attempts at air breathing and reduce their swimming activities (Lefevre, Huong, Wang, et al., 2011) indicating that not all air-breathing is a sign of poor environment and air-breathing is part of the natural behaviours of pangasius needed for good welfare. Farming practices should ensure DO in pangasius holding water is within the recommended levels (see Table 2) and the frequency of air-breathing must be considered when assessing the welfare of pangasius.

Being tropical, freshwater fish, pangasius naturally inhabit waters of 27-30°C (Waycott, 2015c). Pangasius do not tolerate water temperatures below 14°C for long periods of time as they become less resistant to disease and growth rates are reduced (Waycott, 2015b). When rearing pangasius in non-native areas where water drops below 25°C, care must be taken regarding Ichthyophthiriasis, a parasite causing white spot disease, which thrives at such temperatures (Kumar et al., 2018). Pangasius should only be reared in regions native to these species to ensure they are reared at suitable temperatures. However, with climate change, there is a concern about pangasius' capabilities to cope with warmer temperatures. There is an urgent need to examine the effects of increased temperature (> 30°C) on pangasius welfare, as very little is known and results are contradictory as some studies reported an increase in stress at higher temperatures and other studies did not (Ha et al., 2019; Phuong et al., 2017; Shahjahan et al., 2018). The mixed findings of studies investigating pangasius' abilities to adapt to projected warmer water temperatures are likely due to the airbreathing abilities of pangasius which enables them to cope with poor water quality and low DO (Ha et al., 2019; Phuong et al., 2017; Shahjahan et al., 2018). Despite these findings, the drop in DO with increasing temperature was specifically cited to be a source of stress in pangasius (Shahjahan et al., 2018). Water quality is reduced as temperature increases; solubility of oxygen decreases with temperature thereby reducing the DO available to pangasius (Wetzel, 2001).

High ammonia levels have been reported in grow-out ponds (Phuong & Oanh, 2010; Ut *et al.*, 2016) but the sampling depths are not reported and it is unknown if the ammonia is high throughout the depths of the ponds. At high temperatures, the total ammonia nitrogen equilibrium (unionised ammonia + ionised ammonia) is driven towards the toxic unionised ammonia (Thurston *et al.*, 1981).

Unionised ammonia can cause convulsion and coma and is lethal on average at 2.79 mg NH₃/l for freshwater fish (Randall & Tsui, 2002; Wright & Wood, 2012). As pangasius are reared at 27-30° C, unionised ammonia levels must be monitored closely and be maintained well below toxic level and ideally there should be no unionised ammonia in the water. High turbidity, which is common in pangasius rearing environments, is an indicator of poor water quality (Sørensen, 2005). The use of sedimentation ponds can improve the water quality prior to the water entering the rearing ponds (Naturland, 2019). Waycott (2015b) established the recommended water quality ranges for pangasius detailed in the table 2. Surveys of ponds and river outlets have shown that parameters reach levels outside of the recommended range (Phuong et al., 2010; Phuong & Oanh, 2010), demonstrating that pangasius are submitted to unsuitable environments during rearing, causing them physiological stress.

Table 2. Water quality recommendations for pangasius (Waycott, 2015c)

Parameter	Recommended range	
Temperature	27-30°C	
Oxygen	2.5-7.5 mg/l	
pН	6.5-9.5	
Turbidity	10-15cm	
Alkalinity	15-25.7 mg/l	
Ammonia nitrogen	0.7-1 mg/l	
Salinity	<2 ppt	
Hardness	15.3-35.5 mg/	
Chloride	<550 mg/l	

Given the importance of water quality in pangasius, Compassion recommends the regular monitoring of water quality parameters (dissolved oxygen, temperature, pH, turbidity, salinity, ammonia nitrogen and salinity as a minimum) at multiple depths of ponds or in and around nets and cages, using Waycott (2005e) parameters as reference for an ideal range. This data is crucial to understanding how the fish behave and aggregate within the pond/net/cage. When changes in the environment occur which lead to sub-optimal conditions within a pond/net/cage, management steps should immediately be taken to address any welfare impacts upon the fish e.g. by oxygenating the water, increasing the frequency of water changes, reducing biomass within the pond/net/cage.

Enrichment

Barren environment in fish farming leads to a chronic lack of cognitive, sensory and physical stimulation (Näslund & Johnsson, 2016). Although no research has been carried out on the effect of background coloration on grow-out size in pangasius, a study by Mat Nawang et al. (2019) has shown that rearing juvenile pangasius with different coloured backgrounds affects growth and stress levels. Juvenile pangasius are commonly reared using white or blue backgrounds. White backgrounds were found to cause significantly higher number of mucous cells (an indicator of stress) compared to the fish reared with a green background (blue backgrounds were not tested). Although higher, growth rate for fish in the white background was not significantly different from those in the green background (Mat Nawang et al., 2019). Appropriate enrichment has the potential to reduce stress during the rearing of juveniles without reducing production, but research is desperately needed. In fact, so little is known about the natural behaviour and needs of pangasius to experience good welfare that the FishEthoBase does not provide welfare recommendations and highlights the need for research (Castanheira, 2020). When rearing in ponds, ponds must be either earthen ponds which allow for water exchange or if concrete ponds are used, the bottom of the tanks must be made from natural substrate or covered with natural substrate (Naturland, 2019).

GOOD FEEDING

Feeding

Efficient feeding systems need to meet the nutrient requirements of pangasius, minimize water pollution and result in the ability to express natural behaviours. The quantity of feed offered, and the feeding methods used must ensure that all the fish have access to feed and they are satiated in order to remove the need for competition and aggression. Factors such as appetite, number, size variation of fish and how the feed is distributed must be considered. Daily food intake is affected by seasonal and environmental factors such as temperature and day length as well as natural feeding rhythms.

Pangasius are reared using commercial and/or home-made feeds. Pangasius are typically fed 1-3 times a day (up to six times per day) (Phan et al., 2009) depending on life stage, but independent of type of feed (commercial or home-made) (Nguyen, 2013). Younger fish are typically fed commercial feed as they do not feed well on home-made feed (Waycott, 2015a). Methods of dispersing feed differ according to feed type with commercial feeds broadcasted over the pond surface from boats, while home-made feeds are provided at a feeding places or stations (Nguyen, 2013). Reported feeding rates vary between 1-18% body weight/day for commercial and 1-10% body weight/day for home-made feeds (Nguyen, 2013; Phan et al., 2009). Conversion rate for home-made feed tends to be higher than for commercial feed due to the higher water content (Nguyen, 2013). Pangasius are naturally bottom feeders but can be trained to eat at the surface as commercial feed floats while home-made feed sinks (Nguyen, 2013).

Pangasius are a nutritionally low input species and require little animal protein such as fishmeal and fish oil (Waycott, 2015a). Therefore, most feeds utilize low inclusion levels of fishmeal with grain-based proteins ingredients constituting the bulk of the feed. Pond culture typically uses more homemade feed than cage or net culture with fish gaining additional nutrition by consuming sediment, bacteria and other organisms in the pond (Waycott, 2015a).

Feed

Pangasius are fed home-made feed, commercial feed or a combination of the two and the two differ in their composition. The quality of home-made feed is highly variable (Nguyen, 2013). The feed generally consist of grain-based protein sources (i.e., rice bran, soybean meal, corn), an animal protein source (i.e., fishmeal, meat bone meal, poultry meat) as well as nutritional supplements (Phan et al., 2009; Waycott, 2015a). Since 2008, dry, pelleted commercial feeds are increasingly replacing homemade feeds (Phan et al., 2009; Waycott, 2015a). Commercially produced feeds contain 30-33% crude protein (Nguyen, 2013) typically consisting of 5-10% fishmeal and 90% vegetable content. Though more expensive, the quality of commercial feed is more consistent (Nguyen, 2013). Commercial feed also leads to a better feed conversion ratio and their floating quality results in less build-up in cages, nets or the bottom of ponds (Waycott, 2015a). Regardless of feed type, protein content must meet the nutritional needs of pangasius who naturally feed on algae and plants as well as zooplankton and insects or even fruit, crustaceans and fish for larger individuals (Griffiths et al., 2010). Homemade feed should be supplemented with commercial feed if necessary, to ensure the nutritional and energetic needs of the fish are met. Protein content can be anywhere from 19-32% (Nguyen, 2013; Waycott, 2015a). However, fish proteins are expensive and can be successfully replaced with other available proteins (i.e., shrimp head meal, golden apple snail meal, groundnut cake, sweet potato leaf meal and cassava leaf meal) without compromising growth performance, feed utilisation or carcass traits of pangasius (Da et al., 2016). The sustainability of the protein replacements as well as the welfare of the animal involved, and the nutritional value of the resulting feed must be considered when selecting replacement proteins. Wild caught fish are fed directly to pangasius or are caught in so called reduction fisheries for the reduction to fishmeal and fish oil (FMFO) which is then added to feed (Mood & Brooke, 2012). The welfare and sustainability implications of reduction fisheries must be considered. The use of wild caught fish as or in feed represents food wastage as most of these fish are, in fact, human edible and energy is inevitably lost during the process. The welfare of fish caught for direct feed or by reduction fisheries is very poor during capture, landing and killing, as there is no humane slaughter practiced. The FMFO industry has substantial negative welfare and sustainability consequences and should be addressed (CIWF, 2019). Food businesses should look for assurance schemes that regulate feed and feed ingredients in Pangasius production (see Table 3).

Table 3. Pangasius feed regulation

	Species	Feed
Naturland (2019)	Pangasius spp. in ponds, nets, cages	Origin of sources is regulated and FMFO composition in feed limited to 20%
Soil Association (2017)	Pangasius spp.	In the grow-out stages, pangasius must be fed feed which is naturally available in ponds and lakes. When natural feed is not available in sufficient quantity, supplementary seaweed or organic feed of plant origin may be used, preferably grown on the holding. When supplementary feeding is used, a maximum of 10% fishmeal or fish oil derived from sustainable fisheries may be included in the feed. If additional feed is needed, records must be kept and explanation given in the organic plan.
Friends of the Sea (2014)	Generic	Using animal feed certified by Friend of the Sea, when available on the market for the species bred is recommended. Alternatively, using trimmings from processing of edible products is recommended. Using animal feed produced by IFFO certified plants such as Responsible Sourcing / Responsible Production is required. GMO and growth hormones are not allowed.
Aquaculture Stewardship Council (2019) WWF (2010)	P. hypophthalmus and P. bocourti	The use of uncooked or unprocessed fish and/or fish products (including trash fish and pangasius fish processing by-products) is not allowed. Fish products used in feed must not be on the "threatened categories" on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species or in the Convention on International Trade in Endangered Species (CITES) Appendices I, II and III. ISEAL-certified fishmeal and fish oil products must be used in feed within 3 years of availability in the region and within 5 years from the publication date of the ASC Pangasius Standard. Up until these standards can be met, Fishmeal or fish oil products used in feed must been sourced from fisheries deemed satisfactory for sustainability by FishSource.

Compassion recommends that food for pangasius be of adequate quality and nutrition for the life stage and size of the fish to minimize competition and ensure that all the fish have access to feed. The type of feed used must be suitable for the life stage as younger fish do not feed well on home-made feed. Home-made feed should be supplemented with commercial feed if necessary to ensure the nutritional and energetic needs of the fish are met. If commercial food is used, pangasius should be trained to feed at surface from early production stages. For older fish, home-made feed (which sink) is preferable in ponds as it permits the fish to perform natural bottom-feeding behaviour. When using home-made feed which sinks, it is vital to ensure good water quality at all depths of the pond as pangasius avoid areas of poor water quality which tends to be worst at the bottom of ponds. If water quality at the bottom of the pond is poor, sinking feed may not be consumed. This results in fish being hungry and further degradation of the water quality.

Compassion recommends that pangasius should not be fed on wild-caught fish and the amount of fishmeal in pangasius feed be eliminated or minimised as much as feasible while still providing for the nutritional needs of the farmed fish. This can be achieved by replacing fishmeal with substitutes such as shrimp head meal, golden apple snail meal, groundnut cake and others however the sourcing of substitutes must be chosen carefully to avoid unsustainable sources.

Fasting

It is standard practice to fast fish by withdrawing feed prior to certain management practices such as handling, transport and slaughter (Lines & Spence, 2012). Emptying the gut reduces physiological stress by lowering metabolic rate and waste production (Ashley, 2007; Lines & Spence, 2012). Based on industry experience pangasius are typically fasted for two days at 30°C which is considered sufficient time for gut emptying (Sørensen, 2005). Extended feed deprivation may lead to aggression and cannibalism as the fish become more hungry; which can lead to injury and death (Smith & Reay 1991, EFSA2008). Weakened fish are unlikely to have the energy reserves to cope with transport (Davis & Gaylord, 2011; Kakisawa et al., 1995; Moberg & Mench, 2000) resulting in higher mortalities than occurs when fish are transport following standard fasting (typically 2-8%, Sørensen, 2005). Despite the impact of prolonged fasting, Naturland (2018) allows for pangasius to be kept in artificial tanks for a maximum of two weeks at high density (125 kg/m³) without feed for the purpose of conditioning for transport or slaughtering. This practice is unacceptable as it causes prolonged stress and suffering. Conditioning in artificial tanks should be avoided due to the unnecessary handling and transfer required.

Compassion recommends that fasting periods should only be carried out when absolutely necessary and be no longer than the time needed for the gut to empty. Based on industry practice, fasting for 48h at 30°C appears to be sufficient (Sørensen, 2005). Therefore, fasting should not exceed 48h for each fish. Records of the dates and duration of fasting should be kept.

Procedures should be in place to ensure that this maximum time is adhered to for every fish in the pond, net or cage. For example, where multiple harvests/days are required to slaughter/remove all the fish in a pond, the fish should be segregated so that fasting time can be adhered to. Records of dates and duration of fasting should be kept.

GOOD HEALTH

Disease (see Table 4)

Health is a fundamental requirement of good welfare. As a result of intense farming and growing culture area, disease outbreaks in pangasius farms are increasing in frequency (see Table 4) (Poulsen et al., 2008). Poor water quality, handling or low water temperatures can all increase the likelihood of infections (Waycott, 2015b). Water quality management is one of the methods used by farmers to manage diseases (Hossain et al., 2018; Phan et al., 2009) while treatments for diseases include antibiotics and chemicals (see Table 4) (Hossain et al., 2018; Phan et al., 2009). Prevention methods include feed additives (e.g. vitamin C) and water changes, liming (a mineral pond treatment technique to improve water quality) and prophylactic use of antibiotics (Phan et al., 2009; Rico et al., 2013).

A survey of farmers indicated that mortality was high during grow-out with an average of 30% in the early months and 10% during the later months with disease (such as Bacillary Necrosis of Pangasius (BNP) also called Enteric septicaemia of catfish) and poor weather reported as the most common causes of mortality (Phan et al., 2009) (see Table 4). High disease outbreaks coincide with the start of the wet season (June/July) (Phan et al., 2009). The reason for this is unclear but disease and pathogen transfer might be a result of flooding as this has been suggested as causing the spread of disease in people in the Mekong delta where pangasius production is high (Renaud & Kuenzer, 2012). This is likely aggravated by the close proximity of many farms (<1km), the sharing of water between farms (Faruk et al., 2017; Faruk, 2008; Jennings et al., 2016; Phu, Douny, et al., 2015) and farms selling



fish which died as a result of disease to other farmers as feed for other species (Phan *et al.*, 2009). As the majority of pangasius production takes place in the Mekong delta, it is effectively a single epidemiological zone and needs to be managed as such (Jennings *et al.*, 2016). In some cases, mortality rates due to disease outbreaks can be as high as 90% (see Table 4) which is unacceptable and must be urgently investigated and managed, as a systematic pathological and epidemiological matter. In a survey of 50 farms in Bangladesh, 80% of farms had disease outbreaks at the time of the survey and 100% of the farms had disease outbreaks before the survey (Faruk *et al.*, 2017) whereas during another survey from 2018 in Bangladesh which surveyed 15 farms, 60% reported having had disease outbreaks in the past and only 13% reported disease outbreaks at the time of the survey (Hossain *et al.*, 2018). This difference in disease reports highlights the need for disease records to be kept.

Compassion recommends that water systems and distances between farms, as well as transport of fish amongst farms to be tightly managed to prevent the spread of disease and pathogens. The selling of diseased fish for use as fish feed should be stopped. Dead pangasius sold for fish feed must be strictly regulated and cause of death must be confirmed. If the cause of death is disease or pollution, the fish must not be sold for fish feed as per the guidelines set out by WWF (2010). Pangasius used as feed must not have undergone treatments prior to dying/culling to prevent transfer of chemical or antibiotic traces via the feed.

Use of Antimicrobials

Antimicrobial usage is common in Asian aquaculture (Phu, Phuong, et al., 2015). Surveys have shown that 60-100% of farms use antibiotic treatments (Hossain et al., 2018; Rico et al., 2013; Whitehead, 2016) and three groups of antimicrobials which have been banned or restricted (enrofloxacin, oxytetracycline and tetracycline) were found to still be used excessively (Phu, Douny, et al., 2015; Rico et al., 2013). The misuse of antibiotics results in widespread resistance with bacteria showing resistance to multiple drugs (Tu Thanh Dung et al., 2008; Rico et al., 2013). The data gathered by Rico et al. (2013) suggested that although there is a wider range of antibiotic ingredients used, the total quantity of antibiotics per tonne of pangasius harvested (93 g) is lower than the reported amounts for other important production animals such as salmon in Canada (175 g) and Chile (580 g) and for poultry in Europe (18-188 g). Since reporting of chemical residues became obligatory by the EU Rapid Alert System for Food and Feed (RASFF), there was a decrease in alerts until 2018 (18 alerts in 2011 vs 1 alert in 2015, 2016 and 2018 and no alerts in 2017) but there have been 5 alerts in 2019 and 4 alerts in the first 4 months of 2020 (Phu, Phuong, et al., 2016; RASFF, 2020). The recent increase in alerts by RASFF suggests a surge in the use of chemicals during pangasius rearing.

The quality of antimicrobial products used by pangasius farmers has been found to be low when tested. Only 6 of 21 tested products contained active substances within ±10% of the concentration declared on the label and two products did not contain any of the declared antimicrobials (Phu, Phuong, et al., 2015). In addition, quality between batches was not constant and instructions for use were not clear on all products (Phu, Phuong, et al., 2015). Quality of antimicrobials used for pangasius farming needs to be monitored and regulated (Phu, Phuong, et al., 2015). The erroneous information and poor quality control of antimicrobials is important for the fish welfare as the ability to treat diseases in fish will be lost if antibiotic resistant strains develop (Rico et al., 2013), fish will continue to suffer and may be subjected to repeat infections if application levels or quality of the product are too low to be effective (Waycott, 2015b). Finally, if too much of the product or an incorrect product is applied, fish, and other organisms, may be harmed by

residues (Waycott, 2015b). There is evidence that antibiotics used to treat fish can have adverse effects such as suppressing the immune system (Corcoran *et al.*, 2010) therefore the composition and concentration of antibiotics must be accurate to prevent poor health in fish. Some chemicals used to treat parasites may also cause welfare issues. For example, Monir *et al.* (2015) found the application of cypermethrin, a common chemotherapeutic agent used to control ectoparasites, caused erratic swimming, discoloration of the skin, loss of reflex, hyperactivity, surfacing, increasing opercular ventilation and damage to gills and liver as well as mortality in fish even at the low dose of 0.025m/l.

Antibiotics must not be used to compensate for a system with lower welfare potential and a badly managed production system. Compassion recommends that antimicrobials are only used as treatment and not prophylactically. The quality of the antimicrobials and other drugs must be monitored and regulated to minimise the development of resistant pathogens or any other aversive reaction that can cause poor fish welfare. The health status must be assessed and possible cause of disease must be diagnosed prior to treatment. Antimicrobials should only be used upon the recommendations of a vet. Records of antibiotic usage should be kept which include dates, type of antibiotic, reason for use and amount used.

Vaccination

Bacillary Necrosis of Pangasius (BNP), which is caused by the bacterium *Edwardsiella ictaluri* and Motile Aeromonad Septicaemia (MAS) which is caused by *Aeromonas* spp. Are some of the most widespread diseases affecting pangasius and result in high levels of mortality (Table 4) (Griffiths *et al.*, 2010; Phan *et al.*, 2009; Phu, Douny, *et al.*, 2015; Phu, Phuong, *et al.*, 2015, 2016). Vaccine against *E. ictalurid* has been available since 2013 however surveys have showed that the use of vaccines for pangasius is limited due to the associated costs for the farmers (Phu, Phuong, *et al.*, 2016). Since 2016, a vaccine against both *E. ictalurid* and *A. hydrophila* is available (PHARMAQ, 2016) but no surveys since 2016 are available on vaccine usage. The failure to vaccinate pangasius is likely to result in a higher number of fish becoming diseased and dying which is a welfare concern. In addition low levels of vaccination are associated with higher levels of antimicrobial use (Phu, Phuong, *et al.*, 2016) which is known to increase resistance in bacteria thereby lowering the efficacy of antimicrobials (Tu Thanh Dung *et al.*, 2008; Rico *et al.*, 2013).

Vaccination must not be used to compensate for a system with lower welfare potential and a badly managed production system. Compassion recommends vaccination of pangasius whenever vaccines are available. The vaccine against BNP is an injected vaccine (Thompson & Roberts, 2016) – this requires additional handling of the fish, removing the fish from the water and could cause harm and injury if not carried out correctly. Vaccination should be carried out using high management standards where time out of water and handling are limited and vaccines should only be administered by a trained person to prevent poor welfare of pangasius. Compassion recommends the use of vaccines added to feed when it is available rather than using injection vaccines due to the lower associated welfare risks.

Health promotion

The use of bioactive compounds from plants as feed supplements or water treatments is increasing (Nugroho et al., 2019). Benefits of supplements include increased growth, improved feed utilisation, improved immune function and disease resistance as well as higher survival rates (Nugroho et al., 2019; Van Doan et al., 2016). However, dosage must be evaluated carefully as some doses have been found to result in reduced growth and feed utilisation (Nugroho et al., 2019). Further research is needed into the usage of feed supplements and water treatments in pangasius to ensure the welfare of the fish is not negatively affected.

Feed additives must not be used to compensate for a system with lower welfare potential and a badly managed production system. Due to the limited scientific evidence on the use of feed additives for pangasius, and the variability in findings during feeding trials, Compassion cannot provide recommendations on feed supplementation for pangasius. Further research is required in this area.



Table 4: List of common pangasius disease and associated mortalities

Disease	Causative agent	Туре	Syndrome
Bacillary Necrosis of Pangasius spp (BNP) or Enteric septicaemia of catfish	Edwardsiellosis ictaluri High densities, pollutants, health problems & crowding	Bacterium	Immediately before death fish swim slowly at surface of water and look pale Haemorrhages on eyes and fin bases; white spots on kidney, spleen, and liver; some cellular necrosis
Motile Aeromonad Septicaemia (MAS) AKA Haemorrhagic disease	Aeromonas spp. (mainly A. hydrophila)	Bacterium	Haemorrhages on eyes, body and fins; bloody ascites in the peritoneum leasing to swollen body
Jaundice disease	Unknown	Unknown	Yellowing of flesh and sclera of the eye; necrosis of haematopoietic tissue
White spot Disease Aka Ich	Ichthyophthirius spp. Adverse climate, poor water quality & high densities	Parasites	Slow swimming at the surface, disorientation, lesions, fin rot, white spots on body, difficulty breathing, erratic swimming Fish with no scales are more susceptible
Parasite disease	Trichodina spp. Epistylis spp. Adverse climate, poor water quality & high densities	Parasites	Slow swimming at the surface, disorientation, lesions, fin rot, white spots on body, difficulty breathing, erratic swimming
Fluke worm	Clonorchis sinensis	Parasite	Infects liver during grow- out
Red (spot) disease	Pseudomonas fluorescens Aeromonas spp. High densities, pollutants & organic mud; more likely to occur when fish are stressed	Bacterium	Red spot, followed by anal protrusion, tail & fin rot, pop eye, dropsy & gill rot; slow swimming, no food intake, haemorrhages on head, mouth & base of fins & possible gas in the gut

Treatment	Fatality	Occurrence	Reference
Improved water quality; antibiotics Prevention via vaccination	50-90%	98% of surveyed farms	Austin & Austin, 2016; Tu Thanh Dung et al., 2008; Griffiths et al., 2010; Phan et al., 2009; Phu, Phuong, et al., 2015; Waycott, 2015b
Improved water quality and nutrition; antibiotics	60%	83% of surveyed farms	Griffiths <i>et al.</i> , 2010; Phan <i>et al.</i> , 2009; Phu, Phuong, <i>et al.</i> , 2015, 2016
Improved husbandry and nutrition; antibiotics	1-10%	17% of surveyed farms	Griffiths <i>et al.</i> , 2010; Khoi, 2011; Luu, 2013
15-20 ppt formalin for 30 minutes	May reach 100%	58% of surveyed farms	Griffiths <i>et al.</i> , 2010; Kumar <i>et al.</i> , 2018; Phan <i>et al.</i> , 2009; Uawonggul N <i>et al.</i> , 2018; Waycott, 2015b
15-20 ppt formalin for 30 minutes; antibiotics	Low	Unknown	Griffiths <i>et al.</i> , 2010; Waycott, 2015b
Prevention via pond preparation	High	Unknown	Griffiths <i>et al.</i> , 2010; Waycott, 2015b
Antibiotics	Severe losses in fry & fingerlings	61% of surveyed farms	Corneillie, 2014; Phan et al., 2009; Waycott, 2015b

Table 4: List of common pangasius disease and associated mortalities

Disease	Causative agent	Туре	Syndrome
Vibriosis	Vibrio spp.	Bacterium	Weariness with necrosis of skin & appendages leading to body malformation, slow growth, internal organ liquefaction, blindness & muscle opacity
White disease	Myxobolus spp., Henneguya spp.	Parasite	White cysts in kidneys and gills
Slimy disease	Not provided	Not provided	Not provided
Head beriberi	Not provided	Not provided	Not provided

OPPORTUNITIES TO EXPRESS NATURAL BEHAVIOUR

Management practices

Many management practices are likely to cause stress for pangasius including crowding, handling and transport. In a glucan (a polysaccharide derived from D-glucose studied for its stress reduction potential) supplement feeding experiment, stress response to crowding was tested by measuring blood cortisol and glucose levels (Phu, Ha, et al., 2016). Unfortunately, the experimental design did not include a control group for testing the stress response. Nevertheless, blood cortisol and glucose levels were elevated in all treatments following a 4 h crowding stressor and remained elevated during recovery after the stressor (Phu, Ha, et al., 2016); indicating that pangasius experience elevated stress during crowding. Despite the potential stress reducing benefits of glucan, the fish in this study were stressed, it is therefore likely that fish reared under standard conditions without glucan experience even higher levels of stress. Crowding time should therefore be limited and only carried out when necessary. Following fasting, pangasius are harvested by raising nets or cages by hand, netting fish in nets during low tides or by partial draining of the ponds (Griffiths et al., 2010; Lines & Spence, 2014). Pangasius may be netted into dry containers, moved via brailling or pumping (Griffiths et al., 2010; Lines & Spence, 2014). Dry brailling, commonly used for nets and ponds, requires low levels of crowding however results in extremely poor welfare due to the exposure to air and risks of bruising, crushing, puncture and abrasion injuries as a result of the high densities inside the braille (Lines & Spence, 2012). Wet brailles allow fish to be moved short distances with fewer welfare problems (Lines & Spence, 2012). Adult pangasius are commonly transported by road or boat to a processing plant for slaughter (Lines & Spence, 2014). Road transport containers can contain very little water, so often pangasius are too exhausted to swim or maintain their equilibrium on reaching the slaughter unit (Lines & Spence, 2014). Live transport in well boats is normally without aeration (Griffiths et al., 2010). Sørensen (2005) observed transportation in baskets without water to be the most common method for transferring fish from the well boat to the factory and reported that this practice can take up to 20 minutes and resulted in 2-8% mortality. This practice causes suffering in pangasius and must not be used.

Treatment	Fatality	Occurrence	Reference
Antibiotics & chemical disinfectants	Unknown	Found in 25-50% of fish sampled at market	Corneillie, 2014; Gobi <i>et al.</i> , 2016; Ina Salwany <i>et al.</i> , 2019; Noorlis <i>et al.</i> , 2011
No effective treatment known	Unknown	40-70% of sampled fish	Dung et al., 2008; Molnár et al., 2006; Phu, Phuong, et al., 2016
Not provided	Not provided	28% of surveyed farms	Phan et al., 2009
Not provided	Not provided	Not provided	Phan et al., 2009

Compassion recommends that the health status of the fish must be assessed before starting any crowding, handling or transport. It is essential to closely monitor the pangasius for signs of stress and provide oxygenation prior to starting gentle crowding. Gentle crowding includes fish swimming in a calm and leisurely way and only the occasional fish should be breaking the surface (OIE, 2015). Oxygen levels should be monitored continuously, and management of the crowd should be adjusted based on these, plus welfare indicators such as the fish behaviour. Any signs such as red discoloration or signs of skin/snout damage or haemorrhage on individual fish should signal immediate intervention. Crowding pangasius should only be carried out for a maximum of 2 hours with 48h between crowds to allow the fish time to recover. Crowding must be limited to a maximum of two crowding in a week and three in a month. Pangasius must not undergo repeated crowding at harvest. Pangasius must not be out of the water for more than 15 seconds. Compassion recommends the pumping or, if pumping is not possible, wet braille to transfer pangasius from ponds/nets/cages to boats to prevent crowding, injuries, and prolonged air exposure. Road transport must only be carried out in in trucks fitted with tanks. Sufficient water and oxygenation must be provided for boat and road transport to prevent stress and poor welfare in pangasius.

Welfare indicators

Crowding, handling, and transportation are well documented stressors in fish (Wall, 2001). Little research has been carried out on the stress response of pangasius, however when pangasius were netted, held out of water for 5 minutes and then crowded for 3 hours, Yaghobi et al. (2015) found an increase in blood cortisol and glucose levels 1h after the start of the stressor. During recovery after exposure to the stress factor, blood glucose levels were lower than prior to the start of the stressor, indicating that the fish were depleting their energy reserves (Yaghobi et al., 2015). This research shows that pangasius experience stress when undergoing current industry practices. These must therefore be managed to minimise the stress pangasius experience and to prevent poor welfare.

Sørensen (2005) noted a red discolouration of the mouth and belly area, as well as in the fins when fish were heavily crowded or taken out of water roughly indicating stress and injury. Research is needed to better understand the causes of the red discolouration and its potential as an easy stress indicator for farmers. Mortality rates of between 2-8% (by weight) were reported during the transport and harvest of pangasius (Sørensen, 2005). This may be due to high levels of stress, poor water quality and fish dying from asphyxiation once removed from water. These mortality levels are not acceptable, and practices must be improved to reduce the suffering of pangasius during harvest. For a more detailed overview of the welfare issues for pangasius, please see document 5 of this pack: Improving the welfare of farmed pangasius at slaughter.

Ability to express natural behaviours

Little research has been carried out on the natural behaviours of pangasius. Pangasius naturally migrate upstream for breeding (Sokheng et al., 1999). In captivity, pangasius do not breed and spawning is induced via hormonal treatments. There is an urgent need for an assessment into the effects of captivity on the behavioural response of pangasius and to assess the consequences of being unable to migrate. Pangasius are bottom feeders, providing them with feed and an environment that makes it possible to display this behaviour is beneficial to their welfare. Pangasius reared with a green background displayed higher levels of swimming than pangasius reared with a white background (Mat Nawang et al., 2019) and providing pangasius with suitable environments can increase the expression of natural swimming behaviours. During experiments, pangasius have been observed to be sensitive to environmental change such as a change in lighting or movement resulting in fleeing behaviour which caused injuries to the fish (Lefevre, Huong, Wang, et al., 2011). Effects of disturbance during rearing needs to be assessed for their welfare implications and management practices need to be adjusted accordingly to ensure pangasius experience low levels of stress during rearing and can display natural behaviours.

Compassion recommends reducing disturbance of pangasius during rearing to prevent stress behaviours and to enable pangasius to display natural behaviours. Providing Pangasius with opportunities to express their natural behaviour, such as natural substrate at the bottom and feeding at the bottom, also have the potential to improve the welfare potential of the system. Compassion urges for further research into natural behaviours of pangasius.

ANNEX 1

Pangasius is referred to with numerous names in the literature and within the aquaculture industry. Below is a table summarising the names used for Pangasianodon hypophthalmus and Pangasius bocourti.

Latin name	Synonymous Latin name	Misapplied Latin name	Common name
Pangasianodon hypophthalmus	Helicophagus hypophthalmus ^{1,2} Pangasius hypophthalmus ¹⁻⁵ Pangasius sutchi ¹⁻⁵	Pangasius pangasius ^{1,2} Pangasius pleurotaenia ^{1,2}	Iridescent shark-catfish ^{1,2} Pangas catfish ^{1,2} Stripe catfish ^{1,6} Sutchi catfish ^{1,6} Sutchi catfish ^{1,5} Swai ^{1,2} Thailand catfish ^{1,2} Tra Swai ^{4,5,7} Pangasius ⁷ Striped pangasius ⁷ Striped pangasius ⁷ Basa ⁸ Tra ⁸ Vietnamese catfish ⁸ Asian catfish ⁸ River cobble ⁸ Pangus catfishes nei ¹⁰
Pangasius bocourti	Pangasius bacourti ^{11,12} Pangasius altifrons ^{11,12}		Basa ^{6,7} Pangasius ⁶ Tra ⁷ Vietnamese catfish ⁷ Asian catfish ⁷ River cobble ⁷ Basa catfish ^{11,12} Royal Basa ^{11,12} Mekong catfish ¹³

 $^{^1\} http://www.catalogueoflife.org/col/details/species/id/53d4229 bacedae 1311051e7cddd 073d4/source/tree$

² https://www.fishbase.in/summary/Pangasianodon-hypophthalmus.html

https://www.nsnoase.in/summary/Pangasianodon-hypophtnalmus.ntml
http://www.fao.org/fishery/culturedspecies/Pangasius_hypophthalmus/en
https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2603
https://www.fws.gov/fisheries/ANS/erss/uncertainrisk/ERSS-Pangasianodon-hypophthalmus-FINAL-August2018.pdf
http://fishcount.org.uk/studydatascreens2/2015/numbers-of-farmed-fish-CO-2015.php?countrysort=Striped%2Bcatfish%2Fsort2
https://www.seafoodhealthfacts.org/description-top-commercial-seafood-items/pangasius

⁸ https://www.ciwfdocs.org/docs/~D124336

http://fishcount.org.uk/studydatascreens2/2015/numbers-of-farmed-fish-C0-2015.php?countrysort=Pangas%2Bcatfish%2Fsort2
 http://fishcount.org.uk/studydatascreens2/2015/numbers-of-farmed-fish-C0-2015.php?countrysort=Pangas%2Bcatfishes%2Bnei%2Fsort2
 http://www.catalogueoflife.org/col/details/species/id/b480a8154e43e27addfff0a189777965/source/tree

¹² https://www.fishbase.in/summary/Pangasius-bocourti.html

¹³ Nguyen, T. K. H., T. T. H. Do, T. P. Nguyen, B. Mark and B. J. Frank (2019). "Impact and tissue metabolism of nitrite at two acclimation temperatures in striped catfish (Pangasianodon hypophthalmus)." Aquatic Toxicology.

REFERENCES

Algers, B., Blokhuis, H. J., Bøtner, A., Broom, D. M., Costa, P., Greiner, M., Guemene, D., Hartung, J., Koenen, F., Müller-Graf, C., Morton, D. B., Osterhaus, A., Pfeiffer, D. U., Raj, M., Roberts, R., Salman, M., Sharp, J. M., Vannier, P., & Wierup, M. (2008). Scientific opinion of the panel on animal health and welfare on a request from the European Commission on animal welfare aspects of husbandry systems for farmed European seabass and gilthead seabream. The EFSA, 844, 1–21.

 $A quaculture \ Stewardship \ Council. \ (2019a). \ ASC \ Pangasius \ Standard. \ https://asc-aqua.org/wp-content/uploads/2019/09/ASC-Pangasius-Standard_v1.2_Final.pdf$

Aquaculture Stewardship Council. (2019b). Seabass, seabream and meagre standard. https://www.asc-aqua.org/wp-content/uploads/2019/03/ASC-Sea-Bass-Seabream-and-Meagre-Standard_v1.1_Final.pdf

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science, 104(3-4), 199-235. https://doi.org/10.1016/j.applanim.2006.09.001

Austin, B., & Austin, D. A. (Eds.). (2016). Bacterial fish pathogens: disease of farmed and wild fish (6th Editio). Springer International Publishing. https://doi.org/10.1007/978-3-319-32674-0

Azimuddin, K. M., Hossain, M. A., Wahab, M. A., & Noor, J. (1999). Effect of stocking density on the growth of Thai pangas, Pangasius sutchi (Fowler) in net cage fed on formulated diet. Bangladesh Journal of Fisheries Research, 3(2), 173–180.

Belton, B., Haque, M. M., Little, D. C., & Sinh, L. X. (2011). Certifying catfish in Vietnam and Bangladesh: who will make the grade and will it matter? Food Policy, 36(2), 289–299. https://doi.org/10.1016/j.foodpol.2010.11.027

Castanheira, M. F. (2020). Pangasianodon hypophthalmus pangasius, short profile. FishEthoBase. http://fishethobase.net/db/33/shortprofile/#ref FishEthoScore

 ${\it CIWF. (2019). Until the seas run dry. http://changingmarkets.org/wp-content/uploads/2019/04/REPORT-WEB-UNTILL-THE-SEAS-DRY.pdf}$

Conte, F. S. (2004). Stress and the welfare of cultured fish. Applied Animal Behaviour Science, 86, 205–223. https://doi.org/10.1016/j.applanim.2004.02.003

Corcoran, J., Winter, M. J., & Tyler, C. R. (2010). Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. Critical Reviews in Toxicology, 40(4), 287–304. https://doi.org/10.3109/10408440903373590

Corneillie, S. (2014, March). The role of prebiotics in pangasius production. International Aquafeed. https://www.scribd.com/document/214607763/The-role-of-prebiotics-in-pangasius-production

Da, C. T., Lundh, T., Lindberg, J. E., & Berg, H. (2016). Growth performance, feed utilisation and biological indices of Tra catfish (*Pangasianodon hypophthalmus*) cultured in net cages in pond fed diets based on locally available feed resources. International Aquatic Research, 8(4), 309–321. https://doi.org/10.1007/s40071-016-0144-z

Davis, K. B., & Gaylord, T. G. (2011). Effect of fasting on body composition and responses to stress in sunshine bass. Comparative Biochemistry and Physiology, Part A, 158(1), 30–36. https://doi.org/10.1016/j.cbpa.2010.08.019

Dung, T.T., Ngoc, N. T. N., Thinh, N. Q., Thy, D. T. ., Tuan, N. A., Shinn, A., & Crumlish, M. (2008). Common diseases of pangasius catfish farmed in Vietnam. https://www.aquaculturealliance.org/advocate/common-diseases-of-pangasius-catfish-farmed-in-vietnam/?savePDF=cf4883b0b60c43472a1e3165efd56633&article=common-diseases-of-pangasius-catfish-farmed-in-vietnam

Dung, Tu Thanh, Haesebrouck, F., Tuan, N. A., Sorgeloos, P., Baele, M., & Decostere, A. (2008). Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. Microbial Drug Resistance, 14(4), 311–316. https://doi.org/10.1089/mdr.2008.0848

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M., & Gadd, D. (2002). The relationships between stocking density and welfare in farmed rainbow trout. Journal of Fish Biology, 61(3), 493–531. https://doi.org/10.1006/jfbi.2002.2057

Faruk, M. A. R. (2008). Disease and health management of farmed exotic catfish *Pangasius hypopthalmus* in Mymensingh district of Bangladesh. In M. G. Bondad-Reantaso, C. V. Mohan, M. Crumlish, & R. P. Subasinghe (Eds.), Diseases in Asian Aquaculture (pp. 193–204). Asian Fisheries Society.

Faruk, M., Rahman, N., & Patwary, Z. (2017). Risk factors associated with tilapia and pangasius diseases. Journal of the Bangladesh Agricultural University, 15(2), 325–331. https://doi.org/10.3329/jbau.v15i2.35083

- Friend of the Sea. (2014). Aqua marine criteria and indicators for the certification of sustainable marine aquaculture. https://friendofthesea.org/wp-content/uploads/FOS_Aquaculture_Marine_rev2_03112014_en.pdf
- Gobi, N., Malaikozhundan, B., Sekar, V., Shanthi, S., Vaseeharan, B., Jayakumar, R., & Nazar, K. A. (2016). GFP tagged Vibrio parahaemolyticus Dahv2 infection and the protective effects of the probiotic *Bacillus licheniformis* Dahb1 on the growth, immune and antioxidant responses in *Pangasius hypophthalmus*. Fish and Shellfish Immunology, 52, 230–238. https://doi.org/10.1016/j.fsi.2016.03.006
- Graham, J. B. (Ed.). (1997). Air-breathing fishes: evolution, diversity, and adaptation. Elsevier.
- Griffiths, D., Van Khanh, P., & Trong, T. Q. (2010). Cultured aquatic species information programme. *Pangasius hypophthalmus*. http://www.fao.org/fishery/culturedspecies/Pangasius_hypophthalmus/en
- Ha, N. T. K., Huong, D. T. T., Phuong, N. T., Bayley, M., & Jensen, F. B. (2019). Impact and tissue metabolism of nitrite at two acclimation temperatures in striped catfish (*Pangasianodon hypophthalmus*). Aquatic Toxicology, 212, 154–161. https://doi.org/10.1016/j.aquatox.2019.05.008
- Hoang, T. H., Bang, S., Kim, K. W., Nguyen, M. H., & Dang, D. M. (2010). Arsenic in groundwater and sediment in the Mekong River delta, Vietnam. Environmental Pollution, 158(8), 2648–2658. https://doi.org/10.1016/j.envpol.2010.05.001
- Hossain, M., Shikha, F., & Chakrabarty, T. (2018). Studies on the culture condition of pangus (*Pangasius hypophthalmus*) at different farms in Trishal Upazila. Journal of Environmental Science and Natural Resources, 11(1–2), 97–107. https://doi.org/10.3329/jesnr.v11i1-2.43377
- Hung, L. T., Lazard, J., Mariojouls, C., & Moreau, Y. (2003). Comparison of starch utilisation in fingerlings of two Asian catfishes of the Mekong River (*Pangasius bocourti*, *P. hypophthalmus*). Aquaculture Nutrition, 9, 215–222.
- Hvas, M., Damsgaard, C., Gam, L. T. H., Huong, D. T. T., Jensen, F. B., & Bayley, M. (2016). The effect of environmental hypercapnia and size on nitrite toxicity in the striped catfish (*Pangasianodon hypophthalmus*). Aquatic Toxicology, 176, 151–160. https://doi.org/10.1016/j.aquatox.2016.04.020
- Ina-Salwany, M. Y., Al-saari, N., Mohamad, A., Mursidi, F., Mohd-Aris, A., Amal, M. N. A., Kasai, H., Mino, S., Sawabe, T., & Zamri-Saad, M. (2019). Vibriosis in fish: a review on disease development and prevention. Journal of Aquatic Animal Health, 31(1), 3–22. https://doi.org/10.1002/aah.10045
- Isaac, T. (2017). Seafood watch: Rainbow trout *Oncorhynchus mykiss*. https://www.seafoodwatch.org/-/m/sfw/pdf/reports/t/mba_seafoodwatch_farmed_raceways_ponds_troutreport.pdf
- Jennings, S., Stentiford, G. D., Leocadio, A. M., Jeffery, K. R., Metcalfe, J. D., Katsiadaki, I., Auchterlonie, N. A., Mangi, S. C., Pinnegar, J. K., Ellis, T., Peeler, E. J., Luisetti, T., Baker-Austin, C., Brown, M., Catchpole, T. L., Clyne, F. J., Dye, S. R., Edmonds, N. J., Hyder, K., et al. Verner-Jeffreys, D. W. (2016). Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. Fish and Fisheries, 17(4), 893–938. https://doi.org/10.1111/faf.12152
- Kakisawa, S., Kaneko, T., Hasegawa, S., & Hirano, T. (1995). Effects of feeding, fasting, background adaptation, acute stress, and exhaustive exercise on the plasma somatolactin concentrations in rainbow trout. In General and Comparative Endocrinology (Vol. 98, Issue 2, pp. 137–146). https://doi.org/10.1006/gcen.1995.1054
- Khoi, L. (Ed.). (2011). Fish disease prevention and treatment practices at the farm level. In Quality management in the pangasius export supply chain in Vietnam: the case of small-scale pangasius farming in the Mekong River Delta. https://www.rug.nl/research/portal/files/14565722/09_c9.pdf
- Kumar, V., Roy, S., Sarkar, U. K., & Das, A. K. (2018). Occurrence of Ichthyophthiriasis in *Pangasianodon hypophthalmus* (Sauvage, 1878) cultured in net-cages of maithon reservoir, Jharkhand, India. National Academy Science Letters, 41(5), 275–278. https://doi.org/10.1007/s40009-018-0647-9
- Lefevre, S., Huong, D. T. T., Ha, N. T. K., Wang, T., Phuong, N. T., & Bayley, M. (2011). A telemetry study of swimming depth and oxygen level in a Pangasius pond in the Mekong Delta. Aquaculture, 315(3-4), 410-413. https://doi.org/10.1016/j.aquaculture.2011.02.030
- Lefevre, S., Huong, D. T. T., Wang, T., Phuong, N. T., & Bayley, M. (2011). Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (*Pangasianodon hypophthalmus*). Comparative Biochemistry and Physiology A, 158(2), 207–214. https://doi.org/10.1016/j.cbpa.2010.10.029
- Lefevre, S., Wang, T., Huong, D. T. T., Phuong, N. T., & Bayley, M. (2013). Partitioning of oxygen uptake and cost of surfacing during swimming in the air-breathing catfish *Pangasianodon hypophthalmus*. Journal of Comparative Physiology B, 183(2), 215–221. https://doi.org/10.1007/s00360-012-0701-8

- Lines, J. A., & Spence, J. (2012). Safeguarding the welfare of farmed fish at harvest. Fish Physiology and Biochemistry, 38(1), 153–162. https://doi.org/10.1007/s10695-011-9561-5
- Lines, J. A., & Spence, J. (2014). Humane harvesting and slaughter of farmed fish. Revue Scientifique et Technique (International Office of Epizootics), 33(1), 255–264. https://pdfs.semanticscholar.org/bc54/9627077e8876bbaf69928f644295c3a452d7.pdf
- Luu, T. T. T. (2013). Investigation into jaundice in farmed catfish (*Pangasianodon hypophthalmus*, Sauvage) in the Meking delta, Vietnam (Issue March) [University of Stirling]. https://pdfs.semanticscholar.org/3de0/a936f3dc2001c9556beb87ea6655d4765cd2.pdf?_ga=2.50383273.1223974564.1582033527-862326112.1582033527
- Malik, A., Kalhoro, H., Shah, S. A., & Kalhoro, I. B. (2014). The effect of different stocking densities on growth, production and survival rate of pangas (*Pangasius hypophthalmus*) fish in cemented tanks at fish hatchery. International Journal of Interdisciplinary and Multidisciplinary Studies, 1(10), 129–136.
- Mat Nawang, S. U. S., Ching, F. F., & Senoo, S. (2019). Comparison on growth performance, body coloration changes and stress response of juvenile river catfish, *Pangasius hypophthalmus* reared in different tank background colour. Aquaculture Research, 50(9), 2591–2599. https://doi.org/10.1111/are.14215
- Moberg, G., & Mench, J. A. (Eds.). (2000). The biology of animal stress: basic principles and implications for animal welfare. CABI publishing.
- Molnár, K., Székely, C., Mohamed, K., & Shaharom-Harrison, F. (2006). Myxozoan pathogens in cultured Malaysian fishes. I. Myxozoan infections of the sutchi catfish *Pangasius hypophthalmus* in freshwater cage cultures. Diseases of Aquatic Organisms, 68(3), 209–218. www.int-res.com
- Monir, M. S., Ud Doulah, M. A., Rahman, M. K., Akhter, J. N., & Hossain, M. R. (2015). Effect of cypermethrin on the histoarchitecture of gills and liver of a freshwater catfish, *Pangasianodon hypophthalmus*. Asian Journal of Medical and Biological Research, 1(3), 641–647. https://doi.org/10.3329/ajmbr.v1i3.26488
- Mood, A., & Brooke, P. (2012). Estimating the number of farmed fish killed in global aquaculture each year. http://fishcount.org.uk/published/std/fishcountstudy2.pdf
- Murk, A. J., Rietjens, I. M. C. M., & Bush, S. R. (2018). Perceived versus real toxicological safety of pangasius catfish: a review modifying market perspectives. Reviews in Aquaculture, 10(1), 123–134. https://doi.org/10.1111/raq.12151
- Näslund, J., & Johnsson, J. I. (2016). Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish and Fisheries, 17(1), 1–30. https://doi.org/10.1111/faf.12088
- Naturland. (2019). Naturland's Standards organic aquaculture. https://www.naturland.de/images/UK/Naturland/Naturland Standards/Standards Producers/Naturland-Standards Aquaculture.pdf
- Ngoc, P. T. A., Meuwissen, M. P., Cong Tru, L., Bosma, R. H., Verreth, J., & Lansink, A. O. (2016). Economic feasibility of recirculating aquaculture systems in pangasius farming. Aquaculture Economics & Management, 20(2), 185–200. https://doi.org/10.1080/13657305.2016.1156190
- Nguyen, T. P. (2013). On-farm feed management practices for striped catfish (*Pangasianodon hypophthalmus*) in Mekong River Delta, Viet Nam. In On-farm feeding and feed management in aquaculture. http://www.fao.org/tempref/FI/CDrom/T583/root/09.pdf
- Noorlis, A., Ghazali, F. M., Cheah, Y. K., Tuan Zainazor, T. C., Ponniah, J., Tunung, R., Tang, J. Y. H., Nishibuchi, M., Nakaguchi, Y., & Son, R. (2011). Prevalence and quantification of Vibrio species and Vibrio parahaemolyticus in freshwater fish at hypermarket level. International Food Research Journal, 18(2), 689–695.
- Nugroho, R. A., Hardi, E. H., Sari, Y. P., Aryani, R., & Rudianto, R. (2019). Growth performance and blood profiles of striped catfish (*Pangasianodon hypophthalmus*) fed leaves extract of *Myrmecodia tuberosa*. Nusantara Bioscience, 11(1), 89–96. https://doi.org/10.13057/nusbiosci/n110115
- OIE (Ed.). (2015). Welfare aspects of stunning fish and killing of farmed fish for human consumption. In Aquatic Animal Health Code. https://www.oie.int/fileadmin/Home/eng/Health_standards/aahc/2010/en_chapitre_welfare_stunning_killing.htm
- Oppedal, F., Vågseth, T., Dempster, T., Juell, J.-E., & Johansson, D. (2011). Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon (*Salmo salar L.*). Aquaculture, 315(3–4), 361–368.
- Phan, L. T., Bui, T. M., Nguyen, T. T. T., Gooley, G. J., Ingram, B. A., Nguyen, H. V, Nguyen, P. T., & De Silva, S. S. (2009). Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. Aquaculture, 296(3–4), 227–236.

- PHARMAQ. (2016). fish vaccine ALPHA JECT ® Panga 2 received Marketing Authorisation. https://www.pharmaq.no/updates/pharmaq-fish-va/
- Phu, T. M., Douny, C., Scippo, M. L., De Pauw, E., Thinh, N. Q., Huong, D. T. T., Vinh, H. P., Phuong, N. T., & Dalsgaard, A. (2015). Elimination of enrofloxacin in striped catfish (*Pangasianodon hypophthalmus*) following on-farm treatment. Aquaculture, 438, 1–5. https://doi.org/10.1016/j.aquaculture.2014.12.032
- Phu, T. M., Ha, N., Tien, D., Tuyen, T., & Huong, D. (2016). Effect of beta-glucans on hematological, immunoglobulins and stress parameters of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. Can Tho University Journal of Science, 4, 105–113. http://sj.ctu.edu.vn/ql/docgia/download/baibao-28684/15-BE-TRAN MINH PHU(105-113)50.pdf
- Phu, T. M., Phuong, N. T., Dung, T. T., Hai, D. M., Son, V. N., Rico, A., Clausen, J. H., Madsen, H., Murray, F., & Dalsgaard, A. (2016). An evaluation of fish health-management practices and occupational health hazards associated with pangasius catfish (*Pangasianodon hypophthalmus*) aquaculture in the Mekong Delta, Vietnam. Aquaculture Research, 47(9), 2778–2794. https://doi.org/10.1111/are.12728
- Phu, T. M., Phuong, N. T., Scippo, M. L., & Dalsgaard, A. (2015). Quality of antimicrobial products used in striped catfish (*Pangasianodon hypophthalmus*) aquaculture in Vietnam. PLoS ONE, 10(4), e0124267. https://doi.org/10.1371/journal.pone.0124267
- Phuong, L., Huong, D., Nyengaard, J., & Bayley, M. (2017). Gill remodelling and growth rate of striped catfish *Pangasianodon hypophthalmus* under impacts of hypoxia and temperature. Comparative Biochemistry and Physiology Part A, 203, 288–296. https://www.sciencedirect.com/science/article/pii/S109564331630232X
- Phuong, N. T., & Oanh, D. T. H. (2010). Striped catfish aquaculture in Vietnam: a decade of unprecedented development. In S. . De Silva & F. . Davy (Eds.), Success Stories in Asian Aquaculture (pp. 131–147). Springer Science & Business Media. https://doi.org/10.1007/978-90-481-3087-0_7
- Phuong, N. T., Ut, V. N., Tung, V. T., Hang, N. T. T. V., Lien, N. T. K., Oanh, D. T. H., Huong, D. T. T., & Morales, E. J. (2010). Water quality monitoring in striped catfish (*Pangasianodom hypophthalmus*) farms in the Mekong Delta, Vietnam. http://media.sustainablefish.org/Final report_Water Quality2009_final.pdf
- Poulsen, A., Griffiths, D., Nam, S., & Nguyen, T. T. (2008). Capture-based aquaculture of Pangasiid catfishes and snakeheads in the Mekong River Basin. In A. Lovatelli & P. F. Holthus (Eds.), Capture-based aquaculture. Global overview. FAO (Issue No. 508, pp. 69–91). FAO Fisheries Technical Paper.
- Rahman, M. M., Islam, M. S., Halder, G. C., & Tanaka, M. (2006). Cage culture of sutchi catfish, *Pangasius sutchi* (Fowler 1937): effects of stocking density on growth, survival, yield and farm profitability. Aquaculture Research, 37(1), 33–39. https://doi.org/10.1111/j.1365-2109.2005.01390.x
- Randall, D. J., & Tsui, T. K. N. (2002). Ammonia toxicity in fish. Marine Pollution Bulletin, 45(1-12), 17-23. https://doi.org/10.1016/S0025-326X(02)00227-8
- RASFF. (2020). EU Rapid Alert System for Food and Feed. https://webgate.ec.europa.eu/rasff-window/portal/?event=searchResultList
- Renaud, F., & Kuenzer, C. (Eds.). (2012). The Mekong Delta system: interdisciplinary analyses of a river delta. Springer Science & Business Media.
- Republic of the Philippines (2012). Guidelines on the environmentally sound culture of pangasius in the Philippines, Order No.243. https://www.bfar.da.gov.ph/LAW?fi=406
- Rico, A., Phu, T., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Little, D. C., Dalsgaard, A., & Van den Brink, P. J. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture, 412, 231–243. https://doi.org/10.1016/j.aquaculture.2013.07.028
- RSPCA. (2018a). Welfare standards for farmed Atlantic salmon. https://science.rspca.org.uk/documents/1494935/9042554/RSPCA+welfare+standards+for+farmed+Atlantic+salmon+%28PDF+2.56MB%29. pdf/60ae55ee-7e92-78f9-ab71-ffb08c846caa?t=1557668417384
- RSPCA. (2018b). Welfare standards for farmed rainbow trout. https://science.rspca.org.uk/documents/1494935/9042554/RSPCA+welfare+standards+for+farmed+Rainbow+trout+%28PDF+2.29MB%29.pdf/36aeab04-e2f1-8875-d8ae-f7c4ff724c4d?t=1557668422472
- Scottish Salmon Producers Organisation. (2019). Scottish salmon monthly mortality rate. https://www.scottishsalmon.co.uk/reports/monthly-mortality-rate-december-2019

Shahjahan, M., Uddin, M. H., Bain, V., & Haque, M. M. (2018). Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*. Fish Physiology and Biochemistry, 44(5), 1309–1318. https://doi.org/10.1007/s10695-018-0522-0

Soil Association. (2017). Organic aquaculture standards. https://www.soilassociation.org/media/15726/soilassociation-aquaculture-standards-v1-3-may-2017.pdf

Sokheng, C., Chhea, K., Viravong, S., Bouakhamvongsa, K., Suntornratana, U., Yoorong, N., Nguyen, T., Tung, T., Quoc, B., Poulsen, A. F., & Jorgensen, J. V. (1999). Fish migrations and spawning habits in the Mekong mainstream-a survey using local knowledge (basin-wide). http://ifredi-cambodia.org/wp-content/uploads/2004/01/Chan Sokheng et al. 1999 Fish migrations and spawning habits in the Mekong mainsteam.pdf

Sørensen, N. K. (2005). Slaughtering processes for farmed pangasius in Vietnam. In Consultancy surveying slaughter processes and by-products handling in the Vietnamese industry. https://nofima.brage.unit.no/nofima-xmlui/bitstream/handle/11250/2576750/Rapport%2B12-2005%2BPangasius.pdf?sequence=1&isAllowed=y

Tett, P., Verspoor, E., Hunter, D.-C., Coulson, M., Hicks, N., Davidson, K., Fernandes, T., Nickell, T., Tocher, D., Benjamins, S., Risch, D., Wilson, B., Wittich, A., & Fox, C. (2018). Review of the environmental impacts of salmon farming in Scotland. https://www.parliament.scot/S5_Environment/General Documents/20180125_SAMS_Review_of_Environmental_Impact_of_Salmon_Farming_-_Report.pdf

The Humane Society of the United States. (208 C.E.). An HSUS report: the welfare of animals in the aquaculture industry. https://www.humanesociety.org/sites/default/files/docs/hsus-report-animal-welfare-aquaculture-industry.pdf

Thompson, K. D., & Roberts, J. . R. (2016). Fish Vaccines (A. Adams (Ed.)). Birkhauser - Springer.

Thurston, R. V., Russo, R. C., & Vinogradov, G. A. (1981). Ammonia toxicity to fishes. Effect of pH on the toxicity of the unionized ammonia species. Environmental Science & Technology, 15(7), 837–840. https://pubs.acs.org/doi/pdf/10.1021/es00089a012

Uawonggul N, Rattanamalee, C., & Daduang S. (2018). Immunization of Basa fish (*Pangasius bocourti*) against *Ichthyophthirius multifiliis* with live and sonicated trophonts. Iranian Journal of Fisheries Sciences, 17(4), 763–775. https://doi.org/10.22092/ijfs.2018.119247

Ut, V. N., Giang, H. T., Phu, T. Q., & Morales, J. (2016). Assessment of water quality in catfish (*Pangasianodon hypophthalmus*) production systems in the mekong delta. Can Tho University Journal of Science, 3, 71–78. https://doi.org/10.22144/ctu.jen.2016.107

Van Doan, H., Doolgindachbaporn, S., & Suksri, A. (2016). Effect of *Lactobacillus plantarum* and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity and disease resistance of pangasius catfish (*Pangasius bocourti*, Sauvage 1880). Aquaculture Nutrition, 22(2), 444–456. https://doi.org/10.1111/anu.12263

Wall, A.J., (2001). Ethical considerations in the handling and slaughter of farmed fish. Farmed fish quality. Blackwell Science. Bristol, England, pp.108-119.

Waycott, B. (2015a). Pangasius farming: feed and nutrition. The Fish Site. https://thefishsite.com/articles/pangasius-farming-feed-and-nutrition

Waycott, B. (2015b). Pangasius farming: health and disease. The Fish Site. https://thefishsite.com/articles/pangasius-farming-health-and-disease

Waycott, B. (2015c). Pangasius farming: water quality and biosecurity. The Fish Site. https://thefishsite.com/articles/pangasius-farming-water-quality-and-biosecurity

Wetzel, R. G. (Ed.). (2001). Limnology: lake and river ecosystems. Academic press.

 $Whitehead, R. J. (2016, April). Report: almost all Vietnamese cattle and seafood farms use antibiotics. Food Navigator-Asia. Com. https://www.foodnavigator-asia.com/Article/2016/04/21/Report-Almost-all-Vietnamese-cattle-and-seafood-farms-use-antibiotics?utm_source=copyright&utm_medium=OnSite&utm_campaign=copyright$

Wright, P. A., & Wood, C. M. (2012). Seven things fish know about ammonia and we don't. Respiratory Physiology & Neurobiology, 184(3), 231–240. https://doi.org/10.1016/j.resp.2012.07.003

WWF.~(2010).~Pangasius~aquaculture~dialogue~standards.~https://c402277.ssl.cf1.rackedn.com/publications/603/files/original/WWFBinaryitem17873.pdf?1374503595

Yaghobi, M., Paykan Heyrati, F., Dorafshan, S., & Mahmoudi, N. (2015). Serum biochemical changes and acute stress responses of the endangered iridescent catfish (*Pangasianodon hypophthalmus*) supplied with dietary nucleotide. Journal of Agricultural Scoence and Technology, 17, 1161–1170.