

The science driving change in Nile tilapia



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FOREWORD

Although Nile tilapia is resistant to variations in environmental conditions and management, this does not mean that this species is not subject to stress and suffering in inadequate conditions of captivity. Nile tilapia are sentient beings and must be provided with a good quality of life in farmed environments. This document summarises research relevant to the grow-out phase of Nile tilapia 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 natural behaviour. This is in line with the adapted Five Freedoms model of Welfare Quality.

This document reviews research and offers recommendations for the welfare of Nile tilapia (*Oreochromis niloticus*), the most reared and consumed species of tilapia worldwide. We consider that welfare recommendations should be species-specific, but this report is based on general information relative to tilapia and therefore we believe that its conclusions can be extrapolated to other tilapia species reared and consumed around the world (see Annex 1 for a list of other tilapia species).

GOOD ENVIRONMENT

When discussing the welfare of Nile tilapia in a captive environment, it is important to know that the grow-out stage of Nile tilapia culture can be conducted in four different types of systems, which can affect important welfare factors, such as stocking density and water quality. Grow-out of Nile tilapia occurs in ponds, floating cages or hapas, tanks/raceways (FAO, 2009; Saraiva & Volstorf, 2022) and recirculating aquaculture systems (RAS) (FAO, 2009). Extensive and semi-intensive culture (i.e. ponds) usually respect some spatial and habitat needs of Nile tilapia, but most industrial farming, especially in floating cages and RAS, does not (Saraiva & Volstorf, 2022).

What is a hapa?

They are fine meshed nylon cages settled in ponds or lakes, usually used for fingerling rearing or to separate populations of different sizes. The size recommended for a hapa is a square of 3 metres side and at least 1.5 metres deep, although they vary widely in sizes.

Intensive vs extensive systems in aquaculture

Depending on the stocking density applied and their external inputs, rearing systems can be classified according to how intensive they are.

Extensive systems: Low stocking density and rely on natural production for feeding. It can use fertilisation to boost natural production.

Semi-intensive aquaculture systems: Low to intermediate density. Relies on fertilisation and external sources of feed.

Intensive systems: High density. Relies only on external sources of feed.

Nile tilapia culture in ponds is conducted with a variety of inputs, including agricultural by-products (e.g. brans, oil cakes, vegetation and manures), inorganic fertilisers and feed, generally stocked at 1-3 fish/m² (FAO, 2009).

The culture of Nile tilapia at high densities in floating cages is practised in large lakes and reservoirs. Cages vary widely in size and construction materials, varying from 2.7 up to >100 m³ stocked with 25-300 fish/m³ (Rakocy, 2005).

When they are reared in hapas, the area where they are placed is on average 120 m² (Saraiva and Volstorf, 2022).

Tanks and raceways used for Nile tilapia grow-out stage are also of varying sizes (10-1,000 m³) and include many different shapes as circular, rectangular, square and oval tanks. The maximum tilapia density in raceways ranges from 160 to 185 kg/m³ and aeration is employed in such tanks because dissolved oxygen is usually the limiting water quality factor, but water use efficiency is much higher in these systems.

Recirculation systems for Nile tilapia culture are specially used in temperate regions, allowing to culture tilapia year-round under controlled conditions in those regions (Rakocy, 2005). Most of these systems can replace 5-10% of the water volume daily, being composed mainly by fish rearing tanks, a solids removal device, a biofilter, an aerator or oxygen generator and a degassing unit (Rakocy, 2005).

Stocking density

Determining the minimum rearing space per individual to optimize fish welfare is usually more complex for fish than for terrestrial species. Although accessing some resources may be considered a two-dimensional condition for fish, like accessing the bottom for benthic species or water exchange at the surface, fish usually use a three-dimensional environment (Conte, 2004; Ellis *et al.*, 2002). It is also important to consider that stocking density in terms of weight per volume or surface increases as fish grow, therefore it is not easy to measure it accurately at any time under farming conditions. Often stocking density for species reared in ponds is reported in fish per surface unit, or volume unit, making it more complicated to include the growth in the stocking density consideration. Moreover, the suitable stocking density should also consider the water quality conditions and other possible system parameters that are directly affected by densities to keep the fish in good welfare such as ensuring access to food or reduce aggression among others.

Stocking density in tilapia

Stocking density in farmed fish is commonly reported as kg of fish per cubic metre, as fish occupy a three-dimensional space. Nile tilapia stocking density can be reported as kg/m³, individuals/m³ or individuals/m² which we found to be common across literature for this species. It should be considered that the stocking density increases with age as the fish grow. Ponds, nets and cages are of variable depths which is not always reported and varies regionally, as a result, the stocking density per cubic metre cannot always be calculated from the literature and it can only be reported based on the surface area of the pond, net or cage.

With such variable depths the three-dimensional space available for tilapia is unknown and will not be consistent across ponds, nets or cages for identical stocking densities when they are reported in square metres.

An estimation has been calculated to obtain densities in kg/m³. The following assumptions for the calculations have been followed:

- Pond depth: 1 metre. According to personal communication.
- It was considered weight at harvest as the highest biomass achieved by the reared tilapia. Average weight at harvest used was 0.530 kg (Mood *et al.*, 2023).

When aiming to provide adequate housing space and density for fish, it is also important to know the natural aggregation patterns and social behaviours of the species. Juveniles and adults live in groups of unknown, variable sizes in the wild (McConnell, 1959; Turner & Robinson, 2000), and form groupings under captive conditions too (Delcourt *et al.*, 2009; Zhao *et al.*, 2016). Despite that, tilapia is at times a territorial fish, which aggressively defends its territory (Barreto & Volpato, 2006; Corrêa *et al.*, 2003; Philippart & Ruwet, 1980; Pinho-Neto *et al.*, 2014; Volpato *et al.*, 2003). The tilapias get into violent confrontations which are also part of their breeding behaviour. Because Nile tilapia may reach sexual maturity before harvesting in farms and considering that this species is able to spawn throughout the year in the tropics and during the warm season in the subtropics, such confrontations is in fact a problem that should be considered.

Tilapia may become territorial when a territory is available; at other times and when they cannot maintain a territory, they join groups to escape aggression. A very low density can generate opportunities for defending territories and consequently, increase aggressive behaviour as shown by Faller & Debacker (1988) research where aggressive acts were higher in 4-males' groups (2 fish/m³) compared to 16-males' groups (8 fish/m³). Thus, in production systems, if there is a very low density, it is likely that the aggressiveness among the fish will increase, as there will be more territory to defend. In addition, other than injuries caused by confrontations, growth of both dominant and subordinate individuals is reduced, with a greater response in the subordinates (E. M. V. Cruz & Brown, 2007). Despite that, it is important to prevent very high densities too. Nile tilapias seem to be stressed and grow less when they are in crowded environments (Barcellos *et al.*, 1999b). Basal plasma cortisol levels of juveniles were reported to increase at stocking densities of 5-10 individuals/tank compared to single or paired individuals, thus indicating a probable chronic stress response due to social stress (Barcellos *et al.*, 1999b). In fact, there is an apparent direct relationship between stocking density and stress and an inverse relation between stocking density and growth from densities of 50 fish/m³, i.e.: tilapias experienced more stress and grew less when stocking density increased from 50 fish/m³ (Volstorf & Maia, 2019a). As an additional complicating factor, a high density may influence food access, with more dominant fish eating more than non-dominant fish, and therefore increasing differences in size which in turn can increase aggression.

General recommendations for a better survival and good growth are to stock fry at 500 fish/m³, and juveniles at <50 fish/m³, but this may depend on oxygen levels in the water (Volstorf & Maia, 2019b). It is also relevant to note that better densities also depend on the type of production system and the age of the fish. Below (Table 1), Pedrazzani *et al.* (2020) suggest housing densities according to age and production system considering fish welfare for a semi-intensive production:

Table 1: Suggested stocking densities for Nile tilapia according to rearing systems and weight/age of individuals (based on Pedrazzani et al. (2020)) for a better welfare condition considering nutritional indicators.

Raising system	Weight (g)	Age (days)	Stocking density (pond: fish/m ² ; cage: fish/m ³)	
			No aeration or water renewal	With aeration or water renewal
Excavated pond	Fry (1-30)	40-80	20-30	40-50
	Juvenile (30-200)	80-120	4-5	6-10
	Adult (200-1,000)	>120	0.8-1.2	2-3
Cage	Fry (1-30)	40-90	1,200-1,500	
	Juvenile (30-200)	90-120	450-500	
	Adult (200-1,000)	>120	100-150	

Another consideration when deciding or assessing stocking density is the degree of intensity of the rearing system. Pedrazzani *et al.* (2020) differentiates between systems for their recommendations (Table 1) placing big relevance in the degree of water renewal for such differentiation. More confounding is that is easy to find different ranges for stocking densities of each intensity (Table 2).

Table 2: Summary of stocking density (fish/m²) for ponds reared at different intensities.

Stocking density (fish/m ²)	Extensive	Semi intensive	Intensive
SEAFDEC Aquaculture Department (2016)	1-2	3-4	5-10
FAO (2023)	<1	1-2	>2
P. S. Cruz (1997)	0.5 - 2	2-4	>4

Furthermore, as there are many factors that can influence stocking density like loss of space due to structures inside and outside the system or due to territoriality, natural preferences for some places, or formation of shoals, it is important to consider that stocking density is only a part of a complex interaction of factors to affect welfare that should never be considered in isolation (Volstorf & Maia, 2019a). Therefore, despite the fact that stocking density can be an important management tool for optimising the welfare of farmed fish, it is strongly influenced by many factors, such as housing type, management and routine protocols and biological

aspects. Only by properly integrating welfare indicators with biological knowledge and farm-specific procedures can 'optimal' density ranges be defined (Saraiva *et al.*, 2022).

RECOMMENDATION ON STOCKING DENSITY

Compassion recommends that Nile tilapia are given adequate space to meet their physiological and behavioural needs, and that all individual fish have access to adequate foods and be able to avoid competition with other individuals. Adult tilapia (200 - >1000 g) should be kept at a maximum stocking density 3 fish/m² (estimated 1.6 kg/m³) for ponds or 50 fish/m³ (estimated 26.5 kg/m³) for nets or cages.

There is evidence that tilapia can become territorial and increase aggressiveness if they are kept at a very low stocking density. Behaviour should be observed for signs of such behaviour and if they appear the stocking density should be adjusted to reduce aggressions. Environmental factors should be regularly monitored across the enclosure and should inform the stocking density used for that particular system. Poor welfare can occur at any given stocking density, and it should be reviewed after every production cycle, and adjusted according to the behaviour and environmental information collected.

Research on stocking density for tilapia is scarce and in general is not related to welfare. While there are a few indications that can guide our recommendation on maximum stocking density for Nile tilapia, there is not enough information for a minimum stocking density. More research is needed to give more specific recommendations and our recommendations will be reviewed when it is available.

Water quality

One of the main concerns regarding stocking density is that a high density can lead to a quick deterioration in water quality. Poor water quality conditions, such as high ammonia concentration, wide thermal variations, very low (acid) or very high (alkaline) pH, quickly affect the health of fish, who are then highly susceptible to diseases. Moreover, it is important to consider that besides being harmful for fish – even fatal depending on the level and the species – a very high temperature reduces the amount of dissolved oxygen, which can also quickly cause the fish to die. Thus, despite Nile tilapia being adaptable to a variety of freshwater environments and conditions, including brackish water (Peterson *et al.*, 2004), good water conditions are fundamental to a high quality of life for tilapias. Thus, temperature, pH levels, turbidity, oxygen concentration, and toxic ammonia and nitrite concentrations are all important. Pedrazzani *et al.* (2020) established an assessment protocol for semi-intensive production of tilapia recommending water quality ranges for tilapias under farming conditions, which are detailed in Table 3, although it is worth discussing several parameters on more detail.

Considering the water temperature, Nile tilapia have a very wide temperature tolerance range of 11-42°C where they are capable of living (FAO, 2009), although within that range there is a decreasing survival of Nile tilapia at <25°C and at >30°C (Volstorf & Maia, 2019a), which depends on acclimatisation. Despite it, Pedrazzani *et al.*, (2020) proposes a range between 25°C and 32°C (Table 3). It is also relevant to consider that this species performs temperature-related depth displacements (El-Sayed *et al.*, 1996), avoiding temperatures below 21°C by moving to the bottom, given the chance. Thus, considering this natural behavioural expression of this species, it is possible to infer that an optimum range for Nile tilapia welfare should start at 22°C. Moreover, it was already shown that the optimum range for survival is 25-30°C and for growth it is around 30°C (Volstorf & Maia, 2019b).

Nile tilapia are mainly found in freshwater, they can probably be considered as an euryhaline fish as they can adapt to a wide range of salinities. If they are gradually exposed, they are capable of surviving at 35‰ of salinity which is already a seawater salinity and can also easily live in brackish water (Volstorf & Maia, 2019a). They can be found from 0.2 to 0.8 m of water transparency (Volstorf & Maia, 2019a) which means that they do not need clear water and can do perfectly well in high turbidity.

Furthermore, Nile tilapia are found in waters with a dissolved oxygen level of about 0.1-12 mg/L (Volstorf & Maia, 2019a). Despite that, it is recommended to keep oxygen at >4 mg/L or decrease stocking density to compensate (Gilbert, 1996). Dissolved oxygen is maintained in ponds by exchanging 5-15% of the water volume daily, with fishes stocked at 1-3 fish/m² (FAO, 2009). The water flowing through the nets or cages on lakes and reservoirs reduces the risk of water degradation. Despite Nile tilapia being very resistant to ammonia and nitrite concentrations in the water, unionised ammonia and nitrite levels must be monitored closely and maintained below toxic level, ideally with no unionised ammonia in the water, to assure better welfare conditions (see Table 3 for specific parameter values).

Table 3: *Water quality parameters for Nile tilapia, Welfare assessment protocol (Pedrazzani et al., 2020) and CIWF recommendation. Italics highlight the difference between both recommendations.*

Parameter	Welfare assessment protocol recommended range (Pedrazzani et al., 2020)	CIWF recommended range
Temperature	25-32 °C	25-30 °C
pH	6-8.5	6 - 8.5
Turbidity	25-40 (cm)	25 – 40 (cm)
Oxygen saturation	70-95 (%)	> 4 mg/L
Non-ionised ammonia	0.000-0.050 (mg/L)	0.000-0.050 (mg/L)
Nitrite	0.00-0.50 (mg/L)	0.00-0.50 (mg/L)
Alkalinity	30-100 (mg/L)	30-100 (mg/L)

RECOMMENDATION ON WATER QUALITY

Given the importance of water quality in Nile tilapia, Compassion recommends the regular monitoring of water quality parameters (dissolved oxygen, temperature, pH, turbidity and ammonia nitrogen, and salinity if tilapias are reared in brackish water) at multiple depths of the enclosures, using as reference the ranges summarised in Table 3.

This data is crucial to understanding how the fish behave and aggregate within the enclosure. When changes in the environment occur which lead to sub-optimal conditions within an enclosure, 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 enclosure.

Enrichment

In fish farms, rearing environments are usually designed mainly for practical reasons for the farmer, thus usually from a human perspective and based on economic requirements, with little consideration for fish welfare (Arechavala-Lopez *et al.*, 2022). Therefore, barren environments in fish farming are very common and can lead to a chronic lack of cognitive, sensory, and physical stimulation (Näslund & Johnsson, 2016). A good way of offering proper stimulation for fish under captive conditions is through environmental enrichment, which is basically environmental changes that would allow the fish to express more natural behaviours - thus approaching its behavioural repertoire towards their natural range, and, on the other hand, reducing the expression of abnormal behaviours in captivity.

It is worth considering, though, that each fish species has its own particularities and needs that can vary greatly and should be considered when applying environmental enrichments. For example, for a species that does not use the substrate, making it available in the environment may not be as important as it is for Nile tilapia, who build nests in the sand in the reproductive phase (Mendonça *et al.*, 2010) and also use the substrate to forage for food (Bwanika *et al.*, 2006; Oso *et al.*, 2006; Peterson *et al.*, 2006). Therefore, it is fundamental to deeply know the needs and the natural behaviours of Nile tilapia to offer adequate stimuli for this species in captivity conditions.

Below we suggest some practical environmental enrichments for tilapias, considering the life stage, when necessary, since larvae, fry, juveniles and adults of the same species may have different needs:

- **Depth:** it is recommended to provide at least 2-6 m of depth in the environment (Volstorf & Maia, 2019b), with temperatures more suitable for the species, as Nile tilapia swim deeper with decreasing water temperature and shallower with increasing water temperatures (El-Sayed *et al.*, 1996). It is important to consider that the lower the depth, the smaller volume of water and therefore the number of animals that can be kept in the system at optimal welfare decreases.
- **Substrate:** as Nile tilapia live over sand and mud (Volstorf & Maia, 2019a) and use substrate to forage for food (Bwanika *et al.*, 2006; Oso *et al.*, 2006; Peterson *et al.*, 2006), providing these substrates for all life stages is important. In the absence of such substrates, offering gravel can help to improve fish welfare (Delicio *et al.*, 2006). Considering specifically the brood stock in farms, such substrates are even more relevant for breeding males, which build nests in the sand. Tilapia male preference for small-grained gravel compared to stones to dig spawning nests have been already demonstrated, and therefore should be considered in the design of tilapia enclosure (Freitas & Volpato, 2013; Mendonça *et al.*, 2010).
- **Shelters:** Nile tilapia seem to prefer having shelters in the environment (Delicio *et al.*, 2006). Thus, offering submerged branches, bushes, or trees are natural options that can work as shelters (Volstorf & Maia, 2019b). Artificial structures that imitate those structures such as cut PVC pipes can also work as shelters (Maia *et al.*, 2021). The addition of such structures can also provide more complexity in the environment, which is good for fish welfare. Using substrate in a more complex environment, such as gravel with some glass balls and small coloured PVC pipes, was already demonstrated to improve the welfare state of Nile tilapia (Tatemoto *et al.*, 2021).
- **Photoperiod** (light period in 24h period): as the natural photoperiod for this species is 9-15h per day depending on the season (Volstorf & Maia, 2019a) providing these daily hours of light should improve the welfare of this fish.

- **Light intensity and colour:** Nile tilapia tend to be more aggressive when exposed to high light intensities (Carvalho *et al.*, 2013). Intensities up to 280 lux are recommended (Carvalho *et al.*, 2013). In addition, blue light helps reducing the stress response in adverse conditions, such as confinement (Maia & Volpato, 2013; Volpato & Barreto, 2001).

RECOMMENDATION FOR ENCLOSURE AND ENVIRONMENTAL ENRICHMENT

Compassion recommends that any enclosure for farmed fish should be designed according to the biology of the species, and it should include elements that increase the structural and sensory complexity of the enclosure. Providing such increased complexity would reduce the chronic lack of cognitive, sensory, and physical stimulation.

For Nile tilapia, there is a range of options from offering sandy or small-grained substrate, elements similar to branches, shelter and using lighting of the appropriate intensity and wavelength. Some systems where tilapia are reared cannot provide all of these options or any of these options, therefore their welfare potential is greatly reduced.

GOOD FEEDING

Although Nile tilapia can be farmed extensively without the addition of any formulated feed, most of the global farming of this species is carried out in semi-intensive systems which requires the provision of certain amount of feed. Efficient feeding systems for Nile tilapia should meet the nutrient requirements of this species, besides also minimising water pollution. A good feeding system should also result in improving the expression of natural behaviours of Nile tilapia related to feeding. It is crucial that both the quantity of feed offered, and the feeding methods used ensure that all the fish have access to feed, thus better assuring that individuals are satiated and preventing competition and aggression. In this line, variables such as appetite, density, size variation of fish and how the feed is distributed must always be considered. It is also important to consider that daily food intake is affected by seasonal and environmental factors, such as temperature and day length, as well as natural feeding rhythms.

Feeding

According to FAO (2009), Nile tilapia is an omnivorous grazer in the wild that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacterial films associated with detritus. This species is considered an opportunistic feeder, opting for either a predominantly herbivorous diet consisting of phytoplankton and bottom debris, or an omnivorous diet composed basically of macroinvertebrates, insect larvae and detritus (El-Sayed, 1999; Volstorf & Maia, 2019b). Nile tilapia can even filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although their main source of nutrition is obtained by surface grazing on periphyton mats (FAO, 2009). Given the large quantities of mud, detritus and debris found in the stomach of dissected Nile tilapia, they can be considered a bottom grazing species (Bwanika *et al.*, 2004).

Nile tilapia are good candidates to be reared extensively in ponds: rearing the tilapia on low stocking densities and relying solely on the pond natural production, mostly phytoplankton. Farmers of extensive and semi-intensive systems can use inorganic and organic fertilisers to boost natural productivity and ensure correct nutrition for their farmed fish (FAO, 2023). FAO (2023) indicates that ponds managed extensively can sustain yields of 3,000 kg/ha which offers a good idea on how economically sustainable the system is. Initial fertilisation and regular

fertilisation of ponds during the rearing needs to be done with care and must consider soil type and water quality to avoid phytoplankton blooms that can lead to sudden decrease of dissolved oxygen (FAO, 2023). Alternatively, natural production can be boosted by adding structures such as bamboo sticks where the periphyton can grow (Hem *et al.*, 2001) which at the same time can create shelter and structural complexity for the tilapia (Volstorf & Maia, 2019b). As for regular fertilisation, dissolved oxygen must be considered and monitored to ensure that boosted natural production does not create a deficit of oxygen for the fish (FAO, 2023).

Extensive systems like ponds are also candidates for the implementation of polyculture. This type of rearing grows several species and/or crops in the same space, creating an ecosystem where one species benefits from the other improving the area production. Tilapia can be reared in combination with rice fields, common carps and shrimps (FAO, 2023; Fitzsimmons & Shahkar, 2016; Hem *et al.*, 2001). In rice fields, tilapia contribute nutrients for the rice and the rice offers a complex environment where tilapia can find shelter and food (Hem *et al.*, 2001). In semi-intensive systems, where external feed is used together with fertilisation, combining tilapia with shrimp improves feed utilisation efficiency and reduces environmental pollution (Fitzsimmons & Shahkar, 2016).

In most Nile tilapia farms, where pelleted dry or moist feeds are used - either farm-made or commercial feeds - broadcasting by hand is the preferred method of feeding, but the use of automatic feeders is also possible (Kebede, 2019). Feeding fish by hand allows the farmer to monitor the feeding behaviour and general health of the Nile tilapia (Kebede, 2019). In this system, fish should be fed every day, being that fry is fed at least 8-10 times per day, fingerlings 4-6 times per day, and larger fish 2-3 times per day (Kebede, 2019).

Feeding systems usually used in farms can potentially stress Nile tilapia too. ENDO *et al.* (2002) found that fish who choose when to feed had lower levels of plasma cortisol, higher phagocytic activity of their macrophages, higher antibody production and a higher number of lymphocytes than scheduled-fed fish. Moreover, it is worth mentioning that the skin colour of self-fed fish was found to be paler than that of the scheduled feeding fish, which may be an indication that they were not stressed, as skin darkening is associated with stress response in Nile tilapia. These findings indicate that a self-feeding regime is less stressful than scheduled feeding for Nile tilapia, representing a good example of the benefits of adapting management and techniques according to natural behavioural needs of fish. Moreover, even light intensity seems to have a direct relation with stress response in Nile tilapia (Volstorf & Maia, 2019a). Groups of three similar-sized male adults kept in 140 L aquaria expressed a tendency towards fewer aggression under low light intensity of 280.8 lux than under high intensity of 1,394.1 lux (Carvalho *et al.*, 2013). Thus, lower light intensity seems to be better for this species.

Brooders are usually fed with high quality feed at 0.5-2% of body weight daily (FAO, 2009). In ponds, fingerlings are given extruded feed (30% protein) at an initial rate of 8-15% of biomass per day, which is gradually decreased to a final rate of 4-9% per day (FAO, 2009). As tilapias grow, larger juveniles and adults are fed between 1.5 to 3% of their body weight for the rest of the grow-out period (FAO, 2009).

Formulated feed

We strongly recommend rearing tilapia in extensive systems, i.e.: not relying on external feed. However, as mentioned before, most tilapia farming is carried out on semi-intensive systems requiring a certain amount of external feed. It is estimated that 92% of tilapia farms rely on

some combination of commercially formulated, pelleted feed and other feed types to supplement the naturally occurring nutrients produced in the culture systems (Tacon, 2020). Most Nile tilapia farmers use complete diets – that is, diets supplying all the components (protein, carbohydrates, fats, vitamins, and minerals) necessary for the optimal growth and health of the fish (Table 4) (Kebede, 2019).

Table 4: *Ideal composition of a diet for Nile tilapia* (Kebede, 2019).

Feed component	Percentage (%)
Proteins	18-50
Lipids	10-25
Carbohydrates	15-20
Ash	<8.5
Phosphorus	<1.5
Water	<10
Vitamins	Trace
Minerals	Trace

Prepared feeds that provide a diet with an adequate content of protein, lipids, carbohydrates, vitamins, and minerals for Nile tilapia are readily available in developed countries and are also manufactured and available in developing countries with an export market for high quality tilapia products (FAO, 2009). Manures and agricultural by-products are used to produce this species cost effectively, as prepared feeds are often too expensive for the production of Nile tilapia sold in domestic markets in developing countries (FAO, 2009).

The feed conversion efficiency (FCE) for tilapia culture is around 0.59, which means that 1 kg of fish feed leads to 0.59 kg of fish with the remaining 0.41 kg counting as waste load in the culture system (Chatvijitkul *et al.*, 2017). In this same example, the feed conversion ratio (FCR) would be 1.7 ($FCR = 1/FCE$). By considering that fish are close to 75% water (*i.e.*, only 25% dry matter) and that feed is approximately 90% dry matter, on a dry matter basis, the waste load from feed is closer to 0.75 kg and the FCR for dry matter would be closer to 6.12 (Chatvijitkul *et al.*, 2017).

Nile tilapias accept feeds with a high percentage of plant proteins (FAO, 2009). Then, the use of fish meal and fish oil in the feed may be mostly (Al-Feky *et al.*, 2016) or even completely, replaced by non-forage fishery products (El-Saidy & Gaber, 2002; El-Sayed, 1998; Sarker *et al.*, 2016), especially in integrated farming (El-Sayed, 2020), which is important for a more sustainable process. The fish meal and fish oil (FMFO) industry has a substantial negative welfare and sustainability impact that should be addressed (Changing Markets Foundation & Compassion in World Farming, 2019). Thus, the sustainability of the new feed components, as well as the welfare of the animals involved, and the nutritional value of the resulting feed must be always considered when selecting replacement proteins. In this regard, it should be considered that the formulated feed should not contain plant ingredients that are considered edible for humans and instead should use wastes and by-products, or alternative resources that do not cause environmental harm (Lara *et al.*, 2023). Given that the Nile tilapia's need is for dietary essential amino acids rather than for dietary protein, replacement calculations should be carried out thoroughly (Ng & Romano, 2013).

Nile tilapia grow quickly, even on formulated feeds with lower protein levels, and can tolerate higher carbohydrate levels compared to many carnivorous farmed species (FAO, 2009). It is likely that this species has adapted to meet its needs for essential amino acids rather than for protein levels (Teodósio *et al.*, 2020). In general, feeds with 25-35% crude protein content can provide the required nutrients for Nile tilapia brood fish, which is similar for the grow-out stage, and diets with 34-36% crude protein content give the highest growth of Nile tilapia fry (1-5 g size) (Kebede, 2019). High quality feed with up to 35% protein content is used during grow-out stages (FAO, 2009). Furthermore, probiotics and prebiotics are also used in Nile tilapia feed (Kebede, 2019), as probiotics can help build up the beneficial bacteria in the intestine and competitively exclude the pathogenic bacteria. Probiotic bacteria also release enzymes, which help in the digestion of feed (Kebede, 2019).

Feeding practices

Considering the quantity of feed provided for fish, Pedrazzani *et al.* (2020) proposes that if all feed is consumed within 3-5 minutes by Nile tilapia, this can be considered as an indicator of a high score for fish welfare in relation to its nutrition, in a way that quicker or lower times taken to consume all the feed means lower welfare status for this fish. Moreover, the quality of the provided feed and its capacity to reach the nutritional requirements of Nile tilapia, thus reflecting in the condition factor and feed conversion rate of fish, are fundamental. It is also important to consider that one common source of social stress in Nile tilapia, which is a territorial species, is related to resource distribution in the environment. Thus, it is important to consider feeding practices incorporating both food and space, thus applying well-designed feeding regimes, with the potential to greatly increase Nile tilapia welfare, and therefore is recommended. Such feeding regimes go beyond considerations of feed frequency, quantity, or quality, and should focus on adequate spatial distribution of feed, ensuring that all individuals are similarly nourished. In this scenario, for better welfare conditions, not only the amount of feed provided, but also other appropriate nutritional indicators in different production stages should be considered (Table 5).

Table 5: *Indicators for welfare assessment related to the nutrition state of Nile tilapia considering the production stage (extracted from Pedrazzani et al. (2020))*

Welfare Indicator	Production stage		
	Growing/grow-out	Capture	Pre/slaughter
Amount of feed provided	X		
Crude protein (CP)	X		
Feed Conversion Ratio (FCR)	X		
Condition Factor (K)	X		
Feeding handling	X		
Fasting period	X	X	

The CDC tilapia toolkit (CDC, 2021) states the following feeding criteria must be considered for optimal health and welfare of tilapia:

- Feeding is of a quality, quantity and feeding frequency suitable for the fish stage of development.
- Feeds and feeders meet manufacturers' recommendations, good aquaculture husbandry practices and local regulatory requirements, and must provide adequate access for all fish.
- Probiotics are used at the appropriate nutrition and growth stage, to prevent overuse.
- All feeding systems are checked for proper operation daily.
- In the event of a supply failure, the farms can provide feed within 24 hours.
- Food is fed in such a way that fish can eat without undue competition.
- A documented chain of custody and traceability for fisheries products in feed is kept.
- Food type and presentation provides interest and occupation for the aquaculture species.

RECOMMENDATION ON FEED AND FEEDING

Compassion recommends adopting extensive systems to rear tilapia where natural production is the source of feed. Nile tilapia are omnivorous grazers who can feed on natural production of ponds; using an external source of feed increases the environmental impact of rearing tilapia.

When an external source of feed needs to be used, Compassion recommends avoiding the use of human edible ingredients. Compassion recommends that Nile tilapia should not be fed on wild-caught fish and the amount of fishmeal in the feed be eliminated or minimised as much as is feasible while still providing for the nutritional needs of the farmed fish. Tilapia are capable of thriving on high plant protein content diets, with little to no content of fish meal or fish oil, which can be replaced to non-forage fishery products. Using ingredients that are edible for humans further increases the impact of rearing animals in captivity and reduces the efficiency of the food system. They can be replaced by agriculture by-products.

Compassion recommends that feed for tilapia be of adequate quality and nutrition for the life stage and size of the fish to minimise competition and ensure that all fish have access to feed. The feed used must have a composition adequate to the life stage, as younger tilapia require a higher proportion of protein compared to adults. When formulating, feed should be considered to meet the required essential amino acids amount rather than proteins as this species is adapted to meet their needs on an essential amino acids base.

Compassion recommends adapting feeding systems and practices to the biology and behaviour of tilapia such as self-feeding systems. Carefully considering fish behaviour into the design of fish feeding systems and strategies would not only allow them to feed more efficiently but would also reduce stress and allow for their observation.

Fasting

It is a common practice in aquaculture to fast fish by withdrawing feed before certain management practices, such as handling, transport and/or slaughter (Lines & Spence, 2012). This practice is conducted because emptying the gut of fish reduces their physiological stress by lowering metabolic rate and waste production (Ashley, 2007; Lines & Spence, 2012). In fact, the FWI (Fish Welfare Initiative) welfare standards recommends fasting Nile tilapia before transportation, handling, and any stressful procedure (Fish Welfare Initiative, 2021). Extended

feed deprivation may lead to aggression and cannibalism as the fish become hungrier, which can lead to injury and death. Weakened fish are unlikely to have the energy reserves to cope with transport (Davis & Gaylord, 2011; Kakisawa *et al.*, 1995) resulting in higher mortalities than when fish are transported following standard fasting.

However, because studies on appropriate feed withdrawal period for Nile tilapia are still scarce, it is hard to make proper recommendations about fasting time. Despite that, according to the precautionary principle, Rey *et al.* (2019) suggests applying the recommendation for Atlantic salmon by the RSPCA (2018) to Nile tilapia. Such welfare standards for Atlantic salmon states that feed withdrawal should not exceed 48 hours prior to grading or transporting fish and should not exceed 72 hours before harvesting. Moreover, given that the harvest of Nile tilapia – that is a tropical fish - is typically carried out when temperatures are low, feed withdrawal should be considered that under such environmental temperatures, fish probably will not feed anyway and therefore withdrawal should likely be shorter. In a digestibility study, Lanna *et al.* (2004) described that the average time for Nile tilapia to empty their gastric tract was up to 13.5 hours and depended on dietary composition, taking less time the more fibre the feed contained. Despite this time, a survey in Brazil showed that fasting time in a big proportion of facilities was between 10 and 48 hours and a median of 24 hours (Coelho *et al.*, 2022), indicating for a longer time than the 13.5 hours described before. Contrary to Rey *et al.* (2019), Costa, (2019) suggested that fasting should be below 24 hours for Nile tilapia. For comparison, another commonly farmed tropical fish reared at similar temperatures, the pangasius, are typically fasted for two days at 30°C which is considered sufficient time for gut emptying (Sørensen, 2005). Despite the impact of prolonged fasting, Naturland (2022) allows for tilapia 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.

Furthermore, it is worth mentioning that FWI's Nile tilapia welfare standards (Fish Welfare Initiative, 2021) specifies that the production facility should define upper limits for time periods of farming procedures - including fasting - to ensure better welfare practices, while also providing accurate records showing that these limits are in fact respected. Similarly, CDC's tilapia toolkit (CDC, 2021) gives a higher ranking to farms that keep records of how long the fish are fasted before slaughter.

RECOMMENDATION ON FASTING

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. Studies on Nile tilapia feed withdrawal are scarce and therefore our recommendation follows the precautionary principle and withdrawal should be below 24 hours and ideally not exceed 13 hours 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

Health indicators/assessment

Health is a fundamental requirement of good welfare for any animal. There are some welfare indicators specifically for Nile tilapia that can be used to better assess the general health conditions of these fish under farming conditions.

During grow-out stage of production systems, it is possible to observe the physical conditions of the fish and the presence of visible ectoparasites (Pedrazzani *et al.*, 2020). Once present, ectoparasites certainly have a negative impact on the health of the animals, and it is necessary to treat them and seek to avoid a new infestation in future.

Considering the body condition of Nile tilapia, the presence of physical injuries or abnormal body structures that can be easily observed - including in the capture of fish - are good indicators of poor health conditions. Such injuries or deformations include physical damage to the eyes, jaws, operculum, skin, spine, fins, gills, or even loss of scales - which can even be seen while the fish are in the water (Pedrazzani *et al.*, 2020). It is important to note that, in addition to being indicative of management problems, some of these lesions can also be indicative of aggressive interactions between individuals (mainly fin or skin lesions). In this scenario, it is important to review the stocking density (Ibrahim, 2020). Moreover, it is also possible to evaluate blood glucose levels and mortality rate to assess the health status of farmed Nile tilapia (Pedrazzani *et al.*, 2020) in grow-out stage. Table 6, taken from Pedrazzani *et al.* (2020), summarises the descriptions or reference values for the scoring each of the indicators.

Table 6: *Nile tilapia health indicators, adapted from Atlantic salmon (Stien et al., 2013) by Pedrazzani et al. (2020). The lower the score of the indicator, the better health state of the fish.*

INDICATORS	SCORE	DESCRIPTIONS OR REFERENCES VALUES
Eyes	1	Apparently functional and healthy
	2	Haemorrhage, exophthalmos, traumatic injury; unilateral
	3	Haemorrhage, exophthalmos, traumatic injury
	4	Bilateral. Bilateral cataract, chronic condition, impaired vision
Jaws	1	Normal aspect, healthy
	2	Light superior or inferior deformity (aesthetics)
	3	Moderate superior or inferior deformity (affecting feeding)
Operculum	1	Normal aspect, healthy

	2	Partially covering the gills ($\geq 75\%$ covered)
	3	Partially covering the gills ($< 75\%$)
	4	Unilateral or bilateral absence
Skin	1	Normal aspect, healthy
	2	Scar tissue, scale loss, ulcers or superficial injuries $< 1 \text{ cm}^2$
	3	Ulcers or superficial injuries $> 1 \text{ cm}^2$, redness, light necrosis
	4	Severe necrosis, darkening, bleeding, inflammation
Fins	1	Normal, healthy appearance
	2	Scarred or slightly necrotic tissue
	3	Moderate injury or necrosis (thickening/splitting)
	4	Severe necrosis, bleeding, inflammation, exposure of the rays
Gills	1	Normal aspect, healthy
	2	Light injury or necrosis, thickening or splitting
	3	Moderate injury or necrosis, thickening or splitting
	4	Severe necrosis, bleeding, inflammation, pallor, or darkening
Spine	1	Normal structure
	2	Lordosis or scoliosis, normal weight
	3	Lordosis or scoliosis, weight loss
Ectoparasites	1	No infestation
	2	Moderate infestation (≤ 5 parasites)
	3	Intense infestation (> 5 parasites)
Blood glucose (mg/dL)	1	30–59
	2	60–80

	3	81–120
	4	<30; >120
Mortality	1	≤10%
	2	≤25%
	3	≤50%
	4	≥75%

Diseases

Under farming conditions, especially the high-density conditions in intensive systems, disease outbreaks can be a real problem. A first important step considering health issues for fish is to prevent disease, as once the symptoms are noted, it is possible that the disease is already in a very advanced stage, thus making it difficult the effectiveness of any treatment and, consequently, the recovery of sick fish. Therefore, it is always better to work preventively to avoid infestation and maintain the health of Nile tilapia. Diseases can usually be avoided by maintaining a high-quality environment and reducing handling stress (FAO, 2009).

More specifically, the pre-disposing factors that can cause diseases in Nile tilapia include bad handling, thermal shock, external parasitic wounds, immunosuppressive diseases (such as columnaris), faulty management, overfeeding, storing feed in warm and/or humid conditions, food putrefaction or contamination, excessive toxic ammonia in the water, overcrowding, damage of gills, poor hygienic conditions, excessive organic matter (that can be caused by excessive use of nitrogenous fertilisers for fertilising the aquaculture systems), higher water temperature and low dissolved oxygen (Ibrahim, 2020).

It is important to note that stress is usually the first step towards disease occurrence, it is an important conditioning or favour factor since it reduces the resistance of the fish and makes them more susceptible to disease (Everitt & Leung, 1999). Thus, preventing stress responses of Nile tilapia is always a good way to prevent disease. Stress can be caused by several reasons under farming conditions – including some of the pre-disposing factors mentioned above, such as nutritional differences (e.g. vitamin imbalances), poor environmental quality and rearing conditions, and physical, chemical and biological interference (crowding, handling, transportation, pollution, organic enrichment, etc.) (El-Sayed, 2020). Moreover, an important cause of chronic stress in tilapia is the effect of social interactions and hierarchies (El-Sayed, 2020). These involve aggressive interactions culminating in injuries, where the resulting submissive individuals may be constantly under stress caused by dominant fishes. Thus, this is another relevant reason to monitor and optimise fish densities and environmental enrichment to reduce these aggressive interactions or allow submissive fish to avoid them.

Though there have been few recent records of emerging viruses that have drastically impacted the tilapia culture (Ibrahim, 2020), viruses have been implicated in large disease outbreaks with severe mortalities in both farmed and wild tilapia (Bigarré *et al.*, 2009; H. W. Ferguson *et al.*, 2014). These outbreaks featured infection by betanodavirus and herpes-like viruses, causing alterations in central nervous system (Ibrahim, 2020). Betanodavirus is the cause of viral nervous necrosis (VNN) disease and has been recorded in many cultured marine fish species

worldwide, and lately for freshwater species causing high mortalities, especially at the larval and juvenile fish stages (Munday *et al.*, 2002). Viral disease outbreaks in Nile tilapia can also be caused by a novel enveloped RNA-virus leading to ocular, cutaneous and meningeal pathology (Ibrahim, 2020). Tilapia lake virus is currently considered the most critical virus for Nile tilapia (Ibrahim, 2020), which is implicated in recent mass tilapia deaths in Israel and Ecuador (del Pozo *et al.*, 2017; H. W. Ferguson *et al.*, 2014) and that poses a potential threat to the global tilapia industry (Jansen *et al.*, 2019). Tilapia lake virus seems to manifest itself as a problem of the brain in Israel (Eran *et al.*, 2016), while it attacks the fish liver in Ecuador (del Pozo *et al.*, 2017; H. W. Ferguson *et al.*, 2014) and Colombia (Tsofack *et al.*, 2017).

According to FAO (2009), the most common diseases of Nile tilapia caused by bacteria are Motile Aeromonas Septicaemia (MAS), Vibriosis, Columnaris, Edwardsielliosis, Streptococcosis. Saprolegniosis is a common disease caused by fungus (FAO, 2009), while diseases caused by ciliates and monogenetic trematodes parasites are also common (FAO, 2009). The major bacterial, mycotic and parasitic disease problems affecting Nile tilapia are included in the Tables 7, 8, 9, 10, 11 based on Ibrahim (2020).

RECOMMENDATION ON DISEASES

Compassion recommends preventing the infestation and spread of diseases and pathogens by tight management of water systems, distances and wild fauna contact, as well as transport of fish and shared used of equipment amongst farms and enclosures.

Experiencing stress seems to be one of the main factors favouring the spread of diseases, therefore reducing sources of stress such as transport, as well as any other of predisposing factors, will increase the chances to avoid diseases.

Table 7: List of common bacterial diseases of Nile tilapia, based on Ibrahim (2020)

DISEASE	AGENT	SYNDROME	TREATMENT	PREVENTION	REFEREN CES
Motile Aeromonas Septicemia (MAS disease)	<i>Aeromonas hydrophila</i> , <i>Aeromonas sobria</i> , <i>Aeromonas caviae</i>	Peracute (before acute phase): sudden death and high mortalities among young fish Acute (ascitic form): petechial haemorrhages on the skin, bilateral exophthalmia, ascites, detached scales, emaciation and anorexia Chronic (ulcerative form): abscesses or ulcers or both	10 days treatment: Oxytetracycline (OTC), florfenicol: Dose 55 mg kg ⁻¹ b.wt., for 10 successive days (medicated food) 2 weeks treatment: Romet 30® (Sulfamethoxazole-Trimethoprim) (SXT): Initial dose 246 mg kg ⁻¹ fish b.wt./day for 11 days followed by a maintenance dose of 154 mg kg ⁻¹ b.wt., for 3 days External treatment: KMnO ₄ : 3-4 mg L ⁻¹ for 1 h, Formalin (37-40%): 250 mg L ⁻¹ for 1 h Iodophores (Betadine): 2-3 ppm L ⁻¹ as indefinite bath	Prevention: Remove predisposing factors Vaccination: The major problems limiting the development of a commercial vaccine are the heterogenicity of the strain	Austin <i>et al.</i> , 2007; Stojanov <i>et al.</i> , 2010
Pseudomonas septicaemia (Fin Rot Disease)	<i>Pseudomonas Fluorescens</i> , <i>Pseudomonas Putida</i>	Fin rot at all fins septicaemic signs as MAS	Nearly the same as MAS	Nearly the same as MAS	Austin <i>et al.</i> , 2007; Stojanov <i>et al.</i> , 2010
Vibriosis	<i>Vibrio anguillarum</i>	Peracute form (before acute phase): affects stressed young fish. Anorexia, skin darkening and sudden death	Nearly the same as MAS	Prevention: Remove predisposing factors Vaccination: licenced vaccine (polyvalent vaccine)	Ibrahim, 2020

		Acute form: haemorrhages on skin and fins, boil-like (furuncle) lesions all over body surface which break resulting in large open ulcers, ascites, pop eye (Exophthalmia), deep abscesses in muscles and internal haemorrhage.		incorporating both <i>V. anguillarum</i> and <i>V. ordalii</i>). Pasteurisation of marine offals before feeding freshwater fish	
Edwardsiellosis	<i>Edwardsiella tarda</i>	Loss of pigmentation, ascites, corneal opacity, small white nodules may be present in the kidney, liver, spleen, intestine and gills	Nearly the same as MAS	Preventive measures: Elimination of stress factors (sewage pollution etc.), avoiding wild amphibians and reptiles, addition of vitamin C in ration at the rate of 150 mg kg ⁻¹ food. Licensed vaccine: is offered commercially in the form of formalised whole culture vaccine for <i>E. tarda</i> .	Evans <i>et al.</i> , 2006; Ibrahim, 2020
Yersiniosis (ERM)	<i>Yersinia ruckeri</i>	Reddening in the mouth, haemorrhagic gastroenteritis, splenomegaly and yellow discharge from the vent with vent prolapsed In chronic infections: Fish are dark, lethargic, bilateral exophthalmia and may have eye rupture, petechial haemorrhages all over the	Nearly the same as MAS	Preventive measures: Nearly the same as MAS. Licensed vaccine: is offered commercially in the form of formalised whole culture vaccine for <i>Y. ruckeri</i>	Perera <i>et al.</i> , 1994)

		skin, muscles, internal organs			
Streptococcosis	<i>Streptococcus iniae</i> , <i>S. agalactiae</i> and <i>S. parauberi</i>	Suppurative exophthalmia ("pop-eye"), Meningoencephalitis, lethargy, darkened skin and haemorrhages all over internal organs. Some diseased fish exhibit eye opacity without exophthalmia	Erythromycin with dose of 55 mg kg ⁻¹ body weight. Sensitivity test to identify an antibiotic of choice. External treatment and following hygienic measures can be helpful in minimising mortalities	<p>Preventive measures: Nearly the same as in MAS.</p> <p>Vaccination: <i>S. parauberis</i> experimental bacterin gave high levels of protection, for more than 2 years.</p>	Evans <i>et al.</i> , 2000; Toranzo <i>et al.</i> , 2009
Staphylococcosis	<i>Staphylococcus aureus</i> , <i>S. epidermidis</i>	Exophthalmia, congestion and ulcerations on the tail. Splenomegaly with diffusion of numerous white nodules in the gills, liver, gonad, stomach, intestine, and mesentery but not in the heart or brain	Sanitary measures application	<p>Vaccination: Vaccine containing antigens against <i>Staphylococcus</i> spp. Suitable for administration by immersion, injection, and even oral application but the vaccination trial against diseases caused by Gram positive cocci bacteria (staphylococci or micrococci) is not an option yet.</p>	Noga, 2010; Varvarigos, 2001
Mycobacteriosis	<i>Mycobacterium marinum</i> , <i>M. fortuitum</i> and <i>M. chelonae</i>	Young fish infected with mycobacteriosis show no external signs. As fish become older or become stressed, the infection becomes more serious. Emaciation, exophthalmia and/or ulceration, off food, fin and tail rot and scale loss. Internally, grey-white nodules in liver, kidney, spleen, heart and muscles.	Mycobacteriosis of fish is non-treatable	Destroy infected stocks and disinfect facilities before restocking with mycobactericidal agent. Gloves should be worn on handling infected fish or cleaning contaminated tanks or other equipment. Wash hands thoroughly with 70% isopropyl alcohol and a bactericidal soap.	Jernigan & Farr, 2000; Noga <i>et al.</i> , 1990

		Skeletal deformities as spinal curvature may be noticed. Infected fish succumb and die		Mycobacteria are sensitive to 60-85% alcohol	
Columnaris disease (Cotton wool disease)	Flavobacterium columnare	Greyish-white cutaneous foci on the fins, head and trunk. Skin in the affected area may be eroded, resulting in shallow ulcers. On the gills, the lesions appear to radiate from a focal point; the affected gill tissue becomes bleached and necrotic, but fusion of the lamellae does not occur. The pathogen's yellow-pigmented cells may be present in large numbers and colour the lesions yellow or orange. Highly virulent strains of the bacterium lead to fish death without any gross clinical signs	Treating the culture water with therapeutic chemicals legal for use on food fish Potassium permanganate is a commonly used therapy	No commercial vaccines are available. Probiotic, <i>Bacillus subtilis</i> , in water or diet (as prophylaxis) are effective in amelioration the lesions of <i>F. columnare</i> infections	Decostere <i>et al.</i> , 1998; Eissa <i>et al.</i> , 2010
Bacterial gill disease (BGD)	Flexibacta, Aeromonads and pseudomonads	Lethargy, loss of appetite, increased gill activity, extended gill opercula and fusion of gills filaments. A decreased appetite is an early sign. White to grey spots can be seen on the affected gill	Potassium permanganate (KMnO ₄) can be used at 5 mg L ⁻¹ for 1h. Treatment for 3 days is recommended for "flow through" water system. It is important to replace the water to improve the water quality and flush out toxic irritants	Not provided	Abowei & Briyai, 2011

Table 8: List of common mycotic diseases of Nile tilapia, based on Ibrahim (2020)

DISEASE	AGENT	SYNDROME	TREATMENT	PREVENTION	REFERENCES
Saprolegniosis	<i>Saprolegnia</i> spp.	Cotton wool like masses on affected areas of skin and egg, dermal ulcers, abnormal swimming due to skin irritation. In severe cases, death occurs due to osmoregulatory failure	NaCl (3-5 %) as a dip/1-2 min Formalin (37 %) 4 ppm as indefinite bath. Hydrogen Peroxide, KMNO ₄ (5 mg L ⁻¹) 1 h.	Remove all predisposing factors	Eli et al., 2011; Yanong, 2003
Branchiomycosis (Marbling disease)	<i>Branchiomyces sanguinis</i> and <i>Branchiomyces demigrans</i>	Marbling appearance on gills (pathognomonic), asphyxia (surfacing, gasping, rapid opercular movement, accumulation at the water inlet). High mortalities: 50% (2 days post infection)	KMNO ₄ : 4 mg L ⁻¹ as indefinite bath. Benzalkonium chloride: 4 ppm /1 h as bath. NaCl: 3-5% for 1 min as dip. Formalin: 15 ppm as indefinite bath	Remove all predisposing factors. Increase water flow can stop mortalities. 3D system (Drainage, Dryness and Disinfection)	
Ichthyophonosis (Swing disease or Sandpaper disease)	<i>Ichthyophonus hoferi</i>	Abnormal swimming behaviour (swinging, corkscrew like motion), multiple granulomas in the internal organs, sandy picture of the skin, spinal deformities, emaciation, exophthalmia, and ascites	No treatment. Total eradication of infected fish	Avoid feeding infected raw fish flesh or offal except after pasteurization. Avoid introduction of the infected fish into the reared fish 3D system.	

Aspergillo-mycosis	<i>Aspergillus flavus</i> and <i>A. parasiticus</i>	Pale gills, impaired clotting, anaemia, poor growth rate and Hepatoma. In severe cases, death occurs	No treatment	Using good quality feed, storing feed in good hygienic condition	
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Table 9: List of common protozoa diseases of Nile tilapia, based on (Ibrahim, 2020)

DISEASE	AGENT	SYNDROME	REFERENCES
White spot disease (Ichthyophthiriasis, sand grain, gravel or Ich disease)	<i>Ichthyophthirius multifilii</i>	Abnormal swimming behaviour (flashing). Itching against fixed objects which causes erosion and ulcer. Excessive mucus secretion. White spots on gills, skin, fins, and eye may lead to asphyxia and blindness. Emaciation and high mortalities	A. E. Eissa, 2016; I. A. M. Eissa, 2002
Trichodiniasis	<i>Trichodina</i> spp.	Turbid greyish white mucous on fins and skin with swollen gills. Excessive mucous secretions, darkening of skin, flashing behaviour (rolling in water) and anorexia. Rubbing against the bottom, sides of the pond or hard objects. Presence of injuries, haemorrhages and gasping with difficult respiration	
Chilodonellosis	<i>Chilodonella piscicola</i> (<i>C. cyprini</i>) and <i>C. hexasticha</i>	Nearly the same as Trichodiniasis	
Ichthyobodoosis (costiasis)	<i>Ichthyobodo necator</i> (<i>Costia necatrix</i>)	Nearly the same as Trichodiniasis	
Hexamitosis	<i>Hexamita intestinalis</i> .	Intestinal form: severe anaemia and emaciation, shreds of mucous emerging from vent, off-food, catarrhal gastroenteritis Systemic form: Hole-in the head disease: there are small hole-like lesions on the head with yellow, cheesy stringy mucous. Degeneration of liver,	

	Flagellated protozoa Hexamita	kidney and spleen with bloody ascitic fluids in the peritoneal cavity and muscular small ulcers	
Piscine Coccidiosis	Family Eimeriida <i>Eimeria</i> spp.	Nodular raised white areas in the gut due to inflammation of the intestine, trailing faeces and darkening of the body colour	
Trypanosomiasis	Hemoflagellate of genus Trypanosoma. <i>T. tilapiae</i>	Clinical signs and lesions. (Suspicious)	

Table 10: List of common parasitic diseases of Nile tilapia, based on (Ibrahim, 2020)

DISEASE	AGENT	SYNDROME	REFERENCES
Skin Fluke disease	Gyrodactylus (most common species: <i>G. elegans</i>)	Signs of skin irritation. Flashing swimming, scratching their body against fixed objects and sluggish swimming. The heavily infested fishes show different mortality % depending on the age of the infested fishes and degree of infestation. In chronic stages with mild infestations, significant emaciation and low body gain are common	A. E. Eissa, 2016; I. A. M. Eissa, 2002
Gill Fluke disease	Dactylogyrus and Cichlidogyrus (most common species: <i>D. vastator</i> and <i>C. tilapiae</i>)	Signs of gill irritation Surfacing, gasping, accumulation at water inlet and rapid opercular movements	
Yellow grub disease	<i>Clinostomum</i> spp.	Presence of yellow punches, like grapes in gills and underlying muscles causing gill damage associated with signs of asphyxia	
Black spot disease	<i>Posthodiplostomum cuticola</i>	Presence of scattered multiple black spots all over the body, especially scales	

Parasitic cataract	<i>Diplostomum spathicum</i>	Unilateral or bilateral central eye opacity, emaciation, death from being off food	
Sanguinicolidae (Sanguinicolosis)	<i>Sanguinicola</i> spp.	Acute mortality in severe infections, anaemia, and emaciation in chronic infected. Low body gain, signs of asphyxia, excessive blood-tinged gill mucous and marbling appearance of gills	
Diphyllbothriasis	<i>Dipyllobothrium latum</i>	Infested fish float on the water surface while bending the head to one side. Death of fish may be due to penetration of the heart by the larvae. Freshly dead infested fish may appear as S or C-shape.	
Heart worm disease (amplicaeum)	Larval nematodes of Family Ascaridae (<i>Amplicaeum</i> sp.)	Slight abdominal distention, paleness of skin and nervous manifestations may be observed	
Acanthocephala (spiny-headed worms)	<i>Acanthosentis tilapiae</i>	No apparent clinical signs, especially in light infestations. In chronic heavy infestations, emaciation and mortality are common	

Table 11: List of common crustacean diseases of Nile tilapia, based on (Ibrahim, 2020)

DISEASE	AGENT	SYNDROME	REFERENCES
Lerniosis anchor worm infestation	<i>Lernaea elegans</i>	Skin irritation with excessive mucous secretion, abnormal swimming behaviours, scale detachment and presence of focal haemorrhagic granulomatous ulcers. Asphyxia in heavy gill infestations. Excessive blood-tinged gill mucous and the white adult females are seen attached to the gill filaments. High mortality in severe infestations	A. E. Eissa, 2016; I. A. M. Eissa, 2002
Ergasilosis	<i>Ergasilus</i> spp.	Asphyxia in heavy gill infestations. Excessive blood-tinged gill mucous. High mortality in heavy infestations	

Argulosis (Fish Lice)	<i>Argulus</i> spp	Abnormal swimming behaviour. Characteristic linear skin haemorrhages with circular, crater-like depressions surrounded by inflammatory zones and to which the parasites are attached. High mortality in heavy infestations	
Isopodiosis (Nerocila disease)	<i>Nerocila orbignyi</i>	Not provided	

Use of antimicrobials

Antimicrobials as antibiotics have been largely used over the years in aquaculture to prevent the spread of diseases in commercial facilities. In Tables 7, 8, 9 and 10, it is possible to identify the recommended antibiotics for some bacterial diseases, like Erythromycin to treat Streptococcosis and Oxytetracycline or Romet 30® (Sulfamethoxazole-Trimethoprim) to treat several diseases, as Motile Aeromonas Septicemia (MAS), Pseudomonas septicaemia (Fin Rot Disease), Vibriosis, Edwardsiellosis and Yersiniosis (ERM) (Ibrahim, 2020). It is important to note that before using antibiotics, sources of stress should be eliminated or reduced and that treatments must be conducted for the required time period (Ibrahim, 2020). Too high a dose or too long a treatment will increase a danger of toxicity to the Nile tilapia, while too low a dose or too short a treatment time of antibiotic increase the risk to develop antibiotic resistance (Ibrahim, 2020). Two-week withdrawal period is recommended for all chemotherapeutic treatments prior the intended release or harvest date for Nile tilapia (Ibrahim, 2020).

However, antibiotics may be toxic to aquatic animals and/or may accumulate in the environment (El-Sayed, 2020). There is also 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. Furthermore, the misuse of antibiotics can result in widespread bacterial resistance to multiple drugs and some chemicals used to treat parasites may also cause welfare issues.

An alternative to the antibiotic use for Nile tilapia is the use of green-water system, which can control pathogenic bacteria in culture water and reduce their effects (El-Sayed, 2020). Microalgae, such as *Chlorella*, naturally secretes antimicrobial compounds, which inhibit pathogenic bacterial growth in such systems (El-Sayed, 2020).

RECOMMENDATION FOR ANTIBIOTIC USE

Compassion recommends that antimicrobials are only used as a treatment and not prophylactically. Antibiotics must not be used to compensate for a system with lower welfare potential and a badly managed production system.

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 and following vet and manufacturer guidelines without extending or shortening the treatment. Records of antibiotic usage should be kept which include dates, type of antibiotic, reason for use and amount used. [END]

Vaccination

The vaccination process may be stressful for fish, because it involves some handling and air exposition, depending on how the process is conducted. Despite that, vaccination as an alternative to chemotherapeutic treatment for diseases have become an important aspect of aquaculture and are widely used (A. E. Eissa, 2016). Licenced vaccines are already available against some important bacterial diseases that occur in Nile tilapia, such as Vibriosis – caused by *Vibrio anguillarum*, Edwardsiellosis – caused by *Edwardsiella tarda*, and Yersiniosis (enteric red mouth disease) – caused by *Yersinia ruckeri* (Table 7). Vaccines against other pathogens are being developed (Ibrahim, 2020). The major problems limiting the development of a commercial vaccine against some important Nile tilapia diseases like Motile Aeromonas

Septicemia (MAS disease) and *Pseudomonas septicaemia* (Fin Rot Disease) are the heterogeneity of the Nile tilapia strains (Ibrahim, 2020). The differing genetic selection of the different strains means that resistance, response to a disease and immune status will vary and differ, introducing one more factor complicating vaccine design for Nile tilapia.

RECOMMENDATION FOR USE OF VACCINES

Compassion recommends vaccination of tilapia whenever vaccines are available.

Vaccination must not be used to compensate for a system with lower welfare potential and a badly managed production system.

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.

Injected 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 tilapia.

[END]

Health promotion

Concerns over the use of antibiotics in aquaculture has led to the exploration and use of alternatives, such as probiotics and prebiotics in fish diets to promote the health and reduce the need for antibiotics (Defoirdt *et al.*, 2011; R. M. W. Ferguson *et al.*, 2010; Martínez Cruz *et al.*, 2012; Nayak, 2010; Tuan *et al.*, 2013; Welker *et al.*, 2011). It has already been demonstrated that the use of probiotics, prebiotics and alternative feed ingredients has the potential to positively affect growth, intestinal health, nutrient digestibility, water quality and reproduction in aquaculture species, including Nile tilapia (Haygood & Jha, 2018). For instance, using the probiotic *Bacillus subtilis*, in water or diet (as prophylaxis) is effective in amelioration the lesions caused by Columnaris disease (Table 7).

Moreover, reducing the susceptibility of fish to disease by adjusting environmental conditions to reduce adverse effects; regulating water temperatures; properly altering oxygen and other dissolved gas levels; reducing levels of ammonia and nitrite; reducing population densities; improving methods of handling; and using immunostimulants to improve disease resistance, are also important to promote Nile tilapia health. Immunostimulants are natural and synthetic compounds that promote the non-specific immune response and antibody production, including vitamins, trace elements, yeasts, glucans and others (Ibrahim, 2020). Additionally, some measures can help to promote health by preventing Nile tilapia diseases under farming conditions such as:

- Preventing exposure to physical, chemical and biological disease agents;
- Controlling environmental conditions that affect fish by farm site selection, water supply, fish handling, transport systems and waste removal;
- Diet selection, quantities fed and feeding frequency;
- Application of vaccination programmes for licenced vaccines;
- Application of sanitation programmes and egg disinfection for prevention of vertical and horizontal transmission of pathogens;
- Monitoring fish densities and welfare indicators.

OPPORTUNITIES TO EXPRESS NATURAL BEHAVIOUR

Welfare can only be properly assessed, monitored, and eventually incorporated into laws and regulations if there is an objective definition (Broom, 1991). Among the many definitions of welfare that have been debated over (Carenzi & Verga, 2009; Hewson, 2003), the one proposed by Broom (1986, 1991) best fulfils the premises of clarity and objectiveness and can therefore be operationalised (i.e. put into practice). According to this definition, welfare is **the state of the animal as it copes with the environment**. This definition of welfare has important implications:

- (i) Welfare is a characteristic of an animal, not something that is given to it.
- (ii) Welfare will vary along a continuum, from negative to positive.
- (iii) Welfare can be measured independently of ethical considerations.
- (iv) Measures of difficulty in coping with the environment give information about the welfare of the animal concerned.
- (v) Direct measurements of the state of the animal must also be used to assess its welfare, over and above knowledge of its biology.
- (vi) Coping mechanisms may vary among different species, and there are several consequences of failure to cope.

Three distinct approaches are used when addressing animal welfare (*Fraser, 1999, 2009; Huntingford et al., 2006*). A feelings-based approach requires that to be in a state of good welfare the animal should be free from negative experiences and have access to positive ones. This approach works under the assumption that fish are sentient animals, capable of feelings, emotions or equivalent affective or mental states. A function-based approach requires for good welfare that an animal can adapt effectively to its environment, such that all its biological functions are working effectively. Lastly, a nature-based approach assumes that each species has an inherent biological nature and that the ability to express it (particularly to express a natural repertoire of behaviour) is essential for good welfare. Applying each of these approaches separately has led to important improvements in animal welfare (Fraser, 2009). However, suffering, health problems and impairment of natural behaviour often accompany each other. An integrated, multi-disciplinary ethological approach can promote the objective measurements of welfare (Saraiva *et al.*, 2018) and provides answers to two important questions when it comes to welfare:

- 1- Are the animals healthy?
- 2- Do they have what they want (Broom, 2010; Dawkins, 2003, 2004)? Therefore, ensuring that the animals are free from diseases and providing them with the opportunities to express natural behaviours are good management practices to promote good welfare. On the other hand, failure to provide these features will inevitably cause distress to the animals.

The best way of preventing, or at least reducing, Nile tilapia diseases is the adoption of good management practices (El-Sayed, 2020). Diseases can usually be avoided by maintaining a high-quality environment and reducing handling stress (FAO, 2009). Because stress response is commonly the first step towards disease occurrence, it is important to consider that many common management procedures in farms are likely to cause stress for Nile tilapia, such as handling, crowding, transportation, confinement, and even the colour of light (Saraiva & Volstorf, 2022; Wall, 2001).

Management practices

Handling

Considering handling stress, it has already been demonstrated that chasing Nile tilapia for just 60 seconds with a net is already stressful for this fish (Volstorf & Maia, 2019a). Cortisol concentrations of Nile tilapia juveniles who were chased with a net for 60 seconds were higher (75 ng/g body tissue) than those of a control group not chased with a net (15 ng/g body tissue) for at least 2 hours. After 8 hours of handling, the levels went back to normal (Barcellos *et al.*, 2011). When studying chronic handling stress on Nile tilapia, Barcellos *et al.* (1999a) studied the effects of acute stress response of Nile tilapia who were previously exposed to handling stress over time. Fish exposed to such chronic stress had lower growth rates than non-stressed fish, thus indicating a negative effect from the stress originated from constant handling. However, fish previously exposed to chronic stress expressed lower plasma cortisol levels (196 ng/ml) than fish exposed only to acute stress (267 ng/ml) indicating an habituation to handling, although not eliminating their response to additional acute stress (Barcellos *et al.*, 1999a).

Crowding

Confinement is also a potential stressor for Nile tilapia. In an experiment investigating the effect of confinement on Nile tilapia, adults were confined for 30 minutes in one treatment, while for a control group, a partition was dipped in water without really confining the fish (Barreto & Volpato, 2004). A higher ventilatory frequency was observed in the two groups in relation to basal values and, despite a faster decrease in the control group, none of the groups returned to basal frequencies at the end of the observation period, indicating that confinement, real or perceived, stressed the fish, although fish who only perceived the confinement recovered faster (Barreto & Volpato, 2004). However, it has been seen that the use of blue light colour in the environment can reduce the stress response of this fish, as both plasma cortisol levels (Volpato & Barreto, 2001) and ventilatory frequency (Maia & Volpato, 2013) in confined Nile tilapia did not increase when fish were under blue light.

Crowding conditions can also stress Nile tilapia. Increasing fish density leads to what is known as social stress, which can also cause a chronic stress response (El-Sayed, 2020). In fact, decreased growth rates with increased stocking densities of Nile tilapia fry has already been reported (Dambo & Rana, 1993; El-Sayed, 2002). Basal plasma cortisol levels of Nile tilapia fingerlings were reported to increase with higher stocking densities, indicating a chronic stress response attributable to social stress (Barcellos *et al.*, 1999b). Similarly, when stocking density increased from 50 ind/m³, stress increased and growth reduced (Volstorf & Maia, 2019a). Therefore, crowding conditions should be limited and only carried out when necessary.

Treatment

Nile tilapia may also be exposed to secondary stressors as medical (chemical) treatments during management. The response of Nile tilapia to secondary stress after treatments with a mixture of formalin, malachite green and methylene blue (FMC) has already been investigated (Yavuzcan Yildiz & Pulatsu, 1999). This study demonstrated that treating the fish with FMC increased plasma glucose and haematocrits while reduced plasma phosphorus and calcium; magnesium was not affected. The authors attributed such changes in blood chemistry of fish to secondary stress response of Nile tilapia to FMC treatment. Such findings indicate that therapeutic agents, such as FMC, are under-recognised stress sources in management for Nile tilapia and therefore can affect the ability of tilapia to express natural behaviour.

Transport

Considering transportation management, Nile tilapia are usually hauled live to processing plants for slaughtering (FAO, 2009), which can be stressful for fish. Such transportation stress can cause serious welfare issues (Lines & Spence, 2014). It has already been demonstrated that Nile tilapia transported for 6-8h in plastic bags were stressed (Félix *et al.*, 2021; Hohlenwerger *et al.*, 2016; Teixeira *et al.*, 2018). Such stress response can be minimised by using anaesthetics during transportation (Félix *et al.*, 2021; Navarro *et al.*, 2016), but it must be considered that the use of anaesthetics is not allowed in some countries (European Food Safety Authority, 2004). It would be better to reduce the source of stress, meaning reducing journey times and reduce and avoid unnecessary transport.

RECOMMENDATIONS FOR MANAGEMENT PRACTICES

Compassion recommends that the health status of the fish must be assessed before starting any crowding, handling, transport, or treatments. These processes should be reduced or refined whenever possible so that fish experience the least amount of stress and should safeguard their welfare before and during their occurrence.

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. Crowding should only be carried out for a maximum of 2 hours with 48 hours 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. Tilapia must not undergo repeated crowding at harvest. Tilapia must not be out of water for more than 15 seconds.

Compassion recommends the use of pumps when fish are transferred between water bodies or to transport. If it is not possible, wet braille to transfer tilapia from the water system to the means of transport to prevent crowding, injuries, and prolonged air exposure. Compassion also recommends reducing journey times and avoid any unnecessary transport.

Road transport must only be carried out in trucks fitted with tanks. Sufficient water and oxygenation must be provided for boat and road transport to prevent stress and poor welfare in tilapias.

Welfare indicators

Easily visible behaviours that fish express can indicate their welfare state. Unusual or altered behaviours can indicate that fish are very stressed or even sick. Thus, the simple observation of the occurrence of abnormal behaviours is already an indication that a fish is not in a good welfare state. Pedrazzani *et al.* (2020) summarised behavioural indicators of welfare state for Nile tilapia during grow-out stage and capture management on farms (Table 12). It is also important to note that higher expression of natural behaviours considering the biological needs of the species may indicate that Nile tilapia are in a positive welfare condition. Because studies on altered behaviours as indicators of poor welfare state are more common, more research investigating positive behavioural indicators of welfare are needed.

Table 12: Behavioural signs that should be observed during grow-out stage or capture management (extracted from Pedrazzani et al. (2020))

Behavioural indicator	Grow-out stage	Capture
Gulping air at surface	X	X
Respiratory frequency	X	X
Swimming	X	X
Distribution in tank	X	X
Body colouration	X	X
Social behaviour	X	
Foraging behaviour	X	
Response to light	X	X
Response to air exposure		X

Considering indicators related to body and eye colouration, if a Nile tilapia is stressed, its body colour (more specifically the vertical “bands” it has along its body) becomes darker. There are situations of extreme stress in which practically the whole body of the fish becomes dark. Basically, the same happens with eye colouring, which has already been better investigated. The darker the eyes, the more stressed the fish is (Freitas *et al.*, 2014). The colour of the body and the eyes can also darken depending on the hierarchical position of the fish (Barreto *et al.*, 2011; E. M. V. Cruz & Brown, 2007; Evans *et al.*, 2008; Volpato *et al.*, 2003), which can also mean a stressful condition for Nile tilapia. However, body and eye darkening can be related to non-stressful factors such as the colour of the background substrate, which then is probably related to camouflage.

Gulping air at surface is another behavioural indicator mentioned by Pedrazzani *et al.* (2020). If Nile tilapia are breathing close to the surface, it is likely that that dissolved oxygen is very low in the water, which certainly has a negative effect on the welfare state of the fish. The ventilatory frequency, which can be identified by the movement (beating) of the operculum, can be also a good behavioural indicator that the Nile tilapia is stressed (Barreto & Volpato, 2004, 2006). If this species is stressed by confinement (Barreto & Volpato, 2004, 2006) or social stressors (Barreto & Volpato, 2006), the beating of the operculum is faster than normal, as the breathing is faster.

When considering the foraging behaviour of fish in general, if they are not interested in the food offered or feed less than usual, it might be an indicator of bad welfare state. Furthermore, swimming, and spatial distribution in the tank are two other indicators of the welfare state (Pedrazzani *et al.*, 2020). Unusual movement patterns of the fish in the water, both in terms of swimming rhythm and body posture, can be considered indicators of poor

welfare state. In fact, erratic or abnormal swimming patterns of Nile tilapia are common symptoms of diseases (El-Sayed, 2020). In addition, huddling in a corner or having some other type of abnormal spatial distribution is also a behavioural indicator that Nile tilapia are in a poor welfare state. Because Nile tilapia perform temperature-related depth displacements (El-Sayed *et al.*, 1996), that is, moving deeper in colder water and shallower with warmer waters, such movements can indicate that the temperature of the system is inappropriate for the species.

What is a social linear hierarchy?

It is a group structure where the dominant and submissive individuals are found in a linear sequence.

Furthermore, because Nile tilapia is a fish species with a social linear hierarchy when in small groups (Volstorf & Maia, 2019a), aggressive confrontations between individuals can occur at low stocking densities. If there are a lot of confrontations, it certainly means that the animals are very stressed, which negatively impacts their welfare. Thus, social behaviours can be used as a welfare indicator (Pedrazzani *et al.*, 2020). Moreover, besides stocking density, other common practices in aquaculture, such as classifying individuals by matching their sizes, water renewal, and environment lighting, can also affect Nile tilapias' social aggressive interactions and, thus impacting on their welfare state (Gonçalves-de-Freitas *et al.*, 2019).

There are also behavioural indicators that should be observed during feeding and capture managements to better evaluate the welfare conditions of Nile tilapia under farming conditions. Basically, feeding behaviour is considered appropriate if the fish consume the feed within 3-5 minutes, and capture management is adequate if the fish express normal swimming and move up to show a small number of dorsal fins or body parts on the surface (Pedrazzani *et al.*, 2020). Table 13 summarises the descriptions and respective values as references for scoring each of these behavioural indicators.

Table 13: *Behavioural signs that should be observed during feeding or capture management, with scoring and corresponding description (extracted from (Pedrazzani *et al.*, 2020)). The lower the score, the better for fish welfare.*

Management	Score	Criteria
Feeding	1	Apprehension of all food in 180–300 seconds
	2	Apprehension of all food in 120–180 seconds
	3	Apprehension of all food in ≤ 120 seconds
	4	No apprehension of all food or ≥ 360 seconds
Capture	1	Normal swimming, none or low numbers of dorsal fins or body parts on surface
	2	Excited swimming behaviour, >20 dorsal fins or low body parts on surface
	3	Swimming in different directions or decreasing activity, fish stuck against net
	4	Many fish floating on side, body exposure to air, exhaustion

Ability to express natural behaviours

Despite that juveniles and adults of Nile tilapia move considerably between feeding and breeding grounds in the wild, this species is considered typically resident in fresh water (McConnell, 1959; Philippart & Ruwet, 1980). Therefore, apparently no migration pattern is impaired by captive conditions in farms for this species. Because Nile tilapia are found usually at 0-7 m depth in the wild (Bwanika *et al.*, 2004; Komolafe & Arawomo, 2007) - meaning shallow waters - the farming systems can provide part of their natural depth range, which usually varies between 0.5 m and 4 m depth (El-Sayed, 2020). Despite that, as Nile tilapia can be occasionally found up to 30 m depth (Njiru *et al.*, 2006), it is important to note that not the whole natural depth range of this species can be covered under captive conditions. This is even more relevant considering that Nile tilapia move deeper with decreasing water temperatures (El-Sayed *et al.*, 1996). Therefore, the temperature should be checked and kept under optimal conditions for the species in farms.

Moreover, Nile tilapia are also able to naturally reproduce under farming conditions, by spawning easily and spontaneously in hapas, earthen ponds and concrete tanks (El-Sayed, 2020). However, even when these fish can spawn despite a lack of substrate in the environment (El-Sayed, 2020), it is important to provide a substrate, as males naturally dig and defend a pit in sandy substrate, where they perform elaborate behaviours (Castro *et al.*, 2009; Mendonça *et al.*, 2010; Uchida *et al.*, 2005). Additionally, as Nile tilapia is considered a benthic species that also uses substrates for feeding (Bwanika *et al.*, 2004; Oso *et al.*, 2006; Peterson *et al.*, 2006), this is another reason to provide substrate in farms. It was already demonstrated that Nile tilapia juveniles and adults grow better with substrate (Uddin *et al.*, 2009), which is present in earthen ponds (El-Sayed, 2020; Uddin *et al.*, 2009).

RECOMMENDATION ON ABILITY EXPRESS NATURAL BEHAVIOUR

Compassion recommends designing the enclosure to offer Nile tilapia with adequate depth and substrate. An appropriate sandy substrate and a depth of at least 2 m. would provide Nile tilapia with opportunities to express their natural behaviour, such as using the substrate to feed, nesting or avoid uncomfortable temperatures.

ANNEX 1: SPECIES OF TILAPIA

Aquaculture production of species of tilapia reared in 2020 (FAO, 2022) and estimated number of tilapia using Estimated Mean Weight (EMW) (Mood *et al.*, 2023).

Common name	Scientific name if available	2020 Aquaculture production (Tonnes – live weight)	Estimated Mean Weight (Lower–Higher mean)	Estimated number	
				Lower estimate	Upper estimate
Nile tilapia	<i>Oreochromis niloticus</i>	4,514,615	338-530	8,518,141,509.43	13,356,849,112.43
Tilapias nei	Tilapia of non-disclosed species	1,095,566	300-530	2,067,105,660.38	3,651,886,666.67
Blue-Nile tilapia, hybrid	<i>Oreochromis niloticus</i> x <i>Oreochromis aureus</i> , Hybrid	414,042	603-603	686,636,815.92	686,636,815.92
Mozambique tilapia	<i>Oreochromis mossambicus</i>	57,567	300-530	108,616,981.13	191,890,000.00
Tilapia shiranus	<i>Oreochromis shiranus</i>	5,422	300-530	10,230,188.68	18,073,333.33
Three spotted tilapia	<i>Oreochromis andersonii</i>	4,395	300-530	8,292,452.83	14,650,000.00
Longfin tilapia	<i>Oreochromis macrochir</i>	3,836	300-530	7,237,73.85	12,786,666.67
Redbreast tilapia	<i>Coptodon rendalli</i>	3,494	300-530	6,592,452.83	11,646,666.67
Blue tilapia	<i>Oreochromis aureus</i>	1,681	300-530	3,171,698.11	5,603,333.33
Blackchin tilapia	<i>Sarotherodon melanotheron</i>	67	300-530	126,415.09	223,333.33
Mango tilapia	<i>Sarotherodon galilaeus</i>	28	300-530	52,830.19	93,333.33

Redbelly tilapia	<i>Coptodon zillii</i>	6	300-530	11,320.75	20,000.00
Sabaki tilapia	<i>Oreochromis spilurus</i>	-	300-530	-	-

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