



Article An Assessment of the Risk Factors Associated with Disease Outbreaks across Tilapia Farms in Central and Southern Zambia

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Abstract: The study investigated the management practices that contribute to disease outbreaks in farmed tilapia in Lusaka and central and southern provinces in Zambia. It was a cross-sectional qualitative study undertaken from January to March 2021 in which questionnaires were administered to 49 farmers to assess their fish health management and biosecurity competence. Data were analysed using means, percentages, ratios, and logistical regression. The results showed that the majority of the farms had high stocking densities (>8 fish/m², 44.4%), reared Nile tilapia (67.7%), and sourced water for farming from rivers and streams (45.7%). A few farmers measured water quality parameters daily (16.7%) and removed dead fish from ponds daily (20.8%). The stocking density (p = 0.013), fish species (p = 0.031), dead fish disposal methods (p = 0.023), and control of predator birds (p = 0.016) influenced the total mortality recorded on farms, while pond type (p = 0.031 and p = 0.045), water source (p = 0.023), and stocking density (p = 0.027) influenced the duration of a mortality episode. It is evident that some fish health management practices and biosecurity concepts among tilapia farmers in the study area are inadequate and may not contain disease outbreaks or the spread of pathogens.

Keywords: fish health; biosecurity; disease; tilapia; risk analysis; Zambia

1. Introduction

Globally, aquaculture has played a significant role in improving the economic status of farmers and other key players (actors at each node) in the fish value chain [1]. In the past two decades, the aquaculture sector has seen rapid development due to increased demand for fish as an affordable source of animal protein [2]. Due to new technologies, fish culture methods have become more intensive, leading to higher yields per unit area [3]. However, the increase in aquaculture production has been accompanied by huge losses because of high fish mortality caused by disease outbreaks [4]. Outbreaks due to diseases such as tilapia lake virus (TiLV), streptococcosis, and motile aeromonad septicaemia have been reported in several intensive tilapia producing regions around the world [5–7]. The loss of revenue due to fish diseases is estimated at USD 6 billion per year globally [8]. Therefore, fish disease management remains a significant factor in the growth of the sector.

Fish diseases may be divided into infectious diseases, caused by pathogenic organisms present in the environment [9], and non-infectious diseases, caused by environmental



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). problems, nutritional deficiencies, or genetic anomalies [10]. The presence of pathogens in a fish population does not always result in disease and mortality, and individuals can remain asymptomatic under favourable conditions [11]. However, many factors are associated with the development and progress of fish disease in intensive production facilities. These include poor husbandry practices and inadequate biosecurity systems [12]. External stressors, such as high stocking densities, poor water quality and improper nutrition, may exacerbate the development of clinical disease, which sometimes leads to high incidences of mortality and low productivity [13]. Globally, biocide and antibiotic treatments are used widely in intensive fish production systems against infectious pathogens that cause disease and are present in the aquatic environments where fish are reared [3]. In many developed aquaculture-producing countries, the constant exposure of fish on farms to antimicrobials has contributed to increased antibiotic resistance in aquatic animals and adjacent ecosystems, and this resistance has spread to terrestrial animals and humans [14]. The farmers' knowledge of the clinical signs indicating a disease and the relevant biosecurity measures, such as the collection and disposal of dead fish, has an impact on the outcome of disease outbreaks [12]. Furthermore, prevention and control strategies are critical to preventing the onset of disease and reducing losses from disease when it occurs [15]. Therefore, preventing disease outbreaks by managing risk factors remains cardinal for the sustainability and growth of the aquaculture sector.

Similar to many developing countries, Zambia has reported the rapid growth of the aquaculture industry, supported by increased investment in the sector, which has allowed farmers to adopt improved aquaculture practices [16]. With intensified aquaculture production, diseases such as streptococcosis and lactococcosis have already been reported on some fish farms in Zambia [7]. Many fish farms in Zambia face several challenges related to health management practices that make them highly vulnerable to disease outbreaks [17]. Some of the challenges are a lack of knowledge of health management due to inadequate extension services, a lack of basic biosecurity measures and a lack of proper diagnostic tools [17].

Studies on fish diseases conducted in Zambia so far have been limited to the diagnosis and detection of pathogens [7,18,19]. However, so far, there has been no study on the potential risk factors associated with disease outbreaks in aquaculture farms. This study, therefore, investigated the management practices that can contribute to disease outbreaks in farmed fish in Lusaka and central and southern provinces in Zambia.

2. Materials and Methods

2.1. Study Area

The study was conducted in the central and southern regions of Zambia, including Lusaka and central and southern provinces. The selection of the study areas was based on the high number of commercial fish production activities in the regions. The study areas included nine districts: Siavonga (16.5323° S, 28.7111° E), Chirundu (16.0271° S, 28.8509° E), Lusaka (15.3875° S, 28.3228° E), Kafue (15.7644° S, 28.1766° E), Chilanga (15.5702° S, 28.2702° E), Kabwe (14.4285° S, 28.4514° E), Choma (16.8054° S, 26.9970° E), Kalomo (17.0299° S, 26.4784° E), and Livingstone (17.8520° S, 25.8285° E) [Figure 1].

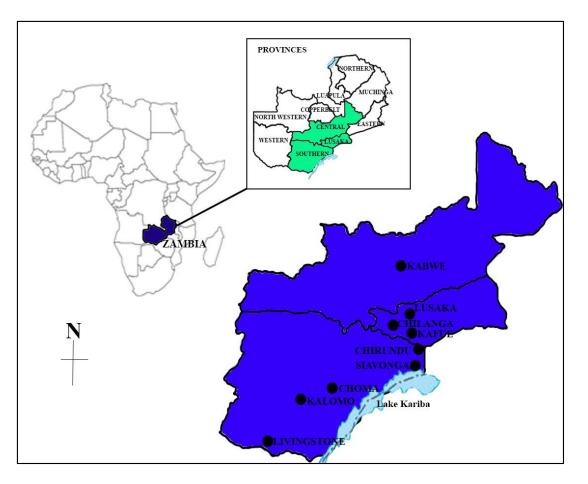


Figure 1. Map of Zambia showing Lusaka and central and southern provinces and nine districts within (Kabwe, Lusaka, Chilanga, Kafue, Chirundu, Siavonga, Choma, Kalomo, and Livingstone) which are a high aquaculture production region in Zambia and the study area.

2.2. Study Design and Data Collection

A cross-sectional qualitative survey of farms in the study area was conducted between January and March 2021. A questionnaire was prepared, reviewed, and pretested, and a final version incorporating the pretest results was produced. The questionnaire was pretested by interviewing four fish farmers and two researchers from the University of Zambia, whose responses were included in the final version. Data were collected from the selected fish farms using face-to-face interview to collect answers for the queries in the questionnaire. The questionnaire assessed the farming system, water management practices, stocking information, mortality data, knowledge of clinical signs, and any disease control measures. A total of 49 grow-out fish farmers were selected from a sample frame of 102 fish farmers registered with the Ministry of Fisheries and Livestock in the study area. For the purpose of this study, a fish farm was defined as an operation that reared fish in ponds for sale. The participating farms were selected on the basis of accessibility and the farmers' willingness to provide responses to the questionnaire.

2.3. Data Analysis

Data were entered into an Excel spreadsheet (Microsoft Excel 2010 version, Redmond, WA, USA) and then exported to DATAtab[™] (Styria, Austria), a Web-App for statistical data analysis, where descriptive statistics (frequencies and proportions) were computed and presented using tables for categorical parameters. The summary tables were prepared in accordance with the objective of the study. To examine the significance of associations of risk factors with the outcome variable, logistic regression analysis with the estimation of odds ratios (OR) was used. To compare the strength of the effect of each individual

independent variable on the dependent variable, a standardised beta coefficient was used. The *p*-values indicated the probability of observing the coefficient value, or in the more extreme cases, whether the null hypothesis is correct.

3. Results

In this study, we selected 49 farms from a sample frame of 102 farms for inclusion in the main analyses as these farmers had provided adequate data of quality.

3.1. Farm Production Characteristics (Pond Type and Size, Stocking Density, and Fish Species)

In the study area, the farmers reared three tilapia fish species, with the majority being Nile tilapia (*Oreochromis niloticus*) (Table 1). All the farmers included in the study practiced the monoculture system of fish production.

	Frequency	Percent (%)	95% CI
Por	nd Type		
Earthen ponds	26	53.1	39–67
Earth ponds with dam liners	18	36.7	23-50
Concrete ponds	5	10.2	2–19
Size of	Ponds (m ²)		
<300	15	30.6	18-44
301 to 500	3	6.1	0–13
501 to 600	23	46.9	33-61
>600	8	16.3	6–27
Stocki	ng Density		
<4 fish/m ²	8	16.3	6–27
4 to 8 fish/ m^2	22	44.9	31–59
>8 fish/m ²	19	38.8	25–52
Fish	Species		
Nile tilapia (Oreochromis niloticus)	33	67.4	54-80
Threespot tilapia (Oreochromis andersonii)	14	28.6	16-41
Greenhead tilapia (Oreochromis macrochir)	2	4.1	0–10

Table 1. Farm characteristics, such as pond type and size, stocking density, and fish species.

Over 50% of the farmers used unlined earthen ponds to rear their fish (Table 1).

For the purpose of this study, the pond sizes were categorised as small ponds ($<300 \text{ m}^2$), medium-sized ponds ($301-500 \text{ m}^2$), large ponds ($501-600 \text{ m}^2$), and extra-large ponds ($>600 \text{ m}^2$). The majority (46.9%) of the farmers in the study area used large ponds. A few farmers (6.1%) used medium-sized ponds (Table 1).

A considerable number of the farmers (38.8%) overstocked (>8 fish/ m^2) their ponds (Table 1).

3.2. Water Sources and Water Quality Monitoring

The majority of the farmers used either rivers/streams or boreholes as the water source for their aquaculture production facilities. A small number of the farmers (14.3%) used lake water for fish production, which they abstracted for their ponds (Table 2).

The monitoring of water quality was poor, with about a quarter of the farmers (26.5%) testing for physicochemical parameters, such as dissolved oxygen and pH. Among the farmers who monitored water quality, the majority (76.9%) carried out this exercise only once a month (Table 2).

	Frequency	Percent (%)	95% CI
	Source	of Water	
River/stream	22	44.9	31–59
Borehole	20	40.8	27-55
Lake	7	14.3	04–24
	Water-Quality-Mo	nitoring Performed	
No	36	73.5	61–86
Yes	13	26.5	14–39
	* Frequency of Wate	r Quality Monitoring	
Monthly	10	76.9	65-89
Weekly	2	15.4	5 to 26
Daily	1	7.7	0 to 15

Table 2. Water sources and water-quality-monitoring trends.

n = 49, * n = 13, CI, confidence interval.

3.3. Mortality Trends and Fish Disease Management

3.3.1. High-Mortality Incidences and Trends

About 90% (87.8%) of the farmers in the study area reported to have observed a high incidence of fish mortality during each production cycle. Two-thirds of the farmers reported mortalities mainly in fingerlings with average body weight between 3 and 20 g (Table 3). The majority (71.4%) of the farmers reported mortality duration of less than 5 days. Half the farmers reported very low cumulative mortality (<5%) at the end of each production cycle (Table 3).

Table 3. Clinical signs and disease outbreak trends as per farmer records.

	Frequency	Percent (%)	95% CI
High-Mor	tality Incidences		
Yes	43	87.8	79–97
No	6	12.2	3–21
Growth Stage with	the Highest Mortali	ty Rate	
3 to 20 g	29	59.2	45-73
21 to 50 g	6	12.2	3–21
51 to 150 g	6	12.2	3-21
251 g and above	2	4.1	0–10
Duration of the M	ortality Episodes (l	Days)	
<5	35	71.4	59-84
5 to 10	6	12.2	3–21
>10	2	4.1	0–10
Total Mortalit	y Rate (%) per Cycl	e	
<5	26	53.1	39–67
5 to 10	5	10.2	2-19
>10	12	24.5	12–37
Clinic	al Signs Seen		
Fish gasping for air at the water surface	15	30.6	18-44
Cotton wool appearance on the skin surface	6	12.2	3-21
Reddish discolouration of the skin	3	6.1	0-13
Fin rot/erosion	3	6.1	0-13
Fish swimming in circles	2	4.1	0-10
Others (lethargy, corneal opacity)	6	12.2	3–21
I don't know	8	16.3	6–27
Medicines Us	ed to Treat Sick Fis	h	
None	19	38.8	25–52
Salt	18	36.7	23-50
Potassium permanganate	4	8.2	0–16
Lime	2	4.1	0-10

n = 49, CI, confidence interval.

3.3.2. Clinical Signs

The most prominent clinical sign 30.6% of the farmers reported was fish gasping for air at the surface of the water. Other clinical signs reported included reddish lesions of the skin, fin rot/erosion, cotton wool appearance on the skin surface, lethargy, and corneal opacity. About 16.3% of the farmers were not able to identify clinical signs in their fish production facilities during high-mortality episodes (Table 3).

3.3.3. Disease Treatment

The common treatment 36.7% of the respondents used during disease outbreaks was salt (sodium chloride) treatment. A significant number of farmers (38.8%) did not treat their fish during high-mortality episodes (Table 3).

3.4. Biosecurity Management

3.4.1. Visitor Access to Fish Production Site

The majority (83.7%) of the farmers in the study area had restricted visitor access to the fish production facility (ponds). Furthermore, 69.4% of the farms had physical barriers, such as a perimeter fence, limiting the access of visitors to the fish production facility. Only one-fifth of the farmers had some form of handwash and footbath stations for disinfection at their production facility (Table 4).

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	Frequency	Percent (%)	95% CI
Restrict Visitor Entr	ry into the Produc	tion Area	
Yes	41	83.7	73–94
No	8	16.3	6–27
Restrict Access to	the Fish Production	on Area	
Yes	34	69.4	56-82
No	15	30.6	18–44
Follow a Cleaning Progr	camme for Tools a	nd Equipment	
Yes	25	51	37-65
No	24	48.9	35–63
Use Disinfectants	for Cleaning Equ	ipment	
Chlorine	19	38.8	25-52
None	16	32.7	20-46
Quaternary ammonium chloride	14	28.6	16–41
Use Footbaths and Handwash at t	he Entrance of the	Fish Production Fa	cility
No	39	79.6	68–91
Yes	10	20.4	9–32
Control Pred	ator Birds (Metho	ds)	
Use bird nets	15	30.6	18-44
Chase them away	15	30.6	18-44
Use scarecrows	10	20.4	9–32
Use fireworks	5	10.2	2–19
Use no method	4	8.2	0–16
Dispose of I	Dead Fish (Method	s)	
Leave the dead fish in water	17	34.7	21-48
Bury the fish	14	28.6	16-41
Dispose of the fish in uncovered pits	7	14.3	4–24
Incinerate the fish	5	10.2	2–19

n = 49, CI, confidence interval.

3.4.2. Equipment Cleaning and Disinfection

About half (51%) of the farmers had a cleaning programme for tools and equipment used in the fish production facility. In all, 38.8% of the farmers used chlorine as the

disinfectant of choice and about one-third of the farmers washed their tools and equipment with water but did not use any chemical to disinfect them (Table 4).

3.4.3. Control of Predator Birds

The predominant methods of controlling predator birds around fish ponds was using bird nets (30.6%) and chasing away the birds physically (30.6%) (Table 4).

3.4.4. Disposal of Dead Fish

About 34.7% of the farmers indicated that they did not remove dead fish from their ponds during mortality episodes (Table 4). The farmers who did use disposal methods reported that they incinerated the dead fish, disposed of the dead fish in uncovered pits or buried the dead fish in pits.

3.5. Fish Health Diagnostic and Extension Services

During disease outbreaks, 79.1% of the farmers reported having received some form of technical assistance from a professional who either visited their facility or gave advice via a phone call (Table 5). The majority of the farmers (67.6%) consulted aquaculturists for assistance in disease diagnosis. For the purpose of this study, an aquaculturist was defined as a person with an academic qualification, a certificate, a diploma, or a degree in fisheries and aquaculture.

Table 5. Fish health diagnostic and extension services.

	Frequency	Percent (%)	95% CI
Seek Professiona	l Help During Mor	tality Episodes	
Yes	34	79.1	56-82
No	9	20.9	8–29
* Profess	sionals Providing Se	ervices	
Aquaculturists	23	67.6	50-77
The source hatchery	7	20.6	6–27
Training institution personnel	2	5.9	0-10
Veterinarian	2	5.9	0–10

n = 43, * n = 34, CI, confidence interval.

3.6. Risk Factors Associated with Fish Mortality

Risk factors associated to tilapia disease outbreaks were addressed here through the analysis of the (a) estimated total mortality rate recorded at the farms and (b) estimated duration of each high-mortality episode.

3.6.1. Estimated Total Mortality Rates Recorded on Farm

When the cumulative mortality rate for the case farms was used as the dependent variable in determining risk factors, the significant independent variables were pond size, fish species, dead fish disposal, and control of predator birds. The independent variable, pond size ($<300 \text{ m}^2$), was significant (p = 0.013), thereby indicating that there was 39% increase in number of farmers in the category per unit increase in the cumulative mortality rate. Farmers rearing Nile tilapia (p = 0.031) increased by 30% per unit increase in the cumulative mortality rate. The number of farmers disposing of dead fish by burying them (p = 0.023) decreased by 46% per unit increase in the cumulative mortality rate. In terms of controlling predator birds, a correlation was revealed of the number of farmers using scarecrows (p = 0.016) and chasing birds away (p = 0.003) with the unit mortality rate increasing by 34% and 45%, respectively (Table 6).

	Beta Coefficient	Odds Ratio	Standard Error	р	R2	
		Pond Size (m ²)				
<300	0.33	1.39	1.45	0.013		
301 to 500	-0.01	0.99	2.59	0.961	0.00	
501 to 600	0.24	1.27	1.29	0.057	0.09	
600>	-0.09	0.91	2.29	0.46		
		Species				
Nile tilapia	0.26	1.30	1.31	0.031		
Threespot tilapia	0.06	1.06	1.56	0.639	0.04	
Greenhead tilapia	0.04	1.04	2.97	0.761		
	Dead 1	Fish Disposal Met	hods			
Bury the fish	-0.62	0.54	2.92	0.023		
Dispose of the fish in uncovered pits	-0.31	0.73	3.38	0.197	0.14	
Leave the dead fish in water	-0.45	0.64	3.09	0.099		
Incinerate the fish	-0.18	0.84	3.25	0.358		
	Control of Predator Birds					
Use scarecrows	0.29	1.34	1.32	0.016		
Use bird nets	-0.16	0.85	1.71	0.202		
Chase them away	0.37	1.45	1.35	0.003	0.25	
Use no method	0.11	1.12	2.17	0.375		
Use fireworks	-0.14	0.87	2.17	0.249		

Table 6. Factors associated with a high total cumulative mortality on farms.

3.6.2. Estimated Duration (Days) of High-Mortality Episodes

The independent factors that were significantly associated with the duration of mortality episodes were pond type, water source, and stocking density. All three pond types, earth ponds with dam liners (p = 0.031), concrete ponds (p = 0.031), and earth ponds (p = 0.045), showed a significant increase, by 1.18, 0.78, and 1.11 units, respectively, in the number of days for mortality episodes. The number of farmers who either reared fish on the lake or used water from the lake (p = 0.023) increased by 0.26. The number of farmers who stocked their fish at the rate 5 to 8 fish/m² (p = 0.027) increased by 0.24 (Table 7).

Table 7. Factors associated with the duration (days) of high-mortality episodes.

	Beta Coefficient	Odds Ratio	Standard Error	p	R2
	Por	nd Type			
Earth ponds with dam liners	1.18	3.25	3.33	0.031	
Concrete ponds	0.78	2.18	2.97	0.031	0.12
Earth ponds	1.11	3.03	3.18	0.045	
	Sourc	e of Water			
Borehole	0.00	1.00	0.63	0.992	
River/stream	0.16	1.17	0.63	0.138	0.06
Lake	0.26	1.30	0.89	0.023	
	Stocking De	ensity (Fish/m ²)			
<4	0.11	1.12	0.86	0.335	
4 to 8	0.24	1.27	0.62	0.027	0.02
>8	0.09	1.09	0.68	0.426	

4. Discussion

This study was conducted to assess management practices and the potential risk factors that contribute to disease outbreaks in tilapia farms in Zambia. The results indicated that

pond size and type, water source, stocking density, fish species, control of piscivorous birds, and disposal methods of dead fish were the main contributing factors to the cumulative mortality rate per batch of fish produced and the duration of high-mortality episodes.

Tilapias are among the most important warm-water fishes used for aquaculture production [20]. In this study, the common tilapia species the respondents reared were Nile tilapia, threespot tilapia, and greenhead tilapia, and the majority of the respondents reared Nile tilapia, indicating that at that time it was probably the most reared fish in that region of the country. In 2019, the Food and Agriculture Organisation of the United Nations (FAO) ranked Nile tilapia ninth among the aquatic species (plants and animals) reared globally, it being the most popular species group farmed in 127 countries [21]. The fish adapts well in water temperatures between 23 and 30 °C, making the tropical and subtropical conditions of Zambia conducive for Nile tilapia production [22]. In our study, we found a positive correlation between the rearing of Nile tilapia and high total mortality rates on the farms. However, this result is in contrast with research findings that have demonstrated that Nile tilapia is less susceptible to disease development compared to other species. Nile tilapia has been found to be more resistant to *Streptococcus* spp. compared to blue tilapia (Oreochromis aureus) and Mozambique tilapia (Oreochromis mossambicus) and their hybrids [23]. Furthermore, Edwardsiella tarda has been shown to cause mild to moderate lesions and mortality in Nile tilapia but not in African catfish (*Clarias gariepnus*) [24]. In 2009, in an infection experiment, Songe demonstrated that compared with threespot tilapia and straightfin barb (Barbus paludinosus), Nile tilapia was more resistant to Aphanomyces invadans infections, not showing any clinical signs even after 32 days of inoculation [19]. Therefore, because of its comparative resistance to a number of disease pathogens, Nile tilapia is an excellent culture species for the developing aquaculture industry in Zambia. Furthermore, the positive correlation of Nile tilapia to cumulative mortality could also be attributed to it being the overrepresented species in this study.

About half of the farmers in this study reared fish in large ponds (501–600 m²), although it has been previously reported that 80% of the farmers in Zambia and 90% of the farmers in Sub-Saharan Africa use medium-sized ponds [25,26]. The correlation between pond size and total or cumulative mortality rates was only seen in small ponds ($<300 \text{ m}^2$). Pond size and water surface area have been shown to have a relationship with the levels of dissolved oxygen. Impaired respiration in fish is more pronounced in small ponds compared to larger ones, where midnight dissolved oxygen readings were lower in the former (1.65 mg/L) than in the latter (3.18 mg/L) [27]. In contrast to the observations of this study, higher incidences of septicaemia and columnaris disease have previously been reported in fish from large ponds, attributed to high intensive culture practices, such as feeding rates [28].

Stocking density has a huge influence on the growth rate, productivity, and incidence of disease outbreak in aquaculture production facilities. The FAO recommends a stocking density of 4 to 8 fish/ m^2 for pond culture [29]. In this study, fewer than half the farmers had the ideal stocking density. Stocking density (4 to 8 fish/ m^2) showed a positive correlation with the duration of a high-mortality episode. This result is in contrast to studies that have demonstrated that fish reared in high stocking densities (>8 fish/m²) are susceptible to high mortalities. High stocking densities have been shown to reduce feeding activity and growth rates in farmed fish as well as increase the level of metabolites, such as urine and faeces, in pond water [30]. Accumulating metabolites change water quality, subjecting fish to chemical stressors in addition to the chronic stress caused by social dominance [31,32]. Several studies have demonstrated that high stocking density exacerbates disease development and transmission and mortality rates [33-35]. A significant relationship exists between high stocking density, infectious dose (cfu/mL) of Streptococcus iniae in Nile tilapia and outbreaks of Streptococcus agalactiae in tilapia cultured in a high-stocking-density environment, poor-quality water, and water temperatures above 28 °C [33,35]. In Ecuador, the severity of Tilapia lake virus outbreak was positively associated with high stocking densities apart from other risk factors [34].

The source of water used in aquaculture production has a direct impact on the quality of the water that the fish is exposed to. In this study, among the water sources, lake water was the only potential risk factor in relation to the duration of disease outbreak. Despite rivers and lakes providing a ready supply of water for fish production, studies have shown that in high-aquaculture-production regions, water quality parameters such as physicochemical parameters (ammonia, phosphates, and heavy metals) and microbiological parameters are high [36,37]. Depending on the number of farms upstream, pathogens might enter the water body (river, stream, or lake), which will serve as a vehicle for transmitting the pathogens to farms downstream. Pathogens such as *Saprolegnia* spp. have shown a strong correlation with fish farms in downstream locations receiving water from infected farms upstream [38]. We can postulate that lake water was a potential risk in this study because of the increasing number of farms on lakes currently growing fish in these lakes, thereby increasing pathogen presence in the areas.

An essential best practice in aquaculture production is the routine monitoring of water quality parameters such as dissolved oxygen and pH. This study revealed that the majority of the farmers in the study region did not monitor water quality in their rearing units at all. In other words, once the fish were stocked in the ponds, dissolved oxygen, temperature, pH, ammonia, turbidity, and others were not measured throughout the production cycle. Only a small proportion of the farmers monitored water quality parameters daily. High levels of chemicals, such as ammonia, have a toxic effect on fish, leading to their inability to extract energy from feed and lethargy among the fish on chronic exposure [39]. High ammonia levels also promote the proliferation of ectoparasites and disease pathogens in the pond water [39,40]. Therefore, the measurement of ammonia and other water-quality variables provides a snapshot of conditions at the time the water sample was collected. Routine monitoring of water quality provides information to the farmers on the suitability of the aquatic environment for fish rearing, allowing the farmers to change the water when the parameters are above permissible limits. Furthermore, it serves as a fish welfare assessment tool that helps prevent fish exposure to chemical stress that may predispose the fish to stress and possible opportunistic disease development and outbreaks.

Globally, disease outbreaks have been reported in many aquaculture production regions. Depending on the aetiology and causative pathogens, disease can be reported at any stage of the growth phase of fish. In this study, the majority of the farmers reported high-mortality episodes in fish when the fish weighed 3 to 20 g. In Zambia, hatcheries supply fingerlings at body weights between 2 and 5 g. This means that the farmers reported high mortality rates during the first 14 days after they stocked fish in their ponds [41]. In one study conducted in Zambia, high early mortalities were reported at a farm on lake Kariba, and the major contributing factor was cumulative stress experienced prior to, during and after the transportation of the fingerlings [42]. The main stressors were (a) mechanical trauma during grading and counting prior to transportation, (b) undulating water temperature, low dissolved oxygen (DO) levels, high density of fish, changes in water salinity and high turbidity during transportation, and (c) abrupt changes in temperature and water quality and high stocking density at stocking in the cages [43]. Mortalities immediately post-stocking can be attributed to stress from handling and transportation and will usually last about 3 to 5 days. If mortalities persist for more than 5 days, it is likely a disease outbreak due to primary or secondary pathogens. Some of the disease pathogens known to cause mortality in tilapia fingerlings are *Flavobacterium columnare* [44], *Gyrodactylus* sp. and *Trichodina* sp. [45], and tilapia lake virus [46].

Among the clinical signs observed during mortality episodes, fish gasping for air at the surface of water was the commonest one reported by farmers. This clinical sign is mainly reported in fish in hypoxic conditions, where the dissolved oxygen is insufficient to support respiration. Other clinical signs reported were erratic swimming, reddish lesions of the skin, fin rot or erosion, cotton-like appearance on the skin, lethargy, and corneal opacity. These clinical signs reported by the farmers are consistent with those seen in tilapia diseases such as columnaris, streptococcosis, lactococcosis, motile aeromonad septicaemia, and saprolegniasis [7,43,47]. In Zambia, disease outbreaks caused by *Aeromonas* spp., *Streptococcus agalactiae*, and *Lactococcus garvieae* have been reported in a number of farms on Lake Kariba rearing Nile tilapia [7,18].

Disease control remains an integral component of aquaculture production as intensification increases the risk of outbreaks. Globally, several methods of controlling fish diseases are available and are categorised as either reactive treatments or proactive disease strategies. In this study, farmers reported using salt (sodium chloride), potassium permanganate, or lime as disease treatment options. This study, therefore, confirms that farmers do not use antibiotics to control disease outbreaks in aquaculture production facilities in the central and southern regions of Zambia. It is important to note that the global antimicrobial consumption in aquaculture in 2017 was estimated at 10,259 tons, with the highest consumers being China (57.9%), India (11.3%), Indonesia (8.6%), and Vietnam (5%) [48]. The non-use of antibiotics in the Zambian aquaculture industry offers an opportunity to develop more sustainable and environmentally acceptable methods of preventing diseases, such as the use of probiotics, vaccines, and ethnoveterinary products. Ethnoveterinary medicine has been used successfully to treat diseases on fish farms in Korea [49].

Due to losses and low quantity of fish available for sale at the end of each cycle, the cumulative mortality rate reported on fish farms has a huge impact on the revenue expected by the farmer. In this study, a larger proportion of farmers recorded cumulative mortality rates of less than 5% in each production cycle. Only 27.3% of the farmers reported higher cumulative mortality rates (>10%). Disease outbreaks by primary or secondary pathogens have been shown to cause mortality rates higher than 10%. Elsewhere, viral pathogens such as tilapia lake virus (TiLV) and infectious spleen and kidney necrosis virus (ISKNV) have been shown to cause mortality rates of up to 90% [5,50]. *Lactococcus garvieae, Streptococcus* spp., *Francisella* spp., *Edwardsiella tarda*, and other bacterial pathogen have recorded mortality rates of up to 50% [7,51–53]. The significant potential risk factors associated with the cumulative mortality rate in this study were pond size, fish species reared, methods of disposing of dead fish, and methods of controlling piscivorous birds.

The prompt removal and appropriate disposal of moribund and dead fish reduce the chances of an infection spread [54]. In this study, about a third of the farmers reported not removing dead fish from the pond, and this poor practice perpetuates the cycle of infection. It should be highlighted that the transmission rate of a bacterial fish pathogen (*Flavobacterium columnare*) has been demonstrated to be higher from a dead host, most likely a consequence of the higher shedding rates of dead fish when compared to living fish [55]. Furthermore, *Edwardsiella ictaluri* can be transmitted to susceptible fish from infected individuals by cannibalism [56]. Therefore, leaving dead fish in a pond exacerbates the transmission of pathogens to the susceptible individuals in the population, leading to more mortalities and losses. In this study, we found a negative correlation between the fish burial disposal method and the total mortality rate. For every unit increase in the number of farmers burying dead fish, there was a decrease in the total mortality rate.

In our study, the majority of the farmers did not allow visitors to access their fish production facility and most had perimeter barriers that prevent people or animals from accessing the site. In contrast to our result, a study conducted in Zambia by Hasimuna et al. (2020) reported that the majority of the small-scale farms did not have barrier fences to prevent animals (otters and monitor lizards) and people that may be vectors of some pathogens from accessing the fish production site [17]. Flores et al. (2015) reported that in Bataan Province in Philippines, 58% of the farmers had perimeter barriers around their ponds [57]. Only 20.4% of the farmers reported managing biosecurity by placing footbath and handwash stations at the access point to the fish production facility. Bacterial pathogens such as *Streptococcus iniae*, *Streptococcus agalactiae*, *Lactococcus garvieae*, and *Mycobacterium marinum* can be transmitted mechanically from fish to humans and vice versa through handling [58]. However, the risk of humans spreading fish disease pathogens is low unless they are workers working in handling live fish and the activities are conducted within a few hours of two different rearing units.

In our study, only about half of the farmers had some form of programme for disinfecting and cleaning their tools and equipment. The only disinfectants the farmers used were chlorine and quaternary ammonium chloride. In Vietnam, a better developed aquaculture industry, 50% of the farmers in the northern region reportedly used disinfectants in the production facilities, which is consistent with the result of this study [59]. Although a similar proportion of farmers in both studies used disinfectants, in Vietnam, the farmers used up to 20 different disinfectants, compared to the 2 disinfectants reported in this study [59]. In a disinfectant susceptibility study, quaternary ammonium compounds and chlorine-based compounds showed mild and poor efficacy towards bacteria isolated from fish farms [13]. Furthermore, the use of chlorine and quaternary ammonium compounds as a disinfectant have been demonstrated to promote the horizontal transfer of plasmids by natural transformation via the exchange of antimicrobial resistance genes across bacterial genera and leading to the emergence of new antimicrobial-resistant bacteria [60,61]. As farmers in this study commonly used quaternary ammonium compounds and chlorine, a susceptibility assessment is warranted to assess the efficacy of these compounds against bacteria on farms.

Piscivorous birds help transmit pathogens between ponds and farms in addition to contributing to economic losses by eating fish from the facility. Pathogens transmitted by birds include digenean parasites, *Francisella* spp., *Edwardsiella tarda*, and viral pathogens [51,62]. Therefore, the methods of controlling these birds will have an impact on disease transmission as well. In this study, the farmers used bird nets, physical chasing, scarecrows, and fireworks to control birds, with the first two being the commonest methods. Furthermore, there was a correlation between two of the control methods (using scarecrows and chasing away the birds) and an increase in the total mortality rate, indicating that these two methods are not effective enough and, therefore, regardless of their being implemented on the farms, the mortality rates increased, most probably since the birds still had access to the fish and kept feeding on them.

The availability of skilled fish health diagnostic and extension services has an impact on the outcome of disease outbreaks. In this study, the majority of the farmers reported receiving some form of technical help when they experienced high fish mortality in their aquaculture production facilities. Furthermore, most farmers reported receiving fish disease diagnostic services from aquaculturists, who in this study were individuals with some academic qualification in fisheries and aquaculture. An overview of the curriculum of academic institutions offering academic qualification in fisheries and aquaculture revealed little content on fish diseases and diagnosis (personal communication). The aquaculture sectors in other countries, such as Bangladesh and Kenya, have also reported low access of farmers to disease diagnostic services due to unavailability of specialised personnel [63,64]. To increase farmers' accessibility to fish health diagnostic and extension services, in China, it was proposed that a call centre will be created that will help fish farmers to access experts in fish disease diagnosis and treatment via mobiles or telephones [65]. With the rapidly growing aquaculture sector in Zambia, the adoption of the fish expert call centre strategy will increase farmers' access to fish disease diagnostic services in the country.

5. Conclusions

This study highlighted the potential risk factors associated with the duration of disease outbreak episodes and the total mortality rate in the production cycle among tilapia farmers in Lusaka and central and southern provinces in Zambia. The risk factors identified in this study were pond size, pond type, stocking density, water source, species of tilapia reared, methods of disposing of dead fish, and methods of controlling predatory birds. The study further highlighted that the biosecurity and fish health management practices among the fish farms were moderate to low. Some of the poor fish health management practices were (1) the lack of a water-quality-monitoring plan among the majority of the respondents, (2) a

low frequency of water quality testing, (3) high fish stocking densities, and (4) low rates of removal and disposal of dead fish from ponds. Other poor practices were treatment of disease with salt, lime, or potassium per manganate regardless of the causative agent. The positive findings of the study were that the majority of the respondents in the central and southern regions of Zambia had access to some form of fish health technical services, were able to identify the clinical signs of fish disease, and did not use antibiotics.

To support the growth of the aquaculture industry in Zambia, deliberate steps should be taken to empower farmers with knowledge of fish health management and basic best aquaculture practices. An integrated planning approach to aquaculture development should be adopted which requires all stakeholders (farmers, scientists, and policy makers) to have knowledge of fish health management and biosecurity. Furthermore, the government of Zambia should consider establishing links among stakeholders in the aquaculture industry in Zambia by creating strong fora for the exchange of information on the fish health status of the industry.

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