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Spatial Risk Estimation of Parasitic Infestations of Pond and Cage Cultured Nile Tilapia (*Oreochromis niloticus*) in the Lake Victoria Crescent, Uganda

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Abstract: A study was conducted to determine the estimated parasitic infestation and associated risk factors (Water quality, Farm management practices and External factors such as Intermediate hosts and Wild fish entry) of farmed Nile tilapia in Pond grow-out, Cage grow-out (lake), Cage grow-out (reservoir) farms and Hatcheries in the Lake Victoria Crescent, Uganda. Sixteen parasite genera and 65% (418/640) infestation rate were obtained. However, Pond grow-out farms and Hatcheries did not only constitute 81% and 63% of the parasite genera, but also had the highest mean number of parasite genera per farm of 1 to 7 and 4 to 8 respectively. Cage grow-out (reservoir) farms and Hatcheries had the highest mean prevalence of >0.7. Water quality parameters, farm management practices and external factors varied across the 4 farming systems, with Cage grow-out farms (lake) with the best water quality parameters, farm management practices and control over intermediate hosts and wild fish entry. Using Spatial Areal Unit Modelling with Conditional Autoregressive Priors, out of 16 risk factors, only Intermediate hosts had a significant effect on estimated parasitic infestation. Estimated parasitic infestation of 0.28 (low) or 0.55 (high) prevalence was obtained. All Cage grow-out (reservoir) farms and Hatcheries, and 78% (14/18) of Pond grow-out farms had high estimated parasitic infestation despite their locations, while 75% (3/4) of Pond grow-out farms that had low estimated parasitic infestation were located in Masaka (an area with many minor and less polluted rivers). Fifty-six percent (5/9) of Cage grow-out (lake) had low estimated parasitic infestation and were located in the Southern part of Wakiso, Southern part of Mukono and Southern part of Jinja but far from the Napoleon Gulf. The Cage grow-out farms with high estimated parasitic infestation were found in the Southeastern part of Buikwe (an area with sugar cane plantations and factories) and Southern part of Jinja in the polluted Napoleon Gulf. Therefore, the type of farming system and its location (spatial component) need to be given at-most importance when coming up with management and sanitary control strategies to encounter parasite infestation.

Keywords; farmed fish; parasites; water quality; farm management; spatial modelling

Key Contribution: A few studies on parasites of farmed Nile tilapia have been carried out in Uganda. However, this study has made use of the parasite occurrence and risk factors to estimate parasitic infestation using a GIS tool. This is first time this tool is being used in Parasitology studies in Uganda.

1. Introduction

Aquaculture production has steadily increased worldwide to approximately 130.9 million tonnes in 2022 [1], in response to the increasing demand for the fish protein amidst reduced supply from capture fisheries. On the global scale, Africa's aquaculture production contributed to 7 per cent of the total fish production in 2022 [1]. In Uganda, fish production also rose with an estimated total production of 95,000 tonnes in 2010 to 139,000 tonnes in 2021 [1]. The increase is attributed to Nile tilapia, a commonly cultured species, that contributes approximately 49% of national annual aquaculture production [2]. The species has intrinsic favourable attributes that promote its rearing not only in Uganda but the world over. These include high nutritional value, easy managerial protocol, highly prolific, indiscriminate appetite and tolerance to poor water quality [3]. Indeed, the culture of *Oreochromis niloticus* contributes to improved food and nutrition security, economic growth, trade and living standards of the human populations [4]. Despite the above benefits, fish production is faced by a number of challenges including limited land for culture, lack of feeds, predators, poor fish seeds, competition from other agricultural sectors, lack of access to financial support, environmental pollution, and poor fish disease management [5,6].

Intensification of aquaculture production in Uganda is believed to cause disease out-breaks in future [7]. Certainly, fish parasites have been reported to be the largest contributors of fish disease problems in the country [7,8]. In pond and cage culture systems, the parasites co-exist with farmed fish if the host-pathogen interaction is not disrupted [9]. Nevertheless, rapid changes in water quality and farm management practices may result in an increase in the parasite populations [9–11]. Parasites attack fish's external surface (skin, eyes, fins) or internal organs (gills, gut, gonads, kidney, liver, swim bladder, muscle tissue, intestines) [12]. As a result, serious economic losses through morbidity, loss of productivity and treatment costs may occur on a farm [13,14]. Therefore, in order to control the direct and indirect effects of these parasites, functional aquatic health management and biosecurity systems are need and yet they are still missing in the country [15]. For instance, farmers often have little knowledge of biosecurity measures and good farm management practices [16]. The few available agricultural extension workers who would provide technical support to farmers are hardly knowledgeable in disease management [15,17]. Furthermore, there have been few disease surveillance programs, lack of well-equipped diagnostic laboratories, shortage of fish health experts, high costs of diagnosis, and absence of disease outbreak reports due to poor record keeping by farmers [15,18]. Therefore, this calls for the employment of better epidemiological approaches like disease risk modelling [19–21].

Currently, environmental DNA, statistical modelling, mathematical modelling and risk mapping are some of the tools being utilized in disease risk modelling [18–22]. Risk mapping, which makes use of Geographic Information Systems (GIS), has been noted to be used in aquatic animal disease management [23,24]. These GIS make use of the combination of computer science and statistics to define the role played by environmental factors, farm management practices and other external factors in parasite proliferation in culture systems [25,26]. In this way, the parasite-fish interaction is defined using evaluation of results on parasites' occurrence and the risk factors that influence their proliferation [20,27]. Simple spatial analyses like production of descriptive parasitological maps are performed as well as more complex analysis like disease risk modelling [28–30]. Hence, the study made use of the Geographical Information Systems incorporated with water quality, farm management practice and external factors (intermediate hosts and wild fish entry) data to identify fish farms in the Lake Victoria Crescent with conditions that may promote parasitic infestation in pond and cage cultured Nile tilapia.

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2. Materials and Methods

2.1. Study Area and Sample Collection

The study was conducted in the Lake Victoria Crescent, Uganda (1° 0′ 0.0000″ S and 33° 0′ 0.0000″ E). In the Crescent, only districts with a relatively high aquaculture development were visited (Figure 1). Nile tilapia fish farms within the selected districts were chosen using block sampling i.e., 5% of the total number of fish farms in the district, in order to overcome denominators between selected districts. This was as follows: eight farms from Buikwe (C), five from Wakiso (A), four from Bugiri (G), three from Masaka (E) and Mukono (B), two from Mpigi (D), Busia (J), Tororo (K), and Namayingo (H), and one from Jinja (F). *A total of 640 live specimens of Nile tilapia, 20 samples per* farm (from different farming units on each farm), were randomly collected from 32 fish farms (18 Pond grow-out farms, 9 Cage grow-out farms (lake), 2 Cage grow-out farms (reservoir) and 3 Hatcheries between mid October and early December, 2019. This sampling period was selected due to the fact that the Crescent receives near normal to above normal rainfall during this period [31] and rainfall has not only a direct influence on parasite life cycle but also an indirect influence on the host population abundance [32]. The collected fish had weight between 56g and 328 g and length ranged between 11 cm and 23 cm. The fish were transported in separate labeled plastic bags to the Parasitology Laboratory at Aquaculture Research and Development Centre, National Agricultural Research Organization for parasitological examination.

2.2. Examination of Fish for Different Parasites

2.2.1. Macroscopic Examination

The external body surface including scales, gills, fins and operculum of freshly harvested live fish specimen were examined with the use of the naked eye and a dissecting microscope for the presence of external parasites. The scales were crushed carefully by a scalpel to identify any macroscopic lesions or cysts [33]. Furthermore, the fish were dissected from anus ventrally along the middle of abdomen to mouth as described by [34] in order to expose the internal organs. Two lateral incisions were done in order to expose the body cavity, alimentary canal and other internal organs and all were examined for presence of macroscopic adult parasites and encysted metacercariae [35].

2.2.2. Microscopic Examination

Scrapings from the body surface, fins and gills of the fish were taken with a cover slip and were smeared onto clean microscope slides and examined microscopically [36]. The digestive tract was dissected along its length using scissors and investigated by parts and its content was emptied into a petri dish and diluted with physiological saline; the films were then prepared and examined [35]. Adult trematodes and cestodes were collected, washed with physiological saline to remove mucus and debris, and left in a refrigerator at 4 °C until complete relaxation. Then, they were fixed according to [37] and stained in acetic acid alum carmine according to [38]. The nematodes were collected in warm 70% ethyl alcohol solution and cleaned in lactophenol for 12 to24 hours. They were then preserved in 70% alcohol plus 5% glycerin solution [39]. All of the prepared slides were examined to identify the parasite genera basing on morphological features such as shape and size, and predilection sites in the fish host as described elsewhere by [40–51].
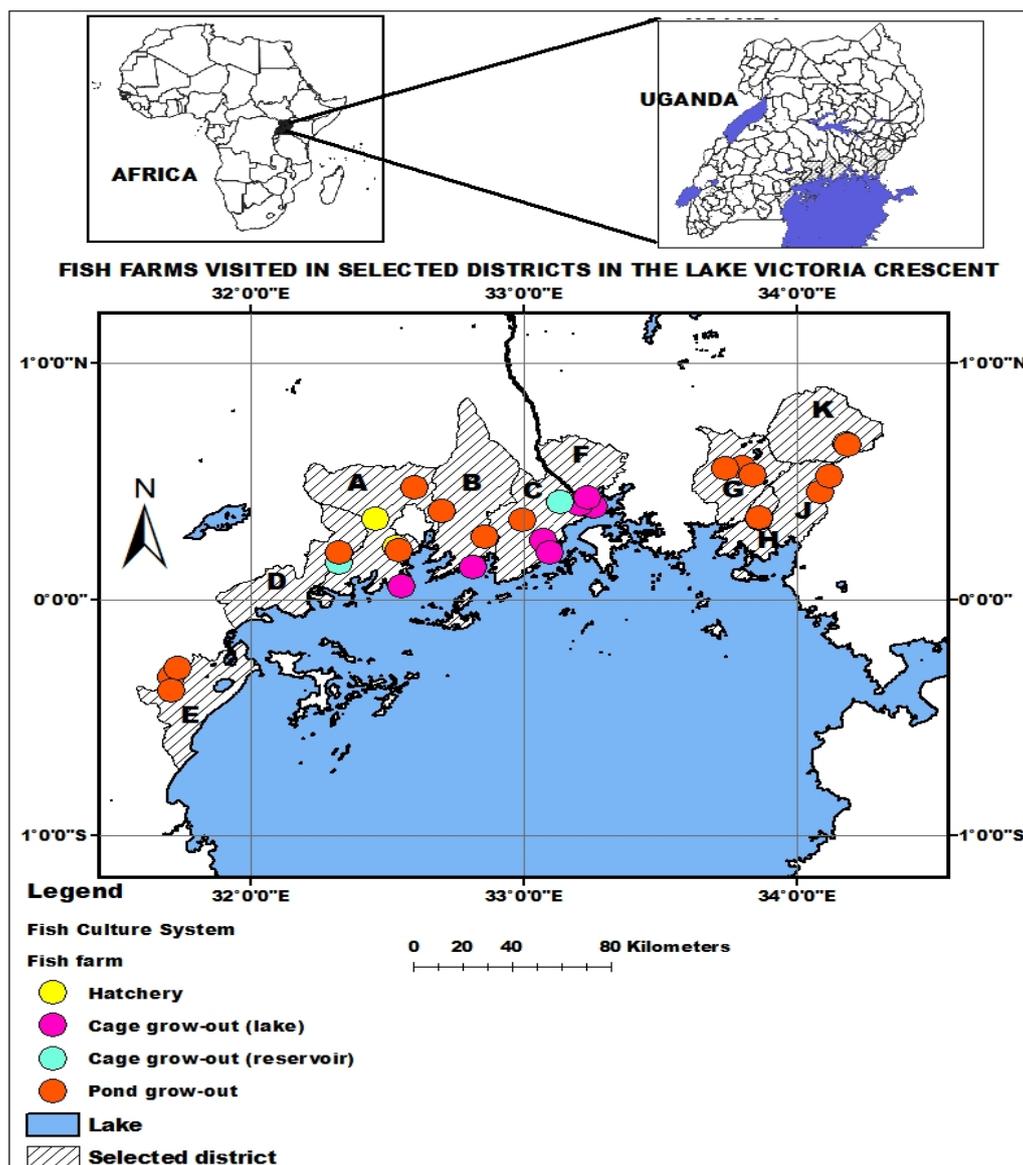
Figure 1. Map showing fish farms visited in selected districts in the Lake Victoria Crescent.

2.3. Collection of Water Quality, Farm Management Practices and Other External Factors (Intermediate Hosts and Wild Fish Entry)

2.3.1. Water Quality

Temperature, pH, salinity, dissolved oxygen, conductivity, and total suspended solids were collected using VuSitu digital water testing kit (In-Situ Inc., United States), while ammonia, nitrite, chloride and hardness were collected using Fresh Water Aquaculture Kit AQ-2 (LaMotte Company, United States).

2.3.2. Farm Management Practices Data and External Factors

Data were collected through direct observation, qualitative open-ended questionnaires and interviews given to the fish farm managers. Management factors; fish seed source, stocking density, feeding and nutrition status (i.e., amount of feed given, frequency of feeding, time intervals of feeding and quality of feed) and disinfection were ascertained by questionnaires and interviews whereas external factors such as wild fish entry and intermediate hosts were ascertained by direct observation as recommended by [52,53].

2.4. Assessment of Geographical Areas for Potential Parasitic Proliferation

The specific latitudes and longitudes of fish farms visited were collected by use of Global Positioning System tool (Garmin International Inc., United States). These coordinates were used to locate the areas with potential parasitic infestation.

2.5. Statistical Analysis

2.5.1. Parasite Diversity and Infestation Levels

The parasite diversity in the study was determined by parasite diversity estimators; Chao and Jackknife. The Shannon– Wiener Index (H′) and evenness (E), and the Berger–Parker Dominance Index (d) (a quantification of the most abundant parasite types in a given sample) were also determined as described by [54]. Infestation levels were analyzed by prevalence, mean intensity and mean abundance using the guidelines outlined by [55].

2.5.2. Effect of Water Quality, Farm Management Practices and External Factors on Parasitic Proliferation

The effect of each risk factor on parasitic infestation was defined by regression analyses performed in R Software version 4.2.1 [56]. Prevalence was used as the dependent variable while the risk factors served as predictor variables. The risk factors included fish seed source, stocking density, feeding and nutrition status, presence of intermediate hosts, wild fish entry, disinfection, temperature, salinity, dissolved oxygen, pH, conductivity, total dissolved solids, ammonia, nitrite, chloride and hardness. Uni-variate regression models were then performed to individually evaluate the effect of each risk factor on prevalence. Upon analysis, only intermediate hosts had a significant effect on the prevalence (Table 8) and this risk factor was used for further analyses.

2.5.3. Assessment of Fish Farms in Lake Victoria Crescent for Potential Parasitic Infestation

Before modelling the estimated parasitic infestation on the fish farms in the Lake Victoria Crescent in R Software version 4.2.1, fish farms were first grouped into 11 hexagons of width 20 km. Each hexagon included at least 1 farm and the average prevalence of fish farms in each hexagon was defined. This was done in order to overcome small denominators among fish farms. Hexagons with no fish farms were excluded from further analyses. The remaining hexagons were then included into the final model as a spatial area-level random effect [57], using a Conditional Auto-regressive (CAR) prior structure [39]. The final model was then performed using the average prevalence of fish farms in each hexagon, the significant risk factor (intermediate hosts) and the obtained spatial area-level random effect. Furthermore, the Moran's I statistic was computed to determine the presence of spatial auto-correlation in the residuals from the final model, and this could quantify if there would be any clusters in the estimated parasitic infestation [58].

2.5.4. Mapping of the Estimated Parasitic Infestation on Fish Farms in the Lake Victoria Crescent

Using ArcGIS software version 10.6, the fitted values obtained from the final model were used to map the estimated parasitic infestation on the fish farms in the Lake Victoria Crescent, Uganda.

3. Results

3.1. Parasite Diversity and Infestation Levels

Out of 640 fish samples, 418 (65%) were infested with a single or multiple parasites belonging to one or many different genera with some shown in Figure 2. A total of 16 different parasite types were identified to genera level. Parasite diversity indices; Shannon index (H′) of 0.961, Evenness (E) of 0.347, Berger–Parker Dominance index (d) of 0.79 and diversity estimators; Chao = 16.125 and Jackknife = 16.998, were obtained as shown in Table 1.

Table 1. Summary of ecological characteristics of *Oreochromis niloticus* parasite diversity.

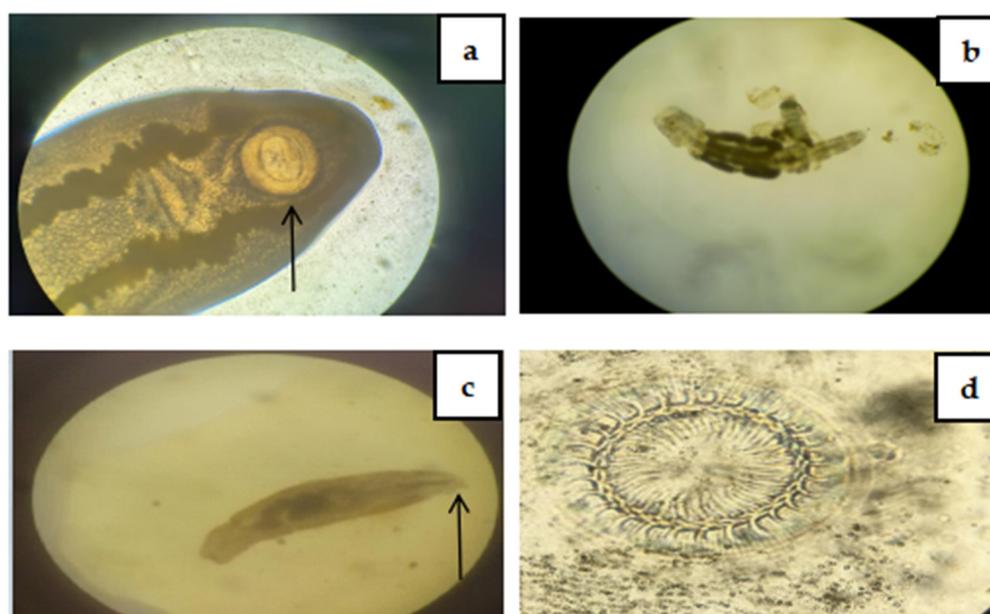| Diversity parameter	Oreochromis niloticus (n = 640)
Total number of genera	16
Shannon index (H')	0.961
Evenness (E)	0.347
Berger–Parker Dominance index (d)	0.79
Diversity estimators	
Chao	16.125
Jackknife	16.998

Figure 2. Micrographs of some of the parasites identified in/ on the fish; a) Dorso-ventrally flattened oval metacercariae of *Clinostomum* sp. with a sucker (arrow) around the anterior mouth, b) Elongated, cyclopoid and segmented *Ergasilus* sp. with elongated antennae, c) Dorso-ventrally flattened *Acanthocephalus* sp. with spiny proboscis (arrow), d) Cylindrical shaped *Trichodina* sp. with numerous denticulate rings as viewed under Electronic Leica microscope at X400.

The most prevalent parasites were the *Trichodina* species with a prevalence of 23.4% followed by *Dactylogyrus* species with a prevalence of 14.2%. Of all parasite genera, 43.8% (7/16) had mean intensities of greater than ten individuals per fish. *Trichodina* species had the highest mean abundance of 9.66, compared to the rest of the parasite genera with mean abundance less than 1 as indicated in Table 2.

Table 2. Prevalence (P), Mean intensities (Mi) and Mean abundance (Ma) of *Oreochromis niloticus* parasites in the study.

No.	Genus	Prevalence (%)	Mean intensity	Mean abundance
1.	*Trichodina*	23.4	41.21	9.66
2.	*Dactylogyrus*	14.2	4.16	0.59
3.	*Neascus*	5.6	4.9	0.28
4.	*Clinostomum*	3.6	3.22	0.12
5.	*Ergasilus*	5.9	4.61	0.27
6.	*Myxobolus*	4.4	13.64	0.60
7.	*Amirthalingamia*	0.6	2.25	0.014
8.	*Acanthocephalus*	1.4	1.56	0.022
9.	*Monobothroides*	0.2	25	0.039

10.	*Contracaecum*	0.3	3	0.0094
11.	*Eimeria*	0.2	35	0.055
12.	*Gyrodactylus*	0.6	4.5	0.028
13.	*Chilodonella*	0.3	17.5	0.055
14.	*Ichthyobodo*	1.9	6	0.11
15.	*Ambiphrya*	0.3	12	0.038
16.	*Diphyllobothrium*	3	11.47	0.34

Pond grow-out farms and Hatcheries consisted of the highest number of parasite genera, with 81% and 63% of the parasite genera identified in the study respectively (Table 3). The Hatcheries had the highest mean number of parasite genera per farm, having between 4 to 8 parasite genera per farm, followed by Pond grow-out with 1 to 7 parasite genera per farm. Cage grow-out (reservoir) farms had the highest parasite frequencies followed by Pond grow-out farms, Hatcheries with moderate and Cage grow-out (lake) farms with the least. Cage grow-out (reservoir) farms and Hatcheries had the highest mean prevalence of >0.7 while Pond grow-out farms had moderate and Cage grow-out (lake) farms with the least (Table 3).

Table 3. Parasite genera found, number of parasite genera and parasite frequencies in the various fish farms.

Fish farming system	Genera present (Number of farms infested (n))	Mean number of genera on farms (range)	Mean parasite frequencies on farms (range)	Mean prevalence on farms (range)
Pond grow-out (N = 18)	Trichodina (n = 16) Dactylogyrus (n =12) Neascus (n = 3) Clinostomum (n = 4) Ergasilus (n = 3) Myxobolus (n = 3) Amirthalingamia (n =1) Contracaecum (n = 1) Gyrodactylus (n =2) Chilodonella (n = 1) Ichthyobodo (n = 4) Ambiphrya (n = 1) Diphyllobothrium (n = 1)	3(1 -7)	118 (12 - 447)	0.49 (0.15 - 0.8)
Cage grow-out (lake) (N= 9)	Trichodina (n = 5) Dactylogyrus (n = 5) Neascus (n = 1) Diphyllobothrium (n = 1)	1(0 - 3)	30(18 - 87)	0.29 (0 - 0.65)
Cage grow-out (reservoir) (N = 2)	Trichodina (n = 2) Dactylogyrus (n =2)	3(2-3)	2555(1021- 4089)	0.73(0.55-0.9)

	Myxobolus (n = 1)		
Hatchery (N =3)	Trichodina (n = 2) Dactylogyrus (n =2) Neascus (n = 3) Clinostomum (n = 3) Ergasilus (n = 1) Myxobolus (n = 2) Amirthalingamia (n =1) Acanthocephalus (n = 2) Monobothro (n = 1) Eimeria (n = 1)	6(4-8) 108(57-193)	0.78(0.65-1)

3.2. Water Quality, Farm Management Practices, External Factors and Parasitic Infestation

3.2.1. Water Quality, Farm Management Practices and External Factors in the Various Fish Farms

The mean and range values of water quality parameters, farm management practices and external factors against recommended limits for fish culture, varied from one farming system to another as shown in Tables 4–7. All the Grow-out pond farms had levels of pH, Salinity, Nitrite and Chloride fall within the optimum range for pond culture (Table 4). In most Grow-out pond farms, Temperature and Ammonia were outside the recommended limits, whereas less than half of pond farms had Dissolved oxygen, Total dissolved solids, Conductivity and Hardness falling outside the recommended limits (Table 4). Furthermore, most of the Grow-out pond farmers did not only obtain seed from either wild catch or other fellow farmers but also had poor feeding and didn't carry out disinfection on the farm. Slightly more than half of the Grow-out pond farms had their ponds overstocked. There was also presence of intermediate hosts in most of the Grow-out pond farms while slightly more than half had wild fish entry (Table 4).

Table 4. Water quality, farm management practices and external factors at Pond grow-out farms.

Pond grow -out farms (N = 18)	Parameter	Mean values (range) values of pond water	Recommended limits*	Percentage (%) above or below recommended limits (N = 18)
Water quality	DO (mg/l)	5.1 (2.1- 9.7[a])	5.5 – 10	38.9% (n = 7)
	T(°C)	24.9 (21.9 - 26.9[a])	26 – 32	72.2% (n = 13)
	pH	7.6 (6.7 - 8.1)	6.5–8.5	0% (n = 0)
	Salinity (PSU)	0.06 (0 - 0.2)	0 -20	0% (n = 0)
	Total dissolved solids (mgl^{-1})	0.08 (0 - 0.27[b])	< 0.13	22.2% (n = 4)
	Conductivity (µS/cm)	121 (5 - 408[a])	100 -2000	44.4% (n = 8)
	Ammonia free nitrogen (mg/l)	1.2 (0.05 - 2[b])	0 – 0.2	94.4% (n = 17)
	Hardness (ppm as CaCO3)	36 (8 - 88[b])	< 50	27.8% (n = 5)
	Nitrite (mgl^{-1})	0.05 (0.005 - 0.5[b])	0 – 0.2	0% (n = 0)
	Chloride (ppm)	17 (4 - 40)	<230	0% (n = 0)
Farm management practices	Fish seed source (1-Certified hatchery, 2-Other: wild catch or from fellow farmers)	2 (1 - 2[b])	Certified hatchery-1	83.3% (n = 15)
	Feeding and nutrition status n status (Rank 1 to 5 according to feed type, ration and intervals)	3(2 - 5[a])	Rank 4, 5	77.8% (n = 14)
	Disinfection (Present-1 , Absent-0)	0 (0 -1[a])	Present-1	77.8% (n = 14)
	Stocking density (1-overstocked, 2-recommended (4 to 8 fish/m^2) or under-stocked	2 (1 - 2[a])	Recommended or under-stocked -2	55.6% (n = 10)

External factors	Intermediate hosts (Present-1 , Absent-0)	1 (0 - 1[b])	Absent-0	77.8 % (n = 14)
	Wild fish entry (Entry-1 , No entry-0)	1 (0 - 1[b])	No entry -0	55.6% (n = 10)

[a]Only lower value was below the optimum range. [b]Only upper value was above the optimum range. [ab]Both the lower value and upper value were outside optimum range. *Recommended limits as reported by [59–61]. N = Total number of fish farms assessed. n is the number of fish farms whose parameter was either below or above or outside recommended limits.

Table 5. Water quality, farm management practices and external factors at Cage grow-out farms (lake).

Cage grow-out farms (lake) (N= 9)	Parameter	Mean values (range) values of lake water	Recommended limits*	Percentage (%) above or below recommended limits (N = 9)
Water quality	DO (mg/l)	5.7 (4.3 - 6.9[a])	5.5 – 10	44.4% (n = 4)
	T(°C)	26.2 (25.2 - 27.7[a])	26 – 32	44.4% (n = 4)
	pH	8.6 (8.0 - 9.5[b])	6.5–8.5	66.7% (n = 6)
	Salinity (PSU)	0.04 (0.02 - 0.05)	0 -20	0% (n = 0)
	Total dissolved solids (mgl[-1])	0.05 (0.02 - 0.07)	< 0.13	0% (n = 0)
	Conductivity (µS/cm)	78 (38 - 109[a])	100 -2000	66.7% (n = 6)
	Ammonia free nitrogen (mg/l)	1.5 (0.5 - 2[b])	0 – 0.2	100% (n = 9)
	Hardness (ppm as CaCO3)	22 (6 - 48)	< 50	0% (n = 0)
	Nitrite (mgl[-1])	0.05 (0 - 0.05)	0 – 0.2	0% (n = 0)
	Chloride (ppm)	13 (5 - 38)	<230	0% (n = 0)
Farm management practices	Fish seed source (1-Certified hatchery, 2-Other: wild catch or from fellow farmers)	2 (1 - 2[b])	Certified hatchery-1	55.6% (n = 5)
	Feeding and nutrition status n status (Rank 1 to 5 according to feed type, ration and intervals)	4 (3 - 4[a])	Rank 4, 5	11.1% (n = 1)
	Disinfection (Present-1 , Absent-0)	1 (0 -1[a])	Present-1	22.2% (n = 2)
	Stocking density (1-overstocked, 2-recommended (60 to 80 fish/m³) or under-stocked)	1 (1 - 2[a])	Recommended or under-stocked - 2	77.8% (n = 7)
External factors	Intermediate hosts (Present-1 , Absent-0)	0 (0 - 1[b])	Absent-0	44.4% (n = 4)
	Wild fish entry (Entry-1 , No entry-0)	0(0 -0)	No entry -0	0% (n = 0)

Table 6. Water quality, farm management practices and external factors at Cage grow-out farms (reservoir).

cage grow -out farms (reservoir) (N = 2)	Parameter	Mean values (range) values of reservoir water	Recommended limits*	Percentage (%) above or below recommended limits (N = 2)
Water quality	DO (mg/l)	3.8 (2.9 - 4.7[a,b])	5.5 – 10	100% (n = 2)
	T(°C)	24.1 (24 - 24.2[a,b])	26 – 32	100% (n = 2)
	pH	7.7 (7.3 - 8)	6.5–8.5	0% (n = 0)
	Salinity (PSU)	0.03 (0.02 - 0.03)	0 -20	0% (n = 0)
	Total dissolved solids (mgl[-1])	0.05 (0.04 - 0.05)	< 0.13	0% (n = 0)
	Conductivity (µS/cm)	62 (54 - 70[a,b])	100 -2000	100% (n = 2)
	Ammonia free nitrogen (mg/l)	2 (2 - 2[b])	0 – 0.2	100% (n = 2)
	Hardness (ppm as CaCO3)	26 (20 -32)	< 50	0% (n = 0)
	Nitrite (mgl[-1])	0.005 (0.005 - 0.005)	0 – 0.2	0% (n = 0)
	Chloride (ppm)	32 (25 - 38)	<230	0% (n = 0)
Farm management practices	Fish seed source (1-Certified hatchery, 2-Other: wild catch or from fellow farmers)	2 (2 - 2)	Certified hatchery-1	100% (n = 2)
	Feeding and nutrition status n status (Rank 1 to 5 according to feed type, ration and intervals)	3 (3 - 3)	Rank 4, 5	100% (n = 2)
	Disinfection (Present-1 , Absent-0)	0 (0 -0)	Present-1	100% (n = 2)
	Stocking density (1-overstocked, 2-recommended (60 to 80 fish/m³) or under-stocked)	2 (2 -2)	Recommended or under-stocked -2	0% (n = 0)
External factors	Intermediate hosts (Present-1 , Absent-0)	1 (1 - 1)	Absent-0	0% (n = 0)

	Wild fish entry (Entry-1 , No entry-0)	1 (0 -1)	No entry -0	50% (n = 1)

Table 7. Water quality, farm management practices and external factors at Hatcheries.

Hatcheries (N = 3)	Parameter	Mean values (range) values of hatchery pond water	Recommended limits*	Percentage (%) above or below recommended limits (N = 3)
Water quality	DO (mg/l)	5.7 (4.3 - 6.5[a])	5.5 – 10	33.3% (n = 1)
	T(°C)	24.7 (24.2 - 25.1[a,b])	26 – 32	100% (n = 3)
	pH	7.8 (7.5 - 8.1)	6.5–8.5	0% (n = 0)
	Salinity (PSU)	0.06 (0.03 - 0.09)	0 -20	0% (n = 0)
	Total dissolved solids (mgl^{-1})	0.09 (0.05 - 0.13[b])	< 0.13	33.3% (n = 1)
	Conductivity (µS/cm)	118 (74 - 192[a])	100 -2000	66.7% (n = 2)
	Ammonia free nitrogen (mg/l)	1.2 (1 - 1.5[a,b])	0 – 0.2	100% (n = 3)
	Hardness (ppm as CaCO3)	32 (14 - 61[b])	< 50	33.3% (n = 1)
	Nitrite (mgl^{-1})	0.05 (0 - 0.05)	0 – 0.2	0% (n = 0)
	Chloride (ppm)	12 (6 - 13)	<230	0% (n = 0)
Farm management practices	Fish seed source (1-Certified hatchery, 2-Other: wild catch or from fellow farmers)	1 (1 -1)	Certified hatchery-1	0% (n = 0)
	Feeding and nutrition status n status (Rank 1 to 5 according to feed type, ration and intervals)	4 (3 - 5[a])	Rank 4, 5	33.3% (n = 1)
	Disinfection (Present-1 , Absent-0)	1 (1 - 1)	Present-1	0% (n = 0)
	Stocking density (1-overstocked, 2-recommended (4 to 8 fish/m²) or under-stocked)	1 (1 - 2[a])	Recommended or under-stocked -2	66.7% (n = 2)
External factors	Intermediate hosts (Present-1 , Absent-0)	1 (1 -1[b])	Absent-0	100% (n = 3)
	Wild fish entry (Entry-1 , No entry-0)	0 (0 - 1[a])	No entry -0	33.3% (n = 1)

All the Cage grow-out farms (lake) had levels of Salinity, Total dissolved solids, Hardness, Nitrite and Chloride fall within the optimum range for cage culture (Table 5). In most lake cage farms, pH, Conductivity and Ammonia were outside the recommended limits, whereas less than half of cage farms had Dissolved oxygen and Temperature falling outside the recommended limits (Table 5). Furthermore, more than half of the Cage grow-out (lake) farmers obtained seed from either wild catch or other fellow farmers while most of them had good feeding, overstocked their cages and carried out disinfection on the farm. Less than half of the Cage grow-out farms had intermediate hosts while no farm had Wild fish entry.

All the Cage grow-out farms (reservoir) had levels of pH, Salinity, Total dissolved solids, Hardness, Nitrite and Chloride fall within the optimum range for cage culture whereas Dissolved oxygen, Temperature, Conductivity and Ammonia fell outside the recommended limits (Table 6). Even though, there was no overstocking in the Cage grow-out farms (reservoir), all of them had poor feeding, didn't carry out disinfection on the farm and obtained seed from either wild catch or other fellow farmers. Although, the two Cage grow-out farms (reservoir) no intermediate hosts one had wild fish entry (Table 6).

All the Hatcheries had levels of pH, Salinity, Nitrite and Chloride fall within the optimum range for seed culture whereas Temperature and Ammonia fell outside the recommended limits (Table 7). Furthermore, Conductivity was outside the recommended limits in most hatcheries while few hatcheries had Dissolved oxygen, Total dissolved solids, and Hardness fall outside the optimum range for seed culture. All Hatcheries produced their own seed and carried out disinfection while they over- stocked their seed production ponds. Intermediate hosts were found in all Hatcheries while one had wild fish entry (Table 7).

3.2.2. Effect of Water Quality, Farm Management Practices and External Factors on Parasitic Infestation

Uni-variate generalized linear models were fitted to individually evaluate the effect of each risk factor on prevalence. Only one of the 16 risk factors showed a statistically significant effect on prevalence and that was intermediate hosts as seen in Table 8

Table 8. Uni-variate analysis of potential risk factors for parasitic infestation on different fish farms.

No.	Model covariates	Estimate	P-value
1.	intercept + dissolved oxygen	-0.0244	0.306
2.	intercept + temperature	-0.0610	0.093
3.	intercept + pH	-0.109	0.147
4.	intercept + salinity	-0.227	0.832
5.	intercept + total dissolved solids	0.0149	0.985
6.	intercept + conductivity	-0.0000569	0.915
7.	intercept + ammonia	-0.0347	0.658
8.	intercept + hardness	0.00236	0.264
9.	intercept + nitrite	-0.194	0.729
10.	intercept + chloride	0.00630	0.130
11.	intercept + fish seed source	-0.116	0.228
12.	intercept + feeding and nutrition	0.0958	0.402
13.	intercept + stocking density	0.0000000152	0.339
14.	intercept + wild fish entry	0.153	0.0931
15.	intercept + intermediate hosts	0.267	0.00465*
16.	intercept + disinfection	-0.0571	0.530

* = statistically significant risk factor.

The fitted model of the intermediate host risk factor and prevalence together with neighbourhood matrix of the hexagonal regions containing fish farms were used to quantify the presence of spatial autocorrelation in the residuals from the model. A Moran's I statistic was computed and a permutation test conducted to assess spatial autocorrelation significance. A Moran's I test statistic of -0.307 with a p-value of 0.823 was obtained, indicating that the residuals did not contain any substantial spatial autocorrelation (p > 0.05). Therefore, there was no need of adding the spatial random effect to the final model. A generalized linear model of prevalence and intermediate hosts was then finally performed. According to the final model, increase in number of intermediate hosts was associated with an increased parasitic infestation, as indicated by an estimate of 0.267 with a P-value of 0.00465.

3.3. Estimation of Parasitic Infestation

The fitted values obtained from the final model corresponded to an estimated prevalence of 0.28 (low) or 0.55 (high) at the fish farms. A risk map of estimated parasitic infestation based on the final model of the visited fish farms in the Lake Victoria Crescent was produced (Figure 3). Estimated parasitic infestation varied across fish farms and farming systems in the Crescent as evidenced in Figure 3. Pond grow-out farms had the highest estimated parasitic infestation of all the farming systems. Of the 18 Pond grow-out farms visited, 78% (14/18) had high estimated parasitic infestation despite their locations. However, 75% (3/4) of Pond grow-out farms with low estimated parasitic infestation were located in Masaka. For the case of Cage grow-out (lake) farms, 56% (5/9) had low estimated parasitic infestation and were located in Southern part of Wakiso, Southern part of Mukono and Southern part of Jinja but far from the Napoleon Gulf of Lake Victoria. The Cage grow-out farms with high estimated parasitic infestation were found in Southeastern part of Buikwe and Southern part of Jinja in Napoleon Gulf of Lake Victoria. All Cage grow-out (reservoir) farms and Hatcheries had high estimated parasitic infestation despite their location.
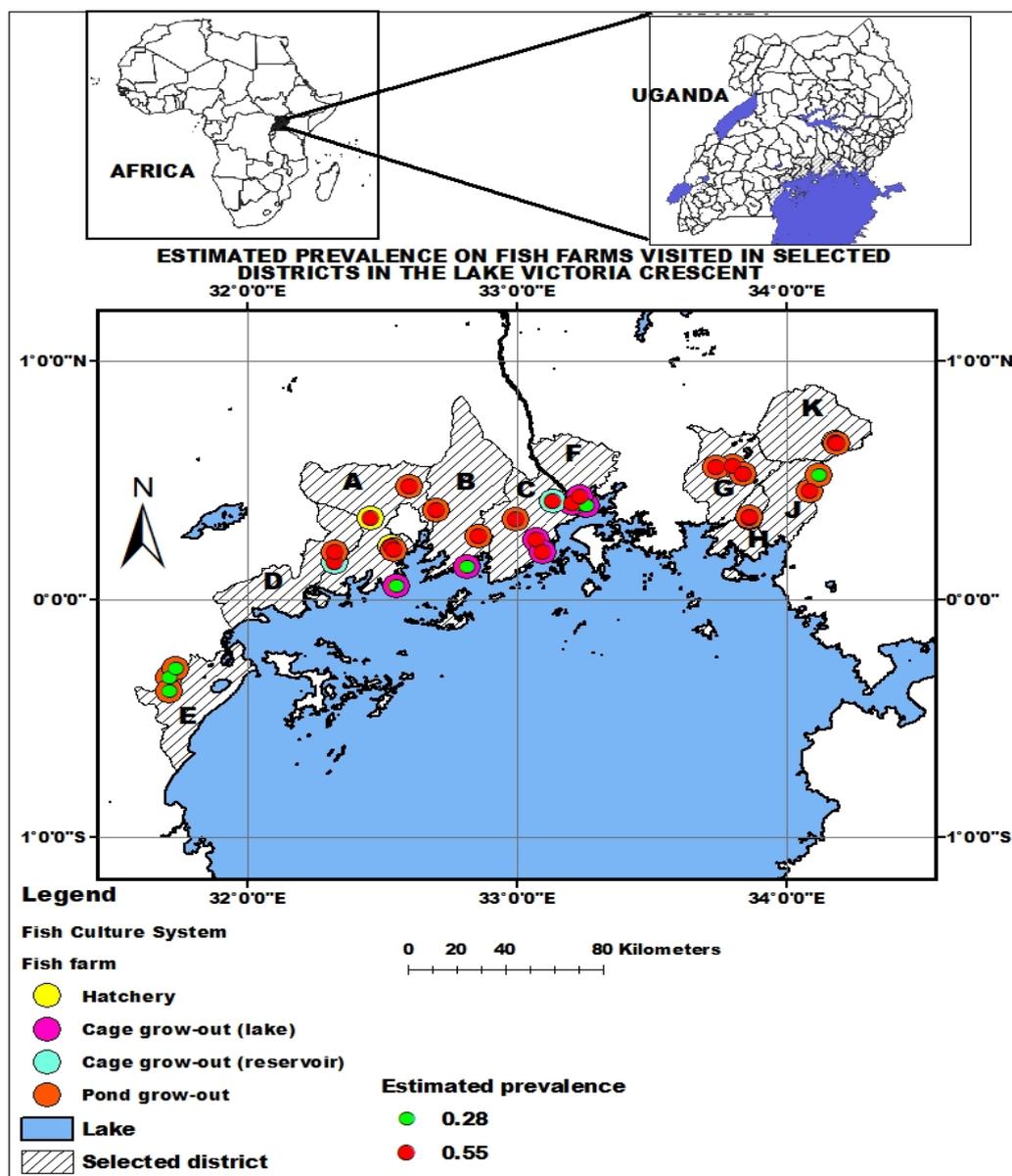
Figure 3. A risk map of estimated parasitic infestation based on the final model.

4. Discussion

4.1. Parasite Diversity and Infestation Levels

The high infestation rate of 65% (418/640) in the various fish farms in the study was largely due to the poor water quality, farm management practices, intermediate hosts and wild fish entry as shown in Tables 4–7. However, this infestation rate in the present study was lesser than the 89% (124/140) rate recorded earlier by [8] in fish farms along the Lake Victoria Crescent. The difference may be due to the different sampling efforts whereby only 25 fish farms, five from each of the districts of Masaka, Mpigi/Mityana, Wakiso, Mukono and Kampala were visited by [8], compared to the 32 fish farms, eight farms from Buikwe, five from Wakiso, four from Bugiri, three from Masaka and Mukono, two from Mpigi, Busia, Tororo, and Namayingo, and one from Jinja visited in the present study. Parasite diversity indices obtained in the study as shown in Table 1 included a high Shannon index *(H′)* of 0.961, indicating that the farmed fish and water environment in the fish farms provided a good host and habitat for the 16 parasites genera [62]. The parasite community had a low Evenness *(E)* of 0.347 which meant that the farmed fish and water environment in the fish farms provided a

good host and habitat respectively to some specific parasitic types (i.e., *Trichodina* sp. *and Dactylogyrus* sp.) and these may have had a greater influence on infestation pattern compared to others [62]. These two parasite genera were found in all of the farming systems as shown in Table 3 and this signified the poor water quality across the Lake Victoria Crescent, as these two parasite genera have been noted to be an indicator of poor water quality in culture systems by some of scholars [27,63]. Furthermore, there was a high Berger–Parker Dominance index (d) of 0.79 for *Trichodina* species and this still was due to the poor water quality [63]. Species estimators, Chao = 16.125 and Jackknife = 16.998, as shown in Table 1 informed that the fish samples were adequate for the study to get the maximum available number of parasite genera. This species richness in the study was higher than that obtained by [8] in the Lake Victoria Crescent i.e., 11 genera. However, despite the differences in sampling efforts in the two studies, the higher species richness in the present study showed an increase in parasite diversity as aquaculture intensifies in the region over years. Ecto-parasite *Trichodina* species had the highest prevalence and mean abundance as reported in an earlier study by [8]. This reaffirmed the continued poor water quality in most fish farms in the Lake Victoria Crescent over years, as *Trichodina* species are commonly associated with heavy parasitic infestations of fish under stress due to poor water quality [63]. The persistent poor water quality may be attributed to overstocking, uncontrolled addition of livestock and poultry manure in ponds, low water exchange, poor siting of cages, overfeeding etc. Unlike, 18.2% (2/11) of the parasites recorded with a mean intensity of five or more individuals per fish by [8], 43.8% (7/16) of all parasite genera in the present study had a mean intensity exceeding 10 individuals per fish. This increase may be attributed to the intensification of aquaculture along the Lake Victoria Crescent [3], which is characterized by high stocking densities in the culture systems [64,65]. The increased fish biomass stocked disrupts the parasite-fish interaction [9] by causing stress, poor feeding ability and injuries, thereby increasing the fish's susceptibility to parasite attack [66].

Fish in Pond grow-out farms and Hatcheries were infested with the highest number of parasite genera and mean number of parasite genera per farm as compared to the other farming systems. Most of the Pond grow-out farms were characterized by high ammonia levels, poor feeding and high stocking densities which may have affected the fish physiological functions and metabolic rate hence increasing their susceptibility to different parasite attack [63,67–69]. Furthermore, these farmers outsourced seed from the wild and fellow farmers, yet these seed may be a reservoir of some of these parasites [70]. Certainly, ponds usually provide favorable environmental conditions and habitat for intermediate hosts like copepods, snails and fish-eating birds which are essential for the life cycles of *Neascus*, *Clinostomum*, *Contracaecum*, *Amirthalingamia* and *Diphyllobothrium* species, thus the high parasite genera numbers [71]. Most of these Pond grow-out farmers are small scale farmers and couldn't carry out disinfection as a bio-security measure on the farm so as eliminate intermediate hosts and fish parasites on farming equipment. The flow-through farming systems in Pond grow-out farms also facilitated the wild fish entry hence leading to the transmission of parasites with free-living stages from the wild fish to the farmed fish [72].

The high parasite genera and mean prevalence in Hatcheries could have been due to different parasite types introduced in the hatcheries through; transfer of infected live brood fish, transfer of eggs from infected to uninfected farm, movement of birds or faeces of infected birds, human movement from infected farms to uninfected hatcheries, movement of wild fish and infected water in case of flooding [73]. Furthermore, Hatcheries were characterized by high stocking densities, which stresses fish, thereby reducing fish immunological functions and increasing the fish susceptibility to parasite attack [74]. The high stocking biomass also increases proximity for transmission of some parasites especially monogeneans which have a direct life cycle, thus increasing the proliferation of the parasites [75].

The low number of parasite genera, frequencies and mean prevalence in Cage grow-out (lake) farms may be largely due to good water quality, good feeding, disinfection and no wild fish entry as shown in Table 5. The good water quality and proper feeding largely improves the health of fish farmed and increases their immune resistance to parasitic [76]. Furthermore, the siting of some cages

into the lakes didn't support the nesting and habitation of piscivorous birds, copepods, and snails which are needed by some parasites to complete their life cycles as mentioned by [77].

The high mean parasite prevalence and frequencies in Cage grow-out (reservoir) farms could be largely due to the poor water quality as fish is enclosed in cages and within a reservoirs with no enough water exchange. The low water exchange not only reduces dissolved oxygen but also increases ammonia levels thus lowering the fish physiological and immunological response towards parasites [66].

4.2. Effect of Water Quality, Farm Management Practices and External Factors on Parasitic Infestation

4.2.1. Water Quality, Farm Management Practices and External Factors on Parasitic Infestation

Water quality parameters, farm management practices and external factors against recommended limits for fish culture, varied from one farming system to another. In Pond grow-out farm, the poor water quality was due to overstocking, uncontrolled addition of livestock and poultry manure to ponds, low water exchange, and overfeeding. These farmers are mainly small scale farmers who couldn't afford buying commercial feed, seed from certified hatcheries, disinfectants to clean farm equipment, hire technical personnel to manage the farm etc due to their financial status, and mainly rely on government support, hence the poor farm management practices [78].

Most Cage grow-out (lake) farms had better water quality and this was due to the fact that this culture system allows free flow of water, permitting water exchange and waste removal into the surrounding water [79]. Furthermore, due to the capital-intensive nature in cage farming, most Cage grow-out (lake) farmers were middle and large - scale farmers and were able to buy commercial feed, buy seed from certified hatcheries, buy disinfectants to clean farm equipment, buy new nets or repair them to prevent wild fish entry,and hire technical personnel to manage the farms, hence the better farm management practices [78].

All Cage grow-out (reservoir) farms had poor water quality due to the fact that most farmers utilize sand mining holes filled with water for cage culture yet they are small and have no inlets and outlets, compared to the large and open reservoirs with many inlets and outlets utilized elsewhere [80]. Just like most Pond grow-out farmers, Cage grow-out (reservoir) farmers were mainly small scale farmers who couldn't afford buying commercial feed, buying seed from certified hatcheries, buying disinfectants to clean farm equipment and hiring technical personnel to manage the farm due to their financial status, and mainly relied on government support, hence the poor farm management practices [78].

Water quality was fair in Hatcheries and this was due to fact that hatchery operators over-stocked the hapas in open ponds, breeding ponds, and resting ponds, and fed fish on protein rich feed, hence leading to an increase in uneaten feed and excreted ammonia from the farmed fish [81]. Just like most Cage grow-out (lake) farms, Hatchery operators were able to buy commercial feed, buy disinfectants to clean farm equipment, buy new happas or repair them to prevent wild fish entry and hire technical personnel to manage, hence the better farm management practices [78].

4.2.2. Effect of Risk Factors on Parasitic Infestation at Various Farms across the Lake Victoria Crescent

Out of the 16 risk factors evaluated, only intermediate hosts showed a statistically significant effect on parasitic infestation as seen in Table 8. This meant that intermediate hosts was a major contributor to the estimated parasitic infestation as the increase in number of intermediate hosts increased the risk of parasitic infestation. These intermediate hosts are needed by some fish parasites to complete their life cycles [8]. Parasites of *Neascus, Clinostomum, Contracaecum, Amirthalingamia and Diphyllobothrium* species needed copepods, snails or/and piscivorous birds to complete their life cycles [77].

Copepods have been noted to be intermediate hosts for *Diphyllobothrium* and *Amirthalingamia* species and the intensification of aquaculture has provided them with conducive environments to

survive and spread disease [82]. [83] noted that increasing temperature, decreasing pH, and eutrophication contribute to the proliferation and parasitism of these intermediate hosts.

Snails have been noted to be intermediate hosts for trematodes like *Clinostomum, Diplostomum* and *Neascus* species in which the parasites develops into sporocysts and finally forms cercariae that infects the fish [84]. According to [85], the distribution of these snails is influenced by temperature, pH, conductivity, precipitation, and substrate composition, and availability of food and these physical, chemical and biology factors were provided for in the study.

Fish eating birds can act as final hosts to nematodes of freshwater fish such as *Contracaecum* species whereby fish-eating birds such as cormorants, eagles and pelicans contain the larval stages [71]. These parasites are then introduced into the production systems by the birds or faeces of infested birds [73], and the ponds really provide a good habitat for these birds [62]. These migratory birds carry parasites from one infested farm to another as evidenced in a study by [86], who revealed that these birds contained a higher nematode species richness of migratory than the resident birds.

4.3. Parasitic Infestation Estimation

All Cage grow-out (reservoir) farms and Hatcheries and most of Pond grow-out farms had high estimated parasitic infestation despite their locations and this could be due to poor water quality, poor feeding, and high stocking densities, as seen in Tables 4 and 6–7, which not only reduce fish's ability to resist stressors and increase fish's susceptibility parasitic infestation [66], but also favour the habitation for intermediate hosts such as fish-eating birds, snails and copepods [85]. However, a few Pond grow-out farms in Masaka had low estimated parasitic infestation and this was because Masaka has many minor rivers which provide good quality water for pond fish farming [87], which rivers are less polluted since most of the population engages in farming and fisheries activities [88]. Therefore, physico-chemical factors such as water temperature, ammonia, DO, pH, conductivity and turbidity will always be kept within the recommended ranges for fish culture and this will influence fish's health and resistance against the parasites hence reduction on parasitic infestation [66,89]. Cage grow-out (lake) farms with high estimated parasitic infestation were located in the Napoleon Gulf of Lake Victoria and Southeastern part of Buikwe. This Gulf has been noted to be persistently eutrophicated and polluted by some authors [90–93], whereas the sugar factories and plantations in Buikwe have continuously released waste-water effluents and soil nutrients especially phosphorus from unprotected crop fields into the lake respectively [94,95]. These chemical pollutants and nutrients released interfere with the flow velocity, pH, dissolved oxygen, light and temperature [96]), hence increasing fish's susceptibility to parasitic attack [62].

5. Conclusions

The utilization of spatial modelling in the study allows us to understand the relationship between farmed fish and parasites in the culture environments at specific locations. In the present study, the difference in estimated parasitic infestation among the four farming systems (Pond grow-out, Cage grow-out (lake), Cage grow-out (reservoir) and Hatchery) at different locations is well defined and the major contributing risk factor is identified. This approach facilitates evidence-based decision making among scientists and policy makers in the face of uncertainties surrounding fish diseases. Therefore, by identifying major risk determinants at a specific farm or farms, spatial modelling can guide on the most effective management strategies and interventions to be utilized in controlling the spread of parasitic infestations in specific locations.

Institutional Review Board Statement: Permission was sought and gotten from the Directorate of Fisheries Resources, Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda, to collect and use live fish samples. All procedures were conducted within the provisions of Section 12 of The Animals (Prevention of Cruelty) Act [50]. Based on these provisions there were no limitations for performing the investigations under this paper.

Informed Consent Statement: Not Applicable

Data Availability Statement: The data presented in this study are available on request from the corresponding author

Conflicts of Interest: The authors disclosed no conflict of interest to anybody or any organization

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