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An overview of fish disease and parasite occurrence in the cage culture of *Oreochromis niloticus*: A case study in Lake Victoria, Kenya

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Cage aquaculture has been on a steady rise in Lake Victoria, Kenya, since 2016, resulting in the current culturing of over 3,600 cages of Tilapia (O. niloticus) *(Orina et al., 2018). Unfortunately, there has been limited, if any, focus on fish health aspects. Rise in intensification and commercialization predisposes fish stocks to disease due to rise in stress levels and consequent reduction in the fish immunity. Nutrient rich surroundings create a conducive environment for rapid proliferation of bacterial and saprophytic fungal growth leading to net clogging and consequently a low biological oxygen demand. Such conditions predispose the stocks to infections. This study was conducted to provide a baseline analysis of the health conditions/status of the cultured fish in this region. It encompassed studies from 2016 to 2018 on tilapia of the genus O. niloticus using both experimental (using standard procedures and protocols) and socio-economic studies (using structured questionnaires, see annexure 1). Results found the following occurrences; bacterial infections (10%), fungal infestations (12.5%), myxosporean parasites in the gills (5%), parasitic copepods (10%) and fin rot (2.5%) in the stocks. There were no significant differences between abiotic parameters in the cage locations and the wild ($p > 0.05$). Additionally, 90% of the respondents had no fish disease training or clue on the treatment action necessary whenever fish diseases struck. Findings from this study put to the fore the significance of fish diseases in a cage culture system in light of commercialization of the industry and the importance of biosecurity and maintenance of optimal environmental conditions within the scope of Blue Economy growth in this region. This study did not detect any disease or parasite of zoonotic importance.*

Keywords: biosecurity, fish health, Tilapia, baseline analysis, water quality

Introduction

Cage culture is viewed as a recent entry into the aquaculture industry in Africa, though it has been in practice in other parts of the world, for instance China where it originated from (Bao-Tong, 1994). Though cage culture is relatively new in Africa, it has been widely applied by a Ugandan private company, source of the Nile (Kashindye et al., 2015). Little information is available on cage

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culture in Kenya (Munguti et al., 2014) albeit the existing huge potential of the venture. Few private investors (e.g. Pioneer Aqua-park and Jak-port farms) have ventured into this practice in the Kenyan portion of Lake Victoria.

As with other ventures, cage culture is faced by a variety of challenges including but not limited to resource use conflicts (since the water is a shared resource by various stakeholders), vandalism, poaching (Basset and Dillard, 1985) and lake turnover (which leads to the mixing of the anoxic bottom layer of the water resulting to fish kills) in cases of poor cage siting and disease. Environmental factors largely determine the extent to which an etiological agent will cause mortality in the system. Fish kills have been reported earlier in Lake Victoria and have been associated with low dissolved oxygen (D. O) and sometimes with nitrite and ammonia poisoning (Ochumba, 1990).

The effect of disease in a cage culture system can be immense due to the high stocking density which necessitates transmission. Additionally, the high stocking density leads to increased stress on the fish making them more vulnerable to disease causing agents. It is therefore of utmost importance to know about the occurrence of pathogenic diseases and fish parasites in this venture in order to put in place the relevant control measures to maximize on production and profit margins by reducing the cost of production and the growth period (Akoll, P., Department of Zoology, Makerere University, Uganda. unpublished, 2005).

Apart from the already existing diseases and parasites affecting caged tilapia, some emerging diseases are causing rising concern since they affect the health status of the stock leading to huge economic losses for instance Tilapia lake virus (TiLV). TiLV is an emerging disease which was first observed in Israel then consequently in Columbia, Ecuador, Egypt and Thailand (FAO, 2017). Due to its negative effects, there have been global concerted efforts to counter it. Findings have shown losses of up to 80-90% of stocks in Israel, Ecuador and Colombia (Bacharach et al., 2016; Del-Pozo et al., 2016; Kembou et al., 2017). The estimated worldwide tilapia trade stood at 9.8 billion USD (FAO, 2017) on an annual scale and considering the possibility of transfer across continents, strict biosecurity measures ought to be put in place in order to prevent transfers. For a successful cage culture venture, the potential effects of parasites and diseases should not be downplayed since they largely affect the economic output of the venture. This study is a preliminary analysis meant to bring to the fore, the diseases and parasites affecting the cage cultured Tilapia (*Oreochromis niloticus*) within the region and correlate the occurrence to the siting of cages.

Methodology

Study area

The study was carried out in selected cage establishments within Lake Victoria, Kenya (Fig. 1) during the dry season (September and October) because the results from respondents indicated that diseases occur most during the dry season. Lake Victoria is the second largest freshwater lake in the world with a surface area of 68,000 km^2 and is divided between three countries; Uganda (43%), Tanzania (51%) and Kenya (6%). Currently, the lake holds a little over 3696 cages across the five riparian counties of the Kenyan side with dimensions ranging from 8 m^3 to circular cages of 20 meters diameter.

Framework development/approach

Multistage sampling was adopted for this study. First, cluster sampling was used to choose Winam gulf and open waters as areas from which fish cages were to be sampled. In stage two, simple random sampling was deployed to select one cage establishment (from 3) at Winam gulf and two cage establishments (from 42) in the open waters. Further, simple random sampling was used to select 3 cages from 18 cages within the selected establishment at Winam gulf and 6 cages from the 40 cages within the selected two establishments at open waters. Fish samples were also collected from each cage using simple random sampling. Randomly selected cage culture establishments (39) were visited and randomly selected respondents interviewed through structured questionnaires for the socio-economic study while physical sampling of water for various parameters and random fish sampling was done for the practical assessment by using standard procedures and protocols.

Fish samples were collected randomly

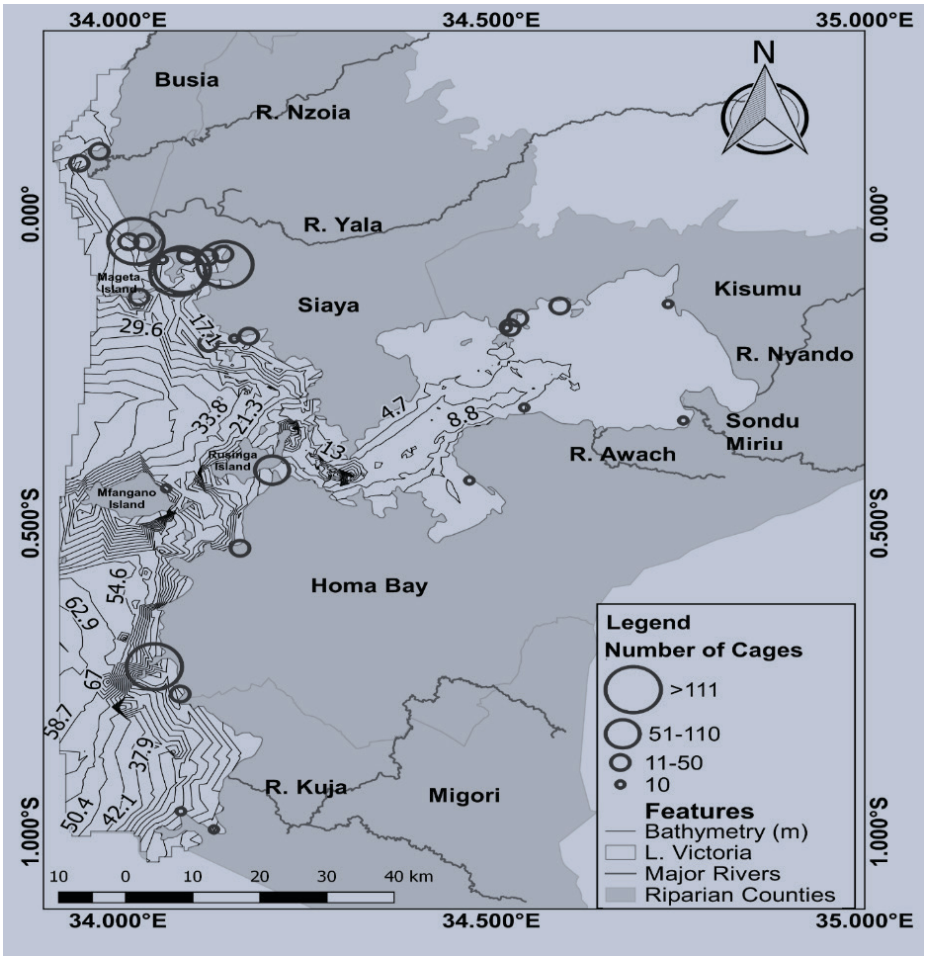

Figure 1. Map of the study site showing various cage establishments and density within the riparian counties of Lake Victoria, Kenya.

from three different cage establishments (one randomly selected establishment among the three establishments located within Winam gulf and two establishments randomly selected from 42 establishments in the open waters). Samples of fish were collected by randomly scooping using a scoop net in 3 different cages per establishment. The three different cages per establishment were also randomly selected. This resulted in a total of 9 cages that were designated for sampling. 10 samples were withdrawn from the three cages at Winam Gulf and 5 samples from each of the 6 cages within the open waters because the open waters were more populated as compared to Winam gulf. This resulted in the total number of samples being 40. The sample size was arrived at due to the availability of fish and the nature of the study at that particular time. The specimens were visually examined at the body surfaces for wounds, lesions, deformities and abrasions. Further on, photos of the samples were taken using a digital camera.

For the analysis of parasite occurrence and abundance, skin smears were made by holding the fish firmly on the head and scrapping the skin on either of both sides from the anterior of the fish (and under the pectoral fin) to its posterior by using glass slides, covered with a cover-slip and examined under a microscope. The eyes, cranial fluid, gills and intestines were examined under a high magnification microscope (*40 to *100) and identified to the lowest level by using identification keys by (Paperna, 1996). Counts were done and recorded for parasites occurring in various organs. Further on, gills were removed individually and samples preserved in 70% alcohol for further examination. The internal organs and tissues

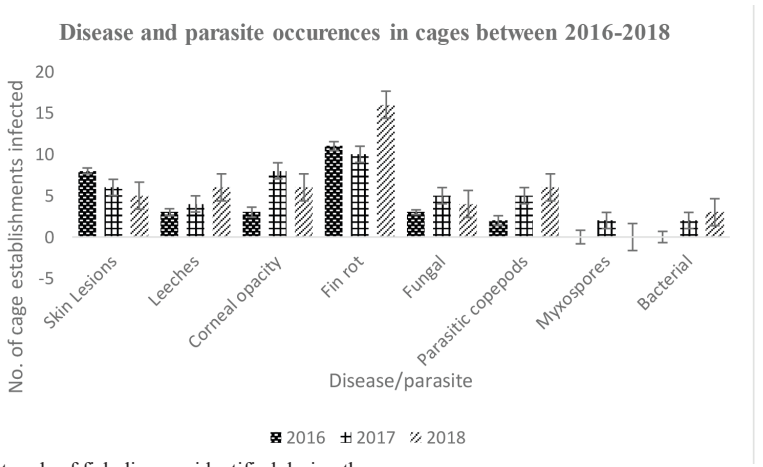

Figure 2. Types and trends of fish diseases identified during the survey.

(kidney, liver, bile and spleen) were then carefully examined for parasites and samples fixed for further analysis and examination in the laboratory.

Bacteriological analysis was conducted on all fish samples at the laboratory. The fish surface was observed for any lesions. Gills and fins were also observed for any signs of infection (see annexure 2). The fish bodies were carefully opened to prevent of puncture any part of the intestinal tract. Swabs were aseptically taken from gills, kidney, liver, and spleen (after searing the surface of the organs with a hot blade). The swabs were inoculated in 7.5% sheep blood agar (Merck, KGaA, Darmstadt, Germany) and further sub cultured in Mac Conkey agar (Merck, KGaA, Darmstadt, Germany). Both the inoculated blood agar and Mac Conkey agar were aerobically incubated at 37˚C for 24 to 48 hours. Colonies were then gram stained, morphology recorded and identification done using identification keys.

The influence of cages on water quality and their possible association with fish diseases and parasites was done in 4 sites. Within the gulf, water samples were taken in triplicates from 4 sampling points; near the cages, mid cages, just outside the cages and 100 m off the cages (which was treated as a control point). Similarly, this was replicated in the open waters where water samples were taken in triplicates from 4 sampling points; near the cages, mid cages, just outside the cages and 100 m off the cages (which was also treated as a control point). In-situ physico-chemical parameters including Dissolved Oxygen (DO), pH and Temperature were determined at the aforementioned sampling points while water samples for nutrients; ammonia, nitrites and nitrates were collected, preserved and stored in ice pending a later analysis in the laboratory.

Data was analysed for normality by using MS Excel 2010. The descriptive and inferential data analysis was conducted by using MS Excel, 2010. One way analysis of variance (ANOVA) was applied to check for any significant differences on the water quality parameters between the cage and non-cage sites. For all analyses, 95% level of confidence was applied as the basis for rejection or accepting the null hypothesis. Mapping involved geo-referencing of cages per station based on the GPS locations established for cages presence in the lake using Arc GIS 10.0 (The Environmental System Research Institute, USA). The length-weight relationship is sufficiently described by the equation $W=aL^b$, where W is total body weight (g), L is the total length (cm), and a and b are the coefficients of the functional regression between W and L. Moreover, condition factor (K) was determined to understand the health condition of fish by using the formula;

$K=100W\ l^{-3}$, where K= condition factor, W=weight (g) and L=Length (cm) (Bannister, 1976).

Results

Out of the 40 specimens examined in the laboratory, 12 specimens were found to be infected

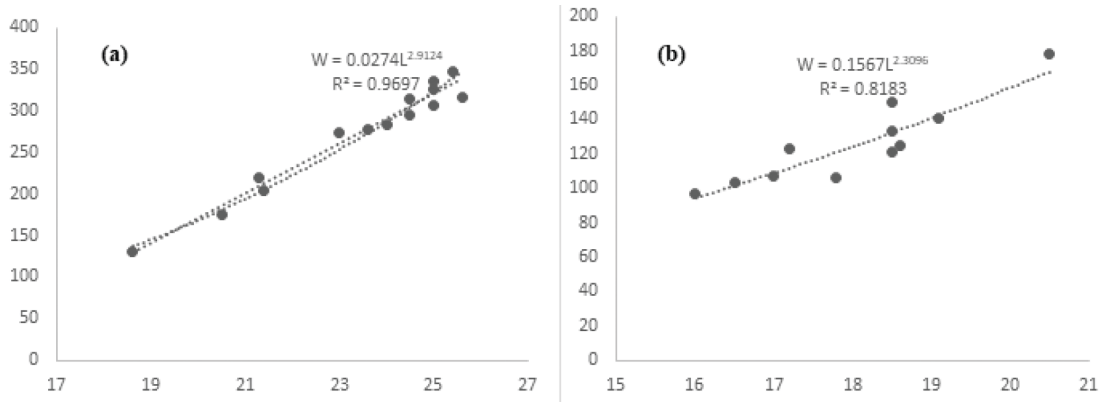

Figure 3. Length weight relationship and condition factor of healthy (a) versus un healthy fish (b).

while 28 specimens were healthy. In the socio-economic survey, 39 respondents from 39 cage establishments were interviewed on the health status of their stocks (Fig. 2). The questionnaires were administered to the respondents in 2016 and 2017 while in 2018, only fish samples were collected for laboratory analyses as guided by feedback from respondents in the 39 questionnaires. Most of the farmers (63%) experienced fish diseases. The farmers confirmed the ailments were caused by fungi or bacteria (38% and 33% respectively) which did deviate much from the findings in the laboratory analyses. Despite the differences in the occurrences of the various diseases and parasites, there were no significant differences ($p > 0.05$) among the years (2016, 2017 and 2018).

Infections and parasites identified and trends between 2016 and 2018

Myxospores were found in 5% of the *O. niloticus* during the study. Colonies of Myxosporea were observed in the mesenteries and in the gills. The infected filaments had between 2 and 5 colonies of spores and both sexes of *O. niloticus* were susceptible to the infection. The gill filaments appeared swollen and filled with the growing cysts in transverse sections. Mycotic infections from suspected *Saprolegnia* spp. were detected. Greyish-white outgrowths were visible with the naked eye and on further examination under a microscope, the findings pointed to the Saprolegnia spp. infection. Identified parasites of this species were mainly ectopasitic crustacean copepods which included: Lernaea *cyprinacea, Argulus africanus, Dolops ranarum,* and *Ergasilus lamellifer* some of which resulted to dermal injuries. Additionally, parasitic protozoans of the groups *Trichodina* spp. and *Ichthiophthirius multifiliis* and monogenean (*Cichlidogyrus spp.)* were noted in the fish at varying intensities.

Occurrence of the various infections

Results found the following occurrences; bacterial infections (10%), fungal infestations (12.5%), myxosporean parasites in the gills (5%), parasitic infections (10%), ocular lesions 20% and fin rot (2.5%) in the stocks.

Length-weight relationship and condition factor of the fish in relation to disease/ parasite occurrence

An investigation into the relationship between the health statuses of the stocks and the well being of the fish pointed to the fact that infections affected the general well-being of the fish. This is illustrated in Fig. 3.

A look into the effect of infections on the well-being of the fish by using the length –weight relationship, it was found that both the healthy and sick fish portrayed negative allometry at b=2.3 and b=2.9 respectively. The fish condition factor was at 1.80 for the healthy fish while the sick fish had 0.01. Further on, the coefficient of variation (R^2) for the healthy and sick fish was approximately 97% and 82% respectively.

Table 1. Water quality parameters in the cages and control/wild sites.

	Site A Gulf control	Site A Gulf cages	Site B Open waters control	Site B Open waters cages
DO (mg l^{-1})	6.21± 0.47	6.33±0.33	3.88±0.48	3.44±0.07
pH	7.81±0.37	8.22±0.21	7.29±0.06	7.2±0.02
Temperature (°C)	26.9±0.94	27.17±0.49	24±0.02	24±0.01
NO_2 (µg l^{-1})	14.51±0.01	19.29±0.46	7.76±0.52	11.68±0.33
NO_3 (µg l^{-1})	16.95±0.01	20.55±0.04	17.49±0.55	25.3±0.38
NH_4-N (µg l^{-1})	57.31±0.03	66.28±0.04	35±0.03	109.6±0.11

Abiotic parameters

According to the summary, the values obtained in both cage sites were within the limits required for the culture of Tilapia. Results indicated that there were no significant differences ($p > 0.05$) in the water quality parameters between the gulf cages and gulf control and none between the open water cages and the open water control. However, slight deviations were observed with for instance the open water cage site, where there was lower DO than that recommended for optimal performance in a Tilapia culture system (Table 1). One conspicuous thing is evident in the relatively elevated ammonia values in the open water cages. In as much as they were within the tolerable levels, their distinct high value could be a disaster in the offing if not checked.

Discussion

The study made use of prevalence data to indicate the presence of various diseases recorded as at that time. In accessibility of far-flung areas with cages was a challenge in this study. Due to this, analysis of some ectoparasites was not ideal since the samples arrived in the laboratory after a day hence the live specimens were not well visible. This could erroneously be recorded as being free of ectoparasites, while in reality they could have died while on transit to the laboratory.

Susceptibility of fish to diseases and parasites is said to be aggravated by stress from nutritional deficiencies, poor handling and poor water quality/high organic loading in the environment (Zago et al., 2014). The stocking density for the cages under investigation was similar at 1,500 fish for an 8 m^3 cage. A study by Chakraborty (2010) on the correct stocking density of *O. niloticus* in a tropical environment recommended density for optimal performance. The current stocking density in majority of the cages is over the recommended, indicating possible stressful conditions due to overstocking/overcrowding. This can consequently lead to ease of disease outbreaks and transmissions. The importance of proper site selection and siting of cages in the mapped regions can be seen by the low dissolved oxygen experienced in the cages within the gulf and in the open water cages that were located close to the shore (< 50 meters). Compromised environmental conditions are a catalyst to diseases and infections in fish. The effect of exposure of *O. niloticus* to un-ionised ammonia concentrations of 0.05 mg l^{-1} for 75 days was studied by El-Sherif and El-Feky (2008) who found gill epithelial hyperplasia developed resulting to a reduction in the amount of oxygen diffusing across the membranes and consequently predisposing the fish to bacterial infections. Moreover, fish exposed to increased metabolic ammonia are said to be more prone to bacterial gill disease.

Bacterial infections were detected in the study with a prevalence of 10%. Of this, fin rot and suspected *Streptoccocus* spp. were observed. Skin lesions, unilateral or bilateral exopthalmia and eye opacity were characteristic observations in the fish with suspected streptococcus infections. Fin rot was characterized by a sloughing off and rotting of the fins (anal, caudal, pectoral and pelvic fins). Most bacterial infections are secondary in nature, arising from a trigger. Examples of triggers can be poor water quality or external abrasions and injuries that create entry routes for the bacteria. The present study found lesions in 80% of the samples that were found to have bacterial infections.

The present study found a Myxosporean parasite prevalence of 5% in the gills of the stocks under the study. These parasites have been said to cause various pathogenic effects including reduction of

host respiratory capacity (Molnar and Szekely, 1999), parasitic castration (Sitjà-Bobadilla and Alvarez-Pellitero, 1993) and myoliquefaction of the tissue after host death (Pampoulie et al., 1999). Microsporean parasites occurring in fish gills could result to reduction of the respiratory capacity of the fish, resulting to detrimental effects (McCraren et al., 1975). Studies have shown that these parasites are transmissible and as such, measures need to be taken to counter their effects within the system.

This study found the occurrence of fungal infections in the stocks to be 12.5%. The respondents reported seeing the fish with 'wooly' extensions from various parts of the body including the mouth. From the laboratory analysis, observations under the microscope showed thread-like extensions which are characteristic of *Saprolegnia* spp. Saprolegnia is one of the phycomycete fungi that results to mycotic infections and act as wound parasites originating from abrasions due to poor handling, confinement in nets and other farming practices typical with cage culture (Paperna, 1996). Routine practices in the cage culture system for instance netting, sorting, grading, lifting from holding containers into the cages, holding and sorting brooding fishes prior to spawning and also constant fighting among male brood fishes during the spawning season. The high stocking density easily aggravates this condition since there is minimal space for movement. Resultant wounds create avenues for pathogens entry into the system of the host fish externally and or internally causing serious infections and eventual death.

Impact of disease or parasite infection on the fish health was established by the length-weight and condition factor analysis. Infected fish were found to exhibit poorer health as compared to those that had neither a disease nor parasite. Laxity, poor feeding and consequently poor conversion of feed to flesh are the consequences of poor health in the stocks. These factors eventually result to poor returns on investment to the farmer.

The study recorded a 20% prevalence of ocular lesions in the stocks under investigation with cases being observed in both the cultured and wild sites. Literature has linked the occurrence of Ocular lesions to various causes among them existence of eye flukes or monogenetic trematodes, bacterial infections (Pretto-Giordano et al., 2010; Chang and Plumb, 1996), poor water quality conditions leading to an upsurge of ammonia and feeding with an aflatoxin contaminated diet (Mehrim and Salem, 2013). The study by Mehrim et al. (2006) on aflatoxicated *O. niloticus* showed fin and tail rot, anorexia, yellowing of the body surface and ocular opacities leading to cataracts and total blindness. It is therefore necessary to conduct feed analysis on the viability of the feed that had been used. Findings from this study will be used as a baseline to direct further research in this field to establish the exact causes of the infections observed

Conclusions

This study found infections of varying intensities from samples of caged fish, as well as from information by the respondents in the case of the socio-economic survey. Cages in the gulf and close to the shore had more cases of infections. However, we did not establish a significant link between fish disease occurrence and siting of the cages, although dissolved oxygen was lower in cages within the gulf as compared to the ones in open waters. Additionally, the relatively elevated ammonia levels in the cages within the gulf could also point to inadequate flushing which could indicate possible accumulation of waste in the longrun if not checked.

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Annex 1: Socio-economic questionnaire

DATA COLLECTION TOOL FOR ASSESSMENT OF CAGE CULTURE TECHNOLOGY COMMERCIALIZATION BY COUNTIES IN LAKE VICTORIA

Kenya Marine and Fisheries Research Institute is carrying out a documentation of Cage culture investment in Lake Victoria by County. The exercise entails identification and documentation of cage technology adaptive levels, stocking densities, fish growth performance, disease incidences, source and quality of seed and feeds as well as markets and market value. The assessment should overally demonstrate the role of cage culture on food security, job creation and poverty alleviation at the food fish production chain node. The outputs of the Assessment will inform the development of a strategy for up scaling and out scaling the identified technological packages to enhancing capacities of value chain actors to adopt and adapt for aquaculture commercialization.

You have been identified as a respondent in this important exercise. The Assessment will last a maximum of 30 minutes. Your support and responses will be highly appreciated.

Thank you.

Section 1: Profile of Enumerator

Name of Enumerator: …..……

Phone no. of Enumerator: ……..

Section 2: Profile of Respondent

1. Name of Respondent:
   ……..
2. Phone no. of Respondent:
   ……
3. County:
   ……
4. Sub County:
   …….
5. Name of Establishment:
   …….
6. GPS Coordinates:
   …….
7. Name of beach or nearby beach:
   ……..

Section 3: Socio-Demographic Information

8. Gender: Male □ Female □
9. Marital: Married □ Single □ Widow □ Widower □
10. Age (Years): Below 18 □ 19-35 □ 36 – 45 □ Above 45 □
11. Education Level: Lower Primary □ Upper Primary □ Secondary □ Diploma/ Certificate □ Degree □ Post graduate □
12. Main Occupation: Full time fish farmer □ Part time fish farmer □ Fisherman □ Others
    (Specify)……

Section 4: Profile of the Cages

13. Organization Level: Individual □ Group □ Cooperative □ Company □ County □ Others
    (Specify)……
14. Size of the Organization[1]:
    …….
15. Employee capacity[2]:
    …….
16. Production Manager Training Level: Lower Primary □ Upper Primary □ Secondary □ Diploma/Certificate □ Degree □ Post graduate □
17. Production Manager Training:
    ……..
18. Employee 1 Training Level: Lower Primary □ Upper Primary □ Secondary □ Diploma/Certificate □ Degree □ Post graduate
19. Employee 2 Training Level: Lower Primary □ Upper Primary □ Secondary □ Diploma/Certificate □ Degree □ Post graduate
20. Employee 3 Training Level: Lower Primary □ Upper Primary □ Secondary □ Diploma/Certificate □ Degree □ Post graduate
21. When was cage culture initiated? Month……Year……..
22. How many cages were installed at inception and the trend over time in growth

Year					
No of Cages					

23. Is the cage locally constructed or imported as complete set. Local □ Imported □
24. What material is used to construct the cage frame? Galvanized metal □ PVC □
25. What is the source of the cage net material: ……
26. What is the size of cages (Length by width by depth / Circular Diameter by depth)
    ……
    ……

1 Enumerator to explain meaning of "Size" in terms of number of Farmers / Members / Groups etc.

2 Enumerator to explain meaning of "Employee capacity" in terms of number of workers / Members / Groups etc.

Section 5: Fish Management

27. What is the stocking density?
……
28. What is the feeding regimes Once □ Twice □ Thrice □ Other (specify)
……
29. On what basis are fish fed with a given quantity per feeding session
Body weight □ …… Satiation □
Other……..
30. What is the source of feeds (Company and CP)
……
……
……
…….
31. What quantities are procured per given session?
……
32. What is the cost of feeds at the different growth stages

Stage	Fingerlings	Post fingerlings	Juvenile	Market size
Price (Ksh) kg^{-1}				

33. What is the source of fingerlings (Name of Hatchery/Hatcheries)
……
……
……
34. What is the size of fingerlings (g) at stocking……..
35. What is the price per fingerling (Ksh)…….
36. Which is the transportation mode of fingerlings from hatchery to cages
……
37. After how long do you harvest the stocked fish
(months)…….
38. At what weight do you harvest the market size fish
(g)……
39. Do you attain uniform size by cage YES □ NO □
40. What is the estimated amount of feed consumed per cage by harvesting time (Kg)
…….
41. What is the survival rate at harvesting time (%)
……
42. How many production cycles do you run per year
……
43. Do you receive extension services? YES □ NO □
44. If YES name them
……
……
……...
45. If yes, How frequent? Weekly □ Monthly □ Annually □ Not at all □

Section 6: Market Profile

46. What is the market price per kilogram (Ksh)/Piece……..
47. Where is the market
……
……
……
……
48. Which is the preferred state of fish; Smoked □ Fried □ Fresh gutted □ Fresh whole □ Chilled □ Frozen
49. What is the transportation mode of harvested fish to the market?
……
……

Section 7: Diseases Incidences

50. Have you reported mortality cases YES □ NO □
51. If yes at what time of growth is mortality first recorded (month 1,2,3 etc)…….
52. How frequent are mortalities recorded Daily □ Weekly □ Monthly □
53. At what time of the day are mortalities recorded? Morning □ Midday □ Evening □
54. What magnitude of mortalities Few □ Mass □
55. How are the dead fish disposed?
……
……
56. Are there reported disease cases YES □ NO □
57. If yes, which disease? Viral □ Bacterial □ Fungal □ Parasitic □
58. Did you identify the source of infection YES □ NO □
59. If YES, what was the source of infection
……
……..
60. How frequent is the infection Weekly □ Monthly □ Annually □
61. Which month (s) of the year does the disease occur?
.....
62. How did you manage the disease?
……
……
63. Do you have any training in disease management YES □ NO □
64. If yes, to what level? Module □ Certificate □ Diploma □ Undergraduate □ Post-graduate □

Section 8: Resource Use Conflict

65. Have you experienced resource conflicts YES □ NO □
66. If yes, which ones

.....
.....
.....

67. Have you experienced theft cases YES □ NO □
68. How often is theft experienced? Daily □ Weekly □ Monthly □ Annually □
69. Have you identified the thieves? YES □ NO □
70. If yes, who are they? Fishermen □ Group members □
    Others…….
71. How have you addressed theft cases?
    ……
    ……
72. Have you experienced fish escapees?
73. If yes? How often? Daily □ Weekly □ Monthly □ Annually □
74. How have you managed the escapee problem?
    ……
    ……
    ……

Annex 2: Images of various infections observed during the laboratory analysis of samples