RESEARCH PAPER

Bacterial and Enterobacterial load of pond water and aquacultured tilapia, *Oreochromis nilotics* (L.), sourced from two major farms in Fiji

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ABSTRACT

Total bacterial load and total Enterobacteriaceae in pond water, gills, skin and gastro-intestinal tract of aquacultured tilapia, *Oreochromis niloticus* (Linnaeus, 1758), raised at Navua Prawn Farm (NPF) and Naduruloulou Research Station (NRS) was estimated. The aerobic mesophilic counts of bacteria in pond water were estimated to be 6.7 x 103 cfu/mL and 2.98 x 105 cfu/mL at NPF and NRS respectively. The Total Enterobacteriaceae Counts in pond water were estimated as 6.40 x 102 cfu/mL and 1.33 x 104 cfu/mL at NPF and NRS respectively. The aerobic mesophilic counts and Total Enterobacteriaceae counts were much higher in gills, gastro-intestinal tract and on skin of tilapia cultured at NRS than at NPF. The purged fish sample from NRS showed much lower Total Enterobacteriaceae and aerobic mesophilic counts. The tilapia pond at NRS may have been contaminated by the daily influx of duck excreta from a cage housed just above the pond for fertilization purpose. The infrequent water change of tilapia pond further led to increased bacterial levels. The daily water change of ponds at NPF contributed to hygienic culture conditions. Proper purging of tilapia is recommended to extend storage life of tilapia and tilapia products and to prevent spread of food-borne diseases.

Keywords: Tilapia, bacterial load, Enterobacteriaceae, pond water, excreta-fertilization, storage life.

1.0 INTRODUCTION

Tilapia has been domesticated in Fiji for human consumption since 1950's (Holmes 1954). Since then the consumption of fresh tilapia is increasing and culture of tilapia is slowly moving towards commercialization. The production volume and value of tilapia has increased by 12.5% from 2007 (Fisheries Department 2008). As products from tilapia aquaculture increase and current sales outlets saturate, attempts are now being made to develop post-harvest processing and value-adding of tilapia to provide additional avenues for marketing of tilapia (Natasha *et al.* 2013). The quality and storage life of tilapia and tilapia products would be highly dependent on the bacterial load of fish and the culture environment. However, there is a dearth of information on the bacterial load in tilapia cultured in Fiji.

Fish production in excreta-fertilized fish ponds is a common practice in Fiji. Chicken manure is broadcast over the pond bottom and/or in a sack floated in the pond to initiate fertilization (Nandlal and Pickering 2004). The fertilizer encourages the growth of plankton that provides natural food for the tilapia and is a relatively in-expensive way to provide food for the fish. Sometimes integrated farming with livestock is also employed with tilapia farming. This system of farming may introduce enteric bacteria into the water system. The enteric bacteria or Enterobacteriaceae is a large family of Gram-negative bacteria that includes many harmless symbionts and pathogens such as Salmonella, *Escherichia coli* (*E. coli*), Yersinia pestis, Klebsiella, Shigella, Proteus, Enterobacter, Serratia, and Citrobacter (Harrigan 1998). While these bacteria are not normal inhabitant of fish flora, they have been isolated from the stomach and intestines of fish (Guzman *et al.* 2004). Freshwater fish and their aquatic environment are known to harbour human and animal pathogenic bacteria (Leung *et al.* 1992). The pathogenic bacteria of the Enterobacteriaceae family may cause contamination of fish resulting in poor quality of fish and fishery products and faster spoilage upon harvest (Kaneko 1971).

To evaluate the sanitary conditions of ponds, either of the indicator pathogenic bacteria; *E. coli*, Faecal Coliforms or Total Enterobacteriaceae, are considered. Some workers have investigated the microbial loading of tilapia reared in brackish water ponds (Al-Harbi and Uddin 2005), freshwater ponds (Al-Harbi 2003), sewage and wastewater-fed ponds (Balasubramanium *et al.* 1992) but microbial loading of tilapias reared in Fiji are unknown. It is important to know the bacterial load on tilapia in order to market tilapia either as live fish or as processed value-added products which are of highest quality possible.

The present study was designed to determine the total bacterial and total Enterobacteriaceae counts in pond water, skin, gills and gastro-intestinal tract of cultured tilapia from two major farms in Fiji; Naduruloulou Aquaculture Research Station (NRS) in Nausori and Navua Prawn Farm (NPF) in Navua , Fiji.

2.0 MATERIALS AND METHODS

The bacterial load of pond water and tilapia project was a preliminary work conducted prior to the processing of value-added products from tilapia as part of a larger study on establishing post-harvest processing mechanisms for potential commercialisation of tilapia in Fiji.

2.1 *Description of the ponds*

Naduruloulou Research Station (NRS) was established in 1975 and is located 7km to the North from Nausori Town, along the Rewa River. Water to the pool site is supplied from the nearby Rewa River by continuous pumping. Tilapia is the only species in the pond under study. This pond is used for grow-out phase and fish is harvested for sale at the end of the grow-out period. A cage is housed just over the pond for rearing ducks. The duck excreta are used for fertilization of the pond water. The depth of pond is ca. 1.5m and the size is on average 1,500m^2. Water change is done once a month and is partially drained out during rainy days to prevent flooding which may cause loss of fish.

The Navua Prawn Farm (NPF) is Fiji's largest commercial farm, operated by the then Dairy Farm Fiji Aquaculture Unit, located in Navua along the Navua River. It was established in 2005 and its main commodities are prawns and tilapia. Water to the pond site is supplied from the nearby Navua River. Tilapia is the only fish species in the pond under study. This pond is used for grow-out phase and fish is harvested for sale at the end of the grow-out period. Fertilization is done by

broadcasting chicken manure packed in cotton sacks over pond water once a month. The depth of this pond is ca. 1.5m and the pond size is on average 1,500m^2.

2.2 *Water and Tilapia Sample collection*

Test case ponds in each farm sites were sampled separately over a two day period in the month of September, 2012. All sampling was done between 0830 to 1030 h. Sterilized glass sample bottles (250 mL) were left capped until used for the collection of pond water samples in triplicates from eight different sites within the pond. Water samples were collected in triplicates 15-20cm below the water surface to avoid surface contamination.

Twenty tilapias were randomly harvested by a cast net from the test case ponds and were placed individually in UV pre-sterilized bags before being placed in sterilized cooler chests containing ice. The temperature inside the cooler chests was 3°C. Direct contact of the fish samples with the ice was avoided to ensure maximal survival and recovery of bacteria. Aseptic procedures were strictly followed during collection, transportation and analysis of fish samples.

All samples were transported to the University of the South Pacific Microbiology Laboratory at the Marine Campus in Laucala Bay, Fiji, within 30 - 50 minutes. Microbial examinations were carried out immediately.

2.3 *Water quality analyses*

The pH, temperature and dissolved oxygen (DO) was measured with a YSI meter and turbidity was measured with a secchi disk. All water parameters were measured in triplicates at the same sites where water samples were collected within the pond.

2.4 *Sample preparation*

Pond water samples: After preparing a composite sample, five-fold dilutions were made using 0.1 % peptone water and 1 ml from each dilution was transferred to petri dishes and Petrifilm™ Enterobacteriacea films. Plates were poured using tempered Plate Count Agar (PCA) maintained at 46°C. The content of the plates were mixed thoroughly and allowed to set. After medium had set, plates were inverted and were incubated at the following temperatures as outlined by APHA (2005):

a) 30 ± 0.5 °C for 72 ± 3 h for Aerobic Mesophilic Count
b) 35 ± 0.5 °C for 48 ± 3 h for Total Heterotrophic Count
c) 35 ± 0.5 °C for 24 ± 3 h for Total Enterobacteriacea Count

Water samples were also sent to Institute of Applied Science (IAS) Microbiology Laboratory at the University of the South Pacific, Marine Campus, in Laucala Bay, Fiji for confirmation of *Vibrio* spp. where procedures outlined by APHA (2005) were used.

Microbial counts were expressed as $\log_{10}$ colony forming units (cfu) per mL of sample.

Fish samples: In the laboratory, each fish sample was placed in a sterile tray disinfected with 75 % ethyl alcohol. Skin surface, gills and the gastro-intestinal regions were used to test for bacterial load. These regions were swabbed separately with sterile 3M™ environmental quick swabs (Thermofisher, NZ). A sterile template was used to swab skin surface area of 25 cm^2 whereas the gills (opened aseptically using sterile forceps) were swabbed from both sides. The gastro-intestinal region was swabbed by making an insertion in the abdomen using a sterile scalpel.

The swab was placed in 100 mL Peptone Saline Water and mixed thoroughly by rotating the bottle on the table in circular motion for at least a minute. Five-fold dilutions were prepared after 30 minutes of resuscitation at room temperature and samples were inoculated on Petrifilms for the following:

a. 35 ± 0.5°C for 48 ± 3 h for Aerobic Mesophilic Count
b. 35 ± 0.5°C for 24 ± 3 h for Total Enterobacteriacea Count

Fish samples were also sent to Institute of Applied Science Microbiology Laboratory at the University of the South Pacific, Marine Campus, in Laucala Bay, Fiji for confirmation of *Vibrio* spp. where procedures outlined by APHA (2005) were used.

2.5 *Data Analysis*

Means and standard deviations (SD) were calculated on version 18 of SPSS. Independent T-test was performed on the same statistical software to determine significant difference

Table 1. Mean (±SD) physicochemical parameters of pond culture water at Naduruloulou Research Station and Navua Prawn Farm.

Pond Site	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Turbidity (cm)	Water colour
Navua Prawn Farm	3.88 ± 2.25[a]	26.0 ± 0.47[b]	6.60 ± 0.03[c]	13.25 ± 1.98[d]	Light brown
Naduruloulou Research Station	2.01 ± 1.16[a]	25.5 ± 0.35[b]	7.89 ± 0.06[c]	9.54 ± 0.87[d]	Medium Brown

Means followed by the same letter in the same column do not differ significantly by Independent T-test ($P < 0.05$). n = 24

Table 2. Microbial load of pond culture water from Naduruloulou Research Station and Navua Prawn Farm.

Type of Count	Navua Prawn Farm (cfu/mL)	Naduruloulou Research Station (cfu/mL)
Aerobic Mesophilic Count	6.70×10^3	2.98×10^5
Heterophic Count	6.97×10^3	7.00×10^4
Total *Enterobacteriacea* Count	6.40×10^2	1.33×10^4
Vibrio spp.	ND	ND

(a = 0.05) between the physiochemical parameters of pond water for the two farms under study.

3.0 RESULTS

3.1 *Physicochemical parameters of pond water at Naduruloulou Research Station and Navua Prawn Farm*

The Dissolved Oxygen (DO) of pond water at NPF was higher than NRS, while not much variation in the water temperatures between the study farms was observed (Table 1). The pond water was slightly acidic at the NPF and slightly alkaline at the NRS. The colour of pond water at NPF was light brown with low turbidity level; while medium brown colour of pond water was observed at NRS with water turbid level beyond 9.54 cm (Table 1). No significant variation (Independent T-test; $P < 0.05$) was observed in the parameters between the two farms.

3.2 *Microbial load of pond water and fish*

The Aerobic Mesophilic Counts, Heterophic Counts and Total Enterobacteriacea Counts were much higher at NRS than at NPF (Table 2). Vibrio spp. was not detected.

The mean Aerobic Mesophilic Count and Total Enterobacteriaceae Count of gills, gastro-intestinal tract and skin of tilapia obtained from NPF, NRS and purged fish are shown in Table 3. Generally, higher counts of Aerobic Mesophilic Counts and Total Enterobacteriaceae Counts were observed on gills and skin of purged and unpurged tilapia fish from the two farms. Slightly lower counts were observed in the gastro-intestinal tract of fish from both farms (Table 3).

Fish samples brought from NPF showed highest AMC on gills followed by skin and gastro-intestinal tracts regions. Similar trend was also noted for TEC for these samples. Fish samples obtained from NRS showed high counts of AMC and TEC on skin followed by gills and gastro-intestinal tract. Marked difference in AMC and TEC was observed between the samples obtained from NRS and NPF. Higher counts of aerobic mesophilic colonies and total Enterobacteriaceae colonies were recorded for samples obtained from NRS (Table 3).

Purged samples showed lower counts of AMC and TEC than samples from NRS and NPF. The gastro-intestinal tract of purged samples had higher counts of AMC and TEC than gills and skin. No Enterobacterial colony formations were observed on skin samples of purged fish (Table 3).

Table 3. Microbial load of gills, gastro-intestinal tract and skin of tilapia sampled at Naduruloulou Research Station and Navua Prawn Farm.

Type of Count	Navua Prawn Farm (cfu/cm^2)	Naduruloulou Research Station (cfu/cm^2)	Purged Fish* (cfu/cm^2)
Gills			
Aerobic Mesophilic Count	9.77×10^3	1.64×10^8	1.64×10^2
Total *Enterobactericeae* Count	2.84×10^2	2.30×10^8	5.91×10^1
Gastro-intestinal tract			
Aerobic Mesophilic Count	2.22×10^3	1.58×10^8	8.05×10^2
Total *Enterobactericeae* Count	2.36×10^2	2.03×10^8	2.75×10^2
Skin			
Aerobic Mesophilic Count	6.09×10^3	7.20×10^8	1.30×10^2
Total *Enterobactericeae* Count	1.68×10^2	2.49×10^8	ND
Inner Flesh			
Aerobic Mesophilic Count	ND	ND	ND
Total *Enterobactericeae* Count	ND	ND	ND

*ND = Not detected. *Results for purged fish could not be obtained from NPF due to time constraints. Results displayed are for purged fish obtained from NRS only.*

4.0 DISCUSSION

The physiochemical parameters of the two tilapia farms studied were not within the range suitable for tilapia culture in the Pacific as per the guidelines provided by Nandlal and Pickering (2004). The dissolved oxygen (DO) was lower at NRS (2.01 ± 1.16 mg/L) than the recommended level of > 3 mg/L. Oxygen dissolves in water from air, by the action of waves created by wind and by addition of new water (Nandlal and Pickering 2004), photosynthetic oxygen generation and total plankton respiration (Smith and Piedrahita 1988).

The amount of plankton (zooplankton and phytoplankton) and algae in the water also affects turbidity (Nandlal and Pickering 2004). The turbidity levels of both farms were also much lower than the recommended levels of 30 - 35cm (secchi disk reading) provided by Nandlal and Pickering (2004). While the turbidity level at NPF was lower than the recommended value, water change of ca. 50 % done every afternoon at NPF, led to lower turbidity and higher DO content than at NRS. More frequent water change at NRS fish ponds is warranted to improve dissolved oxygen level, turbidity and to also flush out bacteria.

The results showed high bacterial load in pond water at both the farms; 6.7 x 103 cfu/mL at NPF and 2.98 x 105 cfu/mL at NRS (Table 2). Similar results were obtained by Al-Harbi and Uddin (2003, 2005) in brackish water ponds for tilapia in Saudi Arabia. Al-Harbi and Uddin (2005) found the total viable count (TVC) as 1.4 ± 1.5 x 103 to 8.6 ± 2.7 x 103 cfu/mL in brackish water ponds for tilapia in Saudi Arabia, while Al-Harbi and Uddin (2003) reported a TVC in earthen tilapia ponds of 5.6 ± 0.8 x 103 to 2.4 ± 1.2 x 104 cfu/mL at temperatures in the range of 27 – 28 °C. Our study also showed similar results for NPF but not for NRS.

The microbial load reported for NRS is relatively higher than as reported by the supra-literature. The continuous daily influx of duck excreta result in increase of bacterial numbers for decomposition of excreta. The nutrients from excreta also support the growth of plankton and other micro-organisms which are consumed by the fish with little additional feeding taking place (Balasubramanium *et al.* 1992). The combined effects of the high ambient temperature of the pond water, which was close to optimum for many mesophilic bacteria in natural systems (Newaj-Fyzul *et al.* 2008), and nutrients from excreta may have caused the high bacterial load in the tilapia pond at NRS. Animal excreta also introduce Enterobacteriaceae into the ponds. Sources of Enterobacteriaceae into tilapia ponds could be varied; for example the initial Enterobacteriaceae level in the water pumped in from the river and animal excreta run-off from land during rain; however, duck excreta used

for fertilization purpose appears to be the most obvious contaminant.

NPF had lower bacterial counts than NRS (Table 2). The daily water change of ca. 50% flushes out excessive bacterial growth and also maintains ideal culture conditions for rearing tilapia. The findings of this study agree with Pullela *et al.* (2000) who demonstrated lower faecal coliform count in tilapia reared in a re-circulating system than tilapia cultured in a non-circulating system.

NPF had lower bacterial load fish and in the pond water than NRS. Al-Harbi and Uddin (2004) reported the intestinal bacterial load of fresh water tilapia in Saudi Arabia as 6.8 x 106 to 7.5 x 107 cfu/g. Al-Harbi and Uddin (2005) reported 8.7 x 105 to 2.1 x 106 cfu/g in gills of tilapia reared in brackish water while 7.1 x 105 to 8.7 x 106 cfu/g was reported in gills of hybrid tilapia reared in earthen ponds in Saudi Arabia (Al-Harbi and Uddin 2003). Al-Harbi and Uddin (2003, 2004 & 2005) reported higher microbial load in gills and intestine than the corresponding pond environment. Intestinal microflora of fish or contamination of fish as a result of enteric bacteria of human or animal origin has been responsible for various food spoilages (Cahill 1990).

The incidence of high bacterial load in gills and intestine of fish than in pond water has been stipulated to be due to high metabolic activity of fish associated with increased feeding rates at higher temperatures (Al-Harbi and Uddin 2004). On the contrary, results of this study show that bacterial load of pond water and on fish tissues to be almost similar at a temperature of 25 - 26 °C.

Intestinal microflora of fish has also been shown to be influenced by the microflora of the ingested feeds (Savas *et al.* 2005). It has also been mentioned that the intestinal flora composition of fish is related to the degree of bacteriological contamination of the food consumed (Pal and Gupta, 1992; Savas *et al.* 2005). Tilapia is a voracious feeder and mainly feeds on the detritus organic matter. Both free-living and particle bound bacteria are usually associated with particulate organic matter and are consumed by fishes such as carp and tilapia (Savas *et al.* 2005). The microflora of pelletized feed was not investigated due to time and budget constraints; however, it would be worthwhile to investigate the microbial load of pelletized feed used for feeding tilapia.

Purged fish from NRS, on the other hand, showed considerable reduction in Aerobic Mesophilic Count and Total Enterobacteriaceae count in gills, gastro-intestinal tract and on the skin of tilapia. It was in depurated fish, that higher bacterial and enterobacterial load was found in gastro-intestinal tract than on gills and skin. The higher count of bacterial load in the gastro-intestinal tract could be due to fish being purged for only two hours. Balasubramanium *et al.* (1992) reported that the bacterial reduction in the gut content of different species reared in wastewater fed ponds ranged between 63 and 78 % after 20 days of depuration. Thus, longer hours of purging may be required to further decrease the bacterial load in fish.

Studies on the microbiological quality of fish raised in wastewater-fed fishponds have indicated that faecal bacteria may penetrate the fish flesh when fish is grown in highly polluted water, whereas other studies found no or little penetration of micro-organisms in aquaculture environments in which the fish were not stressed (Cam *et al.* 2007). The present study showed no penetration of micro-organisms into the fish flesh which indicates that the aquatic environment may not be highly polluted as may occur in wastewater-fed ponds. This study has re-affirmed that bacterial flora of fish reveals the bacteriological conditions of the water where fish inhabit. High bacterial and enterobacterial load of pond water led to establishment of these bacteria in fish gills, skin and gastro-intestinal tract. Normally, to determine the sanitation and hygiene of aquaculture ponds, counts of faecal coliforms and/or *E. coli* are used. The World Health Organization's guideline for fishpond state that water should have a faecal coliform count of $\leq$ 1000 per 100 mL (WHO, 1989). While the counts of faecal coliforms or *E. coli* were not estimated separately, the current levels of Enterobacteriaceae at NRS deem the culture conditions as unhygienic.

This has implications for post-harvest quality and shelf-life as contamination may affect the storage life and quality of the fishery products. Contamination may result from rupturing of the fish intestine during processing and/or from inadequate washing. For future commercialization of tilapia in Fiji, this data is required for the development of preventive measures to safe guard against

infectious agents that could cause diseases and post-harvest losses. Aquatic microorganisms not only influence the water quality but are also known to be closely associated with the physiological status of the fish, disease and postharvest quality. It is recommended that tilapia farmers practice proper purging of tilapia before sale and proper sanitation and hygiene to be maintained during handling of fish to extend storage life of fish and fishery products and to prevent spread of infection to humans. It is important that initial microbial load of fish is at safe levels in order to prevent contamination and food-borne diseases.

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