Part-IV

Ability of bacteriological self-purification of waste fed fish
Fish harvested from domestic sewage fed ponds are frequently contaminated with human enteric bacteria, often to high levels. In fact, they can concentrate bacteria even in high numbers than surrounding aquatic environment. Fish accumulate microorganisms from their environment so that their microbiological safety as a human food is directly related to the quality of the waterways in which they are cultivated. Their cultivation in waterways that have received domestic sewage effluent has been the basis for many outbreaks of enteric diseases and food poisoning. Their safety as a food is also related to the potential of contaminating bacterial species to multiply to infective levels during marketing and retailing operations.

Buras et al., (1987) reported the presence of high numbers of bacteria (including pathogenic micro-organisms: Aeromonas, Pseudomonas, Salmonella etc.) in the digestive tract, mucus, gills, internal organs and flesh of sewage fed fish.

Fish flesh for human consumption must be thoroughly devoid of these bacteria (Niewolak, 2000). The ability of fish to get rid of the micro-organisms by releasing them to water (Morse et al., 1978; Lesel and LeGac, 1983; Buras et al., 1987 Phelps and Stiebel, 1991) may be used in practice to purify fish reared in sewage-supplied ponds. This can be accomplished by a process called depuration.

The term depuration is used to refer to the riding of microbes from the body of fish, after it is placed in uncontaminated water for a short period just prior to sale. Microorganisms accumulated in the alimentary tract of the animal by previous feeding activities are eventually discharged as part of the faecal material and the fish is then considered to have become microbiologically cleansed or purified (Son and Fleet, 1980).

Different tissues depurate at different rates, depending on their physiological roles (Heath, 1995). However, whole body depuration rate is inversely proportional to the body size. So
younger fish are able to do this more rapidly than the larger ones (Newman and Mitz, 1988). Further, sloughing of mucus from skin and gills may also help in depuration.

Depuration has been demonstrated to successfully reduce to low levels the number of bacterial and some viral agents in moderately polluted fish. Several factors influence the degree of depuration. These include system design, initial water quality, oxygenation and flow rates, salinity, temperature, shellfish-to-water ratios, removal and settlement of faecal material, the period of purification, health status of the fish, the type of pathogen and level of contamination (Lee and Younger, 2002).

It is now recognized that the success of depuration is intimately associated with an understanding of the farming environment and an appropriate monitoring program to assess growing water and fish quality. Depuration is a process which will confer a level of additional food safety assurance to fish with intermittent pollution. Depuration will not render fish grown in heavily polluted waters a safe product. A sanitary survey and continuing bacteriological monitoring program is therefore necessary to identify fish samples where depuration will be a useful adjunct to quality assurance.

Thus an attempt has been made in this section to study depuration rate of waste reared fish. Bacteriological contamination of the muscles and digestive tract content of *Oreochromis* spp. from wastewater culture was evaluated in the present study and compared with the results obtained for the same fish kept for 25 days in a cemented tank supplied with uncontaminated water.
In the present study, depuration of TC, FC, E. coli, FS, TVC and Vibrio spp. was measured in a sewage fed fish Oreochromis spp. Sixty individuals of Oreochromis spp., each of 50 g body weights were collected from the sewage fed ponds and transported to laboratory. Five freshly harvested fish were immediately subjected to bacteriological analysis and rest fifty five were kept in a cemented tank (58.2" x 31.1" x 20.2", Length x Breadth x Height) under laboratory conditions containing clean uncontaminated water for twenty five days. The water was replaced daily. The bottom of the tank was cleaned on each alternate day. Feeding activity was encouraged by the addition of Japanese dry food 5 hours after the water was changed.

The purified fish (five fish) were sacrificed at regular intervals of 24 hours for 7 days and at 25th days and the tissue residue analysis were performed to study the amount of bacteria depurated, following its exposure to clean, uncontaminated water. The tissues (muscles and digestive tract contents) were analyzed for the bacteria following the procedure of APHA (1998 and 2001).

Significant reduction of bacterial concentration in fish after depuration was measured by pair data t test in which difference at P<0.05 was considered significant.
4.3. RESULTS AND DISCUSSION

4.3.1. Depuration of fish

Depuration is used to purify fish. This facility actually employed to produce safe fish for human consumption. Fish harvested from sewage fed ponds are naturally contaminated with several human enteric bacteria. The responses of these faecal bacteria as well as the pathogenic bacteria like Vibrios, Salmonella, Shigella etc. are thus a direct indication of their fate in purification of fish for direct marketing to consumers.

4.3.1.1. Depuration of bacteria from Oreochromis spp.

Oreochromis spp. got rid of the majority of faecal bacteria (FC, E.coli and FS) through depuration (Table 4.1). Although pathogenic Vibrio spp. were frequently found in cleaned fish (Table 4.1). Level of purification was, however, different for various bacteria and tissues. For example, faecal coliforms and E.coli were almost entirely eradicated (<3 log MPN100g⁻¹) from muscles and digestive tract contents of fish. Concentrations of faecal streptococci decreased by 100% (<3 log MPN100 g⁻¹) from muscles and 75% from digestive tract contents respectively (Table 4.1). On the other hand, TC and TVC counts increased in fish intestines (Table 4.1) where as significant reduction was reported for these two bacteria from fish muscles (Table 4.1).

4.3.1.2. Depuration and initial bacteriological contamination of fish

Efficiency of self-purification of wastewater-reared fish transferred to pure water depends mainly on the initial bacteriological contamination of fish. According to Buras et al., (1987), the muscles of wastewater-reared common carp kept for 14 days in clean running water contained excessive numbers of bacteria and were not suitable for consumption. Similar studies on tilapia containing low numbers of bacteria in the gut and almost no micro-
organisms in the muscles revealed considerable reduction of gut contamination, and sterile muscles after 14 days in clean running water. Niewolak and Tucholski (2000) reported that this procedure was not effective when bacteria were present in fish muscles, and was effective only when the concentration of bacteria in fish organs was low and running water was used. In present research little beneficial impact of depuration for TVC and TC from digestive tract may be explained by considerable initial contamination of the fish themselves with these two groups of bacteria.

4.3.1.3. Static vs. running water fish cleaning

Static fish cleaning in tanks filled with clean water is usually not sufficient due to bacteriological contamination of water, resulting in a possibility of a secondary fish infection. Bocek et al., (1992) reported Salmonella typhimurium in silver carp 14 days after experimental fish infection, followed by their transfer to clean water. Salmonella panama was isolated from channel catfish intestine after 30 days of fish keeping in clean water. In present study on purification, though water was replaced daily but a continuous running system was not maintained. Thus a better bacteriological result could be obtained by transferring fish into a running water system.

4.3.1.4. Depuration of FS

It is evident from Table 4.1. that difference exists in the relative rates of cleansing of different bacteria. Also, it appears for FS that the greatest reductions in counts occur during day 1 of depuration, the rate of cleansing being significantly reduced after this time. No explanations for these differences can be given, but a more detailed study into the kinetics of elimination of individual bacterial species from fish would be worthwhile.
Table 4.1. Elimination of bacteria from *Oreochromis* spp. during laboratory depuration (n=12)

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Depuration time (Days)</th>
<th>TC log MPN 100g⁻¹</th>
<th>FC log MPN 100g⁻¹</th>
<th>E.coli log MPN 100g⁻¹</th>
<th>FS log MPN 100g⁻¹</th>
<th>TVC log cfu g⁻¹</th>
<th>Vibrio spp. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscles</td>
<td>0</td>
<td>1.4±0.51*</td>
<td>0.75±0.3*</td>
<td>0.75±0.3*</td>
<td>1±0.30**</td>
<td>5.36±0.2*</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2±0.60</td>
<td>2±0</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>5.30±0.37</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.5±0.15</td>
<td>2±0.36</td>
<td>0.25±0.13</td>
<td>0.66±0.28</td>
<td>5.63±0.14</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.5±0.15</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>0.5±0.26</td>
<td>6.21±0.12</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.5±0.26</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>0.5±0.33</td>
<td>5.57±0.12</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.5±0.45</td>
<td>0.66±0.28</td>
<td>1.25±0.39</td>
<td>1.5±0.45</td>
<td>5.96±0.18</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.75±0.39</td>
<td>0.16±0.11</td>
<td>&lt;3</td>
<td>2.16±0.47</td>
<td>4.95±0.09</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1±0.30</td>
<td>0.66±0.36</td>
<td>0.5±0.26</td>
<td>1.91±0.28</td>
<td>6.32±0.17</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.5±0*</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>4.94±0.0*</td>
<td>++</td>
<td></td>
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<tr>
<td>Digestive tract contents</td>
<td>0</td>
<td>1.75±0.53</td>
<td>1±0.52*</td>
<td>0.75±0.3*</td>
<td>2±0**</td>
<td>6.27±0.29</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.25±0.44</td>
<td>2.25±0.44</td>
<td>1.5±0.5</td>
<td>1±0.46</td>
<td>5.79±0.79</td>
<td>++</td>
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<tr>
<td></td>
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<td>2.75±0.25</td>
<td>1.5±0.26</td>
<td>1±0.30</td>
<td>1.5±0.33</td>
<td>5.89±0.47</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>2±0.63</td>
<td>1.5±0.45</td>
<td>1.5±0.45</td>
<td>0.88±0.36</td>
<td>6.86±0.02</td>
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<td>4</td>
<td>2±0.60</td>
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<td>1.25±0.39</td>
<td>1±0.30</td>
<td>5.35±0.41</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.25±0.13</td>
<td>2.75±0.13</td>
<td>1.5±0.45</td>
<td>1.75±0.53</td>
<td>6.61±0.19</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2±0.36</td>
<td>1±0.30</td>
<td>1±0.30</td>
<td>1.83±0.44</td>
<td>6.11±0.19</td>
<td>++</td>
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<tr>
<td></td>
<td>7</td>
<td>1.91±0.45</td>
<td>1±0.42</td>
<td>0.5±0.33</td>
<td>1.66±0.30</td>
<td>6.41±0.21</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2±0.60</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>0.5±0.26**</td>
<td>6.52±0.20</td>
<td>++</td>
</tr>
</tbody>
</table>

# Presence or absence test
++= Most of the tested samples positive
** Tissue wise reduction of a particular parameter in a column significant at P<0.01.
* Tissue wise reduction of a particular parameter in a column significant at P<0.05.

Statistical comparison made between 0 and 25th days of depuration
4.3.1.5. Tissue wise difference of depuration vs. recontamination

The reports have indicated that fish flesh contaminated with faecal bacteria when kept in clean water tanks, *E. coli* and FS titres were generally reduced to undetectable levels within first 24 hours and for FC it happened at 3rd and 4th days of depuration. Bacterial levels there after fluctuated (increased, decreased and sometime undetectable {for *E.coli* only}) and finally completely undetectable at 25th days of depuration. In case of digestive tract contents faecal bacterial levels fluctuates throughout the experimental period and reached to an undetectable state (<3 log MPN100g⁻¹ for FC and *E.coli* and 75% reduction for FS) at 25th days of depuration (Table 4.1).

The variation in results of depuration experiments may be attributable to differences in bacterial loads in two different fish tissues, being higher in intestines than muscles. Further, it is to be considered here that muscle is sterile, not comes in contact directly to water, where as intestine is frequently coming in contact with surrounding aquatic environment of fish. Thus, rise of bacterial level in intestines than muscles might be an indication of recontamination of fish by its own faecal matter which demands better management of the purification system specially bottom of the tank.

This conforms to work conducted by Richards (1988), which indicated that depuration system required a disinfection treatment of circulating water to prevent microbial build-up and recontamination of the fish. This could be attributed that initial cleansing might have occurred, but because the water used for the depuration remained unchanged for the stipulated period, conditions present necessitated recontamination of bacteria.

4.3.1.6. Initial bacterial load vs. depuration time

The time required for the purification of polluted fish depends upon the initial level of contamination, more heavily contaminated fish requiring longer cleansing times. The data obtained in this study suggests that this depuration time (Table 4.1) did not able to eliminate efficiently high load of TVC and *Vibrio* but remove low load of faecal bacteria (*FC*, *E.coli* and FS) from fish.
4.3.1.7. Depuration of indicator vs. pathogenic bacteria

It has been suggested that fish might maintain an indigenous gut flora. This would imply mechanisms for selectively retaining some microbial species in the alimentary tract while eliminating others with the faeces. This concept is supported by the fact that total bacteriological counts of fish intestines did not decrease when fish were allowed to feed for extended periods in sterilized water, as was the case during depuration. This ability of fish to selectively retain some microbial species while eliminating others has profound public health implications when considering the microbiological purification of fish by depuration where the elimination of an indicator organism, such as *E. coli*, is used to judge the elimination of other pathogenic species.

The efficiency of fish purification, by depuration, is monitored by the extent to which indicator bacteria have been cleansed. When indicator levels have been cleansed to acceptable standards, it is considered that any pathogenic contaminants, such as *Vibrio* spp., will have been equally cleansed. The assumption made is that all pathogenic bacterial species likely to contaminate fish will be eliminated or discharged at a rate comparable to that of the indicator organism. However, in present research persistence of *Vibrio* spp. in fish after depuration for the 7 days has considerable public health significance and challenges the long-accepted assumptions correlating the cleansing of indicator bacteria with the cleansing of specific pathogenic bacteria (Table 4.1.).

The investigation agrees with the findings of several authors who reported depuration as a very effective process for the elimination of faecal bacteria, such as *E. coli* (eg. Souness and Fleet, 1979), but is less effective for naturally occurring *Vibrio* spp. (Eyles and Davey, 1984; Rowse and Fleet, 1984; Tamplin and Capers, 1992; Groubert and Oliver, 1994 and Croci et al., 2002). There is also some evidence that *Salmonella* spp. are more difficult to remove by depuration than *E. coli* (Son and Fleet, 1980; Nishio et al., 1981 and Murphree and Tamplin, 1995).

Faecal coliforms interact with both the environment and fish in a manner which is significantly different to that of naturally occurring microbes, such as *Vibrio* spp. It has been clearly established from our research that for the fish faecal coliforms, and more specifically
*E. coli*, are not selectively retained in the gut and are readily eliminated with the faeces during fish purification. The present investigation has now established that, for *Oreochromis* spp. at least, *Vibrio* spp. are retained in the gut of the fish and are not readily cleansed from the fish under conditions which promote cleansing of *E. coli*. Presence of *Vibrio* spp. also indicates that fish may not have been fully active in this depuration system.

The presence of *Vibrio* spp. is of health concern because it can be the causative agent of gastroenteritis (Oliver and Kaper, 1997 and Baffone et al., 2001). Presence *Vibrio* spp. in the samples in which the depuration was particularly efficient in the reduction of FC and *E. coli* reinforced the considerations about the real significance of these two indicators for the evaluation of the microbiological state of foods. Moreover, also in the not depurated samples the presence of this *Vibrio* spp. was not associated with particularly high counts of *E. coli* and FC (Table 4.1.). On the other hand, the use of FC and *E. coli* concentrations as indicators for fish quality has raised several criticisms. In fact, many researchers discussed the limits of this use of FC and *E. coli* counts (Kator and Rhodes, 1994 and Leclerc et al., 2000) because these indices of faecal contamination are not reliable indicators of the survival strategies and distribution of *Vibrios* in fish (Ripabelli et al., 1999; Cavallo and Stabili, 2002 and Croci et al., 2002), which are considered to be the major cause of identifiable illness from fish consumption (Wittman and Flick, 1995).

The fact of reduction of faecal bacterial levels confirms that the depuration process was functional. A significant factor that contributed to this success was careful maintenance of conditions within the facility to minimise stress to the fish, because stressful conditions can impair the elimination of microorganisms during depuration (Power and Collins, 1989).

Comparison of the synoptic responses of the *Vibrios* to the faecal bacteria is convincing evidence of the ineffectiveness of depuration for consistently eliminating *Vibrios* from fish. The *Vibrios* are natural constituents of the microflora of fish (Colwell and Liston, 1960) and may have developed mechanisms that allow them to remain associated with fish tissue and resist depuration. Conversely, faecal-borne bacteria are allochthonous organisms and are thus not adapted for persistence in fish tissue.
4.4. REFERENCES


Heath AG. 1995. Water pollution and Fish Physiology. CRC Press, NC. Florida, USA.


Niewolak S and Tucholski S. 2000. Ability of bacteriological self purification of Common carp, Tench and Crucian carp fingerlings reared in pond receiving the discharge from sewage treatment plant and then kept in running water of different quality. Archives of Polish Fisheries. 8(2), 49-61.


