Development and trial of a freshwater mussel (*Batissa voilacea*) depuration system for a village setting.

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Abstract

Freshwater mussels *Batissa voilacea* known as *Kai* are found in the major rivers of Fiji and a largest fishery in the country and ranked third in the pacific. This fishery is mostly dominated by women who spend two to four hours daily, free diving for *Kai* and selling them in the municipal markets or along the road sides stalls. The revenue earn from selling *Kai* put children to school, provide for household needs and village contribution. Hence it becomes an important fishery for the wetland livelihood.

*Batissa voilacea* are susceptible to microbial contaminants due to their feeding attributes and it is important that study on ways to produce a quality shellfish out in the markets is vital. Producing a quality shellfish depuration method has been studied and implemented in shellfish industries inorder to meet the required standards set by developed countries. This paper, however aims to establish quality assurance for freshwater mussels *Batissa voilacea* through the development of a depuration system applicable in a village setting in Fiji. The system used in this experiment is a simple static system. For experiment one three 45 liter plastic bins with different water-changing time intervals. Water-changing time intervals used in this experiment was every 8 hours (three water-changes), every 12 hours (two water-changes) and 24 hours (only one water –change). Results from the first experiment shows that water-changes every 8 hours indicates no detection level of *E.coli* *B.violacea* tissues however for 12 hours and 24 hours has the same results. The 8 hours water-change was used in the second experiment and it shows reduction in *E.coli* level however it indicates presence of *E.coli*.

Key words: Depuration, Quality, water-change time intervals, Batissa violacea.
1. INTRODUCTION.

The freshwater mussel *Batissa violacea* known as *Kai* in Fijian is an important freshwater bivalve in Fiji in terms of yield and effort. It becomes one source of protein in the menu of Fijian families, since it is cheap and fairly assessable. In Fiji, this fishery is mostly dominated by women who usually spend three to four hours daily free diving for these freshwater mussels and selling them at municipal markets or along road side stalls. It is a significant source of income for these rural communities who live close to the river systems as it meet their household like meeting daily household expenses, paying for school fees and village contribution. Hence it is an important fishery that supports traditional wetland livelihoods.

However, kai has the ability to accumulate microbial organisms in the environment they live in due to its filter feeding attributes. Some of the bacterial organisms are potential in causing diseases human such as *E.coli* and *Salmonella*. Health authorities had to close the *Kai* fishery down to suspected cases of typhoid being related to the consumption of *Kai* (*Fiji Times* (01/03/13). Hence this closure has an impact on the livelihood of the local communities. On the other hand it also limits the potential on adding export value to *Kai*. This can be the result from large number of the population live along the river reaches with and most have no proper sewerage system in place. Livestock and farming done along the river bank although local government regulations dictate that 5m buffer strip be in place (https://www.iucn.org). As a consequence this could lead to the contamination and accumulation of bacteria and other contaminants in freshwater mussel’s meat.

In Fiji, soaking of *Kai* in a bucket for 24hours before cooked or eaten is a traditional method to get rid of grit sand and other microbes that could be in the meat of a *Kai*. This is a simple depurating method practiced by the villagers. Depuration is a natural process of purification by which the mussel cleanses themselves (Blake, Tamplin et al. 1985). It was first introduced in the early 1920s as a response to outbreaks of typhoid that relates to consumption of shellfish in both sides of Atlantic (Lees, D.,et al 2010). This system is practice worldwide within shellfish industries since there are standards need to be met. In some countries like Australia, Spain and France they practice tank based depuration system (Lees, D.,et al 2010).Using such systems they also include the use of sterilizing processes which involves use of ozone, chlorination, UV irradiation and iodophores (Otwell et al. 1991). However such method is costly for *Kai* fishers in
the village setting. Recently a study has been carried out on identifying the type of depuration system that can be used in a village setting, a prototype recirculating depuration system was developed and trialed but has been recommended to reconsider (Waqalevu, 2015). This paper aims to establish quality assurance for freshwater mussel *B. violacea* through the development of a depuration system applicable in a village setting in Fiji.

1.1 Aim

- To develop a *Kai* depuration system that is effective and practical for a village setting.

1.1.1 Objectives

- To construct and trial a *Kai* depuration system in a village setting.
- To determine the construction and operational cost of the system.
- To recommend a suitable depuration system that is suitable in a village setting.

1.2 Expected outputs

The following are some of the likely outputs of this project:

- Construction of a village-scale depuration system in Fiji using village-level technologies
- A report detailing construction and operation of a depuration system.
2. METHODOLOGY

2.1 Study Site
The Toga district was selected as the study site for this project. It is located on the south eastern part of Viti Levu and one of the districts that make up Rewa province. The three main villages that make up Toga District are Muana village, Vunisei village and Navatuyaba village. For this project the study site was Muana village (Figure 1). The village is located close to Nausori town nearly 2 km north and close to the urban center. There are approximately 70 households in Muana village with most of the population centered around the church and community hall. There are four different sub clans (i) Madadredre (Mataqali Liuliu); (ii) Natikoriwaca (Mataqali Sauturaga); (iii) Waisousou (Mataqali Mata Ni Vanua); and (iv) Matai (Mataqali Vunivaivai) (Waqalevu (2015).

Figure 1: District of Toga and the main collection site is Muana Village. (Waqalevu,2015).

2.2 Construction and trial of a static depuration system
A static system design is a very easy and basic setting of a system that is suitable in a village setting. This can just set less that 10 minutes . This small scale static system was chosen in considering water availability in the village and electricity . We used three 45 litre of bins that can hold a large volume of water for this experiement . Three bins were used to set a static
system at the USP lower campus seawater laboratory. Each system has different water-changeover a 24 hour period. Three systems were used, the water-change time interval for was every 8 hours for system one, every 12 hours for system two and 24 hours for system three. The depurated water used is tape water. After 24 hours kai were picked randomly in each system for *E. coli* testing. Each system has its duplicate and using their average to draw up the results.

### 2.2.1 Experiment 1: Construction of a Simple Static depuration system using plastic materials.

**Components for experiment set up**

**Plastic Bins 45 liters**

Forty five litre of plastic bins (purchase from Nausori Rubs Big Bear store) were used this experiement. It is easy to get and handy in a village setting and less cost materials. This can be all be set less than 10 minutes.

![Image of plastic bins](image)

**Figure 2.** The three simple static systems set at the lower campus with different water-change time intervals.

**Trays**

For this experiment three trays were used and it cost FJD$6.75. The trays (bought from Nausori Rubs Big Bear store) were supported by the upper part of the bucket a 10 cm high. It make it easier to remove the *Kai* when do changing of water during the experiment and also avoid must disturbance to the *Kai* while changing the water. For this experimant stocking density of 300 gram per liter and 20 liters of water used in each system. Examples of trays used are shown in the
Figure 3 below. The tap water level was filled so that all kai in the tray within the bucket, were submerged in water.

![Figure 3: Kai stocked in the plastic bins (left) and tray(right) used in the experiment.](image)

**Figure 3:** Kai stocked in the plastic bins (left) and tray(right) used in the experiment.

**Stocking density (300g/liter)**

A scale (Figure 4) was used to measured the stocking density which was 300g/liter and the volume of water use for this experiment is 20 liters.

![Figure 4: Scale used to measure the stocking density for the each system.](image)

**Figure 4:** Scale used to measure the stocking density for the each system.

**Time used**

Before depuration *Kai* samples were selected according to 2 – 3 piles for *E.coli* testing. This was for tessting of *E.coli* presence at zero hours.

Water-change time interval was three times in a 24-hour period.

**System 1:** After every 8 hours (ie.three times water-change)
System 2: Water-change time interval was two times, every 12 hours water-changed

System 3: Water-changing time interval is only one times 24 hours.

After the 24 hours period samples were collected randomly from each system for E.coli testing at the upper campus microbiology lab.

2.2.2 Experiment 2 - Use of 14 liters steel bucket with as holding unit 300g/liter stocking density.

Using the result obtained from experiment one different material was used as a holding unit for depuration. Here we aimed to see the effect of using a different material of the holding unit. The material used was a steel bucket. Tape water is use as the depurate water placed in the system after every eight hours the water is changed until a total of 24 hours and then samples are taken for E.coli testing using the 5 mpm method as done in experiment one. Duplicates of the system is done and no trays were used in experiment two. Also Kai samples were also taken for before depuration zero hours. 3 replicates were used in the E.coli testing before the depuration.

![Steel bucket](image)

Figure 5: Steel bucket used as types of materials that can be used in the experiment.

2.2.3 Microbial analysis of Tissue samples

(i) Enumeration of the Escherichia coli in Tissues samples

The method use for enumeration of E.coli was the 5 tube mpm method. The broths used were lactose broth, EC broth and EMB agar. Kai samples were taken from the village and was placed in a ice box using patty ice to keep it in room temperature. At the campus 10 samples of Kai
were picked randomly for *E.coli* testing before depuration and the rest were used in the depuration process. 3 repilcates were done for before depuration. The weight of the *Kai* used in this experiemnt was 10 ± 2 gram and a volume of 0.1% of peptone water is added to the stomacher bag. The mixture was homogenized for approximately 2 minutes until all the contents were homogenized. This becomes the 1:1 dilution the homogenate and is added to 90mls of peptone water to make the master solution. Other dilutions were prepared to 10⁻² and 10⁻³ in 0.1% peptone 9mL water.

For the 5 tube three stage MPN analysis the first 5 tubes contain double strength of lactose broth. 10 ml of the master solution is added to each of the 5 tubes that contained double strenght broth which is equivalent to 1g of shellfish. To other 5 tube to each tube 1ml of 1:10 dilution of homogenate is added equals to 0.1g shellfish. To the other 5 tube inoculate 1 mL of 1:100 dilution of homogenate equivalent to 0.01 g shellfish. The inoculate tubes were then incubate at 37°C ± 1°C for 24 ± 2 h. Three controls were also used in this experiemnt Blank solution, *E.coli* ( positive ) and *Bacillus* ( negative ).

**Figure 6:** Positive ( Yellow & gas production) on the left and negative (clear solution) in lactose broth on the right.

The presence of *E.coli* is subsequently by subculturing the positive faceal coliforms into EC broth. The tubes are they incubate in 44 ± 1 °C for 22 ± 2 h. Positive *E.coli* indicates production of gas and yellow colouration of the broth. The negative ones don’t change the colour.

The positive test tubes were then subculture on to EMB agar for confirmation of *E.coli* presence. The EMB agar is normally prepare the day before of sampling this is to make it ready to be after
the incubation. The plates were divided according to the number of the positive tubes. A loop was used to streak the according to the labelled section. The three controls were also streaked on the agar onto separate agar plates. The positive result of E.coli shows a green metallic sheen (Figure 8) colour on the agar after incubate at 37°C ± 1°C for 24 ± 2 h and the negative will produce no growth.

**Figure 7:** Streaking of positive tubes on to EMB agar for E.coli sub-culturing and green metallic sheen show positive result for E.coli.

The confirmed EC tubes for *E.coli* for each dilution tested and verified in the plates was then used to calculate the Most Probable Number of E.coli in the amount of tissues analysed using the 5MPN table (Appendix). The dilution was then multiply by the extract number of dilution factors.

2.3 **Construction cost**

Using of excel to calculate the cost of the construction excluding the water and power bills.

2.4 **Maintenance of the system**

Keeping of materials in a clean and dry place of storage to prevent spoilage of materials. Especially the steel material which can easily rust if stays more than a day with water in it.

3. **Results**
3.1 Depuration system trial

3.1.1 Experiment 1- Construction of a Simple Static depuration system using plastic materials.

Water-change time interval every 8 hours over a 24 hour period (i.e three water-changes) showed the lowest detection of *E.coli* in the samples tissues using the Most Probable number method.

Figure 8: A graph show the Level of *E.coli* in Kai tissues samples taken from Rewa River 20th May 2015.
3.1.2 Experiment two using steel bucket instead of plastic materials with 300kg/liter.

Using the results obtained from experiment one steel bucket was used as the holding unit for the depuration process. The graph below shows the number of E.coli reduces when using the steel bucket taken from 19th June 2015. Observation was done over period of 24 hours (1 day) with water-change time interval every 8 hours (i.e three times water-change).

Figure 9: E.coli presence in B.violacea tissue after every 8 hours water-change time interval.
3.2 Construction and operational cost (simple static depuration system)

This was the cost of the simple static system that was set at the lower campus. It only includes the materials used however the water and electricity bills were excluded.

**Table 1:** Estimated cost for a small scale static depuration system.

<table>
<thead>
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<th>Materials</th>
<th>Quantity</th>
<th>Unit Cost</th>
<th>Total Cost</th>
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<td>Steel Bucket</td>
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<td>Trays (S)</td>
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<td>Trays (B)</td>
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<td><strong>TOTAL</strong></td>
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4. Discussion

The Rewa River has supports large kai fishery in Fiji and a great source for the local markets however previous studies show presence of microbial contaminants within the Kai tissues and it exceeds international standards which is not safe for consumption. According to Kelly 1994 findings, Rewa River shows an increase in the bacteria counts, however presence of Salmonella was not determine only E.coli. Also (Waqalevu, 2015) he has found that the E.coli CPU counts exceeds the Environmental Protection Agency (EPA) standards and the lowest count from the three samples he got was found to be 466.67 CFU per 100mL of sample which still above the EPA standard and standards required in the Codex Alimentarius standard 292-2008 revised in 2014. For this study results shows for experiment one the CPU of E.coli was 0.831MPN/g which is lower than the standard while for experiment two the CPU for E.coli was 0.466MPN/g before depuration. Hence this issues arises the need for development of a depuration system at Kai harvesting areas. Thus this study aimed to develop a Kai depuration system that is effective and practical for a village setting. Using static depuration system with plastic bins for experiment one the result shows that water-change time interval for every 8 hours over a period of 24 hour has able to reduce the level of E.coli to no detection limit while for 12 and 24 hours there is a still detection of E.coli in the kai tissues. Changing water for a static system often gives the ability for kai to purify itself since it removes the impurities from the system. Therefore changing of water for a static system over a period of 24 hours is able to reduce the level of E.coli to an acceptance level.

Moreover the result of using different materials steel bucket (14 liters) with no trays shows also shows a decrease in E.coli level from 0.466MPN/g to 0.12PMN/g. Thus trays are important to use in depuration since it avoids reabsorption of bacteria by the kai.

Furthermore the cost of the simple static depuration system shows FJD $128.55 that is set in at the lower campus this don’t include the water and electricity bills which can be affordable by the local communities and can be easily managed. But this system can even be modified by the villagers so that the cost is even cheaper for them, depending on their budget the materials are easily accessible (from Nausori Rups Big Bear store) and should be easy to maintain.

Finally from this experiment a simple static depuration system can be suitable and affordable by the local communities in Fiji. Using up to three water-change time intervals every ( 8 hours ) can
able to reduce the amount of *E. coli* present in *Kai* flesh. However using a plastic materials in depuration system is suitable in a village setting since they can last long and easy to maintained compare to the steel bucket. As this experiment used tap water as the depurate water, it would be interesting to determine the use of boiled (and cooled to room temperature) tape water, to get rid of chlorine.

### 5. Conclusion

In summary depuration method helps to improve the quality and avoids consumers from foodborne diseases. Living mussels soak in water for over 24 hours can able to reduce the level of *E. coli*. For this experiment using a static system in a village setting can able to reduce the *E. coli* level within 24 hours however with the water-change time interval it gives the possibility to greatly reduce the amount of *E. coli* to the no detection limit. Changing of depurated water every 8 hours (during a 24 hours period) has shown no detection of *E. coli* in *Kai* tissues. In terms of using different materials plastic materials are found to be good since they can be easily maintained compare to steel buckets which can rust easily.

Following are few recommendations for these findings

- Firstly different collection sites on the experiment should be done inorder to determine the level of *E. coli* in different location.
- Modification should be done on the types of material used and which depuration system is suitable in a village setting.
- Culturing of freshwater mussels in bacteria solution before depuration should done before depuration, this is to really know how effective the system is in reducing the level of *E. coli*.
- Sample size for Microbial analysis of tissue samples should be increase to get precise results.
6. Reference


Whippy, P. G., Pande, P.A. (1987). Interim report on faecal coliform levels in Rewa River shellfish. Institute of Natural Resources, the University of the South Pacific.
### 7. Appendix

<table>
<thead>
<tr>
<th>No. of Tubes Giving Positive Reactions out of 5 tubes containing 1 g, 0.1 g, 0.01 g, 1 g</th>
<th>95% Confidence Limits</th>
<th>No. of Tubes Giving Positive Reactions out of 5 tubes containing 1 g, 0.1 g, 0.01 g, 1 g</th>
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