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MICROBIAL LOAD OF TWO PALAEMONID PRAWNS AND THE WATER SAMPLE FROM OVIA RIVER, NIGER DELTA, NIGERIA

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Abstract
A comparative study of the Microbial load of two palaemonid prawns and water samples of Ovia river, Edo state were analysed using standard methods. Total viable microbial count ranged between 1.62 x 10^5 and 5.58 x 10^5cfu/g in all samples. The highest mean bacteria count 3.27x 10^5cfu/g was found in the gills of M. felicinum while the lowest mean count 1.27 x 10^5cfu/g was found in the tissue and carapace of M. felicinum and M. vollenhovenii respectively. The highest mean fungi count 3.68 x 10^5cfu/g was found in the gills of M. vollenhorenii while the lowest mean count 3.47 x 10^4cfu/g were in the tissues of M. felicinum. Significant differences (P<0.05) were observed between body parts of prawns. A total of 81 isolates were identified as Bacteria, Fungi and Yeast. The bacteria isolates include Micrococcus sp, Proteus sp, Streptococcus sp, Bacillus sp, Escherichia coli, Klebsiella sp, Staphylococcus aureus while fungi isolates include Aspergillus niger, Penicillium sp, Aspergillus flavus, Rhizopus sp, Mucor sp and Saccharomyces sp. M. vollenhovenii was observed to be more polluted while M. felicinum had more pathogenic organisms. The prawn environment is highly polluted and the fisherfolks contributed to the high microbial counts due to their improper hygienic practices.

Key Words: *Microbial load, M. vollenhovenii, M. felicinum, Ovia River*

Introduction

The lower reaches of tropical waters contain appreciable number of decapods crustaceans (Arimoro and Meye, 2007)). The fresh water prawns, *Macrobrachium"* are widely distributed and abundant macro-invertebrates in most aquatic systems (Cook *et al*., 2002). Fresh water crustaceans including prawns though produced in meager quantities (around 95, 3198 tonnes) have gained significance as high valued species by standing as the fifth highest valued species group. Interest on prawns has increased recently due to the possibility of the commercial culture of some of them (Ajuzie and Fagade, 1992; Marioghae and Ayinla, 1995). They are

highly valued as food sources for humans. In local markets close to freshwater streams of the Niger Delta, they are sold fresh or smoke-dried. *Macrobrachium* species are rare in spite of their widely acknowledged status as species of ecological and economic importance to many fisheries. Most freshwater prawns are found in most inland freshwater areas including rivers, lakes, swamps and irrigation ditches. The most commonly found family of the fresh water prawns in Nigeria belongs to the genus *Macrobrachium.* These include *M. vollenhovenii, M. felicinum, M. macrobrachion and M. equidens*. *Macrobrachium* species offer quality protein, low saturated fat, and may contain omega-3-fatty acids. They also contribute to the cardiovascular stability of adult as well as children's growth and development (Food and Drug Administration, 2007). *M. vollenhovenii* is the largest of the local Palaemonid prawns, with a maximum size of 200mm (Ehigiator and Agbogbo, 2010). It is a handy prawn in many ways, it thrives in many water and can survive in waters with low dissolved oxygen as low as 1ppm. It is an omnivorous detritivore with preference for animal remain (Marioghae, 1982). This species is also quite tasty, although the integument is thick. *M. felicinum* occurs along with *M. vollenhovenii* in non-tidal fresh water zones of White Water Rivers. Its oxygen and pH tolerance will therefore be probably as for *M. vollenhovenii.* It is an omnivore and has a maximum adult size of 80mm which is not good enough to recommend for culture.

Reviews on the microbiological evaluation of aqua-cultured raw fish/shell fish and their growing environments emphasized the need for the assurance of the quality and before sending them to retail and export markets (Reilly and Kaferstein, 1999). Shell fishes, the most importantly traded food of aquatic origin are in the forefront of food safety and quality improvement due to stringent microbial quality and safety regulations, enforced by international regulatory authorities. Rapid spoilage due to higher water content and other issues mentioned in Abubakar *et al*., (2008) are important concern and affect shelf-Iife of the prawn. The shelf life depends on the number and types of microorganisms, mainly bacteria, initially present and their subsequent growth as well as natural sources. Most available information has been basically taxonomic (Nip and Moy, 1988; Naiyanetr, 2001; Mejia *et al*., 2003 Murphy and Austin, 2005), on the migratory behaviour Osuamkpe and Powell, (1981) and on the general ecology. Hence the need for this study is to provide information on the microbial content of the prawn species (*M. vollenhovenii* and *M. felicinum*) and how these related to the microbial content of the water sample of Ovia River. The objective of this study therefore, is to determine qualitatively and quantitatively the microbial flora of *M. vollenhovenii, M. felicinum* and water samples of Ovia River.

Material and Methods

Description of study area

The study area is a stretch of Ovia river flowing North to South from Iguiye through Iguorakhi , Ikoroto Gelegele all in Edo state. The river in its upper reaches flows through a secondary rainforest zone in the vicinity of Ikoro

village (SW of Benin City, Lat. 06° 16′, Long. 05°27′).The area is characterised by high fishing activities and it could be described as a non tidal clear water (Figure 1).

Fig. 1: Map of Ovia Local Government Area Showing Sample Station
Source: Ministry Of Lands And Survey, Benin City 2010

Collection of Prawn and Water Samples

Live *M. felicinum* and *M. vollenhovenii* were purchased from the fisher folks from the Ovia River at Ikoro village between the hours of 10.00am-11.00am during the months of October and December, 2010. Samples were then packed into newly purchased polythene bags containing ice chips then placed in cooler box. The cooler box was transported to the Fisheries department for proper identification before it was sent to the microbiology laboratory both of the Uuniversity of Benin. Water samples were collected monthly with 1 litre plastic bottle immersed below the surface of the water and transported to the laboratory for microbial analysis in replicates from the River.

Preparation of Culture Media

The culture media used were Nutrient Agar (NA) for bacteria and potato dextrose Agar, (PDA) for fungi. In preparing the media, 28g of NA was diluted in 1 litre of sterile water using a conical flask while 39g of PDA was also diluted in 1 litre of sterile water also using a conical flask. The two solutions were autoclaved for proper sterilization.

The preparation and sterilization were done according to Taylor *et al.* (1998).

Preparation of Samples for Culture/Serial Dilution

Three whole prawns from each species were selected and the prawn parts taken were the gills, tissues and carapace. 1.0g of each prawn part was weighed and crushed by homogenizing and were added to a 10mI sterile water to make a stock solution. From this stock solution, 1.0 ml was pipetted into 9.0mI sterile water. This process was repeated with seven other universal bottles each containing 9.0mI of sterile water up to 10^{-7} dilution. Stock 10^{-1}, 10^{-3} and 10^{-5} dilution bottles were then selected for the pour plate method.

Culturing/Inoculation/Incubation and Colony Counts

The dilution bottles selected for the pour plate were then inoculated in Petri dishes containing PDA and NA for fungi and bacteria respectively. Anti-fungi and antibiotic were also added to the plates containing NA and PDA respectively (This was done to inhibit the growth of fungi and bacteria respectively). In all, 108 Petri dishes were inoculated, and incubated. Water samples A, B, C were also inoculated using the pour plate method. 18 Petri dishes were inoculated for water samples and all these were done in the presence of a Bunsen burner to ensure complete sterility.

Characterization and Identification of Isolates

Bacterial isolates were identified on the basis of cultural, morphological and biochemical tests such as Gram-stain, methyl red, voges-prosakaser, indole, oxidase, catalase, hydrogen sulphide, motility, gelatin liquefaction, citrate tests as described by (Buchanan and Gibbons, 1974), while the fungal colonies were identified according to (Barnett and Hunter, 1974).

Statistical Analysis

Analysis of variance was performed to determine the differences between the prawn parts and the water samples.This was done at a 5% probability level using the least significant difference (LSD).

Results

The total viable microbial count (TVC) in Cfu/g isolated from prawns and water samples are presented in Table 1. The total viable microbial count ranged between 1.62 x 10^5 and 5.58 x 10^5 cfu/g in all samples. The highest mean bacteria counts were found in the gills of *M. felicinum* 3.27 x 10^5 cfu/g while the lowest mean bacteria counts were found in the tissue 1.27 x 10^5 cfu/g and carapace 1.27 x 10^5 cfu/g of *M. felicinum* and *M. vollenhovenii* respectively. The highest mean fungi counts 3.68 x 10^5 cfu/g were found in the gills of *M. vollenhovenii* while the lowest mean fungi count 3.47 x 10^4 cfu/g were found in the tissues of *M. felicinum*. The gills had the highest total viable microbial counts while the tissues had the lowest total viable microbial count. However, bacteria counts were generally higher than fungi counts except for *M. vollenhovenii* which had the higher counts for fungi compared to *M. felicinum* and water samples. Significant differences ($P<0.05$) were observed between body parts of prawns

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Table1: Total viable microbial count (TVC) in Cfu/g isolated from prawns and water samples from Ovia river

Samples	Fungi counts	Bacteria counts	Total viable microbial count
***M. felicnum* (A)**			
Carapace	$4.93 \times 10^4 \pm 0.23$	$1.97 \times 10^5 \pm 0.15$	$2.46^{b} \times 10^5 \pm 0.17$
Gills	$1.15 \times 10^5 \pm 0.33$	$3.27 \times 10^5 \pm 0.85$	$4.42^{a} \times 10^5 \pm 1.18$
Tissues	$3.47 \times 10^4 \pm 1.41$	$1.27 \times 10^5 \pm 0.25$	$1.62^{b} \times 10^5 \pm 0.33$
***M. vollenhovenii*(B)**			
Carapace	$5.33 \times 10^4 \pm 1.41$	$1.27 \times 10^5 \pm 0.15$	$1.80^{b} \times 10^5 \pm 0.26$
Gills	$3.68 \times 10^5 \pm 4.81$	$1.90 \times 10^5 \pm 1.22$	$5.58^{a} \times 10^5 \pm 6.00$
Tissues	$4.67 \times 10^4 \pm 1.51$	$1.57 \times 10^5 \pm 0.23$	$2.04^{b} \times 10^5 \pm 0.35$
Water samples			
A	$1.40 \times 10^5 \pm 0.41$	$1.60 \times 10^5 \pm 0.40$	$3.00^{a} \times 10^5 \pm 0.14$
B	$9.32 \times 10^4 \pm 1.21$	$1.60 \times 10^5 \pm 0.40$	$2.53^{a} \times 10^5 \pm 5.46$
C	$7.57 \times 10^4 \pm 0.27$	$1.61 \times 10^5 \pm 0.41$	$2.37^{a} \times 10^5 \pm 2.21$

**Values are means of triplicates*

**Means in the same column with the same superscript are not significantly different (P>0.05)*

Table 2 shows the frequency of occurrence of microbial isolates from the parts of the prawns and water samples. A total of 81 isolates were identified as Bacteria, Fungi and Yeast. The bacteria isolates include *Micrococcus* sp*., Proteus sp, Streptococcus* sp*., Bacillus* sp*., Escherichia coli, Klebsiella* sp*., and Staphylococcus aureus* while fungi isolates include *Aspergillus niger, Penicillium* sp*., Aspergillus flavus, Rhizopus* sp*., Mucor* sp. and *Saccharomyces* sp. The most frequently isolated bacteria include, *Micrococcussp*, *Proteus* sp. and *Escherichia coli* 9(17.95%), *Bacillus* sp. and *Staphylococcus aureus* 7(15.39%), *Streptococcus* sp. and *Klebsiella* sp*.,* 3(7.69%) respectively.

While the most frequently isolated fungi include; *Aspergillus niger* and *Saccharomyces* sp. 9(21.43%) followed by *Candida sp*, 7(16.67%) then, *Rhizopus* sp. 6(14.29%) *Penicillum* sp. 5(11.41%), *Mucor* 4(9.52%) and *Aspergillus flavus* 2(4.76%). However, in all the samples fungi isolates 42(51.85%) was higher than bacteria isolates 39 (48.15%) whereas, the diversity of the bacteria 7(53.85%) was higher than that of fungi 6 (46.15%).The highest number of microbial isolates was obtained from the gills 13(16.05%) and 12(14.82%) of *M. vollenhonenii* and *M. felicinum* while the lowest 7(8.64%) was obtained from water samples B and C respectively.

Table 2: Frequency of occurrence of microbial isolates from prawn and water samplesof ovia river

Isolates	**No (%)**	*M.felicinum*			*M.vollenhovenii*			Water samples		
		C	G	T	C	G	T	A	B	C
Bacteria										
Micrococcus sp.	7(17.95)	√	√	x	√	√	x	√	√	√
Proteus sp.	7(17.95)	√	√	x	√	√	x	√	√	√
Streptococcus sp.	3(7.69)	x	x	x	x	x	x	√	√	√
Bacillus sp.	6(15.39)	√	√	√	√	√	x	√	x	x
Escherichia coli	7(17.95)	√	√	x	√	√	x	√	√	√
Klebsiella sp.	3(7.69)	x	√	x	√	√	x	x	x	x
Staphylococcus aureus	6(15.39)	√	√	√	√	√	√	x	x	x
Total bacteria isolates/diversity	**39(46.84)/ 7(53.85)**	**5(12.82)**	**6(15.39)**	**2(5.13)**	**6(15.39)**	**6(15.39)**	**1(2.56)**	**5(12.82)**	**4(10.26)**	**4(10.26)**
Fungi										
Aspergillus niger	9(21.43)	√	√	√	√	√	√	√	√	√
Candida sp.	7(16.67)	√	√	x	√	√	x	√	√	√
Penicillium sp.	5(11.41)	√	x	√	√	√	√	x	x	x
Aspergillus flavus	2(4.76)	x	√	x	x	√	x	x	x	x
Rhizopus sp.	6(14.29)	√	√	√	√	√	√	x	x	x
Mucor sp.	4(9.52)	√	√	x	√	√	x	x	x	x
Saccharomyces sp.	9(21.43)	√	√	√	√	√	√	√	√	√
Total fungi isolates/ diversity	**42(51.85)/ 6(46.15)**	**6(14.29)**	**6(14.29)**	**4(9.52)**	**6(14.29)**	**7(16.67)**	**4(9.52)**	**3(7.14)**	**3(7.14)**	**3(7.14)**
Total microbial isolates/ diversity	**81(100)**	**11(13.58)**	**12(14.82)**	**6(7.41)**	**12(14.82)**	**13(16.05)**	**5(6.17)**	**8(9.88)**	**7(8.64)**	**7(8.64)**

Key: √**-**Present, x-Absent, C-Carapace, G-Gills, T-Tissue

Discussion

The total viable microbial counts of *M. vollehonvenii, M. felicinum* and water of Ovia river, Edo State were considerably higher than those reported by Green, (1949) who found microbial counts of 1.6 x 10^3 cfu/g to 1.6 x 10^5 cfu/g of fresh shrimp caught in the gulf of Mexico with an average of 4.2 x 10^4 cfu/g and Yousuf *et al*., (2008) who obtained 1.08 x 10^2 to 1.2 x 10^5 cfu/ml for prawn from Bangladesh. This can be attributed to the fact that micro-organisms including coliforms are present as a result of man's activity in tropical waters unlike temperate waters where they are absent except in grossly polluted waters.

However, Jay (1986) and Ekanem and Adegoke (1995) have reported that the level of contamination of shellfish depends on the extent of pollution in the growing waters. Khan (2001) also reported that increase in microbial contamination could have beenfrom water source, poor hygiene and sanitation. International Commission on Microbiological Specification for Foods (1986) and Food and Drug Administration, (1991) have suggested a maximum microbial count IPC of not greater than 1 x 10^5 cfu/ g and coliform level of not greater than 1 x 10^2 cfu/g of shellfishes for consumer safety. The result of the present study agrees with the report of Ekanem *et al*. (1994) who observed unacceptable levels of bacterial contaminants (including pathogens) in clams. Also observed during this study, was *M. Felicinum* with higher bacteria counts than *M. vollenhovenii* and water samples. This might imply that *M. felicinum* probably feeds more on feacal waste and organic matter. Although *M. vollenhovenii* also feeds voraciously on these, it can be inferred that *M. felicinum* concentrates more of these organisms than the latter. The total viable microbial count of 5.58 x 10^5 cfu/g and 4.42 x 10^5cfu/g in gills of *M. vollenhovenii* and *M. felicinum* were the highest when compared to others parts and water sample. Since the gills are the breathing organs, it can be said that most of these pathogenic organisms are gotten from the prawn environment (water). Although the microbial count for the water 3.0 x 10^5 cfu/g was generally lower than that of the prawns. The high amount of microbial load in prawns may be as a result of its environment and improper temporary storing before sales. *M. felicinum* was eventually found to have a higher microbial load than *M. vollenhovenii,* which may be due to its feeding habits.

A total of 81 isolates were identified as Bacteria, Fungi and Yeast. The bacteria isolates include *Micrococcus* sp*., Proteus* sp*., Streptococcus* sp*., Bacillus* sp*., Escherichia coli, Klebsiella* sp*., and Staphylococcus aureus* while fungi isolates include *Aspergillus niger, Penicillium* sp*., Aspergillus flavus, Rhizopus* sp*., Mucor* sp. and *Saccharomyces* sp. The most frequently isolated bacteria include, *Micrococcus sp*, *Proteus* sp. and *Escherichia coli* 9(17.95%), *Bacillus* sp. and Staphylococcus *aureus* 7(15.39%), *Streptococcus* sp. and *Klebsiella sp* 3(7.69%). While the most frequently isolated fungi include; *Aspergillus niger* and *Saccharomyces* sp. 9(21.43%) followed by *Candida* sp., 7(16.67%) then, *Rhizopus* sp. 6(14.29%) *Penicillum sp.* 5(11.41%), *Mucor* 4(9.52%) and *Aspergillus flavus* 2(4.76%). However, in all the samples fungi isolates 42(51.85%)

was higher than bacteria isolates 39 (48.15%). The highest number of microbial isolates was obtained from the gills 13(16.05%) and 12(14.82%) of *M. vollenhonenii* and *M. felicinum* respectively while the lowest 7(8.64%) was obtained from water samples B and C. *Aspergillus niger* and *Saccharomyces* sp. 9(21.43%) the highest occurring species are natural contaminants of food. These are found in soil and indoor environments (Abarca *et al.,* 1994; Klich, 2002).

The composition of the microbial flora from *M. vollenhovenii, M. felicinum* and water samples of Ovia River was similar to that reported by Williams *et al.* (1952) who reported *Achromobacter, Bacillus, Micrococcus* and *Pseudomonas* as the main groups present in fresh shrimp. Ikenebomeh and Elohor (2005) isolated *Staphylococcus aureus*, *Bacillus cereus* and other organisms in a similar study on fresh and roasted edible worms (*Rhynchoporus phoonics*) larvae from 5 locations in Delta state. *Escherichia coli*, *Streptococcus sp* and *Klebsiella* sp. were the most frequently isolated index of water quality and indicators of feacal contamination. *E.coli* implies a risk that one or more other pathogens may be present. The presence of *E.coli* in fresh prawn was not reported for farmed *M. rosenbergii* in Brazil by Leitao and Rios, (2000), this shows the influence of prevailing environment on the bacteria composition in the prawns. Although Hazen, (1988) reported that *E. coli* can be found in unpolluted warm tropical waters and can survive indefinitely. It is not really a reliable indicator of contamination or sewage pollution in tropical waters.

Staphylococcus aureus isolated from *M. felicinum* and *M. vollenhovenii* are also found in waters polluted by sewage though they are related to human i.e. they are normal human flora. Nimrat *et al*. (2005), noted that *S. aureus* and *Bacillus* sp. were present in spermotophores of fresh black tiger shrimp. *Candida sp* isolated was as a result of the activities of the inhabitants who use the river for domestic purposes such as bathing and washing. *Candida albican* is an organism mostly found in women that causes "Candidiasis" or "Thrush" (Ducluzeau and Bensaada 1982). *Penicillum sp, Rhizopus* sp. and *Mucor* sp. are also not natural inhabitants of prawns and its environment. This must have been gotten from the sacks used in keeping the prawns before sales since they are usually found in a variety of organic substrates such as fruits, leathers, sacks and vegetables. Human handlers had a way of influencing the flora of the samples, resulting in their increase.

Conclusions

Prawns being highly nutritious but largely susceptible to the activity of micro-organisms can be kept in a wholesome state if the level of pollutionin the environment can be controlled,since substances from this pollution can be picked by prawn and cause serious infection when consumed by humans. The fisherfolks or prawn sellers should be enlightened on the importance of personal hygiene and maintenanceof environments in order to ensure the safety of the prawn products. The inhabitants of the fishing communities should be enlightened on the importance of observing proper sanitation and shunning improper

practices such as defecatingin the rivers where prawns live. Other activities such as bathing, washing and other commercial activities should also be discouraged to reduce the microbial content of the rivers and the prawns found in them.

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