

Comparison of nutrient composition of *Holothuria atra* (Jaeger, 1833) in three geographically separated locations in Sri Lanka: preliminary investigation

K.P.G.K.P. Guruge¹ and D.H.N. Munasinghe^{2*}

¹Department of Oceanography and marine Geology, Faculty of Fisheries and Marine Sciences and Technology, University of Ruhuna, Matara, Sri Lanka.

²Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka,

Abstract

Although, local consumption of sea cucumbers is limited in Sri Lanka, there is high demand for them in export market. This study was performed to compare the nutrient composition of *Holothuria atra* populations in three different geographical locations in Sri Lanka. Therefore, this study was designed to compare nutrient composition of *H. atra* populations in three different locations in Sri Lanka. Samples were collected from Southern, Northern, and Eastern coastal regions. Ash contents were significantly different among selected three coastal regions. The highest moisture and ash contents were observed in sea cucumbers from the Eastern region. There was also a significant difference in the protein and lipid content of sea cucumbers from the three regions, with the highest crude protein and fat contents were recorded from the Northern coastal region. The moisture content, in the Southern region was significantly different from other two sites ($p < 0.05$). These differences may reflect different diets of the sea cucumbers from these regions. Further investigations are suggested to estimate the influence of environmental characters on the proximate compositions of *H. atra* populations. The value of sea cucumbers depends on their nutrient composition. Therefore, the results of this study will be the basic platform for future research in the field.

Keywords: *Holothuria atra*, Geographical variation, Nutrient composition, Sea cucumbers, Sri Lanka

*Corresponding author: donamunasinghe@gmail.com

Introduction

Holothurians are sedentary marine invertebrates that belong to the phylum Echinodermata and class Holothuroidea. Holothurians, are popularly known as sea cucumbers, are a good nutritional source of protein and their cartilaginous body acts is a rich in mucopolysaccharides (Dharmananda, 2003).

After the sea cucumber species of high economical value have been already over exploited in Sri Lanka, there is an increasing interest in exploiting the middle and low value species from the natural resources in Sri Lanka. *H. atra* (Jaeger, 1833) is considered as low value species. The value of sea cucumbers depends in part on their

nutrient composition. Although, sea cucumbers are harvested in different geographical areas in Sri Lanka, only few studies have been reported on their ecology and fisheries (Dissanayake and Wijeyrathna 2007; Dissanayaka and Athukorala, 2008/2009). This study estimated nutrient composition of *H. atra* living in three different geographical areas, which has not been studied previously

Materials and Methods

Samples were collected from Point Pedro in the Northern region, Kayenkeri in the Eastern region and Ahangama to Dondra Harbour sites from Southern region. The length and size of the collected samples ranged from 20-21 cm. Moisture, Ash, Crude Protein, Crude Fat contents were determined following AOAC standards. Samples were kept dried in an Oven at 105 °C. The ash content of samples was determined by igniting samples heated to 550 °C for four hours. The total nitrogen content of the muscle was determined by Kjeldahl analysis using Kjeltac, Auto analyzer. The crude fat content of body muscles were determined by exhaustive Soxhelt extraction using chloroform-methanol mixture on a Soxtec system HT6 (Barnes and Black-stock, 1973).

Results

The ash and moisture content was significantly different among the sample sites ($p < 0.05$), being highest in the Eastern region. The moisture content in the samples from the Southern region was significantly different ($p < 0.05$) from other two sites. The crude protein and fat contents were also significantly different ($p < 0.05$), with the highest values in the Northern coastal region. The crude fat content Northern coastal region was significantly different ($p < 0.05$) from southern and eastern sites.

*Table 1: Mean (\pm SD) moisture, ash crude protein and crude fat content of *Holothuria atra* from different regions in Sri Lanka. The range of values is provided in parentheses.*

Parameter	Site		
	Southern	Northern	Eastern
Moisture %	86.90 \pm 1.41 (85.49-88.31)	89.98 \pm 2.41 (87.57-92.39)	92.35 \pm 0.47 (91.88-92.82)
Ash %	16.30 \pm 1.28 (15.02-17.58)	20.74 \pm 0.74 (20.00-21.48)	24.15 \pm 0.52 (23.63-24.67)

Parameter	Site		
	Southern	Northern	Eastern
Crude Fat %	1.08±0.33 (0.75-1.41)	1.62±0.05 (1.57-1.67)	1.04±0.03 (1.01-1.07)

Discussion

Significant differences in ash, moisture, crude lipid and crude protein content were observed in the *H. atra* sampled from the different locations. In general, ash content indicates amount of minerals available in their muscle samples and may reflect different mineral composition of the sediments in the different regions. The crude lipid and protein content of some Holothurians may vary due to selective feeding and differences in the food supply (Lee et al, 1989).

Conclusion

Holothuria atra is a sedentary animal that feed on sediments on their living environment. A significant difference in nutrient composition was observed among the three locations in Sri Lanka. The differences observed differences in the body composition of animals that collected from different regions may be a result different diet or diet availability. Further investigations are suggested to analyze environmental factors to determine the influence of environmental factors on proximate compositions of the *H. atra* populations.

Reference

- Dharmananda, S.,(2003). Sea cucumbers: Food and medicine.[on line] Available at: <http://www.itmonline.org/arts/seacucumber.htm> [Accessed 30th September 2013]
- Dissanayaka, D.C.T. and Athukorala, S., (2009).Status and Management of Sea Cucumber Fishery in Sri Lanka. (pdf). Colombo: National Aquatic Resource Development Agency. Available at:<<http://www.nara.ac.lk>.(Accessed 5th March 2013)
- Dissanayake D.C.T. and Wijeyrathna J.M.S., (2007), Studies on the Sea Cucumber fishery in the North Western Coastal region of Sri Lanka, Sri Lanka Journal of Aquatic Sciences. (online) Available at :<<http://www.ljol.info/index.php/SLJAS/article/view/2212> > (Accessed 25th April 2013)

Bacteria in ornamental fish aquarium water: Are they sensitive to commercial anti-bacterial preparations?

D.P.T. Rushanthi¹, E. Pathirana^{1*}, P.D.I.S. Liyanage¹ and I. Pathirana²

¹*Faculty of Fisheries and Marine Sciences, Ocean University of Sri Lanka,*

²*Department of Animal Science, University of Ruhuna, Sri Lanka*

Abstract

Most bacterial infections in freshwater fish are caused by opportunistic bacteria. Although various commercial antibacterial preparations are available in the local market, relatively little is known about the efficacy of these preparations and the bacteria present under local conditions. The objectives of the present study were 1) to assess the efficacy of some selected (n=7) commercial antibacterial preparations and 2) to isolate and identify bacteria present in aquarium water. Commercial preparations containing formalin (A), acriflavine (B and C), flavin (D), methyleneblue (E), potassium permanganate (F), and iodine (G), as the active ingredient, were tested in this study. Water samples (n = 9) were collected aseptically from fish-tanks at the university aquarium, during a period of 3 days. Fifty microliters from each sample was cultured separately on nutrient agar and then one replicate of each was treated with one antibacterial preparation separately, at their manufacturer recommended dose (MRD). As for F and G, trial doses were used. Treated samples were kept at room temperature after mixing well. Sub-samples were collected from each treatment at 10 min, 30 min and 1hr and were cultured separately by spread plate method. All cultures were incubated at 37°C, overnight. The bacterial colonies were then enumerated using the colony count (Rocker, Taiwan). The total plate count (CFU/ml) dropped significantly ($p < 0.05$, $n=3$) 10 minutes after the treatment with potassium permanganate ($7.04 \pm 2.13\%$) and dropped further at 30 minutes ($3.22 \pm 1.16\%$) and at 1 hour ($2.64 \pm 1.19\%$), compared with the control (100%). The acriflavine and flavin preparations (B, C and D) showed differences in antibacterial activity. Anti-bacterial efficacy of the products carrying methylene blue and formalin preparations were not significant ($p > 0.05$) at MRD. Potassium permanganate demonstrated the most promising anti-bacterial efficacy out of all the preparations tested. *Pseudomonas aeruginosa* and *Bacillus* spp. could be identified from the samples.

Keywords: aquarium water, anti-bacterial preparations, bacteria, efficacy, ornamental fish

Corresponding author: erandivet@yahoo.com

Introduction

Colorful aquaria are maintained for recreation and as an enterprise. It is important to maintain ornamental fish under stress-free conditions, in order to maintain fish health. Most of the pathogenic bacteria in freshwater fish are opportunistic (Austin and Austin, 2007). Formalin, malachite green, potassium permanganate, acriflavine and methylene

blue are some of the most commonly used antibacterial compounds for aquarium tanks (Maity et al, 2011). Although various commercial antibacterial preparations are available in the local market, relatively little is known about the efficacy of these preparations and the bacteria present under local conditions. Thus the objectives of the present study were 1) to assess the efficacy of some selected (n=7) commercial antibacterial preparations and 2) to isolate and identify bacteria present in aquarium water.

Materials and Methods

The commercial preparations containing formalin (A), acriflavine (B and C), flavin (D), methylene blue (E), potassium permanganate (F), and iodine (G), were tested in this study. Water samples (n = 9) were collected aseptically, from fish-tanks at the university aquarium, during a period of 3 days. Fifty microliters from a sample on each day was cultured directly on nutrient agar and then one replicate each was treated with one antibacterial preparation separately, at their manufacturer recommended dose (MRD), on each day. As for F and G, treatment doses were decided based on a trial-and-error methods and existing literature. Three different doses were tested separately, for F and G. Anti-bacterially treated samples were kept at room temperature after mixing well. Sub-samples were collected from each treatment at 10 min, 30 min and 1hr and were cultured separately by spread plate method. An additional sub-sample was collected from the replicate treated with A, after 3h, for spread plating method. All cultures were incubated at 37°C, overnight. The bacterial colonies were then enumerated using the colony count (Rocker, Taiwan). Colony count (CFU/ml) of control samples were compared with that of sub-samples collected from different treatment regimens and different time intervals, were compared using generalized linear models (GENLIN) of SPSS version 20.0 (IBM Corporation, Somers, NY, USA).

Furthermore, the colonial morphology of different isolates on nutrient agar was studied. Each different colony was subjected to microscopic examination of Gram-stained smears. Subsequently colonies were sub-cultured on MacConkey agar (HiMedia, India) and Pseudomonas selective agar (HiMedia, India), based on colonial morphology and microscopic appearance. Finally, biochemical tests were performed to identify the genus/species of each type.

Results and Discussion

At the most effective dose, the total plate count (CFU/ml) dropped significantly ($p < 0.05$, $n = 3$) 10 minutes after the treatment with potassium permanganate ($7.04 \pm 2.13\%$) and dropped further at 30 minutes ($3.22 \pm 1.16\%$) and at 1 hour

($2.64 \pm 1.19\%$), compared with the control (100%). The study revealed an effective dosage of $100 \mu\text{l/L}$ of water, for potassium permanganate. The acriflavine and flavin preparations (B, C and D) showed differences in antibacterial activity. Anti-bacterial efficacy of the products carrying methylene blue and formalin preparations were not significant ($p > 0.05$) at MRD. The antibacterial action of Lugol's Iodine was also significant ($p < 0.05$, $n=3$) 10 minutes after the treatment ($18.14 \pm 4.66\%$), and dropped further after 30 minutes ($6.78 \pm 5.58\%$). However, there was rise ($12.48 \pm 3.98\%$) after 1 hour from treatment, compared to the count after 30 minutes post-treatment. Lugol's iodine is not usually used to treat bacterial infections in fish. However, previous studies also have reported safe elimination of surface bacteria in fish by using Lugol's iodine. *Pseudomonas aeruginosa* and *Bacillus* spp. could be identified from the samples.

Conclusion

Potassium permanganate demonstrated the most promising anti-bacterial efficacy out of all the preparations tested. The study revealed an effective dosage of $100 \mu\text{l/L}$ of water, for potassium permanganate. *Pseudomonas aeruginosa* and *Bacillus* spp. could be identified from the samples.

References

- Austin, B. and Austin, D. A. (2007). Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. 4th Edn, p 1. Praxis Publishing, UK.
- Musa, N., Wei, S.L., Shaharom, F. and Wee, W. (2008). Surveillance of bacteria species in diseased freshwater ornamental fish from aquarium shop. World Appl. Sci. J. 3: 903-905.