Bangladesh J. Fish. (2018) **30**(1) : 103-111

Seasonal variation in bacteriological count between native and exotic climbing perch, *Anabas testudineus* (Bloch, 1972) from North-Eastern Bangladesh

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Abstract. The study was conducted to assess the seasonal variation of bacterial population in native and exotic climbing perch, *Anabas testudineus* from the North-Eastern region of Bangladesh. For this purpose, ten fish markets were selected randomly considering two seasons-explicitly in winter (December-February) and summer (March-May). Live fish were collected, and bacterial loads were analysed at 6-12 days interval during the experimental period. The higher bacterial load i.e. total viable count (TVC) was observed during summer whereas lower TVC was observed in winter irrespective of markets and varieties of climbing perch. For both strains, the highest TVC was noted in summer (April: temperature 36.3^0C) and lowest in winter (February: temperature 18^0C) indicating that the seasonal variation of TVC is highly correlated to temperature fluctuation in summer and winter. It is assumed that the optimum temperature with high relative humidity during summer season accelerated the growth of bacterial populations than those of winter. The present study revealed that, although bacterial load in winter did comply with ICMSF (International Commission on Microbiological Specification of Food) standard, however TVC in summer season didn't comply with ICMSF standard. It can be concluded that the higher bacterial count in summer that fails to comply with the ICMSF standard may pose an alarming threat for the export of fishery products and food safety issues in Bangladesh.

Keywords: Total Viable Count, Climbing perch, Seasonal variation, ICMSF standard, Food safety

Introduction

The success of aquaculture depends on water quality which is greatly influenced by aquatic microorganism along with other factors (Perez *et al*. 2003). Usually, aquatic animal including fish takes a large number of bacteria through their food and drinking water which accumulate in their gut (Hansen and Olafsen 1999). Bacterial decomposition has been shown to be a major factor causing rapid deterioration of fish quality and bacterial flora on fish reflects the aquatic environment which affects the quality and storage life of fishery products (Laycock and Regier 1971).

Climbing perch (*Anabas testudineus*) commonly known as koi in Bangladesh is a freshwater fish found in inland water bodies. While intensive and unhygienic culture practices bring some health problems, most of which occurred during post-harvest management of cultured and captured fishes including native and exotic varieties of climbing perch. Bacteria are ubiquitous in the aquatic environment (Allen *et al*. 1983) and poor water quality can cause burn off the slime coat or stress of the koi that making

it more susceptible to bacterial infection (Wendelaar Bonga 1997). *Pseudomonas* spp. and *Aeromonas* spp. are deadly bacteria that invariably present in every climbing perch fish pond causing life-threatening bacterial infection such as ulcer, fin and tail rot (Rahman *et al*. 2010). Apart from other microbes, bacteria are one of the most important microorganisms present in fish responsible for causing diseases, mortality, reducing biodiversity, economic loss etc. (Hossain *et al*. 2014, Hossain *et al*. 2009a, 2009b, Ahmed *et al*. 2009a, 2009b, 2009c).

The intensity and abundance of bacterial flora depend on aquatic environmental conditions including seasonal temperature variation and expected to influence the intestinal microbiota of fishes (Hovda *et al*. 2012). Generally, the bacterial load is higher in other seasons except winter, possibly because the high ambient temperature in the water body is close to optimum for many mesophilic bacteria in natural systems (Rheinheimer 1985). Seasonal variations, explicitly summer and winter temperature variations, influence a wide variety of animal life cycle including various farm (Hossain *et al*. 2014) and wild fishery species (Bari *et al*. 2014 and 2015). Although few studies investigated the health status of farmed climbing perch, *A. testudineus* (Ahmed *et al*. 2009a) however, comprehensive studies on microbial load in fishes are scant. Moreover, there is very few information on seasonal variation in bacterial load i.e. total viable count (TVC) in fishes and there is no information on the seasonal variation of bacterial load in native and exotic varieties of climbing perch, *A. testudineus*. Thus the present study aims to know the seasonal variation of bacterial load in native and exotic varieties of climbing perch, *A. testudineus* at North Eastern region of Bangladesh.

Materials and Methods

Site profile of the study area

North Eastern part of Bangladesh (Sylhet) is very important for freshwater capture fisheries due to the abundance of rivers, *beel* (static lake), *haor* (bowl or saucer shape shallow depression) and canal. (Hossain 2013). For the present study ten (10) fish market of Sylhet Sadar Upazila were selected randomly for the collection of native and exotic climbing perch (*Anabas testudineus*) including *Kazir bazar*, *Bondor bazar*, *Shibgonj*, *Tilagor*, *Major tila*, *Akhalia*, *Subid bazar*, *Amborkhana*, *Shahi Eidghah* and *Baluchor noyabazar*.

Sample collection and preparation

Two seasons winter (December, 2014 to February, 2015) and summer (March, 2015 to May, 2015) were considered for collection of the samples. Two varieties of climbing perch, *A. testudineus* were selected to investigate the seasonal variation of bacterial load in summer and winter by means of Total Viable Count (TVC) values calculated as (log CFU/g$\pm$SD). The fish samples were randomly collected from fish markets by using icebox. The onsite ambient temperature was recorded by using Silver Indoor Digital Thermometer & Hygrometer (LC Technology 302-604S) and time series temperature

data were collected from Sylhet Meteorological center to cross-check with primary data. After the collection, the samples were transported to the laboratory of the Department of Microbiology and Immunology, Sylhet Agricultural University, Sylhet. The samples were prepared by dissecting the fish and taking 25 g of fish muscle, gill and intestine, and homogeneously mixed with 225 ml of distilled water in a Stomacher lab blender (Seward Stomacher 400, UK). Each sample was mixed aseptically with sterile distilled water at the ratio of 1:10. Then the sample was shaken properly to make a homogenous suspension. Later on 10 fold serial dilutions (1:10) were prepared to range from 10^{-2}-10^{-9} according to the recommendation of International Standardization Organization (ISO 1995). Then the diluted samples were taken in nutrient broth and mixed well. Finally, the nutrient broth containing samples were placed into an incubator to incubate the sample for 24 hours.

Calculation of Total Viable Count (TVC)

According to ISO (1995), 1 ml of each tenfold diluted sample was transferred and spread to Plate Count (PCA) agar using a sterile pipette and a sterile glass spreader. The incubated plates were then kept in an incubator at 37^0C for 24-48 hrs. Only plates having 30 to 300 colonies were considered for counting in order to get acceptable values and number of bacteria colony forming unit per gram of the sample (CFU/g) was calculated after Kashem *et al*. (2014) as

$$\text{Number of bacteria (CFU/g)} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{Volume of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Data analysis

After 24 hrs of incubation colonies were counted upon visualization and data were recorded. These values were useful and other statistical analysis and interpretations thereafter by using the computer software Microsoft Excel and SPSS. For the comparisons of seasonal variation in the TVC in native and exotic varieties of climbing perch, Student's t-test and Pearson correlation test were applied where P value of < 0.05 was considered as statistically significant.

Results

Seasonal variation in bacterial load in native and exotic varieties of climbing perch

In summer, mean values of TVC (Log CFU/g $\pm$ SD) were higher and noted as (8.57 ± 0.08) and (8.50 ± 0.06) for native and exotic variety of climbing perch respectively (Table I). The lower mean TVC was recorded during winter as (6.68 ± 0.75) and (6.62 ± 0.74) for native and exotic *A. testudineus* respectively (Table 1).

TVC in the sample of native variety of *A. testudineus* from various markets was found to be higher than that in exotic koi (Fig. 1). On the other hand, in all the studied fish markets, higher values of TVC (log CFU/g) were noted in summer (March-May) than in the winter (December-February) season for both the varieties of *A. testudineus*. Although the TVC varied due to different market conditions e.g. hygiene, sorting, handling etc. however, a higher temperature may be the focal influencing drivers for the mass growth of bacteria in both varieties of climbing perch, *A. testudineus*. One of the striking issues of this study is that bacterial load in winter season complied with the ICMSF (International Commission on Microbiological Specification of Food: acceptable range for raw fish log 5.70 to log 7.70, standard 1986) for both varieties of climbing perch, *A. testudineus* while bacterial load in summer season didn't comply with the ICMSF standard.

Table 1. Seasonal variation and comparison of Total Viable Count (TVC) in native and exotic variety of climbing perch, *A. testudineus*

Season (months)	Varieties of *A. testudineus*	Numbers of samples	TVC (Log CFU/g ± SD)	*P*- value
Summer (March-May)	Native Koi	10	8.57 ± 0.08	< 0.05
Winter (Dec - Feb)		10	6.68 ± 0.75	
Summer (March-May)	Exotic Koi	10	8.50 ± 0.06	< 0.05
Winter (Dec - Feb)		10	6.62 ± 0.74	

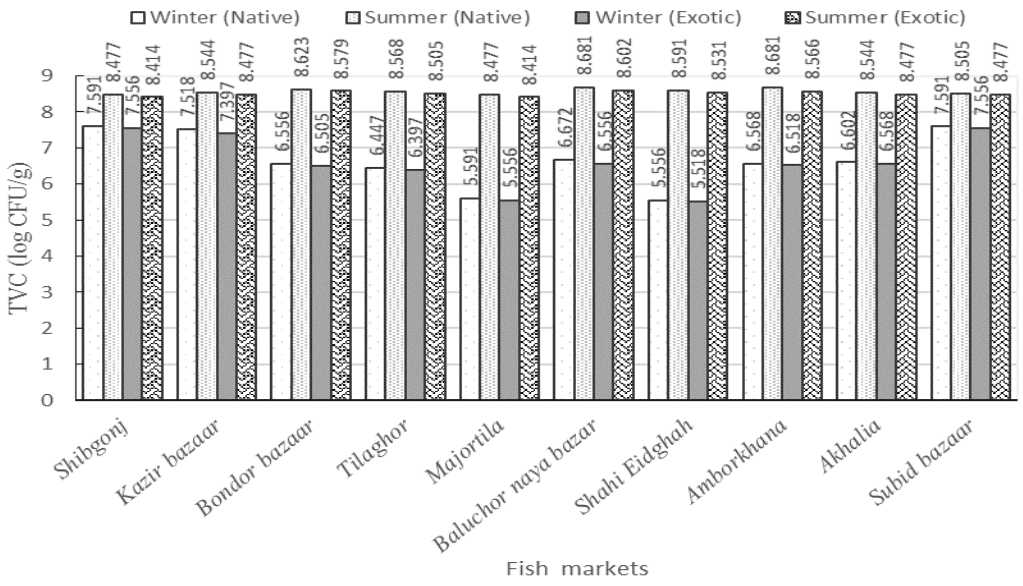

Fig. 1. Total Viable Count (TVC) in native and exotic *A. testudineus* during winter and summer seasons, collected from 10 fish markets of north eastern Bangladesh

Changes in the bacterial load with ambient temperature variation in winter and summer seasons

Anabas testudineus is affected by bacteria in which it lives especially the surrounding the environment and also infected by bacteria during post-harvest management. The range of temperature recorded during the study period was 18-36.3^{0}C. During the winter season lower ambient temperature ranged from 18-28.5^{0}C and the comparatively higher temperature in summer ranged from 30-36.3^{0}C. Highest TVC values were noted at 36.3^{0}C in the month of April 2015, whereas, the lowest was found at 18^{0}C during the month of February 2015. The higher ambient temperature during summer than winter may act as an influential factor for the lower TVC in winter (Fig. 2) and higher bacterial growth in summer (Fig. 3).

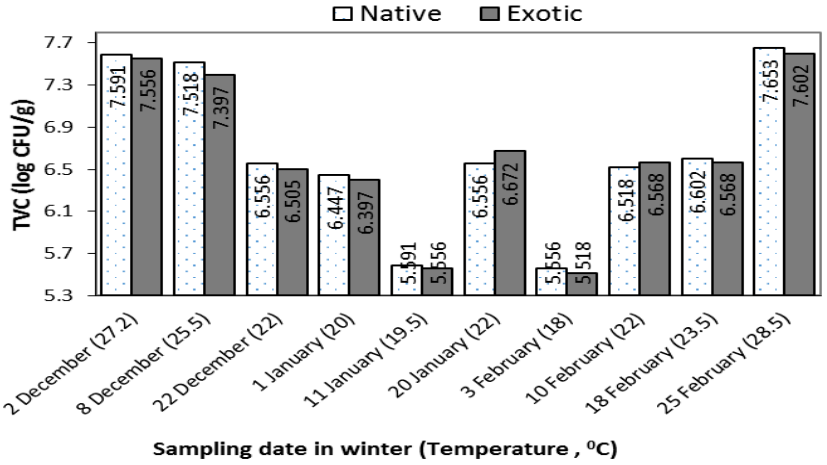

Fig. 2. Fluctuation of the bacterial load with ambient temperature in winter (December, 2014 to February, 2015) season for native and exotic climbing perch, *A. testudineus*.

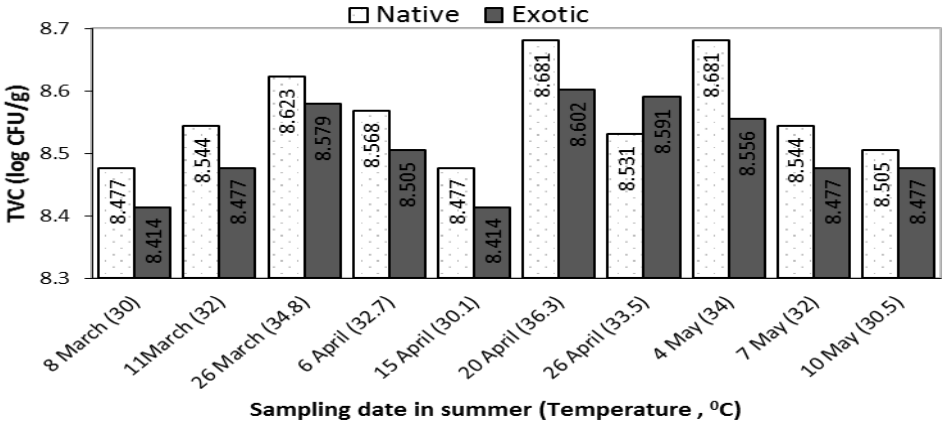

Fig. 3. Fluctuation of the bacterial load with ambient temperature in summer (March-May, 2015) season for native and exotic climbing perch, *A. testudineus*.

The significantly strong positive correlation between bacterial load in native climbing perch and temperature was evident (correlation co-efficient r = 0.949 and 0.937; $p<0.05$) during summer and winter season respectively. Significantly strong positive correlation between TVC in exotic *A. testudineus* and temperature were also evident (r = 0.970 and 0.946; $p<0.05$) in summer and winter season respectively. Although the TVC was positively correlated with ambient temperature in both varieties of climbing perch, *A. testudineus,* however, the comparatively higher bacterial load was occurred in native variety than in exotic variety of climbing perch during both seasons.

Discussion

In summary bacterial load was higher in summer than winter for both varieties of climbing perch. The present study was in line with Al-Harbi and Uddin (2007) who observed bacterial load in hybrid tilapia higher ($3.9\pm1.7\times106$ to $1.1\pm2.4\times107$CFU/g) in summer than ($1.9\pm2.0\times105$ in $1.2\pm2.9\times106$CFU/g) in winter. Al-Harbi and Uddin (2004) reported the seasonal variation of TVC in the intestine of tilapia ranging from ($6.8\pm1.9\times106$ to $7.5\pm1.4\times107$) CFU/g in early summer, ($1.6\pm2.0\times106$ to $5.1\pm2.5\times107$) CFU/g in summer and ($8.9\pm1.8\times105$ to $1.3\pm0.9\times107$) CFU/g in winter. Bisht *et al*. (2014) observed 10 times higher bacterial load in pond sediment (6.40 x 104) CFU/g compared to pond water (6.93 x 103CFU/ml); intestinal bacterial load 100 times higher (6.67 x105CFU/g) during the winter season and 1000 times higher (2.33 x106) CFU/g during summer season in comparison to the superficial skin of fish during winter and summer (3.39 and 8.87 x103) CFU/cm2 respectively. It is also known that the intestinal microbial flora (TVC) of fish changes in parallel with environmental changes as a result summer season contained higher bacterial load than winter (Hagi *et al*. 2004, Holben *et al*. 2002). So the present study is quite similar to all other study.

The bacterial load was found higher in summer than in winter because of high ambient temperature in the water body close to optimum for many mesophilic bacteria in natural systems (Rheinheimer 1985). Some study suggested that the bacterial load in fish might be increased with the rise of water temperature (Fernandes *et al*. 1997, Hossain *et al*. 1999). The changes of total viable counts depending on the changes in water temperature were confirmed that dominant *Lactococcus lactis* in the summer, when water temperatures were over 20^0C, and recessive *Lactococcus raffinolactis* growth confirmed at the temperature of 4-10^0C (Hagi *et al*. 2004). The present study also relates with the findings of Al-Harbi and Uddin (2004) who noted higher bacterial load at the (temperature ^{0}C$\pm$SD) (33.0 ± 2.3) in July and lower load (14.5 ± 1.5) in January. So the impact on bacterial load with temperature is very much evident.

From the present study, it can be concluded that native variety of climbing perch, *A. testudineus* contained higher bacterial load than the exotic variety. In comparison with seasons, the highest amount of bacterial load was found in summer whereas the

lowest was in winter in both varieties of climbing perch, *A. testudineus*. The highest bacterial load in the month of April (at 36.3^0C temperature) whereas lowest in February (at 18^0C) revealed that the seasonal variation of TVC is highly correlated to the temperature fluctuation in summer and winter. Although bacterial load in winter comply with ICMSF (International Commission on Microbiological Specification of Food) standard, however TVC in summer season didn't comply with ICMSF standard for both the varieties of Climbing perch, *A. testudineus*. Findings submit an urgent research and policy attention to avoid any further critical situation towards exporting of fishery products and microbial food safety issue in Bangladesh.

Acknowledgments. The research was supported by Ministry of Science and Technology of Bangladesh as a research fellowship of NST-2015 for their financial support. Thanks go to supportive member of the Laboratory of Microbiology and Immunology, Sylhet Agricultural University for their continuous help to complete the research.

Literature Cited

Ahmed, G.U., M.M. Hossain and M.M. Hassan, 2009b. Seasonal variation of disease and pathology of a perch, *Nandus nandus* (Hamilton) from oxbow-lake fisheries of Bangladesh. *Eco-Friendly Agril. J.*, *2*(8): 761-767.

Ahmed, G.U., M.M. Hossain and M.S. Hossain, 2009a. Histopathology of diseases of an air-breathing teleost, *Anabas testudineus* (Bloch) from freshwater fisheries of Bangladesh. *Int. J. Sustain. Agril. Tech.*, *5*(4): 75-81.

Ahmed, G.U., N. Akter, S.A. Nipa and M.M. Hossain, 2009c. Investigation on the health condition of a freshwater eel, *Monopterus cuchia* from Ailee beel, Mymensingh, Bangladesh. *J. Bangladesh Agril. Univ., 7*(2): 421-426.

Al-Harbi, A.H. and M.N. Uddin, 2004. Seasonal variation of bacterial flora in ponds in Saudi Arabia used for tilapia aquaculture. *J. Appl. Aquac.,* 16(1-2): 53-61.

Al-Harbi, A.H. and M.N. Uddin, 2007. Seasonal trends in gill bacterial flora of hybrid tilapia, *Oreochromis niloticus*×*O. aureus*. *J. Appl. Aquac.,* 19(1): 61-70.

Allen, D.A., B. Austin and R.R. Colwell, 1983. Numerical taxonomy of bacterial isolates associated with a freshwater fishery. *Microbiology,* 129(7): 2043-2062.

Bari, S. M., S.M.I. Khalil, M.A.A. Mamun, M.M. Hossain, S.S. Marine and M.M. Hossain. 2014. Seasonal variation and effects of Helminth infestation on *Clarias batrachus* (Linneaus, 1957) from haor basin of Sylhet region, Bangladesh. *J. Sylhet Agric. Univ.*, 1 (2): 231-237.

Bari, S.M., S.M.I. Khalil, M.A.A. Mamun, M.J. Islam, M.A. Baten and M.M. Hossain, 2015. Seasonal variation in population dynamics of Helminth parasites in *Clarias batrachus* from natural wetlands of Sylhet, Bangladesh. *Int. J. Nat. Sci.*, 5(2): 86-89.

Bisht, A., U.P. Singh and N.N. Pandey, 2014. Comparative study of seasonal variation in bacterial flora concomitant with farm raised fingerlings of *Cyprinus carpio* at tarai region of Uttarakhand. *J. Env. Biol.,* 35(2): 363-367.

Fernandes, C.F., G.J. Flick Jr, J.L. Silva and T.A. McCASKEy, 1997. Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. *J. Fish. Protec.,* 60(1): 54-58.

Hagi, T., D. Tanaka, Y. Iwamura and T. Hoshino, 2004. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. *Aquaculture,* 234(1-4): 335-346.

Hansen, G.H. and J.A. Olafsen, 1999. Bacterial interactions in early life stages of marine cold water fish. *Micro. Ecol., 38*(1): 1-26.

Holben, W.E., P. Williams, M. Saarinen, L.K. Särkilahti and J.H.A. Apajalahti, 2002. Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma phylotype* in farmed and wild salmon. *Micro. Ecol.,* 44(2): 175-185.

Hossain, M.M., 2013. Evaluating the performance of co-management organizations (CMOs) in sustainable benefits sharing in Tanguar haor, Bangladesh. *In*: M.G. Mustafa, N.A. Khan, A.F.M. Akhtaruzzaman, A.K. Harun and R.M. Chowdhury (eds.), Co-Managed and Climate Resilient Ecosystems. USAID's IPAC Project, IRG and the WorldFish, Bangladesh. 182-201.

Hossain, M.M., G.U. Ahmed, M.J. Ferdous, S.M.I. Khalil, S.M. Bari, M.M. Hassan and K. Gosh, 2014. Seasonal variation of clinicopathological changes of a farmed exotic carp *Hypophthalmichthys molitrix* from North-Eastern region, Bangladesh. *An. Vet. Anim. Sci.,*1 (3): 122-135.

Hossain, M.M., G.U. Ahmed, Z. Tazri and M.A. Haque and M.N. Akter, 2009a. Health status of a snakehead (*Channa punctatus*) of two fish markets in Mymensingh, Bangladesh. *Bangladesh. J. Fish. Res.,* 13(1): 11-17.

Hossain, M.M., G.U. Ahmed, Z. Tazri and M.A. Haque, 2009b. Clinical and pathological investigation of diseases in some small indigenous species (SIS) from fish markets of Mymensingh. *Int. J. Biol. Res*., *7*(3): 1-6.

Hossain, M.M., M.N. Uddin, M.N. Islam, S.C. Chakraborty and M. Kamal. 1999. Study on the intestinal bacteria of *Labeo rohita* (Ham.). *Bangladesh J. Fish. Res.,* 3(1): 63-66.

Hossain, M.T., G.U. Ahmed, M.M. Hassan, M.A. Kashem, M.M.M. Alam and M.M. Hossain, 2014. Health status of eels from open waters: a clinical and histopathological approach. *An. Vet. Anim. Sci.,* 1(1): 30-38.

Hovda, M.B., R. Fontanillas, C. McGurk, A. Obach and J.T. Rosnes, 2012. Seasonal variations in the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). *Aquac. Res*., 43(1): 154-159.

ICMSF (International Commission of Microbiological Specification for Food), 1986. Microorganisms in food (Vol. 2). Sampling for microbiological analysis: principles and specific applications. ICMSF, Univ. Toronto Press, Toronto, Canada.

ISO, 1995. Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6.The Netherlands.10-18.

Kashem, M.A., M.N. Uddin, M.M. Hossain, M.T. Hasan, S.A. Haque, M.N.A. Khan, M.M. Hassan and F.M.A. Hossain, 2014. Effect of Oxytetracycline on bacterial load of *Labeo rohita* (Rohu) fish in culture pond. *Glo. Adv. Res. J. Microbiol.,* 3(2): 18-24.

Laycock, R.A. and L.W. Regier, 1971. Trimethylamine-producing bacteria on haddock (*Melanogrammus aeglefinus*) fillets during refrigerated storage. *J. Fish. Res. Bd. Can.,* 28(3): 305-309.

Perez, O.M., L.G. Ross, T.C. Telfer, and L.M. del Campo Barquin, 2003. Water quality requirements for marine fish cage site selection in Tenerife (Canary Islands): predictive modelling and analysis using GIS. *Aquaculture,* 224(1-4): 51-68.

Rahman, M.S., M. Hasan, Z.H. Mahmud and M.S. Islam, 2010. Bacterial load in twelve freshwater fishes of four feeding habits in Bangladesh. *Dhaka Uni. J. Bio. Sci.,* 19(2): 145-150.

Rheinheimer, G., 1985. Aquatic Microbiology, 3rd ed. University of Kiel, West Germany. Wiley, Chichester, New York, Brisbane, Toronto, 257 p.

Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol Rev.*, 77(3): 591-625.

(Manuscript received 25 March 2018)