



## POST MORTEM VARIATION OF TOTAL VOLATILE BASE NITROGEN (TVB-N) AND TRIMETHYLAMINE NITROGEN (TMA-N) IN VETKI (*Lates calcarifer*)

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**Abstract:** Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) contents of Vetki (*Lates calcarifer*) were investigated following Conway's Microdiffusion technique at 4 hour interval in Fish Quality Control Laboratory, Fisheries and Marine Resource Technology Discipline, Khulna University. Observed TVB-N and TMA-N value did not vary significantly among different size classes. At fresh condition, TVB-N content was observed as 11.106±0.8 mg, 11.162±0.8 mg, and 11.162±0.8 mg in small, medium and large size classes of fish respectively and it increased to 59.087±0.8 mg, 68.417±1.5 mg and 75.967±2.7 mg for 100 g<sup>-1</sup> respectively at complete spoilage stage (after 28 hrs). At fresh condition, TMA-N content was 6.7±0.016 mg, 11.069±1.79 mg and 11.172±1.01 mg for 100 g<sup>-1</sup> in small, medium and large size classes of fish respectively and it increased to 48.893±3.01 mg, 52.492±0.089 mg and 57.717±1.95 mg for 100 g<sup>-1</sup> respectively at complete spoilage stage. TVB-N and TMA-N values started to increase gradually in all size groups with the passage of time but the rate of increase became faster after 20th hour and continued till the final stage was reached.

**Keywords:** Vetki (*Lates calcarifer*), total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N).

### Introduction

Vetki (*Lates calcarifer*) is an economically important fish with catadromous habits within its natural distribution. Market demand and price of this fish vary with freshness of the fish. It is also assumed that duration of freshness might have relation with the size of the fish. Trimethylamine nitrogen (TMA-N) and Total Volatile Base nitrogen (TVB-N) concentrations are used for the immediate determination of freshness (Botta 1995). Several chemical tests indirectly related to bacterial activity have been often employed for assessing freshness or levels of spoilage in fish. The proposed tests have been used to establish quantities of different spoilage compounds. According to Howgate (1982) only three have stood the test of time as reliable i.e. determination of trimethylamine (TMA), total volatile bases (TVB) and hypoxanthine (Hx). TMA is a nitrogenous volatile base formed by the reduction of trimethylamine oxide (TMAO) by certain species of aerobic bacteria, which utilize the oxygen in anaerobic conditions. In other words, TMAO acts as the terminal electron acceptor (Huss *et al.* 1997). It has also been hypothesized that some intrinsic enzymes may be partly responsible for its production (Connell, 1990). TMA is only produced in fish that have adequate amounts of TMAO (Howgate, 1982). In view of the above, the present study was undertaken at ambient temperature to observe whether any significant variation occurs in TVB-N and TMA-N contents in different sizes of Vetki.

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## Materials and Methods

**Collection and preparation of sample:** Live Vetki belonging to 3 different size categories (Small: <250g, Medium: 250-500g and Large: >500g) were collected from a local fish farm and immediately brought to the Quality Control Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University. The samples were kept at ambient temperature (20-22°C) in the laboratory up to complete spoilage. TVB-N and TMA-N were measured at 4-hour intervals. Each measurement was taken in triplicate.

**Technique for analysis of sample:** TVB-N (Total volatile base nitrogen) and TMA-N (Tri methyl amine nitrogen) were determined according to the procedure stated in the manual of Siang and Kim (1992).

**Extract preparation:** The extract was prepared by mixing 2 gm of the minced / grinded fish muscle with 8 ml of 4% TCA (Trichloroacetic Acid) in a Mackerty bottle and was homogenized properly. It was left for 30 minutes at ambient temperature with stirring. Then it was filtered through a filter paper. The filtered solution was kept in Mackerty bottle and was labeled. The filtered solution was also stored in a refrigerator at -20°C (to prevent any further chemical, bacterial or enzymatic breakdown of the muscle).

**Moisture determination:** The percentage of moisture of the fish sample was estimated according to the manual of AOAC (Anon, 1988).

**TVB-N determination:** Three Conway's units were taken which were thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer rim of each unit was applied sealing agent (Vaseline) was applied. Using a micropipette, 1 ml of inner ring solution was pipetted into the inner ring of each unit. Into the outer ring of each unit, 1 ml of the sample extract was pipetted. 1 ml of saturated K<sub>2</sub>CO<sub>3</sub> solution was carefully pipetted into the outer ring of each unit, carefully to prevent any entering the inner ring, and immediately the units were covered and closed with clips. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. Then, the units were placed in an incubator at 37°C for 60 minutes. Units' covers were removed and green colored inner ring solution was titrated with 0.02N HCl using a burette (50 ml) until the solution became pink. Average titrate volume of HCl was found from the results of three titrations for each fish species muscle sample. For each value TVB-N values were calculated. A blank test was also carried out using 1 ml of 1% TCA, instead of sample extract.

**TMA-N determination:** Trimethylamine nitrogen in fish muscle was determined by the Conway technique, which is same as TVB-N determination but prior to addition of potassium carbonate, 1 ml of 10% neutralized formalin was pipetted to the extract to react with ammonia and thus allow only the TMA-N to diffuse over the unit. The calculation was done by the same formula as used in the Conway micro-diffusion technique for TVB-N.

**Data Analysis:** Data were analyzed by using SPSS 12.0 and Microsoft Excel. Multiple comparison and ANOVA were as done for testing significance of variation

## Results

**Variation in TVB-N in different size groups:** TVB-N content in different size groups of Vetki are shown in Fig. 1. The relationship between TVB-N and time are given in Fig. 2, 3 and 4, which indicate that the relationship is linear i.e., TVB-N content increased with time in all the size groups. However, TVB-N contents did not vary amongst different size groups.

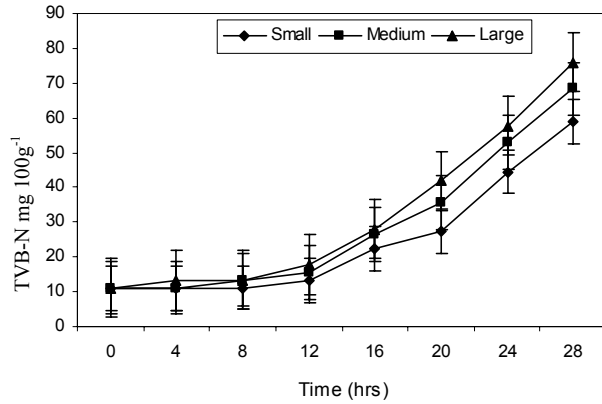


Fig. 1. Changes in TVB- N with time at different size.

TVB-N (Small) = 1.65 + 1.67 \* Time  
R-Square = 0.83

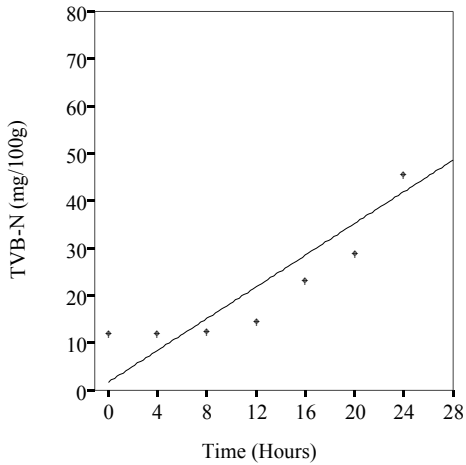


Fig. 2. Regression curve of TVB-N with time (Small)

TVB-N (Medium) = 0.70 + 2.05 \* Time  
R-Square = 0.87

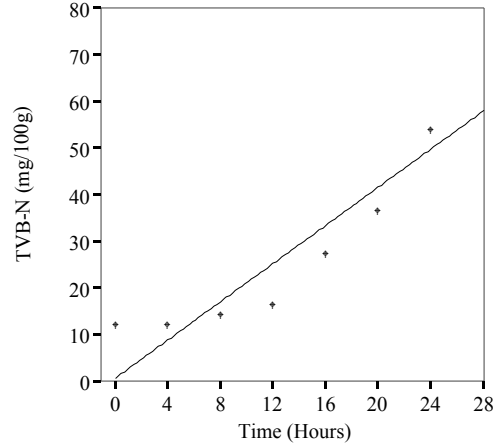


Fig. 3. Regression curve of TVB-N with time (Medium)

TVB-N (Large) = 0.24 + 2.30 \* Time  
R-Square = 0.88

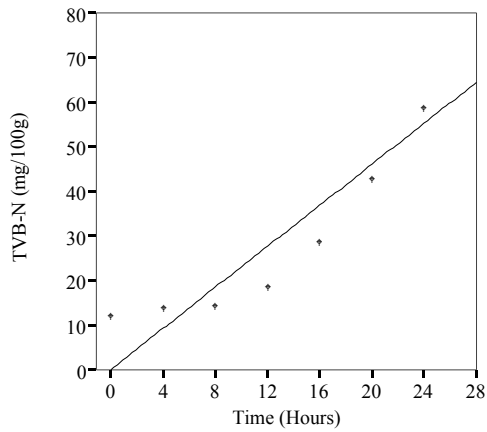


Fig. 4. Regression curve of TVB-N with time (Large)

It is evident from table 1 that TVB-N contents were very similar for all categories of fish at very fresh condition. But as the time progressed, TVB-N value of medium and large fishes increased at a slightly rate faster than that of the small fishes. TVB-N content varied slightly amongst different size groups but it was not statistically significant at 5 % level of significant.

**Table.1** Changes in TVB - N content with time in different size

	0 hrs	4 hrs	8 hrs	12 hrs	16 hrs	20 hrs	24 hrs	28 hrs
Small	11.106±0.8	11.114±0.76	11.181±0.7	13.402±0.03	22.213±2.7	27.544±0.7	44.529±2.8	59.087±0.8
Medium	11.162±0.8	11.181±0.7	13.346±0.06	15.587±0.7	26.525±1.1	35.66±3	53.004±2.2	68.417±1.5
Large	11.162±0.8	13.290±0.2	13.346±0.06	17.826±3.1	7.9362±0.3	41.976±3.3	57.754±3.8	75.967±2.7

**Variation in TMA-N in different size groups:**

TMA-N content in different size groups of Vetki are shown in Fig. 5. The relationship between TMA-N and time are given in Fig. 6, 7 and 8, which indicate that the relationship is linear and TMA-N content increased with time in all the size groups. Although there was a slight variation in TMA-N contents amongst different size groups but the variation is not statistically significant at 5% level of significant.

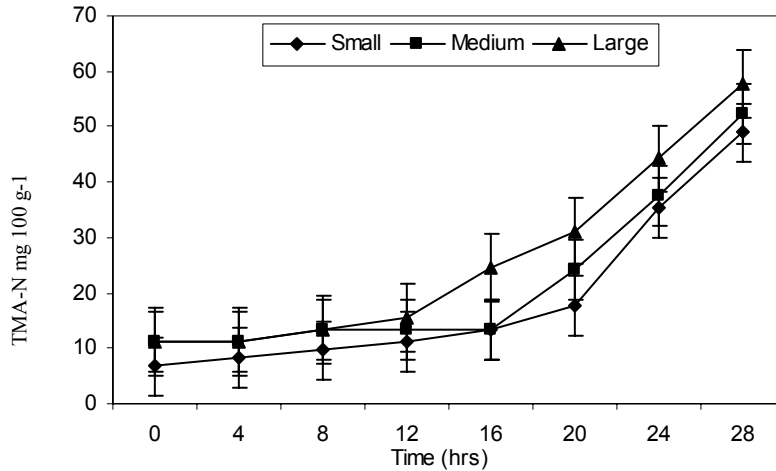


Fig. 5. Changes in TMA-N content with time in different size

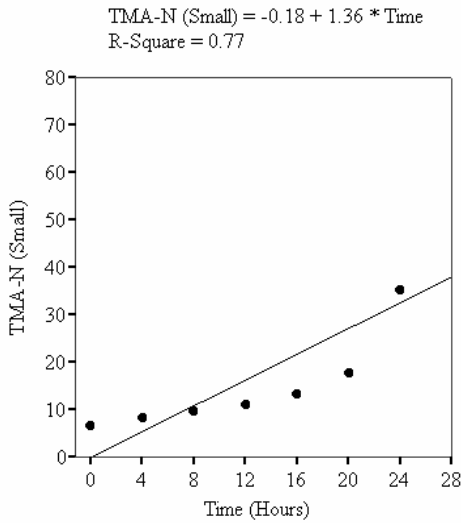


Fig. 6. Regression curve of TMA-N with time (Small)

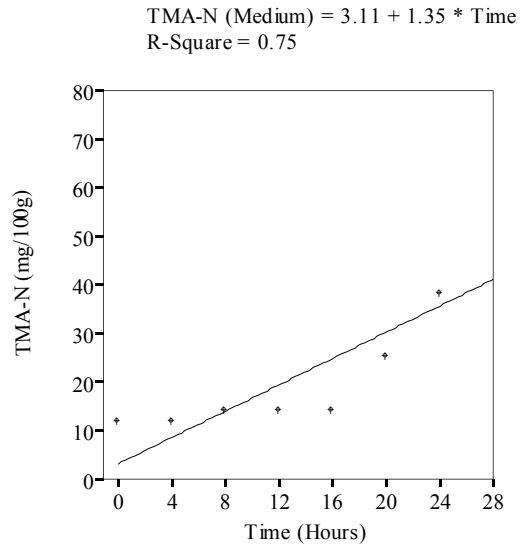


Fig. 7. Regression curve of TMA-N with time (Medium)

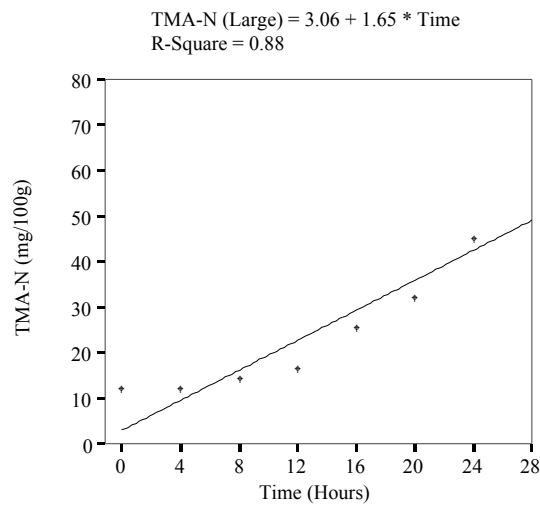


Fig. 8. Regression curve of TMA-N with time (Large)

It is evident from table 2 that at the initial stage when the fish was in highly fresh condition, TMA-N values of fishes changed slowly. But as the time progressed, it increased. At the initial stage, the values in small fishes were less than that of the medium and large size groups.

**Table-2** Changes in TMA – N content with time in different size.

	0 hrs	4 hrs	8 hrs	12 hrs	16 hrs	20 hrs	24 hrs	28 hrs
Small	6.7±0.016	8.21±0.38	9.599±0.41	11.172±1.01	13.346±0.08	17.789±2.06	35.347±1.99	48.893±3.01
Medium	11.069±1.9	11.136±0.74	13.290±2.77	13.330±2.35	13.346±0.06	24.3087±2.98	37.562±2.42	52.492±0.89
Large	11.172±1.1	11.190±1.02	13.365±0.056	15.596±0.75	24.509±2.64	31.200±0.94	44.240±3.96	57.717±1.95

### Discussion

Increase in TVB-N in fishes with time, as observed in the present study, is in agreement with the findings of Curran *et al.* (1981), Jenson *et al.* (1979) Poulter *et al.* (1978) and Nair *et al.* (1971). Bandyopadhyay *et al.* (1986) found TVB-N 13.55 mg 100 g<sup>-1</sup> and 15.65 mg/100 g<sup>-1</sup> in *Catla catla* and *Labeo fimbriatus* respectively which are a bit different from the present findings. This might be due to the variation in species. Haque *et al.* (2008) reported TVB-N values from the range 3.95±0.31 mg to 47.84±0.63 mg 100 g<sup>-1</sup> in *Liza parsia* which is approximately similar to the present findings. The use of TMA-N as an index of fish freshness (Gibbons, 1936) based on the observation that the production of TMA depends on the bacterial activity as well as on endogenous enzyme. Under the local conditions, TMA was also found to be a good indicator of freshness for white Pomfret, Chinese Pomfret and Grouper (Siang and Kim, 1992) as has been found in the present work.

### Conclusion

TVB-N and TMA-N are good indicators of freshness for Vetki. However, titrimetric method was used for TVB-N and TMA-N analysis, so there was a chance of being error during titration. Moreover, the laboratory condition was not controlled, so different experiments had to be conducted under different environmental conditions. Therefore, further study with larger number of variables in controlled environment is suggested.

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