

# Muscle Protein, Carbohydrate and Lipid in Nile Tilapia (*Oreochromis niloticus*) from an Urban Harbour and a Rural Wetland in Kerala, India: A Two-Site Comparison

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**Abstract**—Tissue biochemical composition can provide a low-cost measure of differences among fish sampled from contrasting environments. This study compared water-extractable protein, carbohydrate and lipid in the muscle of Nile tilapia, *Oreochromis niloticus*, obtained from an urban harbour at Kalamukku, Kochi, and a rural wetland at Mepral, Thiruvalla, Kerala, India. Five fish were analysed from each site. A 0.5 g muscle sample was homogenised in 10 ml water and centrifuged at 7000 rpm. Protein was estimated by the Lowry method using bovine serum albumin as the standard, carbohydrate by the anthrone method using glucose as the standard, and lipid by the ferric chloride-sulphuric acid reaction using cholesterol as the standard. Absorbance was measured colorimetrically at 630 nm for protein and carbohydrate and at 540 nm for lipid. Mean values were compared between sites by one-way analysis of variance. Rural fish showed higher extractable protein ( $31.60 \pm 1.85$  mg/100 ml) and carbohydrate ( $3.20 \pm 0.65$  mg/100 ml) than urban fish ( $21.92 \pm 1.52$  and  $2.00 \pm 0.63$  mg/100 ml, respectively), whereas lipid was higher in urban fish ( $7.39 \pm 1.09$  mg/100 ml) than rural fish ( $4.27 \pm 1.05$  mg/100 ml). Protein and lipid differences were statistically strong, while the carbohydrate difference was weaker after adjustment for three comparisons. Because the study included one site in each habitat category and did not measure water chemistry, contaminants, fish size, sex or reproductive condition, the observed differences cannot be attributed specifically to pollution. The findings provide a preliminary biochemical comparison and identify the need for replicated sites and concurrent environmental measurements in future studies.

**Index Terms**—*Oreochromis niloticus*, fish biochemistry, muscle composition, urban-rural comparison, environmental stress, Kerala.

## I. INTRODUCTION

Freshwater and coastal aquatic systems near expanding urban settlements receive a range of anthropogenic inputs, including domestic sewage,

industrial discharges, agricultural runoff and storm-water. Such inputs can modify the physical and chemical conditions of aquatic habitats and can impose physiological demands on resident organisms. Anthropogenic pollution is now recognised as a major pressure on aquatic ecosystems, with sewage, nutrients, heavy metals, oil and other contaminants affecting freshwater and marine environments (Häder et al., 2020). Fish are particularly useful in environmental assessment because they integrate exposure over time and can respond through biochemical, physiological and tissue-level changes (van der Oost et al., 2003).

Protein, carbohydrate and lipid are important biochemical constituents of fish tissues and are closely related to energy storage, growth and maintenance. Stress can alter the allocation and mobilisation of energy substrates. The fish stress response includes mobilisation of energy reserves and redistribution of energy away from growth and reproduction (Wendelaar Bonga, 1997). However, biochemical responses do not necessarily follow a single direction under all forms of environmental stress. The magnitude and direction of change depend on the nature and duration of the stressor, tissue examined, nutritional condition and other biological factors.

The biochemical composition of fish is also influenced by factors unrelated to pollution. Body size, diet, season and other endogenous and environmental variables can affect protein and lipid deposition (Shearer, 1994). In Nile tilapia, salinity and the history of environmental exposure can also influence physiological performance (Watanabe et al., 1985). Therefore, comparisons between fish from contrasting

habitats should distinguish observed differences from causal claims about contamination.

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is widely cultured and is tolerant of a broad range of environmental conditions. Its availability from local fishers makes it a practical species for comparative biochemical studies. Previous work has shown that environmental stress and contaminated water can be associated with changes in carbohydrate, protein and lipid profiles in fish (Javed & Usmani, 2015; Javed et al., 2017).

The present study therefore compared three biochemical fractions—protein, carbohydrate and lipid—in muscle extracts of *O. niloticus* obtained from an urban harbour and a rural wetland in Kerala. The specific objective was to determine whether the biochemical profiles of fish from the two sampling locations differed. The study was designed as a preliminary two-site comparison; it was not intended to identify a particular pollutant or to establish a causal effect of pollution.

## II. MATERIALS AND METHODS

### A. Study Sites

Two sampling locations in Kerala were considered. Kalamukku Harbour is a coastal fishing harbour in Ernakulam District near Kochi, on the Arabian Sea coast (approximately 9°59'1" N, 76°14'32" E). Mepral is a wetland locality in Peringara Panchayat, Thiruvalla taluk, Pathanamthitta District (approximately 9°23'58" N, 76°31'48" E). The former was classified as the urban site and the latter as the rural site on the basis of their setting. No physicochemical or contaminant measurements of the source waters were made during this study.

### B. Fish Collection and Sample Handling

*Oreochromis niloticus* specimens were obtained directly from local fishers at Kochi and Thiruvalla. The fish were placed in an ice box and transported to the laboratory. On arrival, the specimens were washed with tap water before tissue sampling. Five fish from each site were included in the biochemical comparison, giving a total sample size of 10 fish.

A 0.5 g portion of muscle tissue was removed and homogenised with 10 ml of water using a mortar and pestle. The homogenate was centrifuged at 7000 rpm and the resulting supernatant was used for the

colorimetric assays. The original project record did not specify the centrifugation duration, exact anatomical muscle location, fish sex, fish size or reproductive stage; these limitations are considered in the interpretation.

### C. Biochemical Assays

Protein was estimated by the Lowry method (Lowry et al., 1951), using bovine serum albumin as the reference standard. Carbohydrate was estimated by the anthrone method (Roe, 1955), using glucose as the standard. Lipid was estimated using a ferric chloride–sulphuric acid reaction with cholesterol as the standard (Zlatkis et al., 1953). Standard series and reagent blanks were prepared for each assay. Absorbance was recorded at 630 nm for protein and carbohydrate and at 540 nm for lipid.

The biochemical values are reported as mg/100 ml of extract. Because the tissue was extracted with water rather than subjected to a standard whole-muscle proximate analysis, the reported values are treated as concentrations in the prepared extract and are used primarily for relative comparison between the two groups. They should not be interpreted as total muscle protein or total muscle lipid on a wet-weight basis.

### D. Statistical Analysis

Results are presented as mean  $\pm$  standard deviation (SD). Protein, carbohydrate and lipid were analysed separately with site (urban versus rural) as the grouping factor. With two groups, one-way ANOVA is mathematically equivalent to an independent-samples t-test. The reported F statistic, degrees of freedom and P value were calculated from the group means, SDs and  $n = 5$  per site. Statistical significance was assessed at  $P < 0.05$ . Because three biochemical variables were tested, a Bonferroni-adjusted threshold of  $P < 0.017$  was also considered. The small sample size limits the power of formal assumption checks; therefore, the results are interpreted cautiously.

## III. RESULTS

### A. Biochemical Composition

The muscle extracts of *O. niloticus* differed between the two sampling locations for all three measured biochemical fractions. Protein was  $21.92 \pm 1.52$  mg/100 ml in the urban group and  $31.60 \pm 1.85$

mg/100 ml in the rural group. The rural mean was approximately 44% higher than the urban mean.

Carbohydrate was  $2.00 \pm 0.63$  mg/100 ml in the urban group and  $3.20 \pm 0.65$  mg/100 ml in the rural group, corresponding to an approximately 60% higher mean in the rural group. Lipid showed the opposite pattern: the urban group had  $7.39 \pm 1.09$  mg/100 ml compared with  $4.27 \pm 1.05$  mg/100 ml in the rural group, making the rural value approximately 42% lower.

The one-way ANOVA gave  $F(1,8) = 81.7$ ,  $P < 0.001$  for protein;  $F(1,8) = 8.8$ ,  $P = 0.018$  for carbohydrate; and  $F(1,8) = 21.2$ ,  $P = 0.002$  for lipid. Thus, protein and lipid remained statistically significant after the Bonferroni-adjusted threshold of  $P < 0.017$ , whereas carbohydrate was just above that threshold.

The numerical results are summarised in Table 1. The graphical comparison in Fig. 2 shows the direction and magnitude of the differences, with error bars representing SD.

#### IV. DISCUSSION

The present comparison showed a distinct biochemical pattern between tilapia obtained from Kalamukku Harbour and Mepral. Rural fish had higher extractable protein and carbohydrate, whereas urban fish had higher extractable lipid. Similar changes in biochemical profiles have been reported in fish exposed to environmental stress, including alterations in carbohydrate, protein and lipid metabolism (Javed & Usmani, 2015). Fish exposed to heavy-metal-loaded wastewater have also shown multiple biochemical responses, together with oxidative stress and other biological effects (Javed et al., 2017).

The lower protein and carbohydrate values observed in the urban sample could be consistent with increased energetic demand if the fish were experiencing environmental stress. Under stress, fish can mobilise energy substrates to support maintenance and adaptation (Wendelaar Bonga, 1997). However, the higher lipid value in the urban sample does not support a simple interpretation of general depletion of energy reserves. Differences in diet, habitat, body condition or other physiological factors could contribute to the observed lipid pattern.

An important limitation is that Kalamukku and Mepral represent different environmental settings as well as different geographic and hydrological conditions. Kalamukku is a coastal harbour, whereas Mepral is an

inland wetland. Salinity, food availability and other habitat characteristics can influence fish physiology; salinity in particular is an important environmental factor for Nile tilapia (Watanabe et al., 1985). Fish size, sex, reproductive condition, season and nutritional history were not recorded, although these factors can affect tissue composition (Shearer, 1994).

The experimental design also limits the level at which the results can be generalised. There was one sampling site representing each habitat category, with five fish sampled within each site. Thus, the five fish within a site are subsamples from the same location rather than independent replicates of an urban or rural population. The statistical tests establish that the measured fish samples differed, but they do not establish that urban habitats as a class differ from rural habitats, nor do they demonstrate a pollution effect.

The biochemical extraction procedure imposes an additional interpretive limitation. Water extraction of 0.5 g tissue with 10 ml water does not constitute a standard determination of total muscle protein or total lipid. The reported concentrations therefore describe the measured extract fractions rather than complete proximate composition. The lipid assay was calibrated against cholesterol, and the response should consequently not be interpreted as a direct measurement of total tissue lipid.

Taken together, the findings are best regarded as a preliminary biochemical comparison. A stronger environmental assessment would include several independent urban and rural sites, fish matched for size and sex, sampling in the same season, and measurements of pH, temperature, dissolved oxygen, conductivity or salinity and other relevant water-quality variables. Analysis of contaminants in water, sediment or fish tissue would be necessary before the biochemical differences could be linked specifically to pollution.

#### V. CONCLUSION

Muscle extracts of *Oreochromis niloticus* from the rural wetland at Mepral contained higher measured protein and carbohydrate concentrations, while extracts from the urban harbour at Kalamukku contained higher measured lipid concentrations. Protein and lipid showed the strongest statistical evidence of a difference between the two samples. The findings provide a useful preliminary comparison of

fish biochemical composition between two contrasting locations in Kerala, but they do not establish that pollution caused the differences. Future work should use replicated sites, record fish size, sex and season, standardise sample handling, measure water quality and analyse relevant contaminants so that biochemical responses can be related to environmental exposure.

## VI. DECLARATIONS

**Ethical statement:** No live-animal exposure experiment or experimental manipulation was conducted. Fish specimens were obtained from local fishers and processed for biochemical analysis. The authors should ensure that this statement is consistent with the institutional requirements applicable to the study.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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**Data availability:** The individual assay observations underlying the reported means and standard deviations should be retained by the authors and can be made available by the corresponding author on reasonable request.

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cholesterol. Journal of Laboratory and Clinical Medicine, 41(3), 486–492.

#### TABLES AND FIGURES

| Variable     | Urban (Kochi) | Rural (Thiruvalla) | Rural – urban | Difference (%) | F(1,8) | P      |
|--------------|---------------|--------------------|---------------|----------------|--------|--------|
| Protein      | 21.92 ± 1.52  | 31.60 ± 1.85       | +9.68         | +44.2          | 81.7   | <0.001 |
| Carbohydrate | 2.00 ± 0.63   | 3.20 ± 0.65        | +1.20         | +60.0          | 8.8    | 0.018  |
| Lipid        | 7.39 ± 1.09   | 4.27 ± 1.05        | -3.12         | -42.2          | 21.2   | 0.002  |

Table 1. Muscle protein, carbohydrate and lipid in *O. niloticus* extracts (mg/100 ml), with one-way ANOVA results. Values are mean ± SD; n = 5 fish per site.

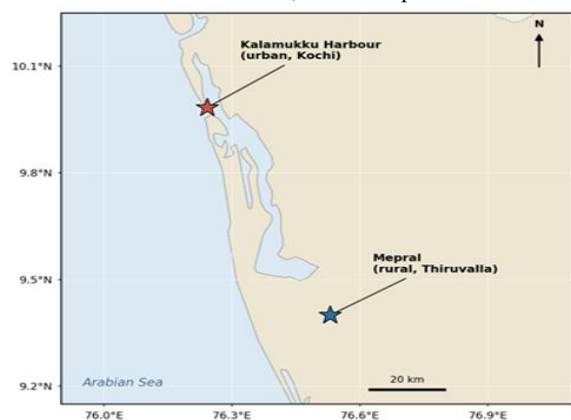


Fig. 1. Location of the two sampling sites: Kalamukku Harbour, Kochi (urban) and Mepral, Thiruvalla (rural), Kerala, India.

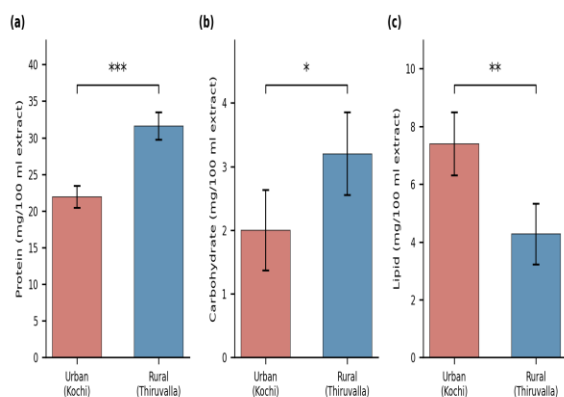


Fig. 2. Protein, carbohydrate and lipid concentrations in muscle extracts of *O. niloticus* from the urban and

rural sites. Bars show means and error bars show SD (n = 5 per site). Significance markers correspond to the reported ANOVA comparisons: \*\*\* P < 0.001, \*\* P < 0.01, \* P < 0.05.