

Biochemical Composition and Nutritional Value of Three Species of Hillstream Fish belonging to the Genus *Garra* from North-Eastern India

A K BHAGOWATI and B K RATHA

Biochemical Adaptation Laboratory, Department of Zoology,
School of Life Sciences, North-Eastern Hill University,
Shillong 793014 (India)

(Received 20 September 1981)

The biochemical constituents like total protein, free amino acids, DNA, RNA and phosphates were estimated in muscle, liver, kidney, brain and gill of three species of hillstream fish belonging to the genus *Garra* (*G. gotyla gotyla*; *G. annandalei*; *G. lissorhynchus*). The fishes were collected from different localities and showed tissue-specific variations in their constituents. Nutritionally, these fishes are as good as other important fishes. These wild fishes have served the people of this hilly region as a good source of protein nutrition and may be exploited for commercial purposes.

Key Words: Biochemical constituents; *Garra* species; Adaptational and nutritional value

Introduction

Fishes are one of the major sources of protein nutrition for human beings. Therefore, efforts are being made all over the world to exploit both the marine and freshwater bodies for fish production. Besides culturable fishes, the wild fishes also provide a major bulk of fish protein. Hence, studies on different sources and nutritive values of wild fishes are necessary. North-Eastern part of India is particularly rich in hill streams whose major fish fauna consists of the *Garra* species. There are about eight species of genus *Garra* reported till now in these

hill streams (Menon 1974 and Majhi 1980). Some of them occur in large numbers and grow to a moderate size. They have been serving as a staple food for the people of this region. Some reports on the biology and ecology have appeared on a few species only (Hora & Mukerji 1936, Hora 1937, Fraser 1937, Jones 1941, Agrawal & Tyagi 1969, Somvanshi 1976, 1980 and Somvanshi & Bapat 1979). However, no information is available on the biochemical composition of these fishes to assess their nutritional values.

The present report deals with some preliminary investigations on some biochemical constituents like the total protein, free amino-acids, DNA, RNA and phosphates in different tissues of the three species of *Garra* (*G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*) from North-Eastern India.

Materials and Methods

Fish

G. gotyla gotyla (length 11–14 cm; wt. 16–19 g) and *G. annandalei* (length 9–10.5 cm; wt. 13–16 g) were collected during April 1979 from Pagladia river, a torrential stream at Uttarkuchi (26°51'N and 91°25'E), Assam, at an altitude of about 265 m. *G. lissorhynchus* (length 6–7.5 cm; wt. 8–10 g) were collected during May, 1979 from Umkhras stream at Shillong (25°34'N and 91°56'E), Meghalaya, at an altitude of about 1400 m. The fishes were collected between 7–10 A.M. when the water temperature was 17–18°C in both the places. The sexes could not be distinguished in these fishes because the gonads were not developed and there was no other morphological difference. The tissues (muscle, liver, kidney, brain and gill) were immediately removed after collection on the spot and were transported to the laboratory in ice. They were kept at –15°C till the analyses were made. All the estimations were done within a week of collection. Some tissues like brain and kidney were very small in size and therefore, tissues from 2–3 fishes were pooled for each set. Six–seven sets of estimations were done from 12–15 fishes.

Analytical procedures

A 10% homogenate was made for each sample with ice-cold 0.012M Tris-HCl buffer (pH 7.4). The tissue fractionation

was done following Schneider (1957) for estimations of different biochemical constituents. The cold acid-soluble fraction was used for free amino acid and phosphate estimation and the final residue after nucleic acid extraction was dissolved in 1N KOH for protein estimation. The DNA and RNA were estimated in the hot TCA soluble nucleic acid extract.

Total free amino acid was estimated colorimetrically following the ninhydrin method of Spies (1957), using glycine as standard. Inorganic, labile, bound and total phosphates were estimated by the method of Fiske and Subbarow (1925) with KH_2PO_4 as standard. DNA and RNA were estimated by diphenylamine and orcinol reagents respectively following Schneider (1957), using calf thymus DNA and yeast RNA as standards. Protein was estimated by Folin-Ciocalteu reagent (Lowry et al. 1951) with bovine serum albumin as standard.

All chemicals and biochemicals were of analytical grade and were obtained from either Sigma Chemical Co., USA or Glaxo Laboratories, India. The data were expressed as mg/g wet wt of tissue.

Results

The concentrations of various chemical components studied have been expressed in mg/g wet wt of muscle, liver, kidney, brain and gill of *G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*. The concentrations of total protein and free amino acids have been shown in table 1, DNA and RNA in table 2 and the phosphates in table 3. There have been variations in the concentrations of different components in different tissues. However, in general, there were no significant interspecific differences among the three species studied for a specific component in a particular tissue except for DNA.

Table 1 Tissue protein (P) and free amino acid (FAA) contents* (mg/g wet wt) in muscle, liver, kidney, brain and gill of *G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*

	Muscle		Liver		Kidney		Brain		Gill	
	P	FAA	P	FAA	P	FAA	P	FAA	P	FAA
<i>G. gotyla gotyla</i>	168.8 ±14.19	0.975 ±0.019	150.33 ±24.26	1.785 ±0.034	109.45 ±8.02	1.37 ±0.04	62.28 ±6.34	0.677 ±0.802	38.37 ±4.04	0.49 ±0.025
<i>G. annandalei</i>	166.3 ±14.56	0.93 ±0.13	151.73 ±14.01	1.6 ±0.018	106.56 ±7.56	1.12 ±0.02	61.41 ±5.59	0.625 ±0.02	37.39 ±5.06	0.32 ±0.01
<i>G. lissorhynchus</i>	164.89 ±15.38	0.908 ±0.044	149.45 ±13.96	1.48 ±0.24	107.5 ±7.05	1.03 ±0.19	51.73 ±5.89	0.542 ±0.015	37.16 ±5.11	0.265 ±0.036

*Values are mean ± standard deviation.

Table 2 DNA and RNA contents (mg/g wet wt.) in muscle, liver, kidney, brain and gill of *G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*

	Muscle		Liver		Kidney		Brain		Gill	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
<i>G. gotyla gotyla</i>	0.983 ±0.05	1.81 ±0.13	0.718 ±0.013	2.36 ±0.07	0.851 ±0.01	1.86 ±0.14	1.07 ±0.02	1.64 ±0.025	0.789 ±0.014	1.399 ±0.127
<i>G. annandalei</i>	1.017 ±0.01	1.79 ±0.12	0.729 ±0.03	2.37 ±0.025	0.867 ±0.05	1.85 ±0.07	1.112 ±0.011	1.72 ±0.019	0.808 ±0.03	1.59 ±0.16
<i>G. lissorhynchus</i>	1.04 ±0.014	1.81 ±0.14	0.795 ±0.016	2.48 ±0.035	0.91 ±0.01	1.87 ±0.04	1.16 ±0.019	1.72 ±0.03	0.823 ±0.019	1.55 ±0.19

Table 3 Inorganic (Pi), labile (Pl), bound (Pb) and total phosphate (Pt) contents* (mg/g wet wt.) in muscle, liver, kidney, brain and gill of *G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*

	Muscle	Liver	Kidney	Brain	Gill
<i>G. gotyla gotyla</i>					
Pi	30.0 ±4.0	45.0 ±5.0	37.33 ±7.64	25.66 ±4.1	63.0 ±14.36
Pl	4.0 ±0.5	7.33 ±1.18	4.0 ±0.5	3.33 ±0.95	3.66 ±1.07
Pb	27.33 ±4.5	24.66 ±12.9	18.0 ±8.5	16.0 ±5.6	20.67 ±7.25
Pt	61.33 ±13.06	77.0 ±13.42	59.33 ±6.42	44.99 ±11.0	87.33 ±21.52
<i>G. annandalei</i>					
Pi	29.66 ±4.22	43.0 ±12.65	36.66 ±4.73	22.33 ±2.13	66.0 ±14.0
Pl	2.83 ±0.6	7.5 ±0.5	3.83 ±1.27	5.17 ±2.24	2.16 ±0.79
Pb	29.18 ±5.4	20.5 ±6.0	17.83 ±6.9	16.5 ±2.2	15.5 ±5.54
Pt	61.66 ±12.0	73.0 ±8.0	58.33 ±9.53	44.0 ±4.36	83.66 ±18.03
<i>G. lissorhynchus</i>					
Pi	33.6 ±4.73	47.0 ±8.54	39.0 ±14.35	25.0 ±4.0	70.0 ±10.0
Pl	3.5 ±1.0	6.0 ±0.59	3.83 ±0.61	3.83 ±0.79	1.66 ±0.79
Pb	23.5 ±5.5	17.0 ±8.0	11.83 ±6.5	19.83 ±6.9	7.67 ±2.26
Pt	60.23 ±13.24	70.0 ±14.35	54.66 ±9.56	48.66 ± 5.6	79.33 ±9.65

*Values are mean ± standard deviation

Among the tissues, protein concentration was highest in muscle followed by liver, kidney, brain and gill. Free amino-acids were maximum in liver followed by kidney, muscle, brain and gill. DNA level, in general, was maximum in *G. lissorhynchus* and minimum in *G. gotyla gotyla*. However, these differences between the species were not significant. The tissue distribution of DNA was in the order of brain > muscle > kidney >

gill > liver. The concentration of RNA was higher in liver and there were not much variations among the other four tissues studied. The tissue distribution of different phosphates was as follows: total and inorganic phosphates: gill > liver > kidney > muscle > brain; labile phosphate; liver > kidney > muscle > brain > gill; and bound phosphate showed highest level in muscle and no definite order in other tissues.

Discussion

The wild fishes constitute one of the major sources of cheap nutrition for the rural population. The nutritional value of different fishes depends on their biochemical compositions like protein, amino-acids, vitamins, mineral contents, etc. The protein concentrations in different tissues of the three species of *Garra* studied by us are well comparable with those of many commercially important fishes (Zaitsev et al. 1969 and Ogino & Takeda 1978). Thus, in spite of their relatively smaller sizes, these wild *Garra* species have served the hill people of this region as a good source of protein nutrition and could be exploited commercially.

The type of tissue distribution of proteins and free amino acids observed in this study, might be due to the structural and physiological differences among the tissues. Muscle contains a large amount of structural proteins with low turnover rate whereas liver and kidney are highly active metabolic tissues rich in functional proteins with higher turnover rates. Hence, these tissues had higher concentrations of protein. The total free amino-acid level in these species seems to be lower than those reported in carp muscles (Yudaev 1950 and Siddiqui et al. 1973). This indicates that the amino-acid pool in these fishes might have been depleted for either a higher rate of protein synthesis or used for energy production due to poor feeding.

DNA content of a tissue might indicate the cell number (Goss 1966), as the DNA content per cell is a constant factor for a given species. It has been earlier reported that these three species of *Garra* have same number of chromosomes ($2N=50$) (Majhi 1980). The DNA concentrations per unit wet weight of tissues are different due to their variable cell sizes. Such

tissue-specific variations have been reported earlier in other fishes (Love 1970, Jafri & Mustafa 1976 and Mustafa 1977).

The level of RNA reflects the rate of metabolic activity of a tissue (Leslie 1955, Bulow 1970). The RNA content as studied by us shows a higher level than that of carp muscle RNA concentration previously reported by Mustafa (1977). This may be an indication of higher rate of metabolism in these fishes. The metabolically active tissues like liver, kidney and muscle have also more RNA than other tissues.

The total phosphate content in tissues was similar in the three *Garra* species. This may be due to their similar feeding pattern. Ogino and Takeda (1978) have shown a direct correlation between the tissue phosphorous content and dietary phosphorous level in rainbow trout. Most of the phosphates in these tissues are present in free or bound form. The labile phosphates contribute to a lesser amount. The free phosphate level indicate the metabolic state of the tissue. But a very high level of free phosphate observed in the gill may be due to the accumulation of phosphate from surrounding water. Such accumulation of phosphorous has been shown in developing embryos of carp by Moroz and Luzhin (1976). The highest level of labile phosphate with a fairly higher amount of free phosphate in the liver of the three species studied, indicate the higher metabolic status of this tissue.

Taking into consideration the observed higher levels of protein, RNA and phosphates with a depleted free amino acid pool in general, it may be suggested that the *Garra* species have a higher metabolic rate as an adaptation for life in the fast-flowing streams, and they could be developed as a source of protein nutrition for the people of this region.

Acknowledgement

The authors thank the Heads of the Department of Zoology and Botany, North-Eastern Hill University, Shillong,

for laboratory facilities. One of us (A K B) thanks the Council of Scientific & Industrial Research for the award of a Junior Research Fellowship during this work.

References

- Agrawal V P and Tyagi A P 1969 Food, feeding habits and the alimentary canal of freshwater fishes of Muzaffarnagar; *Agra Univ. J. Res.* **18** 15-28
- Bulow F J 1970 RNA-DNA ratios as indicators of recent growth rates of a fish; *J. Fish. Res. Bd. Can.* **27** 2343-2349
- Fraser A G L 1937 Fish of Deolali II. Ecological and biological observations; *J. Bombay nat. Hist. Soc.* **39** 689-711
- Fiske C H and Subbarow Y 1925 The colorimetric determination of phosphorous; *J. biol. chem.* **66** 375-400
- Goss R J 1966 Hypertrophy versus hyperplasia; *Science* **153** 1615-1620
- Hora S L 1937 Notes on fishes in the Indian museum. XXVIII. On three collections of fish from Mysore and Coorg, South India; *Rec. Indian Mus.* **39** 5-28
- and Mukerji D D 1936 Fish of the Eastern Doons, United Provinces; *Rec. Indian Mus.* **38** 133-146
- Jafri A K and Mustafa S 1976 Nucleic acids in the dark and white muscles of a freshwater carp, *Barbus stigma* (Cuv. & Val.); *Curr. Sci.* **45** 415-416
- Jones S 1941 An interesting case of migration of stone-licking fish *Garra mullya* (Sykes) for breeding; *Curr. Sci.* **10** 445-446
- Leslie I 1955 The nucleic acid content of tissues and cells; in *The Nucleic acids, Chemistry and Biology*, Vol. 2 pp. 1-50 eds E Chargaff and J N Davidson (New York: Academic Press)
- Love R M 1970 *The Chemical Biology of Fishes* (London and New York: Academic Press) 85 pp
- Lowry O H, Rosenbrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin Phenol reagent; *J. biol. chem.* **193** 265-275
- Majhi A 1980 Studies on biology and chromosome analysis of some teleostean fishes of North-Eastern India; Ph.D. Thesis, North-Eastern Hill University, Shillong
- Menon A G K 1974 *A checklist of fishes of the Himalayan and the Indo-Gangetic Plains*; Inland Fish. Soc. India Spl. Publ. No. 1.
- Moroz I Ye and Luzhin B P 1976 Dynamics of metabolism in the embryonic and early post embryonic development of the carp *Cyprinus carpio*; *J. Ichthyol.* **16** 964-970
- Mustafa S 1977 Nucleic acid turnover in the dark and white muscles of some freshwater species of carps during growth in the pre-maturity phase; *Copeia* **1** 173-176
- Ogino C and Takeda H 1978 Requirements of rainbow trout for dietary calcium and phosphorous; *Bull. Japan Soc. Sci. Fish.* **44** 1019-1022
- Schneider W C 1957 Determination of nucleic acids in tissues by pentose analysis; in *Methods in Enzymology* Vol. III pp 680-684 eds S P Colowick and N O Kaplan (New York: Academic Press)
- Siddiqui A Q, Siddiqui A H and Ahmad K 1973 Free amino acid contents of the skeletal muscle of carp at juvenile and adult stages; *Comp. Biochem. Physiol.* **B44** 725-728
- Somvanshi V S 1976 Biology of *Garra mullya* (Sykes) from Marathwada; Ph.D. Thesis, Marathwada University, Aurangabad
- 1980 Study on some aspects of spawning biology of a hill stream fish *Garra mullya* (Sykes); *Proc. Indian natn. Sci. Acad.* **B46** 105-113
- and Bapat S S 1979 Food and feeding habits of a hillstream fish, *Garra mullya* (Sykes); *J. Inland Fish. Soc. India* **11** 87-93
- Spies J A 1957 Colorimetric procedures for Amino-acids; in *Methods in Enzymology* Vol III pp 468-470 eds S P Colowick and N O Kaplan (New York: Academic Press)
- Yudaev N A 1950 Content of histidine, carnosine and anserine in muscle of some fish; *Dokl. Akad. Nauk, SSSR*, **70**
- Zaitsev V, Kizevetter I, Lagunov L, Makarova T, Minder L and Podsevalov V 1969 *Fish Curing and Processing* translated by A D E Merindol (Moscow: Mir Publishers) 51 pp