

CONTENTS

	<i>Pages</i>
CHAPTER-I: Introduction	1–22
I.1 Introduction	1
I.2 Abundance	2
I.3 Nutritional significance of the fish	3-17
I.3.1 Proximate composition	4
I.3.2 Minerals	8
I.3.3 Fatty acids	13
I.3.4 Amino acids	15
I.3.5 Vitamins	16
I.4. Metagenomics	17
I.5. The 16S rRNA Gene	18
I.6. North-East region of India	19
CHAPTER-II: Literature Review	23–47
CHAPTER-III: Abundance Study of Fish Species from the <i>Hel</i> river, Kokrajhar, Assam	48–57
III.1 Introduction	48
III.2 Materials and Methods	49-50
III.2.1 Sample collection	49
III.2.2 Sample selection and identification	50
III.3 Results and Discussion	53–57
III.3.1 Abundance study	53
CHAPTER-IV: Determination of Nutritional Composition of Fish Species	58-64
IV.1 Materials and Methods	58-60
IV.1.1 Sample collection and preparation	58
IV.1.2 Determination of moisture content	58
IV.1.3 Determination of total solids	58
IV.1.4 Determination of ash content	59

IV.1.5	Determination of crude protein	59
IV.1.6	Evaluation of crude fat	59
IV.1.7	Calculation of total carbohydrate content	59
IV.1.8	Nutritive value	60
IV.1.9	Determination of minerals	60
IV.1.10	Statistical analysis	60
IV.2	Results and Discussion	60–64
IV.2.1	Proximate composition	60
IV.2.2	Mineral contents	62
CHAPTER-V: Fatty Acid Composition of Fish Species		65–77
V.1	Materials and Methods	65
V.1.1	Sample preparation	65
V.1.2	Determination of fatty acid composition	65
V.2	Results and Discussion	73–77
CHAPTER-VI: Amino Acid of Fish Species		78–82
VI.1	Materials and Methods	78
VI.1.1	Sample preparation	78
VI.1.2	Determination of amino acids	78
VI.2	Results and Discussion	78–82
CHAPTER-VII: Cholesterols, Triglyceride and Vitamin Contents of Fish Species		83-87
VII.1	Materials and Methods	83
VII.1.1	Sample preparation	83
VII.1.2	Evaluation of Total Cholesterol, LDL, HDL, VLDL and Triglycerides	83
VII.1.3	Evaluation of Vitamins A and D	83
VII.1.4	Statistical analysis	84
VII.2	Results and Discussions	85–87
VII.2.1	Total Cholesterol, HDL, LDL, VLDL and Triglycerides	85
VII.2.2	Evaluation of Vitamin A and D contents	87

CHAPTER-VIII: Metagenomics Study of <i>Barilius bendelisis</i>	88–104
VIII.1 Introduction	88
VIII.2 Materials and Methods	90–93
VIII.2.1 Sample preparation	90
VIII.2.2 Method for genomic DNA (gDNA) isolation	90
VIII.2.3 DNA confirmation and quantification	91
VIII.2.4 PCR amplification of DNA	91
VIII.2.5 Qualitative and quantitative analysis of PCR products	92
VIII.2.6 Preparation of libraries for 2 × 250 bp (base pair) Run chemistry	92
VIII.2.7 Cluster generation and sequencing	92
VIII.2.8 Wet-Lab Inference	93
VIII.2.9 Bioinformatics analysis (Reads statistics)	93
VIII.2.10 QIIME overview and steps for 16S analysis	93
VIII.3 Results and Discussion	95–104
Conclusion	105–106
Publications	107
References	108–139
Appendices	140–165

LIST OF TABLES

	<i>Pages</i>
Table III.1 : Local name, scientific name, family and order of fish species found in the <i>Hel</i> river, Assam	51
Table III.2 : Relative abundance (RA) study of fish species found in the <i>Hel</i> river of Kokrajhar, Assam	52
Table III.3 : Fish species from the <i>Hel</i> river selected for the present study	56
Table IV.1 : Evaluation of proximate composition of nine fish species per 100 g of dry weight	62
Table IV.2 : Estimation of mineral contents of nine fish species per 100 g of dry weight	63
Table V.1 : Fatty acid composition of <i>Barilius bendelisis</i>	69
Table V.2 : Fatty acid composition of <i>Chagunius chagunio</i>	69
Table V.3 : Fatty acid composition of <i>Garra gotyla</i>	70
Table V.4 : Fatty acid composition of <i>Labeo pangusia</i>	70
Table V.5 : Fatty acid composition of <i>Neolissochilus hexagonolepis</i>	71
Table V.6 : Fatty acid composition of <i>Raiamas bola</i>	71
Table V.7 : Fatty acid composition of <i>Tor putitora</i>	72
Table V.8 : Fatty acid composition of <i>Cyprinion semiplotum</i>	72
Table V.9 : Fatty acid composition of <i>Barilius barna</i>	73
Table V.10 : Fatty acid (methyl ester) composition of nine fish species in the % of total fatty acids by the peak area	75
Table VI.1 : Amino acid composition (% of total area of amino acids) of nine fishes	80
Table VII.1 : Total cholesterol, LDL, HDL, VLDL and triglyceride contents in fish species	84
Table VII.2 : Vitamins A and D contents of fish species	86
Table VIII.1 : Primers used for 16S rRNA in the present study	92
Table VIII.2 : gDNA quality and quantification NanoDrop machine	94
Table VIII.3 : Reads statistics	95
Table VIII.4 : OTUs (Operational taxonomic units) summary	96
Table VIII.5 : The individual samples summary	104

LIST OF FIGURES

	<i>Pages</i>
Fig.I.1 : Map showing the study area	21
Fig.III.1 : Representation of the total number of species, Shannon-Weiner index (H') and Pielou's Evenness (J)	54
Fig.III.2 : Selected fish species of the present study	55
Fig.V.1 : Gas chromatogram of <i>Barilius bendelisis</i>	66
Fig.V.2 : Gas chromatogram of <i>Chagunius chagunio</i>	66
Fig.V.3 : Gas chromatogram of <i>Garra gotyla</i>	66
Fig.V.4 : Gas chromatogram of <i>Labeo pangusia</i>	67
Fig.V.5 : Gas chromatogram of <i>Neolissochilus hexagonolepis</i>	67
Fig.V.6 : Gas chromatogram of <i>Raiamas bola</i>	67
Fig.V.7 : Gas chromatogram of <i>Tor putitora</i>	68
Fig.V.8 : Gas chromatogram of <i>Cyprinion semiplotum</i>	68
Fig.V.9 : Gas chromatogram of <i>Barilius barna</i>	68
Fig.VIII.1 : 1.2% Agarose gel showing extracted DNA of the sample with 1 Kb (Kilo base pair) marker (AE1) and PCR product on 1.5 % Agarose gel (AP1)	94
Fig.VIII.2 : Bioanalyzer 2100 profile of final libraries of 16S metagenome using DNA 1000 chip	95
Fig.VIII.3 : Taxonomy at phylum level (Top 5 phyla shown in Pie diagram)	97
Fig.VIII.4 : Taxonomy at class level (Top 5 classes shown in Pie diagram)	98
Fig.VIII.5 : Taxonomy at order level (Top 5 orders shown in Pie diagram)	99
Fig.VIII.6 : Rank abundance plot of <i>Barilius bendelisis</i> sample	103
Fig.VIII.7 : Rarefaction plot of <i>Barilius bendelisis</i> sample	103
Fig.III.A.1a : ZSI report of morphological identification of fish species	140
Fig.III.A.1b : ZSI report of morphological identification of fish species	141
Fig.III.A.2 : Permission for studying fish diversity of <i>Hel</i> river, Serfanguri, Kokrajhar, Assam	141
Fig. VI.A.1 : HPLC chromatogram of acidic and basic amino acid mixture standard	142
Fig. VI.A.2 : HPLC chromatogram of amino acids in <i>B. Bendelisis</i>	142

Fig. VI.A.3	: HPLC chromatogram of amino acids in <i>C. Chagunio</i>	143
Fig. VI.A.4	: HPLC chromatogram of amino acids in <i>G. gotyla</i>	143
Fig. VI.A.5	: HPLC chromatogram of amino acids in <i>L. Pangusia</i>	143
Fig. VI.A.6	: HPLC chromatogram of amino acids in <i>N. Hexagonolepis</i>	144
Fig. VI.A.7	: HPLC chromatogram of amino acids in <i>R. Bola</i>	144
Fig. VI.A.8	: HPLC chromatogram of amino acids in <i>T. putitora</i>	144
Fig. VI.A.9	: HPLC chromatogram of amino acids in <i>C. Semiplotum</i>	145
Fig. VI.A.10	: HPLC chromatogram of amino acids in <i>B. barna</i>	145
Fig. VII.A.1	: HPLC chromatogram of fat-soluble vitamins in <i>R. bola</i>	146
Fig. VII.A.2	: HPLC chromatogram of fat-soluble vitamins in <i>C. semiplotum</i>	147
Fig. VII.A.3a	: HPLC chromatogram of fat-soluble vitamins in <i>B. bendelisis</i>	148
Fig. VII.A.3b	: HPLC chromatogram of fat-soluble vitamins in <i>B. bendelisis</i>	149
Fig. VII.A.4	: HPLC chromatogram of fat-soluble vitamins in <i>C. chagunio</i>	150
Fig. VII.A.5	: HPLC chromatogram of fat-soluble vitamins in <i>B. barna</i>	151
Fig. VII.A.6	: HPLC chromatogram of fat-soluble vitamins in <i>G. gotyla</i>	152
Fig. VII.A.7	: HPLC chromatogram of fat-soluble vitamins in <i>N. hexagonolepis</i>	153
Fig. VII.A.8	: HPLC chromatogram of fat-soluble vitamins in <i>T. putitora</i>	154
Fig. VII.A.9	: HPLC chromatogram of fat-soluble vitamins in <i>L. Pangusia</i>	155
Fig. VIII.A.1	: Taxonomy at family level (Top 5 families shown in Pie : diagram)	156–157
Fig. VIII.A.2	: Taxonomy at genus level	158–160
Fig. VIII.A.3	: Taxonomy at species level	161–163
Fig. VIII.A.4	: OTU heat map	164
Fig. VIII.A.5	: Krona graph	165