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pH-based Alteration of Electrical Conductivity and Total Protein Profile of Muscle of Fish, *Heteropneustes fossilis* (Bloch) as an Indicator of Aquatic Pollution

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Abstract: Alteration of electrical conductivity and total protein profiles of muscle of *Heteropneustes fossilis* (Bloch) have been assessed as an indicator of responses to change in pH of the aquatic medium. Fish were kept in different aquaria containing tap water maintained at different pH levels (viz., 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 10.5) for 48 hrs and the muscle was exposed to different electrical fields by subjecting them to 5, 10, 15, 20 and 25 volt/cm. Required pH was accomplished by adding conc. HCl (AR grade) and NaOH (LR grade) in tap water. As protein is an organic semiconductor, the responses of electrical conductivity, protein band profiles (number, molecular weight and intensity of bands) and total protein content of muscle, if any, were analyzed at different pH to evaluate the extent of damage/alteration caused by pH at protein level. pH not only modulated the muscle conductivity at the lower and higher levels, but also altered significantly in regard to both protein profiles and total protein content, indicating possibility and relevance of utilizing pH as a potent indicator of extent of damage inflicted on fish muscle protein and thereby to a considerable extent the degree of aquatic pollution.

Key words: pH, muscle conductivity, muscle protein, pollution, fish.

Introduction

Increasing level of pollution and its impact on living aquatic organisms, particularly fish, have become a subject of great concern. In the aquatic environment, life goes on under dynamic and unstable circumstances, forcing fishes to acclimatize to various factors, such as, changes in population density, pressure, temperature, dissolved gases, light, pH, etc. which impose a considerable amount of stress on their lives and predispose them to diseases (Bakde and Niyogi Poddar, 2011). Among these factors, change in pH that expresses the activity of hydrogen ion in a system or a solution has occasionally been reported to have a stressful damaging effect in different organisms (Allan and Maguire, 1992; Burton, 1994) including fish (Baker et al., 2009; Fu et al., 2011). The general effects of pH on the properties of protein-based films from bigeye snapper (*Priacanthus tayenus*) surimi (Chinabhark et al., 2007) and conjointly with calcium on fish and fisheries have been studied earlier (Brown, 1982). As the protein is rich in fish muscle (Bose et al., 1991), and being an organic semiconductor (Gurtmann and Lyons, 1981), the estimation of conductivity of fish muscles has been suggested to be of an immense importance to monitor the effects of various environmental pollutants (Benech and Quattara, 1990; Straetkvern et al., 1991; Bai et al., 1994; Jaramillo et al., 1994; Arockiadoss et al., 1998). Electrical conductivity indicates the amount of ions (electrically charged particles) in the water. Since the proteins are made up of amino acid and carboxylate groups, the pH variation is expected to protonate or deprotonate the selective protein

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centre and is expected to exhibit variation in electrical conductance of the fish muscle proteins (Arockiadoss et al., 1998).

Recently, measurement of total body electrical conductivity has been used as a tool for identification of metal-contaminated fish protein (Arockiadoss et al., 2008), microbiological quality of white croaker, *Micropogonias furnieri* (Montagner et al., 2005) and a non-destructive and reliable means of estimating a variety of body composition in mammals (Presta et al., 1983), birds (Walsberg, 1988) and fish (Brown et al., 1993). The effect of pH on the formation of edible films made from the muscle protein of fish, *Makaira mazara* was reported by Hamaguchi et al. (2007). Earlier solubility of muscle proteins (actinomysin) from some sub-trophic fish and cod muscle protein by pH was evaluated by different authors (Tsai et al., 1989; Stefansson and Hultin, 1994). It was also reported that for fish muscle proteins, adding acid or alkali improves its emulsifying properties, which are mainly correlated with an increase in surface hydrophobicity and surface-interfacial activity (Kristinsson and Hultin, 2003). On the other hand, total protein profile and protein content of different tissues of fish was previously explored as an indicator of genotoxicity by various authors (Manna and Mukherjee, 1986; Guha and Khuda-Bukhsh, 2002, 2003, 2004). The effects of pH on liver protein of *H. fossilis* as biomarker of aquatic pollution have been reported in our earlier studies (Guha et al., 2011). However, to the best of the knowledge of the authors, the effect of change of pH of water to the conductivity using fish muscle protein and the alteration of total protein profiles and total protein content of *Heteropneustes fossilis* as biomarkers has not been done earlier, for which the present study was undertaken.

Materials and Methods

Test Fish

Live specimens of *Heteropneustes fossilis* (Bloch) belonging to the family Clariidae, weighing between 10 and 15 gm and ranging from 12 to 15 cm in length, procured from the local fish market were used for the study. Fishes were acclimatized in a glass aquaria (20 L capacity) containing fresh tap water (stored from deep tube well) at least for 10 days. During acclimatization the fishes were fed with artificial diet (rice bran + wheat + oil cake) in palette form twice in a day. The experiment was conducted in 15 L glass aquaria each containing 10 L tap water (temperature 28-32°C) and five fishes were used for both the control and experimental series.

Maintenance of pH

The maintenance of different pH was accomplished through addition of required amounts of conc. HCl (AR grade) (for acidic pH 3.0-6.0) to fresh tap water, and NaOH (LR grade) was added in different amounts to raise the pH level from 8.0 to 10.5 for making it alkaline. The fishes were kept in different pH mediated water for 48 hrs for the quantitative and qualitative estimations and the electrical conductivity study of muscle protein. The pH of the water was checked twice a day to maintain the desired pH, as it was liable to change slightly owing to food and excreta in the water. The pH values were measured using a digital electronic pH meter (Systronics).

Electrical Conductivity Study

For the study of electrical conductivity, the muscle of the experimental fish remaining in different pH for a period of 48 hr was thinly sliced laterally (about 2.0 × 1.0 × 1.0 cm^3) and two electrodes (thin copper wire) were fixed onto the fish muscle at a distance of about 2.0 mm. The electrodes were connected in a series with a digital ammeter (made by Sreemaster, India) and a D.C. power supply. The conductivity for the muscle protein (μA) derived from the fishes raised in water of different pH values was measured at various electric fields (volt/cm), viz., 5 volt, 10 volt, 15 volt, 20 volt and 25 volt as per the procedure of Arockiadoss et al. (1998).

Total Protein Profile Study

For the study of total protein profile, the muscle of the experimental fishes was dissected out from the fish after 48 hr at different pH levels and homogenized in 0.1% NaCl solution. After centrifugation at 3000 g for 15 minutes, the supernatants were collected. Aliquotes containing known amount of protein for each tissue (5 to 10 micro gram) was diluted in 10× Laemmli's buffer and loaded in separate wells. The Sodium Dodecyl Sulphate-Polyacrulamide Gel Electrophoresis technique (Laemmli, 1970) was followed to estimate the molecular weight and intensity of each protein bands in respect of standard protein samples of known molecular weights. Analysis of molecular weight marker for protein profile was done by Gel Documentation System (Total Lab 2.01, Ultra Lum, 1D image software). For estimating the total protein content the technique of Lowry et al. (1951) using folin-ciocalteu reagent was followed.

Statistical Analysis

The data were subjected to Andersion-Dearling Normality test and found to be within normal range. For the analysis of data, Student's t-test was conducted

between data of control and experimental series of fishes and to test the level of significance the Fisher and Yates statistical tables (Fisher and Yates, 1963) were used.

Results

Electrical conductivity study: The muscle of *H. fossilis* obtained from different pH solutions showed an increase in conductivity in almost all cases as the applied field increased. It is interesting to note that the change of conductivity was more significant as the field increased beyond 5 volt/cm (Figure 1). The variation of the conductivity with pH values at different fields on fish muscle is presented in Figure 2. The graph shows its maximum values corresponding to pH values of 3.0 and 6.5, with a shoulder around pH 5.0 for all the electric fields applied (Figure 2).

Qualitative analysis of protein: Data on gel electrophoretic protein profiles of muscle tissue of the experimental fish at different pH solutions reveal that the number of bands was equal (i.e., 20) at pH 6.0 and 7.0. From pH 6.0 to pH 3.0 (decreasing) and from pH 7.0 to pH 10.0 (increasing) the number of bands increased in all successive pH levels. But at pH level beyond 10.0, the number of bands became decreased (Table 1 and Figure 3). Comparison of the molecular weight and intensity of each band showed a differential response at different pH levels (Table 1).

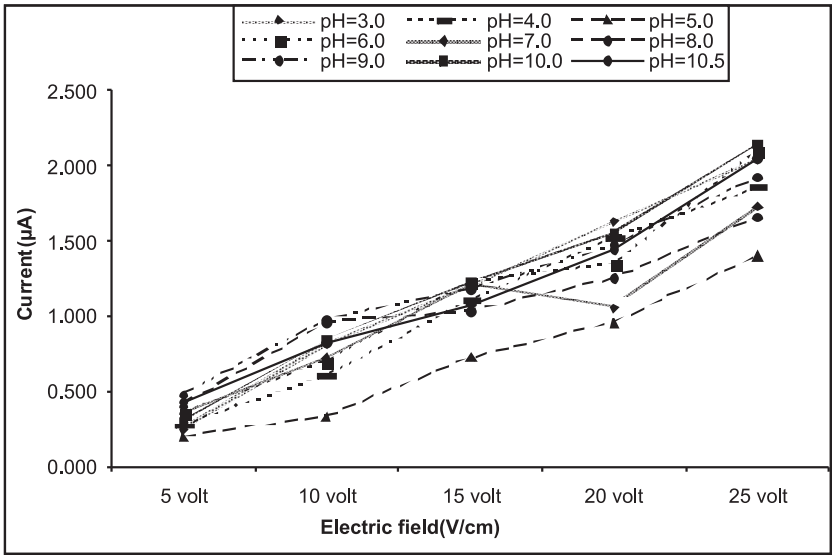

Figure 1: Variation of electrical conductivity in relation to electric fields for various pH in muscle of *H. fossilis*.

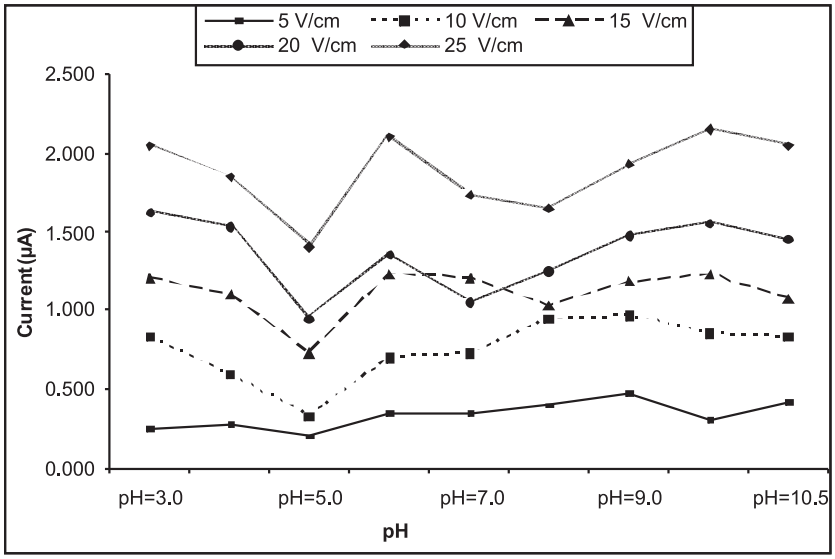

Figure 2: Variation of electrical conductivity in relation to pH levels for different electric fields in muscle of *H. fossilis*.

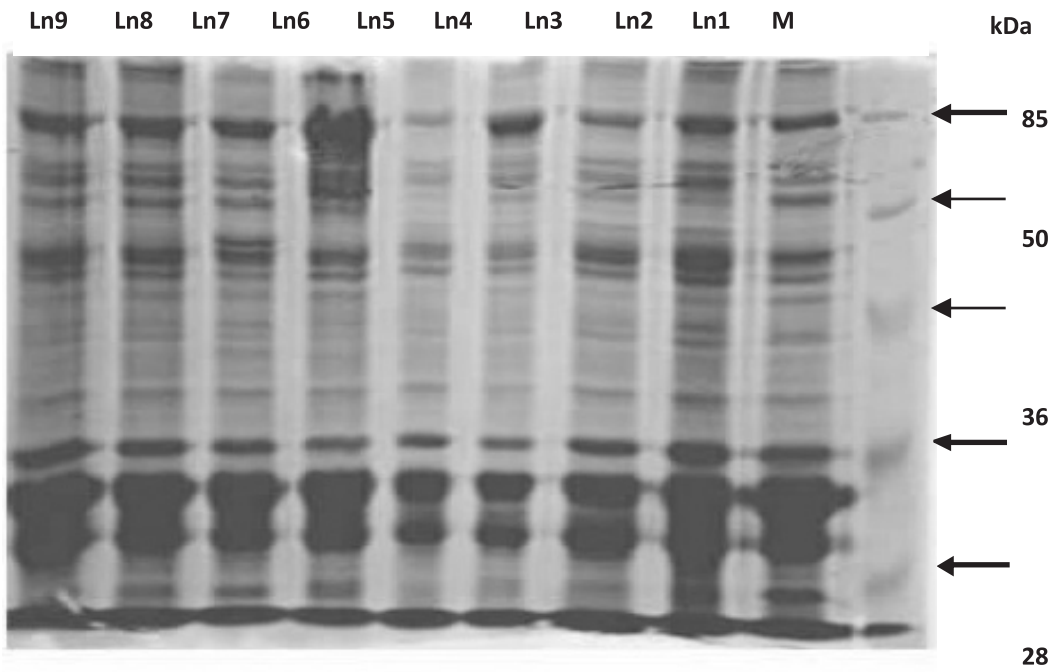

Figure 3: Protein patterns of muscle of *Heteropneustes fossilis* with exposure of different pH. The number of lanes designates the exposure of pH values. Ln-1: pH 3.0; Ln-2: pH 4.0; Ln-3: pH 5.0; Ln-4: pH 6.0; Ln-5: pH 7.0; Ln-6: pH 8.0; Ln-7: pH 9.0; Ln-8: pH 10.0; Ln-9: pH 10.5; M = Molecular Weight Marker.

Quantitative analysis of protein: The total protein contents in the increasing level of pH (pH 8.0, 9.0, 10.0, 10.5) were less, of which pH 8.0, pH 9.0 and pH 10.0 showed statistically significant (p <0.005, 0.01 and <0.001) when compared with pH 7.0 (Table 1). Similar to the result obtained in the increasing level of pH, statistically significant differences (p <0.01 and <0.001) in results were shown for decreasing level of pH (pH 6.0 and 5.0). The interesting feature was that below pH 5.0, the total protein content increased slightly, but this was not statistically significant (Table 1).

Discussion

In the present study, pH was found to have a direct effect on the electrical conductivity, total protein profile and protein content of the muscle tissue of *H. fossilis*. pH influences protein stability in two ways: (i) it influences the charge (positive or negative) on the protein molecule; and (ii) it affects protein solubility. The fish muscle is made up of proteins, and amino acids linked by peptide groups and contain both acidic (COOH) and basic (NH_2) forms. The amino acids in proteins react with alkalis or acids to form salts and thus proton (H^+) conduction occurs (Arockiadoss et al., 1998). In this study, the results showed that in pH 5.0 the conductivity is minimal. pH below or above 5.0 showed an increase of conductivity indicating more positive H^+ and/or protonation (Star and Taggart, 1981). On the other hand, as the pH increases, the protein becomes unstable due to the shortening or folding during muscular contraction by forming actin-myosin complex, resulting in its denaturation and the carboxylic acid groups in this protein form sodium ion and thus induced alteration of electrical conductivity (Frelin et al., 1989). Due to its permeability into the fish membrane (sodium pump) (Shanmugam, 1992), the conductivity of muscle increases reaching its maximum at pH 10.0. In addition to this the changes of conductivity for various electrical fields (Figures 1 and 2) signifies the effects of acidic and/or alkaline solution. It seems that the increase or decrease in concentration of H^+ and/or OH^- as a consequence of changed pH of the aquatic environment results in the change of electrical conductivity of muscle protein of *H. fossilis*. Thus, measuring the electrical conductivity as a function of pH values may indicate the environmental pollution level that affects aquatic organisms.

Similarly, the effect of pH on the total protein profile and protein content of muscle tissue may have an additional support to the conductivity study for monitoring the extent of pollution levels. Fuller et al. (1989) reported that increasing the extracellular pH over

Table 1: Analysis of molecular weight marker for protein profile by Gel documentation System (Total Lab 2.01, Ultra Lum, 1D image software) and changes in total protein (µg/ml) levels from muscle of *Heteropneustes fossilis* after exposure with different pH. Rm = Relative mobility; I = Intensity of band. For amount of protein significance test was done between data of pH 7.0 (control) and the other pH. *P* values: [a]<0.05; [b]<0.01; [c]<0.001

No. of bands	*Range of pH*																	
	3.0		*4.0*		*5.0*		*6.0*		*7.0*		*8.0*		*9.0*		*10.0*		*10.5*	
	Amount of protein: 2.57±0.325[n]		*Amount of protein: 3.20±0.150[n]*		*Amount of protein: 2.51±0.135[b]*		*Amount of protein: 2.00±0.050[c]*		*Amount of protein: 3.27±0.025*		*Amount of protein: 2.17±0.075[c]*		*Amount of protein: 2.56±0.120[b]*		*Amount of protein: 1.87±0.375[a]*		*Amount of protein: 2.57±0.325[n]*	
	Rm	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*	*Rm*	*I*
1	0.003	166.72	0.001	172.98	0.19	114.85	0.197	48.44	0.176	12.57	0.178	42.57	0.202	57.19	0.193	132.50	0.19	116.85
2	0.186	162.02	0.181	68.78	0.24	104.63	0.244	139.39	0.196	53.13	0.2	135.39	0.225	5.06	0.243	111.16	0.239	91.90
3	0.194	161.73	0.188	211.67	0.278	34.16	0.28	18.39	0.24	55.94	0.246	126.28	0.245	121.73	0.283	50.28	0.267	12.38
4	0.241	145.16	0.196	168.30	0.294	36.80	0.294	39.16	0.281	14.88	0.263	80.09	0.285	19.20	0.294	70.79	0.283	39.20
5	0.26	28.31	0.243	101.91	0.301	66.26	0.301	56.63	0.294	24.28	0.267	47.18	0.298	55.70	0.315	62.68	0.292	55.84
6	0.285	54.88	0.282	41.00	0.309	22.42	0.311	27.22	0.31	12.13	0.277	61.16	0.316	60.80	0.327	4.47	0.315	45.43
7	0.294	73.81	0.299	78.48	0.322	3.32	0.335	12.23	0.336	11.73	0.291	63.76	0.352	91.48	0.36	62.08	0.362	47.78
8	0.312	88.06	0.312	16.36	0.338	22.56	0.363	45.44	0.358	45.96	0.305	58.60	0.363	86.70	0.381	38.79	0.382	24.31
9	0.341	11.56	0.327	6.32	0.366	79.95	0.376	29.33	0.377	41.79	0.321	21.62	0.381	29.00	0.4	23.53	0.402	15.88
10	0.365	99.35	0.342	30.88	0.38	39.53	0.396	15.42	0.434	12.84	0.363	92.87	0.401	22.15	0.427	12.59	0.441	10.25
11	0.385	65.70	0.365	51.59	0.399	11.56	0.434	12.64	0.452	7.84	0.383	71.55	0.427	23.77	0.438	23.29	0.497	25
12	0.404	44.43	0.385	28.53	0.427	10.70	0.454	7.20	0.486	35.12	0.401	30.74	0.438	16.97	0.454	5.44	0.545	83.43
13	0.438	46.87	0.404	24.05	0.437	22.58	0.486	16.56	0.534	126.12	0.426	13.30	0.453	26.61	0.491	45.90	0.585	36.37
14	0.461	17.47	0.431	28.69	0.468	6.44	0.537	103.37	0.571	111.94	0.437	7.22	0.471	6.65	0.513	10.52	0.624	13.00
15	0.477	9.99	0.445	34.09	0.491	46.60	0.571	136.55	0.603	10.19	0.457	9.60	0.491	42.56	0.54	125.48	0.667	2.59
16	0.497	53.11	0.461	14.96	0.513	4.56	0.603	13.80	0.617	59.44	0.489	36.25	0.54	120.78	0.576	94.77	0.697	202.81
17	0.518	8.88	0.477	9.21	0.539	163.14	0.619	69.31	0.647	2.35	0.511	7.02	0.578	103.92	0.621	27.02	0.704	198.72
18	0.547	124.12	0.496	53.93	0.576	134.67	0.671	21.72	0.669	8.14	0.537	121.44	0.621	40.74	0.651	10.37	0.967	165.73
19	0.591	78.91	0.516	3.91	0.623	57.07	0.695	262.51	0.697	251.55	0.575	116.65	0.672	70.65	0.672	42.05	0.971	83.97
20	0.63	53.60	0.546	100.89	0.67	25.27	0.958	171.32	0.96	134.15	0.619	48.48	0.693	215.70	0.697	205.89		
21	0.658	16.06	0.58	76.33	0.699	257.35					0.65	7.80	0.963	187.01	0.965	207.84		
22	0.677	74.10	0.625	37.13	0.952	108.29					0.669	55.52	0.966	112.82	0.969	86.29		
23	0.707	227.37	0.675	20.74	0.955	145.62					0.695	223.40						
24	0.722	16.25	0.703	220.88							0.96	127.29						
25	0.949	174.64	0.953	168.73							0.963	111.81						

the range of pH 7.4-8.9 would stimulate protein synthesis by about 60% in the rat heart preparation anterogradely perfuse in vitro. Jaroli and Sharma (2005) reported that the reduction in protein content may be due to increased utilization of protein to meet out the energy demand when the fish is under stress condition. For protein synthesis, it is probable that in our in vitro experiment translation (rather than transcription or RNA processing) is affected, since effects are observed rapidly (Winkler, 1982). However, the increase in total protein content unmistakably substantiated the synthesis of new protein and decrease of protein content may be a full proof of the denaturation of protein due to the effect of pH (Guha and Khuda-Bukhsh, 2002). On the other hand, decline in the protein contents of muscle of *H. fossilis* would suggest an intensive proteolysis which in turn contributes the increase of the free amino acids to be fed into the TCA cycle as keto acids, thus supporting the view of Kabeer et al. (1978). Furthermore, the lower amount of total protein content at below and higher pH values of 7.0 found in the present study possibly is due to protein solubility as a consequence of changed pH.

Solubility of protein is affected by pH and ionic strength, and for *H. fossilis*, it must be tested to propose any process of protein recovery or removal of undesirable components (Fuente-Betancourt et al., 2009). The results of the total protein profile showed that the band characteristics (intensity) differ quite appreciably at different pH levels (Table 1 and Figure 3). This could mean that some difference in the type of proteins could still be there even if a particular band was in the same molecular weight class. But where the band distinctly differed in its molecular weight class, the change could be unmistakably substantiated when the band profile of pH 7.0 was compared with that of the other pH levels (Table 1).

Another interesting finding of the study is that the number of bands was equal (i.e., 20) at pH 6.0 and 7.0 but it increased in the upper and lower pH values except for pH 10.5 (Table 1 and Figure 3). Therefore, the changes in total protein profile recorded after pH treatment were attributed largely to proteotoxic effect of pH in this fish. In fact, Hightower (1991) introduced the term "proteotoxicity" as a central aspect of toxicity, which takes place at the levels of proteins. Since proteotoxicity can also arise by defective functioning of altered DNA, study of total protein profile as an endpoint can also complement other pollution potentials in fish (Guha and Khuda-Bukhsh, 2004). As pH may result in DNA damage or may result from DNA damage, it becomes imperative that there could be some impairment of gene activity as a consequence, either having direct or indirect effects on proteins (Guha and Khuda-Bukhsh, 2002, 2004). The concomitant changes observed in the total protein profile of muscle will amply speak for this. Thus, the overall results of the study would imply that the alteration of conductivity, total protein profile and protein content was due to the changes in pH by creating a hazardous aquatic environment and further in-depth studies will be necessary to understand the precise mechanism by which such changes take place.

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