



## Determination of stress levels of *Otolithoides biauritus* (Cantor, 1849) in selected zones of Indian Sundarbans

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The Indian Sundarbans is one of the three largest single tract mangrove forests of the world. The hydro-geochemical environment of this ecosystem is highly dynamic. Factors such as global warming, heavy siltation and solid waste disposal have caused considerable damage to the ecological status of the Indian Sundarbans and also affected the natural fish population. Here, we determined the stress levels of the amphidromous Sciaenid *Otolithoides biauritus* (Cantor, 1849) in response to water quality parameters of selected zones of Indian Sundarbans which can indicate its capacity to survive environmental stress by potentiating stress response through molecular activities. Samples were collected during the post-monsoon season in February 2023 from four stations namely S1 (22°09.943'N 088°53.325'E), S2 (22°09.661'N 088°54.948'E), S3 (22°00.857'N 088°45.567'E) and S4 (21°34.861'N 088°14.157'E) with different salinity regimes and the complete protein profile of muscles was established by SDS-PAGE. The concentrations of stress protein Hsp70 (pg/mL) and cortisol hormone (ng/mL) were determined via quantitative ELISA method. Physicochemical parameters such as water temperature, salinity, pH, dissolved oxygen, total dissolved solids (TDS), transparency and depth were recorded *in situ*. Nutrients such as nitrate, phosphate and silicate concentrations were estimated *via* standard spectrophotometric methods. A comparative account of the findings was established between S1, S2, S3 and S4. Highest average cortisol concentrations were observed at S1, while highest Hsp70 at S3. A 97 kDa protein was found exclusively at S2 while two proteins (26 kDa and 23.5 kDa) found at S1, S3 and S4, were absent at S2. S2 recorded least cortisol and Hsp70 levels. A long term monitoring is recommended for better understanding of the spatial variation of protein profile under fluctuating hydrodynamic conditions of the Indian Sundarbans.

**Keywords:** Aquatic pollution, Cortisol, Hsp70

The Sundarbans mangrove-dominated Ganges delta is one of the three largest single tract mangrove forests of the world<sup>1</sup>. It is a complex ecosystem comprising a transitional zone between the freshwater of the rivers originating from the Ganges and the saline water of

the Bay of Bengal. Thus, with the numerous drainage channels and significant coastal processes, the hydro-geochemical environment of the Indian Sundarbans is highly dynamic in nature. The salinity condition of this ecosystem is largely dependent on the volume of freshwater coming from the upstream on the one hand and the nature of tide on the other. The energy flux of this ecosystem has greatly changed over the years due to changes in the tidal behaviour, the intensity of the annual floods and its associated sediment loads, the fluctuating salinity levels and industrial and maritime pollution<sup>2</sup>. The footprint of global warming is also being felt through an increase in surface water temperatures and increased evaporation, eventually leading to an increase in salinity<sup>3</sup>. Additionally, heavy siltation and solid waste disposal which clog the connections of the estuaries with the main channel of the Ganges, salt water intrusions from Bay of Bengal and reduction in the water spread area due to reduced tidal action facilitate salinity increase<sup>3</sup>. Apart from salinity, other factors like dissolved oxygen and inorganic nutrient concentrations are also influenced by the degree of horizontal and vertical mixing of water in the mangrove waterways. Apart from salinity, other factors like dissolved oxygen and inorganic nutrient concentrations are also influenced by the degree of horizontal and vertical mixing of water in the mangrove waterways. The increase in the saturation of suspended solids due to erosion and sedimentation processes and increased levels of subsequent churning action is leading to a decrease in transparency. Thus, these factors are not only the cause of the declining ecological status of the Indian Sundarbans but are greatly affecting the natural fish population which are increasingly subjected to multiple stressors threatening their sustainability and subsequent survival.

Sciaenids are small to moderately sized fishes belonging to the Sciaenidae family which occur worldwide and form a significant part of commercial fisheries of India. *Otolithoides biauritus* is a commercially important Sciaenid occurring along the Indian coast<sup>4</sup>. It exhibits a high phylogenetic similarity with *Otolithoides pama* commonly found in the Hooghly-Matla estuarine system<sup>5</sup>. The hypersaline environment and the unavailability of freshwater

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in the Matla-Bidyadhari regions of the estuary challenges the survival of economically important fin fishes like *O. biauritus* as opposed to upper reaches of the Gangetic delta<sup>6</sup>. The present study was conducted to determine the stress levels in *O. biauritus* (Cantor, 1849) in response to water quality parameters from selected zones of Indian Sundarbans. *Otolithoides biauritus* is an amphidromous teleost found both in coastal and inshore waters. It regularly migrates between freshwater and sea in both directions, but not for breeding<sup>7</sup>. It is a carnivore and an active predator feeding on other teleost fishes and crustaceans. This fish has a high demand for its meat (as food), swim bladder (used for making glass in the beverage industry) and skin (alternate leather source) in the international market<sup>8</sup>. Although the IUCN status of *O. biauritus* is not assessed<sup>9,10</sup> but reported as rare in the Sundarbans<sup>9</sup>. This fish was selected for the present study as it was found to be a common catch at all the four spots selected for sampling. Based on Direct Catch Sampling (in which fishermen were made to catch fish at the selected sites of sampling based on GPS coordinates using appropriate fishing methods), Fishermen Catch Sampling (in which we searched for local fishing trawlers in which the major fish caught by the fishermen from various sites were recorded), information from local fishermen and local fish market surveys, this fish proved to be an important food-fish, sold fresh, dried and salted, in the markets.

In the present study, we have made an attempt to determine the effect of salinity and other physicochemical parameters on the cortisol, Hsp70 and the total protein level of *O. biauritus*. Cortisol the major stress hormone in fishes has proven to be a reliable indicator of stress<sup>11,12,13</sup> and, thus a crucial hormone in the physiology and biology of fishes. The hypothalamus pituitary inter-renal tissue (HPI) axis of fishes responds to increased concentrations of various types of stress by an increase in the cortisol levels. Apart from its role in controlling the stress responses, cortisol is a regulatory hormone for both metabolism and osmoregulation in fishes<sup>14</sup>. Heat shock proteins are also natural biomarkers that play a vital role in the adaptive survival of fishes and other organisms under environmental stress. This study focused on the stress-relieving effect of heat shock protein 70 expressed in this fish and its associated physicochemical factors in this estuarine ecosystem. Environmental assessments using suitable biomarkers can help determine the magnitude of the impact

caused by the stressors<sup>15</sup> by inducing biological responses, such as homeostasis and repair systematics in fish.

## Materials and Methods

### Sampling sites

Samples were collected during the post-monsoon season in February, 2023 from four stations namely Satjelia (S1), Kumirmari (S2), Dobanki (S3) and Frasersgunj (S4) with different salinity regimes. The sampling stations extended from Satjelia (S1) [22°09.943'N 088°53.325'E] and Kumirmari (S2) [22°09.661'N 088°54.948'E] in the north-eastern part of the Indian Sundarbans to Dobanki (S3) [22°00.857'N 088°45.567'E] located to the southwest of S2 and lastly Frasersgunj (S4) [21°34.861' N 088°14.157' E] in the southernmost fringe of the Indian part of the Sundarbans. The first sampling site, S1, was located along Satjelia which is situated in the middle of a ring of islands, including Sajnekhali to the southwest, Gosaba to the west, Rangabelia to the northwest, Mollakhali to the north, and Kumirmari to the northeast. The island of Satjelia (615 sq. km) is the biggest island under Gosaba block. S2 is offshore along the village Kumirmari, a small village island with a geographical area of 292.86 hectares, flanked by dense mangrove forests of Marichjhaapi in the south. S3, along Dobanki area is the mixing layer of the confluence of five rivers (Bidyadhari, Matla, Datta, and minor rivers Chadabida and Hedokhanga) with river Bidyadhari being the predominant channel. This area surrounded by a dense mangrove cover is a nutrient rich zone which experiences vertical upwelling and rapid mixing of suspended materials. It is also a prominent conveyance route for nearby tourist attractions like Netidhopani, Dobanki Nature Camp, and Watch Tower. S4, located near Frasersgunj fishing harbour is the southernmost tip of the Indian Sundarbans where it opens into the Bay of Bengal, flanked by the island Jambu Dweep on the west. Apart from dual tidal amplitudes, this region is characterised by high saltwater intrusions, direct wave action from the Bay of Bengal and shoreline shifts<sup>16</sup>.

### Water quality analysis

All the oceanographic equipment necessary for the investigation was installed on boat for *in situ* measurements. The GPS coordinates at each location were noted using a handheld GPS device (make: Garmin, Model: eTrex 10). Water quality and environmental parameters were measured *in situ*

using a handheld multi-parameter probe (make: Hanna Instruments, Model no.: H198194) which included surface water temperature ( $^{\circ}\text{C}$ ), pH, salinity (practical salinity unit), dissolved oxygen (DO) in  $\text{mgL}^{-1}$  and total dissolved solids (TDS) in  $\text{mgL}^{-1}$ . The depth at each site was recorded using a depth sounder (make: Hondex PS-7) and the transparency of the water column was measured using a Secchi disc. The maxima and minima of light intensity were recorded at each station using a Luxmeter (make: Lutron, Model No.: LX-1102). All sites were sampled during day time with S1, S2 and S3 being covered at high tide and S4 at low tide, as S4 is a highly turbulent region owing to its proximity to the Bay of Bengal.

#### Nutrient analysis of water

For nutrient analysis of dissolved nitrate ( $\text{NO}_3^{3-}$ ), dissolved phosphate ( $\text{PO}_4^{3-}$ ) and dissolved silicate ( $\text{SiO}_3^{2-}$ ), the water samples were collected in triplicate and filtered on the boat using GF/F ( $45\mu$ ) filter paper and the filtrate was brought back to the laboratory on ice. Within 48 hours of water collection, nutrient contents were measured following the standard procedures; Silicate – IS: 3025 (Part: 35): 1988; Nitrate – IS: 3025 (Part: 34): 1988; Phosphate – APHA 23<sup>rd</sup> Edition 4500 P<sup>17</sup>.

#### Fish sampling

Live fish were caught and collected from the sampling sites (maintaining the GPS way point) with the help of local fishermen on dinghy boats. The live fishes were immediately transferred to an ice-box to be safely transferred to the laboratory. The specimens were then preserved at  $-20^{\circ}\text{C}$  for further studies. All fish specimens have been identified by the Zoological Survey of India, Kolkata.

#### Protein extraction and estimation

Protein was extracted from fish muscle tissue by homogenising in 0.2 M Tris HCl buffer (pH 7.4) at  $4^{\circ}\text{C}$ . The extract was clarified by centrifugation at  $12000\times g$  for 20 min at  $4^{\circ}\text{C}$ . The supernatant was collected and stored at  $-20^{\circ}\text{C}$ . The protein concentration in the extract was estimated by the modified method of Lowry<sup>18</sup>. A calibrated solution of bovine serum albumin was used as a standard.

#### SDS-PAGE

SDS-PAGE was performed to study the total protein profile of muscles. Gel Documentation System (make: Biotech, Model No.13-01-03) has been utilised for the determination of molecular weights. The protein sample was heated with an equal

amount of sample buffer [0.06M Tris HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5%  $\beta$ -mercaptoethanol, 0.01% Bromophenol blue] at  $100^{\circ}\text{C}$  for 3 min. The sample was loaded in the well of a 10% T mini-gel ( $8\times 7$  cm) and the gel was run using Laemmli<sup>19</sup> buffer system [0.05 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.4] at room temperature ( $27^{\circ}\text{C}$ ) for 2h 30 min at 70V. The gel was calibrated using a marker mixture [Protein Molecular Weight Marker (Broad Range), make: Genei, Cat. No. 3110475001730] consisting of Myosin, Rabbit Muscle (205 kDa), Phosphorylase b (97.4 kDa), Bovine Serum Albumin (66 kDa), Ovalbumin (43 kDa), Carbonic Anhydrase (29 kDa), Soyabean Trypsin Inhibitor (20.1 kDa), Lysozyme (14.3 kDa), Aprotinin (6.5 kDa) and Insulin chains (3.5 kDa). After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R 250 and de-stained with methanol:acetic acid:water (4:1:5) mixture.

#### Cortisol hormone and Hsp70 assay

Cortisol hormone assay from samples was done using ELISA kit [Fish cortisol ELISA Kit Catalog Number: CSB-E08487f, make – Cusabio], and Heat Shock Protein 70 was assayed using ELISA kit [Fish Heat Shock Protein 70 (Hsp70) ELISA Kit Catalog Number: CSB-E16327Fh, make – Cusabio], according to the manufacturer's recommendations. Tissue samples (0.1 g) were suspended in 1ml of 1M Phosphate buffer (pH 7) overnight, subjected to two freeze-thaw cycles, vortexed for 30 min and centrifuged for 10 min at  $12000\times g$ . The supernatant (50  $\mu\text{L}$ ) was then used directly in the assay without dilution. Cortisol and Hsp70 concentrations were measured for a total of 5 individuals of *O. biauritus* from each site. Each of these samples was analysed in duplicate, across 96-well plates. Cortisol and Hsp70 concentrations were calculated based on a standard curve run on each plate using the software Cusabio Curve Expert 1.4.

## Results and Discussion

The pattern of average water chemistry and other physical parameters in the four areas of sampling and fish collection in the Sundarbans during February 2023 is presented in Table 1. Tides in the Bay of Bengal are semidiurnal, exhibiting two highs and two lows per day. There was a gradual gradient in salinity regime from the North-east to the South with the lowest salinity recorded at Kumirmari (15.82) and the highest recorded at Frasergunj (20.47). The TDS level

Table 1 — Physical parameters at four sampling sites of the Sundarbans during February, 2023

Sampling site	Waypoint		Distance from Bank		Light intensity (Lux)		Temp °C	Depth m	Salinity	pH	TDS mg/L	DO mg/L	Transparency inch
			Left (yd)	Right (yd)	Max	Min							
	Satjelia	22°09.943'N	088°53.325'E	494	559	50000	37200	22.71	3.8	17.26	8.14	14050	6.72
Kumirmari	22°09.661'N	088°54.948'E	563	130	3897	2936	22.6	6.2	15.82	8.17	12950	6.9	12.24
Dobanki	22°00.857'N	088°45.567'E	270	728	95000	78800	22.7	6.9	19.35	8.21	15520	6.61	24
Frasergunj	21°34.861'N	088°14.157'E	264	367	39620	35770	23.54	1.6	20.47	6.86	16580	6.79	26

also showed a similar gradient with the highest at Frasergunj (16580 mg/L) and the lowest at Kumirmari (12950 mg/L). The pH values ranged between 6.86 to 8.21 while, DO levels had a narrow range between 6.61-6.79 mg/L across all sites. The transparency was the highest at Satjelia (S1) [30 inches] which may be attributed to the highest light intensity observed at S1 during sampling. The average surface water temperature at the sampling sites ranged from 22.6-23.54°C with the highest at S4 (Frasergunj). The nutrient concentrations of nitrate, phosphate, and silicate have been presented in Table 2, which shows the highest nutrient load at Satjelia. Cortisol level in the fish samples of *O. biauritus* collected from the four sites ranged between 4.8 and 32 ng/mL while the Hsp70 protein ranged from 26.20 pg/mL to 39.45 pg/mL (Table 3). On an average, the fish sample collected from Satjelia had the highest level of cortisol whereas, elevated levels of Hsp70 were found in the specimens captured from Dobanki. The protein concentration in the muscle ranged between an average of 20563.06 and 35617.41 µg per gram of tissue. The protein profile as studied by SDS-PAGE revealed a few variations among the fishes collected from the four sampling sites (Table 4).

Water levels and circulation in the river network of the mangrove ecosystem in the Sundarbans are largely dependent simultaneously on the tidal oscillations and the freshwater inflow from the upstream. The water quality in turn controls the chemistry and biology of the estuaries. Among the four sites, the depth, salinity and TDS values show that Satjelia is the most affected by erosion and breaching of embankments as the construction of these embankments have not been done in a planned manner due to dearth of technical knowledge. A higher tidal amplitude and heavy siltation was quite obvious at Satjelia with a depth of just 3.8 m at the site selected for sampling. A somewhat alkaline pH of 8.14, high TDS of 14050 mg/L can be attributed to the semi-urban nature of this region which receives high inorganic and organic nutrient load from various natural and anthropogenic sources. Multifunctioning factors like hydraulic

Table 2 — Nutrient analysis of water samples collected from four sampling sites of the Sundarbans

Sampling site	Silicate (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Satjelia (S1)	10.4	12.4	0.034
Kumirmari (S2)	8.9	14.6	0.028
Dobanki (S3)	9.8	15.1	0.032
Frasergunj (S4)	9.2	14.2	0.03

Table 3 — Average cortisol, Hsp 70 and total muscle protein content of fish collected from four sampling sites of the Sundarbans

Sampling site	Specimen no.	Average cortisol level (ng/mL)	Average Hsp 70 protein level (pg/mL)	Average total protein conc. (µg/g of muscle tissue)
Satjelia (S1)	S1011	39.9	26.20	20605.87
Kumirmari (S2)	S2012	4.80	22.02	20563.06
Dobanki (S3)	S3014	15.4	39.45	54257.34
Frasergunj (S4)	S4014	32.0	36.67	35617.41

Table 4 — Comparative protein data of *Otolithoides biauritus* showing molecular weights of the proteins collected from four sampling sites of the Sundarbans

Molecular weight of Proteins in kDa	Satjelia (S1)	Kumirmari (S2)	Dobanki (S3)	Frasergunj (S4)
174.3	+	+	+	+
143.6	+	+	+	+
97.0	-	+	-	-
94.3	+	+	+	+
78.6	+	+	+	+
70.0	+	+	+	+
66.0	+	+	+	+
57.6	+	+	+	+
41.5	+	+	+	+
32.0	+	+	+	+
28.3	+	+	+	+
26.0	+	-	+	+
23.5	+	-	+	+
20.1	+	+	+	+
12.7	+	+	+	-
4.4	+	+	+	+

action, abrasion or corrosion, attrition and undercurrents responsible for erosion are working day and night in this part of the Sundarbans<sup>20</sup>. Due to the elevated levels of suspended solids in surface waters, re-aeration can be hampered, and hence DO is just 6.72 mg/L at Gadkhali. Dissolved oxygen concentration above 6.5-8.0 mg/L is considered healthy water for living organisms to survive<sup>21</sup>. Fish however requires DO concentration of up to >10 mg/L<sup>22</sup>. The high salinity and TDS (20.47 and

16580 mg/L, respectively) at Frasergunj with slightly acidic pH of 6.86 is obvious from its proximity to the sea. This area has been reported to experience a high salinity range, flooding, erosion and saline water intrusion<sup>15</sup>.

The nutrient concentrations of phosphate and silicate were also found to be highest at Satjelia, while Kumirmari recorded the highest nitrate concentration. While both Satjelia and Kumirmari experiences anthropogenic pressure from human settlements, Kumirmari faces a large amount of leaf litter deposition from the adjacent densely forested Morichjhaapi region. Increased level of pH has been found to favour the release of nitrogen and phosphorus nutrients in the water<sup>24</sup> during energy metabolism. Dobanki and Kumirmari recorded the highest pH of 8.17 among the four sampling sites. All aquatic organisms including fish depend directly on nutrients for their survival, growth and reproduction and the nutrient availability is directly related to the productivity of the water body. Nutrient input may occur anthropogenically or naturally through physical, chemical and biological processes.

An increase in the cortisol hormone concentrations of *O. biauritus* when exposed to increased salinity confirm the adaptation and acclimatisation of this fish to salted water and thus increase salinity tolerance. Apart from controlling the stress responses, cortisol is also a regulatory hormone for both metabolism and osmoregulation in fish. This study shows that the overall cortisol level was higher in fishes sampled from areas with higher salinity levels. Cortisol level was lowest in the specimens collected from Kumirmari where the salinity level and TDS was low when compared to the other sites, and with the highest DO among the sampled sites. Although highest salinity and TDS was recorded at Frasergunj, the cortisol level was highest (39.9 ng/mL) in the fish sampled from Satjelia, an area with comparatively high salinity and TDS. The high levels of silt and clay arising from the erosion of the embankments in this region and the low depth of the channel may contribute to the high TDS levels observed. Thus, increased cortisol levels may not only be induced by the high salinity but also by increased pH and TDS levels in these areas. High proportions of nutrients and suspended solids concentration showed maximum cortisol expression in fishes in this study. Although there are no references of basal level of cortisol in teleost fish, there are some reports of basal levels of

plasma cortisol in unstressed salmonid fish which normally lies in the range of 0-5 ng/mL<sup>24</sup>. The recorded level of cortisol in *O. biauritus* was quite high when compared to serum cortisol concentration under salinity stress in other fish. A similar study in the Brown-marbled grouper *Epinephelus fuscoguttatus*, a food fish in high demand in Asia, changed from 0.2972 to 0.4379 ng/mL at low changes in salinities (from 20 to 25 ppt)<sup>25</sup>. The mean serum cortisol level in another species of *Epinephelus* (*E. malabaricus*) fish when transferred to 29 and 34% seawater increased significantly to 84.246 and 97.465 nmol/L, respectively<sup>26</sup>. A similar study on plasma cortisol of *Oreochromis mossambicus* fry reared in seawater was found to be 2.44-fold higher than that of fry reared in freshwater (55.8ng/mL)<sup>27</sup>. The role of cortisol is unique to fish as it functions as a mineralocorticoid. In other vertebrates ranging from amphibians to mammals, aldosterone serves this function by acting through the mineralocorticoid receptor<sup>28</sup>. Since aldosterone is absent in fish<sup>29</sup>, this is compensated by the ability of the cortisol to bind to both glucocorticoid receptors (GR) and mineralocorticoid receptors in fish<sup>30,31</sup>. This corticosteroid in the teleost fish regulates both chloride cell differentiation and function. As *O. biauritus* is an amphidromous teleost migrating between fresh water and salt water, cortisol stimulates an increase in the number of chloride cells and activity of Na<sup>+</sup>-K<sup>+</sup> ATPase enzymatic activity in the gills during seawater acclimation thus increasing the overall salinity tolerance in synergy with insulin-like growth factor-I (IGF-I) and growth hormone<sup>32</sup>. On the other hand, when the fish is in a freshwater environment, cortisol presumably cooperates with prolactin (PRL) to ensure ion-osmotic homeostasis<sup>29</sup>. Although variation in cortisol level is associated with adaptation to negative stressors, all cortisol fluctuations in fish may not be indicative of distress. Changes in cortisol concentrations may be related to environmental cycles, circadian rhythms, sex, maturity and reproductive stages also<sup>33,34,35,36</sup>.

Environmental exploitation and stresses in this aquatic ecosystem are also evidenced by the level of Hsp70 protein, which is a natural biomarker that plays a vital role in the adaptive survival of environmental stress in fish. Physicochemical conditions in the four sampling sites were crucial in the Hsp70 induction and relative stress response. The Hsp70 level was highest in the fish captured from Dobanki (39.45

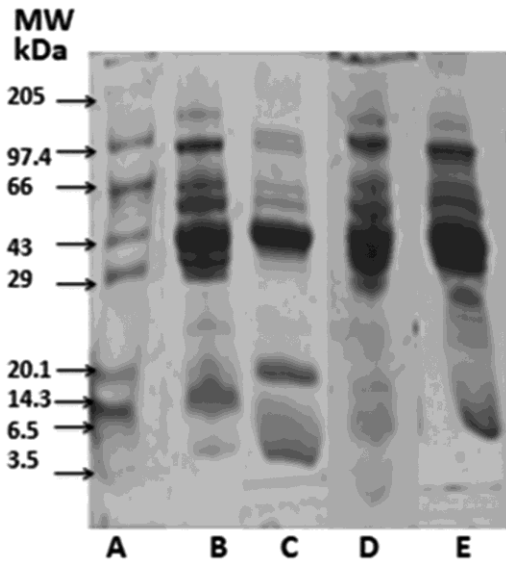


Fig. 1 — SDS-PAGE protein profile of *Otolithoides biauriatus* collected from the 4 sampling sites of the Indian Sundarbans; (A) Marker Protein; (B) Satjelia; (C) Kumirmari; (D) Dobanki; and (E) Frasersgunj

pg/mL) followed by Frasersgunj (36.67 pg/mL). Kumirmari fish sample showed minimum expression of Hsp70. Comparatively lower DO levels, probable prevalence of interspecific competition, niche overlap and competition for resources at the mixing layer of the Dobanki confluence zone may prove to be stressors for Hsp70 induction apart from the salinity factor. These results confirm a similar work done to study the effects of environmental salinity on 70 kDa heat shock protein (Hsp70) gene expression using divergent stickleback ecotypes (*Gasterosteus aculeatus*) from marine and freshwater habitats. In both ecotypes, the Hsp 70 of fish acclimated to marine water (20 ppt) was significantly higher compared to fish acclimated to freshwater (2 ppt)<sup>37</sup>.

The SDS-PAGE protein profile of the muscle tissue revealed a total of 16 protein bands ranging between the molecular weight of 4.4 kDa to 174.3 kDa (Table 3 and Fig. 1). The 94.3, 66, 57.6, 41.5 and the 32 kDa were the most dominant bands. A 97 kDa protein band was detected uniquely in the Kumirmari sample. However, the 26 and 23.5 kDa protein bands were absent, which were contrastingly present in the rest three samples. The 12.7 kDa protein band was absent in the fish collected from Frasersgunj. There was a notable overexpression of the 32 kDa protein in the samples collected from all sites except Kumirmari.

## Conclusion

The current study highlighted cortisol and Hsp70 as suitable biomarkers to analyse the scalable stress response of fish in the stressful aquatic ecosystem of the Indian Sundarbans. It indicates the capacity of fish to survive environmental stress by potentiating stress response through molecular activity and energy defence mechanisms. Water quality reflected through the physicochemical factors as the influencing stressors at the four sampling sites revealed Kumirmari (S2) as the least stressful zone. The spatial variations in protein profile of the fish populations suggest a probable effect of the stressors on the muscle protein too. Fish populations of *O. biauriatus* survived the synergistic effects of salinity, suspended solids, low DO and moderate concentration of pollutants through molecular alterations via cortisol hormone and Hsp70 activities.

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## Conflict of interest

Authors declare no competing interests.

## References

- 1 Blasco F, The Mangroves of India. In: *Institut Francis de Pondichery, Travaux de las Section Scientifique et Technique*, Tome XIV (Facicule 1. Pondichery), 1975. 80.
- 2 Syed MA, Hussain YA & Weir M, Detecting fragmented mangroves in the Sundarbans, Bangladesh using optical and radar satellite images. In: *The 22<sup>nd</sup> Asian Conference on Remote Sensing (Proceedings)*, Singapore, 2001.
- 3 Mitra A, Gangopadhyay A, Dube A, Schmidt ACK & Banerjee K, Observed changes in water mass properties in the Indian Sundarbans (northwestern Bay of Bengal) during 1980–2007. *Curr Sci*, 97 (2009) 1445.
- 4 Bhakta D, Das SK, Das BK, Behera BK, Nagesh TS, Johnson C & Chakraborty HJ, Morphological and molecular analysis of *Otolithoides pama* (Hamilton, 1822) (Perciformes: Sciaenidae) from Hooghly-Matlah estuarine system of West Bengal, India. *Indian J Geo-Mar Sci*, 50 (2021) 219.
- 5 Bhakta D, Das SK, Das BK, Behera S & Nagesh TS, Relationship between otolith morphometry and fish size of *Otolithoides pama* (Hamilton, 1822) from Hooghly-Matlah estuary, India. *Indian J Geo-Mar Sci*, 49 (2020) 1636
- 6 Mitra A, Zaman S, Pramanick P & Mitra S, Monitoring condition factor of the dominant fin fishes in the estuaries of lower gangetic delta in the backdrop of climate change. In:

- Climate Change Challenge (3C) and Social-Economic-Ecological Interface, Environmental Science* (Ed. Nautiyal S., Springer International Publishing, Switzerland), 2016, 347.
- 7 Froese R & Pauly D (Eds.). Fish Base. World Wide Web electronic publication. [http://www.fishbase.org/Country/CountrySpeciesSummary.php?c\\_code=356&id=4570](http://www.fishbase.org/Country/CountrySpeciesSummary.php?c_code=356&id=4570), 2013.
  - 8 Kizhakudan JK, Kizhakudan SJ & Megarajan S, *Otolithoides biauritus* (Cantor, 1849). *CMFRI Repository* (2017) 251.
  - 9 Pal M, Kar S & Mishra SS, An overview of the fishes of Indian Sundarbans and their conservation status. *J Environ & Sociobiol*, 11 (2014) 171.
  - 10 Habib KA, Neogi AK, Nahar N, Oh J, Lee YH & Kim CG, An overview of fishes of the Sundarbans, Bangladesh and their present conservation status. *J Threat Taxa*, 12 (2020) 15154.
  - 11 Gorissen M & Flik G, The endocrinology of the stress response in fish—An adaptation-physiological view. *Fish Physiol*, 35 (2016) 75.
  - 12 Mommsen TP, Vijayan MM & Moon TW, Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Rev Fish Biol Fish*, 9 (1999) 211.
  - 13 Wendelaar Bonga SE, The stress response in fish. *Physiol Rev*, 77 (1997) 591.
  - 14 Samaras A, A Systematic Review and Meta-Analysis of Basal and Post-Stress Circulating Cortisol Concentration in an Important Marine Aquaculture Fish Species, European Sea Bass, *Dicentrarchus labrax*. *Animals*, 13 (2023) 1340.
  - 15 Tejada-Vera R, López-López E & Sedeño-Díaz JE, Biomarkers and bioindicators of the health condition of *Ameioba splendens* and *Goodea atripinnis* (Pisces: Goodeidae) in the Ameioba river, Mexico. *Environ Int*, 33 (2007) 521.
  - 16 Thakur S, Dey D, Das P, Ghosh PB & De TK, Shoreline change detection using Remote Sensing in the Bakkhali Coastal Region, West Bengal, India. *Indian J Geosci*, 71 (2017) 611.
  - 17 Rice EW, Baird RB & Eaton AD, *Standard Methods for the Examination of Water and Wastewater* 23<sup>rd</sup> Edn. (American Public Health Association, American Water Works Association, Water Environment Federation VA, USA), 2017, 4500.
  - 18 Lowry CH, Rosebrough NJ, Farr AL & Randall RI, Protein measurement with folin phenol reagent. *J Biol Chem*, 193 (1951) 265.
  - 19 Laemmli UK, Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature*, 227 (1970) 680.
  - 20 Gour J, Pisciculture - A Changing Trend in Matla-Bidyadhari Interfluvium in Indian Sundarbans. *JGEE*, 2 (2012) 327.
  - 21 Bozorg-Haddad O, Delpas M & Loáiciga HA, Water quality, hygiene, and health. In: *Economical, Political, and Social Issues in Water Resources*, (Ed. Bozorg-Haddad O; Elsevier), 2021, 217.
  - 22 Torrans L & Steeby J, Effects of dissolved oxygen concentration on oxygen consumption and development of channel catfish eggs and fry: implications for hatchery management. *N Am J Aquac*, 70 (2008) 286.
  - 23 Hou D, He J, Lü C, Sun Y, Zhang F & Otgonbayar K, Effects of Environmental Factors on Nutrients Release at Sediment-Water Interface and Assessment of Trophic Status for a Typical Shallow Lake, Northwest China. *Sci World J*, (2013) 1.
  - 24 Pickering AD & Pottinger TG, Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiol Biochem*, 7(1989) 253.
  - 25 Tahir D, Shariff M, Syukri F & Yusoff FM, Serum cortisol level and survival rate of juvenile *Epinephelus fuscoguttatus* following exposure to different salinities. *Vet World*, 11 (2018) 327.
  - 26 Tsui WC, Chen JC & Cheng SY, The effects of a sudden salinity change on cortisol, glucose, lactate, and osmolality levels in grouper *Epinephelus malabaricus*. *Fish Physiol Biochem* 38 (2012) 1323.
  - 27 Ron B, Zohar Y, Borski R, Young G & Grau EG, Effects of dorsal aorta cannulation on cortisol and other stress parameters in the euryhaline tilapia, *Oreochromis mossambicus*. *Aquaculture*, 135 (1995) 213.
  - 28 Agarwal MK & Mirshahi M, General overview of mineralocorticoid hormone action. *Pharmacol Ther*, 84 (1999) 273.
  - 29 Prunet P, Sturm A & Milla S, Multiple corticosteroid receptors in fish: from old ideas to new concepts. *Gen Comp Endocrinol*, 147 (2006) 17.
  - 30 Bury NR, Sturm A, Le RP, Lethimonier C, Ducouret B, Guiguen Y, Robinson-Rechavi M, Laudet V, Rafestin-Oblin ME & Prunet P, Evidence for two distinct functional glucocorticoid receptors in teleost fish. *J Mol Endocrinol*, 31 (2003) 141.
  - 31 Sturm A, Bury N, Dengreville L, Fagart J, Flouriot G, Rafestin-Oblin ME & Prunet P, 11-Deoxycorticosterone is a potent agonist of the rainbow trout (*Oncorhynchus mykiss*) mineralocorticoid receptor. *Endocrinology*, 146 (2005) 47.
  - 32 McCormick SD, Endocrine control of osmoregulation in teleost fish. *Am Zool*, 41 (2001) 781.
  - 33 Sánchez-Vázquez FJ, López-Olmeda JF, Vera LM, Migaud H, López-Patiño MA & Míguez JM, Environmental cycles, melatonin, and circadian control of stress response in fish. *Front Endocrinol*, 10 (2019) 279.
  - 34 Strange RJ, Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. *Trans Am Fish Soc*, 109 (1980) 298.
  - 35 Hwang IJ, Kim SY, Kim HB & Baek HJ, Changes in plasma sex steroid and cortisol levels during annual reproductive cycle of ribbed gunnel, *Dictyosoma burgeri*. *Dev Reprod*, 16 (2012) 279.
  - 36 Pottinger TG, Balm PHM & Pickering AD, Sexual maturity modifies the responsiveness of the pituitary-interrenal axis to stress in male rainbow trout. *Gen Comp Endocrinol*, 98 (1995) 311.
  - 37 Metzger DCH, Healy TM & Schulte PM, Conserved effects of salinity acclimation on thermal tolerance and hsp70 expression in divergent populations of three spine stickleback (*Gasterosteus aculeatus*). *J Comp Physiol B*, 186 (2016) 879.