Island; so the number of misclassified pixels is also very high leading to 1.49% accuracy for SI and 0.73% accuracy for NDSI. The proposed index has identified saline blank pixels with an accuracy of 73.92%. The salinity of pixels representing different types of saline soils has also been physically tested and analysed, and then compared with the SBDI values for assessment of accuracy (Table 3).

It may be concluded after ground validation that SBDI detects saline blanks most accurately compared to the other indices.

In this study we have developed an index on hyperspectral image data for automatic detection of saline blanks amidst dense mangrove forests of the Sunderban Biosphere Reserve. We have successfully detected the hyperspectral bands that indicate the presence of minerals present in saline blank areas. The developed index has been compared with existing salinity indices (NDSI and SI). It is found that the former performs better than the latter, and accurately detects the saline blank areas of Henry Island of the Sunderbans Delta.

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Contrasting observation in culturable aerobic and micro-aerophilic heterotrophic fish gut-bacteria: intestine-breathing *Lepidocephalichthys guntea* (Hamilton Buchannan) versus gill-breathing *Labeo rohita*

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Lepidocephalichthys guntea **exhibits exceptional adaptive characters and can survive in flowing or stagnant waters as well as muddy hypercarbic condition resembling a desiccating habitat. This study was conducted to relate aerophilic (both aerobic and micro-aerophilic) bacterial density in the gut of** ***L. guntea*** **with dissolved oxygen (DO_2) content. Aerophilic bacterial density in the gut of** ***L. guntea*** **was found independent of DO_2 content, as the air pocket(s) present in the gut balances the deficit of oxygen obtained through gill respiration. This phenomenon was found to be reversed in gill-breathing fish like** ***Labeo rohita*** **because the additional mechanism to breathe air via gut is absent. The density of both the categories of**

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culturable aerophilic bacteria was found to be significantly reduced in the gut of *L. rohita* gut in low DO_2 condition of its habitat.

Keywords: Dissolved oxygen, gut bacteria, *Labeo rohita*, *Lepidocephalichthys guntea*.

THE loach, *Lepidocephalichthys guntea* displays unique adaptive characters and can survive in flowing or stagnant waters as well as semi-aquatic (muddy, low dissolved oxygen, hypoxic and hypercarbic condition) habitat. It has been observed that *L. guntea* uses intestine as an accessory respiratory organ, in addition to its original function in digestion[1]. The fish comes to the air–water interface with swift movement to gulp atmospheric air before returning back into the water[2,3]. The air which is gulped through the mouth after being used for oxygenation is voided out through the anus. The distance between the blood capillary and intestinal lumen has been found to be lower than the value reported for the secondary lamellae of this fish[4]. Hence, it was hypothesized that the aerophilic component of gut microbiome in *L. guntea* should respond differently in changed DO_2 condition compared to the de-stability of aerophilic bacterial population in gill-breathing fishes under DO_2 stress. The above hypothesis was thus tested and the results have been discussed in this communication.

A set of two glass aquariums [60 cm × 29 cm × 34.5 cm ($l \times b \times h$)] for each fish type was used; one was fitted with an aeration pump and the other was devoid of it. To each aquarium 7.8 litre water along with 600 g of river sediment was added such that the sediment height became 0.8 cm after settlement and total height of the water column, including sediment was 4.5 cm. When such a set-up was used to keep 18 gill-breathing fingerlings of *Labeo rohita* (mean length 12.4 ± 2.7 cm, mean weight 25.8 ± 3.7 g) or 60 *L. guntea* fishes (mean length 5.7 ± 1.0 cm, mean weight 2.0 ± 0.5 g), the DO_2 concentrations fell gradually from an initial value of 3.9 ± 0.3 mg l^{-1} to as low as 1.3 ± 0.2 mg l^{-1} in a span of one week. Each time when 150 ml volume of water was pipetted out (using a mechanical sucker attached to a glass pipette) in a small BOD bottle for DO_2 analysis, the same volume of tap water was refilled in the aquarium. In the other aquarium where the aeration pump was fitted, the air-sparger disc (diameter 2.0 cm, height 1.5 cm) was just immersed up to 1.5 cm from the water surface. Under such a set-up, DO_2 concentration was maintained (withdrawal of 150 ml water followed by refilling of the same volume of tap water each time during DO_2 analysis) at 5.0 ± 0–5.3 ± 0.3 mg l^{-1}. After the fishes were kept in the aquarium, live fishes were withdrawn from day 3 onwards for necessary sacrifice. For quantification of heterotrophic aerobic gut bacteria, plates were kept at room temperature (30 ± 2°C) for 24–48 h in an incubator (ICI, India). For quantification of micro-aerophilic heterotrophic gut bacteria, candle jar method was used. Inoculated plates were placed inside a thick-walled, transparent, heat-tolerant, gas-impermeable desiccator (diameter, 300 mm) (Tarson, India) and a candle was lit and kept in it before the lid was sealed. Sealing was done with grease between lid and base of the desiccator and then the joined area was covered externally with para-film along the circumference to enable micro-aerophiles to grow in an elevated concentration of dissolved free CO_2 along with diminished (very low) concentration of oxygen[5].

L. guntea and *L. rohita* were captured from the experimental aquariums and their guts were carefully removed. Each gut was cut into anterior, mid and posterior parts and fixed in Bouin's fluid containing 10% formaldehyde. Then the tissues were dehydrated in increasing ethanol grades and embedded in paraffin (MT 56–58°C)[6]. Then 6 µm thick sections of tissues were cut with the aid of a microtome (Lipshaw-type rotary microtome, York Scientific Industries Pvt Ltd, India), and stained with hematoxylin and eosine stain. Stained sections were observed under a light microscope (Dewinter Technologies, Model DEW/002, Dewinter Optical Inc., India) and photographed (DIGIEYE powered through USB 2.0-equipped desktop computer). The results were statistically validated using online (in-silico.net/tools/statistics/t-test) and SPSS 16 software.

Live samples of *L. guntea*, collected from the stream water during different months (variable DO_2 content), revealed insignificant differences in net culturable heterotrophic gut-bacterial load (data not shown). We hypothesized that this could be a phenomenon in case of gut-breathing *L. guntea* but not in the case of gill-breathing fish like *L. rohita*. Hence, we simulated DO_2 content (as of the field condition) by manipulating water column height over the stream sediments (from the same stream) deposited in the experimental aquariums setup in the laboratory. Influence of DO_2 content on the culturable heterotrophic (both aerobic and micro-aerophilic) bacterial load in the gut of both gut-breathing (in addition to gill) and solely gill-breathing fishes, reared separately in different aquariums, was studied. Two different DO_2 conditions in the aquarium water, i.e. (i) 5.0 ± 0 to 5.3 ± 0.3 mg l^{-1} and (ii) 3.9 ± 0.3 to as low 1.3 ± 0.2 mg l^{-1} were maintained. Homogenized gut content of the two different fishes, reared in two different DO_2 conditions, was dilution-plated to enumerate aerobic heterotrophic (plates kept in the bacteriological incubator) as well as micro-aerophilic (plates kept in the candle jar) bacterial load (Table 1). Both sets of plates were incubated at room temperature because the fishes (being poikilothermic) were also reared at room temperature in the aquarium. Total culturable aerobic as well as micro-aerophilic bacterial count of *L. guntea* gut showed no statistically significant difference between samples reared in high and low DO_2 conditions (Table 2). The result was just the opposite in case of *L. rohita*; aerobic or micro-aerophilic

Table 1. Aerobic and micro-aerophilic bacterial density in the gut of *Lepidocephalichthys guntea* and *Labeo rohita* reared in aquariums having different dissolved oxygen (DO_2) conditions

Lepidocephalichthys guntea			*Labeo rohita*		
DO_2 content	Total aerobic bacterial count ($\times 10^5$ cfu g^{-1} gut tissue)	Total micro-aerophilic bacterial count ($\times 10^5$ cfu g^{-1})	DO_2 content	Total aerobic bacterial count ($\times 10^5$ cfu g^{-1} gut tissue)	Total micro-aerophilic bacterial count ($\times 10^5$ cfu g^{-1})
High	(A) 232 ± 33	(B) 125 ± 9.7	High	(C) 708 ± 11.8	(D) 565 ± 29
Low	(A′) 272 ± 47	(B′) 115 ± 7.0	Low	(C′) 25 ± 4.4	(D′) 40 ± 7.9

Each value is the mean of five sample observations and standard deviation (±).

Table 2. Testing significant difference, if any, in gut aerobic and micro-aerophilic bacterial density under two different states of dissolved oxygen content of water in case of an intestine-breathing fish (*L. guntea*) and a gill-breathing fish (*L. rohita*) with the aid of two-sample *t*-test

Lepidocephalichthys guntea		*Labeo rohita*	
Sample combination[#]	Results	Sample combination[#]	Results
A and A′	*t*-value = 1.5575 d*f* = 7.17 *P* value ≤ 0.1623 Two samples are not significantly different	C and C′	*t*-value = –121.2702 d*f* = 5.0912 *P* value ≤0.0001 Two samples are significantly different
B and B′	*t*-value = 1.8693 d*f* = 7.2774 *P* value = 0.1022 Two samples are not significantly different	D and D′	*t*-value = –39.0573 d*f* = 4.5904 *P* value ≤ 0.0001 Two samples are significantly different

[#]Sample combinations represent total count of aerobic bacteria at high (A) and low (A′) DO_2 conditions as well as micro-aerophilic bacteria at high (B) and low (B′) DO_2 conditions in the gut of *L. guntea*; and total count of aerobic bacteria at high (C) and low (C′) DO_2 conditions as well as micro-aerophilic bacteria at high (D) and low (D′) DO_2 conditions in the gut of *L. rohita*.

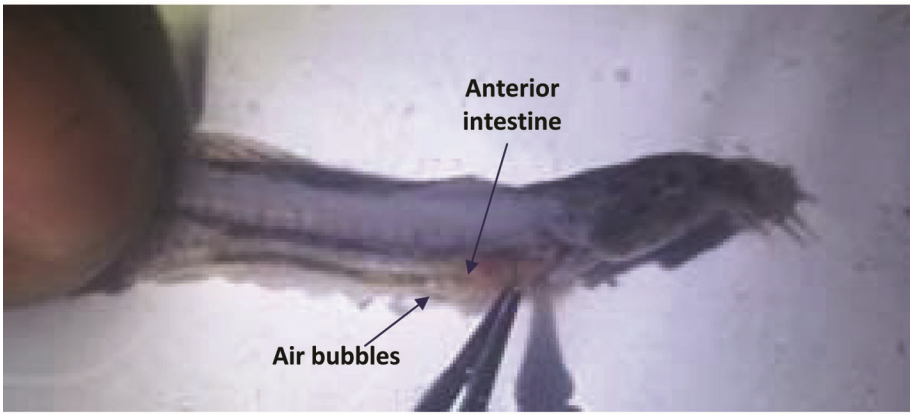

Figure 1. Anatomical observation of whole gut of *Lepidocephalichthys guntea*.

gut-bacterial load in high DO_2 condition was significantly different from that in low DO_2 condition (Table 2). Higher DO_2 content showed higher incidence of aerobic or micro-aerophilic load and vice versa. Therefore, it is plausible that the intestine (gut)-breathing mechanism in *L. guntea* supports the growth of aerobic heterotrophs in its gut (heterotrophic bacterial density remained unaffected) under low DO_2 condition in the surrounding water, while under the same prevailing low and high DO_2 conditions, the difference in aerobic and micro-aerophilic bacterial load is significant in gill-breathing fish like *L. rohita*. The results of this study (Tables 1 and 2) thus support our hypothesis.

Earlier studies have shown that posterior intestine of *L. guntea* is probably adapted to suit its role for aerial respiration because of considerable reduction of the absorptive area and penetration of blood capillaries between the columnar epithelial cells[7]. However, in the study, we have anatomical and histological evidences to prove that air-breathing facility in the gut of *L. guntea* is not restricted to the posterior part, but also extends to the anterior part. Two to three air bubbles, in live dissections,

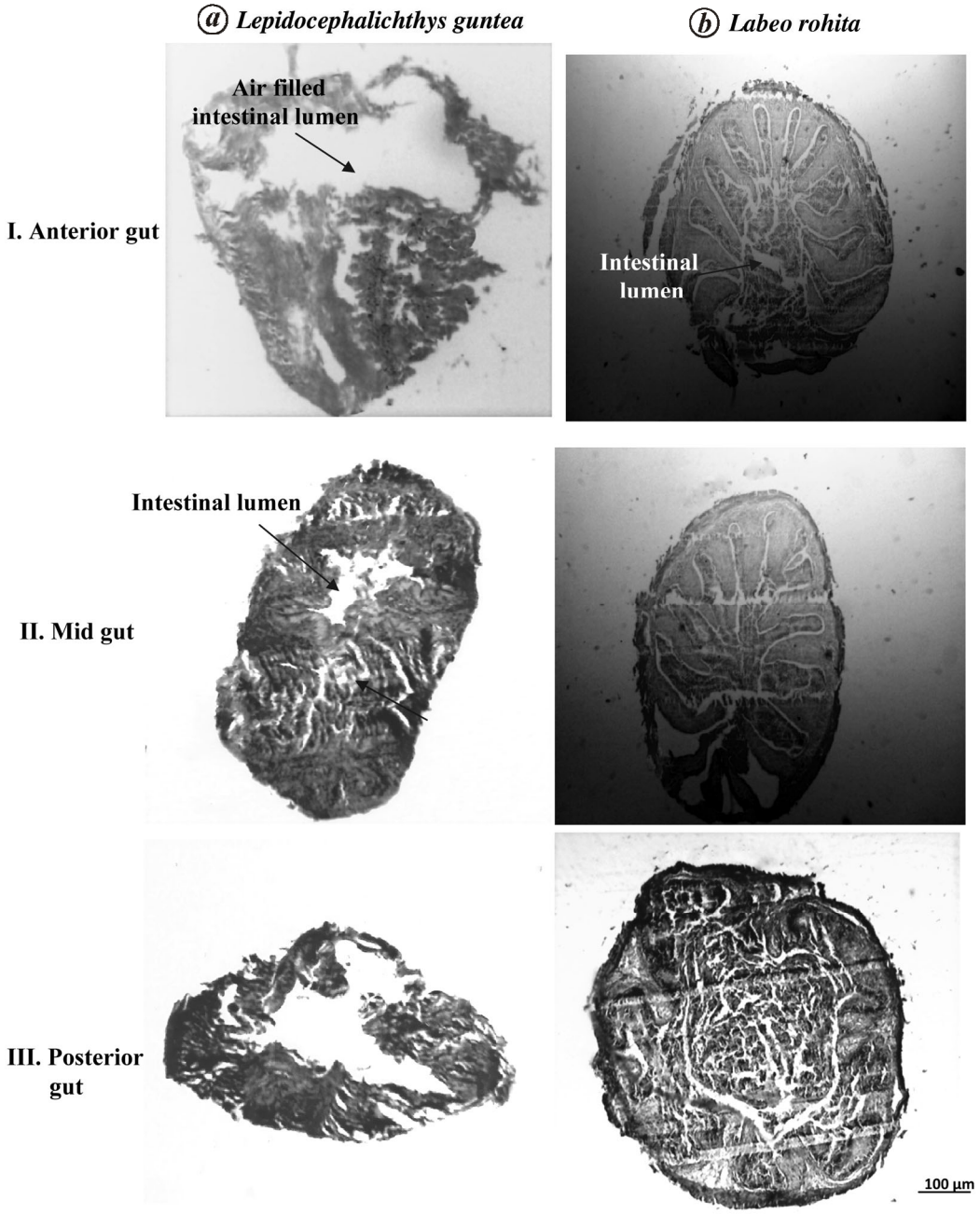

Figure 2. Comparative histological observation of different gut regions of *Lepidocephalichthys guntea* (intestinal breather) and *Labeo rohita* (gill breather) observed at high magnification (40×).

were always found to remain prominently visible in the anterior region of the gut. The entire gut is fortified with rich blood supply. The blood vessels are highly branched in the anterior region and throw out capillaries up to the epithelial layer; this makes the gut reddish in appearance (Figure 1). A sub-intestinal plexus in the anterior region of the gut was also present (Figure 2 *a*(II)). Thus the anterior intestine has two distinct functional regions – the upper respiratory and the lower nutritive part (Figure 2 *a*(I)), which is markedly different from *L. rohita* (Figure 2 *b*(I)). The mid intestine of *L. guntea* has an architecture similar to the anterior one (Figure 2 *a*(II)). Histology of the posterior intestinal region (Figure 2 *a*(III)) is much simpler in nature. These observations clearly indicate that the anterior region of the gut is involved in aerial respiration. Thus not only the posterior intestine, but the entire gut is involved in aerial respiration of *L. guntea*.

To the best of our knowledge, this is the first report where the plausible effect of intestinal air-breathing

phenomenon in *L. guntea* on the aerophilic and micro-aerophilic heterotrophic gut microflora has been studied taking exclusive gill-breathing fish as control to examine the differences in terms of microbiology and gut-architecture (revealed through histology).

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

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Response of fast ice to ground penetrating radar and backscattering coefficient from scatterometer in Larsemann Hills, East Antarctica

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The study presents inter-annual variations in the backscatter response of fast ice (sea ice attached to the coast) to C band Advanced Scatterometer (ASCAT) (2012–2016). It also analyses the Ground Penetrating Radar (GPR) observations collected during the 35th Indian Scientific Expedition to Antarctica (ISEA, 2015–16) for identification of different fast ice features and to measure fast ice depth in the Larsemann Hills area, East Antarctica. Apart from clear demarcation of features like melt water channels, frozen icebergs within fast ice and underlying topography near island, GPR provided fast ice depth information, which was used to understand backscatter response. The seasonal variations of C band backscatter were caused due to changes in snow thickness, time of freezing and sporadic melt/freeze events apart from summer melt. The backscatter response to NOAA high resolution blended daily sea surface temperature (SST) variations indicate that sudden rise/fall in backscatter during winter is probably due to sporadic melt/freeze events caused by rise/fall in SST. The results show volumetric contribution from sheet ice and domination of snow metamorphism towards increase in backscatter over fast ice. This study highlights the importance of monitoring backscatter response of fast ice to determine its state and condition. Depending on the characteristics of backscatter inter-annual curve, information about time of freeze up, melt season, ice build-up, and sporadic freeze/thaw events can be inferred which play an important role in the energy budget of Antarctica.

Keywords: Antarctica, ASCAT, fast ice, GPR, Larsemann Hills.

THE Larsemann Hills area is located on the south-eastern coast of Prydz Bay, Princess Elizabeth Land, in East Antarctica. Because of its ice free area of approximately 40 sq. km, this region is environmentally, scientifically and logistically significant. The ice-free area consists of two major peninsulas (Stornes and Broknes), four minor peninsulas, and approximately 130 near-shore islands[1]. Indian research base 'Bharati' is located between Thala Fjord and Quilty bay, east of Stornes Peninsula in Antarctica at 69°24.41′S, 76°11.72′E. Fast ice formation in this area varies every year with varying ice thickness surrounding Bharati station. The fragmented land is bounded by open sea in the North and polar sheet ice in the South. Strong winds blow from NE to SW[2]. Temperature during the study period (2012–2017) reached as high as 6.23°C in summer and dipped to –36.26°C in winter. The fast ice in this area is single year ice which states disintegrating upon the arrival of supply ship to Bharati station. This leads to the development of flaws in ice, which act as zones of high backscatter.

Researchers studied the snow on Antarctic sea ice using data collected over 10 years to characterize snow thickness, snow type and their geographical and seasonal variations; snow grain size, density, and salinity; frequency of occurrence of slush; thermal conductivity, snow surface temperature, and temperature gradients within snow; and the effect of snow thickness on albedo.

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