DEVELOPMENT OF FRESHNESS PREDICTION MODELS FOR *LABEO ROHITA* (HAMILTON,1822) AND *PENAEUS VANNAMEI* (BOONE,1931) STORED IN ICE

Dissertation submitted in partial fulfillment of the

requirements for the degree of

M.F.Sc. (Fish Processing Technology)

By

AMAN KUMAR MISHRA, B.F.Sc.

FPT-MB2-02

ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION

(University Established Under Section 3 of UGC Act 1956)

Panch Marg, Off Yari Road, Versova,

Andheri (W), Mumbai – 400 061

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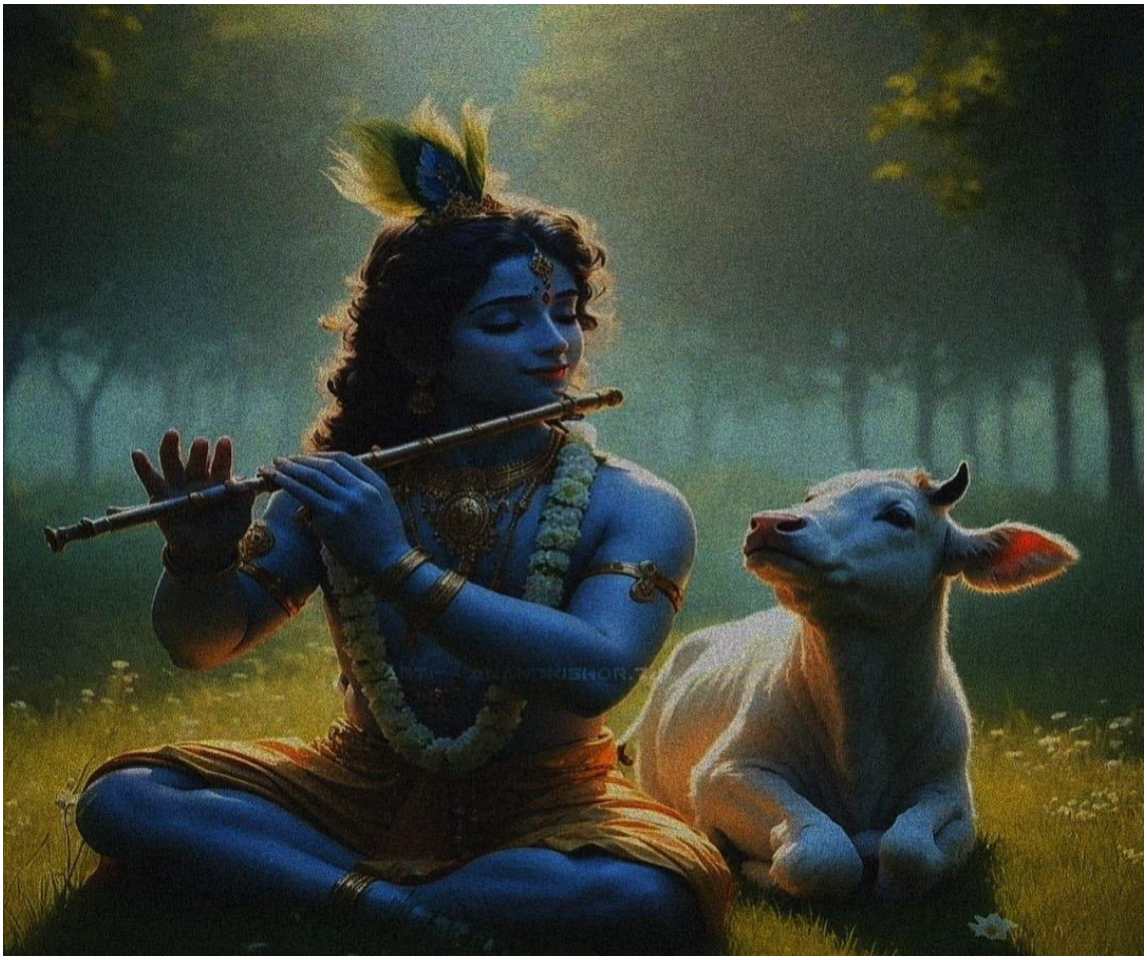
Dedicated to LORD KRISHNA,

My beloved family &

friends



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(A University Established Under Sec. 3 of UGC Act 1956)
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Govt. of India.

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CERTIFICATE

Certified that the dissertation entitled "**DEVELOPMENT OF FRESHNESS PREDICTION MODELS FOR *LABEO ROHITA* (HAMILTON,1822) AND *PENAEUS VANNAMEI* (BOONE,1931) STORED IN ICE**" is a bonafide record of independent research work carried out by **Mr. Aman Kumar Mishra** during the period of study from January, 2024 to September, 2024 under our supervision and guidance for the degree of **Master of Fisheries Science (Fish Processing Technology)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

Major Advisor

(Layana P.)
Scientist,
FRHPHM Division,
ICAR-CIFE, Mumbai

Advisory Committee

(B. B. Nayak)
Principal Scientist and Head
FRHPHM Division
ICAR-CIFE, Mumbai

(Karankumar Ramteke)
Scientist, FRHPHM
Division, ICAR-CIFE,
Mumbai

(Rabi Narayan Sahoo)
Principal Scientist,
Division of Agricultural
Physics, ICAR-IARI,
New Delhi

पंच मार्ग, ऑफ यारी रोड, वरसोवा, अंधेरी (प), मुंबई - ४०० ०६१. (भारत)
Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061. (India)
कार्यालय / Office : 022-26361446/7/8,
Fax : 022-26361573
Website : www.cife.edu.in

DECLARATION

I hereby declare that the dissertation entitled **"DEVELOPMENT OF FRESHNESS PREDICTION MODELS FOR *LABEO ROHITA* (HAMILTON, 1822) AND *PENAEUS VANNAMEI* (BOONE, 1931) STORED IN ICE"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

Aman mishra.

Date: 30 September, 2024

Place: Mumbai

(AMAN KUMAR MISHRA)

M.F.Sc. Student

ICAR-CIFE Mumbai

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Aman mishra.

(AMAN KUMAR MISHRA)

सारांश

ताजगी की भविष्यवाणी करने के लिए एक मॉडल विकसित करने के उद्देश्य से, जो मछलियों की ताजगी और क्षरण के बीच अंतर कर सके, इस अध्ययन में कई प्रयोग किए गए। चुनी गई मछलियां लाबियो रोहिता (हैमिल्टन, 1822) और पेनीअस वन्नामेई (बून, 1931) थीं। चूंकि मछली का क्षरण संचयी और तेजी से बढ़ने वाली प्रक्रिया है, प्रारंभिक प्रयोगों का उद्देश्य प्रत्येक क्षरण सूचकांक (spoilage index) के लिए महत्वपूर्ण अस्वीजन बिंदु (Critical Rejection Point - CRP) निर्धारित करना था, जिसे तात्कालिक और विलंबित बर्फीकरण द्वारा प्रभावित किया गया। इस उद्देश्य के लिए ताजे रोहू को तापमान दुरुपयोग के तहत रखा गया, जहां मछलियों को बर्फ में रखने से पहले कमरे के तापमान पर 4 घंटे (T1), 8 घंटे (T2), 12 घंटे (T3), और 16 घंटे (T4) तक रखा गया, जबकि नियंत्रण समूह (C) को पकड़ने के तुरंत बाद बर्फ में रखा गया। मछलियों के सिर, मध्य और पूंछ के हिस्सों पर जैव रासायनिक परीक्षण किए गए, जबकि सूक्ष्मजीवविज्ञान परीक्षण के लिए संयुक्त नमूनों का उपयोग किया गया। चार गुणवत्ता मापदंड – TVB-N, TBARS, pH, और TPC को भंडारण के दौरान प्रतिदिन मापा गया। मछली और उसके विभिन्न अंगों की प्रतिदिन तस्वीरें ली गईं ताकि ताजगी का अनुमान लगाने वाला मॉडल विकसित किया जा सके। तापमान दुरुपयोग के कारण TVB-N का CRP 16 घंटे पर पहले दिन, 12 घंटे पर दूसरे दिन, 8 घंटे पर तीसरे दिन, और 4 घंटे पर चौथे दिन निर्धारित किया गया। pH प्रारंभ में स्थिर रहा लेकिन पहले दिन के बाद तेजी से बढ़ा, जो क्षरण का संकेत देता है। TPC सभी उपचारों में दिन 0 से दिन 4 तक लगातार बढ़ता रहा, जबकि TBARS मानों में अनियमित उतार-चढ़ाव देखा गया। सबसे तेज क्षरण मछलियों के मध्य भाग में पाया गया। इसी प्रकार का प्रयोग P. vannamei के लिए भी किया गया। संयुक्त नमूनों पर जैव रासायनिक और सूक्ष्मजीविकीय परीक्षण किए गए। पेनीअस वन्नामेई में तापमान दुरुपयोग के कारण TVBN का CRP 16 घंटे और 12 घंटे पर पहले दिन और 8 घंटे और 4 घंटे पर चौथे दिन निर्धारित किया गया। TPC लगातार बढ़ा और pH प्रारंभ में स्थिर रहा लेकिन भंडारण के पहले दिन अचानक बढ़ गया, जिससे क्षरण का संकेत मिला। रोहू पर तापमान दुरुपयोग प्रयोग के दौरान ली गई छवियों से बनाए गए भविष्यवाणी मॉडल की सटीकता 12.7% पाई गई, जबकि सामान्य बर्फीकरण में ली गई छवियों से यह सटीकता 73.7% रही। पेनीअस वन्नामेई के लिए सटीकता 99% से अधिक पाई गई। इस अध्ययन द्वारा विकसित मॉडल तीसरे दिन की ताजा मछली को दसवें दिन की खराब मछली से अलग करने में सहायक होगा।

ABSTRACT

In order to develop a model for predicting freshness based on images that can distinguish between fresh and spoiled fish, several experiments were conducted in this study. The fishes chosen were *Labeo rohita* (Hamilton, 1822) and *Penaeus vannamei* (Boone, 1931). Given that spoilage is accumulative and accelerative, the initial experiments focused on establishing critical rejection points (days) for for each spoilage index as affected by immediate and delayed icing. For this purpose, fresh Rohu were subjected to temperature abuse by holding the fish at room temperature for 4hr (T1), 8hr (T2), 12hr (T3), and 16hr (T4), prior to their ice storage, while control (C) lot was iced immediately after harvest. Biochemical analyses were conducted on head, mid and tail sections of the fish while pooled samples were used for microbiological test. Four quality parameters i.e., TVB-N, TBARS, pH, and TPC were measured daily during storage. Images of the whole fish and its body sections were captured daily by using digital camera to develop freshness prediction models. The CRP (Critical rejection point) for TVBN as affected by temperature abuse was determined as day 0 for 16hr, day 1 for 12 hr, day 2 for 8hr and day 3 for 4 hr. The pH initially remained stable but showed a sharp increase after day 1, indicating spoilage. TPC showed a continuous rise from day 0 to day 4 for all treatments, whereas TBARS values exhibited irregular fluctuations. Rate of spoilage was found to be highest in the mid-section. Similar experiment was conducted for *P. vannamei*. Biochemical and microbiological parameters were performed on pooled sample. The CRP for TVBN as affected by temperature abuse for *L. vannamei* was determined as day 1 for 16hr and 12 hr and day 4 for 8hr and 4 hr. TPC increased continuously and pH remained stable initially, then increased abruptly on day 1 of storage, indicating spoilage. The prediction model developed with images captured during temperature abuse experiment of Rohu showed very less accuracy of 12.7%. However, images taken during normal ice storage of Rohu showed good accuracy rate of 73.7%. For *P. vannamei* excellent accuracy over 99% was obtained. The model developed in the present study will help to differentiate a fresh fish on 3rd day of storage from a spoiled one on 10th day of storage.

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%	Percentage
°C	Degree Celsius
Fig.	Figure
g.	Gram
h.	Hours
ml	Milli litre
min	Minutes
nos	Numbers
cfu	Colony forming units
mAP	Mean average precision
CRP	Critical rejection point
TVB-N	Total Volatile Basic Nitrogen
TMA	Trimethylamine
TBARS	Thiobarbituric Acid Reactive Substances
FFA	Free Fatty Acids
WHC	Water Holding Capacity
TPC	Total Plate Count

1. INTRODUCTION

Globally, a significant portion of food is wasted or spoiled during transportation and storage (FA0, 2020). Fish and seafood are highly perishable commodities, thus maintaining a cold chain during distribution and storage is critical to prevent quality degradation and associated health hazards. Temperature abuse and microbial contamination can cause fish to spoil rapidly. The growing consumer demand for high quality seafood fuelled by the desire for better nutrition and health, has put further strain on the distribution chain. As cloud market chains continue to expand their reach, real-time monitoring of fish freshness has become crucial to meet the growing demand for quality seafood while preventing spoilage and post-harvest losses. This demands the development of innovative systems that can accurately assess fish freshness in real-time while providing consumers dependable information about the quality of the seafood they purchase.

In India, fresh fish isn't always easy to find due to the long distances between key fishing areas and consumer markets, as well as the seasonal nature of the fishing industry. To address this, chilling has become a common method to preserve the fish's nutritional value and quality. By lowering the temperature using ice, the growth of bacteria responsible for food spoilage and foodborne illnesses is reduced, also slows down the natural biochemical changes that occur after the fish is caught. (Gandotra *et al.,* 2017.). A reduction in fish temperature by 5 ^{o}C could reduce the rate of fish spoilage by half (Heen, 1982). However, spoilage is accumulative and accelerating. Temperature abuse or delay in icing will accelerate spoilage at a later stage in the distribution and can cause significant reduction in quality and shelf life of fresh seafood. It is well known that both enzymatic and microbiological activity are greatly influenced by temperature. However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth. A close view of data published by (Huss, 1995) states that one hour at normal air temperature will cause as much spoilage as 15 hrs at 0^{o}C.

The quality loss of fish is traditionally determined by methods involving organoleptic evaluation, enumeration of spoilage bacteria and biochemical quality evaluation. Although these methods have been successfully employed in freshness studies, they are time consuming, tedious, requires lots of consumables, equipment, labor and cost intensive, and are less efficient. To overcome this situation nondestructive modern analytical techniques which involves machine learning and artificial intelligence is used (Lytou *et al.,* 2024).

Machine learning, the core technology of artificial intelligence, utilizes a diverse set of algorithms capable of analysing and processing information, such as images uploaded to a system. Through this process, raw data is transformed into valuable insights that can be leveraged to make predictions or estimations. Machine learning models need to be trained using datasets (Cui *et al.,* 2024). The predictive model is built using several features, and as such, parameters of the models are determined using historical data during the training phase. For the testing phase (20-30%) dataset is used, which is part of the historical data that has not been used for training phase is used for the performance evaluation purpose (Van Klompenburg *et al*., 2020).

In the context of food quality assessment, machine learning proves to be highly beneficial. It facilitates the evaluation of food quality without the need for physical contact or destructive testing methods, preserving the integrity of the food product (He *et al.,* 2015). This non-invasive technique allows accurate, efficient, and automated quality control, making it an essential tool in modern food safety and quality assurance practices. By continuously learning from vast datasets, machine learning systems can adapt and improve, further enhancing their predictive accuracy (Yavuzer & Köse, 2022).

Application of image-based AI models are gaining attention in food processing quality control for freshness and shelf-life prediction, defect detection and product inspection (Wu *et al.,* 2022; Rayan *et at.,* 2021). It is applicable to a wide range of products, from perishable goods to pharmaceuticals, enhancing its versatility across industries. Image based quality and shelf-life prediction model brings efficiency, accuracy and proactive management for industries that handle perishable goods (Ismail *et al.,* 2021, Chu et al., 2021).

The usual image-based methods employed in food quality assessment are hyperspectral imaging, fluorescence imaging, multispectral imaging, standard visual imaging, and RGB imaging. Both hyperspectral and multispectral cameras, often mounted on drones, are frequently utilized for assessing the quality of crops during on-site monitoring (Nia *et al.,* 2019; Momin *et al.,* 2023; Hardy *et al.,* 2024). For RGB imaging, a DSLR camera is typically used to capture high-resolution images. These methods collectively provide a wide range of spectral data, allowing for detailed analysis of various aspects of the subject, whether it be crop health or other quality indicators in fisheries and agricultural practices (Ulhmann *et al.,* 2020).

Despite having many advantages over the traditional method, very limited work has been done till now in the field of fish quality assessment and monitoring. Non-destructive spectroscopic and imaging techniques for fish quality evaluation was developed by He *et al.,* (2015), fluorescence spectroscopy, RGB, and multispectral imaging for assessing white meat quality was employed by Fan and Su., (2022) and a portable multispectral imaging device was developed for Seabream quality monitoring (Lytou *et al.,* 2024)

In this context the aim of our study is to develop an image-based freshness prediction model for commercially important species. The model species chosen in the study are *Penaeus vannamei* and *Labeo rohita* because of their high market value and export demand. With this background, the following objectives were set for the present study

1) To determine critical rejection points for spoilage indices of *Labeo rohita* and *Penaeus vannamei* during ice storage
2) To develop image-based quality prediction models for *Labeo rohita* and *Penaeus vannamei* during ice storage

2. REVIEW OF LITERATURE

2.1 Chilled storage of fish

Chilled storage involves keeping samples in ice for a limited period, which slows the rate of spoilage compared to storing at room temperature. The principle of preservation by chilling involves lowering the temperature of food to slow down the microbial growth, biochemical reactions, and enzymatic activity within the organism, thereby delaying spoilage and extending the shelf life of the product. By maintaining food at temperatures just above freezing, between 0°C and 5°C, chilling reduces the growth and reproduction of microorganisms and minimizes the degradation of food quality. This method is effective in preserving the freshness, texture, and nutritional value of perishable items without significant alterations to their chemical structure, making it one of the most widely used and convenient techniques for short-term food preservation.

The shelf life of ice stored Pacific white shrimps was determined to be 8 days (Okpala *et al.,* 2014). Shelf-life of catla stored in ice immediately after death was 20 days but shelf-life reduced to 12 days when icing was delayed for 10 hr after death. Similar trend was observed for two other fish species of magur and tilapia (Nabi *et al.,* 2001).

2.2 Quality changes in fishes and shrimps during chilled storage

During chilled storage, fish and shrimp undergo a range of quality changes due to biochemical, microbiological, and physical processes. Biochemically, enzymes continue to break down proteins and lipids, leading to off-Flavour and textural degradation. Lipid oxidation, particularly in fatty fish, causes rancidity. Microbial spoilage, mainly from psychrotrophic bacteria which leads to the production of unpleasant odors, slime formation, and further spoilage. Physical changes such as moisture loss, tissue softening, and drip loss contribute to textural and visual deterioration. Over time, these processes result in a loss of freshness, indicated by changes in appearance, texture, and flavour. Effective temperature control and storage conditions are essential to minimizing these quality changes and extending the shelf life of chilled stored fish and shrimp.

Liu *et al.* (2013), Studied physical and biochemical parameters of grass carp (*Ctenopharyngodon idella*) fillets stored at −3 and 0 °C. This study aimed to examine the impact of super chilling at −3 °C compared to ice storage at 0 °C on the biochemical and physical properties of grass carp fillets. Fillets stored at −3 °C experienced significant alterations in whiteness, drip loss, and textural hardness, while shifts in pH, total volatile basic nitrogen, and TCA-soluble peptides were slower. Over the 21-day storage period, both benefits and drawbacks were identified for fillets stored at −3 °C compared to those at 0 °C. Proteolytic degradation and microbial spoilage were notably delayed at −3 °C, but the increased tissue damage caused by super chilling led to quicker deterioration in appearance, water retention, and texture.

Hozbor *et al.* (2006), studied the microbiological changes and their relationship with quality indices during aerobic iced storage of sea salmon. They observed the variations in the microbial flora of sea salmon (*B. sandperch*) stored at 0°C under aerobic conditions. Psychrotrophic bacteria exhibited similar behaviour to mesophilic bacteria, although their counts were consistently higher at 5°C, reaching 12 log10 cfu/g by the end of the storage period. These findings indicate the significant role of these bacteria in determining the product's shelf life.

The sensory attributes and levels of biochemical freshness indicators in ungutted rainbow trout *(Oncorhynchus mykiss)* stored on ice for up to 12 days were analysed. The findings indicate that both the K1 index and Hx ratio are reliable indicators of trout freshness when stored on ice, whether gutted or not; a K1 value above 70% and an Hx ratio exceeding 13% signal unacceptability. However, these indicators are not effective for assessing freshness in vacuum-packed trout stored under refrigeration (Rodriguez *et al.,* 1999).

In a research, fresh Mediterranean horse mackerel *(Trachurus mediterraneus)* were exposed to gamma irradiation at doses of 1 and 2 kGy and stored on ice for 18 days. Quality changes during storage were assessed by evaluating microbial counts, trimethylamine (TMA), and volatile basic nitrogen levels. Additionally, lipid composition and sensory characteristics were analysed. The irradiation treatment effectively reduced bacterial counts throughout the storage period. Although TMA and total volatile basic nitrogen (TVB-N) levels increased in all samples over time, irradiation notably restricted these increases, even at the lower dose of 1 kGy.

According to the quality index method, the control group had a sensory shelf life of 4 days, while the irradiated samples had an extended shelf life of up to 9 days. Similarly, thiobarbituric acid-reactive substances (TBARS) initially rose after irradiation on the first day but were lower at the end of storage compared to the control group. The results highlight that gamma irradiation is an effective supplement to chilled storage, enhancing microbiological quality and prolonging the shelf life of small pelagic fish species (Mbarki *et al.,* 2020).

A study was conducted on the biochemical changes in *P. monodon* and *M. rosenbergii* during 10 days of ice storage. The TVB-N value of 1 day ice stored shrimp was 10.5 mg/100g of sample. The TVB-N values gradually increased over the storage period, reaching up to 60 mg/100g by the end of the 10 days. The tiger head-on shrimp remained organoleptically acceptable for up to 8 days in ice storage, during which the TVB-N values were recorded at 32.2 mg/100g, slightly exceeding the recommended export limit for TVB-N (Kamal *et al.,* 2000).

Kamal *et al.* (2000), conducted a study on biochemical changes *in P. monodon* and *M. rosenbergii* during ice storage for 10 days. At the end of 10 days of ice storage, moisture content of freshwater prawn slightly decreased from 78.34 to '77.35%.

The study examined the quality changes in whole ungutted golden gray mullet *(Liza aurata)* during storage either on ice or in a refrigerator (without ice). The analysis focused on microbiological quality (including total viable and psychrophilic counts, lactic acid bacteria, and Enterobacteriaceae), chemical quality (pH, total volatile basic nitrogen, trimethylamine, peroxide value, thiobarbituric acid, and free fatty acids), and sensory attributes of the raw fish over 16 days. The shelf life of the golden gray mullet was found to be 10 days on ice and around 14 days in the refrigerator. Initial TVC levels for fresh fish were 3.10 log CFU/g muscle for both storage methods, which increased steadily to 7.63 and 7.11 log CFU/g muscle by day 16 for refrigerator and iced storage, respectively ($P < 0.05$). Considering that 7 log CFU/g is often the upper limit for the acceptability of freshwater and marine fish (ICMSF, 1978), the shelf life was approximately 14 days in the refrigerator and 10 days on ice (Bahmani *et al.,* 2011).

Duarte *et al.* (2020), noted that under refrigeration, fish generally have a shelf life of 14 days when stored on ice at 0 °C. However, this shelf life decreases to 8 days

if the ice-to-fish ratio is 1:1 (w/w) at 2 °C and to 10 days when kept between 0–1 °C in plastic bags with the same ratio. When stored in polystyrene boxes with ice at 2–4 °C, the shelf life drops to 5 days, and at 5 °C under similar conditions, the shelf life is limited to 7 days. The same 7-day limit is reached when fish are refrigerated at 2–4 °C. The shelf life of fish is influenced by various factors such as species, habitat, diet, and the capture and transport processes, all of which must be considered when optimizing freezing and refrigeration methods.

According to (Andrade *et al.,* 2014), quality indices based on the concentration of ATP degradation products and the level of biogenic amines are commonly used to evaluate fish freshness and shelf life due to their strong correlation with changes that occur during the period of storage. However, factors such as fish species, the type of tissue sampled, stress experienced during capture, storage temperature, and other variables must be taken into account to establish reliable reference values.

2.2.1 Quality changes in *Labeo rohita* (Hamilton, 1822) during chilled storage

TVB-N and TVC levels in stored Rohu were measured every 4 days over a 24-day period. The initial and final TVB-N values were 4.57 mg/100g (fresh sample), 19.88 mg/100g (after 24 days at 5°C), and 7.10 mg/100g (after 24 days at 0°C). The corresponding TVC values were 2.29 log (cfu/g) (fresh sample), 9.5 log (cfu/g) (after 24 days at 5°C), and 8.1 log (cfu/g) (after 24 days at 0°C) (Prabhakar *et al.,* 2021).

Dhanapal *et al.,* (2013), found that the organoleptic quality of Rohu fish stored in ice was significantly influenced ($P < 0.01$) by factors such as Total Volatile Base Nitrogen (TVBN), Trimethylamine (TMA), Thiobarbituric Acid (TBA), Free Fatty Acids (FFA), Water Holding Capacity (WHC), and microbial counts. The results from organoleptic quality tests indicated that the maximum shelf life of Rohu fish in ice storage was 17 days.

The moisture content of Rohu fish stored in ice increased from an initial value of 78.55% to 80.16% by the 10th day, likely due to the absorption of melted ice water by the fish muscle. Subsequently, a gradual decrease in moisture content was observed, reaching 77.69% by the 18th day (Dhanapal *et al.,* 2013).

The parameters of Indian major carp Rohu (*Labeo rohita*) were analyzed during eight days of iced storage. The pH of the fish flesh was measured using a pH

meter throughout the storage period. An increase in pH was observed, starting from 6.10 on the first day, rising to 6.6 by the sixth day, and reaching 6.90 on the eighth day (Jain *et al.,* 2007).

The content of TVB-N and TMA-N in Rohu stored under limited refrigerated conditions (5°C and 0°C) and frozen storage (-5°C) for 42 days showed a consistent increase. The formation of TVB-N and TMA-N was higher at elevated temperatures, with initial and final values recorded at 4.57 ± 0.321 and 0.14 ± 0.047 mg/100g, and 46.56 ± 0.994 and 3.24 ± 0.112 mg/100g, respectively. The volatile compound formation in Rohu was slower at -5°C, as freezing significantly reduces enzymatic and microbial activity compared to storage at 0°C and above (Prabhakar *et al.,* 2019).

2.2.2 Quality changes in *Penaeus vannamei* during chilled storage

Huang *et al.* (2016), found that the levels of total volatile basic nitrogen (TVB-N), NH_3, and trimethylamine (TMA) in *P. vannamei* increased with storage time at both 25°C and 4°C. TVB-N, NH_3, TMA, inosine, hypoxanthine, and the K-value were identified as reliable indicators of white shrimp freshness during storage. However, the total plate count did not align with the recommended acceptability limits for white shrimp. Sensory evaluation, in conjunction with TVB-N, TMA, and K-value measurements, indicated that the shrimp's quality became unacceptable after 6 hours of storage at 25°C and after 7 days at 4°C.

Kim *et al.* (2020), investigated the quality assessment and acceptability of *P. vannamei* during chilled storage using biochemical parameters. They found that the K-value rose from 9.96% to 12.32%, reaching a maximum of 75.14%. Similarly, TVB-N increased from 1.86 mg/100 g to 34.71 mg/100 g.

Liu *et al.* (2024), observed that the pH of *P. vannamei* during storage initially decreased and then increased. This decline was attributed to the degradation of sugars into acids during the early stages of low-temperature storage, which lowers the pH. Later, microorganisms decompose proteins and amino acids into substances like ammonia, causing the pH to rise. Results showed that on the seventh day, the pH of shrimp in the 1°C cold-storage group (pH = 7.86) was nearing the end of its shelf life, while shrimp in the frozen-thawed (F-T) group approached the end of its shelf life on the 14th day (pH = 7.78), with a significant reduction in quality.

2.2.2.1 Formation of black spot in *Penaeus vannamei*

Nurhayati *et al.* (2018), found that the spread of black spots on shrimp increases with storage time, making it a useful parameter for assessing shrimp quality deterioration. Their study revealed that the pre-rigor phase occurs shortly after death (0 to 2 days of storage), rigor mortis sets in between 3 to 11 days, post-rigor lasts from 12 to 17 days, and deterioration (decay) begins from the 18th day and continues until the 23rd day. Black spots first appear on the cephalothorax and spread over time. During the pre-rigor phase, muscle fibers remain intact and compact. In the rigor mortis phase, muscle fibers start to shrink, and by the post-rigor phase, they are fragmented into pieces.

2.3 Effect of delayed icing on quality of fish and shell fish

The impact of delayed icing on the quality of *Penaeus vannamei* was assessed using microbiological, chemical and sensory analyses. The shrimp were separated into three groups: one was immediately iced, while the other two were subjected to delayed icing after being held at ambient temperature (30 ± 2°C) for 2 and 4 hours, respectively. Sensory evaluation revealed that the shelf life of the shrimp iced immediately and those with a 2-hour icing delay was about 9 days. Whereas, the shrimp with a 4-hour icing delay had a reduced shelf life of 6 days (Annamalai *et al.,* 2015).

Experiment on Delayed Icing in Rainbow Trout was conducted by Dawood *et al.* (1986), they conducted the experiment to examine the effect of delayed icing on Rainbow trout (*Salmo irideus*). The fish were divided into six groups:

(1) Iced immediately.
(2) Held at 10 °C for 6 hours.
(3) Held at 20 °C for 6 hours.
(4) Held at 20 °C for 18 hours.
(5) Held at 30 °C for 4 hours.
(6) Held at 30 °C for 6 hours.

Groups 2 through 6 were iced after their respective holding periods at the specified temperatures. Fish quality was evaluated every 2 days for 14 days during iced storage. The results indicated that bacterial deamination and fat hydrolysis increased

with higher temperatures and longer pre-icing durations, leading to spoilage. However, fish iced immediately or after being held at 10 °C for 6 hours remained of acceptable quality even after 14 days.

The study investigated the impact of delayed icing on the quality of *Penaeus monodon* shrimp stored in plastic and bamboo baskets after a three-hour harvest at ambient temperatures (28°-32°C). Shrimp were iced at a 1:1 ratio and stored for 21 hours. Quality was assessed through visual, biochemical, and microbial analyses over 24 hours. Biochemical results showed acceptable quality throughout the experiment. Microbial counts ranged from log10 3.99±0.12 cfu/g to log10 4.33±0.21 cfu/g in bamboo baskets and log10 4.01±0.12 cfu/g to log10 4.83±0.19 cfu/g in plastic baskets. The results of this experiment suggest that shrimp quality remains stable during the first three hours after harvest, indicating that rural farmers may not need to ice the shrimp right away. Therefore, it is recommended that immediate icing harvesting after may not be essential for rural farmers (Rahi *et al.,* 2008).

Holding fish at room temperature (25°C) for 4 hours is equivalent to keeping in ice (0°C) for around 2.5 days. Similarly keeping fish at room temperature (25°C) for 8, 12 and 16 hours is equivalent to storing in ice (0°C) for around 5, 7.5 and 10 days respectively (Huss H. H.,1995).

2.4 Traditional methods for quality evaluation of fish

Traditional methods for evaluating fish quality primarily involve sensory, chemical, and microbiological analyses. Sensory evaluation assesses freshness based on appearance, texture, odour, and taste, but it can be subjective. Chemical methods include measuring pH, total volatile basic nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) to monitor spoilage and lipid oxidation within the food product. Microbiological techniques such as Total plate count (TPC) is used for determination of microbial content. Though effective, these methods are often labor-intensive, time-consuming, and may require destructive sampling, making them less suitable for real-time, large-scale monitoring in the seafood industry.

2.4.1 Sensory methods for quality evaluation

Various sensory evaluation methods are used by Huang *et al.* (2021) to assess quality of mackerel fish, such as the Palatability and Spoilage test, Torry scheme, EU scheme, Quality Index Method, Catch damage index, and Processed fish damage index. Other approaches include Affective, Discriminative, and Descriptive tests. Each method having unique advantages and limitations. Although sensory protocols for mackerel have been partially harmonized, careful attention is required during sample processing to minimize changes that could affect results. Proper handling ensures accurate and reliable assessment of fish quality throughout the sensory evaluation process.

2.4.2 Biochemical methods for quality evaluation

In an experiment, Cheng *et al.* (2015) discuss about recent improvements in biochemical methods for assessing fish freshness, considering the impact of factors before and after harvest. They highlight several techniques for determining freshness by biochemical measurements such as moisture content, volatile compounds like TVB-N, protein degradation, and lipid oxidation (measured by TBARS and peroxide value). Additionally, they address the importance of tracking ATP breakdown (K value), physical assessments, and detecting foreign material contamination. These methods offer a comprehensive approach to ensuring fish quality.

2.4.3 Microbiological methods for quality evaluation

Microbiological methods for fish quality evaluation involve monitoring key microorganisms, including the total aerobic count (TAC), coliform bacteria, E. coli, Salmonella spp., and Vibrio parahaemolyticus. These indicators help assess microbial contamination levels and overall safety, providing critical information on spoilage and potential health risks in fish products (Cwikova, O, 2016).

2.5 Non-destructive method for quality evaluation

Non-destructive methods for fish quality assessment have gained importance due to their ability to evaluate freshness and spoilage without damaging the product. These techniques allow for continuous monitoring throughout storage and transportation, ensuring better quality control. Key non-destructive methods include

spectroscopy, artificial neural network, Infrared reflectance spectroscopy, hyperspectral imaging, multispectral imaging etc.

Recently, increasing focus of consumers on food quality and safety has led to a growing demand for faster, more sensitive analytical technologies. Traditional methods, such as physicochemical, textural, sensory, and electrical techniques, have been commonly used to evaluate the freshness and authenticity of fish and seafood. While effective, these methods are destructive, costly, time-consuming, and prone to variability. In contrast, emerging techniques like spectroscopy offer significant advantages, including rapid analysis, minimal sample preparation, high repeatability, and lower costs. Most importantly, these methods are non-invasive and non-destructive, making them ideal for monitoring and freshness evaluation. Hassoun and Karoui, (2017).

According to Cheng *et al.* (2013), traditional techniques and methods for evaluation and detection of fish quality and safety are very tedious, laborious, expensive and time-consuming while new advanced technology like spectroscopic techniques have successfully overcome some of these disadvantages and can supplement or replace them. Spectroscopic techniques are gaining attention due to their high specificity, convenience, non-destructive nature, and cost-effectiveness, along with their very quick response time. These methods show significant potential for detecting pathogens, foreign contaminants, changes in protein structure, lipid oxidation, and monitoring spoilage inside the fish. They are particularly useful for determining whether the fish sample is safe for consumption and meets international trade standards.

Dutta *et al.* (2015), developed an automated, efficient, and non-destructive image processing method for assessing fish freshness. Their results established a correlation between specific features and freshness levels using training data. Based on this correlation, they proposed a framework for identifying freshness from images of Rohu fish. This framework is considered significant, and the prototype could have practical applications in the fish industry and for consumers.

2.5.1 Fish quality assessment by Artificial neural network

A real-time, non-destructive approach for assessing the freshness of common carp *(Cyprinus carpio)* using advanced vision-based intelligent modeling was

developed. In this research, Garavand *et al.* (2019) investigated an artificial neural network for evaluating the freshness of common carp during ice storage. The process began with capturing and pre-processing images of the fish samples. Channels such as R, G, B, H, S, I, L*, a*, and b* were calculated, followed by extracting six types of texture features from each channel. Over a two-week period, 1344 images were obtained from 48 fish samples, with images taken daily. Preprocessing steps, including filtering, segmentation, auto-cropping to remove the background, and color space conversion, were applied, resulting in 54 texture features extracted per image from the R, G, B, H, S, I, L*, a*, and b* channels.

2.5.2 Fish quality assessment by Spectroscopy

Spectroscopy has emerged as a valuable tool for assessing fish quality by providing rapid, non-destructive measurements of biochemical, microbiological and physical changes during spoilage. Techniques such as near-infrared (NIR), Fluorescence Spectroscopy, Hyperspectral imaging and Multispectral imaging detect variations in water content, protein degradation, lipid oxidation, and microbial activity in fish tissues. These spectral methods offer precise insights into freshness, shelf-life, and safety, surpassing traditional sensory and chemical tests. Spectroscopy's real-time analysis capability allows for the continuous monitoring of fish quality, making it an effective method for quality control and quality monitoring in the seafood industry.

2.5.2.1 Infrared reflectance spectroscopy

Evaluation of freshness in freshwater fish based on near infrared reflectance spectroscopy and chemometrics was developed by (Zhou *et al.* 2019). In their study, 150 samples were analysed in reflectance mode across the spectral range of 1000–1799 nm to determine freshness indicators such as pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and ATP-related compounds (K value). Prediction models for these freshness parameters were created and validated through partial least-squares regression (PLSR) paired with a competitive adaptive reweighted sampling (CARS) algorithm and optimal pre-processing techniques. The models achieved satisfactory prediction coefficients (Rp) of 0.945, 0.932, 0.954, and 0.807, along with root mean square errors of prediction (RMSEP) of 0.081, 2.099, 0.107, and 6.509 for pH, TVB-N, TBARS, and K value, respectively.

2.5.2.2 Fluorescence Spectroscopy

Momin *et al.* (2023), utilized fluorescence spectroscopy to identify the optimal wavelength for developing an optimized fluorescence imaging system aimed at quality assessment of agricultural products. The study demonstrated the application of fluorescence imaging for detecting peel defects across various citrus fruits as an example of this technique's potential.

2.5.2.3 Hyperspectral imaging

Hardy *et al.* (2024), utilized hyperspectral imaging combined with machine learning to assess freshness of fish. In this study, salmon fillets were monitored over four consecutive storage days using a hyperspectral camera (400–1000 nm) and absorption spectroscopy. Optimized K-Nearest Neighbors (KNN) analysis was applied across all wavelengths, resulting in an average classification accuracy of 77.0% for predicting storage days. The mean spectrum analysis revealed that the reduced absorbance around 600 nm was the key indicator differentiating fresh from spoiled samples. Further examination of the hyperspectral data highlighted variations in fillet freshness, with accelerated spoilage observed in the tail sections. Notably, this research is the first to employ histogram analysis in evaluating seafood freshness using the hyperspectral data.

2.5.2.4 Multispectral imaging

This study focuses on predicting different freshness indicators in fish fillets using a multispectral imaging system. A straightforward imaging system (430–1010 nm) combined with linear and non-linear regression models was employed to monitor spoilage indicators during a 12-day storage period at 4 ± 2 °C. The non-linear models proved to be more effective in accurately predicting all three freshness indicators. Among the spoilage indices, this model showed the highest predictive accuracy for the PPC value, while the TVB-N content prediction was less precise. The development of a multispectral imaging system based on the LS-SVM model appears to be appropriate for simultaneously estimating all three indicators ($R^2_p > 0.862$ and RPD > 2.678). Given that the traditional PPC evaluation takes up to 10 days to complete, using the multispectral imaging method offers significant advantages as it is a non-destructive and rapid technique for estimating this indicator in fish fillets (Nia *et al.,* 2019).

2.6 Artificial intelligence

Artificial intelligence (AI) in fish quality assessment enables rapid, accurate, and non-invasive evaluation of freshness and spoilage. AI techniques, such as machine learning and deep learning, process data from imaging systems (e.g., computer vision), spectroscopy, and electronic noses (E-nose) to analyse physical, chemical, and sensory quality of fish. AI models can identify patterns, predict quality degradation, and classify fish based on freshness or spoilage levels. These systems enhance decision-making in seafood quality control, reduce human error, and streamline processes, contributing to more efficient supply chain management and improved product quality assurance.

2.6.1 Artificial intelligence in fish quality assessment

Detection of fish freshness using artificial intelligence methods was explored by (Yasin *et al.,* 2023), This study introduces a new approach for assessing fish freshness using deep learning techniques. While sensory analysis has traditionally been used by humans to determine fish freshness, achieving an objective evaluation has been difficult. The study addresses this challenge by utilizing deep learning algorithms, specifically SqueezeNet and InceptionV3, to classify fish freshness based on a dataset of 4,476 images categorized as fresh or stale. The results show that the SVM, ANN, and LR models each achieved 100% accuracy with these deep learning methods. The study's significance lies in its potential applications in the food industry, providing a reliable solution for quality control and food safety.

Freshness of fisheye was detection using common deep learning algorithms and machine learning methods with a developed mobile application. This study employed artificial intelligence techniques to classify the freshness of fish based on eye features. The primary aim was to develop a model that accurately categorizes fish into three classes: highly fresh, fresh, and not fresh. To achieve this, two deep learning algorithms, SqueezeNet and VGG19, were used to extract features from a dataset of 8,780 images. The results showed that the combination of the VGG19 model for feature extraction and an Artificial Neural Network (ANN) for classification achieved the highest success of 77.3% for the FFE dataset. The application was

designed to provide instant freshness detection for any of the fish sample (Yildiz *et al.,* 2024).

2.6.2 Machine learning

In an experiment, images of sea bream, sea bass, anchovy, and trout were captured using a digital camera over 7 days of refrigerated storage. The images were uploaded to a web-based machine learning tool called Teachable Machine (TM), which was trained to recognize the fish's pupils and heads. Additionally, images of each species from different angles were included to enhance TM's ability to identify the fish species. The study demonstrated that image processing could accurately differentiate fish species and distinguish between fresh and spoiled fish. The model's accuracy improves when specific colour changes are observed during storage (Emre Yavuzer & Memduh Kose, 2022).

He *et al.* (2015), highlighted the advancements of three non-destructive optical techniques—spectroscopy, computer vision, and hyperspectral imaging in evaluating and determining fish quality, while also discussing future trends in these technologies for enhanced quality assessment of fish and fish products. The study revealed that these optical methods offer clear benefits in terms of increasing efficiency and reducing the labor involved in manual inspections, allowing for rapid and straightforward fish quality evaluations compared to traditional sensory analysis and destructive testing methods.

2.6.3 Deep learning

Wu *et al.* (2022), investigated methods for predicting salmon freshness under variable temperature conditions. While existing microbial kinetic models work well for stable temperatures, they lose accuracy when temperatures fluctuate. To overcome this limitation, the researchers employed deep learning to analyze the complex dynamics of temperature variations during storage. They developed a new model, CNN_LSTM (convolutional neural network combined with long short-term memory), capable of accurately predicting total viable counts (TVC) despite temperature fluctuations. The model performed exceptionally well, with a determination coefficient (R^2) over 0.95 and a root mean square error (RMSE) below 0.2. Additionally, it showed promise for forecasting freshness under conditions beyond temperature variability, opening new avenues for freshness prediction. This study underscores the

effectiveness of the CNN_LSTM model in managing temperature variations, thanks to the robust feature extraction and data fitting capabilities of deep learning techniques.

Rayan *et at.* (2021), developed a fully automated model to classify Fish freshness based on deep learning models and image processing techniques. The method uses the pre-trained VGG-16 deep neural network as a feature extractor in conjunction with Bi-LSTM (Bi-directional Long Short-Term Memory) for classification. This study used the sample species of Nile Tilapia. When combined, the model showed excellent results predicting fish freshness achieving accuracy of 98% on the testing dataset.

2.7 Image based Prediction models for fish quality assessment

Prediction models in fish quality assessment use mathematical and computational techniques to forecast fish freshness and spoilage. These models integrate data from various quality indicators such as microbial growth, temperature, pH, and chemical changes, collected via non-destructive methods like spectroscopy. Machine learning and artificial neural networks (ANNs) are commonly used to develop these models, allowing for accurate predictions of shelf life and quality degradation. By providing real-time, data-driven insights, prediction models optimize storage, reduce waste, and improve decision-making in seafood supply chains, ensuring better quality control from harvest to consumption.

2.7.1 Prediction model using Python and AI

Spectral equipment such as spectrometers, spectral analysers, spectrographs, or spectrophotometers are used to determine spectral values. In spectroscopy, identifying the sample or analyte can be challenging, but this issue can be addressed with a prediction model for spectroscopy implemented in Python. The prediction model for this project involved data preprocessing and identifying the most effective model based on the collected datasets. The study found that the accuracy of the prediction model exceeded 90%. Ultimately, the research demonstrated that the prediction model is capable of predicting spectroscopy-based data formats (Ismail *et al.,* 2021).

Hosseini *et al.* (2008), present a method for assessing fish freshness using support vector machines (SVMs) to enhance fish identification systems. The approach involves evaluating fish freshness through artificial neural networks (ANNs) after processing images of various fish species. Data was collected from four selected fish species over a ten-day period. The study concluded that using individually trained ANNs for each species improves performance. The accuracy of freshness assessment for the sampled fish was 95.83%, 93.75%, 91.67%, and 95.83% for the respective species. The findings suggest that the proposed method is effective and reliable for assessing fish freshness.

2.7.2 Shelf-life prediction model

Cui *et al.* (2024), developed machine learning models for predicting the shelf-life of various marine fish species and established a real-time prediction platform. This study was the first to utilize four machine learning algorithms to create a multi-objective model capable of predicting the shelf-life of five marine fish species across different storage temperatures. The model used 14 input features, including species, temperature, total viable count, K-value, total volatile basic nitrogen, sensory evaluation, and E-nose-GC-MS/MS data. By employing machine learning algorithms, particularly the GA-BP and RBF neural networks, the models achieved highly accurate predictions of shelf-life for the five species under varying temperatures.

2.7.3 Quality prediction model

Zhang *et al.* (2011), developed quality prediction models for grass carp (*Ctenopharyngodon idella*) stored at various temperatures. This study established kinetic models to forecast freshness changes in grass carp during storage at different temperatures. The stability in quality of grass carp was improved at lower temperatures, as indicated by changes in sensory assessments, microbial growth (TAC), and chemical indices (K value, TVB-N, and TBA value). The findings suggest that lower temperatures result in higher quality. Freshness indicators consistently followed a first-order correlation with storage time and temperature, showing high regression correlations. The relative errors between the predicted and observed values of TAC, K value, and TVB-N were within ±10%. Thus, the quality changes of grass carp can be reliably predicted using the models for TAC, K value, and TVB-N within a temperature range of -3 to 15 °C.

2.7.4 Sensor based quality prediction model

Gracia *et al.* (2017) developed a smart sensor to predict the quality of fresh fish stored under ice conditions. This sensor measures quality by combining data on biochemical and microbial spoilage indicators with dynamic models to predict quality according to the Quality Index Method (QIM) and European Union (EU) grading standards. The sensor can also account for variability within a batch when spoilage indicators are measured in multiple fish samples. It was specifically designed and tested for fresh Atlantic cod (*Gadus morhua*) under commercial ice storage conditions. The sensor required only two spoilage indicators i.e. psychrotrophic bacterial counts and total volatile base-nitrogen content to provide accurate estimations of quality, comparable to established sensory methods. Further research and technological advancements are underway to develop faster, non-destructive measurement methods. An iterative approach was used to continually refine and improve the predictive capabilities of the sensor.

Multiple sensors were utilized to detect compounds determining odors and, consequently, product quality. Accurate food quality assessment depends on proper sensor function. This study introduces a machine learning-based failure tolerance system that bypasses faulty sensors. A Single Plurality Voting System (SPVS) classification approach was used, wherein individual classifiers are trained on distinct features, and their outputs are combined into a composite classifier. K-Nearest Neighbor (kNN), Decision Tree, and Linear Discriminant Analysis (LDA) are used as base classifiers. The method is applied to predict beef cut quality using a public dataset with 11 sensors and 12 beef cut types. Automatic quality prediction is critical for price determination, as freshness impacts market value (Kaya *et al.,* 2020).

3. MATERIAL AND METHODS

3.1. Material

3.1.1 Experimental fish (*Labeo rohita*)

Fresh Rohu fish were purchased from a freshwater fish farm in Raigad district, Maharashtra. The fishes were transported to Post-Harvest Technology pre-processing laboratory in ice boxes with fish to ice of 1:1. The fishes had an average length of 35.5 ± 4.5 cm and an average weight of 750 ± 70 grams.

Plate 1: *Labeo rohita*

3.1.2 Experimental Shrimp (*Penaeus vannamei*)

Fresh Vannamei shrimp were brought from brackish water shrimp farm in the Palghar district of Maharashtra. They were transported to Post harvest technology department in ice boxes, maintaining a fish-to-ice ratio of 1:1. The count of the shrimp was 40 -50 per kilogram, with an average length of 14 ± 2 cm and an average weight of 23 ± 2 grams.

Plate 2: *Penaeus vannamei*

3.1.3 Chemicals and glasswares

All chemicals utilized were of analytical grade and were sourced from Hi-Media, Qualigens, and Merck, India. All the glassware employed in the study were manufactured by Borosil. Centrifuge tubes and other plastic wares were procured from Tarsons and Axygen, U.S.A.

3.1.4 Instruments

The equipment used in this study included a Kjeldahl distillation apparatus (Pelican, Kelplus-kelvac VA, Mumbai, India) for TVB-N analysis, an electronic balance (GR-200, A and D Ltd., India) for weighing chemicals and samples, Polytron system PT 2100 homogenizer (Kinematica AG, Germany) for sample homogenization, while a digital pH meter (Eutech Tutor, Eutech Instruments, Singapore) was used for measuring muscle pH of fish and shrimp samples. For microbiological analysis, including Total Plate Count, a laminar air flow system (Don Whitley Scientific Equipments Pvt. Ltd., Thane, Maharashtra, India) was used. Incubation was carried out in a bacterial incubator (Nuaire, Mumbai,India). Lastly, a UV-Vis spectrophotometer (LABINDIA UV3092, Delhi, India) and steam distillation apparatus (Borosil, India) were used for TBARS analysis.

3.1.5 Imaging setup

The images were taken inside a specially designed photography cabinet, i.e. a black box equipped with optimal lighting to ensure consistent illumination (white light, illumination of 544 lux). The interior of the cabinet was set against a black background to prevent reflection and to provide a clean, contrast-enhancing backdrop for the images. At the top of the cabinet, a small circular opening was built to accommodate the camera lens, ensuring proper focus and stability during image capture. Additionally, a drawer located at the bottom of the cabinet provided a convenient space to place and retrieve samples, streamlining the photography process. The dimensions of the photography cabinet were 30 inches in length, 19 inches in width, and 30 inches in height. The images were captured from a consistent height of 30 inches.

Plate 3: Front view of imaging cabinet

Plate 4: Top view of Imaging cabinet

3.1.6 Digital camera

Canon EOS 80D digital (DSLR) camera was used for taking photographs of fish and shrimp daily at different angles from a fixed position. The Canon EOS 80D comes with a 24.2 MP APS-C CMOS sensor, 45-point cross-type autofocus, and a DIGIC 6 processor. It uses Dual Pixel CMOS AF for quick, smooth auto focus in live

view and video mode. Users can frame shots with an optical viewfinder or a 3" rotating touchscreen.

Plate 5: Canon EOS 80D digital camera

3.2 METHODS

3.2.1 Experimental Design

To establish critical rejection points of spoilage indices during chilled storage, the experiment involved immediate and delayed icing of Rohu. The fishes were divided into five lots, each with 30 nos. Lot 1 was iced immediately after capture without subjecting to room temperature and was designated as Control (C). The remaining four lots were subjected to temperature abuse by holding them at room temperature for different time period i.e., 4 hr, 8 hr, 12 hr and 16 hr and were designated as T1, T2, T3 and T4, respectively.

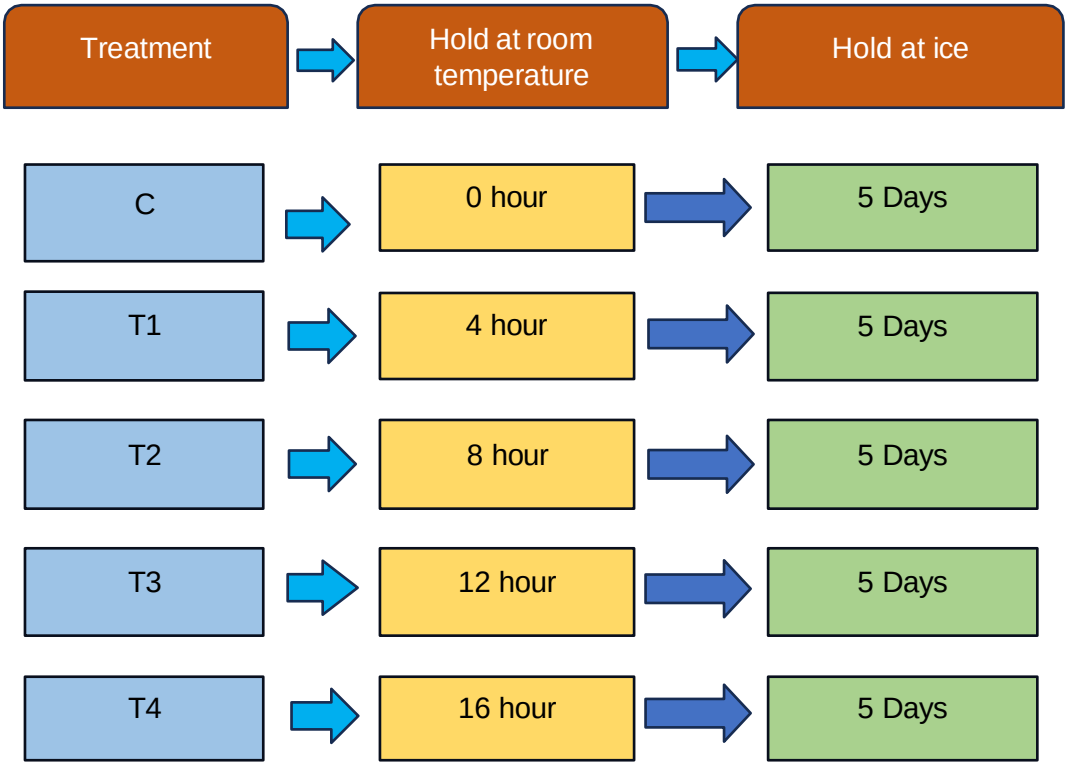

Plate 6: Flow diagram of experimental design

The treatment lots after temperature abuse were stored in ice in an insulated box at 1:1 ratio. All the biochemical (TVBN, TBARS and pH) and microbiological tests were conducted on three sections of the fish body, i.e., head, mid and tail. Standard images of the whole fish and its sections (head, mid, and tail) were captured daily for developing freshness prediction models

Similar experiment was employed for *P. vannamei.* Unlike Rohu, all biochemical (TVBN, TBARS and pH) and microbiological tests were conducted on pooled samples. Images of whole shrimp was taken daily for developing freshness prediction models

Table:1 Equivalent quality loss of temperature abused fish to ice stored fish (Huss, 1995)

Holding time at room temperature (25°C)	Ice storage (0°C)
4 hours	2.5 days
8 hours	5 days
12 hours	7.5 days
16 hours	10 days

3.2.2 pH measurement (sallam, 2007)

A 10 g portion of fish mince was homogenized with 50 mL of distilled water using a homogenizer (Polytron system PT 2100, Kinematica, AG, Germany) for a duration of 30 seconds. The pH of the resulting fish homogenate was subsequently measured using a digital pH meter (Eutech Instruments, Singapore), which had been previously calibrated with standard buffer solutions at pH 4.8 and pH 9.2.

3.2.3 Total volatile basic nitrogen (TVBN) (Malle and Tao,1987)

The total volatile base nitrogen (TVBN) content was measured using a modified version of the official European steam distillation method (EU Official Journal, 2008). This method involves the extraction of TVBN using an alkaline solution followed by the titration of the liberated ammonia. Initially, 25 g of flesh from a fresh or iced fish sample was weighed and homogenized with 100 mL of 7.5% trichloroacetic acid (TCA) for 2 minutes. The homogenate was then filtered through Whatman No. 1 filter paper, and the filtrate was adjusted to a total volume of 100 mL in a volumetric flask.

Subsequently, 25 mL of the filtrate, along with 6 mL of 10% NaOH, was placed into a distillation tube and subjected to steam distillation using a distillation unit (Pelican Kelplus Classic DX VATS (P), India). The apparatus was sealed, and the distillation process was initiated. The steam distillate was collected in a flask containing 15 mL of 4% boric acid and a few drops (0.04 mL) of a mixed indicator (methyl

red/bromocresol blue in a 2:1 ratio). The distillation continued until a total of 100 mL of distillate was collected.

The alkaline distillate was then titrated against 0.025 N sulfuric acid (H_2SO_4) until the end point was reached, as indicated by a color change from green to pink. The TVBN content of the fish sample was calculated using the following formula.

$$\text{TVBN (mg N/100 g)} = \frac{\text{TV}\times\text{N}\times 14\times\text{V1}\times 100}{\text{V2}\times\text{W}}$$

Where,

TV = Titre value

N = Normality of acid used for titration

V1 = Total volume of TCA extract

V2 = Volume of TCA extract taken for distillation

W = Weight of the sample

3.2.4 Thiobarbituric acid reactive substances (TBARS) (Tarladgid *et al.,* 1960)

A 10-gram sample of fish and shrimp was macerated using a mortar and pestle with 50 mL of distilled water. The homogenate was transferred into a 500 mL round-bottom flask through a funnel, followed by the addition of 47.5 mL of distilled water and 2.5 mL of 4 N HCl, adjusting the pH to 1.5. The flask was heated using an electrical heating mantle, and the first 50 mL of distillate was collected in a measuring cylinder within 10 minutes after the mixture began boiling. From the collected distillate, 5 mL was pipetted into a stoppered tube, and 5 mL of thiobarbituric acid (TBA) reagent was added. The tubes were then placed in a boiling water bath at 100°C for 35 minutes. Simultaneously, a blank was prepared using 5 mL of distilled water and 5 mL of TBA reagent. After boiling, the tubes were cooled for 10 minutes in a beaker containing water. The optical density (OD) of the sample was measured at 538 nm using a spectrophotometer.

$$\text{TBARS (mg-MDA equivalent/kg fat)} = \frac{A\times 390}{V\times W}$$

Where,

A = Absorbance at 538nm

V = Volume (ml) of distillate taken

W = Mass of sample

3.2.5 Total plate count (TPC) (BAM, 2004)

The total plate count of the pooled sample from *Labeo rohita* and *Penaeus vannamei* was determined using the spread plate method. A 10 g portion of the pooled sample was aseptically taken and mixed with 90 mL of sterile saline solution. The mixture was thoroughly homogenized using a mortar and pestle. Serial decimal dilutions were then prepared, and 100 µL of each dilution was spread onto sterile plate count agar plates. The inoculated plates were incubated at 37˚C for 24 hours. After incubation, colonies were enumerated. The results were expressed as logarithmic colony-forming units per gram (log CFU/g).

3.2.6 Process of developing image-based prediction model

The process of development of image-based prediction model began with capturing images of the species from various angles under standardized lighting and background conditions. Subsequently, the images were annotated (labelled) using the Roboflow platform. The model was then trained using a pre-existing dataset based on freshness. Species and day wise labelling was done to differentiate fresh from spoiled one. Predictions were made using Python code and machine learning implemented with YOLOv8. Finally, the accuracy of the model was evaluated using YOLOv8.

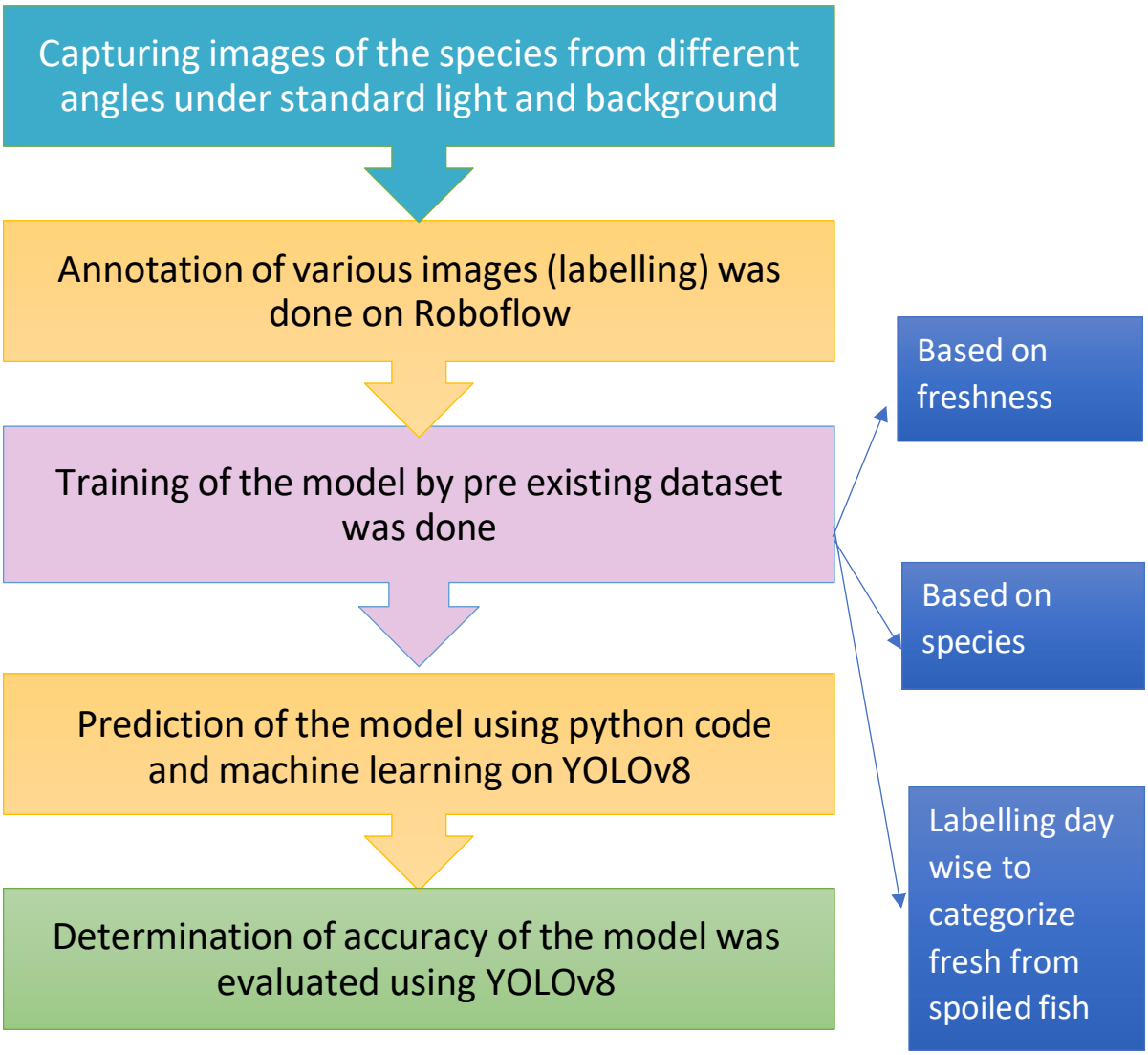

Plate 7: Flowchart of fish freshness prediction model development

3.2.6.1 Capturing Images of *Labeo rohita* and *Penaeus vannamei*

Two sets of experiments were conducted to develop image based prediction model for Rohu. The first experiment involved temperature abuse of fish followed by ice storage for 5 days. A total of 1500 images were taken from five treatments mentioned in section 3.2.1. Images were captured from different sections of the fish body, i.e. head, mid and tail as well as whole fish, daily. The second experiment involved immediate icing of fish and images were captured on daily basis for 16 days. A total of 480 images were captured. For shrimp, the images of both immediately iced and temperature abused shrimps were taken for 5 days. A total of 2015 images were taken from different sections of the shrimp, i.e. head, mid and tail.

3.2.6.2 Annotation of images using Roboflow software

Roboflow is a popular platform that provides tools for building, training, and deploying computer vision models. It simplifies the process of working with datasets, labelling images, and training machine learning models. It is often used for tasks such as object detection, image classification, and segmentation.

The fish and shrimp images were annotated for object detection. This dataset was hosted on Roboflow, which allows labelling of images with bounding boxes around the objects. Roboflow's annotation tools were utilized to systematically label the species in each image. To further enhance model performance, particularly when dealing with a small dataset, data augmentation techniques such as rotation, flipping, and brightness adjustments were employed. The dataset after adequate annotation and augmentation were exported in the YOLO (You Only Look Once) format, with Roboflow providing direct support for exporting to YOLOv8 format. This structured approach ensures a well-prepared dataset that is conducive to effective model training.

3.2.6.3 Training of the predefined model in YOLOv8

YOLOv8 is usually employed for predicting image data and represents the latest advancement in the YOLO series of object detection models developed by Ultralytics. This iteration enhances both accuracy and processing speed, while integrating novel techniques to optimize performance for real-time applications.

To set up YOLOv8 for the training process, the configuration file must be tailored to the specific dataset being utilized. Essential modifications included specifying the

number of classes for species, providing the paths to the training and validation images and labellling. Once the configuration was prepared, the training of the YOLOv8 model was commenced using the dataset sourced from Roboflow. The number of epochs were adjusted according to the size of the dataset and the capabilities of the hardware being used.

3.2.6.4 Validation of the model in YOLOv8

Following the training phase, model evaluation involved analysing validation metrics generated by YOLOv8, which included determination of precision, recall, mean Average Precision (mAP), and loss. These metrics are crucial for assessing the model's overall performance. Furthermore, YOLOv8 facilitated the visualization of results by outputting predicted bounding boxes and confidence scores on validation images. This enabled effective detection of fish and shrimp species. The model performance was evaluated using several key metrics.

Mean Average Precision (mAP): serves as the primary indicator for computer vision models, representing the mean of the Average Precision (AP) across all classes within the model. This metric allows comparison between different models or variations of the same model on identical tasks, with values ranging between 0-1.

Precision: precision was derived from the confusion matrix, measuring the accuracy of the model's positive predictions by calculating the proportion of true positives relative to the total number of positive predictions, including false positives.

$$\text{Precision} = \frac{\text{True positives}}{\text{True positive} + \text{False negative}}$$

Recall: Also known as sensitivity or the true positive rate, is another metric from the confusion matrix that evaluated the model's effectiveness in identifying all true positive instances, calculated as the ratio of true positives to the sum of true positives and false negatives.

$$\text{Recall} = \frac{\text{True positives}}{\text{True positive} + \text{False negative}}$$

3.2.7 Statistical Analysis

All the data were statistically analysed using one-way and two-way analysis of variance (ANOVA) using Software Statistical Package for Social Sciences (SPSS) version 25.0. Duncan's multiple range test (DMRT) was used for post hoc comparison of the mean at 5% probability level. The data in the text, figures, and tables are presented as mean ± standard error.

4. RESULTS

To develop an image-based freshness prediction model which could differentiate a fresh fish from a spoiled one, a few sets of experiments were undertaken under the present study. As spoilage is accumulative and accelerative, firstly, the effect of immediate and delayed icing on Rohu and shrimp was assessed by monitoring the biochemical and microbiological spoilage indices during storage. Prior to icing, the fishes were subjected to temperature abuse by holding them at room temperature for different time period. Biochemical and microbial quality evaluation was carried out during storage. From the data obtained, the study aimed to establish critical rejection points (days of storage) for each spoilage index as affected by temperature abuse. The images of both fish and shrimp were captured on daily basis, as the spoilage progressed. These images were analysed using machine learning algorithms to develop image-based freshness prediction models. This section presents the experimental results obtained, graphically or in tabular form.

4.1 Establishing critical rejection points for spoilage indices of *Labeo rohita* during ice storage

The fishes were divided into five lots: Control (C, i.e. immediately iced), and T1, T2, T3 and T4 were the lots exposed to room temperature for 4, 8, 12, and 16 hours respectively. All five lots were stored in ice for five days. Biochemical analyses (TVB-N, TBARS, pH) were performed on different sections of Rohu (Head, mid and tail), and microbiological tests were done on pooled samples daily. The data were analysed to determine the progression of spoilage during storage at different parts of fish body and to establish the key rejection points for each treatment lot as affected by temperature abuse.

4.1.1 Effect of temperature abuse on Total Volatile Basic Nitrogen (TVB-N) content of *Labeo rohita* during ice storage

TVB-N levels in Rohu subjected to five different treatments over five days of ice storage (day 0 to day 4), are displayed in figure.1,2,3,4, and 5. The data were analysed day wise using 2-way ANOVA.

4.1.1.1 Total Volatile Basic Nitrogen (TVB-N) of *Labeo rohita* on day-0 of ice storage

The Fig 1. shows the TVB-N content of different sections of *Labeo rohita* subjected to immediate icing and temperature abuse on initial day of storage (Day-0). The middle region exhibited the highest values across sections, with significant differences observed between the control and treated groups ($p<0.05$). TVBN levels were highest (35.3 mg%) in mid-section of 16 hr lots (T4) and crossed the limit of acceptability (35 mg%) on day 0. The control lot had lowest TVBN content (19.8 mg%).

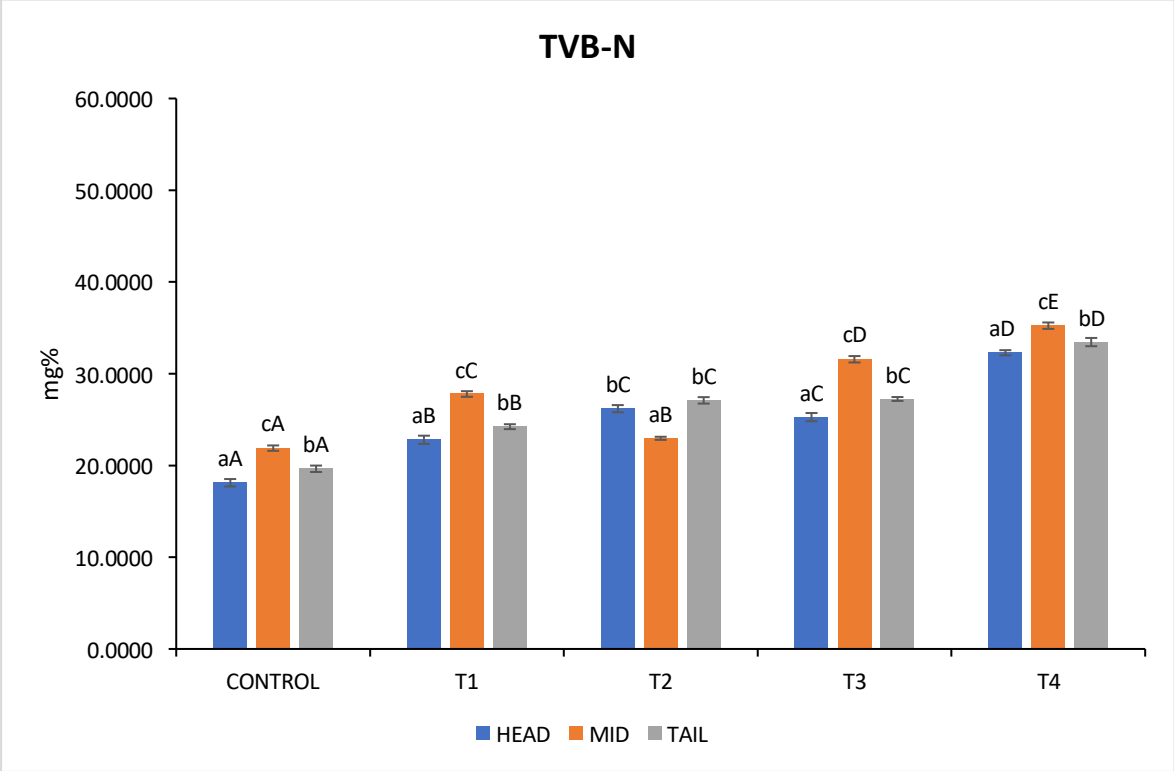

Figure 1. TVB-N content of *Labeo rohita* on day-0 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference ($p\leq0.05$). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.1.2 Total Volatile Basic Nitrogen (TVB-N) of *Labeo rohita* on day-1 of ice storage

Fig 2. shows that on day 1, the TVB-N content in Rohu increased across all treatments (T1–T4) when compared to the control, indicating relatively high spoilage

in temperature abused lots. The TVB-N level of mid-section of T4 reached 41.41 mg% TVB-N levels of mid sections of control, T1, T3 and T4 were significantly different from head and tail section *(p≤0.05).* On day 1, T3 crossed the acceptable limit

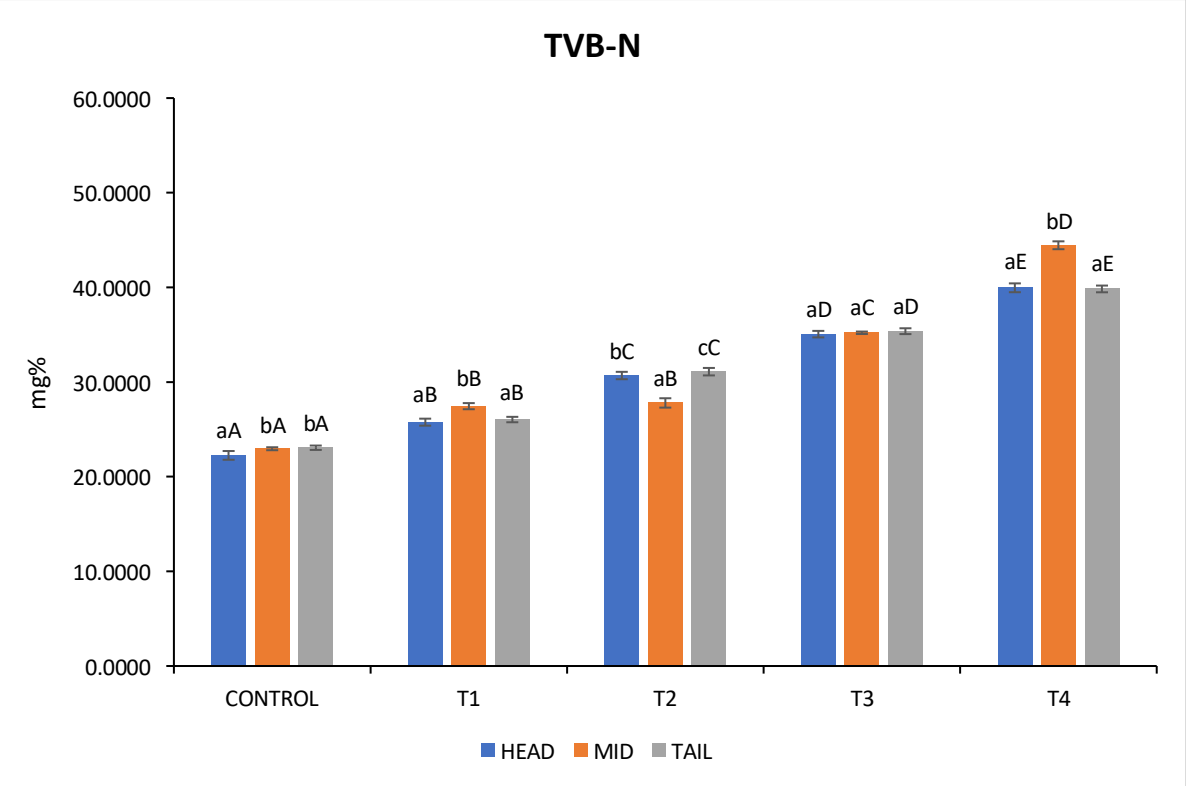

Figure 2. TVB-N content of *Labeo rohita* on day-1 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.1.3 Total Volatile Basic Nitrogen (TVB-N) of *Labeo rohita* on day-2 of ice storage

Fig 3. shows that on day 2, the highest TVB-N levels are observed in T4, particularly in the middle (45.17mg%) and head portions. TVB-N levels of head, mid and tail portions of T1, T2 and T3 were significantly higher than control and followed the order T3 > T2 > T1 > C. A spike was observed in T2 from day 1 (31mg%) to Day 2 (39mg%) and crossed the limit of acceptability

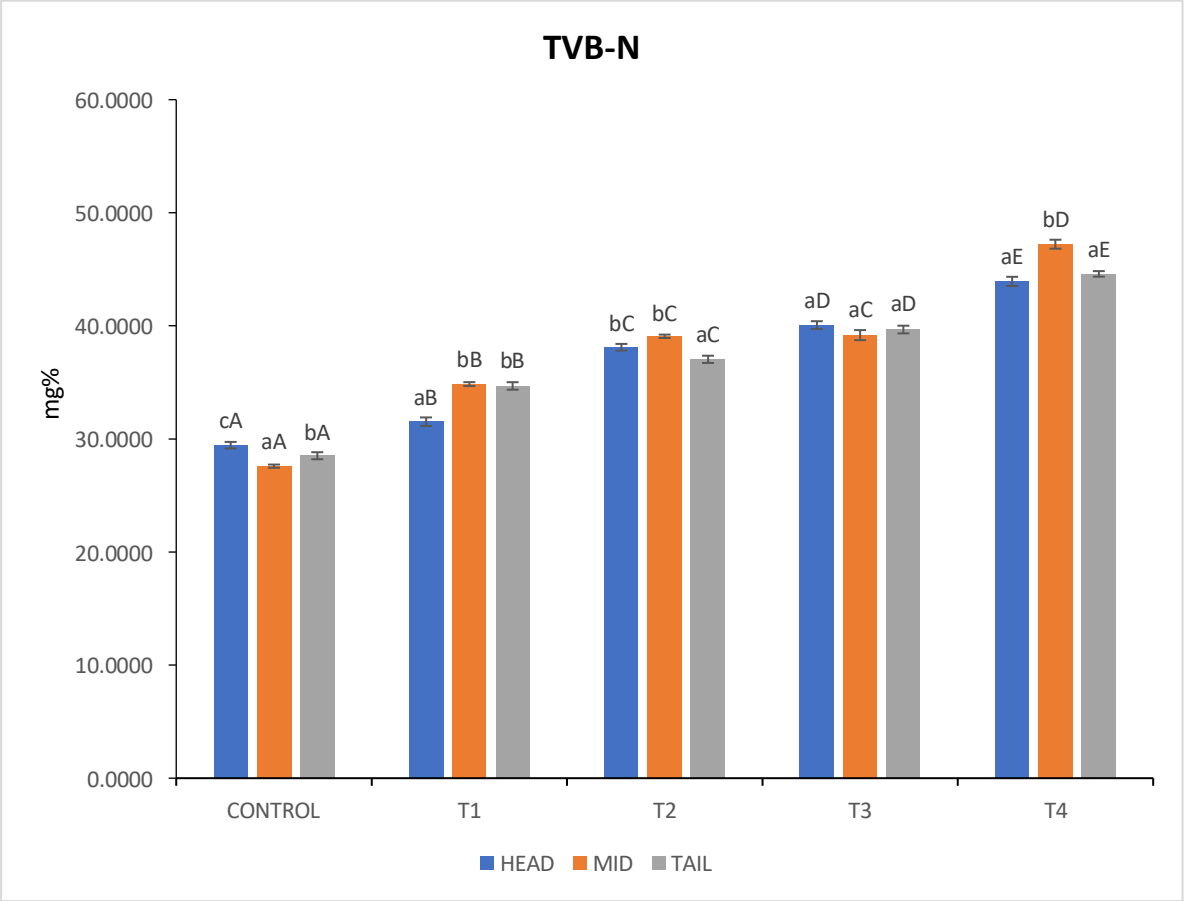

Figure 3. TVB-N content of *Labeo rohita* on day-2 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.1.4 Total Volatile Basic Nitrogen (TVB-N) of *Labeo rohita* on day-3 of ice storage

On day 3, TVBN content of all three sections of T3 (12 hr) crossed the acceptable limit. The data are depicted in Fig:4. TVB-N levels of mid and tail section of T2 were significantly higher than that of T1 and C. Although control remained within the acceptable range, the head section of T1 (36.46) crossed the limit. TVBN content of mid-section of T4 continued to increase and reached 49.78 mg% for 16hr (T4) treatment.

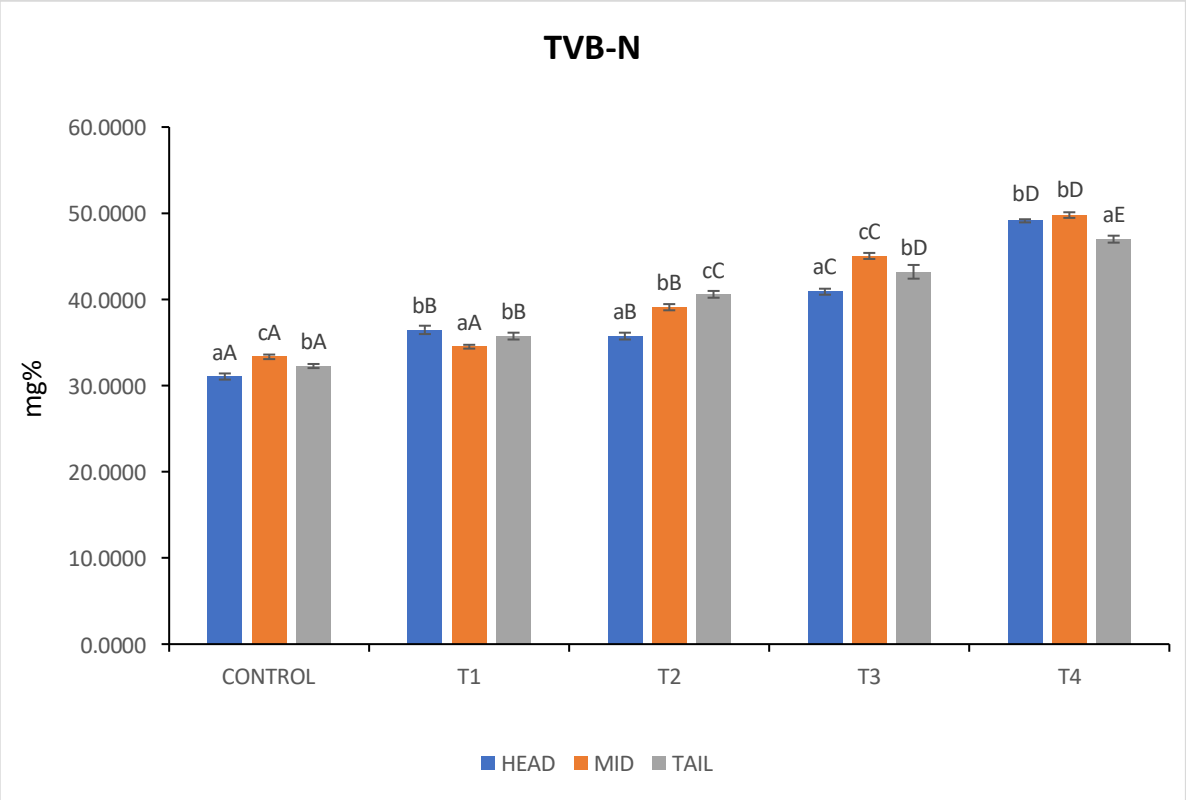

Figure 4. TVB-N content of *Labeo rohita* on day-3 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.1.5 Total Volatile Basic Nitrogen (TVB-N) of *Labeo rohita* on day-4 of ice storage

The Fig 5. shows that the TVB-N content in Rohu continued to increase across the treatments. On day 4, TVBN content of the head and mid-section of T1 (4hr) reached 41.19mg% and 41.37mg% and that of T2 (8 hr) were 49.56mg%, and 55.05mg% respectively. The mid-section of all treatments except control recorded significantly higher amount of TVBN compared to head and tail sections. Mid-section of C, T1, T2, T3 and T4 recorded 31.6mg%, 42.2mg%, 43.36mg%, 52.5mg%, and 56.23mg% TVBN, respectively. Control continued to remain within the limit of acceptability.

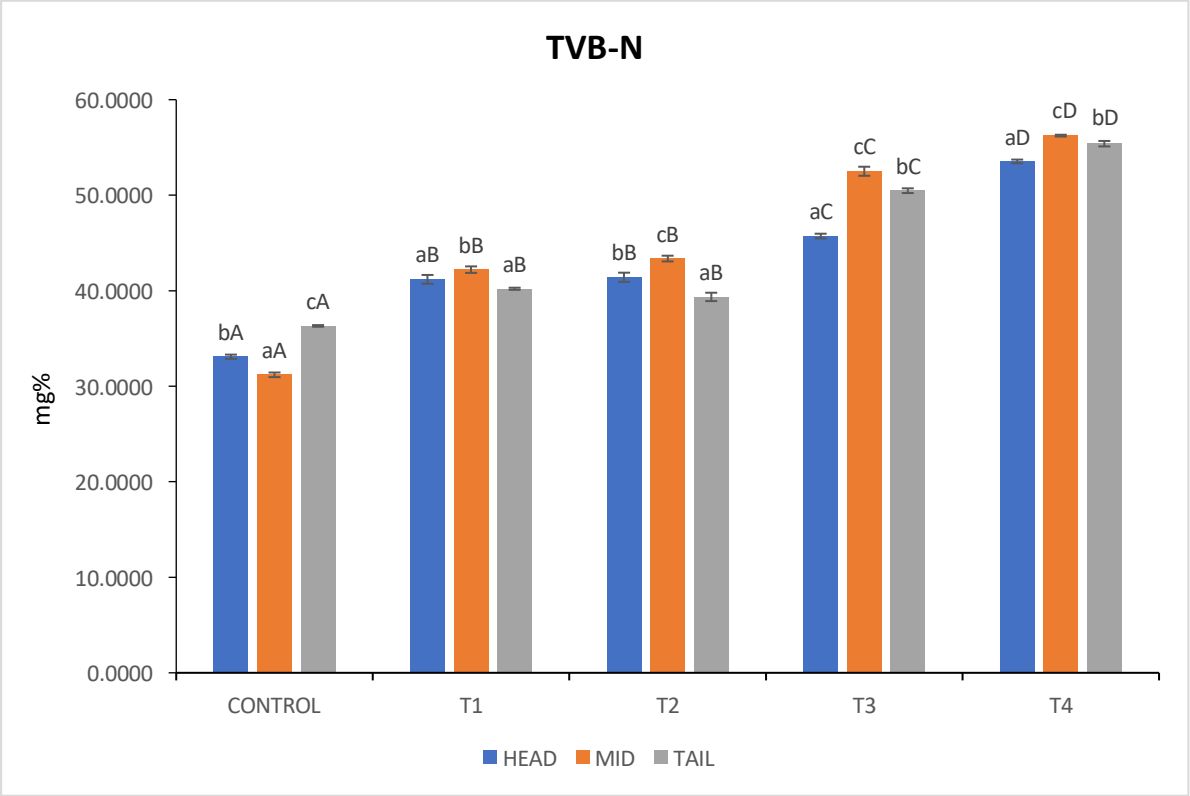

Figure 5. TVB-N content of *Labeo rohita* on day-4 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.2 Effect of temperature abuse on Thiobarbituric Acid Reactive Substances (TBARS) content of *Labeo rohita* during ice storage

TBARS (Thiobarbituric Acid Reactive Substances) is a widely used test to assess lipid oxidation in food, particularly fats and oils. It measures malondialdehyde (MDA), a by-product of lipid peroxidation, which indicates the level of fat degradation and oxidative rancidity. In fish, TBARS values below 2 mg MDA/kg are generally considered acceptable, showing minimal oxidation. Higher values suggest increased lipid oxidation, leading to a decline in the quality of the food product. Although specific limits may vary based on regional standards and fish type, a TBARS value above 5 mg MDA/kg typically signals rancidity and spoilage. This test is crucial in determining the freshness and shelf life of fish and other fatty foods.

4.1.2.1 Thiobarbituric acid reactive substances (TBARS) of *Labeo rohita* on day-0 of ice storage

The Fig 6. illustrates the TBARS values of Rohu (head, mid, and tail sections) on Day 0 across different treatments such as control, 4hr, 8hr, 12 hr, 16 hr (Control, T1, T2, T3, T4). Among treatments, the highest value was observed in T4(16hr) i.e., 2.63 mg MDA/kg, in the mid-section.

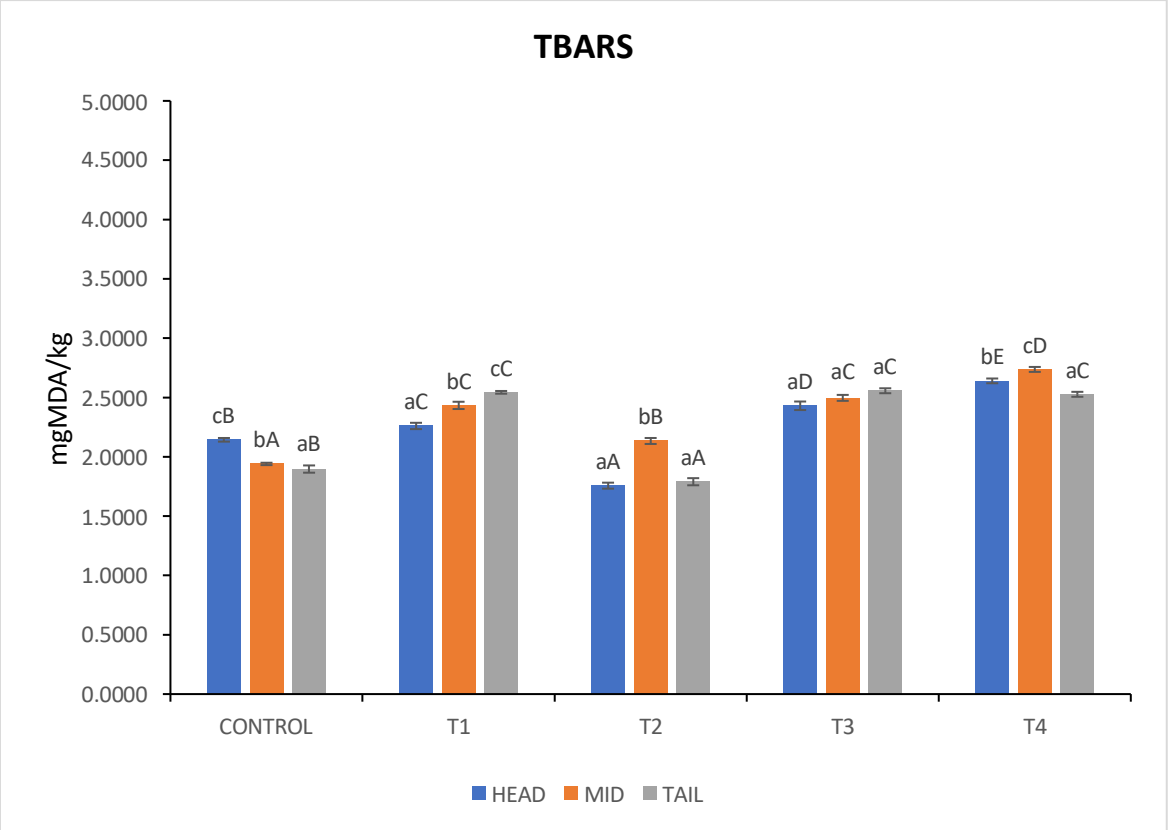

Figure 6. TBARS content of Rohu on *Labeo rohita* of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.2.2 Thiobarbituric acid reactive substances (TBARS) of *Labeo rohita* on day-1 of ice storage

In Fig:7, a spike in TBARS content was observed in T4 (16hr) and the value reached 4.13 mg MDA/kg). Except T3, control (1.67mg MDA/kg), T1 (1.93mg MDA/kg) and T2 (1.51mg MDA/kg) remained within the acceptable limit.

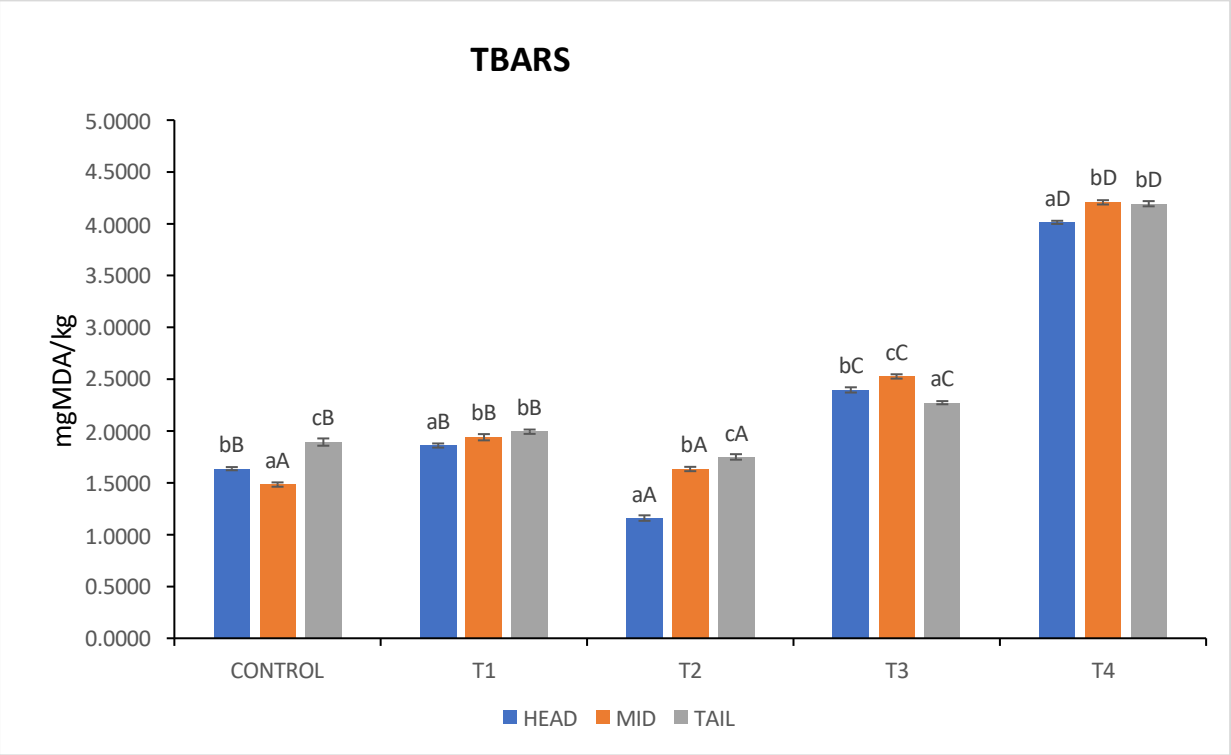

Figure 7. TBARS content of *Labeo rohita* on day-1 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.2.3 Thiobarbituric acid reactive substances (TBARS) of *Labeo rohita* on day-2 of ice storage

In Fig 8. The TBARS content of T1, T2, T3 including control (2.06mg MDA/kg) crossed the acceptable limit of 2 mg MDA/kg. Significant differences were noted between portions within the same treatment.

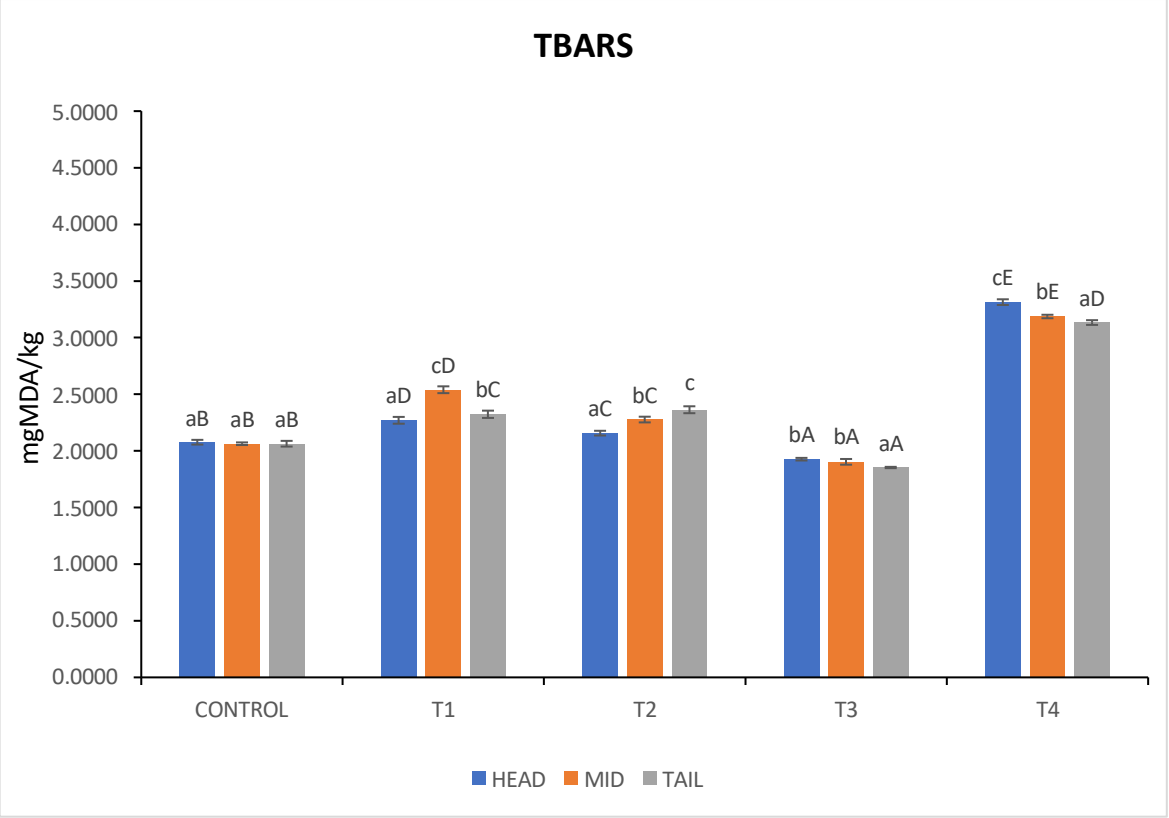

Figure 8. TBARS content of *Labeo rohita* on day-2 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.2.4 Thiobarbituric acid reactive substances (TBARS) of *Labeo rohita* on day-3 of ice storage

TBARS content (3.36mg MDA/kg) of different sections of T4 was significantly higher than that of other treatments. In Fig 9. the TBARS content of other treatments including control remained below 2.5mg MDA/kg on Day 3 as well.

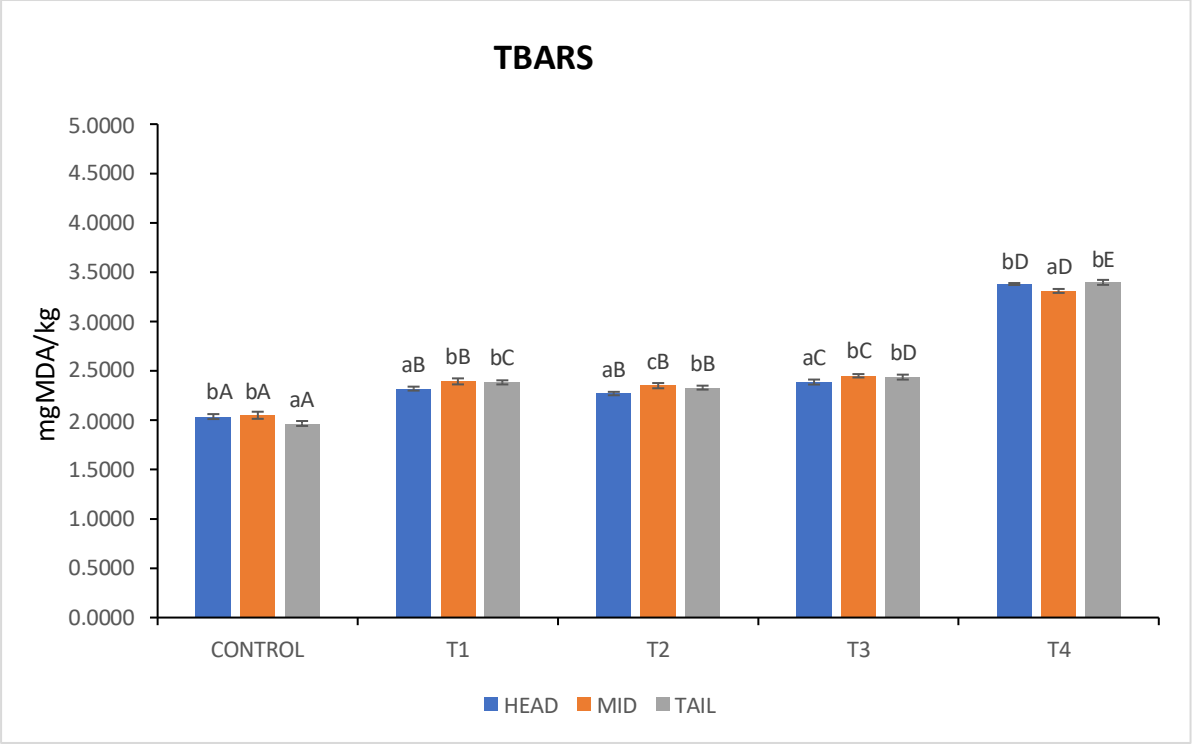

Figure 9. TBARS content of *Labeo rohita* on day-3 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.2.5 Thiobarbituric acid reactive substances (TBARS) of *Labeo rohita* on day-4 of ice storage

In Fig 10, An increase in TBARS content was observed in C, T1, T2, T3 and T4 and reached 2.7, 2.83, 2.89, 2.99, 4.16mg MDA/Kg respectively.

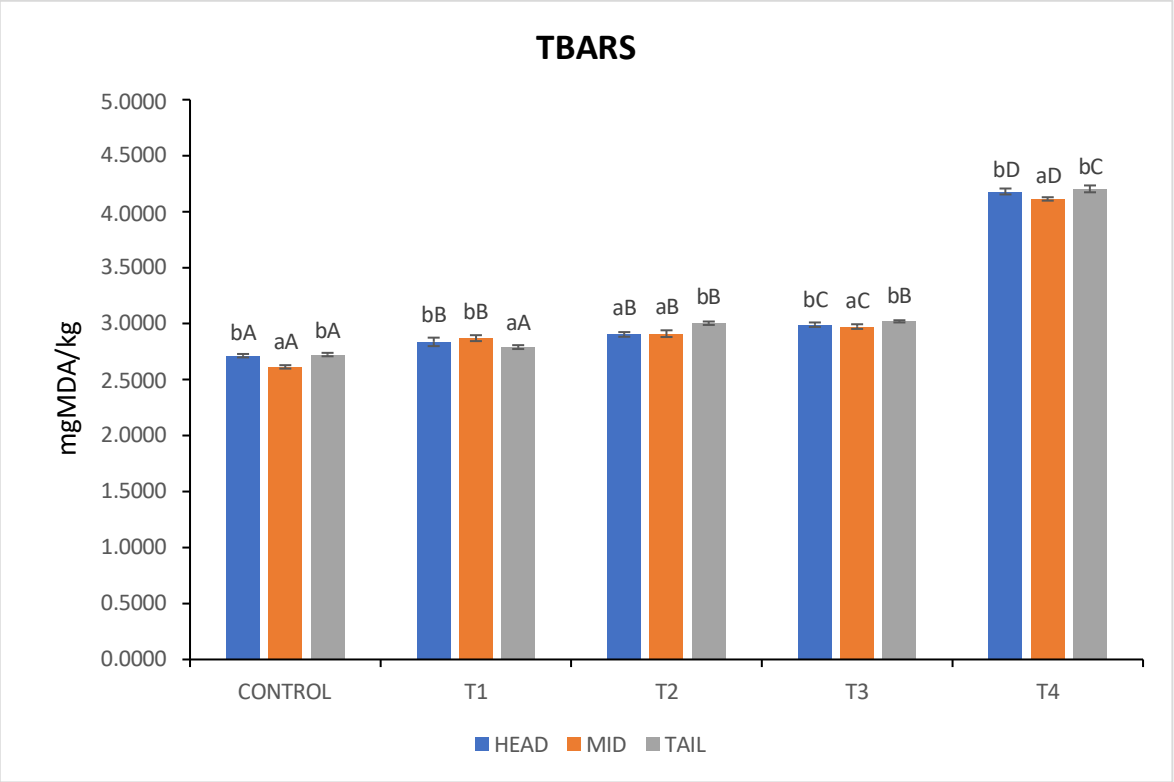

Figure 10. TBARS content of *Labeo rohita* on day-4 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.3 Effect of temperature abuse on muscle pH of *Labeo* rohita (Hamilton, 1822) during ice storage

Fresh fish generally have a pH around 6.2 to 6.5. During storage of fish, the pH typically increases as spoilage progresses. As storage time extends, microbial activity and enzymatic breakdown of proteins produce alkaline compounds like ammonia, raising the pH. A rise in pH indicates spoilage, leading to deterioration in quality and reduced freshness.

4.1.3.1 pH content of *Labeo rohita* on day-0 of ice storage

The Fig 11. displays the pH levels of different sample parts (Head, Mid, Tail) across five treatments on Day 0. pH content of the head and mid portion of control, T1 and T2 were found to be significantly different from tail. pH of all treatments ranged

between 6.27 to 6.6. Control (6.54) and T1 (6.51) exhibited higher pHs, while T4 had the lowest, 6.27.

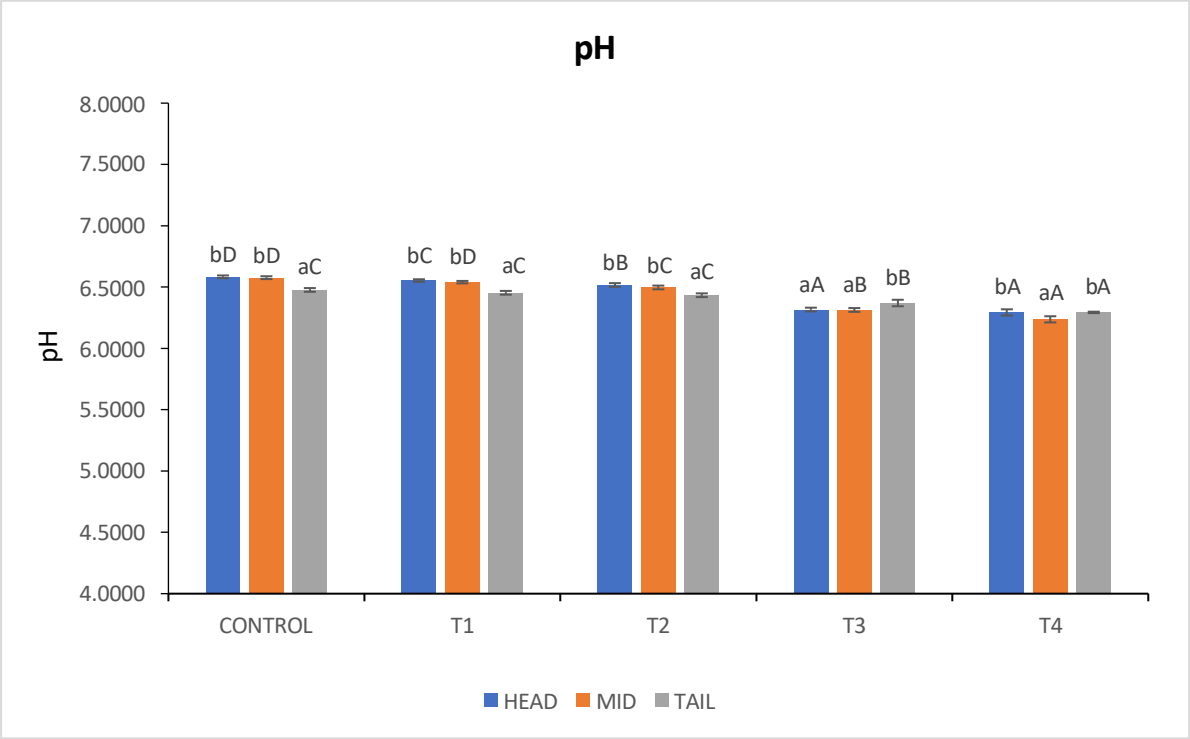

Figure 11. pH content of *Labeo rohita* on day-0 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significant difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.3.2 pH content of temperature abused *Rohu* on day-1 of ice storage

The fig 12. illustrates pH variation across five treatments- control, 4hr, 8hr, 12 hr, 16 hr (control, T1, T2, T3, T4) and three body parts (Head, Mid, Tail) on the 1st day of storage. A slight decline in pH is observed from the control group to T4. Control exhibited the highest pH of 6.54, followed by T1, T2, T3 and T4 respectively, with T4 showing the lowest pH of 6.31. Despite this trend, no significant difference in pH is noted between the different body parts within any treatment.

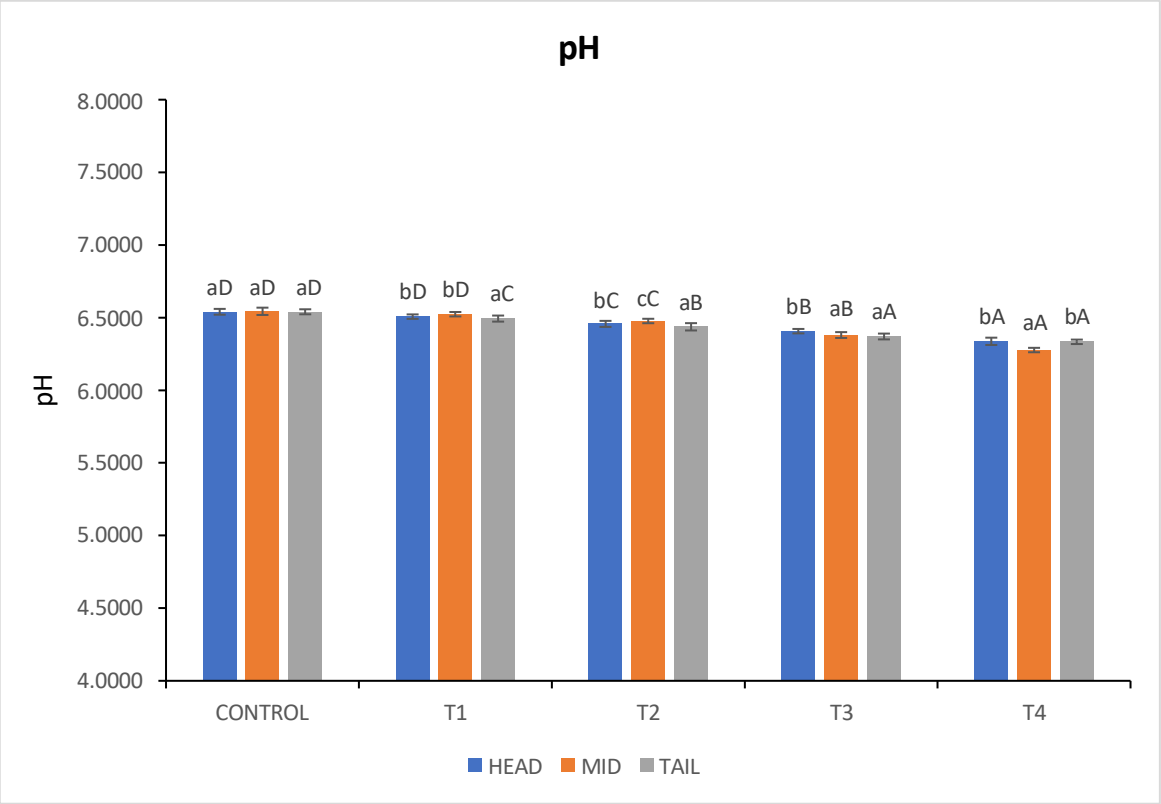

Figure 12. pH content of *Labeo rohita* on day-1 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.3.3 pH content of *Labeo rohita* on day-2 of ice storage

An abrupt rise in pH for all the treatments was observed in Fig 13. Control had 7.59, followed by T1 (7.6), T2 (7.6), T3 (7.31) and T4 (7.26) respectively, with T2 showing the highest pH 7.44. On day 2, head and tail portions had higher pH in comparison to mid-portion shows relatively lower value for pH.

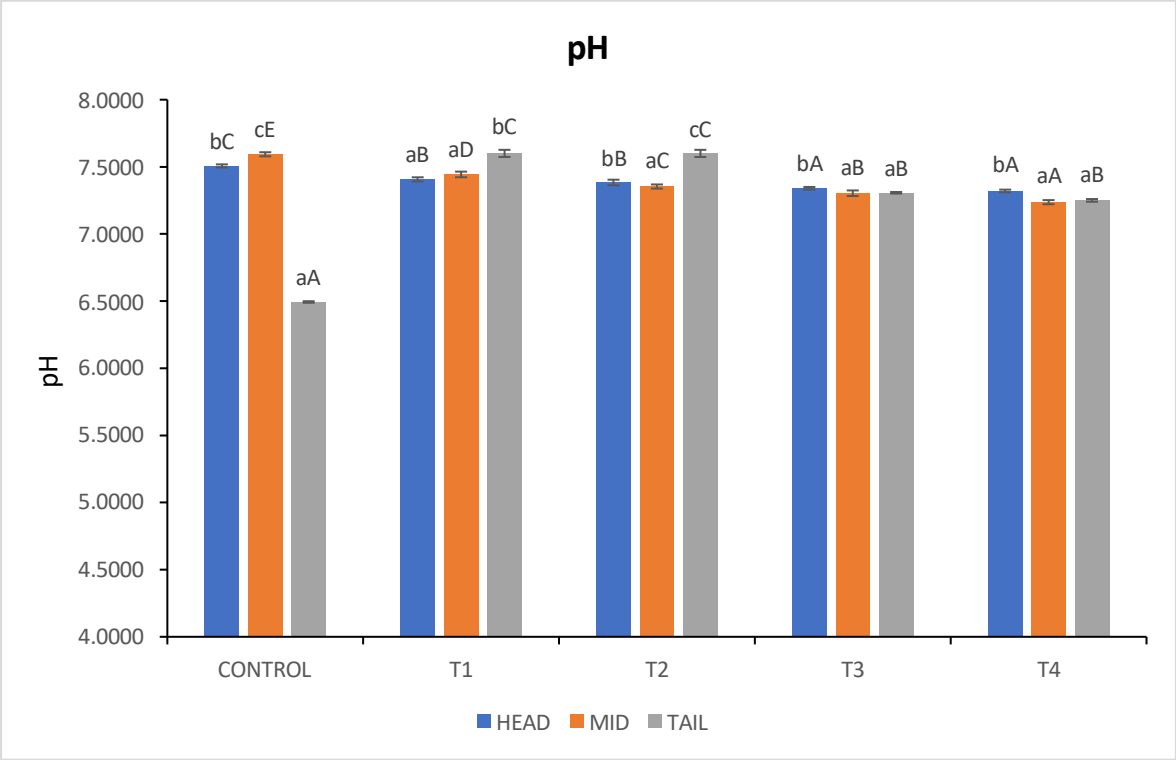

Fig 13. pH content of *Labeo rohita* on day-2 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.3.4 pH content of *Labeo rohita* on day-3 of ice storage

In Fig 14, pH remains fairly constant in comparison to day 2 for each treatment. No any further changes in pH were seen on day 3 in treatments. Control exhibits the highest pH 7.51, followed by T1, T2, T3 and T4 respectively. With T4 showing the lowest pH 7.31, and continuous decline in pH is observed from the control group to T4. There is no substantial variation in pH levels between the different body parts Head, Mid, and Tail within any individual treatment.

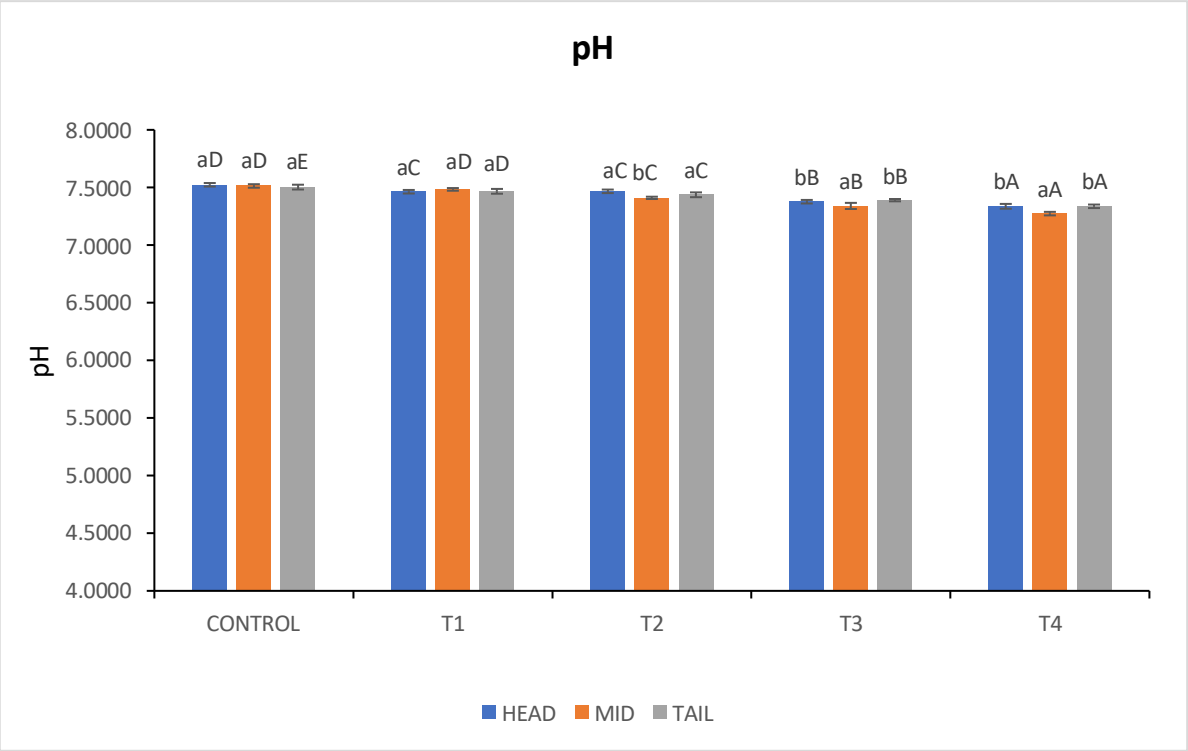

Fig 14. pH content of *Labeo rohita* on day-3 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.3.5 pH content of *Labeo rohita* on day-4 of ice storage

In Fig:15 the pH remains stable resembling Day 2 and Day 3, with no further changes observed in any treatment or across the days. Control and T1 exhibit the highest pH of 7.50 and 7.48 respectively, followed by T2, T3, and T4, with T4 having the lowest pH of 7.39. Despite the overall decrease in pH from control to T4, there is no significant difference in pH between the Head, Mid, and Tail regions within each treatment.

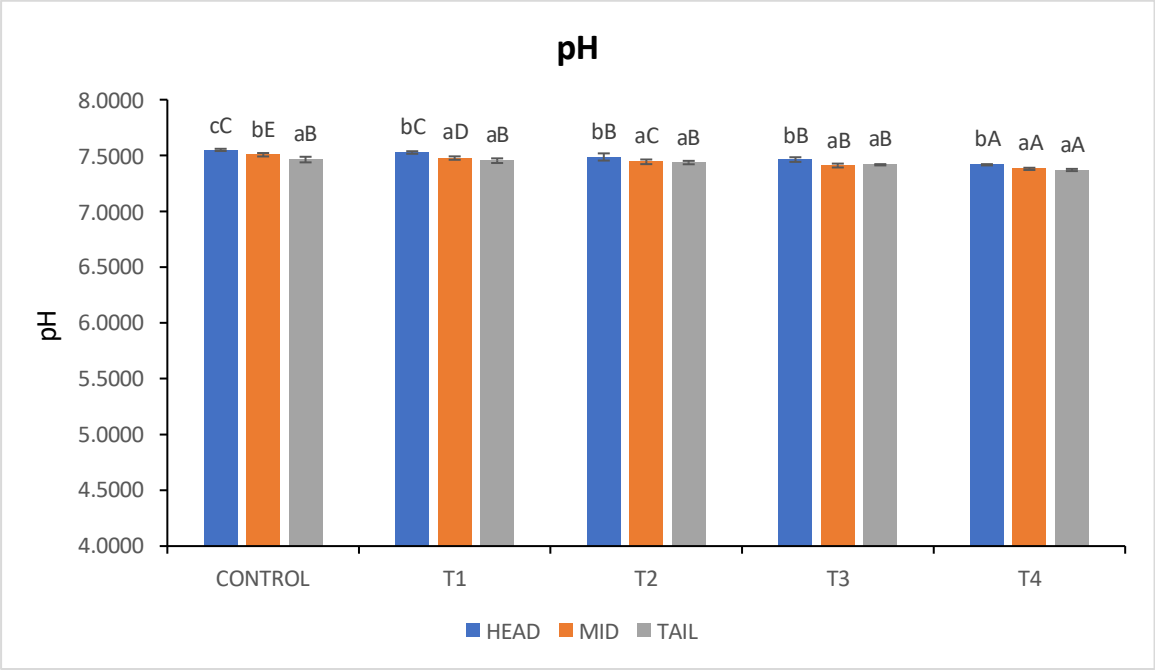

Fig 15. pH content of temperature abused *Labeo rohita* on day-4 of ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

The superscripts on error bars represent significance difference (p≤0.05). The uppercase letters denote significant difference between the treatments and lowercase letters denote the significant difference between the portions.

4.1.4 Effect of temperature abuse on microbiological quality of *Labeo* rohita (Hamilton, 1822) during ice storage

The total plate count of immediately iced and temperature abused *Labeo rohita* over a 5-day storage period is detailed in Table 2. An increase in Total Plate Count (TPC) was observed across nearly all treatments. On Day 0, control had the lowest TPC and T4 had the highest count of 6.2 log CFU/g. TPC of control, T1 and T2 increased slightly towards day 5. TPC of T3 and T4 showed a decreasing trend as storage progressed.

Table 2. Total plate count (log cfu/g) of *Labeo rohita* during ice storage

Treatments	Day-0	Day-1	Day-2	Day-3	Day-4
Control	4.12	4.94	4.22	4.22	4.88
T1	4.91	5.92	5.00	5.01	5.48
T2	5.29	5.90	4.80	4.15	5.91
T3	5.85	5.44	5.06	4.40	4.99
T4	6.20	5.83	5.48	5.00	5.38

Here T1, T2, T3 and T4 is holding sample at 4,8,12 and 16 hours respectively,

4.1.5 Determination of Critical rejection points (CRP) for spoilage indices of Rohu

As per table 3. for TVBN, the control group stayed within the acceptable limit throughout the storage. The 16-hour lot (T4) all sections exceeded the limit on the day 1, with the middle section surpassing it on day 0. Day -0 is taken as CRP for 16hr lot. For the 12-hour, 8-hour, and 4-hour treatments, the value crossed the critical limit on 1, 2, and 3, respectively. The TBARS test revealed that the control group passed the spoilage threshold by day 4. The 8-hour and 16-hour treatments exceeded the critical limit on days 2 and 0, respectively, while the 4-hour and 12-hour treatments crossed the limit on day 0 but initially showed a decrease before rising again. This suggests that the spoilage rate varies depending on the treatment duration, with some treatments experiencing a temporary drop in spoilage before increasing. The pH initially remained stable but showed a sharp increase after day 1, indicating spoilage. TPC showed a continuous rise from day 0 to day 4 for all treatments. Fig 3. Indicate the specific day when each treatment went beyond the critical limit of rejection for different measured parameters.

Table 3. Critical rejection points (CRP) for each treatment (parameter wise) of *Labeo rohita*

Parameter	Treatments				
	Control	**T1**	**T2**	**T3**	**T4**
TVB-N (35 mg%)	-	Day 3	Day 2	Day 1	Day 0
TPC (log 5)	-	Day 1	Day 0	Day 0	Day 0
TBARS (2mg MDA/kg)	Day 2	Day 0	Day 2	Day 0	Day 0

4.2 Establishing critical rejection points for spoilage indices of *Penaeus vannamei* during ice storage

4.2.1 Estimation of Total Volatile Basic Nitrogen (TVB-N) in *Penaeus vannamei* during ice storage

The Fig 16. shows the Total Volatile Basic Nitrogen (TVB-N) levels (mg%) of treatments (Control, T1, T2, T3, T4) during storage. On Day 0, TVB-N content was low for Control (7.56mg%), whereas T4 recorded the highest (34.14mg%). A slight increase was observed in all treatments on day 1. On Day 2, T4 (41.98 mg%) and T3 (35.42mg%) crossed the acceptable limit of TVBN. Whereas, control and T1 had 19.03mg% and 21.20mg% respectively. On day 3, C, T1 and T2 continued to remain within the limit. Finally, on Day 4, a notable increase was observed for C, T1 and T2 with T1 and T2 lots exceeding the limit of acceptability, while the control had TVBN within the acceptable limit.

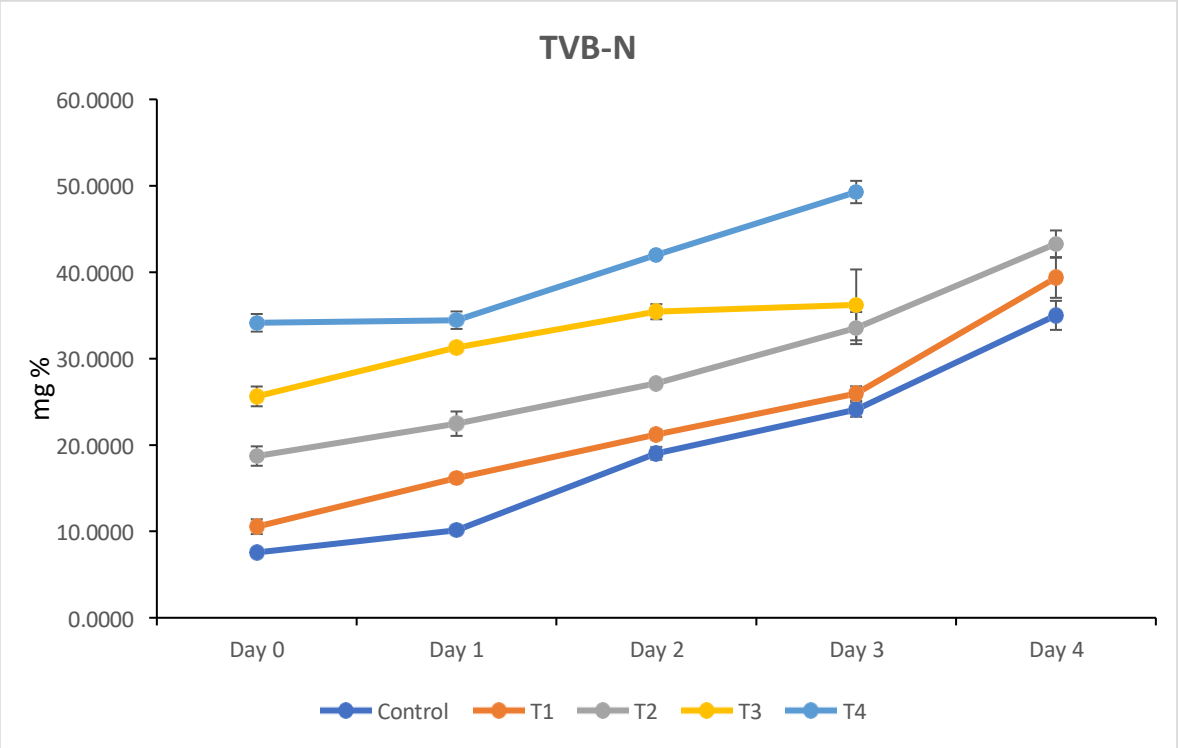

Fig 16. TVB-N content of *Penaeus vannamei* during ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

4.2.2 Estimation of pH content in *Penaeus vannamei* during ice storage

Figure 17 illustrates the pH trend of treatments (control, T1, T2, T3, T4) from day 0 to day 4. On day 0, control (6.79), T1 and T2 exhibited comparable values with no significant differences observed between them, whereas T3 and T4 (6.93) demonstrate a slightly higher values. On Day 1, a notable increase in value was recorded for all treatments, with T2, T3, and T4 demonstrating a more substantial rise. The control exhibited a pH of 6.84, while that of T4 was 7.52. On Day 2, the control (7.09) and T1 (7.19) showed a steady increase, whereas T2, T3, and T4 (7.31) faced a significant decrease in comparison to Day 1. On Day 3, the control had 7.26 and T4 had 7.39 and T3 and T4 were discontinued. By Day 4, the values for the control decreased to 6.96, T1 to 7.05, and T2 to 7.14.

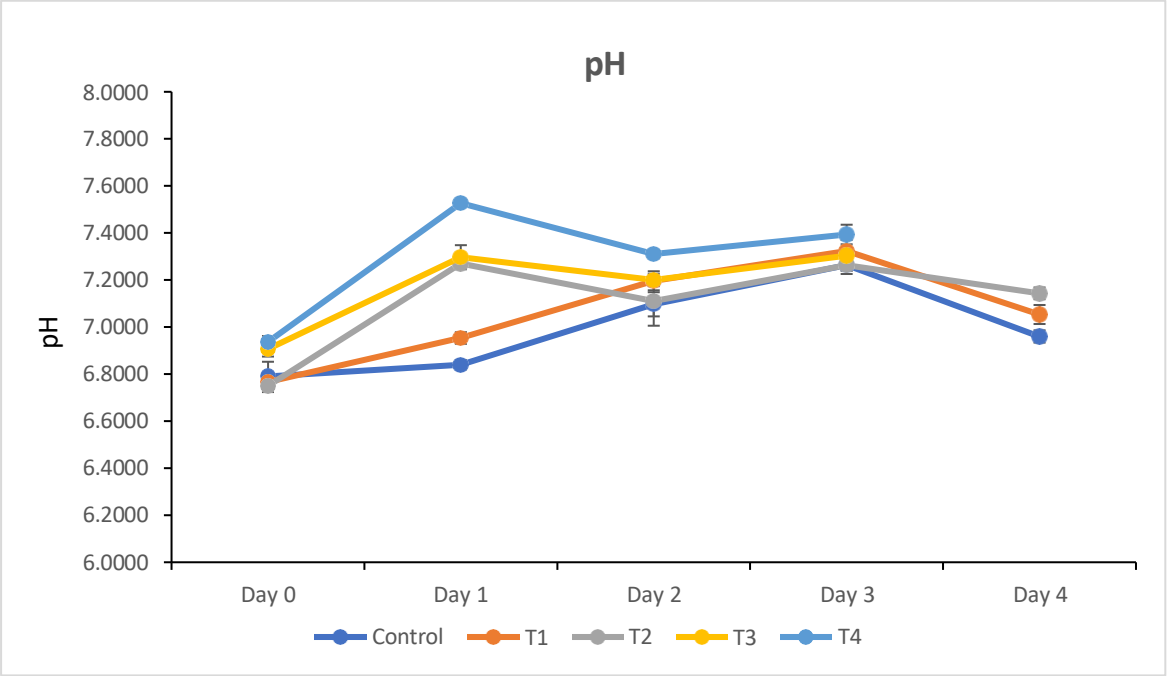

Fig 17. pH content of *Penaeus vannamei* during ice storage

Data expressed as mean ± standard deviation, (n=3). Control- immediately iced lot; T1, T2, T3 and T4 are fishes exposed to room temperature for 4,8,12 and 16 hours respectively.

4.2.3 Microbiological quality of *Penaeus vannamei* during ice storage.

Table 3. shows the microbiological profile of *Penaeus vannamei* over a 5-day storage period, presented as log CFU/g. A consistent increase in Total Plate Count (TPC) was noted among the treatments. TPC of T4 started with 7.25 log CFU/g) on day 0 and continued to increase and recorded 8.14 on day 2. All treatments exhibited an increase in bacterial count throughout storage.

Table 4. Total plate count (log cfu/g) of *Penaeus vannamei* during ice storage

Treatment	Day-0	Day-1	Day-2	Day-3	Day-4
Control	5.11	5.76	5.96	7.01	7.04
T1	5.29	7.02	7.39	7.88	8.34
T2	6.16	6.76	7.05	7.92	9.32
T3	6.34	6.88	7.69	8.69	
T4	7.25	7.92	8.14		

Here T1, T2, T3 and T4 is holding sample at 4,8,12 and 16 hours respectively

4.2.4 Determination of Critical rejection points (CRP) for spoilage indices of Rohu

The table 3. shows CRP for each spoilage index as affected by temperature abuse. The 4-hour and 8-hour treatments exceeded the critical limit by Day 4, control continued to remain within the limit. 16-hour and 12-hour lots crossed the threshold on Day 2 itself. The microbiological quality followed a similar trend, with T4 sh owing the highest total plate count (TPC) values. By Day 2, the TPC in T4 exceeded acceptable limits, making it unfit for consumption. Treatment 12 hr (T3) and 16 hr (T4) was discarded after day 3 due to very high bacterial count. This indicates that prolonged exposure to temperature abuse accelerates spoilage in shrimp.

Table 5. Critical rejection points (CRP) of each treatment (parameter wise) of *Penaeus vannamei*

Parameter	Treatments				
	Control	**T1**	**T2**	**T3**	**T4**
TVB-N (35 mg%)	Day 4	Day 4	Day 4	Day 2	Day 2
TPC (log 5)	Day 0	Day 0	Day 0	Day 0	Day 0

4.3 Image based freshness prediction model of *Labeo rohita*

4.3.1 Data classification of Experiment-1

The experiment involved temperature abuse prior to icing and lots were stored for 5 days. A total of 1500 images were captured from different sections of the fish body, i.e. head, mid and tail. The images were then categorized into three groups, i.e., training, validation and testing. As given in table 4, 70% (1050 nos.) of the images were taken for training purpose, 15% (225 nos.) for validation purpose and rest 15% (225 nos.) for testing purpose. The predefined YOLO model was customized and a separate model was developed using the image dataset. The model was trained for 100 epochs, with the entire training process taking approximately 110 minutes.

Table 6. Split of the image data into training, validation and test dataset

Dataset	Augmentation	Images
Training	Yes	1050
Validation	No	225
Test	No	225

4.3.1.1 Data Loss during model training

The Plate 8. displays graphs depicting various performance metrics for both the training and validation datasets, illustrating the progressive reduction in box loss and objectness loss throughout the training process.

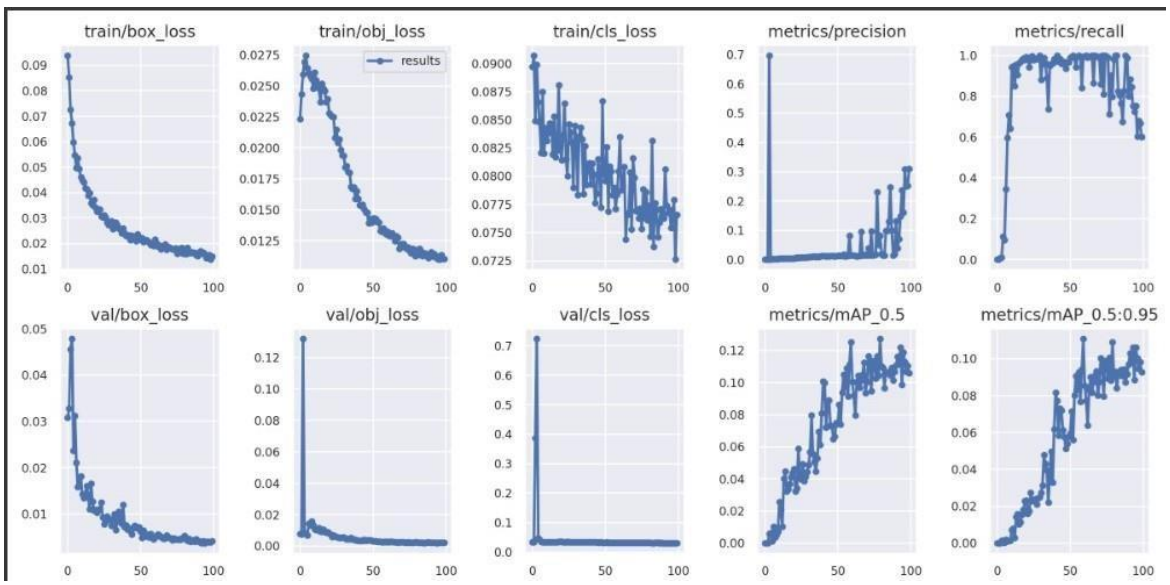

Plate 8. Plot of box loss, objectness loss, classification loss and mean average precision over the training epochs for the validation dataset for *L. rohita.*

Plate 8. visually depicts the progressive increase in mean Average Precision (mAP) across the training epochs, highlighting a notable upward trend. Notably, mAP50-95 began to plateau around the 100-epoch mark; however, 100 epochs were not sufficient to accurately detect the Rohu fish species. As the number of epochs increased, the model's performance and precision of the model continued to improve. Based on this observation, the decision was made to increase the number of epochs to 150 to further enhance the model's ability to detect fish species effectively.

4.3.1.2 Confusion matrix

The confusion matrix illustrates the model's performance in classifying the data. The darker diagonal elements indicate that the model has successfully classified some instances with relatively high accuracy. However, the presence of lighter shades off the diagonal suggests there are several misclassifications across different categories.

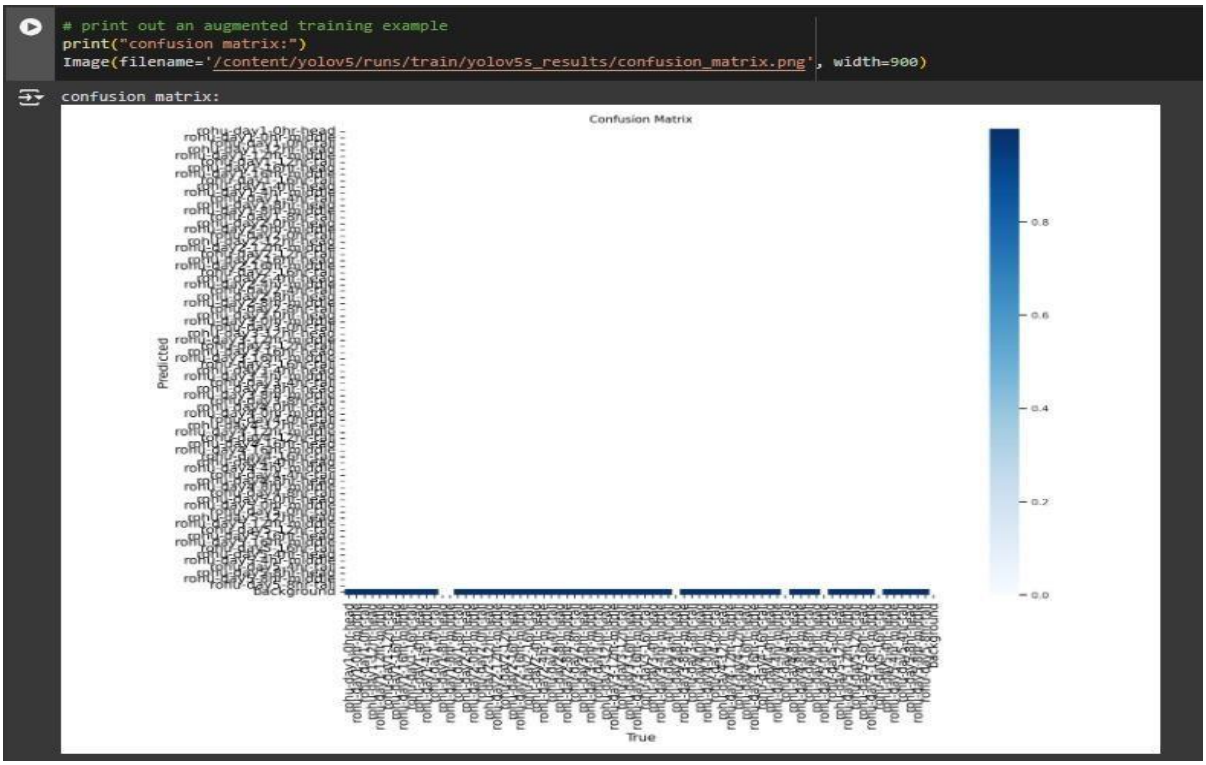

Plate 9. Confusion matrix

Plate 10. Images of *Labeo rohita* obtained after training (experiment-1)

4.3.1.3 Estimation of accuracy of the model

```
                 Class     Images  Instances          P          R      mAP50  mAP50-95: 100% 6/6 [00:02<00:00,  2.84it/s]
                   all        161        161     0.0127      0.995      0.125      0.111
      rohu-day1-0hr-head        161          3     0.0135          1     0.0704      0.065
    rohu-day1-0hr-middle        161          4     0.0188          1     0.0767     0.0698
      rohu-day1-0hr-tail        161          4     0.0179          1     0.0865       0.06
      rohu-day1-12hr-head        161          2     0.0126          1     0.0538     0.0499
    rohu-day1-12hr-middle        161          1    0.00571          1     0.0262     0.0236
      rohu-day1-12hr-tail        161          1    0.00575          1     0.0711     0.0569
      rohu-day1-16hr-head        161          3     0.0156          1      0.123      0.111
    rohu-day1-16hr-middle        161          3     0.0169          1     0.0786     0.0747
      rohu-day1-16hr-tail        161          3     0.0144          1      0.142      0.114
      rohu-day1-4hr-head        161          1    0.00578          1     0.0199     0.0199
    rohu-day1-4hr-middle        161          2     0.0121          1     0.0226     0.0219
      rohu-day1-4hr-tail        161          2     0.0114          1     0.0713     0.0474
      rohu-day1-8hr-tail        161          3      0.016          1     0.0533     0.0426
      rohu-day2-0hr-head        161          7      0.029          1      0.256      0.228
    rohu-day2-0hr-middle        161          8       0.04          1      0.262      0.204
      rohu-day2-0hr-tail        161          3    0.00971          1      0.264      0.203
      rohu-day2-12hr-head        161          2     0.0118          1      0.287      0.186
    rohu-day2-12hr-middle        161          2     0.0123          1     0.0327     0.0327
      rohu-day2-12hr-tail        161          1    0.00556          1     0.0255     0.0179
      rohu-day2-16hr-head        161          2    0.00995          1      0.142      0.128
    rohu-day2-16hr-middle        161          1    0.00588          1     0.0524     0.0524
      rohu-day2-16hr-tail        161          5     0.0279          1      0.108      0.091
      rohu-day2-4hr-head        161          1    0.00585          1    0.00716    0.00501
    rohu-day2-4hr-middle        161          4     0.0219          1     0.0577     0.0519
      rohu-day2-4hr-tail        161          3     0.0123      0.667     0.0169     0.0094
      rohu-day2-8hr-head        161          1    0.00633          1     0.0124     0.0112
    rohu-day2-8hr-middle        161          1    0.00629          1     0.0284     0.0256
      rohu-day2-8hr-tail        161          1    0.00595          1    0.00816    0.00489
      rohu-day3-0hr-head        161          3     0.0166          1      0.125      0.123
    rohu-day3-0hr-middle        161          2     0.0113          1     0.0408     0.0349
      rohu-day3-0hr-tail        161          5     0.0215          1      0.173      0.126
      rohu-day3-12hr-head        161          3     0.0165          1     0.0355     0.0326
    rohu-day3-12hr-middle        161          2     0.0126          1     0.0666      0.054
      rohu-day3-12hr-tail        161          2     0.0168          1     0.0199     0.0139
      rohu-day3-16hr-head        161          1    0.00613          1     0.0226     0.0204
    rohu-day3-16hr-middle        161          4      0.024          1      0.255       0.23
      rohu-day3-16hr-tail        161          1    0.00571          1     0.0452     0.0317
      rohu-day3-4hr-head        161          2     0.0125          1     0.0224     0.0224
    rohu-day3-4hr-middle        161          1    0.00629          1      0.497      0.448
      rohu-day3-4hr-tail        161          2      0.012          1      0.176      0.154
      rohu-day3-8hr-head        161          1    0.00599          1      0.249      0.224
    rohu-day3-8hr-middle        161          6     0.0326          1      0.266      0.224
      rohu-day3-8hr-tail        161          5     0.0292          1      0.168      0.159
      rohu-day4-0hr-head        161          1    0.00581          1     0.0243     0.0218
    rohu-day4-0hr-middle        161          1    0.00461          1      0.995      0.895
      rohu-day4-0hr-tail        161          3     0.0161          1     0.0543     0.0543
      rohu-day4-12hr-head        161          4     0.0219          1      0.359       0.32
    rohu-day4-12hr-middle        161          4     0.0194          1     0.0543     0.0387
      rohu-day4-12hr-tail        161          3     0.0182          1     0.0728     0.0693
      rohu-day4-16hr-head        161          1    0.00585          1      0.124      0.124
    rohu-day4-16hr-middle        161          2     0.0108          1     0.0631     0.0544
      rohu-day4-16hr-tail        161          1    0.00633          1     0.0114     0.0114
      rohu-day4-4hr-head        161          2     0.0126          1      0.527      0.527
    rohu-day4-4hr-middle        161          2     0.0126          1     0.0252     0.0227
      rohu-day4-8hr-head        161          2     0.0127          1     0.0812     0.0693
```

Plate 11. Estimation of model accuracy

The displayed image in Plate 11. provides the metrics for various classes from a model's training run, indicating its performance for the *L. rohita* dataset. The observations in the key metrics are given below:

Precision (P): Precision values are generally high, close to 1 for most classes, meaning the model has a strong ability to correctly identify the positive instances from all instances.

Recall (R): Recall values for the different classes are consistently at 1, indicating that the model is retrieving all relevant instances effectively.

mAP50 (Mean Average Precision @ 50%): This column shows mAP50 values, indicating how well the model performs in detecting objects within 50% overlap. Most values are high (close to 0.9–1.0) across different classes, showing good detection performance.

mAP50-95: This is a stricter metric as it considers overlap from 50% to 95%. The values range from 0.2 to 0.6 for most classes, with some reaching 0.8, suggesting there is room for improvement in precision at higher overlap thresholds.

The model demonstrated very low precision about 0.127 across all classes. This implies that the model is less accurate when it comes to detecting and classifying the objects. While, the mAP50 scores indicated that the model struggles with the identification of object, the mAP50-95 values highlight a further decline in accuracy when more stricter localization criteria were applied.

The developed model demonstrated very less accuracy, near about 12.7%, underscoring its reliability in classifying data. Its consistent low performance across multiple test scenarios validated that the model is not suitable for tasks demanding precise predictions.

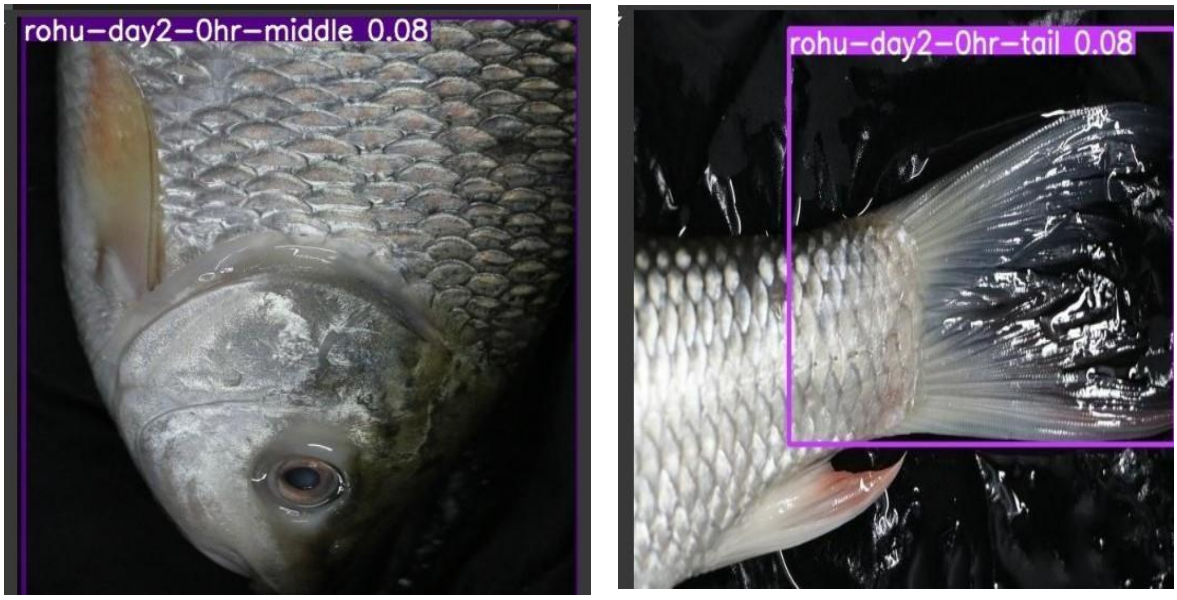

Plate 12. Predicted Result of *L. rohita* image

Plate 12. Displays a real example of the model's predictions. The model was trained with the images of Rohu, treatment wise and day wise from day 1 to day 5. When an unknown input is provided, and the system identifies which day it corresponds to. The confidence scores, ranging from 0 to 1, offer a numerical measure of the model's certainty in its classifications. This comparison effectively highlights the model's ability to correctly identify and classify objects while simultaneously providing a quantifiable level of confidence for each prediction.

4.3.2 Data classification of Experiment-2 of *Labeo rohita*

This experiment involved capturing images of different sections and whole body of Rohu during ice storage for 16 days. A total of 480 images were captured. 70% (336 nos.) of the images were taken for training purpose, 15% (72 nos.) for validation purpose and rest 15% (72 nos.) for testing purpose.

Table 7. Split of the data into training, validation and test dataset

Dataset	Augmentation	Images
Training	Yes	336
Validation	No	72
Test	No	72

The model was trained over 25 epochs, with the entire training process taking approximately 35 minutes. The figure displays graphs depicting various performance metrics for both the training and validation datasets, illustrating the progressive reduction in box loss and objectness loss throughout the training process.

4.3.2.1 Data Loss during model training

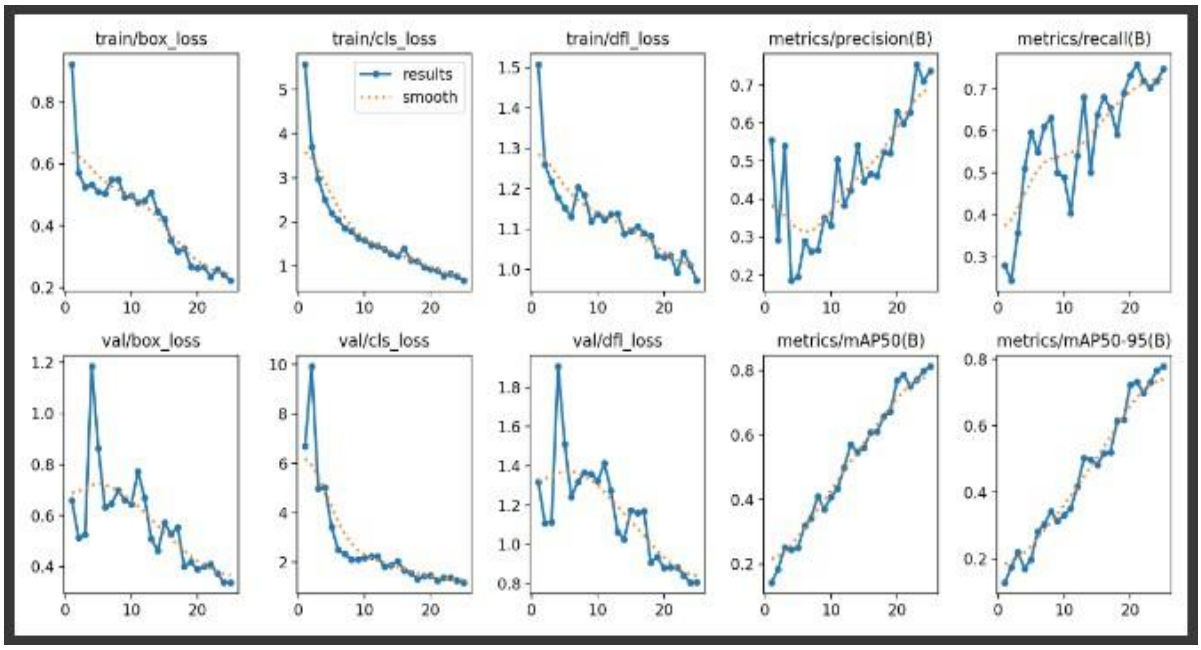

Plate 13. Plot of box loss, objectness loss, classification loss and mean average precision (mAP) over the training epochs for the validation dataset.

Plate 13. visually depicts the progressive increase in mean Average Precision (mAP) across the training epochs, highlighting a notable upward trend. Notably, the mAP50-95 begins to plateau around the 25-epoch mark, suggesting that further training doesn't improve performance much. Based on this, it was decided to stop training at 25 epochs to balance improving the model's accuracy while avoiding unnecessary extra training that offers little benefit.

4.3.2.2 Confusion matrix

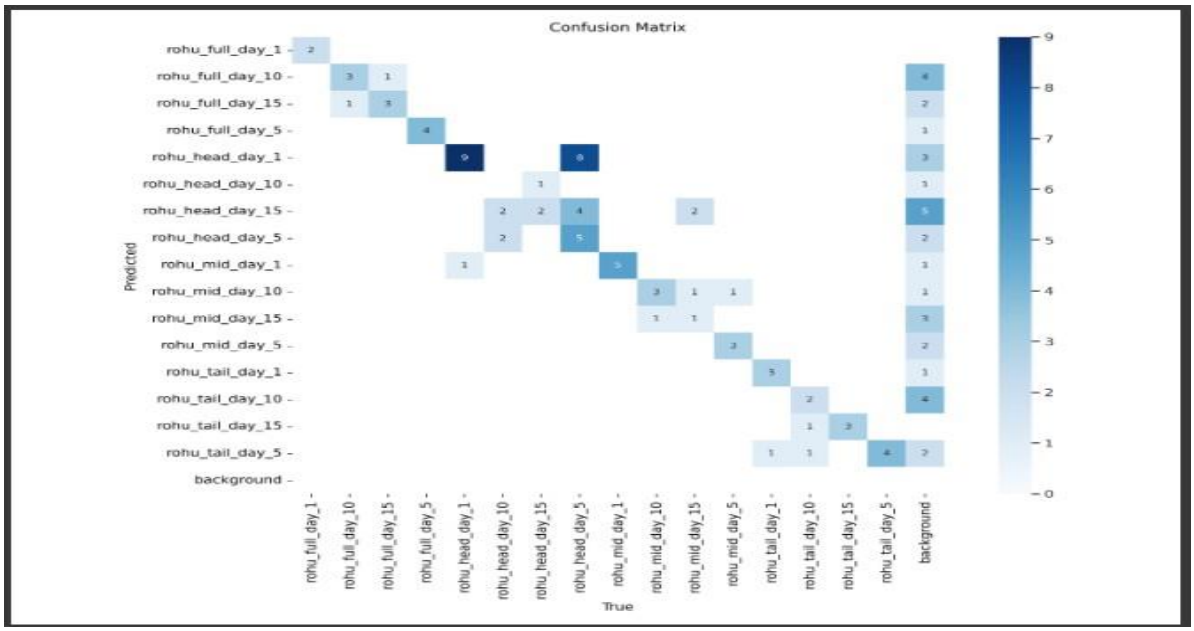

Plate 14. Confusion matrix

The confusion matrix illustrates the model's performance in classifying Rohu fish samples across different day intervals. The diagonal elements, represented by the darker blue shades, indicate the correctly classified instances for each category. For example, categories like " Rohu_Head_DAY5" and " Rohu_tail_DAY5" have 7 correct classifications, demonstrating good accuracy for those classes.

On the other hand, the lighter blue off-diagonal elements show instances of misclassification. For example, "Rohu_tail_DAY2" was misclassified as" Rohu_tail_DAY1" in 8 cases. Similar misclassifications were observed for other categories, such as "Rohu_Head_DAY10", indicating that the model occasionally confuses closely related time intervals.

Overall, the model shows good classification accuracy, successfully identifying most categories, especially the background class. However, it struggles with some overlap between samples from adjacent day intervals, indicating there is some room for improvement in distinguishing specific stages of Rohu fish degradation.

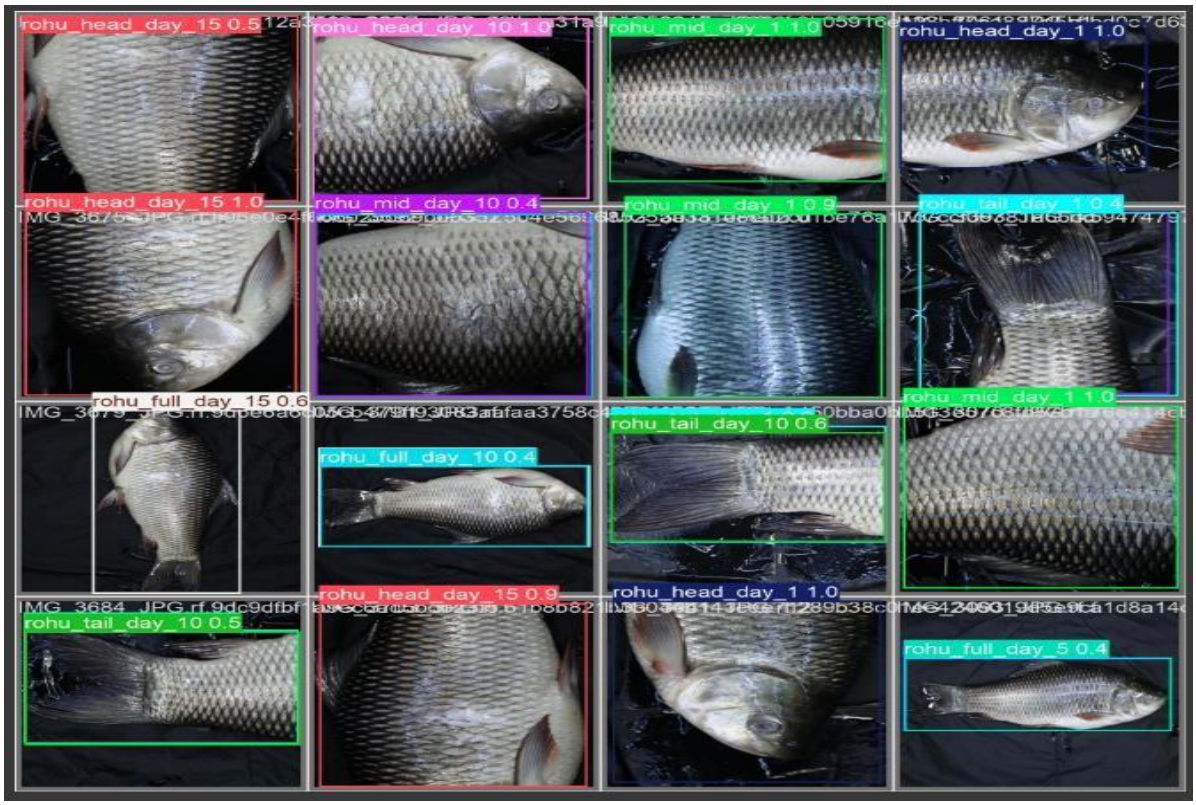

Plate 15. *Labeo rohita* after training of experiment 2

4.3.2.3 Estimation of accuracy of the model

```
[10] !yolo task=detect mode=val model={HOME}/runs/detect/train/weights/best.pt data={dataset.location}/data.yaml

Ultralytics 8.3.7 🚀 Python-3.10.12 torch-2.4.1+cu121 CUDA:0 (Tesla T4, 15102MiB)
YOLO11s summary (fused): 238 layers, 9,418,992 parameters, 0 gradients, 21.3 GFLOPs
val: Scanning /content/datasets/rohu_head-3/valid/labels.cache... 80 images, 0 backgrounds, 0 corrupt: 100% 80/80 [00:00<?, ?it/s]
                 Class     Images  Instances      Box(P          R      mAP50  mAP50-95): 100% 5/5 [00:02<00:00,  1.78it/s]
                   all         80         80      0.737      0.748      0.812      0.782
         rohu_full_day_1          2          2      0.904          1      0.995      0.995
        rohu_full_day_10          4          4      0.867        0.5      0.745      0.745
        rohu_full_day_15          4          4      0.658          1      0.912      0.912
         rohu_full_day_5          4          4      0.886          1      0.995      0.952
         rohu_head_day_1         10         10      0.538          1      0.794      0.716
        rohu_head_day_10          4          4      0.426       0.25      0.235      0.235
        rohu_head_day_15          3          3      0.173      0.667      0.465      0.465
         rohu_head_day_5         17         17      0.758      0.294      0.604      0.583
          rohu_mid_day_1          5          5      0.796          1      0.995      0.995
         rohu_mid_day_10          4          4      0.917       0.75       0.87       0.87
         rohu_mid_day_15          4          4      0.715       0.75      0.849      0.849
          rohu_mid_day_5          4          4          1      0.463      0.895      0.874
         rohu_tail_day_1          4          4          1      0.828      0.995       0.81
        rohu_tail_day_10          4          4      0.424        0.5      0.661      0.639
        rohu_tail_day_15          3          3          1      0.968      0.995      0.962
         rohu_tail_day_5          4          4      0.729          1      0.995      0.908
Speed: 2.3ms preprocess, 12.6ms inference, 0.0ms loss, 8.2ms postprocess per image
Results saved to runs/detect/val
💡 Learn more at https://docs.ultralytics.com/modes/val
```

Plate 16. Estimation of accuracy of the model

The results provided for the validation process using the YOLOv8 model are as follows:

Classes: The classes evaluated are "Rohu_full_DAY1", "Rohu_Head_DAY5", "Rohu_mid_DAY10", etc.

Metrics:

Precision (P): For the overall dataset (all classes), the precision was found to be 0.737

Recall (R): The overall recall was 0.748

Mean Average Precision (mAP50): For all classes combined, the mAP at IoU=0.50 is 0.812

Mean Average Precision (mAP50-95): For all classes, mAP at IoU=0.50-0.95 is 0.782

These metrics indicate good performance in both precision and recall, meaning the model accurately detecting the objects (Rohu) with a high rate of correct classifications across different day intervals. The mAP values suggest strong object detection capability, particularly at the 0.50 threshold, with slightly lower performance when averaged across multiple IoU thresholds (0.50 to 0.95).

Here we have given an unknown input and the system shows that the given input belongs to which day. The model developed was found have 73.7 %, accuracy, highlighting its reliability in classifying data with minimal mistakes. The model's high performance in diverse tests demonstrated its robustness and suitability for tasks needing precise predictions. Increasing the number of images could further enhance the accuracy of the model.

Plate 17. Predicted Result of *L. rohita* image of experiment-2

Plate 17. illustrates a realistic example of image predictions. Due to nominal changes in Rohu could be seen day to day, images were grouped into 4 different labels each having 4 days. Day 1 to day 4 was labelled as day-1, day 5 to day 8 was labelled as day-5, day 9 to day 12 was labelled as day-10. And day 13 to day 16 was labelled as day-15. Image of all the portions and whole body was captured for 15 days, few unknown images from same experiment were uploaded to the model to predict which day they were from. Based on the images, two unknown images from day 1 head portion and day 5 full image of rohu was uploaded, and the model accurately predicted both. It identified the day 1 head image with 98% accuracy and the day 5 full image of rohu with 70% accuracy. The top images showing the true class labels, while the bottom images show the model's predictions along with confidence scores

(0 to 1). This visual comparison illustrates how accurately the model categorizes items and how confident it is in each prediction.

By comparing both the experiment of rohu, Experiment-2 gave better results compared to Experiment-1 due to having more images per label across the portions and days. Experiment-1 had fewer images per label, leading to lower accuracy.

4.4 Image based freshness prediction model of *Penaeus vannamei*

4.4.1 Data classification

Total no. of images captured was 2015, from that splitting into three parts was done, 70% for training purpose i.e. 1410 images, 15% for validation purpose i.e. 302 images, 15% for testing purpose i.e. 303 images. The model was trained over 25 epochs, with the entire training process taking approximately 45 minutes. The figure displays graphs depicting various performance metrics for both the training and validation datasets, illustrating the progressive reduction in box loss and objectness loss throughout the training process.

Table-8 Split of the data into training, validation and test dataset

Dataset	Augmentation	Images
Training	Yes	1410
Validation	No	302
Test	No	303

4.4.1.1 Data Loss during model training

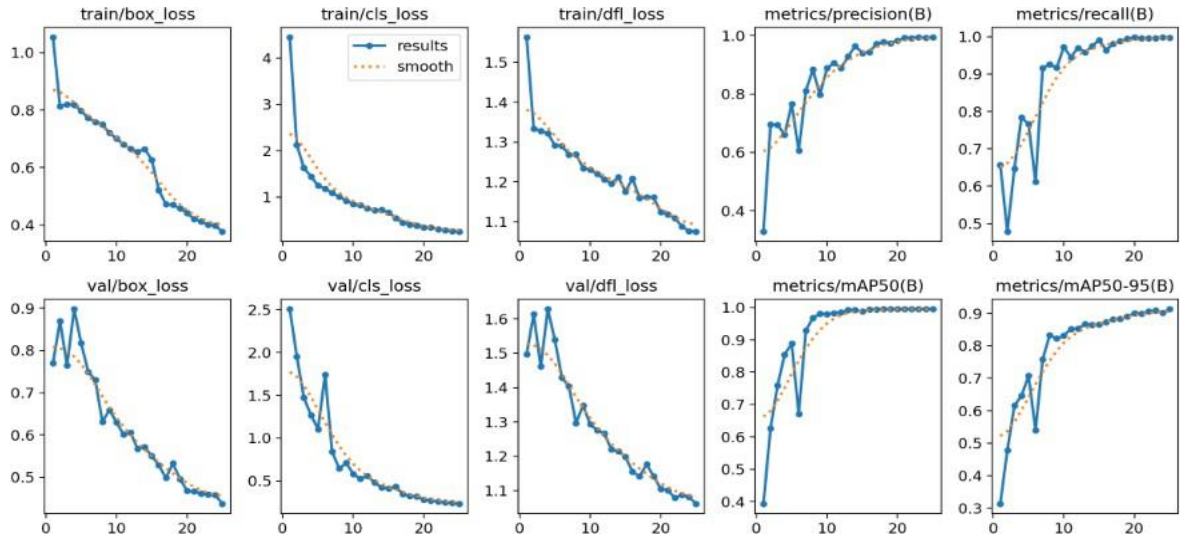

Plate 18. Plot of box loss, objectness loss, classification loss and mean average precision (mAP) over the training epochs for the validation dataset.

Plate 18. visually depicts the progressive increase in mean Average Precision (mAP) across the training epochs, highlighting a notable upward trend. Notably, the mAP50-95 begins to plateau around the 25-epoch mark, suggesting diminishing returns in performance improvement. This observation guided the decision to cap the training duration at 25 epochs, striking a balance between optimizing the model's accuracy and mitigating the diminishing returns associated with prolonged training.

4.4.1.2 Confusion matrix

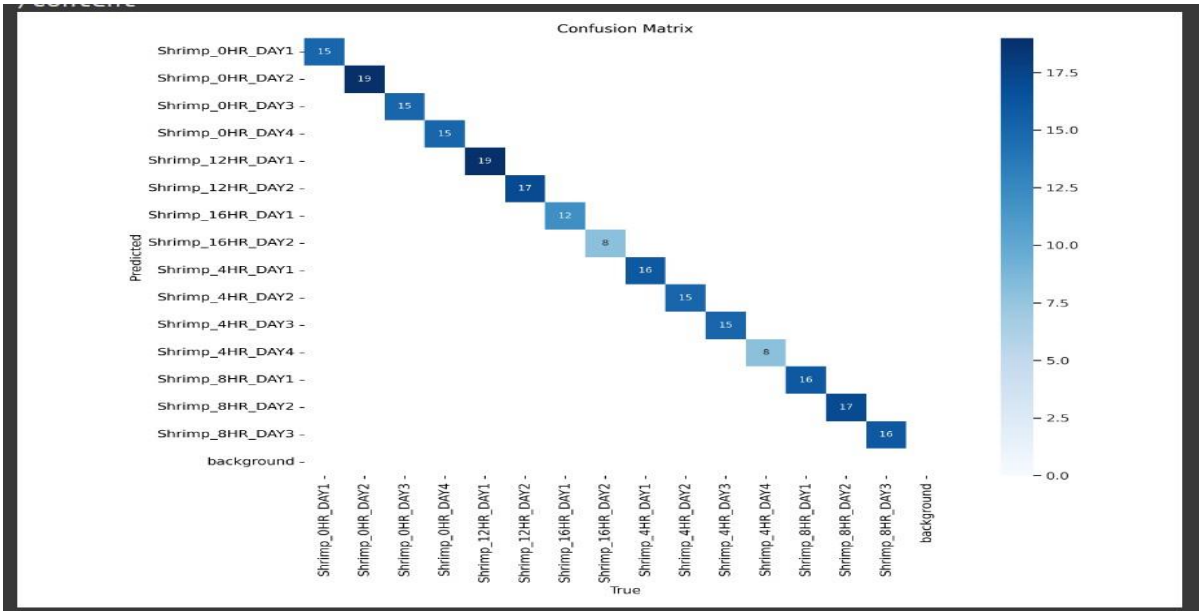

Plate 19. Confusion matrix

The confusion matrix illustrates the model's performance in classifying shrimp samples across different time intervals. The diagonal elements, represented by the darker blue shades, indicate the correctly classified instances for each category. For example, categories like "Shrimp_0HR_DAY2" and "Shrimp_12HR_DAY1" have 19 correct classifications, demonstrating high accuracy for those classes.

On the other hand, the lighter blue off-diagonal elements show instances of misclassification. For example, "Shrimp_16HR_DAY2" was misclassified as "Shrimp_16HR_DAY1" in 8 cases. Similar misclassifications are observed for other categories, such as "Shrimp_4HR_DAY4", indicating that the model occasionally confuses closely related time intervals.

Overall, the model demonstrates strong classification accuracy, as most categories are correctly identified, with the background class being handled particularly well. However, there is some confusion between samples from neighbouring time intervals, particularly around the 16-hour mark, suggesting room for improvement in distinguishing between certain degradation stages of the shrimp samples.

Plate 20. *Penaeus vannamei* after training

4.4.1.3 Estimation of accuracy of the model

```
[ ] %cd {HOME}

    !yolo task=detect mode=val model={HOME}/runs/detect/train/weights/best.pt data={dataset.location}/data.yaml

/content
Ultralytics YOLOv8.0.196 🚀 Python-3.10.12 torch-2.3.1+cu121 CUDA:0 (Tesla T4, 15102MiB)
Model summary (fused): 168 layers, 11131389 parameters, 0 gradients, 28.5 GFLOPs
val: Scanning /content/datasets/SHRIMPDAYHRWISEJULY-1/valid/labels.cache... 234 images, 11 backgrounds, 0 corrupt: 100% 234/234 [00:00<?, ?it/s]
WARNING ⚠️ Box and segment counts should be equal, but got len(segments) = 29, len(boxes) = 223. To resolve this only boxes will be used and all segments will be remo
                 Class     Images  Instances      Box(P          R      mAP50  mAP50-95): 100% 15/15 [00:06<00:00,  2.20it/s]
                   all        234        223      0.995      0.997      0.995      0.913
        Shrimp_0HR_DAY1        234         15      0.992          1      0.995      0.964
        Shrimp_0HR_DAY2        234         19      0.994          1      0.995      0.963
        Shrimp_0HR_DAY3        234         15      0.995          1      0.995      0.907
        Shrimp_0HR_DAY4        234         15      0.995          1      0.995      0.929
       Shrimp_12HR_DAY1        234         19      0.995          1      0.995      0.882
       Shrimp_12HR_DAY2        234         17      0.997          1      0.995      0.936
       Shrimp_16HR_DAY1        234         12      0.994          1      0.995      0.879
       Shrimp_16HR_DAY2        234          8      0.988          1      0.995      0.859
        Shrimp_4HR_DAY1        234         16      0.997          1      0.995      0.895
        Shrimp_4HR_DAY2        234         15      0.994          1      0.995      0.916
        Shrimp_4HR_DAY3        234         15      0.997          1      0.995      0.855
        Shrimp_4HR_DAY4        234          8       0.99          1      0.995      0.922
        Shrimp_8HR_DAY1        234         16          1      0.958      0.995      0.909
        Shrimp_8HR_DAY2        234         17      0.997          1      0.995      0.935
        Shrimp_8HR_DAY3        234         16      0.995          1      0.995      0.939
Speed: 2.5ms preprocess, 10.6ms inference, 0.0ms loss, 4.9ms postprocess per image
Results saved to runs/detect/val
💡 Learn more at https://docs.ultralytics.com/modes/val
```

Plate 21. Estimation of accuracy of the model

The results provided for the validation process using the YOLOv8 model are as follows:

Classes: The classes evaluated are "Shrimp_0HR_DAY1", "Shrimp_0HR_DAY2", "Shrimp_0HR_DAY3", etc., all related to shrimp samples collected at different times.

Metrics:

Precision (P): For the overall dataset (all classes), the precision is 0.995.

Recall (R): The overall recall is 0.997.

Mean Average Precision (mAP50): For all classes combined, the mAP at IoU=0.50 is 0.995.

Mean Average Precision (mAP50-95): For all classes, mAP at IoU=0.50-0.95 is 0.913.

These metrics indicate very high performance in both precision and recall, meaning the model is accurately detecting the objects (shrimp) with a high rate of correct classifications across different time intervals. The mAP values suggest strong object detection capability, particularly at the 0.50 threshold, with slightly lower performance when averaged across multiple IoU thresholds (0.50 to 0.95).

Here we have given an unknown input and the system shows that the given input belongs to which day. The model developed was found to be highly accurate, and the accuracy is found to be more than 99%, Highlighting its reliability in accurately classifying data with minimal error. This high performance across various test scenarios confirms the model's robustness and suitability for applications requiring precise predictions.

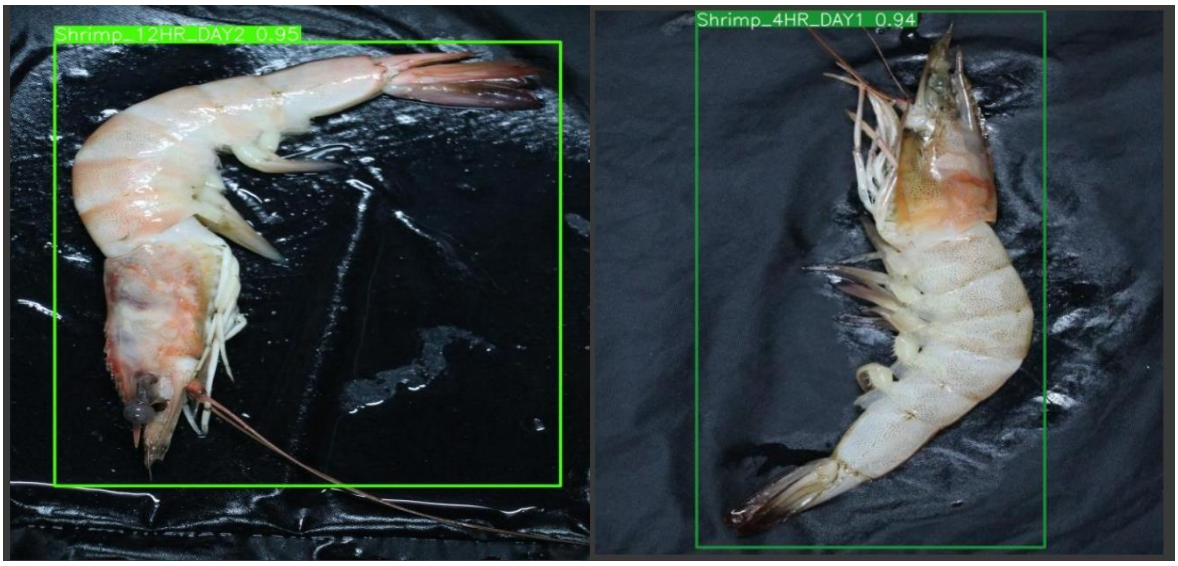

Plate 22. Predicted Result of *P. vannamei* image

Plate 22. illustrates a real-world example of image predictions. Here we have trained the image of shrimp treatment wise apportioned in days from day 1 to day 5. A few unknown images from same experiment were uploaded to the model to predict which day they were from. Based on the images, two unknown images from day 1 and day 2 were uploaded, and the model accurately predicted both. It identified the day 1 image with 94% accuracy and the day 2 image with 95% accuracy. The top images accurately display the class labels, serving as a visual reference for the ground truth. In contrast, the bottom images depict the model's predictions, which are presented as class names accompanied by confidence scores. These scores, ranging from 0 to 1, quantify the model's certainty in its classifications. This visual representation effectively highlights the model's ability to correctly categorize items while providing a measure of confidence for each prediction.

5. DISCUSSION

In order to create a model for predicting freshness based on images that can distinguish between fresh and spoiled fish, several experiments were conducted in this study. Given that spoilage is both accumulative and accelerative, the initial assessment focused on the impact of immediate and delayed icing on Rohu and shrimp by tracking the biochemical and microbiological spoilage indices throughout the storage period. The data collected focused on identifying key rejection points (storage days) for each spoilage index influenced by temperature abuse. The images of the fishes taken as spoilage progressed, were analysed using machine learning algorithms to create models for predicting freshness based on visual data. This section discusses the experimental results acquired during the study.

5.1 Critical rejection points for TVBN

TVB-N is a chemical indicator often used to assess the freshness and quality of meat, seafood, and other protein-rich foods. Higher levels of TVB-N indicate a greater degree of spoilage, making it an important parameter in food safety and quality control, particularly for seafood and meat products. The established standards for TVB-N concentrations in fish are 5–10 mg/100 g for fresh fish, 15–25 mg/100 g for moderately fresh, 30–40 mg/100 g indicating the onset of spoilage, and 50 mg/100 g or higher for spoiled fish.

During the storage study of *Labeo rohita*, on day 0, the TVB-N levels were highest (35.3 mg%) in mid-section of 16 hr lots (T4) and crossed the limit of acceptability. The control lot had lower TVBN (19.8 mg%) compared to temperature abused lots. A spike was observed in 8hr lot from day 1 (31mg%) to Day 2 (39mg%) and crossed the limit of acceptability. On day 4, mid-section of C, T1, T2, T3 and T4 recorded 31.6mg%, 42.2mg%, 43.36mg%, 52.5mg%, and 56.23mg% TVBN, respectively. The CRP for TVBN as affected by temperature abuse was determined as day 0 for 16hr, day 1 for 12 hr, day 2 for 8hr and day 3 for 4 hr.

Prabhakar *et al.* (2021) measured TVB-N levels in ice stored *Labeo rohita* and reported initial and final TVB-N values as 4.57 mg/100g (fresh sample), 19.88 mg/100g (after 24 days at 5 °C), and 7.10 mg/100g (after 24 days at 0 °C). This study

is in concordance to our result that the TVBN content is affected by exposure to higher temperature.

In an experiment on delayed icing, conducted by (Surti *et al.,* 2001), 2-hour delay in cooling reduced the storage life of Grouper by about half, and each additional hour of improper storage further decreased shelf-life by roughly 1 day. In contrary to this, the TVBN content of 4 hr lot on day 0 was similar to that of control on Day 2. It can be concluded that the Rohu fish exposed to room temperature for 4 hrs had a quality reduction equivalent to 3 days in Ice.

The CRP for TVBN as affected by temperature abuse for *L. vannamei* was determined as day 1 for 16hr and 12 hr and day 4 for 8hr and 4 hr. On day 0, the TVBN content of 4hr, 8hr, 12 hr and 16 hr were found to be equivalent to that of control on day 1, day 2, day 3 and day 4, respectively. This could be concluded as the shrimp exposed to room temperature abuse for 4hr had a quality reduction equivalent to 2 days in Ice. Quality reduction in 8hr lot is equivalent to 3 days, 12 hr is equivalent to 4 days and 16 hr was found to be equivalent to 5 days.

Similar result was recorded by Kamal *et al.* (2000), they found that the initial TVB-N value of ice stored *P. monodon* and *M. rosenbergii* was around 10 mg/100g of sample. The TVB-N values gradually increased over the storage period, reaching up to 60 mg/100g by the end of the 10 days.

Comparable study by Naderi *et al.* (2017), found that the impact of delay in icing on the quality of banana shrimp (*Fenneropenaeus merguiensis*) indicated that fresh shrimp iced immediately after being caught (lot I) remained organoleptically acceptable for 12-13 days, whereas a 2-hour delay in icing (lot II) reduced the shelf life to 8-9 days. The findings suggest that immediate icing extends the shelf life of banana shrimp to 12-13 days, while shelf life of 2-hour delay iced shrimp shortens to 8 days.

5.2 Critical rejection points for TBARS

Thiobarbituric Acid Reactive Substances is a common test used to measure lipid oxidation in food products, also indicating the extent of oxidative rancidity. It quantifies malondialdehyde, a byproduct of lipid peroxidation, which reflects the level of deterioration in fats and oils. For fish, acceptable TBARS values are below 2mg

MDA/kg, with higher levels indicating lipid oxidation and quality decline. Specific limits can vary slightly depending on regional standards or the type of fish, but a TBARS value exceeding 5mg MDA/kg is typically considered a sign of rancidity and spoilage.

TBARS values in *Labeo rohita* varied across sections and treatments on day 0, with the highest value of slightly greater than 4 mg MDA/kg was recorded in T4 for all sections, it crossed critical limit of spoilage on day o itself. Control had approximately 1.5 mg MDA/kg. T1 showed a slight increase, T2 had the lowest values around 1.3 mg MDA/kg, followed by a significant rise in T3 and a sharp surge in T4. On Day 1, TBARS peaked at around 4.2 mg MDA/kg in T4, with the control at about 1.5 mg MDA/kg, an increase from T3 to T4 was observed. On Day 2, T4 again had the highest values (3.2 mg MDA/kg) but value decreased significantly as compared to day, while T3 exhibited the lowest. By Day 3, T4 recorded the highest at around 3.2 mg MDA/kg, with the control near 2 mg MDA/kg. On Day 4, T4 surpassed 4 mg MDA/kg, and the control remained the lowest at around 2.5 mg MDA/kg. Control and T2 crossed the limit for acceptability on day 2 of storage, while T1, T3 and T4 crossed the acceptability limit on 0^{th} day, indicating consistent increasing trends of TBARS values with delayed icing across all days among all the portions. Significant differences were noted between portions within the same treatment ($p<0.05$).

The study by Chaijan *et al.* (2005), found changes in the lipids of sardine (*Sardinella gibbosa*) muscle during 15 days of iced storage. Throughout this period, lipid deterioration, including both lipolysis and lipid oxidation, was observed. A significant increase ($P < 0.05$) in thiobarbituric acid reactive substances (TBARS), which are indicators of lipid oxidation, was detected during the entire storage time. After 15 days of storage, the TBARS value in sardine muscle increased by 97% compared to that in fresh muscle, suggesting lipid oxidation occurred during post-mortem handling. From the result, TBARS levels showed a slight increase within the first 3 days of storage, indicating the formation of secondary lipid oxidation products.

5.3 Critical rejection point for Total Plate count

During chilled storage, fish and shrimp undergo significant microbial changes as bacteria proliferate over time, impacting the quality of the organism. Initially, microbial growth is slow, but it accelerates as storage progresses, leading to an

increase in Total Plate Count (TPC). Psychrotrophic bacteria, which thrive in cold environments, play a key role in spoilage. As bacterial counts rise, the freshness and safety of fish and shrimp deteriorate, limiting their shelf life and rendering them unsuitable for consumption after extended storage period. The International Commission on Microbiological Specification for Food (ICMSF, 1986) recommends that the acceptable limit for TPC in fish muscle for good quality fish is 5×10^5 CFU g^{-1}. TPC levels reaching or exceeding 10^7 CFU g^{-1} are considered to indicate poor or unacceptable quality.

The microbiological status of *Labeo rohita* during a 5-day storage period, expressed in log CFU g^{-1}. An increase in Total Plate Count (TPC) was observed in most of the treatment. On Day 0, T4 showed the highest TPC at 6.20 log CFU g^{-1}, while T2 had the highest on Day 1 at 5.90 log CFUg−1. The highest TPC on Days 2, 3, and 4 was recorded in T4, T1, and T2, respectively. Overall, T4 consistently having the highest TPC across all treatments and days. The microbiological profile of *Penaeus vannamei* during a 5-day storage period showed a consistent rise in Total Plate Count (TPC) across treatments. T4 had the highest TPC on Days 0 and 1, while T2 peaked on Day 2. T1 crossed the critical limit of spoilage on day 1 of storage, while T2, T3 and T4 crossed the critical limit on 0^{th} day itself. Control did not cross the limit of acceptability during 5 days of ice storage.

Lilabati and Vishwanath (1996) recorded the total bacterial load ranged only between 10^5 and 10^6 CFU g^{-1} during the iced storage of freshwater fish *Notopterus chitala*, which agrees with the present findings.

Relatively similar result recorded by Liu *et al.,* (2024), the TPC of the samples reached 6.4 log $CFUg^{-1}$, indicating spoilage between the 8th and 10th days. The TPC values highlighted a significant difference between the frozen-thawed (F-T) and cold-storage groups ($p < 0.05$), indicating that the F-T group may have retained slightly better freshness compared to the cold-storage group throughout the storage period.

In an experiment by Lan *et al.* (2020), found that TPC value of shrimp ≤5.0 log $CFUg^{-1}$ represents freshness, a TPC value between 5 and 6 log $CFUg^{-1}$ represents sub-freshness, and when the TPC value is more than 6 log $CFUg^{-1}$, the shrimp is considered to have reached a degree of spoilage and does not have the condition of being edible.

The effect of delayed icing on the quality of banana shrimp (*Fenneropenaeus merguiensis*) was investigated by determining sensory, physicochemical (pH, TVB-N), microbial (TPC) and microstructure aspects over 20 days during ice storage. The fresh shrimp stored in the immediately iced after caught (lot I) were organoleptically acceptable for 12-13 days while a 2-hr delay in icing (lot II) shortened the shelf life up to 8-9 days. Result indicates, immediately iced banana shrimp (*P. merguiensis*) has a shelf life 12-13 days whilst 2-hr delayed in icing has shortens shelf life of 8 days during ice storage.

Related work was performed by Parashideh *et al.* (2015), found that the total plate counts in shrimp subjected to a 2-hour delay in icing were higher compared to those iced immediately after catch. The chemical parameters in the immediately iced shrimp exhibited slower changes than in the shrimp with a 2-hour delay. Findings of this study was found to be equivalent, demonstrate that immediate icing significantly impacts the shelf life of *Penaeus vannamei*, with shrimp lasting 9 days under a 2-hour icing delay and 12 days when iced immediately.

The effect of delayed icing on the bacteriological quality of a commercially important tropical fish, barracuda (*Sphyraena barracuda*), was examined by Jeyasekaran *et al.* (2006). The fish was divided into two groups. One group was iced immediately, while the other group was left at ambient temperature. (32 ± 2 °C) for 6 h and then iced. Immediate icing extended the shelf life of barracuda by 6 days. In immediate iced fish, the total bacterial count increased from 10^3 to 10^6 colony-forming units (CFU)g−1. While fish which was kept at room temperature for 6 hours the total bacterial count remained at 10^6 CFUg−1

5.4 Prediction model of *Labeo rohita*

mAP50 (Mean Average Precision @ 50%): This column shows mAP50 values, indicating how well the model performs in detecting objects within 50% overlap. Most of the values are high (close to 0.9–1.0) across different classes, showing good prediction performance.

Similar experiment was performed on big eye tuna by Mahajan *et al.* (2024) on prediction model, The research is geared towards training an object detection model on the fishnet dataset. The model's extensive training routine produced outstanding results, with a mean Average Precision at IoU(Intersection over Union)

50 (mAP50) of 0.90351 and a mAP50-95 of 0.69167. These metrics are strong indicators of the model's ability to detect and localize fish in the given dataset.

According to Huang *et al.* (2016), they employed computer vision and NIR spectroscopy to capture image data related to organoleptic changes and spectral data for structural changes in fish samples during storage. The findings revealed that computer vision surpassed NIR spectroscopy, as the BP-ANN model achieved a 94.17% accuracy in the training set and 90.00% in the prediction set with computer vision, whereas the NIR spectroscopy model attained 86.67% and 80.00% accuracy in the training and prediction sets, respectively. When combining data from both techniques, the BP-ANN classification model achieved the best performance, with accuracy rates of 96.67% in the training set and 93.33% in the prediction set.

5.5 Prediction model of *Penaeus vanammei*

The confusion matrix illustrates the model's performance of *P. vannamei* in classifying shrimp samples across different time intervals. The elements, indicate the correctly classified instances for each category. For example, categories like "Shrimp_0HR_DAY2" and "Shrimp_12HR_DAY1" have 19 correct classifications, demonstrating high accuracy for those classes. The model displays very high classification accuracy which was more than 99%, with most categories accurately recognized and the background class managed effectively. Nevertheless, there is some ambiguity between samples from adjacent time intervals, especially around the 16-hour mark, highlighting the need for improvement in differentiating certain degradation stages of the shrimp samples.

A comparable outcome was reported by Mahajan *et al.* (2024), who developed a prediction model for bigeye tuna. The confusion matrix they produced offers a detailed visualization of the prediction results across four different classes, as well as the background category. This matrix is an important tool for evaluating the model's classification accuracy and identifying areas for enhancement. Their findings demonstrated exceptionally high precision and accuracy.

An experiment on sea bream (*Sparas aurata*) by Calanche *et al.* (2020), developed a predictive model for the freshness index (%) and ice storage time. Result exhibited an accuracy close to 90% following practical validation. The findings of this study suggest that the predictive tools designed may be proposed as a valuable

alternative to monitor spoilage by assessing freshness as a key aspect of seabream quality.

Du *et al.* (2015) detected the odor of shrimp stored at 5°C using an electronic nose. By incorporating sensory evaluation and TVBN, they developed a model to assess shrimp freshness. Principal component analysis revealed that the first three components accounted for 86.97% of the total variation, which were used to build a freshness estimation model using Fisher Linear Discriminant. The model demonstrated discriminant rates of 98.3% for 120 training samples and 91.7% for 36 test samples, showing that it could reliably assess shrimp freshness with high accuracy rate.

6. SUMMARY

This study aimed to investigates the effects of delayed icing on spoilage indicators of *Labeo rohita* (Rohu) and *Penaeus vannamei* (shrimp), as well as the development of a machine-learning-based freshness prediction model. The experiment was designed to analyze the impact of temperature abuse, where both species were held at room temperature for varying durations (4, 8, 12, and 16 hours) before being iced. The control group for both species was iced immediately after harvest. Holding fish at room temperature (25°C) for 4 hours is equivalent to storing in ice (0°C) for around 2.5 days. Similarly keeping fish at room temperature (25°C) for 8, 12 and 16 hours is equivalent to storing them in ice (0°C) for approximately 5, 7.5 and 10 days respectively.

Over the storage period, several spoilage parameters were assessed, including Total Volatile Basic Nitrogen (TVB-N), Thiobarbituric Acid Reactive Substances (TBARS), pH, and Total Plate Count (TPC). Additionally, images of different portion as well as whole fish was captured daily, and based on these images machine learning models were trained to predict the freshness of the product based on visual characteristics.

In *Labeo rohita,* TVB-N, a key indicator for spoilage, showed a clear upward trend over the storage period, with higher values observed in samples subjected to longer periods of temperature abuse. On Day 0, the TVB-N content in the control group (iced immediately) was the lowest, at around 19.88 mg%, while the group exposed to 16 hours of room temperature (T4) had the highest levels of TVB-N at 33.66 mg%. This trend continued over the storage period, and by Day 4 of storage, the TVB-N content in the T4 group had increased to 56.23 mg%, far exceeding the acceptable limit for freshness (35-40 mg%). The mid-section of the fish consistently showed the highest TVB-N levels compared to the head and tail, indicating that this part of the fish was more prone to spoilage under these conditions.

TBARS, indicating lipid oxidation, was significantly impacted by temperature abuse. On Day 0, the middle section of T4 had the highest TBARS value, showing greater lipid oxidation than the control. Throughout storage, TBARS levels rose in all

temperature-abused groups, with T4 peaking above 4 mg MDA/kg by Day 4, signaling fat degradation and reduced fish quality.

The pH levels of *Labeo rohita* reflects the progression of spoilage, with fresh fish typically having a pH between 6.2 and 6.5. As spoilage advanced, microbial and enzymatic activities produced alkaline compounds, raising the pH. On Day 0, the control and temperature-abused groups had pH values between 6.27 and 6.54, but by Day 4, all groups showed a significant increase in pH, with the control group reaching 7.50, indicating spoilage.

Microbiological analysis (TPC) revealed a rapid rise in bacterial counts over the 5-day storage period. The control group maintained the lowest bacterial levels, while T4 had the highest, peaking at 6.2 log CFU/g on Day 0. The control group stayed within acceptable limits for most of the storage period, highlighting the effectiveness of immediate icing in preventing microbial spoilage.

The TVB-N results indicated that after 5 days of storage, the control group's value was within the critical spoilage rejection range. For the 16-hour treatment, all three portions exceeded the critical spoilage threshold on the first day, with the mid-section surpassing the rejection limit on day 0. Treatments of 12 hours, 8 hours, and 4 hours exceeded the spoilage rejection threshold on days 1, 2, and 3, respectively. From the result of TBARS, control exceeded the critical limit for spoilage on 4^{th} day of storage. While 8hr and 16 crossed the critical range on day 2 and day 0 respectively. Whereas 4 hr and 12 hr exceeded the limit on day 0 itself but value decreases and value show increasing trend thereafter.

The same spoilage trends were observed in *Penaeus vannamei*, but rate of spoilage was bit faster as compared to *Labeo rohita*. TVB-N levels in shrimp increased rapidly in temperature-abused groups, particularly in T4. On Day 0, TVB-N levels in T4 were already high at 34.14 mg%, and by Day 4, they had risen to 43.28 mg%, well beyond the acceptable limit. pH levels in *Penaeus vannamei* also showed a clear increase over time, with the control group maintaining a relatively stable pH of 6.96 by Day 4, while T4 had reached a pH of 7.39, indicating significant spoilage. The microbiological quality of the shrimp followed a similar pattern to the fish, with T4 exhibiting the highest TPC values. By Day 2, the TPC in T4 had exceeded acceptable levels, leading to its rejection for consumption.

The TVB-N results indicates that after 5 days of storage, the control group remained within the critical spoilage range. The 4-hour and 8-hour treatments exceeded the critical spoilage limit by Day 4, while the 16-hour and 12-hour treatments surpassed this threshold earlier, on Day 2 itself. This indicates that prolonged exposure to temperature abuse accelerates spoilage in fish and shrimp.

In addition to these biochemical and microbiological assessments, the study developed a machine-learning-based image analysis model to predict the freshness of the fish and shrimp. The model was trained on thousands of images captured over the storage period, with the goal to differentiate between fresh and spoiled samples. The YOLO (You Only Look Once) algorithm was used for prediction, and the model was trained for 100 epochs for rohu and 25 epochs for shrimp, achieving promising results. For *Labeo rohita*, the model reached a mean Average Precision (mAP50) of around 0.9, indicating a good level of accuracy in detecting spoilage based on image data. The model's performance in predicting the freshness of *Penaeus vannamei* was very good, with an accuracy of over 99% in classifying shrimp samples into different spoilage groups.

In conclusion, delayed icing significantly affected the biochemical and microbiological quality of both fish and shrimp, with delayed treatments showing faster spoilage compared to controls. With high accuracy and minimal error, this machine learning model could be employed to monitor the quality of seafood in real-time, ensuring better management of storage conditions and helping to prevent spoilage. Accuracy of the model can be improved by training more related images into different labels and portions.

7. REFERENCES

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