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Effect of Stunning on Stress Responses and Quality of Farmed Bigeye Trevally (*Caranx sexfasciatus*) during Ice Storage

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ABSTRACT

A study was conducted during May–July, 2023 at ICAR-CIFT, Cochin, Kerala, India to evaluate the effect of the stunning by immersion in ice water slurry, on the flesh quality of Bigeye trevally (*Caranx sexfaciatus*) during ice storage. The changes in tissue stress/quality changes were monitored immediately after catch and during the shelf life of the fish. The major stress/quality parameters were escaping behaviour, muscular and vestibulo-ocular reflexes, pH at death, onset of rigor, and muscle pH, TVB-N, K-value, microbial, and sensory parameters during ice storage. The results indicated that the initial muscle pH was lower in conventional samples compared to fish stunned by ice immersion. The total volatile base nitrogen (TVB-N) increased significantly during storage and within an acceptable limit up to 21 days of storage for both conventional and stunned fish. K value increased considerably during storage, from 4.8% to 56.01% for conventional samples and 4.76% to 35.07% for stunned samples. Microbiologically, the conventional fish was rejected on the 17th day, and the stunned fish was rejected on the 21st day of storage. Sensory attributes were gradually diminished with the storage time which were in agreement with microbial counts. Based on the results of this study, the flesh quality of stunned big eye trevally could be maintained up to 21 days and conventional samples up to 17 days during ice storage.

KEYWORDS: *Caranx sexfaciatus*, bigeye trevally, stunning, ice storage

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

The aquaculture industry has expanded rapidly, surpassing capture fisheries in aquatic animal production with an output of 94.4 mt, accounting for 51% of global production and a record 57% intended for human consumption (Anonymous, 2024). Concurrently, public awareness of animal welfare has increased, highlighting the need for improved understanding of farmed fish welfare, particularly stress associated with handling, stunning, slaughtering and transportation, Recent studies have demonstrated that stress during the death of fish can adversely affect muscle quality by triggering hormonal, biochemical, metabolic, immune, and osmoregulatory responses (Poli et al., 2005; Zhang et al., 2023; Yan et al., 2025). Moreover, stress induces the release of plasma cortisol into the blood, promotes anaerobic glycolysis, leading to lactic acid accumulation, early onset of rigor and rigor resolution which negatively affects muscle quality (Simitzis et al., 2014; Zhang et al., 2017). Some authors also reported it affects the organoleptic properties such as colour, texture etc (Robb et al., 2000; Lefevre et al., 2016; Bordignon et al., 2024). Several recommendations have been proposed to reduce stress before and after slaughter (Anonymous, 2000; Anonymous, 2009a; Anonymous, 2009b); however, no specific, universally adopted guidelines are currently available. Therefore, it is essential to identify suitable slaughtering methods that effectively reduce animal stress while maintaining fish quality, and that are easy to operate and cost-effective (Van de Vis et al., 2003). Stunning is one such method, as it induces the immediate loss of consciousness without recovery prior to death, thereby reducing stress and improving animal welfare (Erikson et al., 2012). Several stunning and slaughtering procedures are used in the fish industry, among which the most commonly practiced methods include asphyxia in air, percussion, immersion in ice water slurry, electrical stunning and gill cutting (Yan et al., 2025; Brijs et al., 2025; Mohammadi Dehcheshmeh et al., 2025) There are differing opinions regarding the efficacy of various stunning methods on fish stress and meat quality, and no consensus exists on the optimal approach. Bordignon et al. (2024) reported that stunning by immersion in cold saline water (–6°C, 5% NaCl) is a viable alternative to percussion for rainbow trout, as it reduces peripheral cortisol levels while maintaining comparable post-mortem quality and freshness during storage. Gradient cooling has been reported as the most favourable slaughter method for grouper, as it minimizes stress, oxidative damage, and apoptosis (Zhao et al., 2025). Live chilling is a widely adopted method in which fish are directly transferred to an ice–water slurry; the chilled and unconscious fish are then transported to the processing facility and processed immediately. The main advantage of cold–shock stunning is that it is relatively inexpensive and convenient for handling large quantities of fish and require no specialized equipment (Bermejo-Poza et al., 2021). However, limited information is available on live chilling and its effects on quality and shelf life of fish (Qin et al., 2016). Bigeye trevally (*Caranx sexfasciatus*) is a fast-growing and commercially important fish species widely distributed in the tropical and subtropical waters of India and the Pacific Ocean (Violante-González et al., 2020). It belongs to the family Carangidae and fetches a high market price owing to its desirable taste and good meat quality (Madgett et al., 2022). In India, the species is widely cultured along the coastal regions of South India due to its favourable sensory attributes and high nutritional value (Rajesh et al., 2016). Considering its economic importance and increasing market demand, the present study evaluated the effect of stunning by immersion in an ice–water slurry on the quality of farmed Bigeye trevally (*C. sexfasciatus*) during ice storage, in comparison with the conventional method of asphyxia in air.

2. MATERIALS AND METHODS

The study was conducted during May–July 2023 at ICAR–CIFT, Cochin. Sixty live Farmed Bigeye Trevally (*Caranx sexfaciatus)* (Average Size: 704.57 g±13; length 36.57±1.2 cm) were procured from a commercial cage fish farm, Kodugalloor, Thrissur, Kerala. Live trevallies were divided randomly into two lots (30 individuals in each group). One lot of live fish was immersed into ice water slurry in the ratio 1:1 (2±1 °C) until no opercular movements (SI) were observed. Another lot of fish was exposed to air until death (asphyxia) simulating the procedure adopted by the traditional fisherman (C).

2.1. *Fish stress evaluation*

The behaviour, stimuli and reflexes of stunned (SI) and struggled fish (C) were studied by observing the duration of escape behaviour and the vestibulo-ocular reflex (VOR) by following the method developed by Kestin et al., 2002. The onset of rigor mortis was determined by observing and measuring tail curvature according to the method described by Diouf and Rioux (1999), using five fish selected from each batch. After assessment, the fish were placed in polystyrene boxes with ice water slurry (SI)and ice (C) at 1:1 ratio and transported to the laboratory and stored in ice for 17 and 21 days.

2.2. *pH measurement*

The pH of the gills and muscle was measured immediately after death. Gill pH was determined by collecting mucus scrapings from the gill region and its pH was measured using a digital pH meter (Hanna instruments, HI2221 pH/OHP meter). Muscle pH was determined according to Binsi et al., 2015.

2.3. *Proximate composition and biochemical analysis*

The proximate composition of bigeye trevally was determined following the method described by Laly et al., 2025. Moisture content was measured by drying the samples in a hot-air oven (Esco Isotherm) at 105°C until a constant weight was obtained. Total protein content was estimated using the micro-Kjeldahl distillation method. Crude fat was extracted using petroleum ether in a Soxhlet apparatus. Ash content was determined by incinerating the samples in a muffle furnace at 550°C for 4–5 h.

For biochemical analyses, the fish samples were filleted and homogenized using a blender maintained at 4–6±1°C. The homogenates were stored at −20°C until further analysis. The pH of the fish muscle was detailed in the section 2.3 The TVB-N was determined by Conway's (1957) method.

2.4. K-value

K-value was determined by the method described by Ryder (1985) using HPLC system. About 5 g of fish muscle was homogenized with 25 ml of perchloric acid (0.6M) at 4°C for 1 min in a homogenizer. The homogenate was centrifuged at 6500 rpm for 10 min and the resulting supernatant was immediately neutralized to pH 6.5–6.8 using potassium hydroxide (1M). The neutralized extract was kept at 0°C for 30 min, then the precipitated potassium perchlorate was removed by filtration. After filtration, the sample was injected into HPLC system. K-value was calculated using the concentrations (moles g^{-1} wet muscle) of the major adenine nucleotides and their degradation products in the following equation.

$$K\ value = \{(HxR+Hx)/(ATP+ADP+AMP+IMP+HxR+Hx)\} \times 100$$

Where,

ATP-Adenosine triphosphate, ADP-Adenosine diphosphate,
AMP-Adenosine monophosphate, IMP-Inosine monophosphate,

HxR-Inosine, Hx-Hypoxanthine.

2.5. Microbiological analysis

For microbiological enumeration, 25 g of fish muscle were aseptically homogenized with 225 ml of phosphate buffer in a stomacher (Seward Limited, UK). The homogenate was serially diluted in 9 ml aliquots of sterile phosphate buffer. Three consecutive dilutions were plated, in triplicate, on Plate Count Agar (Difco) for mesophilic and psychrotrophic counts. Plates for mesophiles were incubated at 35°C±2°C for 48±2 h, (Ryser and Schuman, 2013) while psychrotrophs were incubated at 10°C for 7 days (Cousin et al., 2013). *Pseudomonas* spp. was enumerated on Pseudomonas Agar Base supplemented with cephaloridine–fucidin–cetrimide (CFC) and incubated at 25±1°C for 44±4 h (Ntzimani et al., 2023). All microbial counts were expressed as $\log_{10}$ colony-forming units per gram ($\log_{10}$ cfu g^{-1}).

2.6. Sensory evaluation

The sensory evaluation of the fish was carried out by a panel of five trained and experienced laboratory personnel, following the method described by Amerine et al. (1965). A nine-point hedonic scale was used by the panellists to score the fish (9=like extremely; 1= dislike extremely). The sensory scores were given based on the appearance, colour, odour, flavour, and texture of the fish. The overall acceptability score was calculated by adding the scores of all attributes divided by the total number of attributes. Samples with an overall acceptability score of 4 or below were considered rejected.

2.7. Statistical analyses

All analyses were carried out in triplicate, and the results were expressed as mean± standard deviation. Experimental data were analysed using SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed at a 95% confidence level ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Fish stress evaluation

The results on behaviour and stress responses of both conventional and stunned fish were presented in Table 1. The conventional fish exhibited significantly longer escape behaviour (17±0.7 min) than the stunned fish (4.4±0.4 min). Sudden immersion in ice slurry water rapidly reduced the metabolic rate of the stunned fish, leading to an immediate loss of swimming activity, escape responses, and opercular movements. Zampacavallo et al. (2015) similarly reported that seabass immersed in a water–ice (2:1) mixture lost equilibrium within 3 min, with reduced breathing amplitude and movement. Consequently, the stunned fish lost VOR within 6.3±0.3 min, while the conventional fish took 19.06±1 min, accompanied by intense escape behaviour and excessive mucus secretion, indicating a higher level of stress during death.

The pH of the gill mucus was significantly lower in the conventional fish (5.9±0.07,) than in the stunned

Table 1: Escape behaviour, VOR, Gill and muscle pH, onset of rigor of conventional and stunned fish during death of fish

Parameters	Conventional	Stunned
Duration of Escape behaviour (min)	17±0.7	4.4±0.4
Vestibulo-ocular reflex (VOR) (min)	19.06±1	6.3±0.3
Gill slime pH	5.9±0.07	6.96±0.05
Muscle pH	6.28±0.3	6.67±0.04
Onset of rigor (min)	50±4.4	75±5

Values were given as mean±standard deviation, (n = 3) fish (6.96±0.05). The increased mucus secretion during struggling was known to reduce pH due to the release of glycoproteins in the mucus (Baldi et al., 2018). Similarly, the conventional fish showed lower muscle pH (6.28±0.3) compared to the stunned fish (6.67±0.04), which might be attributed to vigorous muscular activity during the perimortem phase that depletes muscle glycogen results lactate accumulation. The lower pH was due to the formation of H^+ ions associated with lactate production and ATP depletion.

The onset of rigor mortis occurred within 50±4.4 min (Table 1) in the conventional fish, whereas it was slightly delayed in the stunned fish (75±5 min). The reduced struggling in stunned fish leads to lower muscle activity, thereby extending the onset of rigor mortis (Lyu et al., 2015). Early rigor onset might influence key biochemical processes, including glycogen breakdown and the rate of ATP depletion, which in turn affected the freshness of fish. Overall, the early onset of rigor mortis and reduced muscle pH observed in the control fish negatively impact the sensory quality, an important determinant of overall fish quality.

3.2. Nutrient composition of big eye trevally

Farmed bigeye trevally had a moisture content of 71.54±1.48%, protein content of 20.62±0.95%, fat content of 5.87±0.38%, and ash content of 1.45±0.04%. The proximate composition of farmed trevally differed from that of wild counterparts, particularly in terms of fat and moisture content (Chandrashekar and Deosthale, 1993; Ahmed et al., 2022). Farmed fish typically exhibit higher fat levels than wild fish, which might be attributed to the high dietary fat content in commercial feeds and the reduced physical activity associated with cultured conditions (Fallah et al., 2010).

3.3. Changes in the quality attributes of fish during storage

3.3.1. Muscle pH

The changes in pH of conventional and stunned fish during storage are presented in Figure 1. The pH of the conventional fish ranged from 6.28±0.04–6.31±0.01, while the stunned fish showed a pH range of 6.67±0.02 – 6.41±0.01 from day 0 to day 21 of ice storage. Both samples exhibited a rapid drop in pH within the first 48 h, and no significant difference was observed between the samples at the end of the storage period. A similar trend was reported by Bermenjo-Poza et al. (2021) in rainbow trout stunned using ice–slurry and electronarcosis, where the pH of both groups decreased within 48 h and reached almost similar values at the end of storage. Likewise, Cavali et al. (2023) observed that the pH of air-asphyxiated and hypothermia-treated fish declined from 6.49–6.43 and 6.62–6.32, respectively, within 15 days of storage. The pH of fish muscle could vary depending on species, season, diet, and capture method. In the present study, both conventional and stunned fish exhibited a gradual increase in pH after 48 h of storage and remained within the acceptable range of 7.0 (Massa et al., 2005) throughout the storage period.

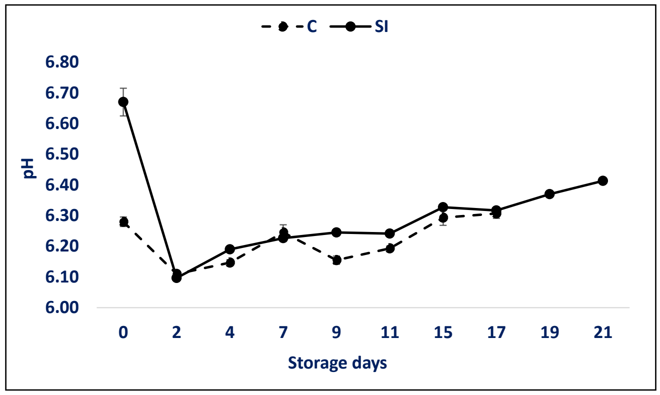

Figure 1: Changes in pH of conventional and stunned fish during ice storage

3.3.2. TVB-N

TVB-N was widely used as an indicator of freshness in fish and fishery products. It was produced through the accumulation of volatile nitrogenous compounds generated from the degradation of both proteinaceous and non-proteinaceous substances, primarily due to bacterial activity (Moosavi-Nasab et al., 2021). The changes in the TVBN of conventional and stunned fish was given in the Figure 2. On day 0, the TVB-N value of conventional and stunned fish was 9.47±0.06 and 9.27±0.05 mg %. During storage, the TVB-N content of both the conventional and stunned fish increased steadily, reaching 22.94±1.09 mg % on day 17 and 20.67±0.29 mg % on day 21, respectively. Throughout the study period, the TVB-N values did not exceed the acceptable limit of 30–35 mg % recommended for fish and fishery products (Connell, 1995). The stunned fish exhibited comparatively lower TVB-N values than the

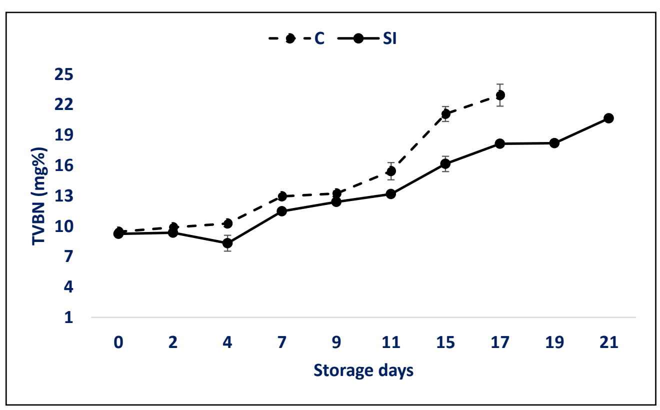

Figure 2: Changes in TVBN of conventional and stunned fish during ice storage

conventional at the end of storage. This reduction might be attributed by the immersion in ice slurry water caused rapid chilling effect, which exerted an initial bacteriostatic action which delayed the formation of volatile amines during storage. Similar findings were reported by Lyu et al. (2015), who observed that fish immersed in ice water slurry have lower TVBN values (25.06±0.42%) than fish subjected to air asphyxia (29.82±0.28 mg %) during refrigerated storage.

3.3.3. K-value

K-value was widely used as an indicator of fish freshness, and a K-value below 20% was considered to represent very fresh fish suitable for consumption (Fan et al., 2014). The K-value increases during fish deterioration due to the formation of inosine and hypoxanthine resulting from ATP degradation (Agustini et al., 2009). The initial K-values of the conventional and stunned fish were 4.80±0.07 % and 4.76±0.06%, respectively and showed a significant increase (p<0.05) in both during storage (Figure 3). The K-values of conventional reached to 56.01±1.06% on day 17 and 35.07±0.72% on day 21, respectively, but remained below the acceptability limit of 60% (Kunjulakshmi., 2020). Stunned fish consistently exhibited significantly lower K-values than the conventional, indicating delayed ATP degradation. Similar trends have been reported in silver carp subjected to ice–water slurry stunning (Zhang et al., 2017). Variations in K-value development were influenced by factors such as pre-slaughter stress, struggling during death, bleeding of fish, size of fish, and storage temperature (Agustini et al., 2009; Laly et al., 2025).

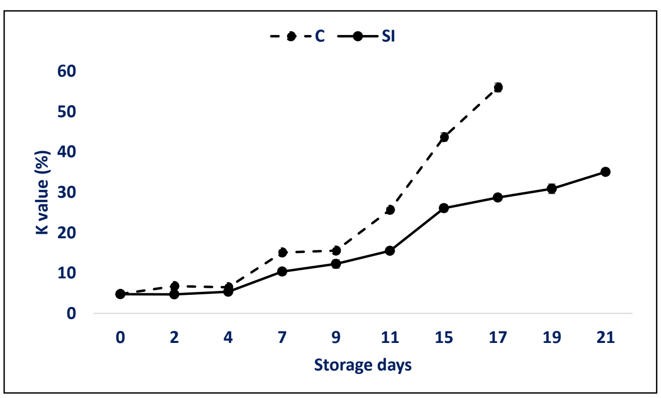

Figure 3: Changes in K-value of conventional and stunned fish during ice storage

3.3.4. Microbiological analysis

The changes in mesophilic and psychrotrophic bacterial counts of the conventional and stunned fish were shown in Table 2. The initial mesophilic counts of the conventional and stunned fish were 3.47±0.03 and 3.29±0.05 $\log_{10}$ cfu g^{-1}, respectively, while the initial psychrotrophic counts were 2.77±0.07 and 2.45±0.2 $\log_{10}$ cfu g^{-1} indicating that the fish used for the study were of good microbiological quality. Both mesophilic and psychrotrophic bacterial counts increased linearly during storage and reached values of 7.52±0.03 and 7.60±0.02 $\log_{10}$ cfu g^{-1} (mesophilic) and 7.21±0.04 and 7.8±0.18 $\log_{10}$ cfu g^{-1} (psychrotrophic) in the conventional and stunned fish, respectively. For fresh fish, the acceptability limit proposed by the Anonymous, 1986 was 7 $\log_{10}$ cfu g^{-1}. Both mesophilic and psychrotrophic counts exceeded this limit on the 17th and 21st days of storage for the conventional and stunned fish, respectively. Based on the microbiological analysis, the shelf life of the conventional and stunned fish was found to be 17 and 21 days, respectively, which correlated well with the sensory evaluation results. Ntzimani et al. (2023) reported that seabass slaughtered and transported using different mixtures of conventional flake ice and slurry ice exhibited shelf life of 19, 21, and 25 days. In the present study, no statistically significant differences were observed between mesophilic and psychrotrophic counts (p>0.05), indicating a co-dominance of mesophilic and psychrotrophic bacteria in trevally during ice storage. Similar observations were reported by Scherer et al. (2006) for grass carp subjected to ice-water slurry and electrical stunning and stored in ice for 20 days.

Pseudomonas spp. was specific spoilage bacteria commonly found in fish and fishery products stored at low temperatures (Jeyasekaran et al., 2006). Changes in *Pseudomonas* counts in conventional and stunned fish stored in ice were shown in Table 2. *Pseudomonas* was detected from the 2nd day of storage in both conventional and stunned fish, with initial counts of 1.46±0.15 and 1.4±0.00 $\log_{10}$ cfu g^{-1}, respectively. The counts increased steadily during storage and reached 6.88±0.06 and 6.86±0.07 $\log_{10}$ cfu g^{-1} in conventional and stunned fish, respectively. The initially low levels of *Pseudomonas* observed in both trevally samples might be attributed to the chilling effect, which retards bacterial growth. Scherer et al. (2006) reported that psychrotrophic bacteria such as *Pseudomonas* and *Acinetobacter* were the dominant spoilage bacteria in grass carp during ice storage. *Pseudomonas* and H_2S-producing bacteria are the two major specific spoilage organisms in fish from both tropical and temperate waters (Lima, 1981) and were capable of producing volatile amines such as methyl mercaptan and dimethyl sulfide, as well as ketones, esters, and aldehydes.

3.3.5. Sensory evaluation of fish

Sensory analysis involves an overall evaluation of meat quality based on appearance, odour, texture, and morphological features (Ozdogan et al., 2021). It was a powerful tool for estimating fish quality and showed strong correlation with various spoilage indices (Anupama et al., 2018). The results presented in (Table 2) indicated that conventional and stunned fish stored in ice had a shelf life of 17 and 21 days, respectively, with sensory scores showing a significant

Table 2: Changes in total mesophilic, psychrotrophic, Pseudomonas count and sensory analysis of conventional and iced fish during ice storage

Days of storage	Conventional				Stunned			
	Total mesophilic count (log_{10} cfu g^{-1})	Total psychrotrophic count (log_{10} cfu g^{-1})	Pseudomonas count (log_{10} cfu g^{-1})	Sensory analysis	Total Mesophilic count (log_{10} cfu g^{-1})	Total psychrotrophic count (log_{10} cfu g^{-1})	Pseudomonas count (log_{10} cfu g^{-1})	Sensory analysis
0	3.47±0.03	2.77±0.07	0.00±0.00	9±0.00	3.29±0.05	2.45±0.2	0.00±0.00	9±0.00
2	4.40±0.01	3.37±0.03	1.46±0.15	8±0.00	3.63±0.02	2.63±0.07	1.40±0.00	8.8±0.45
4	4.46±0.15	3.61±0.15	1.77±0.07	7.6±0.55	3.65±0.16	2.69±0.1	1.67±0.07	8±0.00
7	4.96±0.01	4.55±0.07	2.87±0.08	7±0.00	3.64±0.02	3.41±0.12	2.35±0.04	7.6±0.05
9	5.14±0.02	5.65±0.02	3.96±0.25	6.6±0.55	4.17±0.19	3.58±0.11	3.30±0.11	7.4±0.05
11	6.79±0.18	6.33±0.2	4.46±0.08	5.4±0.55	4.59±0.02	4.54±0.06	3.43±0.15	7±0.00
15	6.89±0.02	6.42±0.03	6.08±0.04	4.4±0.45	5.47±0.03	5.69±0.09	4.31±0.2	6±0.00
17	7.52±0.03	7.21±0.04	6.88±0.06	3.8±0.55	6.12±0.02	5.67±0.03	5.22±0.14	5.4±0.55
19	-	-	-	-	6.41±0.03	6.69±0.15	5.30±0.11	4.6±0.55
21	-	-	-	-	7.60±0.02	7.8±0.18	6.86±0.07	3.6±0.55

Values were given as mean±standard deviation, (n=3)

decrease ($p<0.05$) during the storage period. On day 0, both conventional and stunned fish exhibited a strong seaweedy odour, transparent and protruding eyes, firm and resilient texture, vibrant red gills with transparent mucus, and a shiny appearance, indicating a high degree of freshness. These quality attributes were better retained in stunned fish than in conventional samples during ice storage. The sensory characteristics of both groups remained more or less similar during the first three days of storage. However, as storage progressed, noticeable changes were observed in eye appearance, gill colour, and muscle texture. At the point of rejection, the fish exhibited a milky white or opaque cornea, dark brown gills covered with thick, waxy slime, softened muscle texture, and pronounced off-odours and off-flavours. An overall acceptability score of 4 or below was considered the rejection limit. Accordingly, control and stunned fish were rejected by the sensory panellists on the 17th and 21st day of storage, respectively, which was in good agreement with the microbiological analysis. Similar observations were reported by Zhang et al. (2017), who found that fish stunned in ice-water slurry exhibited superior sensory quality compared with fish stunned by percussion and gill cutting.

4. CONCLUSION

Conventional fish showed greater stress, evidenced by behavioural responses, pH changes, and earlier rigor mortis. Stunned fish maintained superior quality during ice storage and exhibited significantly lower K-values. Shelf lives were 17 and 21 days for conventional and stunned fish, respectively. Ice–water immersion stunning is a simple, cost-effective method that improves fish quality during storage.

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