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Comparative analysis of freshness between pelagic and demersal marine fish

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Abstract Sensory examination of pelagic fish had higher average overall sensory acceptance score (5.93±1.42 points) compared to demersal fish (5.64±1.53 points) ($p<0.05$). Physicochemical analysis average total volatile basic nitrogen (TVB-N) of pelagic fish was lower than demersal fish ($p<0.05$), measuring at 11.71±2.40 and 13.26±3.53 mg of nitrogen/100 g, respectively. The average trimethylamine (TMA-N) in pelagic fish exceeded that in demersal fish ($p<0.05$), registering at 11.15±3.98 and 9.70±1.34 mg of nitrogen/100 g, respectively. Pelagic fish exhibited a higher average pH in muscle compared to demersal fish ($p<0.05$), measuring at 7.16±0.75 and 6.99±0.20, respectively. Microbiological examination revealed a statistically significant increase ($p<0.05$) in the quantity of every microorganism type tested across all fish types over a span of 4 days. It is concluded that pelagic fish tend to retain freshness and sustain longer storage durations in comparison to demersal fish. This may be influenced by factors such as muscle structure, physiological characteristics, and environmental conditions.

Keywords: Freshness quality, Habitat, Marine fish, Storage duration

Introduction

Marine fish can be categorized into two main groups—pelagic and demersal—based on their habitat, water depth, and behavior, all of which affect their metabolism (Drazen and Seibel, 2007; Baxter *et al*., 2022). Pelagic fish typically inhabit the upper layers of water, where they swim extensively and tend to have narrow, slender bodies. In contrast, demersal fish live closer to the sea bottom, occasionally hiding in the rocks or reefs and tend to have wider bodies. Moreover, the metabolic processes in pelagic and demersal fish are influenced not only by their habitat depth but also by their dietary habits (Yang and Somero, 1993). Pelagic fish species, such as *Euthynnus affinis*, *Sphyraena jello*, and *Pampus argenteus*, mainly feed on crustaceans, molluscs, teleosts, and zooplankton (copepods, megalopa, cephalopods, etc.) (Abdurahiman *et al*., 2006; Bharathi and Ponn, 2018; Vigneshwaran *et al*., 2018), while demersal fish species, such as *Epinephelus coioides* and *Nemipterus japonicus*, consume macroinvertebrates such as

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polychaetes, small shrimp, amphipods, and fish (Dolbeth *et al*., 2008; Chuaykaur *et al*., 2020; Saraswati and Perdhana, 2020). Pelagic fish are most active during the daytime, descending between dusk and midnight and resurfacing at dawn (Prihartato *et al*., 2015), while demersal fish species, such as threadfin bream (*Nemipterus randalli*) and dusky grouper (*Epinephelus marginatus*), also exhibit group foraging behavior at night (Gibran, 2007; Stern *et al*., 2014).

Marine fish is often regarded as difficult to handle in culinary contexts because it spoils rapidly, with deterioration and beginning to spoils ranging from 12 hours to 25 days which depends on the postharvest handling methods (Abraham-Olukayode and Oramadike, 2015; Ntzimani *et al*., 2023), as a result of different postharvest handling methods from slaughtering and using different temperatures for preservation. It is also highly susceptible to oxidation (Passi *et al*., 2005; Nathanailides *et al*., 2011; Susanto *et al*., 2011) and develops off-flavors when improperly handled (Mohammadi *et al*., 2021; Zhang *et al*., 2021). The preservation of its sensory and nutritional quality is strongly affected by several factors. In Thailand, fishermen commonly store fresh fish on flaked ice immediately after capture, and the fish are usually transported and sold on flaked ice. Currently, various preservation methods are used, including super chilling, deep chilling, and gamma irradiation (Moini *et al*., 2009; Mozuraityte *et al*., 2021; Zhao *et al*., 2021; Jeong *et al*., 2022; Ando *et al*., 2024). However, maintaining fish on flaked ice remains the most convenient and widely used method in traditional Thai markets. While cooling or refrigeration can help maintain freshness, they do not kill or eliminate microorganisms nor stop enzymatic activity (Rasul *et al*., 2022; Zhong *et al*., 2023). Moreover, the freshness of fish is influenced by various factors, and the type of fish species is one of the reasons (Lougovois and Kyrana, 2005). The muscle structure of different types of fish significantly influences their freshness quality, as reported by Cheng *et al*. (2014). A nutritional profile analysis of pelagic fish muscles has revealed their lower moisture content and higher fat levels compared to demersal fish muscles (Nurnadia *et al*., 2011). Therefore, a comparative analysis of the freshness of pelagic and demersal fish sold on flaked ice in traditional markets, along with determination of the optimal storage period for each type, is imperative for ensuring food safety, quality control, and extending shelf life.

Thailand possesses a high diversity of fish species, some of which are exclusively dominant in its waters; however, the lack of data on their quality and freshness represents a critical knowledge gap that hinders sustainable fisheries management, value-chain development, and informed consumer choice. In this study, a comparative analysis of the quality and freshness of popular pelagic and demersal fish species in the Gulf of Thailand was conducted to obtain the traditional fish markets, the most commonly used market style. The assessment involved sensory tests, physicochemical analyses, and microbiological evaluations. Moreover, particular emphasis was placed on determining the duration during which the fish remains suitable as high-quality raw material.

Materials and methods

Sample collection

Three pelagic fish species, including eastern little tuna (*E. affinis*), pickhandle barracuda (*S. jello*), and white pomfret (*P. argenteus*), and three demersal fish species, including orange spotted grouper (*E. coioides*), yellowtail fusilier (*C. cuning*), and threadfin bream (*N. japonicus*), were obtained from a traditional fish market in Bangkok, Thailand, which receives a variety of fish types. Twelve fish of each species were randomly selected. Each fish was individually placed into a polyethylene bag containing crushed ice and transported to the laboratory. The samples were transferred to new polyethylene bags and stored on crushed ice in a 400 L plastic cooler tank with a cover, mimicking the storage method used by local Thai restaurants for seafood. All samples were kept in the tank with crushed ice at an ice-to-fish (w/w) ratio of 1:1, and this storage condition was maintained for a duration of 4 days. Any water resulting from melted ice was drained and replenished with fresh crushed ice in the same proportion. For freshness quality assessment, the samples were randomly selected from a plastic cooler tank for sensory testing, physicochemical analysis, and microbiological examination, with 3 replicates per sample taken on days 1, 2, 3, and 4 of the experiment. The experimental procedures were approved (approval no. CC-KMITL/2023/001) by the Institutional Animal Care and Use Committee of King Mongkut's Institute of Technology Ladkrabang, Thailand.

Sensory freshness quality analysis

Samples of each fish type were subjected to freshness quality assessment through a sensory evaluation, which involved rating the freshness of fish based on external characteristics (eyes, gills, and body), odor, and texture using the 9-point hedonic scaling method (Sinthusamran *et al*., 2016). This method assigns a score ranging from 1 to 9 based on the evaluator's preference or acceptance of consumption. A score of 9 indicates the highest value, signifying very fresh fish that the evaluator highly prefers and finds suitable for consumption. Conversely, a score of 1 represents the lowest value, indicating fish that is not fresh and least preferred for consumption. This study was conducted in accordance with the international guidelines for human research protection, as approved by the Research Ethics Committee of King Mongkut's Institute of Technology Ladkrabang, Thailand (approval no. EC-KMITL_67_039).

Physicochemical freshness quality analysis

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N) levels were determined using Conway's micro-diffusion method (Siang and Kim, 1992) and expressed in mg/100 g of the sample. For the analysis, a fish sample weighing 5 g was put into the beaker with 50 mL of distilled water and homogenized for 2 min. The pH of the homogenized mixture was measured using a pH meter (Yong, 1992).

Microbiological freshness quality analysis

Microbiological freshness quality was assessed using the plate count method (spread plate method) by mixing 25 g of fish meat sample with 225 ml of Butterfield's Phosphate-Buffered Dilution Water (pH 7.2). After sample dilution, 0.1 ml of the sample suspension was spread onto Plate Count Agar (PCA) for the isolation of the total viable count (TVC) (AOAC, 2000), Xylose Lysine Deoxycholate (XLD) agar for the isolation of *Salmonella* spp. (adapted from Speck, 1976), Baird-Parker Agar for the isolation of *Staphylococcus aureus* (Bacteriological Analytical Manual, 1998c), Mannitol-egg yolk-polymyxin (MYP) agar for the isolation of *Bacillus cereus* (Bacteriological Analytical Manual, 1998a), Tryptose-sulfite-cycloserine (TSC) agar for the isolation of *Clostridium perfringens* (Bacteriological Analytical Manual, 1998b), and Coliform agar for the isolation of *Escherichia coli* (AOAC, 2000), respectively. After the incubation at 35±2 °C for 24-48 hrs, characteristic colonies were observed. Colonies were counted and reported as log CFU/g. Finally, biochemical tests were performed to confirm the pathogenic identification.

Statistical data analysis

Differences in sensory scores among fish types and experimental dates, TVB-N, TMA-N, pH, and microbial contents were analyzed using two-way analysis of variance (ANOVA). The average sensory scores, TVB-N, TMA-N, pH and microbial contents between pelagic and demersal fish groups, as well as among different fish species over the four experimental days, were tested using the non-parametric one-way ANOVA/Kruskal-Wallis H-test. Additionally, comparisons between experimental days were conducted using the Mann-Whitney U-test. All statistical analysis were performed using the SPSS version 26 program.

Results

Sensory analysis

The overall sensory acceptance score was slightly higher for pelagic fish (5.93±1.42) than for demersal fish (5.64±1.53). Significant differences in sensory acceptance scores were observed between the pelagic and demersal fish groups throughout the 4-day period ($F=7272.002$, $df=1$, $p=0.000$) (Table 1). Furthermore, the overall sensory acceptance scores of both pelagic and demersal fish showed a significant decrease ($p<0.05$) from the 1st day to the 4th day of the experiment, with significant differences observed between each day of the experiment except for the 1st and 2nd days (Table 2). Specifically, the scores decreased from 7.10±0.19 and 7.12±.46 to 4.29±0.73 and 3.66±0.05 in pelagic and demersal fish, respectively. On day 1, pelagic fish exhibited minimal mucus, slightly cloudy eyes, softened meat, dark red gills, and a faint fishy odor, with the highest score of 7.55±0.17 points observed for pickhandle barracuda. In contrast, demersal fish displayed abundant mucus, cloudy eyes, collapsing stomach, softened meat, dark red gills, and a pronounced fishy odor, with the lowest acceptance score of 6.6±0.29 points recorded for threadfin bream (Table 2). By the 4th day of the experiment, all fish exhibited signs of spoilage, such as yellow mucus, sunken eyes, and muscle breakdown, dry and bruised skin, and dark black gills. Consequently, sensory scores for all fish species fell below 5 points, indicating spoilage and rendering them unsuitable for consumer acceptance.

Physicochemical analysis

Determination of TVB-N content

The average TVB-N levels in pelagic and demersal fish were 11.71±2.40 and 13.26±3.53 mg of nitrogen/100 g of sample, respectively. Differences in TVB-N levels between pelagic and demersal fish were significant across the 4-day experimental period ($F=1809.710$, $df=1$, $p=0.000$), with significant disparities observed within both groups on the 1st and 4th days ($p<0.05$) (Figure 1A). The TVB-N levels increased significantly in both pelagic and demersal fish throughout the experiment (Figure 1A). By the end of the experiment, pelagic fish exhibited a TVB-N level of 14.37±0.32 mg of nitrogen/100 g of sample, which was lower than that of demersal fish, recording a TVB-N level of 17.93±4.89 mg. of nitrogen/100 g of. sample. Significant differences in TVB-N levels were observed between days 1 and 3, 1 and 4, and 2 and 4 of the experiment for pelagic fish and between days 1 and 4, 2 and 4, and 3 and 4 for demersal fish (Table 3). Additionally, the TVB-N values of all fish species differed significantly across the 4 days (Table 3). Moreover, at the end of the experiment, threadfin bream showed the highest TVB-N level (23.54±0.03 mg of nitrogen/100 g of sample), while white pomfret exhibited the lowest TVB-N level (14.00±0.03 mg of nitrogen/100 g of sample).

Table 1. Two-way analysis of variance (ANOVA) results on the influence of the group of fish (pelagic and demersal) and experimental day on sensory acceptance scores (A), physicochemical freshness indicators (B-D), and microbiology freshness indicators (E-J)

Parameter	Source	F	df	significant
(A) Sensory	Intercept	7272.002	1	0.000
	Typ of fish	4.602	1	0.033
	Day	111.538	3	0.000
	Typ_fish * Day	1.094	3	0.351
Physicochemical				
(B) TVB-N	Intercept	1809.710	1	0.000
	Typ of fish	6.959	1	0.010
	Day	24.794	3	0.000
	Typ_fish * Day	1.710	3	0.174
(C) TMA-N	Intercept	2450.712	1	0.000
	Typ of fish	11.770	1	0.001
	Day	38.991	3	0.000
	Typ_fish * Day	10.681	3	0.000
(D) pH	Intercept	45521.132	1	0.000
	Typ of fish	80.087	1	0.000
	Day	28.117	3	0.000
	Typ_fish * Day	5.676	3	0.002
Microbiology				
(E) TVC	Intercept	298321.782	1	0.000
	Typ of fish	52.310	1	0.000
	Day	453.259	3	0.000
	Typ_fish * Day	4.606	3	0.006
(F) *Salmonella* spp.	Intercept	3840.599	1	0.000
	Typ of fish	3.124	1	0.082
	Day	13.741	3	0.000
	Typ_fish * Day	1.371	3	0.260
(G) *S. aureus*	Intercept	2665.796	1	0.000
	Typ of fish	1.353	1	0.249
	Day	20.821	3	0.000
	Typ_fish * Day	0.389	3	0.761
(H) *B. cereus*	Intercept	20702.379	1	0.000
	Typ of fish	0.030	1	0.864
	Day	36.575	3	0.000
	Typ_fish * Day	1.206	3	0.315
(I) *C. perfringens*	Intercept	8323.614	1	0.000
	Typ of fish	3.333	1	0.073
	Day	19.714	3	0.000
	Typ_fish * Day	0.704	3	0.553
(J) *E. coli*	Intercept	13334.221	1	0.000
	Typ of fish	3.907	1	0.077
	Day	16.265	3	0.000
	Typ_fish * Day	1.446	3	0.238

Table 2. Overall acceptability of sensory scores of all fish species over the course of the 4-day experiment

Day	Overall acceptability of sensory score							
	Easternlittle tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demersal fish
1	7.25±0.23^{a}	7.55±0.17^{a}	7.10±0.19^{a}	7.30±0.23^{a}	7.25±0.21^{a}	7.50±0.45^{a}	6.60±0.29^{a}	7.12±0.46^{a}
2	6.50±0.43^{a}	7.25±0.46^{a}	7.00±0.32^{a}	6.92±0.38^{a}	7.00±0.52^{a}	6.25±0.52^{b}	6.25±0.55^{a}	6.50±0.43^{a}
3	4.61±0.24^{b}	6.00±0.29^{b}	5.06±0.64^{b}	5.22±0.71^{b}	5.33±0.17^{b}	5.28±0.36^{bc}	5.17±0.24^{b}	5.26±0.0^{b}
4	3.50±0.36^{b}	4.43±0.51^{b}	4.93±0.30^{b}	4.29±0.73^{c}	3.71±0.30^{b}	4.14±0.56^{c}	3.14±0.30^{c}	3.66±0.50^{c}
p-value	<0.001	<0.001	<0.001	0.000	<0.001	<0.001	<0.001	0.000

Remarks: a,b,c and d = Significant (p<0.05) in the column.

Table 3. Fish quality assessment of different physicochemical parameters, including the Average±SD values of (A) total volatile basic nitrogen (TVB-N), (B) trimethylamine (TMA-N), and (C) pH, for all fish species across the 4-day experiment

Total volatile basic nitrogen (TVB-N) Unit : mg of nitrogen/100 g of sample

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	9.52±0.02^{a}	7.84±0.01^{a}	8.96±0.03^{a}	8.77±0.86^{a}	11.20±0.03^{ab}	10.08±0.05^{a}	9.52±0.06^{ab}	10.27±0.86^{a}
2	11.20±0.00^{ab}	11.65±0.10^{ab}	10.05±0.03^{ab}	10.97±0.83^{ab}	14.05±0.06^{ac}	11.68±0.09^{ab}	6.65±0.08^{a}	10.79±3.78^{a}
3	12.91±0.08^{bc}	12.88±0.06^{bc}	12.36±0.18^{bc}	12.72±0.31^{bc}	10.04±0.04^{b}	12.35±0.05^{bc}	19.71±0.10^{bc}	14.03±5.05^{a}
4	14.56±0.02^{c}	14.56±0.05^{c}	14.00±0.03^{c}	14.37±0.32^{c}	14.58±0.09^{cd}	15.68±0.06^{c}	23.54±0.03^{c}	17.93±4.89^{ab}
P-value	0.014	0.016	0.016	0.000	0.016	0.015	0.015	0.000

A. Trimethylamine (TMA-N) Unit : mg of nitrogen/100 g of sample

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	7.28±0.00^{a}	8.96±0.03^{a}	6.16±0.04^{a}	7.47±1.41^{a}	7.84±0.04^{a}	8.40±0.02^{a}	7.84±0.04^{a}	8.03±0.32^{a}
2	8.96±0.02^{ab}	10.63±0.02^{ab}	7.84±0.01^{ab}	9.14±1.46^{ab}	8.95±0.03^{ab}	9.50±0.02^{ab}	9.75±0.01^{ab}	9.40±0.41^{b}
3	11.75±0.02^{bc}	12.84±0.05^{bc}	9.52±0.00^{bc}	11.37±1.69^{b}	9.54±0.02^{bc}	10.06±0.03^{bc}	10.95±0.03^{bc}	10.18±0.71^{bc}
4	22.40±0.04^{c}	14.00±0.05^{c}	13.44±0.03^{c}	16.61±5.02^{c}	10.08±0.03^{c}	11.20±0.03^{c}	12.32±0.06^{c}	11.20±1.12^{dc}
P-value	0.015	0.016	0.014	0.000	0.016	0.015	0.016	0.000

B. pH

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	5.43±0.03^{a}	7.28±0.05^{a}	6.39±0.03^{a}	6.37±0.93^{a}	7.26±0.03^{ab}	6.81±0.02^{a}	6.45±0.04^{a}	6.84±0.41^{a}
2	5.80±0.02^{ab}	8.96±0.02^{ab}	6.44±0.02^{ab}	7.07±1.67^{ab}	7.16±0.04^{ac}	6.85±0.03^{a}	6.72±0.00^{ab}	6.91±0.23^{a}
3	6.34±0.03^{bc}	11.75±0.02^{bc}	6.43±0.04^{ab}	8.17±3.10^{b}	6.94±0.06^{c}	6.90±0.03^{ac}	6.94±0.05^{bc}	6.93±0.02^{a}
4	7.14±0.03^{c}	7.23±0.02^{c}	6.78±0.05^{b}	7.05±0.24^{c}	7.56±0.03^{b}	7.21±0.03^{bc}	7.07±0.04^{c}	7.28±0.25^{b}
P-value	0.015	0.015	0.04	0.000	0.016	0.019	0.015	0.007

Remarks: a,b,c and d = Significant (p<0.05) in the colum.

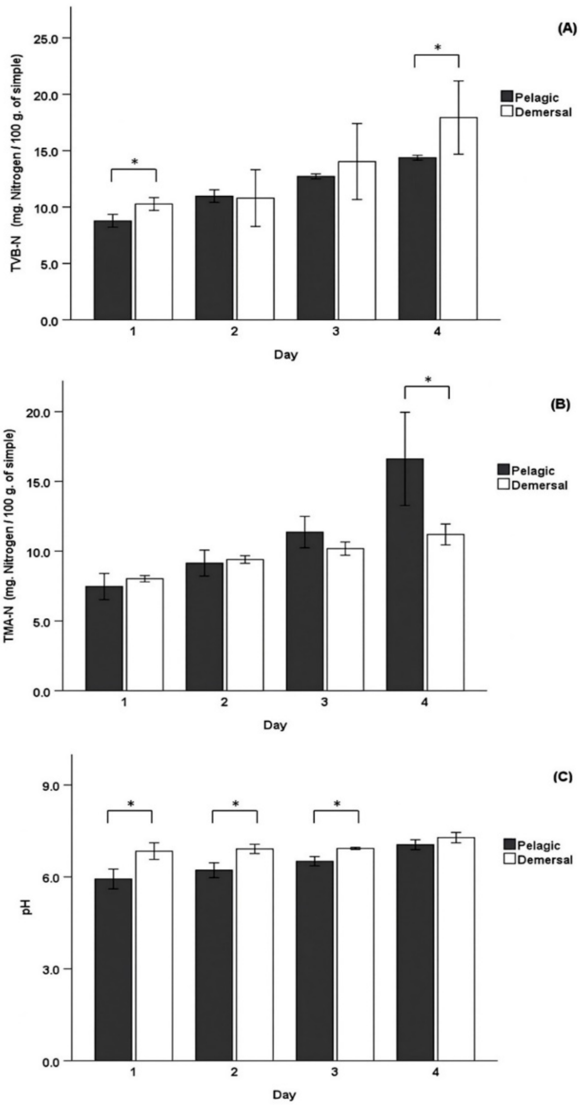

Figure. 1 Physicochemical freshness quality of pelagic and demersal fish across the 4 days of experiment: (A) Total volatile basic nitrogen (TVB-N), (B) Trimethylamine (TMA-N), and (C) pH

Determination of TMA-N content

The average TMA-N content in pelagic fish was 11.15±3.98 mg of nitrogen/100 g of sample, whereas in demersal fish, it was 9.70±1.34 mg of nitrogen/100 g of sample. Significant differences in TMA-N levels were observed between pelagic and demersal fish across the 4-day experimental period ($F=2450.712$, $df=1$, $p=0.000$), with both groups exhibiting significant differences on the 4th day ($p<0.05$) (Figure 1B). The TMA-N levels of both pelagic and demersal fish increased significantly throughout the experiment (Table 3). By the end of the experiment, pelagic fish had a TMA-N level of 16.61±5.02 mg. of nitrogen/100 g of sample, which was higher than that of demersal fish, recording a TMA-N of 11.20±1.12 mg of nitrogen/100 g of. sample. Significant differences in TMA-N levels were observed between various days of the experiment, except for between days 1 and 2 and 2 and 3 for the pelagic fish and between days 2 and 3 and 3 and 4 for the demersal fish (Table 3). Additionally, significant differences were observed in the TVB-N values of all fish species across the 4 days (Table 3). Moreover, at the end of the experiment, eastern little tuna exhibited the highest TMA-N content at 22.40±0.04 mg nitrogen/100 g of sample, while yellowtail fusilier had the lowest TMA-N content at 10.08±0.03 mg of nitrogen/100 g of sample.

Determination of pH

The average pH levels of pelagic and demersal fish were 7.16±0.75 and 6.99±0.20, respectively. Significant differences in pH were observed between pelagic and demersal fish across the 4-day period ($F=45521.132$, $df=1$, $p=0.000$), with both groups displaying significant differences every day except for the 4th day (Figure 1C). The pH levels of both pelagic and demersal fish increased significant throughout the experiment (Table 3). By the end of the experiment, pelagic fish had a pH of 7.05±0.24, which was lower than that of demersal fish, recording a pH of 7.28±0.25. Significant differences in pH levels were observed between various days of the experiment except for between days 1 and 2 and 2 and 3 for pelagic fish and between days 1 and 4, 2 and 4, and 3 and 4 for the demersal fish. Additionally, significant differences in pH values were observed among all fish species across the 4 days (Table 3). Notably, at the end of the experiment, yellowtail fusilier exhibited the highest pH value at 7.56±0.03, while white pomfret displayed the lowest pH value at 6.78±0.05.

Microbiological analysis

The average TVC of pelagic and demersal fish were 3.8±0.28 log CFU/g and 3.9±0.32 log CFU/g, respectively. Significant differences were observed in TVC between pelagic and demersal fish across the 4-day experimental period ($F=298321.782$, $df=1$, $p=0.000$), with both groups exhibiting significant differences ($p<0.05$) on the 2nd, 3rd, and 4th days (Figure 2A). On day 4, the TVC of pelagic fish was 4.0±0.03 log CFU/g, while that of demersal fish was 4.1±0.03 log CFU/g. Significant differences in TVC were observed between days 1 and 3, 1 and 4, and 2 and 4 of the experiment for both pelagic and demersal fish (Table 4). Moreover, significant differences in TVC values were observed among all fish species across the 4 days (Table 4). At the end of the experiment, threadfin bream exhibited the highest TVC at 4.1±0.03 log CFU/g, while white pomfret showed the lowest TVC at 4.0±0.02 log CFU/g.

Table 4. Changes in microbiological quality indices for freshness assessment of all fish species during the 4 days of experimentation: (A) Total viable count (TVC), (B) *Salmonella* spp., (C) *Staphylococcus aureus*, (D) *Bacillus cereus*, (E) *Clostridium perfringens*, and (F) *Escherichia coli*

A. TVC (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	3.4±0.0^{a}	3.3±0.0^{a}	3.4±0.0^{a}	3.4±0.1^{a}	3.3±0.0^{a}	3.4±0.1^{a}	3.5±0.0^{a}	3.4±0.1^{a}
2	3.8±0.0^{ab}	3.8±0.0^{ab}	3.6±0.0^{ab}	3.7±0.1^{ab}	3.9±0.0^{ab}	3.8±0.0^{ab}	3.9±0.0^{ab}	3.9±0.1^{ab}
3	3.9±0.0^{bc}	3.9±0.0^{bc}	3.8±0.0^{bc}	3.9±0.0^{bc}	4.0±0.0^{bc}	4.0±0.0^{bc}	4.1±0.0^{bc}	4.0±0.1^{bc}
4	4.1±0.0^{b}	4.0±0.0^{c}	4.0±0.0^{c}	4.0±0.0^{c}	4.1±0.0^{c}	4.1±0.0^{c}	4.2±0.0^{c}	4.1±0.0^{c}
P-value	0.015	0.016	0.016	0.000	0.016	0.015	0.015	0.000

B. *Salmonella* spp. (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	2.8±0.1^{a}	2.7±0.2^{a}	1.6±1.4^{a}	2.4±0.9^{a}	2.0±0.0^{a}	2.3±0.3^{a}	2.5±0.0^{a}	2.3±0.3^{a}
2	2.9±0.1^{ab}	2.6±0.2^{ab}	2.8±0.2^{b}	2.7±0.2^{b}	3.1±0.1^{ab}	3.0±0.2^{ab}	2.6±0.2^{a}	2.8±0.3^{bc}
3	2.9±0.0^{b}	2.5±0.4^{b}	2.8±0.3^{ab}	2.7±0.3^{ab}	3.1±0.0^{bc}	3.1±0.0^{b}	3.1±0.1^{ab}	3.1±0.1^{c}
4	3.1±0.2^{bc}	2.8±0.1^{ab}	3.0±0.1^{ac}	3.0±0.2^{ac}	3.2±0.0^{c}	3.2±0.0^{bc}	3.3±0.0^{b}	3.2±0.1^{cd}
P-value	0.055	0.517	0.090	0.024	0.043	0.051	0.022	0.000

C. *S. aureus* (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	1.4±1.3^{a}	2.6±0.2^{a}	2.4±0.4^{a}	2.1±0.9^{a}	1.4±1.3^{a}	2.4±0.1^{a}	2.3±0.3^{a}	2.1±0.8^{a}
2	3.0±0.1^{ab}	2.8±0.0^{ab}	2.7±0.2^{a}	2.8±0.2^{ab}	2.1±0.2^{ab}	2.6±0.3^{a}	2.7±0.1^{ab}	2.5±0.4^{a}
3	3.2±0.1^{bc}	2.9±0.2^{ab}	3.1±0.0^{ab}	3.1±0.2^{bc}	2.9±0.1^{b}	3.1±0.1^{ab}	3.2±0.1^{bc}	3.1±0.2^{bc}
4	3.3±0.2^{c}	3.0±0.2^{b}	3.2±0.1^{b}	3.2±0.2^{c}	2.9±0.1^{bc}	3.2±0.1^{b}	3.3±0.0^{c}	3.1±0.2^{c}
P-value	0.022	0.124	0.021	0.000	0.036	0.028	0.017	0.000

D. *B. cereus* (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	2.9±0.2^{a}	2.6±0.2^{a}	2.5±0.5^{a}	2.7±0.3^{a}	2.5±0.2^{a}	2.8±0.3^{a}	2.8±0.2^{a}	2.7±0.2^{a}
2	3.0±0.2^{ab}	3.0±0.1^{bc}	3.2±0.1^{a}	3.1±0.1^{b}	2.7±0.1^{ab}	2.9±0.1^{ab}	3.2±0.1^{ab}	2.9±0.2^{ab}
3	3.2±0.0^{b}	3.2±0.1^{c}	3.1±0.1^{bc}	3.2±0.1^{c}	3.2±0.0^{b}	3.2±0.0^{bc}	3.1±0.2^{ab}	3.2±0.1^{bc}
4	3.2±0.0^{bc}	3.1±0.2^{cd}	3.2±0.0^{c}	3.2±0.1^{bd}	3.2±0.0^{bc}	3.2±0.1^{c}	3.3±0.1^{b}	3.3±0.1^{c}
P-value	0.029	0.069	0.063	0.000	0.022	0.036	0.046	0.000

E. *C. perfringens* (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	2.7±0.2^{a}	2.3±0.3^{a}	2.3±0.2^{a}	2.4±0.3^{a}	2.2±0.2^{a}	2.9±0.2^{a}	2.4±0.4^{a}	2.5±0.4^{a}
2	2.7±0.2^{ab}	2.4±0.4^{ab}	2.6±0.3^{ab}	2.6±0.3^{a}	2.9±0.1^{ab}	3.2±0.1^{a}	2.5±0.4^{a}	2.8±0.4^{ab}
3	2.8±0.2^{bc}	3.1±0.0^{bc}	2.8±0.1^{ab}	2.9±0.2^{bc}	2.9±0.1^{ab}	3.0±0.1^{bc}	3.1±0.1^{ab}	3.0±0.1^{b}
4	3.0±0.0^{c}	3.1±0.1^{c}	3.1±0.0^{b}	3.1±0.1^{c}	3.0±0.3^{b}	3.0±0.1^{b}	3.3±0.1^{b}	3.1±0.2^{bc}
P-value	0.088	0.031	0.031	0.000	0.085	0.081	0.029	0.004

F. *E. coli* (Unit: log CFU/g)

Day	Eastern little tuna	Pickhandle barracuda	White pomfret	Pelagic fish	Yellowtail fusilier	Orange spotted grouper	Threadfin bream	Demarsal fish
1	2.3±0.2^{a}	2.8±0.1^{a}	2.4±0.1^{a}	2.5±0.3^{a}	2.5±0.3^{a}	2.7±0.2^{a}	3.0±0.1^{a}	2.7±0.3^{a}
2	2.9±0.1^{ab}	2.8±0.1^{b}	2.7±0.2^{ab}	2.8±0.1^{a}	2.7±0.2^{b}	3.0±0.1^{bc}	3.1±0.1^{b}	3.0±0.2^{ab}
3	3.1±0.1^{b}	3.0±0.1^{ab}	2.9±0.1^{bc}	3.0±0.1^{bc}	2.9±0.2^{bc}	3.1±0.1^{c}	3.2±0.1^{bc}	3.1±0.2^{b}
4	3.2±0.1^{ab}	3.1±0.2^{ab}	3.0±0.1^{c}	3.1±0.1^{c}	2.9±0.4^{c}	3.1±0.2^{cd}	3.1±0.1^{bd}	3.0±0.2^{bc}
P-value	0.024	0.070	0.027	0.000	0.237	0.288	0.335	0.043

Remarks: a,b,c and d = Significant (p<0.05) in the column

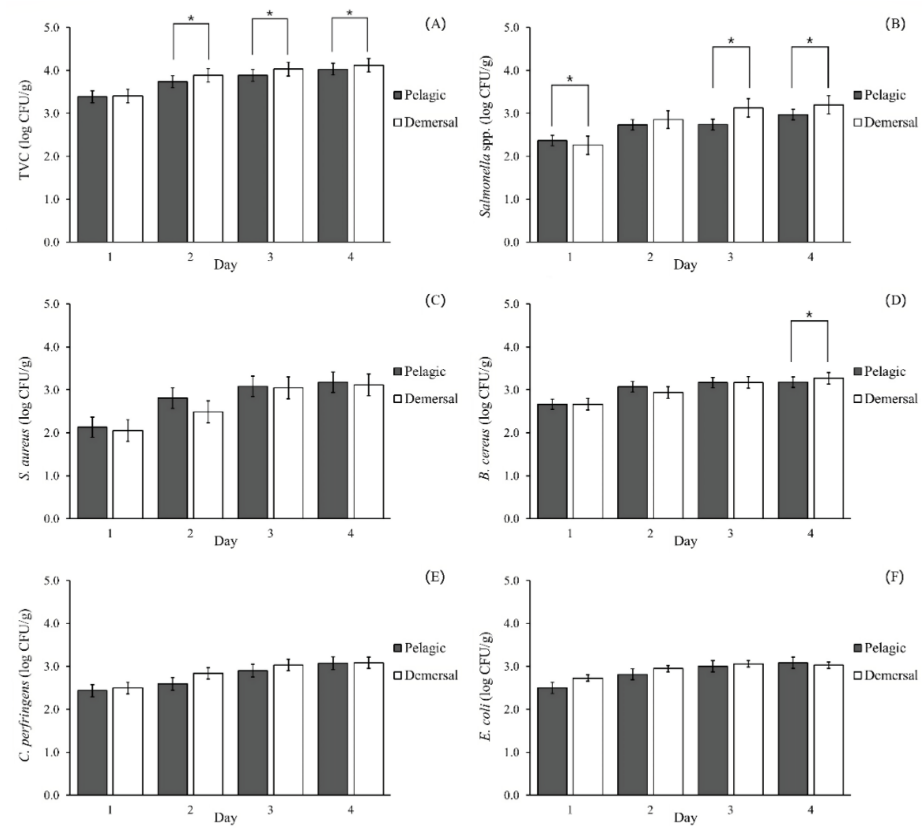

Figure 2. Microbiological assessments of freshness quality represented by (A) total viable count TVC, (B) *Salmonella* spp., (C) *Staphylococcus aureus*, (D) *Bacillus cereus*, (E) *Clostridium perfringens*, and (F) *Escherichia coli*.) of pelagic and demersal fish across all 4 days of the experiment

Salmonella spp. was detected in all fish species on every day of the experiment. The average *Salmonella* spp. count in pelagic fish was 2.7±0.25 log CFU/g, whereas in demersal fish, it was 2.9±0.43 log CFU/g. Significant differences were observed in *Salmonella* spp. counts between pelagic and demersal fish across the 4 days of the experiment (F=3840.599, df=1, p=0.000), with both groups showing significant differences (p<0.05) on days 1, 3, and 4 (Figure 2B). On day 4, pelagic fish had a count of 3.0±0.19 log CFU/g, with significant differences (p<0.05) observed between days 1 and 2 and 2 and 4 of the experiment, while demersal fish had a count of 3.2±0.07 log CFU/g, with significant differences (p<0.05) observed between all days of the experiment except for between days 2 and 3 and 3 and 4 (Table 4). Significant differences in *Salmonella* spp. count were observed among all fish species across the 4 days (Table 4). Furthermore, at the end of the experiment, threadfin bream showed the highest *Salmonella* spp. count at 3.3±0.01 log

CFU/g, while pickhandle barracuda exhibited the lowest count at 2.8±0.10 log CFU/g.

The average *S. aureus* count in pelagic fish was 2.8±0.47 log CFU/g, whereas in demersal fish, it was 2.7±0.50 log CFU/g. Significant differences were observed in *S. aureus* counts between pelagic and demersal fish across the 4 days of the experiment (F=2665.796, df=1, p=0.000), with both groups showing non-significant differences (p>0.05) on all 4 days (Figure 2C). On day 4, pelagic fish exhibited an *S. aureus* count of 3.2±0.18 log CFU/g, with significant differences (p<0.05) observed between days 1 and 3, 1 and 4 and 2 and 4 of the experiment, while demersal fish displayed a count of 3.1±0.21 log CFU/g, with significant differences (p<0.05) observed between all days of the experiment except for between days 1 and 2 and 3 and 4. (Table 4). Significant differences in *S. aureus* counts were observed among all fish species across the 4 days (Table 4). Notably, at the end of the experiment, threadfin bream showed the highest *S. aureus* count at 3.3±0.01 log CFU/g, while yellowtail fusilier showed the lowest count at 2.9±0.11 log CFU/g.

The average *B. cereus* count in pelagic fish was 3.0±0.27 log CFU/g, whereas in demersal fish, it was 3.0±0.24 log CFU/g. Significant differences were observed in *B. cereus* counts between pelagic and demersal fish across the 4 days of the experiment (F=20702.379, df=1, p=0.000), with both groups showing significant differences (p<0.05) on days 4 (Figure 2D). On day 4, pelagic fish had *B. cereus* counts of 3.2±0.12 log CFU/g, with significant differences (p<0.05) observed between days 1 and 2, 1 and 3 and 1 and 4 of the experiment, while demersal fish exhibited the counts of 3.3±0.06 log CFU/g, with significant differences (p<0.05) observed between days 1 and 3, 1 and 4, and 2 and 4. (Table 4). Moreover, significant differences were observed among all fish species across the 4 days (Table 4). Notably, at the end of the experiment, threadfin bream exhibited the highest *B. cereus* count at 3.3±0.06 log CFU/g, while pickhandle barracuda displayed the lowest count at 3.1±0.16 log CFU/g.

The average *C. perfringens* count in pelagic fish was 2.8±0.29 log CFU/g, while in demersal fish, it was 2.9±0.27 log CFU/g. Significant differences were observed in *C. perfringens* counts between pelagic and demersal fish across the 4 days of the experiment (F=8323.614, df=1, p=0.000), with both groups showing non-significant differences (p>0.05) on all 4 days (Figure 2E). On day 4, pelagic fish exhibited a *C. perfringens* count of 3.1±0.05 log CFU/g, with significant differences (p<0.05) observed between all days of the experiment except for between days 1 and 2 and days 3 and 4, while demersal fish had a *C. perfringens* count of 3.1±0.20 log CFU/g, with significant differences (p<0.05) observe between days 1 and 3 and days 1 and 4 of the experiment (Table 4). Moreover, significant differences were observed among all fish species across the 4 days (Table 4). At the end of the experiment, threadfin bream showed the highest *C.*

perfringens count at 3.3±0.07 log CFU/g, whereas yellowtail fusilier exhibited the lowest count at 3.0±0.25 log CFU/g.

The average count of *E. coli* in pelagic fish was 2.8±0.26 log CFU/g, whereas in demersal fish, it was 2.9±0.15 log CFU/g. Significant differences were observed in *E. coli* counts between pelagic and demersal fish was across the 4 experimental days (F=13334.221, df=1, p=0.000), with both groups showing non-significant differences (p>0.05) on all 4 days (Figure 2F). On day 4, pelagic fish exhibited an *E. coli* count of 3.1±0.12 log CFU/g, with significant differences (p<0.05) observed between all days of the experiment except for between days 1 and 2 and days 3 and 4, while demersal fish showed an *E. coli* count of 3.0±0.24 log CFU/g, with significant differences (p<0.05) observed between days 1 and 3 and days 1 and 4. (Table 4). Furthermore, significant differences were observed among all fish species across the 4 days (Table 4). At the end of the experiment, eastern little tuna showed the highest *E. coli* count of 3.2±0.11 log CFU/g, while yellowtail fusilier exhibited the lowest *E. coli* count of 2.9±0.35 log CFU/g.

The TVC and the bacterial counts of *Salmonella* spp., *C. perfringens*, and *E. coli* were lower in pelagic fish than in demersal fish, whereas the *S. aureus* and *B. cereus* count was higher in pelagic fish than in demersal fish. Significant differences were observed in TVC of all fish species across the 4 days (p<0.05). *Salmonella* spp. counts showed significant differences (p<0.05) across the 4 days for yellowtail fusilier and threadfin bream. Similarly, *S. aureus* counts differed significantly (p<0.05) over the 4 days for eastern little tuna, white pomfret, orange spotted grouper, yellowtail fusilier, and threadfin bream. Similarly, *B. cereus* counts differed significantly (p<0.05) over the 4 days for eastern little tuna, orange spotted grouper, yellowtail fusilier, and threadfin bream, while *C. perfringens* counts showed significant differences (p<0.05) across the 4 days for pickhandle barracuda, white pomfret, and threadfin bream. Additionally, *E. coli* counts displayed significant variations (p<0.05) among the four days for eastern little tuna and white pomfret.

Discussion

Pelagic fish are considered fresher than demersal fish as they consistently receive higher overall acceptance scores determined using 9-point hedonic scales. A score closer to 9 indicates that the fish is very fresh and highly preferred for consumption, while a score closer to 1 indicates the opposite, rendering it unsuitable for consumption (Everitt, 2009). Employing the 9-point hedonic scale method provides a systematic approach to assess the freshness of Indian mackerel (*Rasrelliger kanagurta*) across diverse storage conditions (Chudasama *et al*., 2018) as well as aids in evaluating changes in the freshness of threadfin bream (*N. japonicus*) subjected to different handling methods before storage on ice over time (Sarve *et al*., 2019). Some species in this experiment exhibited lower scores in the early days of the experiment, along with sunken stomachs and bruised skin, indicative of fish deterioration. The daily spoilage and sensory changes in fish are contingent upon post-harvest handling and market hygiene management. The cleanliness and hygiene standards of fish markets directly influence fish deterioration and the risk of pathogenic microorganism contamination (Revathy and Shibu, 2023; Roy *et al*., 2023). Furthermore, cleanliness and market hygiene have been reported to affect consumer sensory acceptance scores (Sari *et al*., 2020). Sensory assessment is a valuable method to detect fish freshness, although it should be complemented with other detection techniques (Solanki *et al.,* 2016).

The examination of fish freshness quality using physicochemical methods revealed that the TVB-N, TMA-N, and pH values of all fish species increased steadily throughout the experiment. The increase in TVB-N levels can be attributed to various enzymatic processes, free amino acid contamination, nucleotide degradation, amine oxidation, or microbial activities (Alçiçek, 2011). These processes may lead to the formation of alkaline substances, such as ammonia, consequently elevating the TVB-N in fish meat during storage. Similarly, the increase in TMA-N levels results from the breakdown of trimethylamine oxide (TMAO). Changes in these compounds significantly impact the odor and taste of fish. When TMAO decomposes into TMA-N, it imparts a sweet taste to the fish; however, the presence of TMA-N causes a fishy smell. Moreover, TMA-N is more commonly found in red muscle fish compared to white muscle fish. Fish with a high proportion of red muscle tissue typically inhabit shallower depths, from the surface to the middle of the water column. This distribution correlates with the decreasing proportion of red muscle tissue with depth, corresponding to the habitat preferences of each fish species (Drazen *et al*., 2013). Consequently, pelagic fish, such as eastern little tuna, which possess a significant amount of red muscle, exhibited higher levels of TMA in their meat by the end of the experiment. Moreover, the quantity and availability of TMA are influenced by factors such as fish type, size, gender, and habitat (Tsigarida *et al*., 2003). The pH analysis conducted in this study revealed that the pH gradually increased from the 1st to the 4th day of the experiment, which correlated with the increase in TVB-N and ammonia accumulation. This pH variation is attributed to the enzyme- and ATP-mediated release of inorganic phosphate and ammonium (Viji *et al.,* 2014), consistent with the findings of Amitha *et al.* (2018), where a continuous increase in TVB-N, TMA-N, and pH values was observed during threadfin bream preservation in ice under refrigeration. Pelagic fish, such as eastern little tuna, pickhandle barracuda, and white pomfret exhibit lower TVB-N and pH values compared to demersal fish. However, pelagic fish tend to contain higher levels of TMA-N because of its accumulation in fish with red muscle tissue. Therefore, pelagic fish may emit a stronger fishy odor compared to demersal fish.

Upon microbiological examination and comparison with the Ministry of Public Health of Thailand standard values (2020) for the analysis of pathogenic microorganisms in seafood consumed fresh or raw, it was observed that the bacterial levels in all types of fish studied exceeded the set standards from the first day of testing. This aligns with the sensory assessment conducted on the first day of the experiment, where all fish exhibited signs of spoilage, including mucus, slightly cloudy eyes, sunken stomachs, fishy odor, and softened meat, indicating a state of deterioration. Therefore, fish exhibiting such characteristics should be avoided, as they indicate poor quality and may present potential health risks if consumed raw; proper cooking is recommended to minimize these risks. Furthermore, prolonged storage leads to increased microbial levels in fish. This trend is consistent with the findings of Amitha *et al*. (2018), who studied microbial changes of threadfin bream stored in ice. They found that the initial microbial count in fish meat was 3. 98 log CFU/g, which gradually increased ($p<0.05$) to 6.98 log CFU/g by the end of the experiment, indicating a rise in microbial load over time. In addition, the increased microbial load may be influenced by contamination during fish transport, packaging, and post-harvest area sanitation (Edirisinghe and Wickramasinghe, 2019). Liao *et al.* (2023) observed contamination of ice with *S. aureus, Vibrio parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Shigella* spp. upon contact with food, which may lead to increased microbial levels in fish. Such contamination contributes to food spoilage and significant economic losses.

In conclusion, pelagic fish species, such as eastern little tuna, pickhandle barracuda, and white pomfret, tend to exhibit fresher qualities and have longer shelf lives compared to demersal fish species, such as threadfin bream. These distinctions are evident in both physicochemical and sensory parameters, suggesting that pelagic fish generally maintain freshness better than demersal fish. Factors influencing this difference may include muscle structure, internal muscle composition, and various biological aspects such as spawning season and stomach contents. In addition, fish freshness can be compromised by both internal deterioration and external factors like microbial contamination during handling and storage. These findings can be used as valuable information for the development of the fisheries industry and as a database for use in fisheries resource management in Thailand, contributing to enhanced fishing practices in the future.

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Conflicts of interest

The authors declare no competing financial interests.

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