

Review comments

1. The reference to fish composition as aspects of fish quality is not right in my considered scientific opinion. However, the reference of pH and peroxide value variations with seasons as aspects of fish quality is scientifically correct. So, if the authors can do that in the document, discussions by restricting quality to those 2 parameters, then that is scientifically sound.
2. The reference to fish safety is not necessary because peroxide values and pH are not good determinants of food quality. Fish quality aspects should not be discussed in the document at all. They should be avoided in my opinion.
3. I advise the authors to do grammar check of the text as there are considerable errors of grammar in the article.
4. The article is not ready for publications as it is now until the corrections as guided above are done.

Original Research Article

Annual variation in proximate chemical composition of fish sold in the market of Ludhiana market, Punjab, and its behavior in seasons of the year

ABSTRACT

A study was carried out to investigate the nutritional composition and quality of four different fish species (*Wallago attu*, *Labeo rohita*, *Pangasianodon hypophthalmus*, and *Rastrelliger kanagurta*) sourced from the Ludhiana fish market spanning from September 2021 to August 2022, encompassing four distinct seasons: post-monsoon, winter, pre-monsoon, and monsoon. The proximate parameters were analysed through following the standard methods of AOAC (2019). Peroxide value (PV) was recorded by titration method and pH was recorded using pH meter. One-way ANOVA ($p \leq 0.05$) was performed to evaluate the relationship among proximate composition and quality parameters in fish flesh. Statistical analysis was performed by using SPSS ver. 20 and Microsoft Excel software packages for the evaluation of proximate composition and quality parameters. The findings of the present study showed that all of the fish species recorded the lowest levels of moisture content in pre monsoon season, while the highest were recorded during the monsoon season. The selected fish species displayed seasonal fluctuations in their protein content. The lipid content of *P. hypophthalmus* was observed significantly greater (10.28%) in comparison to in other species. Significant statistical differences in the average ash content were observed throughout various seasons in *L. rohita* and *R. kanagurta*. The peroxide value exhibited variations ranging from 2.15 to 4.17 milliequivalents of O₂/kg throughout various seasons in different species. The fish species *R. kanagurta* had the lowest pH values, whereas *L. rohita* recorded the highest pH levels. In conclusion, the quality parameter served as a comprehensive framework for ensuring the safety of fish consumption. In India, fish markets have experienced significant growth, driven in part by a rise in per capita consumption of fish in recent years. Changing dietary preferences and increasing disposable incomes are expected to further boost fish consumption in the foreseeable future. A comprehensive scientific analysis of the nutritional composition and quality parameters of selected distinct fish species, sourced from the Ludhiana fish market, revealed intriguing seasonal variations. However, it's imperative

to highlight that data related to various critical aspects of the fish industry in Punjab are documented.

Keywords: Fish market; Commercially important fish; Proximate composition; Quality parameters; Consumer safety

1. INTRODUCTION

Fish emerges as a remarkably valuable source of high-quality animal protein and stands as one of the most abundant reservoirs of essential minerals. The fishery and aquaculture sector exhibit immense potential in addressing concerns related to food security and nutrition [1]. As of the latest data for the year 2021-22, India's total fish production reached an impressive 162.48 lakh tonnes. Furthermore, it is worth noting that per capita fish consumption in India currently stands at 6.31 kg, a figure that highlights the significance of further interventions, particularly in regions like Punjab, where the per capita consumption stands at a mere 0.4 kg, significantly lower than the national average [2]. In the landscape of Asian countries, fish protein alone contributes a substantial 31% to the pool of animal protein sources [3].

The comprehensive analysis of the proximate composition of fish muscles offers valuable insights into the composition of different species. This allows for meaningful comparisons of nutritional differences both within and among species. Fish, as a dietary source, presents a rich array of essential nutrients, including noteworthy quantities of protein, vitamins, minerals, and healthy fats. It is renowned as a globally accepted source of low-fat, high-quality protein and minerals, in addition to being a rich source of polyunsaturated fatty acids (PUFA) [4; 5].

Ensuring the nutritional integrity and safety of fish available in the market relies heavily on maintaining the highest standards of product quality. A multitude of factors come into play, including the duration between harvesting and consumption, as well as the meticulous control of temperatures during all stages of handling, processing, and storage. These factors exert profound influences on the biochemical composition, microbial load, and sensory attributes of the fish. Parameters such as pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), and sensory evaluation serve as critical indicators of fish spoilage, reflecting alterations in protein degradation, lipid oxidation, and microbial proliferation over time. By monitoring these parameters, stakeholders can gauge the overall quality and safety of fish products, facilitating informed decisions regarding their consumption and commercial viability [6]. The objective of this research endeavor was to conduct a comprehensive examination into the nutritional composition and quality parameters of four diverse fish species, namely *Wallago attu*, *Labeo rohita*, *Pangasianodon hypophthalmus*, and *Rastrelliger kanagurta*, procured from the Ludhiana fish market to discern the variations in fish muscle composition across different seasons.

2. MATERIAL AND METHODS

The present study focused on the selection of fish markets in five districts of Punjab, India, namely Amritsar (31°37' 50.969"N, 74°52' 30.93"E), Jalandhar (31°20' 2.605"N, 75°34' 13.205"E), Ludhiana (30°55' 17.501"N, 75°54' 5.642"E), Mohali (30°43' 49.67"N, 76°42' 2.46"E), and Bathinda (30°12' 29.399"N, 74° 57' 56.785"E). The data collection spanned a period of one year, from September 2021 to August 2022, with sampling conducted at regular intervals corresponding to different seasons, including post-monsoon, winter, pre-monsoon, and monsoon. The selection process took into consideration the marketing of fish obtained from both culture and capture fisheries in Punjab, as well as fish imported from other states. *L. rohita* (culture fishery), *P. hypophthalmus* (culture fishery), *W. attu* (capture fishery) and *R. kanagurta* (capture fishery) muscle samples were collected in fresh condition from markets in sterile containers and transported in an insulated box to the Department of Fisheries Resource Management, College of Fisheries Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Collected samples were stored at -20°C for further analysis. Analysis of the proximate composition encompassing parameters such as moisture, protein, lipid, carbohydrate, and ash content, along with quality parameters Peroxide value (PV) and pH were performed using fish muscle after thawing at room temperature.

All the proximate parameters were analysed following the standard methods [7]. Moisture content was recorded by the standard hot air method using the hot air oven. The crude protein level of the fish meat was recorded by estimating total nitrogen by the Kjeldahl method using an electrically heating digestion and distillation unit. The crude lipid content of the fish meat was recorded by the Soxhlet extraction method using the Soxhlet fat extraction unit. The total carbohydrate content of the fish meat was recorded by the Anthrone method using the spectrophotometer. Ash content of the fish meat was recorded by the dry method at high temperature ($550 \pm 25^\circ\text{C}$) using a muffle furnace. Peroxide value (PV) was determined by titration method using 0.02 N sodium thiosulfate for titration [8]. The level of pH in fish flesh was estimated by the use of a pH meter. One way ANOVA ($p=0.05$) was performed using Tukey's-b Post Hoc multiple comparisons method to evaluate the relationship among proximate composition and quality parameters. Statistical analysis was performed by using SPSS ver. 20 software package.

3. RESULTS AND DISCUSSION

3.1 Proximate composition of selected fish species

3.1.1 Moisture content in fish flesh

Among the four selected fish species, namely *W. attu*, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta*, their lowest moisture content was recorded during the pre-monsoon season (79.18%, 77.01%, 73.02%, and 72.48%, respectively), contrasting with their highest levels observed during the monsoon season at 81.08%, 79.92%, 74.96%, and 74.94%, respectively. These fluctuations highlight the pronounced influence of seasonal variations on fish moisture levels in the market. The moisture content across the four species ranged from 72.48% to 81.08% across different seasons (Table 1). Significant variations in average moisture content were observed across seasons ($p<0.05$) among the selected species, with *R. kanagurta* recording the minimum and *W. attu* the maximum moisture content. According to FAO [12], fish fillets typically exhibit an inverse relationship between moisture and lipid content, with their combined sum constituting approximately 80% of the proximate composition, while the remaining 20% comprises other components.

In the study conducted by Naher et al. [9], an examination was carried out to determine the moisture levels of *W. attu* samples exposed to fresh and frozen storage conditions in the Mymensingh district, Bangladesh. The moisture content recorded for *W. attu* under fresh and frozen conditions was determined to be $79.00 \pm 0.45\%$ and $75.00 \pm 1.01\%$, respectively. Similarly, Dayami and Sarojnalini [10] documented an average moisture content of $79.18 \pm 0.43\%$ in *L. rohita* sourced from Loktak Lake, Manipur, which closely corresponds to the findings of the current investigation. Moreover, Dayami and Sarojnalini [10] noted the highest moisture content in *P. hypophthalmus* during the monsoon season compared to other periods. Sonavane et al. [10] reported a moisture content of 72.24% in *R. kanagurta* and emphasized the inverse relationship between moisture and fat content in fish, suggesting that fatty fish types typically exhibit relatively lower moisture content.

Table 1: Moisture content of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	80.44 ^{ab} ±0.28	78.23 ^b ±0.29	74.61 ^{ab} ±0.28	73.41 ^b ±0.29
Winter	79.53 ^b ±0.25	77.32 ^b ±0.29	73.66 ^{bc} ±0.25	72.79 ^b ±0.27
Pre-monsoon	79.18 ^b ±0.29	77.01 ^b ±0.32	73.02 ^c ±0.28	72.48 ^b ±0.27
Monsoon	81.08 ^a ±0.32	79.92 ^a ±0.29	74.96 ^a ±0.30	74.94 ^a ±0.33
Average	80.05±0.25	78.12±0.36	74.07±0.26	73.40±0.31

The values (mean ± standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.1.2 Protein content in fish flesh

During the study period, *W. attu*, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta* exhibited their lowest protein content in flesh during the monsoon season, measuring at 14.62%, 14.35%, 9.72%, and 16.48%, respectively, whereas their highest levels were recorded during the pre-monsoon season, reaching 16.49%, 16.05%, 11.59%, and 18.36%, respectively. These fluctuations underscore the significant impact of seasonal variations on protein levels among diverse fish species. Notably, protein levels ranged from 9.72% to 18.36% across different seasons (Table 2). Furthermore, the average protein content values exhibited significant variations across various seasons ($p < 0.05$) within the four selected species. Specifically, *P. hypophthalmus* displayed the lowest protein content, while *R. kanagurta* exhibited the highest. The observed increase in protein contents during the post-monsoon season suggests a recuperation of fish from the rigors associated with breeding activities.

Mishra [13] documented an average protein content of 15.95% during the monsoon season and 16.99% during the post-monsoon season in *L. rohita* sourced from various fish markets in Sultanpur, Uttar Pradesh. The observed increase in protein content during the post-monsoon season suggests a recovery phase for the fish following the demanding breeding activities. Rahman *et al.* [14] recorded a protein content of approximately 13.58% in *P. hypophthalmus*. They also noted significant distinctions between live and deceased fish specimens, underlining the impact of extended storage on fish quality, encompassing chemical composition and nutritional attributes. Mishra [13] additionally highlighted the seasonal variability in fish protein content, typically showing lower values during breeding seasons like the monsoon. Sonavane *et al.* [11] evaluated an average protein content of 19.14% in *R. kanagurta* harvested from the Ratnagiri coast, concluding that mackerel serves as an excellent protein source across diverse societal strata. In the present study, the protein levels of fish were higher during the pre-spawning period (pre-monsoon) and declined during the spawning period (monsoon), mirroring the trend [13]. In fish muscle, water content reached its peak levels while muscle protein content decreased during the monsoon. The decline in protein and the rise in water content of the muscle were attributed to gonadal development and maturation, which depleted muscle protein reserves during the monsoon season [15].

Table 2: Protein content of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	15.74 ^b ±0.11	15.21 ^b ±0.10	10.65 ^b ±0.11	17.54 ^b ±0.11
Winter	15.76 ^b ±0.14	15.70 ^{ab} ±0.17	11.20 ^{ab} ±0.15	18.13 ^{ab} ±0.18
Pre-monsoon	16.49 ^a ±0.23	16.05 ^a ±0.17	11.59 ^a ±0.16	18.36 ^a ±0.18
Monsoon	14.62 ^c ±0.19	14.35 ^c ±0.19	9.72 ^c ±0.18	16.48 ^c ±0.20
Average	15.65±0.22	15.33±0.20	10.79±0.22	17.63±0.23

The values (mean ± standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.1.2 Lipid content in fish flesh

W. attu, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta* exhibited their lowest lipid content in flesh during the monsoon season, measuring at 0.69%, 1.59%, 9.33%, and 2.47%, respectively, contrasting with their highest levels during the winter season at 1.07%, 2.05%, 11.04%, and 4.05%, respectively. The lipid content across different species

varied from 0.69% to 11.04% in various seasons (Table 3). Notably, lipid values were minimal in *W. attu* and maximal in *P. hypophthalmus*, with the lowest values recorded during the monsoon and the highest during the winter season. According to FAO [12], moisture and lipid content in fish muscle exhibit an inverse correlation and escalated moisture content and rapid decline in lipid content in studied fish species could be linked to the spawning season. Ganeshwade and Jadhav [16] conducted a study on seasonal biochemical variations in *W. attu* from the Krishna River near Audumber, Maharashtra. They reported a lipid content of 0.64% during the winter, which gradually decreased until the monsoon period. Mishra [13] documented high lipid content in *L. rohita* during the post-monsoon season, attributed to active fish feeding. Subsequently, this lipid content declined in the pre-spawning season, indicating its utilization during gonadal development and as an energy source during ovulation and spawning.

Rahman *et al.* [14] documented a lipid content of approximately 8.0% in *P. hypophthalmus*, while Murthy *et al.* [17] reported a lipid content of 3.83% in *R. kanagurta* sourced from various fish outlets in Navi Mumbai, Maharashtra. Sonavane *et al.* [11] observed an inverse relationship between moisture and fat content, indicating that fatty fish species tend to possess relatively lower moisture content. A similar inverse relationship was noted between *W. attu* and *P. hypophthalmus* in the current study.

Table 3: Lipid content of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	0.78 ^a ±0.06	1.62 ^a ±0.07	9.78 ^b ±0.80	3.09 ^b ±0.07
Winter	1.07 ^a ±0.11	2.05 ^a ±0.14	11.04 ^a ±0.16	4.05 ^a ±0.17
Pre-monsoon	1.02 ^a ±0.14	1.94 ^a ±0.05	11.00 ^a ±0.13	3.98 ^a ±0.14
Monsoon	0.69 ^a ±0.12	1.59 ^a ±0.13	9.33 ^b ±0.13	2.47 ^c ±0.15
Average	0.89±0.06	1.80±0.07	10.28±0.23	3.40±0.21

The values (mean ± standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.1.3 Carbohydrate content

Carbohydrate levels in fish flesh reached their lowest points during the monsoon season in all four species *W. attu*, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta* measuring at 0.54%, 1.46%, 0.62%, and 0.24%, respectively. Conversely, they peaked during the pre-monsoon season at 0.78%, 2.04%, 1.13%, and 0.36%, respectively (Table 4). Across different seasons, carbohydrate content varied from 0.23% to 2.04%. *R. kanagurta* exhibited the lowest carbohydrate values, while *L. rohita* displayed the highest. Carbohydrate values were minimal during the post-monsoon period and maximal during the pre-monsoon season.

Rahman *et al.* [14] reported a carbohydrate content of approximately 0.73% in *P. hypophthalmus*, aligning closely with the values recorded in the present study. Sahu and Parida [18] highlighted the relatively low levels of carbohydrates compared to other proximate compositions in fish, noting a carbohydrate level of 0.51% in *R. kanagurta*. In marine fishes, glycogen does not serve as a significant reserve energy source. Lower levels of carbohydrate content in the total proximate composition of fish muscle, indicating that most of the glycogen in freshwater fishes does not significantly contribute to body reserves [19]. These findings are consistent with the results observed in the current study.

Table 4: Carbohydrate content of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	0.60 ^a ±0.03	1.64 ^b ±0.04	0.72 ^b ±0.04	0.23 ^a ±0.03
Winter	0.74 ^a ±0.07	2.01 ^a ±0.06	1.04 ^a ±0.08	0.32 ^a ±0.06
Pre-monsoon	0.78 ^a ±0.08	2.04 ^a ±0.07	1.13 ^a ±0.07	0.36 ^a ±0.05
Monsoon	0.54 ^a ±0.06	1.46 ^b ±0.08	0.62 ^b ±0.07	0.24 ^a ±0.06
Average	0.66±0.04	1.79±0.07	0.88±0.07	0.29±0.03

The values (mean \pm standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.1.4 Ash content

Ash content reached its nadir during the monsoon in all four species *W. attu*, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta* measuring at 2.54%, 1.65%, 1.62%, and 1.71%, respectively, and peaked during the pre-monsoon season at 3.78%, 2.28%, 2.04%, and 3.04%, respectively (Table 5). Across different species, ash content varied from 1.62% to 4.34%, with the lowest observed in *P. hypophthalmus* and the highest in *W. attu*. Hasan *et al.* [20] documented an ash content ranging from 1.04% to 2.95% in *P. hypophthalmus*, with an average value of 1.75%, which closely aligns with the findings of the current study. Sonavane *et al.* [11] reported an ash content of 1.42% in *R. kanagurta* harvested from the Ratnagiri Coast, Maharashtra. Mishra [14] observed higher ash content during the post-monsoon season and lower levels during the monsoon season. The association between higher ash content and increased mineral metabolism is notable. The availability of food substantially increases during the post-monsoon season following the receding water levels during the monsoon. The findings of the present study also indicate a similar trend, with ash content peaking during the pre-monsoon season and reaching its lowest during the monsoon season.

Table 5: Ash content of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	3.61 ^b \pm 0.07	1.79 ^{ab} \pm 0.07	1.66 ^a \pm 0.08	1.74 ^b \pm 0.07
Winter	4.28 ^a \pm 0.13	2.24 ^a \pm 0.13	1.98 ^a \pm 0.14	2.98 ^a \pm 0.13
Pre-monsoon	4.34 ^a \pm 0.13	2.28 ^a \pm 0.12	2.04 ^a \pm 0.12	3.04 ^a \pm 0.13
Monsoon	3.14 ^b \pm 0.14	1.65 ^b \pm 0.13	1.62 ^a \pm 0.14	1.71 ^b \pm 0.14
Average	3.84 \pm 0.15	1.99 \pm 0.10	1.83 \pm 0.08	2.37 \pm 0.20

The values (mean \pm standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.2 Quality assessment of selected fish during seasonal interval

3.2.1 Peroxide value

The average peroxide values across different species ranged from 2.15 to 4.17 milliequivalents of O₂/kg, varying by season (Table 6). While seasonal fluctuations in peroxide values were statistically significant (p<0.05) in *L. rohita*, no significant seasonal variations were observed in *W. attu*, *P. hypophthalmus*, and *R. kanagurta*. Specifically, peroxide values were lowest in *W. attu* and highest in *R. kanagurta*, with minimum values recorded during winter and maximum values during the pre-monsoon season. Peroxide value is indicative of fat oxidation, with products considered rancid when peroxide values range between 20 and 40 milliequivalents of O₂/kg [21]. The current study observed significantly lower peroxide content in four different fish species collected from the Ludhiana fish market across various seasons compared to the permissible limit.

Table 6: Peroxide value of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	2.55 ^c \pm 0.10	2.62 ^b \pm 0.11	3.49 ^a \pm 0.22	3.99 ^a \pm 0.13
Winter	2.15 ^{bc} \pm 0.11	2.57 ^b \pm 0.14	3.70 ^a \pm 0.11	3.53 ^a \pm 0.24
Pre-monsoon	3.88 ^b \pm 0.13	4.03 ^b \pm 0.10	4.07 ^a \pm 0.12	4.17 ^a \pm 0.13
Monsoon	2.88 ^a \pm 0.13	2.92 ^a \pm 0.12	4.01 ^a \pm 0.11	4.06 ^a \pm 0.14
Average	2.86 \pm 0.20	3.03 \pm 0.18	3.82 \pm 0.09	3.94 \pm 0.10

The values (mean \pm standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

3.2.2 pH value of fish flesh

The pH content in the four distinct fish species ranged from 6.46 to 6.87 across different seasons (Table 7). The mean pH values exhibited significant variations across various seasons ($p < 0.05$). Notably, the lowest pH values were recorded in *R. kanagurta*, while the highest was observed in *L. rohita*. Across all four species, the pH content showed a consistent pattern of being lowest during the pre-monsoon season and highest during the monsoon season. The pH patterns observed in this study align with the findings of Rahman *et al* [14], who suggested that variations in fish pH are influenced by seasonal changes, dietary habits, fish activity levels, and environmental conditions. Post-mortem, fish muscle pH undergoes initial declines due to anaerobic lactic acid formation, followed by rises in later stages due to the generation of basic compounds, as indicated by Hossain *et al.* [22]. Importantly, throughout the study period, the pH levels in all four species *W. attu*, *L. rohita*, *P. hypophthalmus*, and *R. kanagurta* consistently remained within the acceptable limit (<7), as defined by ElShehawey *et al.* [23], reaffirming the safety of these fish species for consumption.

Table 7: pH of selected fish during seasonal interval

Season	<i>W. attu</i>	<i>L. rohita</i>	<i>P. hypophthalmus</i>	<i>R. kanagurta</i>
Post-monsoon	6.59 ^c ±0.03	6.63 ^c ±0.02	6.57 ^c ±0.03	6.55 ^c ±0.02
Winter	6.75 ^b ±0.03	6.77 ^b ±0.03	6.73 ^b ±0.03	6.71 ^b ±0.03
Pre-monsoon	6.48 ^d ±0.03	6.49 ^d ±0.02	6.47 ^d ±0.02	6.46 ^c ±0.03
Monsoon	6.85 ^a ±0.02	6.87 ^a ±0.02	6.86 ^a ±0.02	6.85 ^a ±0.02
Average	6.67±0.04	6.69±0.04	6.66±0.05	6.64±0.05

The values (mean ± standard error) with different alphabetical superscripts (a, b, c...) differ significantly within the seasons from different sites (in a column).

4. CONCLUSIONS

The Ludhiana fish market presents a diverse array of fish sourced from both inland and marine sectors. An analysis of proximate composition revealed significant variations influenced by species-specific characteristics and seasonal fluctuations. Notably, the quality parameters exhibited intriguing seasonal differences, with locally available species like *W. attu* and *L. rohita* demonstrating superior quality compared to fish imported from distant states such as *P. hypophthalmus* and *R. kanagurta*. These quality assessments not only illuminate seasonal variations but also provide a robust framework for ensuring the safety of fish consumption, thereby enhancing consumer awareness and confidence in the offerings available at the market.

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