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# Biochemical and Microbial Changes in Ice-stored Three Small Indigenous Fish of North-East (India)

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ABSTRACT

The biochemical, microbial and organoleptic changes in three commercially important small indigenous fishes (Mystus gulio, Puntius sophore and Gudusia chapra) during ice storage has been studied. Proximate analysis revealed the moisture, ash, protein and lipid contents were 74.76%, 1.58%, 16.87% and 5.69%in M. gulio, 72.55%, 1.72%, 18.21% and 4.49%in P. sophore, and, 73.18%, 1.77%, 16.24% and 4.43% in G. chapra. Based on the biochemical, microbiological and organoleptic evaluation, the shelf-life of these fishes in ice-stored condition was considered to be 13 days, 10 days and 13 days respectively. The TVBN was increased to 29.87 mg% on 10th day of ice-storage in P. sophore, whereas, it crossed the border line of spoilage on 13th day in M. gulio and G. chapra. The aerobic plate count was reached to 6 log cfug-1 on 13th day in M. gulio and G. chapra and 10th day for P. sophore. Sensory analysis was well-correlated with the biochemical changes in fish. From this, it was concluded that, the edible quality of ice-preserved M. gulio and G. chapra remain upto 13 days and P. sophore upto 10 days.

## 1. Introduction

Freshness is an important quality index of aquatic products. But the freshness of aquatic products deteriorates faster after post-mortem due to upshot of biochemical and microbial breakdown mechanisms (Olafsdottir et al., 1997). Thus it is essential to minimize the deterioration and extending shelf life via using high hydrostatic pressure (Erkan and Uretener, 2010), irradiation (Erkan and Ozden, 2007), ozonized slurry ice (Campos et al., 2005), super chilled vacuum pack (Duun and Rustad, 2008), edible films and coatings (Vasconez et al., 2009) etc. Low temperature treatment and minimizing microbiological and biochemical changes at decreased temperature is another important method to maintain fish freshness (Wang et al., 2003). .Fish being highly perishable commodity, suffers huge loss due to improper post-harvest care. Thus, proper processing and storage of it are vital to maintain the quality. In spite of following established preservation techniques, the expected nutritional value is not reached in stored fish. The commonest mean of fish preservation is icing. Ice lowers the temperature of the fish and thus decreases the microbial or biochemical activities to minimize spoilage (Siddique et al., 2011). Icing merely slows down microbial activity since fish support a population of cold-tolerant bacteria (Alberto et al., 1987) which through predominance at the surface of the fish, secrete enzymes into the tissues and bring out a complex series of chemical changes (Shewan, 1971). A number of works has been reported on the quality of the ice-stored medium to big-sized fish. But such reports on small indigenous fishes are limited. M. gulio, P. sophore and G. chapra are small-sized high-valued food fish of North-East India and no information is available on its quality during ice-preserved. Thus, the study has been undertaken so that the shelf-life of these fish during ice-storage could be established.

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## 2. Materials and Methods

Fresh sample of M. gulio (13.3±0.3 cm; 26.6±1.6g), P. sophore (9.0±0.5 cm; 12.26±0.99g) and G. chapra (8.57±0.69 cm; 6.95±0.54 g) were collected from the local markets, washed thoroughly in tap water and then stored species-wise in insulated boxes with alternate layer of flake ice and fish at 1:1. The boxes were kept at ambient temperature (23.5 – 34.60C). Sampling was done before icing (Day-1) and at two days interval after icing. Ice was partial replenished during each sampling.

***Sampling:*** Eight to ten P. sophore and G. chapra and four to five M. gulio were randomly sampled from different places of the box (bottom, centre and top) and its entrails and gills were removed. Then the former two fish were de-scaled and later one skinned and flesh samples were collected, mixed and grinded to make composite sample for biochemical and microbial analyses.

***Biochemical analyses:*** Moisture, ash, total lipid and crude protein content of the fishes were analyzed following the AOAC (2000), total volatile basic nitrogen (TVBN) by the Conway's micro-diffusion method (Conway, 1947), peroxide value (PV) by the chloroform extraction method (Jacob, 1958) and thiobarbituric acid reactive substances (TBARS) by Benjakul and Bauer (2001). The sample (0.5 g) was dispersed in 2.5 ml 0.0375% thiobarbituric acid, 15% trichloroacetic acid and 0.25N HCl. The mixture was heated in boiling water for 10 min and then cooled in running tap water. Then, it was centrifuged at 8000 rpm for 20 min in room temperature. The supernatant was collected and its absorbance was measured at 532 nm in a spectrophotometer (SHIMADZU, UV 2550). TBARS were calculated and expressed as mg malonaldehyde/kg fish meat.

***Total aerobic plate counts***: To determine the total number of viable microorganisms in the fish muscle, 10 g of sample was added to 90 ml sterile physiological saline (0.85% NaCl), mixed thoroughly in stomacher (Stomacher 80, Seward, UK) and further dilutions were carried out in the same diluent (APHA, 2001). Pour plating method was followed using plate count agar for enumerating the bacteria. The plates were incubated at 370C for 48 h and counts were taken. All the constituents of the media used were acquired from Hi Media Laboratories Pvt. Limited, Mumbai, India.

***Sensory analysis:*** Sensory characteristics and overall acceptability were assessed by a panel of six members belonging to the College of Fisheries, Lembucherra, Tripura on the basis of ten-point scale on each sampling (Sukumar et al., 2007). Sensory characteristics included general appearance, odour and texture of fish. Scale employed for evaluating sensory quality is given in Appendix. The scores were given in the decreasing order scale with 10 – 9 for excellent, 8 – 7 for good, 6 – 5 for fair and acceptable, 4 – 3 for poor and 2 – 1 for very poor. The mean of the scores given by the panel has represented the overall sensory quality. A score of 3 to 4 was considered unacceptable.

***Statistical analyses:*** Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.0 for Windows). The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at $p<0.05$.

## 3. Results and Discussion

The proximate composition as well as Changes of biochemical and microbial parameters of ice stored *fishes* is presented in Table 1. This is almost similar to earlier finding of moisture-72–74%, crude protein-16–18% and total lipid-4–5% in the fish. 4. The moisture content was registered a steady increase over a period of 13 days in *M. gulio* and *G. chapra* and 10 days in *P. sophore*. The moisture content increase was 6.18% in *M. gulio* and 4.92% in *G. chapra* which were significantly different ($p < 0.05$) and in *P. sophore*, the increase (3.6%) was insignificant ($p > 0.05$). The correlation (r) between the period of storage and change in moisture content was found 0.69, 0.86 and 0.66, ($p < 0.01$) in *M. gulio, G. chapra* and *P. sophore* respectively. Joseph *et al*. (1988) has also reported a similar trend in iced *Labeo rohita*. The uptake of moisture was due to exposure of fish to ice melt water and it was found to be higher in *M. gulio* than *P.sophore* and *G. chapra*. This difference was due to having scales in *P. sophore* and *G. chapra* whose barrier effect is more than the scale-less *M. gulio*. In *M. gulio*, the ash content has showed a steady decrease ($p < 0.05$) of about 43% from the initial value of 1.58% with the increase of storage period of 13 days. Whereas, only 27% and 29% decrease ($p < 0.05$) of ash were observed in *P. sophore* and *G. chapra* from the initial value of 1.72% and 1.77% respectively. The decrease of ash content after day-4 was insignificant ($p > 0.05$). The correlation ($r$) between the period of storage and change in ash content was -0.90, -0.53 and -0.78 in *M. gulio, G. chapra* and *P. sophore* respectively. The loss of minerals during ice storage could be attributed to the leaching of minerals in the ice melt water. Like moisture uptake, loss of minerals in *M. gulio* was found more than *P. sophore* and *G. chapra* which could be due to lack of scales in the former. The crude protein (TN% x 6.25) content showed a gradual decrease to 11.87% (16.87% of initial value), 15.60% (18.21 of initial value) and 15.07% (16.24% of initial value) at the end of ice storage period in *M. gulio*, *P. sophore* and *G. chapra* respectively. The correlation ($r$) between the period of storage and change in protein were found -0.64, -0.91 and 0.59 in *M. gulio*, *P.*

*sophore* and *G. chapra* respectively. This can be attributed to leaching out of soluble nitrogenous components in the ice melt water. The loss of nitrogenous components was lowest in *G. chapra* followed by *P. sophore* and this may be attributed to the presence of scale. These changes are due to breakdown of the cellular structure and growth of microorganisms that either naturally associated with the fish or contamination from handling (Ehira and Uchiyama, 1987).

TVB-N increases curvilinearly or linearly as spoilage progresses in fish and a level of 30 mg TVB-N/100g is considered unfit for human consumption (Gokodlu *et al.*, 1998). In this study, TVB-N showed a significant increase ($p < 0.05$) to 38.27 mg in *M. gulio,* 29.87 mg in *P. sophore* and 36.07mg/100g of muscle in *G. chapra*. This increase may be due to formation of ammonia and other volatile amines during storage (Mazorra-Manzano *et al.*, 2000). The correlation (r) between the period of storage and change in TVB-N levels was found to be 0.98, 0.97 and 0.99 ($p < 0.01$) in case of *M. gulio, P. sophore and G. Chapra* respectively. Total lipid content was declined ($p < 0.05$) from 5.69 to 4.21% in *M. gulio* over 13 days, 4.49 to 4.21% in *P. sophore* over 10 days and 4.43 to 3.79% in *G. chapra* over 13 days during ice storage.

Rancidity development was measured by primary (PV) and secondary (TBARS) lipid oxidation compounds formation. PV value was low (*M. gulio* – 3.55, *P. sophore* – 2.61 and *G. chapra*– 2.60) in early raw fish and it registered a significant ($p < 0.05$) increase during ice storage. This was as a result of the presence of pro-oxidant enzymes (lipoxygenases, peroxidases, and so on) and chemical pro-oxidant molecules (namely, hemoproteins and metal ions) (Erickson, 1997; Sikorski and Kolakowski, 2000). The peroxide value reached to 18.22 and 19.07 on day-13 in *M. gulio* and *G. chapra* respectively, and 21.22 on day-10 for *P. sophore*. Although the inherent fat content of all the three fishes were moderate, no taint of rancidity was noticed till the fishes were in acceptable organoleptically. This may be because of the final PV which was below the critical level of giving any rancid smell to the fish. Thiobarbituric acid reactive substances (TBARS) in muscle of all the three fish increased as the storage time increased. The rate of increase varied depending upon species.

**Table 1.** Proximate composition as well as biochemical and microbial quality aspects of ice stored fishes

| Composition | Species | Day-1 | Day-4 | Day-7 | Day-10 | Day-13 |
|---|---|---|---|---|---|---|
| Moisture | *M. gulio* | $74.76^{a}$(1.01) | $78.25^{b}$ (0.69) | $79.2^{b}$ (1.06) | $79.16^{b}$ (0.86) | $79.38^{b}$ (0.23) |
| | *P. sophore* | $72.55^{a}$(1.03) | $74.36^{a}$ (0.98) | $75.55^{a}$ (0.78) | $75.16^{a}$ (1.42) | |
| | *G. chapra* | $73.18^{a}$ (0.15) | $75.22^{b}$ (0.21) | $76.12^{bc}$(0.60) | $76.65^{c}$ (0.31) | $76.78^{c}$ (0.33) |
| Ash | *M. gulio* | $1.58^{c}$ (0.03) | $1.24^{b}$ (0.08) | $1.02^{a}$ (0.05) | $0.91^{a}$ (0.02) | $0.89^{a}$ (0.05) |
| | *P. sophore* | $1.72^{c}$ (0.17) | $1.58^{b}$ (0.12) | $1.18^{a}$(0.09) | $1.15^{a}$ (0.03) | |
| | *G. chapra* | $1.77^{b}$ (0.27) | $1.78^{ab}$ (0.53) | $1.45^{a}$ (0.27) | $1.32^{a}$ (0.24) | $1.10^{a}$ (0.15) |
| Protein | *M. gulio* | $16.87^{b}$ (1.15) | $11.97^{a}$ (0.30) | $12.14^{a}$ (0.09) | $12.08^{a}$ (0.13) | $11.87^{a}$ (0.12) |
| | *P. sophore* | $18.21^{b}$ (0.16) | $17.75^{b}$ (0.45) | $15.94^{a}$ (0.36) | $15.60^{a}$ (0.30) | |
| | *G. chapra* | $16.24^{a}$ (0.24) | $16.20^{a}$ (0.76) | $15.81^{a}$ (0.56) | $15.27^{a}$ (0.18) | $15.07^{a}$ (0.13) |
| Lipid | *M. gulio* | $5.69^{d}$ (0.03) | $5.26^{c}$ (0.05) | $4.93^{b}$ (0.13) | $4.78^{b}$ (0.12) | $4.21^{a}$ (0.06) |
| | *P. sophore* | $4.49^{b}$ (0.20) | $4.14^{ab}$ (0.47) | $3.97^{a}$ (1.87) | $4.21^{ab}$ (1.23) | |
| | *G. chapra* | $4.43^{c}$ (0.13) | $4.31^{bc}$ (0.05) | $4.05^{ab}$ (0.05) | $3.95^{a}$ (0.09) | $3.79^{a}$ (0.11) |
| TVB-N | *M. gulio* | $14.0^{a}$ (0.12) | $20.53^{b}$ (0.50) | $24.73^{c}$ (0.93) | $29.87^{d}$ (0.42) | $38.27^{e}$ (1.92) |
| | *P. sophore* | $13.07^{a}$(1.23) | $19.13^{b}$ (0.47) | $26.13^{c}$ (1.87) | 29.87 (1.23) | |
| | *G. chapra* | $12.27^{a}$ (0.33) | $18.77^{b}$ (0.32) | $25.80^{c}$ (0.78) | $28.63^{d}$ (0.20) | $36.07^{e}$ (1.19) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *M. gulio* | $3.55^{a}$ (0.27) | $6.22^{b}$ (0.29) | $9.44^{c}$ (0.46) | $14.11^{d}$ (0.83) | $18.22^{e}$ (1.15) |
| PV | *P. sophore* | $2.61^{a}$ (0.45) | $6.11^{b}$ (0.29) | $11.11^{c}$ (0.56) | $21.22^{d}$ (1.24) | |
| | *G. chapra* | $2.60^{a}$ (0.35) | $6.17^{b}$ (0.43) | $9.52^{c}$ (0.29) | $15.25^{d}$ (0.28) | $19.07^{e}$ (0.77) |
| | *M. gulio* | $0.68^{a}$ (0.02) | $0.87^{b}$ (0.04) | $1.15^{c}$ (0.09) | $1.39^{d}$ (0.05) | $1.74^{e}$ (0.05) |
| TBA | *P. sophore* | $0.87^{a}$ (0.03) | $1.42^{b}$ (0.01) | $1.49^{c}$ (0.02) | $1.5^{c}$ (0.01) | |
| | *G. chapra* | $0.66^{a}$ (0.04) | $0.89^{b}$ (0.04) | $1.10^{c}$ (0.09) | $1.46^{d}$ (0.06) | $1.78^{e}$ (0.03) |
| | *M. gulio* | $3.51^{a}$ (0.01) | $4.43^{b}$ (0.01) | $5.18^{c}$ (0.02) | $5.83^{d}$ (0.01) | $6.32^{e}$ (0.03) |
| APC | *P. sophore* | $4.36^{a}$ (0.02) | $5.2^{b}$ (0.02) | $5.73^{c}$ (0.01) | $6.45^{d}$ (0.01) | |
| | *G. chapra* | $3.47^{a}$ (0.01) | $4.37^{b}$ (0.01) | $5.18^{c}$ (0.02) | $5.81^{d}$ (0.02) | $6.33^{e}$ (0.02) |
| | *M. gulio* | $9.05^{d}$ (0.06) | $8.78^{d}$ (0.08) | $7.76^{c}$ (0.15) | $6.82^{b}$ (0.23) | $6.11^{a}$ (0.15) |
| Sensory | *P. sophore* | $9.12^{d}$ (0.07) | $8.35^{c}$ (0.13) | $7.15^{b}$ (0.16) | $6.27^{a}$ (0.06) | |
| | *G. chapra* | $9.05^{d}$ (0.03) | $8.84^{d}$ (0.14) | $7.78^{c}$ (0.21) | $6.84^{b}$ (0.28) | $6.17^{a}$ (0.20) |

Values are means ± S.E. Mean values bearing different superscripts (a, b, c, etc.) in a row are significantly different ($p<0.05$) with respect to sampling.

TBA value (mg malonaldehyde/Kg meat) was increased by 2.5-folds, 2.7-folds and 1.7-folds in *M. gulio*, *G. chapra* and *P. Sophore* respectively. The differences in lipid oxidation possibly resulted from the varied fatty acid compositions (Benjakul *et al*., 2005). Polyunsaturated fatty acids are more prone to oxidation when compared with saturated fatty acids and lipid oxidation occurred during ice storage might caused the denaturation of proteins. TBA concentration, aerobic mesophilic, psychrophilic, enters bacteria and coliform counts, and all attributes related to the sensory analysis are most sensitive to variations during storage (Hernandez *et al*., 2009). The microbial growth in the fish during storage in ice is shown in Figure 1. The bacterial count (log *cfu*$g^{-1}$ meat) was gradually increased ($p < 0.05$) from 3.51 to 6.32 and 3.47 to 6.33 by the 13th day of ice storage in *M. gulio* and *G. chapra* respectively, whereas, in *P. sophore* the same was increased from 4.36 to 6.45 by the 10th day of storage. The microbial growth was almost 1.8 fold in *M. gulio* and *G. chapra,* whereas, 1.5 fold in case of *P. sophore*. However, in any of the fish the APC did not exceed the acceptable limit of $10^{7}$*cfu*$g^{-1}$ (Capell *et al*., 1997). Overall sensory scores of the fish are shown in Figure 2.

Initially the fish exhibited fresh muddy odour, shiny appearance, elastic in texture, bright red gills and perfectly fresh eyes with convex black pupil. No significant changes ($p > 0.05$) were observed till second sampling (Day 4) for *M. gulio* and *G. chapra,* whereas, sensory scores during all the sampling days were found significantly different ($p < 0.05$) in *P.* sophore. On 13th

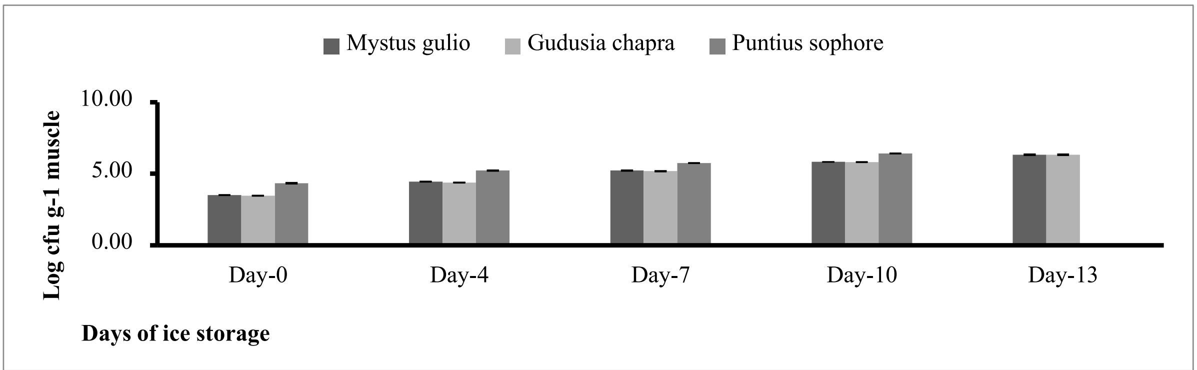


**Fig. 1.** Change of total aerobic plate count during ice storage of *M. gulio*, *P. sophore* and *G. chapra*.

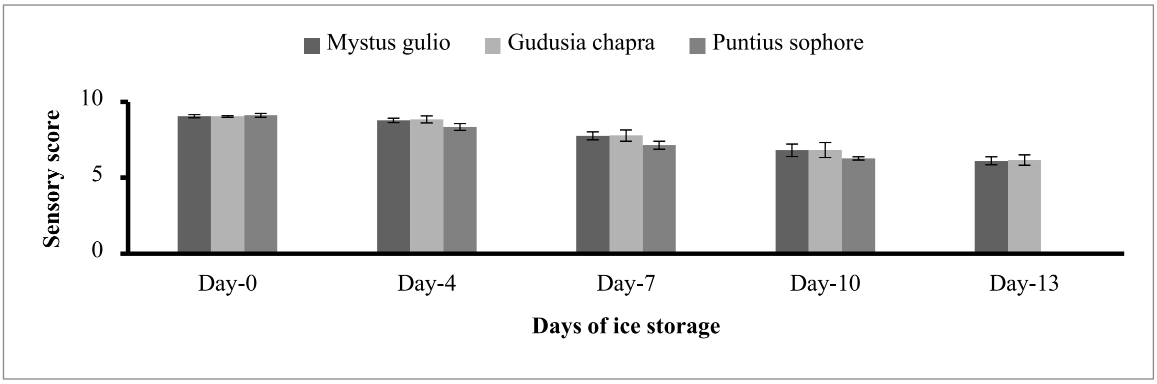


**Fig. 2.** Change of sensory score during ice storage of *M. gulio*, *P. sophore* and *G. chapra*.

day (for *P. sophore*) and 16th day (for *G. chapra* and *M. gulio*), the fish lost its fresh odour and there was a slight decayed odour of weed. The texture became soft and scale was easily removable. Eyes were completely concave and gills exhibited bleaching with dark brown discoloration. Based on the sensory quality, the shelf life of *M. gulio* and *G. chapra* could be considered as 13 days and for *P. sophore* 10 days. The panel of judges reported off-odour from all the fishes on the day of rejection.. However, shelf-life of iced fish depends upon some inherent qualities, such as lipid content, NPN, moisture *etc.* Biochemical indices like TVB-N, PV, TBA *etc.* and also the state of the fish and do not always correlate with the organoleptic assessment which finally determines the shelf-life of fish when preserved with ice or frozen stored. TVB-N was found poor indicator of spoilage (Kyrana *et al*., 1997; Tejada and Huidobro, 2002) and do not show any significant increase when fish is considered as spoiled according to the organoleptic evaluation (Chytiri *et al*., 2004). Although, there were not much differences in the values of quality indices observed in all the fishes on the last sampling days, but shelf-life was fixed based on the organoleptic evaluation. *P. sophore* showed less shelf-life than other two species studied. The fishes were procured from the local markets where they are kept without ice. The factors like time gap between harvest and arrival of fish into the market and period in which the fish remains without ice also influence the shelf-life of fish when preserved with ice or frozen stored. However, such study could give some preliminary idea to the farmers and traders for safe handling of fish. Since many small size fish are of immense commercial importance especially in the Northeast region, assessment of their shelf-life in ice storage condition is equally important. Present work indicated that under proper ice storage condition the shelf life could be about13 days in *M. gulio* and *G. chapra* and 10 days in *P. sophore*.

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**Appendix.** Scale employed for sensory evaluation of *M. Gulio*, *P. sophore* and *G. chapra* stored in ice (Sukumar *et al.,* 2007)

| **General appearance** | **Texture** | **Odour** | **Score** |
|---|---|---|---|
| Bright opalescent sheen, cornea transparent, eyes perfectly fresh, convex black pupil, bright red gills, slime transparent, no bleaching | Very firm, elastic to finger touch, fish not yet in rigor, scale very firmly attached to skin | Muddy odour | 10 |
| Eye fresh, slightly convex, black pupil, red gills, slime translucent, cornea translucent | Moderately firm, elastic, fish in pre rigor | Muddy odour | 9 |
| Eyes flat, very slight gray pupil, dull red gills, slightly translucent cornea | Firm, moderately elastic, scale firmly attached to skin, fish in rigor | Slightly muddy odour, no off odour | 8 |
| Slime translucent, eyes flat, slight gray pupil, loss in red colour of gills | Slightly firm, slightly elastic, fish in rigor | Odour of seaweed and mud | 7 |
| Eyes slightly, gray pupil, slight opalescent cornea, discoloration of gills, some mucus, outer slime slightly opaque | Slight soft, some grittiness near tail, fish passing out of rigor | No off odour | 6 |
| Eyes sunken, pale pupil, opaque cornea, slime opaque, some mucus, light brown gills | Moderately soft, moderate grittiness, slightly loose scales, fish in post rigor | Slight decayed odour of weed | 5 |
| Eyes completely sunken, milky white pupil, opaque cornea, brown gills | Soft, definite grittiness, slightly loose flesh, scale easily removable | Decayed odour of weed | 4 |
| Eyes completely concave, head sunken with thick slime, gills exhibit bleaching and dark brown discoloration | Very soft, marked grittiness, loosened flesh, scales, easily rubbed off the skin | Stale cabbage, phosphine like odour | 3 |
| Eyes completely concave, sunken head and body, cornea and pupil milky white, body covered with yellowish mucus or slime | Very soft flabby, slight retaining of finger indentation, flesh easily torn | Ammoniacal with strong odour | 2 |
| Eyes loose and completely concave, body and head sunken and discoloured, bloom completely gone, thick yellowish slime or mucus | Extremely soft and flabby, strong retention of marks, flesh very easily torn | Indole, faecal, $H_2S$, strong ammoniacal and putrid odours | 1 |