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Seasonal Variation of Sardine (*Sardinops melanosticta*) Muscle Lipids and other Components

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Abstract

Compositional variations of food chemical components of sardine muscle during the fishing seasons were studied.

Overall compositional change (ratio of the amount of protein, lipid, water, Na, Ca and Fe) seemed to characterize each shoal.

Lipid composition represented by the amount of triglyceride and phosphatidylethanolamine seemed to characterize the subpopulation. And composition of phosphatidylethanolamine molecular species was considered to reflect the physiological condition of sardine.

Introduction

Seasonal variation of proximate composition of abundantly caught fish restricts the constant commercial utilization of them. Therefore, characterization of the compositional change of fish sources is becoming important for their effective utilization.

Ueda¹⁾ studied the lipid seasonal changes of Japanese horse mackerel (*Trachurus japonicus*), chub mackerel (*Scomber japonicus*), yellowfin tuna (*Thunnus albacares*), brown-backed toadfish (*Lagocephalus lunaris spadiceus*) and filefish (*Monacanthus tomentosus*). Hayashi et al²⁾ studied those of sardine (*Sardinops melanosticta*). Although these studies were carefully done, discussions were exclusively on fatty acid compositional changes.

The purpose of this study was to investigate the multiple compositional change collectively of a representative abundantly caught fish, the sardine, throughout the seasons.

Procedure

Materials

Sardine (*Sardinops melanosticta*) samples were captured 11 times from June 4th to December 19th, 1984 at the coast of Kamiiso-chō, Hokkaido Japan (Table 1). Set net that has a mesh size of 20 mm was used throughout the year.

Methods

Dorsal muscle of at least 20 sardines were collected and separated into dark

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Table 1. Size of the sardine (*Sardinops melanosticta*) examined and the yield of the muscle.

Captured date	Body length cm	Body weight g	Yield %
June 4th '84	18.5±0.8	63.6± 7.1	44
June 18th '84	18.7±1.0	89.5± 7.1	50
July 2th '84	18.2±1.2	81.5± 7.8	48
July 16th '84	19.3±1.0	78.4± 7.8	48
July 30th '84	16.4±1.6	51.8±14.3	50
Aug. 15th '84	15.0±1.9	52.4±18.3	46
Aug. 27th '84	15.0±1.8	41.7± 8.8	47
Sep. 12th '84	12.2±1.1	29.4± 9.0	50
Sep. 26th '84	13.0±0.9	23.6± 5.6	49
Oct. 8th '84	14.6±1.5	33.6±10.1	49
Oct. 24th* '84	—	—	—
Dec. 19th '84	10.3±**	9.1±**	44

* This sample was obliged to obtain from the market.

** Number of fish examined was too small to calculate the standard deviation.

(DM) and white (WM) muscle. Moisture content was measured by the AOAC method. Crude protein content was determined according to the Kjeldahl method. Extractive protein was measured by the Hashimoto's³⁾ procedure. Total lipid was extracted from the muscle according to the method of Bligh-Dyer. Lipid composition was determined by the densitometric method (Ozumor Densitometer model 82, Tokyo) after charring the thin layer chromatographic plate (TLC) at 150-160°C. Spray reagent used for TLC was 3% cupper acetate in 8% phosphoric acid. Developing solvent used for TLC were n-hexane/ethyl ether/acetic acid (80:20:0.5, v/v) for non-polar lipids and chloroform/methanol/acetic acid/water (25:15:4:2, v/v) for polar lipids. Purification and identification of phosphatidylcholine (PC) molecular species were done in the same manner as previously reported⁴⁾. Na, Ca and Fe content were measured using Hitachi 170-30 atomic absorption spectrum. All the components analyzed were calculated as mg/100 g muscle for the principal component analysis⁵⁾ (PCA).

Results and Discussion

Compositional Change of Sardine Muscle

Fig. 1 shows the result of seasonal change analysis of the main components analyzed by PCA. It is obvious from this figure that lipid content is in inverse proportion to water content, and the plots of DM shows large dispersion along these two eigenvectors, i.e. lipid content and water content are in contrast to those of WM which shows far smaller dispersion. So, it can be considered that the seasonal variation of lipid content in sardine muscle is mostly caused by DM. This was also supported by the results in Fig. 2. The movement direction that is shown by the arrow from June 4th to July 30th is nearly parallel to the eigenvector of DM total

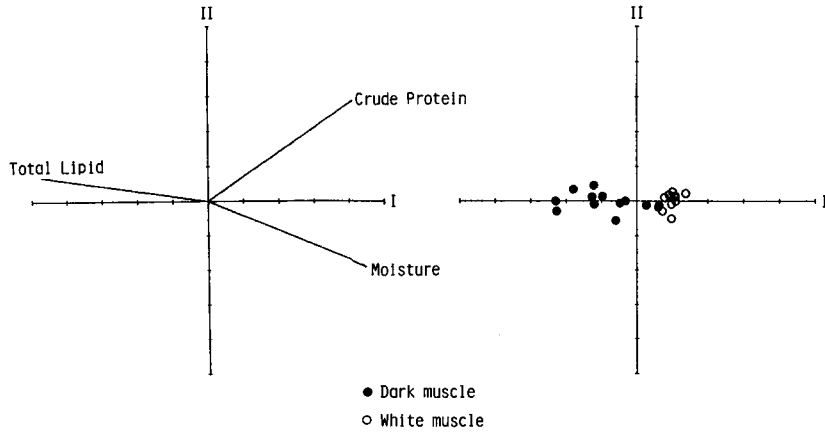


Fig. 1. Dispersion of the principal loadings of the plots of each sampling date on the first (I) and second (II) PCA plane. The left plane illustrates the eigenvectors on the same plane.

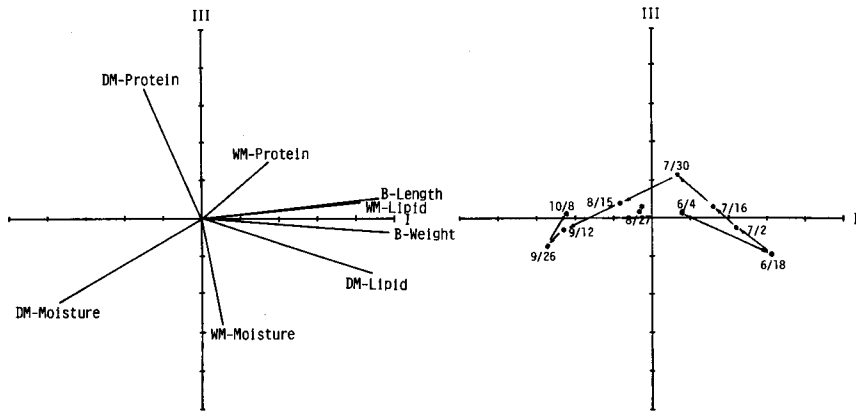


Fig. 2. Movement of the plot of principal loading of each sampling date on the first (I) and third (III) PCA plane.

*Samples were obtained from the different set net.

Slash shows the sampling date, for example, 6/4 stands for June 4th.

Abbreviations of eigenvectors ;

DM-Protein : Crude protein of dark muscle,

WM-Protein : Crude protein of white muscle,

DM-Lipid : Total lipid of dark muscle,

WM-Lipid : Total lipid of white muscle,

DM-Moisture : Moisture of dark muscle,

WM-Moisture : Moisture of white muscle,

B-Length : Body length, B-Weight : Body weight.

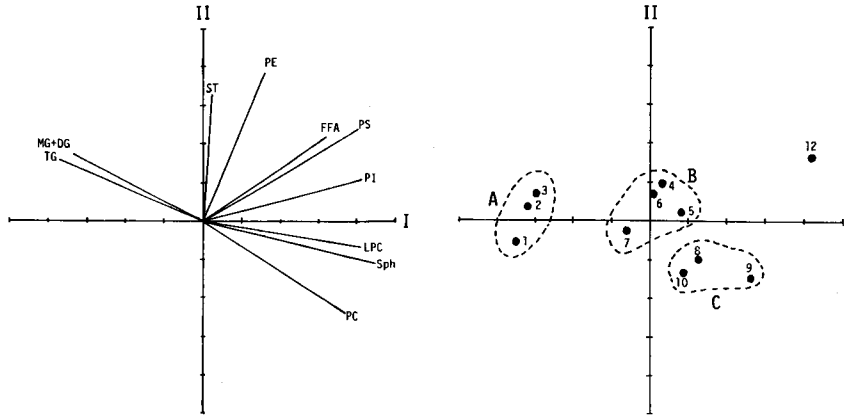


Fig. 3. Classification of shoal by lipid composition.

No. of the plots are as follows ;

- | | | |
|----------------|--------------------------------------------------------------------------------------|----------------|
| 1 : June 4th, | 2 : June 18th, | 3 : July 2th, |
| 4 : July 16th, | 5 : July 30th, | 6 : Aug. 15th, |
| 7 : Aug. 27th, | 8 : Sep. 12th, | 9 : Sep. 26th, |
| 10 : Oct. 8th, | 11 : Oct. 24th (This is not plotted since it was obliged to obtain from the market.) | |

12 : Dec. 19th.

Abbreviations of eigenvectors ;

- | | |
|---------------------------|---------------------------------|
| TG : Triglyceride, | ST : Sterol, |
| FFA : Free fatty acid, | MG + DG : Mono and Diglyceride, |
| PC : Phosphatidylcholine, | PE : Phosphatidylethanolamine, |
| PS : Phosphatidylserine, | PI : Phosphatidylinositol, |
| Sph : Sphingolipid, | LPC : Lysophosphatidylcholine. |

lipid content and the movement direction from July 30th to Oct. 8th is also parallel to the eigenvector of DM moisture content that is nearly in inverse proportion to lipid content. This Fig. 2 also shows that the direction of eigenvectors of body length, body weight, lipid content of WM and lipid content of DM is close to the axis of the first principal component. From this aspect, it can be said that there is a positive correlation between the size of the fish and the lipid content of the fish muscle. These results coincide with the results of Aizawa *et al.*⁶⁻⁸⁾.

Fig. 3. illustrates the lipid compositional change of WM during the fishing season using PCA. The plots enclosed by the broken lines seem to exhibit the analogous shoal. It was considered that group A is the Japan Sea subpopulation and B and C were considered the Pacific subpopulation⁹⁾, one that has a spawning ground offshore of Akita prefecture, Japan. Lapse from A to C that may be shown by the vector from A to C has the analogous angle with the eigenvectors of TG and PC, and it nearly makes a right angle against the eigenvectors of PE and ST. This suggests that the amount of TG and PC is mostly affected by the subpopulation captured, namely, the Pacific subpopulation is rich in TG and poor in PC while the Japan Sea subpopulation exhibits the inverse relation. PE and ST is considered to be constant between these two subpopulations.

Results of the analysis of overall compositional change is illustrated in Fig. 4

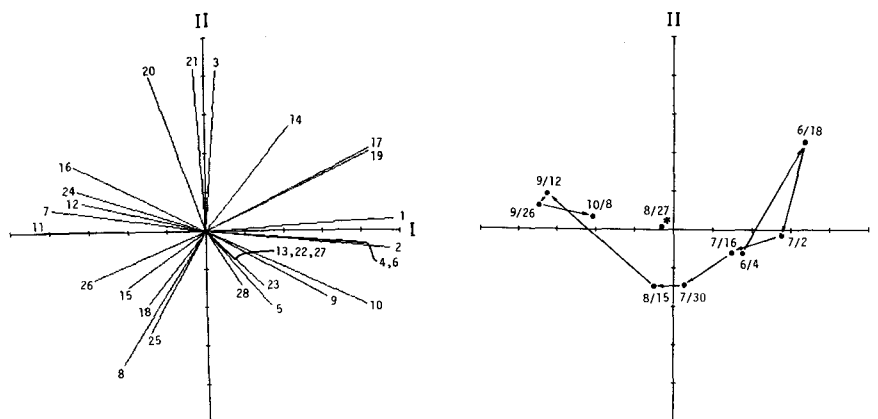


Fig. 4. Movement of the plot of principal loading of each sampling date on the first (I) and second (II) PCA plane.

Eigenvectors are ;

- 1: Body length, 2: Body weight,
- 3: Moisture of white muscle,
- 4: Total lipid of white muscle,
- 5: Crude protein of white muscle.
- 6: Triglyceride of white muscle,
- 7: Phosphatidylcholine of white muscle,
- 8: Phosphatidylethanolamine of white muscle,
- 9: Sarcoplasmic protein of white muscle,
- 10: Myofibrillar protein of white muscle,
- 11: Alkaline soluble protein of white muscle,
- 12: Insoluble protein of white muscle,
- 13: Sodium of white muscle,
- 14: Calcium of white muscle,
- 15: Iron of white muscle,
- 16: Moisture of dark muscle,
- 17: Total lipid of dark muscle,
- 18: Crude protein of dark muscle,
- 19: Triglyceride of dark muscle,
- 20: Phosphatidylcholine of dark muscle,
- 21: Phosphatidylethanolamine of dark muscle,
- 22: Sarcoplasmic protein of dark muscle,
- 23: Myofibrillar protein of dark muscle,
- 24: Alkaline soluble protein of dark muscle,
- 25: Insoluble protein of dark muscle,
- 26: Sodium of dark muscle,
- 27: Calcium of dark muscle,
- 28: Iron of dark muscle.

using PCA. Plots of the lapse from the first sampling date i.e. June 4th to Oct. 8th on this plane showed a crescent-shaped movement. This indicates that multiple ingredients change consecutively with the lapse of time.

Seasonal Change of PC Molecular Species

Though the amount of PC is usually smaller than that of TG, it is often said

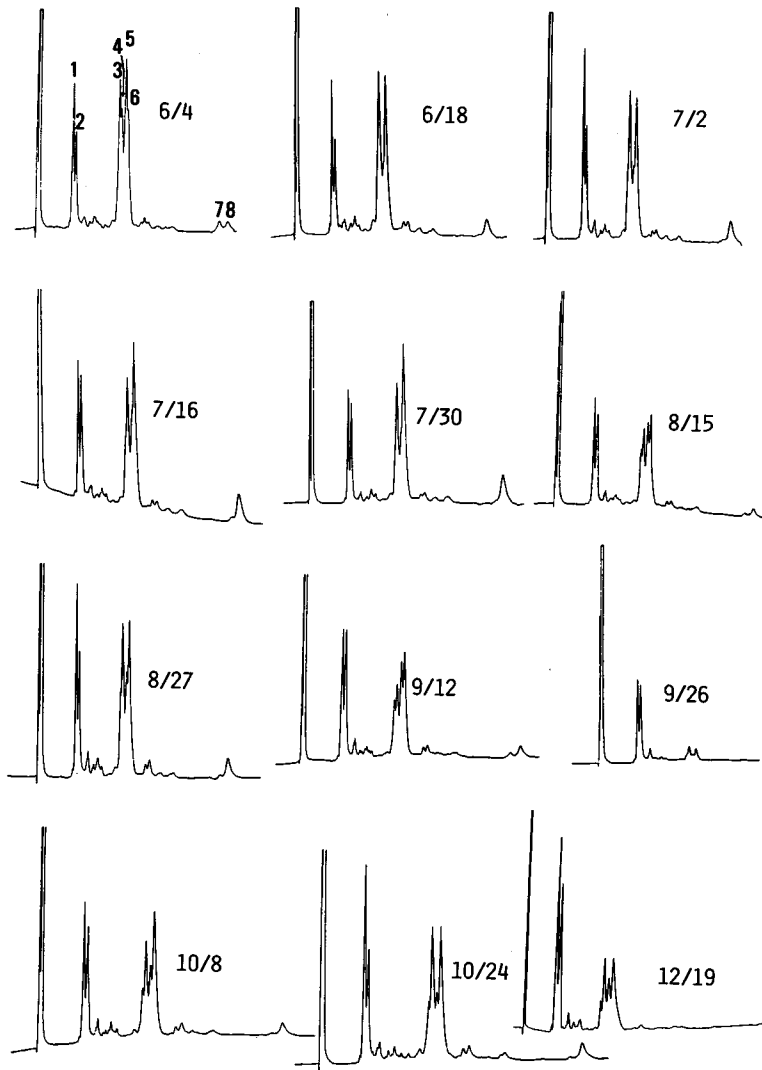


Fig. 5. HPLC chromatograms of sardine dark muscle PC.

Slash shows the sampling date.

Peak No.s are ;

1 : (20 : 5) (22 : 6), 2 : (22 : 6) (22 : 6), 3 : (20 : 5) (16 : 0),

4 : (16 : 0) (20 : 5), 5 : (22 : 6) (16 : 0), 6 : (16 : 0) (22 : 6),

7 : (18 : 1) (16 : 0), 8 : (16 : 0) (18 : 1).

Condition was as follows ;

Sample : Acetyldiglyceride derived from PC,

Equipment : Hitachi 638-50 Liquid Chromatograph,

Column : Chemcosorb I-5C18, 4 ϕ \times 300 mm,

Mobile phase : acetonitrile/water (100 : 1, v/v),

Flow rate : 1.0 ml/min,

Temperature : ambient,

Detector : RI, 8 \times 10.

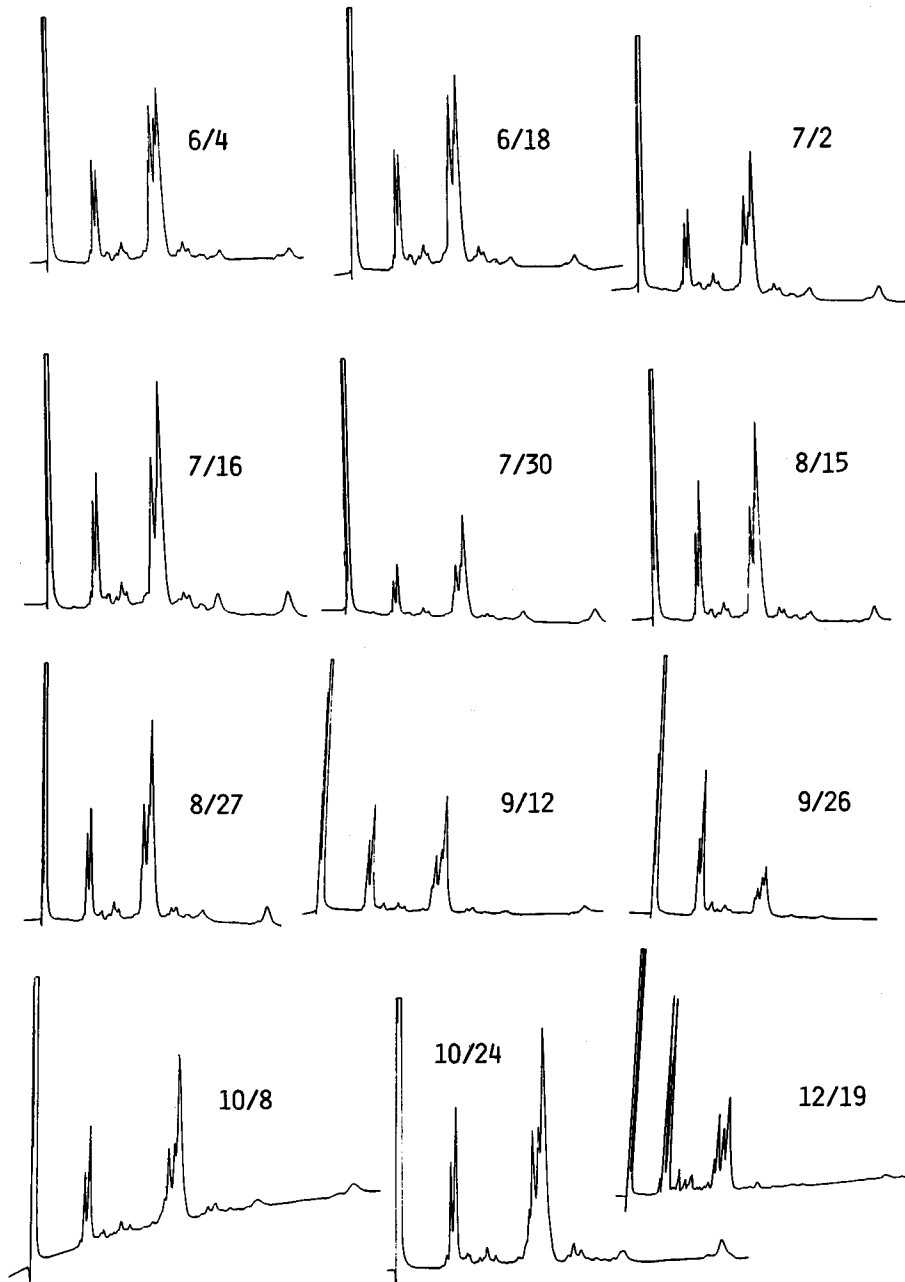


Fig. 6. HPLC chromatograms of sardine white muscle PC.
Slashes, Peak No.s and Condition as in Fig. 5.

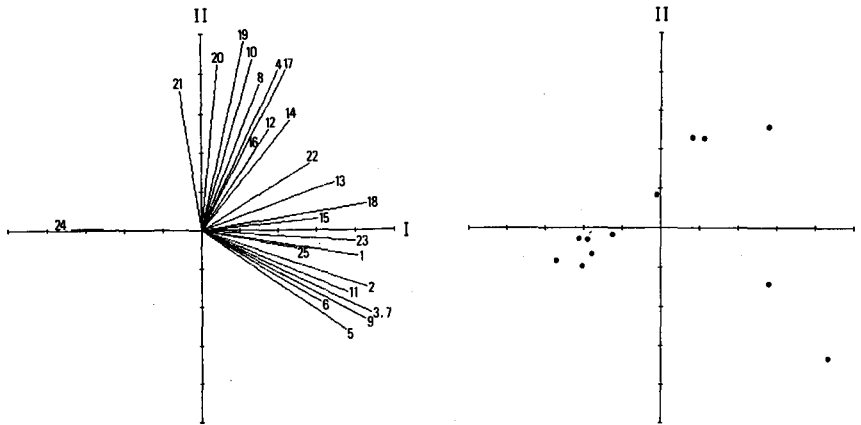


Fig. 7. Seasonal variation of PC molecular species of sardine dark muscle.

Eigenvectors are ;

- | | | |
|-------------------------|-------------------------|-------------------------|
| 1 : (20 : 5) (20 : 5), | 2 : (20 : 5) (22 : 6), | 3 : (22 : 6) (22 : 6), |
| 4 : (22 : 5) (22 : 6), | 5 : (20 : 3) (22 : 6), | 6 : (22 : 4) (22 : 6), |
| 7 : (16 : 1) (20 : 5), | 8 : (22 : 6) (16 : 1), | 9 : (16 : 1) (22 : 6), |
| 10 : (14 : 0) (22 : 6), | 11 : Unknown, | 12 : (20 : 5) (16 : 0), |
| 13 : (16 : 0) (20 : 5), | 14 : (22 : 6) (16 : 0), | 15 : (16 : 0) (22 : 6), |
| 16 : (22 : 5) (16 : 0), | 17 : (16 : 0) (22 : 5), | 18 : (16 : 0) (22 : 4), |
| 19 : Unknown, | 20 : (18 : 1) (16 : 0), | 21 : (16 : 0) (18 : 1), |
| 22 : Others, | 23 : Moisture, | 24 : TG, |
| 25 : PC. | | |

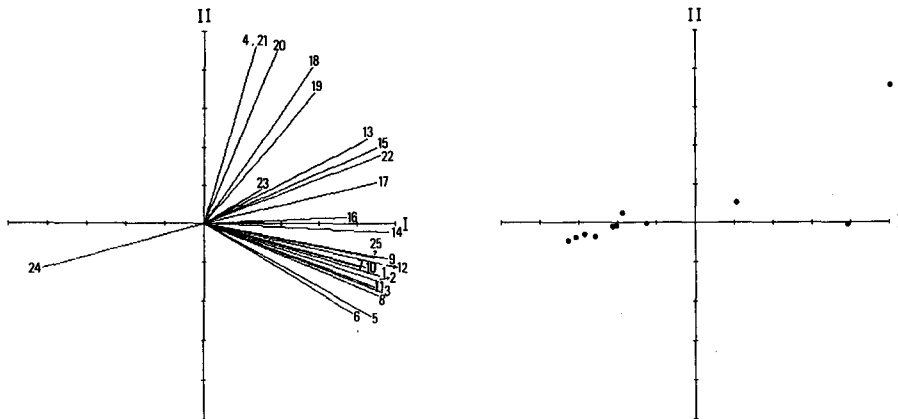


Fig. 8. Seasonal variation of PC molecular species of sardine white muscle.
Nos. of eigenvectors as in Fig. 7.

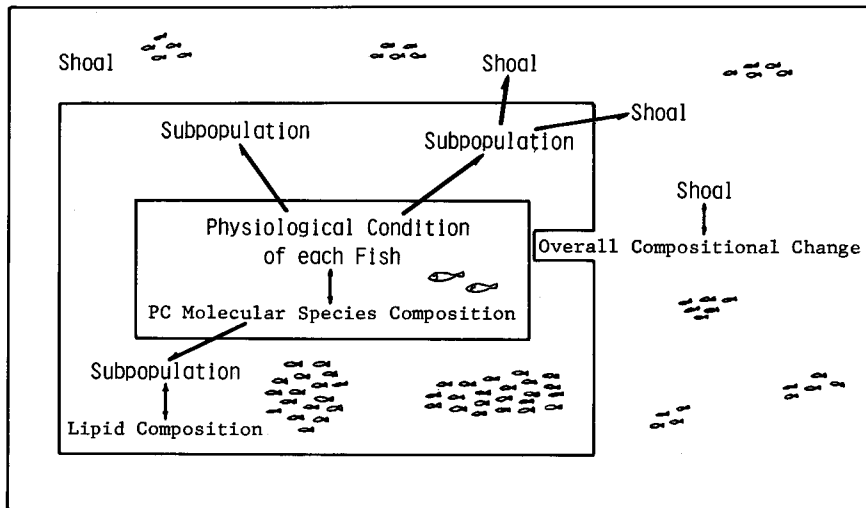


Fig. 9. Mass classification of sardine from a food chemical point of view.

that PC affects the quality of fish in a considerable degree especially when it is stored under low temperature. The susceptibility of attack from hydrolytic enzymes or oxygen is affected not only by the fatty acid composition but also by the molecular species of PC¹⁰⁻¹²). Therefore, the compositional change of the PC molecular species was studied. Results are shown in Fig. 5-8. By observing these figures throughout, it was suggested that there is significant variation in the PC molecular species composition of sardine muscle. Namely, these variations are the variance direction of the plots on PCA plane laid almost parallel to the eigenvectors of TG as well as PC molecular species of (16:0) (20:5), (16:0)(22:6) and (16:0) (22:5) as specifically observed in WM (Fig. 8). But the direction of the eigenvectors between TG and PC molecular species of (16:0) (20:5), (16:0) (22:6) and (16:0) (22:5) were completely the opposite. Though supplementary studies should be done to be conclusive, it was suggested that the amount of these PC molecular species is almost in inverse proportional to the amount of TG. Another observation was made from Fig. 7 and Fig. 8. It was suggested that compositional variation of the molecular species of PC becomes large in proportion to the decrease in TG amount.

Overall Observations

Fig. 9 illustrates the mass classification of sardine from a food chemical point of view by summarizing the results mentioned above. Composition of PC molecular species was considered to reflect the physiological condition of migratory fish. Lipid composition represented by the amount of TG and PC seems to characterize the subpopulation. And overall compositional change (i.e. ratio of the amount of protein, water, lipid, Na, Ca, Fe) seem to characterize each shoal. This model illustrated in Fig. 9 is expected to be verified by a more systematic study.

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