Bull. Nansei Reg. Fish. Res. Lab. (8) 11~20 (1975)

Some Haematological Properties of Matured Red Sea Bream, *Chrysophrys major* TEMMINCK et SCHLEGEL

Hiroko ISHIOKA and Tohru FUSHIMI*

Many haematological properties in the fish have been studied to utilize to the diagnosis of diseases, the evaluation of effects of pollution, and the fundamental physiology.

Prominent changes in certain of the blood properties of human and other higher vertebrates often occur under the condition of disease and are successfully distinguished from those of healthy ones. On the other hand, it has been well known that the haematological characteristics of the healthy fish were ranged widely, and also the physiological characteristics affected by the external environmental conditions change the values considerably. Although the need has been stressed for the establishment of the standard range of normal haematological values, the achievement is not so notable regardless of the efforts put into the research.

In the case of standardizing the haematological values for the diagnosis of disease, the physiological range of variation of these values of the fish should be established. It is a very important subject of haematological studies that how the blood values are influenced by the change of external or internal conditions such as the osmotic pressure of the medium or maturation and spawning.

As one of these subjects, the present study had the purpose to clarify the effects of maturation on the haematological characteristics, especially on the electrophoretic patterns of the serum proteins.

The authors would like to acknowledge the continuing guidance and encouragement of Dr. Masaru Fujiya, Freshwater Fisheries Research Laboratory and Dr. Seiji Sakaguchi, Nansei Regional Fisheries Research Laboratory. We are also grateful to Dr. Hisao Ozaki, the professor of Tokyo University of Fisheries, for providing related reports and a critical reading of the manuscript.

Materials and Methods

The present study was carried out from March, 1972 to February, 1973.

Red sea breams applied for this investigation were hatched at the same time, reared and cultured in fish preserves under the routine culture techniques at Hiroshima Prefectural Fisheries Experimental Station for 3 years. For growth of these fish, mixture of 49.5 % minced raw fishes (sand eel or mysid shrimps) and 49.5 % synthetic diet for rainbow trout and 1 % Halver type vitamin mixture were fed. In spawning season, from March through June, tocopherol drugs were added at 1 % to the diet.

Received: June 12, 1975. Contribution No. 47
from the Nansei Regional Fisheries Research Laboratory.
* Hiroshima Prefectural Fisheries Experimental Station

Specimens were anesthetized in about 1/5000 tricaine methane sulfonate (MS 222) sea water solution for the collection of the blood samples and the dissection.

After the measurement of the gonad weight and morphological characteristics the gonads were removed and fixed in Bouin's solution for histological observation. The blood samples were collected by excising the caudal fin in 5 ml syringe contained anticoagulant solution (Anglot ET) and were put in plane glass tubules. The blood in the syringe was applied for the analysis of values on haemoglobin, haematocrit and protein of the plasma. The bloods in the plane glass tubules were prepared for electrophoresis. The serum was separated with the aid of gentle centrifugation after clotting of the blood. Then, the sera were refrigerated at $-20°C$ untill the operation of electrophoretic analysis.

The haemoglobin contents were determined with the simplified method of haemometer[1], and the haematocrit values were determined by the micro-haematocrit method using micro-capillary centrifuge. The values of protein concentration of the serum were determined by Hitachi-Protein-meter (refractometer).

The electrophoretic analysis was primarily carried out following the standard method of the Society of Electrophoresis[2].

For electrophoresis, a Toyo Model SE-2 microzone chamber was employed using cellulose acetate membrane. It was powered by Toyo Electric Currentmeter Model No. III BA 8-2. Before the application of sera on the cellulose acetate membrane, they were concentrated to 10 - 12 % of protein on refractometer by air vacuum pump, then, 1 μl of the serum was employed. All runs were for 65 min. at a constant current of 0.8 mA/cm with the veronal buffer.*

The cellulose acetate membranes were stained with Ponceau 3 R reagents and decolorized in 1 % acetic acid solution and then dried in the customary manner. They were scanned with the Toyo Densitrol.

Results

The sampling of the blood was carried out once a month, and in the spawning season, twice a month. In principle, 12 fish were randomly supplied for dissection from the fish preserve in the sea.

The seasonal changes of gonad index and the state of the ovary are represented in Fig. 1. The state of ovary is divided into two phase, developmental and immature. The former ovary had the oocytes with yolk globule and the latter only had the oocytes without yolk globule under histological observation.

The comparisons of condition factors and some blood properties of male and female are presented in Table 1.

Haematocrit values and haemoglobin contents seem to be higher at the begining of the maturation and then decrease gradually to August and then also increase slightly to November. However, these changes are not so significant.

---

* Veronal 2.76g
Veronal-Na 15.45g
in 1000 ml of distilled water (pH: 7.725, μ:0.075)

Table 1. Seasonal changes in some blood characteristics, condition factor and gonad weight in red sea breams.

Date	No. of Fish Examined		Body Length (cm)	Body Weight (g)	Condition*1 Factor	Gonad Weight (g)	Hematocrit Value (%)	Hemoglobin Content (g/dl)	Protein Value (g/100ml)
'72, 3,27	F*2	6	30.6±1.85	947.5±124.6	32.3±2.64	26.2±7.97	48.3±4.5	9.3±1.40	8.4±0.8
	M*3	5	31.1±1.94	944.0±147.6	29.8±1.69	40.5±7.48	47.5±4.7	9.4±0.82	6.2±0.8
4,14	F	6	30.5±1.67	892.5±149.0	29.7±2.56	47.6±27.4	44.0±4.5	8.4±0.85	7.3±0.5
	M	6	28.8±2.23	766.7±106.7	30.7±3.74	35.5±11.9	41.5±6.3	8.3±1.18	5.7±0.9
4,27	F	6	30.8±2.23	900.0±110.1	29.5±4.93	49.4±25.4	38.0±4.1	7.0±0.47	7.1±0.7
	M	6	31.2±1.65	905.8±151.1	28.2±1.72	45.1±12.4	45.2±5.4	7.2±1.96	5.7±1.0
5,15	F	7	31.8±4.72	1059.3±417.3	29.5±1.55	73.1±31.0	43.8±7.1	8.2±0.91	6.1±1.7
	M	5	31.2±3.71	976.3±334.7	29.2±1.30	60.2±28.8	43.5±7.5	8.2±0.82	5.4±1.7
5,29	F	7	28.8±1.99	741.3±125.2	28.8±3.10	52.2±35.7	41.2±6.1	7.6±0.85	5.2±1.1
	M	5	28.7±1.82	754.0±143.3	30.1±1.57	35.3±8.50	40.5±0.9	7.9±0.82	4.3±0.2
6,15	F	6	29.4±2.23	752.5±170.4	27.3±0.70	53.9±19.6	38.3±5.4	7.2±1.12	4.9±0.6
	M	5	30.1±1.88	765.0±119.4	27.0±0.89	22.2±6.14	40.2±4.1	7.7±0.98	4.8±0.8
6,29	F	6	32.8±4.09	1070.8±387.6	29.0±3.08	13.7±12.8	42.6±4.4	8.1±0.72	4.9±0.8
	M	6	30.7±2.57	879.2±241.4	29.3±3.74	17.8±7.94	39.3±3.6	8.1±0.86	5.2±0.9
7,17	F	7	32.2±3.03	1025.7±279.6	29.9±2.69	6.2±1.60	41.4±2.7	8.0±0.60	4.9±0.5
	M	5	30.3±1.36	814.0±106.6	28.9±1.14	3.6±0.76	44.1±2.3	8.4±0.70	5.4±0.5
8,14	F	4	33.2±2.70	1215.0±225.3	33.2±6.34	7.4±0.97	36.9±1.2	7.8±1.13	6.1±0.7
	M	8	29.9±2.94	827.5±175.6	30.8±2.61	2.0±0.75	39.7±2.1	8.1±0.81	5.9±0.7
10,17	F	4	32.7±2.54	1100.0±230.5	31.3±3.53	7.1±3.28	35.8±2.2	8.6±1.11	5.6±0.6
	M	8	31.2±1.40	1020.0±179.3	33.1±1.89	2.5±0.97	37.9±3.8	8.9±0.70	6.2±0.8
11,16	F	1	32.1	1060	31.8	7.9	40	8.7	5.0
	M	11	32.2±3.66	959.4±294.2	28.0±2.58	2.4±0.80	43.4±3.2	9.1±0.54	5.2±0.5
'73, 2,16	F	2	33.6±0.35	1220.0± 70.7	32.2±2.86	6.6±1.06	41.3±1.8	9.6±0.57	5.4±0.1
	M	4	31.1±3.01	921.3±316.4	29.5±2.10	7.1±0.85	41.8±1.6	9.2±0.24	4.4±0.8

*1Condition Factcr : $\frac{(\text{Body Weight})^3}{(\text{Body Length})} \times 1000$ F*2 : Female M*3 : Male

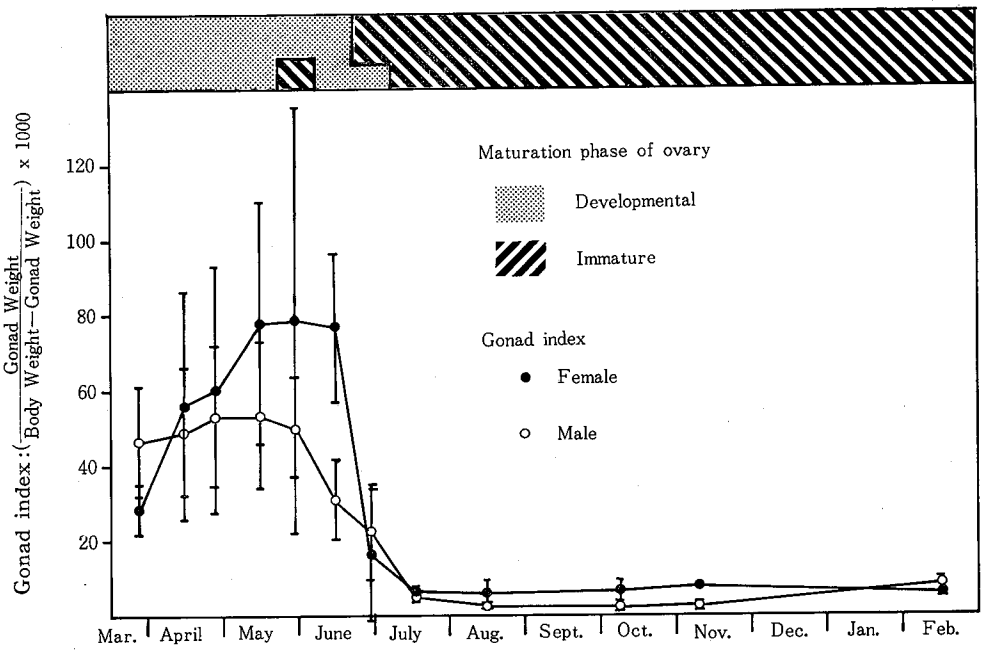

Fig. 1. Seasonal changes in gonad index and histological phase of ovary in red sea breams.

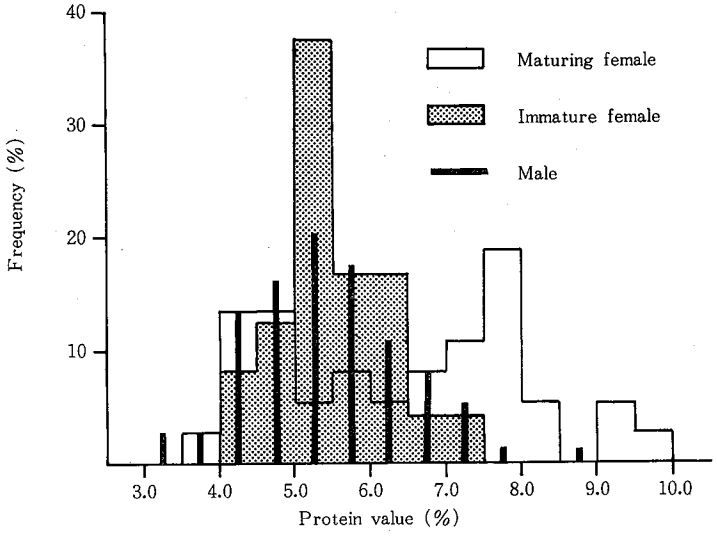

Fig. 2. Distribution of protein values determined by refractometer in red sea breams.

Among examined blood properties, the values of serum protein concentration measured by refractometer showed the great changes and variations, especially the females' was higher than that of the male in maturing period. The values of

protein concentration are presented in Fig. 2 on male, immature female and matured female following the developmental stages of gonads judged from histological observation.

The results of maturing female showed the peculiar feature of the distribution of protein concentration with the wider range of variation.

The results of electrophoresis are shown in Table 2 and a representative film and its corresponding densitometer profile are illustrated in Fig. 3. No attempt was made to identify the protein components of the sera studied as albumin or globulins, and the electrophoretic fractions were numbered in order of increasing mobility to the positive pole. As illustrated in Fig. 3, this profile is divided into 6 fractions.

Table 2. Results of electrophoretic analyses on serum proteins of red sea breams.

	Fraction	I	II	III	IV	V	VI
Migration distance from origin (cm)	Female 1* (n=37)	2.76 0.35	1.64 0.35	0	1.10 0.28	2.40 0.30	3.02 0.31
	Female 2** (n=24)	2.80 0.24	1.61 0.13	0	1.02 0.16	1.99 0.19	2.92 0.24
	Male (n=74)	2.67 0.35	1.55 0.28	0	0.95 0.19	1.90 0.24	2.84 0.29
Relative distance***	Female 1 (n=37)	91.2 9.2	53.9 9.8	0	35.9 8.0	78.0 7.3	100
	Female 2 (n=24)	95.2 6.9	54.9 4.9	0	34.5 4.9	68.4 5.5	100
	Male (n=74)	93.7 8.9	54.4 7.8	0 0	33.4 5.3	66.5 5.0	100
Relative area composition (%)	Female 1 (n=37)	1.2 0.8	28.0 4.5	45.2 6.3	7.0 3.0	14.3 4.3	3.1 1.4
	Female 2 (n=24)	3.0 1.9	22.1 4.4	54.7 7.1	6.9 3.7	6.2 2.5	6.6 3.3
	Male (n=74)	2.3 1.8	24.9 5.1	52.6 5.3	7.4 3.1	6.9 2.6	5.5 2.9

(n= : No. of fish examined)

For each component, mean values are represened as the value of first reading and standard deviation as one of second reading.

Female 1* : Females with developing oocytes.

Female 2**: Females with immature oocytes.

*** : The relative migration distance was calculated as the ratio of migration distance to that of fraction VI.

Fraction I contains the faint band most rapidly migrating to the positive pole and the fraction VI the band most rapidly migrating to the negative pole. The relative mobility of these bands migrated from the starting point are almost consistent. The fraction V of the maturing females, however, as presented in Table 2, is slightly different in relative mobility from those of the males and the immature females. This band of maturing female, numbered V', may probably be

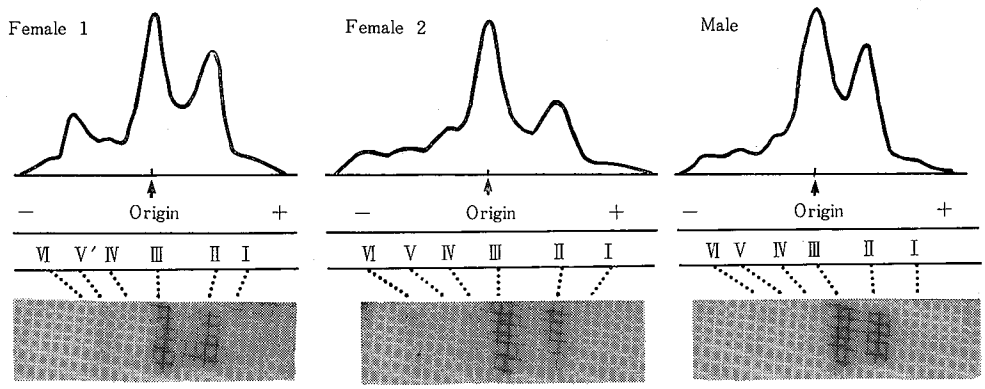

Fig. 3. The representative electrophoretical patterns and their corresponding densitometer profiles of serum protein in red sea breams.

composed of band V and other different protein fraction relating to the yolk formation.

As illustrated in Fig. 1, the histological observations were made from the standpoint of the appearance of yolk globules and these results were closely related with the patterns of sera proteins on electrophoresis. Although the patterns of males and immature females without yolk globules in the oocytes are almost constant all the year round, those of females with yolk globules in the oocytes are characteristic, and the characteristic feature is appeared on fraction V.' Further, during these investigations, 2 fish had the seminal glands with small ovarian tissues. These ovaries had not the oocytes accumulating yolk substances, and the electrophoretic patterns of serum proteins from those fish showed the same pattern as male showed.

Discussion

Recently, basic informations of the spawning of red sea breams have been published.[3)4)] They have a long spawning period over two monthes from April to June and the maturation process begins on late March.

There are some papers about the problems on the blood properties on maturation process. Gelineo[5)] observed the high haemoglobin concentration on several fishes during maturation period. On the other hand, it was reported by Sano[6)7)] that haematocrit values and haemoglobin contents fell remarkably with the development of gonads in both sexes of cultured rainbow trout, and was considered that these phenomena were due to the depletion of nutritive substances by spawning.

In the present study, condition factors, haemoglobin contents and haematocrit values are on the decrease slighly during the latter half period of the spawning season. These results may support the assumption that a small range of variation in these values results from active feeding even in spawning season in red sea breams.

Some research results on the electrophoretic patterns of serum proteins of red sea breams have been reported by Saito[8)9)] on Tiselius method and on paper electrophoresis. According to the results, five fractions, f, I, II, III and IV were identified while authors' obtained are six fractions. The fraction I is a faint band and often looks like the tailing of fraction II. The patterns also different from that of Saito, and it is supposed that these discrepancies may be depended upon the difference of the method applied.

Matsuura[10)] represented the maturation stage of ovarian eggs of red sea breams with the histological observation, and the maturation of ovary was divided into three phases, such as immature, developmental and maturation. These phases correspond to peripheral nucleolus stage, phase from yolk vesicle stage to later yolk globule stage and that from migration nucleus stage to maturation stage respectively. Red sea breams spawn several times and have all stages of oocytes in the ovary throughout the spawning season. In the present study, it was clarified that the characteristics of the electrophoretic patterns of serum proteins of female depend on the developmental phase of ovary. In spawning season, the fish with ovary having only peripheral nucleolus oocytes did not show such characteristic pattern of serum protein.

In red sea breams, hermaphrodism is observed on several times. Kitajima, C. and T. Fushimi[11)] reported that this hermaphrodism was mostly observed in younger matured fish, about 2-3 years old, in the appearance rate of 20 - 30 % under cultured and natural condtions. These fish always had the seminal glands with various sized ovaries and these ovaries had no oocytes in developing phase. These facts may be the results from deficient supplies of substances necessary for yolk formation by blood stream.

Studies on the pattern changes of electrophoresis relating to the gonad maturation showed positive or negative results. Sindermann C, J, and D. F. Mairs[12)] had found no major changes in serum patterns attributable to reproduction or to fresh water migration on anadromous alewives, *Alosa pseudoharengus*. Drilhon A. et J. M. Fine[1,3)], however, reported the fraction a little to the anode on starch gel electrophoresis concerning the female gonad maturation on the salmon, *Salmo salar*. Yamashita[14)] reported that on scorpion fish, *Sebastiscus marmoratus*, the fraction V, migrating to the negative side, was higher on the electrophoretical pattern by cellulose acetate membrane on the female before the extrusion of fries.

Although this resembles to the results obtained by the present study, it is uncertain whether the physiological changes occurring in the maturation process of viviparous fish are similar to that of the oviparous fish. To know the pattern variations attributable to sex and maturity, the gel electrophoretic analyses of sera from rainbow trouts, *Salmo gairdneri*, were made by R. V. Thurston,[15)] who obtained ten fractions numbered from I, the fraction nearest to the positive pole, to X, the fraction nearest to the negative pole. Primarily there were not pattern variations attributable to sex but the maturity had the characteristic effects on the patterns in band intensities of fraction VI and fraction IX. W. E. Vanstone

and F. Chung-Wai Ho[16] reported on coho salmon, *Onchorhynchus kisutch,* that on the paper zone electrophoresis, bands were divided into six fractions from I of the positive side to VI of the negative side and the fraction IV was appeared only in the sera of maturing female. Aida, K., Phan-Van-Ngan and T. Hibiya[17] also reported on the starch gel electrophoresis that there are 15 fractions on females of Ayu, *Plecoglosus altivelis,* and fraction nine and eleven showed the distinct increase in maturation period, and both fractions became stainable with Sudan Black B, as these increased on the concentration. On *Onchorhyncus keta, O. nerka.* and *O. masou,* Hara[18] reported the appearance of the new fraction at the position of β-globulin on cellulose acetate membrane.

In spite of the differences of the methods, the results show the similar intensity increase patterns of a fraction in the position of inclination towards negative side in the maturing female when the changes on electrophoretical patterns of serum attributable to the ovary maturation are recognized on Saimonina.

In the present study, the values of the protein concentration by refractometer showed the characteristic bimodal distribution on matured females and the higher mode may contain the lipid component. It seems that the fraction V' is almost the same as the serum protein specific for female by Aida et al. concerning the state of appearance and the position of inclination towards negative pole.

From the discussions described above, it is assumed that the appearance of the female-specific protein with the development of oocytes supports that the yolk substances are synthetized in the organ other than ovary and provided into the oocytes with blood streams. Further, it may be indicative of the analogous mechanism on yolk accumulation in the oocytes among fishes that there are something in common on the electrophoretical characteristics of female-specific protein during maturation, whereas there are differences in species and in analytical methods. These are the subjects for future studies.

Summary

Some blood properties, haemoglobin contents, haematocrit values, values of protein concentration by refractometer and electrophoretic patterns of the serum protein were determined relating to the maturity process of gonads on red sea breams, *Chrysophrys major* TEMMINCK et SCHLEGEL. While condition factors, haemoglobin contents and haematocrit values decreased slightly in the late spawning season, the values of protein concentration showed the extreme increase in the begining and the decrease in the late of the spawning season on females. Electrophoretic patterns on cellulose acetate membrane of blood serum proteins disclosed the characteristic feature in the female with the oocytes of developing phase.
This was the appearance of the fraction V' a little to the anode, and it may be identified as the female-specific serum protein as that seen on Salmonina concerning to the state of appearance and its position.

References

1) Matsusato, T., & C. Kitajima, 1972: The Comparison of Haemoglobin Determination Methods for Diagnosis of Fish Disease (in Japanese). The Aquiculture, 19 (3), 121—127.
2) The Society of Electrophoresis, 1967: The Experimental Method for Electrophoresis (in Japanese). the 4 th edition, pp. 443, Bunkodo
3) Kitajima, C., & T. Fushimi, 1969: On the Spawning of Red Sea Breams Cultured for 2 Years (in Japanese). The Aquiculture, 17 (1), 11—18.
4) Koga, F., Y. Tanaka, & A. Nakazono, 1971: Observation on Spawning of Red Sea Bream, *Chrysophrys major* TEMMINCK et SCHLEGEL, and Black Sea Bream, *Mylio macrocephalus* (Basilewsky),in Aquarium (in Japanese). Report of Fishery Research Laboratory Kyushu University, (1), 83—89.
5) Gelineo, S., 1957: Le taux l'hemoglobine sanguine de pleusieurs espéces de poisson de mer. C. R. Soc. Biol. Paris, 151, 1594.
6) Sano, T., 1960: Haematological Studies of the Culture Fishes in Japan 3. Changes in blood constituents with growth of rainbow trout. The Journal of the Tokyo University of Fisheries, 46 (1—2), 77—87.
7) Sano, T., 1963: Blood Properties of Cultured Fish (in Japanese). Bull. Japan. Soc. Sci. Fish., 29 (12), 1113—1118.
8) Saito, K., 1957: Biochemical Studies on the Fish Blood-VIII. On the Electrophoretic Specificity of Serum Proteins (in Japanese). ibid, 22 (12), 752—759.
9) Saito, K., 1957: Biochemical Studies on the Fish Blood-IX. On the Paper Electrophoretic Analysis of Serum Proteins (in Japanese). ibid, 22 (21), 760—767.
10) Matsuura, S., 1972: Fecundity and Maturation Process of Ovarian Eggs of Red Sea Bream, *Pagrus major* (TEMMINCK et SCHLEGEL) (in Japanese). Sci. Bull. Fac. Agr. Kyushu Univ., 26 (1—4), 203—215.
11) Kitajima, C., & T. Fushimi, 1970: Studies on the Culture of maturing fish on Red Sea Breams (in Japanese), Reports on the Study on Fish Seedling Production (Hiroshima Prefectural Fisheries Experimental Station), 2—13.
12) Sinderman, C. J., & D. F. Mairs, 1961: Blood Properties of Pre-spawning and post-spawning Anadromous Alewives. Fish. Bull. U. S. Fish & Wildlife Service, 61, 144—151.
13) Drilhon, A., & J. M. Fine, 1963: Dimorphisme Sexuel dems les Proteines Seriques de *Salmo salar* Etude Electrophoretique. C. R. Soc. Biol. Paris, 157 (11), 1897. (translated by Dr. H. Ozaki)
14) Yamashita. H., 1968: Haematological Study of a Species of Rock-fish, *Sebastiscus marmoratus*-IV. Change of the Amount of Blood Elements and the Electrophoretic Pattern of Serum Protein under the Influence of Stress. Bull. Japan. Soc. Sci. Fish., 34 (12), 1066—1071.
15) Thurston, R. V., 1967: Electrophoretic Patterns of Blood Serum Proteins from Rainbow Trout, *Salmo gairdneri*. J. Fish. Res. Bd. Canada, 24 2169—2188.
16) Vanstone, W. E., & F. Chung-Wai Ho, 1961: Plasma Proteins of Coho Salmon, *Onchorhyncus kisutch*, as Separated by Zone Electrophoresis. ibid, 18, 393—399.
17) Aida, K., Phan-Van-Ngan, & T. Hibiya, 1973: Physiological Studies on Gonadal Maturation of Fishes-I. Sexul Difference in Composition of Plasma Protein of Ayu in Relation to Gonadal Maturation. Bull. Japan. Soc. Sci. Fish., 39 (11), 1091—1106.
18) Hara, A., 1975: Electrophoretical and Immunological Studies of Fish Serum Proteins. ibid, 41 (1), 105—113.

養殖マダイの産卵期における血液性状について

石岡　宏子・伏見　　徹

同一時期に産卵され，飼育された養殖3年生マダイについて，毎月，採血を行ない，ヘモグロビン量，ヘマトクリット値，蛋白質含量，血清蛋白電気泳動像について測定を行なった。養殖マダイの産卵期は3月から6月までであり，その間，ヘモグロビン量，ヘマトクリット値の変動は極めて小さかったが，蛋白質含量は産卵期初期に高く後期に低下する傾向が認められ，特に雌魚で顕著であった。さらに，産卵期の雌魚の血清蛋白電気泳動像は，卵細胞の成熟と密接な関係があることが明らかになった。すなわち，卵細胞の発育状態が卵黄球期以後の卵巣を持つ雌魚の場合には，血清蛋白パターンは，陽極側から数えて5番目のピークの位置変化，濃度増加という特異性を示し，雄魚および卵黄球期以前の発育段階にある卵細胞だけをもつ雌魚では，周年，ほぼ一定の泳動像を示した。このことは卵黄形成のための物質が卵巣以外の器官で生合成され，これが血流によって卵細胞まで運搬されるためであろうと推考した。