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COMPARATIVE STUDY OF SOLUBLE GONAD PROTEIN FOR BOTH SEXES OF *SIGANUS RIVULATUS* (FAMILY: SIGANIDAE) IN RELATION TO MATURITY STAGES BY ISOELECTRIC FOCUSING.

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ABSTRACT

Soluble gonad protein for both sexes of *Siganus rivulatus* was analysed by isoelectric focusing in seven maturity stages. Sex- specific electrophoretic and isoelectric focusing patterns were found. More protein fractions are identified for stage II of both sexes than for the other stages. The total number of protein bands for female was greater than those of male. In female, spawning stage has the maximum number (4) of specific band while in male the maximum ones (6) were separated from stage (III). The number of common bands in both female and male was (3 & 2) respectively. Clear differences in protein patterns of the seven stages for both sexes can be used to characterize the breeding season.

INTRODUCTION

Using thin layer polyacrylamide isoelectric focusing, complex genetic constitution can be obtained clearly with species specific protein profiles.

Lundstrom (1979; 1980; 1981 and 1983); Yamada & Suzuki (1982) ; Ukishima *et.al.* (1984); Ng *et.al.* (1986) and Basaglia (1992) used the technique of thin layer isoelectric focusing (IEF) with good separation and reproducible patterns of sacroplasmic protein.

Kamel (1987) reported that *Sigaus rivulatus and S. luridus* can be identified by IEF patterns of their eye lens protein.

Thrustom (1967) used electrophorasis on acrylamide gels of blood protein of *Salmo gairdnei* and their patterns showed marked interspecific differences and revealed characteristic band for ripe females. Borchard (1978) found characteristic patterns of serum protein corresponding with maturation stages, and protein fractions increased with spermatogenesis activity and development of the ovaries of *Salmo gairdnei.*

The objective of the present study is to evaluate the possibility of using IEF method for determination of differences between the protein patterns of gonad for both sexes of *Siganus rivulatus.*

MATERIAL AND METHODS

Samples of *Siganus rivulatus* were obtained from Alexandria Western Harbour in (1997). Samples of gonads were taken at different maturity stages. The maturity stages of ovaries and testes were classified into seven stages according to Niklosky (1963).

1-Preparation of extracts:

Gonad protein was extracted from both sexes by blending 0.5 gram of gonad with 5ml 0.1M Tris-HCL, PH 8.6 followed by mechanical homogenization. Samples of gonad protein were centrifuged for ten minutes at 4 ℃ at 6,000 rpm. The supernatant liquid was pipetted into vials and stored at -18 ℃ until assayed.

2-Isoelectric Focusing:

Phast system apparatus (Pharmacia LK B.S, 75182,1987) was used to separate and stain the protein bands. Isoelectric focusing of soluble gonad protein was carried out on 0.35 mm. thick polyacrylamide gels of plastic film produced by LKB. The pH range used was 4.0-6.5 using

sample applicator (0.5µL), following the instructions in LKB leaflet N° 17-0518-01 by the method described by Hrukeshoven and Dernick (1985). Gels were dried, photographed and scanned at 620 nm.by LKB Ultrascan XL.

Relative quantitative and qualitative protein bands were analyzed on the basis of the number, position, density and width of each band. Values of isoelectric points (PI'S) were calculated according to the Pharmacia PI clibaraion Kit (N° 11-B-045-02) at pH gradient from 4.0-6.5 (Laas *et.al.*; 1979 & 1980) and EL-Gharabawy & Zaki (1990). Three replicates for each sample were used for protein analysis.

RESULTS

Comparison of the separation pattern of the soluble protein of gonads obtained by isoelectric focusing showed that both female and male of *Siganus rivulatus* can be differentiated by this technique and exhibited sex-specific patterns and unmistakably characterize both sexes. In fact, in certain regions of the gel, considerable intrspecific variations are present. (Figs. 1& 2).

a-Female.

In the seven maturity stages of female (Fig.1a) the protein patterns showed 20 to 27 protein fractions varying in number and arrangement of insensitivity colored and light bands.

The number of total bands in the following patterns, immature stage (A), stage I (B), stage II (C), stage III (E), ripe stage (F), spawning stage (G), and spent stage (H) are 20, 24, 26, 22, 22, 25 and 22 bands respectively (table 1).

The arrangement and number of specific bands differ from one pattern to another, i.e. one specific band is present in immature stage (A) at PI 4.95, while in stage I (B) two bands are detected at PI's 5.10 and 5.40. In stage II (C) three specific fractions are found at PI's 4.58, 4.60 and 4.75. From stage III (E), two specific bands are separated at PI's 5.30 and 6.00. Ripe stage (F) has two specific bands at PI's 4.80 & 5.95. Four specific fractions are present in the spawning stage (G) at PI's 4.28; 4.70, 4.93 & 6.38 respectively, where in spent stage (H) one specific fraction is separated at PI 6.30. In general there are three common bands at PI's 6.15; 6.23 & 6.45 (table 1).

Figure (2) shows the densitometric scan of electophoretic patterns of soluble protein of the gonad, which revealed the presence of different mobility's, intensities and migration of the bands. It is obvious that in the patterns A; B; C; E; and G, most of bands have high cathodic mobility, whereas pattern H shows higher anodic migration. Most of bands of pattern F (ripe stage) are situated between the anode and cathode. It is also clear that stage II has the maximum number of bands (26) and spawning stage is characterized by having the maximum number of specific bands (4), while the minimum one (20) are present in the immature stage (table1).

From table (1) and figure (2A) it is obvious that in immature stage, the maximum percentage of protein fractions (13.1, 13 & 8.2) are found toward the cathode at PI's 5.98, 6.03 & 6.50. The densitogram of stage I figure (2B) shows the peaks of the bands which spread from the cathode toward the anode and the maximum percentage of protein (7.1 & 8.2) are found at PI 's 5 and 6.03 (table 1). The mature stage II which is illustrated at figure (2C) shows that the maximum percentage of soluble gonad protein (11.2) is found at PI 5.98 and the protein fractions are concentrated at the cathode. In stage (III), the maximum values of protein

fractions (12.1, & 10.6) are found at the cathode and at PI's 6.50 & 6.68 (Fig.2E & Table 1). Figure (2F) which refers to the electro-phoretogram of ripe stage shows that the maximum percentage of protein band (11.2) lies at cathode direction and the peaks of the bands are found between PI 5.68 & 6.23 (table1).

The soluble protein of the spawning stage for female is maximum (12.9%) in the cathodic region at PI 6.58 and the densitogram of this stage shows that most of the band peaks are found at the cathode (Fig.2G). In spent stage the maximum percentage of protein fraction (16.3) is found at the anode direction at PI 5.33 (table1). From figure (2H) it is obvious that the most peak of bands in this stage are located in the anode region.

b-Male.

The maturity stages of the testes are represented by seven patterns (Fig.1b: I; J; K; L; N; O and P). The total number of bands ranges from 18 to 25. Two common bands are present in all patterns, the isoelectric points of these bands are estimated at PI 4.15 & 6.23. The first common band can hardly be detected in immature (I) and spent (P) stages (Fig.1b). One band is common in five patterns (I; J, K, N, & P) at PI 5.10. At PI 5.73 there is also one common band for five patterns (I, J, K, L, N & O) at PI 6.20 (table 2).

Each stage has its own distinct protein fraction and can be identified by a band at certain isoelectric points as in immature stage (I) which is characterized by the specific bands at PI's 4.93; 5.20 & 6.30, however stage I (J) is identified by five distinct bands at PI's 4.18, 4.53, 4.73, 5.33 & 5.95. Stage II (K) exhibits four specific fractions at PI's 4.43, 4.68, 5.88 &6.03. Six specific fractions are separated from stage III (L) at PI's 4.75, 4.95, 5.03, 5.18, 5.55, 5 & 6.43. Two specific fractions are separated from ripe (N) and spawning (O) stages at PI's values 5.68 &

6.50 and 4.20 & 6.28 respectively. At spent stage (P) there are four specific bands at PI's 4.60, 4.65, 5.85 & 5.98 (table 2).

The densitograms taken from this gel give support to the previous results deduced from figure (1b) especially for quantitative analysis. Densitograms have to be considered as a fine reflection of the results obtained by IEF.

The densitograms scan for males reveal the different mobilities and intensities of the bands. From figure (2) it is clear that in the patterns I; J ; K; L & O, bands migrated towards the cathodic region except pattern P which represents the spent stage as the bands migrated towards the anodic region.

Table (2) represents the percentage of protein fractions in the seven stages of testis maturation. It is evident that the maximum values of the protein fraction (8.4, 8.4 & 9.8%) of the immature stage are found towards the cathode between PI 6.20 & 6.30 and the minimal values (1.1%) at PI 5.43 (Fig.2 I). In the mature stage (I) the maximum quantitative values of the protein bands (9.7, 10.5, 7.1 & 8.5%) are found towards the cathode between PI 5.53 & 6.60 (Fig.2 J). The maximum values (8.6 & 7.2%) of scanning pattern of protein fraction for the stage II are separated at PI 5.70 & 6.23 toward the cathodic region (Fig.2K). In stage III (table2 & Fig.2L) the maximum percentages of protein bands are concentrated at the cathodic region (PI 6.23 &6.35), and the minimal one (0.8) at PI 4.88.

The densitogram of the ripe stage (N) shows that the maximum quantitative values (13.3 & 12.6) of scanning patterns of band are found to be at the cathodic region where their peaks between PI 5.58 & 6.23 (Fig.2 N). The minimum percentage of protein (2.2) is found at PI 4.90 & 5.23. Figure (2 O) shows the soluble testes protein for the spawning stage (O) which is characterized by having maximum percentages (13.3, 12.6 &

7.5) at PI 5.58, 5.60 & 6.20 respectively toward the cathodic region. For the spent stage (P) it is observed that the maximum value of protein fraction (22.8) appear at PI 5.98, however this stage is characterized by having most of the peaks at the anodic region (Fig.2 P & table 2).

DISCUSSION

Isoelectric focusing offers several advantages in problems of sex identification from small tissue samples or diluted tissue extracts. Resolution of protein phenotype can be excellent, with 20-36 scorable proteins from 10 or 20 µl of extract. Comparisons between sex are facilitated by protein migration to specific pH points which correspond to their isoelectric points (Bsaglia and Marchetti 1991).

In the present study, the maturity stages for both sexes of *S. rivulatus* can be identified by IEF patterns of gonad tissue protein. However, the protein patterns obtained are found to be more specific between the two sexes. Collection of photographs of IEF patterns for the maturity stages for this species of fish on a polyacrylamide gel can serve as a reference.

The obtained results show that each female and male has a characteristic specific electrophoretic and isoelectric focusing pattern. However, in the pattern of spent stage for both sex, there are characteristic anodic bands which show a clear differentiation between the other maturity stages.

In the present work it is clear that the maximum number of specific bands (4) are present in spawning stage for female *S. rivulatus*. This is in a good agreement with Moharram (1994), who reported that the same stage and the same sex for the same species had the maximum number of specific bands in the plasma protein. Also, this study shows that each sex has a characteristic sex-specific electrophoretic and isoelectric focusing

patterns. However, in all fractions, characteristic sex zones are recognized. In female, there is characteristic cathodic bands showing a clear sex specific relation-ship between the seven maturity stages (Fig.2 & Table 1).

The present results show that the electrophoretic patterns of gonad protein of *S. rivulatus* reveal great differences with advancing maturation. Similar results are also reported by EL-Garabawy *et al.* (1995) for *Mugil seheli,* Abdallah (1996) for *Diplodus vulgaris,* and Zaki *et al.* (1997) for *Chrysicthys rueppelli & Chrysicthys auratus.*

Herzberg and Pasteur (1974) reported that it is possible to identify the mature and immature stages of five species of grey mullet (*Liza saliens, Liza provensalis, Liza ramada, Liza aurata and Mugil cephalus)* caught off the Mediterranean coast of Israel. They showed that a fast moving band occurred in the pattern of all immature stages of these species, also they found that these fractions decrease during maturation of all species, it became faint in adult of *L. ramada, L. provensalis* and *L. saliens* while it was not observed in adult of *L. aurata* and *M. cephalus.*

The present study revealed that stage II for both sexes has a maximum number of bands. This is in accordance with El-Gharabawy and Zaki (1990) for *Mugil capito.*

The results in the present study indicated that the number of protein fractions during vitellogenesis process (stage III) and ripe stage in *S. rivulatus* decreased. These findings are in a good agreement with Thorsen and Fyhn (1991) who reported a significant decrease in protein content during hydration process in the oocyte of some marine fishes and Carnevali *et al.*(1992) who determined the changes in the electrophoretic patterns of yolk proteins during vitellogenesis for *Sparus aurata..*

Generally, it is obvious that the patterns of female has a greater number of protein fractions than in male (Table 1 & 2). This result is

considered due to cell activities of the ovary and its function differs from those of testis (Basagila, 1992).

Book, (1964) and Thurston, (1967) declared that not only soluble protein of gonads is used for differentiation between sexes but also protein serum.

It can be concluded that the collection of photographs of IEF patterns for maturity stages for *Siganus rivulatus* on a polyacrylamide gel can serve as a reference for differentiation between sexes.

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Table 1. The isoelectric point (PI's) values and the corresponding percentage of quantitative values of scanning pattern in female ovary for *Siganus rivulatus*.

PI Values	Percentage of concentrations for maturity stages.						
	A	B	C	E	F	G	H
4.15	----	----	2.7	2.7	3.1	4.7	6.2
4.28	----	----	----	----	----	3.8*	----
4.48	----	----	----	----	----	2.9	5.4
4.50	----	----	----	----	----	1.6	1.7
4.58	----	----	1*	----	----	----	----
4.60	----	----	2.7*	----	----	----	----
4.65	----	4	2.8	----	----	----	----
4.68	----	----	----	----	----	2.4	5.3
4.70	----	----	----	----	----	6.4*	----
4.75	----	----	7.2 *	----	----	----	----
4.80	----	----	----	----	1.9*	----	----
4.85	----	----	----	----	----	3	10
4.88	----	----	----	----	----	4.9	3.2
4.90	----	----	4.1	4.8	----	5.6	7.3
4.93	----	----	----	----	----	6.6*	----
4.95	4*	----	----	----	----	----	----
5.00	----	7.1	2.2	----	4.1	5.6	----
5.05	----	----	3.2	4.3	1.6	4.7	7.1
5.10	----	4.6*	----	----	----	----	----
5.18	3.3	----	----	----	3.2	----	----
5.20	3.3	5.1	2.6	1.1	----	----	3.4
5.23	5.3	----	----	1.7	----	----	1.7
5.28	----	2.6	3.5	----	----	----	----
5.30	----	----	----	1.9*	----	----	----
5.33	2.5	5.2	3.7	----	3.7	----	16.3
5.35	----	----	----	3.9	2.8	----	----
5.38	5.7	3.3	8.9	2.9	----	6.6	----
5.40	----	2*	----	----	----	----	----
5.45	2.1	----	3.8	3	----	4.6	4.5
5.48	----	3.2	----	4.5	----	----	----
5.50	----	2.7	2.7	4.5	----	----	7.6
5.58	----	1.9	2.2	5.3	3.9	----	----

Table 1. cont.

5.65	----	6.8	----	----	----	4.6	----
5.68	----	----	----	12.1	7	2.1	----
5.70	3.5	----	5	----	----	----	----
5.73	----	5.5	----	----	11.2	----	----
5.75	3.5	----	----	----	6.2	----	----
5.78	----	2.2	----	----	4.8	----	----
5.85	----	----	----	----	3	2.3	7.8
5.88	----	4.1	5.7	4.9	3.8	2.8	----
5.93	3.5	3.5	2.8	----	----	----	----
5.95	----	----	----	----	8.9*	----	----
5.98	13.1	2.5	11.2	3.6	8.9	3.1	----
6.00	----	----	----	2.9*	----	----	----
6.03	13	8.2	----	----	----	----	----
6.10	5.2	----	----	----	----	----	3.4
6.13	5.2	1.3	4.1	----	3.4	----	----
6.15	5.2	2.4	4.4	8.4	3.7	3.1	2.8
6.18	5.2	----	----	10.1	8.9	----	3.4
6.20	7.3	4.5	1.8	----	----	----	3.3
6.23	8.1	2.3	3.7	5.8	10.8	2.5	2.3
6.25	----	---	3.7	1.7	----	3.9	----
6.30	----	----	----	----	----	----	0.6*
6.38	----	----	----	----	----	4.2*	----
6.45	2.9	5.9	6.4	5.4	1.4	4.2	5.4
6.50	8.2	1.3	1.7	10.6	----	----	6.2
6.58	----	----	----	----	1.4	12.9	----

* Specific band. A = immature stage; B = stage I; C= stage II; E= stage III; F= ripe; G= spawning; H= spent.

Table 2. The isoelectric point (PI's) values and the corresponding percentage of Quantitative values of scanning pattern in male testis for *Siganus rivulatus*.

PI Values	Percentage of concentrations for stage of maturity						
	I	J	K	L	N	O	P
4.15	3.30	1.60	5.40	2.30	2.60	2.6	5.5
4.18	.....	1.30*	------	------	------	------	------
4.20	------	-----	------	------	------	3.7*	------
4.25	----	1.5	----	----	3.70	----	----
4.43	----	----	2.3*	----	----	----	----
4.53	----	5.8*	----	----	----	----	----
4.60	----	----	----	----	----	----	14*
4.63	----	----	2.3	----	----	1.6	----
4.65	----	----	----	----	----	----	3.6*
4.68	----	----	4.00*	----	----	----	----
4.73	----	2.40*	----	----	----	----	----
4.75	----	----	----	1.30*	----	----	----
4.83	1.5	2.8	----	----	----	----	----
4.88	----	----	----	0.80	----	2.20	----
4.90	----	----	4.30	----	2.20	----	5.8
4.93	7.20*	----	----	----	----	----	----
4.95	----	----	----	1.6*	----	----	----
5.00	----	----	2	----	----	2.5	----
5.03	----	----	----	1.6*	----	----	----
5.10	5.2	4	1.1	----	2.5	----	7.2
5.18	----	----	----	8.6*	----	----	----
5.20	----	4.1	----	----	----	2.2	----
5.23	----	1.9	----	----	2.2	----	----
5.25	5	2.1	2.2	----	----	----	----
5.28	----	----	3.6	----	----	6.3	----
5.30	3.7	4.3	5.1	----	6.3	----	----
5.33	----	3.8*	----	----	----	----	----
5.38	----	----	3.9	1.1	----	----	7.9
5.40	3	3.1	----	----	----	----	----
5.43	1.1	----	3	----	6.1	----	----
5.45	5.2*	----	----	----	----	----	----
5.48	----	1.6	5.9	----	----	----	5.2
5.50	7.2	----	----	----	----	----	4
5.53	----	9.7	3.5	----	----	----	----
5.55	----	----	----	3.9*	----	----	----
5.58	----	----	4.5	2	13.3	13.3	----
5.6	5	2.5	3.9	----	----	12.6	----

*Specific bands. I= immature stage; J= stage I; K= stage II; L= stage III; N= ripe; O= spawning; P= spent .

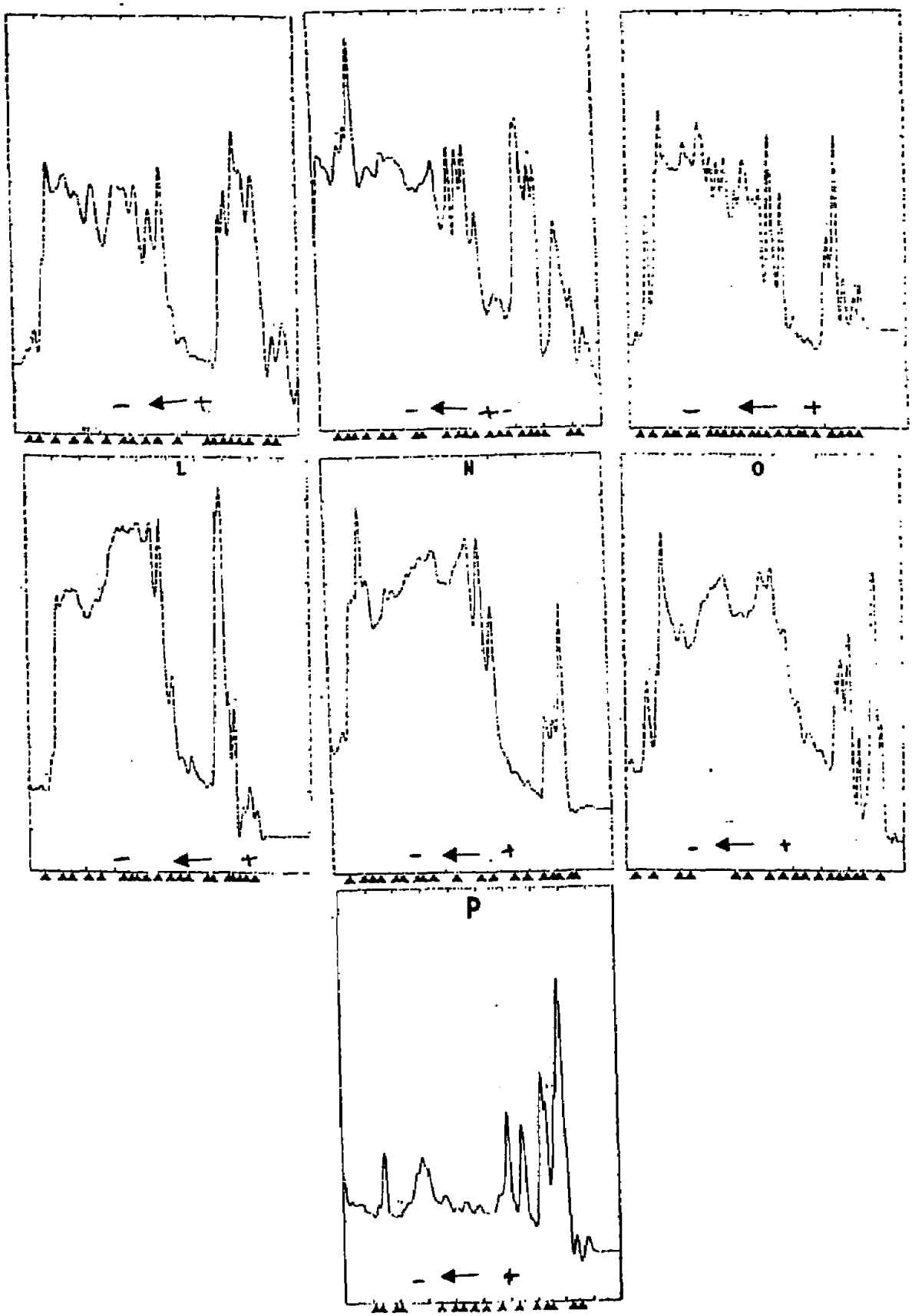

Figure (2). The densitograms of the gonad of Siganus rivulatus.

Male: I , immature stage; J, stage I; K , stage II; L, stage III; N, ripe stage; O, spawning; P, spent. +,anode -, cathode

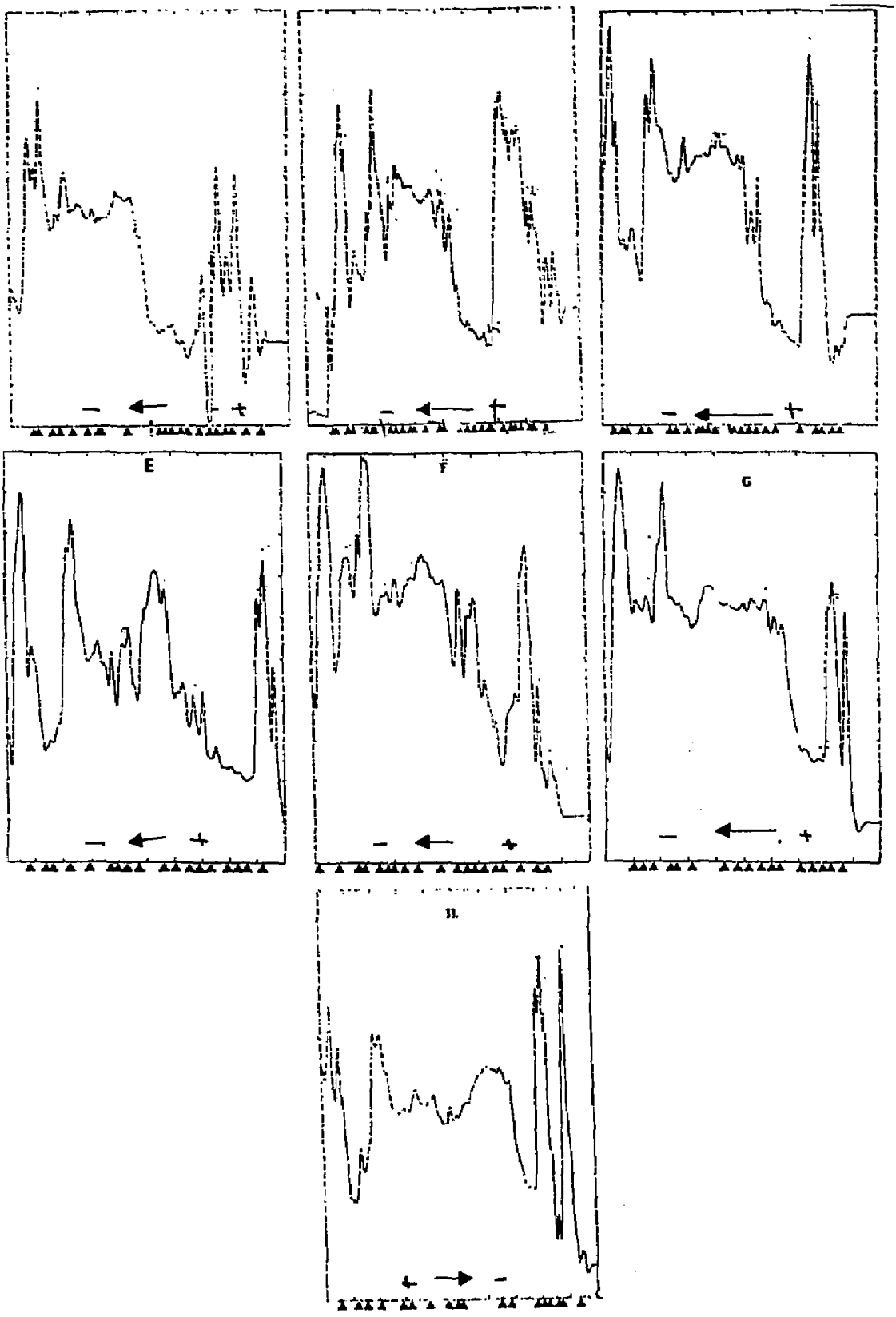

Figure (3). The densitograms of the gonad of Siganus rivulatus.
Female: A, immature stage; B, stage I; C, stage II; E, stage III; F, ripe stage; G, spwaning; H, spen -, cathode +,anode

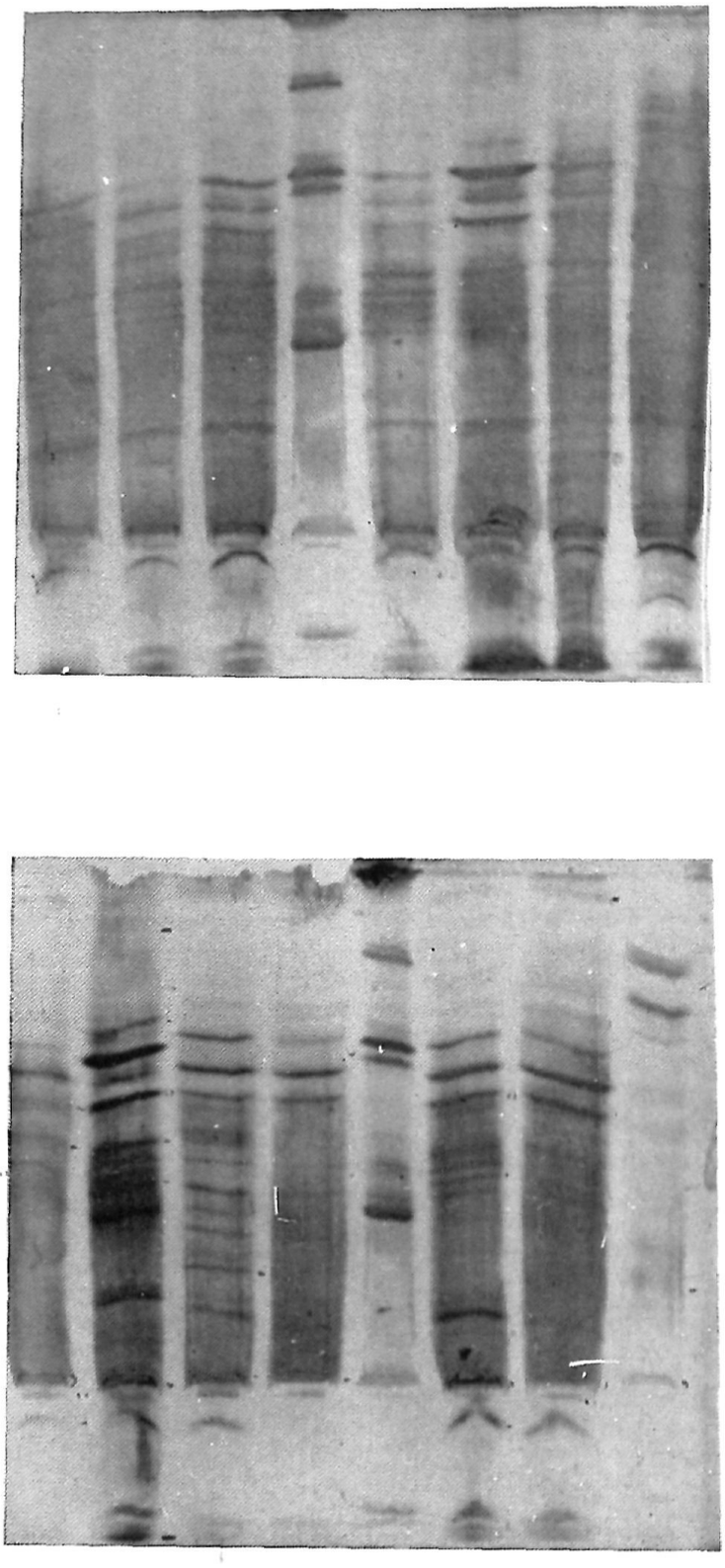

Figure (1) : Isoelecric focusing patterns of the soluble gonad proteins at all maturity stages of Siganus rivulatus on polyacrylamide gel, at PH 4.0-6.5.; +, anode; -, cathode.

a - Female : A, immature; B, stage I; C, stage II; D, protein marker; E, stage III; F, ripe stage; G, spawning; H, spent stage.

b - Male : I, immature; J, stage I; K, stage II; L, stage III, M, protein marker; N, ripe stage; O, spawning; P, spent stage.