Bolm. Zool., Univ. S. Paulo
8:77-91, 1984

EFFECT OF VARIATION OF SALINITY ON PROTEIN, RNA AND DNA CONTENTS OF LIVER, MUSCLE AND OVARY OF FEMALE SINGI FISH, HETEROPNEUSTES FOSSILIS (BLOCH), AT TWO PHASES OF REPRODUCTIVE CYCLE

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RESUMO - Investigou-se as mudanças causadas nos teores de proteínas e ácidos nucleicos do fígado, músculo, ovários e peso dos órgãos (HSI, GSI) pela variação de salinidade do meio (65, 135 e 225 mOsm, NaCl/litro, e salinidade zero ou água destilada) em fêmeas não vitelogênicas (NV) e vitelogênicas (V) do peixe *Heteropneustes fossilis* (Bloch) às temperaturas de 25°C e 30°C, 30 dias após o período de aclimação nos respectivos meios. Obteve-se valores máximos de HSI tanto nos peixes (NV) e (V) quando mantidos em água destilada e valores mínimos quando em solução de 225 mOsm. O aumento de temperatura de 25°C para 30°C causou uma redução nos valores de HSI somente nos peixes (V) Salinidade de 135 e 65-135 mOsm produziu o valor mais alto de GSI nos dois grupos de peixes: (NV) e (V) O efeito estimulante da alta temperatura (30°C) no GSI foi encontrado somente nos peixes (NV) em todos os meios salinos. O teor hepático de proteínas e RNA foi máximo nos peixes (NV) e (V) mantidos em meios de 65 mOsm de NaCl e mínimo nos de 225 mOsm de salinidade. O aumento de temperatura de 25°C para 30°C não alterou o teor protéico hepático mas aumentou e diminuiu o teor de RNA nas fases (V) e (NV). O teor muscular de proteínas e RNA foram máximos a salinidades zero e 65 mOsm e mínimos a 225 mOsm tanto a 25°C, como 30°C. A alta temperatura (30°C) aumentou o teor protéico, mas não alterou o teor de RNA. Salinidade de 135 mOsm causou o maior aumento nos teores de proteínas, RNA e DNA do ovário das fêmeas (NV) a 25°C ou 30°C. Salinidade zero reduziu bastante o teor desta substância no ovário. No caso do GSI o efeito estimulador da alta temperatura (30°C) no acúmulo de proteínas, RNA e DNA se fez sentir em todas as concentrações salinas do meio. Os peixes (V) apresentaram teor máximo de proteínas ovarianas a salinidade de 135 mOsm e mínimos em água destilada. Os ovários (V) tiveram a taxa mais alta de DNA em 65 e 135 mOsm de salinidade do que a zero ou 225 mOsm. A influência das altas temperaturas não foi senti-

da nas fêmeas (V) O teor de RNA ovariano dos peixes (V) também não se alterou em todas as condições experimentais.

ABSTRACT - The changes in protein and nucleic acid contents of liver, muscle and ovary and organ weight (HSI, GSI) caused by variation of salinity of the medium (65, 135 and 225 mOsm NaCl/liter, zero salinity or distilled water) were investigated on non-vitellogenic (NV) and vitellogenic (V) female Singi fish (*Heteropneustes fossilis* Bloch) at 25° and 30° after 30 days acclimation in the respective medium. The HSI was maximum in (NV) and (V) fish kept in distilled water and minimum in 225 mOsm NaCl solution. Rise of temperature from 25°C to 30°C caused reduction in HSI only in (V) fish, but not in (NV) one. The salinity of 135 and 65-135 mOsm produced highest GSI in (NV) and (V) fish respectively. A stimulating effect of higher temperature (30°) on GSI was found in only (NV) fish in all saline media. The amount of liver protein and RNA was maximum in (NV) and (V) fish kept in 65 mOsm NaCl medium and minimum in 225 mOsm salinity. Rise of temperature from 25°C to 30°C did not alter the liver protein content, but increased and decreased the RNA content at (V) and (NV) stage respectively. The muscle protein and RNA contents were maximum in zero salinity and 65 mOsm NaCl medium, and minimum in 225 mOsm saline group at 25°C or 30°C Higher temperature (30°C) increased the muscle protein content, but not the RNA. The salinity of 135 mOsm caused maximum increase in protein, RNA and DNA contents of the (NV) ovary at 25°C or 30°C. The zero salinity has a very depressing offect on these ovarian substances. As in case of GSI, a stimulating effect of higher temperature (30°C) on the accumulation of protein, RNA and DNA in (NV) ovary was noted in all saline media. The (V) fish showed maximum ovarian protein content in 135 mOsm salinity and minimum in distilled water. The (V) ovary had higher DNA content in 65 and 135 mOsm salinity than that in zero or 225 mOsm salinity. The influence of higher temperature on (V) ovary was absent. The ovarian RNA amount of (V) fish was not altered under the experimental conditions.

INTRODUCTION

In fish, an effective mechanism for osmotic regulation is necessary for maintaining the normal life processes. Salinity of water is one of the most important environmental factors which control metabolism, survival and distribution of fish (Holliday, 1969). Investigations have been made on the salinity tolerance and osmotic and ionic regulation in which there is a good deal of metabolic involvement of different kinds (Bashamohideen and Parvatheswararao, 1972; Venkatachari, 1974; Bhan and Mansuri, 1978; Love, 1980). Several reports are also available on the effects of different concentrations of saline on the nitrogen metabolism in euryhaline fish (Lecal, 1958; Jones, 1959; Cowey *et al.*, 1962; Cowey and Parry, 1963; Parvatheswararao, 1967; Huggins and Colley, 1971; Venkatachari, 1974; Mansuri and Bhan, 1978). Since the fresh water fishes are hypertonic to the surrounding water, their body organs are also likely to be affected or influen-

ced by the change in osmotic concentration of the medium.The salinity tolerance of Singi fish (*Heteropneustes fossilis* Bloch), a stenohaline fish, has been reported (Al Daham and Bhatti, 1977; Parwez *et al.*, 1979). But to our knowledge,the informations on the changes in different cellular constitu - ents of different organs, such as liver, muscle and ovary of fresh water or stenohaline fish after adaptation in diffe - rent concentrations of sodium chloride solution are inadequa te.

In view of these facts, investigations have been under taken on the effects of different concentrations of sodium chloride on different cellular components of liver, muscle and ovary of female Singi fish (*Heteropneustes fossilis* Bloch). The Singi fish of two phases of reproductive cycle , non-vitellogenic and vitellogenic, were used for the experi- ments in order to show the influence of reproductive stage on the changes in cellular components of different organs.In the present communication, the data on the changes in organ weight and protein, RNA and DNA contents of liver, muscle and ovary are presented.

MATERIALS AND METHODS

The female Singi fishes (*Heteropneustes fossilis* Bloch) of body weight 40-60 g (length 20-22 cm) were purchased from a local supplier and acclimatized in the laboratory condi - tions at 25°±1°C for 7 days before the experiments. The ova- ry of the non-vitellogenic (NV) fish contained only Stage I oocytes, whereas that of the vitellogenic (V) fish was loa - ded with Stage III oocytes. The experimental non-vitelloge - nic (December-January) and vitellogenic (June-July) fishes were distributed at random in different groups of 15 each for acclimatizing them to four different concentrations of sodium chloride solution, e.g., 65 mOsm, and 225 mOsm/liter. The same number of fish (15) were kept in distilled water (0 salinity) Each fish was kept in 1 liter of the respective medium in a glass jar with 12L : 12D photoperiod and fed *ad libitum* with *Tubifex tubifex*. The medium was changed at eve- ry alternate day. The fishes were acclimatized in this way in the respective medium (sodium chloride solution of each concentration or distilled water) at 25°C and 30°C for 30 days, after which they were sacrificed for the estimation of protein, RNA and DNA contents of liver, skeletal muscle and ovary. Tissue protein was estimated by following the method of Lowry *et al.* (1951), RNA by the method of Mejbaum (1939) as modified by Munro and Fleck (1966) and DNA by the method of Burton (1956) as modified by Croft and Lubran (1965). The hepatosomatic index (HSI) and the gonadosomatic index (GSI) were also calculated: weight of the organ x 100/body weight.

Since the weight of the liver and ovary of Singi fish undergoes a seasonal variation (Dasmahapatra and Medda, in press) and is also affected by change of environmental tempe rature (Dasmahapatra, 1980), the results of the amounts of protein, RNA and DNA of liver and ovary were first calcula - ted per 100 mg of fresh tissue, and then the final results of the amounts of these cellular substances were expressed

Table 1 - Effect of variations of salinity (0, 65, 135, 225 milliosmole NaCl per liter) on the hepatosomatic index (HSI) and gonadosomatic index (GSI) of female (non vitellogenic and vitellogenic) Singi fish (*Heteropneustes fossilis* Bloch). The fishes were fed and kept in the respective medium at 25°C or 30°C for 30 days. Each group consisted of 15 fishes.

	Temperature	Reproductive phase							
		Non-vitellogenic (fishes of December-January)				Vitellogenic (fishes of June-July)			
		Salinity (milliosmole per liter)							
	°C	0 (Distilled water)	65	135	225	0 (Distilled water)	65	135	225
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
HSI	25°	1.49 ± 0.04	1.25^{b} ± 0.05	1.08^{bA} ± 0.06	$0.94^{bB\alpha}$ ± 0.02	2.01 ± 0.05	1.94^{a} ± 0.04	1.70^{aB} ± 0.03	$1.50^{bB\beta}$ ± 0.06
	30°	1.32*** ± 0.03	1.19^{a*} ± 0.04	1.07^{bA*} ± 0.04	$0.93^{bB\alpha*}$ ± 0.04	1.86** ± 0.04	1.70^{a***} ± 0.05	1.52^{bA***} ± 0.05	$1.35^{bB\beta**}$ ± 0.02
GSI	25°	0.72 ± 0.14	1.35^{a} ± 0.15	2.01^{bA} ± 0.28	$1.22^{aN\alpha}$ ± 0.18	8.16 ± 0.28	11.20^{b} ± 0.15	11.25^{bN} ± 0.24	$9.00^{nB\beta}$ ± 0.41
	30°	0.98* ± 0.20	1.86^{b**} ± 0.12	3.45^{bB***} ± 0.19	$1.52^{aN\alpha*}$ ± 0.16	8.71* ± 0.46	13.22^{b***} ± 0.38	14.62^{bN***} ± 0.83	$9.20^{nB\beta*}$ ± 0.33

S.E. Standard error. 't' test probability difference : 'P' value : a,A,α,** = P<.05; b,B,β,*** P<.01; n,N,*, Not significant. Alphabets denote comparison between different saline groups (including distilled water) at a particular temperature (25°C or 30°C) and stars denote comparison between different temperature groups (25° and 30°C) at a particular saline concentration or distilled water medium. Small English alphabets are used for comparison between distilled water and 65 mOsm or 135 mOsm or 225 mOsm NaCl groups, capital English alphabets for comparison between 65 mOsm and 135 mOsm or 225 mOsm groups and Greek alphabets for 135 mOsm and 225 mOsm groups.

Table 2 - Effect of variations of salinity (0, 65, 135, 225 milliosmole NaCl per liter) on the protein content of liver, muscle and ovary of female (non-vitellogenic and vitellogenic) Singi fish (*Heteropneustes fossilis* Bloch). The fishes were fed and kept in the respective medium for 30 days at 25°C or 30°C temperature. Each group consisted of 15 fishes.

	Tempe-rature	Reproductive phase							
		Non-vitellogenic (fishes of December-January				Vitellogenic (fishes of June-July)			
		Salinity (milliosmole per liter)							
	°C	0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.	0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.
Liver protein (mg/100g of body weight)	25°	70.30 ± 2.65	81.54^{a} ± 3.47	56.95^{bB} ± 2.58	$45.74^{bB\alpha}$ ± 3.82	150.28 ± 5.78	166.38^{a} ± 2.61	131.45^{aB} ± 5.35	$119.56^{bB\gamma}$ ± 4.68
	30°	74.38* ± 4.70	88.54^{a*} ± 3.48	53.83^{bB*} ± 4.68	$35.48^{bB\beta*}$ ± 2.21	157.48* ± 2.19	171.88^{b*} ± 2.52	142.45^{bB*} ± 3.52	$124.71^{bB\beta*}$ ± 2.67
Muscle protein (mg/100mg of fresh tissue)	25°	12.97 ± 0.43	12.40^{n} ± 0.39	10.48^{aB} ± 0.50	$8.68^{bB\beta}$ ± 0.37	14.88 ± 0.46	14.38^{n} ± 0.52	12.48^{bA} ± 0.44	$8.82^{bB\beta}$ ± 0.35
	30°	14.08** ± 0.28	14.44^{n**} ± 0.56	10.89^{bA*} ± 0.36	$8.46^{bB\beta*}$ ± 0.45	16.82** ± 0.34	16.48^{n**} ± 0.17	12.88^{bB*} ±0.32	$8.02^{bB\beta*}$ ± 0.41
Ovary protein (mg/100 of body weight)	25°	49.78 ± 3.87	202.53^{b} ± 10.15	384.48^{bB} ± 17.40	$192.28^{bN\beta}$ ± 13.19	789.00 ± 34.38	1682.45^{b} ± 76.63	1888.00^{bA} ± 53.83	$1207.55^{bB\beta}$ ± 70.48
	30°	65.45*** ± 2.16	482.45^{b***} ± 27.57	611.44^{bB***} ± 17.14	$322.36^{bB\beta***}$ ± 22.90	882.45* ± 71.80	1792.00^{b*} ± 56.95	2064.45^{bB*} ± 77.64	$1282.82^{aB\beta*}$ ± 95.48

S.E. = Standard error. 't' test probability difference : 'P' value : a,A,α,** P<.05; b,B,β,*** = P<.01; n,N,γ,* = Not significant. Alphabets denote comparison between different saline groups (including distilled water) at a particular temperature (25°C or 30°C) and stars denote comparison between different temperature groups (25°C and 30°C) at a particular saline concentration or distilled water medium. Small English alphabets are used for comparison between distilled water and 65 mOsm or 135 mOsm or 225 mOsm NaCl groups, capital English alphabets for comparison between 65 mOsm and 135 mOsm or 225 mOsm groups and Greek alphabets for 135 mOsm and 225 mOsm groups.

per 100 g of body weight of fish to eliminate the error, if any, caused by change in organ weight. But, since it was difficult to estimate the total amount of skeletal muscle in the body, the results of protein, RNA and DNA contents of muscle were expressed per 100 mg of fresh tissue. Statistical analysis of the data were made using student's 't' test. In each case, the mean data were the average from 15 fishes.

RESULTS

1. Changes in weight of liver (HSI) and ovary (GSI),Table 1.

The vitellogenic (V) fish had higher HSI and GSI than non-vitellogenic (NV) fish. Increase in NaCl concentration of the medium from 65 to 135 mOsm/liter and from 135 to 225 mOsm/liter caused a reduction in HSI in each case in both(NV) and (V) fish at 25°C and 30°C. The HSI was consequently the lowest in both (NV) and (V) fish at 25°C and 30°C when the salinity was raised to 225 mOsm. The increase in temperature from 25°C to 30°C exerted no significant influence on HSI in (NV) fish kept in different concentrations of NaCl. But the (V) fish showed less HSI at 30°C in each saline medium in comparison to that at 25°C. Surprisingly, Singi fishes of both the reproductive stages kept in distilled water showed maximum liver weight, the HSI being higher at 25°C than 30°C.

Addition of NaCl or increase in osmolarity of NaCl in the medium within certain limits caused enhancement in GSI in (NV) and (V) fish. At (NV) stage, the GSI was minimum in fish kept in distilled water, maximum in fish kept in 135 mOsm NaCl solution and intermediate in 65 and 225 mOsm NaCl solutions at 25°C and 30°C. In (V) fish, GSI increased to the maximum level when they were maintained in 65 and 135 mOsm NaCl solutions, it was the lowest in distilled water and in 225 mOsm NaCl solution. The rise of temperature from 25°C to 30°C enhanced the GSI in (NV) as well as (V) fish maintained in 65 and 135 mOsm NaCl solutions.

2 Changes in protein content of liver, muscle and ovary (Table 2)

The protein content of liver and ovary was at higher level in (V) fish in comparison to (NV) one. The muscle protein content was also higher in (V) fish, except in fish kept in 225 mOsm NaCl solution. The amount of liver protein increased to the maximum level in (NV) and (V) fish kept in 65 mOsm NaCl solution both at 25°C and 30°C, while its amount was minimum in fish maintained in 225 mOsm NaCl solution at these temperatures. The level of liver protein in (NV) and (V) fish kept in distilled water was higher in comparison to that in fish maintained in 135 and 225 mOsm NaCl solutions. Although there was a reduction in protein content of liver with the increase in concentration of NaCl solution from 65 mOsm, the rise of temperature from 25°C to 30°C had no effect on liver protein amount in fish maintained in any medium.

Table 3 - Effect of variations of salinity (0, 65, 135 and 225 milliosmole NaCl per liter) on the RNA content of liver, muscle and ovary of female (non-vitellogenic and vitellogenic) Singi fish (*Heteropneustes fossilis* Bloch). The fishes were fed and kept in the respective medium at 25°C or 30°C for 30 days. Each group consisted of 15 fishes.

	Temperature	Reproductive phase							
		Non-vitellogenic (fishes of December-January)				Vitellogenic (fishes of June-July)			
		Salinity (milliosmole per liter)							
	°C	0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.	0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.
Liver RNA (mg/100 g of body weight	25°	7.69 ± 0.37	9.58^{b} ± 0.34	6.55^{nB} ± 0.67	$3.70^{bB\beta}$ ± 0.13	13.45 ± 1.26	17.28^{a} ± 0.48	11.48^{nB} ± 0.45	$7.45^{aB\beta}$ ± 0.80
	30°	5.48*** ± 0.36	7.45^{b**} ± 0.32	4.99^{nB**} ± 0.36	$1.78^{bB\beta**}$ ± 0.90	17.66** ± 1.45	20.88^{a**} ± 1.18	16.06^{nA**} ± 1.75	$11.28^{bB\beta**}$ ± 1.19
Muscle RNA µg/100 mg of fresh tissue	25°	84.28 ± 4.76	86.45^{n} ± 3.47	52.48^{bB} ± 3.02	$40.10^{bB\alpha}$ ± 3.42	52.44 ± 4.28	49.28^{n} ± 3.31	33.88^{bB} ± 1.02	$29.00^{bB\beta}$ ± 1.45
	30°	88.44* ± 3.48	90.46^{n*} ± 4.46	60.48^{bB*} ± 3.46	$45.88^{bB\alpha*}$ ± 4.61	51.87* ± 3.00	46.45^{n*} ± 3.64	36.12^{bA*} ± 2.19	$25.01^{bB\beta*}$ ± 1.62
Ovarian RNA (mg/100 g of body weight)	25°	12.72 ± 0.93	15.71^{a} ± 0.81	19.35^{bB} ± 0.18	$16.21^{bN\alpha}$ ± 0.88	35.19 ± 2.81	37.84^{n} ± 2.86	37.06^{nN} ± 1.95	$36.45^{nN\gamma}$ ± 3.75
	30°	13.25* ± 0.82	18.27^{b**} ± 0.45	22.00^{bB***} ± 0.76	$18.81^{aA\beta**}$ ± 0.86	39.10* ± 1.44	44.48^{n*} ± 3.04	42.45^{nN*} ± 2.35	$38.20^{nN\gamma*}$ ±2.45

S.E. Standard error. 't' test probability difference : 'P' value : a,A,α,** P<.05; b,B,β,*** = P<.01; n,N,γ,* Not significant. Alphabets denote comparison between different saline groups (including distilled water) at a particular temperatures (25°C or 30°C) and stars denote comparison between different temperature groups (25°C and 30°C) at a particular saline concentration or distilled water medium. Small English alphabets are used for comparison between distilled water and 65 mOsm or 135 mOsm or 225 mOsm NaCl groups, capital English alphabets for comparison between 65 mOsm and 135 mOsm or 225 mOsm groups and Greek alphabets for 135 mOsm and 225 mOsm groups.

Increase in saline concentration from 65 to 135 mOsm and from 135 to 225 mOsm caused reduction in muscle protein content in each case both at 25°C and 30°C in (NV) and (V) fish. The protein content of muscle was about the same in fish kept in distilled water and in 65 mOsm NaCl solution at 25°C or 30°C at these two reproductive stages, but the hi - gher temperature (30°C) increased the muscle protein content in these fishes. The muscle protein was at the lowest level in (NV) and (V) fish kept in 225 mOsm NaCl solution at 25°C and 30°C.

The (NV) fish had markedly less protein content of ova ry than (V) fish kept in all media. The ovarian protein le - vel was the lowest in 0 salinity at 25°C or 30°C in both(NV) and (V) fish. It then gradually increased when the salinity of the medium was raised to 65 mOsm and 135 mOsm, but with the rise of salinity to 225 mOsm from 135 mOsm the ovarian protein amount was reduced in both (NV) and (V) fish at 25°C and 30°C. The increase in temperature from 25°C to 30°C caused enhancement of protein content of ovary in (NV)fish kept in all media, while in (V) fish such influence of higher tem perature (30°C) was not found.

3. Changes in RNA content of liver, muscle and ovary (Table 3)

Like protein, the RNA content of liver and ovary was found to be higher in (V) fish. But the muscle RNA content was more in (NV) fish. Addition of NaCl in the medium to a concentration of 65 mOsm caused an increase in liver RNA con tent in (NV) and (V) fish at 25°C or 30°C in comparison to the distilled water group. Increase in saline concentration from 65 to 135 mOsm decreased the liver RNA content in (NV) and (V) fish. The lowest amount of liver RNA was found at both (NV) and (V) stage in 225 mOsm NaCl medium at 25°C or 30°C. The rise of temperature from 25°C to 30°C caused reduc tion and enhancement of liver RNA content in (NV) and (V) fish respectively in all media.

The RNA content of muscle remained at about the same level in (NV) or (V) fish kept in 0 salinity and in 65 mOsm NaCl solution at 25°C or 30°C. Increase in saline concentra- tion from 65 to 135 mOsm and from 135 to 225 mOsm decreased the muscle RNA content in each case in both (NV) and (V) fish at 25°C or 30°C. The rise of temperature from 25°C to 30°C did not cause any change in muscle RNA content in any fish in any medium under study.

The RNA content of ovary gradually increased with the increase in salinity of the medium up to 135 mOsm in (NV) fish at 25°C or 30°C. At V stage the ovarian RNA content was almost at the same level in all media at 25°C and 30°C. The influence of higher temperature (30°C) on the enhancement of ovarian RNA content was observed in (NV) fish in all NaCl me dia, but in (V) fish there was no significant change in ova- rian RNA by higher temperature.

Table 4 - Effect of variations of salinity (0, 65, 135 and 225 milliosmole NaCl per liter) on the DNA content of liver, muscle and ovary of female (non-vitellogenic and vitellogenic) Singi fish (*Heteropneustes fossilis* Bloch). The fishes were fed and kept in the respective medium at 25°C or 30°C for 30 days. Each group consisted of 15 fishes.

	Temperature °C	Reproductive phase: Non-vitellogenic (fishes of December-Jan.) — Salinity (milliosmole per liter): 0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.	Vitellogenic (fishes of June-July) — 0 (Distilled water) Mean ± S.E.	65 Mean ± S.E.	135 Mean ± S.E.	225 Mean ± S.E.
Liver DNA (mg/100 g of body weight)	25°	3.79 ± 0.08	3.80^{n} ± 0.14	3.04^{nN} ± 0.25	$3.97^{nN\gamma}$ ± 0.40	4.78 ± 0.40	4.50^{n} ± 0.46	3.98^{nN} ± 0.42	$4.00^{nN\gamma}$ ± 0.20
	30°	4.01* ± 0.14	3.98^{n*} ± 0.05	3.94^{nN*} ± 0.10	$3.35^{nN\gamma*}$ ± 0.12	4.91* ± 0.24	4.28^{n*} ± 0.60	4.57^{nN*} ± 0.48	$4.25^{nN\gamma*}$ ± 0.32
Muscle DNA (µg/100 mg of fresh tissue)	25°	65.32 ± 4.48	67.1^{n} ± 4.15	58.45^{nN} ± 7.42	$60.45^{nN\gamma}$ ± 4.00	48.44 ± 5.36	42.85^{n} ± 4.12	50.20^{nN} ± 3.47	$47.77^{nN\gamma}$ ± 5.80
	30°	68.00* ± 5.00	60.48^{n*} ± 4.40	59.44^{nN*} ± 5.45	$55.55^{nN\gamma*}$ ± 4.46	40.88* ± 7.22	38.44^{n*} ± 5.21	42.22^{nN*} ± 4.00	$36.40^{nN\gamma*}$ ± 5.88
Ovarian (mg/100 g of body weight)	25°	0.80 ± 0.03	1.17^{b} ± 0.06	1.39^{bB} ± 0.04	$1.12^{aN\alpha}$ ± 0.10	7.41 ± 0.28	9.03^{a} ± 0.60	9.08^{bN} ± 0.44	$6.75^{nA\alpha}$ ± 0.90
	30°	1.12* ± 0.08	1.52^{a***} ± 0.04	1.82^{bB***} ± 0.06	$1.40^{aN\beta**}$ ± 0.10	6.78* ± 0.70	10.47^{b*} ± 0.65	9.51^{aN*} ± 0.77	$8.43^{nA\alpha*}$ ± 0.38

S.E. Standard error. 't' test probability difference : 'P' value : a,A,α, ** = P<.05; b,B,β, *** P<.01, n,N,γ,* Not significant. Alphabets denote comparison between different saline groups (including distilled water) at a particular temperature (25°C or 30°C) and stars denote comparison between different temperature groups (25°C and 30°C) at a particular saline concentration or distilled water medium. Small English alphabets are used for comparison between distilled water and 65 mOsm or 135 mOsm or 225 mOsm NaCl groups, capital English alphabets for comparison between 65 mOsm and 135 mOsm or 225 mOsm groups and Greek alphabets for 135 mOsm and 225 mOsm groups.

4. Changes in DNA content of liver, muscle and ovary (Table 4)

The DNA content of liver remained unchanged with the increase in saline concentration as well as in temperature from 25°C to 30°C in both (NV) and (V) fish. The same nature of results (unchanged) was obtained in case of muscle DNA in all these fishes. As expected, the DNA content of ovary was higher at (V) stage. In (NV) fish, increase in NaCl concentration up to 135 mOsm was associated with an increase in each case in the amount of ovarian DNA at 25°C or 30°C. When the saline concentration was raised from 135 to 225 mOsm, reduction in ovarian DNA content was noticed in (NV) fish This reduced level of DNA was significantly higher in comparison to that in fish kept in distilled water at 25°C or 30°C. In (V) fish kept in distilled water and in 225 mOsm NaCl, the DNA content of ovary was almost at the same level at 25oC or 30°C, but this level of ovarian DNA was found to be less than that of fish maintained in 65 mOsm and 135 mOsm NaCl at either of these two temperatures. The rise of temperature from 25°C to 30°C had no influence on the ovarian DNA content in (V) fish kept in all media, but significantly increased the ovarian DNA amount in (NV) fish kept in all NaCl solutions.

DISCUSSION

Singi fish can survive in media which are considerably hypertonic to its natural fresh water habitat containing about 5 mOsm salinity (Al Daham and Bhatti, 1977; Parwez *et al.*, 1979). In some representative experiments we have found that they also survive 45-50 days in distilled water but only 12-16 days in 270 mOsm NaCl medium, this osmolarity is about the same as that of its body fluid (Parwez *et al.*, 1979). We have, therefore, choosen for experiments different NaCl concentrations having osmolarity less than that of the body fluid of Singi fish, such as 65, 135 and 225 mOsm/liter corresponding to about 75%, 50% and 17% less osmolarity than that of body fluid, and also distilled water to study the adaptive changes in the liver, muscle and ovary with respect to protein, RNA and DNA contents and organ weight (HSI and GSI) at both non-vitellogenic and vitellogenic stages at 25°C and 30°C. Singi fishes show better metabolic efficiency, particularly the ovarian activity, at a temperature range from 28.6°C to 32°C (Vasal and Sundararaj, 1976) We have also observed the aggravated activity of the ovary of (NV) Singi fish at 30°C (Dasmahapatra and Medda, 1982a)

It is evident from our results that the cellular metabolism of liver, muscle and ovary were markedly affected or influenced by changes in the concentration of NaCl in the medium in which the Singi fishes were maintained for 30 days A number of such induced metabolic changes were temperature dependent (Dasmahapatra, 1980) The HSI was maximum in fish kept in distilled water and minimum in fish kept in 225 mOsm NaCl. But the GSI was minimum in fish kept in distilled wa-

ter, maximum in fish kept in 135 mOsm NaCl and intermediate in fish kept in 65 mOsm and 225 mOsm NaCl at (NV) stage. The reduction in HSI in (NV) and (V) fish with the rise of salinity of the medium at 25°C or 30°C might be due to the depletion or decreased synthesis of some cellular constituents of liver. The higher temperature (30°C) caused a decrease in HSI particularly in (V) fish. The concomitant increase in GSI in (NV) fish with the rise of salinity of the medium up to 135 mOsm NaCl both at 25°C and 30°C might be the result of influenced maturation of the ovary. The highest GSI was found in (V) fish maintained in 65 and 135 mOsm NaCl solutions. But the GSI was almost the same in (V) fish kept in 0 salinity and 225 mOsm NaCl solution. Higher temperature favoured ovarian growth (more GSI) at a suitable saline concentration. The results further indicated that higher saline concentration (225 mOsm) retarded ovarian growth in (NV) fish. But this medium appeared to be better than distilled water so far as the GSI of the (NV) fish was concerned. The higher GSI values of (V) fish in an environmental salinity of 65 mOsm and 135 mOsm may be explained as the result of better maintenance of the gravid ovary and the lower GSI values in 225 mOsm NaCl and distilled water as the result of regression of the ovary. It seems, therefore, that a saline concentration ranging from about 25% to 50% of the osmolarity (65-135 mOsm per liter) of the body fluid of Singi fish may be suitable for the formation and maturation of the oocytes in the ovary (Dasmahapatra and Medda, 1982a) possibly through some concomitant contributions of the metabolities from the liver (and muscle) leading to a decrease in weight of the latter organ (liver)

Further, suitability of NaCl medium having osmolarity from 65 to 135 mOsm for ovarian activity in preparation for the favourable conditions for the formation and maturation of eggs can be supported by the enhanced levels of protein, RNA and DNA contents of ovary of (NV) fish kept in these NaCl media (65 and 135 mOsm), the higher concentration of 135 mOsm NaCl being considered as the best medium in these respects. The combined stimulating effect of salinity and higher temperature (30°C) was evidenced from the higher values of these cellular constituents of ovary at 30°C in (NV) fish kept in these saline media. Although a saline concentration of 225 mOsm had some depressing action on the maturation of eggs in (NV) fish (Dasmahapatra and Medda, 1982a)and also on the cellular constituents of ovary studied, this saline medium was better than distilled water so far as the metabolic functions of this organ were concerned. This was evident from the higher levels of protein, RNA and DNA contents of (NV) ovary in 225 mOsm NaCl group in comparison to those of distilled water group. The data further indicated that in (V) fish the salinity range from 65-135 mOsm NaCl was favourable for the maintenance of gravid ovary. This could be supported by the enhaced levels of protein and DNA (glycogen and lipid also, unpublished) and unchanged number of mature eggs (Dasmahapatra and Medda, 1982a) in (V) ovary in such saline range. The reduction in the amounts of protein and DNA (glycogen and lipid also, unpublished) in the ovary of (V) fish kept in distilled water and 225 mOsm NaCl solution in comparison to those of the fish kept in 65 and

135 mOsm NaCl solutions might be interpreted as the initial preparatory changes for the regression of mature eggs.

The gradual fall of the level of protein and RNA in liver and muscle of (NV) and (V) fish with the rise of salinity of the medium from 65 to 135 or 225 mOsm may possibly be the result of induced breakdown and release of these substances for the development of maturing oocytes and/or for maintaining the osmolarity of the body fluid in a salinity higher than that of the natural habitat of Singi fish. It has been reported that some euryhaline fishes exposed to higher saline concentration than their natural habitat show some degradation of protein in liver and muscle and the resultant increase in the amino acid level in blood (Venkatachari, 1974; Bhan and Mansuri, 1978) possibly to adjust the osmolarity of the body fluid. Thus a constant difference in osmolarity between the body fluid and the surrounding medium is probably maintained. It may be assumed that such difference in osmolarity is maintained in Singi fish when kept in the saline of increasing osmolarity and the resultant degradation of protein helps not only for supplying the metabolites for the growth and maturation of oocytes but also for adjusting the osmolarity of the body fluid possibly to maintain a constant difference. It may be noted further that the DNA content of liver and muscle was not changed by the change in salinity of the medium at 25°C or 30°C. That means the environmental salinity has no direct effect on DNA synthesis or degradation in liver and muscle of Singi fish.

The induced changes in different organs at the variation of salinity of the medium may be argued as the results of the changes in hormonal levels, particularly of gonadotrophin, prolactin and/or estrogen at different saline concentrations. Although the endogenous hormonal levels of Singi fishes kept in different saline media have not been studied, the ovarian changes including formation and maturation of eggs may be assumed to be due to enhacement of gonadotrophin level in different salinity. It has been reported that the gonadotrophin content of the pituitary of *Mugil* species (Brackish water) held in fresh water is considerably lower than that of pituitaries from sea water fish and the area of the pituitary containing the gonadotrophic cells is reduced in size in fresh water specimens (Blanc and Abraham, 1968 ; Blanc-Livni and Abraham, 1970)

The higher estrogen level in blood of (V) Singi fish than (NV) fish (Lamba *et al.*, 1982) may be responsible for higher levels of protein, RNA and DNA contents of liver of (V) fish (Dasmahapatra and Medda, 1982b) Estrogen administration in Singi fish also increased the protein, RNA, lipid and water contents and decreased the glycogen content of liver, but failed to cause any change in muscle and ovary (Medda *et al.*, 1980; Dasmahapatra and Medda, 1982c) The reduction in the amount of protein and RNA contents of liver and muscle in (NV) and (V) fish with the rise of salinity of the medium may, therefore, be due to the direct effect of salinity, rather than indirectly through the mediation of estrogen. The variation of salinity of the medium may cause an alteration in the prolactin concentration in Singi fish. It has

been reported that the transfer of sticklebacks from fresh water to sea water causes the appearance of intercellular cysts among the prolactin cells and also the diminution in number of these cells with shrunken nuclei and poorly granulated cytoplasm which indicate their decreased secretory activity (Benjamin, 1978). Prolactin is also involved in osmoregulation in fresh water fish (Brett, 1979; Gallis *et al.*, 1979). Moreover, prolactin has an antigonadal effect (de Vlaming and Vodicnik, 1977) The possibility of the involvement of prolactin in the changes of liver, muscle and ovary of Singi fish with the change in salinity of the medium is yet to be assessed.

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