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Seasonal changes in the biochemical composition of the muscle and liver of bib (*Trisopterus luscus* L.) (Pisces, Gadidae) from the Cantabrian Sea (N Spain)*

C. R. MERAYO

Departamento de Biología de Organismos y Sistemas, Facultad de Biología, 33071 Oviedo, Spain.

SUMMARY: Seasonal variations in major biochemical components (water, lipids, protein, and ash) in the muscle and liver of bib, *T. luscus* L. were followed using samples of fish taken from commercial catches from November 1987 to October 1988. Analysis of variance (ANOVA) showed that there were significant differences between sexes and between months for all the components considered. Therefore, males and females were studied separately in subsequent analysis. Water content of muscle and liver increased during spring, reached the highest values in April and fell gradually in summer. Protein levels remained fairly constant with slight increases occurring during winter and a decline in March, reaching minimum values in April. Seasonal changes in liver lipid content followed virtually the opposite pattern of that of the liver water content. Thus, lipid content of the liver decreased through the spring coinciding with the spawning season and reaching minimum values in April. A recovery of liver lipids was observed in May. It is concluded that the main source of lipid for gonadal development in this species is the liver reserves.

Key words: bib, fish biochemical composition, *Trisopterus luscus*.

RESUMEN: CAMBIOS ESTACIONALES EN LA COMPOSICIÓN QUÍMICA DEL MÚSCULO Y EL HÍGADO DE LA FANECA (*TRISOPTERUS LUSCUS* L.) DEL MAR CANTÁBRICO. – Se examinaron las variaciones estacionales de la composición química (agua, lípidos, proteínas y cenizas) en el músculo e hígado de la faneca, *T. luscus* L., utilizando muestras de peces recogidas de pescas comerciales desde Noviembre 1987 hasta Octubre 1988. Un análisis de varianza (ANOVA) mostró que había diferencias significativas entre los sexos y entre los meses para todos los componentes considerados. Por tanto, machos y hembras fueron estudiados por separado en los siguientes análisis. El contenido en agua del músculo e hígado aumenta durante la primavera, alcanzando los valores mas altos en Abril y desciende gradualmente en verano. Los niveles de proteína permanecen bastante constantes con un ligero incremento durante el invierno y un descenso en Marzo, alcanzando los valores mínimos en Abril. Los cambios estacionales en el contenido en lípidos del hígado presentan un modelo opuesto al contenido en agua. El contenido en lípidos del hígado desciende durante la primavera coincidiendo con la estación de desove y alcanza los valores mínimos en Abril. En Mayo se observa una recuperación de los lípidos del hígado. En conclusión la mayor reserva de lípidos para el desarrollo gonadal en esta especie está en el hígado.

Palabras clave: faneca, composición química de peces, *Trisopterus luscus*.

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INTRODUCTION

Seasonal changes in water, lipid and protein content of muscle and liver are known to occur in several fish species including gadoids, and these changes have been related to the growth of gonads and other processes associated with spawning (Muñoz and Herrera, 1959; Love, 1970; Dawson and Grimm, 1980; Dabrowski, 1982).

The bib, *Trisopterus luscus*, is an important commercial fish species, along the coast of the Central Cantabrian Sea region. Little is known about the chemical composition of this species and only one report, which presented the analysis of two muscle samples from the Galician coast population, has been published (Oliver, 1949). A detailed study of the changes in body components of *Gadus capelanus* Risso was undertaken by Muñoz and Herrera (1959); the relationship between body components and gonadal cycle in other gadoids has been described by Turuk, 1972; Lucena et al.,1980; and Krivobok and Tokareva,1972.

The reproductive biology of the bib on the Asturian coast has been described (Rodriguez-Merayo, 1991) and it is known that the period of reproductive activity is from December to April. The present study was carried out in order to examine whether there were seasonal changes in water, lipid, protein and ash levels in the bib, and whether any such changes could be related to the event of the breeding period. In this study the muscle and liver were analysed in order to estimate the mobilisation of reserves for the gonadal growth and reproduction.

MATERIALS AND METHODS

For this study 137 individuals of *T. luscus* of total length between 200-340 mm and 1-4 years old were obtained from commercial catches taken from the central region of the Asturias Sea (Northern Spain) from November 1987 to October 1988. From every catch, the length to the nearest mm and total and eviscerated weight (± 0.1 g), as well as the liver and gonad weights (± 0.1 g) were recorded for each fish. Each month, the entire ground muscle and liver of 5-6 fish of each sex were minced and homogenised, and random samples of muscle and liver were taken for chemical analysis for water, ash, protein and lipid content. The water content was estimated by 24 h drying at 80-90ºC to constant weight. Ash levels were determined by combustion in a muffle furnace at 500ºC. Lipid was determined by the Soxhlet method of total extraction with diethyl ether. Nitrogen was measured after digestion by Kjeldahl's method, protein being calculated as Nx6.25. Samples were analysed in duplicate.

TABLE 1. – Mean eviscerated weight (g); water, protein, lipid and ash contents (% wet weight) of muscle for males and females according to date of capture. (Values given are means ± standard deviations).

Date	Sex	n	Weight	Water	Protein	Lipid	Ash
18/11/87	male	5	195±24	79.17±0.10	18.12±0.08	0.91±0.02	1.45±0.26
	female	6	206±17	79.16±0.04	17.87±0.24	0.98±0.02	1.40±0.21
15/12/87	male	5	294±35	79.60±0.30	17.81±0.12	0.90±0.03	1.21±0.14
	female	6	312±18	78.50±0.30	18.78±0.19	0.95±0.03	1.18±0.10
18/01/88	male	5	271±47	78.76±0.56	19.12±0.08	0.84±0.04	1.27±0.15
	female	6	343±51	78.57±0.33	19.16±0.09	0.86±0.03	1.22±0.10
21/02/88	male	6	220±16	79.36±0.28	18.29±0.16	0.95±0.03	1.28±0.13
	female	6	214±10	80.37±0.10	17.35±0.29	1.08±0.08	1.18±0.10
11/03/88	male	5	287±46	79.10±0.12	18.72±0.31	0.73±0.02	1.29±0.13
	female	6	302±23	80.01±0.22	17.99±0.24	0.66±0.03	1.24±0.10
15/04/88	male	5	299±22	81.13±0.12	16.86±0.14	0.64±0.03	1.15±0.09
	female	5	266±19	81.71±0.24	15.79±0.20	0.79±0.02	1.11±0.10
16/05/88	male	6	301±19	80.15±0.12	18.01±0.15	0.65±0.03	1.27±0.10
	female	5	464±71	80.33±0.17	17.96±0.23	0.72±0.03	1.25±0.10
06/06/88	male	6	387±16	79.48±0.17	17.53±0.18	0.54±0.04	1.32±0.10
	female	6	274±17	79.60±0.96	17.03±0.25	0.69±0.04	1.29±0.10
07/07/88	male	7	333±46	79.36±0.22	17.91±0.15	0.68±0.05	1.21±0.07
	female	6	314±26	79.59±0.13	19.20±0.34	0.67±0.03	1.19±0.10
01/08/88	male	6	291±18	80.86±0.17	17.06±0.11	0.86±0.03	1.20±0.08
	female	5	316±32	80.90±0.19	17.05±0.22	0.82±0.02	1.01±0.08
15/09/88	male	6	256±90	79.94±0.05	17.95±0.15	0.90±0.04	1.17±0.07
	female	6	289±14	79.91±0.10	18.00±0.21	0.92±0.04	1.13±0.10
27/10/88	male	6	283±21	80.62±0.32	17.20±0.20	0.93±0.05	1.21±0.08
	female	6	296±28	80.25±0.21	17.30±0.29	0.91±0.03	1.20±0.10

Several authors have suggested that when body components are used as a percentage of dry matter, the results may be statistically suspect. Thus this problem has been deceived by transformation of the data with arcsin $\sqrt{x}$ (Zar, 1984). Mean water, protein, lipid and ash contents were examined to test for normality and homogeneity of variance with Kolmogorov-Smirnov and Cochran tests previous to analysis of variance. Two-way ANOVA was used to analyze the sex and date effects on muscle and liver water, lipid, protein and ash content.

Relationships between water, lipid and protein values in muscle and liver and the gonadosomatic index (expressed as the proportion of gonad weight to eviscerated body weight) were evaluated by means of correlation and regression analysis in order to determine the relationship between maturation and water, lipid and protein levels.

RESULTS

Muscle

In Table 1 the average data of the chemical composition of bib muscle are given. Water content oscillated between 78.5 % and 81.7 %; lipid levels ranged from 0.5 % to 1.08 %; protein levels were between 15.79 % and 19.20 %, and ash content was between 1.01 % and 1.49 %.

Water content of muscle (Table 1) was low in samples taken from November to January, and was highest in samples taken in April, especially for females. Apart from an increase between November to February, muscle lipid levels declined in both sexes from March (Table 1). The highest levels of lipid were achieved in late February but were considerably lower in April when the water content was maximal. Protein levels remained almost constant, with slight decreases in April (Table 1). Ash content presented a consistent pattern, rising to a maximum in November (Table 1).

There were significant differences (ONEWAY, $F_{2,410}$= 394788.7, p=0.0001) in the mean water, lipid and protein content of muscular biochemical components during the annual cycle.

There were significant differences between sexes and between months for all the variables considered (Table 2). Interaction effects (sex x month) were also significant. This indicated that the magnitude of these differences varied with time.

Analysis of correlation indicated that there was a clear inverse correlation between muscle water and muscle protein of male (r=-0.82; p<0.001, n=68) and female (r=-0.77, p<0.001, n=69) bib. There was a clear inverse correlation between lipid and eviscerated weight of male (r=-0.68, p < 0.001) and female (r=-0.43, p < 0.001).

The relationships between the eviscerated weight (W) and muscle water (Wt), protein (Pr) and lipid (Lp) of female was investigated by a step-wise multiple regression which gave the following equation:

TABLE 2. – Results of TWO-WAY ANOVA on mean eviscerated body weight, mean muscle water, protein, lipid and ash contents by sex and month.

Variable	Source of Variation	DF	SS	F	Signif of F
BODY WEIGHT	Sex	1	63.84	6.88	.010
	Month	11	280144.3	27.44	p<.001
	Sex x month	11	129791.2	12.71	p<.001
	Residual	113			
WATER	Sex	1	.000	9.45	.003
	Month	11	.012	138.96	p<.001
	Sex x month	11	.002	17.98	p<.001
	Residual	113			
PROTEIN	Sex	1	.000	4.88	.029
	Month	11	.012	148.92	p<.001
	Sex x month	11	.003	34.03	p<.001
	Residual	113			
LIPID	Sex	1	.000	51.03	p<.001
	Month	11	.007	167.98	p<.001
	Sex x month	11	.001	13.73	p<.001
	Residual	113			
ASH	Sex	1	.000	7.42	.007
	Month	11	.002	6.156	p<.001
	Sex x month	11	.000	.604	.882
	Residual	113			

$$W = -3001.36 - 3308.01 * Lp + 2943.6 * Pr + 2097.5 * Wt$$

(Multiple r=0.56, n=65, $p < 0.001$).

and for male:

$$W = 700.98 - 4668.11 * Lp$$

(Multiple r=0.68, n=66, $p < 0.001$).

Lipid was the most important explanatory variable for the female (β values were 0.37, 0.54 and 0.34 for lipid, protein and water respectively). For the male only lipid entered as predictor variable.

Gonadosomatic index (GSI) oscillated between 8.18 and 0.59 for females, and 1.39 and 0.13 for males (Table 3). In April it fell to a low level indicating that spawning had occurred. The mean GSI values were significantly different between sexes (TWO-WAY ANOVA, $F_{1,113}=99.687$, $p < 0.001$) and months ($F_{1,113}=18.238$, $p < 0.001$).

There was no correlation between muscle lipid and GSI in either sex. However, muscle water content of male was significantly correlated with GSI ($r = -0.49$, $p < 0.001$), and muscle protein content was also significantly correlated with GSI ($r = 0.63$, $p < 0.001$).

The relationship between the GSI and muscle water, protein and lipid of female was:

$$GSI = -25.378 + 64.901 * Pr$$

($r = 0.25$; $n = 67$; $p < 0.001$).

Thus, although the GSI may vary with the muscle protein content, no variation could be demonstrated in the water and lipid content of the muscle.

The relationships between the hepatosomatic index (HSI) and muscle water, protein and lipid content of females were:

$$HSI = 104.499 - 96.131 * Wt + 62.562 * Lp$$

(Multiple $r = 0.72$; $n = 67$; $p < 0.001$).

Muscle water was the most important explanatory variable (β values were 0.68 and 0.30 for water and lipid respectively).

TABLE 3. – Changes in mean liver weight (g), liver water and lipid contents (% wet weight), and hepatosomatic and gonadosomatic indices of males and females with month of capture. (Values given are means ± standard deviations)

Date	Sex	n	Liver weight	Water	Lipid	HSI	GSI
18/11/87	male	5	9.00±0.66	31.67±1.26	58.12±0.38	4.67±0.69	0.26±0.10
	female	6	11.15±2.20	28.26±2.68	61.80±0.57	5.38±0.62	0.62±0.19
15/12/87	male	5	4.53±1.47	35.55±0.95	49.07±0.24	1.52±0.36	0.41±0.11
	female	6	21.49±1.82	26.95±1.35	51.20±0.37	6.90±0.61	3.84±0.90
18/01/88	male	5	5.57±1.40	29.32±0.54	51.40±0.41	2.04±0.20	1.07±0.44
	female	6	14.56±1.75	24.71±0.46	61.45±0.60	4.29±0.54	8.18±2.61
21/02/88	male	6	4.11±1.13	53.21±1.10	30.71±0.41	1.86±0.47	1.39±0.42
	female	6	6.55±1.37	57.39±0.47	22.61±0.63	3.04±0.51	6.05±4.92
11/03/88	male	5	5.36±1.50	52.66±0.38	35.89±0.33	1.84±0.25	1.32±0.40
	female	6	9.66±2.71	59.56±0.97	24.10±0.35	3.17±0.68	7.45±2.59
15/04/88	male	5	2.74±0.48	75.93±0.18	10.53±0.35	0.91±0.20	0.29±0.15
	female	5	2.67±0.44	73.15±1.00	12.01±0.62	1.00±0.11	1.86±0.48
16/05/88	male	6	8.94±0.65	46.40±1.73	42.17±0.78	2.97±0.11	0.47±0.18
	female	5	15.54±6.82	46.59±1.52	40.61±0.40	3.27±1.18	1.85±0.50
06/06/88	male	6	13.05±4.08	48.39±1.11	46.21±0.54	3.37±1.03	0.35±0.14
	female	6	9.73±1.89	41.53±0.93	48.33±0.48	3.54±0.59	1.18±0.43
07/07/88	male	7	9.18±1.95	39.89±0.81	31.35±0.58	2.75±0.37	0.27±0.08
	female	6	11.99±3.21	41.65±0.43	33.90±1.03	3.78±0.77	0.88±0.24
01/08/88	male	6	10.24±2.41	38.89±0.72	54.70±0.57	3.52±0.72	0.26±0.02
	female	5	8.54±1.81	39.95±1.14	54.87±0.62	2.69±0.46	0.94±0.08
15/09/88	male	6	11.02±3.22	26.31±0.50	66.67±0.44	4.29±1.19	0.13±0.03
	female	6	14.87±2.82	24.12±0.52	68.10±0.44	5.13±0.79	0.59±0.28
27/10/88	male	6	9.51±1.86	32.53±0.31	64.14±0.74	3.35±0.50	0.27±0.05
	female	6	13.14±3.29	27.54±0.46	66.18±0.93	4.40±0.75	0.75±0.20

Liver

Water content of liver increased during late winter and spring, and reached the highest values in April (Table 3). Seasonal changes in lipid content of the liver showed virtually the opposite pattern, they decreased from January to March due to the development of the gonads, and reached minimum values in April, coinciding with the spawning season. A recovery of lipids was observed in late spring and summer, after spawning.

There were significant differences in liver water and liver lipid content, and hepatosomatic index (HSI) between males and females and between months (Table 4). Interaction effects (sex x month) were also significant.

A clear inverse correlation between water of liver and HSI was found in males (F=-0.58, p<0.001) and females (F=-0.76, p<0.001). There was a highly significant correlation between liver lipid and HSI of males (F=0.67, p<0.001) and females (F=0.62, p<0.001), but the correlation between liver lipid and GSI was not significant.

The relationship between the GSI and HSI of female and liver water and liver lipid gave the following equation:

$$GSI = 5.129 - 0.049 * Lp$$

(Multiple r= 0.27; n = 67; P < 0.001)

$$HSI = 14.523 - 0.169 * Wt - 0.081 * Lp$$

(Multiple r = 0.82; n = 66; P < 0.001).

Liver weight also varied seasonally (Table 3) remained relatively high in winter and autumn, but declined from February to April. There was a highly significant correlation between liver weight and liver water content of males (r=-0.42, p<0.001) and females (r=-0.66, p<0.001); and between liver weight and liver lipid of males (r=0.51, p<0.001) and females (r=0.52, p<0.001).

The relationship between the liver weight (Wli) and water (Wtli) and lipid (Lpli) of the liver gave the following equation for females:

$$Wli = 46.25 - 0.54 * Wtli - 0.28 * Lpli$$

(Multiple r=0.72, n=66, P<0.001)

and for males:

$$Wli = 2.55 + 0.12 * Lpli$$

(Multiple r=0.51, n=66, P<0.001)

TABLE 4. – Results of TWO-WAY ANOVA on mean liver weight, mean liver water and lipid contens and gonadosomatic and hepatosomatic indices for different sex and months.

Variable	Source of Variation	DF	SS	F	Signif of F
LIVER WEIGHT	Sex	1	499.36	79.90	p<.001
	Month	11	1207.01	17.56	p<.001
	Sex x month	11	856.05	12.45	p<.001
	Residual	113			
WATER	Sex	1	.011	88.99	p<.001
	Month	11	2.799	2040.63	p<.001
	Sex x month	11	.071	51.95	p<.001
	Residual	113			
LIPID	Sex	1	.000	6.28	.014
	Month	11	4.194	9869.45	p<.001
	Sex x month	11	.112	262.78	p<.001
	Residual	113			
GSI	Sex	1	174.93	99.69	p<.001
	Month	11	352.06	18.24	p<.001
	Sex x month	11	180.14	9.33	p<.001
	Residual	113			
HSI	Sex	1	42.74	102.97	p<.001
	Month	11	143.63	31.46	p<.001
	Sex x month	11	71.25	15.61	p<.001
	Residual	113			

DISCUSSION

Spawning of bib in the Asturian region takes place from December to April (Rodriguez-Merayo, 1991). Peak values of muscle lipid were attained in February (Table 1), at which time ovarian growth was also maximal (Table 3). During maturation of the gonad in bib, the muscle water content increases to the highest levels and muscle protein content decreases. This was also found in cod, *Gadus morhua*, by Love, (1970) who suggested that the decrease in protein and the increase in water content of the muscle were the results of gonadal growth taking place at the expense of muscle protein reserves.

The liver is the major storage depot in gadoids, therefore special attention was devoted to its study. The liver lipid level changes were delayed in relation to those in muscular lipid levels and the muscle lipid store proved to be very low relative to the lipid levels in the liver. Liver weight varied seasonally and was related to liver lipid and liver water contents. In cod, seasonal variations in liver weight have been reported by Jangaard *et al.* (1967). The liver lipid content in bib was at a maximun from September to January, before the spawning season (Table 3). By the end of the spawning period, the liver lipid levels of females decreased from the highest values in January to the values in April. Thus, liver lipid stores appear to be a critical factor for reproductive success, since their reduction probably reflects mobilisation in response to spawning. Ovarian growth occurs at the same time as the fish liver lipid decreases. Liver weight reduction occurred during the same period. It has been determined that liver lipid are mobilized for the ovary, and that lipid mobilization from muscle is two months later (Table 1). Liver and not muscle is the main source of lipid reserves of the bib, thus the bib can be classified as a "non-fatty" fish.

Differences between sexes have been observed during maturation. The male gonad is relatively smaller than that of the female. Thus, the male fish can restore their reserves of protein more quickly than the female (Table 1). The female fish have larger livers than males for the greater part of the year and the liver and muscle lipid reserve underwent higher decrease before and during spawning in females than in males.

The present results are in accordance with previous investigations in other Gadoid species such as *Gadus capelanus* (Vives and Suau, 1956 and Muñoz and Herrera, 1959), *Gadus morhua* (Love, 1960; Turuk, 1972; Krivobok and Tokareva, 1973 and Shatunovskii et al., 1975), and *Micromesistius poutassou* (Lucena *et al.*, 1980). The storage of lipids in the liver and an increase in both body and liver weights in the early period of maturation was also found in *G. morhua* (Shatunovskii *et al.*, 1975). An increase in liver lipids during the early ripening of the gonads, followed by a decrease, has also been noted in *G. morhua* (Turuk, 1972).

The seasonal changes in liver lipid reserves have been discussed by Vives and Suau (1956), who concluded that for *G. capelanus* changes were probably due to seasonal variations in food supply. In the case of bib, seasonal food changes have not been observed (Rodriguez-Merayo, 1991) and because of this do not have a direct effect. A good correlation between liver weight and liver lipid reserves in Baltic cod was found by Podrazhaskaya and Iarzhombek, (1970).

The high ash levels in November coincide with those observed in other fish (Herrera and Muñoz, 1957: *Sardina pilchardus*; Muñoz and Herrera, 1959: *Gadus capelanus;* Herrera and Muñoz, 1963: *Mullus barbatus*). These reserve stores are, probably, for future growth in spring. However, comparison of biochemical components must be made with caution, because variations in the quality of analytical data may be due to differences among species, the sampling season, size, age and other biological factors.

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