Research Article

Observation on the biochemical constituents of threadfin bream *nemipterus japonicus* during gonad maturation from suez gulf, red sea, Egypt

Abstract

The present study revealed that the biochemical constituent with respect to different maturity stages of *Nemipterus japonicus* fish is associated with reproductive cycle, storage and utilization of reserves. Gonadosomatic index *N. Japonicus* recorded high values (3.5 and 0.51) in May and low values (0.4 and 0.25) in February for female and male, respectively. The maturity stages was classified into 5 maturity stages as follow, 1) immature stage, 2) maturing stage, 3) mature stage, 4) ripe stage and 5) spent stage. The spawning season of *N. japonicus* extend from May to September. The biochemical composition in muscle, liver and gonad in both sexes are found higher in early stages of maturity and decreases during gonad maturation. The muscle had more fat and crude protein content during the immature stage. Early stages of maturation, hence offer greater food value in these species. With the advancement of maturity, a drop in the fat and soluble crude protein was observed, whereas a linear relationship with moisture was noted from immature to mature stage. The composition of muscle always demonstrated an inverse relationship with hepatic and gonadal composition. Highest crude protein percentage was in stage IV (72.0 ± 2.22) in ovary. The total crude lipid percentage of the ovary showed a gradual increase from Stage I (5.1 ± 0.47) to Stage IV (21.8 ±1.02).

Keywords: Biochemical composition, Crude lipid, Crude protein, Maturity cycles, *Nemipterus japonicus,* Gonadosomatic index, Suez Gulf

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Introduction

Threadfin bream *Nemipterus japonicus* is a species of the family *Nemipteridae* which has an economic importance in the Gulf of Suez. They are valued food fishes in many parts of the world and are caught commercially by hook and line and bottom trawl.[1] Kerdgari et al.[2] reported that *N. Japonicus* is a demersal fish; very abundant in muddy and sandy coastal waters of depths between 5 to 80 m. It has a wide distribution from Gulf of Suez, the Red Sea and eastern shores of Africa to the Philippines and Japan .[3] The present of this fish in the Mediterranean is evidently due to migration from the Red Sea via the Suez Canal.[4] Summary of the spawning time of *Nemipterus japonicus* in different regions (Table 1). The biochemical composition of the fish depends on the season, habitat and the type of food consumed by the fish. Moisture, fat, crude protein and minerals are the main components of fish meat. The analysis of the component is referred to a proximate composition. Crude protein content of fish varies not only in relation to species, but in relation to individuals of a same species.[5-8]

Table 1 Summary of the spawning time of *Nemipterus japonicus* in different regions

Source	Date of Spawning	Locality
At Waltair	December-February and June-July	Dan.[40]
In WS Hoar	In Summer	Sheridan .[26]
Kakinada	August-April(Peak February-December	Murty .[24]
Madras	June-March(Peak December-March)	Vivekanandan & James .[43]
Jizan area	Year round (peak November-May)	Bakhsh.[44]
Off Cochin	July-August	Joshi [41]
Gulf of Suez	September-May	Amin. [12]
The eastern Mediterranean	April – November (peak in July)	Elhaweet.[16]

In general, the biochemical composition of the whole body indicates the fish quality. Therefore, biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units compared to other species. Variation of biochemical composition of fish flesh may also occur within same species depending upon the fishing ground, fishing season, age and sex of the individual and reproductive status. The spawning cycle and food supply are the main factors responsible for this variation.[9,10] The present study was undertaken to study the biochemical constituents in different body tissues like muscle, liver and gonad and their variation in relation to sex and maturity cycle in *Nemipterus japonicus*.

Materials and methods

Total 230 specimens of *Nemipterus japonicus* were collected from the fish landing center at attaka Suez fish port during fishing season which extend from September 2012 to May 2013. Fresh fish samples of *N. Japonicus* were collected for biochemical analysis. To determine sexual maturity, gonads from males and females were weighed and morphologically examined according to Hajort scale (1910) with modifications in order to determine time of reproduction. Specimens of various maturity stages were stage I (immature), stage II (maturing and recovering spent), stage III (mature), stage IV (ripe), and Stage V (spent) of female and male were constitutes of muscle, liver and gonad.

Gonado-somatic index values for each month were used according to the following formula: GSI % = gonad weight (g) X 100 /Body weight (g)

For histological examination of gonads, a piece of male and female gonad at different maturity stages were fixed in Bouin solution, dehydrated in alcohol, embedded in paraffin and sections, 5-7 µ thick, were prepared. Sections were stained with haematoxylin and eosin. Slides were examined by Leica microscope. Different stages of maturity of each sex of *N. Japonicus* were collected and their total weight was taken. The weight of tissues was measured at the nearest 0.01 mg. Estimation of the moisture content was carried out by drying the pre-weighted wet samples of muscle, liver and gonad in a hot air-ovan for 24 hours until a constant weight was obtained. The difference between wet weight and dry weight was taken as the moisture content. Then the dried samples were finely powdered and stored in desiccators for estimation of biochemical constituents. Biochemical analysis for crude protein, crude lipid and carbohydrate were carried out using standard methods by using the standard methods.[11] All the values of biochemical components were expressed in percentage dry weight basis (%DWB). The caloric content was determined by multiplying the concentration of various components with conversion factors 4.15, 9.40 and 5.65 for carbohydrate, crude lipid and crude protein respectively. The caloric values were expressed as calories per gram (cal g-1 DWB). For each maturity stage mean value for five samples were taken.

Statistical analysis

The data on biochemical analysis of liver, ovary and body tissue for different maturity stages were analysed by one way ANOVA using the SYSTAT software version 7.0.1 and the significance was tested ($P < 0.05$). The analysis of variance was worked out for each biochemical parameter in muscle, liver and gonad to test significant changes (I) between different tissues at various stages of maturity and (II) between different stages of maturity in various tissues (Figure 1).

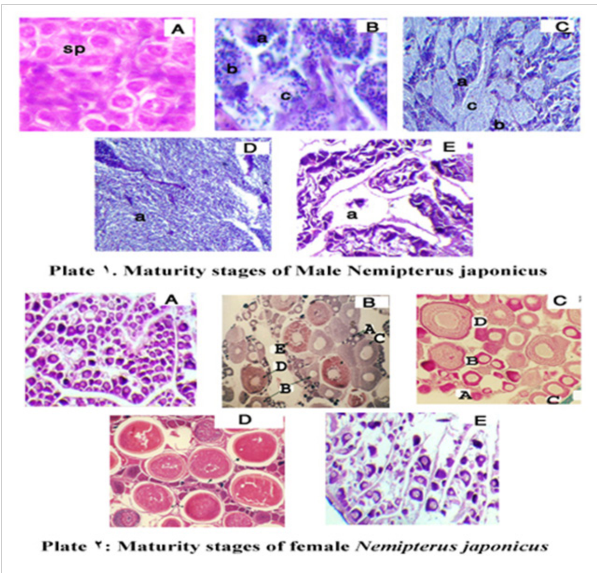

Figure 1 Photomicrograph of gonad developmental stages.

Plate 1 Male

Figure A: Cross section in testis of *N. japonicus* at immature stage showing spermatogonia cells (Heamatoxylen & Eosin X 40).

Figure B: Cross section in testis of *N. japonicus* at maturing stage showing (a) spermatogonia cells, (b) primary spermatocyte, (c) secondary spermatocytes. (Heamatoxylen & EosinX 20)

Figure C: Cross section in testis of *N. japonicus* at mature stage showing (a) primary spermatocyte, b) secondary spermatocytes, (C) sperm. (Heamatoxylen & EosinX Figure D. Cross section in testis of *N. japonicus* at ripe stage showing lobule full of sperms (a). (Heamatoxylen & EosinX 20)

Figure E: Cross section in testis of *N. japonicus* at spent stage demonstrating residual of spawning. (Heamatoxylen & EosinX 20).

Plate 2 Female

Figure A: Cross section in ovary of *N. japonicus* at immature stage have oocytes at perinucleolus stage, (Heamatoxylen & Eosin X20).

Figure B: Cross section in in ovary of *Nemipterus japonicas* showing matureing stage, early perinucleolus (A), vacoulized stage (B), cortical alveoli (C) and permaturation stage (D). (Heamatoxylen & Eosin X10).

Figure C: Cross section in in ovary of *Nemipterus japonicas* showing mature stage, ovarian wall (OW), perinucleolus stage (A), cortical alveoli stage (B) and permaturation stage (D). (Heamatoxylen & Eosin X10).

Figure D: Cross section in in ovary of *Nemipterus japonicas* showing ripe stage, (Heamatoxylen & Eosin X4)

Figure E: Cross section in in ovary of *Nemipterus japonicas* showing spent stage , (Heamatoxylen & Eosin X10)

Results

Gonadal somatic index and spawning season

Monthly average variation in GSI of both sexes of *Nemipterus japonicus* was quite apparent in Figure 2. Females had the maximum values in September (2.4) and May (3.5) then gradually decreased to reach a minimum value in February (0.87). The maximum values of GSI for males were recorded in September (0.44) and May (0.51) and reached minimum value in February (0.25). This means that spawning season extend from May to December.

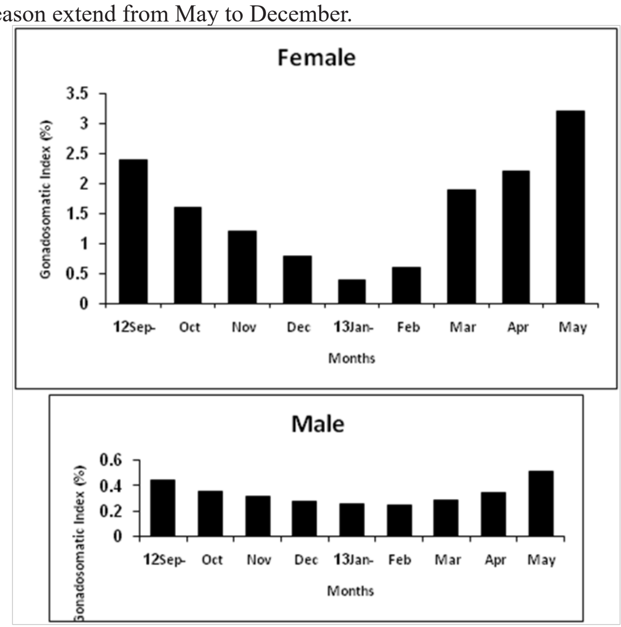

Figure 2 Seasonal variation of the average Gonadosomatic index of *N. japonicus* in the Gulf of Suez.

Macroscopic and Microscopic of maturity stage of gonad.

A)Male

Figure 2A: Macroscopic and Microscopic of maturity stage of gonad (Male).

Table: 2A

Maturity stage	Morphologically	Histologically
Immature	Testes are small, thin, and thread-like in shape.	The testes are small in size and contain spermatogonia which are the only cellular Components. (Plate 1A).
Maturing	Testes are semitransparent, dark gray, and extending upto less than ½ of the abdominal cavity.	The testes exhibit active spermatogenesis more than in the preceding stage (spermatogonia and primary spermatocytes). (Plate 1B).

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Table Continued...

Maturity stage	Morphologically	Histologically
Mature	Testes are large, opaque, well developed and white or ivory in colour. They are ribbon shaped, occupying about ⅔-¾ of the body cavity.	In this stage the cells of all stages of development could be seen spermatogonia, spermatides and sperms. (Plate 1C).
Ripe	They look like mature testes thick, flat but more swollen, creamy white, extending in the entire body cavity.	In this stage the parachute shape is broken and the testes appear filled with sperms. (Plate 1D).
Spent	Testes are shrunken, opaque and strap-like. They turned back upto ⅔ body cavity.	Residual spermatozoa and spermatogonia present towards the spermatic duct. (Plate 1E).

Figure 2B: Macroscopic and Microscopic of maturity stage of gonad (Female).

B)Female

Table: 2B

Maturity Stage	Morphologically	Histologically
Immature	The ovaries are narrow tube like bands and transparent in colour.	The most advanced oocyte is at the perinucleolus stage .the cytoplasm of the oocyte is strongly stained by heamatoxylin and a few nucleoli are observed in the nucleus. The diameter of the oocytes is smaller than 70 µm. (Plate 2A).
Maturing	The ovary is small, pink in colour and translucent.	The most advanced oocyte is at the cortical alveoli stage. Cortical alveoli, which are randomly distributed in the peripheral region of the cytoplasm, are found. The diameter of the oocytes is 65-290 µm. (Plate 2B).
Mature	The ovaries occupy 2/3 of body cavity, swell, reddish yellow in colour, eggs visible and numerous.	The most advanced oocyte is at the migration nuclear. Yolk globules increase in size and many oil droplets are situated around the nucleus located of the center of the oocytes. The nucleus migrates toward the animal pole. The diameter of oocytes 410-600 µm. (Plate 2C).
Ripe	Ovary is long and broad, filling all the body cavity and yellow in colour.	The most advanced oocyte reach the migratory and pre maturation. Oil droplets fuse with one another and become a single large oil globule located at the center of the oocyte. The diameter of oocytes is 630-900 µm. (Plate 2D).
Spent	Ovary is flaccid but not fully empty, deeper in colour.	Irregular convoluted ovigerous folds containing large atretic follicles and net-shape connective tissue besides few numbers of perinucleolus stage. (Plate 2E).

Biochemical changes of female during maturation

A) Muscle

The moisture content of the muscle was minimum (72.0±2.03 %) in immature stage and high (80.0±2.91%) in ripe which was significantly (P<0.05) higher among all the maturity stages, but decreased to (76.5±2.52 %) in the spent stage. Crude protein content in muscle gradually declined from (70.5±1.83 %) in immature stage to ripe stage (53.0±1.01 %) fishes produced insignificant (P>0.05) intermediate crude protein values and increased again (80.0±2.94 %) with higher significantly (P<0.05) when the fishes reached to spent stage. The maximum level (19.3±1.08 %) with significantly (P<0.05) highest of muscle fat in immature stage fishes declined to a minimum level (4.3±0.09 %) whereas significantly (P<0.05) lowest on the attainment of ripe maturity stage. The muscle carbohydrate content declined from immature stage (0.85±0.007 %) to low percentage in ripe maturity stage (0.70±0.005 %) which was not significantly different (P>0.05). Ash content of the whole flesh of all maturity stages could not produce any significant differences among each other. Ash content of the flesh decreased progressively from maximum value (1.57±0.083 %) where significantly (P<0.05) higher ash content was recorded in immature stage to minimum in ripe stage (1.32±1.01 %) (Table 3).

Table 3 Biochemical constituents (% DWB) in muscle of female *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture	protein	Lipid	Carbohydrate	Ash
I (Immature)	72.0 ± 2.03	70.5 ± 1.83	19.3 ± 1.08	0.85 ± 0.007	1.57 ± 0.083
II (Maturing)	74.0 ± 2.47	68.3 ± 2.07	17.3 ± 0.75	0.78 ± 0.006	1.45 ± 0.073
III (Mature)	77.0 ± 2.64	61.3 ± 1.74	10.6 ± 0.64	0.75 ± 0.005	1.40 ± 0.074
IV (Ripe)	80.0 ± 2.91	53.0 ± 1.01	4.3 ± 0.09	0.70 ± 0.005	1.32 ± 0.031
V (Spent)	76.5 ± 2.52	80.0 ± 2.94	6.2 ± 0.34	0.79 ± 0.007	1.46 ± 0.070

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

B) Liver

The highest moisture content (72.0±2.01 %) with high significant (P < 0.05) was recorded in immature fishes, which decreased progressively to 58.8±0.56 % as fish reached to mature stage then slightly increased in the ripe stage (60.8±1.64 %) and the spent stage (64.7±1.12 %), respectively. However, crude protein content showed a positive relationship with gonad maturation. Highest crude protein content was observed in ripe stage (73.0±1.64 %) and significantly (P<0.05) higher and lowest in immature stage (60.0±2.01 %), while crude lipid content of the liver showed positive relationship with ovarian maturation from stage I, the value was 8.9±0.61 % and increased progressively to highest value 19.9±1.04 % in stage III with higher significant (P < 0.005) and return to decrease in stages IV and V with values 14.33±0.66 and 13.4±0.72 %, respectively. Liver carbohydrate content produce insignificant (P>0.05) intermediate in stage I showed 4.3±0.075 %, which increased to 5.7±0.045 % in ripe stage and further decreased to 4.6±0.025 % in spent stage. Ash content increased with increasing maturity from 1.57±0.041 % in immature stage to 1.62± 0.056 % in mature stage followed with slightly steady decrease in ripe and spent stages which could not produce any significant differences (Table 4).

C) Ovary

The moisture content of the ovary showed a steady decrease from immature stage (82.5±2.97 %) which was significantly (P<0.05) to mature stage (72.0±2.12 %) and elevation in values 78.8±2.52 % and 77.0±2.47 % with low significantly showed in ripe and spent stages, respectively. Total crude protein content exhibited a positive relation with the maturation of gonads. In immature stage, it was 60.3±1.87 %, where as in ripe stage, it was 72.0±2.22 % with high significant (P<0.05). Total crude lipid content showed a positive relationship with a gradual increase in maturation from 5.1±1.87 % whereas significantly (P<0.05) lowest in immature stage to 21.8±2.22 % with high significant (P<0.05) in ripe stage but return to decrease with spent stage 13.3±1.47 %. Low carbohydrate and a significantly (P>0.05) lower content observed in stage I (3.5±0.048 %), increased to 5.0±0.048 % in mature stage with high significant (P < 0.05) and decreased in value to 4.1±0.051 % in spent stage. Also, ash content could not produce any significant differences among each stage where values increased from minimum content 1.45±0.027 in immature stage to maximum content 1.63±0.032 % in mature stage and returned to decreased to reach 1.56±0.047 % in spent (Table 5).

Table 4 Biochemical constituents (% DWB) in Liver of female *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture	protein	Lipid	Carbohydrate	Ash
I (Immature)	72.0 ± 2.01	60.0 ± 2.01	8.9 ± 0.61	4.3 ± 0.075	1.57 ± 0.041
II (Maturing)	64.0 ± 1.55	64.0 ± 1.55	10.2 ± 0.75	4.0 ± 0.064	1.58 ± 0.055
III (Mature)	58.8 ± 0.56	66.0 ± 0.56	19.9 ± 1.09	4.6 ± 0.068	1.62± 0.056
IV (Ripe)	60.8 ± 1.64	73.0 ± 1.64	14.3 ± 0.66	5.7 ± 0.045	1.60 ± 0.064
V (Spent)	64.7 ± 1.12	65.0 ± 1.12	13.4 ± 0.72	4.6 ± 0.025	1.60 ± 0.012

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

Table 5 Biochemical constituents (% DWB) in gonad of female *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture	protein	Lipid	Carbohydrate	Ash
I (Immature)	82.5 ± 2.97	60.3 ± 1.87	5.1 ± 0.47	3.5 ± 0.048	1.45 ± 0.027
II (Maturing)	75.0 ± 2. 45	61.0 ± 1.35	6.4 ± 0.55	4.7 ± 0.057	1.58 ± 0.035
III (Mature)	72.0 ± 2.12	70.0 ± 2.02	16.3 ± 0.82	5.0 ± 0.048	1.63 ± 0.032
IV (Ripe)	78.8 ± 2.52	72.0 ± 2.22	21.8 ±1.02	4.5 ± 0.052	1.59 ± 0.022
V (Spent)	77.0 ± 2.47	63.3 ±1.47	13.3 ± 0.97	4.1 ± 0.051	1.56 ± 0.047

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

Biochemical changes of male during maturation

A) Muscle

The flesh moisture content percentage gradually increased from 70.0±1.94 % in immature stag to 78.0±2.84 % in ripe stage fishes with high significant (P < 0.05). While crude protein content showing inverse relationship with gonad maturation where start with maximum value 70.3±1.84 % and significantly (P<0.05) higher in immature stage and minimum 54.3±0.74 % and insignificant (P>0.05) in ripe stage. Crude lipid content in the muscle was 14.8±1.84 % and significant (P<0.05) in immature stage and it decreased slightly to 3.4±0.74 % in stage IV. Even though the crude lipid content of muscle decreased from immature stage to ripe stage and increased to 5.6±0.97 % produce significantly (P<0.05) higher in spent stage. Muscle carbohydrate content declined from immature stage 0.80±0.007 % with high significant (P<0.05) to low content in mature stage fishes 0.63±0.005 % whereas less significant (p>0.05) and return to elevate 0.71±0.006 % and 0.74±0.005 % in ripe and spent stages respectively. Ash content could not produce any significant differences among each stages of the flesh decreased from 1.60±0.084 % in immature to 1.35±0.024 % in ripe stage (Table 6).

B) Liver

In the liver, moisture content showed an inverse relation with the gonad maturation. It decreased from 73.5±2.05 % with high significant (P<0.05) in stage I to 59.3±0.97 % with insignificant (P>0.05) in mature stage (Table 7). However, crude protein percentage showed a positive relationship with gonad maturation. Highest crude protein percentage and significant (P<0.05) was observed in ripe stage 65.0±1.45 % and lowest value and insignificant (P>0.05) in immature stage 55.0±0.75 %. Crude lipid content of the liver showed positive relationship with ovarian maturation. In immature stage, the value was 7.1±0.75 % whereas less significant and increased to 17.5±1.27 % with high significant (P<0.05) in mature stage. Liver carbohydrate content in immature stage showed 3.2±0.051 %, which increased to 5.4±0.097 % with high significant (P<0.05) in ripe stage and further decreased to 4.4±0.075 % and insignificant (P>0.05) in spent stage. Ash content of the liver increased from 1.5±0.075 % in immature to 1.63±1.45 % where significantly (P<0.05) higher in ripe stage.

Table 6 Biochemical constituents (% DWB) in muscle of male *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture %	protein %	Lipid %	Carbohydrate %	Ash
I (Immature)	70.0 ± 1.94	70.3 ± 1.84	14.8 ± 1.84	0.80 ± 0.007	1.60 ± 0.084
II (Maturing)	73.0 ± 2.07	67.2 ± 1.17	10.3 ± 1.17	0.73 ± 0.006	1.57 ± 0.072
III (Mature)	76.0 ± 2.42	59.4 ± 0.92	8.9 ± 0.92	0.63 ± 0.005	1.42 ± 0.032
IV (Ripe)	78.0 ± 2.84	54.3 ± 0.74	3.4 ± 0.64	0.71 ± 0.006	1.35 ± 0.024
V (Spent)	77.5 ± 2.67	59.0 ± 1.07	5.6 ± 0.97	0.74 ± 0.005	1.5 ± 0.071

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

Table 7 Biochemical constituents (% DWB) in Liver of male *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture	protein	Lipid	Carbohydrate	Ash
I (Immature)	73.5 ± 2.05	55.0 ± 0.75	7.1 ± 0.75	3.2 ± 0.051	1.50 ± 0.075
II (Maturing)	63.0 ± 1.21	59.0 ± 1.01	9.8 ± 0.91	3.5 ± 0.052	1.54 ± 0.061
III (Mature)	59.3 ± 0.97	61.0 ± 1.27	17.5 ± 1.27	4.7 ± 0.082	1.56 ± 0.064
IV (Ripe)	60.1 ± 1.05	65.0 ± 1.45	12.9 ± 1.09	5.4 ± 0.097	1.63 ± 0.075
V (Spent)	65.2 ± 1.74	60.0 ± 1.04	11.2 ± 1.02	4.4 ± 0.075	1.56 ± 0.054

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

C) Testis

The moisture percentage content of the ovary showed a steady decrease from immature stage (84.5±2.95 %) where significantly (P<0.05) higher to ripe stage (77.1±2.24 %), whereas the significantly (P<0.05) lowest followed by slightly increased 79.0±2.23 % in spent stage (Table 8). Total crude protein exhibited a positive relation with the maturation of gonads. In immature stage, it was 59.0±0.95 % with insignificant (P>0.05), whereas in ripe stage, it was 71.0±2.64 % where found to be significantly (P<0.05) higher. Total crude lipid content showed a positive relationship with a gradual increase from 5.08±0.095 % in stage I to in 20.7±2.040 % stage IV where produced significantly (P<0.05) highest. However, total carbohydrate content showed a fluctuating trend. High carbohydrate content observed in stage III (4.5±0.064 %), but started with 3.0±0.039 % in stage I and it was stable in stage IV and V with insignificant (P>0.05). The variations in the mean ash content in liver of the five stages were not significant (p>0 05).

D) Caloric content

Total caloric content of muscle, liver and gonad for the five stages of male and female are show in Tables 9 & 10. In male muscle, liver and testis, maximum caloric value in spent stage with high significant

(P<0.05) and minimum caloric value in ripe stage were noticed. In case of female, maximum caloric value in immature stage which was significantly (P<0.05) higher and minimum in spent stage were noticed for both muscle and liver tissues while in ovary, maximum caloric value in mature stage and minimum in spent stage were recorded where produced insignificant (P>0.05) intermediate values of their content among each other.

Table 8 Biochemical constituents (% DWB) in testes of male *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stage	Biochemical Constituents				
	moisture	protein	Lipid	Carbohydrate	Ash
I (Immature)	84.5 ± 2.95	59.0 ± 0.95	5.08± 0.095	3.0 ± 0.039	1.40 ± 0.045
II (Maturing)	79.0 ± 2.73	60.2 ± 1.35	7.09 ± 0.350	4.0 ± 0.043	1.53 ± 0.055
III (Mature)	78.5 ± 2.81	69.0 ± 2.01	15.6 ± 1.610	4.5 ± 0.064	1.55 ± 0.061
IV (Ripe)	77.1 ± 2.24	71.0 ± 2.64	20.7 ± 2.040	4.0 ± 0.031	1.56 ± 0.064
V (Spent)	79.0 ± 2.23	56.8 ± 1.23	12.4 ± 1.230	4.0 ± 0.029	1.52 ± 0.057

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

Table 9 Biochemical constituents (% DWB) in testes of male *Nemipterus japonicus* during maturity stages (Mean ± SD).

Stages	Tissue (Calg-1)	Protein (Calg-1)	Carbohydrate (Calg-1)	Lipid (Calg-1)	Total Caloric Value (Calg-1)
	Muscle	3361	134	584	4079
I (Immature)	Liver	2982	1287	2674	6943
	Testis	1385	642	3254	5281
	Muscle	3207	121	556	3884
II (Maturing)	Liver	3112	1209	2516	6837
	Testis	1382	487	3053	4922
	Muscle	3245	118	472	3837
III (Mature)	Liver	2716	1073	2402	6191
	Testis	1452	448	2704	4602
	Muscle	3373	126	604	4103
IV (Ripe)	Liver	2841	1244	3049	7134
	Testis	1500	404	3198	5102
	Muscle	3485	129	743	4357
V (Spent)	Liver	3062	1289	3132	7483
	Testis	1621	448	3417	5486

(% DWB): Percentage dry weight basis, (Mean±SD): Mean ± Standard Deviation, (n=5 samples for each stage)

Table 10 Caloric value of the biochemical composition of muscle, liver and gonad in different stages of maturity stages of female *Nemipterus japonicus* (dry weight basis)

Stages	Tissue (Calg-1)	Protein (Calg-1)	Carbohydrate (Calg-1)	Lipid (Calg-1)	Total Caloric Value (Calg-1)
	Muscle	3424	120	507	4051
I	Liver	2766	781	2288	5835
	Ovary	3168	271	2055	5494
	Muscle	3345	106	433	4884
II	Liver	2246	627	1424	4297
	Ovary	3365	315	2248	5928
	Muscle	3232	96	365	3693
III	Liver	2124	585	1501	4210
	Ovary	2589	249	2254	5092
	Muscle	2812	81	273	3166
IV	Liver	1620	550	1311	3481
	Ovary	2301	247	2278	8307
	Muscle	3359	112	471	3942
V	Liver	2175	607	1408	4190
	Ovary	2200	296	2362	4858

Discussion

In the present study, ripe fishes (stage IV) were observed from late April to December with the highest GSI in July, indicating a prolonged spawning season with one peak at warming months of the year in Egypt. *N. japonicus* in the Gulf of Suez had prolonged spawning season extending from September to February and from May to July.[12] Earlier studies have indicated that peak spawning was during September–April off Indian Coast.[13-15] Spawning activity of the thread fin bream populations along the Indian Ocean coast has a major peak during the post monsoon months from September to December. Amin.[12] and Elhaweet.[16] concluded that threadfin breams spawn over extended periods varies from one region to another according to some factors such as wind, current and not directly to temperature.

Histological maturation and G.S.I. values for the ovaries examined, all the fish collected in September to May were spawning with G.S.I about (3.2) for female and (0.51) for male having gonad in mature and ripe stages. Ovaries maturation was almost all at the oil droplets stages. The presence of oil droplets among oocytes in the maturation stages suggests that, spawned eggs are pelagic this suggestion is confirmed with results obtained from Takashi & Robert.[17] From October to January a decrease in oocyte development occurred, this means complementation of spawning season and the G.S.I. values decreased. The appearance of ovulatory follicles was observed through the period from December to February and suggests the end of spawning season. Peaks in spawning occur in September and May months based on the highest G.S.I values and the highest occurrence of ovaries in the maturation stages. It is considered that spawning activity encompasses the time period from May to September in the summer season this period is a closed fishing season in the Suez Gulf. Histological observations of ovarian development reported by Takashi & Rober.[17] and Ramadan.[18] were similar to our observation, maturity stage are applicable for discrimination between the different condition of the gonads whether in the female grow larger towards the spawning season.

Moisture is a major constituent in animal body which plays an important role in regulating osmotic function. It also serves as a medium by which nutrients and biochemical constituents are transported to various organs. The amount of moisture in fish is higher than that of all other higher vertiberates. In *N. Japonicus* flesh moisture percentage gradually increased from immature to the ripe fishes. Many authors reported that the water level of the muscle tissue increases with maturation. In the liver, the highest water content was recorded in immature fishes, which decreased as fish reached maturity and increased in the spent stage. The advancement towards maturity was associated with a very rapid fall of moisture in the ovaries and the lowest values coincided with peak ripeness as observed by Craig et al.[19] in perches. It was also found that in *N. japonicus*, the water content in the gonad generally showed a slightly higher value for the males where as in the muscle of the females had higher values. Masurekar & Pai .[20] noticed fluctuating muscle water content during the maturation of gonads in *Cyprinus carpio*. In other side Rao & Krishnan .[21] demonstrated that moisture content decreased in the ovary lines and muscle with the imaturation of gonads.

The hepatic fat content in *N. japonicus* gradually increased from immature to mature stages fishes. But then, it showed a decline during ripe and spent stages in both the species. Highest values of liver fat preceded peak ripeness of gonads and with the onset of spawning, a drain in the liver fat seemed to occur. The advancement of maturation was accompanied by increase in gonadal fat, reaching maximum values during ripe stage (Stage IV). Utilization of the stored crude lipids for the growth of sexual cells and energy metabolism had been reported by several authors.[22,23] The decline in muscle fat from immature to ripe in *N. japonicus* fishes indicates that the buildup of fat occurs first in the muscle and was perhaps subsequently diverted to gonads. The depletion of liver fat from mature to spent fishes suggests that the utilization of muscle fat alone did not fulfill the demands of gonadal maturation and hence liver fat was also utilized at final stages of maturation.

Murty.[24] observed minimum liver total crude lipid content with the progress of gonadal maturation in *Tilapia nilotica* and *Sparus auratus*. Kozlova.[25] reported utilization of crude lipids in the liver of pelagic sculpins for gonadal development and its subsequent depletion after maturation. The total crude lipid content of the liver was observed to have decreased with the maturation of gonads of steelhead trout.[26] and red drum, *Sciaenops ocellatus* [19] In common dentex (D. dentex), variation of liver crude lipid levels with the maturation of gonads was noticed.[27]

The maturation and the enrichment of gonads in crude lipid coincided with a decline of the crude lipid content in muscles and liver. The production of very large numbers of gametes, particularly eggs, during the relative short period of reproduction is very energy intensive .[28] Jobling .[29] stated that although the energetic investment in eggs is greater than that in sperm, the energetic costs of engaging in reproductive behavior may be markedly higher for the males, and the overall investment in reproduction may be similar for the two sexes. Zaboukas et al.[30] reported that the more intense reduction in the crude lipid content in the somatic tissues of females compared to males indicates that the reproductive cost is higher in females than in males in *Sarda sarda*.

At the time of maturation of gonads and spawning, crude lipid in fish is utilized mainly for three purposes, first) as endogenous source of energy for sustaining the fish, since most of them are known to abstain from feeding during spawning and for increased muscular activity of fish that have spawning migratory behavior, second) for the synthesis of generative materials (eggs and sperms) and yolk deposition and third) for the synthesis of steroid hormones.[31]

The changes in fat of in all the three tissues showed an inverse relationship with the moisture content in the nemipterids. This relationship has been noted in various other fishes and also in nemipterids itself by Krishnaveni .[23] Earlier works in *Sparus auratus*, pelagic sculpins [25] However, muscle crude lipid content increased with the maturation of gonads in *C. Carpio* [32] and in the common dentex .[27]

The crude protein content in all the three tissues of both male and female *N. Japonicus* followed a similar trend as that of fat, during the maturity cycle. The crude protein content in muscle gradually declined from immature to ripe fishes and increased again when the fishes reached spent stage. The crude protein content in liver increased from immature to ripe fishes. The increase in the crude protein content of liver and gonads toward ripeness in nemipterids could possibly be due to the increased feeding during maturation of these fishes. The decline in soluble liver crude protein from mature or ripe to spent stage was evident in both sexes. Expenditure of soluble liver crude protein for the purpose of germ building at later stages of maturation has been observed by Brown & Murphy .[33]

In both the sexes the highest crude protein content level in gonads was recorded in mature and ripe fishes. Its steep rise in the gonads of maturing or mature fishes indicated a peak period of synthesis and mobilisation of soluble crude proteins as gonad build up advances. An inverse relationship was discernible also between moisture and crude protein content in all the tissues. Medford & Mackay .[22] showed that muscle crude protein and crude lipid of the northern pike, Esox lucius were high before spawning and low after spawning. This could be due to the fact that these constituents might have been utilized for gonadal development. During the prespawning period, change occurs in the endocrine system that monitors supply of nutrients to gonads from all parts of body including liver and muscle [34,35]

Similar observations in other teleosts were also made by Nuriyal & Singh.[36] and Sivakami et al. [32] Also, they reported that maximum crude protein content in the ripe fishes and the minimum in spent and early maturation stages. Also, he demonstrated that crude protein content can be correlated with the stages of maturity and spawning, with high values when the gonads are ripe. In the animals studied, gonad crude protein was the highest and muscle crude protein was the least. Crude protein was the highest in the prespawning stage during the monsoon period which may be due to its ready supply by the liver .[37] The high content of crude protein values in the gonads may be due to the fundamental nitrogen demands required for maturation.[38] Venkatesan et al.[31] reported that crude protein level in the butterfish muscle of female decreased from maturing stage to spent stage as the maturation of various advanced and in male crude protein content decreased from immature stage to ripe stage indicating utilization of crude protein for the development of testis.

The muscle carbohydrate declined from immature stage to mature stage fishes. This may be possibly due to the conversion of muscle fat into carbohydrate through glyconeogenesis to maintain an adequate glucose level, necessitated both by the greater muscular activity and maturation of the gonads, as evidenced by the decrease of muscular fat during the ripe stage. In nemipterid liver the carbohydrate percentage increased from immature to ripe stage and declined during spent stage. Bligh & Dyer .[39] concluded that, though much of the energy for fishes comes from glyconeogenesis, a greater vigour for this mechanism has been found in the males, and the onset of spawning causes expenditure of crude lipid from the body of females and glycogen from the males.

The gonadal carbohydrate content which increased from immature to mature fishes decreased during ripe and spent stages. A possible explanation at this juncture would be that when fat and crude protein has accumulated in the gonad, the role of carbohydrate becomes negligible. However, Dan.[40] and Joshi .[41] report that glycogen and glucose both have been found to accumulate in the ovary during maturation. But the changes in the carbohydrate reserves of the fish seem mostly to reflect the requirements of the developing ovaries. The carbohydrate percentage did not exhibit any distinct relation to the moisture content in general, but a slightly inverse relationship in gonads could be observed. Rao & Krishnan.[21] observed that carbohydrate content in liver showed high fluctuations with the maturation of gonads. In the nemipterids studied ash content of the flesh decreased from immature to mature or ripe stage but increased in the liver and gonad. The decrease from ripe to spent gonads indicates the withdrawal and utilization of minerals for egg build up. Another probable reason could be that the Nemipterus feed maximum during mature and ripe stages. The ash content showed inverse relationship with the moisture percentage in the present study. Maturation was accompanied by an increase in the water content of the muscle. The water content in the muscle, liver and gonad showed an inverse relationship with the ash content in male nemipterids. There is little information on the energetic costs of reproduction in *N. japonicus.* Studies on other families of marine and freshwater fishes suggest, however, that the energetic costs associated with reproduction are significant and can have important consequences affecting growth and mortality rates after sexual maturation .[42]

Jobling .[29] believes that somatic and gonadal growth can be considered to be in competition for limited resources, and a decrease is commonly seen in somatic growth rate when fishes mature. In male muscle, liver and testis, maximum caloric value in spent stage and minimum in ripe stage were noticed. In case of female, maximum caloric value in immature stage and minimum in spent stage were noticed for both muscle and liver tissues while in ovary, maximum caloric value in mature stage and minimum in spent stage were recorded. The present study revealed that the utilization of biochemical constituents with respect to different maturity stages of *N. Japonicus* fish is associated with reproductive cycle, storage and utilization of reserves. Moisture showed a negative relationship with crude lipid and carbohydrate content of muscle, liver and gonad irrespective of their development stages. The biochemical composition in muscle, liver and gonad in both sexes are found higher in early stages of maturity and decreases during gonad maturation.

Acknowledgments

None.

Conflicts of interest

None.

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