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Lipid Changes in Relation to Maturation and Spawning of Tropical Double Spotted Queenfish, *Scomberoides lysan* (Forsskål, 1775)

[1]S. Sutharshiny, [2]K. Sivashanthini and [2]W.S. Thulasitha
[1]Department of Fisheries,
[2]Department of Zoology, University of Jaffna, Jaffna, Sri Lanka

Corresponding Author: K. Sivashanthini, Department of Zoology, University of Jaffna, Jaffna, Sri Lanka Tel: 00 94 21 2218199 Fax: 00 94 21 2222685

ABSTRACT

The present study was undertaken to understand the lipid changes in gonad, muscle and liver tissues of tropical double spotted queen fish, *Scomberoides lysan* (Family: Carangidae) in relation to sexual maturity and spawning. Cholesterol (CS), phospholipid (PL) and triacylgycerol (TAG) were determined in gonad, muscle and liver tissues with respect to maturity stages of both sexes as well as months. Fish were periodically caught from waters around Sri Lanka throughout the year 2010 to 2011. Fish length, weight, sex and maturation status were recorded. Content of CS, PL and TAG in gonad, muscle and liver tissues were determined at the laboratory. The values of CS, PL and TAG in the ovary increased to 2-5 fold throughout the ovarian maturation and decreased to 2-8 fold after spawning, whereas in liver and muscle tissue, increased up to maturation and decreased during spawning. Similar dynamics was recorded in males. The main lipid constituents in the liver and muscle of mature fish were TAG and PL, respectively. Lipid constituents in gonads showed higher value, whereas muscle and liver showed lower value in June and September, which represent the spawning time of *S. lysan*. It has been concluded that the values of lipid in tissues of *S. lysan* influence the cycle of maturation and time of spawning. This new information can be used for the determination of the fishing season for *S. lysan,* when it is not reproductively active and has high nutritional value in terms of lipid. The knowledge gained on CS, PL and TAG in different maturity stages of *S. lysan* can also be utilized in broodstock diet formulation in the future culture trials of *S. lysan*.

Key words: Cholesterol, phospholipid, S*comberoides lysan*, triacylglycerol

INTRODUCTION

In fish, lipids are known to be an important energy source for reproduction, since large amounts of lipids are required both for female egg production and for male breeding activities, such as enhanced swimming activity, competition, courtship, parental care and nesting (Goda *et al*., 2007; Ebrahimnezhadarabi *et al*., 2011).

When a spawning migration is involved, the adult fish generally deplete all their reserves and die after spawning as in the case of Sockeye Salmon (*Oncorhynchus nerka*) (Hinch *et al*., 2006) and eel (*Anguillia anguillia*) (Fricke and Kaese, 1995). If no migration is involved, fish are capable of building their energy reserves completely after spawning.

The lipid storage tissue varies depending on the fish species; some species deposit in liver (most gadoids; Alonso-Fernandez and Saborido-Rey, 2012) while some in muscle (Antarctic fish; Clarke *et al*., 1984) and some in both liver and muscle (Jezierska *et al*., 1982; Hedayatifard and Yousefian, 2010).

Fluctuation of lipid classes in gonad, muscle and liver of adult fish is directly associated with sexual maturity and spawning of fish (Mourente *et al*., 2002; Huynh *et al*., 2007). Knowledge gained from lipid changes in different tissues of species would be helpful to understand the physiology and ecology of that species. Due to higher investment of resources for reproduction, lipid reserves in liver and muscle are mobilized and transferred to the gonad during maturation and spawning (Zaboukas *et al*., 2006; Sutharshiny and Sivashanthini, 2011a; Singh *et al*., 2012). Further, variations in lipid composition in fish tissues depend on environmental conditions (Lund *et al*., 2000) and seasonal variations (Kandemir, 2010).

The length of the spawning season and spawning frequencies varies greatly between species. Some species for example *Salmoniform, Atheriniform* and *Tetraodontiform* have a marked seasonal periodicity in gonadal maturation (Taylor, 1984), while species of *Blennius pholis* has ripe ovaries throughout the year (Qasim, 1957). Some fish spawn only once a year or once in their lifetime (e.g., most *Oncorhynchus* spp. and *Anguilla* spp., De Vlaming, 1983) while others spawn several times a year for example Black and White bream (Jacques and Patrick, 2003), *Latris lineate* (Bransden *et al*., 2007) and *Scombroides lysan* (Thulasitha and Sivashanthini, 2013).

Lipids are complex classes of compounds, can be broadly divided into two groups, one is polar lipids composed principally of phospholipids and the other one is non polar lipids composed principally of triacylglycerols and cholesterol (Tocher, 2003). These components affect the biochemical processes of organism at different level. Phospholipid is the main lipid of cellular membranes and important constituents of egg yolk in fish (Johnson, 2009). It can also be an important source of energy (fatty acids) in fish, particularly during embryonic and early larval development in species that produce phospholipid rich eggs (Tocher, 1995). Triacylglycerol is the major energy storage form in fish (Shulman, 1974) and stored in liver, muscle and mesenteric fat (Sheridan, 1994). Cholesterol is a precursor for the steroid hormones and bile acids (Scott, 1987) and used for additional cellular functions in the testis (Sharpe *et al*., 2006).

The Double spotted queenfish (*Scomberoides lysan*) is a tropical fish and broadly distributed throughout the Indo-Pacific region (Froese and Pauly, 2010; Varghese *et al*., 2011). It is an economically important food fish in Sri Lanka. The species is popular for dry fish production with export demands and especially consumed by mothers during pregnancy and immediately after delivery. Thus, it is highly prized, continues to maintain a high market demand and marketed preserved, dried or salted (Sutharshiny and Sivashanthini, 2011b; c) and hold an important position towards the economy of the fisheries of Sri Lanka.

Though there are several studies on lipid dynamics in different tissues related to reproduction were recorded for temperate fish species (Fiorin *et al*., 2007; Lloret *et al*., 2008) only few studies are available for tropical fish species (Arrington *et al*., 2006; Hiroaki, 2012; Ovie *et al*., 2007; Talat *et al*., 2006) especially for carangids (Ramadan, 2002; Assem *et al*., 2005). Few research works related to lipid composition of fish were carried out by different authors in Sri Lanka (Anas *et al*., 2009; Thilakarathne and Attygalle, 2009; Ubhayasekera *et al*., 2012). However no studies were performed on lipid changes in *S. lysan* and therefore the present study on variation in lipid classes of *S. lysan* is the first pilot study in Sri Lanka.

The knowledge gained from the present study on lipid changes of *S. lysan* based on lipid class constituents-cholesterol, triacylglycerol and phospholipids can be considered and applied in the future studies, contributing to economic and health development and sustainable management of *S. lysan* in Sri Lanka.

MATERIALS AND METHODS

Sample collection: Regular field visits were made once a month to the landing centers at Jaffna, Trincomalee, Mannar and Puttalam (Fig. 1). From the landed marketed fish, size selective samples were collected monthly from January 2010 to December 2011 with the assistance of fishermen co-operative society's Union of each landing site. The fish samples collected were actually caught mainly by 17.78 cm 21 ply mesh size, drift nets used particularly for queen fish (Katta valai). Fish samples were also collected from the by catch species caught using 6.35 and 8.89 cm mesh size drift net and seine net. Immediately after collection, fish were chilled before freezing (Graham *et al*., 1992) and brought to the laboratory in an ice box (Giostyle, Ole 25; Italy).

Morphometric analysis: Fish were allowed to thaw slowly and Standard Length (SL) was determined using measuring tape to the nearest 0.1 mm and Body Weight (BW) was measured using top loading balance to the nearest 0.01 g before conducting lipid analysis.

Sex determination: Sex and gonad maturity stages were determined for each specimen using macroscopic examination of gonad and recorded.

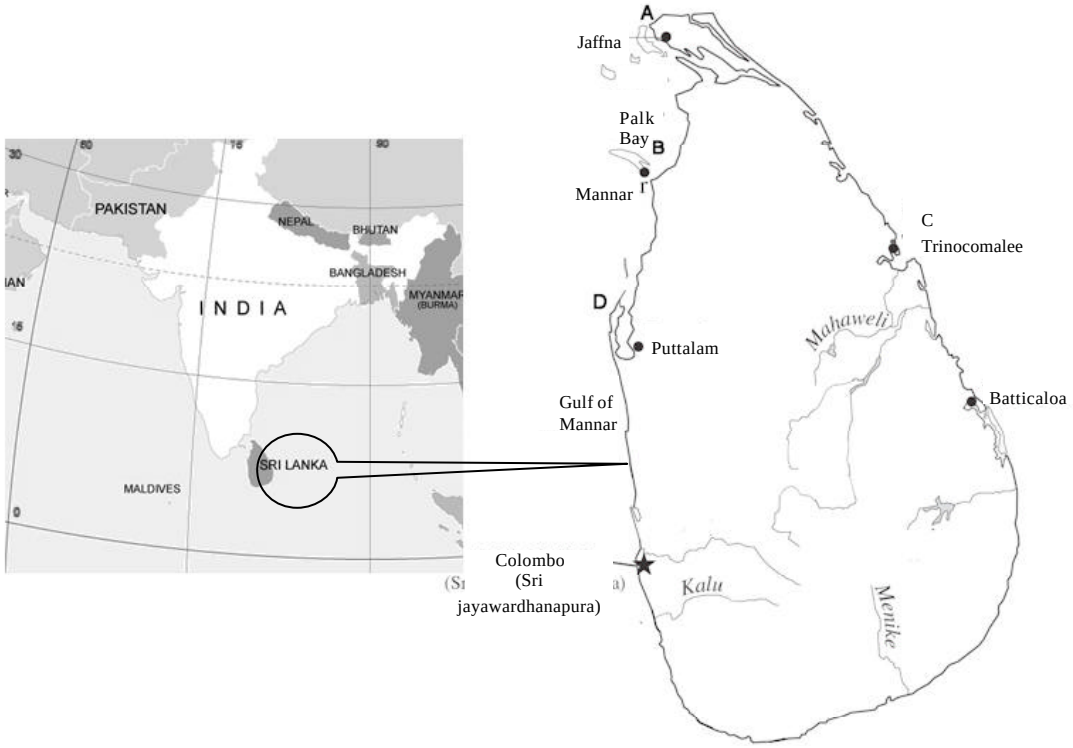

Fig. 1: Sampling stations of *S. lysan*. A: Jaffna, B: Mannar, C: Trincomalee and D: Puttalum

Tissues analysis: Fish were dissected and whole gonad and liver were removed and weighed using an electronic balance (OHAUS; USA) to the nearest 0.01 g. The muscle tissue from dorsal side that is directly under the dorsal fin and well above the lateral line was removed. Tissues were dried in an Oven (YCO-010; Germany) at 60°C for 24 h. The tissues were covered with filter paper to prevent accidental weight loss of lipid from tissues and to stop droplets erupting out of the container. The dried tissues were reweighed and ground twice in an electric grinder (Preett XT- 97; India).

Total lipid extraction: Total lipid in each tissue was analysed according to the Bligh and Dyer (1959) method. All chemicals were purchased from standard sources Sigma chemical company, USA. A weight of 10 g dried tissue powder was homogenized with 200 mL of chloroform/methanol mixture that prepared as in the ratio 2:1 (v/v). After dispersion, the whole mixture was agitated for 20 min at 2000 rpm in vortex mix (Karl Hecht KG; Germany) at room temperature. The whole mixture was filtered (funnel with a folded filter paper; Diameter-11 cm). The solids on filter paper were washed with 40 mL of distilled water, then the fluid mixture was vortexed for 1 min and centrifuged (Sigma; Germany) at low speed (2000 rpm) for 10 min to separate the two phases and allowed to stand. A biphasic system was obtained. The upper phase was siphoned, the lower chloroform phase containing lipids was filtered off and the water was removed from the extract by passing it through a folded filter paper containing anhydrous sodium sulphate. The interface was rinsed twice with methanol/chloroform (1:1 v/v). The lower phase containing individual lipids were recovered after evaporating under vacuum in a rotary evaporator (1 KA HB 10 basic; Germany). The dried lipid extracts with a small volume of chloroform-methanol mixture in Kjldhal flask were left to evaporate in the fume chamber. The resulting extract of total lipid was stored in a sealed vial at -20°C for further analysis.

Lipid class analysis: Cholesterol (CS) (Zlatkis *et al*., 1953), phospholipid (PL) (Zilversmit and Davis, 1950) and triacylglycerol (TAG) (Foster and Dunn, 1973) in different tissues were estimated. Standard curve for each lipid class constituents was plotted using the attached LABOMED, INC software in UV Visible spectrophotometer (LABOMED, UVD-3000). The concentrations of lipid classes in tissues were quantified.

Cholesterol analysis: A weight of 0.1 g extracted lipid was taken. Five milliliter of ferric chloride (in acetic acid) reagent was pipetted into lipid and mixed. Then 3 mL of concentrated sulphuric acid was pippetted into it, mixed again and allowed to stand for 20 min. 0.1 mL of glacial acetic acid was used for blank. The concentration of CS in tissues was read against the blank at 560 nm.

Phospholipid analysis: A weight of 0.1 g extracted lipid was taken into a 150 mL of kjeldhal flask and 1.0 mL of 5 N sulphuric acid was added to digest the lipid in a digestion rack (Sigma; Germany) till the appearance of light brown colour. Three drops of concentrated nitric acid were added to flask and continued the digestion till the brown colour changed into colourless. The Kjeldhal flask was cooled. 0.1 mL of distilled water was added to it and heated in a boiling water bath for 5 min. 1.0 mL of ammonium molybdate and 0.1 mL of amino-2-naphthol-4-sulphonic acid were added and it was transferred to 5 mL volumetric flask and total volume was made upto 5 mL with distilled water. Distilled water was used for blank. The concentration of PL in tissues was read against the blank at 660 nm within 10 min.

Triacylglycerol analysis: A weight of 0.1 g extracted lipid was taken. Four milliliter of isopropanol was added and mixed well. 400 mg of washed alumina was added. The mixture was placed in a mechanical rotator for 15 min and centrifuged. Two milliliter of supernatant was transferred into 15×100 mm of screw-capped tubes. A volume of 0.6 mL of potassium hydroxide was added into supernatant solutions, stoppered and incubated at 70°C for 15 min. Tubes were allowed to cool. 1 mL of metaperiodate solution and 0.5 mL of acetone reagent were added and mixed well; stoppered and incubated at 50°C for 30 min. Tubes were allowed to cool. One milliliter of distilled water was used for blank. The concentration of TAG in tissues was read against the blank at 405 nm.

Data analysis: All data were statistically analyzed by Micro soft Excel (Version 2007) and STATISTICA Soft ware (Version 6; Statsoft Inc.,Tulsa, USA). The data were checked for normal distribution by one-sample kolmogorov-smirno test and the variances were tested by the Levene's test for homogeneity. Lipid class concentrations in tissues were compared among gonad maturity stages as well as months. Lipid class contents in tissues were first analyzed by one way Analysis of Variance (ANOVA). When the results of the one way ANOVA show the mean values of the samples are significantly different, the ANOVA was followed by Post hoc comparison of means and Duncan's Multiple Range Test (DMRT) using STATISTICA 6.0 software. The level of statistical significance was set at $p<0.05$. Monthly analysis of lipid class content in different tissues was conducted only for adult fish (maturing stage to spent stage), which was collected throughout the year except January, November and December for female whereas March, November and December for male. Monthly lipid class data in different tissues for both years were pooled together and the average values for each month were computed.

RESULTS

One thousand four hundred and nineteen fish samples ranging from 10.7 to 67.8 cm in SL were examined and BW of individuals ranged from 21.10 to 2925.00 g. Reproductive status of individual fish was examined and the gonad developmental stages were classified as immature unsex (stage I), immature (stage II), maturing (stage III), mature (stage IV), spawning (stage V) and spent (stage VI) (Table 1).

Table 1: Macroscopic features of different gonad maturity stages (GMS) in *S. lysan*

Gonad maturity stages	Female	Male
Stage I	No differentiation of the gonad	No differentiation of the gonad
Stage II	Small ovaries, pinkish to translucent in colour with tapering ends. 25 to 35 mm in length	Small strap/thread like opaque testis with smooth appearance
Stage III	Flattened ovaries with pink colour. Oocytes are not visible externally and 30-100 mm in length	Larger than stage II, milt produced when squeezed
Stage IV	Rounded ovaries; yellow to orange in colour. Small oocytes can be visible through ovarian wall and 70-120 mm in length	Large opaque, bone colour testis. Exterior dorsal blood vessel are present and prominent
Stage V	Large, rounded and yellow colour ovaries with visible oocytes. Blood capillaries are also visible. Eggs may be released when pressure applied and 100-150 mm in length	Testis is large in size, but more have swollen with larger exterior blood vessels. Milt is released with little or no pressure on the abdomen or no pressure on the abdomen or when the tests is cut.
Stage VI	Ovaries are severely shrunken, flaccid, reddish yellow to grey in colour with large lumen and 100-130 mm in length	Testis is small and shrunken

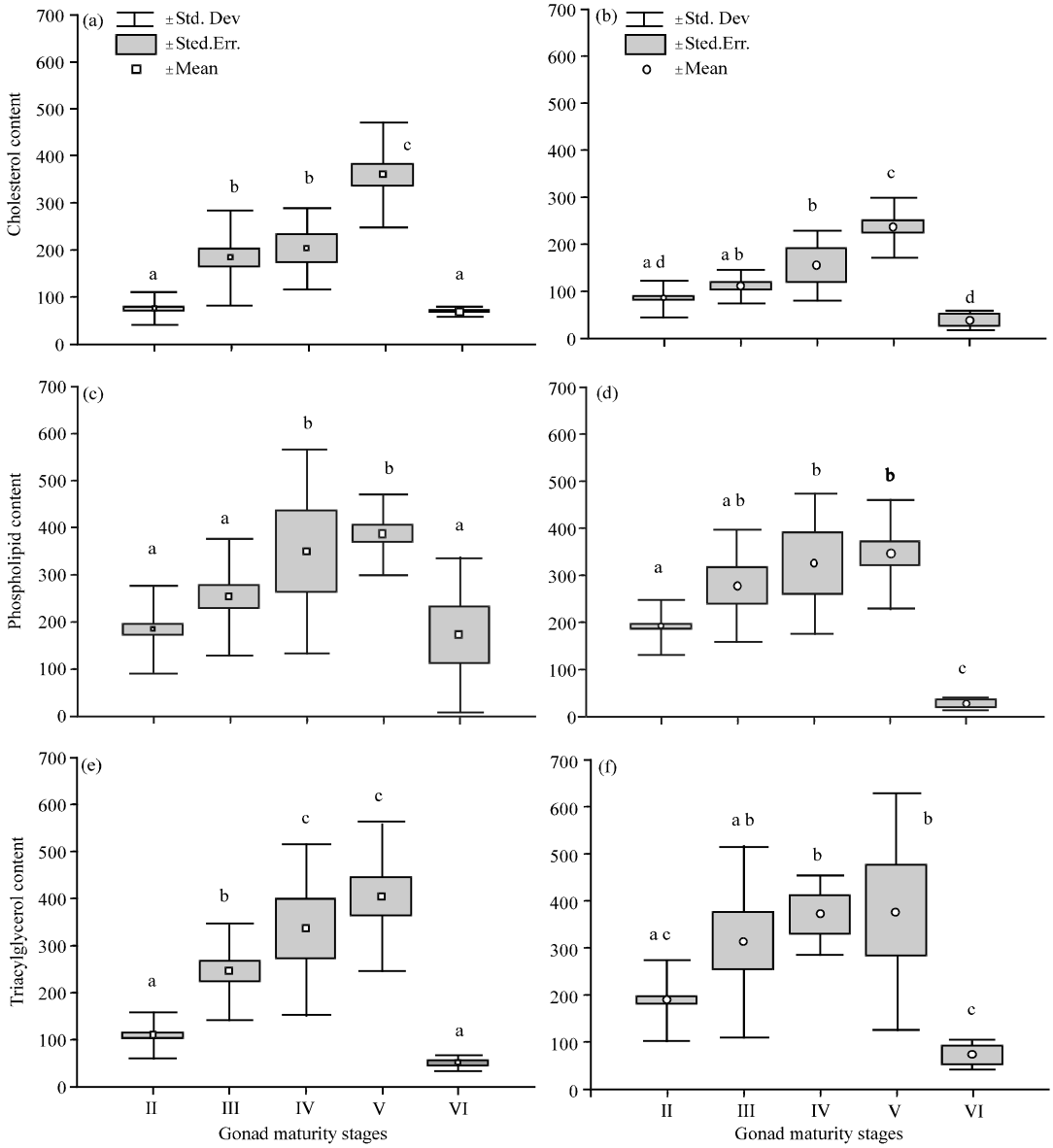

Fig. 2(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g^{-1}) in gonad tissue of *S. lysan* in different gonad maturity stages, II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent. a, c and e-Gonad in female; b, d and f-Gonad in Male. Mean values for each gonad maturity stages with the common letters indicate not significantly difference (p>0.05)

Lipid changes in tissues with gonad maturity stages

Lipid class content in gonad tissues: The changes in lipid content of tissues for gonad maturity stages were analyzed for the entire data set (2010/2011). The amounts of CS, PL and TAG in the ovary increased throughout the ovarian maturation (stage I-V).

CS content in ovary showed 5 fold increase (p = 4.57E - 05) from stage II to stage V while 5 fold decrease (p = 2.86E-05) from stage V to VI (Fig. 2a). CS content in testis showed approximately

3 fold increase from stage II to V whereas a 6 fold decrease thereafter (Fig. 2b). The mean PL levels of both sexes showed 2 fold increases up to stage V and decreased thereafter (Fig. 2c, d). Content of TAG in ovary showed 4 fold increase ($p = 4.68E-05$) up to stage V whereas 8 fold decrease ($p = 2.94E-05$) from stage V to VI (Fig. 2e). Content of TAG in testis showed approximately 2 fold increase at stage V when compared to stage II while a 5 fold decrease from stage V to VI (Fig. 2f).

Lipid class content in muscle tissues: Lipid class concentration in immature unsex (stage I) of *S. lysan* was higher in muscle tissue compared with liver. PL content of muscle and liver tissues in stage I was higher than that of other lipid classes. The content of CS in muscle of female significantly ($p = 0.004$) increased from stage I to stage III and significantly ($p = 4.05E-06$) decreased beyond that (Fig. 3a). In the case of male, CS content in stage II is significantly ($p = 0.01$) increased from stage I and decreased therafter (Fig. 3b). The PL levels in the muscle of female were ($p = 0.008$) increased from stage I to IV (Fig. 3c). The content of PL was higher in females than males at stage IV. The highest contents of PL in male muscle tissue was observed at stage III and it was decreased ($p = 3.65E-05$) beyond stage IV (Fig. 3d). Content of TAG in muscle of females significantly ($p = 4.29E-06$) increased from stage I to stage IV and decreased ($p = 4.05E-06$) upto stage VI (Fig. 2e). In male, the content of TAG significantly ($p = 4.05E-06$) increased from stage I to III and decreased thereafter (Fig. 3f).

Lipid class content in liver: In female liver tissue, content of CS significantly ($p = 4.50E-06$) increased from stage I to III and significantly ($p = 4.3E-06$) decreased thereafter (Fig. 4a). Although the increase in CS content in male was moderately increased ($p = 4.05E-06$) from stage I to IV, it sharply decreased from stage IV to VI (Fig. 4b). PL content in female showed a slight fluctuation among maturity stages (Fig. 4c), significant ($p = 0.09$) difference was not observed from stage I to IV but a considerable ($p = 0.01$) drop was recorded at stage V. However, PL content in male increased from stage I to II and significantly ($p = 4.05E-06$) decreased from stage II to stage VI (Fig. 4d). Changes in the content of TAG was higher when compared to the moderate changes in PL and CS in the liver tissues of female (Fig. 4e). The mean TAG content of female liver tissues showed a 2 fold increase ($p = 1.09E-05$) from stage II to IV and then a 4 fold decrease ($p = 4.29E-06$) from stage IV to VI. A similar trend was also observed for TAG in male liver tissues (Fig. 4f) .

Monthly lipid changes in tissues: All three constituents of lipid class, CS, PL and TAG, showed higher and lower values corresponding to the maturation stage and annual spawning events (Table 2, 3). CS content of testis was significantly ($p = 0.003$) different from CS of ovary, whereas PL and TAG were not significantly different between male and female. Mean CS, PL and TAG content in ovary of females fluctuated throughout study and attained higher value in June and September (Table 2). Male gonads also followed a more or less similar pattern for CS, PL and TAG as that of female adult fish (Table 3).

The highest mean CS content in ovary of females collected in June and September months were 226.4 mg.100 g^{-1}±78.21 and 292.00 mg.100 g^{-1}±156.08, respectively (Table 2). Similar trend of CS content in male testis was observed (Table 3). CS content of testis significantly ($p = 0.046$) increased from July to September and attained a peak at September (226.3 mg.100 g^{-1}±69.92). The mean PL content in ovary of female reached the highest amount in June as 393.54 mg 100 g^{-1}±73.00

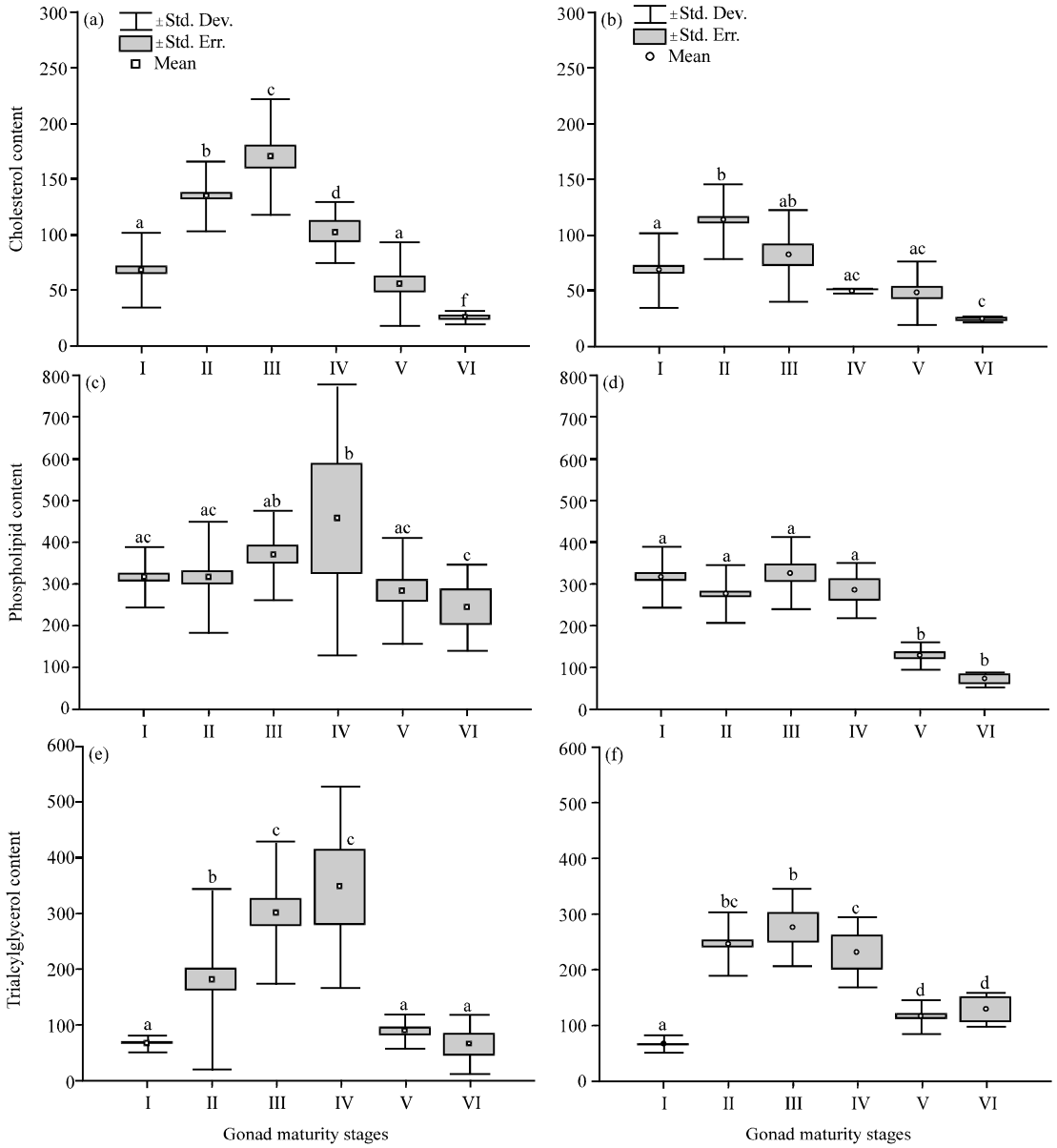

Fig. 3(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g^{-1}) in muscle tissue of *S. lysan* in different gonad maturity stages, I: Immature unsex; II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent. a, c and e-in female; b, d and f-in Male. Mean values for each gonad maturity stages with the common letters indicate not significantly difference (p>0.05)

(Table 2). Similarly, mean PL content in testis of male significantly (p = 0.040) increased from April to June, attained the highest amount as 475.8 mg.100g^{-1}±153.6 and decreased in July (Table 3). Significantly (p = 0.034) highest TAG content in ovary of female was recorded in September (Table 2). The highest amount of TAG in testis of adult male fish was recorded in June as 453.1 mg.100g^{-1}±77.64 (Table 3).

Muscle tissue of both sexes contained low values of CS than the PL and TAG in all months (Table 2, 3). Fluctuations of CS content in muscle tissues of female were significantly lower in June

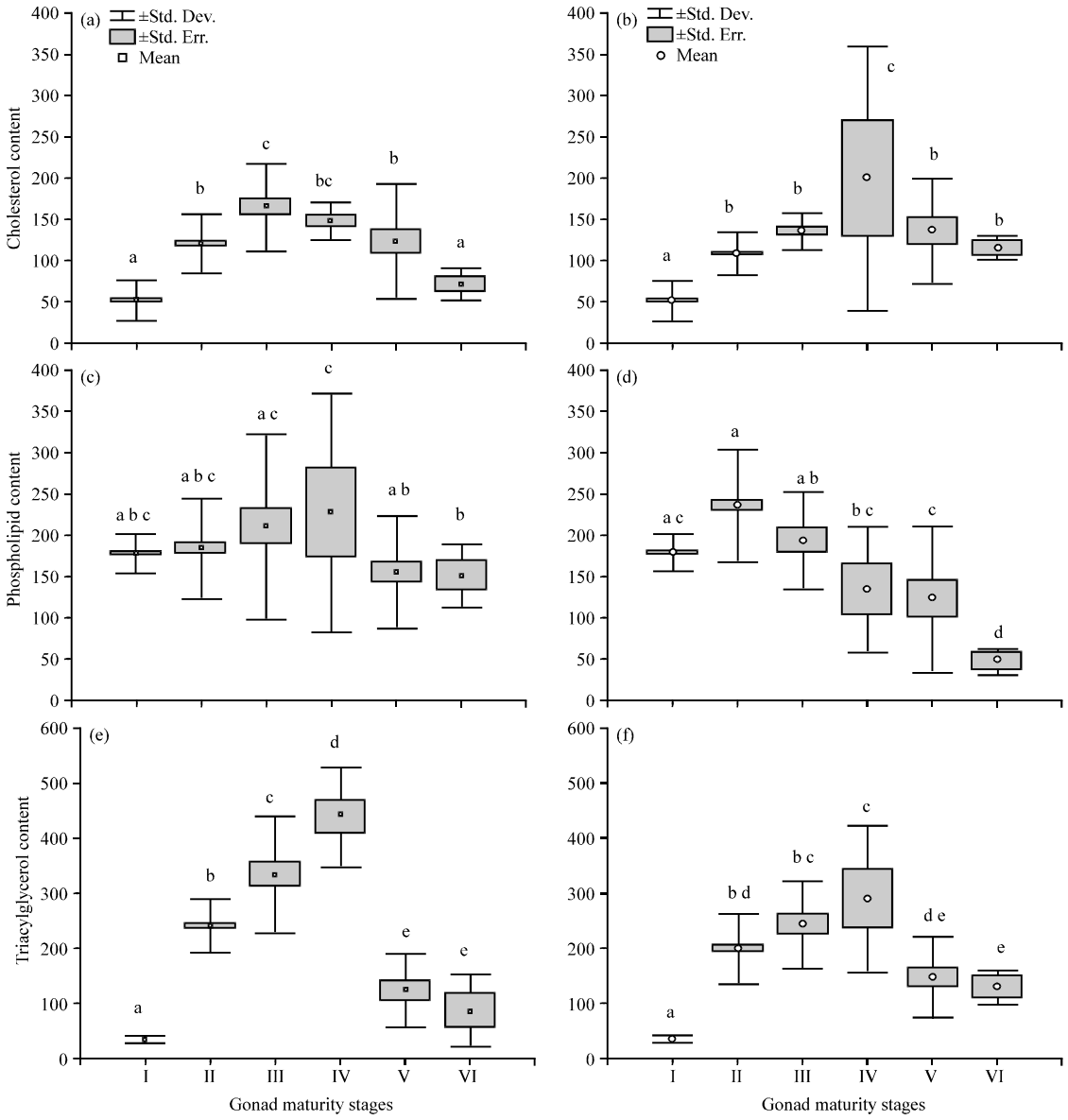

Fig. 4(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g^{-1}) in liver tissue of *S. lysan* in different gonad maturity stages, I: Immature unsex; II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent, a, c and e -in female; b, d and f -in Male. Mean values for each gonad maturity stages with the common letters indicate not significantly difference (p>0.05)

(p = 3.21E -05) and September (p = 1.8E-05) when compared to May. PL content in female muscle tissue significantly (p = 0.004) decreased from April to June. TAG content in muscle tissues of female was significantly (p = 0.001) decreased from August to September (Table 2). But, monthly changes of CS, PL and TAG in male muscle tissue was not significantly fluctuated (Table 3).

Changes in liver CS and PL in both sexes was not significantly predictable (Table 2, 3). But Fluctuation of TAG content in liver was evident in both sexes. Female liver TAG significantly (p = 0.0017) declined from May to June and significantly (p = 0.0012) increased from June to

Table 2: Lipid class content of gonad, muscle and liver tissues in adult female *S. lysan* throughout the year 2010/2011

	Gonad			Liver			Muscle		
Month	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacyl glycerol
January	ND	ND	ND	ND	ND	ND	ND	ND	ND
February	100.89±58.56^{a}	336.94±25.47^{ac}	152.01±53.87^{a}	133.32±15.91^{ab}	179.26±38.54^{a}	297.81±33.43^{ad}	171.66±8.79^{a}	368.16±96.75^{a}	267.93±153.75^{abc}
March	141.19±43.82^{a}	355.48±90.03^{ac}	225.15±90.07^{ab}	154.17±2.02^{ab}	201.31±126.50^{a}	353.13±209.11^{bd}	175.01±8.37^{a}	467.98±94.51^{ab}	273.70±58.27^{abc}
April	200.96±34.59^{a}	230.72±7.75^{cb}	205.27±72.18^{ab}	275.17±42.34^{c}	302.14±70.71^{ab}	307.02±7.77^{abd}	273.74±11.99^{b}	616.20±35.35^{b}	234.37±30.90^{abc}
May	220.30±7.20^{a}	383.23±82.00^{a}	292.23±73.56^{ab}	205.61±7.78^{ac}	350.68±0.79^{b}	456.50±62.93^{b}	283.50±1.95^{b}	464.71±28.97^{ab}	331.73±2.14^{ab}
June	226.38±78.21^{a}	393.54±72.98^{a}	327.19±81.41^{ab}	147.55±85.40^{ab}	154.70±52.76^{a}	190.02±116.69^{ac}	145.31±46.43^{a}	286.55±56.86^{ac}	153.84±130.90^{ac}
July	153.06±47.41^{a}	185.28±4.13^{b}	246.37±3.03^{ab}	140.74±7.75^{ab}	199.67±64.42^{a}	265.31±12.43^{cd}	134.44±11.18^{a}	345.35±56.56^{ac}	331.59±28.29^{ab}
August	267.78±117.76^{a}	349.34±131.54^{ac}	367.48±95.44^{ab}	167.42±24.38^{ab}	242.73±159.72^{ab}	451.86±100.23^{bc}	136.82±41.36^{a}	399.29±232.77^{abc}	400.59±161.18^{b}
September	292.03±156.08^{a}	402.51±75.59^{a}	387.76±175.53^{b}	112.52±62.65^{b}	150.04±61.52^{a}	129.37±72.00^{c}	42.92±22.58^{c}	281.02±120.19^{ac}	97.78±61.03^{c}
October	135.18±53.28^{a}	218.40±100.53^{cb}	321.30±129.39^{ab}	134.89±15.55^{ab}	188.03±46.55^{a}	348.18±51.45^{bd}	87.77±25.15^{d}	185.67±79.80^{c}	263.04±64.86^{abc}
November	ND	ND	ND	ND	ND	ND	ND	ND	ND
December	ND	ND	ND	ND	ND	ND	ND	ND	ND

Mean values for each month with the common letters indicate not significantly difference (p>0.05). ND: Not detected, Values are Means±SD

Table 3: Lipid class content of Gonad, Muscle and Liver tissues in adult male *S. lysan* throughout the year 2010/2011

	Gonad			Liver			Muscle		
Month	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacyl glycerol
January	100.16±0.07^{a}	285.67±34.72^{ab}	147.16±6.99^{a}	110.12±14.14^{a}	123.36±1.49^{a}	169.71±26.16^{a}	163.66±11.95^{a}	305.10±7.06^{ab}	175.10±49.49^{a}
February	98.88±19.30^{a}	345.71±115.23^{ab}	190.94±108.48^{ab}	102.45±0.06^{a}	128.40±3.96^{a}	176.32±28.10^{a}	164.85±19.22^{a}	315.74±20.52^{ab}	182.26±58.35^{a}
March	ND	ND	ND	ND	ND	ND	ND	ND	ND
April	100.78±0.82^{a}	213.70±4.94^{a}	366.93±1.02^{ab}	151.26±1.49^{a}	310.35±14.34^{b}	354.90±6.63^{a}	41.37±1.63^{b}	342.65±3.59^{ab}	143.72±0.69^{a}
May	128.03±36.10^{ab}	384.55±148.42^{ab}	426.83±137.60^{ab}	99.36±66.69^{a}	214.21±66.77^{ab}	260.48±8.30^{a}	41.22±7.91^{b}	199.32±00.1^{ab}	163.43±30.82^{a}
June	133.42±28.84^{ab}	475.75±153.62^{b}	453.10±77.64^{b}	91.62±57.63^{a}	206.04±136.86^{ab}	218.31±39.48^{a}	38.96±10.62^{b}	202.09±34.90^{ab}	170.62±27.51^{a}
July	113.65±14.52^{a}	319.08±161.01^{ab}	367.73±105.00^{ab}	137.98±26.90^{a}	168.10±18.92^{ab}	259.60±00.2^{a}	57.01±7.68^{b}	304.87±117.29^{ab}	305.23±78.30^{a}
August	195.37±98.64^{ab}	229.00±96.50^{ab}	446.59±47.84^{b}	256.94±160.78^{a}	203.24±90.92^{ab}	339.20±178.65^{a}	50.62±0.70^{b}	275.16±38.65^{ab}	261.36±0.001^{a}
September	226.27±69.91^{b}	332.15±120.84^{ab}	424.13±225.51^{ab}	125.27±99.24^{a}	124.51±80.11^{a}	175.16±125.11^{a}	49.15±26.05^{b}	192.08±101.51^{a}	140.63±83.87^{a}
October	102.06±51.63^{a}	259.91±5.07^{ab}	412.96±115.53^{ab}	131.87±32.95^{a}	225.23±62.44^{ab}	220.88±65.37^{a}	97.60±29.24^{c}	376.71±63.83^{b}	222.04±76.17^{a}
November	ND	ND	ND	ND	ND	ND	ND	ND	ND
December	ND	ND	ND	ND	ND	ND	ND	ND	ND

Mean values for each month with the common letters indicate not significantly difference (p>0.05). ND: Not detected, Values are Means±SD

August and again significantly ($p = 0.0002$) decreased in September. Liver lipid in both sexes attained maximum value in August, whereas minimum values were obtained in June and September.

DISCUSSION

Analysis of lipid classes in gonad, muscle and liver tissues are a widely applied methodology in the study of reproduction (Shearer and Swanson, 2000; Das and Sahu, 2001).The results of the present study suggest that the *S. lysan* showed an important relationship between lipid classes and gonad maturity stages as well as with different months. It further shows that *S. lysan* undergoes major changes in lipid contents in gonad, muscle and liver tissue.

Lipid changes in tissues with gonad maturity stages: The findings of lipid classes in immature stages of *S. lysan* are in agreement with the observations made by Litvin *et al.* (2011), who reported that PL was the predominant lipid class in muscle of juvenile weakfish *Cynoscion regalis*, while TAG was in low concentrations. Yet, liver of juvenile fish contained low amount of TAG than that of muscle.

Total lipid content in ovary of *S. lysan* was higher in spawning stage while lowest in spent stage. Similar observation was shown in *Trachinotus ovatus,* where total lipid content of ovaries attained the highest value at spawning stage and lowest value at spent stage (Assem *et al.*, 2005).

The results of the present investigation reveal that the mean value of lipid class composition in ovary has relatively higher amount of TAG and PL than the CS. Ovary of red drum (Vetter *et al.*, 1983) and gilthead sea bream (Mourente and Odriozola, 1990) also contain highest amount of TAG and PL than the CS (proportions of total lipid content). Hilton *et al.* (2008) also noticed that the PL in the brood stock egg of yellowtail kingfish (*Seriola lalandi*) was higher whereas the triacylglycerol value was lower in egg of yellowtail kingfish. In contrast, sand eel has higher value of TAG than PL (proportions of total lipid content) (Tocher and Sargent, 1984).

Muscle lipid content of *S. lysan* attained a maximum value during mature stages and minimum value during spawning stage. Bransden *et al.* (2007) also identified a similar pattern, they specified that the fat content in muscle of male and female striped trumpeter *Latris lineate* were decreased by 25 and 40%, respectively during the spawning period. Contents of PL in muscle of *S. lysan* increased from immature stage to mature and decreased thereafter. Similar trend was demonstrated by Rao (1965), who recorded that concentration of inorganic phosphorous compounds in muscles of *Caranx sexfasciatus* increased with maturity. Yagana (1982) also reported that the value of phospholipid in muscle of catfish *Clarias bairachus* declined during spawning period and the low phosphorous content was observed in post-spawning period. In contrast, Thakur *et al.* (2009) reported that the polar lipid in muscle of yellowtail (*Seriola quinqueradiata*) was minor constituents throughout the maturation. Muscle tissues of mature *S. lysan* fish shows low CS content in the present study. Further the present study describes that *S. lysan* can be included under 'low fat fish' category (Sutharshiny and Sivashanthini, 2011a, c) Hence, consumption of *S. lysan* fish posses no risk to human health.

TAG content in liver tissues of mature *S. lysan* exhibited higher values. Seiichi *et al.* (1993) also identified that the major lipid component of the liver in amberjack and striped jack were triglyceride. Content of TAG in liver tissues of *S. lysan* was decreased after spawning. The observation is consistent with the findings by Phleger (1971), who found that the total liver lipid content of pink salmon *Onchorhychus gorbuscha* decreases in the spent fish. Phleger (1971)

further explained that the liver of fish do not to synthesize the triglyceride after spawning. The cholesterol content of the liver of *S. lysan* exhibited a low variation during the maturation cycle and highest value was recorded at mature stage, while the minimal value was recorded at spent stage. Findings of the present investigation on *S. lysan* is in agreement with the work of Idler and Bitners, 1960), who reported that the total cholesterol content in liver declined and deposited in ovary of migratory salmon, *Oncorhynchus nerka*, during the spawning phases. In contrast, Phleger (1987) identified that the CS content in liver of pink salmon (*Onchorhychus gorbuscha*) remain constant after spawning.

Lipid changes in tissues through out the year: From the present study, it is obvious that the lipid class constituents CS, PL and TAG of gonad, muscle and liver tissues of tropical *S. lysan* show a variation throughout the year, corresponding to the maturation stage and annual spawning events. Peak spawning of adult female *S. lysan* was reported in June and September months (Thulasitha and Sivashanthini, 2013).

The lipid class content in ovary of *S. lysan* fluctuated throughout the year and attained a noticeable peak value during the spawning period while, muscle and liver lipid content of *S. lysan* attained the lower amount. Arrington *et al.* (2006) also proposed similar pattern of seasonal changes in lipid content of muscle, liver and gonad of three neo tropical fish. Similarly, Bustamante (1989) recorded that the body fat accumulated before the spawning of bar jack (*Caranx ruber*) and decreased during the spawning period. At the same time, the lipid content in ovary increased during the spawning time.

Major fluctuations of PL and TAG content in muscle of *S. lysan* were noticed during the period of spawning. Likewise, Thakur *et al.* (2009) showed TAG content in muscle of amberjack (*Seriola dumerili*) varied considerably with season. Although, Polar Lipid (PL) content in muscle remained almost constant over the study period.

Fluctuation of liver TAG content was evident in both sexes of *S. lysan*. Similarly, liver lipid composition of red drum (*Sciaenops ocellatus*) varied throughout the year (Craig *et al.*, 2000). The mean TAG content in liver tissues of both sexes of *S. lysan* was higher during the maturation and declined thereafter. This is in confirmation with the findings of Craig *et al.* (2000).

CONCLUSION

From the present study it has been concluded that the variation in cholesterol (CS), phospholipid (PL) and triacylglycerol (TAG) in gonad, muscle and liver tissues in different gonad maturity stages, confirm a strong link between lipid profile and reproductive strategies of tropical *Scomberoides lysan*. Knowledge of the lipid dynamics of *S. lysan* throughout the year assist to determine the non spawning period and therefore it is the fishing season of *S. lysan*. Range of muscle CS, PL and TAG value obtained in the present study signifies that *S. lysan* recommended as one of the healthiest food fish for human consumption. Determination of CS, PL and TAG content in ovary, muscle and liver tissues of different maturity stages of *S. lysan* further provide information on nutrition of lipid in terms of diet formulation in future culture trails of *S. lysan*. The present study provides fundamental information to successful formulation and implementation of policies, strategies and plans in fisheries management and future aquaculture trials.

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