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**LIPID CHANGES IN RELATION TO SEXUAL MATURITY AND  
SPAWNING IN *Scomberoides lysan* (CARANGIDAE) FROM  
WATERS AROUND JAFFNA PENINSULA, SRI LANKA**

By

**SUTHARSHINY SATHYARUBAN, B.Sc (Hons.)**

**M. Phil**

**DEPARTMENT OF FISHERIES SCIENCE  
FACULTY OF SCIENCE  
UNIVERSITY OF JAFFNA  
THIRUNELVELY, JAFFNA  
SRI LANKA**

**2012**

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**THESIS SUBMITTED TO THE UNIVERSITY OF JAFFNA THROUGH THE  
FACULTY OF GRADUATE STUDIES FOR THE AWARD OF THE DEGREE  
OF MASTER OF PHILOSOPHY IN FISHERIES SCIENCE**

**DEPARTMENT OF FISHERIES SCIENCE**

**FACULTY OF SCIENCE**

**UNIVERSITY OF JAFFNA**

**THIRUNELVELY, JAFFNA**

**SRI LANKA**

**2012**

## CANDIDATES'S DECLARATION

I hereby declare that the entire work embodied in this thesis has been carried out by me. The extent of this information derived from the existing literature has been documented and fully acknowledged at the appropriate places, the work is original and has not been submitted in part or full for any Diploma or Degree in this or any other University.

26-12-2012  
.....

Date

Sathyaruban  
.....

(Sutharshiny Sathyaruban)

Signature of the candidate



මගේ අංකය  
எமது இல.  
My Number

ඔබේ අංකය  
உமது இல.  
Your Number

දුරකථන  
தொலைபேசி  
Telephone

Department of Fisheries 021 222 2307

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ශාචනය.

த. பெ. எண் 57,  
திருநெல்வேலி,  
யாழ்ப்பாணம்.

P. O. Box 57,  
Thirunelvely,  
JAFFNA

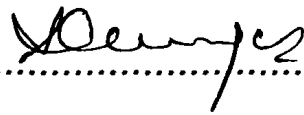


### Certificate

This is to certify that the thesis entitled “Lipid changes in relation to sexual maturity and spawning in *Scomberoides lysan* (Carangidae) from waters around Jaffna peninsula” submitted to the University of Jaffna in fulfilment of the requirements for the award of the Degree of Master of Philosophy in Fisheries Science is a record of original research work done by the candidate Mrs. Sutharshiny Sathyaruban under my supervision.

..... 26. 07. 2012 .....

Date

.....  .....

Research Supervisor

Prof. Dr. Mrs. S. J. Kuganathan Prof. Mrs. Sivashanthini Kuganathan  
B.Sc (Hons), B.L., M.A., U.K., Ph.D. Ind.  
Head, Department of Fisheries Associate Professor in Zoology,  
University of Jaffna, Sri Lanka.

Head/ Department of Fisheries,  
Faculty of Science,  
University of Jaffna  
Sri Lanka.

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*Sathyaruban*  
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**List of publications arose from the project**  
(Copy of each paper enclosed as appendices)

**In peer reviewed Journal**

- **Sutharshiny, S.** and Sivashanthini, K., 2011. Total lipid and cholesterol content in the flesh of the five important commercial fishes from waters around Jaffna peninsula, Sri Lanka. **International Journal of Biological Chemistry**, Vol.5, no.2, pp.161-169.
- **Sutharshiny, S.** and Sivashanthini, K., 2011. Lipid reserves of *Scomberoides lysan* (Forsskål, 1775) from the Sri Lankan waters. **International Journal of Biological Chemistry**, Vol. 5, no.3, pp.170-183.
- **Sutharshiny, S.** and Sivashanthini, K., 2011. Proximate composition of three species of *Scomberoides* fish from Sri Lankan Waters. **Asian Journal of Clinical Nutrition**, Vol.3, pp.103-111.
- **Sutharshiny, S.** Sivashanthini, K. and Thulasitha, W.S., 2013. Lipid changes in relation to sexual maturity and spawning of tropical double spotted queenfish, *Scomberoides lysan* (Forsskål, 1775). **Asian Journal of Animal and Veterinary Advances**, 8(4), pp. 555-570

**Abstract**

- **Sutharshiny, S.** and Sivashanthini, K., 2011. Changes in total lipid in the flesh of different size *Scomberoides lysan* from waters around Jaffna Peninsula. **Proceedings of Jaffna Science Association**, Vol.18, no.01, pp.10.
- **Sutharshiny, S.** and Sivashanthini, K., 2012. Allocation of total lipid and water content at different tissues of *Scomberoides lysan*. **Proceedings of International Conference on Fisheries & Marine Sciences (Marine Fish 2012)**, pp. 23.
- **Sutharshiny, S.** and Sivashanthini, K., 2013. Monthly changes in gonad, muscle and liver lipid of *Scomberoides lysan* from Northern waters of Sri Lanka. **Proceedings of Ruhuna Science Symposium**, pp. 22.

## ABSTRACT

The present study was undertaken to understand the lipid changes in gonad, muscle and liver tissues of tropical double spotted queenfish (*Scomberoides lysan*; Pisces: Carangidae) in relation to sexual maturity and spawning. Total lipid, cholesterol (CS), phospholipid (PL) and triacylglycerol (TAG) were determined in tissues with respect to maturity stages of both sexes as well as months. Samples were periodically caught from landing centres around Jaffna peninsula from January 2010 to December 2011. Fish length, weight, sex and maturation status were recorded. Content of total lipid, CS, PL and TAG in tissues were determined in the laboratory. Extracted total lipid from the liver tissues of all fish was higher than that of muscles. Range of muscle lipid content predicts that *S. lysan* fall under 'low fat fish' category across the entire study period and thus, it is one of the healthiest food fish for human consumption. Correlations between total lipid content in tissues and standard length of fish as well as body weight are significant. The values of total lipid, 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, it increased up to maturation and decreased during spawning. Similar dynamics were observed in males. The main lipid constituents in the liver and muscle of mature fish were TAG and PL respectively. Total lipid and lipid constituents in gonads showed higher value, whereas muscles and livers showed lower value in June and September, which represent the spawning time. It is evident that the values of lipid in tissues of *S. lysan* were influenced by the cycle of maturation and time of spawning. This information is useful 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 present study provides fundamental information to successful formulation and implementation of policies, strategies and plans in fisheries management and future aquaculture trials.

Key words: Cholesterol, phospholipid, *Scomberoides lysan*, triacylglycerol, total lipid

# 1. INTRODUCTION

## 1.1. Lipids in fish

Lipids are known to be the building blocks of fats or fatty substances found in animals and plants. Lipids and specifically their constituent fatty acids play an important role in the life histories and physiology of fish (Sargent, 1989). Knowledge gained from lipid changes in a species would be helpful to understand the physiology and ecology of that species. Lipid fluctuates considerably according to season, age, physiological condition (including the degree of maturity of the genital products), food supply and other environmental factors (Shulman, 1974; Anthony *et al.*, 2000).

Lipids entered into the composition of living matter from the very beginning of life on earth, appeared around 3.5 billion years ago, being composed from bacteria-single cell organisms. These early forms of lipids have evolved with animal and plant species leading to different forms of animal and vegetable fats, used by people in ancient times (Simon *et al.*, 2007 cited in Flavia, 2010).

Lipids are complex classes of compounds, which can be 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 organisms at different levels. Quantity of lipid is used as biochemical index of trophic condition for fresh and marine water fish (Novotony and Beeman, 1990).

Phospholipid is the main lipid constituent of cellular membranes and important constituents of egg yolk in fish (Sorbera *et al.*, 2001; 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). Phospholipids in artificial diets could improve the growth of larvae and early juveniles of various fish species (Tocher *et al.*, 2008).

Triacylglycerol is the major energy storage form in fish (Shulman, 1974). It is a measure of bioenergetics related to ecology and physiology of fish and therefore it can be used to estimate the overall fish condition (Fraser, 1989).

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.*, 2008). Biosynthesis of cholesterol in the liver accounts for approximately 10 % (King, 1996; Mayes, 2012).

Fish rely on both exogenous (dietary) and endogenous lipid sources for energy (Sheridan, 1988). When the lipid content exceeds the maximum that can be metabolized for energy purposes, the remainder will be deposited in different tissues (Huss, 1988). When required, deposited lipids are hydrolyzed by lipases and transported to peripheral tissue for utilization (Sargent *et al.*, 2002). According to Bromlei, (1934), Kizevetter, (1942) and Kleimenov, (1962), the fat depots in fishes are located in the subcutaneous connective tissue (tunas, eels, carp, certain herrings), in skeletal muscles and between the muscle fibers (mackerels, horse mackerels, anchovies, certain Clupeidae, Salmonidae, Acipenseridae), in the abdominal cavity: mesentery (Cyprinidae; certain Clupidae, e.g., "Kilka," Percidae: pike perch, perch; white fishes; pikes), in the liver (Gadidae, Sharks, rays), interosseous tissue (Salmonidae) in the bones and at the base of fins (Pleuronectidae) (Shulman, 1974).

Fish that do not have appreciable amounts of fat in the muscles (Elasmobranchii: Shark, rays, Gadidae, Percidae: pike perch, gobiidae) are classified as “lean fish”. Yet the physiological role of fat is important also in the lean fish. Their fat is concentrated either in the liver or in the abdominal cavity. The seasonal fluctuations of fat reserves in these fish are very pronounced. The fat content of the liver of lean fish varies from 10 - 30% to 40 - 70 % over the year. As for example the fatness of the liver is above 80% in certain sharks, which can be classified as lean fish (Perepletchik *et al.*, 1967). In some fish, the fat is stored in the flesh. These fish are called as “fatty fish” (Anguillidae: freshwater eels, Clupeidae: shad, and certain other herrings) (Love, 1970; Clarke *et al.*, 1984; Alonso-Fernandez and Saborido-Rey, 2012). Some fish species contain intermediate fat content between lean and fatty fish. They are called intermediate fat fish (Salmonidae: Brook trout) (Swift, 1955; Jezierska *et al.*, 1982).

Energy assimilated by fish may be allocated to one of four “compartments”, growth, maintenance, reproduction and storage, among which trade-offs, may occur (Shulman and Love, 1999; Larson, 1991) (Table 1).

Table 1. Examples of study indicates various primary functions for fat reserves in fish

Primary Functions	Study
Maintenance	Guillemot <i>et al.</i> , (1985); Shulman, (1974)
Reproduction	Shulman, (1974); Love, (1970); Lasker, (1970); Tyler and Dunn, (1976); Delahunty and De Vlaming, (1980); Patzner, (1980); Hunter and Leong, (1981); Quast, (1985); Nelson and McPherson, (1987)
Reproduction and Maintenance	MacKinnon, (1972); Iles, (1974); Newsome and Leduc, (1975); Adams <i>et al.</i> , (1985); Reznick and Braun, (1987)
Migration, Reproduction and Maintenance	Rebertson and Wexler, (1960); Dotson, (1978); Beamish, (1979); Saldana and Venables, (1983)

Reproduction has been recognized as an activity which affects the energy allocation processes, since large amounts of reserves are required both for female egg production, and for male breeding activities, such as enhanced swimming activity, competition, courtship, parental care and nesting (Henderson *et al.*, 1984; Chellappa *et al.*, 1989; Coleman and Fisher, 1991; Ballantyne *et al.*, 1996; Mackereth *et al.*, 1999). Females have generally higher energy requirements than males, due to the high energetic cost of eggs (Robards *et al.*, 1999; Guijarro *et al.*, 2003) and to supply essential nutrients such as fatty acids and lipid soluble nutrients (Morris and Chulkin, 2000; Okuda, 2001). 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), Atlantic salmon (*Salma truta*) (Aarestrup and Jepsen, 1998) and eel (*Anguillia anguillia*) (Fricke and Kaese, 1995). If no migration is involved, fish are capable of building their energy reserves completely after spawning.

Patterns of energy allocation in fishes may change in accordance with the degree of sexual maturity of the individual and timing of reproductive activity as well as maturation of the gonads. This energy requires materials obtained from ingested food and energetic reserves deposited in various parts of the body (Tytler and Calow, 1985). Quantifying the energy allocations of organisms have been frequently used to evaluate the magnitude of the allocation for reproduction (Huntingford *et al.*, 2001; Jonsson and Jonsson, 2005).

Inadequate reserves have been implicated in the reduced reproductive potential of several fish species through reduced fecundity and hatchability of eggs and larvae or delayed maturation (Lambert and Dutil, 1997; Koops *et al.*, 2004).

Maturation is a process of allocating energy during development for the growth and differentiation of germinal tissue into gonads and to the ultimate production of ripe gametes (Thorpe, 2007). In teleost, it starts within hours or days after fertilization of the egg, and in the Atlantic salmon *Salmo salar* L. for example, gonadal tissue begins to appear during the early embryo stage ( in the first hour after fertilization) (Adams and Thorpe, 1989).

Lipid mobilization is reported to be very active in fish during the period of gonad growth and maturation (Nikolskii, 1969; Mourente *et al.*, 2002). During the maturation, lipid levels decrease in the liver and muscle tissue of fish and increase in the gonad tissues (Reznick and Brawn, 1987; Brown and Murphy, 1995; Zaboukas *et al.*, 2006; Andrew, 2011; Singh *et al.*, 2012).

## 1.2. *Scomberoides lysan*

The Doublespotted queenfish (*Scomberoides lysan*) is a tropical fish included under family Carangidae.

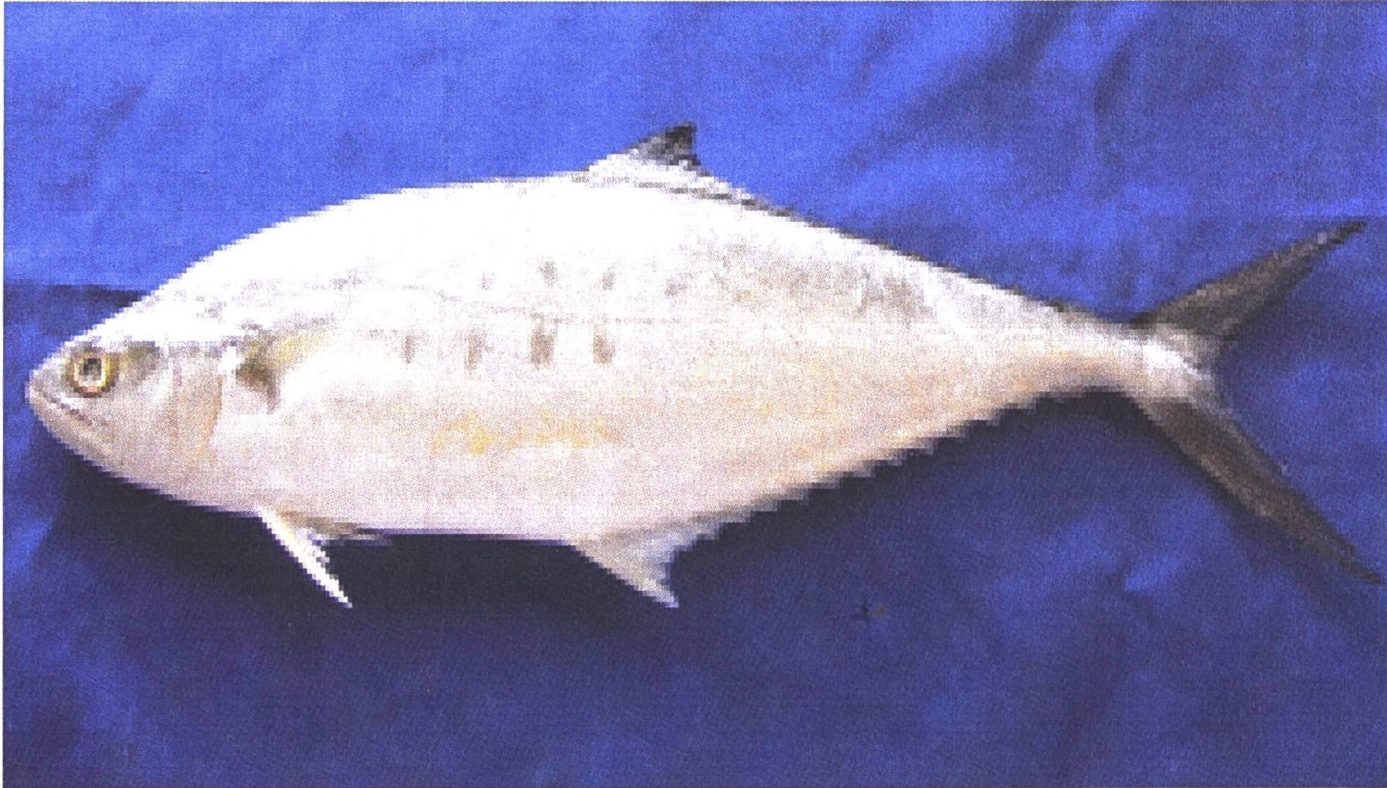


Plate 1. *Scomberoides lysan*

### 1.2.1. Classification (Myers *et al.*, 2012)

Kingdom: Animalia

Phylum : Chordata

Class : Actinopterygii

Order : Perciformes

Genus : *Scomberoides*

Species : *S. lysan*

Binomial name: *Scomberoides lysan* (Forsskål, 1775)

### 1.2.2. Synonyms

A list of names for *Scomberoides lysan* was recorded by several researchers (Table 2);

Source: [www.fishwise.co.za](http://www.fishwise.co.za)

Table 2. List of names for *Scomberoides lysan*

No	Synonym	Author
1	<i>Chorinemus aculeatus</i>	non Bloch, 1973
2	<i>Chorinemus lysan</i>	Forsskål, 1775
3	<i>Lichia lysan</i>	Forsskål, 1775
4	<i>Scomber lysan</i>	Forsskål, 1775
5	<i>Scomber forsteri</i>	Schneider and Forster, 1801
6	<i>Scomber madagascariensis</i>	Shaw, 1803
7	<i>Lichia tolooparah</i>	Rüppell, 1829
8	<i>Chorinemus tolooparah</i>	Rüppell, 1829
9	<i>Scomberoides tolooparah</i>	Rüppell, 1829
10	<i>Chorinemus moadetta</i>	Cuvier, 1832
11	<i>Scomberoides moadetta</i>	Cuvier, 1832
12	<i>Chorinemus exoletus</i>	Cuvier, 1832
13	<i>Chorinemus mauritanus</i>	Cuvier, 1832
14	<i>Chorinemus sanctipetri</i>	Cuvier, 1832
15	<i>Scomberoides sanctipetri</i>	Cuvier, 1832
16	<i>Scomberoides orientalis</i>	Schlegel and Temminck, 1844
17	<i>Chorinemus orientalis</i>	Schlegel and Temminck, 1844

### 1.2.3. Species identification

*S. lysan* fish were identified using the FAO species identification guide (De Bruin *et al.*, 1995). It is primarily silver in color, with dark coloration on the dorsal and caudal fins, double row of black spots on side of body, upper jaw only just reaching vertical from posterior margin of eye, no scutes, scales on the mid body below lateral line partially embedded and lanceolate in shape and tip of 2<sup>nd</sup> dorsal fin sharply pigmented (Plate 1) (Fischer and Bianchi, 1984). It is known to reach up to 110 cm in total length (Daget and Smith-Vaniz, 1986); common length: 60.0 cm total length and body mass up to 11.0 kg (24 lb) (Frimodt, 1995).

### 1.2.4. Distribution

*S. lysan* is associated with reefs and ranges widely throughout the Indian and Pacific Oceans (Figs. 1 and 2). This species ranges eastward from the Red Sea and eastern Africa to Hawaii, the Marquesas, and the Tuamotu Islands. It is found as far north as southern Japan and south to New South Wales and Rapa (Fischer and Bianchi, 1984; Froese and Pauly, 2010). In the Indian Ocean, these are densely distributed in southeast coast of India, Gulf of Mannar and in northern and southern coast of Sri Lanka (Varghese *et al.*, 2011). It occupies relatively clear waters from the surface to about 100 m (330 ft.) (Daget and Smith-Vaniz, 1986), Juveniles and sub adults significantly utilize estuaries (Blaber and Cyrus, 1983; De Bruin *et al.*, 1995).

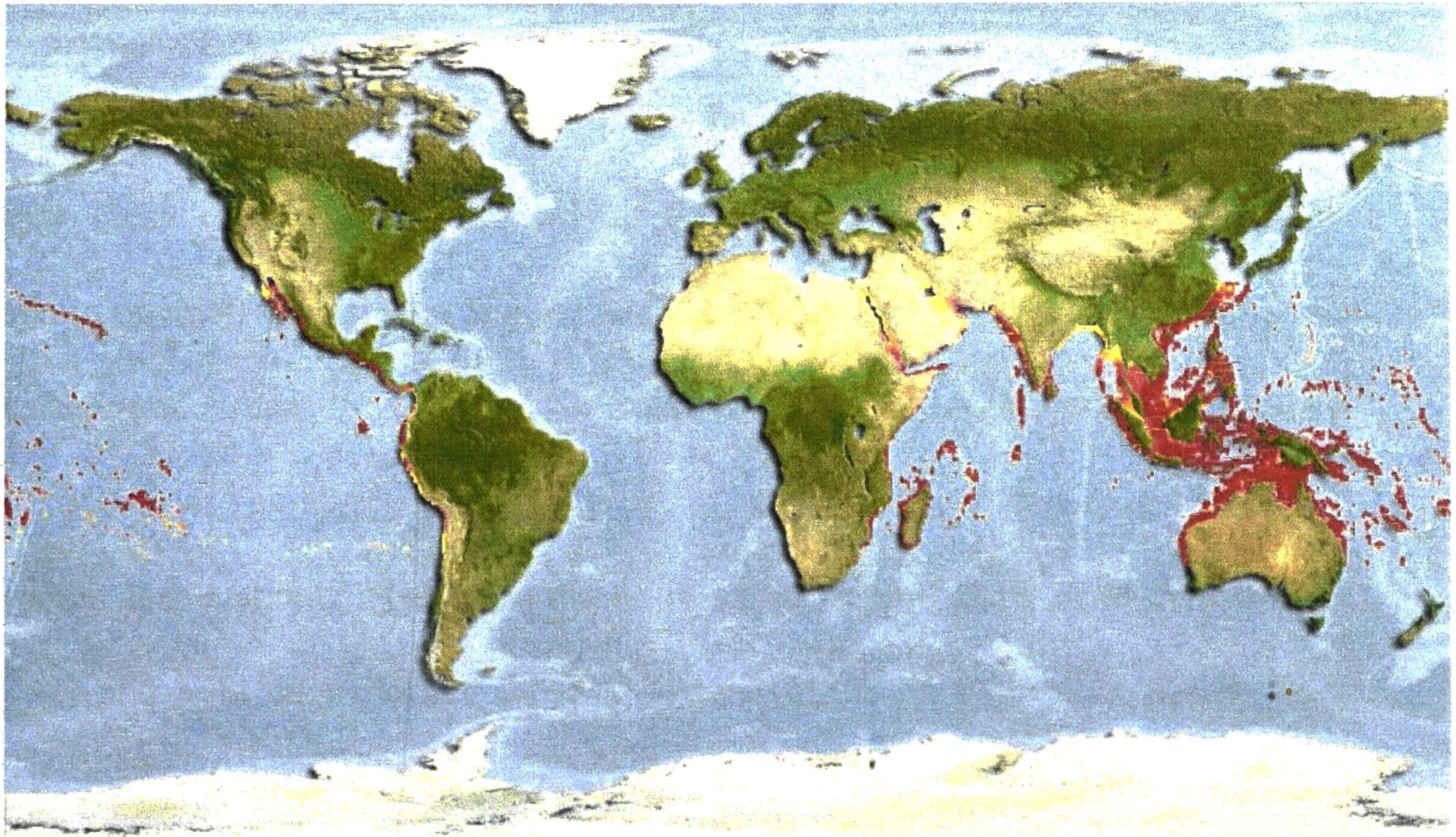


Fig.1. Distribution of *Scomberoides lysan* in the world;  
 (Red colour indicates the distributions)  
 (Sources: Froese and Pauly (2010). ([www.fishbase.org](http://www.fishbase.org)))

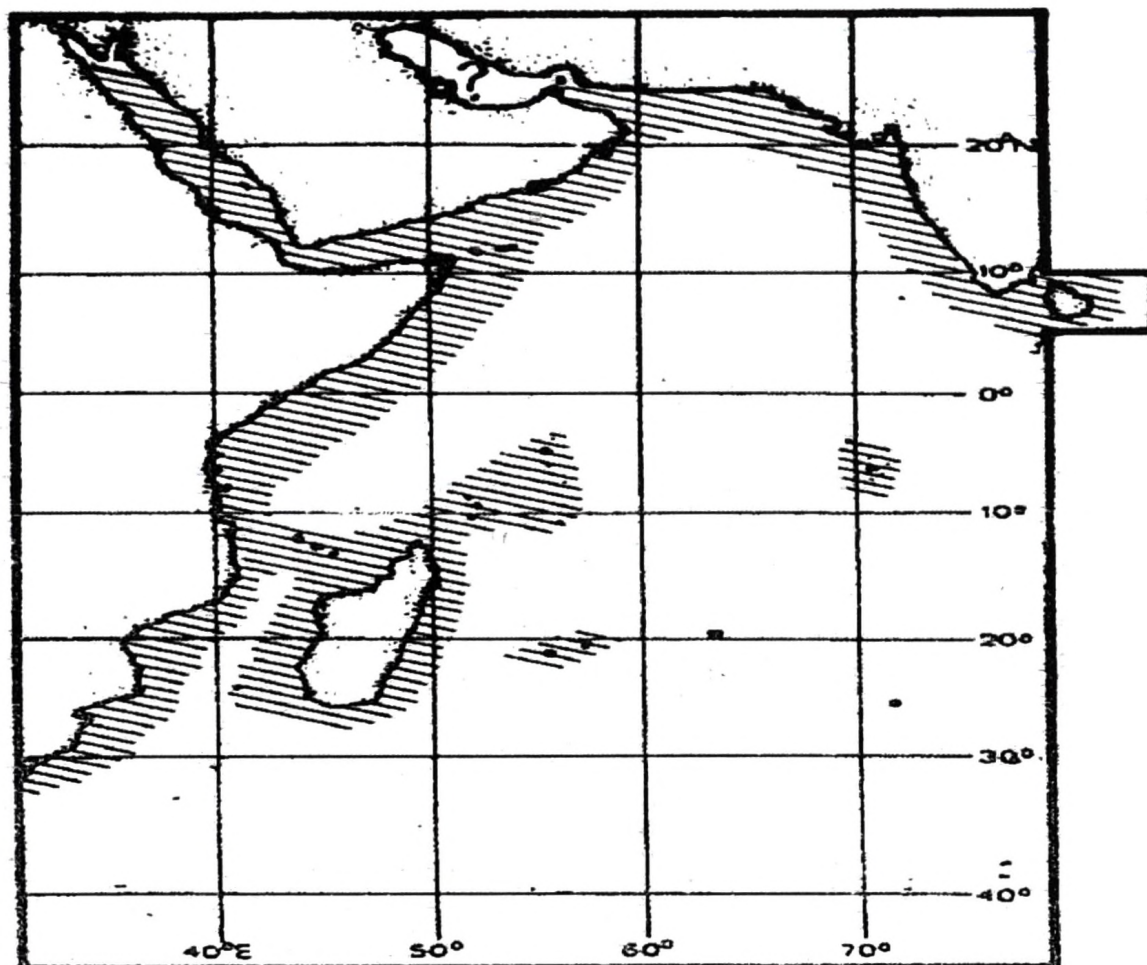


Fig. 2. Distribution of *S. lysan* in Indo- West Pacific Ocean  
 (Source: Fischer and Bianchi, 1984)

### 1.2.5. Economic importance of *S. lysan*

It is an economically important food fish in Jaffna Peninsula. The flesh of the fish is meaty. Flesh: carcass ratio is high so that these fish have high consumer demand and market potential. The species is popular for dry fish production (Plate 2) with export demands and especially consumed by mothers during pregnancy and immediately after delivery. Therefore, it is highly prized, continues to maintain a high market demand and marketed preserved-dried or salted (Sutharshiny and Sivashanthini, 2011a) and hold an important position towards the economy of the fishers of Sri Lanka.



Plate 2. Dry fish of *Scomberoides lysan* from Jaffna fish market.

Double spotted queenfish is also a popular sportfish on ultra light tackle, whipping lures or baits just below the surface. Their tough skin is often stripped off, dried and used for trolling lures (Honebrink, 2000).

Few research works related to lipid composition of fish had been undertaken in Sri Lanka. Jayasinghe *et al.*, (1996) recorded omega-3-fatty acids in some fish and prawns, collected from Fisheries Department retail shop in Colombo. Lipid content of edible flesh in some freshwater species was studied by Wimalasena and Jayasuriya, (1996). Thilakarathne and Attygalle, (2009) recorded the lipid composition in skin and muscle of the Indo-Pacific sailfish, *Istiophorus platypterus*. Anas *et al.* (2009) analyzed the lipid composition and fatty acid profiles of wild caught and fattened mud crab *Scylla serrata*, collected from Negombo and Kalpitiya lagoons. High cholesterol oxidation in pickled mackerel (*Rastrelliger kanagurta*) was recorded by Ubhayasekera *et al.*, (2012). However no studies have been 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 total lipid and lipid class constituents- cholesterol, triacylglycerol and phospholipids could form the basis for future studies, contributing to economic development, health development and sustainable management of *S. lysan* in Sri Lanka.

### 1.3. Objectives of the study

- To quantify the lipid content in gonad, muscle and liver tissue in different sized fish in different months.
- To understand the variation of lipid content of *S. lysan* in order to predict the spawning season of the fish.
- To gain adequate knowledge about the nutritional value of *S. lysan* in terms of cholesterol, triacylglycerol and phospholipids.
- To provide adequate information for development of feeding strategies of *S. lysan* for future culture trials.

## 2. LITERATURE REVIEW

### 2.1. Lipid study on fish

Though there are several studies on lipid dynamics in different tissues related to reproduction for temperate fish species (Dygert, 1990; MacFarlane *et al.*, 1993; Fiorin *et al.*, 2007; Lloret *et al.*, 2008) only a few studies are available for tropical fish species (Victor *et al.*, 1978; Montgomery and Galzin, 1993; Arrington *et al.*, 2006; Hiroaki, 2012) especially for carangids (Ramadan, 2002; Assem *et al.*, 2005). Seasonal changes of lipid and lipid classes in different tissues of carangid fish species have been observed by Bustamante, (1989) and Thakur *et al.*, (2009).

### 2.2 Lipid deposition in carangid fish

Liver tissues have been reported as an energy storage site for several carangid fish species (Shao-ning *et al.*, 2010; Hiroaki, 2012; Rodriguez-Barreto *et al.*, 2012). Some authors identified that the muscle tissues of carangid fish are also a storage site of lipids (Eduardo *et al.*, 1996; Njoku *et al.*, 2004; Manal, 2009; Chedoloh *et al.*, 2011; Nurnadia *et al.*, 2011; Marichamy *et al.*, 2012) and some species store lipids in both muscle and liver tissues (Seiichi *et al.*, 1993; Seiichi and Yusuke, 1993). Phleger, (1987) reported that the bone lipid content of *Alectis ciliaris* ranged from 2.1 to 8.7 % and specified triglyceride was the major lipid in the bones of *A. ciliaris*.

Neutral lipid and phospholipids are major constituents in muscle tissues of two carangid fish species, *Seriola dumerili* and *Seriola rivoliana* (Hiroaki, 2012). Seiichi *et al.*, (1993) examined the characteristics of lipid accumulation in the muscle and liver in five species of commercially important fish, wild puffer (*Takifugu rubripes*), cultured flounder (*Paralichthys olivaceus*), red sea bream (*Pagrus major*), amberjack (*Seriola*

*dumerili*), and striped jack (*Caranx delicatissimus*) and found triglyceride is a major constituent in muscle and liver of these five species. However, polar lipid is reported as a major constituent in the eggs of yellowtail kingfish, *Seriola lalandi* (Hilton *et al.*, 2008).

Aoki *et al.*, (1991) reported that the Docosahexaenoic acid (DHA) content in the muscle tissues of non-migratory carangid fish, *Seriola dumerili* and *Caranx delicatissimus* was less than 20% of the total lipid.

### 2.3. Proximate composition of carangid fish species

Many investigators from different regions of the world studied the proximate composition of muscle tissues in carangid fish (Table 3).

Table 3. Details of proximate composition of carangid fish species

Fish	Moisture content (%)	Protein content (%)	Lipid content (%)	Carbohydrate content (%)	Ash content (%)	Region	Source
<i>Carangoides fulvoguttatus</i>	77.82	19.97	0.24	—	1.50	Saudi	Manal, (2009)
<i>Scomberoides tol</i>	—	17.00	1.20	15.98	—	Thanjavur	Patterson and Ranjitha, (2009)
<i>Trachinotus carojinus</i>	74.76	20.31	5.17	—	1.16	Florida	Gall <i>et al.</i> , (1983)
<i>Scomberoides sp</i>	—	—	2.00	—	—	NorthWest Australia	Sinclair <i>et al.</i> , (1983)
<i>Elagatus bipinnulatus</i>	—	—	1.00	—	—	Malaysia	Gibson <i>et al.</i> , (1984)
<i>Decapterus punctatus</i>	74.06	21.64	2.52	—	3.12	Florida	Hale, (1984)
<i>Caranx georgianus</i>	75.00	21.50	2.64	—	1.34	New Zeland	Hughes <i>et al.</i> , (1980)
<i>Selaroides leptolepsis</i>	—	—	2.90	—	—	Malaysia	Gibson <i>et al.</i> , (1984)
<i>Seriola lalandi</i>	71.50	22.20	4.30	—	1.30	Gansbaai	Andrew, (2011)

(—) - Not indicated

#### **2.4. Diet with fish lipid in aquaculture of carangid species**

Lipids are one of the important components when formulating diets for aquaculture species. Most carangids are important candidates for recreational and aquaculture fisheries (Gunn, 1990; Katsuragawa and Matsuura, 1992; Hiroshi *et al.*, 2004; Hilton, 2008; Thakur *et al.*, 2009; Hiroaki, 2012).

Rodriguez-Barreto *et al.*, (2012) found the percentage of total lipid, lipid class composition and their associated fatty acids in muscle, liver and ovary of wild and cultured mature females of greater amberjack (*Seriola dumerili*) in order to formulate a suitable diet for the broodstock of this species. Watanabe *et al.*, (1996) observed improved egg production in broodstock yellowtail fish, *Seriola quinqueradiata* when fed with raw fish diets of chopped Pacific mackerel *Scomber japonicas*, jack mackerel and sardines.

Vassallo-Agius, (2001a, b) showed improved fecundity and egg quality of broodstock striped jack fish (*Pseudocaranx dentex*) when fed with fish meal and squid meal diets. In a study, Hiroaki, (2012) observed higher triacylglycerol content in muscle and liver tissues of cultured *Seriola dumerili* and *S. rivoliana* than the wild fish when fed with fishmeal. Damian *et al.*, (2007) showed that the body condition and fatty acid compositions of Mediterranean horse mackerel (*Trachurus mediterraneus*) reared in coastal sea cages changed when fed with formulated food pellets containing terrestrial plant food.

## **2.5. Influence of lipid changes in muscle texture**

Thakur *et al.*, (2009) showed that the breaking strength in the muscle of the cultured amberjack (*Seriola dumerili*) and yellowtail (*Seriola lalandi*) had not been correlated with the biochemical constituents such as proximate composition, lipid class composition and collagen content of muscle. These researchers also found that the variations in the meat texture of cultured amberjack were not directly influenced by the changes in the muscle biochemical constituents.

In 2003, Thakur *et al.* showed that the deposition of lipid in myosepta in the muscle of cultured yellowtail (*Seriola quinqueradiata*) proportionally increased with increasing muscle lipid content and the excess lipid deposited sparsely along the thin connective tissue in muscle. Researchers also found that the higher lipid deposition in the connective tissue influence weakening of the muscle structure.

## **2.6. Influence of muscle lipid changes on other activities of carangid fish**

Pritchard *et al.*, (1971) reported that the swimming activity of the Jack Mackerel (*Trachurus symmetricus*) influenced the biochemical content in red and white muscle, and liver tissues of the Jack Mackerel.

Aranda *et al.*, (2006) reported that the lipid content in the jack mackerel, *Trachurus symmetricus symmetricus* body damaged after 120 days during storage in deep freezer. Eymard *et al.*, (2008) investigated oxidation of lipid content in minced horse mackerel (*Trachurus trachurus*) during storage at 5°C for 96 h.

Bae *et al.*, (2011) found that the dark muscles of yellowtail fish, *Seriola quinqueradiata* inhibit cancer development and cellular oxidation.

Moran *et al.*, (2007) measured the changes in oxygen consumption and metabolite concentration (ammonia, free amino acids, glucose, lipid, glycogen, ninhydrin positive substances and protein) in embryos of yellowtail kingfish (*Seriola lalandi*) incubated at different temperatures (17°, 19°, 21° and 23°C). Further the researchers observed that the carbohydrates, lipid and protein are relatively minor importance compared to the catabolism of free amino acids for energy production during embryogenesis.

### 2.7. Other studies on *Scomberoides* species

Agusa *et al.*, (2007) reported the presence of 20 types of trace elements in the liver tissues of *S. lysan* collected from coastal areas of Malaysia. The researchers stated that the accumulation of the trace elements in the liver tissues was higher than that in the muscle (Table 4).

Table 4. Concentrations of trace element (mg/g dry weight) in muscle and liver of *S. lysan*. Source: Agusa *et al.*; (2007).

Trace Elements	Muscle	Liver
V	0.052	0.1
Cr	0.46	0.83
Mn	0.25	5.24
Co	0.006	0.11
Cu	1.37	13.3
Zn	26.8	162
Se	1.8	9.0

Rb	4.00	2.32
Sr	1.54	4.16
Mo	0.008	0.82
Ag	< 0.001	0.045
Cd	< 0.001	0.245
Sn	0.581	Not analysed
Sb	< 0.01	< 0.01
Cs	0.13	0.05
Ba	0.011	0.039
Hg	0.09	0.36
Tl	< 0.001	< 0.001
Pb	0.014	0.079
Bi	< 0.001	0.003

Many investigators from different regions studied the biological parameters in *Scomberoides lysan* (Table 5).

Table 5. Details of biological parameters of carangid fish species

Biological parameters	Region	Source
length-weight relationship	San Miguel Bay	Cinco (1982)
length-weight relationship	Northern waters of Sri Lanka	Thulasitha and Sivashanthini (2011a)
Fork length-weight, Girth-weight, Fork length-gill girth, Fork length-maximum girth ratio, Condition factor Length at first maturity	Kanyakumari coast of Tamil Nadu, India	Balasubramanian <i>et al.</i> , (2008)

*S. lysan* is a major commercially important species in Fiji (America) due to their marketability and exportability (Jansen *et al.*, 1990). Muraleedharan and Gopakumar, (1997) found that the *S. lysan* is used for the surimi production in Cochin, India (Table 6). It is a much-enjoyed food product in many Asian cultures (Anonyms, 2012).

Table 6. Characteristic features of surimi prepared from *S. lysan*  
(Source: Muraleedharan and Gopakumar, 1997)

Description	Result
Colour of mince	White
Yield%	39.7 ± 5.5
Moisture%	76.78 ± 1.11
Protein%	19.59 ± 1.2
Fat%	0.39 ± 0.91
Ash%	1.36 ± 0.75

Arfat and Benjakul, (2012) reported that the yellow stripe travelly collected from Sinkhole coast along the Gulf of Thailand is also used for surimi preparation.

Microbial quality of salted and sun dried *S. lysan* fish from Tuticorin fish market, southeast coast of India was analyzed by Sinduja *et al.*, (2011). Authors reported that the dried fishes have been contaminated with pathogenic bacteria and fungal agents in different seasons. Similarly, Sivashanthini *et al.*, (2012) reported that the experimentally prepared dry fish, *Scomberoides commersonianus* was more hygienic than traditionally prepared one.

Morphology of the venom apparatus in the *Scomberoides sanctipetri* (Cuvier) (*Scomberoides lysan*) was identified by Halstead and Danieison, (1972).

Ramasamy *et al.*, (1995) identified the monogenea parasites (*Vallisia indica*, *Allodiscocotyla chorinemi*, *Heterapta chorinemi*, and *Dionchus remorae*) in the gills of *Scomberoides commersonianus*, *S. tol*, *S. lysan* and *S. tala*. Pillai, (1962) identified the copepod parasite (*Lernanthropodes chorinemi*) on gills of *Scomberoides lysan*, caught off Trivandrum, India. Seven digenean parasites in *Scomberoides* fish species were listed out by Sheng-fa *et al.*, (2010) (Table 7).

Table 7. The parasitic species, host, geographical location of the records and reference to the records

Digenean	Host	Locality	Reference
<i>Stephanostomum ditrematis</i>	<i>S. lysan</i>	South China Sea	Parukhin, (1966; 1976)
<i>Bucephalus fragilis</i>	<i>S. lysan</i>	SCS	Parukhin, (1966)
<i>Lintonium vibex</i>	<i>S. lysan</i>	SCS	Parukhin, (1966)
<i>Phyllodistomum carangis</i>	<i>S. lysan</i>	SCS	Parukhin, (1966; 1976)
<i>Erilepturus hamati</i>	<i>S. lysan</i>	SCS	Gu and Shen, (1978); Shen, (1990)
<i>Parahemiurus merus</i>	<i>S. lysan</i>	SCS	Parukhin, (1966; 1976)
<i>Lecithochirium trichiuri</i>	<i>S. commersonianus</i>	Taiwan Strait	Shih <i>et al.</i> , (2004)

### 3. MATERIALS AND METHODS

#### 3.1 Sampling

##### 3.1.1 Materials and reagents

All chemicals were purchased from Sigma chemical company, USA; unless otherwise stated. Solvents without any impurities were used in the present study.

The glassware were washed thoroughly in teepol solution (Glass cleaner, Sri Lanka) and running tap water and then rinsed well with distilled water. Cleaned glasswares were dried in an oven at 100° C (YCO - 010; Germany). Pipettes were immersed overnight in chromic acid (mole ratio  $K_2Cr_2O_7$ :  $H_2SO_4$  = 1:1), washed well with running tap water and distilled water and then dried. A 60 ml pipette pillar with three valves was used to pipette the chemicals. All the analysis were carried out in triplicate and mean values were reported in the thesis; unless otherwise stated.

##### 3.1.2. Sampling locations

Sampling locations are presented in Fig. 3. Samples from marine waters around Jaffna Peninsula were collected from fish landing stations at Point Pedro, Delft, Passaiyoor and Karainagar.

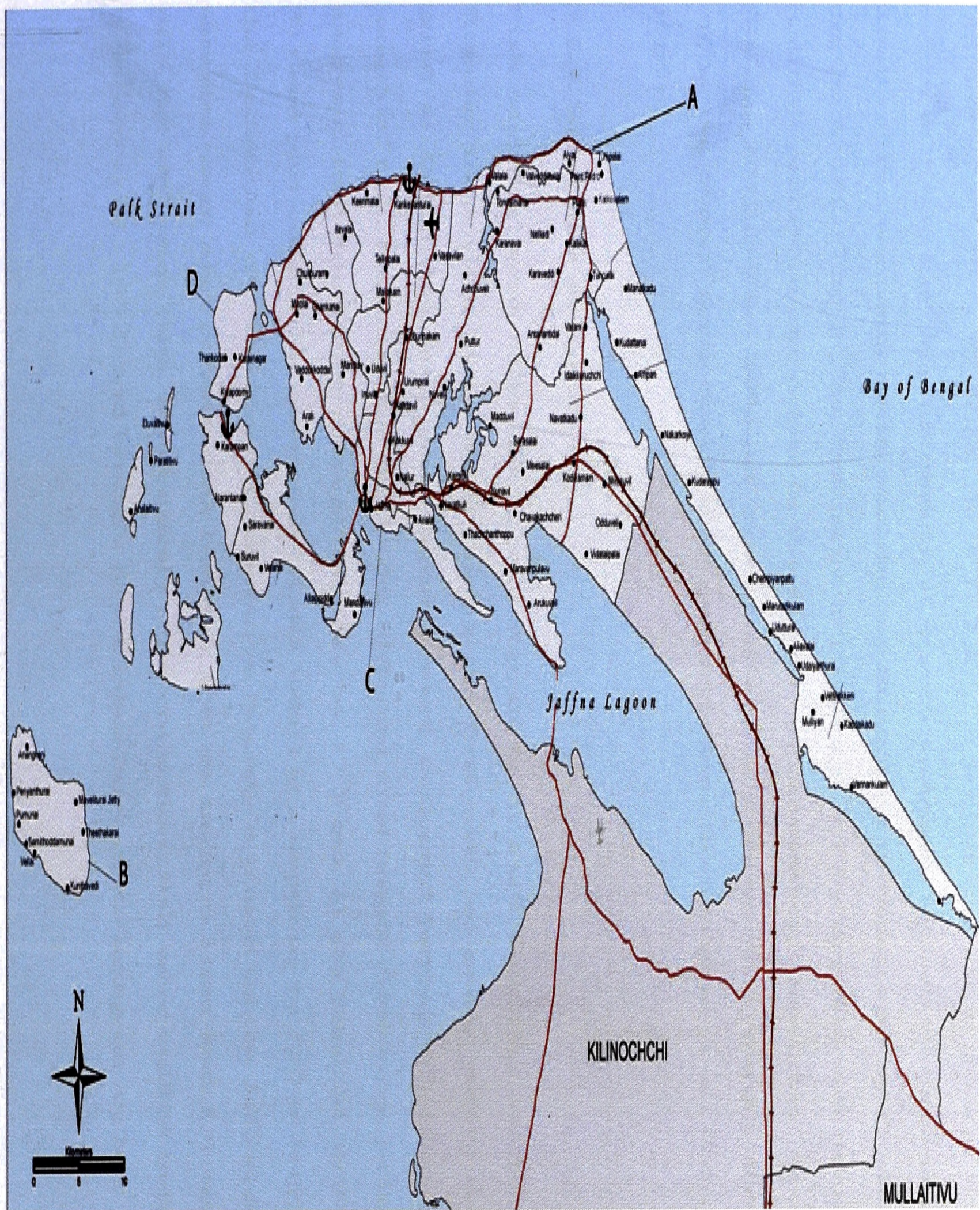


Fig. 3. Sampling sites of fish from waters around Jaffna Peninsula.  
 (A) Point Pedro, (B) Delft, (C) Passaiyoor and (D) Karainagar  
 (Source: <http://reliefweb.int/map/sri-lanka/jaffna-district-sri-lanka-administrative-map>)

The Jaffna Peninsula is located between the longitude of 79° 54' - 80° 2'E and latitude of 9° 30' - 9° 50'N. It is 103 km in length and 10 to 36 km wide with an extent of 1,035 km<sup>2</sup> approximately. It is surrounded by the sea on three sides: the Palk Strait on the west and north, the Bay of Bengal on the east, and by the Jaffna lagoon on the south. The Jaffna Peninsula is characterized by dry and wet distinct periods. Rainfall precipitations occur in the months of November, December, April and May (Russi, 2012). The 80% of precipitations is concentrated during the north-east monsoon, even if there could be several variations from year to year (Thambyahpillay, 1965).

### **3.1.3. Sampling protocol**

Fish samples were collected once a month from each sampling locations (Fig. 3). Regular field visits were made during the first week of every month from January 2010 to December 2011 and samples were collected paying attention to the total length of fish. Fish samples fall under different total length class were collected for the analysis. Individuals were caught mainly by 17.7 cm 21 ply meshed, drift nets (Plate 3) used particularly for queenfish (Katta valai) at a depth up to 100 m with the help of the Federation of Fishermen Cooperative Society's Union of the Jaffna district. Fish samples were also collected as bycatch species using 6.3 cm and 8.8 cm mesh size drift net and seine nets. Immediately after collection, fish were chilled before freezing (Graham *et al.*, 1992) and brought to the laboratory in ice using an ice box.

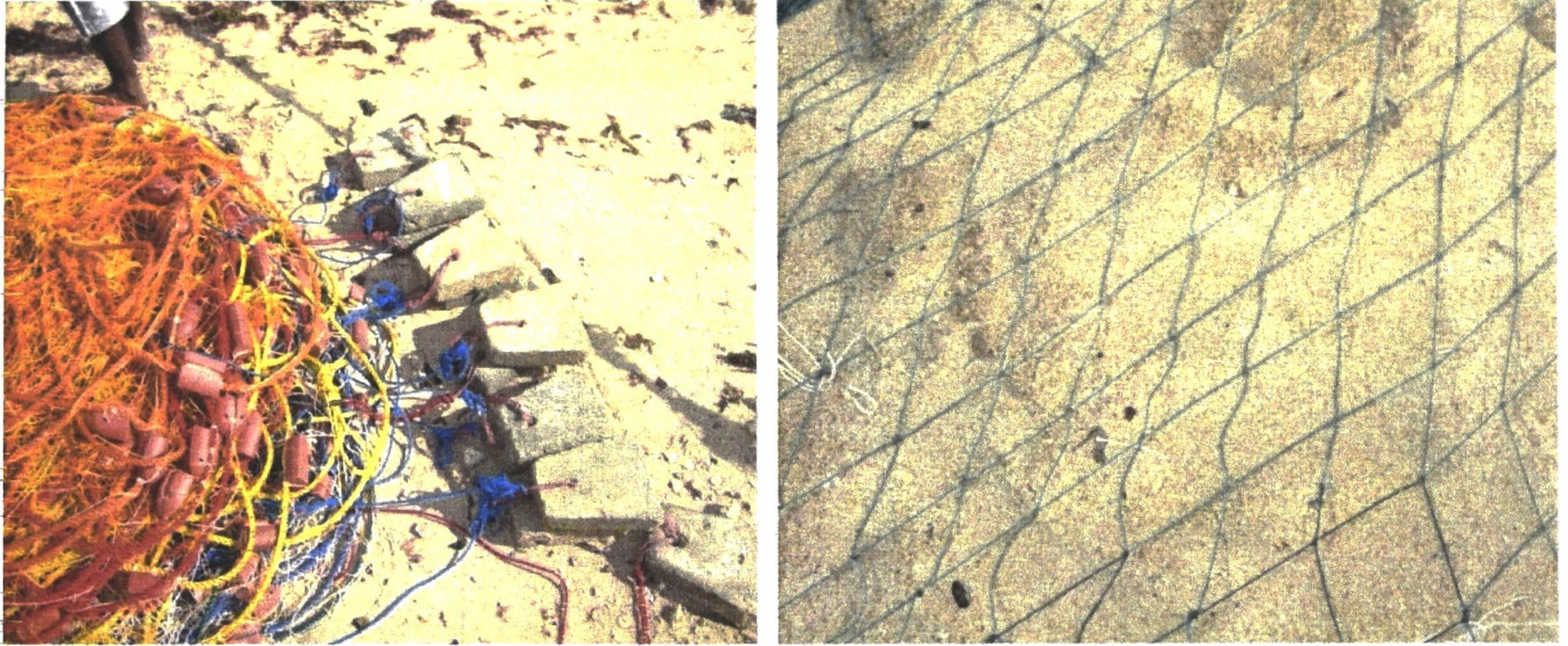


Plate 3: Drift gill net

### **3.2. Morphometric study**

Fish were allowed to thaw slowly at room temperature and standard length (SL) was determined using measuring board to the nearest 0.1 cm and total body wet weight (BW) was measured using top loading balance to the nearest 0.01 g before conducting lipid analysis.

### **3.3. Sex determination and gonad maturity stages**

Sex was determined for each specimen using macroscopic examination of gonad and gonad maturity stages (GMS) were recorded for each specimen as defined by Thulasitha and Sivashanthini, (2012b).

### **3.4. Tissue analysis**

Gonad, muscle and liver tissues were analyzed.

#### **3.4.1. Dissection of fish and evisceration of tissues**

Fish samples were dissected and gonads and livers were removed. Muscle (approximately 100 g) from dorsal side directly under the dorsal fin and well above the lateral line was removed from the fish. Scales, bones and skin were removed from muscle tissues. The gonad, liver and muscle tissues were rinsed with distilled water. Eviscerated gonad, liver and muscle were placed in sterilized petri dish (60 mm Diameter) and labeled.

#### **3.4.2. Drying tissues**

Eviscerated gonad, liver and muscle tissues were weighed using an electronic balance (OHAUS; USA) to the nearest 0.01 g and placed in an Oven (YCO - 010; Germany) at 60° C for 24 hours. The tissues were covered with filter paper to prevent accidental weight loss and to stop droplets erupting out of the petri-dish. The weight of the filter paper was included with that of the container. The components were dried to a constant mass and the dried tissues were reweighed using electronic balance to the nearest 0.1 g. The dried muscle tissues were transferred carefully to polythene packed bag and labeled, then ground twice in an electric grinder (Prett XT- 97; India), stirred, bagged and stored in a freezer at - 20°C until further investigation. Ground muscle tissues were used for lipid analysis within one month.

The dried gonad and liver tissues were transferred carefully into test-tubes with screw caps (15 x 100 mm) and labeled. If the dried tissue particles were stuck along the petri dish, it was dissolved using chloroform reagent and transferred into the test-tubes. The dried gonad and liver tissues was ground in a white mortar and pestle using additional chloroform and methanol mixture without loss of any tissue particles.

### 3.4.3. Total lipid extraction

Bligh and Dyer, (1959) method was used for the extraction of total lipid in tissues

#### Principle of this method:

In Bligh and Dyer, (1959) method, where extraction and portioning were simultaneous, the precipitated non-lipids were isolated between the two liquid phases.

Lipids were extracted in one phase solvent system chloroform/methanol/water 2:1:0.8 (v / v / v).

#### Reagents:

- 01) Chloroform
- 02) Methanol (AnalaR NoR; Leuven Belgium)
- 03) Anhydrous sodium sulphate

A weight of 10 g dried tissue powder was placed into 50 x 500 mm screw cap test tube and weighed. Chloroform: methanol mixture (Volume / Volume) was prepared in the ratio of 2:1. The tissue was homogenized with 200ml of chloroform/methanol mixture (1:20 = Tissue: Chloroform methanol mixture). 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; 11 cm in Diameter). 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. It was found that the approximate

proportions of chloroform, methanol and water in the upper phase were 3: 48: 47 by volume and in the lower phase, the respective proportion was 86: 14: 1. 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 1 g of anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ). The interface was rinsed twice with 10 ml of 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 Kjeldhal flask were transferred into another 5 ml of clean vial and any remaining solvents were left to evaporate in the fume chamber. The lipids extracted from the gonad, muscle and liver were weighed. Lipid extracts were filled in well stoppered glass vials, the cap being secured with a wide length of self sticking tape and then stored in a refrigerator at  $-4^\circ\text{C}$  (Bligh and Dyer, 1959).

### 3.5 Analysis of lipid composition in tissues

Cholesterol (CS), phospholipids (PL) and triacylglycerol (TAG) in gonads, muscles and livers tissue were estimated. The concentration of CS, PL and TAG were quantified using UV Visible spectrophotometer (LABOMED, UVD-3000).

#### 3.5.1 Cholesterol analysis

Zlatkis *et al.*, (1953) method was used to estimate the cholesterol in tissues.

##### Principle of this method:

In the Zlatkis *et al.*, (1953) method, cholesterol converted into highly coloured substances after addition of solvents. The reactions consist of dehydration, oxidation and sulphonation.

##### Reagents:

01) Ferric Chloride – 0.05% in acetic acid (W/V) (HIMEDIA; India)

A weight of 0.05 g of ferric chloride was dissolved in 100 ml of glacial acetic acid.

02) Concentrated sulphuric acid (Analytical Reagent; India)

03) Standard cholesterol solution (579 mg/ dl)

A weight of 579 mg of purified cholesterol (Fluka Chemie; Frei) was measured in an electronic balance. It was dissolved in 95 ml of glacial acetic acid. It was transferred to 100 ml volumetric flask and total volume was made up to 100 ml

with glacial acetic acid (AnalaR Nor; Leuven Belgium). Seven hours was needed to complete the reaction. It was protected from evaporation by keeping in glass stoppered bottles. Serial dilutions were made from 579 mg/dl of standard solution.

**Preparation of standard curve and estimation of the cholesterol concentration:**

A volume of 0.1 ml of diluted standard solution was taken into a dried 13 x 100 mm of test tube. 5 ml of ferric chloride (in acetic acid) reagent was pipetted into the sample and mixed. Pipetted 3 ml of concentrated sulphuric acid into it, mixed again and allowed to stand for 20 minutes. 0.1 ml of glacial acetic acid was used for blank. The contents of the tubes were transferred to dried cuvettes and read the absorbance against the blank at 560 nm in UV Visible spectrophotometer (LABOMED, UVD-3000). Standard curve was plotted using the attached LABOMED, INC software. Similarly, extracted lipid was treated. Subsequently the concentration of cholesterol in different tissues was estimated using the attached LABOMED, INC software (Zlatkis *et al.*, 1953).

### 3.5.2. Phospholipid analysis

Zilversmit and Davis, (1950) method was used to analyze the phospholipid in tissues.

#### Principle of this method:

In the Zilversmit and Davis, (1950) method, phospholipids extracted and digested with a suitable solvent. Precipitated phospholipids oxidized into inorganic phosphate.

#### Reagents:

01) 5 N sulphuric acid

A volume of 13.5 ml of concentrated sulphuric acid (36.8 Normality) was added carefully to about 95 ml of distilled water. It was transferred to 100 ml of volumetric flask and total volume was made upto 100 ml with distilled water.

02) 2.5 % of ammonium molybdate (HIMEDIA; India)

A weight of 2.5 g of ammonium molybdate was dissolved with 95 ml of distilled water. It was transferred to 100 ml of volumetric flask and total volume was made upto 100 ml with distilled water.

03) Amino-2-naphthol-4-sulphonic acid (ANSA) (Fluka Analytical; USA)

A weight of 0.2 g of amino-2-naphthol-4-sulphonic acid, 1.2g of sodium bisulphate and 1.2g of sodium sulphite was mixed well. A weight of 0.25 g of mixture was dissolved with 9 ml of distilled water. It was transferred to 10 ml of volumetric flask and total volume was made upto 10 ml with distilled water.

#### 04) Standard phosphorous solution

A weight of 35.1 mg of potassium dihydrogen phosphate was dissolved in 95 ml of distilled water and 1 ml of 10 N sulphuric acid was pipetted into it. Solution was transferred to 100 ml of volumetric flask and total volume was made upto 100 ml with distilled water. 10 ml of standard solution was transferred into 100 ml of volumetric flask and total volume was made upto 100 ml with distilled water to prepare a working standard containing 800 mg/dl. Serial dilutions were made from standard solution.

#### **Preparation of standard curve and estimation of the phospholipid concentration:**

An aliquot of standard solution was taken into a 150 ml of Kjeldhal flask and 1.0 ml of 5 N sulphuric acid was added to digest 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. 1.0 ml of distilled water was added and heated in a boiling water bath for about 5 minutes. 1.0 ml of ammonium molybdate and 0.1 ml of ANSA were added and it was transferred to 5ml of volumetric flask and total volume was made upto 5 ml with distilled water. Distilled water was used for blank. The contents of the tubes were transferred to dried cuvettes and read the absorbance against the blank at 660 nm within 10 minutes in UV Visible spectrophotometer (LABOMED, UVD-3000). Standard curve was plotted using the attached LABOMED, INC software. Similarly, extracted lipid was treated. Subsequently the concentration of phospholipid in tissues was estimated using the attached LABOMED, INC software (Zilversmit and Davis, 1950)

### 3.5.3. Triacylglycerol analysis

Foster and Dunn, (1973) was used to analyze the triacylglycerol content in tissues.

#### Principle of this method:

In the Foster and Dunn, (1973) method, phospholipids were removed first using isopropanol and alumina then glycerol liberated using potassium hydroxide. Glycerol oxidized into formaldehyde by metaperiodate.

#### Reagents:

01) Isopropanol (AnalaR Nor; Leuven Belgium)

02) Alumina

It was washed with distilled water until all the fine particles were removed and dried in an oven at 100°C – 110°C overnight and stabilized for at least six months at room temperature.

03) Saponifying agent

A weight of 50 g of potassium hydroxide was dissolved in 600 ml of distilled water. It was transferred to 1000 ml of volumetric flask and total volume was made up to 1000 ml with isopropanol and the mixture was stabilized for six months at room temperature.

04) Sodium metaperiodate reagent

A weight of 77 g of anhydrous ammonium acetate was dissolved in 700 ml of distilled water. 60 ml of glacial acetic acid and 650 mg of metaperiodate were added. It was transferred to 1000 ml of volumetric flask and total volume was

made upto 1000 ml with distilled water and the mixture was stabilized for at least six months at room temperature.

05) Acetyl acetone reagent (AnalaR Nor; Leuven Belgium)

A volume of 7.5 ml of acetyl acetone was added into 200 ml of isopropanol and mixed well. It was transferred to 1000 ml volumetric flask and total volume was made upto 1000 ml with distilled water and the mixture was stabilized for at least six months at room temperature.

06) Standard triolein solution

A weight of 1 g of triolein was dissolved in 100 ml of isopropanol. 1 ml of stock standard was transferred to 100 ml of volumetric flask and total volume was made upto 100 ml with isopropanol and the mixture was stabilized for at least six months at 4° C in a tightly sealed container. Serial dilutions were made from standard solution.

**Preparation of standard curve and estimation of the triacylglycerol concentration:**

A volume of 0.1 ml of standard solution was taken into 15 x 100 mm of screw-capped tube. 4 ml of isopropanol was added and mixed well into tube. 400 mg of washed alumina was added by a calibrated scoop. The mixture was placed in a mechanical rotator for 15 min and centrifuged. 2 ml of supernatant was transferred into 15 x 100 mm of screw-capped tubes. 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. 0.1 ml of distilled water was used for blank. The contents of the tubes were transferred to dried cuvettes and read the absorbance against the blank at 405 nm in UV Visible spectrophotometer (LABOMED, UVD-3000). Standard curve was plotted using the attached LABOMED, INC software. Similarly, extracted lipid was treated. Subsequently the concentration of triacylglycerol in tissues was estimated using the attached LABOMED, INC software (Foster and Dunn, 1973).

### **3.6. Data analysis**

Results are presented as mean  $\pm$  standard deviation. Values are displayed in the tables 8 and 9 and figures 4 - 40. The total lipid content in gonads, muscles and livers tissue was expressed as percentage of the total dry mass and lipid classes were expressed as milligram of the dry mass. All data were statistically analyzed by Microsoft Excel 2007 and STATISTICA Software (Version 6; Statsoft Inc., Tulsa, USA). The data were checked for normal distribution with one-sample Kolmogorov - Smirnov test and the variances were checked in the Levene's test for homogeneity.

### **3.7. Total lipid content in tissues**

Cumulative lipid content in liver, muscle and gonad tissues for different gonad maturity stages (GMSs) were computed and plotted as a graph to compare the lipid compartmentalization among these tissues.

### **3.8. Correlation between lipid content in tissues and standard length as well as body weight**

Correlation coefficient and curve fittings were determined using Microsoft Excel. Regression analysis was performed for the pooled data of *S. lysan*, irrespective of sex. It was used to determine the following relationships for *S. lysan*:

- (1) Lipid content in tissues and standard length of irrespective of sex
- (2) Lipid content in tissues and and body weight of irrespective of sex

Furthermore, regression was used to examine the potential relationship between tissue lipid content (gonad, muscle and liver), body size and weight.

### **3.9. Differences between total lipid and lipid class content in different tissues and gonad maturity stages**

Lipid content and lipid class content among sex and gonad maturity stages were compared using STATISTICA 6 Software. Data obtained for total lipid content in liver, muscle and gonad tissues for gonad maturity stages of both sexes 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 and the level of significance was set at  $p < 0.05$ .

### **3.10. Changes of total lipid and lipid class content in different tissues throughout the two years**

Monthly lipid and lipid classes (CS, PL and TAG) were evaluated only for adult fish, which included maturing, mature, spawning and spent stages. Monthly total lipid and lipid class data in different tissues for both years were pooled together and the average values for each month were computed. Fluctuations were examined to assess the potential relationship between lipid content of tissues (gonad, muscle and liver) and spawning. The dependent variable in the analysis was the percentage of lipid (DW) and the independent variable was month (January 2010 to December 2011).

## **4. RESULTS**

### **4.1. Sampling**

A total of 1175 fish were collected during the two-year study period from January 2010 to December 2011 from the selected landing stations.

### **4.2. Morphometric study**

Standard length of fish ranged from 10.7 to 68.8 cm and total wet weight of fish ranged from 21.10 to 2925.00 g.

### **4.3. Sex determination and gonad maturity stages (GMS)**

A total of 458 fish were identified as females, 446 fish were identified as males and others were identified as immature unsex. Reproductive status of individual fish was examined and the gonadal development was classified as immature unsex (stage I), immature (stage II), maturing (stage III), mature (stage IV), spawning (stage V) and spent (stage VI) as described by Thulasitha and Sivashanthini (2012b).

### **4.4. Tissue analysis**

Extracted lipid from the gonad, muscle and liver tissues of all fish were used for lipid class analysis.

### **4.5. Analysis of lipid class composition in tissues**

Standard curves obtained for cholesterol, phospholipids and triacylglycerol are shown in Figs. 4 – 6.

#### 4.5.1 Standard curve for cholesterol

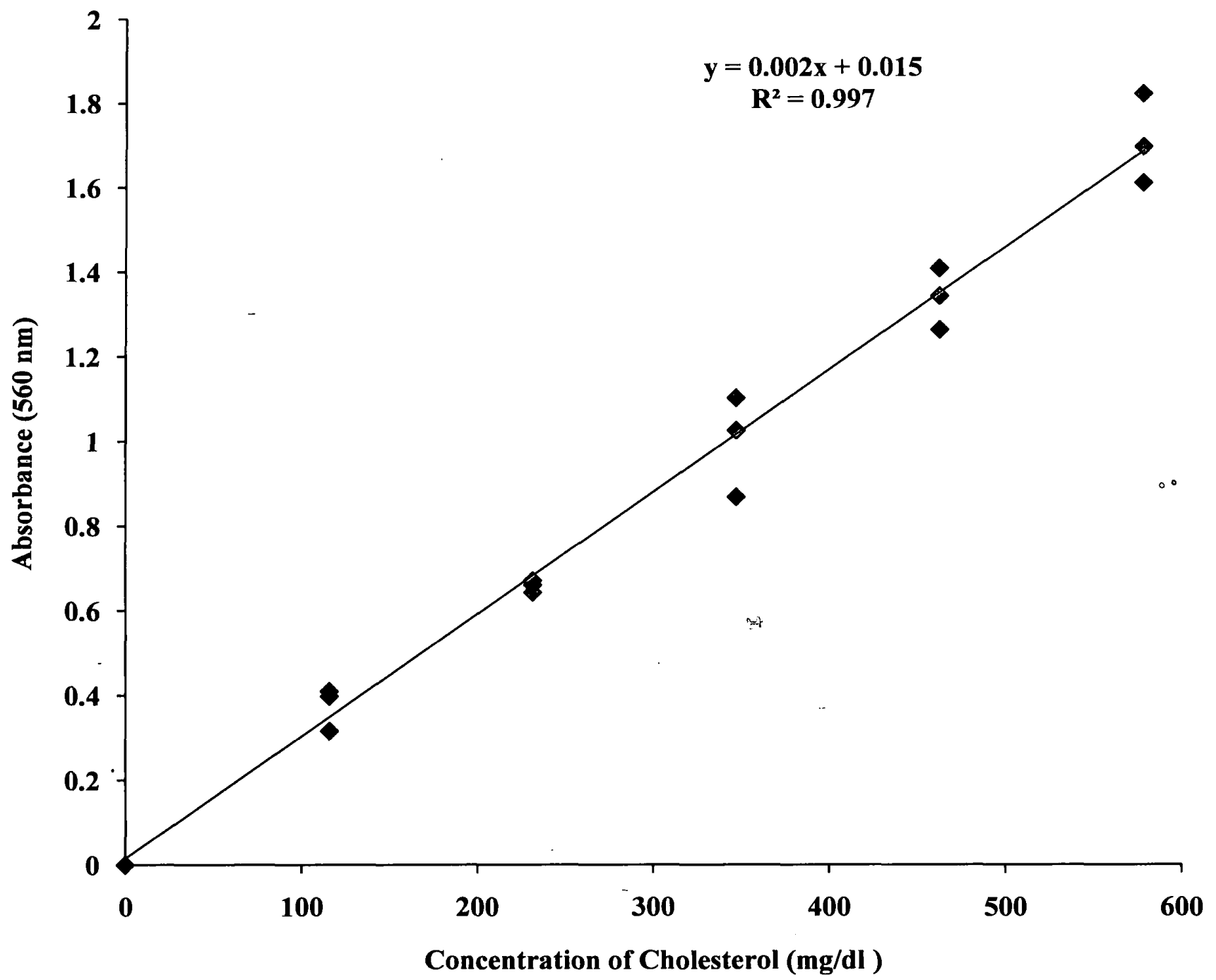


Fig. 4. Standard curve for cholesterol

#### 4.5.2. Standard curve for phospholipid

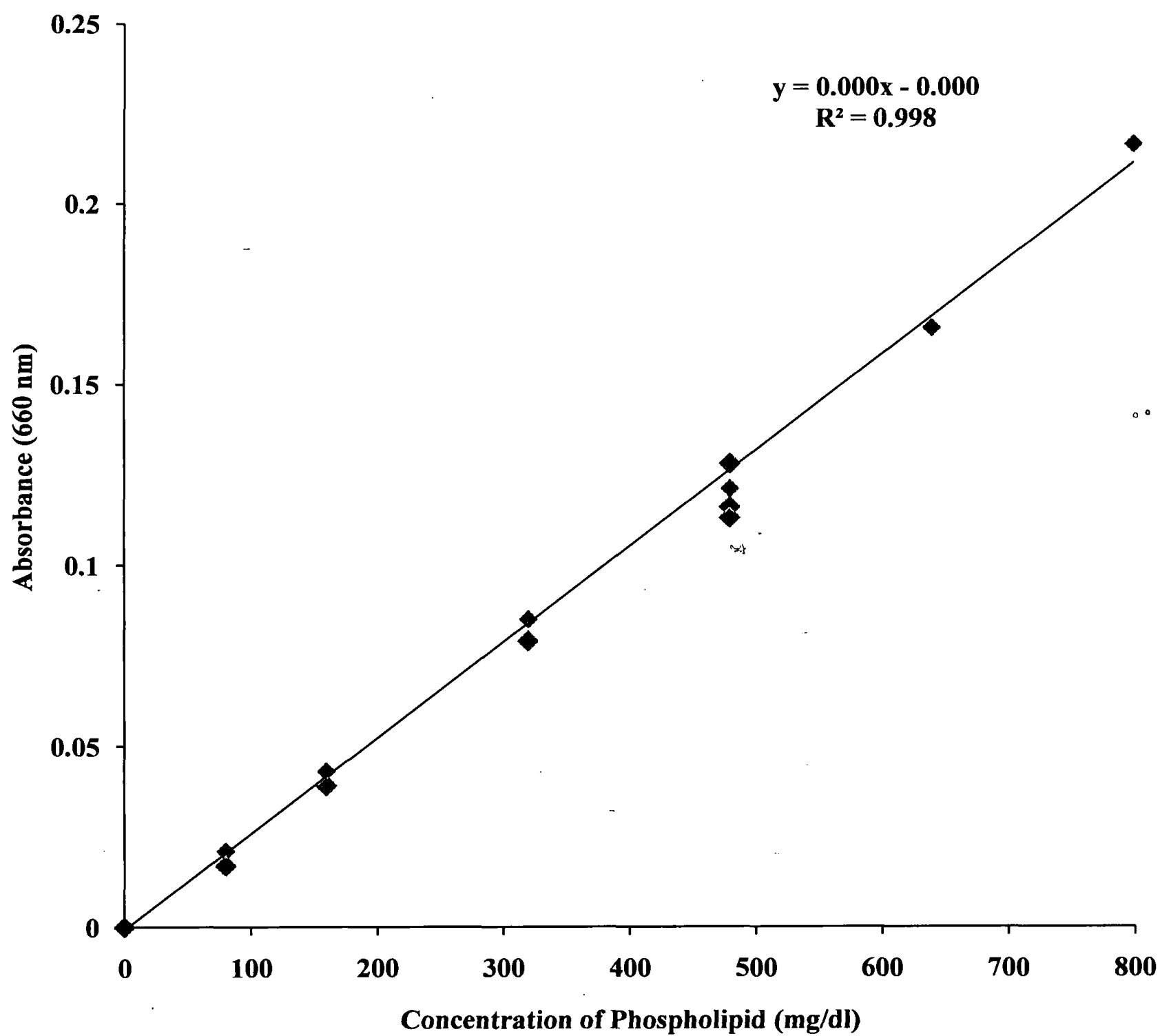


Fig. 5. Standard curve for phospholipid

### 4.5.3. Standard curve for triacylglycerol

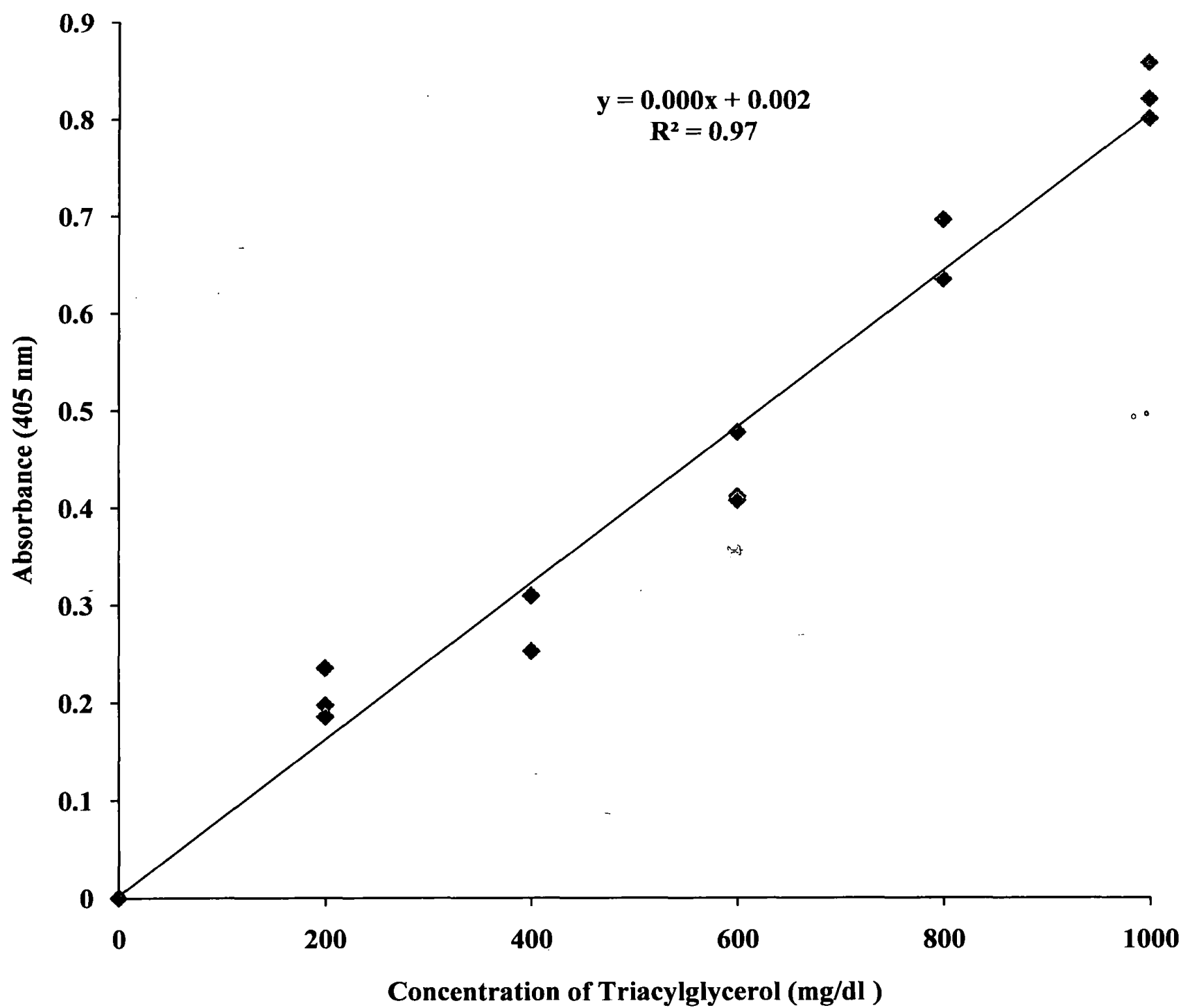


Fig. 6. Standard curve for triacylglycerol

#### 4.6. Data analysis

Results obtained from the statistical analysis for total lipid and lipid class content in gonad, muscle and liver tissues are presented below.

#### 4.7. Total lipid content in tissues

The mean ( $\pm$  SD) total lipid content (percentage of dry weight) in gonad, muscle and liver tissues for all fish revealed that the liver ( $24.34 \pm 10.55$  %) and gonad lipid content ( $15.21 \pm 10.58$  %) were significantly ( $p < 0.05$ ) higher than the muscle lipid content ( $4.68 \pm 1.67$  %) (Fig. 7).

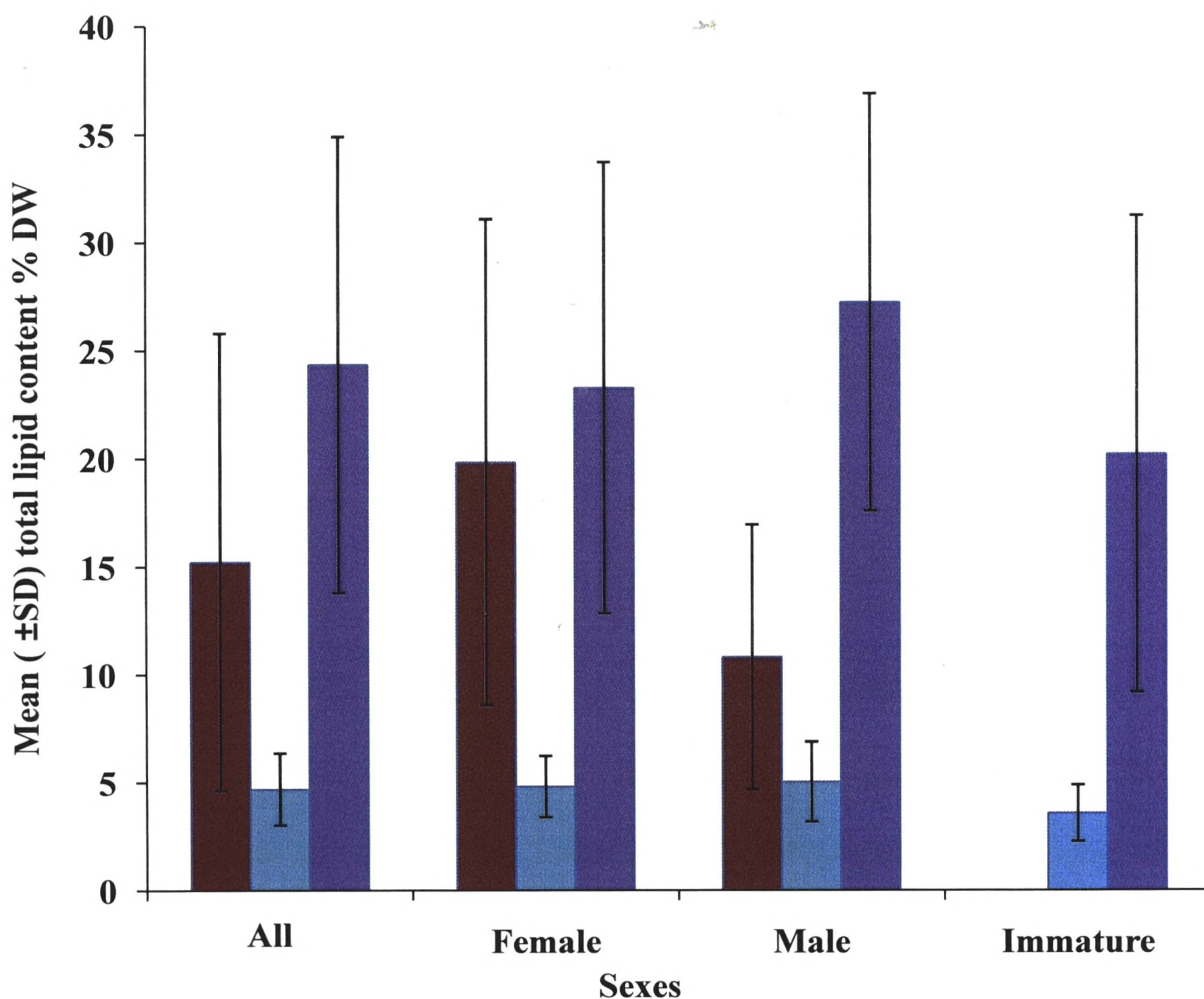


Fig. 7. Total lipid content in tissues

■ Gonad   ■ Muscle   ■ Liver

In females, the mean lipid content of liver and ovary were  $23.27 \pm 10.44$  % and  $19.82 \pm 11.25$  % respectively, while the muscle lipid content was  $4.80 \pm 1.42$  % (Fig. 7).

The mean lipid content of male liver tissues was significantly ( $p < 0.05$ ) higher ( $27.22 \pm 9.65$  %) than the testis lipid content ( $10.80 \pm 6.12$  %). The muscle lipid content was significantly ( $p < 0.05$ ) lower ( $5.02 \pm 1.85$  %) than the above values (Fig. 7).

In immature unsex fish, muscle lipid content was  $3.56 \pm 1.31$  %, whereas the liver lipid content was  $20.19 \pm 11.03$  % (Fig. 7).

Among the tissues of fish, extracted total lipid content was high in liver tissues followed by gonad comprising low values for muscle tissues (Fig. 7).

#### **4.8. Correlation between lipid content in tissues and standard length as well as body weight**

##### **4.8.1. Relationships between lipid content in tissues and standard length (SL)**

During the experimental period, changes of lipid content in gonad, muscle and liver tissues of queenfish varied with the SL. The relationships between lipid in tissues and SL derived for immature unsex, male and female are presented in Figs. 8 - 10.

#### 4.8.1.1. Relationship between lipid content in gonads and standard length

The relationships between lipid content in gonad tissues against SL of *S. lysan* are plotted in Figs. 8a and b.

The percentage of lipid content in ovary tissues (DW), which is positively related to the SL of fish showed a linear relationship between them. In other words, the percentage of gonad lipid content increased significantly with increasing SL. Male *S. lysan* showed a linear relationship between SL and testis lipid.

There was a significant correlation in the females ( $y = 0.715x - 3.511$ ;  $R^2 = 0.612$ ;  $N = 267$ ; Fig. 8a) compared with males ( $y = 0.394x - 6.460$ ;  $R^2 = 0.611$ ;  $N = 207$ ; Fig. 8b).

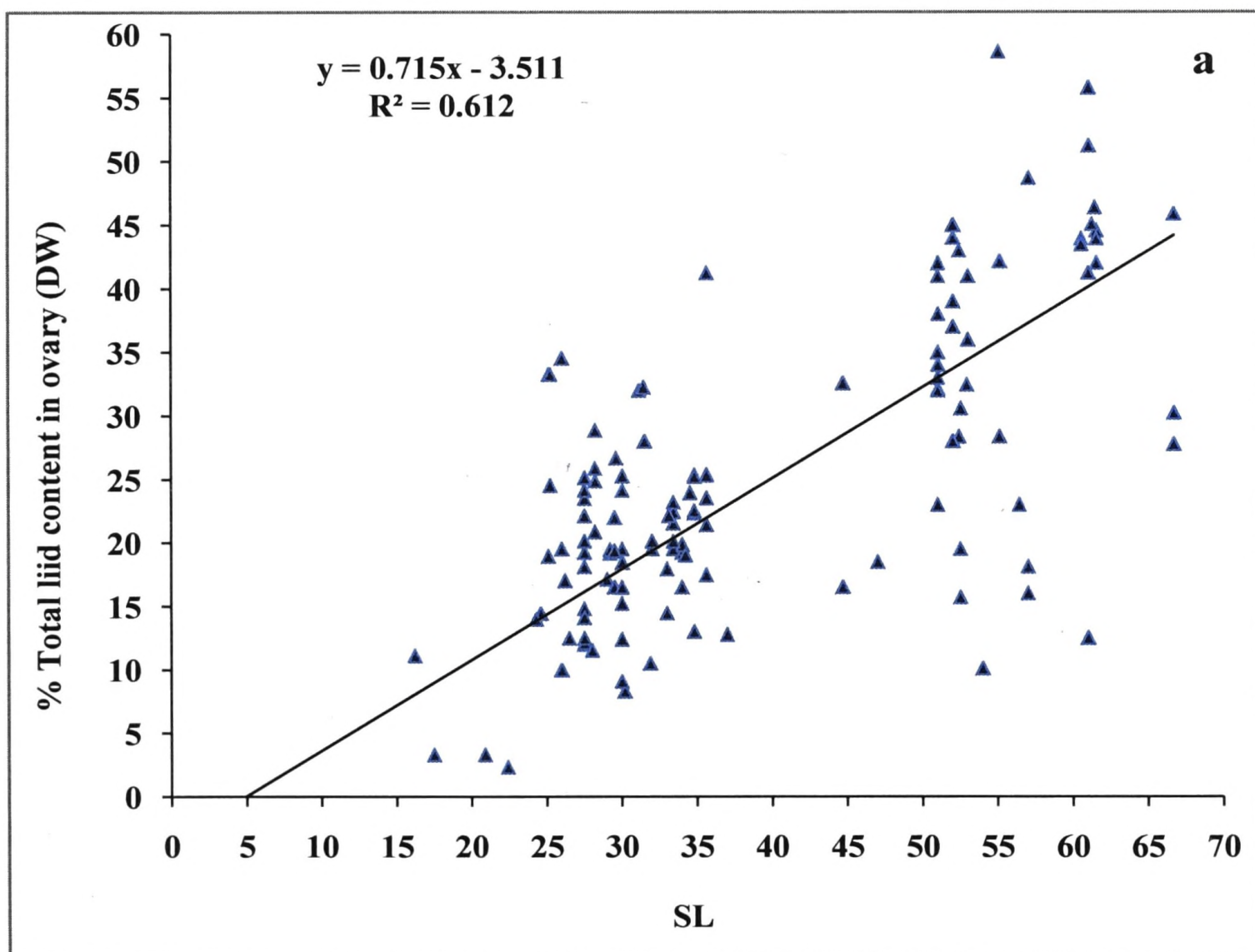


Fig. 8. Relationships between lipid content in gonads and SL of ;  
a: Females

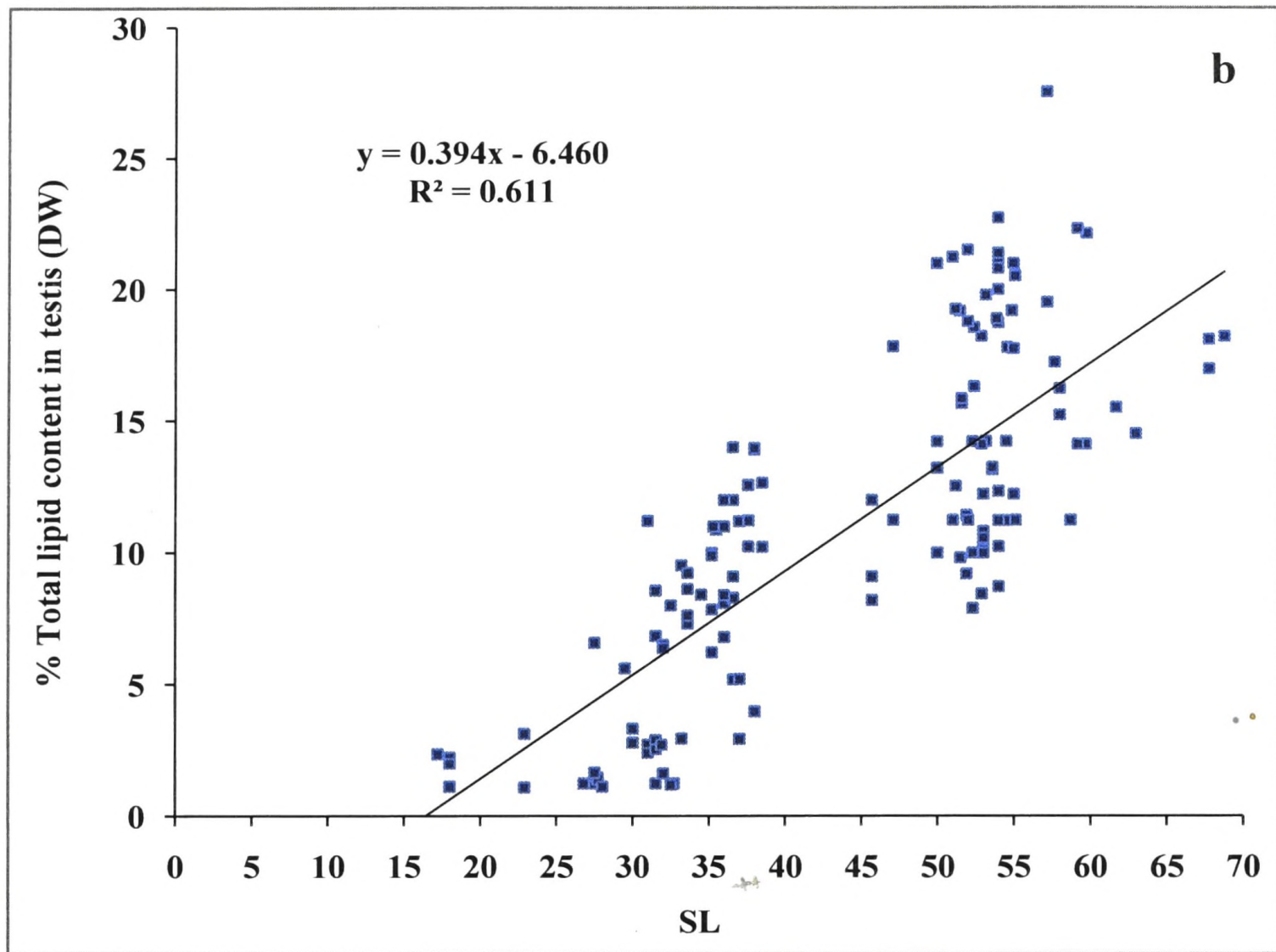


Fig. 8. Relationships between lipid content in gonads and SL of ;  
b: Males

#### 4.8.1.2. Relationship between lipid content in muscles and standard length

The relationship between lipid content in muscle tissues and SL of *S. lysan* plotted in Figs. 9 a, b and c.

The percentage of lipid content in muscle tissues (DW) exhibited a positive significant curvilinear relationship with the standard length of fish. However, a higher significant correlation was observed in the males ( $y = -0.002 x^2 + 0.314 x - 1.876$ ;  $R^2 = 0.617$ ;  $N = 444$ ; Fig. 9a) compared with the females ( $y = -0.002 x^2 + 0.290 x - 1.315$ ;  $R^2 = 0.606$ ;  $N = 456$ ; Fig. 9b).

Adult queenfish (> 37.00 cm in SL) had the highest lipid contents (mean =  $8.34 \pm 2.70$  %) in muscles and the lowest lipid content (mean =  $3.78 \pm 1.15$  %) was observed in immature fish (Figs. 9a, b and c).

When considering immature fish, a positively significant linear relationship ( $y = 0.211x - 0.609$ ;  $R^2 = 0.530$ ;  $N = 255$ ; Fig. 9c) was observed between SL and lipid content.

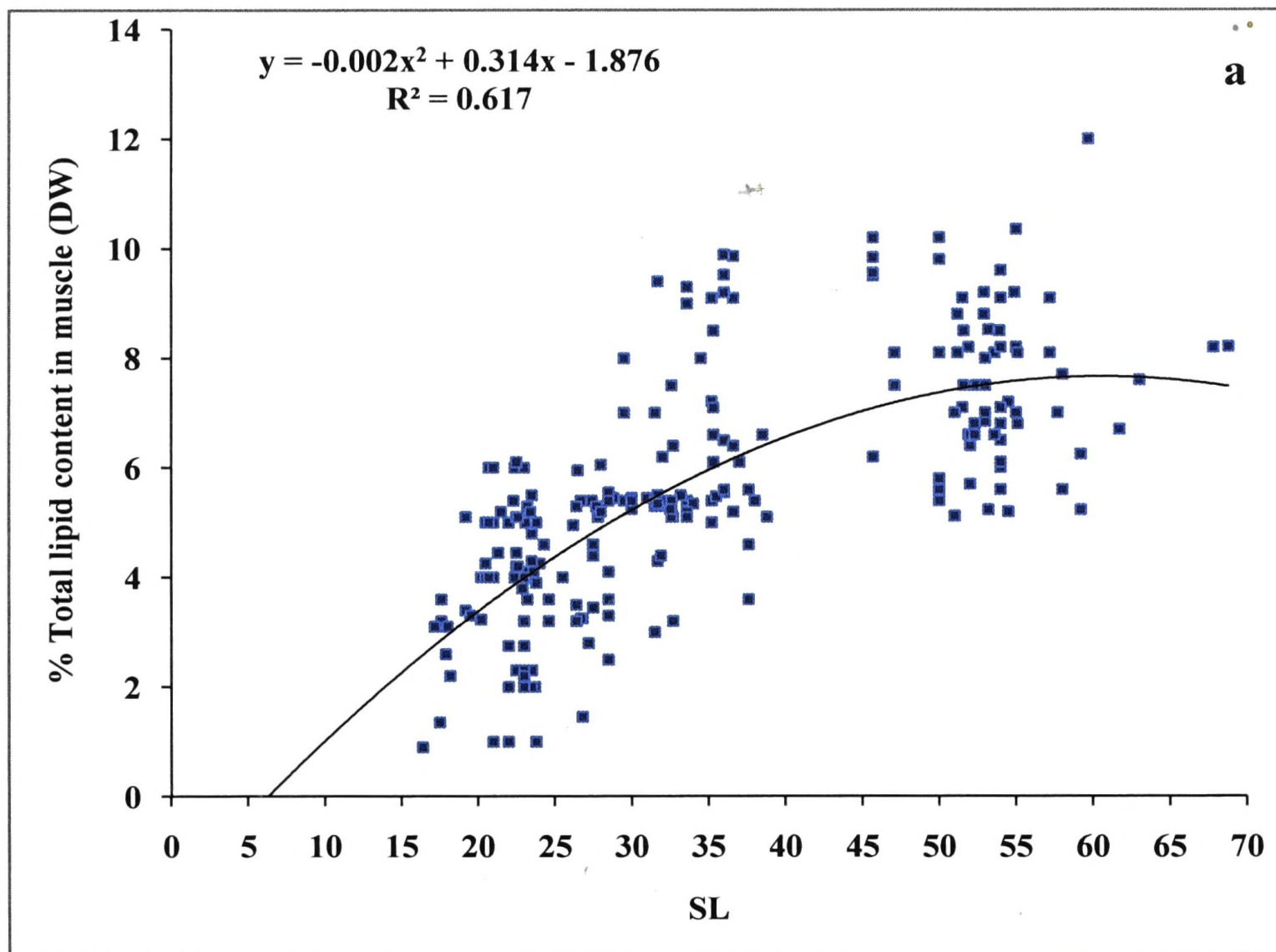


Fig. 9. Relationships between lipid content in muscles and SL of ;  
a: Males,

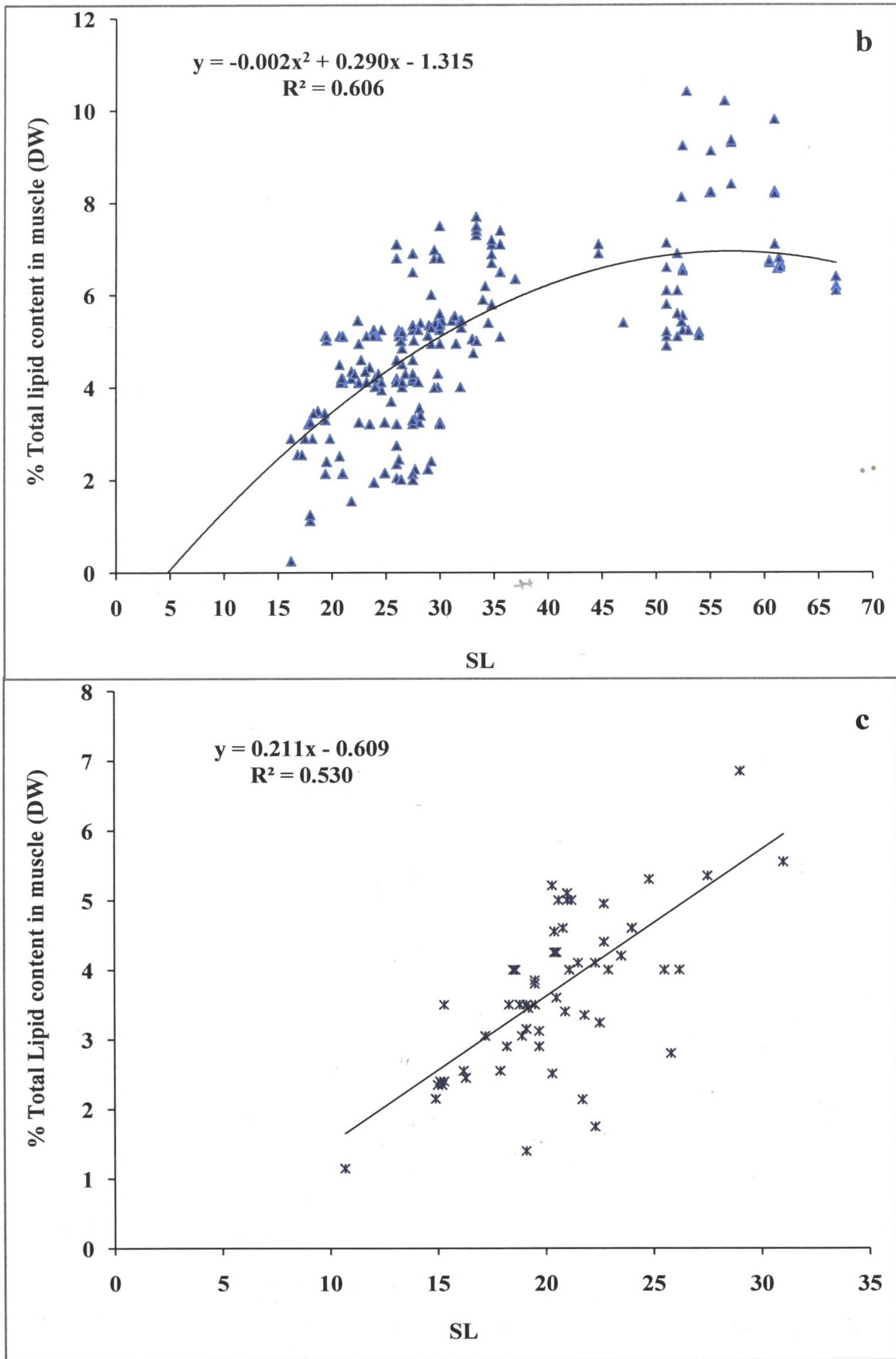


Fig. 9. Relationships between lipid content in muscles and SL of;  
 b: Females, c: Immature

#### 4.8.1.3. Relationship between lipid content in livers and standard length

The relationship between lipid content in liver tissues and SL of *S. lysan* plotted in Figs. 10 a, b and c.

The percentage of lipid content in liver tissues (DW) related to the SL showed a weak correlation between them. The coefficient of determination was higher in males ( $y = -0.054x^2 + 4.209x - 40.77$ ;  $R^2 = 0.453$ ;  $N = 372$ ; Fig. 10a) than females ( $y = -0.017x^2 + 1.771x + 11.42$ ;  $R^2 = 0.429$ ;  $N = 373$ ; Fig. 10b).

A significant curvilinear relationship ( $y = -0.046x^2 + 3.392x - 31.42$ ;  $R^2 = 0.665$ ;  $N = 243$ ; Fig. 10c) was found between muscle lipid content and the SL of immature *S. lysan*.

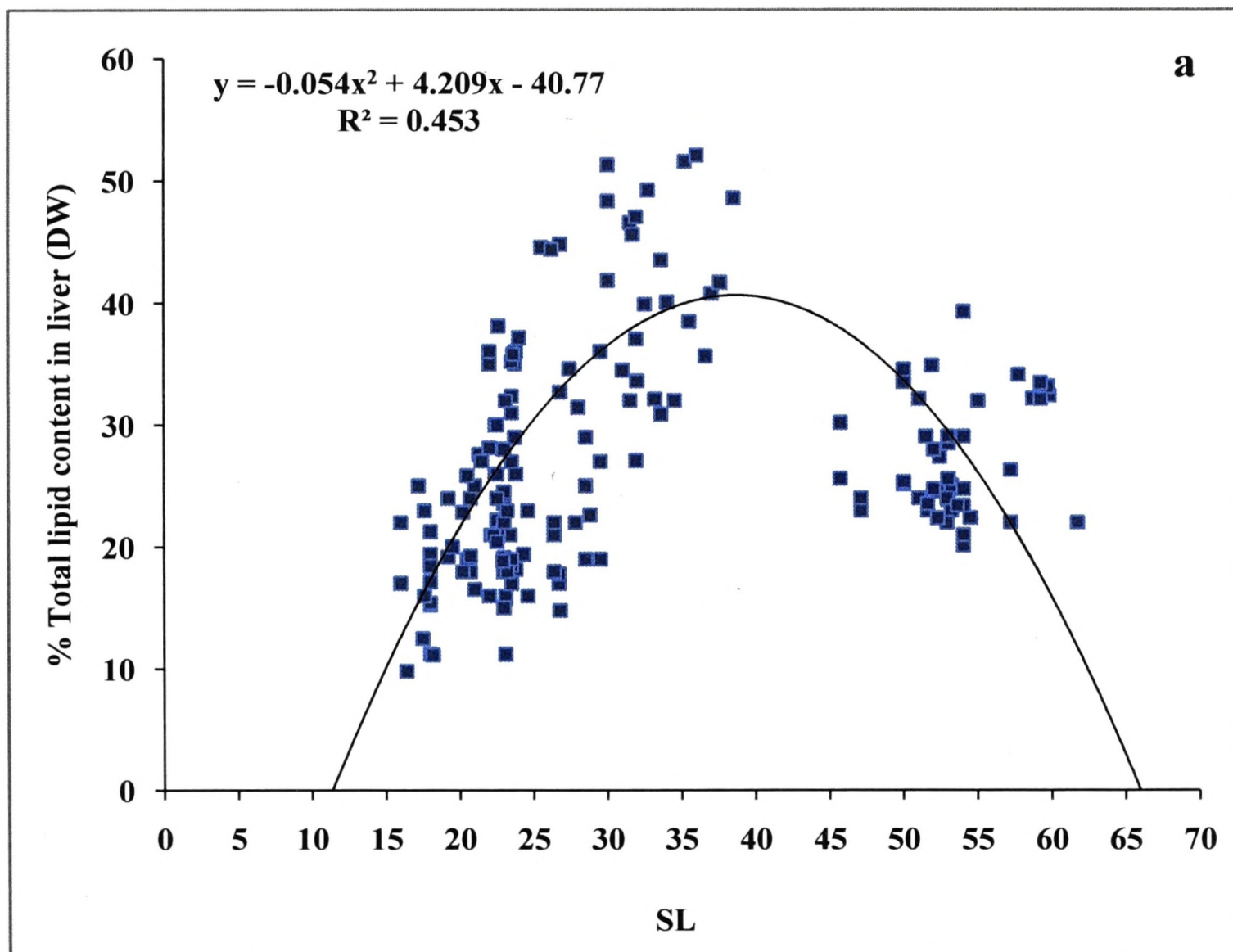


Fig. 10. Relationships between lipid content in livers and SL of ;  
a: Males,

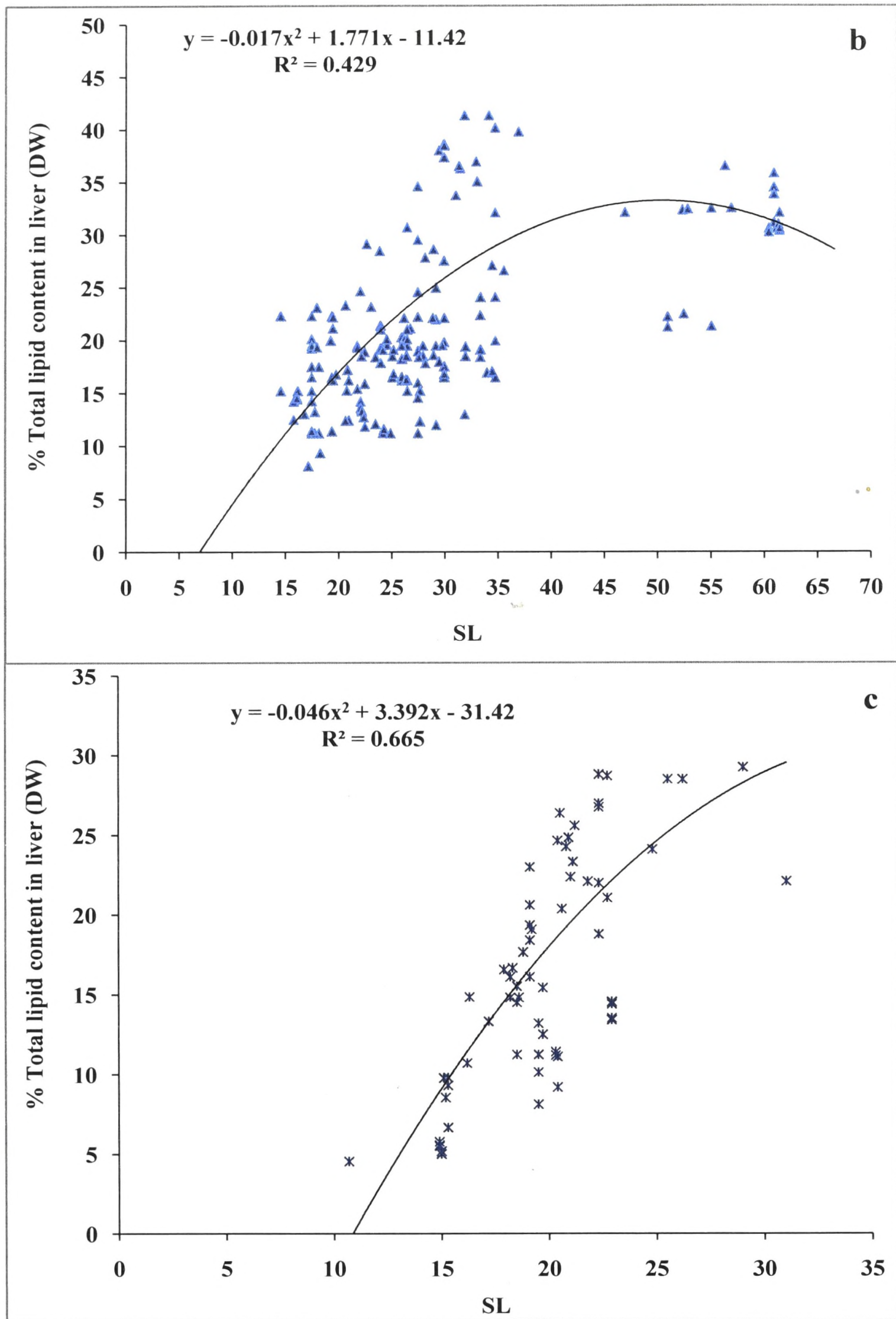


Fig. 10. Relationships between lipid content in livers and SL of ;  
 b: Females, c: Immature

#### 4.8.2. Relationships between lipid content in tissues and body weight (BW)

Relationships between lipid content in gonads, muscles and livers and BW of *S. lysan* fish are shown in Table 8. Body weight of queenfish has a correlation with lipid content of different tissues of fish.

The coefficient of determination for lipid content of muscle tissues against BW were found to be higher in females ( $R^2 = 0.577$ ) than in males ( $R^2 = 0.569$ ) (Table 8).

Weak correlations were observed between lipid content of liver tissues and BW in females ( $R^2 = 0.443$ ) and males ( $R^2 = 0.409$ ) (Table 8).

Significant linear relationships were found between lipid content of gonad tissues of both sexes and BW. The coefficient of determination was higher in females ( $R^2 = 0.537$ ) than the males ( $R^2 = 0.479$ ) (Table 8).

A significant curvilinear relationship was showed between lipid content of liver ( $R^2 = 0.508$ ) and BW in immature fish (Table 8).

Table 8. Relationships between lipid content in muscle, liver and gonad and BW of ;

$R^2$  - Coefficient of determination, p - Probability value

Tissues	sex	n	equation	relation	$R^2$	p value
Muscle	Female	456	$y = -3E-06 x^2 + 0.007 x + 2.811$	Polynomial	0.577	$1.4 \times 10^{130}$
	Male	444	$y = -2E-06 x^2 + 0.005 x + 3.102$	Polynomial	0.569	$3.98 \times 10^{74}$
	Immature	255	$y = -0.000x^2 + 0.311x - 5.291$	Polynomial	0.508	$2.11 \times 10^{85}$
Liver	Female	373	$y = -3E-05 x^2 + 0.062 x + 11.32$	Polynomial	0.443	$4.84 \times 10^{52}$
	Male	372	$y = -4E-05 x^2 + 0.069 x + 15.10$	Polynomial	0.409	$2.8 \times 10^{122}$
	Immature	243	$y = -3E-05x^2 + 0.023x + 1.390$	Polynomial	0.490	$1.68 \times 10^{56}$
Gonad	Female	267	$y = 0.019 x + 9.801$	Linear	0.537	$6.32 \times 10^{38}$
	Male	207	$y = 0.011 x + 4.100$	Linear	0.479	$7.88 \times 10^{31}$

## **4.9. Changes of total lipid and lipid class content in gonad, muscle and liver tissues with gonad maturity stages**

### **4.9.1. Changes of total lipid content**

The percentage of lipid content in gonad, muscle and liver tissues (dry weight) for different gonad maturity stages (immature unsex- stage I, immature sex- stage II, maturing – stage III, mature – stage IV, spawning – stage V and spent stages- Stage VI) of queenfish are shown in Table 9.

#### **4.9.1.1. Gonad tissues**

Percentage of lipid content in the gonad tissues of immature unsexed *S. lysan* was significantly ( $p < 0.05$ ) lower ( $1.51 \pm 0.529$  %) than the lipid content in the other stages (Table 9).

Both sexes showed an increase in gonad lipid content throughout sexual maturation, the highest values were obtained at spawning stage (Table 9). In spawning stage, the lipid content of ovary in females had double the amount of lipid as that of spawning males. The lipid content in ovary showed 7-fold decrease at spent stage when compared to spawning stage while a 4 fold decreases found in the lipid content of males from spawning to spent stages.

Females with spent ovaries ( $5.29 \pm 2.06$  %) and immature ovaries ( $10.89 \pm 7.50$  %) had the lowest lipid content, while the highest values were found in the spawning ovaries ( $37.07 \pm 10.15$  %) and followed by mature ovaries ( $30.99 \pm 13.52$  %) (Table 9). In female fish, lipid content in all the gonad maturity stages (immature, maturing,

mature, spawning and spent stages) were significantly different ( $p < 0.05$ ) between each other.

Similar trend was also observed in males. The highest values of lipid content found in spawning testis ( $16.11 \pm 4.74\%$ ) followed by mature testis ( $14.59 \pm 5.57\%$ ) whereas the lowest values found in immature ( $4.13 \pm 3.49\%$ ) and spent testis ( $4.14 \pm 3.26\%$ ) (Table 9). Lipid content in mature testis was not significantly different ( $p = 0.99$ ) from spawning testis.

#### **4.9.1.2. Muscle tissues**

In the lipid content of immature, maturing, mature, spawning and spent stages of both sexes, the muscle tissues of males had the highest lipid content than that of females (Table 9). Lipid content of muscle in females showed a 30% decrease from mature stage to spawning stage, whereas a 3% decrease in the lipid content of muscle in males was observed from mature stage to spawning stage.

In females, the lipid content in muscle tissues was lower in immature unsexed ( $3.63 \pm 1.23\%$ ) whereas higher in mature stages ( $8.49 \pm 1.21\%$ ) which was significantly different ( $p < 0.05$ ) from that of all other stages (Table 9). Lipid content in the spawning females ( $5.84 \pm 0.97\%$ ) decreased from mature stages. The lipid content of spent stage was  $4.55 \pm 0.53\%$ , which was significantly different ( $p < 0.05$ ) from other stages (Table 9).

For males, the lipid content of muscles in immature ( $4.07 \pm 1.19\%$ ) and spent stages ( $4.38 \pm 0.42\%$ ) was not significantly different ( $p = 0.45$ ) (Table 9). Lipid content of muscles in maturing ( $6.15 \pm 1.57\%$ ) and mature stages ( $7.65 \pm 2.79\%$ ) was significantly different ( $p = 0.0006$ ) between each other. No significant differences ( $p = 0.52$ ) were observed between mature and spawning stages ( $7.38 \pm 1.45\%$ ).

#### **4.9.1.3. Liver tissues**

Immature unsexed fish exhibited low lipid value in liver tissues ( $16.13 \pm 6.84 \%$ ). The lipid content of liver tissues was highest in mature females ( $31.18 \pm 7.00 \%$ ) and mature males ( $35.28 \pm 5.48\%$ ) than that of other stages (Table 9). The lipid content of liver tissues in both sexes decreased during spawning.

In females fish, the mean lipid content of liver tissues in immature stages were significantly ( $p = 0.0004$ ) lower ( $18.58 \pm 6.29 \%$ ) than that of maturing fish ( $28.02 \pm 10.67 \%$ ) (Table 9). There were no significant differences ( $p = 0.16$ ) between lipid content of liver in maturing and mature stages of female. The spawning fish exhibited lower lipid content ( $16.94 \pm 8.81 \%$ ), that differed significantly ( $p = 2.56 \times 10^{-6}$ ) from mature stage.

In male fish, the mean lipid content of liver tissues in immature stages were significantly ( $p = 0.0002$ ) lower ( $23.45 \pm 7.55 \%$ ) than that of maturing fish ( $33.79 \pm 12.86 \%$ ) (Table 9). The lipid content was the highest in mature stage of males ( $35.28 \pm 5.48 \%$ ), which was significantly different ( $p = 9.29 \times 10^{-6}$ ) from that of spawning stage.

Table 9. Percentage of total lipid content in gonad, muscle and liver tissues in gonad maturity stages of *S. lysan*

Gonad Maturity Stages	Total lipid in gonads		Total lipid in muscles		Total lipid in livers	
	Female	Male	Female	Male	Female	Male
I- Immature unsex	1.51 ± 0.56	1.51 ± 0.56	3.63 ± 1.23	3.63 ± 1.23	16.13 ± 6.84	16.13 ± 6.84
II - Immature	10.89 ± 7.50	4.13 ± 3.49	3.68 ± 1.18	4.07 ± 1.19	18.58 ± 6.29	23.45 ± 7.55
III- Maturing	20.47 ± 6.12	11.44 ± 4.21	5.59 ± 0.87	6.15 ± 1.57	28.02 ± 10.67	33.79 ± 12.86
IV- Mature	30.99 ± 13.52	14.59 ± 5.57	8.49 ± 1.21	7.65 ± 2.79	31.18 ± 7.00	35.28 ± 5.48
V - Spawning	37.07 ± 10.15	16.11 ± 4.74	5.84 ± 0.97	7.38 ± 1.45	16.94 ± 8.81	22.49 ± 5.13
VI - Spent	5.29 ± 2.06	4.14 ± 3.26	4.55 ± 0.53	4.38 ± 0.42	10.32 ± 3.45	11.83 ± 5.88

#### **4.9.2. Changes of cholesterol, phospholipids and triacylglycerol content**

The changes in lipid content in gonad, muscle and liver tissues for immature unsex- stage I, immature sex- stage II, maturing – stage III, mature – stage IV, spawning – stage V and spent stages- Stage VI are described in Figs. 11 - 28. There was substantial variability in the cholesterol (CS), phospholipids (PL) and triacylglycerol (TAG) content for different gonad maturity stages.

##### **4.9.2.1. Changes of lipid class content in ovary tissues of females**

The lipid class contents in the ovary throughout the maturation period are presented in Figs. 11 - 13. The amounts of CS, PL and TAG in the ovary increased throughout the ovarian maturation (stage II - V) and significantly declined ( $p < 0.05$ ) thereafter.

##### **4.9.2.1.1. Cholesterol content**

In stage II, CS content was low ( $74.69 \pm 34.19$  mg/100g) and exhibited a least amount compared with other lipid classes (PL =  $184.76 \pm 92.76$  mg/100g and TAG =  $110.54 \pm 49.07$  mg/100g) (Fig. 11). CS content varied from stage III ( $183.16 \pm 101.19$  mg/100g) to stage IV ( $202.41 \pm 86.29$  mg/100g), significantly ( $p = 0.0001$ ) increased in stage V ( $359.04 \pm 111.85$  mg/100g) and significantly ( $p = 2.86 \times 10^{-5}$ ) decreased rapidly to almost  $68.05 \pm 9.56$  mg/100g in stage VI.

CS Content in ovary showed approximately 5 fold increase at stage V when compared to stage II while a 5 fold decrease in ovarian CS content was evident from stage V to VI (Fig. 11).

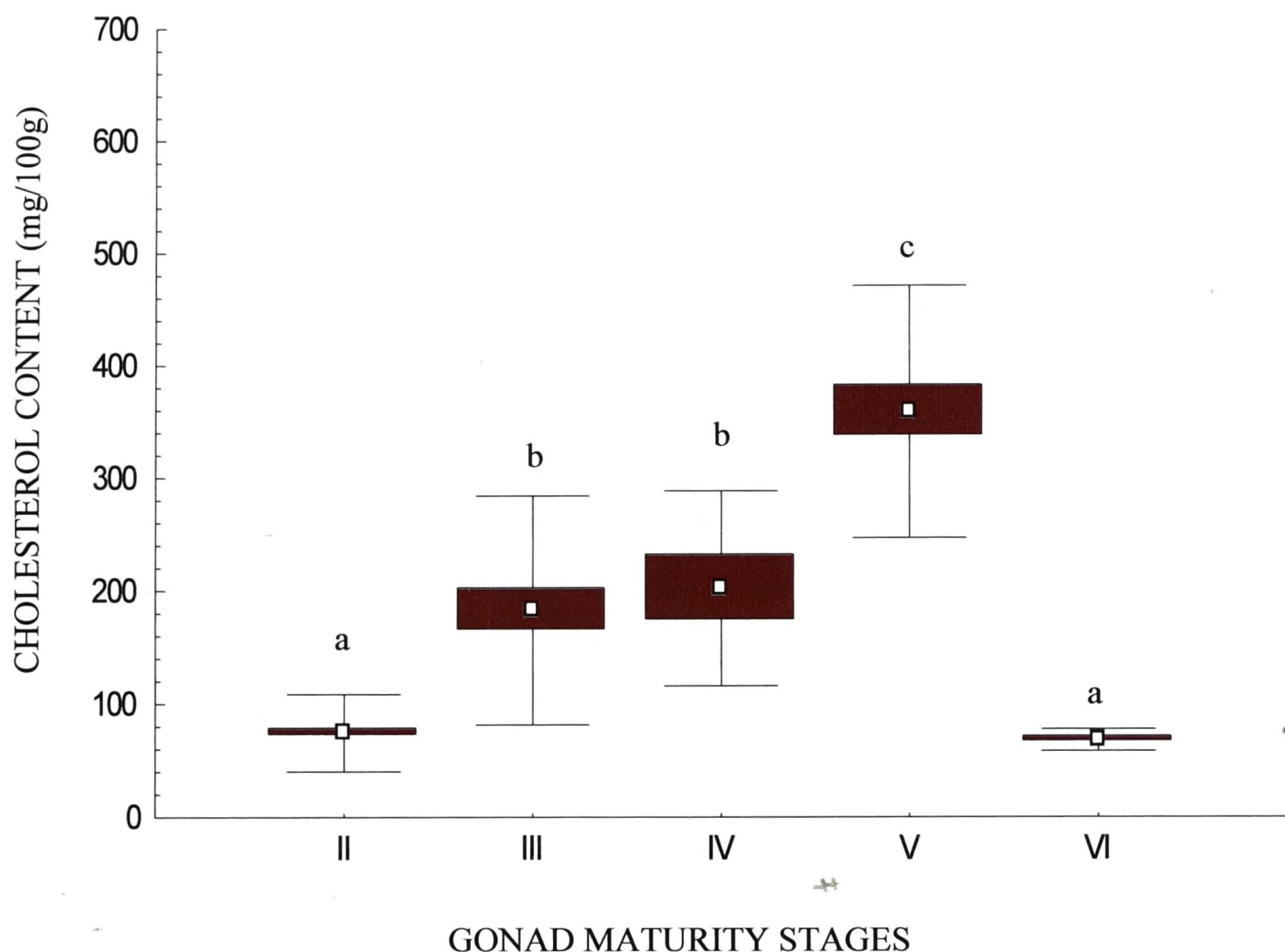


Fig. 11. Variation of cholesterol content in ovarian tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.1.2. Phospholipid content

The mean PL levels were not significantly ( $p = 0.12$ ) different between stage II and III, and fluctuated up to stages VI (Fig. 12). The mean PL content at stage III was  $254.03 \pm 124.92$  mg/100g, which is more or less similar to the TAG content ( $246.53 \pm 102.26$  mg/100g) at stage III while, higher than that of CS content. The PL content significantly ( $p = 0.003$ ) increased upto stage V from stage III, attained the highest value ( $388.49 \pm 86.70$  mg/100g). It significantly ( $p = 7.32 \times 10^{-6}$ ) decreased to  $172.87 \pm 164.08$  mg/100g in stage VI. The mean PL content in ovary was not significantly ( $p = 0.39$ ) different between stage IV and stage V.

PL content in ovary showed approximately 2 fold increase at stage V when compared to stage II and a 2 fold decrease from stage V to VI (Fig. 12).

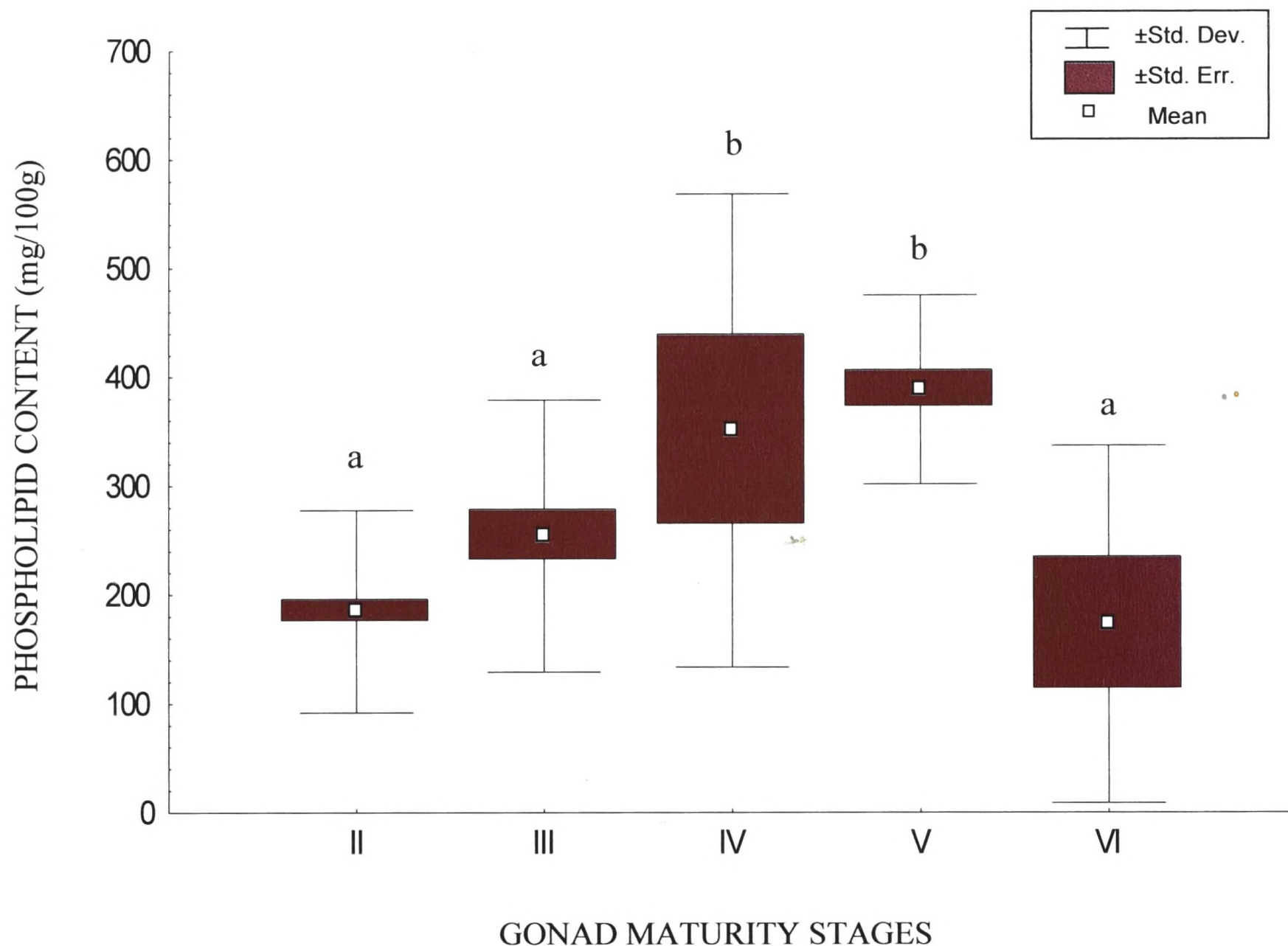


Fig. 12. Variation of phospholipid content in ovarian tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.1.3. Triacylglycerol content

Mean TAG levels fluctuated up and down during the maturation period and the lowest level was found at stage VI (Fig. 13). The TAG content doubled in stage III than stage II and continued to increase significantly throughout maturation. Stage V fish had predominant high level of TAG ( $406.96 \pm 161.22$  mg/100g) than the other stages then

significantly ( $p = 2.94 \times 10^{-5}$ ) decreased in stage VI ( $50.48 \pm 16.38$  mg/100g). Content of TAG in ovary showed approximately 4 fold increase at stage V when compared to stage II whereas a 8 fold decrease in ovarian TAG content was evident from stage V to VI (Fig. 13).

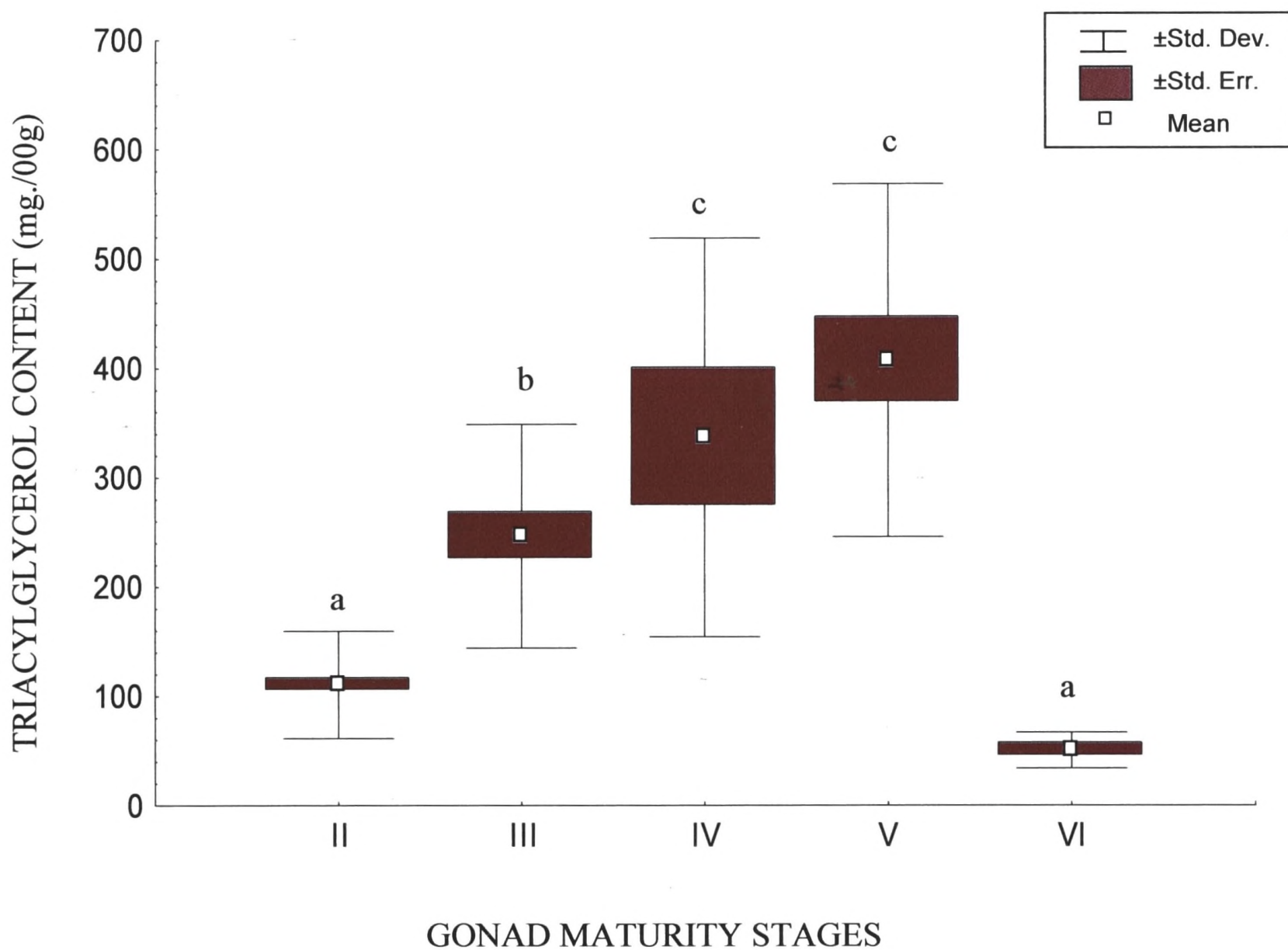


Fig. 13. Variation of triacylglycerol content in ovarian tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### **4.9.2.2. Changes of lipid class content in testis tissues of males**

Changes in lipid classes in testis tissues of males also followed a similar trend as that of ovarian tissues of females during maturation and spawning (Figs. 14 – 16.). The amounts of CS, PL and TAG increased during maturation of testis. Lipid content increased from stage II to V, reached its maximum value in stage V and decreased significantly ( $p < 0.05$ ) after spawning (stage VI). Almost high amount TAG was found in fully mature testis.

##### **4.9.2.2.1 Cholesterol content**

Mean CS values for testis did not vary significantly ( $p = 0.32$ ) between stage II ( $84.37 \pm 39.20$  mg/100g) and III ( $110.97 \pm 35.80$  mg/100g) (Fig. 14) In fully mature spawning testis at stage V,  $235.68 \pm 64.26$  mg/100g of CS content was recorded. This was the highest level whereas lowest level of CS ( $38.47 \pm 19.72$  mg/100g) was recorded in stage VI.

CS content in testis showed approximately 3 fold increase at stage V when compared to stage II whereas a 6 fold decrease in the CS content was evident from stage V to VI.

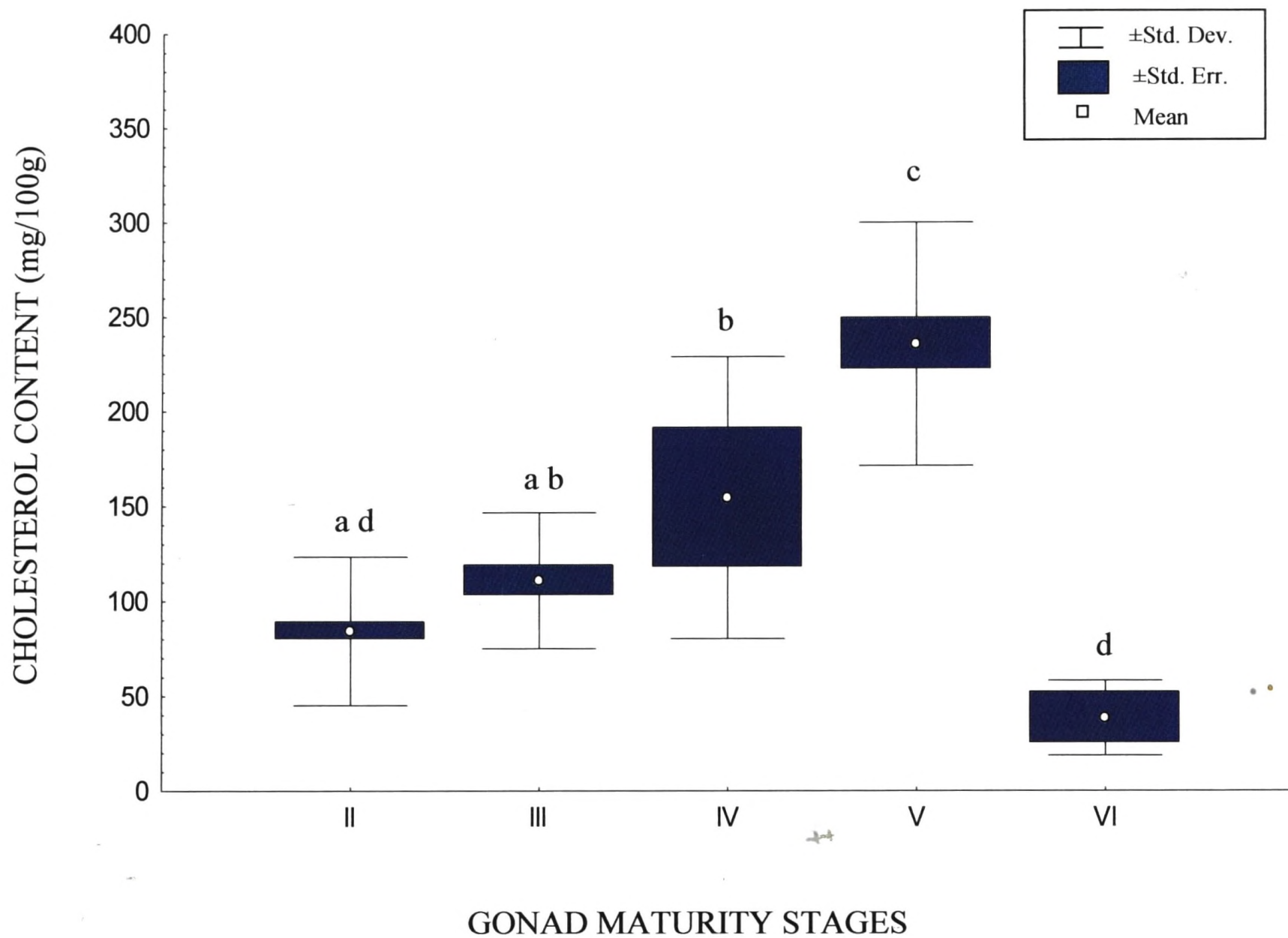


Fig. 14. Variation of cholesterol content in testis tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.2.2 Phospholipid content

Highest PL content ( $345.88 \pm 115.27$  mg/100g) was recorded in spawning testis (stage V) whereas significantly ( $p = 2.89 \times 10^{-5}$ ) lowest level of PL ( $28.85 \pm 13.36$  mg/100g) was recorded in stage VI (Fig.15.). The mean PL content of testis was not significantly ( $p = 0.67$ ) different between stage IV and V.

PL content in testis showed approximately 2 fold increase at stage V when compared to stage II while a 10 fold decrease in the PL content was evident from stage V to VI.

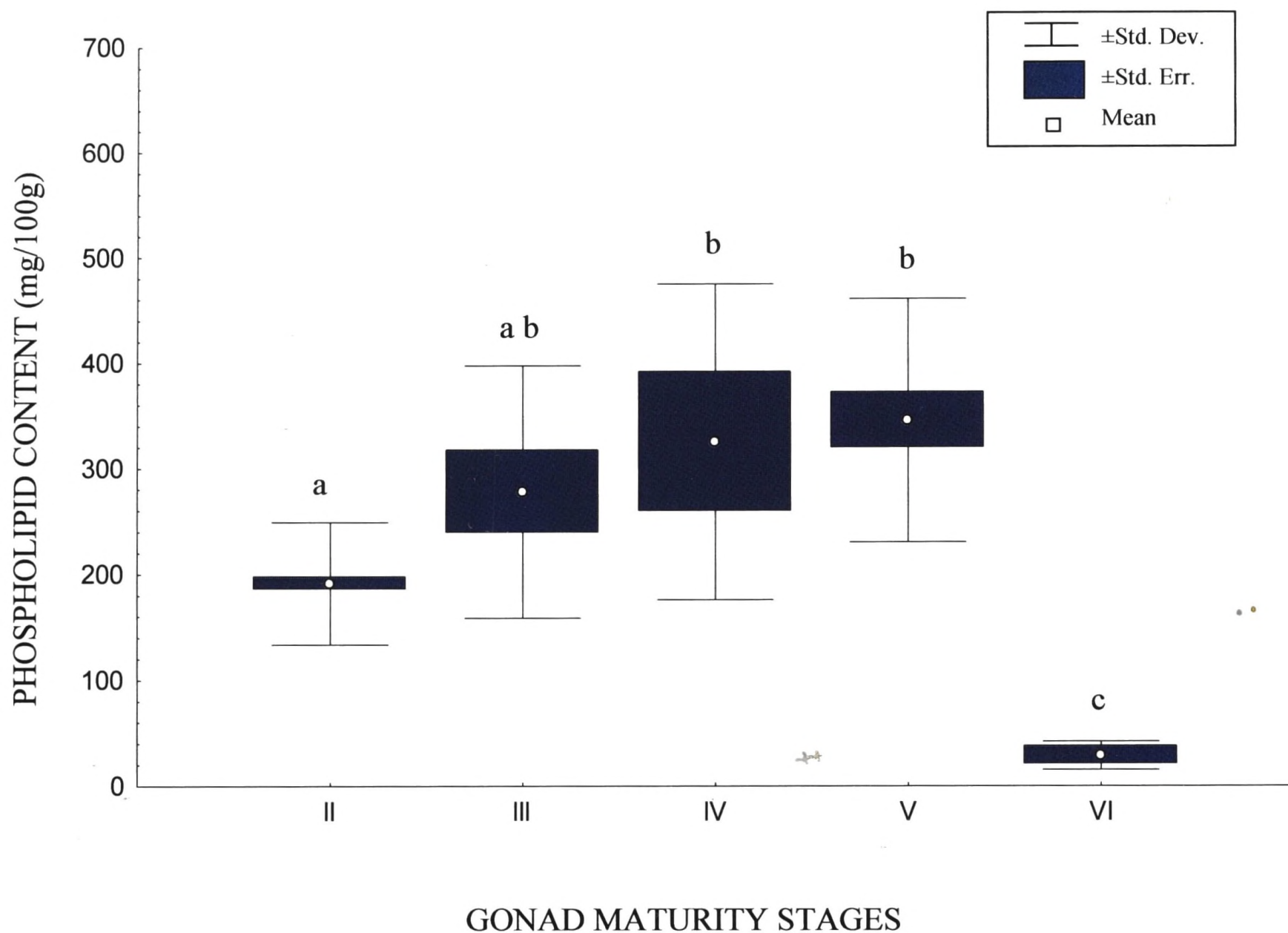


Fig. 15. Variation of phospholipid content in testis tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.2.3. Triacylglycerol content

TAG in testis tissues significantly ( $p = 0.02$ ) increased from stage II ( $189.82 \pm 85.85$  mg/100g) to stage V. In spawning testis of male (stage V),  $379.45 \pm 251.89$  mg/100g of TAG was recorded whereas  $74.27 \pm 31.45$  mg/100g of TAG was recorded in stage VI (Fig. 16). TAG content in testis showed approximately 2 fold increase at stage V when compared to stage II while a 5 fold decrease in the PL content was evident from stage V to VI.

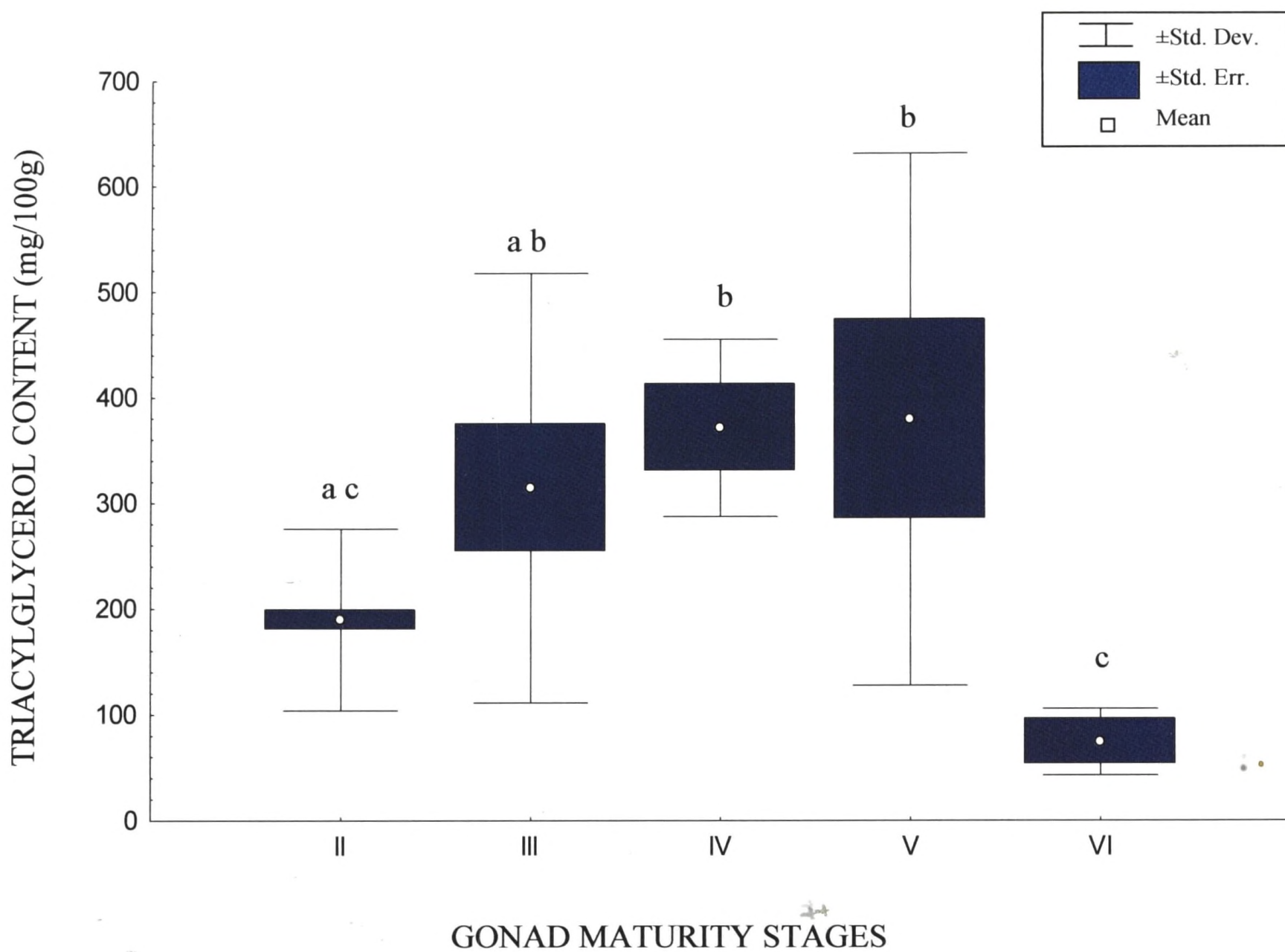


Fig. 16. Variation of triacylglycerol content in testis tissues of *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.3. Changes of lipid class content in muscle tissues of females

The amount of lipid class of the muscle tissues are plotted in Figs. 17 – 19. Unlike the ovary, the mean PL content was more abundant than TAG in all stages.

##### 4.9.2.3.1. Cholesterol content

The CS content in muscle tissues of female was the lowest when compared with other lipid class. The CS content significantly ( $p = 0.004$ ) increased up to stage III ( $169.60 \pm 52.05$  mg/100g) from stage II ( $133.73 \pm 31.53$  mg/100g) and decreased beyond that (Fig. 17). The CS content was low in stage V ( $55.19 \pm 37.49$  mg/100g) and decreased

further in stage VI ( $25.48 \pm 6.05$  mg/100g). No significant difference ( $p = 0.31$ ) was identified between stage I and V.

CS content in muscle showed approximately 2½ fold increase at stage III when compared to stage I while a 6 fold decrease in the CS content was evident from stage III to VI (Fig. 17).

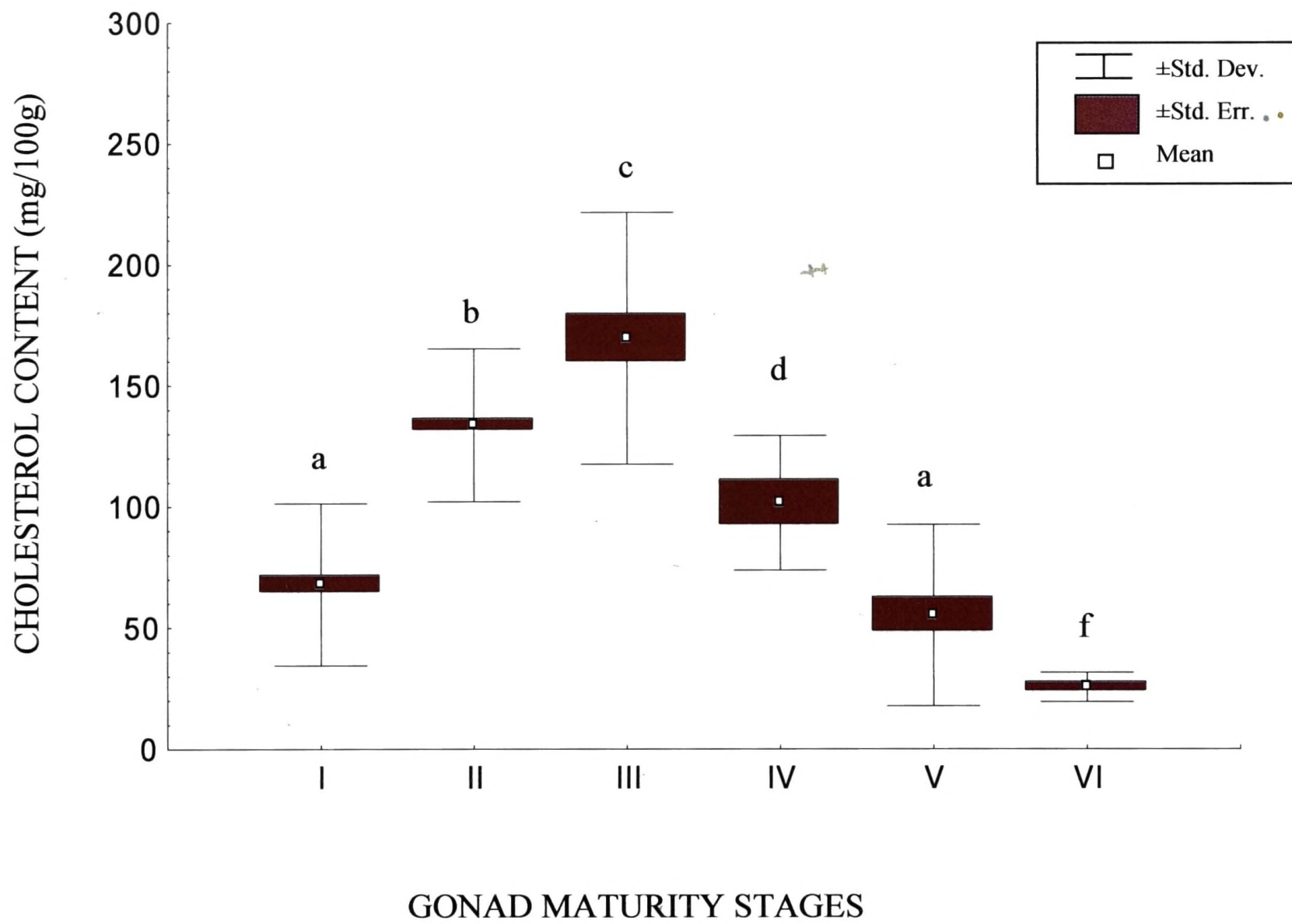


Fig. 17. Variation of cholesterol content in muscle tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.3.2. Phospholipid content

The mean PL levels ( $455.04 \pm 324.05$  mg/100g) in the muscle at stage IV were higher than the other stages (Fig. 18). PL content in muscle increased ( $p = 0.008$ ) from stage I to IV and decreased thereafter until stage VI. Significant difference ( $p = 0.001$ ) was observed between stage IV and V. The PL content was higher in females than males at stage IV (Fig. 18).

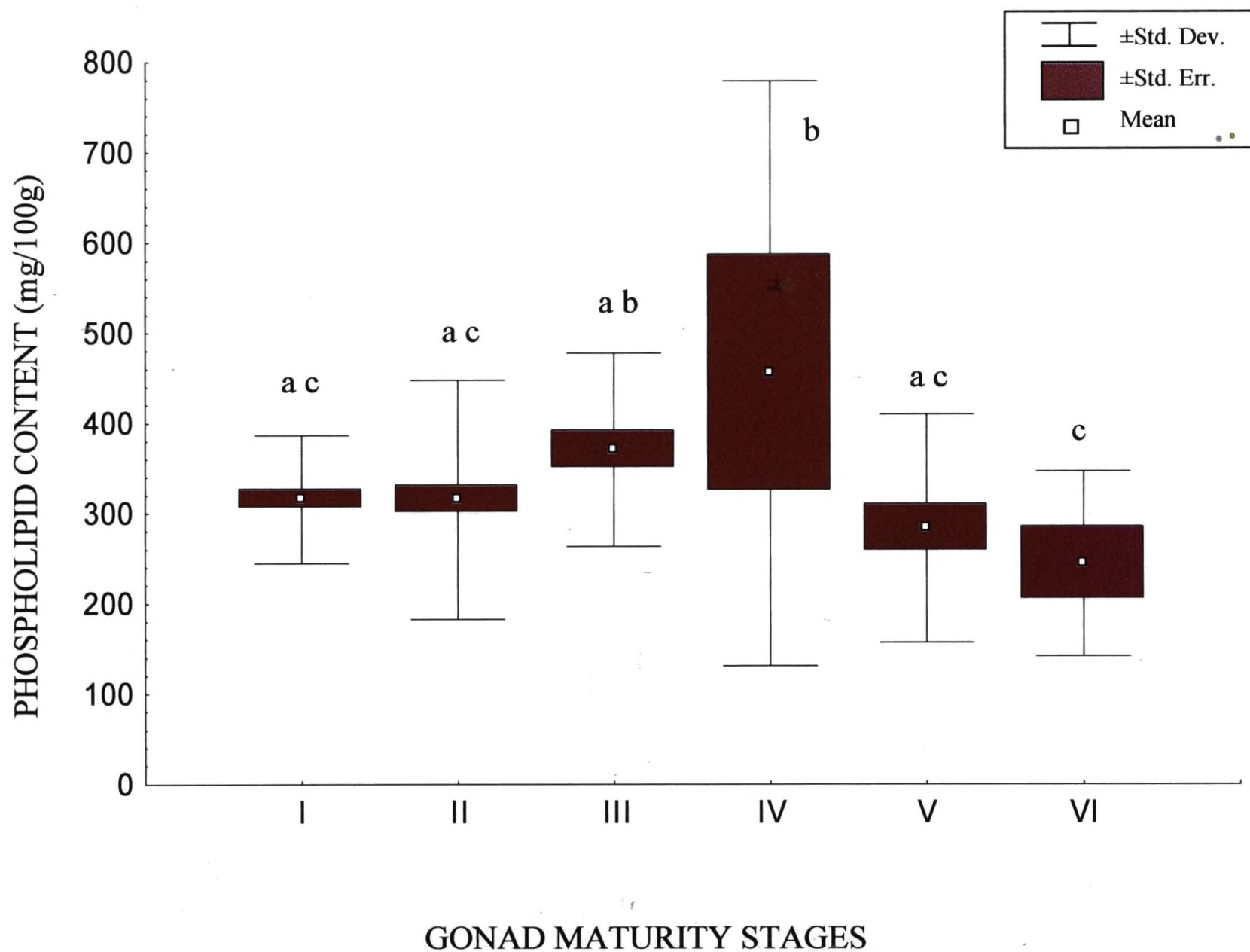


Fig. 18. Variation of phospholipid content in muscle tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.3.3. Triacylglycerol content

Mean TAG in females significantly ( $p = 4.29 \times 10^{-6}$ ) increased from stage I ( $65.73 \pm 15.25$  mg/100g) to stage IV ( $346.11 \pm 180.36$  mg/100g) and decreased ( $p = 4.05 \times 10^{-6}$ ) upto stage VI (Fig. 19). The amounts of TAG in the muscle tissues were the highest in stage IV and showed a decrease from stage IV onwards. No significant difference ( $p = 0.61$ ) was observed between stage V and VI. The mean TAG was lowest in stage VI ( $65.18 \pm 53.73$  mg/100g) (Fig. 19).

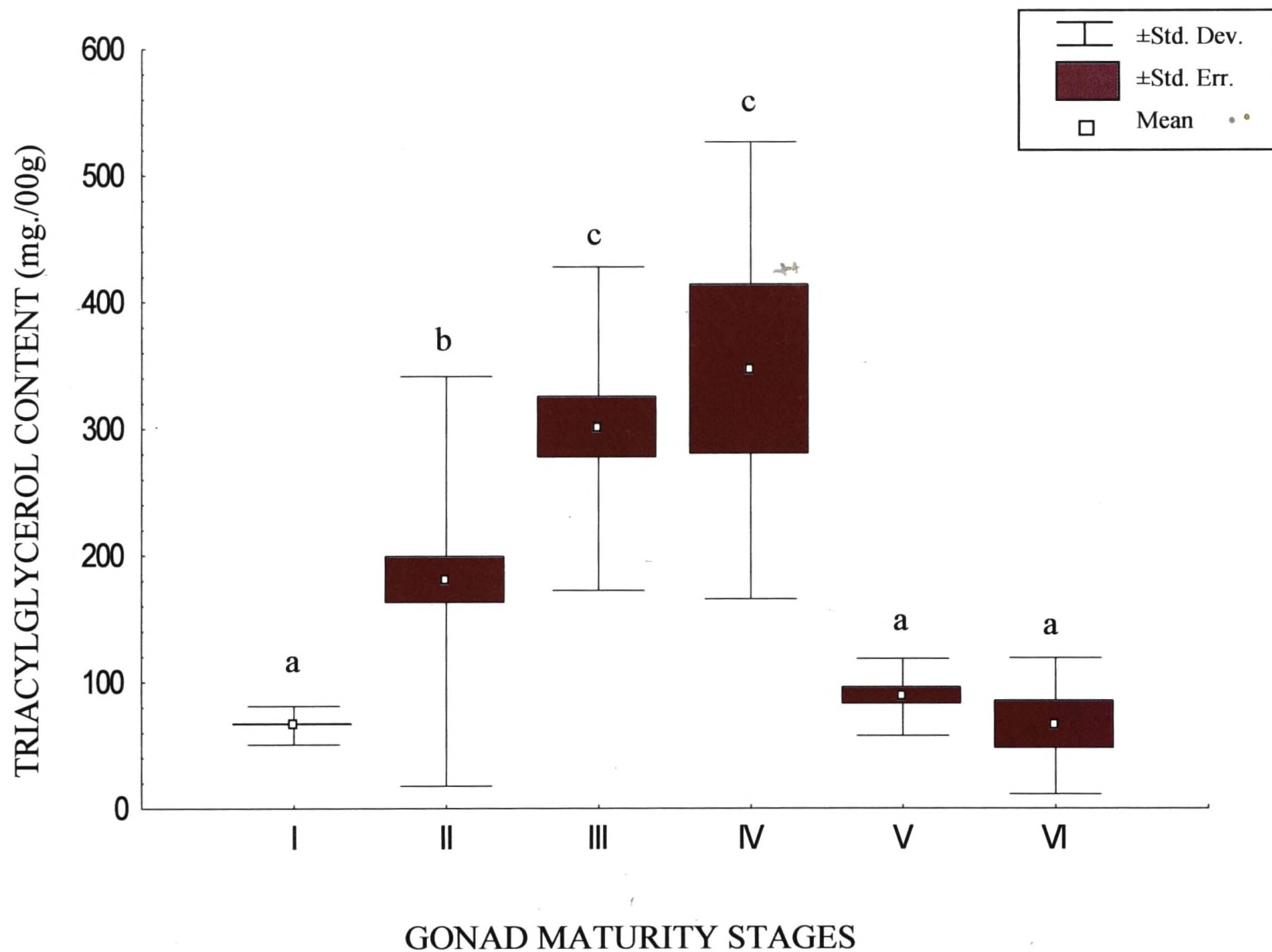


Fig. 19. Variation of triglyceride content in muscle tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### **4.9.2.4. Changes of lipid class content in muscle tissues of males**

The amount of lipid class of the muscle tissues in male throughout the developmental stages are plotted in Figs. 20 – 22. Mean values of PL was predominant in the muscle tissues and followed by TAG content and CS content.

##### **4.9.2.4.1. Cholesterol content**

In the case of muscle tissues of male, CS content increased ( $p = 0.01$ ) from stage I ( $67.89 \pm 33.45$  mg/100g) to II ( $111.81 \pm 34.54$  mg/100g), highest content being recorded in stage II and decreased thereafter (Fig. 20). Values obtained for CS content in stage III was  $80.50 \pm 41.48$  mg/100g, in stage IV was  $49.66 \pm 1.64$  mg/100g, in stage V was  $47.00 \pm 28.90$  mg/100g and in stage VI was  $22.45 \pm 2.24$  mg/100g. The CS content in stage II was significantly different from stage IV ( $p = 0.0008$ ) and stage V ( $p = 0.0005$ ). No significant difference ( $p = 0.88$ ) was observed between stage IV and V.

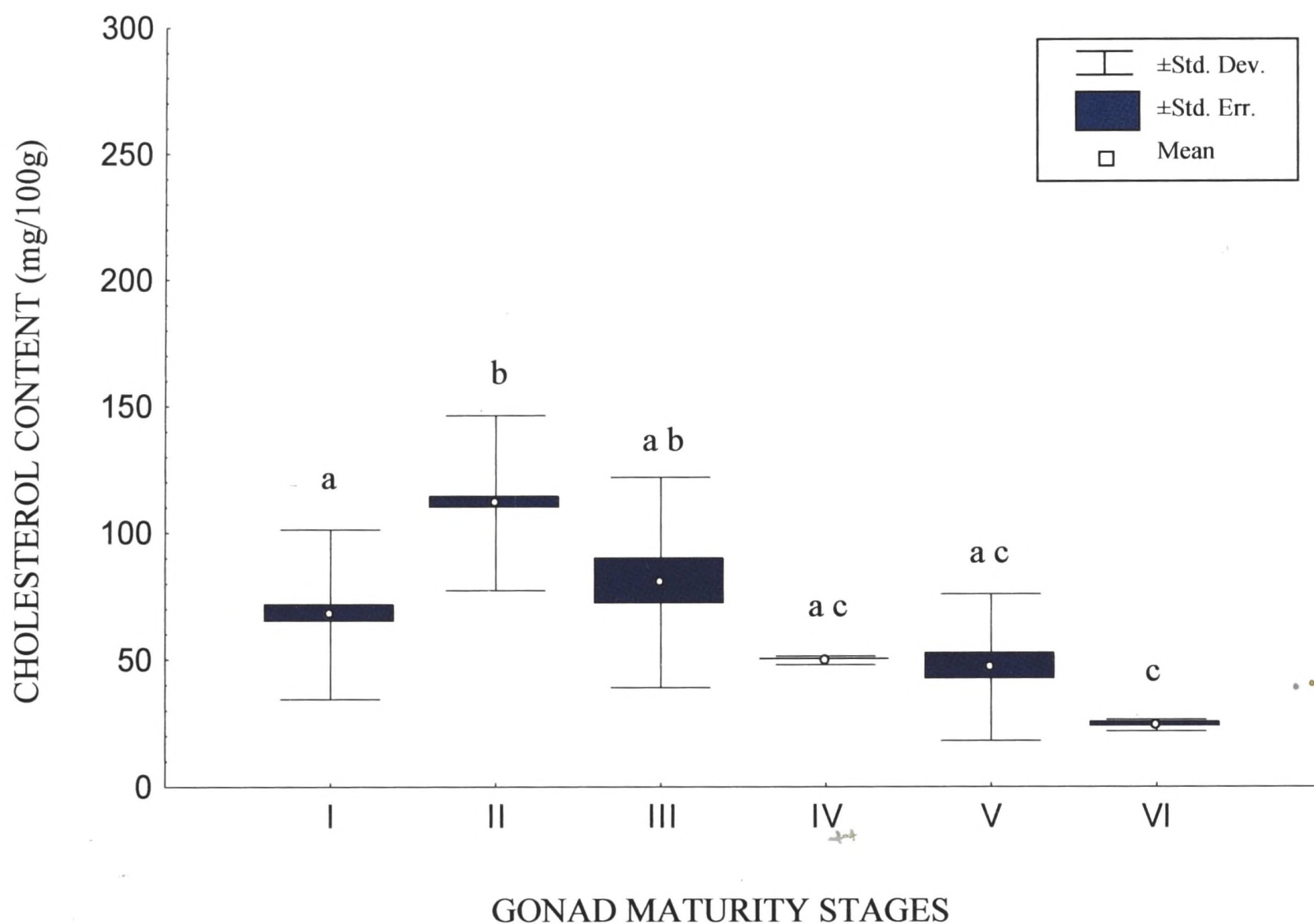


Fig. 20. Variation of cholesterol content in muscle tissues of males *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.4.2. Phospholipid content

The highest PL content in male muscle tissues was observed at stage III ( $325.26 \pm 86.25$  mg/100g) (Fig. 21). PL content was significantly decreased ( $p = 3.65 \times 10^{-5}$ ) from IV ( $285.65 \pm 65.99$  mg/100g) to V ( $128.54 \pm 33.83$  mg/100g). There was no significant difference ( $p = 0.12$ ) between stages V and VI.

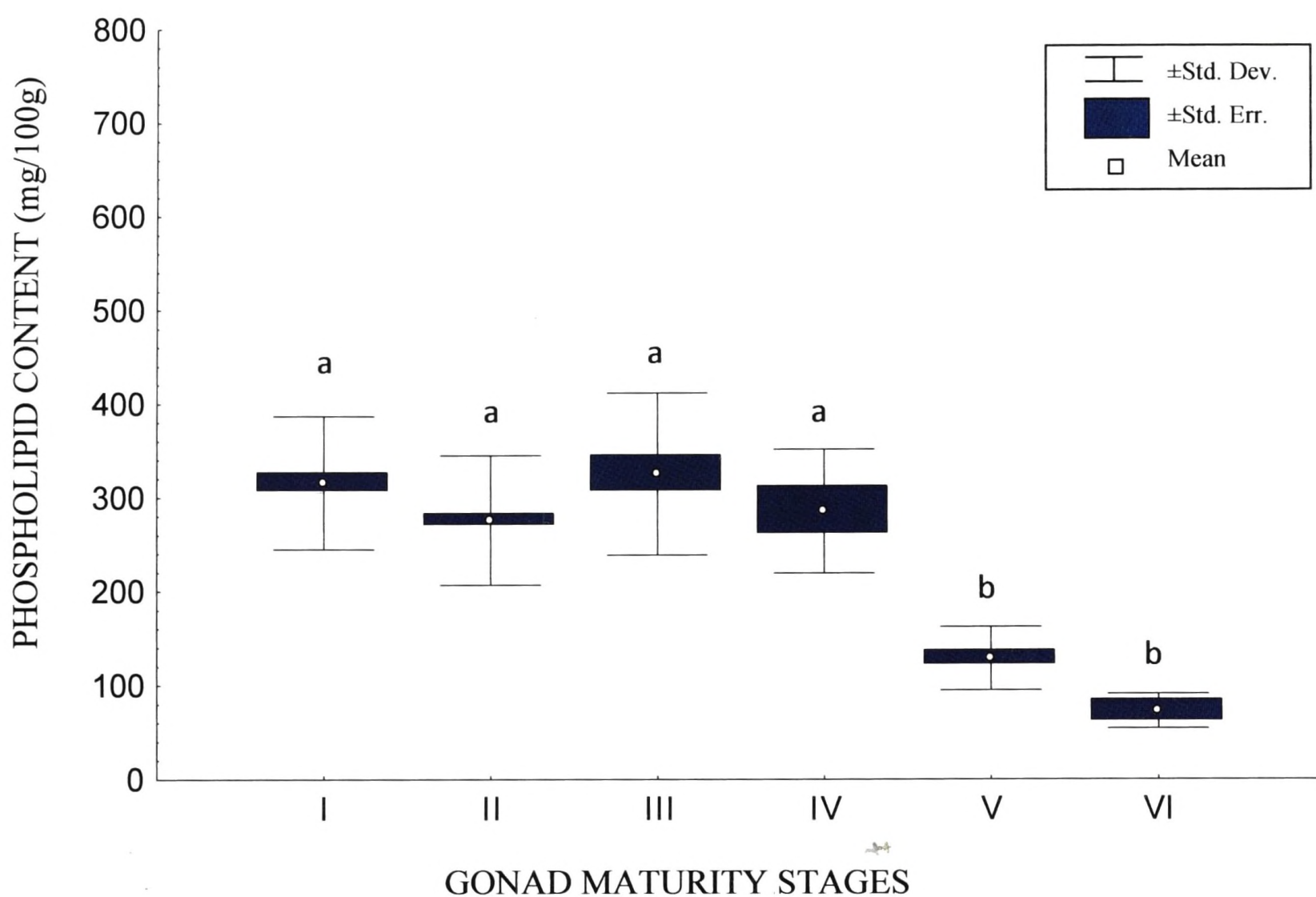


Fig. 21. Variation of phospholipid content in muscle tissues of males *S. lysan* throughout the year. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.4.3. Triacylglycerol content

The mean TAG values significantly increased ( $p = 4.05 \times 10^{-6}$ ) from stage I to III but decreased ( $p = 0.03$ ) from stage III to IV. TAG content was higher at stage III of male ( $284.99 \pm 69.09$  mg/100g) than in the stage III of female (Fig. 22). Significant difference ( $p = 1.38 \times 10^{-5}$ ) in TAG content was detected between stages IV and V. TAG content did not vary ( $p = 0.56$ ) from stage II to IV.

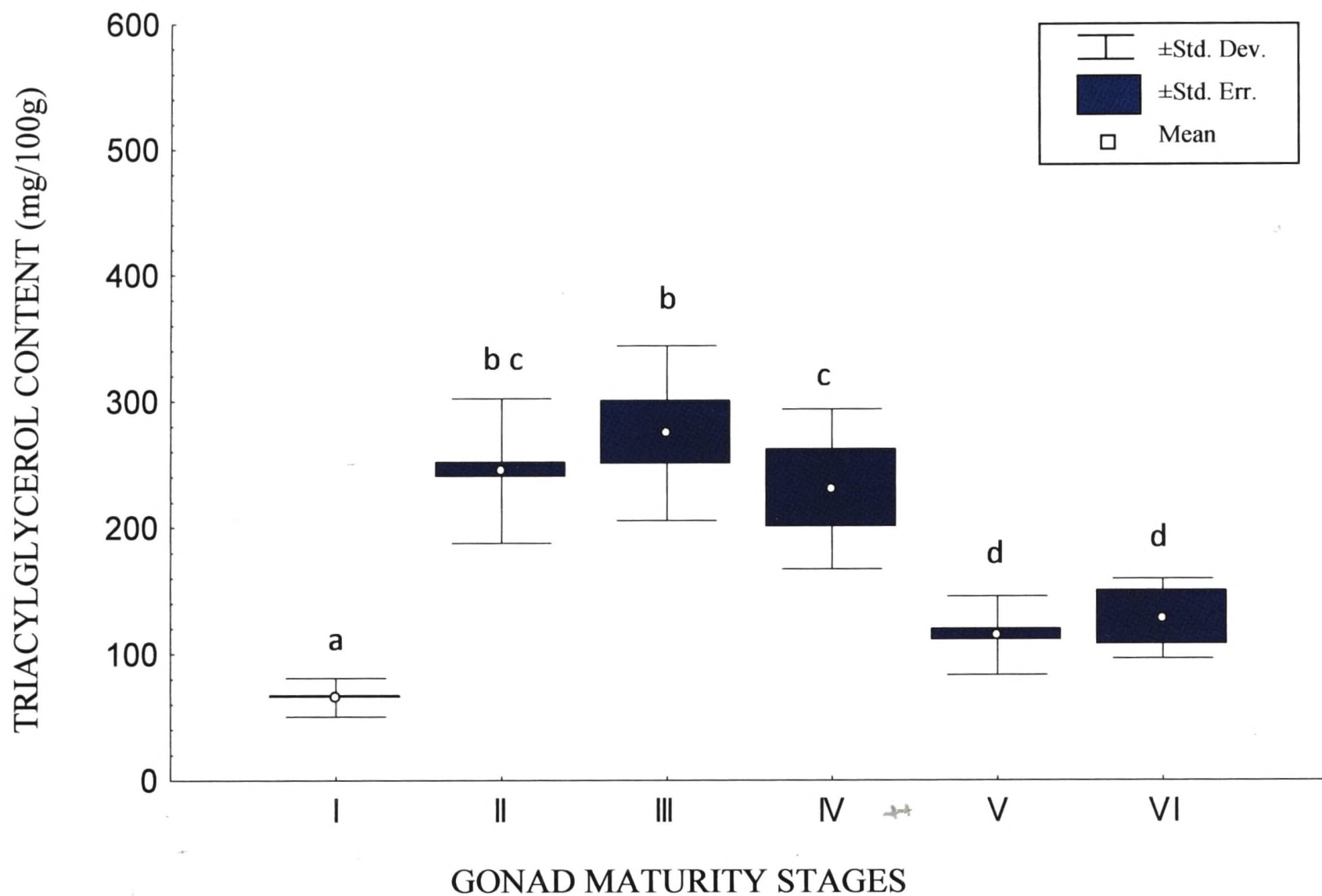


Fig. 22. Variation of triglyceride content in muscle tissues of males *S. lysan* throughout the year. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.5. Changes of lipid class content in liver tissues of females

The lipid classes in liver tissue of females exhibit marked changes during the gonadal maturation. The results of statistical analysis showed a link between lipid class content and maturity stages (Figs. 23 –25).

#### 4.9.2.5.1. Cholesterol content

CS content was significantly ( $p = 4.50 \times 10^{-6}$ ) increased from stage I ( $51.42 \pm 25.10$  mg/100g) to III ( $164.57 \pm 52.69$  mg/100g) and significantly ( $p = 4.3 \times 10^{-6}$ ) decreased to stage VI ( $70.97 \pm 19.54$  mg/100g) (Fig. 23). No major changes in the CS content in females ( $p = 0.26$ ) was found between stages III and stage IV.

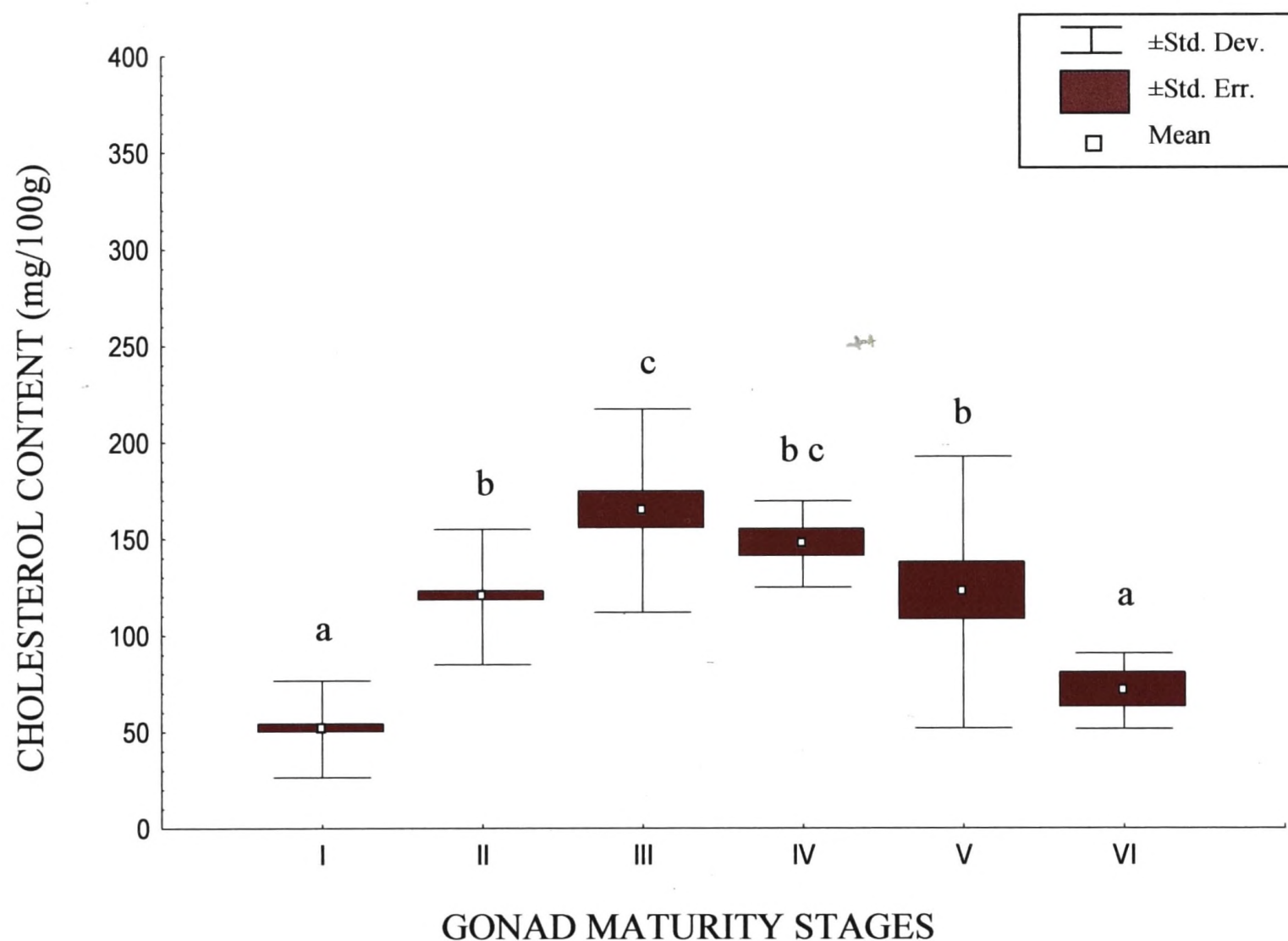


Fig. 23. Variation of cholesterol content in liver tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.5.2. Phospholipid content

Results obtained with PL content in liver tissue of females showed a slight fluctuation among maturity stages (Fig. 24). The mean PL content increased from stage I ( $178.21 \pm 23.09$  mg/100g) to stage IV ( $227.61 \pm 144.83$  mg/100g) and followed a drop at stage V ( $155.78 \pm 68.47$  mg/100g). At stage IV, the mean PL value was considerably higher than at other stages. Significant difference ( $p = 0.09$ ) was not observed from stage I to IV but a significant ( $p = 0.01$ ) drop was recorded at stage V when compared to stage IV.

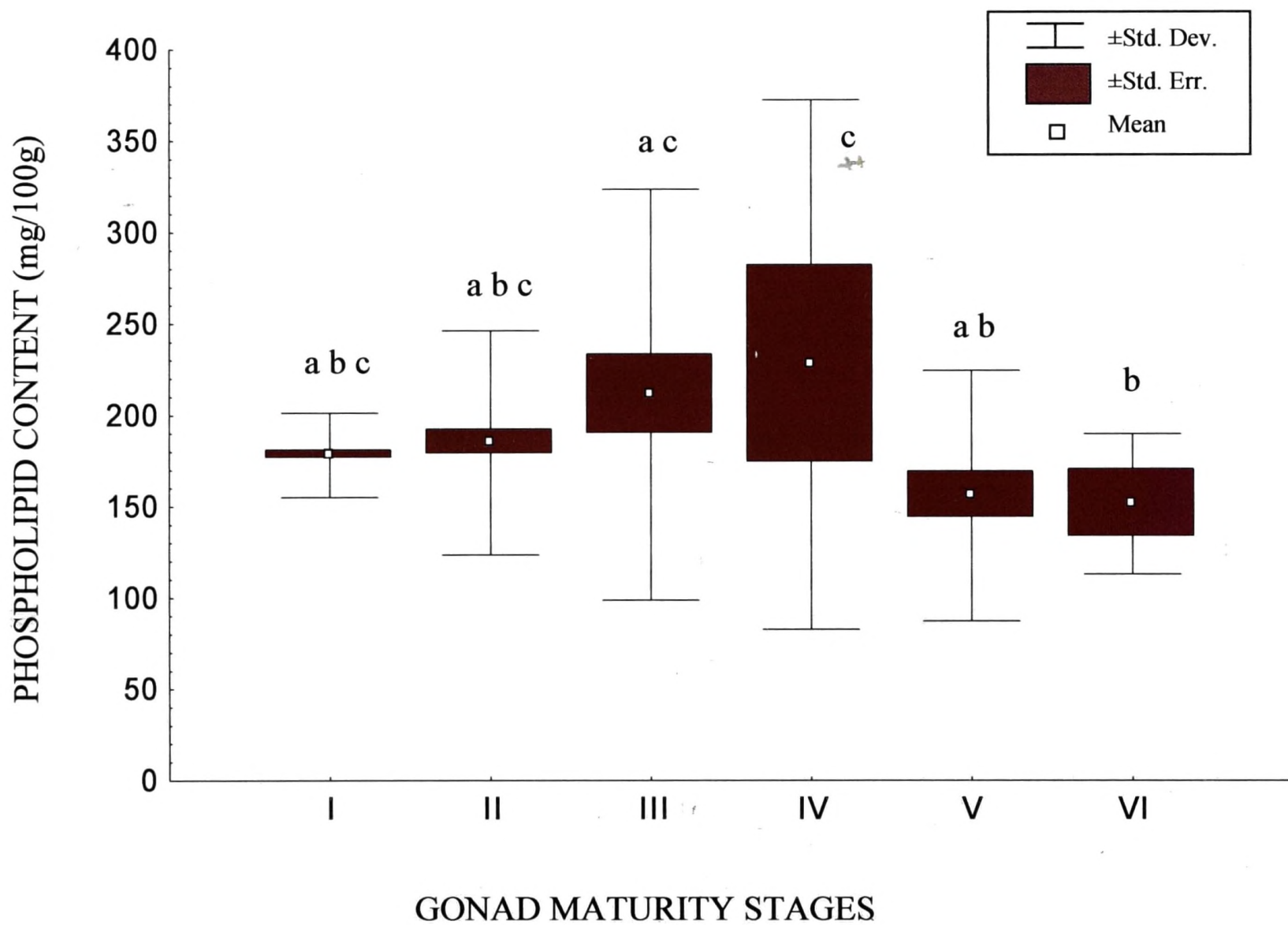


Fig. 24. Variation of phospholipid content in liver tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.5.3. Triacylglycerol content

TAG was the dominant lipid class (Fig. 25) during the gonad maturation. Changes in the TAG content were higher when compared to those of PL and CS in the liver tissues. The mean TAG content showed a 2-fold increase ( $p = 1.09 \times 10^{-5}$ ) from stage II ( $240.36 \pm 48.45$  mg/100g) to IV ( $437.49 \pm 90.46$  mg/100g) and then a 4 - fold decrease ( $p = 4.29 \times 10^{-6}$ ) from stage IV to VI ( $87.46 \pm 65.89$  mg/100g). No significant difference ( $p = 0.11$ ) was observed between stages V and stage VI.

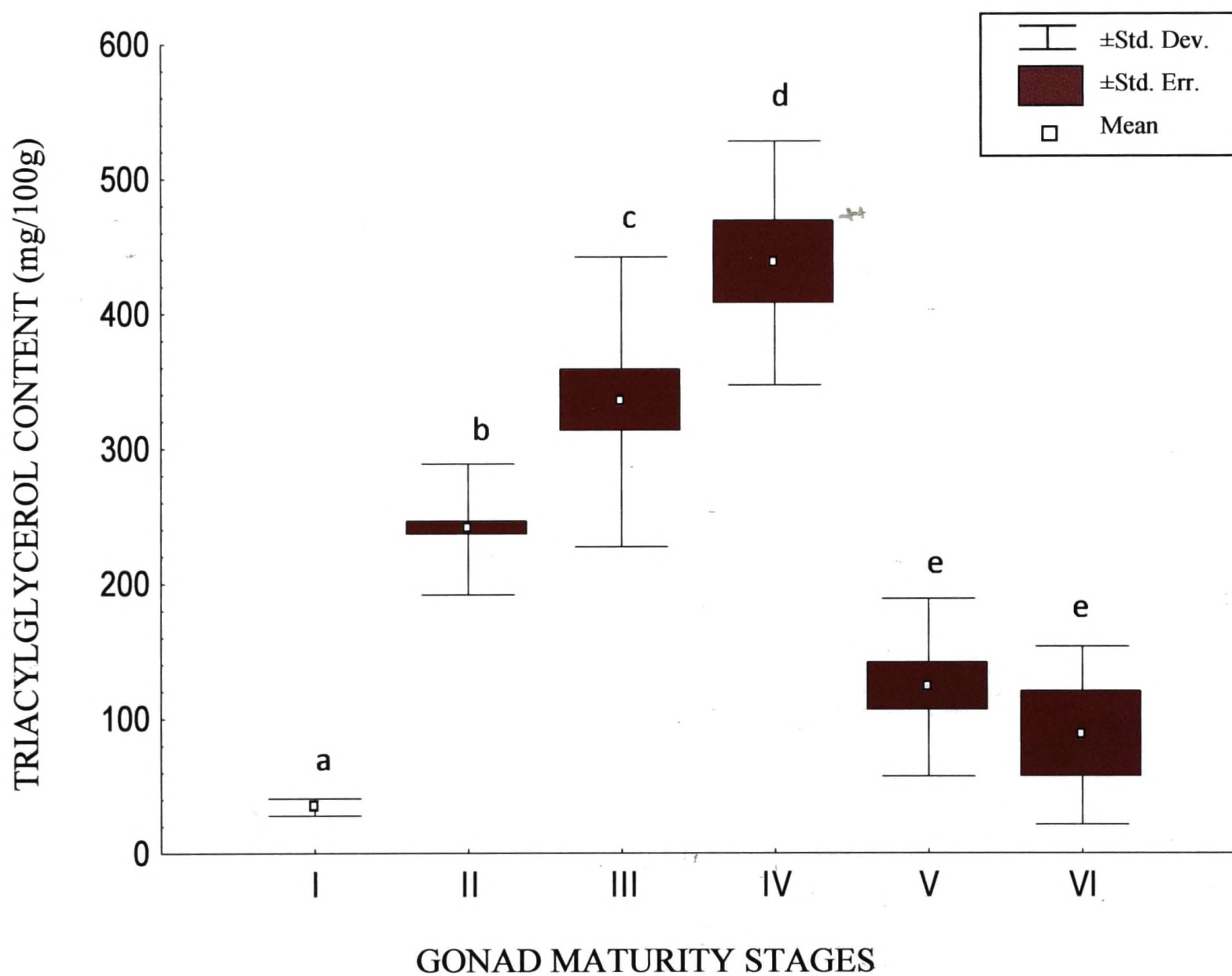


Fig. 25. Variation of triglyceride content in liver tissues of females *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.6. Changes of lipid class content in liver tissues of male

Changes in CS, PL and TAG content in liver tissues of male are shown in Figs. 26 - 28.

##### 4.9.2.6.1. Cholesterol content

CS content liver significantly ( $p = 4.05 \times 10^{-6}$ ) increased from stage I ( $51.42 \pm 25.10$  mg/100g) to IV ( $199.56 \pm 160.15$  mg/100g) and then significantly ( $p = 3.53 \times 10^{-5}$ ) decreased upto stage VI ( $115.13 \pm 13.99$  mg/100g) (Fig. 26). The highest level was recorded at stage IV. Significant difference ( $p < 0.05$ ) was observed between stage IV and other stages (Fig.25).

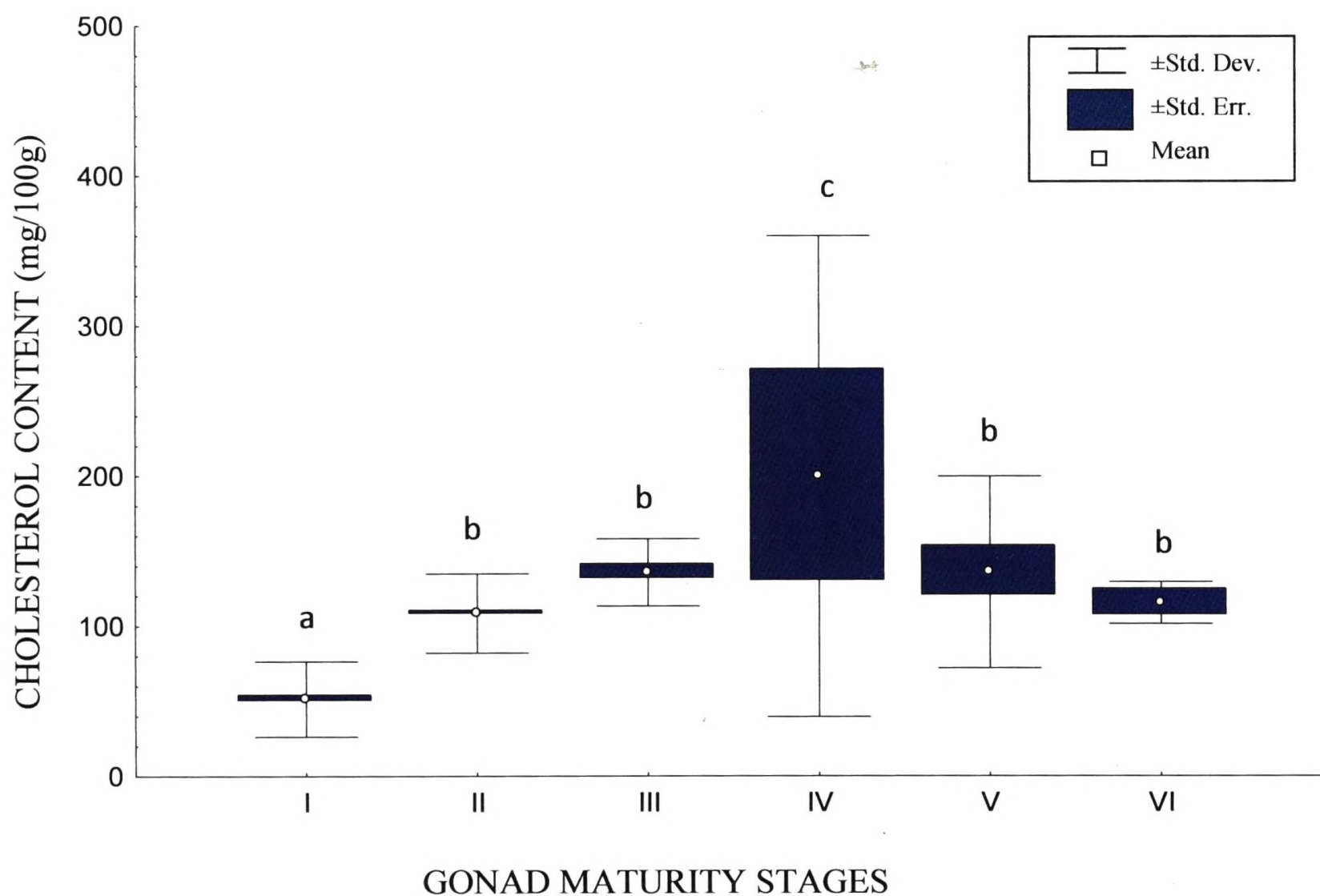


Fig. 26. Variation of cholesterol content in liver tissues of males *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.6.2. Phospholipid content

Although the increase in PL content in male liver tissue was not significantly from stage I ( $178.21 \pm 23.09$  mg/100g) to II ( $234.75 \pm 68.15$  mg/100g), it significantly ( $p = 4.05 \times 10^{-6}$ ) decreased from stage II to stage VI ( $46.86 \pm 18.05$  mg/100g). The PL content in stage VI was significantly different from that of all other stages (Fig. 27).

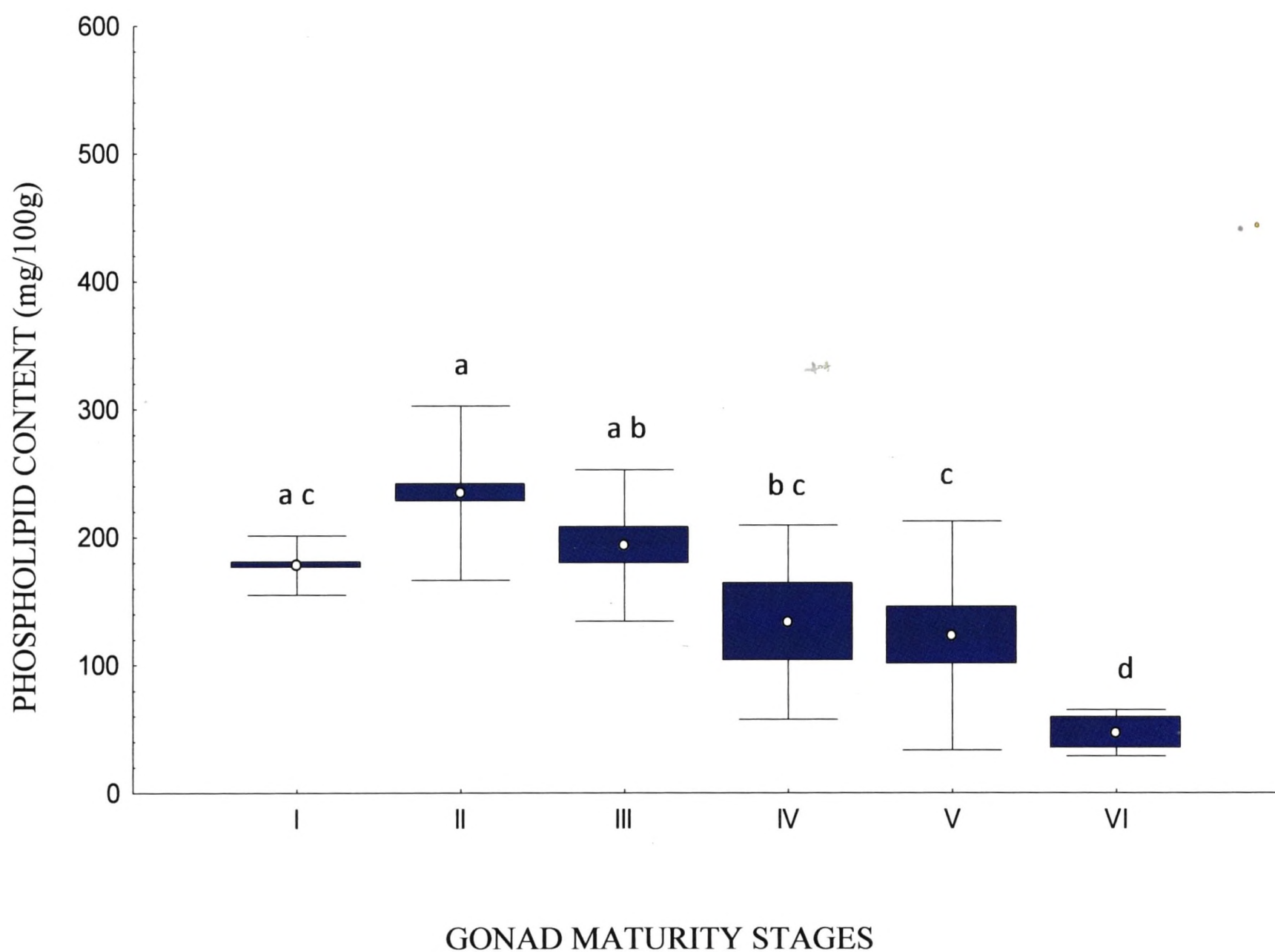


Fig. 27. Variation of phospholipid content in liver tissues of males *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### 4.9.2.6.3. Triacylglycerol content

In liver tissues of males, TAG was the dominant lipid class and the changes in TAG in the liver tissue was moderate. The TAG content increased from stage I ( $34.44 \pm 6.42$  mg/100g) to stage IV ( $289.20 \pm 133.61$  mg/100g) and dropped thereafter in stage VI ( $127.82 \pm 31.53$  mg/100g) (Fig. 28). When compared with changes in PL and CS in the liver tissues, changes in TAG content was higher in males.

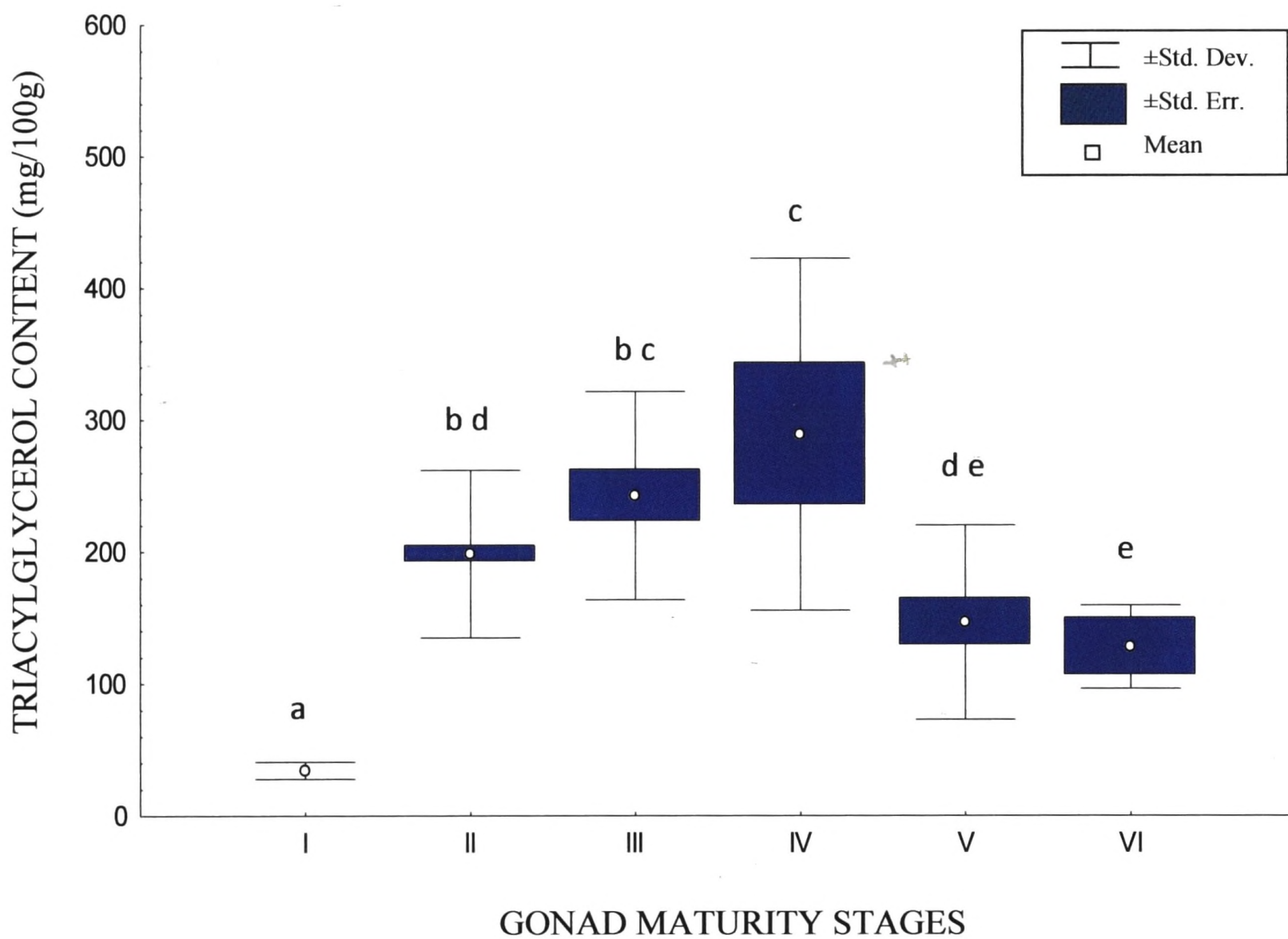


Fig. 28. Variation of triglyceride content in liver tissues of males *S. lysan* in different maturity stages. Gonad maturity stages; I, Immature unsex; II, Immature; III, Maturing; IV, Mature; V, Spawning; VI, Spent. Mean values for each gonad maturity stages with the common letters indicate no significant difference ( $p > 0.05$ ).

#### **4.10. Total lipid and lipid class changes in tissues through out the two years**

In the present study, total lipid, CS, PL and TAG content of the gonad, muscle and liver tissues were investigated monthly for adult *S. lysan* (maturing - stages III, mature - stage IV, spawning - stage V and spent - stage VI). The female adult fish were not collected in the months of January, November and December whereas males were not collected in the months of March, November and December in both years from 2010 to 2011.

Monthly total lipid content and lipid class content data in different tissues for both years were pooled together and the average values for each month are represented in Figs. 29 – 40, since a similar trend of total lipid and lipid class constituents in gonads, muscles and livers were obtained for both years.

Lipid and lipid classes in the tissues of *S. lysan* showed significant monthly variations. Trend of lipid changes in tissues showed fluctuations corresponding to lipid storage and utilization process.

##### **4.10.1. Changes of total lipid content in tissues throughout the two years**

Monthly fluctuation of total lipid content in gonad tissues of adults *S. lysan* are shown in Fig. 29. The mean total lipid values (% Dry weight : DW) in ovary ranged from  $16.01 \pm 4.09$  in October to  $33.36 \pm 10.42$  in September respectively (Fig. 29). During the study period, the highest lipid content was observed in June and September. The mean ovary lipid content (% DW) significantly ( $p = 0.0014$ ) increased from February ( $20.18 \pm 2.91$ ) to June ( $33.14 \pm 11.64$ ), then decreased in July ( $22.15 \pm 5.87$ ), again

increased significantly ( $p = 0.005$ ) upto September (to almost  $33.36 \pm 10.42$ ), and then significantly ( $p = 2 \times 10^{-5}$ ) dropped in October ( $16.01 \pm 4.09$ ). The mean lipid content in testis (% DW) was lower than that of lipid content in ovary throughout the study period (Fig. 29). The mean lipid content ranged from  $8.43 \pm 5.36$  to  $15.57 \pm 4.85$ , from July to September respectively. The data showed that the lipid content in testis was relatively higher in June and September months.

Monthly fluctuations of total lipid content in muscle tissues of adults *S. lysan* are shown in Fig. 30. Lipid content in muscle tissues of the female *S. lysan* was the lowest ( $4.36 \pm 1.22$ ) in February and the highest ( $7.29 \pm 0.83$ ) in August (Fig. 30). The amount of lipid content in muscle tissues significantly ( $p = 0.005$ ) increased from February to May, attained the lowest amount in June and September months. In males, increasing trend in the lipid contents of muscle tissue was recognized from January to May as  $4.88 \pm 0.79$  and  $10.36 \pm 2.58$  respectively (Fig. 30) and attained lower values in June ( $7.08 \pm 1.58$ ) and September ( $6.63 \pm 1.70$ ).

Monthly fluctuations of total lipid content in liver tissues of adults *S. lysan* are recorded in Fig. 31. The lipid content in liver tissues of females exhibited significant ( $p = 0.049$ ) increasing trend from February to May, reached the highest value of  $31.78 \pm 8.69$  and then significantly ( $p = 0.01$ ) dropped in June. Again significantly ( $p = 9.3 \times 10^{-5}$ ) increasing trend was observed from June to August and then significantly ( $p = 2.5 \times 10^{-5}$ ) decreasing thereafter. *S. lysan* utilized their liver lipid content in June and September months arrived a value of  $19.97 \pm 10.33$  and  $18.50 \pm 8.67$  respectively (Fig. 31). The similar trend was observed in liver lipid of male adult fish (Fig. 31).

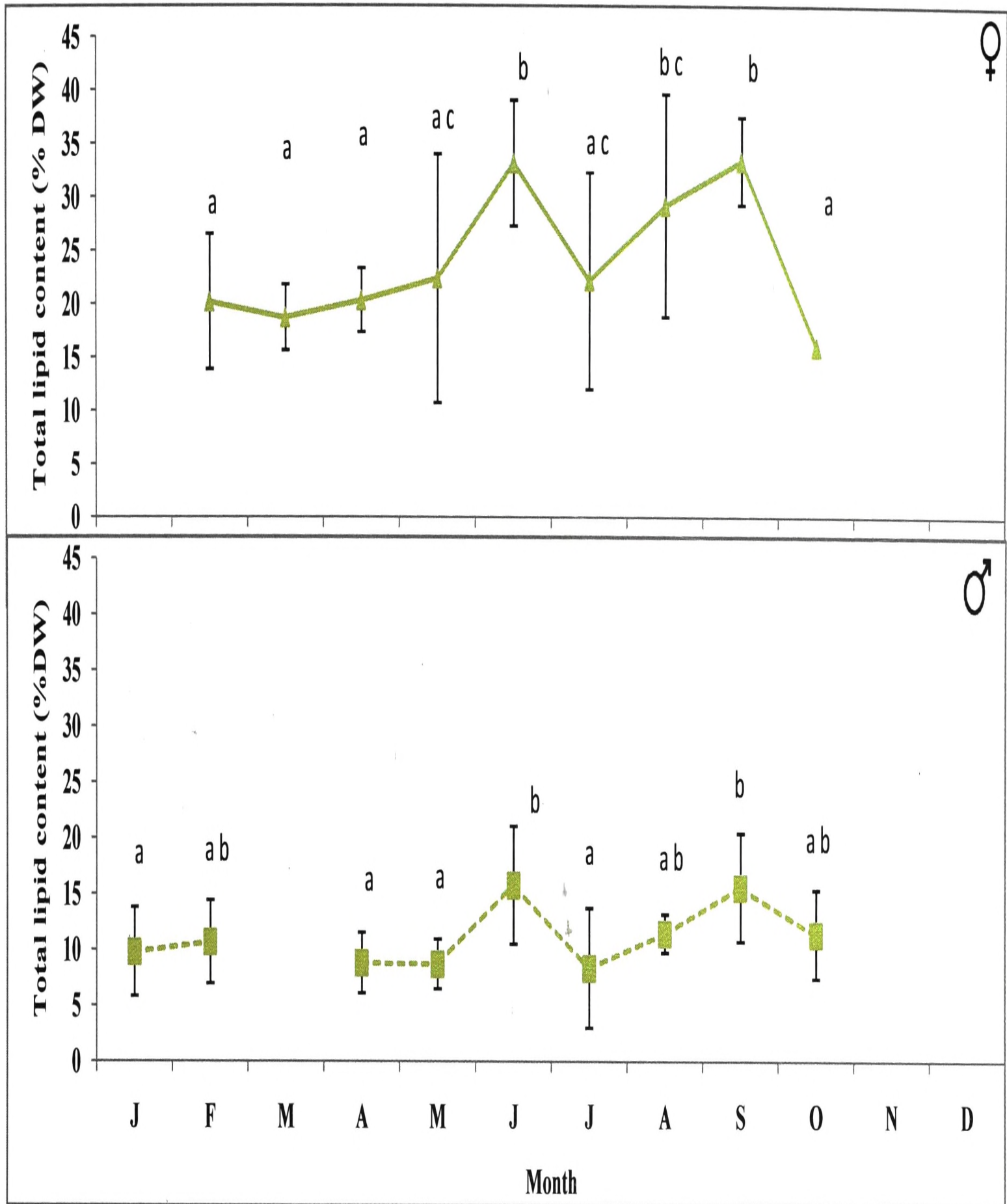


Fig. 29. Monthly fluctuations of percentage of gonad lipid content (DW) in adults *S. lysan*. Values are means  $\pm$  SD; Solid line, females; broken line, males. Mean values for each month with the common letters indicate no significant difference ( $p > 0.05$ ).

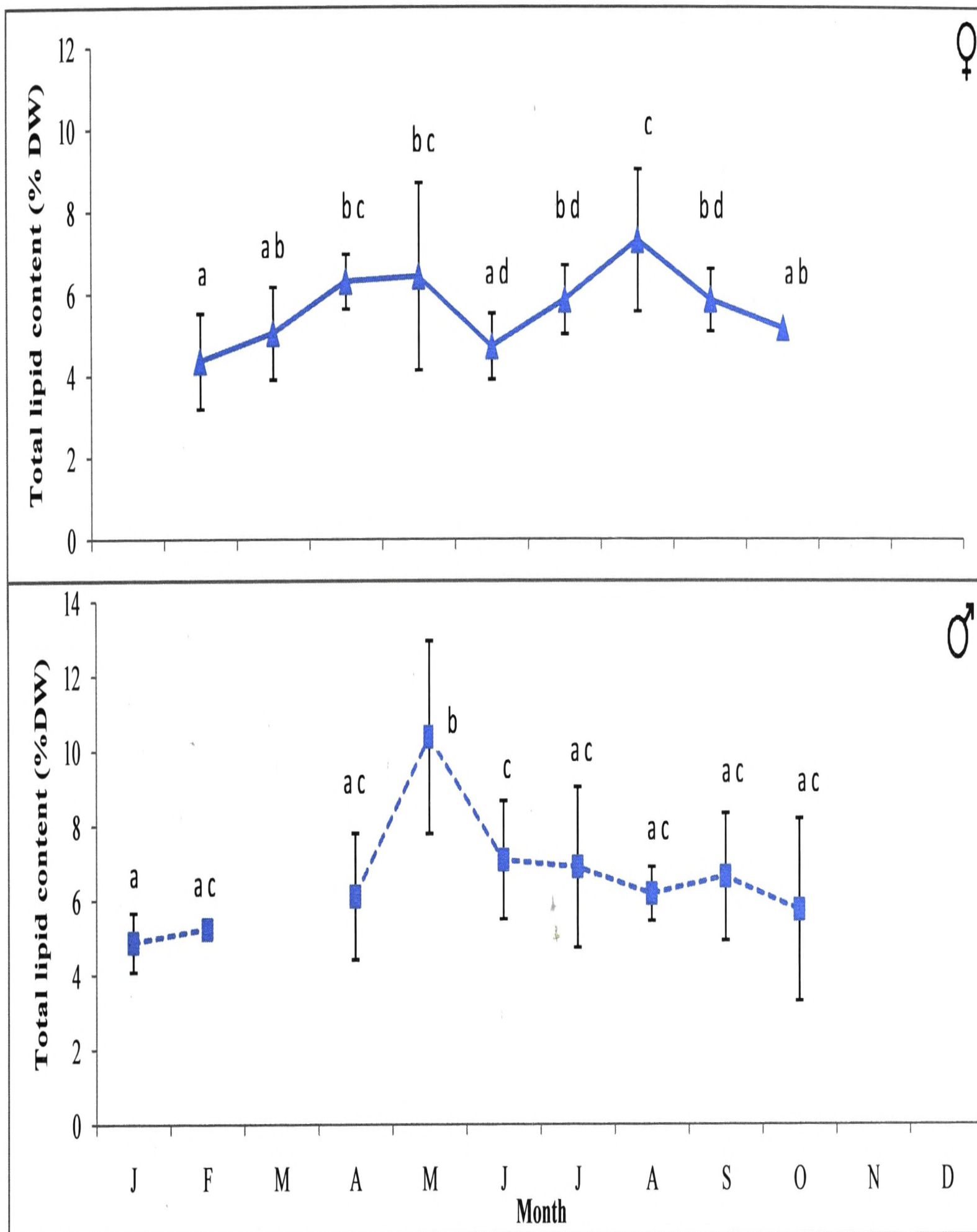


Fig. 30. Monthly fluctuations of percentage of muscle lipid content (DW) in adults *S. lysan*. Values are means  $\pm$  SD; Solid line, females; broken line, males. Mean values for each month with the common letters indicate no significant difference ( $p > 0.05$ ).

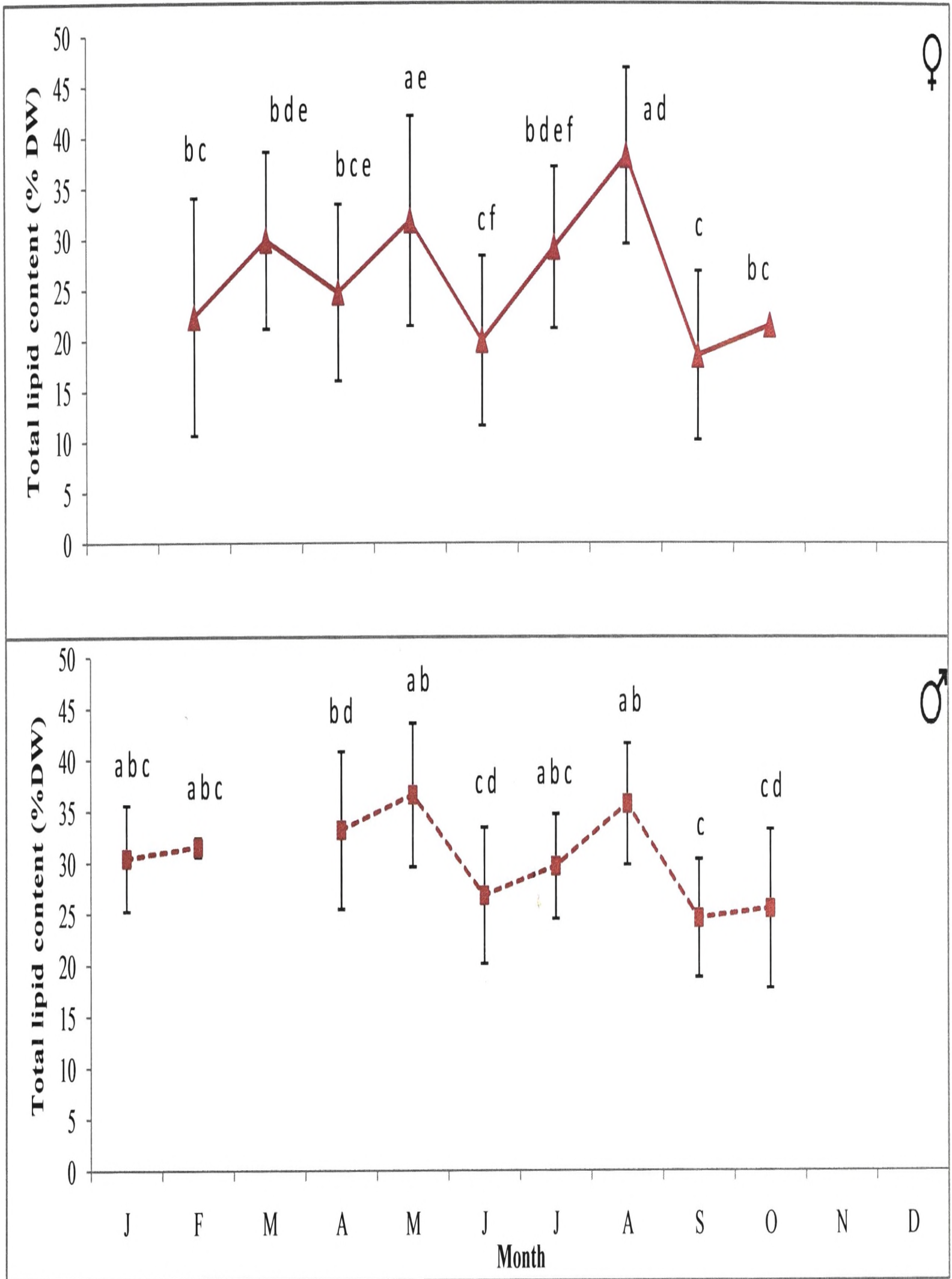


Fig. 31. Monthly fluctuations of percentage of liver lipid content (DW) in adults *S. lysan*. Values are means  $\pm$  SD; Solid line, females; broken line, males. Mean values for each month with the common letters indicate no significant difference ( $p > 0.05$ ).

#### **4.10.2. Changes of lipid class content in tissues throughout the two years**

As a whole, PL and TAG contents were higher than that of CS content in gonads of adult *S. lysan*. However, substantial variations in CS, PL and TAG content for gonad, muscle and liver tissues of adults were observed for both sexes during the study period.

##### **4.10.2.1. Gonad tissues**

Monthly changes occurred in the lipid classes of gonad tissues. The lipid classes predominating in the gonads differed in the final stages of maturation of both sexes. Throughout the study period, 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 females fluctuated throughout this study and attained higher peaks in June and September. Male gonads also followed a more or less similar pattern for CS, PL and TAG on female adult fish (Figs. 32- 34).

Monthly variations of CS content in gonad tissues are displayed in Fig. 32. The highest mean CS content in ovary of females collected in June and September months were  $226.4 \pm 78.21$  mg/100g and  $292.00 \pm 156.08$  mg/100g respectively. Similar trend of CS content in male testis was observed. CS content of testis were significantly ( $p = 0.046$ ) increased from July to September and attained a peak at September ( $226.3 \pm 69.92$  mg/100g).

Monthly variations of PL content in gonad tissues are displayed in Fig. 33. The mean PL content in ovary of female reached the highest value in June as  $393.54 \pm 73.00$  mg/100g. Similarly, mean PL content in testis of male significantly ( $p = 0.040$ ) increased from April to June, attained the highest amount as  $475.8 \pm 153.6$  mg/100g and decreased in July.

Monthly variations of TAG content in gonad tissues are displayed in Fig. 34. The significantly ( $p = 0.034$ ) high TAG content in ovary of females was recorded in September when compared to February. The highest amount of TAG in testis of adult male fish was recorded in June as  $453.1 \pm 77.64$  mg/100g.

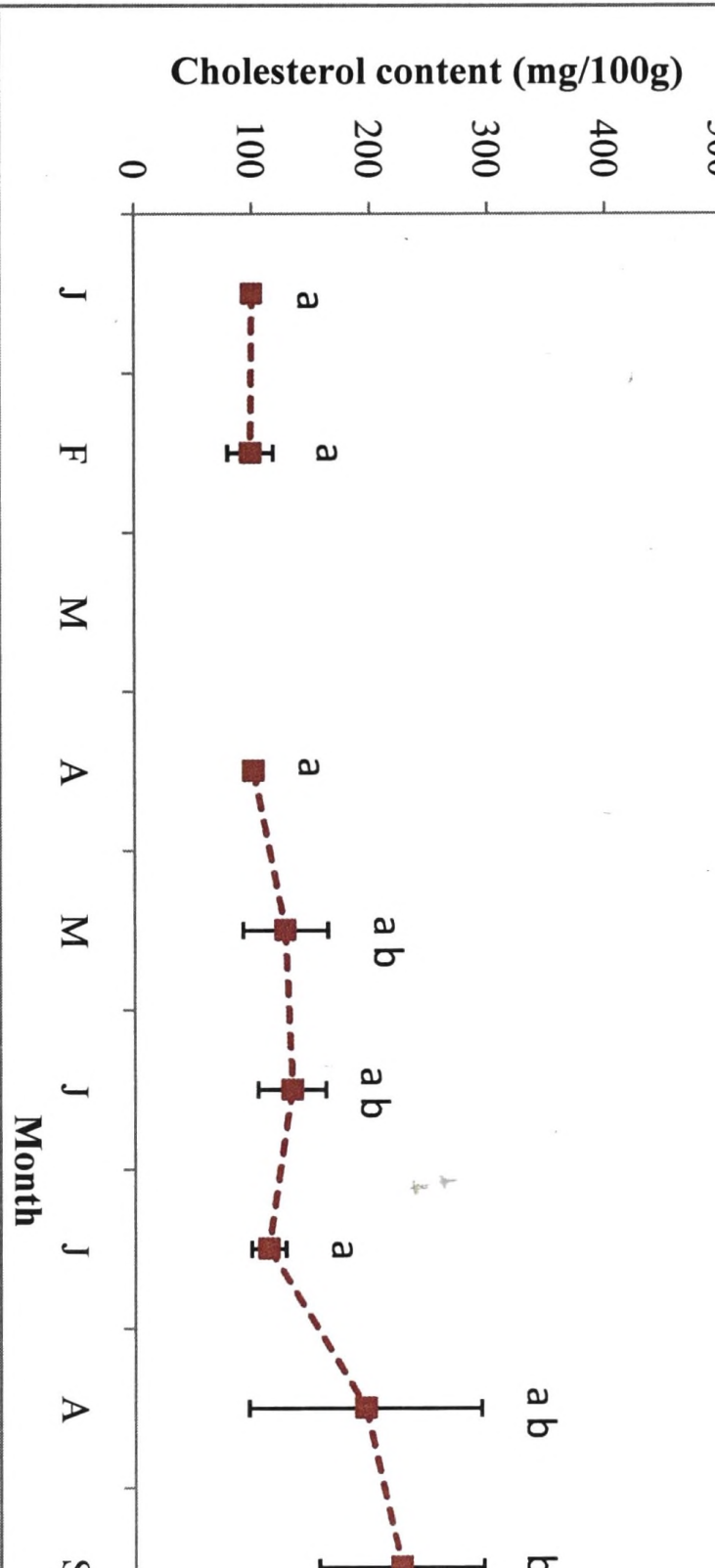


Fig. 32. Monthly fluctuations of cholesterol content of gonad tissues in adults *S. lysan*. Va broken line, males. Mean values for each month with the common letters indicate no

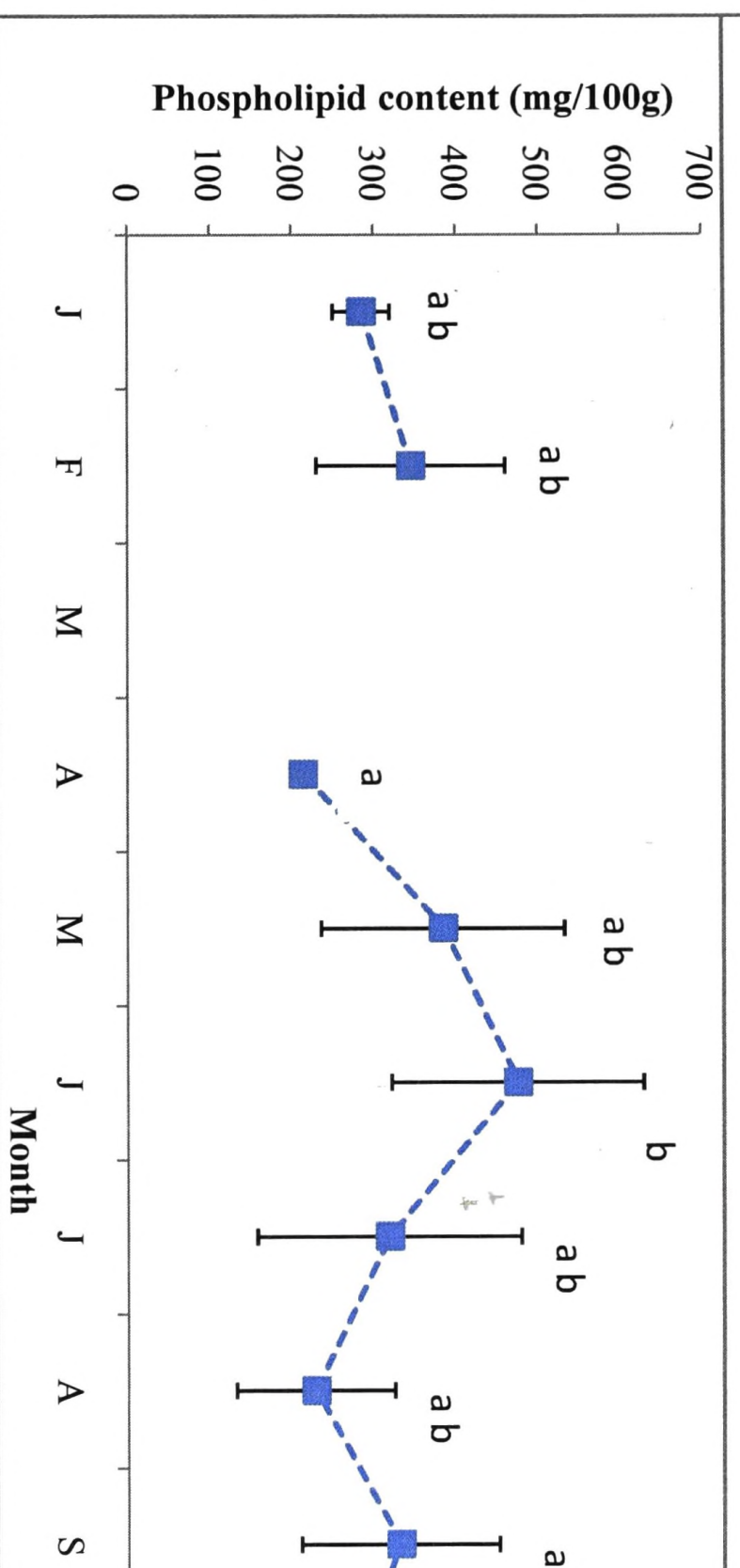


Fig. 33. Monthly fluctuations of phospholipid content of gonad tissues in adults *S. lysan*. Value line, males. Mean values for each month with the common letters indicate no significant

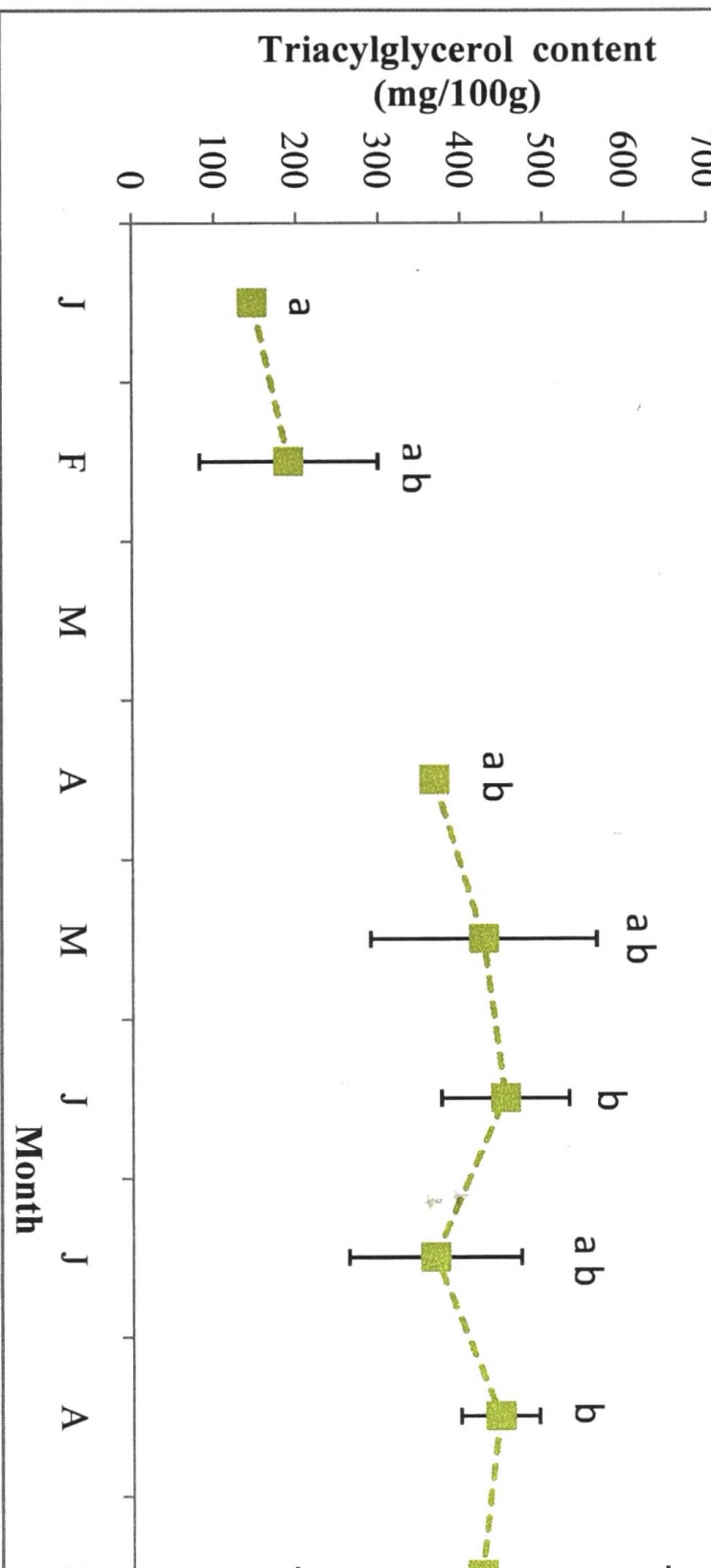


Fig. 34. Monthly fluctuations of triacylglycerol content of gonad tissues in *S. lysan* adults. broken line, males. Mean values for each month with the common letters indicate no s

#### 4.10.2.2. Muscle tissues

Muscle tissue of both sexes contained relatively low values of CS than the PL and TAG during the study period (Figs. 35 - 37). All three lipid constituents CS, PL and TAG were identified in decreased level during June and september months. Mean CS and PL contents in females were higher over those in males.

Monthly variations of CS content in muscle tissues of both sexes are displayed in Fig. 35. Fluctuations of CS content in muscle tissues of female adults *S. lysan* were significantly evident after May month ( $283.5 \pm 1.95$  mg/100g). Lowest mean CS content in female was recorded as  $42.93 \pm 22.58$  mg/100g in September. Lowest mean CS content in males was of  $38.96 \pm 10.62$  mg/100g in June, whereas highest was in February ( $164.9 \pm 19.22$  mg/100g).

Monthly variations of PL content in muscle tissues of both sexes are displayed in Fig. 36. In females, the PL content decreased significantly ( $p = 0.004$ ) from April to June, attained a low value of  $286.6 \pm 56.86$  mg/100g. Decreasing trend was observed in muscle tissues of males from August to October. The lowest mean PL content in muscle tissues of *S. lysan* was recorded in September,  $192.10 \pm 101.5$  mg/100g.

Monthly variations of TAG content in muscle tissues of both sexes are displayed in Fig. 37. TAG content in muscle tissues of females *S. lysan* was decreased significantly ( $p = 0.001$ ) from August to September. Male muscle consisted  $140.16 \pm 83.87$  mg/100g TAG in September. There are no significant ( $p < 0.05$ ) differences in the TAG values between each months.

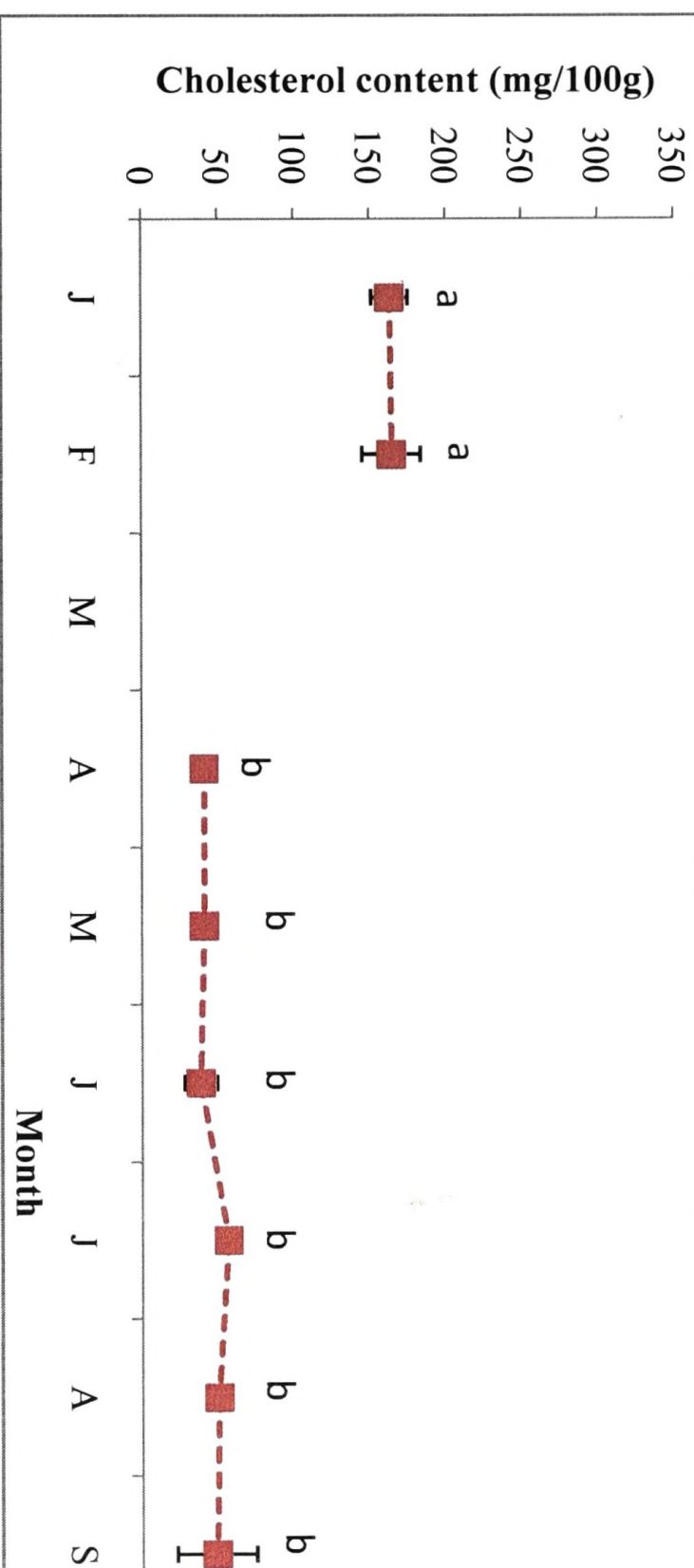


Fig. 35. Monthly fluctuations of cholesterol content of muscle tissues in adults *S. lysan*.   
 broken line, males. Mean values for each month with the common letters indicate no

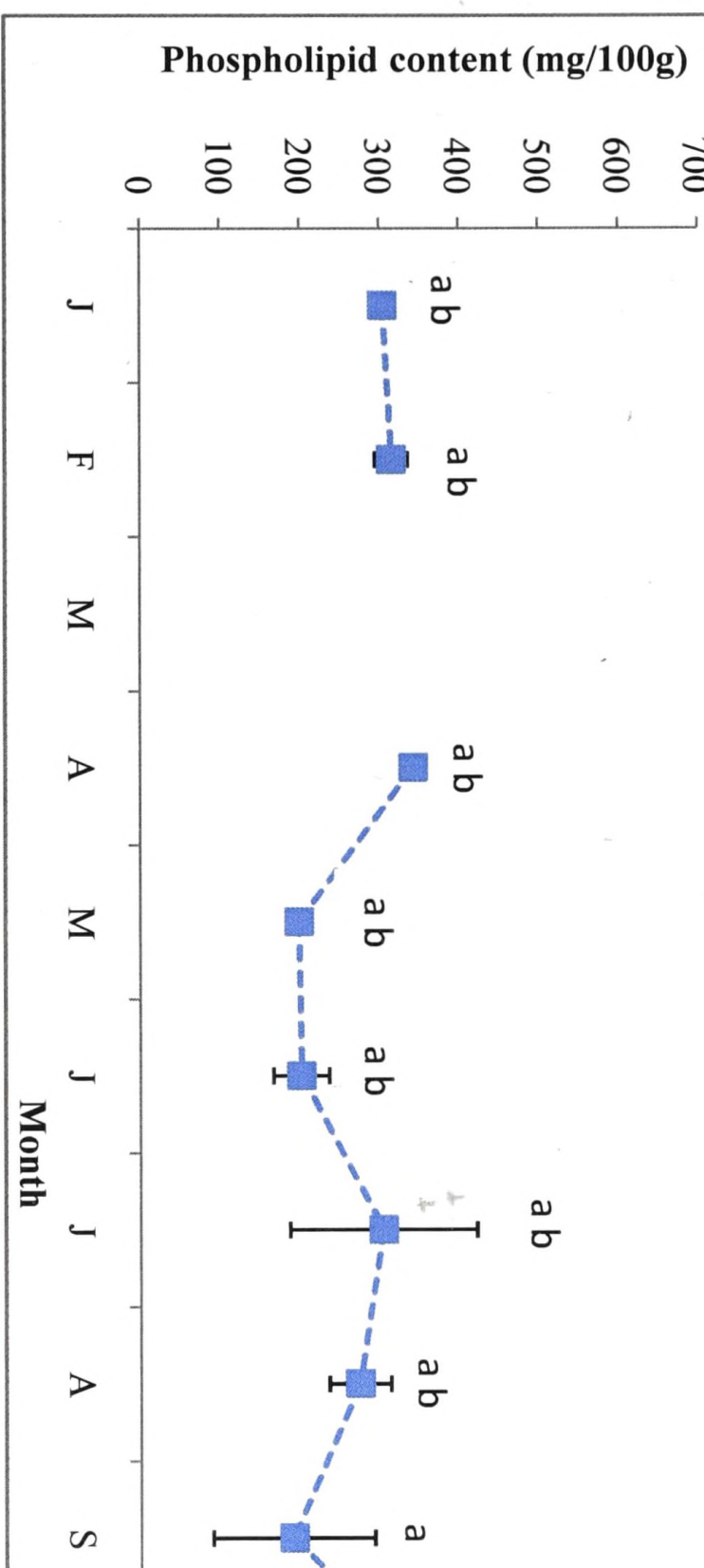


Fig. 36. Monthly fluctuations of phospholipid content of muscle tissues in adults *S. lysan*.  
 broken line, males. Mean values for each month with the common letters indicate no

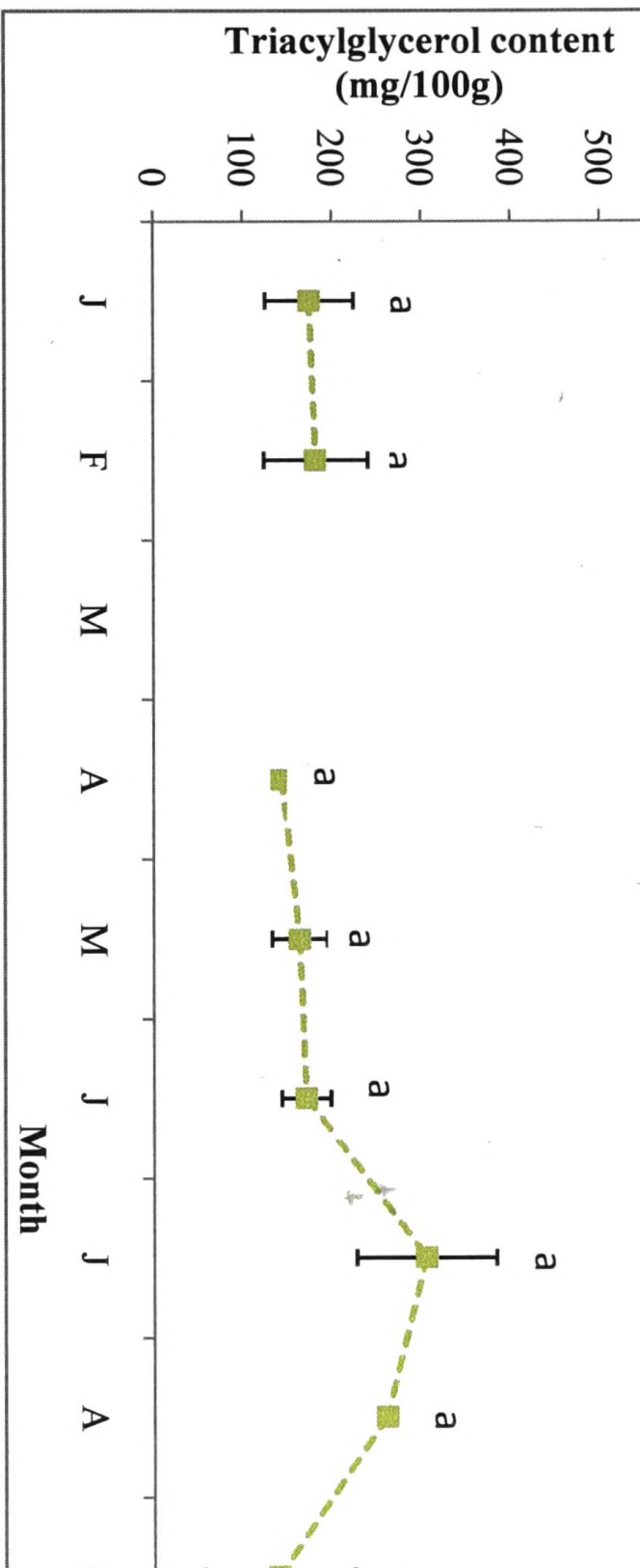


Fig. 37. Monthly fluctuations of triacylglycerol content of muscle tissues in adults *S. lyciscus* females: broken line, males. Mean values for each month with the common letter (0.05).

#### 4.10.2.3. Liver tissues

The mean TAG content in liver tissues of both sexes of *S. lysan* was higher when compared with the mean PL and CS contents.

Monthly variations of CS content in liver tissues of both sexes are displayed in Fig. 38. The lowest monthly mean value of CS in female *S. lysan* was recorded as  $112.52 \pm 62.65$  mg/100g in September, whereas in male *S. lysan*, it was recorded in June as  $112.52 \pm 62.65$  mg/100g.

Monthly variations of PL content in liver tissues of both sexes are displayed in Fig. 39. The PL value obtained from Liver tissues of female in June and September months had the low value of  $154.70 \pm 52.76$  mg/100g and  $150.04 \pm 61.52$  mg/100g respectively, and in male *S. lysan*, it was also recorded in September as  $124.52 \pm 80.11$  mg/100g.

Monthly variations of TAG content in liver tissues of both sexes are displayed in Fig. 40. The trend of TAG content in females was significantly ( $p < 0.05$ ) differ from that of males throughout the years. Female liver TAG content significantly ( $p = 0.001$ ) decreased from May to June and significantly ( $p = 0.0012$ ) increased from June to August and again significantly ( $p = 0.0002$ ) decreased in September. Liver lipid in both sexes fish reached maximum value in August, whereas minimum values were obtained in June and September.

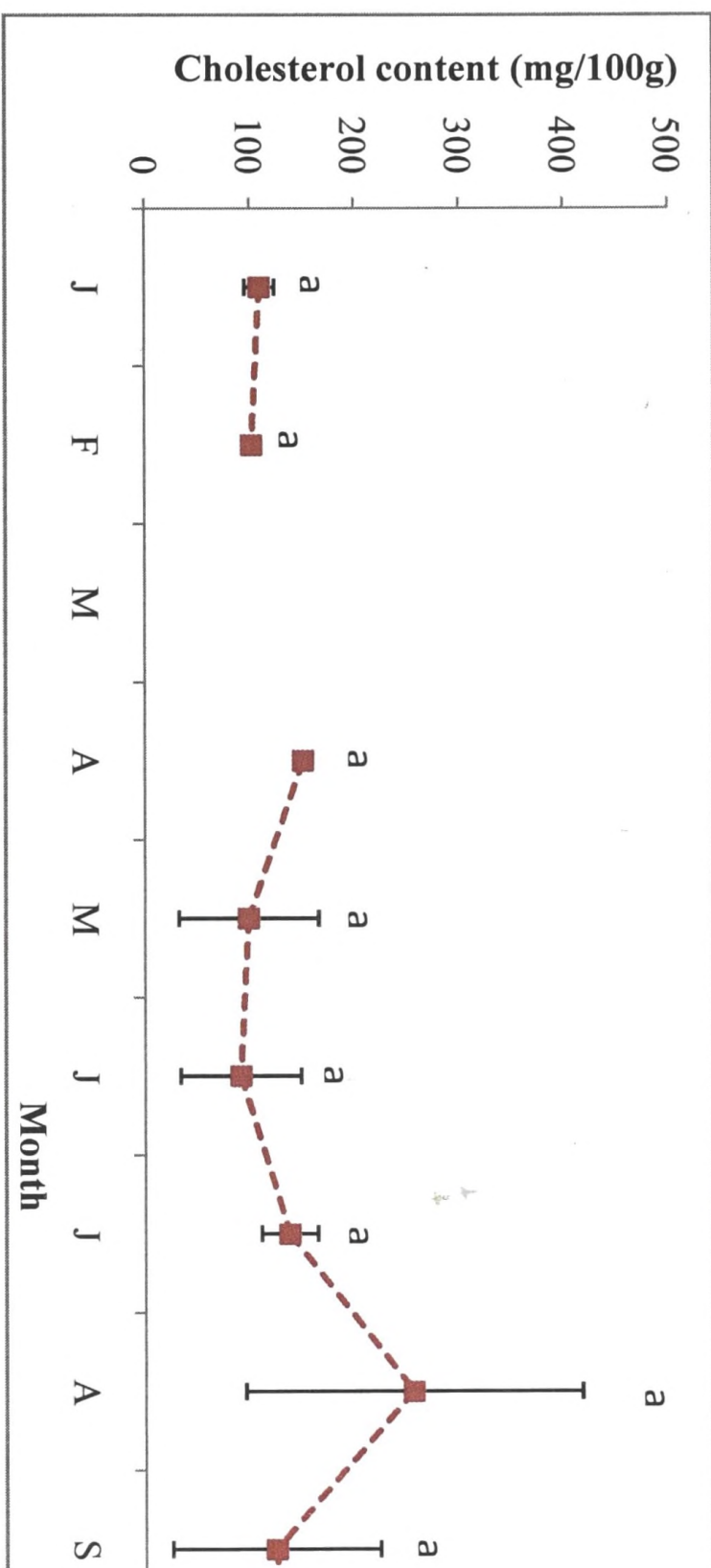


Fig. 38. Monthly fluctuations of cholesterol content of liver tissues in adults *S. lysan*. Values are given as mean ± SD. Different letters indicate significant differences (p < 0.05) between months. Error bars represent standard deviation. A broken line, males. Mean values for each month with the common letters indicate no significant differences.

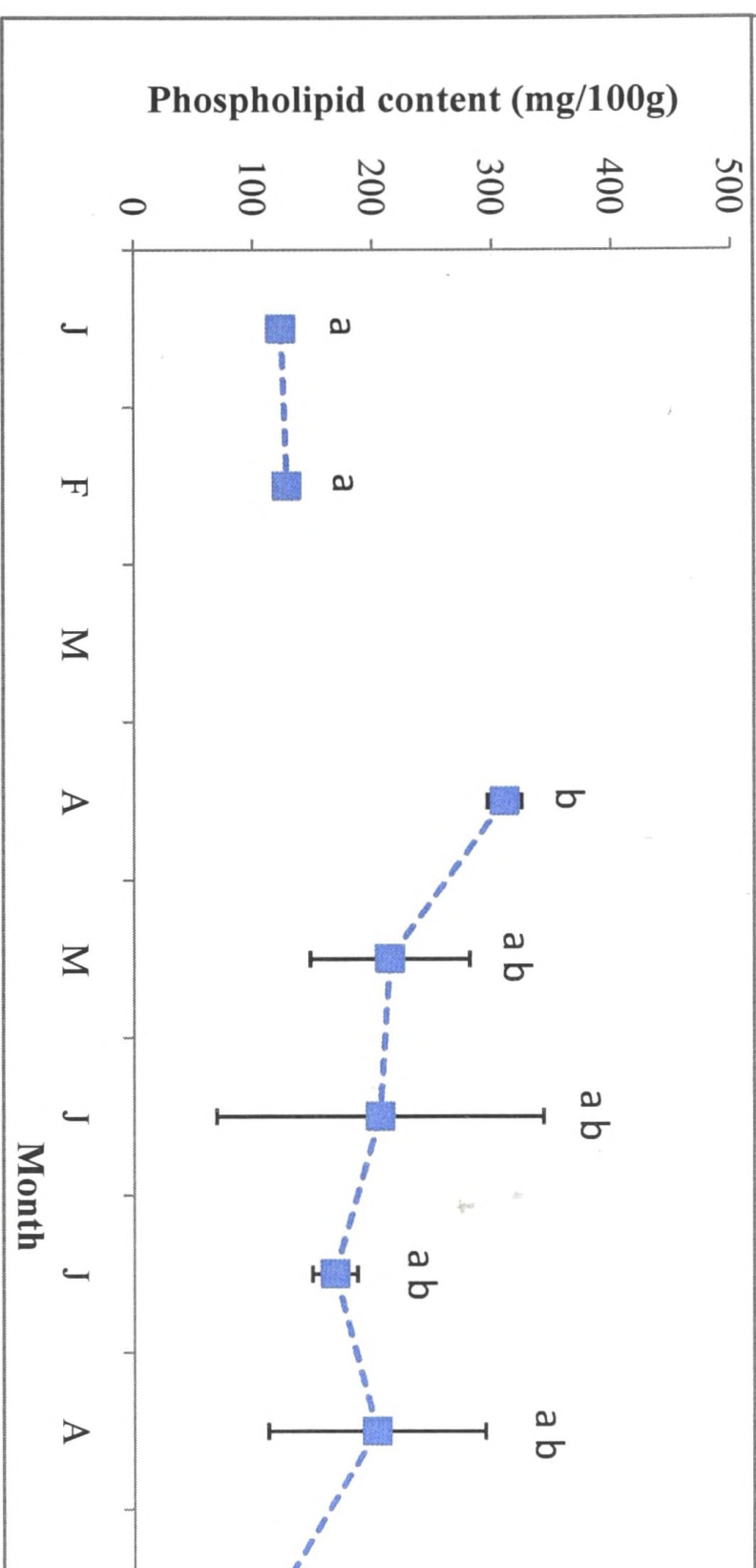


Fig. 39. Monthly fluctuations of phospholipid content of liver tissues in adults *S. lysan*. Values are given as mean  $\pm$  SD. Different letters indicate significant differences ( $P < 0.05$ ) between months. Broken line, males. Mean values for each month with the common letters indicate no significant difference.

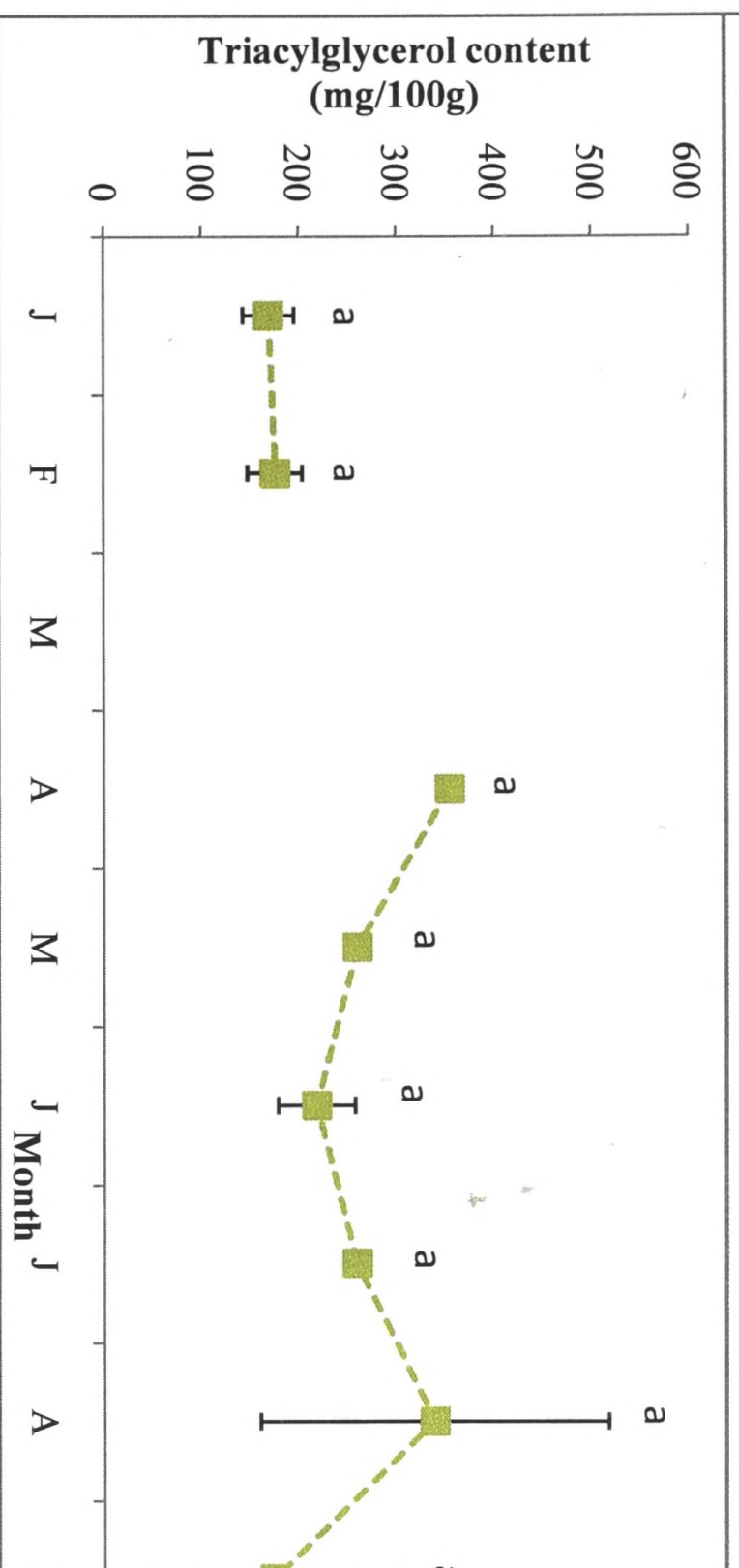


Fig.40. Monthly fluctuations of triacylglycerol content of liver tissues in adults *S. lysan*. Va broken line, males. Mean values for each month with the common letters indic

## 5. DISCUSSION

Analysis of lipid and lipid classes in gonad, muscle and liver tissues is 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.

### 5.1. Lipid compositions in tissues of tropical species

Information on lipid compositions of muscle, liver and gonad tissues in tropical species is relatively scarce. A few studies had been done in several tropical countries as described below. Seasonal changes of lipid content in muscle, liver and gonad tissues of tropical surgeonfishes representing 5 genera such as *Acanthurus*, *Ctenochaetus*, *Naso*, *Prionurus*, *Zebrasoma*) were estimated by Montgomery and Galzin, (1993) from Western Mexico, Hawaii, French Polynesia, Great Barrier Reef and the Red Sea. Lipid composition of juveniles and adult fish species (*Heterotis niloticus*, *Brycenus nurse*, *Gnathonemus cyprinoides* and *Sarotherodon galilaeus*) were recorded by Achionye-Nzeh and Omoniyi, (2002) from a small lake in Nigeria. Suloma *et al.*, (2008) reported the fatty acid composition of muscle tissues in Nile Tilapia (*Oreochromis niloticus*) collected from Philippines. Abdullah and Lohar, (2011) reported substantial increase in the muscle lipid content with gonadosomatic index for three species of tropical carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) caught from river Tapi.

## 5.2. Influence of fish length on lipid content of tissues

*S. lysan* shows a significant curvilinear correlation between standard length and lipid content of muscle and gonad tissues (Figs. 8 and 9). Most of the teleost fish exhibit a correlation between body length and lipid content of tissues as recorded by several investigators. Some fish species show a linear relationship between body length and total lipid content, for example *Channa* fish caught from Basti Ratan Wali (Ali *et al.*, 2001) and white Perch (*Phragmites*) from the Hudson River estuary (Weinstein *et al.*, 2010). Gallagher *et al.*, (1989) reported that the fatty acid composition of the striped bass (*Morone saxatilis*) significantly varied in relation to size of the fish. Cargnelli and Gross, (1997) reported that the adult male bluegills *Lepomis macrochirus* reveal a positive linear relationship between body size and energy reserve. Hence, it is evident that the result of the present study is in conformity with those of the previous studies.

Conversely, lipid content was inversely correlated (negative linear) with size of some fish species for example, Pacific tomcod (*Microgadus proximus*). At the same time some fish showed no correlation between size and lipid content of muscle tissues, for example in walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) (Anthony *et al.*, 2000).

## 5.3. Influence of body weight on lipid content of tissues

Lipid content of tissues in *S. lysan* also varied with the body weight (Table 8). A significant curvilinear correlation between body weight and lipid content of muscle in *S. lysan* was observed whereas a significant linear correlation was observed between body weight and lipid content of gonad in the present study. Similar findings in other

fish species were reported by Neumann and Murphy, (1992), who investigated the relationships between relative weight (Tissues weight/ Body weight) and body composition in white crappie (*Pomoxis annularis*, Rafinesque) from two Texas lakes during the pre spawning period. The coefficient of correlation of body composition in white crappie to relative weight was variable among different components (Table 10).

Table 10. Linear relationship of relative weight (Wr) to body components for white crappie collected during the pre-spawn period. Source: Neumann and Murphy, (1992).

Component	Linear relationship	n	r
Gonad <sup>1</sup>	- 4.260 + 0.084 (Wr)	18	0.293
Liver <sup>1</sup>	- 1.387 + 0.022 (Wr)	24	0.468
Visceral fat <sup>1</sup>	0.475 – 0.001 (Wr)	24	0.063
Moisture <sup>1</sup>	65.143 + 0.057 (Wr)	24	0.187
Ash <sup>2</sup>	54.233 – 0.350 (Wr)	24	0.539
Protein <sup>2</sup>	35.956 + 0.273 (Wr)	24	0.298
Lipid <sup>2</sup>	- 40.353 + 0.553 (Wr)	24	0.549

<sup>1</sup> Expressed as a percentage of wet body weight

<sup>2</sup> Expressed as a percentage of dry body weight

r – Coefficient of correlation

Hassan *et al.*, (2010) identified that the total lipid content in the liver tissues of *Catla catla* collected from Trimu Head, Jhang increased significantly with body weight whereas *S. lysan* show a weak correlation between liver lipid content and body weight in the present study.

#### 5.4. Lipid content in gonad, liver and muscle tissues

Muscle lipid content in *S. lysan* attained maximum value of 12.98% in dry weight and 3.24% wet weight in mature stages, which ranged in standard length from 50 to 61cm.

Childs and King, (1993 ) classified the fish into four categories according to the amount of lipid deposited in their muscle tissues (Table 11).

Table 11. Classification of fish. Source : Childs and King, (1993)

Type of fish	Range of lipid content (% in wet weight) deposited in the muscle tissues
Lean fish	< 2
Low fat fish	2 - 4
Intermediate fat fish	4 - 8
Fatty fish	> 8

According to the classification by Childs and King (1993), *S. lysan* can be classified as low fat fish (Table 11) since it possess the maximum of 3.24 % (in wet weight) lipid content in their muscle tissues. Shulman (1974) also recorded that the lean and low fat fish store lipid primarily in their liver tissues. Liver lipid content of *S. lysan* attained a maximum value of 64.79% in dry weight and 16.19% wet weight in mature stages. It further confirms that the *S. lysan* is a low fat fish (Sutharshiny and Sivashanthini, 2011b).

The results of the present study show that in *S. lysan*, lipids are stored primarily in the liver tissues (Fig. 7). Arrington *et al.*, (2006) recorded that the liver is known to function as a reservoir of lipid dynamics. Sheridan (1988) identified that the fish are capable of synthesizing lipid in their liver and mobilize it into the muscle.

Several other studies confirm that the liver acts as an important organ for lipid reserve in carangid species. Seiichi *et al.*, (1993) reported the lipid content of liver and

dorsal muscle of the cultured adult striped jack and amber jack (Family: Carangidae) collected from Japan (Table 12). They identified that higher lipid content accumulated in the liver tissues than in the muscle tissues.

Table. 12. Body length (cm), body weight (g) and lipid contents in wet weight (g/100g) of muscle and liver in both species. Source : Seiichi *et al.*, (1993)

Description	Amber Jack	Striped Jack
Body Length (cm)	53	36
Body Weight (g)	2074	796
Muscle lipid (g/100g)	2.73	4.86
Liver lipid (g/100g)	10.90	18.78

Similarly Shao-ning *et al.*, (2010) reported the lipid content of different tissues such as white muscle, red muscle, liver and spleen in both sexes of *Trachinotus ovatus* (Family: Carangidae) in China. These researchers identified that the highest and lowest total lipid content were in liver and white muscle respectively. In addition, significant differences were not recorded in total lipid content of the same tissues between male and female. Researchers concluded that the liver of *Trachinotus ovatus* is an important lipid storage organ.

Most studies were done with the lipid contents of muscle (Love, 1970; Kunisaki *et al.*, 1986; Nakagawa *et al.*, 1991) because the quality of fish is associated with the lipid levels of muscle. Lipid content of most carangid species fall within the range reported in the present study while some are deviated. In the present study, total lipid content in muscle tissues of *S. lysan* ranged from 0.9 % to 12 % in dry weight (Fig. 9)

and the mean total lipid content (% of dry weight) in muscle for all *S. lysan* reported as  $4.68 \pm 1.67$  % (Fig. 7).

The lipids extracted from the edible portions of *Caranx stellatus* from Sri Lanka were determined by Liyanage *et al.*, (1989). Mean total lipid content was recorded as 1.3g/100g (range from 1.2 to 1.5) in both months September 1988 and January 1989. Information on the lipid content of the round scad (*Decapterus punctatus*) was estimated by Hale, (1984) from Atlantic and Gulf regions. The average values of fat content of round scad were determined for the raw, headed and gutted fillet and whole body as listed in Table 13.

Table 13. Lipid content (%) of raw, headed and gutted, fillet and whole body

Source: Hale, (1984).

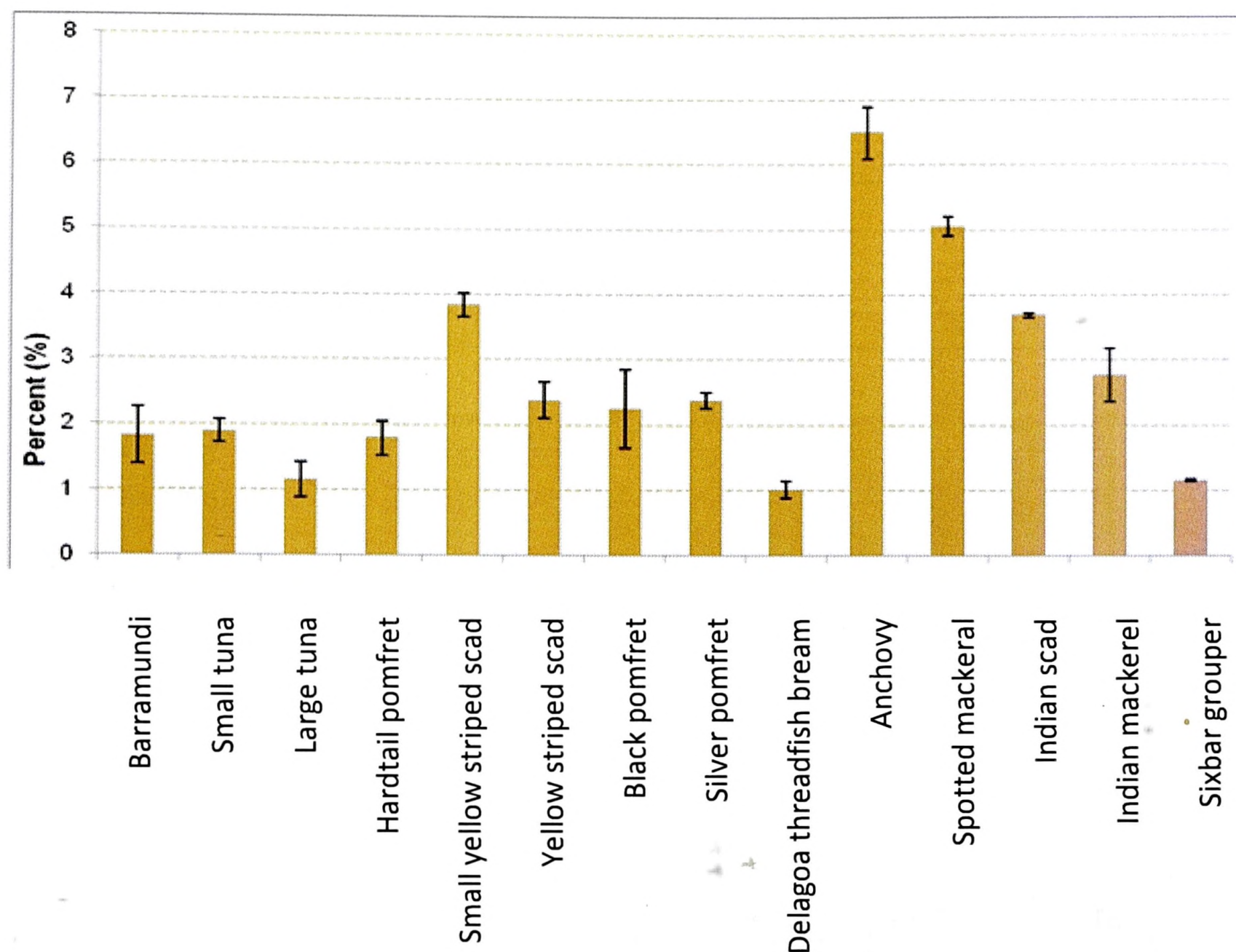
Source	Number of samples	Average weight (g)	Raw Fat (%)	Headed and Gutted Fat (%)	Fillet Fat (%)	Whole Fat (%)
Atlantic	11	35.1	2.52	2.52	1.9	2.96
Gulf	3	36.8	2.60	-----	-----	-----

Lipid content of fleshy parts of some carangid fish obtained from fishing grounds along the east coast of the Gulf of Thailand was recorded by Chedoloh *et al.*, (2011) (Table 14). In this study, lipid content ranged from 1.89 to 3.36%. Hence all carangid species in this study were categorized as low fat fish.

Table 14. Fat level of some carangid fish species. Source: Chedoloh *et al.*, (2011).

Scientific Name	Common name	Fat level %
<i>Parastromateus niger</i>	Black pomfret	2.58 ± 0.51
<i>Atule mate</i>	Yellowtail scad	2.13 ± 0.27
<i>Selar crumenophthalmus</i>	Bigeye scad	3.31 ± 0.25
<i>Carangoides gymnostethus</i>	Bludger	2.12 ± 0.35
<i>Pampus argenteus</i>	Silver pomfret	3.22 ± 0.67

Yazdan *et al.*, (2009) recorded that the fat content of raw fillet in black pomfret (*Parastromateus niger*) collected from Pasar Brong, Serdang Malaysia (Body weight: 340 ± 15 g and Total length: 20 ± 3 cm) was 1.5 g/100g. The lipid content of head and muscle tissues of yellow spotted travelly (*Carangoides fulvoguttatus*) from Saudi Market was recorded as 1.8 % and 0.24 % respectively (Manal, 2009). Wan Rosli *et al.*, (2012) recorded the total lipid content of some fish and shell fish from east coast region of peninsular Malaysia (Fig. 41). Of these species, small yellow striped scad, yellow striped scad and black pomfret exhibited lipid content less than 4%.



**Fig. 41.** Total fat content of selected marine fish species from east coast of peninsular Malaysia. Source: Wan Rosli *et al.*, (2012)

Similar range of lipid content in fillet of pompano (*Parona signata*) in Rio de la Plata, Uruguay was recorded as 0.56 - 13.6 % (Eduardo *et al.*, 1996). Further, Masamichi *et al.*, (1976) recorded that the range of muscle lipid content in horse mackerel (*Trachurus japonicas*) from Fukuoka, Japan, ranged from 1.1% to 11.2%.

The muscle lipid content in some carangid fish species recorded by other researchers was greater than that of *S. lysan* (Fig. 9). The percentages of crude lipid and purified lipid of Aji-Aji fish (*Seriola nigrofasciata*) were recorded as 18.3% (dry

weight) and 16.8 % respectively (Sharina and Jumat, 2006). The results showed that the Aji-aji fish contains high lipid content. Marichamy *et al.*, (2012) investigated the lipid content in edible part of some of the carangid species from Parangipettai coastal waters. Variation in lipid content was observed for four fish species as indicated in Table 15. The lowest percentage of 1.17 % was obtained for *Carangoides malabaricus* while the highest percentage of 18 % was obtained in *Scomberoides commersonianus*.

Table 15. Lipid content of some carangid fish species. Source : Marichamy *et al.*, (2012).

Fish Species	Body length (cm)	Body Weight (g)	Edible Portion (g)	% of edible Portion	Lipid content (% of edible portion)
<i>Scomberoides commersonianus</i>	28.5	142.9	80.4	56.2	18
<i>Carangoides chrysophrys</i>	20.3	98.3	51.6	52.4	16.27
<i>Caranx para</i>	13.8	64.7	32.5	50.2	11.32
<i>Carangoides malabaricus</i>	13.6	19.10	8.2	42.9	1.17

In female *S. lysan*, the maximum mean value of lipid content in ovary was  $37.07 \pm 10.15$  % in dry weight and 9.26% in wet weight at the spawning stage (Table 9). Values reported in the literature show that the value obtained at the present study for lipid content in ovary of *S. lysan* is more or less closer to the earlier reported values. Shirai *et al.*, (2001) expressed that the lipid content of ovary in Japanese catfish (*Silurus asotus*) was  $7.3 \pm 1.6$  g in 100 g wet weight at spawning stage. Similarly,

Zaboukas *et al.*, (2006) recorded that the maximum lipid content (as % wet weight) in *Sarda sarda* (Atlantic bonito) was 14.08 % for ovary and 10.63% for testis.

### 5.5. Lipid classes in gonad, muscle and liver tissues

In the present study, the phospholipids and triacylglycerol content in muscle tissues of *S. lysan* increased significantly from immature to spawning stage and decreased thereafter. Masamichi *et al.*, (1976) showed the changes of fatty acid composition, neutral lipid and phospholipids of muscle tissues in horse mackerel (*Trachurus japonicas*) from Fukuoka, Japan. These researchers recorded that the neutral lipid content increased with the increasing total lipid content while phospholipid content was almost constant without showing any relation to total lipid content.

It is evident from the present study that the phospholipid and triacylglycerol are the predominant lipid classes in the muscle tissue of *S. lysan*. Njoku *et al.*, (2004) reported that the triglyceride and cholesterol were the major lipid class in the muscle tissues of *Trachurus trachurus* collected from Nsukka market, Enugu state, Nigeria (Table 16).

Table 16. Lipid composition of *Trachurus trachurus*. Source: Njoku *et al.*, (2004)

Lipids	Values
% Yield	17.10 ± 0.14
Triacylglycerol mg %	250.0 ± 0.36
Cholesterol mg %	235.0 ± 0.46
Phospholipid mg %	2.4 ± 0.2

## 5.6. Changes of lipid and lipid class with maturity Stages

In the present study, changes in tissue lipid composition were identified throughout the course of maturation and spawning of *S. lysan*.

In immature *S. lysan*, content of lipid and lipid classes was lower than that of adult fish. This is consistent with the findings for other fish species for example *Comephorus baicalensis* (Se-Jong *et al.*, 1997) and *Heterotis niloticus* (Achionye-nzeh *et al.*, 2006).

Lipid class concentration (mg/100g) in immature stage of *S. lysan* was higher in muscle tissue (Cholesterol (CS),  $67.77 \pm 32.67$ ; Phospholipids (PL),  $332.16 \pm 85.08$ , Triacylglycerol (TAG),  $65.47 \pm 12.87$ ) compared with liver (CS,  $50.4 \pm 25.6$ ; PL,  $178.21 \pm 23.09$ ; TAG,  $34.14 \pm 0.78$ ) (Figs. 17 – 28). PL content of muscle and liver tissues in immature stage of *S. lysan* were higher than that of other lipid classes. The results of the present investigation on lipid classes of immature stages are in conformity with the observations made by Litvin *et al.*, (2011), who reported the lipid class dynamics and deposition in juvenile weakfish *Cynoscion regalis* -young of the 1-year in New Jersey shore of Delaware Bay. Litvin *et al.*, (2011) showed that PL was the predominant lipid class in juveniles, which ranged from 8.1 mg/g to 40.0 mg/g, while the TAG content was the lowest value, which was less than 15 mg/g. Yet, liver of juvenile fish contained low amount of TAG ( $< 10$  mg/g) than the muscle. Similarly, Hiroshi *et al.*, (2004) identified that the neutral lipid and phospholipid content of nearly hatched larvae of striped jack (*Caranx fulvoguttatus*: Carangidae) were of higher value,  $46.7 \pm 6.3\%$  and  $53.4 \pm 6.3\%$  of total lipid respectively.

### 5.6.1. Gonad lipid content and spawning

Total lipid content in gonad of *S. lysan* was higher in spawning stage while it was the lowest in spent stage (Table 9). Similar trend was recorded by Assem *et al.*, (2005) for another carangid fish, *Trachinotus ovatus*. They reported that the total lipid content of ovaries in *Trachinotus ovatus* attained the minimum value of  $7.86 \pm 1.08$  % in dry weight at immature stage. It increased progressively to reach a maximum value of  $14.02 \pm 3.69$  % at nearly ripe stage.

The results of the present investigation reveal that the mean value of lipid class composition in spawning ovary has relatively higher amount of TAG, 406.96 mg/100g and PL, 388.49 mg/100g than the CS (359.04 mg/100g) (Figs. 11 – 13). Wigand, (1996) clarified and tabulated the egg lipid composition of several species. Some fish species under perciformes exhibited a considerable variability in the egg lipid composition. For example, TAG and PL content as proportions of total lipid content in egg of red drum were 31.9% and 32.8% respectively (Vetter *et al.*, 1983). Similarly, eggs of gilthead sea bream contain TAG and PL levels of 22.6% and 33.0% (Mourente and Odriozola, 1990). Hilton *et al.*, (2008) also noticed that the phospholipid content in the brood stock egg of yellowtail kingfish (*Seriola lalandi*) was higher (60%) whereas triacylglycerol value (15%) was lower in egg of yellowtail kingfish. Similarly, eggs of some fish species under Gadidae and Pleuronectidae (righteye flounders) contain higher phospholipid value, which ranged from 61 to 77 % of the total lipids than the triglyceride value, which ranged from 7.3 to 14.3% of the total lipids, while sand eel has higher value of TAG, 45.7% than PL 23.4 % in proportions of total lipid content (Tocher and Sargent, 1984).

### 5.6.2. Muscle lipid content and spawning

Muscle lipid content of *S. lysan* attained a maximum value in mature stages and decreased at spawning stage (Table 9). Bransden *et al.*, (2007) identified a similar trend; they found that the fat content in muscle of male and female striped trumpeter *Latris lineate* decreased by 25% and 40% respectively during the spawning period.

PL content in the muscle of *S. lysan* increased from immature stage to mature and decreased thereafter (Fig. 18 & 21). Yagana, (1982) also reported that the value of phospholipid in muscle of catfish *Clarias batrachus* declined during spawning period.

TAG content in muscle and liver tissues of mature *S. lysan* was higher than the CS content (Figs.19, 22, 25 & 28). Further, Seiichi *et al.*, (1993) identified that the major lipid component of the muscle and liver in amberjack and striped jack was triglyceride. Thakur *et al.*, (2003) reported that the neutral lipid was the main constituent in muscle of yellowtail fish (*Seriola quinqueradiata*).

Low CS content was observed in the muscle tissues of mature *S. lysan*, in the present study (Figs 16 & 19) (Sutharshiny and Sivashanthini, 2011c). The Institute of Medicine (IOM) of the U.S. National Academy of Sciences recommended that the Dietary Reference Intake (DRI) of cholesterol for male and female was 300 mg per day (Anonyms, 2013). Considering the IOM's note it could be mentioned that consumption of *S. lysan* fish at appropriate quantity possess no risk to human health.

### 5.6.3. Liver lipid content and spawning

The highest value of lipid in the liver of *S. lysan* was recorded when the fish attain mature stage (Table 9). Notable decrease in lipid content of liver tissue of *S. lysan* was observed in spawning stage. This statement is in agreement with other studies. According to Huynh *et al.*, (2007), the liver lipid content in wet weight of Pacific herring, *Clupea harengus pallasii* was higher,  $10.54 \pm 0.70$  % in the non-spawning period whereas lower  $1.92 \pm 0.42$  % in the spawning period. Similarly, Lal and Singh, (1987) identified that the liver lipid of freshwater catfish *Clarias batrachus* decrease in spawning period and they stated lipids were transferred from the liver to ovary.

Changes in the TAG content in liver tissues of *S. lysan* showed a two-fold increase from immature stage to spawning stage and then a four-fold decrease thereafter (Figs. 25 and 28). This observation is in consistent with the findings by Phleger, (1971), who found that the total liver lipid content of pink salmon *Onchorhynchus 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 low variation during the maturation cycle. The highest value of cholesterol in the liver of *S. lysan* was recorded at mature stage, while the minimal value was recorded during the spent stage. Findings of the present investigation on *S. lysan* is also confirmed by the previous works carried out by Idler and Bitner, (1960), who reported that the total cholesterol content in liver declined and deposited in ovary of migratory salmon, *Oncorhynchus*

*nerka*, during the spawning phases. However, Phleger, (1987) expressed that the CS content in liver of pink salmon (*Onchorhynchus gorbuscha*) remain constant after spawning.

### **5.7. Lipid changes in tissues through out the year**

From the present study, total lipid, CS, PL and TAG in gonad, muscle and liver tissues of tropical *S. lysan* show a significant variation throughout the year, corresponding to the maturation stage and annual spawning events. Peaks spawning of adult female *S. lysan* (Maturing, Mature, Spawning and Spent stages) were in June and September months (Thulasitha and Sivashanthini, 2012c).

Some tropical fish show a marked seasonal variation in tissue lipid composition which corresponds with key stages of the sexual cycle (Watts, 1957; Ito and Watanabe, 1968; Watanabe, 1971). However, Hale, (1984) recorded that lipid content of the round scad (*Decapterus punctatus*) did not vary with the season.

Lipid content of tissues in *S. lysan* varied with the degree of ovarian maturity (Figs. 29 – 40). The CS, PL and TAG content in ovary of *S. lysan* fluctuated throughout the year and attained a noticeable peak value during the spawning period whereas muscle and liver lipid content of *S. lysan* was attained the lower amount during the spawning months (Figs. 32 – 40). Similarly, Bustamante, (1989) recorded that the maximum value of body fat was accumulated before the spawning peak in bar jack (*Caranx ruber*) and decreased during the spawning period. At the same time, the lipid content in ovary of bar jack increased during the spawning time.

Arrington *et al.*, (2006) also proposed similar pattern of seasonal changes in lipid content of muscle, liver and gonad of three neotropical fish. According to gonad maturity stages, they identified the spawning period as February to May. In addition, lipids in both dorsal muscle and liver tissues were stored during June to February and then depleted during the spawning period - February to May.

In *S. lysan*, monthly fluctuations of liver lipid content was observed and the lowest lipid content was reported in the spawning months (June and September) (Figs. 37 – 40). Deka *et al.*, (2012) also found that the lowest value of liver lipid in *Labeo gonius* captured off river of Brahmaputra, India during the breeding season. However, the muscle lipid of *Labeo gonius* attained the highest value during the breeding season. Similarly, liver lipid composition of red drum (*Sciaenops ocellatus*) varied dramatically throughout the year (Craig *et al.*, 2000) and the TAG content in liver was higher during summer months, declined in September and October (Spawning period).

The trend of the muscle lipid content in *S. lysan* fluctuated throughout the year (Figs. 35 – 37). However, the highest values of TAG content in *S. lysan* were observed in May and August, whereas the lowest values were observed in June and September. Thakur *et al.*, (2009) showed unclear trend of seasonal variation in muscle lipid content of amberjack (*Seriola dumerili*).

## 6. CONCLUSIONS

Variation in total lipid, cholesterol, phospholipid and triacylglycerol 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 in gonads, muscles and livers of *S. lysan* throughout the year provide information to determine the non-spawning period or fishing season of *S. lysan* in the waters around the Jaffna Peninsula. Based on the present study peak spawning months of *S. lysan* are June and September and therefore it could be harvested during the other months. Range of muscle lipid value obtained in the present study signifies that *S. lysan* fall under 'low fat fish' category throughout its maturation. Thus, it could be recommended as one of the healthiest food fish for human consumption as it has low fat content. Determination of total lipid, cholesterol, phospholipid and triacylglycerol 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 trials 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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## **Total Lipid and Cholesterol Content in the Flesh of the Five Important Commercial Fishes from Waters Around Jaffna Peninsula, Sri Lanka**

<sup>1</sup>S. Sutharshiny and <sup>2</sup>K. Sivashanthini

<sup>1</sup>Department of Fisheries, University of Jaffna, Jaffna, Sri Lanka

<sup>2</sup>Department of Zoology, University of Jaffna, Sri Lanka

*Corresponding Author: K. Sivashanthini, Department of Zoology, University of Jaffna, Jaffna, Sri Lanka  
Tel: 00 94 21 222 5925 Fax: 00 94 21 222 2685*

### **ABSTRACT**

The present investigation was carried out to understand the total lipid and cholesterol content of flesh of five commercially important adult fishes such as *Siganus lineatus*, *Gerres oblongus*, *Scoliodon laticaudus*, *Scomberoides lysan* and *Hemirhamphus marginatus* collected from waters around Jaffna peninsula. People living in Jaffna peninsula consume fish as the main source of nutrition in their diet. However they have no definite knowledge, pertaining to which species can be intake with their food without harming their health condition. Therefore studies on the nutritional value of marine food fish is a prime theme of research for fish consuming people. In the northern province of Sri Lanka, no study on lipid and cholesterol content of fish has been done so far and therefore the present investigation was initiated. Total lipid in the flesh of five different fishes was extracted and the cholesterol content was estimated in the present study. Mean total lipid and cholesterol content of five commercially important fishes ranged from 2.63 to 4.41% and 54.2 to 104.5 mg/100 g, respectively. Values obtained for total lipid and cholesterol content of five different fishes were found to be good indication of nutritional values. Highest total lipid content was found in muscle tissue of *Gerres oblongus* lowest was in *Hemirhamphus marginatus*. However, highest cholesterol content was recorded for *Scoliodon laticaudus* and the lowest for *Scomberoides lysan*. Analysis of Variance (ANOVA) for total lipid and cholesterol content of five different adult fishes showed that there is significant difference ( $p < 0.05$ ) between fishes.

**Key words:** Lipid, cholesterol, *Siganus lineatus*, *Gerres oblongus*, *Scoliodon laticaudus*, *Scomberoides lysan*, *Hemirhamphus marginatus*

### **INTRODUCTION**

Lipids are the predominant source of energy for fish. The mechanisms by which fish allocate energy from lipids for metabolism, development, growth and reproduction are critical for understanding key life-history strategies and transitions (Leaver *et al.*, 2008). Lipids are the major source of nutrition in marine fishes (Sargent, 1976; Sargent *et al.*, 1989). They are considered an efficient biochemical means of concentrating large amounts of stored energy at small space. The cells of white fat tissue, called adipocytes, are responsible for lipid synthesis, release and storage in

the organism (Szkudelski *et al.*, 2009). The major storage sites of fish are mesenteric fat, muscle and Liver (Sheridan, 1988).

The fat content in fish muscle is highly variable. It depends on species, age, spawning season, fish diet, and muscle type (Gehring *et al.*, 2009). Qualitative studies on the composition of fish flesh have been investigated frequently (Love, 1970). The live weight of majority of fish usually consists roughly of lipid 2-12% (Love, 1980; Weatherley and Gill, 1987). The seasonal storage and utilization of lipid reserves are important in the metabolic activities and overall life histories of many animals including fish (Love, 1970; Shulman, 1974). Constancy in tissue or organ function is reflected in the composition and structure of polar lipids, while neutral lipids vary widely as a result of a balance between dietary intake, anabolism and catabolism (Sargent and Henderson, 1986). There are several classes of lipids, all having similar and specific characteristics due to the presence of major hydrocarbon portion in their molecule.

Lipids can be used as an assay of physiological condition and may reflect resources availability, metabolic activity or recent stress (Fraser, 1989). Lipids are important fuel for marine organisms especially for those living in high latitudes (Clarke, 1983). When maternal diets are deficient, insufficient transfer of lipids to developing ovaries may reduce fecundity and the viability of the progeny (Watanabe, 1985; Luquet and Watanabe, 1986; Heming and Buddington, 1988). The role of lipids in reproduction may be just as critical, supplying energy for activities such as egg development, nest building, courtship, or protection of young (Meffe and Snelson, 1993). Slobodkin (1962) and Calow (1977) noted that fat deposition may actually detract from reproduction, particularly when fat deposition and reproduction are concurrent.

Triglycerides are compounds where most of the fat calories are stored. The reserve fats of fish have a mechanical function, maintaining the elasticity of the outer covers and creating a soft lining for the internal organs (Stroganov, 1962). The major lipids that have direct role in buoyancy of marine fish are wax esters, squalene and alkyldiacylglycerols. Triacylglycerols and cholesterol have an indirect role in buoyancy of marine fish. Many fishes in the ocean have oil-filled bones (mostly triacylglycerols) (Phleger, 1998). Phospholipids are the main constituents of biological membranes (Bhouri *et al.*, 2010) and it provides sheaths surrounding the nerve cells (Sargent and Whittle, 1981; Farkas *et al.*, 1988). It plays a central role in the embryonic metabolism.

Fish is a major source of food for human nutrition providing an important amount of dietary protein and lipid diet in many countries (Bouriga *et al.*, 2010). Compared with red meat, fish flesh is easily digestible (Pirestani *et al.*, 2010). Fish and fish oil are the rich source of omega 3- fatty acids. Fish oils have moved into the center stage of fatty acids in nutrition, it helps to prevent brain aging and Alzheimer's disease (Whelan, 2008). Cod liver oil taken for vitamins A and D. Fish oil were used as industrial chemical based on paints and linoleum.

The Jaffna Peninsula is an area in Northern Sri Lanka. Jaffna peninsula is surrounded by sea water but connected to mainland via an isthmus called Elephant pass. Jaffna is situated within ten degrees of latitude to the north of the equator. It is in close proximity to the sub-continent of India and separated from it by the Palk Strait and the Bay of Bengal. In addition to agriculture and livestock, fishery sector is an important industry in Jaffna provides major source of food and income for society. Jaffna district alone contributed 26% of the total fish production and 57% of the total dry fish production of Sri Lanka, in 1983.

The fishes selected in the present study are high consumer demand food fishes found in the Sri Lankan coast having high flesh content and good taste. Among the selected five fishes *Siganus lineatus*, *Gerres oblongus* and *Hemirhamphus marginatus* are relatively cheaper than the other two and therefore people living in coastal regions of Sri Lanka frequently consume these fishes even though they did not have any idea about the nutritional composition of these fishes. *Scoliodon laticaudus* is a delicious food fish in Sri Lanka the whole part of shark can be utilized as food. Shark meat is used for the production of minced fish products such as fish balls, fish cake, fish sausage, fish ham and fish paste and particularly appreciated in other parts of Asia. Sharks fins are also processed and exported to other Asian Countries by Sri Lankans. Shark liver oil is also an important by product of shark. *Scomberoides lysan* is also an important food fish in Sri Lanka mostly exported to other parts of the world as dry fish.

People living in coastal region of Sri Lanka provide these fishes to pregnant women and feeding mothers. However they have no definite knowledge, pertaining to which species can be intake with their food without harming their health condition. Therefore studies on the nutritional value of marine food fish is a prime theme of research and the results obtain in the present study will provide a detailed understanding on prevention of lipid oriented diseases for a healthy life for the fish consuming people.

In the northern province of Sri Lanka, no study on lipid and cholesterol content of fish has been done so far. As such the present investigation was carried out to understand the lipid and cholesterol content of five commercially important fishes from waters around Jaffna peninsula.

## MATERIALS AND METHODS

**Sampling of fishes:** The commercially valued fishes namely *Siganus lineatus*, *Gerres oblongus*, *Scoliodon laticaudus*, *Scomberoides lysan* and *Hemirhamphus marginatus* were selected for the present study. Samples of five commercially valued adult fishes were collected from Point Pedro, Pasaioor and Delft landing centers (Locations of sampling stations are presented in Fig. 1) from March 2010 to July 2010 and brought to laboratory in an ice box. Total body weight was weighed to the nearest 0.1 g and standard length was measured to the nearest 0.1 mm. The fish samples were dissected and the stage of maturation was determined, macroscopically. Only matured adult fishes from the selected species were considered for the present research. Size range of adult fishes and number of observations are provided in Table 1.

**Identification of fishes:** Collected fishes were brought to the laboratory and species identification was confirmed using the FAO species identification guide (De Bruin *et al.*, 1994).

**Total lipid analysis:** Total lipids in tissue sample were extracted according to the method of Bligh and Dyer (1959), that is modified method of Folch *et al.* (1957). One hundred gram muscle tissue was cut from the fresh fish, rinsed with distilled water and dried to constant weight in a drying oven (60°C, 24 h). Dried samples were minced in a glass blender, homogenized with chloroform: methanol mixture (2:1 V/V), mixed in a vortex mix in 2800 rpm and filtered. The extract was shaken and equilibrated with ¼ of its volume of a saline solution. The extracted lipids were

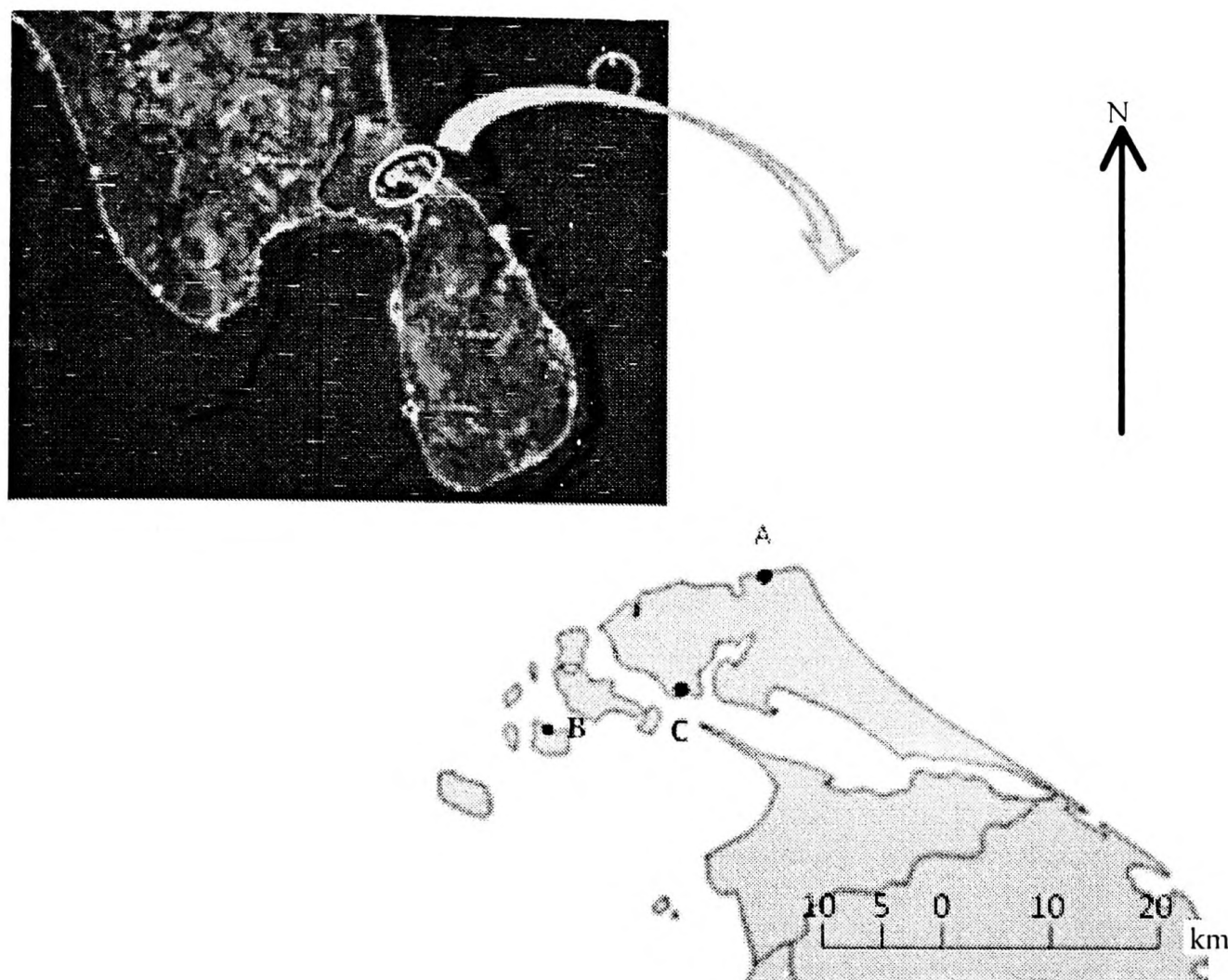


Fig. 1: Sampling sites (A, B and C) of fishes from waters around Jaffna peninsula (A) Point Pedro (B) Delft (C) Passaiyoor

Table 1: Size range and number of observations of fishes examined

Fishes analyzed	Size range (standard length in cm)	No. of fish examined	Degrees of freedom
<i>Siganus lineatus</i>	22 - 25	24	23
<i>Gerres oblongus</i>	15-18	24	23
<i>Scoliodon palasorrah</i>	50-53	24	23
<i>Hemirhamphus marginatus</i>	40-43	24	23
<i>Scomberoides lysan</i>	42-45	24	23

concentrated by a rotary evaporator (IKA RV 10 basic). Lipids were stored in sealed vials. Extracted lipids were weighed in vials using a micro electronic balance ( $\pm 0.001$  mg) in order to calculate the total lipid content. The same procedure was repeated with 24 replicates of each fish species and the mean value was computed.

**Cholesterol analysis:** Cholesterol content of fish was estimated by the Zlatkis *et al.* (1953) method. Extracted lipids were treated with ferric chloride, acetic acid mixture and sulphuric acid and the colour developed was observed. After 20 min absorbance was read at 560 nm in a spectrophotometer (LABOMED, UVD-3000) at the department of Zoology, University of Jaffna. The absorbance readings were plotted in a calibration curve and the relevant cholesterol concentrations were computed. The same procedure was repeated with 24 adult fish samples of each fish and the mean value was computed.

**Statistical analysis:** The total lipid and cholesterol content obtained for five different adult fishes were first analyzed by one way analysis of variance (ANOVA). When a single factor ANOVA rejects the null hypothesis i.e., when the mean of the samples was significantly different, ANOVA was followed by Post hoc comparison of means: Duncan's multiple range test (DMRT) using STATISTICA software in the computer. The level of statistical significance was set at  $p < 0.05$ .

## RESULTS

**Total lipid and cholesterol content:** Total lipid and cholesterol content of five different fish species are presented in Table 2, total lipid and cholesterol content of twenty four adult fish samples of five different fishes were subjected in the computation of mean value in the present study. Mean total lipid content ranged from  $2.6300 \pm 0.060\%$  (*Hemirhamphus marginatus*) to  $4.4117 \pm 0.058\%$  (*Gerres oblongus*) whereas mean cholesterol content ranged from  $54.20 \pm 1.005$  (*Scomberoides lysan*) to  $104.05 \pm 0.900$  mg/100 g (*Scoliodon laticaudus*). Values obtained for total lipid and cholesterol content of five different fishes were found to be good indication of nutritional values. Highest total lipid content was found in muscle tissue of *Gerres oblongus* lowest was in *Hemirhamphus marginatus*. However, highest cholesterol content was recorded for *Scoliodon laticaudus* and the lowest for *Scomberoides lysan*.

**Statistical analysis:** Analysis of Variance (ANOVA) for total lipid and cholesterol content of five different adult fishes showed that there is significant difference ( $p < 0.05$ ) between treatments. Results of Post hoc-Duncan's test is expressed as superscripts in Table 2. Post hoc-Duncan's Multiple Range Test expressed significant different ( $p < 0.05$ ) between five different fishes at all instances. Box-Whisker plots showing significant difference in total lipid content and cholesterol content between five different fishes are shown in Fig. 2 and 3. Significant differences in mean,

Table 2: Mean total lipid and cholesterol content of five fish species from waters around Jaffna peninsula, Sri Lanka. (Values in the column indicate mean  $\pm$  Standard deviation)

Scientific names of fishes analyzed	Mean total lipid (%)	Mean cholesterol content (mg/100 g)
<i>Siganus lineatus</i>	$3.3667 \pm 0.108^a$	$74.400 \pm 0.252^f$
<i>Gerres oblongus</i>	$4.4117 \pm 0.058^b$	$93.917 \pm 0.870^e$
<i>Scoliodon laticaudus</i>	$3.9150 \pm 0.048^c$	$104.05 \pm 0.090^b$
<i>Scomberoides lysan</i>	$3.5767 \pm 0.071^d$	$54.200 \pm 1.005^i$
<i>Hemirhamphus marginatus</i>	$2.6300 \pm 0.060^e$	$71.267 \pm 0.920^j$

Value in the same column with different superscript letters (a-e) (f-j) within a same nutritional component are significantly different ( $p < 0.05$ )

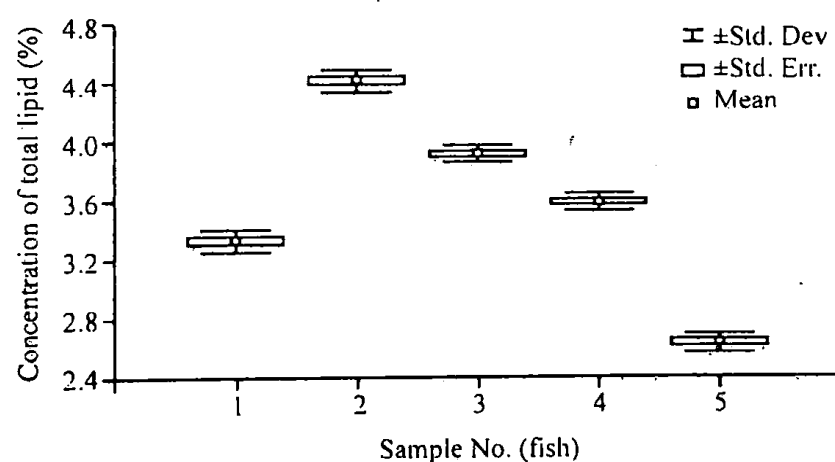


Fig. 2: Box-Whisker plot showing significant difference in total lipid content between five different fishes

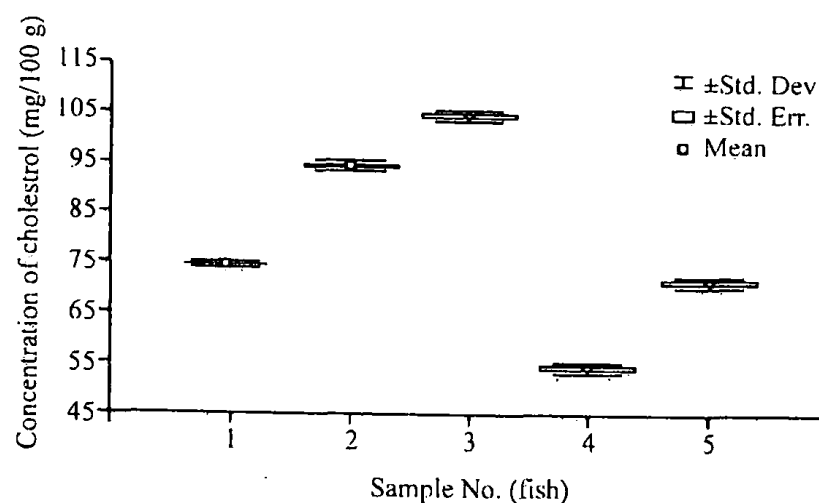


Fig. 3: Box-Whisker plot showing significant difference in cholesterol content between five different fishes

standard error for each mean value and standard deviation for each mean are clearly expressed in Fig. 2 and 3.

## DISCUSSION

Very few studies were carried out on the selected tropical fish species from other parts of the world. In a study by Al-Jedah *et al.* (1999), total lipid content of congeners of the three species of fishes studied in the present investigation was presented from the coast of Qatar. The estimated total lipid content for *S. commerson* was 7.46% (Al-Jedah *et al.*, 1999) which is higher than the value estimated for *S. lysan* in the present study. Al-Jedah *et al.* (1999) estimated lowest total lipid content for *G. filamentosus* than the value obtained for *G. oblongus* in the present study. Total lipid content obtained for *S. canaliculatus* from Quatar waters (Al-Jedah *et al.*, 1999) was 3.18% which tally with the present work i.e. for *S. lineatus* in the present study was 3.36%. In another study, Zhao *et al.* (2007) reported a total lipid value of  $2.79\% \pm 0.15$  for adult *Siganus guttatus* from the South China Sea. The recorded value in the South China Sea is lower than the present estimate computed for a congener of the *Siganus* species. The above differences may have been attributed due to different environmental conditions and nutritional status of those fishes in different topographical regions.

Further, Childs and King (1993) classified fish into three categories according to the content of fat, namely the low-fat category with 0.6-3.0% fat, the medium fat category with 3.5-7.0% fat and the high fat category with levels that range from 8.1-15.3% fat. The fish caught from the Northern waters of Sri Lanka tend to fall into the low and medium fat categories, although it should be noted the fat contents may vary between individual fish or groups of fish of the same species caught at different times or under different conditions.

Among the important five fish species from waters around Jaffna peninsula, the richest total lipids were observed in *Gerres oblongus* and the highest cholesterol content was observed in *Scoliodon laticaudus*. Bykov (1996) reported that the fat content for *Scoliodon* sp. varied between 18 to 23%. Bakes and Nichols (1995) studied about the liver oils from the deep-sea sharks *Somniosus pacificus*, *Centroscymnus plunketi*, *Centroscymnus crepidater*, *Etmopterus granulosus*, *Deania calcea* and *Centrophorus scalpratus*. They analyzed lipid, fatty acid and squalene compositions of each shark species and found high squalene content (50-82% of oil) in all species, except *Centroscymnus plunketi* and *Somniosus pacificus* and suggested that the oil from these deep-sea sharks collected in southern Australian waters is suitable for industrial uses.

The nutritive value of *Scomberoides tol* was found to be good and it had 1.6% of lipid and 0.008% of free fatty acid (Patterson and Ranjitha, 2009). Total lipid content of *Siganus fuscescens* showed seasonal variations and were high in winter and low in summer (Osako *et al.*, 2006). No previous study was done on *Hemirhamphus marginatus* and the present investigation is the first record for this species. The present study indicated that all five species of fish studied presently are intuitively good and they could be exploited successfully for food and for preparing various fish by products.

#### ACKNOWLEDGMENT

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## Lipid Reserves of *Scomberoides lysan* (Pisces: Carangidae) from the Sri Lankan Waters

<sup>1</sup>S. Sutharshiny and <sup>2</sup>K. Sivashanthini

<sup>1</sup>Department of Fisheries, University of Jaffna, Jaffna, Sri Lanka

<sup>2</sup>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 21 222 5925 Fax: 00 94 21 222 2685

### ABSTRACT

The present investigation was carried out to quantify the lipid reserves and to find out the correlation with standard length and water content for total lipid content in different body tissues of *Scomberoides lysan*. Energy allocation of lipid reserves into liver, gonad and muscle were analyzed for the first time for different gonad maturation stages of *S. lysan* from the Sri Lankan waters. Relationships of energy storage with body size and gonad maturation stages and percentage of water content and lipid content of muscle, liver and gonad were computed and compared. Results showed that lipid content in the liver varied between 4.54 and 66.96% (Mean  $31.11 \pm 14.10\%$ ) and revealed liver is the major energy storage site in *S. lysan*. Mean values for percentage of lipid reserve in ovary and liver for maturing female was 20.42 and 41.35% whereas for pre-spawning female was 44.2 and 30.79%, respectively. Similar trend was also obtained for males and it emphasize that the lipid stored in the liver during the maturing stage is mobilized towards the gonad during pre-spawning for gonad development, gamete production and other reproductive purposes. Regression analysis showed that the percentage of lipid of liver, gonad and muscle for all individuals showed a significant curvilinear relationship with standard length. Moreover, a curvilinear relationship was obtained between standard length and lipid hepatosomatic index but linear relationship was obtained between standard length and lipid gonadosomatic index. Interestingly, inverse linear relationship was obtained between percentage of water content and lipid content of muscle, liver and gonad. The results gained from the present study provide information on nutritional status of different maturity stages, reproductive potential and broodstock nutrition of *S. lysan*.

**Key words:** Energy allocation, lipid hepatosomatic index, lipid gonadosomatic index, lipid content, muscle, liver, gonad, pre-spawning

### INTRODUCTION

Energy storage in animals is typically in the form of lipids. Lipid storage and dynamics are particularly important aspects of fish health and population success (Lloret *et al.*, 2008) because they have a large influence on growth, reproduction and survival (Sutharshiny and Sivashanthini, 2011). Now-a-days, fish lipids have been highlighted as being beneficial for human health (Hedayatifard and Yousefian, 2010). Quantity of energy reserves in a fish influence the metabolic activities of it. When energy reserves becomes low, it will lead to a situation of increased natural mortality, less survival, low fecundity, low quality of eggs, belated maturation and inferior hatching

rates and fertilization (Sogard and Olla, 2000; Lambert *et al.*, 2003; Koops *et al.*, 2004; Wanless *et al.*, 2005). This concept has not only been studied in temperate fish but also in other vertebrates and invertebrates. For example, a study on changes in the concentrations of liver total lipids and serum total lipids during the development has been performed by Ali *et al.* (2011) for broiler chicks. Another study on lipid changes specifically fatty acid variation was carried out seasonally in bivalve mollusc *Saccostrea cucullata* by Sajjadi *et al.* (2009).

Lipid content of fish is highly variable and provides indications on the fish quality (Van der Lingen and Hutchings, 2005; Shamsan and Ansari, 2010), affected by habitat characteristic and the food supply (Levi *et al.*, 2005; Shulman *et al.*, 2005). Usually a lipid content of 2-12% in live weight was recorded for majority of fish and fish related organisms (Mahin *et al.*, 2011; Farhoudi *et al.*, 2011; Tawfik, 2009) and 21% in crab, *Portunus sanguinolentus* eggs (Sounndarapandian and Dey, 2008). In a study with sturgeon fish (*Huso huso*). Ebrahimnezhadarabi *et al.* (2011) reported that fat incorporated in juvenile's diet increase the growth and enhance the nutritional indices.

In a fish once the lipid content exceeds the needed quantity of metabolizable energy the balance will be deposited in its tissues. As a result the fish will show high lipid content and categorised as high lipid content fish. Generally, when lipids are stored only in the liver of fish they are termed as lean fish. If the lipids are stored in other body tissues they are termed as fatty fish (Huss *et al.*, 2003).

Lipid storage sites are primarily liver, muscle in the mesentery and along the lateral line or at the base of fish (Arrington *et al.*, 2006). In most cases energy is stored in the liver of the fish and therefore, liver indices becoming as an important indication of the overall fish condition (Lambert and Dutil, 1997).

During breeding, lipid reserves stored in the body of fish will be significantly reduced. This is because energy and essential nutrients has to be supplied to the ovary for its rigorous generative synthesis (Morris and Culkin, 2000; Okuda, 2001).

As a fish mature, gonad development takes place and the fish must produce gametes. The related processes are very energy intensive and require lipids. During these phases, lipid levels decrease in the liver and muscle tissue and increase in the gonads (James and Elizabeth, 2003; Zaboukas *et al.*, 2006).

The changes that take place in the tissue of fish, at the onset of maturation, are often different in males and females. Generally in several fishes, male gonad is smaller than the female. This is caused by difference in size of the fish, fish behaviour and the size of the gonads (Andrew, 2011). Lipids stored in the ovary of fish is the principal source of energy as it provide the energy for larval stages before first feeding and persuade the survival of fish larva (Rainuzzo *et al.*, 1997). For estimating the energy reserves of fishes determination of lipid content has been extensively used among the biochemical parameters (Adams, 1999). Meffe and Snelson (1993) studied the partitioning of the total lipid budget in muscle, liver and ovary in mosquito fish. Reznick and Brown (1987) proposed that somatic lipid storage is an adaptation that by shifting stored energy into the ovary.

The doublespotted queenfish *Scomberoides lysan* (Forsskal 1775) is an economically important food fish in coastal as well as offshore fisheries in Sri Lanka included under family Carangidae. The flesh of the fish is meaty and flesh: carcass ratio is high so that these fish have high consumer demand and market potential. The species is popular for dry fish production with export demands and especially consumed by mothers during pregnancy and immediately after delivery. Therefore, it's highly prized and continues to maintain a high market demand. It is found along the Indian

and Pacific oceans with the reefs. Juveniles are found in shallow waters whereas adults in clear waters (De Bruin *et al.*, 1994). Vary rarely form small groups, usually found as single individuals. *S. lysan* is a popular sport fish. Their tough skin is often stripped off, dried and used for trolling lures (Honebrink, 2000).

Several studies on lipid changes of different organs of fish have been studied for different temperate fishes (MacFarlane *et al.*, 1993; Fiorin *et al.*, 2007; Lloret *et al.*, 2007) whereas no such study is available for tropical fishes, so far. Size-based variation for the level of stored lipids, reporting a positive relationship between body size and lipid content was studied by various researchers (Cargnelli and Gross, 1997; Jonsson and Jonsson, 1997; Saito *et al.*, 1999; Mackereth *et al.*, 1999). The strategy of the energy allocation among different body compartments and the relationships of energy storage with body size and Gonad Maturation Stages (GMS) could change according to their reproductive potential and status.

Information on the energy reserves of *S. lysan* is still lacking and the present investigation was originated as a consequence of it in order to find out the nutritional status and also to understand the energy allocation for reproductive strategies. In the present investigation three different aspects were studied in detail: (1) total lipid content in liver, muscle and gonad was quantified and compared between immature, maturing and pre-spawning stages, (2) relationships between standard length and total lipid content in different organs were computed and compared and (3) relationships between percentage of water content and lipid content in different organs were computed and compared.

## MATERIALS AND METHODS

**Sample collection:** Fishes were collected at monthly regular intervals from January 2010 to December 2010 by means of 7" 21 ply mesh size drift gill nets set in the Point Pedro and Puttalam Sea, Sri Lanka with the help of Federation of Fishermen Cooperative Society's Union of the respective district. Every month, sub samples from the catch were taken to the laboratory for further analysis. Selected fishes representing the full size range of each sex and different GMS were taken for the analysis (Table 1), every month. Standard length ( $L_s$ ) and total wet weight (M) were measured to the nearest 0.1 mm and 0.01 g using calipers and an electronic balance, respectively.

The fish samples were dissected, sex was determined and sexual maturity of gonads was examined macroscopically. Immature stages were very small, flat and thread like. Maturing and pre-spawners were first classified according to sex by visual inspection of the gonads, through an incision made in the ventral mid line. All males and females included in the analysis were then classified microscopically (Mackie and Lewis, 2001). Maturing stage ovaries were signified with the following characters: pinkish in colour, oocytes present, cortical alveoli and oil droplets are distinct and zona radiata well formed. Late pre-spawning stages were distinguished by observing the following characters: Ovaries were large with blood capillaries, yellow or orange coloured and with visible but still non hydrated oocytes and sometimes (if previously spawned) with postovulatory follicles. The maturing males were classified microscopically to be in the maturing stage: Yellow brown bodies, connective and muscle tissues prominent, spermatocytes were the dominant tissue and pre-spawning maturity stage: abundance of spermatozoa and central sperm sinus was present.

Observations of the present study showed that all size classes as well as all GMS were found in the collection of every month's sample which emphasizes *S. lysan* is a multi spawner and spawns throughout the year in Sri Lankan waters perhaps have a peak spawning period. For carangids Thresher (1984) reported repeated, periodical spawning pattern in Hawaiian waters whereas

Griffiths *et al.* (2005) emphasized talang queenfish *Scomberoides commersonnianus* have a protracted spawning season.

Individuals were thawed and eviscerated and muscle, liver and gonads were removed. These sections were chosen because of their reported potential as storage sites for lipids (Shulman, 1974; Sheridan, 1988). Eviscerated weight ( $W_E$ ) and muscle weight were measured to the nearest 0.01 g the gonad and liver were blotted dry and weighed to nearest 0.001 g. Each specimen's sex,  $L_S$ ,  $M$ ,  $W_E$  and weight of muscle, liver and gonad were recorded.

**Lipid determination and estimation of lipo-somatic indices:** Percentage of lipid content in muscle, liver and gonad were analyzed as a measure of energy reserves. The whole liver and gonad were taken for lipid analysis, whilst for the muscle a portion or sub sample of 10 g was taken dorsally directly under the anterior dorsal fin and well above the lateral line. The collected tissues were cleaned by removing skin, scales and bones. They were rinsed with distilled water. Total lipid content of *S. lysan* in muscle, liver and gonad were extracted using chloroform, methanol and water (2: 2: 1.8) according to the method of Bligh and Dyer (1959).

Lipid content in liver and gonad expressed as a percentage of dry mass were given and lipid hepatosomatic index ( $LI_{HS}$ ) and lipid gonadosomatic index ( $LI_{GS}$ ) were computed with the following formula (Lloret *et al.*, 2008):

$$LI_{HS} = \frac{ABSLL}{W_E} \times 100$$

$$LI_{GS} = \frac{ABSLG}{W_E} \times 100$$

Where:

ABSLL = Absolute lipid content in the liver

ABSLG = Absolute lipid content in the gonad

Absolute lipid content was computed by multiplying the respective lipid contents by total tissue weights.

**Data analysis:** Data obtained for total lipid content in liver, muscle and gonad tissues for immature, maturing and pre-spawning male and female were first analyzed by two way Analysis of Variance (ANOVA). When the results of the two way ANOVA show the mean values of the samples are significantly different, ANOVA was followed by Post hoc comparison of means: Duncan's Multiple Range Test (DMRT) using STATISTICA 6.0 software. The level of statistical significance was set at  $p < 0.05$ .

Regression analysis was performed for the pooled data of *S. lysan*, irrespective of sex. Cumulative lipid content in liver, muscle and gonad tissues for different GMSs were computed and plotted as a graph to compare the lipid compartmentalization.

Data obtained for total lipid content in the muscle, liver and gonad against  $L_S$  for all individuals were tested for regression statistics. The relationship between lipid content and  $L_S$  was investigated by least square linear regression analysis using MINITAB 14 software. Similar regression analysis was also performed for the  $LI_{HS}$  and  $LI_{GS}$  data with  $L_S$ . The whole fish lipid levels were compared

in terms of  $LI_{HS}$  and  $LI_{GS}$  index of condition to access whether these indices are a diagnostic measure of nutritional status.

Regression analysis of percentage of water content and lipid content of muscle, liver and gonad of mature and pre-spawning individuals were also computed, irrespective of sexes.

## RESULTS

Three hundred and sixteen specimens were collected from January 2010 to December 2010 from the Point Pedro and Puttalam Sea, Sri Lanka. Among the sub sample collected 42 maturing male, 57 maturing female, 16 pre-spawning male and 7 pre-spawning female were distinguished and recorded whilst the immature remained unsexed since it was difficult to distinguish their sex.

Mean values (Mean±Standard deviation (SD)) of  $L_S$ , M,  $LI_{HS}$ ,  $LI_{GS}$  and percentage of lipid content of muscle, liver and gonad for different GMS of male and female *S. lysan* individuals are presented in Table 1. The total extractable lipid content was determined from the differences between the dry mass before extraction and the dry mass following extraction.

**Partitioning of lipid content in different tissues:** Two way ANOVA revealed significant interaction between lipid content in liver, muscle and gonad of male and female (ANOVA,  $n = 205$ ,  $p < 0.05$ ) and therefore, differences between liver, muscle and gonad of male and female were examined in detail.

No significant difference (DMRT,  $p > 0.05$ ) was observed for lipid content of the liver, muscle and gonad among the maturing male and maturing female. Pre-spawning and maturing individuals exhibited no significant differences (DMRT,  $p > 0.05$ ) for lipid content of liver in both sexes. Pre-spawning male showed no significant differences (DMRT,  $p > 0.05$ ) for lipid content of gonad with that of maturing male and maturing female. These results are given in Table 1.

Percentage of lipid content in the gonad tissues of immature fish was significantly (DMRT,  $p < 0.05$ ) lower than the lipid content in the other stages. It clearly express that low lipid level present in immature fish due to small sized gonads present. The percentage of lipid reserve in ovary

Table 1: Mean values of standard length ( $L_S$ ), body weight (M), Lipid hepato somatic index ( $LI_{HS}$ ), Lipid gonado somatic index ( $LI_{GS}$ ) and percentage of lipid content of muscle, liver and gonad for different maturity stages of *S. lysan* individuals

Sex	Description	LS (cm)	M (g)	Muscle (%)	Liver (%)	Gonad (%)	$LI_{HS}$	$LI_{GS}$
IM	Mean±SD	19.67±2.84	104.88±40.70	3.42±1.13a	19.27±8.16a	1.51±1.08a	0.63±0.02	$2 \times 10^{-4}$ - $3.0 \times 10^{-4}$
		n = 143	n = 143	n = 83	n = 70	n = 10	n = 70	n = 10
		(10.7-26.8)	(21.1-225.53)	(0.51-5.45)	(4.54-38.09)	(0.23-3.33)	(0.002-0.11)	( $5 \times 10^{-4}$ - $4.4 \times 10^{-5}$ )
MM	Mean±SD	30.11±4.10	343±142	4.95±1.02 <sup>bd</sup>	44.19±9.07 <sup>bd</sup>	19.36±8.64 <sup>bd</sup>	0.13±0.08	0.013±0.014
		n = 71	n = 71	n = 42	n = 33	n = 23	n = 33	n = 23
		(23.0-38.0)	(139.0-728.0)	(1.95-6.85)	(30.84-61.31)	(8.33-37.61)	(0.02-0.44)	(0.001-0.052)
PSM	Mean±SD	53.25±0.99	1409±126.8	9.81±1.77 <sup>c</sup>	25.96±4.44 <sup>ce</sup>	20.37±1.414 <sup>bd</sup>	0.035±0.005	0.008±0.002
		n = 26	n = 26	n = 16	n = 16	n = 16	n = 16	n = 16
		(51.5-55.0)	(1286.0-1531.0)	(6.84-13.0)	(18.81-34.88)	(17.76-23.16)	(0.026-0.047)	(0.005-0.011)
MF	Mean±SD	9.15±3.78	310.2±138.6	4.94±0.91 <sup>bd</sup>	41.35±11.30 <sup>bd</sup>	20.42±7.21 <sup>bd</sup>	0.12±0.09	0.017±0.019
		n = 65	n = 65	n = 57	n = 47	n = 36	n = 47	n = 36
		(23.0-38.5)	(136.2-900.0)	(1.95-6.85)	(11.62-66.96)	(9.09-33.33)	(0.01-0.51)	(0.0008-0.09)
PSF	Mean±SD	61.16±0.46	1641±54.76	6.68±0.08 <sup>c</sup>	30.79±0.62 <sup>ce</sup>	44.2±1.35 <sup>c</sup>	0.038±0.01	0.09±0.003
		n = 11	n = 11	n = 7	n = 7	n = 7	n = 7	n = 7
		(60.5-61.5)	(1548.0-1689.0)	(6.57-6.8)	(30.25-32.1)	(42.02-46.39)	(0.01-0.038)	(0.08-0.09)

IM: Immature, MM: Maturing male, MF: Maturing female, PSM: Pre-spawning male, PSF: Pre-spawning female, SD: Standard deviation, n: Sample size, range given in parenthesis

of pre-spawning female exhibited a significantly (DMRT,  $p < 0.05$ ) higher value (mean 44.2%,  $\pm 1.35$ ) than the pre-spawning male (mean 20.37%,  $\pm 1.41$ ). Similarly the percentage of lipid reserve in the liver of pre-spawning female (mean  $30.79 \pm 0.621$ ) was significantly (DMRT,  $p < 0.05$ ) higher than the pre-spawning male (mean  $25.96 \pm 4.44$ ). Respective results explained in this paragraph are tabulated in Table 1.

Lipid compartmentalization for the cumulative percentage of lipid content in liver, muscle and gonad tissues for all individuals revealed lipid content in the muscle (as percentage of dry weight) constituted between 0.51 and 13.00% (mean = 4.76%,  $\pm 2.02$ ) whereas lipid content in the liver between 4.54 and 66.96% (mean 31.11%,  $\pm 14.10$ ).  $LI_{HS}$  ranged between 0.002 and 0.512 (mean =  $0.07 \pm 0.08$ ), lipid content in gonads varied between 0.22 and 46.39% (mean = 20.03%,  $\pm 10.85$ ) and  $LI_{GS}$  ranged between  $5 \times 10^{-42}$  and 0.09 (mean =  $0.02 \pm 0.01$ ). Highest variation for lipid content in liver suggests that liver is the major energy storage site in *S. lysan*. The results explained in this paragraph are given in Table 1.

There is a progressive accumulation of liver lipid content of maturing fish ( $42.52\% \pm 10.47$ ) than the muscle (4.95%,  $\pm 0.95$ ) (Table 1) but the absolute amount of lipid stored in this organ was small (0.077 g,  $\pm 0.08$ ) compared to that stored in the muscle (1.056 g,  $\pm 0.573$ ).

Mean values for percentage of lipid reserve in ovary and liver for maturing female was 20.42 and 41.35%, respectively. For pre-spawning female lipid reserve in ovary and liver was 44.2 and 30.79%, respectively. Similar trend was also obtained with male gonads (Table 1). These results clearly explain that the lipid stored in the liver during the maturing stage is mobilized towards the gonad during pre-spawning for gonad development, gamete production and other reproductive purposes.

**Relationships between  $L_S$  and lipid content:** Relationships obtained by regression analysis for  $L_S$  against  $LI_{HS}$ ,  $LI_{GS}$ , total lipid content in the muscle, liver and gonad for all individuals are shown in Fig. 1. In all individuals, muscle lipid, liver lipid and gonad lipid and the value of  $LI_{HS}$  and  $LI_{GS}$  were size dependent with small individuals have less lipid in all tissues than the larger individuals (Fig. 1).

The percentage of lipid of all individuals (Dry Weight, DW) is positively related to the  $L_S$  showed a significant curvilinear relationship (Fig. 1) between them that is better in the muscle than liver and gonad. Regression analysis of standard length versus percentage of muscle lipid, liver lipid and gonad lipid gave significant ( $p < 0.01$ ) curvilinear equations of  $Y = -0.002x^2 + 0.292x - 1.708$ ,  $Y = -0.062x^2 + 4.958x - 51.04$  and  $Y = -0.011x^2 + 1.515x - 18.30$ , respectively. A significant ( $p < 0.01$ ) curvilinear relationship of  $Y = -0.847x^2 + 12.116x - 3.952$  was also obtained between percentage of muscle lipid content and percentage of liver lipid content of all individuals of *S. lysan*. Moreover, a significant ( $p < 0.01$ ) curvilinear relationship was obtained between  $L_S$  value of  $LI_{HS}$  with the equation of  $Y = -0.000x^2 + 0.024x - 0.319$  but a significant ( $p < 0.01$ ) linear relationship was obtained between  $L_S$  and value of  $LI_{GS}$  with the equation of  $Y = 0.001x - 0.023$ .

Relationships obtained by regression analysis between percentage of water content and lipid content of muscle, liver and gonad of mature and pre-spawning individuals irrespective of sexes are illustrated in Fig. 2. Regression analysis of water content versus percentage of muscle lipid, liver lipid and gonad lipid gave linear equations of  $Y = -0.105x + 12.62$ ,  $Y = -0.73x + 8.42$  and  $Y = -0.493x + 57.85$ , respectively.

Water is one of the most important components for the quality of food matrices including fish muscle. Water influences quality attributes such as appearance, texture and storage stability

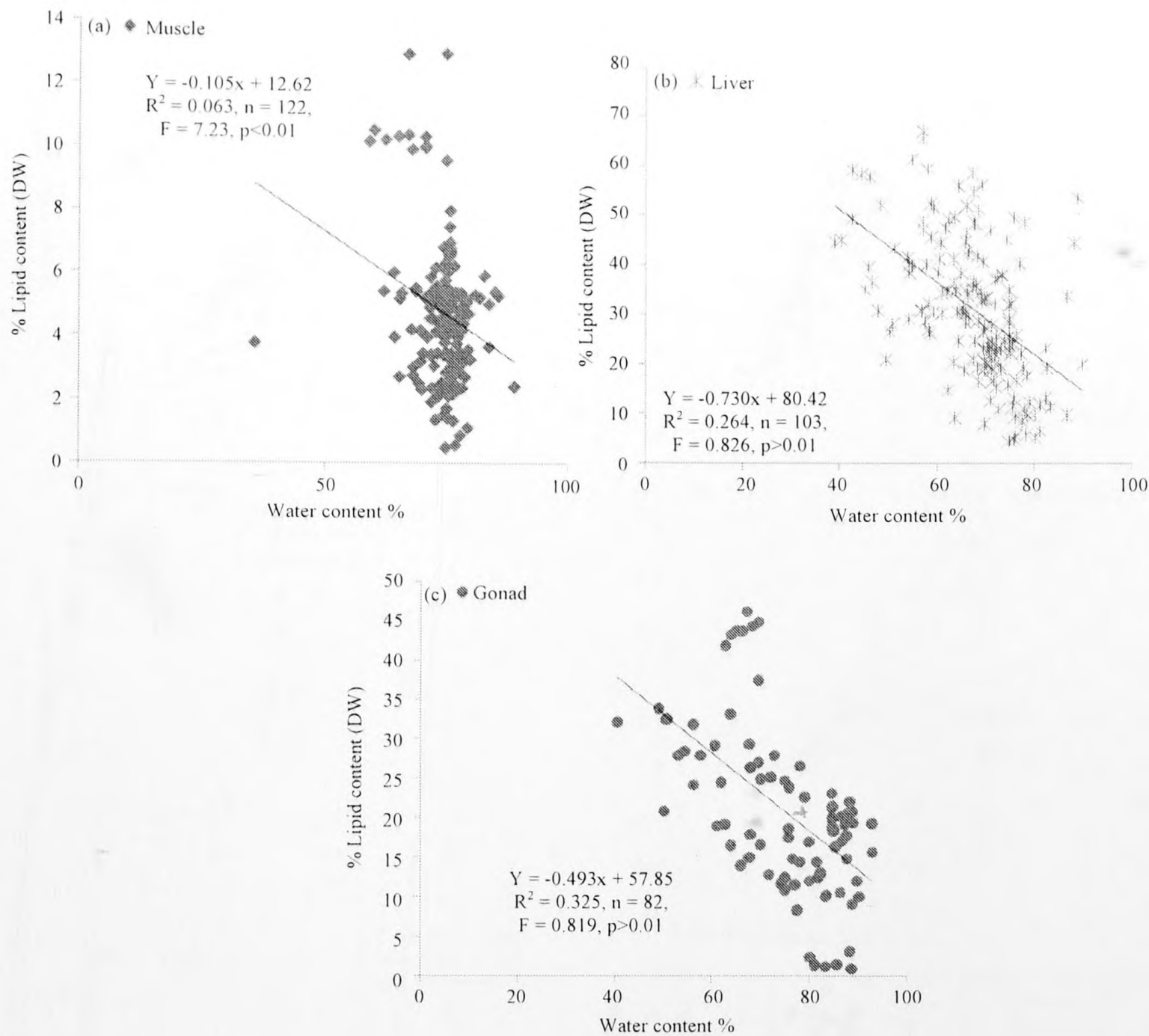


Fig. 2 (a-c): Relationships between % water content and lipid content (% dry weight, DW) of maturing and pre-spawning individuals in (a) muscle, (b) liver and (c) gonad

## DISCUSSION

Even though lipid concentrations varied greatly among individuals, results show that in the *S. lysan* lipids are stored mainly in the liver, varied between 18.81 and 61.31% dry weight in males and between 11.62 and 66.96% dry weight in females. Shao-Ning *et al.* (2010) identified from their research that the liver is an important energy storage organ in *Trachinotus ovatus* (Family: Carangidae). Seiichi and Yusuke (1993) reported that liver lipid and muscle lipid of matured striped jack (Family: Carangidae) was 18.78 and 4.86% and of wild puffer was 76.79 and 1.36%, respectively. These results are in consistent with the values obtained in the present study for *S. lysan*. These findings also confirming the important role of the liver for energy reserve in family Carangidae fishes.

Previous records of lipid content of some other species of family Carangidae are shown in Table 2. Present study revealed muscle lipid content of 0.51-13.00% (mean = 4.76%,  $\pm 2.02$ ) for *S. lysan*. Most of the earlier studies provide information only on muscle lipid of other carangid fishes. For *Parastromateus niger*, Chedoloh *et al.* (2011) reported muscle lipid of 2.58% whereas,

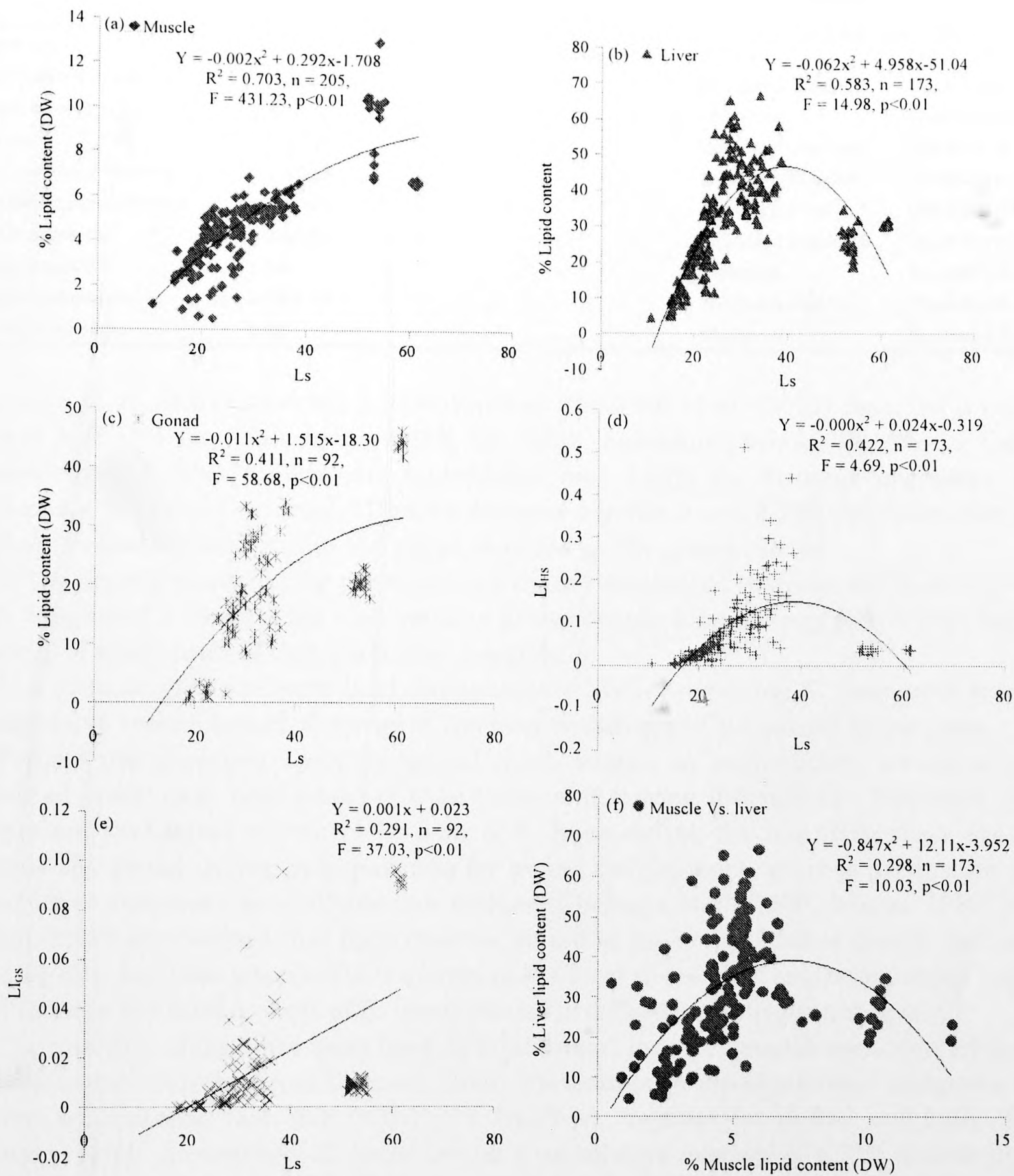


Fig. 1 (a-f): Relationships between standard length ( $L_s$ ) of all individuals and lipid content (% dry weight, DW) in (a) muscle (b) liver (c) gonad (d)  $L_{HS}$  + and (e)  $L_{GS}$  x and (f) relationship between the muscle lipid content (% dry weight, DW) and the liver lipid content (% dry weight, DW)

(Andersen and Rinnan, 2002). A correlation can be noted from the results where a decrease in percentage of water content in liver, muscle and gonad of maturing individuals is linked to an increase in fat content, even though the relationship is better in gonad than in the liver and muscle.

Table 2: Mean values obtained for percentage of lipid of fish species included under family Carangidae from the world

Fish	Muscle	Liver	Gonad	Region	Source
<i>Striped jack</i>	-----	18.78%	4.86%	Japan	Seiichi and Yusuke (1993)
<i>Parastromateus niger</i>	2.58±0.51	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Parastromateus niger</i>	2.33	--	--	Malaysia	Nurnadia <i>et al.</i> (2011)
<i>Atule mate</i>	2.13±0.27	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Selar crumenophthalmus</i>	3.31±0.25	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Carangoides gymnostethus</i>	2.12±0.35	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Pampus argenteus</i>	3.22±0.67	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Pampus argenteus</i>	2.09	--	--	Malaysia	Nurnadia <i>et al.</i> (2011)
<i>Elagatis bipinnulata</i>	2.74±0.74	--	--	Southern Thailand	Chedoloh <i>et al.</i> (2011)
<i>Selaroides leptolepis</i>	2.12	--	--	Malaysia	Nurnadia <i>et al.</i> (2011)

Nurnadia *et al.* (2011) reported 2.33%. Further Chedoloh *et al.* (2011) reported a muscle lipid content of 2.13% for *Atule mate*, 3.31% for *Selar crumenophthalmus*, 2.12% for *Carangoides gymnostethus*, 2.74% for *Elagatis bipinnulata* and 3.22% for *Pampus argenteus*. However, Nurnadia *et al.* (2011) reported 2.09% for *Pampus argenteus* and 2.12% for *Selaroides leptolepis*. All these values fall well within the range recorded in the present study.

In the present study during pre-spawning lipids represented between 42.02-46.39% of the dry ovary weights of *S. lysan*. This high value in pre-spawning females may reflect high reproductive potential of individuals of that particular population.

The increase of the relative lipid content in the liver of maturing *S. lysan* with size indicates a progressive accumulation of energy in the liver as fish grows till sexual maturation.

Female pre-spawners seem to expend much energy on reproductive activities since they presented lower liver lipid reserves than those of maturing individuals. Therefore, it could be emphasized that lipids stored in the liver of *S. lysan* during the maturing stage are mobilized towards the gonad during pre-spawning for gonad development, gamete production and other reproductive purposes. Generally various authors (Chelappa *et al.*, 1989; Adams, 1999; Morris and Culkin, 2000) emphasized that lipid reserves stored in the body of fishes greatly reduced during breeding as it had been supplied to the ovary in the form of energy. The present study has detected differences in the lipid content of *S. lysan* tissues in different maturation stages.

The quantity of lipid has been used as biochemical index of trophic condition for fresh water and marine fish (Novotony and Beeman, 1990). Fish can be grouped into four categories according to their fat contents: lean fish (<2%), low fat (2-4%), medium fat (4-8%) and high fat (>8%) (Ackman, 1989). Accordingly *S. lysan* having a cumulative average of 4.76% muscle lipid in DW (1.281% in wet weight) can be classified under the first category, lean fish. This finding is in consistent with the previous study for other carangids (Chedoloh *et al.*, 2011). From the inferences of the present study being a lean fish *S. lysan* can be recommended as a good nutritional source of food fish for the human health. In all maturation stages lipids are stored chiefly in the liver except pre-spawning female than that of muscle and gonad further confirms liver is the major energy depot in *S. lysan*. This finding could be supported by the earlier observations reported by Huss *et al.* (2003).

Ramadan (2003) reported that the fat content was affected by maturation and the depletion of fat reserve in muscle accompanied by a rise of fat content in gonad for gilthead seabream *Sparus aurata* along the Tunisian coast.

The change in percentage of gonad lipid from maturing to pre-spawning stage is higher in female than the male imply that the energy expended for gamete production in female was higher than male. The gender related differences in lipid depletion reveal the different energy expenditure for gamete production between the sexes.

In a previous study for *Trachinotus ovatus* (Assem *et al.*, 2005) minimal value of ovarian lipid (in dry weight) of  $7.868 \pm 1.081\%$  was obtained for immature stage and the lipid storage increased progressively and a maximum value of  $14.021 \pm 3.697\%$  was obtained for mature stage. Similar trend was also recorded in the present study with tropical *S. lysan*.

Ali *et al.* (2001) identified a linear relationship between log total length and log total body lipid of *Channa* fish. Dumas *et al.* (2007) showed that the body weight chemical composition of rainbow trout can be expressed mathematically and these equations show the linear link between fish size and lipid content of body. Peters (2003) observed a positive curvilinear relationship between percent lipid in the muscle and percent of lipid content in the whole fish. The present results are also in support with the earlier studies.

A negative correlation obtained between the concentration of water and lipid in the liver, muscle and gonad can be supported by the earlier studies by Shulman and Love (1999) and Shulman *et al.* (2005). The negative correlation could be a substitute to specify the condition of fish indirectly if the water content is known and it could be a rapid indicator of lipid reserve of this species. This phenomenon can be referred as the fat water line and is common in many fish species (Love, 1988).

One previous study proved that post larvae of *Macrobrachium rosenbergii* fed with cod liver oil enriched feed had the highest weight gain ( $225.72 \pm 9.05\%$ ), highest specific growth rate ( $2.95 \pm 0.07\%$ ) and highest survival ( $77.14 \pm 4.28\%$ ) as well as low food conversion ratio ( $0.87 \pm 0.03$ ) compared to the post larvae fed with the vitamin E, Vitamin D and astaxanthin (Parakarma *et al.*, 2009). The liver of *S. commersonianus* had high amount of omega-3-fatty acids followed by muscle and head, respectively (Zibae-Nezhad *et al.*, 2010). It would be useful to carry out further studies on the composition of fatty acids and other lipid profile in the liver of *S. lysan* as the results gained could be utilized in the preparation of formulated nutritional feeds for cultured species. Moreover, the knowledge gained on lipid dynamics in relation to gonad maturation stages for *S. lysan* provides information for diet formulation during culture trials as it endow with parallel evidence for broodstock nutrition.

## CONCLUSION

*S. lysan* having a cumulative average of 4.76% muscle lipid (in dry weight) can be classified as lean fish. Being a lean fish it can be recommended as a good nutritional source of food fish for the human health. From the present study it can be inferred that lipids are stored mainly in the liver of *S. lysan*. High value of lipids in ovary of pre-spawning females reflects high reproductive potential. Female pre-spawners possessed lower liver lipid reserves than maturing individuals confirm that lipids stored in the liver of *S. lysan* during the maturing stage are mobilized towards the gonad during pre-spawning for gonad development. The percentage of lipid of *S. lysan* individuals positively related to the size of the fish showed a significant curvilinear relationship. Negative correlation was observed between percentage of water content and lipid content. Findings obtained from the pilot study are good indication on energy allocation and broodstock nutrition.

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## Proximate Composition of Three Species of *Scomberoides* Fish from Sri Lankan Waters

<sup>1</sup>S. Sutharshiny and <sup>2</sup>K. Sivashanthini

<sup>1</sup>Department of Fisheries, <sup>2</sup>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 21 222 5925, 00 94 21 222 2685

### ABSTRACT

Evaluating the proximate composition of food fish is the most important aspect in fish nutrition. The present study was carried out to determine the flesh quality of *Scomberoides lysan*, *S. tol* and *S. commersonianus* fish species which correspond to different grade of inclination of the Sri Lankan consumers. Major nutrient compositions of raw muscle like protein, lipid, moisture, carbohydrate and ash were estimated. Proximate compositions were varied among the species. The highest moisture content was present in *S. lysan* (75.67%) and the lowest in *S. commersonianus* (72.57%). The ash content estimated in *S. lysan*, *S. tol* and *S. commersonianus* were 1.42, 1.49 and 1.6%, respectively. Carbohydrate was present in very low level (<0.3%) in all fish species. Protein content was estimated as 19.47±0.16%, 18.99±0.51% and 21.68±0.65% in *S. lysan*, *S. tol* and *S. commersonianus* respectively. Lipid content for *S. lysan*, *S. tol* and *S. commersonianus* was recorded as 0.89±0.005%, 0.594±0.113% and 1.00±0.12%, respectively. The results revealed that the highest protein content, lipid and ash content were recorded in *S. commersonianus* (21.68, 1.00 and 1.6%, respectively) whereas the lowest lipid content was reported in *S. tol* (0.59%). Marked significant differences (p<0.05) were observed among *Scomberoides* fish species for the mean moisture, protein, lipid, carbohydrate and ash contents. From the results *S. commersonianus* can be suggested as an ideal dietetic food among the three fish analyzed.

**Key words:** Lipid, moisture, protein, ash, carbohydrate, *Scomberoides lysan*, *S. tol*, *S. commersonianus*

### INTRODUCTION

Fish is a favorite foodstuff for the majority of societies. Fish meal contains most important nutritional components and serve as a source of energy for human beings (Ojewola and Annah, 2006; Sutharshiny and Sivashanthini, 2011). Fish is also a vitamin and mineral rich food for young as well as old age people (Edem, 2009; Moghaddam *et al.*, 2007).

Majority of the nutritionists recommend that human beings should eat fish every day (Blanchet *et al.*, 2000; Nestel, 2000; Balk *et al.*, 2004). An increasing amount of evidences suggest that, fish meat and oil contains high amount of polyunsaturated fatty acid that are valuable in decreasing the serum cholesterol to prevent a number of coronary heart diseases (Nordoy *et al.*, 2001; Turkmen *et al.*, 2005). Regular consumption of fish can promote the defense mechanism for protection against invasion of human pathogens because fish food has antimicrobial peptide

(Ravichandran *et al.*, 2010). Ingesting fish can reduce the risk of heart diseases and lower the risk of developing dementia, including Alzheimer's diseases (Grant, 1997).

Breastfed babies of mothers who eat fish have better eyesight perhaps due to the omega-3-fatty acid transmitted in breast milk. Fish oil may be useful in treating dys-lipidemia in diabetes (Friedberg *et al.*, 1998). Eating fish during pregnancy may help to reduce the risk of delivery of a premature baby (Olsen and Secher, 2002). Fishmeal is popular in a large segment of the Sri Lankan population who actively select foods for health maintenance and disease prevention since Sri Lanka is an island and surrounded by Indian Ocean (Anonymous, 2008).

Biochemical composition of flesh is a good indicator for the fish quality (Hernandez *et al.*, 2001), physiological condition of fish and habitat of fish (Aberoumad and Pourshafi, 2010; Shamsan and Ansari, 2010; Ravichandran *et al.*, 2011). Fish of various species don't provide the same nutrient profile to their consumer (Takama *et al.*, 1999) and the nutritive value of a fish varies with season (Varljen *et al.*, 2003).

Moisture, dry matter, protein, lipids, vitamins and minerals are the most important components that act as sources of nutritive value of fish meat (Steffens, 2006).

Quantifying proximate composition is important in ensuring the requirements of food regulations and commercial specifications (Waterman, 2000). Moisture content of flesh is a good indicator of its relative content of energy, protein and lipid (Aberoumad and Pourshafi, 2010). Fish meat contains significantly low lipids and higher water than beef or chicken and is favoured over other white or red meats (Nestel, 2000). The total lipid and ash content of fish vary with the increasing weight or length of the fish; it may also vary with the season and varied habitats (Hassan, 1996). Among the proximate composition, protein in fish is the excellent source, because of the amino acid composition and degree of digestibility (Louka *et al.*, 2004).

Several studies on proximate composition of fish have been made from different parts of the world, so far. De silva and Rangoda (1979) mentioned the information on some chemical characteristics of fresh and salt dried product of *Tilapia mossambica* from Colombo Lake, Sri Lanka. Nutritional analysis of some fresh water fish were determined by Wimalasena and Jayasuriya (1996) from Narammala, Ibbagamuva and Nikaveratiya in Sri Lanka. Proximate composition of different fresh water fishes specifically Magur (*Clarias batrachus*), Shingi (*Heteropneustes fossilis*), Koi (*Anabas testudineus*), Foli (*Notopterus notopterus*), Royna (*Nandas nandas*), Taki (*Channa punctatus*) and Tangra (*Mystus vittatus*) in Bangladesh was estimated by Kamal *et al.* (2007). For a non piscine organism, bivalve *Saccostrea cucullata* proximate composition was estimated by Sajjadi *et al.* (2009). Farhoudi *et al.* (2011) studied the proximate composition of *Cyprinus carpio* in Caspian Sea during larval development. Sounndarapandian and Dey (2008) estimated the proximate composition for matured eggs of crab *Portunus sanguinolentus* (Herbst) in Indian coastal waters. They reported that the protein, lipid and carbohydrate content in matured eggs of *Portunus sanguinolentus* were found to be 59.70, 21 and 7.58%, respectively. Tawfik (2009) studied the proximate composition and fatty acid profile in most commonly available fish species in Saudi market.

In Sri Lanka, *Scomberoides lysan*, *S. tol* and *S. commersonianus* are the importantly marketed food source among the *Scomberoides* fish. These are frequently caught by fishermen and often bought by people because of the good quality and unique meat texture. These are normally utilized fresh, preserved, dried or salted. Pregnant mothers and lactating mothers often intake these fishes in Sri Lanka. These are marketed all over the country and transported from one landing centre to another with the assistance of cooler. Knowledge of nutritional value of these fishes are little

known. Hence, it is essential to know the proximate composition of the fish to report their nutrient composition from the public health point of view.

The exact proximate composition of the *Scomberoides* fishes are not yet determined in Sri Lanka. There is only one study that expresses only the total lipid and cholesterol content of *Scomberoides lysan* of adult fish from Sri Lanka (Sutharshiny and Sivashanthini, 2011). In the present study an attempt has been made to determine the proximate composition of three *Scomberoides* fish species in Sri Lanka.

## **MATERIALS AND METHODS**

**Sample collection:** Fresh fish were collected from the landing sites in Jaffna, Mannar, Trincomale and Puttalam (Fig. 1) with the assistance of the Fishermen Cooperative Society Union of the respective districts. They were transported to the laboratory of Department of Fisheries, University of Jaffna in ice box. Samples were collected monthly for a period of 10 months (May 2010 to February 2011). Their length was measured to the nearest centimeter and body weight was weighed to the nearest gram. The Samples were packed in separate polyethylene bags, labeled and stored in the freezer at -20°C for further laboratory analyses. Fishes were thawed and the bone and skin were separated from the flesh to achieve proximate analysis. Moisture, ash and fat content were analysed at the Department of Fisheries laboratory of University of Jaffna whereas, protein and carbohydrate content were analyzed at the Department of Biochemistry laboratory, Faculty of Medicine, University of Jaffna, Sri Lanka.

**Chemical analysis:** The percentage of proximate composition of fish was determined by conventional method of AOAC (2000). Triplicate determinations were carried out on each chemical analysis.

**Estimation of moisture:** The initial weight of the sample was taken then samples were dried in an oven at about 105°C for about 8 to 10 h until constant weight was reached and the samples were minced in an electric grinder. The percentage of moisture content was determined.

**Protein determination:** The protein content of the fish was determined by micro Kjeldahl method (AOAC, 2000). It involves the conversion of organic nitrogen to ammonium sulphate by digestion of flesh with concentrated sulphuric acid in a micro kjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and total nitrogen was determined titrimetrically. The percentage of protein in the sample was calculated.

**Estimation of fat:** For the estimation of fat content, the dried samples left after moisture determinations were finely grinded and the fat was extracted with chloroform and methanol mixture (AOAC, 2000). After extraction, the solvent was evaporated and the extracted materials were weighed. The percentage of the fat content was calculated.

**Estimation of ash:** The ash content of a sample is residue left after ashing in a muffle furnace at about 550-600°C till the residue become white. The percentage of ash was calculated by subtracting the ash weight from initial weight.

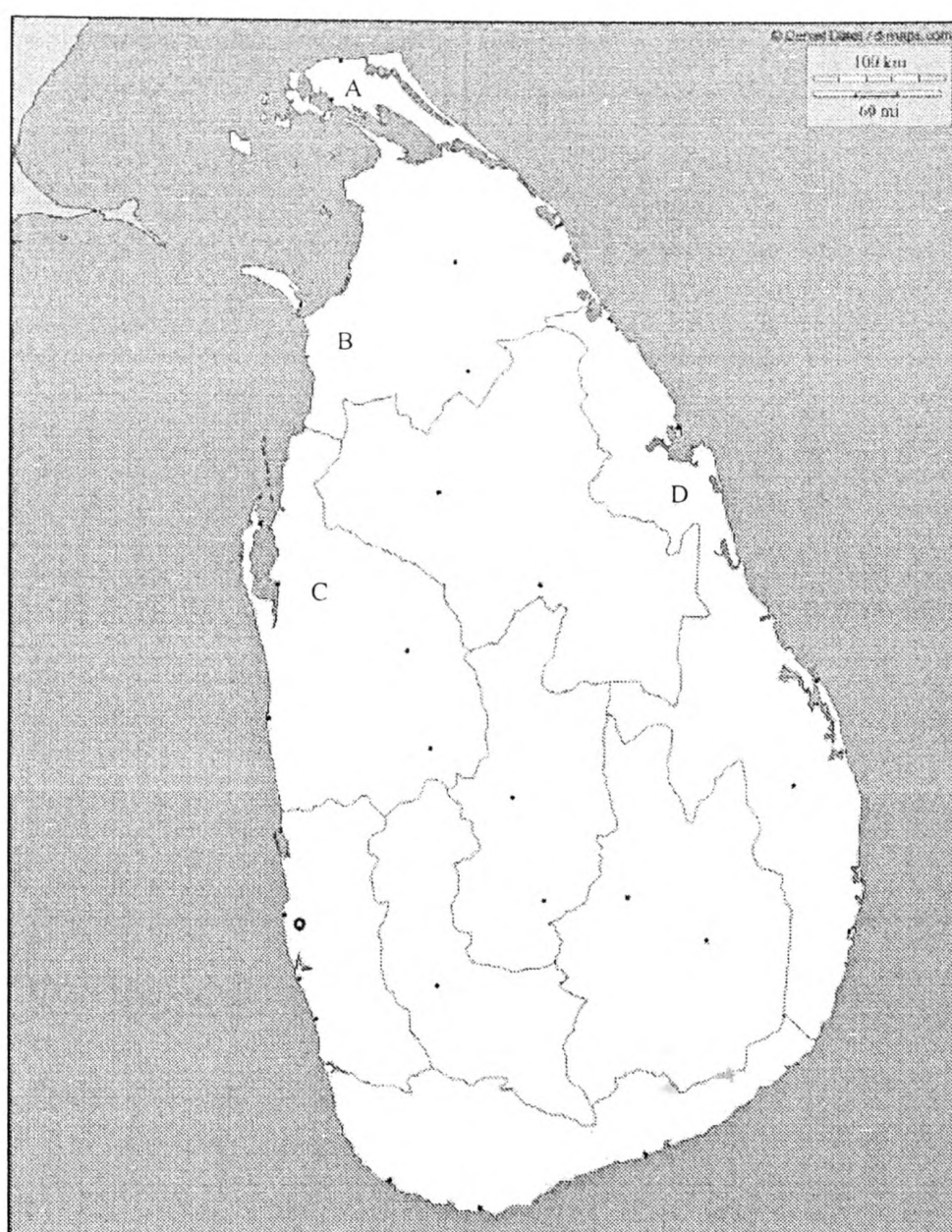


Fig. 1: Sampling sites of *Scomberoides* fishes from Sri Lankan waters. A: Jaffna B: Mannar C: Puttalam D: Trincomalee

**Estimation of carbohydrate:** The carbohydrate was hydrolyzed with acid and the absorbance was read in spectrophotometer (LABOMED, UVD-3000) at the specific wavelength of 550 nm (AOAC, 2000).

**Statistical analysis:** Moisture, ash, fat, protein and carbohydrate content of three species of fish were first analyzed by one way analysis of variance (ANOVA). When a single factor ANOVA rejects the null hypothesis i.e., when the mean of the samples was significantly different, ANOVA was followed by Post hoc comparison of means: Duncan's Multiple Range Test (DMRT) using STATISTICAL software in the computer. The level of statistical significance was set at  $p < 0.05$ .

## RESULTS

The collected samples ranged from 38.5-45.0 cm standard length and body weight ranged from 728.27-1100 g. The moisture, protein, lipid, ash and carbohydrate contents in the muscle of *S. lysan*, *S. tol* and *S. commersonianus* fish species examined are presented in Table 1. Each value indicates the Mean $\pm$ SD of twenty four replicate determinations.

Table 1: Mean values obtained for proximate compositions of three different *Scomberoides* fish species in Sri Lanka

Proximate compositions (%)	<i>S. lysan</i>	<i>S. tol</i>	<i>S. commersonianus</i>
Moisture	75.67±0.12 <sup>a</sup>	74.42±0.75 <sup>b</sup>	72.57±1.03 <sup>c</sup>
Protein	19.47±0.16 <sup>a</sup>	18.99±0.51 <sup>b</sup>	21.68±0.65 <sup>c</sup>
Lipid	0.89±0.005 <sup>a</sup>	0.594±0.113 <sup>b</sup>	1.003±0.12 <sup>c</sup>
Carbohydrate	0.07±0.003 <sup>a</sup>	0.300±0.05 <sup>b</sup>	0.220±0.06 <sup>c</sup>
Ash	1.42±0.006 <sup>a</sup>	1.490±0.044 <sup>b</sup>	1.600±0.006 <sup>c</sup>

Values are Mean±SD from six replicates. Values within the same row, not sharing a common superscript differ significantly at p<0.05

The results (Table 1) showed that *S. commersonianus* consists high protein 21.68±0.65% than the others *S. lysan* (19.47±0.16%) and *S. tol* (18.99±0.51%). Among the three fish species lipid content is also high in *S. commersonianus* (1.00±0.12%) than the others which is 0.89±0.005% and 0.594±0.113% in *S. lysan* and *S. tol*, respectively. But the moisture content is high in *S. lysan* (75.67±0.12%) when compared to the other two. In *S. tol* and *S. commersonianus* water content was 74.42±0.75% and 72.57±1.03%, respectively. As a whole the results showed that *S. commersonianus* is a high protein and lipid content fish with the lowest moisture content. The ash content was high in *S. commersonianus* which is 1.6±0.006%. Carbohydrate compounds are present in negligible amount.

One way analysis of variance (ANOVA) showed that there is marked significant differences (p<0.05) among *Scomberoides* fish species in mean moisture, protein, lipid content, carbohydrate and ash content. Comparison of mean (Mean±SD) values of moisture, protein, lipid, carbohydrate and ash content by Duncan's multiple range tests for three different *Scomberoides* species showed that there is significant difference between each component and it is expressed as superscripts in Table 1.

## DISCUSSION

According to Love (1970), principal composition of fish is 16-21% protein, 0.2-5% fat, 1.2-1.5% mineral, 0-0.5% carbohydrate and 66-81% water. The results obtained in the present study fall well within the earlier reported values. Fish can be grouped into four categories according to their fat content lean fish (<2%), low (2-4%), medium (4-8%) and high fat (>8%) (Ackman, 1989). In terms of the lipid content, fish species examined can be considered to be in the lean fat fish category. The result obtained from the present study is in consistent with the proximate test results for fish species included under family carangidae (Table 2) and other fishes.

Fish received increased attention as a potential source of animal protein and essential nutrients for human diets (Fawole *et al.*, 2007). Protein forms the largest quantity of dry matter in fish (Steffens, 2006). The high protein supply aids in the regulation of blood sugar. Fish is a good low calorie, high protein choice to assist in weight loss for human beings. The fish of *Scomberoides* species examined belongs to high protein (21.68±0.65%), low lipid (0.89±0.005%, in wet weight) category. They contain lower caloric content per unit of protein than do lipid and they were an ideal source of animal protein for use in controlling diets. The concentration of the protein content and lipid were within the range previously reported in *S. tol* (Patterson and Ranjitha, 2009). Fishes with lipid content 0.89% are considered as lean fish (Stansby, 1982; Ackman, 1989). The total lipid and ash contents of fish are reported to vary significantly with gradual increase in the weight and length of the fish and also due to seasonal changes aside from the available nutrients in varied habitats (Hassan, 1996). Whenever, there is a low percentage of water the lipid and protein content

Table 2: Mean values (mean±SD) obtained for proximate compositions of fish species included under family Carangidae from the world

Fish species	Moisture content (%)	Protein content (%)	Lipid content (%)	Carbohydrate content (%)	Ash content (%)	Region	Source
<i>Carangoides fulvoguttatus</i>	77.82	19.97	0.24	--	1.50	Saudi	Tawfik (2009)
<i>Scomberoides tol</i>	--	17.00	1.20	15.98	--	Thanjavu or, India	Patterson and Ranjitha (2009)
<i>Trachinotus carojinus</i>	74.76	20.31	5.17	--	1.16	Florida, Atlantic	Gall <i>et al.</i> (1983)
<i>Scomberoides sp.</i>	--	--	2.00	--	--	NW Australia	Sinclair <i>et al.</i> (1983)
<i>Elagatis bipinnulatus</i>	--	--	1.00	--	--	Malaysia,	Gibson <i>et al.</i> (1984)
<i>Decapterus punctatus</i>	74.06	21.64	2.52	--	3.12	Florida, Atlantic	Hale (1984)
<i>Caranx georgianus</i>	75.00	21.50	2.64	--	1.34	New Zealand	Hughes <i>et al.</i> (1980)
<i>Selaroides leptolepis</i>	--	--	2.90	--	--	Malaysia	Gibson <i>et al.</i> (1984)
<i>Seriola lalandi</i>	71.5±0.4%	22.2±0.44	4.3±0.23%	--	1.3±0.02	Gansbaai (South Africa)	Andrew (2011)

would be high and the energy density also would be high in fishes (Gopakumar, 1998). The low values of carbohydrates recorded in the present study also suggest that glycogen in many marine animals does not contribute significantly to the total reserves in the body (Jayasree *et al.*, 1994). The moisture, protein, fat and ash content were 77.82, 19.97, 0.24 and 1.50%, respectively in the tissue of Yellow-spotted Trevally, *Carangoides fulvoguttatus* (Tawfik, 2009). The nutritive value of *Trachinotus carojinus* fish was found to be 74.76 moisture, 20.31 protein, 1.16 ash content and 5.17% lipid from Florida (Gall *et al.*, 1983). Hale (1984) observed 74.06 moisture, 21.64 protein, 2.52 lipid and 3.12% ash content for round scad from Florida. The proximate composition of fish species greatly varies during the catching season due to physiological reasons and changes in environmental conditions (Boran and Karacam, 2011). Earlier studies for other carangoides are obviously in consistent with the present results obtained for *Scomberoides sp.*

## CONCLUSION

From the present study it can be concluded that the three species of fish studied *S. lysan*, *S. tol* and *S. commersonianus* are the lean fat fish. Among the three fish analyzed *S. commersonianus* is the most preferable food for human consumption because of its relatively high value of lipid and protein content in the flesh. Considering the fact of comparatively low fat content than other fin fishes *S. commersonianus* is an ideal dietetic food for human beings.

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

<sup>1</sup>S. Sutharshiny, <sup>2</sup>K. Sivashanthini and <sup>2</sup>W.S. Thulasitha

<sup>1</sup>Department of Fisheries,

<sup>2</sup>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 triacylglycerol (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, *Scomberoides 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 (*Anguilla anguilla*) (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 (Jeziarska *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 (*Scombroides 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.

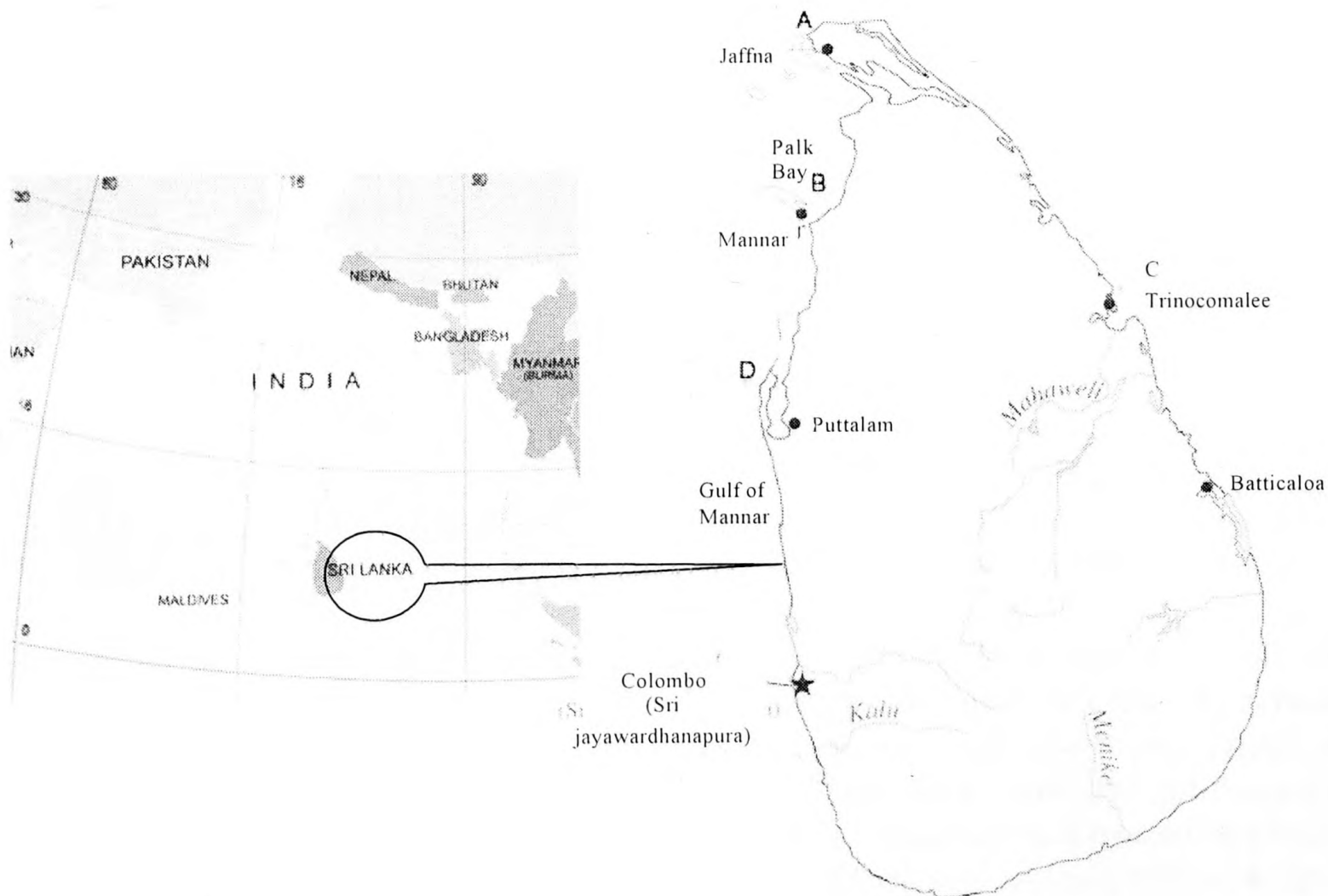


Fig. 1: Sampling stations of *S. lysan*. A: Jaffna, B: Mannar, C: Trincomalee and D: Puttalam

**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 Kjeldhal 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 pipetted 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

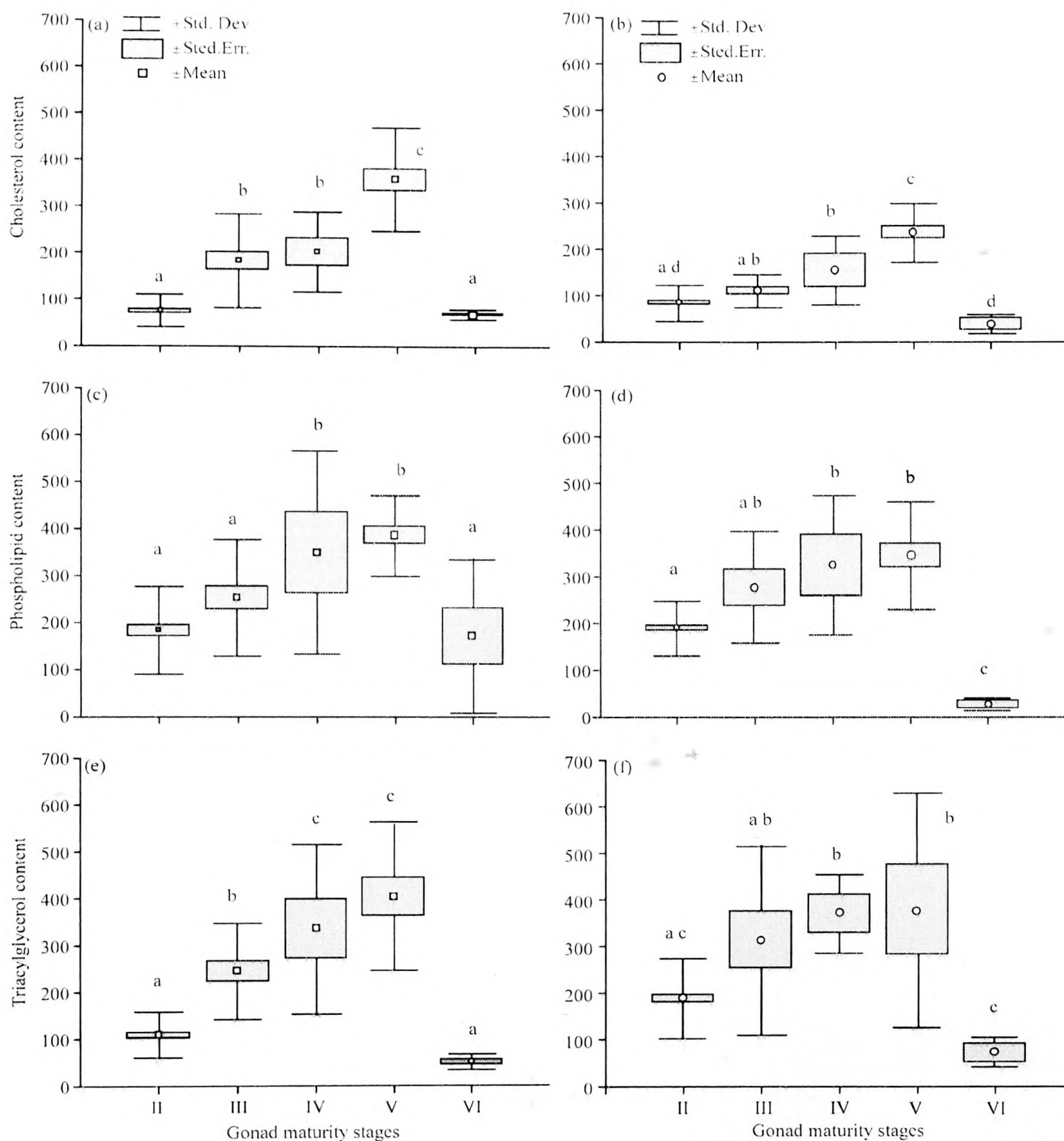


Fig. 2(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g<sup>-1</sup>) in gonad tissue of *S. lysan* in different gonad maturity stages, II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent. a, c and e-Gonad in female; b, d and f-Gonad in Male. Mean values for each 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 thereafter (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  $226.4 \text{ mg} \cdot 100 \text{ g}^{-1} \pm 78.21$  and  $292.00 \text{ mg} \cdot 100 \text{ g}^{-1} \pm 156.08$ , 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 ( $226.3 \text{ mg} \cdot 100 \text{ g}^{-1} \pm 69.92$ ). The mean PL content in ovary of female reached the highest amount in June as  $393.54 \text{ mg} \cdot 100 \text{ g}^{-1} \pm 73.00$

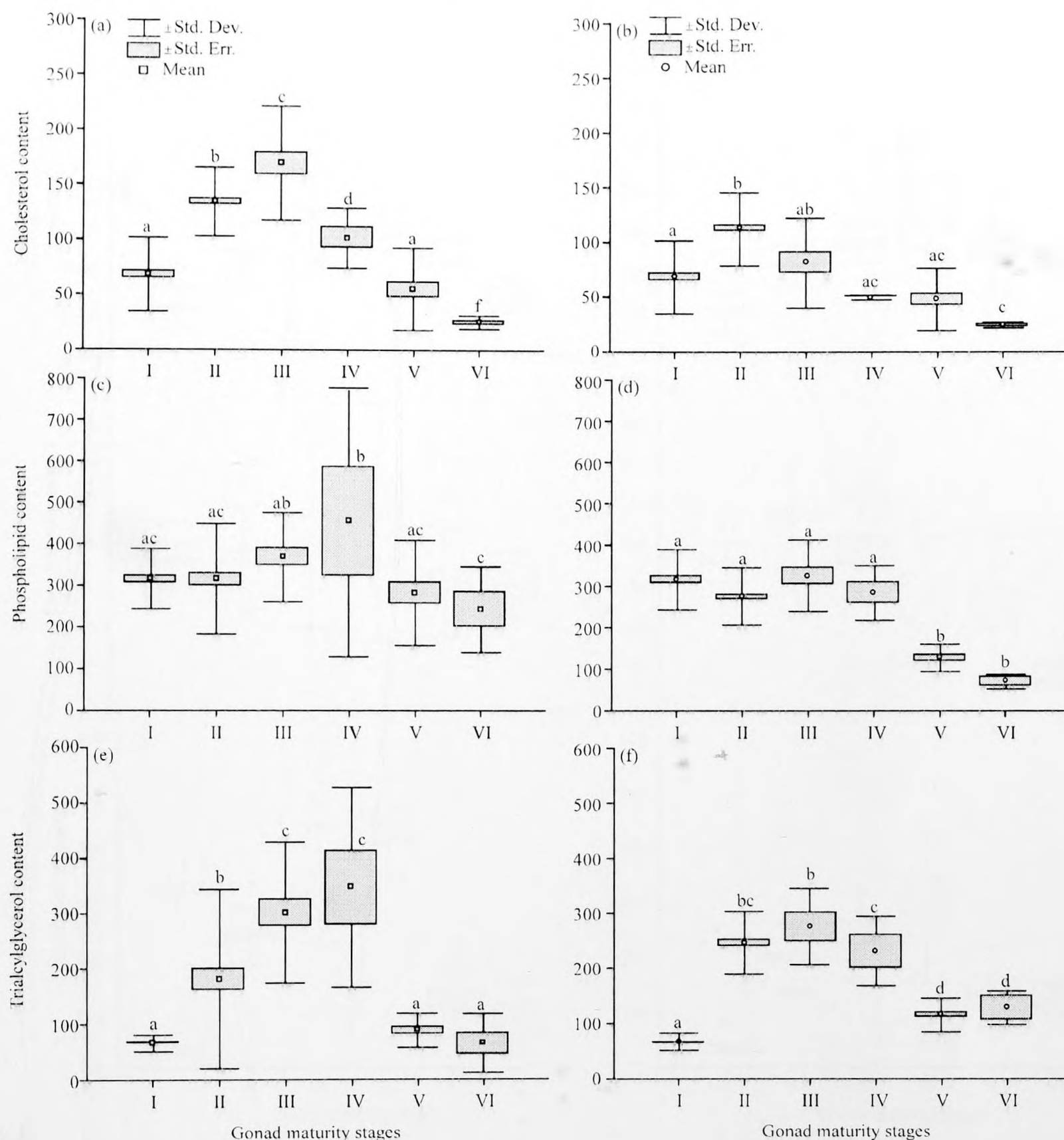


Fig. 3(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g<sup>-1</sup>) in muscle tissue of *S. lysan* in different gonad maturity stages, I: Immature unsex; II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent. a, c and e-in female; b, d and f-in Male. Mean values 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<sup>-1</sup>±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<sup>-1</sup>±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

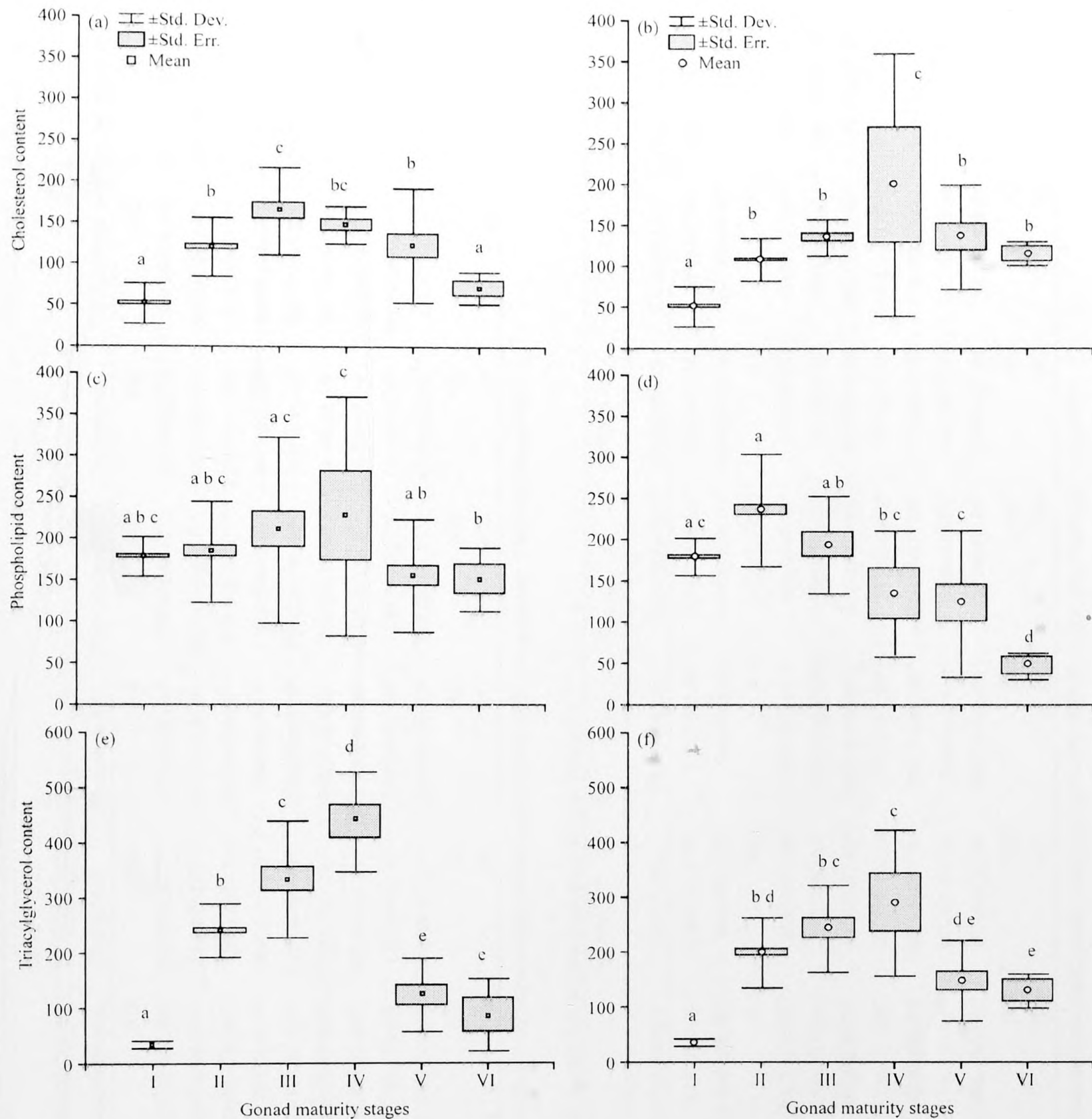


Fig. 4(a-f): Box and Whisker plot showing changes of lipid class content (mg.100g<sup>-1</sup>) in liver tissue of *S. lysan* in different gonad maturity stages, I: Immature unsex; II: Immature; III: Maturing; IV: Mature; V: Spawning; VI: Spent, a, c and e -in female; b, d and f -in Male. Mean values 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

Month	Gonad					Muscle				
	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	
January	ND	ND	ND	ND	ND	ND	ND	ND	ND	
February	100.89±58.56 <sup>a</sup>	336.94±25.47 <sup>ac</sup>	152.01±53.87 <sup>a</sup>	133.32±15.91 <sup>ab</sup>	179.26±38.54 <sup>a</sup>	297.81±33.43 <sup>ad</sup>	171.66±8.79 <sup>a</sup>	368.16±96.75 <sup>a</sup>	267.93±153.75 <sup>abc</sup>	
March	141.19±43.82 <sup>a</sup>	355.48±90.03 <sup>ac</sup>	225.15±90.07 <sup>ab</sup>	154.17±2.02 <sup>ab</sup>	201.31±126.50 <sup>a</sup>	353.13±209.11 <sup>bd</sup>	175.01±8.37 <sup>a</sup>	467.98±94.51 <sup>ab</sup>	273.70±58.27 <sup>abc</sup>	
April	200.96±34.59 <sup>a</sup>	230.72±7.75 <sup>cb</sup>	205.27±72.18 <sup>ab</sup>	275.17±42.34 <sup>c</sup>	302.14±70.71 <sup>ab</sup>	307.02±7.77 <sup>abd</sup>	273.74±11.99 <sup>b</sup>	616.20±35.35 <sup>b</sup>	234.37±30.90 <sup>abc</sup>	
May	220.30±7.20 <sup>a</sup>	383.23±82.00 <sup>a</sup>	292.23±73.56 <sup>ab</sup>	205.61±7.78 <sup>ac</sup>	350.68±0.79 <sup>b</sup>	456.50±62.93 <sup>b</sup>	283.50±1.95 <sup>b</sup>	464.71±28.97 <sup>ab</sup>	331.73±2.14 <sup>ab</sup>	
June	226.38±78.21 <sup>a</sup>	393.54±72.98 <sup>a</sup>	327.19±81.41 <sup>ab</sup>	147.56±85.40 <sup>ab</sup>	154.70±52.76 <sup>a</sup>	190.02±116.69 <sup>ac</sup>	145.31±46.43 <sup>a</sup>	286.55±56.86 <sup>ac</sup>	153.84±130.90 <sup>ac</sup>	
July	153.06±47.41 <sup>a</sup>	185.28±4.13 <sup>b</sup>	246.37±3.03 <sup>ab</sup>	140.74±7.75 <sup>ab</sup>	199.67±64.42 <sup>a</sup>	265.31±12.43 <sup>cd</sup>	134.44±11.18 <sup>a</sup>	345.35±56.56 <sup>ac</sup>	331.59±28.29 <sup>ab</sup>	
August	267.78±117.76 <sup>a</sup>	349.34±131.54 <sup>ac</sup>	367.48±95.44 <sup>ab</sup>	167.42±24.38 <sup>ab</sup>	242.73±159.72 <sup>ab</sup>	451.86±100.23 <sup>bc</sup>	136.82±41.36 <sup>a</sup>	399.29±232.77 <sup>abc</sup>	400.59±161.18 <sup>b</sup>	
September	292.03±156.08 <sup>a</sup>	402.51±75.59 <sup>a</sup>	387.76±175.53 <sup>b</sup>	112.52±62.65 <sup>b</sup>	150.04±61.52 <sup>a</sup>	129.37±72.00 <sup>c</sup>	42.92±22.58 <sup>c</sup>	281.02±120.19 <sup>ac</sup>	97.78±61.03 <sup>c</sup>	
October	135.18±53.28 <sup>a</sup>	218.40±100.53 <sup>cb</sup>	321.30±129.39 <sup>ab</sup>	134.89±15.55 <sup>ab</sup>	188.03±46.55 <sup>a</sup>	348.18±51.45 <sup>bd</sup>	87.77±25.15 <sup>d</sup>	185.67±79.80 <sup>c</sup>	263.04±64.86 <sup>abc</sup>	
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

Month	Gonad					Muscle				
	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	Cholesterol	Phospholipid	Triacylglycerol	
January	100.16±0.07 <sup>a</sup>	285.67±34.72 <sup>ab</sup>	147.16±6.99 <sup>a</sup>	110.12±14.14 <sup>a</sup>	133.36±1.49 <sup>a</sup>	169.71±26.16 <sup>a</sup>	163.66±11.95 <sup>a</sup>	305.10±7.06 <sup>b</sup>	175.10±49.49 <sup>a</sup>	
February	98.88±19.30 <sup>a</sup>	345.71±115.23 <sup>ab</sup>	190.94±108.48 <sup>ab</sup>	102.45±0.06 <sup>a</sup>	128.40±3.96 <sup>a</sup>	176.32±28.10 <sup>a</sup>	164.85±19.22 <sup>a</sup>	315.74±20.52 <sup>ab</sup>	182.26±58.35 <sup>a</sup>	
March	ND	ND	ND	ND	ND	ND	ND	ND	ND	
April	100.78±0.82 <sup>a</sup>	213.70±4.94 <sup>a</sup>	366.93±1.02 <sup>ab</sup>	151.26±1.49 <sup>a</sup>	310.35±14.34 <sup>b</sup>	354.90±6.63 <sup>a</sup>	41.37±1.63 <sup>b</sup>	342.65±3.59 <sup>ab</sup>	143.72±0.69 <sup>a</sup>	
May	128.03±36.10 <sup>ab</sup>	384.55±148.42 <sup>ab</sup>	426.83±137.60 <sup>ab</sup>	99.36±66.69 <sup>a</sup>	214.21±66.77 <sup>ab</sup>	260.48±8.30 <sup>a</sup>	41.22±7.91 <sup>b</sup>	199.32±0.1 <sup>ab</sup>	163.43±30.82 <sup>a</sup>	
June	133.42±28.84 <sup>ab</sup>	475.75±153.62 <sup>b</sup>	453.10±77.64 <sup>b</sup>	91.62±57.63 <sup>a</sup>	206.04±136.86 <sup>ab</sup>	218.31±39.48 <sup>a</sup>	38.96±10.62 <sup>b</sup>	202.09±34.90 <sup>ab</sup>	170.62±27.51 <sup>a</sup>	
July	113.65±14.52 <sup>a</sup>	319.08±161.01 <sup>ab</sup>	367.73±105.00 <sup>ab</sup>	137.98±26.90 <sup>a</sup>	168.10±18.92 <sup>ab</sup>	259.60±0.2 <sup>a</sup>	57.01±7.68 <sup>b</sup>	304.87±117.29 <sup>ab</sup>	305.23±78.30 <sup>a</sup>	
August	195.37±98.64 <sup>ab</sup>	229.00±96.50 <sup>ab</sup>	446.59±47.84 <sup>b</sup>	256.94±160.78 <sup>a</sup>	203.24±90.92 <sup>ab</sup>	339.20±178.65 <sup>a</sup>	50.62±0.70 <sup>b</sup>	275.16±38.65 <sup>ab</sup>	261.36±0.001 <sup>a</sup>	
September	226.27±69.91 <sup>b</sup>	332.15±120.84 <sup>ab</sup>	424.13±225.51 <sup>ab</sup>	125.27±99.24 <sup>a</sup>	124.51±80.11 <sup>a</sup>	175.16±125.11 <sup>a</sup>	49.15±26.05 <sup>b</sup>	192.08±101.51 <sup>a</sup>	140.63±83.87 <sup>a</sup>	
October	102.06±51.63 <sup>a</sup>	259.91±5.07 <sup>ab</sup>	412.96±115.53 <sup>ab</sup>	131.87±32.95 <sup>a</sup>	225.23±62.44 <sup>ab</sup>	220.88±65.37 <sup>a</sup>	97.60±29.24 <sup>c</sup>	376.71±63.83 <sup>b</sup>	222.04±76.17 <sup>a</sup>	
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 poses 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 *Onchorhynchus 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 (*Oncorhynchus 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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**A10**

**Changes in total lipid content in the flesh of different size *Scomeberoides lysan* from waters around Jaffna Peninsula**

Sutharshiny.S and Sivashanthini. K  
*Fisheries Unit, Faculty of Science, University of Jaffna, Sri Lanka*

The present investigation was carried out to estimate the total lipid content of flesh in different size *Scomeberoides lysan* and to understand the lipid changes with size variation. Regular field visits were made to the Point Pedro, Pasaioor and Delft landing centers from January 2010 to June 2010;

The standard length of fishes was categorized in to 10 cm class intervals and twenty six fish samples from each length class were collected and brought to the laboratory. The standard length and weight measurements were taken for each sample and 100 g muscle tissue was dissected from the lateral region of the body, the portion behind the gill operculum and under the dorsal fin. It was subjected to total lipid analysis. Total lipid was extracted by the standard method using chloroform:methanol mixture (2:1 V/V). Percentage of total lipid was computed for each sample. Among the one hundred and fifty eight *S. lysan* analyzed mean total lipid content of flesh varied from 1.15 to 5.10 % for *S. lysan* representing 0 - 10 and 30.1 – 40 cm standard length class interval, respectively.

Statistical analysis for correlation between mean total lipid content of muscle and different size class interval exhibited a positive curvilinear correlation ( $r^2 = 0.936$ ). It explains that the lipid deposition in the muscle increased with the increasing standard length, reached its maximum at 30.1 – 40.0 cm standard length *S. lysan*

**Key words:** Total lipid, *Scomeberoides lysan*, checklist, Jaffna peninsula

## Study on metal concentration of some sea cucumber species in Sri Lanka

Jinadasa B.K.K.K.<sup>1</sup>, Samanthi R.I.<sup>2</sup> and Wicramsinghe I.<sup>2</sup>

<sup>1</sup> Institute of Post Harvest Technology, National Aquatic Resources Research and Development Agency.

<sup>2</sup> Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka.

Sea cucumbers (Holothurians) are consumed in some Southeast Asian countries in processed and many other forms. In Sri Lanka only the body wall of sea cucumber is processed which is called Beche-de-mer and mainly export without consuming locally. The metal concentration of sea cucumbers in Sri Lanka has not been studied sufficiently. In this study metal concentration of some selected sea cucumber species were analyzed. Fresh samples were collected from North-western sea of Kalpitiya and Dutch Bay area during October- November 2008 and 2009 and they were eviscerated immediately after harvesting. Metals like Copper (Cu), Iron (Fe), Zinc (Zn), Lead (Pb), Cadmium (Cd), Cobalt (Co), Chromium (Cr) and Mercury (Hg) in species *Holothuria edulis* (Keels attaya), *H. atra* (Nari attaya), *Thelenota anax* (Poona attaya), *H. scabra* (Jafna attaya), *H. spinifera* (Disco attaya), *Bohadschia* sp. (Sudu nool attaya), *Bohadschia similis* (Line nool attaya), *B. marmorata* (Dumburu nool attaya) and *Stichopus chloronotus* (Dambalaya) were analyzed. Results were statistical analyzed by MINITAB 14, a one - way Analysis of Variance (ANOVA) was performed, followed by Tukey's test for comparisons of significant difference. When consider a single metal all the mean concentrations among ten species were significantly different ( $p < 0.05$ ) and the same result was obtained for all analyzed eight metals. From results obtained the iron showed the highest accumulation (56.7 mg/kg dry weight) and mercury showed the lowest accumulation (24.6 µg/kg dry weight).

**Key words:** Sea cucumber, Sri Lanka, Trace metals

## Allocation of total lipid and water content in different tissues of *Scomberoides lysan*

<sup>1</sup>Sutharshiny, Sathyaruban; <sup>2</sup>Sivashanthini, Kuganathan

<sup>1</sup> Department of Fisheries, University of Jaffna, Sri Lanka

Allotment of total lipid and water content were estimated for skin, intestine, liver, gonad, head, vertebral column, gill, kidney and muscle tissues from fifty matured *Scomberoides lysan*, sampled in June to October 2011 from the Northern waters of Sri Lanka. Total lipid and water content was determined by standard methods. The highest lipid content (g of the dry weight) was in the head followed by vertebral column recorded as  $5.4398 \text{ g} \pm 0.003$  and  $2.947 \text{ g} \pm 0.002$  respectively. The lowest value was recorded in the Kidney tissue ( $0.084 \text{ g} \pm 0.0001$ ). The lipid content of muscle above the lateral line region was significantly higher ( $1.23 \text{ g} \pm 0.0023$ ) than the lower portion. Duncan's test showed that there were significant difference ( $p < 0.05$ ) in the lipid content between different tissues examined except dark muscle versus liver ( $p = 0.8590$ ), vertebral column versus skin ( $p = 0.6873$ ), gill versus gonad ( $p=0.1825$ ) and kidney versus gonad ( $p = 0.057$ ). The tissues of gill and Intestine were rich in water content recorded as  $83.95242 \% \pm 0.001475$  and  $64.46067 \% \pm 0.008302$  respectively. The knowledge gained for *S. lysan* could be a fundamental to understand the nutritional status of tissues.

**Key words :** Lipid, water, *Scomberoides lysan*



## Monthly changes in gonad, muscle and liver lipid of female *Scomberoides lysan* from Northern waters of Sri Lanka

S. Sutharshiny<sup>1</sup> and K. Sivashanthini<sup>2</sup>

<sup>1</sup>Department of Fisheries, University of Jaffna, Jaffna, Sri Lanka

<sup>2</sup>Department of Zoology, University of Jaffna, Jaffna, Sri Lanka

The present study was carried out to assess the monthly fluctuation of lipid content in gonad, muscle and liver tissues of *Scomberoides lysan*. Monthly fish samples of 442 adult female fish captured from the northern waters of Sri Lanka in 2010 and 2011 were used. Tissues of ovary, liver and muscle were taken from each fish for lipid extraction by Bligh and dyer (1959) method. Tissue lipid contents in fish showed temporal fluctuations that correspond to lipid storage and utilization processes. The highest amount of lipid content in ovary was observed in June and September of both years. Lipid content (% dry weight) from the ovarian tissues of fish collected in June and September months for year 2010 were  $34.72 \pm 13.36$  and  $29.01 \pm 9.66$ , respectively whereas it was  $26.13 \pm 3.77$  and  $35.00 \pm 10.36$  for year 2011. Lipid content in muscle tissues was discernibly ( $p < 0.05$ ) lower when compared to that in liver and ovarian tissues. Significantly ( $p < 0.05$ ) increased amount of lipid content in liver tissues was recorded in May and August, and decreased thereafter. Liver lipid content attained the lowest amount in June and September months. Liver and gonad lipid showed a considerable monthly fluctuation during the two year period, while the changes in the amount of muscle lipid were much smaller. Understanding the lipid changes in gonad, muscle and liver tissues of *S. lysan* can predict the time of spawning (June and September), which would help to develop a management plan for this species in Sri Lanka.

**Keywords:** Liver tissues, muscle, ovarian lipid, *Scomberoides lysan*, Temporal variation

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\*sutharshiny12@gmail.com, sivashanthini@gmail.com

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