Studies on the Biology, Morphometrics and Biochemical composition of the Ommastrephid squid, *Sthenoteuthis oualaniensis* (Lesson,1830) of the south west coast of India

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Cochin University of Science and Technology
In partial fulfillment of the requirements
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Doctor of Philosophy

Under the Faculty of Marine Sciences

By

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This work is dedicated to my wife Augusta,
daughter J.Joshibha and Son J.Joewinn for their moral
support and encouragement throughout this study.

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Certificate

This is to certify that the Doctoral Thesis entitled **"Studies on the Biology, Morphometrics and Biochemical composition of the Ommastrephid squid, *Sthenoteuthis oualaniensis* (Lesson,1830) of the south west coast of India"** is an authentic record of work carried out by **Shri A. John Chembian** under my supervision and guidance at the School of Industrial Fisheries, Cochin University of Science and Technology, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** of the Cochin University of Science and Technology and that no part thereof has been submitted before for any degree.

Prof. (Dr.) Saleena Mathew
(Supervising Teacher)

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08/01/2013

Declaration

I, A. John Chembian do hereby declare that this Doctoral Thesis entitled **"Studies on the Biology, Morphometrics and Biochemical composition of the Ommastrephid squid, *Sthenoteuthis oualaniensis* (Lesson,1830) of the south west coast of India"** is an authentic record of the original research work carried out by me under the guidance and supervision of **Dr. Saleena Mathew**, **Professor**, School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**, of the Cochin University of Science and Technology and that no part of it has been submitted earlier for the award of any degree, diploma, associate ship, fellowship or other similar recognition in any University or Institution

(A. John Chembian)

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A.John Chembian.

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General introduction

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1.1 Introduction

With the stabilization of world finfish catches in general, and the depletion of a number of fish stocks that used to support industrial-scale fisheries, increasing attention is now being paid, to the so-called unconventional marine resources. This includes cephalopods such as squids, cuttlefishes, octopuses, and chambered nautiluses, with a total number of living species fewer than 1000 distributed in 43 families. During the last 56 years the global landings of cephalopods rose from 580,435 tonnes to 4,253,046 tonnes, in spite of a substantial decline in the rate of increase in the total world production of fish species (FAO, 2009). The explanation of this constant increase of the captures of cephalopods worldwide is complex. One of the proposed hypotheses is that the stocks of cephalopods have increased, whereas groundfish stocks have diminished (Caddy and Rodhouse, 1998). This is supported by the biological characteristic of cephalopods which are undoubtedly ecological opportunists (Guerra, 2006).

As reviewed by Boyle and Rodhouse (2005) and Pierce et al. (2010), cephalopods were historically important, equally as target species and bycatch, in the coastal artisanal fisheries of numerous countries of the world. The importance of cephalopods in fisheries is increasing year by year. Now the major international fisheries have also directly focused on them. Besides, research on the ecology of the oceans has also revealed the importance of cephalopods in the trophic networks of marine ecosystems, both as prey and subdominant predators, which tend to increase in biomass when other species (particularly their predators and competitors for food) become depleted as a result of heavy fishing (Roper et al,1984). However, as the importance of cephalopods continues to increase world-wide as a fisheries resource, their short life cycles and variable growth rates makes cephalopod stocks volatile and vulnerable. Pierce and Guerra (1994) reviewed the stock assessment methods used in cephalopod fisheries worldwide and found that despite a multitude of assessment techniques, only few cephalopod fisheries were well managed. This scenario has not changed substantially in the last 16 years.

Among the cephalopods the squids of the order Teuthoidea, is the major contributor to Cephalopod fishery. The two Suborders, Myopsida, “covered-eyed" and the Oegopsida, “Open eye” of the squid have formed the basis of the major cephalopod fisheries worldwide and both together contributes more than 71% of the cephalopod catches (Roper et al,1984). The suborder Oegopsida or oceanic squids comprises twenty five families (Sweeney and Roper ,2001), several of which support the major cephalopod fisheries of the world. These are one of the most diverse groups of cephalopods, with more than 240 species described, occupying key trophic roles as predators in the open ocean ecosystem (Clarke, 1996; Jereb and Roper, 2010). Some of these species undergo high fishing pressure and their catches represent half of the total cephalopod world captures (Boyle and Rodhouse, 2005; FAO, 2010). The "flying squids" (Roper et al. 1984) of the family Ommastrephidae (suborder Oegopsida) account for about 65 percent of the world's commercial cephalopods (Brunetti 1990). Although less widely recognized than the inshore Loliginidae (suborder Myopsida)- which are subjects of commercial and artisanal fisheries globally, Ommastrephids are important in commerce and ecology. Six of the 10 genera of this family currently support a fishery, and although much of the catch is not broken down between inshore squids and flying squids, it can reasonably be assumed that this family alone accounts for more than half of the world cephalopod catch.

The effective and rational utilization of the Oceanic fishery resources is of great importance, especially during the time of depletion of shallow water resources. One of such important Oceanic resource is the tropical Indo-Pacific Ommastrephid squid *Sthenoteuthis oualaniensis* (Lesson, 1830). It is the most abundant large sized squid in the Indo-Pacific region with an estimated biomass of 8-11 metric tons (Nigmatullin, 1990). Its biomass in the Indian Ocean has been estimated to be about two million tons (Zuyev et al., 1985) with high concentration of 12 to 42 ton per square km in the Arabian sea during the month of November-January (Nesis, 1993). This greatest abundance in biomass, is due to its broad ecological valency, complicated intraspecific structure, great fecundity and short life cycle with high growth rate (Zuyev and Nesis, 1971; Nesis, 1977; Zuyev et al., 1985). These features brings the *S oualaniensis* into the spectrum of species, most interesting from the scientific and practical point of view.

Sthenoteuthis oualaniensis, commonly known as purple squid, is a member of the family Ommastrephidae and thought to be the most abundant large squid in the tropical and subtropical waters of the Indo-Pacific region (Young and Hirota, 1998; Dunning, 1998). The northern boundary of its range in the Indian Ocean is limited by the Asian continent. The southern boundary reaches the southernmost point of Africa (about 35^{o}S), then is narrowed a little to the central area of the southern Indian Ocean and passes on to the Pacific Ocean approximately along the Tropic of Capricorn to the continental slope of South America. However, near America, westward of the Peru Current, the abundance of *S. oualaniensis* is low and here the Jumbo squid *Dosidicus gigas* predominates (Alexandronetz et al., 1983; Nigmatullin et al., 1988). The reproductive area of *S. oualaniensis* is located in the Indian Ocean northward of 18–22^{o}S, and in the Pacific Ocean between 20–25^{o}N and 16–20^{o}S. The range boundaries are mobile and are displaced toward high latitudes with warming during summer of the appropriate hemisphere and moved to lower latitudes with the fall of water temperature.

It has local commercial significance in the Arabian Sea and the Indian Ocean (Aravindakshan and Sakthivel, 1973; Silas et al., 1982; Roper et al., 1984), but its resources are poorly exploited. The most comprehensive earlier information on *S. oualaniensis* from the tropical zone of the Indian Ocean was presented by Zuyev and Nesis (1971) and Zuyev et al. (1985). On the basis of size differences, dorsal photophore and gladius morphology, five forms of *S. oualaniensis* has been distinguished: (1) dwarf early-maturing equatorial form without dorsal photophore; (2) dwarf late-maturing Red Sea form with dorsal photophore; (3) and (4) middle-sized late-maturing form with dorsal photophore and with single lateral axis of the gladius (3), distributed in the Red and Arabian seas and the Gulf of Aden, and with double lateral axes of the gladius (4), most common in the tropical Indian and Pacific oceans; (5) the giant form with dorsal photophore and single lateral axes, inhabiting Red Sea, Arabian Sea and the Gulf of Aden (Bizikov, 1991, 1996). The dwarf equatorial form is found roughly within 10^{o} latitude of the equator where it co-occurs with the typical *S. oualaniensis*. The dwarf form has several morphological characters that separate it from the typical *S.oualaniensis* showing absence of the dorsal photophore patch, slightly different hectocotylus and slight differences in the spermatophore structure and in the gladius structure (Nesis, 1993).

Ecological differences were also observed between these two forms (Nigmatullin et al., 1983c; Pinchukov, 1983). Researchers have disagreed on whether or not the dwarf form is a distinct species (Clarke, 1966 and Wormuth, 1976). On the other side, research continues to reveal that squid growth can be greatly modified by temperature or seasons of hatching (Rodhouse & Hatfield, 1990; Jackson & Choat, 1992; Forsythe, 1993; Brodziak & Macy, 1996; Dawe &

Beck, 1997; Jackson et al. 1997; Hatfield, 2000; Forsythe et al. 2001) as well as food supply (Jackson & Moltschaniwskyj, 2001). Okutani and Tung (1978) found *S. oualaniensis* in Taiwanese waters to consist of three different seasonal cohorts: a June-spawning group, a September-October spawning group and a February-March spawning group. In the Indian waters Mohamed et al, (2006) has reported the occurrence of medium form with double axis rachis off the south west coast. Meanwhile, a study based on Random Amplified Polymorphic DNA (RAPD) analysis has been done in the Marine Biological Laboratory of College of Marine Science and Technology of Shanghai Fisheries University. Its preliminary findings suggest that a large variation in biology among the groups (Xinjun et al ,2007). Roeleveld (http://swr.nmfs.noaa.gov/pir/feis/ Appendix%20B.pdf) considered the dwarf form to be a separate species that could only be identified as an adult. However, if the dwarf form proves itself to be a nascent divergent species, it will serve as a fascinating example of sympatric speciation, since its range is completely within that of the typical form, and would merit further study for that reason alone.

Though the phenotypic plasticity of cephalopods is widely accepted as one of their main characteristics (Boyle and Boletzky, 1996), our understanding of the causes of plasticity in squid growth is still far from complete. In view of that, in this study the two forms of the *Sthenoteuthis oualanienesis,* dwarf and medium form have been separately studied and compared to understand the difference in its biology, if any, for further research and to evolve future management strategy for the resources.

1.2 Systematics

Class : Cephalopoda
  Sub Class : Coleoidea
    Order : Teuthida
      Suborder : Oegopsina
        Family : Ommastrephidae
          Subfamily : Ommastrephinae
            Genus : *Sthenoteuthis* Verrill, 1880
              Species : *oualaniensis* (Lesson, 1830)*;*
                pteropus (Steenstrup, 1855).

Description: Mantle long, muscular and cylindrical up to the point of origin of fins and tapers to a narrow point at the posterior end (Plate 1.1(b)). Dorsal margin is slightly produced in the middle (Plate 1.1(a)). Fins short, muscular, broad with convex anterior margin. Head large and as wide as mantle and bears comparatively short arms. Funnel short, compact and set in a deep pit present on the ventral side of the head; foveola (Plate 1.1 (b) and Plate 1.2 (b)) with 7-9 longitudinal folds in the central pocket and 3-5 lateral pockets on either side. Funnel locking apparatus inverted T-shaped and fused in its middle portion with the mantle groove (Plate 1.2 (c)). Arms large, strong in the order III>.II>.I>VI and compressed with the third pair strongly keeled (Plate 1.1). Arm sucker biserial; the protecting membranes have prominent trabeculae; the larger arm suckers are provided with about 7-12 sharp teeth around the entire rim of the horny rings (Plate 1.2 (f)). Left arm IV in males thick, longer than the right arm and hectocotylized. Two rows of 14-15 suckers protected by flap-like membranes present on the basal portion of the hectocotylized arm. Suckers and papillae absent on about one half of its distal part. A series of pits present in a single row along the base of the protective membranes.

Tentacles are short, muscular and laterally compressed. Clubs small, slightly expanded; suckers quadriserial with the inner rows on the manus larger (Plate 1.2 (g)). Larger suckers of the club bear about 20 sharp teeth on the rims of which four are larger and located one in each quadrant (Plate 1.2 (e)). Gladius (Plate 1.3 (a-c)) thin and very slender; rachis stout anteriorly, uniformly narrowing to the posterior tip, and with median rib and two marginal ribs along the edges (Plate 1.3.b); posterior end with a small vane about one-seventh of the total gladius length. Beaks (Plate 1.3(d & e)) strong. Radula with seven transverse rows of teeth; rachidian tooth tricuspid; first lateral tooth bicuspid, outer cusp small; second and lateral marginal teeth single and slightly curved. Spermatophore long and small, sperm mass comprises 50-60% of total length; cement body oval, slightly constricted at the posterior quarter of the body; ejaculatory apparatus coiled at oral end. Head, dorsal mantle, fins and arms are uniformly of chestnut brown colour. An oval photophoric patch is present on the antero-dorsal surface of mantle. A unique character of *S. oualaniensis* is mantle element of T-shaped locking apparatus curved with an anterior bifurcation, fused to funnel element along the posterior third of the longitudinal groove.

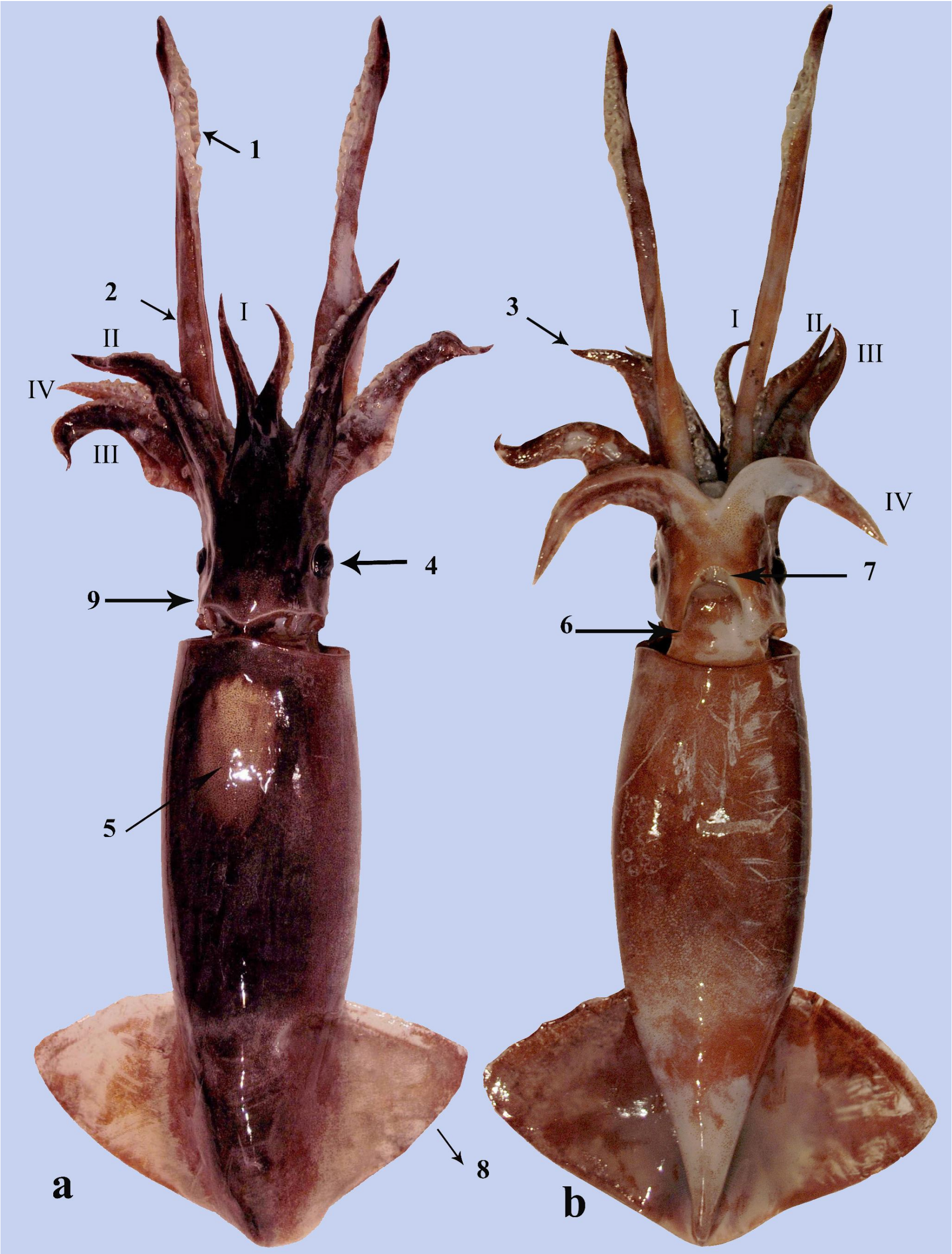

Plate 1.1. *Sthenoteuthis oualaniensis* female (a) Dorsal view (b) Ventral view

1 - Tentacular club, 2 - Tentacle, 3 – Arms (I,II,III & IV), 4- Eye, 5- Photophore, 6 - Funnel , 7 – Foveola, 8- Fin, 9- Head.

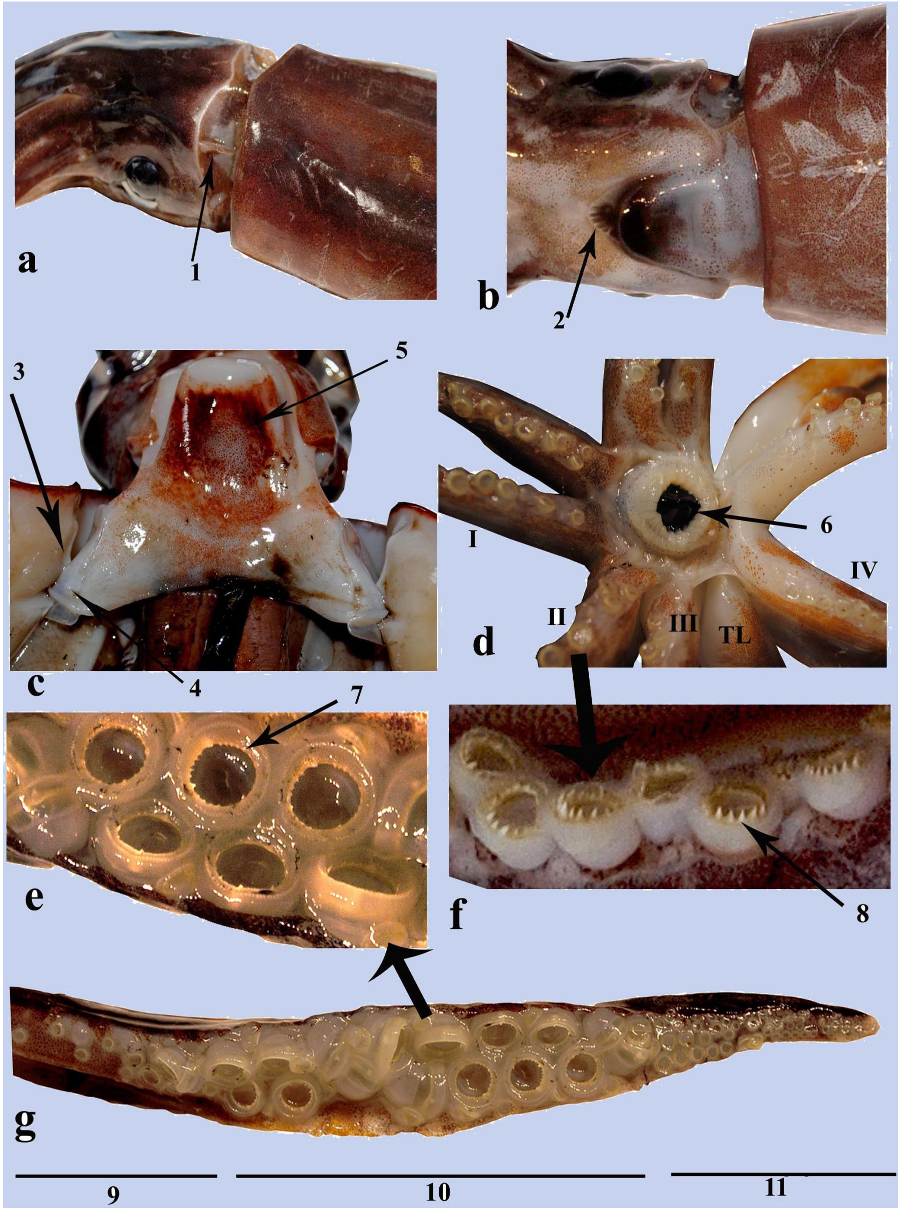

Plate 1.2. *Sthenoteuthis oualaniensis* female . (a) Head and its membrane, (b) Foveola, (c) Mantle and Funnel locking cartilage, (d) Oral view, (e) Enlarged club suckers with rings, (f) Enlarged arm suckers with rings , (g) Tentacular club.

1–Head membrane, 2-Foveola, 3–Mantle locking cartilage, 4–Funnel locking cartilage, 5 – Funnel, 6- Beak, 7- Club ring, 8 – Arm ring, 9-Carpus , 10- Manus, 11- Dactylus portion of the club region.

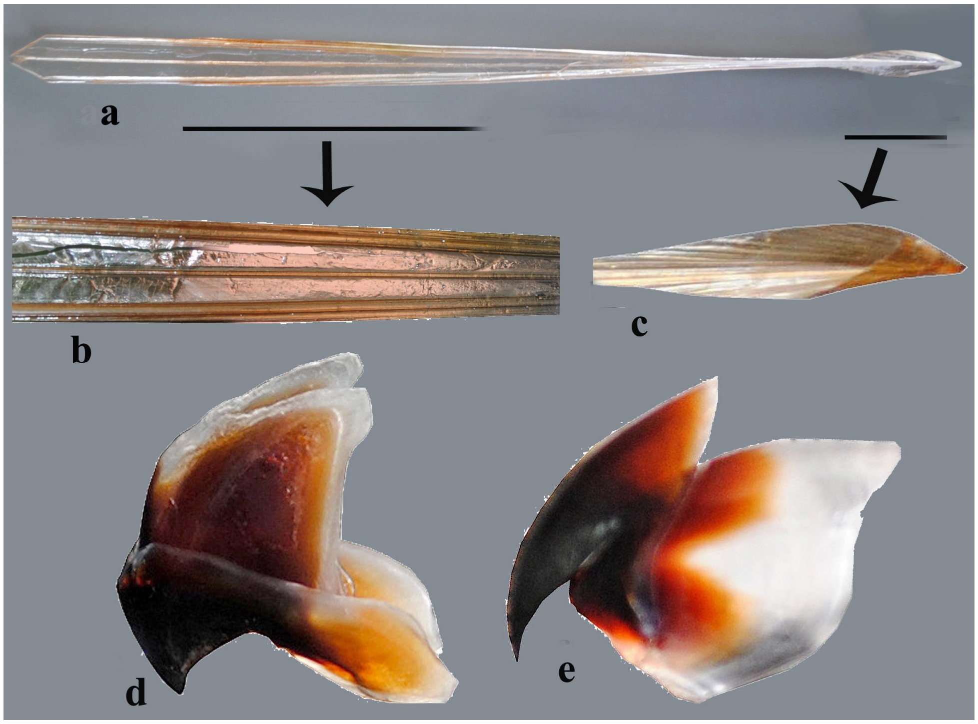

Plate 1.3. *Sthenoteuthis oualaniensis* female (a) Gladius, (b) Enlarged central part of the rachis, (c) Enlarged posterior cone, (d) Lower beak, (e) Upper beak.

1.3 Review of literature

At first, the purpleback squid was described under the name *Loligo oualaniensis* by Lesson in 1830, then the orangeback squid was described by Steenstrup as *Ommastrephes pteropus* in 1855. Both descriptions were rather brief, the size and sex of the holotype specimens were not indicated, and both are now lost. Both species were included in the genus *Ommastrephes* d'Orbigny, 1835, but then Verrill (1880) established a new genus, *Sthenoteuthis*, for these ommastrephids. Soon, Pfeffer (1900) separated purpleback squid into a new genus *Symplectoteuthis*. However, the taxonomy of the genus *Ommastrephes* was in a confused condition, without any precise criteria for species identification. Representatives of this genus were described under different names even from the northern Atlantic (Verrill, 1882; Pfeffer, 1912; Naef, 1923; Rees, 1950; Adam, 1952; Jaeckel, 1958; Clarke, 1966).

In 1950-1970s the majority of specialists accepted the following system: genus *Ommastrephes* d'Orbigny, 1835 with three species: *O. bartramii* (Lesueur, 1821), *O. pteropus* Steenstrup, 1855, and *O. caroli* (Furtado, 1887), and genus *Symplectoteuthis* Pfeffer, 1900 with two species *S. oualaniensis* (Lesson, 1830) and *S. luminosa* (Sasaki, 1915). It was considered that *O. caroli* is endemic of North Atlantic, *O. pteropus* is the tropical Atlantic species and *O. bartramii* is widely distributed in the North Atlantic, North Pacific and South Atlantic (Clarke, 1966; Roper et al., 1984). The main reason for such taxonomic complexity lies in the lack of rich comparative material. The casual and isolated specimens, which fell into specialist's hands, were usually collected from storm strandings or caught near the coast. Development of the oceanic fishery of tunas and other large fishes started from mid- 1950 activated national and international scientific programs, and many vessels began work in open oceanic waters. Observations and fishing for squid were included in the routine procedures of oceanographic expeditions.

First observations and collections of oceanic nektonic squids were conducted by Baker, (1957, 1960), Clarke (1965, 1966), Voss (1956, 1966, 1973), Wormuth (1970, 1976) , Okutani (1977). From the 1970s significant interest in oceanic resources arose due to the introduction of 200-mile exclusive economic zones and the exhaustion of fish stocks in traditional fishing grounds. The subfamily Ommastrephinae was put in order and revealed the main features of their evolution (Nigmatullin, 1979) and delineated the boundaries of species ranges (Zuyev et al., 1976). As a result of the revision of the subfamily Ommastrephinae in the genus *Ommastrephes,* only one species *O. bartramii* was left with *O. caroli* as junior synonym, orangeback and purpleback squids were united in the genus *Sthenoteuthis*, and *Symplectoteuthis luminosa* Okada, 1927 was transferred to *Eucleoteuthis* (Zuyev et al., 1975). Independently Wormuth (1976), using method of numerical classification, had come to a similar conclusion about the association of *O. pteropus* and *S. oualaniensis* in one genus *Symplectoteuthis*. However, this was found to be incorrect later because *Symplectoteuthis* Pfeffer, 1900 is junior synonym of *Sthenoteuthis* Verrill, 1882.

New methods to count squids on the water surface (Zuyev and Nigmatullin, 1974; Zuyev et al., 1980, 1988), studying quantitative distribution in relation to abiotic and biotic environmental factors (Zuyev, 1967, 1971, 1973; Filippova, 1971, 1975; Nesis, 1974; Korzun et al., 1989; Alexandronets et al., 1983; Nigmatullin and Parfenjuk, 1988) were developed. Besides, different aspects of squid biology: reproduction (Zuyev, 1976; Klyuchnik and Nigmatullin, 1974; Burukovsky et al., 1977, 1979; Zalygalin et al., 1977), behavior (Nigmatullin, 1972, 1987; Vovk and Nigmatullin, 1972a,b; Zuyev and Nigmatullin, 1975; Parfenjuk et al., 1983; Nigmatullin and Parfenjuk, 1986), feeding and parasite fauna (Nigmatullin et al., 1977; Gaevskaya, 1977; Gaevskaya and Nigmatullin, 1981; Naidenova and Zuyev, 1978; Nigmatullin and Toporova, 1982; Filippova, 1974), population structure (Zuyev and Nigmatullin, 1977; Zuyev and Shevchenko, 1973; Nigmatullin et al., 1983a,c; Pinchukov, 1983), including also biochemical methods (Koval, 1977) were studied. Research into their morphology and faunistic composition (Okutani, 1970; Roper, 1977; Voss, 1966; Wormuth, 1970; Young, 1972), and, to a lesser degree, their ecology (Alverson, 1963; Clarke, 1966; Ashmole and Ashmole, 1967; Suzuki et al., 1986; Young, 1975; Yamanaka et al., 1977; Hixon et al., 1980) were done.

Main results of these multidisciplinary researches of squid were systematized in the monograph “Nektonic oceanic squids (genus *Sthenoteuthis*)” by Zuyev et al. (1985). Many papers were devoted to their physiological and biochemical features (Abolmasova, 1984, 1985; Abolmasova and Belokopytin, 1987; Abolmasova and Stolbov, 1991; Abolmasova et al., 1990; Belokopytin, 1982; Epstein, 1992; Grigorjeva, 1987; Rosengart et al., 1994; Shulman and Nigmatullin, 1981; Shulman et al., 1984, 1992a; Timonina, 1980). Wide investigations were carried out on their reproductive biology (Nigmatullin and Sabirov, 1987; Laptikhovsky and Murzov, 1990; Chesalin and Giragosov, 1993; Nigmatullin and Laptikhovsky, 1994; Laptikhovsky, 1995; Sabirov, 1995). Contributions to knowledge of squid biology arose from researches on their growth and age, using statoliths and gladii (Mikheev, 1988; Arkhipkin, 1988; Arkhipkin and Bizikov, 1991; Arkhipkin and Mikheev, 1992; Bizikov, 1991, 1996; Laptikhovsky et al., 1993). Great interest in squid was excited after the discovery, with manned submersibles, of large schools of *S. oualaniensis* in the depths of Arabian Sea in 1986 (Chesalin, 1993; Gutsal, 1989, 1991;Korzun, 1990; Korzun et al., 1992; Ruchkin, 1988; Volkov et al., 1988; Zuyev and Gutsal, 1989, 1994).

In 1980–1990s some interesting ecological publications appeared about *S. oualaniensis* based on local data (Silas et al., 1982; Harrison et al., 1983; Dunning, 1988; Young, 1994; Yatsu et al., 1998; Snyder, 1998). The most detailed information on *S. oualaniensis* in the Arabian Sea was published by Chesalin (1993) and Nesis (1993). Recently Mohamed et al. (2006) has reported the occurrence of medium form with double axis rachis off the south west coast of India and have done preliminary studies on its biology. Xinjun et al. (2007) has studied and reported the biology of the species in the northwest Indian Ocean. However, detailed study on the phenotypic composition of *S. oualaniensis* of the Arabian Sea as well as many features of its distribution, biology, productivity and life cycle are still poorly known, so that any new information of this species in the waters off the south west coast of India has important scientific significance.

1.4 Sample collection

Specimens of *S. oualaniensis* were collected between January 2007 and December 2008 in the area Lat 07 ^{o} N to 11 ^{o} N and Long 74 ^{o} E to 77 ^{o} E along the south west coast of India in the depth range of 180 – 2601 m. (Table 1.1, Figure 1.1). Samples were collected from different sources to get the continuity of data, as the specimens are of oceanic in nature and do not have a regular fishery.

Table 1.1. Details of the sampling stations and sample size of the species *Sthenoteuthis oualaniensis* along the South west coast of India . M-medium form, D- dwarf form.

Period	Lat^{o}N	Long^{o}E	Depth range	Sample size (Nos)		
				Total	Medium	Dwarf
January’07	8 – 10	75	193 – 2450	45	27	18
February’07	8 – 10	75	300 - 2534	29	18	11
March’07	9 &10	74 & 75	350 - 2228	38	33	5
April ’07	8 &10	74 & 75	1400- 2400	19	9	10
May’07	9 &10	75	350 - 1800	14	4	10
June’07	9 &10	75	400 - 1505	22	10	12
August’07	8 & 9	75	300 - 2203	42	24	18
September’07	8 – 10	75	250 – 810	50	37	13
October’07	7 & 9	74 & 76	820 - 2403	386	164	222
November’07	10	75	344 – 2120	18	18	0
December’07	9 &10	75	180 – 2000	25	25	0
January’08	8 & 9	75 & 76	400 - 1450	26	16	10
February’08	8 & 9	75 & 76	250 - 1209	30	16	14
March’08	10	75	550 - 2402	18	18	0
April’08	8 - 10	75	300 - 1780	19	7	12
May’08	9 &10	75	330 - 2601	11	4	7
June’08	9 &10	75	280 - 2010	18	9	9
August’08	9	75	200 - 826	36	14	22
September’08	7 - 10	74 & 75	405 - 2220	33	23	10
October’08	7 - 10	75 & 76	378 - 2050	98	56	42
November’08	7 - 9	75 & 76	450 - 1653	14	14	0
December’08	7 - 9	75	399 - 1578	24	19	5
Total				1015	565	450

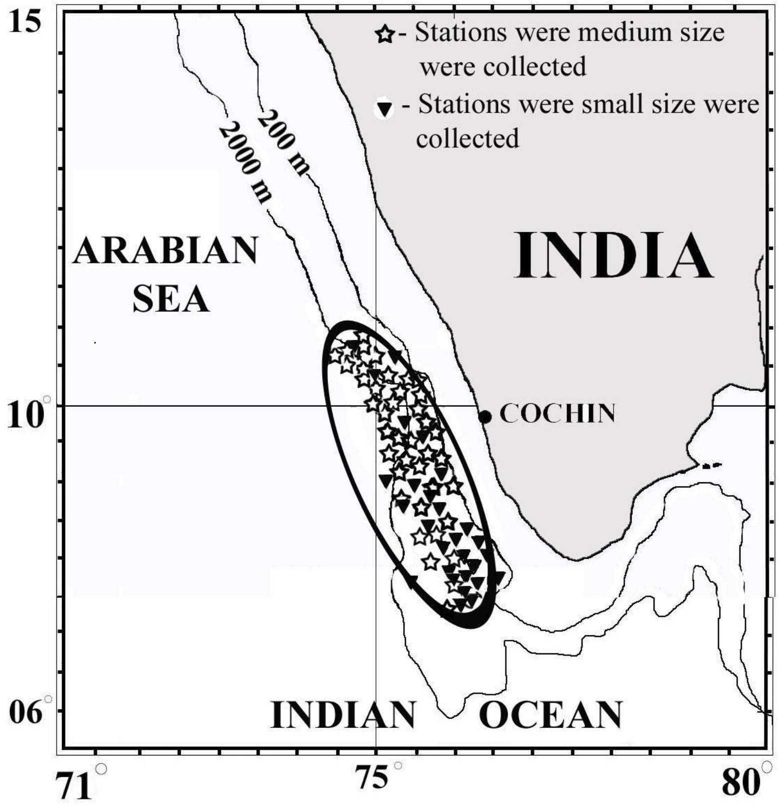

Figure 1.1. Map of the South west coast of India showing the oceanic squid sampling area during the study period.

M V Matsya Sugundhi (31.5m OAL, 245.8 GRT and 650 BHP), a long liner-cum-squid jigger of the Fishery Survey of India (FSI) and the 44-60 footer Gill netters/ Tuna long liners of the Cochin based Colachal fishing boats were of the prime source of collection. Besides, squids were also collected by hand jigging operation from onboard the FSI trawler Matsya Varshini during its night drifting. Matsya Sugundhi employed automatic squid jigging machine (Figure 1.2) to collect the samples, while the fishing boats and Matsya Varshini employed hand line with jigs to collect the samples. No sample could be collected in the month of July due to the 45 days seasonal fishing ban in the west coast of India, from June

15th to July 30th. Specimens of *Sthenoteuthis oualaniensis* in both the phenotypic forms and other oceanic squids such as *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii* were immediately preserved on board the vessel at -20° C by blast freezing. At shore lab, specimens were brought to the ambient temperature by allowing it to be thawed gradually.

Figure 1.2. Squid jigging in action

1.5 Objectives of the study

The main objectives of the study are

- To investigate and identify the presence of different types of plastic phenotype of the species based on the morphometric data collected in the study area .

- To understand the growth and mortality parameters and life span of the different plastic phenotypes identified.
- To Investigate the maturity, fecundity and spawning period of the squid plastic phenotypes in the study area.
- To understand the food and feeding habit of the species in the study area.
- To investigate the biochemical composition of the mantle tissue of the available plastic forms of the species in comparison to the co existing other oceanic squid species.

Chapter 2

Morphometrics of the purple squid Sthenoteuthis oualaniensis (Lesson, 1830)

Contents

2.1 Introduction

Morphometric variation has been used to discriminate forms of fish over a century. With the development of morphometric techniques the morphometric characters of organisms have become the principal indices for taxonomy. Statistical analysis of morphometric characters gives a better idea of relationship within the species of a particular geographic area as well as to compare it with the same species of different geographical area. Recent studies on several species have amply demonstrated the importance of the range of variation in characters used in the identification. Even with a relatively fixed taxonomy, a study of morphometric variation can be of great value in researching the biology of a fisheries resource. In the Pacific sardine, *Sardinops caerulea* (Clark, 1947) and in the northern anchovy, *Engraulis mordax* (McHugh, 1951) the variation in number of vertebral elements have been useful in indicating possible subpopulations.

Similarly, the studies of morphological variation in cephalopods were mostly undertaken to resolve systematic ambiguities as it ascertain the uniqueness of the species. Haefner (1964) studied the morphology of *Loligo pealei* and *Lolliguncula brevis* to distinguish the two species in various size classes. LaRoe (1967) clarified the systematics of the loliginid squids of the tropical western Atlantic Ocean by developing a key based on his studies of morphological variation. Cohen (1976) continued the work on the systematics of these squids by investigating morphological variation with respect to geographical location and sex. Some morphological studies of *Loligo opalescens* were conducted to distinguish subpopulations. Evans (1976) found some statistical differences in morphology in comparing *L. opalescens* from Monterey Bay and southern California. However, before a morphological comparison of squid from different areas can be made, there must be an investigation of other factors, which are likely to cause, differences in morphology.

Rapid growth rates along with physiological strategies aimed at maintaining ‘life in the fast lane (Jackson & O’Dor, 2001) placed squid in a unique position to respond to environmental or climatic changes. It has been suggested that the life-styles of squid promote faster changes in gene frequency than many other organisms and because of this, squid should be able to track changes in climate or biological conditions more efficiently (O’Dor,1998). Indeed, recent work (Jackson & Domeier, 2003) has proposed that squid are effective ecological indicators, with phenotypic variability in *Loligo opalescens* rapidly changing in response to upwelling and productivity variation in the California Current as a result of short-term El Niño/La Niña influences. A substantial body of work is available to demonstrate the marked influence of temperature or season on size, growth rates and life spans of both *loliginid* (Jackson et al. 1997, Hatfield, 2000, Forsythe et al. 2001, Jackson & Moltschaniwskyj, 2001, 2002, Macy & Brodziak, 2001) and *ommastrephid* (Dawe & Beck ,1997, Arkhipkin et al. 2000, Arguelles et al. 2001) squids.

This study investigates the morphological variation of the *Ommastrephid* squid *Sthenoteuthis oualaniensis* (Lesson, 1830) in the south west coast of India to determine the plastic phenotypes, if any, and the morphological characters that would be useful in the field to distinguish the plastic phenotypes and thereby the population structure. Little has been known about this species and its population structure in the south west coast of India, although some surveys have previously documented the distribution of some of its plastic phenotypes in the North west Arabian Sea (Bizikov, 1991; Nesis, 1993). Nesis (1993) has described a complex population structure to this squid, that incorporates three major and two minor forms. A giant form with dorsal photophore and single lateral axes occurring only in the northern Indian Ocean region of the Red Sea, Gulf of Aden and Arabian Sea north of 11-12°N (modal sizes of 400-500mm ML in the Arabian Sea with a maximum size of 650 mm ML). The females of giant form in the Red Sea are smaller than in the Arabian Sea. A medium form – the "typical" one – (modal sizes of 120-150 mm for mature males and 190-250 mm for mature females) occurs throughout the range of the species. The medium form is further subdivided into two forms based on features of the gladius (double or single lateral axes of the rachis). One of them with single lateral axes of the rachis occurs predominantly in the Red Sea, the Gulf of Aden and the Arabian Sea north of 15°-17° N. The other one with double lateral axes of the rachis commonly occurs in the areas south of 10-11° N . But, both forms observed to overlap between 10-11° and 15-17°N. A dwarf form with the maximum size of 140-150 mm ML occurs roughly within 10° latitude of the equator, where it co-occurs with the smaller form. This is similar to the medium form but maturing at a smaller size (mode for females is 120-140 mm with a range of 90-160 mm ML) that is nearly the same size as that of dwarf form and found in the Western Indian Ocean. In the NW Indian Ocean, the dwarf form is absent to the north of 10-11°N.

The dwarf form has several morphological characters that separate it from the typical *S.oualaniensis* particularly in the lack of dorsal mantle photophore patch characteristic of the species, a slightly different hectocotylus, slight differences in the spermatophore structure and in the gladius structure. Researchers have disagreed on whether or not the dwarf form is a distinct species (Clarke, 1966; Wormuth 1976; Nesis ,1993). If the dwarf form proves itself to be a nascent divergent species, it will serve as a fascinating example of sympatric speciation, since its range is completely within that of the typical form, and would merit further study for that reason alone. Complicating this picture is the uncommon occurrence of small early-maturing form with dorsal photophore , similar to the medium form. This matures at a smaller size (mode for females is

120-140 mm with a range of 90-160 mm ML), nearly the same size as the dwarf form and found in the Western Indian Ocean and the eastern tropical Pacific Ocean. Of the five possible forms, giant, medium with single axis, medium with double axis (the typical *S. oualaniensis*), small and dwarf, the latter three occur in the Pacific Ocean. *S. oualaniensis* are sexually dimorphic, with females growing much lager than males. The dimorphism in sucker ring dentition, and the differences in size, also suggests a difference in the feeding spectrum of males and females (Snyder, 1998)

Mid water ecosystems are becoming increasingly studied and commercial fishermen are intensifying the use of squid as a major source of income. This necessitates a working knowledge of the population structure of *S. oualaniensis* in the south west coast of India to understand its huge ecological impact and possible future importance for commercial fishing. In view of that, an attempt has been made to understand the existence of any significant morphological characters that would be useful to distinguish the plastic phenotypes, if any, instantaneously in the field and thereby the population structure.

2.2 Materials and Methods

Samples contained two forms of *S. oualaniensis.* They were identified as of dwarf, early-maturing form, without dorsal photophore and a late-maturing medium form with dorsal photophore as indicated by Clarke (1965) and Nesis (1993). However, the specimens were further subjected to careful morphometric analyses for further confirmation of the plastic phenotypic form.

Altogether 1015 specimens were collected during the period of study, out of which, 565 were of medium form and 450 were of dwarf form.

Measurements were taken following Cohen (1976). Dimensions of measurements have been depicted in Plate 2.1. The sex wise measurements for Dorsal mantle length (DML),Tentacle length (TL), Tentacular club length (TCL), Fin width (FW),Fin length (FL), Mantle width (MW), Head width (HW) and Arm length (AL-1, AL-2, Al-3 and AL-4) were taken in a straight line to the nearest millimeter with the squid lying in its natural position. Whereas, Total weight (TWt) and Mantle weight (MWt) were measured to the nearest gram. The details of the sample size and the parameters collected have been furnished in the Table 2.1.

Length weight data collected were log-transformed (natural logarithms)- as this has been demonstrated to be in exponential relation (Tıraşın 1993)- and predictive regression equations were calculated correlating lnDML to lnTwt, and lnDML to lnMwt, for both medium and dwarf form and compared. lnDML was used as the independent variable, to study the variation between the medium and dwarf forms. The relationship between mantle length (DML) and total body weight (TWt) has been described by Bagenal and Tesch (1978), Ricker (1973,1975) and Le Cren (1951). However, Le Cren's (1951) least square method, employing the equation stated below was used in this study.

$$W = aL^{b} \quad \text{(or)}$$

$$\text{in the linear form} \quad \text{Log } W = \log a + b * \text{Log } L$$

where 'W' is the weight in 'g', 'L' the total length in cm, and 'a' (Intercept) and 'b' (Slope) are fitted constants. If the 'b' value is >3 then the squid considered to have grown in weight or other variable at a rate faster than the DML, called positive allometric growth. Whereas, If b = 3 then the two variables were considered to have grown isometrically. Similarly, if b< 3 then the variable could have grown at a rate slower than the DML called negative allometric growth (Zeidberg, 2004).

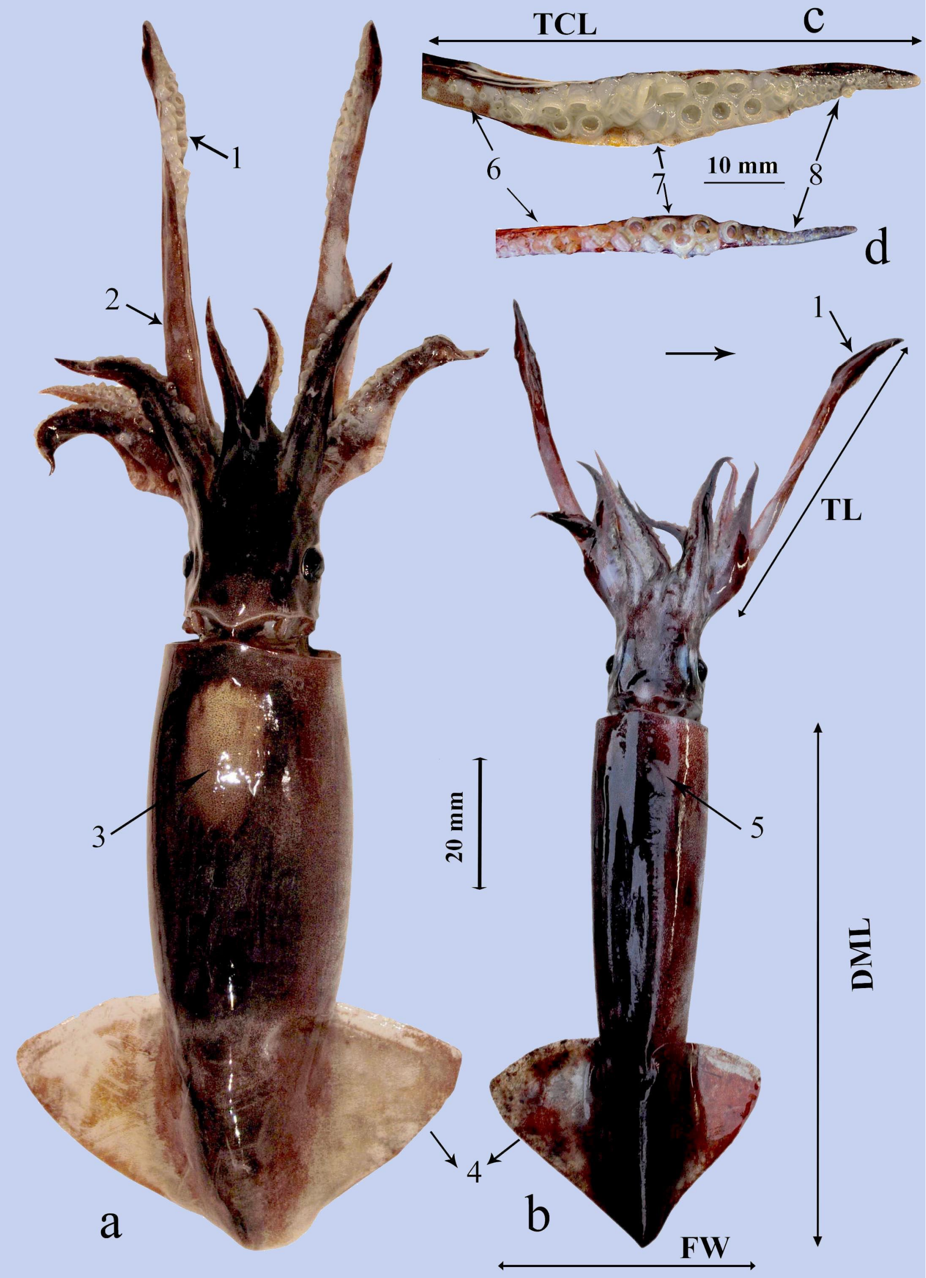

Plate 2.1. Dorsal view of *Sthenoteuthis oualaniensis*. (a) Medium form, (b) Dwarf form, (c) Tentacular club of Medium form, (d) Tentacular club of Dwarf form. 1 - Tentacular club, 2 - Tentacle, 3 – Photophore, 4 - Fin , 5 – Absence of photophore, 6, 7 & 8 – Carpus , Manus and Dactylus portion of the club region.

Table 2.1. Sample size ,Data range, Mean and SD of different parameters collected on *Sthenoteuthis oualaniensis* during the study .

Parameters	Males				Females				Total			
	N	Range	Mean	SD	n	Range	Mean	SD	n	Range	Mean	SD
DWARF SIZE												
Dorsal Mantle Length	77	9.2-13.4	11.05	1.11	373	9.1-16.5	12.92	1.26	450	9.1-16.5	12.6	1.42
Total Weight	77	20-63	37.49	10.8	373	26-111	58.23	16.5	450	20-111	54.65	17.5
Mantle Weight	40	12-33	17.7	4.8	105	17-46	29	6.20	145	12-46	25.8	7.7
Tentacle Length	37	12.6-16.3	14.36	1.04	99	13.8-20.4	17.4	1.67	136	12.6-20.4	16.58	2.04
Tentacle Club Length	37	3.2-4.2	3.65	0.25	99	3.8-5.7	4.6	0.47	136	3.2-5.7	4.3	0.6
Fin Width	29	5.9-9.0	7.09	0.72	47	7.7-11.6	9.5	0.98	76	5.9-11.6	8.6	1.48
Fin Length	-	-	-	-	-	-	-	-	23	3.5-6.1	4.67	0.61
Arm Length-I	-	-	-	-	-	-	-	-	29	3.5-6.2	4.75	0.7
Arm Length-II	-	-	-	-	-	-	-	-	29	4.2-7.6	5.85	0.8
Arm Length-III	-	-	-	-	-	-	-	-	29	4.4-7.8	6.4	0.98
Arm Length-IV	-	-	-	-	-	-	-	-	29	4.1-7.8	5.57	0.8
Mantle Width	-	-	-	-	-	-	-	-	24	2.6-4.8	3.46	0.56
Head Width	-	-	-	-	-	-	-	-	22	1.5-3.0	2.1	0.36
MEDIUM SIZE												
Dorsal Mantle Length	237	9.8-19.5	13.97	2.06	328	10-27	16.61	3.81	565	9.8- 27	15.50	3.5
Total Weight	237	43-322	127.8	58.05	328	39-794	227.8	156.71	565	39-794	186	134.5
Mantle Weight	94	39-179	92.3	34.7	102	38-420	153.7	91.43	196	38-420	124	76.5
Tentacle Length	86	15.8-34.3	22.02	3.7	94	15.4-44.8	27.62	7.0	180	15.4-44.8	24.94	6.28
Tentacle Club Length	86	4.2-11.5	6.84	1.39	94	4.8-15.3	9.07	2.5	180	4.2-15.3	8.0	2.34
Fin Width	39	10.9-18.6	12.63	1.38	63	10.9-20.5	15.03	2.6	102	10.9-20.5	14.11	2.5
Fin Length	-	-	-	-	-	-	-	-	67	3.6-10.5	5.38	1.45
Arm Length-I	-	-	-	-	-	-	-	-	69	3.8-12.1	5.8	1.5
Arm Length-II	-	-	-	-	-	-	-	-	69	5.0-14.5	7.2	1.89
Arm Length-III	-	-	-	-	-	-	-	-	69	5.8-15.0	8.65	1.86
Arm Length-IV	-	-	-	-	-	-	-	-	69	5.0-12.9	6.8	1.65
Mantle Width	-	-	-	-	-	-	-	-	63	3.5-9.5	4.7	1.33
Head Width	-	-	-	-	-	-	-	-	63	2.0-7.0	3.0	1.00

The relationship between various linier variables such as DML, TL, TCL, FW, FL, MW, HW , AL and between TWt and MWt were worked out employing the formula of simple linear regression to understand the relationship of different linear parameters and compare it between the medium and dwarf forms

$$y = a + b. x$$

where y - is the dependent variable , x - the independent variable, and a and b are constants.

Sokal and Rohlf (1981) explains about the preferability of this type of regressions when correlating continuous variables distributed according to the normal distribution. Confidence intervals were calculated at 95% level for the slope coefficient b. Hypothesis for statistical significance has been tested for both the zero slope parameter and slope parameter equal to 3 and 1 respectively using the p-value approach. The regression slope of Medium and Dwarf forms were compared by ANCOVA to determine whether any significant variation exist between them. Wherever the regression lines were parallel and the slopes were equal, then elevation (y-intercept) was compared to study the significance, as these lines have demonstrated to have no significant statistical difference in slope. The correlation coefficient (r) was determined to know the strength and pattern of association between the two variables.

2.3 Results

2.3.1 Length Weight Relationship

The regression equations derived for the dwarf and medium forms along with the goodness of fit, slope confidence interval and P values have been presented in Table 2.2. The ANOVA and the ANCOVA of the parameters analysed has been furnished in the Appendix-I. The DML has been highly correlated with the variable TWt and MWt in both the dwarf and medium form. The DML–TWt curve of dwarf form has a low slope value of less than the expected cubical value of '3' resulting in a highly significant negative allometric growth curve. Whereas, the medium form has a slope value of greater than '3' resulting in a significant positive allometric growth curve (Figure 2.1). This pattern of growth with negative allometry was also observed for the dwarf female, male, and positive allometry for the medium female. However, the medium males grew isometrically (Figure 2.2 & 2.3). The DML–TWt regression curve of dwarf and medium form differed significantly between them ($F=51.89$, $P < 0.05$). Similarly the DML–TWt regression curve between the females of dwarf and medium ($F=56.54$, $P < 0.01$) and males of dwarf and medium ($F=8.82$, $P < 0.01$) were also significantly different from each other indicating a significant variation in the growth pattern between the plastic phenotypes. In variation to the DML-TWt curve, the DML– MWt curve of both dwarf and medium forms has a low slope value than the DML-TWt curve resulting in the highly significant negative allometric growth for both the phenotypes (Figure 2.4).

Table 2.2. Inter population Length Weight relationship of Dwarf and Medium forms of *Sthenoteuthis oualaniensis* in the south west coast of India. D-Dwarf, M- Medium, R^2 – Correlation coefficient. a L^b - equation, CI (b) – Confidence interval of the co efficient b (95%),

Curve	Form	n	R^2	a L^b	CI (b)	P (b)	Growth
DML- TWt	D	450	0.88	0.0613 x $^{2.67**}$	(2.6, 2.8)	0.00	- Allomet
	M	565	0.97	0.0379 x $^{3.05**}$	(3.0, 3.1)	0.04	+ Allomet
DML–FemTWt	D	373	0.84	0.0717 x $^{2.63**}$	(2.5, 2.7)	0.00	- Allomet
	M	328	0.97	0.0296 x $^{3.13**}$	(3.1, 3.2)	0.00	+ Allomet
DML–MaleTWt	D	77	0.86	0.0699 x $^{2.57**}$	(2.4, 2.8)	0.00	- Allomet
	M	237	0.95	0.0466 x $^{2.98**}$	(2.9, 3.1)	0.59	Isomet
DML–MWt	D	145	0.94	0.0546 x $^{2.42**}$	(2.3, 2.5)	0.00	- Allomet
	M	196	0.97	0.041 x $^{2.82**}$	(2.7, 2.9)	0.00	- Allomet
DML–FemMWt	D	105	0.89	0.0618 x $^{2.37**}$	(2.2, 2.5)	0.00	- Allomet
	M	102	0.97	0.0383 x $^{2.83**}$	(2.7, 2.9)	0.00	- Allomet
DML–MaleMWt	D	40	0.86	0.0342 x $^{2.62**}$	(2.3, 3.0)	0.03	- Allomet
	M	94	0.94	0.0289 x $^{2.96**}$	(2.8, 3.1)	0.52	Isomet

** b coefficient is highly significant at o level; P(b)- Hypothesis testing for slope parameter equal to 3 using the p-value approach

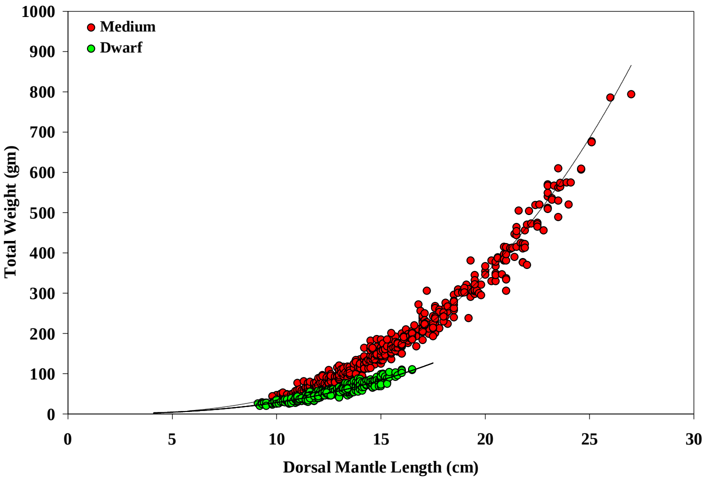

Figure 2.1. Comparison of the relationship between the dorsal mantle length (DML) and total weight (TWt) of Medium and Dwarf form

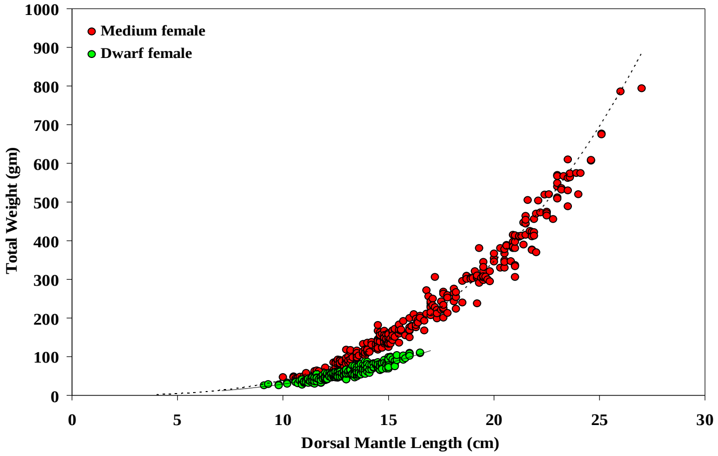

Figure 2.2. Comparison of the relationship between the dorsal mantle length (DML) and total weight (TWt) of the females of Medium and Dwarf form

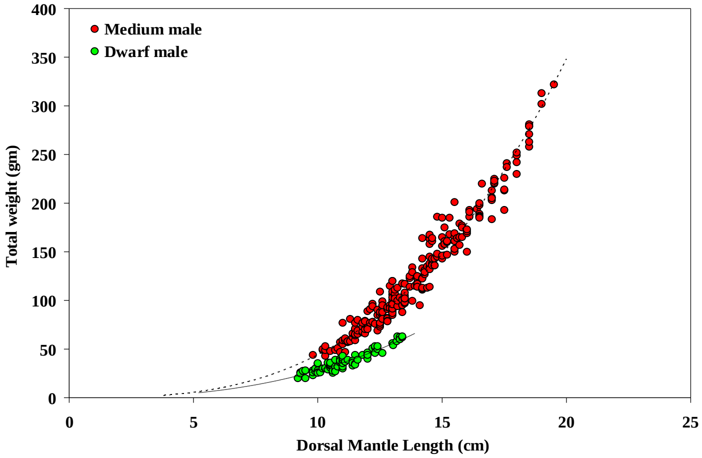

Figure 2.3 . Comparison of the relationship between the dorsal mantle length (DML) and total weight (TWt) of the males of Medium and Dwarf form

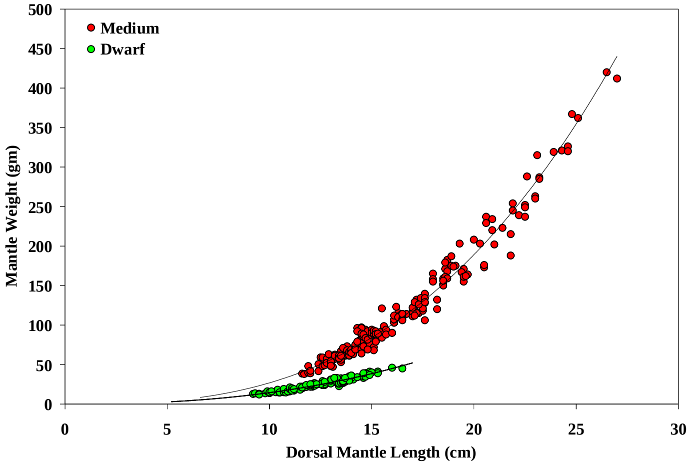

Figure 2.4. Comparison of the relationship between the dorsal mantle length (DML) and mantle weight (MWt) of Medium and Dwarf form

This type of growth pattern persists between the females of dwarf and medium forms (Figure 2.5). Similarly, dwarf male grew with negative allometry, whereas, the medium male grew isometrically (Figure 2.6). However, the slope values of male dwarf and medium were distinctively higher than its female. The relationship between the DML–MWt curve of dwarf and medium form were significantly different (F=31.32, $P < 0.01$) with relatively a low slope value in the dwarf when compared to the medium form (Figure 2.7). Likewise, the DML–MWt regression lines between the females of dwarf and medium were also highly significant (F=16.92, $P < 0.01$). However, the difference between the DML – MWt curve of dwarf and medium male was not significant (F=3.17, P = 0.07).

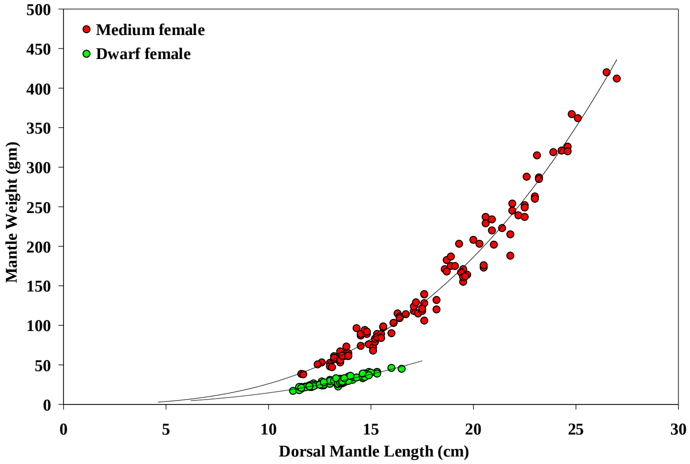

Figure 2.5. Comparison of the relationship between the dorsal mantle length (DML) and mantle weight (MWt) of the females of Medium and Dwarf form

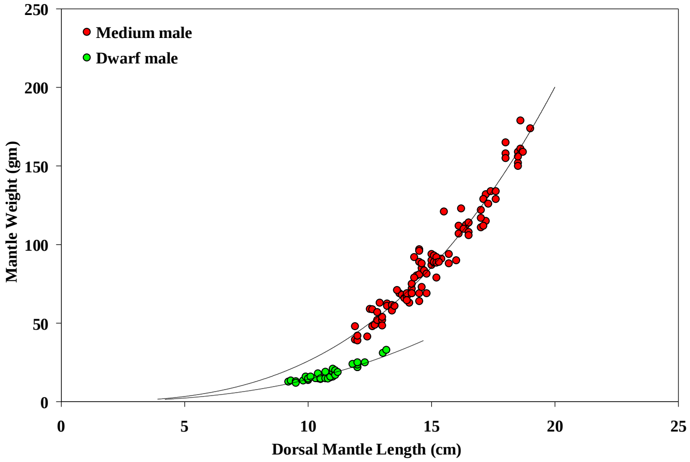

Figure 2.6. Comparison of the relationship between the dorsal mantle length (DML) and mantle weight (MWt) of the males of Medium and Dwarf form

2.3.2 Linear Morphometric relationships

The equations for the relationship between the linear variables such as DML- TL, DML-TCL, DML-FW, DML-MW, DML-HW, DML-AL1, DML-

AL2, DML-AL3, DML-AL4, FL-FW , TL-TCL and TWt- MWt along with its goodness of fit, slope confidence interval and P values were derived for both the dwarf and medium form and presented in Table 2.3. The ANOVA and the ANCOVA of the parameters analysed has been furnished in the Appendix-II.

Table 2.3. Inter population Morphometric relationship of Dwarf and Medium forms of *Sthenoteuthis oualaniensis* in the south west coast of India. D-Dwarf, M- Medium, R^2 – Correlation coefficient. a+bx – equation, CI (b) – Confidence interval of the co efficient b (95%).

Curve	Form	n	R^2	a+ b x	CI (b)	P (b)	Growth
DML- TL	D	136	0.8	1.920 +1.159**x	(1.06, 1.26)	0.00	+ Allometric
	M	180	0.93	- 4.949 +1.804**x	(1.73, 1.88)	0.00	+ Allometric
DML– TCL	D	136	0.86	- 0.116 +0.352**x	(0.33, 0.38)	0.00	- Allometric
	M	180	0.89	- 2.889 +0.658**x	(0.62, 0.69)	0.00	- Allometric
DML- FW	D	76	0.94	- 0.944 +0.767**x	(0.72, 0.81)	0.00	- Allometric
	M	102	0.95	1.748 +0.760**x	(0.73, 0.79)	0.00	- Allometric
DML–MW	D	24	0.56	0.403 +0.305**x	(0.19, 0.42)	0.00	- Allometric
	M	63	0.91	- 0108 +0.356**x	(0.33, 0.38)	0.00	-Allometric
DML–HW	D	22	0.57	- 0.497 +0.207**x	(0.13, 0.29)	0.00	- Allometric
	M	63	0.83	- 0.456 +0.255**x	(0.23, 0.28)	0.00	- Allometric
DML- AL1	D	29	0.91	- 0.498 +0.428**x	(0.39, 0.48)	0.00	- Allometric
	M	69	0.89	0.083 +0.422**x	(0.39, 0.46)	0.00	- Allometric
DML- AL2	D	29	0.75	0.305 +0.453**x	(0.35, 0.56)	0.00	- Allometric
	M	69	0.89	0.024 +0.525**x	(0.48, 0.57)	0.00	- Allometric
DML- AL3	D	29	0.68	0.151 +0.511**x	(0.37, 0.65)	0.00	- Allometric
	M	69	0.90	0. 917 +0.520**x	(0.48, 0.56)	0.00	- Allometric
DML- AL4	D	29	0.78	- 0.082 +0.462**x	(0.37, 0.56)	0.00	- Allometric
	M	69	0.92	0.470 +0.467**x	(0.44, 0.50)	0.00	- Allometric
FL- FW	D	23	0.81	2.909 +1.259**x	(0.99, 1.53)	0.06	Isometric
	M	67	0.92	2.202 +1.745**x	(1.62, 1.82)	0.00	+Allometric
TL- TCL	D	136	0.69	0.316 +0.242**x	(0.21, 0.27)	0.00	-Allometric
	M	180	0.87	-0.699 +0.349**x	(0.32, 0.37)	0.00	- Allometric
TWt-MWt	D	145	0.90	1.503 +0.468**x	(0.44, 0.49)	0.00	- Allometric
	M	196	0.98	4.959 +0.530**x	(0.52, 0.54)	0.00	- Allometric

** b coefficient is highly significant; P (b)- Hypothesis testing for slope parameter equal to 1 using the p-value approach

The relationship between the variables such as DML- TL, DML-TCL, DML-FW, DML-AL1, FL-FW and TWt- MWt of dwarf forms were having high degree of correlation with R^2 greater than 0.8. On the other hand the correlation between the other variables such as DML-HW, DML-MW, DML-AL2, DML-AL3, DML-AL4 and TL-TCL were though correlated well, found to vary between 0.56 to 0.78. However, in the medium form, relationship between the variables were having high degree of correlation with R^2 greater than 0.8. The regression slopes of all the set of parameters were highly significant indicating the influence of one variable on other. The P value test for the hypothesis equal to one indicates the allometric pattern of growth amoung the variables of both medium and dwarf form except the relationship between FL – FW of dwarf forms. This was not significantly different from one and considered to have grown isometrically.

The dwarf and medium regression lines of DML–TL and DML-TCL differed significantly between the forms (TL: F=52.32, P < 0.01; TCL: F=59.63, P < 0.01) with medium having higher slope and lower intercept than the dwarf (Figure 2.7 and 2.8).

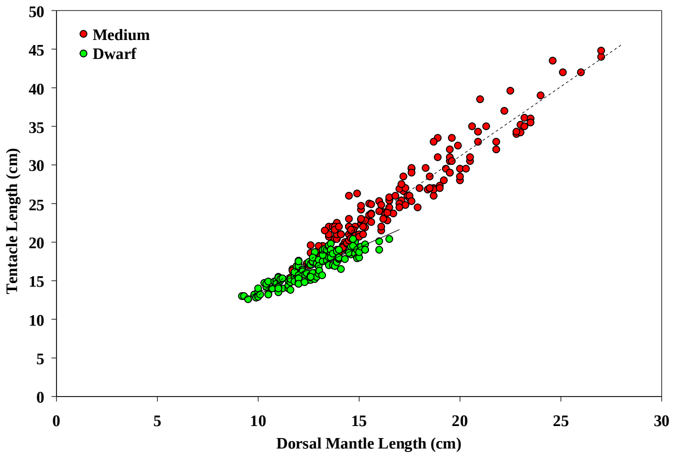

Figure 2.7. Comparison of the relationship between the dorsal mantle length (DML) and tentacle length (TL) of Medium and Dwarf form.

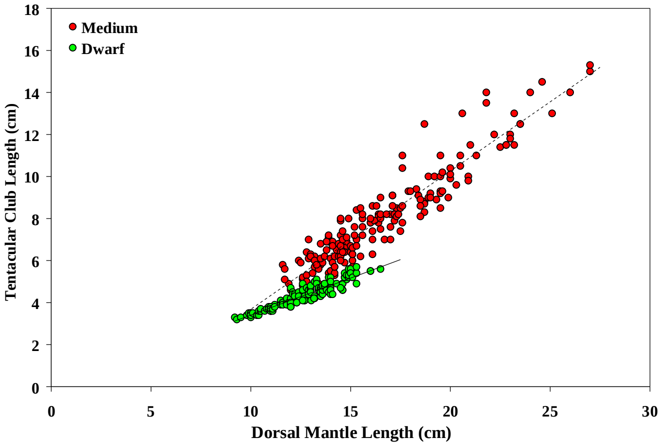

Figure 2.8. Comparison of the relationship between the dorsal mantle length (DML) and tentacular club length (TCL) of Medium and Dwarf form

The difference in the size of the TCL between the dwarf and medium forms were clearly evident in the scatter diagram (Figure 2.8) and was highly significant (F=59.63; P < 0.01). The dwarf TCL was relatively smaller in size then the medium forms. The TCL:TL has a size ratio of 1:4 for dwarf and 1: 3 for the medium form. Dwarf form has 12 % lesser fin width than the medium form of same fin length with length width ratio of 1: 1.6 for dwarf and 1: 2 for medium. Besides the dwarf fin posterior margin is more concave in shape than the medium forms (Figure 2.9).

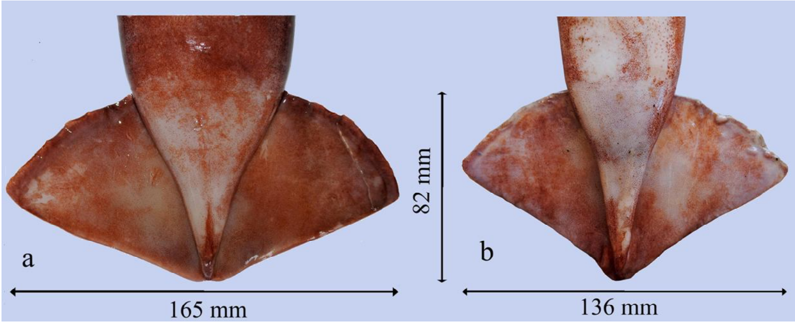

Figure 2.9. Comparison of the female *Sthenoteuthis oualaniensis*, fin shape between (a) Medium and (b) Dwarf form.

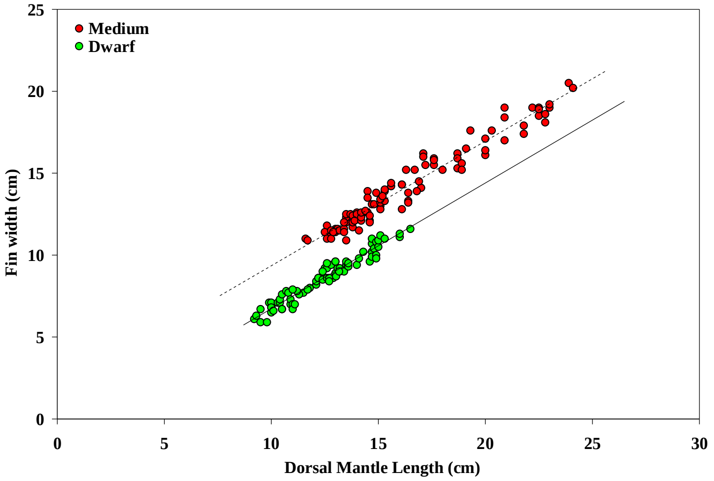

Figure 2.10. Comparison of the relationship between the dorsal mantle length (DML) and fin width (FW) of Medium and Dwarf form

The FW (Figure 2.10), MW (Figure 2.11), HW (Figure 2.12) and ALs of medium form has a higher slope and intercept than the dwarf form in relation to the DML and significantly differs in intercept between them (FW: F=830.87, $P < 0.01$; MW: F=102.53, $P < 0.01$; HW: F=49.12, $P < 0.01$; AL1: F=27.29.87, $P < 0.01$; AL2: F=22.31, $P < 0.01$; AL3: F=46.01, $P < 0.01$; AL4: F=40.09, $P < 0.01$). The armature were in the ascending order of 1<4<2<3 both in dwarf and medium forms (Figure 2.13 and 2.14) with 3rd arm having higher slope followed by 2, 4 and 1. Regression line fitted between the TWt and MWt for both the dwarf and medium forms (Figure 2.15) showed that the medium form has a higher slope and elevation than the dwarf form and were significant at the intercept level (F=45.62; $P < 0.01$).

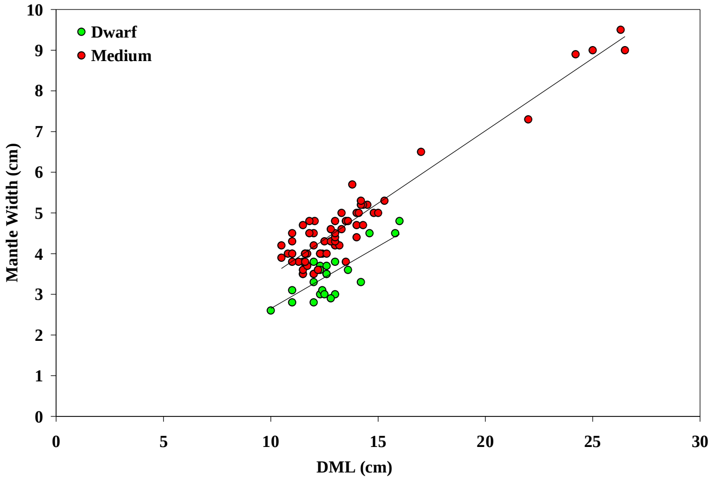

Figure 2.11. Comparison of the relationship between the dorsal mantle length (DML) and mantle width (MW) of Medium and Dwarf form

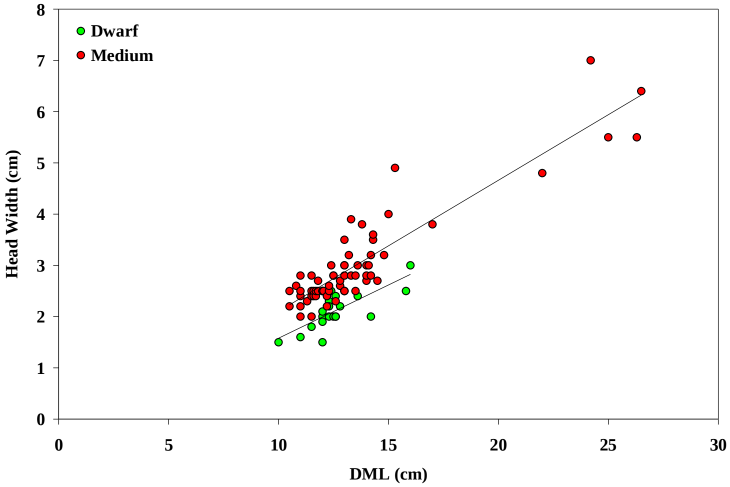

Figure 2.12 . Comparison of the relationship between the dorsal mantle length (DML) and head width (HW) of Medium and Dwarf form

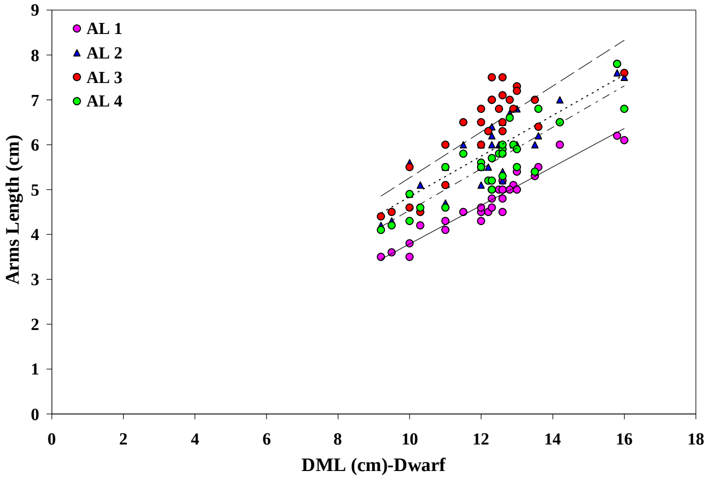

Figure 2.13. Comparison of the relationship between the dorsal mantle length (DML) and Arms length (AL 1-4) of dwarf form

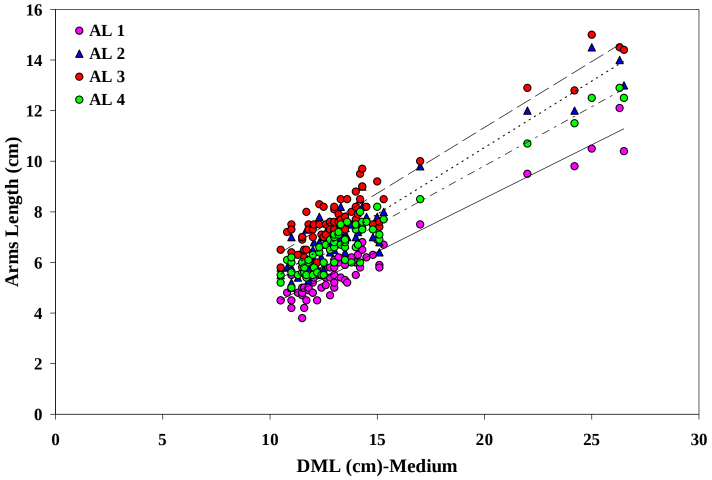

Figure 2.14. Comparison of the relationship between the dorsal mantle length (DML) and Arms length (AL 1-4) of medium form

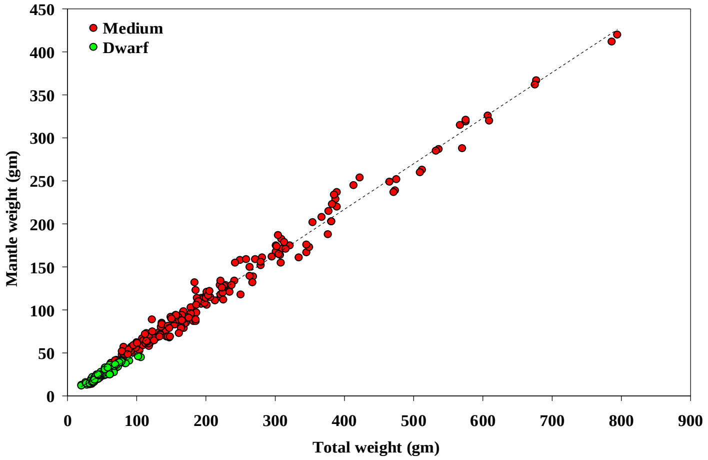

Figure 2.15. Comparison of the relationship between the Total weight (Twt) and Mantle weight (Mwt) of dwarf and medium form.

2.3.3 Sexual Dimorphism

Both in the dwarf and medium form, female attain larger size than the males. In the medium form, female were in the length group of DML 10 to 27 cm with the mean value of 16.61 cm, wherein, males were in the length group of DML 9.8 to 19.5 cm with the mean value of 13.97 cm (Figure 2.16).

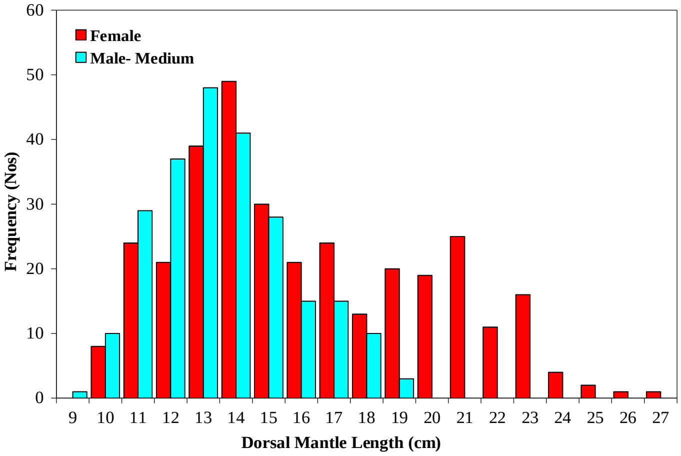

Figure 2.16. Sex wise length frequency chart of the medium form

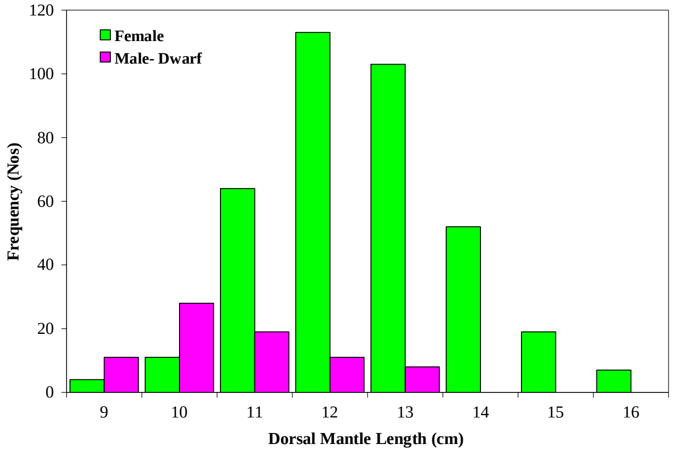

Figure 2.17. Sex wise length frequency chart of the dwarf form

Similarly, dwarf females were in the length group of DML 9.1 to 16.5 cm with the mean value of 12.92 cm and the males were in the length group of DML 9.2 – 13.4 cm with the mean value of 11.05 cm (Figure 2.17). Females tend to grow faster than the males with higher slope value in both the dwarf and medium form to attain the larger size than the male. Tentacle, Tentacular club and Fin width were also larger in female than the male for both the dwarf and medium form in relation to DML.

The sex wise regression equations derived for the dwarf and medium forms along with the goodness of fit, slope confidence interval and P values has been presented in Table 2.4 and 2.5. The ANOVA and the ANCOVA of the parameters analysed has been furnished in the Appendix-III and IV. In dwarf form females have higher slope and lower intercept than the male in both the DML-TL and DML -TCL regression.

In Dwarf the DML-TL regression lines of the sexes were not significant, both in the slope and intercept level, whereas, the difference between the DML-TCL regression line of male and female were significant at the intercept level

(F=7.51, $P < 0.01$). In medium form the male and female regression lines of DML- TL and DML -TCL were parallel with each other with males having lower slope and intercept value than the medium and significantly different from the female at the intercept level. (TL: F=8.05, $P < 0.01$; TCL: F=17.14, $P < 0.01$).

Table 2.4. Sexual dimorphic relationship of Dwarf forms of *Sthenoteuthis oualaniensis* in the south west coast of India. R^2 – Correlation coefficient. a+bx – equation, CI (b) – Confidence interval of the co efficient b (95%).

Curve	Sex	n	R^2	a+ b x	CI (b)	P (b)
DML–TWt	F	373	0.84	$0.072\ x^{2.63**}$	(2.5, 2.7)	0.00
	M	77	0.86	$0.069\ x^{2.57**}$	(2.4, 2.8)	0.00
DML–TL	F	99	0.65	$2.437 +1.125^{**}x$	(0.96, 1.29)	0.14
	M	37	0.65	$5.136 +0.848^{**}x$	(0.64, 1.06)	0.15
DML–TCL	F	99	0.74	$0.036 +0.342^{**}x$	(0.30, 0.38)	0.00
	M	37	0.90	$1.057 +0.239^{**}x$	(0.21, 0.27)	0.00
DML- FW	F	47	0.87	$- 0.415 +0.731^{**}x$	(0.65, 0.81)	0.00
	M	29	0.74	$- 0.108 +0.683^{**}x$	(0.52, 0.84)	0.00
TL- TCL	F	99	0.45	1.250 +0.192**x	(0.14,0.23)	0.00
	M	37	0.60	-0.990+1.860**x	(0.13,0.23)	0.00

Table 2.5. Sexual dimorphic relationship of Medium forms of *Sthenoteuthis oualaniansis* in the south west coast of India.

Curve	Sex	n	R^2	a+ b x	CI (b)	P (b)
DML–TWt	F	328	0.97	$0.0296\ x^{3.13**}$	(3.1, 3.2)	0.00
	M	237	0.95	$0.0466\ x^{2.98**}$	(2.9, 3.1)	0.59
DML–TL	F	94	0.92	$- 4.039 +1.769^{**}x$	(1.66, 1.88)	0.00
	M	86	0.90	$- 3.944 +1.716^{**}x$	(1.60, 1.84)	0.00
DML–TCL	F	94	0.87	$- 2.135 +0.627^{**}x$	(0.58, 0.68)	0.00
	M	86	0.87	$- 2.651 +0.627^{**}x$	(0.57, 0.68)	0.00
DML- FW	F	63	0.95	$2.346 +0.735^{**}x$	(0.69, 0.78)	0.00
	M	39	0.91	$2.193 +0.713^{**}x$	(0.64, 0.78)	0.00
TL- TCL	F	94	0.84	- 0.170 +0.334**x	(0.30, 0.36)	0.00
	M	86	0.85	-0.737 +0.344**x	(0.31, 0.37)	0.00

** b coefficient is highly significant; P (b)- Hypothesis testing for slope parameter equal to 1 using the p-value approach

The TL-TCL regression line of male and female were also parallel with each other. However, female were having higher slope and intercept value than the male for both the dwarf and medium form and were significantly different from the male at the intercept level (Dwarf: F=19.1, $P < 0.01$; Medium; F=6.57, $P < 0.01$). Similarly the DML-FW regression line of medium male and female were of parallel in nature with significant difference between them (F=17.00, $P < 0.01$), while, the same was not parallel and not significant between the sexes in dwarf.

2.4 Discussion

2.4.1 Length Weight Relationship

The scatter diagram plotted for the length weight data collected on the medium and dwarf form clearly differentiate the presence of two population with different growth trend. It has been seen that medium form has grown in weight (b 3.05) with positive allometry, whereas the dwarf form has grown with (b 2.67) negative allometry. The regression line fitted speaks of the trend in the growth of these populations. The sexes of these populations were also clearly distinct from each other in their growth in weight. Females of the medium form had a 'b' value of 3.13 resulting in the positive allometric growth, whereas the dwarf had a 'b' value of only 2.61 with negative allometric growth. This indicates that medium has a different weight than dwarf at the same mantle length, and that medium increases in weight more per unit gain in mantle length than the dwarf form. Among the males, the medium form has grown isometrically with the 'b' value of 2.98, while the dwarf has grown with negative allometry (b 2.6). The 'b' value derived for the *S. oualaniensis* medium male and female was consistent with the findings of Suzuki et al.(1986) in Hawaiian waters. He has given the b value as 3.15 for female maturing between 158 to 205 mm DML and has reported that the curve for males is as essentially the same as females. This has also been corroborated by Siriraksophon et al. (2000) with 3.052 for female and 2.943 for male of the 9.0 cm to 25.0 cm DM length group. The values compare well with the value of 3.1056 obtained for the medium female and male together by Mohamed et al (2006) in the Arabian sea. However, in contrast, Xinjun et al (2007) has obtained a 'b' value of 2.9 for females and 2.58 for males of the *S. oualaniensis* in the northwest Indian Ocean. Perhaps different forms of *S. oualaniensis* could have been included while arriving at this b value, as the value obtained was for the sample with the DML range of 11.0 to 61.0 cm.

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The regression line fitted between the DML and MWt explains the growth in MWt as allometrically negative in both the medium (b 2.8) and dwarf forms (b 2.4). Similar trend follows in the females of medium (b 2.8), dwarf (b 2.4), and in the dwarf male (b 2.6). However, the growth in MWt of the medium male was isometric with its 'b' value as 2.96. It was found that the dwarf and medium form MWt, particularly of the female does not grow similarly with the TWt. Perhaps the female reproductive organs of the squid might be growing disproportionately more in weight resulting in less growth in weight to mantle. The results of this study agree with some of the observations on the use of mantle muscle as an energy source during gonadal development in the cuttlefish, *Sepia dollfusi* by Gabr et al. (1999). In *Lologo forbesi*, when effects of *DML*, month and nutritional state are factored out, there remained a negative effect of gonad size on mantle weight in females, with lighter mantles associated with large gonads, appearing to imply mobilisation of mantle tissue to produce ovary tissue (Smith et al.,2005). Similarly, the mantle mass of female *Illex argentinus* decreases in relation to ML with maturity (Hatfield et al., 1992; Rodhouse and Hatfield, 1992; Clarke et al.,1994). *Loligo opalescens* apparently utilizes mantle protein as a food reserve during spawning; both mantle thickness and width become significantly reduced in spent squid (Fields, 1950 and 1965; Evans, 1976). However, the MWt of the male dwarf and medium forms grows in near uniformity with the total weight, indicating possible proportionate growth of the MWt and male reproductive organs.

2.4.2 Linear Morphometric relationships

The growth of TL and TCL was directly proportional to the growth of the DML in both the dwarf and medium forms. However, the TL has grown with positive allometry in both the dwarf and medium forms while TCL has grown with negative allometry in relation to DML. However, Zuyev et al (2002) has observed that arms and tentacles are characterized by negative allometric growth in the large-sized adult with the DML from 36–40 and 60–65 cm. Perhaps, the growth difference may be due to the distribution of the large form in the comparatively cooler higher latitude away from the equator when compared to the warmer lesser latitude medium form. Such trends have been found in other species sampled over large geographical distances. Cooler zone, bigger, slower-growing squid vs warmer zone, smaller, faster-growing squid have also been observed in *Todarodes sagittatus* populations from the north Atlantic (Rosenberg et al., 1981) compared to tropical Africa (Arkhipkin et al., 1999). Arkhipkin (1996) also found geographical differences in size and growth rates in different populations of *Illex coindetii* in tropical waters off western Africa.

The relationship of DML with the TL and TCL was not similar between the dwarf and medium forms. The TL and TCL of medium form has grow longer and faster than the dwarf form of same DML resulting in the divergence in the growth trend of medium form from the dwarf. The regression of TCL against TL of dwarf and medium form also shows similar trend in the relationship. It is understood that the length of the TL and TCL of both the medium and dwarf form were near equal in the smaller squid with a DML of below 12 cm. The divergence happens during the course of the squid growth. The TCL:TL has a ratio of 1:4 for dwarf and 1: 3 for medium. This indicates the smaller size of the TCL in the dwarf form and readily differentiate it from the comparatively larger medium forms. The size of the TL and TCL has been used by Kashiwada and Recksiek (1978) to differentiate the sub population of the *Loligo opalescens.*

The fin of the dwarf form was distinctively different from the medium form in shape and size. A comparison between the fin of dwarf and medium form of same fin length to its width indicates that the dwarf form was having 12 % lesser width than the medium one. This characteristic shortness of the fin width than the medium form fin of the same fin length and the relative concaveness of the posterior margin of the fin of the dwarf form could be used as the distinct morphological trait to differentiate the dwarf form from the medium form. The regression lines fitted between the DML and FW for the dwarf and medium forms were parallel to each other with sufficient gap in between through out its growth exhibiting clear difference in the growth trend of dwarf fin from the medium. The length width ratio of 1: 1.6 for dwarf and 1: 2 for medium also indicates the distinctiveness of the fin size between dwarf and medium forms. The growth of MW and HW in relation to the DML is directionally proportional with negative allometry and parallel between dwarf and medium forms. The growth of MW and HW was significantly different between the dwarf and medium form. This has been supported by Nesis (1977), who has found distinctions in the proportions of the fin and mantle size between the dwarf, early-maturing form without dorsal photophore and a late-maturing form with dorsal photophore.

The scatter plot and the regression line fitted for DML against AL 1, AL 2, AL 3 and AL 4 indicates that the growth of the arm lengths are directionally proportional to the DML with negative allometry and significantly different between the dwarf and medium. However the regression line of these relationship shows parallel relationship between dwarf and medium form indicating similar growth trend with difference in the size of the arm between dwarf and medium form. Arms were large, strong and compressed with the third pair strongly keeled and arranged in the ascending order of 1<4<2<3 in both the dwarf and medium form as reported by Nateewathana et al. (2002). The scatter plot and the regression line fitted for MWt against the TWt indicates that the mantle of the medium form was heavier and more thicker than the dwarf form mantle of the same DML.

2.4.3 Sexual Dimorphism

Sexual dimorphism in *Sthenoteuthis* is rather well expressed in body size with females of both the dwarf and medium form were distinctively larger than males. Females grew faster in both the dwarf and medium form to attain the larger size in Weight, Tentacle, Tentacular club length and Fin width than the male in relation to DML. Higher growth rates in females have been demonstrated in a number of cephalopods, e.g. *Berryteuthis magister* (Oegopsida: Gonatidae) (Natsukari et al., 1993), *Illex coindetii* (Oegopsida: Ommastrephidae) (Arkhipkin, 1996), and *Loliolus noctiluca* (Myopsida: Loliginidae) (Dimmlich and Hoedt 1998). Moreover, in several species females reach a much larger size than males, which is indirect evidence of faster growth in females, e.g. in *Rossia macrosoma* (Sepiolioidea: Sepiolidae), *Eledone cirrhosa* (Octopoda: Octopodidae), and *Ocythoe tuberculata* (Octopoda: Ocythoidae).

In dwarf though the growth of Tentacle and Tentacular club length was similar in both the male and female around 10 cm DML , the trend diverges with female growing faster and larger than the male subsequently leading to difference between male and female. Changes in the growth trajectories over a small DML range has also been observed by Shea and Vecchione (2002) in three species of oegopsid squids. However, in the medium form the difference in the growth of the Tentacle and Tentacular club length of female and male in relation to DML is parallel and persists more or less evenly through out the growth with female being larger in length than the male. The growth of TCL against TL is parallel between male and female of both the dwarf and medium form and significantly different between the sexes with female having a higher TCL for the same length of TL than the male indicating a dimorphic trend in the growth. Bello and Piscitelli (2000) showed that females of *S.orbignyana* have longer tentacular clubs than males and ingest larger quantities of food than males at any given size, and hypothesised the existence of a cause–effect relationship between sex-related club size and growth. Similarly, both the dwarf and medium female had a wider fin width than the male of the same DML . However, the difference was significant only for medium form. The present results corroborate Mangold-Wirz’s (1963) hypothesis and Ragonese and Jereb’s (1991) results, that is to say, in *S. oualaniensis*, growth rates of females are higher than those of males

Though the dwarf form was clearly distinguishable from the medium form in its growth and morphological traits, the shape and size of its fin width may hold the key to differentiate the dwarf population from the medium form of *Sthenoteuthis oualaniensis* in the southwest coast of India on field.

Chapter 3

Growth and Mortality

Contents

3.1 Introduction

Significant advances have been made in the last decade with regard to our understanding of the rates and form of squid growth. These are short-lived animals characterized by fast growth with year-round spawning strategy. Their populations therefore usually consist of several seasonal cohorts or groups (Caddy, 1991), which helps to avoid drastic decreases in abundance by increasing the chances of survival of at least one of the most numerous cohorts (as, for example, in winter-spawned *Illex illecebrosus*, O'Dor and Coelho, 1993). Spawning, development, and growth of these sub-annual cohorts occur in different seasons and different environmental conditions (Nigmatullin, 1989). The novel form of growth along with short lifespans and resulting rapid turnover in populations make squids unique marine models for studying the relationship between growth and population dynamics.

Although our knowledge has increased in regard to squid growth, questions remain as to how applicable any model of growth may be if a squid grows under differing environmental conditions resulting in different plastic phenotypic forms as in the case of most of the oceanic squids including *Sthenoteuthis oualaniensis*. Research continues to reveal that squid growth can be greatly modified by temperature or seasons of hatching (Rodhouse & Hatfield 1990; Jackson & Choat 1992; Forsythe 1993; Brodziak & Macy 1996; Dawe & Beck 1997; Jackson et al. 1997; Hatfield 2000; Forsythe et al., 2001) as well as food supply (Jackson & Moltschaniwskyj, 2001). Forsythe (1993) was instrumental in developing theories about the influence of temperature on squid growth. His working hypothesis based on laboratory data suggest that relatively small changes in temperature (especially during the juvenile exponential growth phase) can have dramatic consequences on adult body size with a small increase in temperature dramatically increasing growth rate.

However, our understanding of the causes of plasticity in squid growth is still far from complete, though the phenotypic plasticity of cephalopods is widely accepted as one of their main characteristics (Boyle and Boletzky, 1996). Field studies on *Lolliguncula brevis* in the Gulf of Mexico (Jackson et al., 1997) and *Loligo gahi* on the Patagonian Shelf (Hatfield, 2000) tested the Forsythe (1993) hypothesis. In both of these studies, the influence of seasonal temperature variation on the growth of different cohorts was assessed. Both of these studies support Forsythe's hypothesis (1993) and show that squid hatching in warmer periods do ‘out grow’ their cooler-hatched counterparts. Forsythe et al. (2001) furthermore unequivocally demonstrated this same temperature effect on growth using temperature-controlled grow-outs of known age squid hatched and grown in captivity. Grist & des Clers (1998) extended the Forsythe hypothesis and developed a model that explains how differing seasonal growth rates could affect the size distribution in populations of *L. gahi*. It is also important to consider how seasonal differences in growth may reflect important parameters in squid growth models.

Existing studies on the growth function of cephalopods are not consistent, and exponential, linear, and asymptotic functions with and without seasonal oscillation (Masuda et al., 1998; Pauly, 1998; Semmens et al., 2004; Miyahara et al., 2006). For adult squid, nonasymptotic (Jackson et al., 2000), linear, or quasi-linear growth has been postulated or can be identified in the literature (e.g. Masuda et al., 1998; Yatsu, 2000). This has been attributed to the paedomorphic (subadult maturation) life history of cephalopods (Rodhouse, 1998; Hatfield, 2000). The information on the linear growth of cephalopod is collected by counting the growth ring of the hard parts such as statolith. These rings are dark, proteinaceous in nature and formed in response to feeding activity that often has a regular diel basis (Radtke, 1983).

Factors such as temperature (Durholtz and Lipinski, 2000; Villanueva, 2000; Chung and Lu, 2005), age (Bettencourt and Guerra, 2000, 2001), body size (Hussy, 2008a), activity level, oxygen availability, pH (Morris, 1991), and feeding level (Spratt, 1979; Hussy, 2008b) are all known to influence the accretion process of statoliths and their legibility. Oxygen limitation leads to blurred areas without rings towards the margins of the statoliths of older squid, so making it possible to underestimate the real age of squid (Pauly, 1998). Knowing that oxygen levels do influence the formation of statoliths (Morris, 1991; Lipin ski, 1993; Pauly, 1998), we assume that frequent visits by the squid to the Oxygen Minimum Layer (Bazzino et al., 2010) can certainly affect the formation and readability of their statoliths, i.e. that such behavior may result in missing statolith rings in bleached areas (Pauly, 1998). The consequence would be a serious underestimation of the real age and overestimation of the growth rate of the squid determined from statolith analysis. Indeed, this may be the very reason why “linear” growth curves often emerge from size at age data based on age readings presumed to be daily.

Growth curves may be interpreted as having two phases (i) increasing growth rates in the juvenile phase, mimicking ‘logarithmic growth and (ii) declining growth rates as the asymptotic is approached. Owing to mortality and possibly to paedomorphosis and the aging bias, the second phase, however, is often not visible in field sample specimans, although it is apparent in the growth curves of cephalopod raised in captivity (Forsythe and Van Heukelem, 1987). Besides, in many squids and other cephalopods, a strong post spawning mortality eliminates many of the older specimens, leaving the initial linear or log-linear segments of growth curves, to which almost any function can be fitted (Bigelow 1994). This method also requires special expensive equipment and a lot of manpower.

The other method of length based studies are mainly based on Modal Progression Analyses (MPA) and use the von Bertalanffy growth function (VBGF). This methods construct a growth curve by connecting modal or mean length values for successive time intervals. Verrill (1882) first used this modal analysis method for estimating growth rates of cephalopods. The use of von Bertalanffy growth curves in conjunction with cephalopods in general and squid in particular is fraught with controversy, as it was earlier concerning fish. However, the asymptotic growth is an inescapable prediction model for the growth, as it is the net result of a volume to surface relation (von Bertalanffy, 1934,1951). Moreover, if oxygen supply limits the growth of the squid and other cephalopods, then their growth should confirm to an asymptotic curve, ie, either to the familiar von Bertalanffy or to one of its variants.

Many of those working with the cephalopods have argued, on the other hand that the von Bertalanffy growth model cannot accommodate the variety of growth patterns observed in cephalopods. Besides, it is stated that the length based methods have been found useful only in cases where one or several well–defined and short term spawning events take place in the investigated area with no changes in population structure due to migration. This situation is very rare in practice and in most cases, the spawning of the species extends throughout the year with peaks lasting 1–2 months. Besides, several generations may occur in one area simultaneously and the general growth curve obtained by connecting modal or mean length values for successive generation may not reflect the growth rates of each generation. Another problem is the interpretation of length frequency modes. Growth curves may vary considerably in shape time intervals depending on the interpretation of modal sizes. However, Pauly (1985) have shown the variety of growth curve that can be generated with a von Bertalanffy growth curve incorporating seasonal growth oscillations. It is therefore argued that use of length frequency data to estimate the rates of squid growth should be treated with caution, and verification of these data using one of the direct methods is necessary (Jereb *et al*., 1991).

Natural mortality includes all sources of mortality not related to fishing activities and is one of the most difficult parameters to quantify since it may vary due to age-specific predation including cannibalism, disease, spawning stress, starvation, senescence and inter or intra specific competition. Although the evidence is still sketchy, *S. oualaniensis* is probably important in the diet of many, if not most, large fishes, marine mammals and many sea birds that forage within its habitat. Perhaps a more satisfying rationale for studying the natural mortality of squid has been given by Clarke (1983) who has estimated that sperm whales alone consume >100 million tonnes of squid annually. One other major predator for which quantitative estimates are possible is the squid itself (Mohan,1981). They meet one third of their maintenance food requirement by cannibalism. Post-spawning mortality is an another important contributor for the reduction of the cephalopod population.

It is generally assumed that post-spawning mortality in cephalopods is very high. Roper et al. (1984) have reported that many species die after spawning , but the phenomenon is apparently not universal. Post-spawning mortality of squids of both sexes has been established in the case of the Japanese flying squid *Todarodes pacificus*, and the Opalescent inshore squid *Loligo opalescens*, while there is strong evidence that some species may spawn more than once (Juanico, 1983). Cuttlefish lives for approximately two years and exhibits mass mortality of adults apparently at the end of the spawning period, occurs on the Atlantic coast (Boletzky, 1983). The conventional practice in stock assessment work for short-lived species such as squid species of commercial importance has been to assume a single indicative value for adult death rate: the constant natural mortality rate (Caddy, 1996). Estimates of total instantaneous mortality (Z) has been obtained by applying various length based methods. It is assumed that due to the absence of commercial exploitation of the *Sthenoteuthis oualaniensis* in the study area, estimation of fishing mortality (F) doesn't arise. Therefore the assumption is that, in the absence of fishing mortality F, the total mortality Z is due entirely to natural causes Z = M (Sinclair, 2001).

Apart from a preliminary estimate and indication by Mohamed *et al* (2006), no published works are available on the growth and mortality of the *Sthenoteuthis oualaniensis* and its plastic phenotypic variants of the south west coast of India. Therefore, this study attempts to indicate the growth model and mortality of the purple squid with the available means of length based routine of FiSAT software to obtain basic scientific information relevant to future management of its fishery.

3.2 Materials and Methods

The specimens collected as indicated in Chapter one were thawed to the room temperature and then subjected to the measurements for Dorsal Mantle length (DML), from the tip of dorso-posterior end to the tip of the dorso-anterior end of the squid mantle. Altogether 1015 specimens were collected and measured during the period of study. Out of which, 565 were of medium form and 450 were of dwarf form. The measurements were made separately for the dwarf and medium form to the nearest millimeter. Though *S. oualaniensis* are sexually dimorphic, with females growing much lager than males due to the poor representation of male in the sample, sex wise analyses could not be done and only indicative study on the growth of the dwarf and medium were conducted with the available sample. In some of the months, sufficient samples could not be collected due to the non availability of species in the operation areas and to the gear. Besides, the oceanic nature of the specimen and its availability in the distant deeper waters also limited the accessibility. In view of that, the data collected for the year 2007 and 2008 were merged month-wise to increase the date strength for meaningful analyses. Size-frequency distributions were plotted for each month in 1.0 cm interval, sequentially and entered in the FiSAT II (FAO - ICLARM Stock Assessment Tool) software version 1.1.0 for creation of grouped length frequency files for further analyses.

The data of each month were decomposed by using Bhattacharya's method. This splits the composite distribution into separate normal distributions, each representing a cohort of squid, from a mixture of distributions, starting on the left-hand side of the overall distribution. Once the first normal distribution identified, it was subtracted from the total distribution and the procedure was repeated as long as it was possible to identify distinct normal distributions following the functions as below.

$\ln(N_{i+1}) - \ln(N_i) = a_j + b_j \cdot L_i$

where

N_i and N_{i+1} are successive frequencies of the same component of a group of fish in a sample (i.e., representing age group j) and where L_i is the upper class limit of $N_{i.}$

From this, the mean of the normal distribution was

$$\bar{L}_j = a_j/b_j$$

while its standard deviation (s) was

$$\sigma j = (-\Delta L/b_j)^{1/2}$$

where DL was the constant class size.

a separation index (SI) was also computed.

$$SI = \Delta\bar{L}_j / \Delta s_j$$

where

$\Delta\bar{L}_j$ was the difference between two successive means, and Ds_j was the difference between their estimated standard deviations.

The separation of length-frequency samples into their component was an iterative process in that every identified component was subtracted from the remainder of the sample using the Gaussian function,

$$N2i+ = Ni+ - \left\{ \left(\frac{1}{\sigma_j \sqrt{2n}} \right) EXP \left[- \left(\frac{\left[Li\text{-}L_j \right]^2}{2\ \sigma_j^{\ 2}} \right) \right] \right\}$$

where

N_{i+} refers to the previous set of frequencies and N_{2i+1} was the new set of frequencies, less the component identified so far. The output of the Bhattacharya's analyses was the mean lengths, population sizes (in numbers), standard deviations and separation index (SI) for the age groups identified. These results were saved as the "mean and standard deviation" file for further analyses.

The mean lengths of the components obtained were plotted against the sampling months and those mean lengths which were believed to belong to the same cohort were linked to create growth increment file (GIN) and length at age file (LAA). The linking process was highly subjective, so that previous knowledge of the growth pattern of the squid was used in performing the analysis.

The asymptotic length ($L\infty$) and Z/K (Z = total mortality and K = growth coefficient) was estimated by using the Powell-Wetherall method (Powell, 1979; Whetherall, 1986), perhaps the best way of estimating $L\infty$ (Sparre and Venema,1996). This was based on the well-known equation of Beverton and Holt (1957) and estimates the total instantaneous mortality coefficient (Z), in a steady-state population with constant exponential mortality and von Bertalanffy growth, from the mean length (L) of a random sample of fish above length L'(cut off length) (Gayanilo and Pauly,1997). The approach was an improvement on the Beverton-Holt method, allowing the estimation of $L\infty$ in addition to Z/K by taking advantage of the linear relationship between sample mean length and the selection length (Gayanilo and Pauly, 1997).

The growth increment file created by linking of means was used as input for the Gulland and Holt plot, Munro's method and Fabens method routine of FiSAT to calculate the K value and the $L\infty$. Munro's method, like the Gulland

and Holt plot, gives estimates of growth parameters when the standard Gulland and Holt plot fails because of an insufficient range of lengths. This applies particularly to K, which can be estimated from a single pair of Lt, Lt+t values, when an estimate of L_∞ is available. Fabens' method, although rigorous mathematically and much used by fisheries scientists, shares with Munro's method the disadvantage that it cannot be used to fit seasonally oscillating growth increment data. The length at age (LAA) file was also used as input in the FiSAT routine to study the age at length.

L_∞ and K value obtained through the Gulland and Holt plot routine was used in the following von Bertalanffy growth equation. The equation for growth in length is given by:

$$L_t = L_\infty (1 - \exp^{-k(t-t_0)})$$

Growth performance index (ϕ) was computed using the following equation (Pauly and Munro, 1984):

$$\phi = \log_{10}K + 2\log_{10}L_\infty$$

Longevity was obtained from the following equation:

$$t_{max} = t_0 + 3/K$$

where t_{max} is the approximate maximum age the squid of a given population would reach.

The total instantaneous mortality coefficient (Z) was estimated using Length converted catch curve analysis, Jones & van Zalinge plot, Beverton & Holt and Ault and Ehrhardt routine of FiSAT with the required input parameters. However, for the dwarf form the Length converted catch curve analysis was not applied for estimating the total instantaneous mortality coefficient (Z) due to insufficient data. Length converted catch curve analysis was done with the following functions

$$\ln(N_i/\Delta t_i) = a + b \cdot t_i$$

where N is the number of fish in length class i, Δt is the time needed for the fish to grow through length class i, t is the age (or the relative age, computed with $t_o = 0$) corresponding to the mid-length of class i, and where b, with sign changed, is an estimate of Z. As there was no commercial fishing done for this species, fishing mortality is considered as nil. As a result of that the total mortality rate Z was considered as equivalent to natural mortality.

3.3 Results

The growth parameters were estimated using different methodology and compared to arrive at a nearby acceptable value of these parameters separately for the dwarf and medium form. The result obtained in the Gulland and Holt plot, Munro's and Fabens' methods along with the length at age value have been presented in Table 3.1. During the study, specimans with the Dorsal Mantle Length range of 9.1 to 16.5 cm with mean length of 12.6 cm for dwarf and 9.8 to 27 cm with the mean length of 15.50 cm for medium were analysed.

3.3.1 Asymptotic length (L∞)

Length frequency data was analysed by the Powell - Wetherall method (Figure 3.1 (a)) and estimated the L∞ value for dwarf as 17.62 cm with cut off length (L') ranging from 12.50 cm to 15.50 cm and mean length – cutoff length, ranging from 0.50 – 1.21 cm with the regression equation of Y= 4.18 + (-0.237) x X. The mean length – cutoff length and the cutoff length have been highly correlated with r^2 as -1.00. The L∞ arrived for the medium from was of 29.5 cm with cut off length (L') ranging from 13.50 cm to 26.50 cm and mean length – cutoff length, ranging from 0.50 – 3.68 cm with the regression equation of Y= 7.04 + (-0.239) x X. The mean length – cutoff length and the cutoff length have been highly correlated with r^2 as -0.951 (Figure 3.1 (b)). The L∞ arrived by other methods including Powell - Wetherall method varied from 17.37 to 18.05 cm with the average of 17.89 cm for the dwarf and 29.5 to 30.49 cm with the average of 30.08 cm for medium form as stated in the Table 3.1.

Table 3.1. Growth parameters estimated for *Sthenoteuthis oualaniensis* of south west coast of India by various methods.

Forms	Parameters	Models					
		Powell Wetherall	Gulland and Holt	Faben	Munro	Length at age	Average
Dwarf	L∞ in cm	17.62	18.05	18.05	18.37	17.37	17.89
	K / year		3.48	4.07	3.24	3.82	3.65
	ϕ		3.05	3.12	3.04	3.06	3.06
	to		0.0	0.0	0.0	0.0	0.0
	t_{max} (days)		314	269	337	286	302
Medium	L∞ in cm	29.50	30.2	30.2	29.65	30.49	30.08
	K / year		2.33	2.68	2.35	2.19	2.38
	ϕ		3.33	3.39	3.3	3.3	3.33
	to		0.0	0.0	0.0	0.0	0.0
	t_{max} (days)		470	408	465	500	461

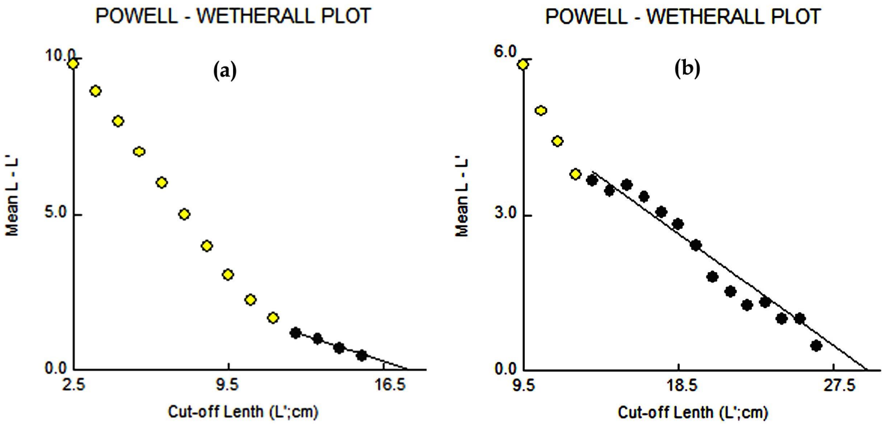

Figure 3.1: *Sthenoteuthis oualaniensis* (a) dwarf form , (b) Medium form

3.3.2 Growth parameters

Growth increment data derived as a result of the linking of means of the component believed to belong to the same cohort were used to calculate the growth co-efficient (K) . Gulland and Holt plot was used to compute the growth rate. The L∞ and the K value of the Gulland and Holt plot were taken as standard due to its closeness to the average and used as the input in the von Bertalanffy's growth equation and generated the VBGF Plot of the FiSAT routine .

3.3.2.1 Dwarf form

A growth coefficient of (K value) 3.48/ year was computed by Gulland and Holt plot with the L∞ of 18.05 cm for the dwarf form. Similarly, in Fabens and Munro methods, the computed K and L∞ values for the dwarf form were of 4.07 / 18.05 and 3.24 / 18.37/year respectively. Besides, the K and L∞ was also studied by using the length at age routine of the FiSAT. This indicates a similar trend with K value as 3.82/year and L∞ as 17.37 cm. The average L∞ of all the five methods was of 17.89 cm and K- 3.65 cm / year from the four methods. Growth performance index (ϕ) estimated ranged from 3.04 – 3.12 with the average of 3.06 and the t_{max} ranged from 269-337 days with an average of 302 days. Growth rate of the dwarf form computed from Gulland and Holt plot was of at the rate (dl/dt) of 0.11 for the mean length of 6.34 cm, 0.061 for the mean length of 10.61 cm, 0.058 for the mean length of 12.42 cm, 0.055 for the mean length of 14.55 and 0.011 for the mean length of 15.50cm. The von Bertalanffy's growth equation for *S. oualaniensis* is expressed as

$$\text{Dwarf : } L_t = 18.05(1- \exp(-3.48 (t + t_0)))$$

and the VBGF was plotted and placed below (Figure 3.2) along with the Faben's growth curve (Figure 3.3) to approximately model the growth and life span of the dwarf form. Wherein, the oscillation parameter (C) and winter point were assumed to be 0 as it is a tropical species. The generated non seasonalized restructured length frequency histogram with growth curve is shown in Figure 3.2.

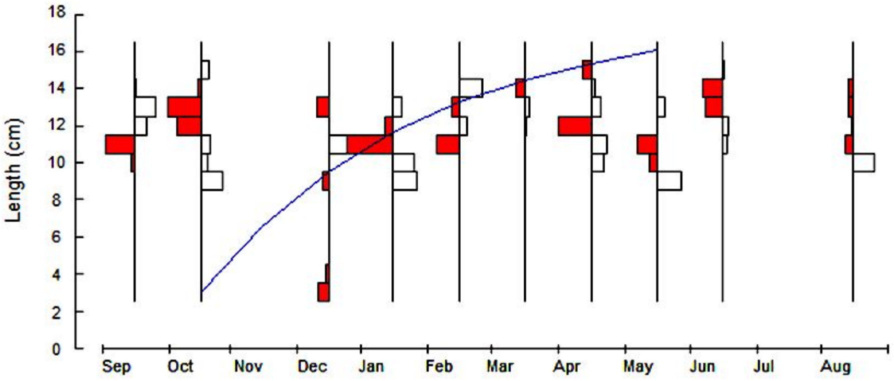

Figure 3.2. von Bertalanfy growth curve for S oualanienesis dwarf form with superimposed histograms. The red and white bars are positive and negative deviations from the weighed model classes representing psuodo-cohorts (Months have been rearranged to get the model curve).

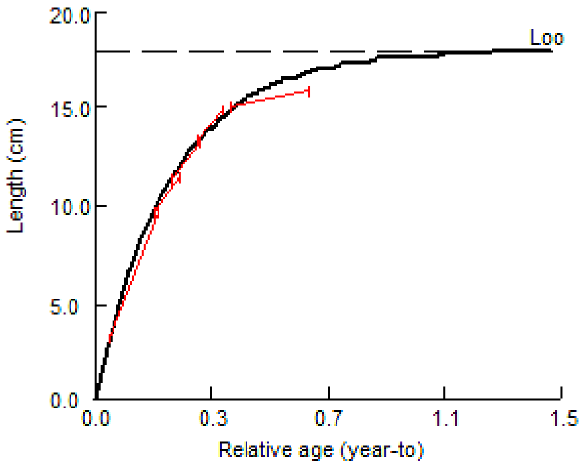

Figure 3.3. Fabens growth curve for *S oualanienesis* dwarf form

3.3.2.2 Medium form

In medium form the computed value of growth coefficient by Gulland and Holt plot was of 2.33/year with the L∞ of 30.20 cm. Similarly, the K and L∞ value computed in Fabens and Munro methods for the medium form were of 2.68 / 30.20 and 2.35 / 29.65 cm respectively. The K and L∞ computed by using the length at age routine of the FiSAT were of 2.19/year and 30.49 cm respectively with K value little lesser than the other methods. The average L∞ of all the five methods was of 30.08 cm and K- 2.38 cm / year from the four methods. Growth performance index (ϕ) estimated ranged from 3.3 – 3.39 with the average of 3.33 and the t_{max} were of from 408-500 with the average of 461 days. Growth rate of the medium form computed from Gulland and Holt plot was of at the rate (dl/dt) of 0.083 for the mean length of 16.89 cm, 0.068 for the mean length of 20.32 cm, 0.057 for the mean length of 22.23 cm and 0.029

for the mean length of 24.13 cm. The von Bertalanffy's growth equation for *S. oualaniensis* is expressed as

$$\text{Medium form : } L_t = 30.2(1- \exp(-2.33\ (t + t_0)))$$

The VBGF was plotted and placed below (Figure 3.4) along with the Faben's (Figure 3.5) growth curve to approximately model the growth and life span of the medium form. The oscillation parameter (C) and winter point were assumed to be 0 as it is a tropical species.

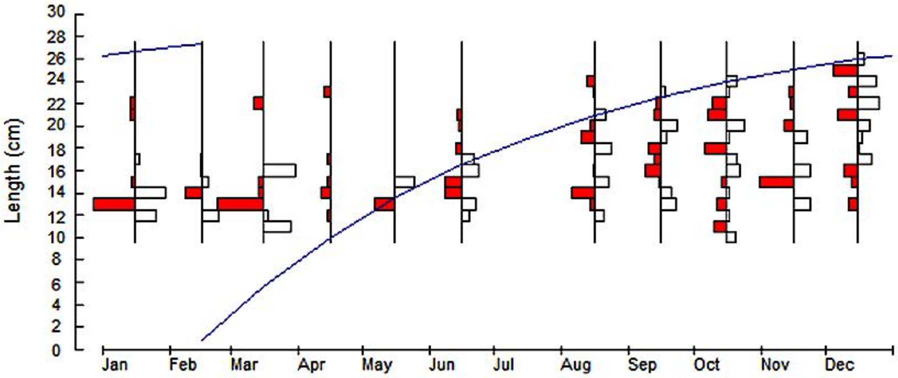

Figure 3.4. von Bertalanfy growth curve for *S oualanienesis* medium form with superimposed histograms. The red and white bars are positive and negative deviations from the weighed model classes representing pseudo-cohorts.

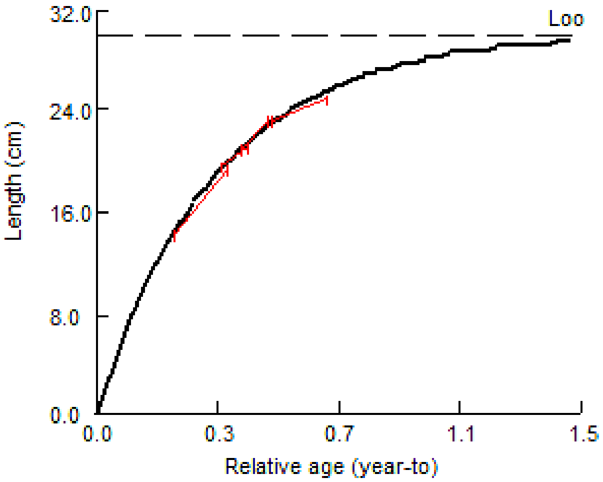

Figure 3.5. Fabens growth curve for *S oualanienesis* medium form

3.3.3 *Mortality parameters*

3.3.3.1 Dwarf form

Due to the insufficiency of data the Length converted catch curve method was not applied to estimate the total instantaneous mortality (Z) for the dwarf. However, the same was estimated by using all the other methods used for medium form to arrive on the Z (Table 3.2). The total instantaneous mortality (Z) was estimated as of 4.010/year (confidence interval of Z = 3.445 - 4.57; standard deviation of the slope = 0.131; r = 0.99) by the Jones and van Zalinge method (Figure 3.6).

Table 3.2. Mortality parameters estimated for *Sthenoteuthis oualaniensis* of south west coast of India by various methods.

Forms	Parameters	Models					
		Powell Wetherall	Length converted catch curve	Jones & van Zalinge plot	Beverton & Holt	Ault & Ehrhardt	$\bar{X}$
Dwarf	Z/ year	-	-	4.01	5.42	4.299	4.58
	Z / K	3.215	-	-	-	-	-
Medium	Z/year	-	6.80	4.906	6.23	6.177	6.027
	Z / K	3.191	-	-		-	-

-

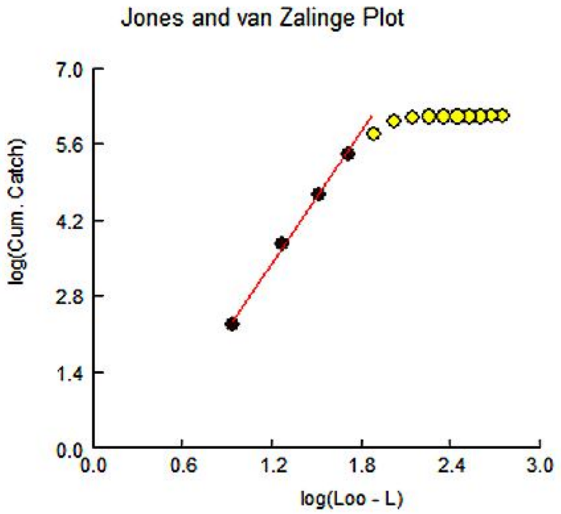

Figure 3.6. Length based Jones and van Zalinge plot of *Sthenoteuthis oualaniensis* dwarf form for estimating total mortality.

The total instantaneous mortality (Z) estimated using Beverton & Holt and Ault and Ehrhardt were of 5.419 and 4.299/year respectively. The average total mortality was computed as of 4.57/ year. Obtaining Natural mortality coefficient (M) is not relevant as there is no fishing mortality due to the non exploitation of this resource for commercial propose till date. Therefore, the total mortality is equivalent to natural mortality here.

3.3.3.2 Medium form

In the medium form the total mortality coefficient (Z) was estimated as of 6.80/year using length-converted catch curve (Figure 3.7) (confidence interval of Z = 5.0 – 8.60; standard deviation of the slope = 0.142; r = 0.99). While Jones and van Zalinge method (Figure 3.8) gave a value of 4.906/year (confidence interval of Z = 4.480 - 5.33; standard deviation of the slope = 0.153; r = 0.99). The Beverton & Holt and Ault and Ehrhardt gave total mortality value of 6.227 and 6.177/year respectively. The average total mortality rate was of 6.02/year. Like dwarf form there was no exploitation of the squid. In view of that, obtaining natural mortality coefficient (M) was not considered relevant and the total mortality rate was considered equivalent to natural mortality rate.

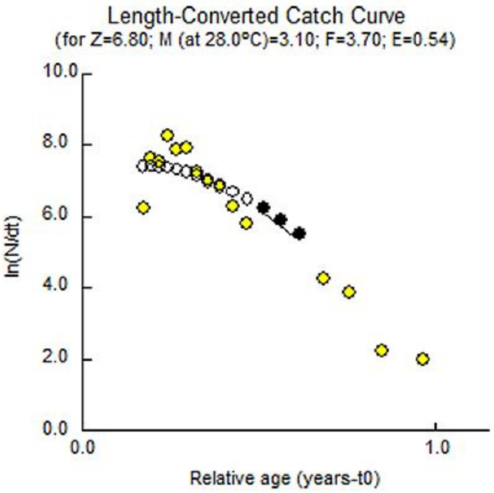

Figure 3.7. Length converted catch curve of *S oualaniensis* medium form

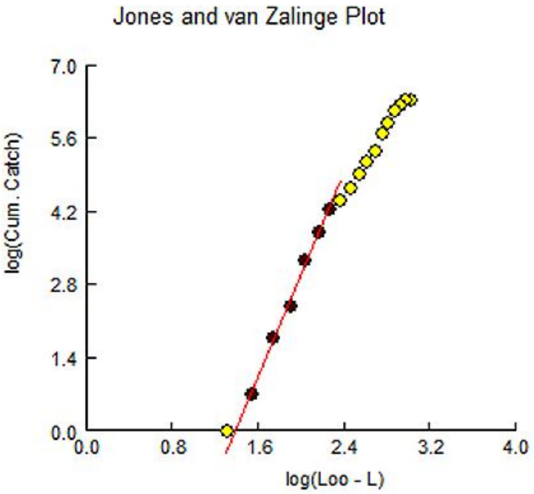

Figure 3.8. Length based Jones and van Zalinge plot of *S. oualaniensis* medium form for estimating total mortality.

3.4 Discussion

3.4.1 Asymptotic length (L∞)

Recognized two forms of *S. oualaniensis* in the present study from the size distribution and other traits following Nesis (1993). First has been the early maturing dwarf form of 9.1- 16.5 cm ML without dorsal photophore and second form has been the middle-sized medium form of 9.8-27.0 cm ML with dorsal photophore. Besides, the study indicates that the dwarf form has the average length infinity at 17.89 cm, while the medium form has the length infinity at 30.08 cm. This is in near conformity with the previous results from the former USSR research of the West Indian Ocean, wherein, the ML was mainly in the range of 9.0-18.0 cm ML, and 8.0-27.0 cm ML, (Trotsenko and Pinchukov, 1994). However, Nesis (1993) says that medium form has a maximum model size of 25.0

cm and dwarf has a maximum size of 15.0 cm only. Nesis (1993) had described the complex population of *S. oualaniensis* as three major and two minor forms. Based on the differences between the plastic phenotypes, attempts had been made times and again to describe these forms as separate species (Clarke, 1965 and Wormuth, 1976). The dwarf form was also suggested to be a separate species that could only be identified as an adult (Xinjun et al., 2007). Snyder (1998) suggested that the giant form resulted from a plastic phenotype in the species. A new study based on RAP DNA (Random Amplified Polymorphic DNA) analysis is being done in Marine Science and Technology of Shanghai Fisheries University, whose preliminary findings suggest a large variation in biology among the groups (Xinjun et al., 2007).

3.4.2 Growth parameters

The growth coefficient (K value) computed using various methods stated in the methodology indicate that, dwarf and medium form has different range of K values.

3.4.2.1 Dwarf form

The K value for the dwarf form ranged from 3.24 to 4.07/ year. The high growth rate of the species has already been acknowledged (Zuyev and Nesis, 1971; Nesis, 1977; Zuyev et al., 1985; Xinjun et al., 2007). It is also indicated that the price for maintaining high metabolic and growth rates is high rate of food consumption in *S. oualaniensis* (Young and Hirota,1998). As there is no previous length based study is available on the growth rate of this species, the obtained K value could not be verified. However, Sparre and Venema (1996) indicates that the higher K is related to the higher metabolic rate of the species, as well, pelagic species are often more active than the demersal species and have a higher K. The metabolic rate is also a function of temperature; tropical species have higher K value than the cold- water species. The K value of 3.48/year and the L∞ of 18.05 cm obtained by the Gulland and Holt plot was applied in the VBGF plot of the FiSAT routine, to generate the growth curve along the model classes representing psuodo-cohorts. The generated growth curve and the Fabens curve indicates that, dwarf form may attains the length infinity in around ten months. This corroborate the results obtained from the t_{max} method, where the average longevity obtained was of ten months (302 days) with the average K value of 3.48/year. In total the longevity of the dwarf form ranged from 269-337 days amoung the four methods applied (Table 3.1).

This is corroborated by Zuyev et al. (1985), who has informed that the growth parameters estimated for the small early-maturing form of genus *Sthenoteuthis* through the von Bertalanffy growth equation indicates its life span to be 1 year. Besides, many studies also indicate that *S. oualaniensis* had its life span less than 1 year (Nesis, 1993; Dong, 1991; Trotsenko and Pinchukov,1994). However, the study based on the gladius micro structure indicates that the duration of life cycle in dwarf early-maturing equatorial form of *S.oualaniensis* was at about 6 months only (Zuyev at al.,2002). The dwarf approximately grows to 10.5 cm ML in three months (91 days) , 14.9 cm in six months (182 days) and 16.7 cm in nine months (274 days). Zaidi bin Zakaria (2000) determined the growth of the smallest oceanic squid *S. oualaniensis* of 10.7 cm as 102 day old. However, Yatsu (2000) determined growth curves for both sexes and reported a female of 12.0 cm ML at 51 days old only. This may suggests that environmental conditions such as temperature and food availability are the main factors influencing the growth rates, lifespan, and fluctuations of relative gonad investment.

It is inferred from the growth curve that the growth is faster in the first half of the life when compared to the second half, as 75% of the growth is achieved in the first five months time. However, it takes another four months to achieve the remaining 25% of the growth indicating a slower later phase of growth. Besides, growth rate of the dwarf form computed from Gulland and Holt plot also indicate a decreasing rate of growth from the juvenile to adult. Lipinsky and Roeleveld (1990) says that squid displays a form of asymptotic growth well represented by the standard von Bertalanffy model with growth rates that declined linearly with length. As there is no previous record available on the growth performance index of the species, the present estimated growth performance index (Ø) of 3.06 could not be compared with the earlier one. However, the growth performance index (Ø) obtained from different methods were compared and found that they did not show much variation and ranged from 3.04 – 3.12 for this form. This clearly shows the reliability of the estimates of K and L∞ in the present study. The growth performance index 2.58 - 3.28 calculated for *Loligo duvaucelii* by Mohamed (1996) off south west coast of India may be an indication of the squid growth in the south west coast of India.

3.4.2.2 Medium form

Medium form growth co-efficient ranged from 2.19 to 2.68/ year. A growth curve for the medium form was generated in the VBGF plot of the FiSAT routine with the K value input of 2.33/year and the length infinity 30.2 cm. This growth curve and the Fabens curve indicates a slightly longer than a year life span for medium form to attains the length infinity. This is substantiated by the longevity study of t_{max} method, which indicate the average longevity of the medium form as around 15 months (461 days) with the K value of 2.38/year. In total the longevity of the medium form ranged from 408-500 days amoung the four methods applied. Zuyev et al.(1985) has indicated that the growth parameters estimated through the von Bertalanffy growth equation for the genus *Sthenoteuthis,* large late-maturing forms for 2 yrs. However, the study based on the gladius microstructure indicates that the duration of life cycle of middle-sized tropical, Red Sea and Arabian Sea forms about 1 yr (Zuyev at al.,2002).

Medium form approximately grows to 13.3 cm ML in three months (91 days), 20.8 cm in six months (182 days) and 25.0 cm in nine months (273 days). Zaidi bin Zakaria (2000) has estimated that female *S. oualaniensis* of 21.7 cm ML was of 275 day old and male of 16.1 cm ML was of 259 day old. It is inferred from the growth curve that the growth is faster in the first half of the life when compared to the second half, as 63% of the growth is achieved in the first seven months time. However, it takes another seven months to achieve the remaining 37% of the growth indicating comparatively a slower later phase of growth. Growth rate of the medium form computed from Gulland and Holt plot indicate a decreasing rate of growth from the juvenile to adult with a slight increase between the mean length 20.0 cm to 23.0 cm. The growth rates, determined by growth marks on statoliths and gladii, of the middle and dwarf forms are nearly the same, but the life span is short in the small forms, probably not more than 6 months (Nesis,1993). As there is no previous record on the growth performance index available, the present estimated growth performance index (Ø) of 3.33 could not be compared.

3.4.3 Mortality parameters

The total instantaneous mortality rate (Z) estimated for the dwarf form varies from 4.010/year of Jones and van Zalinge method to 5.419/year of Beverton & Holt method with an average of 4.576/year. In the medium form the total instantaneous mortality rate (Z) varied from 4.906/ year of Jones and van Zalinge method to 6.80/year of length converted catch curve with the average value of 6.027/year.

In short-lived species such as squids both growth and mortality are quite high (Caddy, 1983), Meiyappan and Srinath (1989) have reported the total mortality of the Indian squid *Loligo duvaucelli* as high as 10.6/ year in the Cochin area based on length converted catch curve method. The higher total mortality rate estimated may possibly be due to faster growth , short life span, high level of cannibalism and possibly higher post spawning mortality in the *Sthenoteuthis oualaniensis*. As there is no directed fishing for this species, fishing mortality is considered as nil and as a result, the reduction of number in the cohort is considered purely due to natural causes.

Chapter 4

Maturation and Spawning Studies

Contents

4.1 Introduction

Cephalopod life-cycles have been considered as somewhat enigmatic (Calow,1987). Some of this enigmas in their life-cycles, such as apparent ubiquity of breeding once and post-reproductive mortality have been attributed by Boletzky (1981) to a combination of r- and K-factors and presumably r- and K-selection (MacArthur & Wilson, 1967) operating in one species. However, it is probably more appropriate to depict life-cycles as complex sets of co-varying traits, some combinations of which might involve so-called r-, others K, and yet others a combination of r- and K-traits (Sibly & Calow, 1985). Thus, cephalopods exhibit a full range of reproductive modes from semelparity to non-seasonal iteroparity (Mangold et al. 1993) to subsume different tactics. Until recently, practically all living cephalopods were considered semelparous marine mollusks that lay their eggs in one single spawning, after which they would die (Boyle, 1983; Calow, 1987; Rodhouse, 1998). The only exception to this pattern was assumed to be *Nautilus*, considered as iteroparous because of its females spawning once in a year, survive, feed, grow and regenerate their gonads for a further reproductive event in the following year (Ward, 1987). Iteroparous organisms are said to have more than one reproductive event in their lifetime and return, after every spawning event, to the preparatory phase through a reproductive resting phase (Cole's,1954).

However, Boletzky (1981, 1986), Rodaniche (1984), Mangold (1987), Harman et al. (1989), and Mangold, Young & Nixon (1993) had cautioned that more variation could exist in the reproductive traits of coleoid cephalopods than was previously realised. Cephalopod species, namely the sepioids, many teuthids, the epipelagic incirrate octopods and the cirrates spawn repeatedly in one spawning season, which could produce confusion when identifying iteroparous or semelparous patterns. In such cases, it will be necessary to distinguish if the egg capsules are released simultaneously (semelparous) or with a significant time interval between successive eggs spawned one by one or successive egg batches (iteroparous). One example of iteroparity according to the definition proposed by Cole (1954) has been observed in the *Sthenoteuthis oualaniensis*.

Harman et al. (1989) stated that, after spawning once, *S. oualaniensis* apparently continues to feed, grow, and mature additional oocytes before spawning again, as the gonads do not regress and subsequently redevelop. Snyder (1998) found a large proportion of maturing and pre-vitellogenic oocytes in the ovary of mature females of this species, which suggests that eggs are laid periodically. However, in both papers mentioned above this reproductive strategy was named `multiple spawning. Harman et al. (1989) indicated that multiple spawning includes all types of non-semelparous reproduction. Based on these variations, several reproductive strategies have been defined for cephalopods with regard to the oocytes maturation process and the type of spawning in each species (Boletzky, 1981, 1986; Harman et al., 1989; Villanueva, 1992; Mangold et al., 1993; Gonza!lez, 1994; Rocha, 1994; Boyle et al 1995; Nesis, 1996; Rasero, 1996; Rocha et al., 1996; Rocha & Guerra, 1996; Nigmatullin & Laptikhovsky, 1994, 1999b and Rocha et al., 2001; Chembian and Mathew,2010).

Rocha et al. (2001) proposed to replace the term semelparity with `spawning once' and iteroparity with `spawning more than once', to avoid the confusion. In other words, to refer exclusively to the spawning pattern (Kirkendall & Stenseth, 1985). It is also necessary to consider in greater detail the dynamic aspects of oocyte growth in the ovary and how oocytes develop. In this regard, it is interesting to distinguish between ovaries with synchronous, group-synchronous and asynchronous ovulation (Wallace & Selman, 1981). During the process of maturation and spawning, the matured oocytes are transferred from the ceolomic cavity of the ovary and accumulated in oviducts until those are full and ready to ovulate. Once the eggs are released during spawning (in single or multiple batches), the oviducts receive a fresh influx of ripe oocytes. It was suggested that the oviducts represent a compromise between the inherited ancestral asynchronous pattern of yolk accumulation and the subsequently evolved intermittent batch spawning strategy (Burukovsky et al. 1977).

This principle was used to develop a universal maturity scale by Lipinski (1979) and its later revision (Lipinski and Underhill, 1995). Based on the oocyte formation and development, ovaries were distinguished as synchronous, group synchronous and asynchronous ovulation. Following Marza (1938, in Wallace & Selman, 1981), in a synchronous ovary `all oocytes, once formed, grow and ovulate from the ovary in unison, further replenishment of one stage by an earlier stage does not take place'. In a group-synchronous ovary, `at least two populations of oocytes can be distinguished at some time; a fairly synchronous population of large oocytes and a more heterogeneous population of smaller oocytes from which the clutch is recruited. In an asynchronous ovary, `oocytes of all stages are present without dominant populations' (Wallace & Selman, 1981).

Besides, the presence or absence of somatic growth between the productions of egg batches was used as a third criterion to obtain a suitable classification of reproductive patterns. Taking these considerations into account, Rocha et al., (2001) proposed five reproductive strategies for cephalopods such as (i) *Simultaneous terminal spawning* (Spawning once- egg laying occurs simultaneously- eg. *Loligo opalescens), (ii) Polycyclic spawning* (Egg-laying occurs in separate batches during different spawning seasons with animal surviving, feeding and growing between each year and ovary regeneration – eg. *Nautilus*), (iii) *Multiple spawning* (Spawning is monocyclic and egg-laying occurs in separate batches, somatic growth continuing between separate spawning events and egg-laying occurs in separate batches during the same spawning (no ovary regeneration) – *eg. Sthenoteuthis oualaniensis*) (iv) *Intermittent terminal spawning* (Spawning is monocyclic and egg-laying occurs in separate batches during the spawning period, somatic growth does not take place between spawning events- *Illex argentinus*) and (v) *Asynchronous ovulation* (Monocyclic spawning- egg-laying occurs in an extended and continuous spawning period in relation to the animal's life. eg. *Argonauta boettgeri).*

It is stated that teuthoids found reproducing on the shelf, slope and in epi-mesopelagic layers of the open ocean (Loliginidae, Ommastrephidae, Enoploteuthidae, Thysanoteuthidae, Ancistrocheiridae, Architeuthidae and Berryteuthis, Gonatidae) are characterized by asynchronous vitellogenesis (Burukovsky and Vovk, 1974; Burukovsky et al. 1977; Nigmatullin et al. 1996; Nigmatullin and Arkhipkin, 1998; Laptikhovsky, 1999a; Hoving et al. 2004). However, casual reports on squid spawning in deep waters (Onychoteuthidae, Gonatidae, Histioteuthidae, and Cranchiidae) revealed evidence of synchronous ovary maturation (Laptikhovsky et al., 2007). In maturing and mature females of *Teuthowenia megalops* and *T. pellucida*, all oocytes were found to be of the same size (Muus, 1956). Synchronous egg development has also been discovered in immature and maturing tropical and subtropical bathypelagic spawners *Onychoteuthis banksi* and different *Histioteuthis spp*. (Arkhipkin and Nigmatullin, 1997; Laptikhovsky, 2001a). It was suggested that in some deepwater-spawning squid, oocytes start to develop synchronously at the very late stages of vitellogenesis, which allows the species to release its eggs in separate batches (Nigmatullin and Piatkowski ,1997; Nigmatullin, 2004).

Development and functioning of the reproductive system in males and females of *Sthenoteuthis oualaniensis* have been investigated in detail (Burukovsky et al., 1977, 1979; Zalygalin et al., 1977; Hixon et al., 1980; Zuyev et al., 1985; Nigmatullin and Sabirov, 1987; Nigmatullin and Laptikhovsky, 1994; Laptikhovsky, 1987, 1995; Sabirov, 1995). The development of oocytes and the structure and development of ovaries in *Sthenoteuthis spp* have been described (Burukovsky et al., 1977, 1979; Zuyev et al., 1985; Nigmatullin and Laptikhovsky, 1994; Laptikhovsky, 1995). The process of the preparation of reproduction for this species falls into 2 parts: (1) the development of the ovary and accessory organs and maturation of the ovary (physiological maturation - maturity stages I-IV), (2) production and accumulation of ripe eggs in the oviducts (functional maturation - stages V). Ova are easily distinguished from immature oocytes by their orange color and smooth texture. Ripe eggs are relatively small and don’t depend on female size (Zuyev et al., 2002). In *S. pteropus*, eggs are relatively uniform. However, in *S.oualaniensis* there are geographical and intraspecific variations in the egg size, eggs of the Indian Ocean females of all intraspecific groups are significantly smaller than of females from eastern Pacific (Zuyev et al.,2002). In three intraspecific groups of *S. oualaniensis* from the Indian Ocean, egg size is not significantly different. But, the eggs of the dwarf early-maturing form are slightly smaller than of middle sized late-maturing and giant forms, the eggs size of the two latter are not different (Zuyev et al., 2002). In mature squids, the nidamental glands are relatively large, opaque and white, whereas the glands of young squid are colorless and small.

Maturation of *Sthenoteuthis oualaniensis* belonging to the different plastic forms begins at different sizes (Zuyev et al., 1985) and proceeds rapidly. As most females live for about one year, it is possible to assume that the process of female maturation lasts about 9–10 months. Oceanic ommastrephids (subfamily Ommastrephinae) show the highest potential fecundity among cephalopods (Harman et al., 1989; Nigmatullin & Laptikhovsky, 1994; Rocha et al., 2001; Nigmatullin, 2002; Laptikhovsky & Nigmatullin, 2005; Zuyev et al., 2002). Potential fecundity depends on adult female size and varies between the phenotypic forms. Spawning activity lasts for 1–3 months at a relatively stable level and without a decrease in feeding rate (Zuyev et al., 2002).

Harman *et al*., (1989) has reported that there is sufficient evidence to indicate that *Sthenoteuthis oualaniensis* is monocyclic with multiple spawning and lays egg in separate batches with group-synchronous ovulation. Somatic growth continues between separate spawning events, while, the species apparently continue to feed, grow, and mature additional oocytes before spawning again (Harman et al., 1989; Nigmatullin and Laptikhovsky, 1994). During the individual spawning period, females continue to show significant somatic growth (Nigmatullin and Laptikhovsky, 1994). At the species level, spawning takes place all-year-round, but at different intensities and different intraspecies forms (plastic phenotypic forms) differ in their time of spawning (Zuyev et al., 2002). A study based on RAP DNA (Random Amplified Polymorphic DNA) analysis is being done in Marine Science and Technology of Shanghai Fisheries University. Preliminary findings of this study suggest variation in biology among the plastic phenotypic groups of the *S oualaniensis* (Xinjun et al., 2007).

The present study is a description of the reproductive biology of the species based on the samples collected. The study aims to provide information on the distribution of mature individuals, sex ratio, size at maturity, the spawning season and discuss the reproductive strategies of the squid in the south west coast of India.

4.2. Materials and Methods

Specimans were collected on a monthly basis. Out of a total of 1015 specimens of *S. oualaniensis* subjected to maturity and spawning studies, 565 were identified (Clarke, 1965 and Nesis, 1993) as of late-maturing medium form with 328 numbers (58 %) of female and 237 numbers (42 %) of male. The remaining 450 were identified as of dwarf, early-maturing form, without dorsal photophore, wherein, 373 (83 %) were of female and 77 (17 %) were of male. The measurement for dorsal mantle length (DML) was taken in a straight line to the nearest millimeter after thawing the specimen to the room temperature from the frozen condition. The total weight (TWt) was measured to the nearest gram. Sex ratio was determined on a monthly basis and tested for significant departures from the expected 1:1 female : male ratio using Chi square test (Sokal & Rholf, 1969) .

Specimens were dissected, sexed and assigned a maturity stage according to the universal scale proposed by Lipinski (1979). However, female scale was slightly modified according to Burukovsky *et al.* (1977). The substages indicated in the fifth stage of the female maturity scale developed by Burukovsky *et al.* (1977) for *Sthenoteuthis pteropus* were merged in this scale as intermittent spawners like *Sthenoteuthis* spp (Harman *et al.,* 1989; Nigmatullin and Laptikhovsky, 1994), substages V-2 and V-3 do not always occur in sequence. Stage V-3 (when a female is ready to spawn) differs from stage V-2 only by the presence of the maximum fullness in the oviducts. After an egg release the fullness of the oviducts decreases, and the female returns to substage V-2. When she accumulates enough oocytes she enters spawning substage V-3 again. Spent stage was not included in the scale as the same was not observed in the sample.

In total five stages were considered for both female and male in the study as follows (Plate 4.1, 4.2 and 4.3).

For female *Sthenoteuthis oualaniensis.*

Juvenile – I The sexual organs are very hard to find with the naked eye. The oviducts and NG appear (if at all) as very fine transparent stripes. The ovary is translucent, membranous.

Immature – II The sexual organs translucent or whitish. The oviducts and nidamental glands form clearly visible, translucent, or whitish strips. NG small; all viscera behind them easily observed. The ovary clearly visible, in most cases without structures observable with the naked eye.

Preparatory- III. The sexual organs are not translucent. The NG grayish white, enlarged, covering some internal organs. Ovary grayish cream and occupies posterior third of the mantle cavity. Structure inside the ovary (immature ova) clearly visible. Oviduct as translucent flat bands and easily distinguishable. Oviductal gland thickened and whitish grey.

Maturing- IV. The NG large, leaf shaped, milky- white, slight increase in size with noticeable increase in volume and weight, covers kidneys and distal part of the liver. Secretion does not exude when cut. Ovary creamy coloured. Occupy approximately ½ length of mantle cavity. Oviductal gland well defined, fleshy, swollen, white and resilient. Eggs are not transparent (roughly 95%) and are pressed together at least in the proximal part of the oviduct. There may or may not be many different stages of eggs in the distal part of the oviduct.

Matured -V. NG large, leaf shaped, milky- white, large than the IV stage and length nearest to half of the mantle length. During spawning the NG bulges and when cut open secrets a viscous substance. Ovary creamy coloured and occupy approximately ½ length of mantle cavity with well defined reticulate pattern on the surface giving mosaic pattern indicating the increased number of matured eggs in the ovary. The appearances of dots on the surface of the ovary indicate the transparent matured eggs. Oviduct red in colour, large, resilient, filled with transparent matured eggs. Its septa's are clearly visible. Oviductal gland large, resilient, and white in colour.

for male *Sthenoteuthis oualaniensis*

Juvenile-I. The sexual organs are very hard to find with the naked eye. Spermatophoric complex appears (if at all) as a transparent or translucent spot. The testis is transparent, membranous.

Immature –II. The sexual organs translucent or whitish; the separate parts of the spermatophoric complex are clearly visible; the testis small; its structure invisible.

Preparatory- III. The sexual organs are not translucent; the vas deferens whitish or white, so with white streak; the testis in most cases is white or pink; its structure is invisible.

Maturing –IV. The vas deferens white, meandering enlarged; SS long with structure less whitish particles inside, but without formed S; the testis tight, crispy ; the testis surface covered with structure.

Mature-V. As above, except that S are present in the SS

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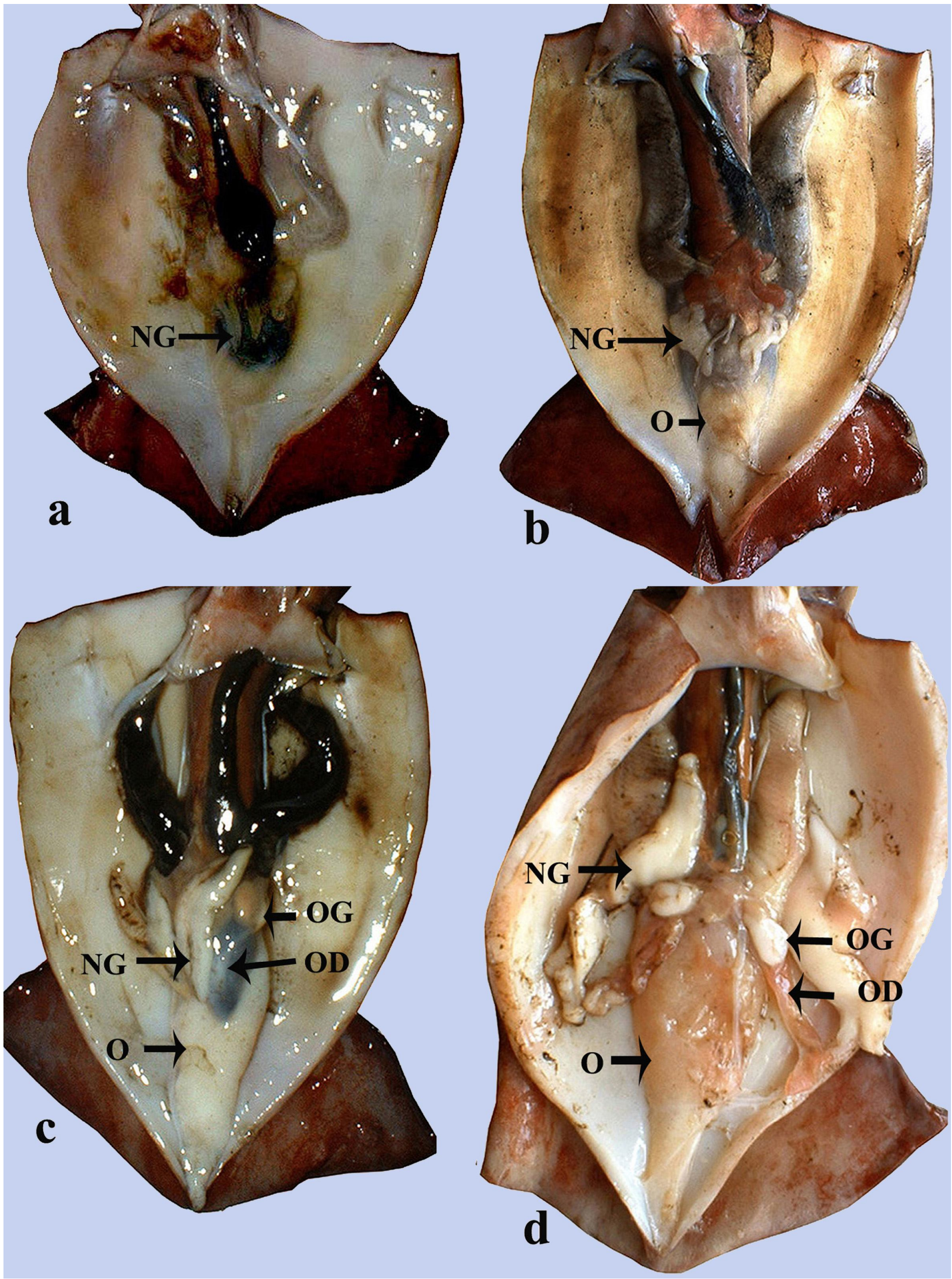

Plate 4.1

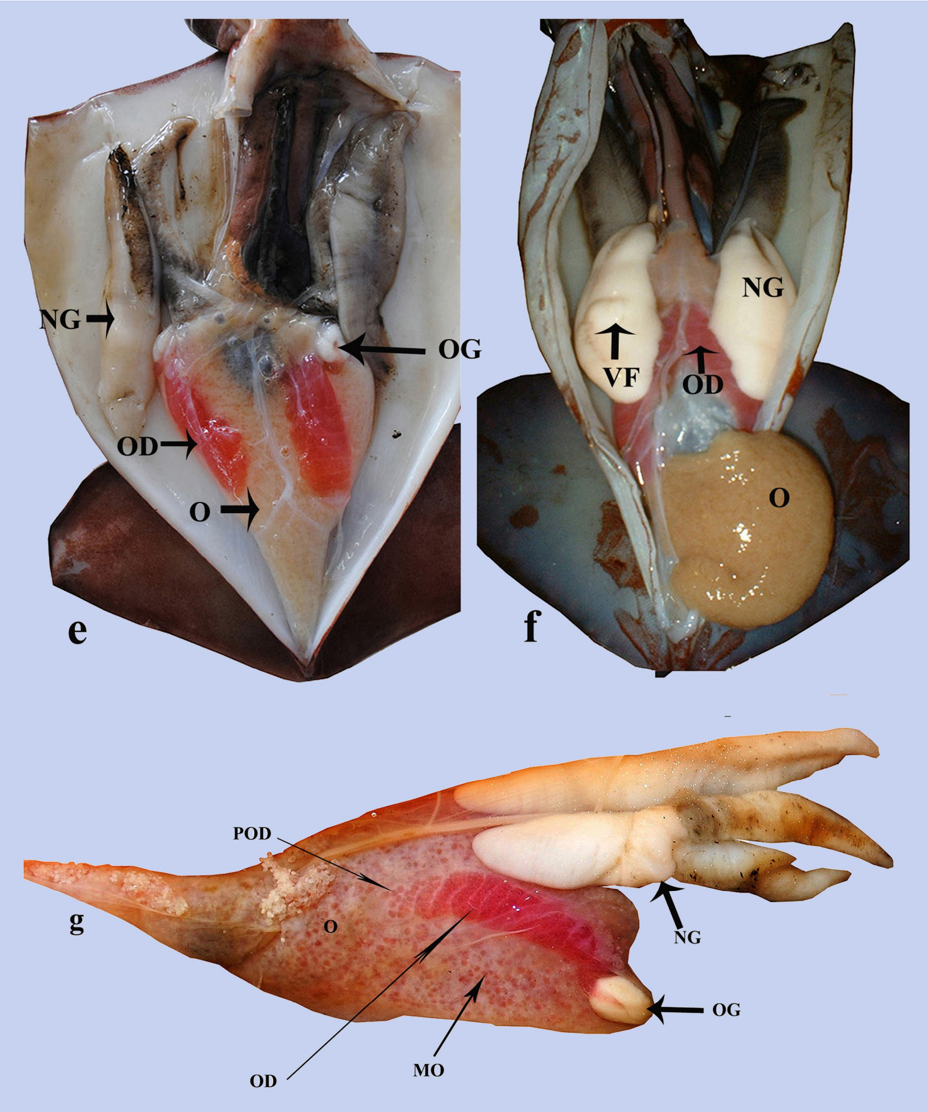

Plate 4.2.

Legend for Plate 4.1 and Plate 4.2;Female *Sthenoteuthis oualaniensis*, indicating various maturity stages and reproductive system (a) Maturity stage -I, (b) Maturity stage -II, (c) Maturity stage -III, (d) Maturity stage –IV,(e) and (f) Maturity stage –V, (g) Fully matured total reproductive system. NG – Nidamental gland, O - Ovary, OD- Oviduct, OG- Oviductal gland, MO- Matured Oocyte (Transparent), POD- Proximal oviduct, VF- Viscous fluid.

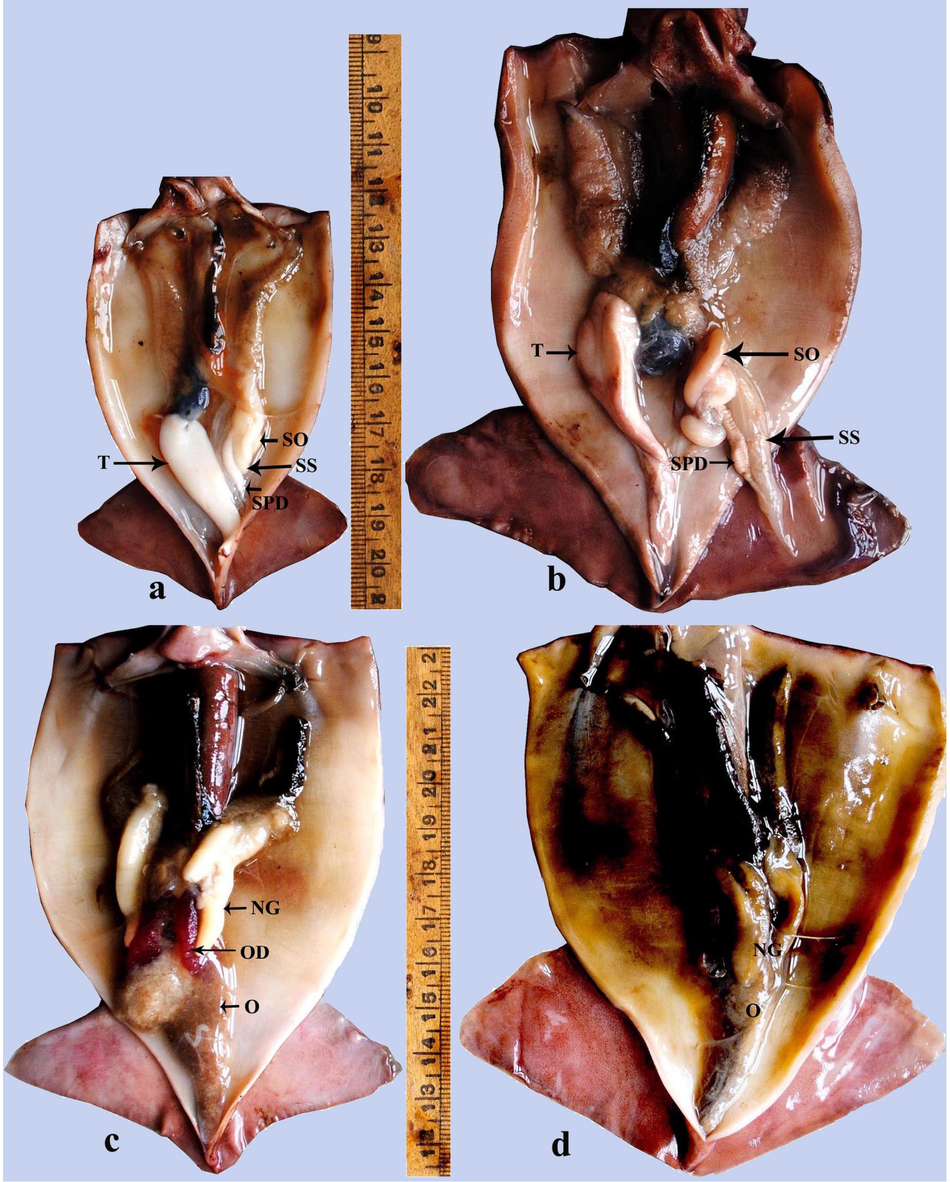

Plate 4.3. A comparison of the Various components of the *Sthenoteuthis oualaniensis* reproductive system between medium and dwarf form (a) Fully matured dwarf male, (b) Fully matured medium male, (c) Fully matured dwarf female, (d) Stage III medium female, NG – Nidamental gland, O - Ovary, OD- Oviduct, T-Testis, SS- Spermatophoric sac, SO- Spermatophoric organ, P-Penis.

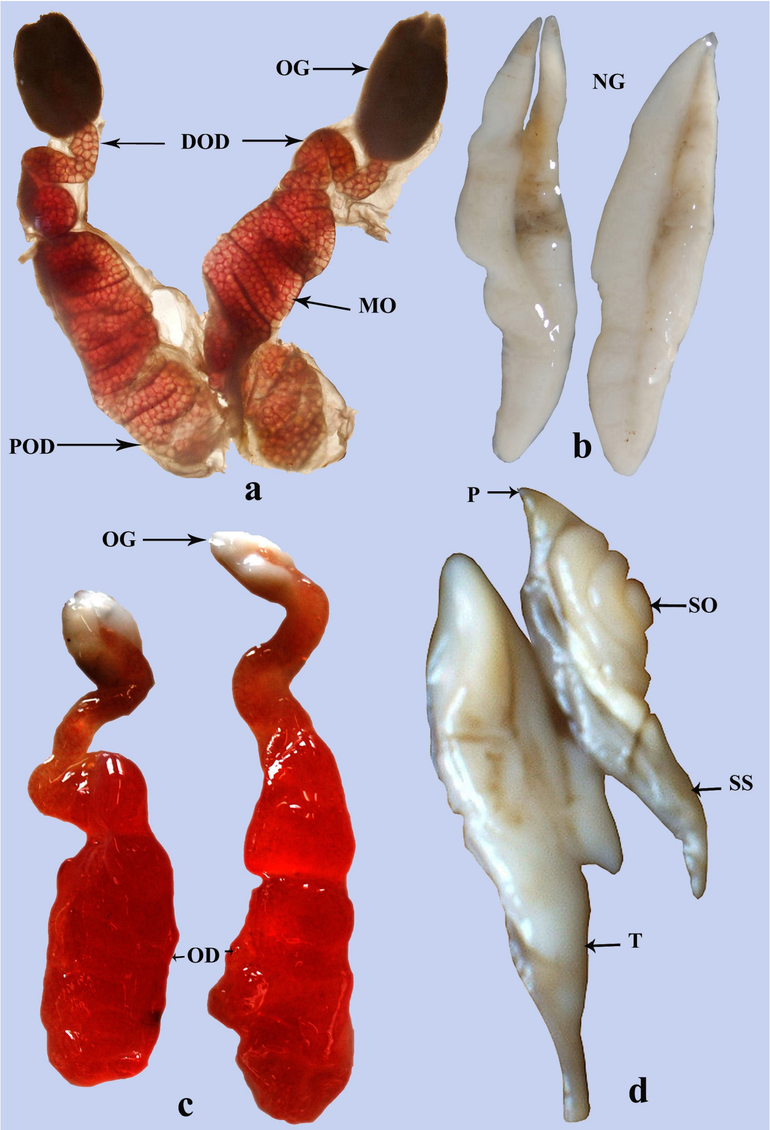

Plate 4.4. Various components of the *Sthenoteuthis oualaniensis* reproductive system. (a) Oviduct and Oviductal gland indicating the transparent matured Oocyte inside the oviduct, (b) Nidamental gland, (c) Oviduct and Oviductal gland, (d) Male reproductive system. NG – Nidamental gland, O - Ovary, OD- Oviduct, OG- Oviductal gland, MO- Matured Oocyte (Transparent), POD- Proximal oviduct, DOD- Distal oviduct, T-Testis, SS- Spermatophoric sac, SO- Spermatophoric organ, P-Penis.

Maturity stages were analysed for size range to understand the variation in the size of each maturity stage and length at which 50 % of the squid attain maturity. Maturity stages were further grouped month wise to study the monthly change in the abundance of each maturity stage for understanding the seasonal fluctuation in the various maturity stages. Different parts of the reproductive system (Plate 4.4), such as oviductal complex, nidamental gland, ovary, oviduct and testis were removed and oviductal complex weight (OCW) (including ovary, both the right and left oviduct and oviductal gland), nidamental gland weight (NGW) (both left and right) , ovary weight (OW) and testis weight (TW) were weighted to the nearest 0.1g to investigate the following maturity indices. The Nidamental gland length (NGL) was measured to the nearest mm.

The maturity indices were calculated by following Markaida and Nishizaki (2001) as follows.

For females

Nidamental Gland Weight Index (**NGWI**)

$$= \frac{\text{Nidamental Gland Weight (NGW)}}{\text{Body Weight – Nidamental Gland Weight (BW- NGW)}} \text{ X } 100$$

$$\text{Ovary Weight Index } (\textbf{OWI}) = \frac{\text{Ovary Weight (OW)}}{\text{Body Weight – Ovary weight (BW-OW)}} \text{ X } 100$$

Oviductal Complex Weight Index (**OCWI**)

$$= \frac{\text{Oviductal Complex Weight (OCW)}}{\text{Body Weight – Oviductal Complex Weight (BW-OCW)}} \text{ X } 100$$

Nidamental Gland Length Index (**NGLI**)

$$= \frac{\text{Nidamental Gland Length (NGL)}}{\text{Mantle Length (DML)}} \times 100$$

Reproductive System Weight Index (**RSWI**) =

$$\frac{\text{(Ovary Weight + Oviductal Complex Weight + Nidamental Gland Weight)}}{\text{(Body Weight– Ovary Weight - Oviductal Complex Weight-Nidamental Gland Weight)}} \times 100$$

For males.

$$\text{Testis Weight Index (TWI)} = \frac{\text{Testis Weight (TW)}}{\text{Body Weight – Testis Weight (BW-TW)}} \times 100$$

The maturity indices were analysed for the overall period of study and month wise to have an understanding of the different maturity stages and spawning period of the species. The non – parametric Mann-Whitney U-test was used to test for differences between maturity index means for each maturity stage.

Fecundity was estimated only at specimens with maturity stages IV and V. The ovary and oviduct were removed from the specimen and fixed in the Gilson' fluid for two days for hardening of the oocytes. Subsamples ranging from 25 mg and 50 mg were taken from three areas in the ovary (anterior, centre and posterior) and two areas in the oviduct (anterior and posterior). They were shaken vigorously in the fluid until the hardened oocytes were separated from the surrounding tissues. Then these separated oocytes were counted one by one, under an illuminated magnifying glass/ dissection microscope. All the oocytes of the sub-sample were counted. The number of oocytes in the ovary and the oviducts contained in the weight of the samples was extrapolated to the total ovary and oviduct weights, respectively. Batch fecundity was arrived by counting the oocytes in the oviduct, wherein, ovarian fecundity was arrived from the ovary. Potential fecundity was estimated by adding the batch and ovarian fecundity of the specimen. To have an understanding of the size of the ripe oocytes 20 ripe oocytes were collected from different subsamples and measured in the longest axis with the micrometer placed in a horizontal position on the eye-piece, and the diameter parallel to the graduation on the micrometer were measured.

4.3. Results

4.3.1. Sex ratio

Medium form of the *Sthenoteuthis oualaniensis* had a total of 328 female against 237 males. This corresponds to an overall female : male ratio of 1.4 : 1. However, on a monthly basis this ratio varied between a maximum of 26: 1 (X^2 test; < 0.05) in the month of January and a minimum of 0.2 : 1 (X^2 test; < 0.05) in the month of September (Table 4.1, Figure 4.1). Females significantly out numbered males in the months of January, February, December and November'7 (X^2 test; < 0.05). However, males out numbered females only in the month of September (X^2 test; < 0.05) with significant departure from the expected 1: 1 ratio. Dwarf form had a total of 373 female against 77 males corresponding to an overall female : male ratio of 4.8 : 1. However, on a monthly basis this ratio varied between a maximum of 14: 1 (X^2 test; < 0.05) in the month of February and a minimum of 0.4 : 1 in the month of May, which was not significant. However, the sex ratio was significantly lower (2 : 1) only in the month of

October (X^2 test; < 0.05) with Except in the month of May, female out numbered the males in all the months. However, only during the months of January’07 to April '07, Jun’07, August’07 to October’07, February’08 and April’08 (X^2 test; < 0.05) females out number the males with significant departure from the expected 1; 1 ratio.

Table 4.1. Sex Ratio of medium and dwarf form in % with the Chi test value

Months	Medium Form				Chi test	Dwarf Form				Chi test
	Female		Male			Female		Male		
	Nos	%	Nos	%		Nos	%	Nos	%	
Jan’07	26.0	96.0	1.0	4.0	0.00	15.0	83.0	3.0	17.0	0.00
Feb’07	15.0	83.0	3.0	17.0	0.00	11.0	100.0	0.0	0.0	0.00
Mar’07	15.0	45.0	18.0	55.0	0.60	5.0	100.0	0.0	0.0	0.03
Apr'07	3.0	33.0	6.0	67.0	0.32	10.0	100.0	0.0	0.0	0.00
May’07	3.0	75.0	1.0	25.0	0.32	5.0	50.0	5.0	50.0	1.00
Jun’07	4.0	40.0	6.0	60.0	0.53	12.0	100.0	0.0	0.0	0.00
Jul-07	- -	- -	- -	- -	- -	- -	- -	--	- -	--
Aug’07	10.0	42.0	14.0	58.0	0.41	15.0	83.0	3.0	17.0	0.00
Sep’07	10.0	27.0	27.0	73.0	0.01	13.0	100.0	0.0	0.0	0.00
Oct’07	91.0	55.0	73.0	45.0	0.16	197.0	89.0	25.0	11.0	0.00
Nov’07	14.0	78.0	4.0	22.0	0.02	0.0	00	0.0	00	0.00
Dec’07	25.0	100.0	0.0	0.0	0.00	0.0	00	0.0	00	0.00
Jan’08	15.0	94.0	1.0	6.0	0.00	7.0	70.0	3.0	30.0	0.21
Feb’08	13.0	81.0	3.0	19.0	0.01	14.0	100.0	0.0	0.0	0.00
Mar’08	8.0	44.0	10.0	56.0	0.64	0.0		0.0		0.00
Apr’08	2.0	29.0	5.0	71.0	0.26	10.0	83.0	2.0	17.0	0.02
May’08	3.0	75.0	1.0	25.0	0.32	2.0	29.0	5.0	71.0	0.26
Jun’08	3.0	33.0	6.0	67.0	0.32	7.0	78.0	2.0	22.0	0.10
Jul-08	--	--	--	--	--	--	--	--	--	--
Aug’08	5.0	36.0	9.0	64.0	0.29	14.0	61.0	9.0	39.0	0.30
Sep’08	4.0	17.0	19.0	83.0	0.00	5.0	56.0	4.0	44.0	0.74
Oct’08	34.0	62.0	21.0	38.0	0.08	28.0	67.0	14.0	33.0	0.03
Nov’08	11.0	73.0	4.0	27.0	0.07	0.0	00	0.0	00	0.00
Dec’08	14.0	74.0	5.0	26.0	0.04	3.0	60.0	2.0	40.0	0.65
Total	328	58.0	237	42.0		373	83.0	77	17.0	

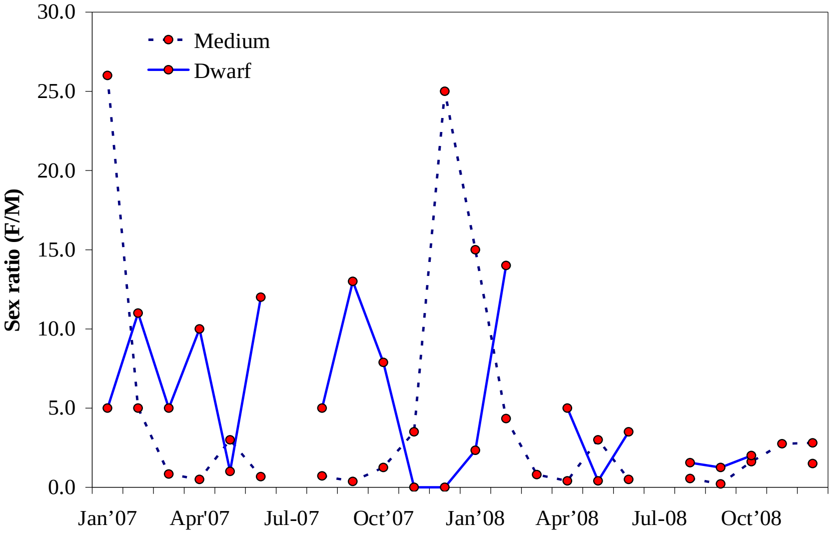

Figure 4.1. Monthly sex ratio for the medium and dwarf form of *S. oualaniensis*

4.3.2. Size of various maturity stages

4.3.2.1. Medium form

Out of the 328 females examined from the medium form, 21 (6%) were of stage-I, 61 (19%) were of stage –II, 61 (19%) were of stage- III, 41 (13%) were of stage IV and 144 (44%) were of stage V (Fully matured). Males were in 237 numbers, out of which 2 (1%) were of stage –I, 6 (3%) were of stage-II, 40 (17%) were of stage –III, 31 (13) were of stage- IV and 158 (67) were of stage V, fully matured. The total mantle length varied between 10 – 27 cm for medium female (10 -14 cm for stage-I, 10-16 cm for stage –II, 12 – 18 for stage –III, 11-19 cm for stage -IV and 12- 27 cm for stage –V fully matured) and 10 – 19 cm for medium male (stage –I & II 10 – 12 cm, stage –III , 10 – 14 cm , stage- IV, 11-15 cm and stage V, 12 – 19 cm). The length-wise distribution of the stages have been given in the Table 4.2. No spend female or male was observed during the sampling period.

Table 4.2. Length wise distribution of different maturity stages of Medium form

DML	Female Maturity Stages (%)					Total	Male Maturity Stages (%)					Total
(cm)	I	II	III	IV	V	(Nos)	I	II	III	IV	V	(Nos)
10	20	80				5.0	14.3	14.3	71.4			7
11	20	70		10		10.0	18.2	68.2	13.6			22
12	12	47	29	6	6	17.0	3.3	3.3	36.7	36.7	20	30
13	26	41	23	8	3	39.0			13.6	20.3	66.1	59
14	14	33	35	7	12	43.0			2.7	5.4	91.9	31
15		22	51	14	12	49.0				9.7	90.3	31
16		5	18	50	27	24.0					100	21
17			9	41	50	22.0					100	14
18			7	33	60	15.0					100	12
19				4	96	25.0					100	4
20					100	13.0						
21					100	22.0						
22					100	17.0						
23					100	15.0						
24					100	5.0						
25					100	5.0						
26					100	2.0						
27					100	2.0						
Total	6	19	19	13	44	328	1	2	17	13	67	237

4.3.2.2. Dwarf form

Dwarf had a total of 373 females, wherein 10 (3%) were of stage-II, 55 (15%) were of stage –III, 82 (22%) were of stage- IV and 226 (60%) were of stage V (Fully matured). Males were only 77 in numbers, out of which stage III and IV animals were 13 (17%) in numbers and stage V, fully matured was 51 in numbers (66%). No female stage I animal and male stage I and II animal and spend in both the sex was observed during the study. Total mantle length varied between 9 – 16 cm for dwarf female (9 -12 cm for stage-II, 9-13 cm for stage –III, 10 – 14 for stage –IV and 11- 16 cm for stage –V fully matured) and 9-13 cm for dwarf male (stage –III , 9 – 10 cm , stage- IV, 9- 11 cm and stage V, 9 – 13 cm). The length wise distribution of the stages have been given in the Table 4.3.

Table 4.3. Length wise distribution of different maturity stages of Dwarf form

DML	Female Maturity Stages in %				Total	Male Maturity Stages in %			Total
(cm)	II	III	IV	V	(Nos)	III	IV	V	(Nos)
9	50.0	50.0	0.0		2	66.7	16.7	16.7	6
10	0.0	80.0	20.0		5	39.1	39.1	21.7	23
11	9.8	37.3	25.5	27.5	51		11.1	88.9	27
12	4.3	17.3	43.5	34.8	92			100	12
13		13.6	20.9	65.5	110			100	9
14			7.6	92.4	66				
15				100.0	35				
16				100.0	12				
Total	3	15	22	60	373	17	17	66	77

4.3.2.3 Size at maturity

The size range of the mature females of the medium form was very wide, varying between 12 cm and 27 cm, than the male with 12 cm and 19 cm. However, in dwarf, the variation between females and males were not so pronounced as medium, wherein, the female varied between 11 and 16 cm and male varied between 9 and 13 cm mantle length. This suggests that male medium attained 100% maturity at a faster rate from the onset of maturity than the medium female. In the medium form, 50 % of the female attained maturity at 17 cm and 100 maturity at 20 cm, while 50 % maturity was attained in the male at 12.7 cm and 100 % at 16.0 cm. However, in dwarf, 50 % of female attained maturity in the size of 12.6 cm with 100% maturity at 15 cm and male at the size of 10.4 cm with 100% at 12 cm. The size at which 50 % of the animals matured for the medium and dwarf form has been indicated in Figure 4.2 and 4.3.

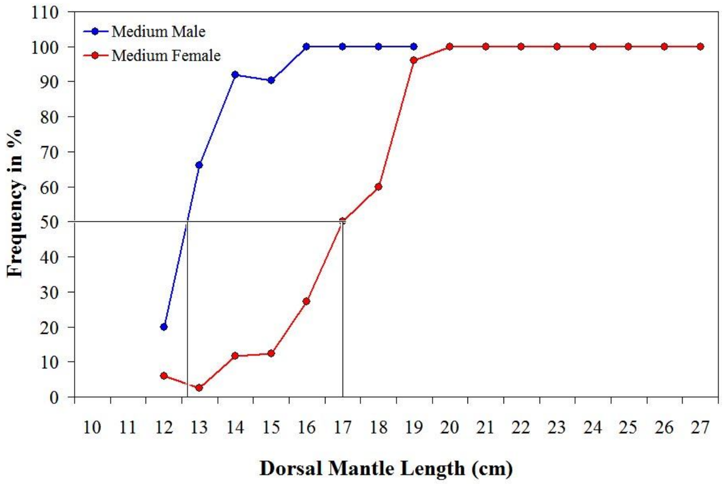

Figure 4.2. Percentage of medium mature male and female for each mantle length class with length at which 50 % attained maturity.

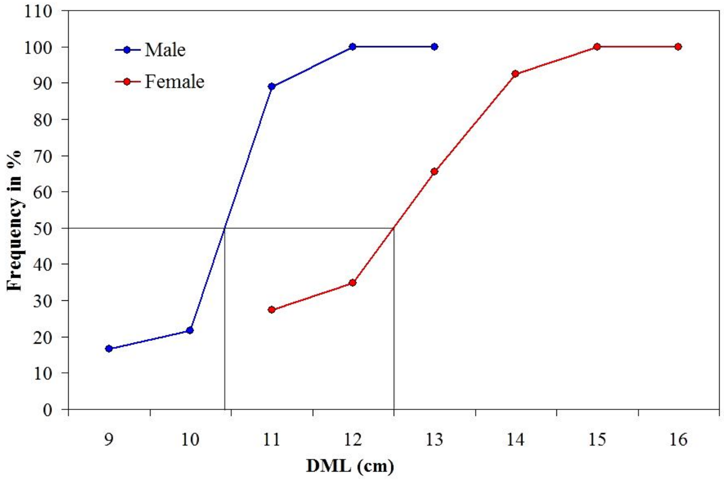

Figure 4.3. Percentage of dwarf mature male and female for each mantle length class with length at which 50 % attained maturity.

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4.3.3. Monthly variation of maturity and spawning season

To determine the spawning season, the occurrence and distribution of mature females and males were examined separately for both the medium and dwarf form along with other maturity stages throughout the period of study.

4.3.3.1. Medium form

Mature male and females were observed throughout the sampling period except in the month of May. More than 50 % of the males were found matured through out the study period except in the months of October and May'2008. However, among females more than 50 % were found matured in the months of April, June and between August to December. Squids in other stages were found in most of the months with relatively less number of stage I juvenile. No squid in stage I, stage II and stage IV were found in more than 50 % of the sample collected in any month. However, in January'2007 females in stage III were found in more than 50 % of the sample collected (Table 4.4). Male in stage I and II were rare and occurred only in the month of October. Similarly, stage III were few in numbers and occurred only during the months of February, March, August, October and December with limited numbers.

Table 4.4. Month wise distribution of different maturity stages of medium form

Months	Female Maturity Stages (%)					Total	Male Maturity Stages (%)					Total
	I	II	III	IV	V	(Nos)	I	II	III	IV	V	(Nos)
Jan’07	0	29.2	58.3	0	12.5	26	0	0	0	0	100	1
February’07	6.3	37.5	6.3	31.3	18.8	15	0	0	33.3	0	66.7	3
March’07	7.7	23.1	46.2	7.7	15.4	15	0	0	5.9	11.8	82.4	18
April ’07	0	0	0	33.3	66.7	3	0	0	0	16.7	83.3	6
May’07	0	33.3	33.3	33.3	0	3	0	0	0	0	100	1
Jun’07	0	0	0	0	100	4	0	0	0	16.7	83.3	6
July'07	-	-	-	-	-	-	-	-	-	-	-	-
August’07	0	0	0	10	90	10	0	0	7.7	7.7	84.6	14
September’07	0	11.1	11.1	0	77.8	10	0	0	0	0	100	27
October’07	5.3	19.7	11.8	11.8	51.3	91	1.4	4	39.2	19	36.5	73
Nov’07	6.7	13.3	26.7	6.7	46.7	14	0	0	0	0	100	4
December’07	3.8	0	15.4	15.4	65.4	25	-	-	-	-	-	-
January’08	0	45.5	36.4	0	18.2	15	0	0	0	0	100	1
February’08	16.7	25	16.7	16.7	25	13	0	0	0	33.3	66.7	3
March’08	0	28.6	42.9	0	28.6	8	0	0	11	0	89	10
Apr’08	0	0	0	0	100	2	0	0	0	0	100	5
May’08	33.3	33.3	0	33.3	0	3	0	0	0	100	0	1
June’08	0	0	0	0	100	3	0	0	0	16.7	83.3	6
July'08	-	-	-	-	-	-	-	-	-	-	-	-
August’08	0	0	0	0	100	5	0	0	11	11	78	9
Sep’08	0	0	0	25	75	4	0	0	0	15.8	84.2	19
October’08	9.7	12.9	9.7	29	38.7	34	4.8	14.	19	19	42.9	21
November’08	9.1	9.1	9.1	18.2	54.5	11	0	0	0	0	100	4
December’08	14.3	21.4	21.4	7.1	35.7	14	0	0	20	20	60	5
Total	6.4	18	19	13	43.6	328	1	2.5	16.5	13	67	237

4.3.3.2. Dwarf form

In dwarf form, matured male and female were found during the entire period of study and constituted more in numbers than other maturity stages. Matured females constituted more than 50 % of the sample in all most all the months except January’07, May’08, October, and December. From the limited sample of dwarf male, could be collected during the study, it was observed that matured male squid constituted more than 50% of the sample in all the months except in October. Stage I of female and stage I & II of male could not be collected during the study. Besides stage II & III of female and stage III & IV of male were limited in occurrence. Though the pre matured stages were limited in frequency and occurrence, the stage III male constituted around 43 and 50 % of the sample in the month of October and December respectively (Table 4.5).

Table 4.5. Month wise distribution of different maturity stages of dwarf form

Months	Female Maturity Stages (%)				Total	Male Maturity Stages (%)			Total
	II	III	IV	V	(Nos)	III	IV	V	(Nos)
Jan’07	0	35.7	35.7	28.6	15	0	33.3	66.7	3
February’07	0	0	27.3	72.7	11	-	-	-	-
March’07	0	0	0	100	5	-	-	-	-
April ’07	0	0	0	100	10	-	-	-	-
May’07	0	20	40	40	5	0	20	80	5
Jun’07	0	0	8.3	91.7	12	-	-	-	-
July'07	-	-	-	-	-	-	-	-	-
August’07	0	0	20	80	15	0	0	100	3
September’07	0	23.1	15.4	61.5	13	-	-	-	-
October’07	8	24	26	42	197	24	24	52	25
Nov’07	-	-	-	-	-	-	-	-	-
December’07	-	-	-	-	-	-	-	-	-
January’08	0	25	0	75	7	0	33.3	66.7	3
February’08	0	0	28.6	71.4	14	--	-	-	-
March’08	0	0	0	100	1	-	-	-	-
Apr’08	0	0	10	90	10	0	0	100	2
May’08	0	0	50	50	2	0	0	100	5
June’08	0	0	14.3	85.7	7	0	0	100	2
July'08	-	-	-	-	-	-	-	-	-
August’08	0	0	9.1	90.9	14	0	11.1	88.9	9
Sep’08	0	0	0	100	5	0	0	100	4
October’08	5.6	27.8	27.8	38.9	28	42.9	21.4	35.7	14
November’08	-	-	-	-	-	-	-	-	-
December’08	0	33.3	33.3	33.3	3	50	0	50	2
Total	4.4	16.8	21.1	57.7	373	17	17	66	77

4.3.4. Maturity indices and maturity stages

4.3.4.1. Medium form

The stage wise number of specimen subjected to different maturity study along with its minimum and maximum value and SD has been given in Table 4.6 and Figure 4.4. Corresponding to the increase in the size of the nidamental gland the mean value of the NGWI has increased from 0.23 in stage I squid to 3.65 in stage V. Similarly the NGLI also increased corresponding to the increase in the length of the nidamental gland from the mean value of 6.75 in stage I to 34.74 in the stage V squid. The high standard deviations of nidamental gland length index (NGLI) indicate that there was a wide range in the size of the nidamental gland within different maturity stages; thus, this parameter was not useful in differentiating maturity stages. Ovary weight index followed the same course by increasing corresponding to the maturity stage of the squid from the mean value of 0.3 in stage I to 4.63 in stage V. The OCWI and RSWI was estimated only in stage III, IV and V squids. The OCWI showed an increase from 0.08 in stage III to 3.66 in stage V squids as well the RSWI from a mere 2.73 of the mean value to 13.69 corresponding to the increased reproductive activity of the squid. In male testis weight index (TWI) was studied. This indicates an increase in the mean value from 0.65 in stage II to 3.57 in the stage V. It is found that the highest indices were from the spawning individuals. The relation between the mantle length and the maturity index (RSWI) were also studied to find the correlation between the two and presented in the Figure 4.5.

Table 4.6. Summary of Maturity Indices for the Medium Female form.

Indices	Maturity Stages	Minimum	Maximum	Mean	S.D	n	Significance
NGWI	I	0.10	0.70	0.23	0.22	7.0	
	II	0.31	2.00	0.94	0.56	10.0	<0.05
	III	0.62	1.97	1.26	0.40	13.0	<0.05
	IV	1.16	3.04	2.03	0.62	10.0	<0.05
	V	1.87	5.82	3.65	1.15	51.0	<0.05
NGLI	I	5.17	7.86	6.75	1.05	5.0	
	II	9.09	14.62	11.93	2.38	5.0	<0.05
	III	12.50	20.69	17.09	3.09	11.0	<0.05
	IV	20.00	28.02	23.96	3.09	6.0	<0.05
	V	21.94	45.22	34.74	5.89	38.0	<0.05
OCWI	I	-	-	-	-	-	
	II	-	-	-	-	-	
	III	0.05	0.15	0.08	0.03	10.0	
	IV	0.12	0.17	0.14	0.02	5.0	<0.05
	V	0.53	8.53	3.66	2.26	40.0	<0.05
OWI	I	0.2	0.43	0.3	0.1	8.0	
	II	0.45	2.62	1.0	0.6	19.0	<0.05
	III	0.78	2.14	1.3	0.4	16.0	<0.05
	IV	1.7	4.38	2.4	0.6	19.0	<0.05
	V	2.43	7.2	4.63	1.2	66.0	<0.05
RSWI	I	-	-	-	-	-	
	II	-	-	-	-	-	
	III	2.06	3.67	2.73	0.61	7.0	
	IV	4.20	5.41	4.71	0.62	3.0	<0.05
	V	5.58	19.33	13.69	4.77	35.0	<0.05
TWI	I	-	-	-	-	-	
	II	-	-	0.65	-	1.0	
	III	0.99	4.03	2.83	0.78	33.0	<0.05
	IV	1.36	5.17	2.91	0.92	32.0	<0.05
	V	2.45	5.85	3.57	0.83	102.0	<0.05

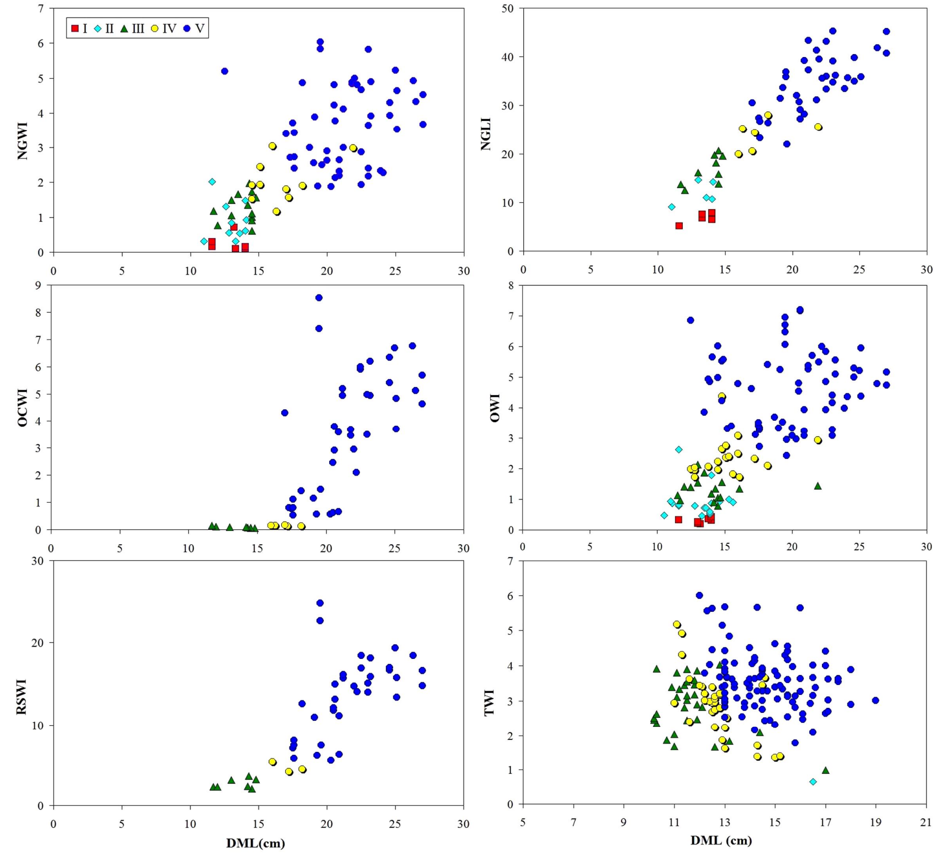

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Figure 4.4. Maturity indices for Medium female and male reproductive organs.

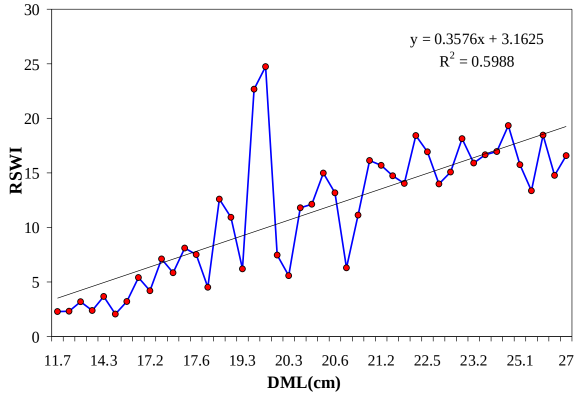

Figure 4.5. Relationship between DML and RSWI of Medium female.

4.3.4.2. Dwarf

The various maturity indices along with its minimum and maximum value and SD pertaining to different stages have been summarized in Table 4.7 and Figure 4.7. Squids in stage I and II could not be studied due to its non availability in the sample. Similar to the medium form all the indices were directionally proportional to the maturity stages indicating a clear positive correspondence to the reproductive activity of the squids. However, similar to the medium form the NGLI has wide variation in the SD indicating wide variation in the length of the gland especially in stage III and V than the medium form. The NGWI varies from 2.19 in stage III to 3.81 stage V. Similarly the NGLI varies from 20.9 in stage III to 33.6 in stage V squid. Ovary weight index varies from 5.19 in stage III to 7.28 in stage V. OCWI and RSWI varies from 0.5 and 8.21 in stage III to 3.12 and 15.2 respectively in stage V. Testis weight index estimated in the male indicates a steady increase in the mean value from 3.55 in stage III to 5.45 in the stage V. This was significantly higher than the TWI of 3.57 estimated in the medium form and indicates that the dwarf testis has been relative larger in size than the medium form. Similar to the medium form the highest indices were obtained from the spawning individuals. Similar to the medium form the relation between the mantle length and the maturity index (RSWI) was studied in dwarf also to find the correlation between the two and presented in the Figure 4.6.

Table 4.7. Summary of Maturity Indices for the Dwarf form.

	Maturity Stage	Minimum	Maximum	Mean	S.D	n	Significance
NGWI	III	1.93	2.68	2.19	0.23609	8	
	IV	2.72	3.04	2.88	0.23195	2	<0.05
	V	2.6	5.55	3.81	0.65799	47	<0.05
NGLI	III	15.7	33	20.9	5.26527	8	
	IV	25.2	27.1	26.2	1.36716	2	<0.05
	V	19.1	42.5	33.6	4.01644	42	<0.05
OCWI	III	0.19	1.38	0.5	0.387097	8	
	IV	0.5	0.63	0.57	0.089243	2	<0.05
	V	0.57	7.3	3.12	2.083363	42	<0.05
OWI	III	2.04	6.48	5.19	1.307722	10	
	IV	3.53	8.73	6.01	1.495813	12	<0.05
	V	3.26	15.69	7.28	2.157599	94	<0.05
RSWI	III	4.36	10.34	8.21	1.788301	8	
	IV	9.76	11.45	10.6	1.196633	2	<0.05
	V	7.99	24.48	15.2	4.489975	42	<0.05
TWI	III	1.85	5.02	3.55	0.229347	13	
	IV	2.78	6.23	4.69	1.065178	11	<0.05
	V	3.26	7.53	5.45	0.705641	42	<0.05

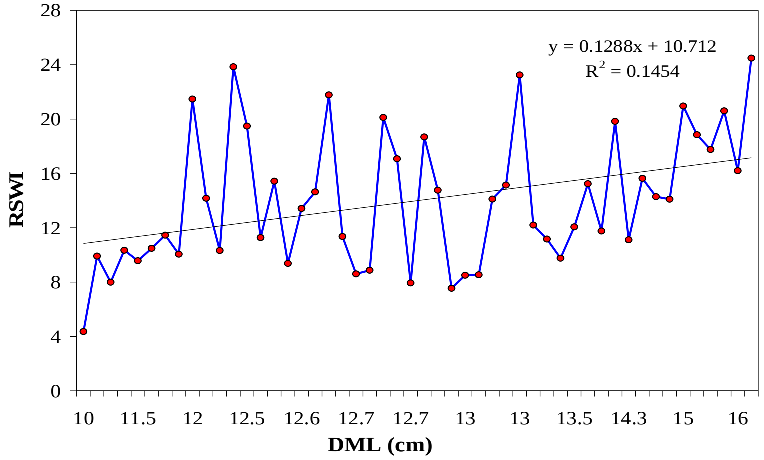

Figure 4.6. Relationship between DML and RSWI of dwarf female.

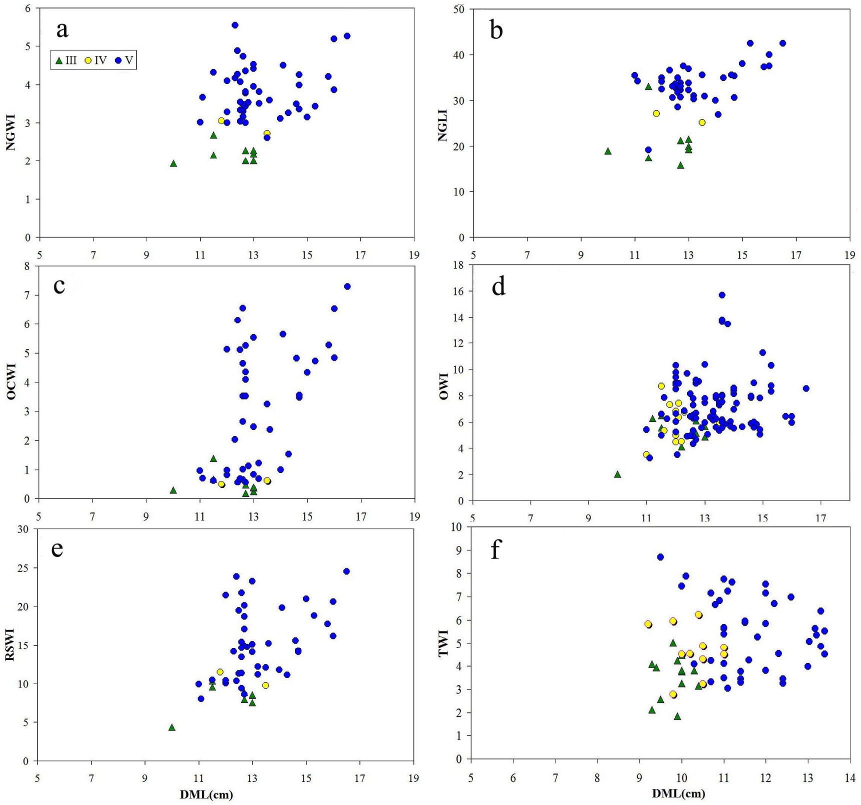

Figure 4.7. Maturity indices for Dwarf female and male reproductive organs.

4.3.5. *Maturity indices and spawning season*

4.3.5.1. Medium

The month wise mean index values of different maturity indices and its SD values have been summarized in Table 4.8. It was seen that a clear pattern of seasonal change in the maturity index was emerging. During most of the months ovary maturity index (OWI) fluctuated between the mean value of 2.0 and 4.0 and attaining the highest value of > 4 during April, August, September and

December followed by the value of > 3 in the months of February, March, June and November. The highest value of April and August had less deviation from the mean value (Figure 4.8). Similarly NGWI mean value also fluctuated between 2.0 and 4.0 with the highest value of > 4, obtained during the months of November and December followed value of > 3 obtained during March , April and September . Both the OWI and NGWI were found to be <1 only in the month of May. Sufficient monthly samples were not available for OCWI and RSWI.

Table. 4.8. Month wise maturity indices for medium form

Months	OWI		NGWI		ODCI		RSWI		TWI	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Jan-07	2.69	2.44	2.33	2.00	0	0	0	0	2.32	1.00
Feb-07	2.68	1.39	1.64	0.24	0	0	0	0	1.92	0.29
Mar-07	2.27	3.1	3.47	2.22	3.22	2.94	18.42	0	3.57	0.65
Apr-07	4.23	1.09	3.14	1.05	1.15	0.50	10.93	0	3.99	0.44
May-07	1.8	0.99	1.56	0.95	0	0	0	0	3.18	1.00
Jun-07	3.51	0.26	2.95	0.07	0	0	0	0	3.09	0.19
Jul-07	0	0	0	0	0	0	0	0	0	0
Aug-07	3.19	0.7	2.11	0.33	0.56	0.10	5.58	0	3.25	0.55
Sep-07	3.9	3	3.77	1.82	3.78	1.20	14.98	0	3.44	0.54
Oct-07	2.33	1.88	2.81	0.92	1.80	2.27	9.1	4.79	3.25	0.80
Nov-07	3.3	1.5	4.80	1.23	0.62	0.30	11.8	0	3.68	0.43
Dec-07	4.47	1.93	4.12	1.23	4.63	2.31	15.21	5.52	4.13	0.81
Jan-08	0.4	0.1	0.31	0.00	0	0	0	0	2.51	1.00
Feb-08	3.04	3.7	2.16	0.89	1.09	1.81	0	0	3.35	2.28
Mar-08	3	1.5	3.60	2.16	1.87	1.72	14.03	0	3.77	0.90
Apr-08	4.4	1.59	2.18	1.56	0	0	0	0	3.94	1.37
May-08	1.53	1.16	1.32	0.62	0	0	0	0	3.21	1.00
Jun-08	3.17	0.14	2.61	0.05	0	0	0	0	2.28	0.37
Jul-08	0	0	0	0	0	0	0	0	0	0
Aug-08	4.29	0.73	2.09	0.26	0.57	0.20	6.2	0	2.90	0.49
Sep-08	4.19	2.62	2.88	0.33	2.19	1.01	10.32	6.12	2.46	1.49
Oct-08	2.78	1.69	2.50	1.43	3.15	2.49	11.09	5.37	2.72	0.94
Nov-08	3.28	0.02	3.66	0.99	1.13	1.20	8.21	3.49	2.98	0.60
Dec-08	3.3	2.2	2.31	2.08	3.19	2.97	10.99	7.59	0	0

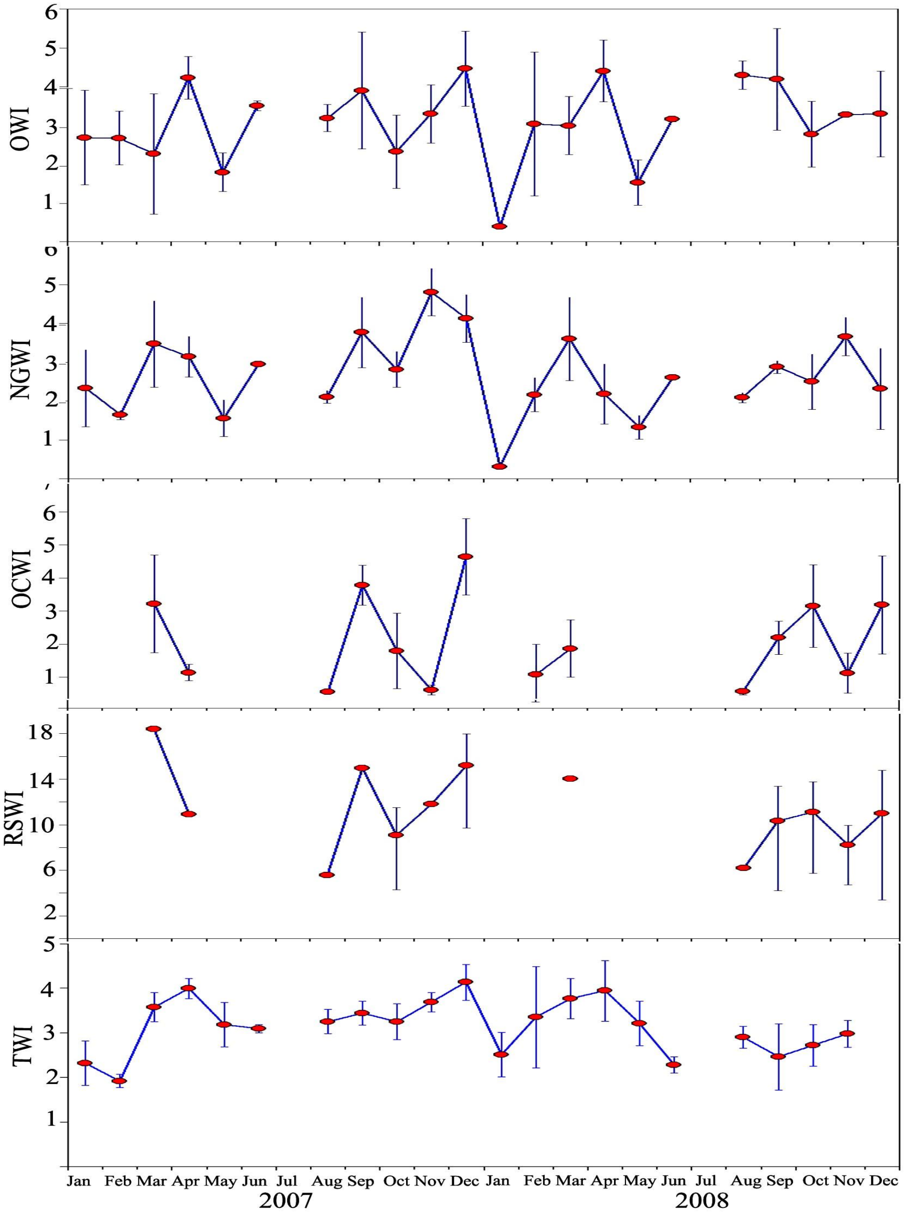

Figure 4.8. Monthly variation in the maturity indices of Medium female and male indicating the SD at each point.

However with the available sample, it was observed that OCWI was highest in the month of December (>4) followed by March, September, October (>3). Whereas, RSWI was highest in the month of March followed by September and December. TWI was highest in the month of April and December and the remaining months the difference was not so pronounced.

4.3.5.2. Dwarf

Dwarf samples were not available in all the months during the study period. In view of that the maturity index estimation could not be conducted for all the months to have a continuity. However, with the available sample an attempt has been made to indicate the spawning period and its periodicity. The month wise mean index values of different maturity indices and its SD values have been summarized in Table 4.9 and Figure 4.9. Among the months studied OWI was highest in the month of May, June and August with a mean value of >8. During the remaining study period ranged between 6 and 7 except in the month of October, when it was 5.7. NGWI, OCWI and RSWI indicates a higher value in the month of March and April. However, due to non availability of data in most of the months, a viable correlation and inference could not be done from these results. TWI ranged between 3 and 7, with a higher value in the month of June followed by May and September.

Table 4.9. Month wise maturity indices for Dwarf form

Months	OWI		NGWI		ODCI		RSWI		TWI	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Jan-07									3.45	
Feb-07										
Mar-07	6.11	1.19	4	0.71	5.23	0.59	17.73	2.98		
Apr-07	7.67	1.66	3.36	0.09	4.12	0.85	17.14	2.41		
May-07									5.69	1.51
Jun-07	8.36	2.55								
Jul-07										
Aug-07	7.22	2.48	3.82	0.74	2.51	2.61	14.74	7.61	5.4	2.23
Sep-07	6.09	1.18	3.4	0.39	2.88	3.36	14.26	3.98		
Oct-07	5.7	1.31	3.33	0.97	1.42	2.05	11.22	4.61	4.54	1.14
Nov-07										
Dec-07										
Jan-08									3.86	0.95
Feb-08										
Mar-08	6.04		3		0.83		10.42			
Apr-08	7.62	1.81	3.34	0.27	2.52	2.56	16.12	6.85	3.5	
May-08	8.73								6.9	1.74
Jun-08	8.37	1.81							7.53	
Jul-08										
Aug-08	8.38	2.74	3.47	0.44	2.55	2.17	15.02	5.18	5.15	1.51
Sep-08	6.67		3.23	0.27	3.53		14.91		6.49	1.12
Oct-08	6.23	1.11	3.4	1.07	1.97	1.99	12.49	3.99	4.19	1.31
Nov-08										
Dec-08	6.32	1.99	3.86	0.85	3.39	2.13	15.46	5.34	5.85	2.68

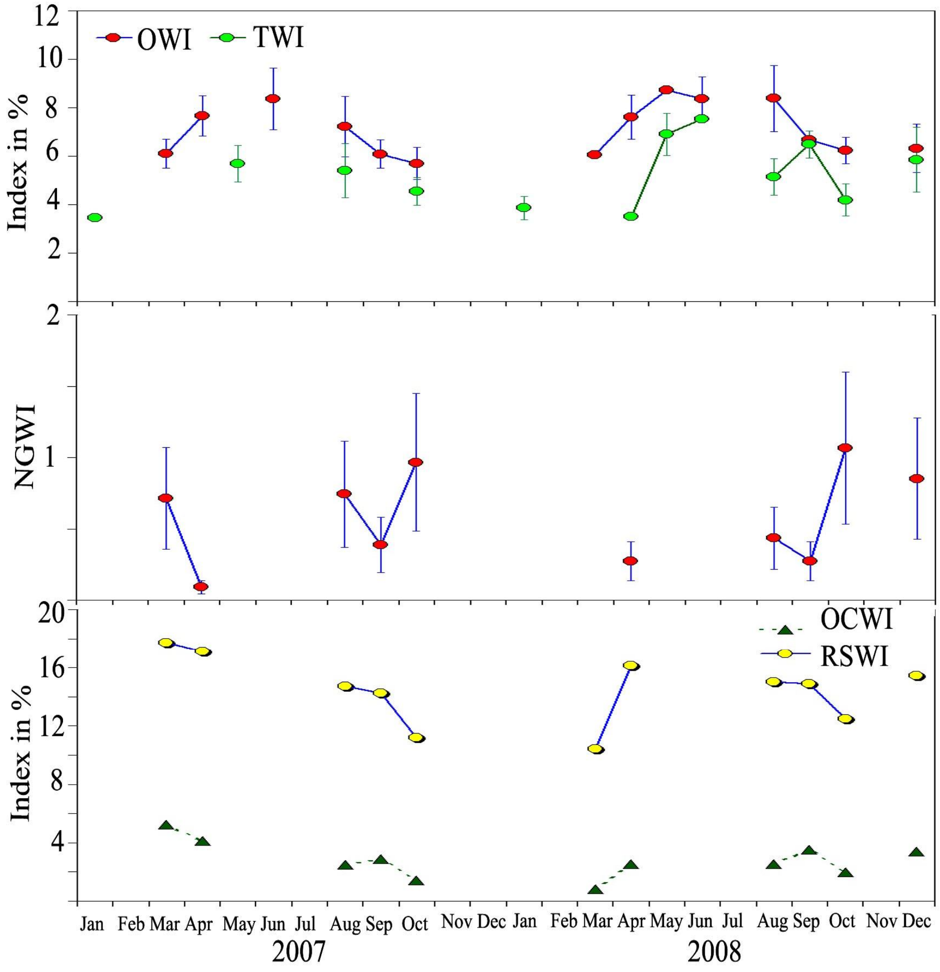

Figure 4.9. Monthly variation in the maturity indices of Dwarf female and male indicating the SD at each point.

4.3.6. Ova and Fecundity

The ripe ova measurements ranged from 0.73 to 0.85 mm in diameter along the long axis of the ova with the mean value of 0.78 mm for the medium form and 0.73 mm for the dwarf form. Medium form is more fecund than the dwarf form. To obtain an estimate of fecundity, the number of maturing and mature ova was counted from females at Stage IV and V of the medium form ranging from the size of 14 to 27 DML. It was assumed that the immature ova would not be spawned. The relationship between the mantle length and the number of ova in the ovary and oviduct of medium form has been shown in Table 4.10. The total number of ova for the squid of mantle length 17 cm and 27 cm were between 40,000 and 2,47,059 for batch fecundity of the oviduct, 1,92,728 and 28,02,555 for the ovarian fecundity and 2,51,202 and 28,90,270 for potential fecundity.

The length wise details of the fecundity for the dwarf have been summarized in Table 4.11. Specimens of 9 cm DML to 16 cm DML were subjected to the fecundity study of the dwarf. However, in the smallest length group of 9 cm DML only one sample was studied for fecundity, which has an batch fecundity of 4,417, ovarian fecundity of 33,091 and corresponding potential fecundity of 37,507. Besides, for the length of 10 cm and 16 cm squid, the batch fecundity was of 5,833 and 50,833; ovarian fecundity 1,06,364 and 4,82,727 and the potential fecundity was of 1,07,727 and 5,28,106 respectively. The relation ship between the mantle length and fecundity of both the form of the *Sthenoteuthis oualaniensis* has been presented in the Figure 4.10.

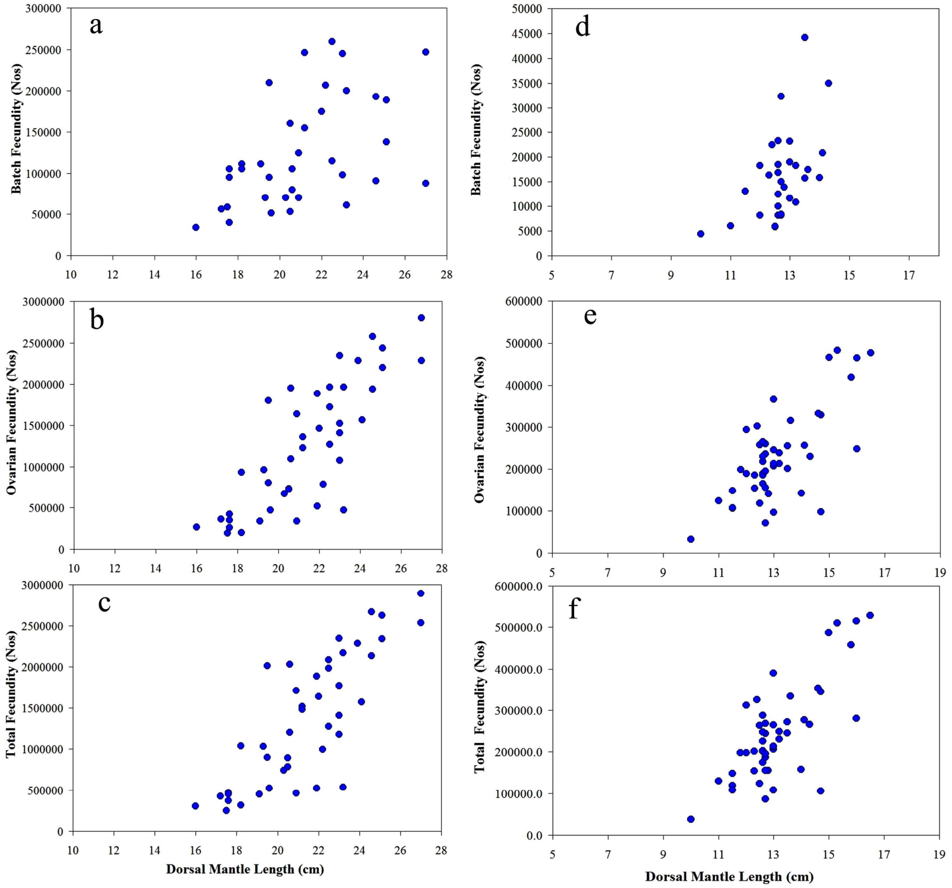

Figure 4.10. Relationship between mantle length and fecundity. (a) to (c) medium form and (d) to (f) dwarf form.

Table. 4.10. Length wise details of the medium form fecundity

Class		Batch Fecundity (Nos)				Ovary Fecundity (Nos)				Potential Fecundity (Nos)			
Interval	f	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
14-16	1			34000				268600				302600	
16-18	7	40000	111176	81598	28991	192728	932823	390247	254472	251202	471846	471846	260865
18-20	8	51205	210000	102578	56297	339623	1801528	813073	442908	450799	2011528	915650	442908
20-22	13	70000	259272	153732	66759	336294	1967779	1325355	530122	461159	2082822	1443610	536879
22-24	8	61000	245000	151062	85868	473583	2345543	1581049	626199	534583	2345543	1656580	621638
24-26	4	90650	193200	152647	48300	1940663	2575755	2288764	278971	2133863	2666405	2441411	251631
26-28	2	87715	247059	167387	112673	2286690	2802555	2544623	364772	2533749	2890270	2712010	252099

Table. 4.11. Length wise details of the dwarf form fecundity

DML (cm)	f	Batch Fecundity (Nos)				Ovary Fecundity (Nos)				Potential Fecundity (Nos)			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
08-10.	1			4417				33091				37507	
10-12.	12	5833	22500	12021	6503	106364	302545	182159	69888	107727	325045	190173	74848
12-14.	27	8167	44167	18146	9136	71364	366364	209729	61959	86363	389613	224514	64522
14-16	9	8489	50833	29332	14627	97572	482727	368730	130946	106061	528106	398062	140627

4.4. Discussion

4.4.1. Sex ratio

This study indicate that, *Sthenoteuthis oualanienesis* female found to outnumber the male in both the medium and dwarf form with the overall sex ratio of female to male as 1.4:1 for medium and 4.8 : 1 for dwarf form. This ratio fluctuates through out the study period for both the forms from a maximum of 26: 1 in the month of January to a minimum of 0.2 : 1 in the month of September. Whereas, in dwarf form this ratio varies between a maximum of 14: 1 in the month of February and a minimum of 0.4 : 1 in the month of May. However, females significantly out number the males in the months of November, December, January and February and males out number female only in the month of September. The months where females dominates is not corresponding with the peak reproductive activity period of the medium form. In contrast, the dwarf female out numbers the males in all the months except in the month of May, when male dominates. This may indicate the continues spawning activity and the absence of clear spawning peak. However, this skewed sex ratio is common with jig caught ommastrephid squids (Young and Hirotta, 1998). The occurrence of large number of females to males in the samples of both the medium and dwarf forms might also be due to the selectivity of the catching method. The size of the jigs may play a direct role on the size of the squid attracted and caught as it emulates the prey. Siriraksophon and Nakamura (2001) has reported that *S. oualaniensis* ratio of females to males caught by jigging machines was 4:1 in Philippines. This indicate the fact that the smaller the size, the lesser the vulnerability of the squid to the jigs. Perhaps, due to this reason the sample size of the dwarf collected during this study was comparatively smaller than the medium form. Likewise, as male being smaller in both the forms it has a fair chance of escaping the jigs.

However, according to the results of the former USSR research in the northwest Indian ocean (Trotsenko and Pinchukov, 1994), the sex ratio had a seasonal variation, and the ratio (F:M) was 1:0.24 in summer, but changed to 1:0.52 in winter. Nesis (1993) found that the sex ratio is nearly equal among young squids but strongly shifted to females among sub adults/adults. The shape of the size frequency curves of the squid caught during this study suggests that males were dominant until 13.0 cm DML for medium forms and 10.0 cm DML for the dwarf form. Beyond this size frequency, the sex ratio was dominated by the females in view of the larger size range of females. Okutani and Tung (1978) report a similar situation off Taiwan and Okinawa. The sex ratio is 1:1 at 10-13.0 cm ML while the relative abundance of males decreases beyond 14.0 cm ML. In Hawaiian waters among all the squid captured larger than 10.0 cm DML, females exceeded males in abundance by 3:1 ratio. However, after examining several alternatives Young and Hirota (1998) concluded that sexes are about equally abundant until males start to mature and the reason for the predominance of females in the catches was unresolved.

4.4.2. Size of various maturity stages

The fully matured late maturing medium form constituted 44 % of the female with a size range 12 to 27 cm DML and 67 % of the male ranging from 12 to 19.5 cm. This wide size range of the matured squid indicate the growth of the squid after attaining the maturity thereby indicating the continuous growth and feeding in between maturation. The presence of large percentage of matured squid may probably due to the fishing method and the size of the jigs used to catch these squids as indicated earlier. In general, the proportion of the squids at preparatory and immature stage was much less than the matured squid in both female and male. The presence of relative low percentage of the squid at the preparatory stage suggests that the transition from immaturity to maturity occurs in a short period of time (Gonzalez & Guerra, 1996). No spent female or male was observed during the sampling period, probably due to post spawning exhaustion these ommastrephid squid may not attack jigs, as it stops feeding (Markaida and Nishizaki, 2001). Similar to the medium form, matured squids dominated the dwarf form with 60 % amoung females and 66 % amoung males. The proportion of the female preparatory stage was around 1/3 of the matured sample size and the immature stage III and II 's were 15 % and 3% respectively to the total. Preparatory and III stage of male constituted only 17 % of the total. No stage I & II squid in the dwarf and male stage I squid in the medium and spent in both the sex was observed during the study. The analyses of the maturity indices in relation to the mantle length indicates that the maturity is directionally proportional to the dorsal mantle length in both the medium and dwarf form. In view of that, it may safely be presumed that, the larger the squid caught by the fishing method, the more greater will be the share of the matured ones in the sample.

4.4.3. Size at maturity

Maturation of squid belonging to the different forms begins at different sizes. The size parameters of mature males and females of the middle-sized forms are not though exactly defined, Chesalin et al.,(1995) has stated that the ML of middle size males squids ranged from 11–25 cm and females 16–28 cm. However, in this study the size range of the mature females of the medium form was found to be very wide, varying between 12 cm and 27 cm, than the male with

12 cm and 19 cm. This suggests that male medium form attained 100% maturity at a faster rate from the onset of maturity than the medium form female .In dwarf form, the variation between females and males were not so pronounced as medium, as the female varied between 11 and 16 cm and the male between 9 and 13 cm mantle length. Zuyev et al., (1985) says that mantle length of mature males in the dwarf equatorial form of *S. oualaniensis was* 7.5–11 cm, of mature females 10–15 cm.. In the medium form, 50 % of the female attained maturity at 17 cm and 100 % maturity at 20 cm, while for male 50 % maturity was attained at 12.7 cm and 100 % at 16.0 cm. This is substantiated by the study in Hawaiian waters, where *S. oualaniensis* females mature between 158 and 205 mm ML with 50 percent mature at 166-175 mm ML and 90 percent mature at 200 mm ML. Males matured mostly by 140 mm ML (Young and Hirota 1998, Suzuki et al. 1986). However, in dwarf, 50 % of female attained maturity in the size of 12.6 cm with 100% maturity at 15 cm and male at the size of 10.4 cm with 100% at 12 cm. Nesis (1977 in Dunning 1998) found males mature over 110 mm ML and females at 180-190 mm ML.

4.4.4. Monthly variation of maturity stages and spawning season

The prevalence of mature male and females throughout the sampling period except in the month of May indicate the existence of reproductive activity throughout the year. It is further strengthened by the fact that more than 50 % of the males were found matured through out the study period except in the months of October and May'08. However, among females more than 50% were mature in the months of April, June and between August to December indicating the period of probable spawning peak of the squid. Squids in other stages were found in most of the months with relatively less number. No squid in stage I, II and IV were found in more than 50 %, of the sample collected in any months. However, only in January'07 female in stage III were found more than 50 % of the sample collected. This may probably be due to the continuous development of matured ova in the ovary and replenishment of the matured ova in the oviduct of the squid, while the squid grows and sustains itself until it is replaced in the spawning stock, at its possible exhausted demise. Male in stage I and II were rare and occurred only in the month of October. Similarly, stage III were few in numbers and occurred only during the months of February, March, August, October and December with limited numbers.

Similar to the medium form matured, dwarf form male and female were found during the entire period of study and constituted more in numbers than other maturity stages. Dwarf matured females constituted more than 50 % of the sample in all most all the months except January'07, May'08, October, and December indicating the two probable spawning peaks between February to April and August to November. It was observed that matured male squid constituted more than 50% of the sample in all the months except in October, suggesting that they are available for reproduction all year around. Though the pre matured stages were limited in frequency and occurrence, the stage III male constituted around 43 and 50 % of the sample in the month of October and December respectively.

4.4.5. Maturity indices and maturity stages

There is a gradual increase of the various maturity indices proportionate to the maturation of the squid. These maturity indices were significantly correlated to maturation and correspond well to the descriptive maturity stages assigned resulting in the clear demarcation of the maturity stages. It is found that the highest indices were from the spawning individuals. The almost doubling of NGWI and OWI from stage IV to stage V is attributable to the abrupt increase in both ovary and nidamental gland volume due to the accumulation of secretions in of nidamental gland and increase in the percentage of yolk oocytes respectively. The OCWI increases multifold from 0.14 of stage IV to 3.66 of stage V due to the ripe eggs accumulation in the oviducts. The multifold increase in the RSWI from 4.71 of stage IV to 13.69 of stage V is also contributable to all the above factors. The RSWI is well with in the estimated maximal maturity index (weight of reproductive system as % of BW) of between 11 and 15 % for *S. oualaniensis* (Zuyev et al., 1985; Harman et al., 1989; Chesalin and Giragosov, 1993; Laptikhovsky, 1995). The relative weight of the entire reproductive system of mature females typically varies between 10-20 % (Burukovsky *et al*., 1977).

Nidamental gland length index (NGLI) indicates from its high standard deviations, the occurrence of a wide range in the size of the nidamental gland within different maturity stages. In the dwarf form, the testis weight index estimated in the male indicates a steady increase in the mean value from 3.55 in stage III to 5.45 in the stage V indicating a relatively larger testis in the dwarf form.

4.4.6. Maturity indices and spawning season

A clear pattern of seasonal change in the maturity index has emerged. The maturity indices such as OWI, NGWI and TWI of the medium form indicates that spawning is a continues process through out the year with a ebb in the month of January and May. Zuyev et al. (2002) says that spawning in *Sthenoteuthis* takes place all-year-round, but at different intensities and different intra-species forms differ in their time of spawning. In this study two probable spawning peaks have been identified, One in the month of March and April and another in the month of August to December. This is further strengthened by the OCWI and the RSWI, which indicates that the spawning activity is at its highest in the month of December followed by March, September, October. This corresponds to the finding of Sukramongkol et al., (2008), which estimate the spawning season of *S oualaniensis* based on the back calculation from July to October in the eastern Bay of Bengal. This is further corroborated by the fact that the spawning peak of the middle sized form, in the northern hemisphere is during autumn and winter (Okutani and Tung, 1978; Zuyev, 1971; Zuyev et al., 1985). In southeastern Pacific the squids of middle-sized form spawn all-year-round with peak in warm season (December–February) (Nigmatullin et al., 1983a). However, Okutani and Tung (1978) found *S. oualaniensis* in Taiwanese waters to consist of three different seasonal cohorts: a June-spawning group, a September-October spawning group and a February-March spawning group.

Dwarf form maturity index estimation could not be conducted for all the months due to non-availability of samples. However, with the available sample an attempt has been made to indicate the spawning period and its periodicity. Maturity index values were highest for all indices when compared to the medium form and there is no considerable difference between the values of the months in case of OWI, NGWI and TWI. This may probably indicate the continuous spawning behavior of the dwarf form through out the year with out any major fluctuation as stated by Harman et al. (1989), Young and Hirota, (1998) and Rocha et al. (2001). However, the OWI and TWI indicates a peak between the months of May and August. This corroborate the study indicating the spawning peak of dwarf equatorial form of *S. oualaniensis* in summer (Nesis, 1975; Pinchukov, 1983; Zuyev et al., 1985). The higher value of the OWI and the TWI than the corresponding medium form indicates that the ovary and testis of the dwarf are relatively larger than the medium form.

4.4.7. Fecundity

Medium form was found to be more fecund than the dwarf form probably as the potential fecundity (PF) depends on adult female size (Laptikhovsky *et al*., 2005). The results of this study corroborate the earlier study of Zuyev et al., (2002). Apart from *Dosidicus gigas* and *S. pteropus* (Nigmatullin *et al*., 1999; Laptikhovsky *et al*., 2005), the PF in *S.oualaniensis* is one of the highest amongst all squid species (Zuev et al., 2002). Actual fecundity in *Sthenoteuthis* is about half as much, but it is still very high and exceeds the total number of yolk oocytes simultaneously present in the female ovary and oviducts combined (Laptikhovsky et al., 2005). Given this enormous fecundity, it is not surprising to find that the egg size in this squid is among the smallest known for oceanic cephalopods. The correlation between the egg weight and female size indicates that the species has an extreme r-strategy with the smallest possible egg size (Laptikhovsky et al., 2005). Using, even partially, this huge number of eggs could only be achieved by an extended intermittent spawning, which has been suggested earlier for this species and *S. pteropus* (Harman *et al*., 1989; Snyder, 1998; Laptikhovsky *et al*., 2005), and for other ommastrephid squid (Laptikhovsky and Nigmatullin, 1993; Nigmatullin and Laptikhovsky, 1994, 1999a; Rocha et al., 2001). As females become mature at an age of 9-10 months and the life span of this species is about one year (Arkhipkin and Mikheev, 1992, Zuyev et al., 2002), spawning in the *Sthenoteuthis* squid could last for up to 3-4 months (Harman *et al*., 1989; Snyder, 1998; Laptikhovsky et al., 2005). During these months, the female may release several cohorts of ripe eggs, which have accumulated in the oviducts.

A large female can have up to one million ripe eggs in her oviducts, but these would not necessarily be laid in one night as a single egg mass (Laptikhovsky *et al*., 2005). More likely, the egg stock in the oviducts could be released by a series of small egg masses, such as the one found by Laptikhovsky and Murzov (1990), which contained about nineteen thousand, eggs (Laptikhovsky and Murzov, 1990). Such a long reproductive period is possible because of continuous feeding, which only decreases slightly with maturation, but not to the same extent as in the ommastrephid squid genus *Illex*, (Laptikhovsky et al., 2005). The *Illex* spp. stops feeding almost completely during the relatively short spawning period (Laptikhovsky and Nigmatullin, 1993). Ovulation is group-synchronous, spawning pattern is monocyclic and egg-laying occurs in separate batches, somatic growth continuing between separate spawning events. (Harman et al., 1989). *Sthenoteuthis* spp. is among the most oceanic squid species in the Ommastrephidae, which is “oceanic” itself when compared with the low fecundity Loliginidae and Sepiolidae that inhabit shelf waters.

The main direction of the evolution of this family was from initial slope-shelf habitats to high sea habitats (Nigmatullin, 1979). It is hypothesised that the penetration of *Sthenoteuthis* spp. in to offshore pelagic waters of open ocean provoked a drastic increase in mortality because of the dispersal of the early staged squid by the complicated system of superficial currents and counter-currents found in equatorial waters (Laptikhovsky et al., 2005). A strategy of intermittent spawning over a relatively long reproductive period would ensure a higher probability that some egg masses would hatch when conditions were favourable for offspring survival (Laptikhovsky et al., 2005). The necessary increase in fecundity co-evolved with the production of eggs of the smallest size known for cephalopods (Nigmatullin and Laptikhovsky, 1994). This hypothesis is supported by the fact that other cephalopod families reproducing in the oceanic epipelagial also have very high fecundity with intermittent spawning of small eggs (Nigmatullin and Arkhipkin, 1998; Laptikhovsky, 1999b; Laptikhovsky and Salman, 2003).

In summary, *Sthenoteuthis oualaniensis* female was found to outnumber the male in both the medium and dwarf form. However, the period of out numbering was not corresponding with the peak reproductive activity period of the medium form. Male of the medium form attained maturity at a faster rate from the onset of maturity than the medium female. Maturity indices were proportionate to the maturation of the squid and were highly correlated to maturation and corresponded to the descriptive maturity stages assigned. A clear pattern of seasonal change in the maturity index has emerged. In medium form spawning was an continues process through out the year with two spawning peaks, One in the months of March and April and another in the months of August to December and with a ebb in the months of January and May. Dwarf maturity index indicated the continuous spawning behavior of the dwarf form through out the year with out any major fluctuation or peak. However, it may have a slight peak between the months of May and August. Medium form found to be more fecund than the dwarf form. The huge number of eggs estimated in this squid along with the longer, continuous period of reproductive activity and wider size range of fully matured stage indicate the continuous extended intermittent spawning and feeding behavior with somatic growth in between. The non availability of spent squid in the sample may indicate the reluctance of the spent squids in taking the jigs, thereby indicating the avoidance of feeding.

Chapter 5

Food and Feeding Behavior

Contents

5.1 Introduction

Cephalopods play an important role in the trophic structure of the world’s marine ecosystems (Rodhouse & Nigmatullin, 1996). They are the subdominant predators and tends to increase in biomass when other species competing for its food and its predator get eliminated by way of heavy fishing (Roper et al.,1984). Cephalopods prefer live animals to the dead one and prey on fast swimming animals such as prawn, crab, smaller cuttle fish, squids and fishes. Its prey attack is visually controlled (Messenger, 1977). Fast swimming animals are captured by shooting the tentacles. Tentacular attack have had very high success rate in the laboratory (Messenger, 1968); 90% on first attack and 100% on second attack. However, the success of predatory attacks per prey encounters diminishes when the prey occurs in a school (Neil and Cullan, 1974). The slow moving animals such as crabs are attacked only with arms without the use of tentacles. Cannibalism is found to be prevalent in cephalopods, this far from being a counter productive mechanism can also be seen in some circumstances as providing competitive advantage (Caddy,1983). Most of the cephalopod juveniles feed on the planktonic crustaceans. At a certain point in the life history, the intensive feeding dictated by high metabolic rate requires a changeover to larger prey items. In *Illex* species O’Dor et.al.,(1980) has estimated that; roughly 5% body weight was consumed per day resulting in growth rates of 1.5% body weight per day.

Amoung the cephalopods, squids, specially ommastrephid, usually stand at a high trophic level in the ocean (Okutani,1998). They are rapid, active predators and feed on live prey, mainly crustaceans, fish and other cephalopods (Nixon, 1987; Hanlon & Messenger,1996; Rodhouse & Nigmatullin, 1996). Body size dictates predator-prey relationships in many marine systems (Lundvall et al. 1999, Jennings et al. 2001, Shin & Cury 2004). Squid often exhibit ontogenetic, size dependent patterns in predation (Collins et al. 1994, Scharf et al. 2000, Juanes 2003, Hunsicker & Essington 2006). Knowledge of the trophic ontogeny of the squids and the size-structure of their feeding relationships is paramount for determining their ecological role. This information can be valuable for assessing the potential consequences of squid feeding habits and for exploring how the strength of their trophic interactions may change if predator and (or) prey body sizes are altered by ecosystem perturbations (Woodward et al. 2005).

Watanabe et al (2004) has reported that neon flying squid *Ommastrephes bartramii* shift their dietary preferences between their large-sized autumn cohort and a small-sized winter–spring cohort This diet shift is not only due to the high abundance of particular prey, but also to the increasing ability of growing squids to catch micronektonic prey. *Myctobum imperatorius* was the smallest fish species found in the stomachs of neon flying squid; this means that it was also available to juveniles as a main prey. Similar size-related diet shifts from crustacean zooplankton to micronekton have been reported for other ommastrephid squids such as *Illex argentinus*, *I. illecebrosus*, *Nototodarus gouldi*, and *Todarodes sagittatus* (Vinogradov & Noskov 1979, O'Sullivan & Cullen 1983, Froerman 1984, Breiby & Jobling 1985, Ivanovic & Brunetti 1994). Mature squids were found to feed mainly on nektoepipelagic myctophids, although the dietary composition varied geographically, but not by sex (Shchetinnikov, 1986a, b). However, dietary dominance by myctophids was related to productive waters, while in oilgotrophic waters the incidence of less important prey or cannibalism increased (Shchetinnikov, 1986b).

Because of their high feeding rates and generalist feeding strategy, squid can potentially exert trophodynamic control on the recruitment of the early life stages of fishes (Dawe 1988, Rodhouse & Nigmatullin 1996, Hunsicker & Essington 2008). Moreover, their importance in food webs may be changing in response to fisheries-induced alterations to trophic structures and climate change (Field et al. 2007, Zeidberg & Robinson 2007).

Sthenoteuthis oualaniensis is one of the major abundant ommastrephid squid in the Indian waters. It is large, fast moving and a key member of the pelagic ecosystem (Roper et al. 1984). It preys on a wide range of meso- and epipelagic species. The feeding behaviors of this species have been previously investigated in some habitats. (Filippova,1974; Tung, 1976; Wormuth, 1976; Nigmatullin et al., 1977 ; Nigmatullin and Toporova, 1982; Zuyev et al., 1985; Chesalin, 1987, 1988, 1994, 1996; Shchetinnikov, 1988). Shchetinnikov (1988 and 1992) studied feeding habits of *Sthenoteuthis oualaniensis* in the Eastern Pacific and found that they preyed heavily on young *Dosidicus gigas*, a member of the same subfamily. However, in Hawaiian waters, the mean size of *S. oualaniensis* commonly caught at jigging stations was about 40% of the mantle length (ML) of the smallest *O. bartramii* females (Young & Hirota 1998). This size difference places much of the *S. oualaniensis* population well within the size range of potential squid prey for *O. bartramii*. Because *O. bartramii* and *S. oulaniensis* are members of the same subfamily, heavy predation by one on the other is expected (see Shchetinnikov 1992). Trophic interactions between these two species-such as those between a predator and prey or between competitors-could strongly affect their life histories. Chesalin, (1994) has done extensive studies on the gut contends of *S. oualaniensis* in the north western part of the Indian Ocean. It was found that, a total of 65 prey taxa had formed the constituents of the stomach contents. Most common are fishes (26 species, including 14 myctophids), followed by crustaceans (16 species) and cephalopods (16 species).

Its sympatric species *S. pteropus* are active predators with a wide food spectrum. On examination of the stomachs *S. pteropus,* it was found to contain a total of 105 species of bony fishes and pelagic invertebrates in the stomachs. Out of that 54 fish species were from 17 families, 30 species of crustaceans were from 16 families and 15 species of cephalopods were from 10 families (Zuyev et al, 2002). Most abundant in the squid diet were fishes of the families Myctophidae (22 species), Exocoetidae (6), Gonostomatidae (4), Gempylidae and Paralepididae (3 in each). Among crustaceans the most frequent were copepods, amphipods, decapods, and euphausiids, among cephalopods squids prevailed, including own juveniles, *Onychoteuthis banksii* and various Enoploteuthidae (Chesalin, 1994). Besides, the representatives of tunicates, chaetognaths, ostracods, etc., were also found. Food species spectrum changes during *Sthenoteuthis* ontogeny. Paralarvae found to feed on crustaceans (copepods, amphipods) and squid as the fragments of crustaceans and lens of squid were found in its alimentary canal (Vecchione, 1992), hence, their main food items were likely to be mesozooplankton. In juveniles it is dominated by meso- and macroplanktonic invertebrates, mainly copepods, amphipods and euphausiids, in adulthood it includes micronektonic and nektonic fish, such as plankton-eating myctophids, flying fishes and *Vinciguerria nimbaria,* and predatory fish such as gempylids, paralepidids, etc. and squids (mainly own juveniles, *Onychoteuthis banksii* and enoploteuthids) (Zuyev et al. 2002).

The strategy of squid feeding behavior significantly changes during its growth. Early juveniles can be referred to active-grazing predators, late juveniles and middle-sized squids are predator-pursuers, while large-sized adults are attacking predators. It is important to note that giant females of *S. oualaniensis* in the Arabian Sea consume mainly myctophids, which are numerous and inactive in the layer 100–200 m (Zuyev et al. 2002). So, these giants do not pass to trophic level of large nektonic predators (as attacking predators) and by their type of behavior they remain active-grazed predators. Experiments with squids in captivity have shown, that the digestion rate in the squid stomach is high and the time to full digestion of meal takes approximately 3–5 h in early juveniles; 5–8 h in late juveniles and 8–10 h in middle-sized squids (Nigmatullin, 1981; Zuyev et al., 1985; Chesalin, 1994). To determine the daily food rations many calculations were made for different size-age group of squids using field data on daily dynamics of stomach content and experimental data on the rate of food digestion (Nigmatullin, 1981; Nikolsky and Chesalin, 1983; Lipskaya, 1986; Chesalin, 1988, 1994, 1996). The daily food ration varies from 28% BW in juveniles to 3–4% BW in adult males. The estimations of daily food ration for adult *S. oualaniensis* were based on decrease of their liver weight during experimental starvation (Shulman and Nigmatullin, 1981). From data on nitrogen excretion rates, the ration size of *S. oualaniensis* was estimated at 6.5% BW, and for *S. pteropus* of same size 6.7% BW (Shulman et al., 1984).

The results of all research show very high food rations of these squids are needed to maintain their active mode of life. The average daily food ration for middle-sized squids (15 to 35 cm ML) was calculated 5–10% BW, while the rations for the majority of adult pelagic fishes is 1–5% BW (Chesalin, 1994). In some areas, such as the Arabian Sea, large oceanic predators rarely occur, therefore *S. oualaniensis*, owing to its high abundance, large size, short life span, fast growth, and high food ration almost completely monopolize the trophic niche of top predators and become the ‘master’ of the Arabian Sea (Chesalin, 1994). The main food chain in the epipelagic ecosystems is: mesoplanktonic crustaceans to myctophids to *Sthenoteuthis.*

Identifying the feeding habits of squid is challenging, owing to their masticating feeding behavior and rapid digestion of prey. Despite that, few studies have been done on the stomach contents of this species on other parts of the ocean and Arabian sea. However, no published reports are available on the diet of this species in the south eastern part of the Arabian sea particularly off the south west coast of India. In view of that this study attempts to identify the food components of this spices and its preference for the food at different stages of its growth in the south west coast of India.

5.2 Materials and Methods

A total of 559 *S. oualaniensis* stomachs were collected for this study on a monthly basis during the study period from the south west coast of India. Out of this, 343 were of late-maturing medium form and the remaining 216 were of dwarf, early-maturing form. The measurement for dorsal mantle length (DML) was taken in a straight line to the nearest millimeter after thawing the specimen to the room temperature from the frozen condition. The total weight (TWt) was measured to the nearest gram.

A subjective visual stomachs fullness index was assigned as 0-for empty stomach, 1-scarce remains, 2- for half full, 3- almost full and 4, completely full (Breiby & Joblin, 1985). Month wise percentage of each index in total was estimated for the medium and dwarf form separately for comparison. The wet weight of both the entire stomach and the contents were recorded to the nearest 0.1 g. The contends were washed gently in tap water over 0.5 mm sieve. Recognizable prey remains were then identified under a binocular microscope for the presence and absence of major prey taxa using reference guides. The prey macerated or digested beyond visual recognition were identified with reference guides. Fishes were identified from their otoliths and other hard structures such as bones, scales, eye-lenses, through specialized guides for this purpose (Miller and Jorgensen, 1973). Number of otoliths in the stomachs helped in the estimation of the number of fish preys. In some cases skin and flesh were also used to differentiate the fish components in the diet. The fish Trichiurus was identified only by the presence of silvery substance in the stomach as well the Mictophid mostly by the presence of photophore with dark tinted flesh and comparing it with the species collected from/ near the area of operation.

Cephalopods components were identified from beaks, (Clarke, 1996; Wolff, 1984) gladius, colour of the undigested and semi digested flesh / skin and eye-lenses. They were usually dominated by sucker balls with the sucker rings and tentacular hooks. Eye lens of the fish was easily differentiated from the cephalopods from its shape, as the fish lens was of round and spherical compared to squid lens, which were hemispherical and semi-circular. The remains of crustaceans were usually consisted of fragments of exoskeleton such as the carapace, telson and appendages. They were identified with its pinkish eyes, grooves, crest, spines, rostral teeth and sutures along with its prominent colour to distinguish the prey item and using the guide of Brusca (1980). Pelagic gastropods were identified based on McGowan (1968). Fish otoliths, selective fish bones, pieces of crustacean exoskeleton and sucker rings of cephalopods were removed and kept in dry bottle for further identification and confirmation.

As most of the stomach contents were specific to the area of operation, inference on the stomach contents were substituted from the species prevalent at the area of operation after examining the sampled prey species and past records carefully such as library of hard parts formed from the species caught during the earlier cruises (Plate 5.1).

The prey constituents of each stomach were separated and counted stomach wise. A fragmented prey count was based on the number of eyes, heads, mouth parts, tails, telsons, paired otoliths or other anatomical parts traceable to a single specimen. However, the number of consumed cephalopods was estimated as the maximum number of upper or lower cephalopod beaks. The advanced degree of digestion of stomach content generally impedes the exact and complete separation of prey for the purpose of weighing (Breiby & Jobling,1985). Hence, only the most conspicuous prey item were weighed to the nearest 0.1g after the removal of surface water with blotting paper. The stomach and its some of the food components have been shown in the Plate 5.1. Numerous indexes have been described to quantify the importance of different prey items in the diets of aquatic species (Hyslop,1980 ; Tirasin and Jorgensen, 1999).Those used in the present study were: The percentage of frequency of occurrence (Fi %), referred as the frequency of occurrence of prey items within the total number of non-empty stomachs:

$$\text{Fi (\%)} = \frac{\text{no. of stomachs including food items i}}{\text{total number of stomachs}} \times 100$$

The numerical percentage of abundance (Cn %), referred as the prey item abundance within the total number of prey items identified in the total number of non-empty stomachs:

$$\text{Cni (\%)} = \frac{\text{total no. of food items i}}{\text{total no. of food items identified in stomachs}} \times 100$$

The gravimetric composition percentage (Wi %) is the wet weight of prey items found within the total wet weight of non empty stomachs.

$$\text{WWi (\%)} = \frac{\text{total wet weight of food items i}}{\text{total wet weight of stomach contents}} \times 100$$

The hierarchization of food items was established using the Index of Relative Importance (IRI) of Pinkas *et al.* (1971), as modified by Hacunda (1981), which provided an optimal balance of the three indexes (Liao et al., 2001):

$$\text{IRIi} = (\text{Cni} + \text{WWi}) \times \text{Fi}.$$

The IRIi index was again converted to % of the total IRIi as

$$\text{IRI\%} = (\text{IRI} / \text{sumIRI}) \times 100$$

The above indices were estimated for the medium and dwarf form separately to understand the general food preference. Similarly size wise prey preference were also estimated for both the medium and dwarf form separately to understand the prey preference at different stages of growth.

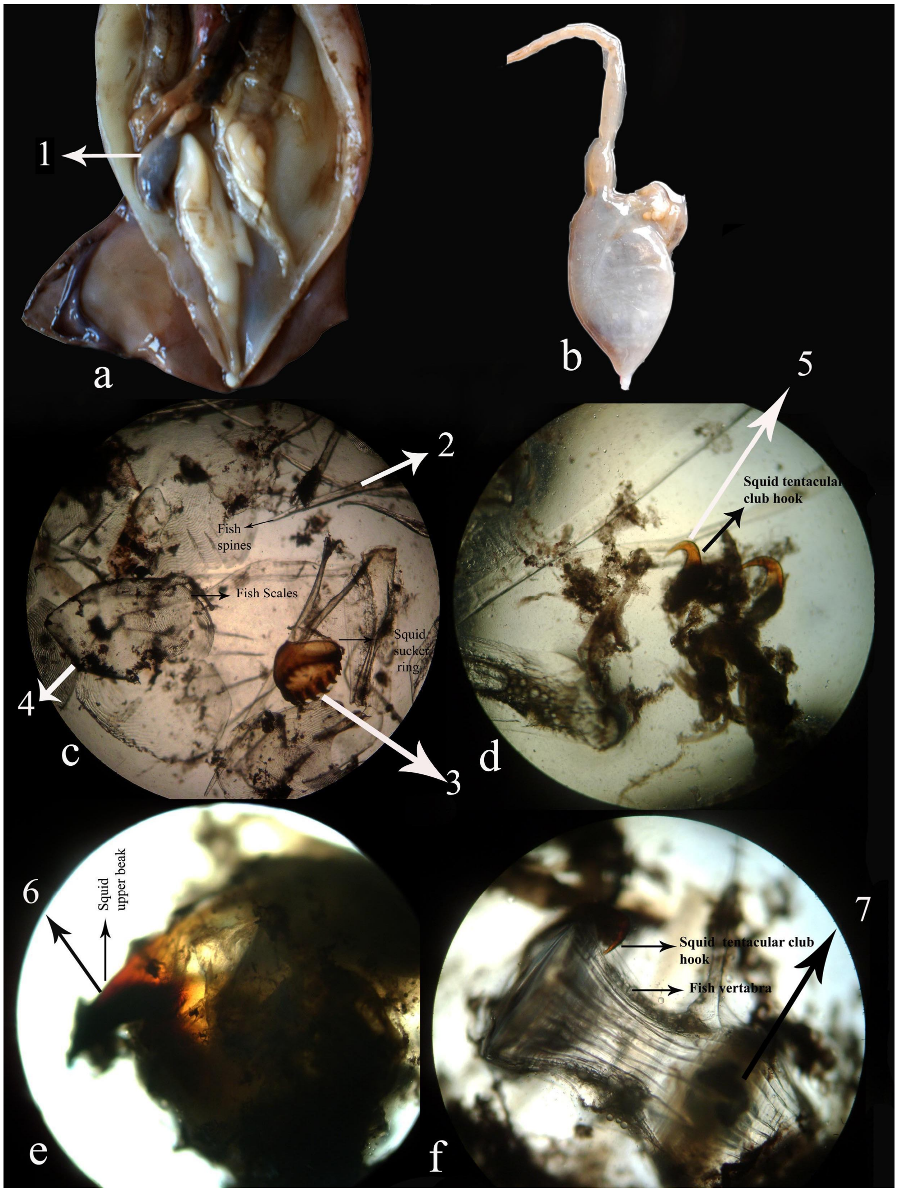

Plate 5.1. Stomach and its various food constituents. (a) Stomach inside the mantle cavity, (b) Stomach, (c) to (f) Microphotograph depicting various food componenets. 1. Stomach, 2. Fish fin ray, 3. Squid tentacular club sucker ring, 4. Fin fish ctenoid scale, 5. Squid tentacular club hook, 6. Squid upper beak, 7.Fish vertebra.

5.3 Results

5.3.1 Stomach fullness

A stomach was considered empty if there was no weighable material. Out of the 343 stomachs of medium form examined, 23.9 % were empty, 21.5 % were of scarce , 28.2 were of half full, 1.6 were of almost full and 24.8 % were of completely full. Whereas in the dwarf form out of the 216 stomachs examined, empty stomach comprised of 44.5 % the total stomachs. The remaining were of scarce (11.8%), almost full (4.6 %) and half and complete full (19.5%) each (Table 5.1). The stomach fullness index fluctuated widely for both the medium and dwarf form during the period of study. Medium, form were observed to have more percentage of empty stomachs in the month of January (45%) followed by September (34.8%), October (31.2%) and December (28.6%). Whereas full stomach were present in higher percentage in the month of October (38.2%) followed by December (35.3%) and January (30.85) (Figure 5.1(b)). In the dwarf, stomach could not be analysed for all the months for want of sample. However, amoung the months analysed, higher percentage of empty stomach were found in the month of May (85.7%) followed by October (57.8%) and September (50 %). Percentage of full stomach were more during the month of March (66.7%) followed by October (40.5%) (Figure 5.1(a)).

Table 5.1. Month wise percentage of stomach fullness in the *S. oualaniensis.* 0-for empty stomach, 1-scarce remains, 2- for half full, 3- almost full and 4, completely full

Months	Stomach Fullness (%)									
	Medium form					Dwarf form				
	0	1	2	3	4	0	1	2	3	4
January’07	45	0	35	0	20	42.9	0	42.9	0	14.3
February’07	16.7	33.3	38.9	5.6	5.6	27.3	9.1	45.5	18.2	0
March’07	0	50	50	0	0	0	0	33.3	0	66.7
April,07	0	62.5	37.5	0	0	11.1	0	44.4	33.3	11.1
May’07	25	25	50	0	0	83.3	16.7	0	0	0
June,07	25	25	25	0	25	20	0	80	0	0
August,07	14.3	47.6	23.8	0	14.3	12.5	12.5	43.8	18.8	12.5
September,07	34.8	17.4	39.1	0	8.7	50	33.3	0	16.7	0
October,07	31.2	12.7	16.6	1.3	38.2	57.8	6.2	9.5	2.4	24.2
November,07	0	16.7	83.3	0	0	0	0	0	0	0
December,07	28.6	19	28.6	0	23.8	0	0	0	0	0
January’08	15.4	0	53.8	0	30.8	0	40	20	20	20
February’08	6.7	40	46.7	0	6.7	14.3	50	21.4	14.3	0
March’08	0	50	50	0	0	0	0	0	0	0
April,08	0	85.7	14.3	0	0	11.1	22.2	55.6	0	11.1
May’08	25	25	50	0	0	85.7	0	14.3	0	0
June,08	25	37.5	12.5	0	25	14.3	42.9	42.9	0	0
August,08	15.4	38.5	23.1	0	23.1	0	33.3	60	6.7	0
September,08	33.3	9.5	52.4	0	4.8	50	0	50	0	0
October,08	17	20.8	22.6	5.7	34	40.5	16.7	2.4	0	40.5
November,08	0	33.3	50	16.7	0	0	0	0	0	0
December,08	17.6	23.5	23.5	0	35.3	40	20	40	0	0
Average	23.9	21.5	28.2	1.6	24.8	44.5	11.8	19.5	4.6	19.5

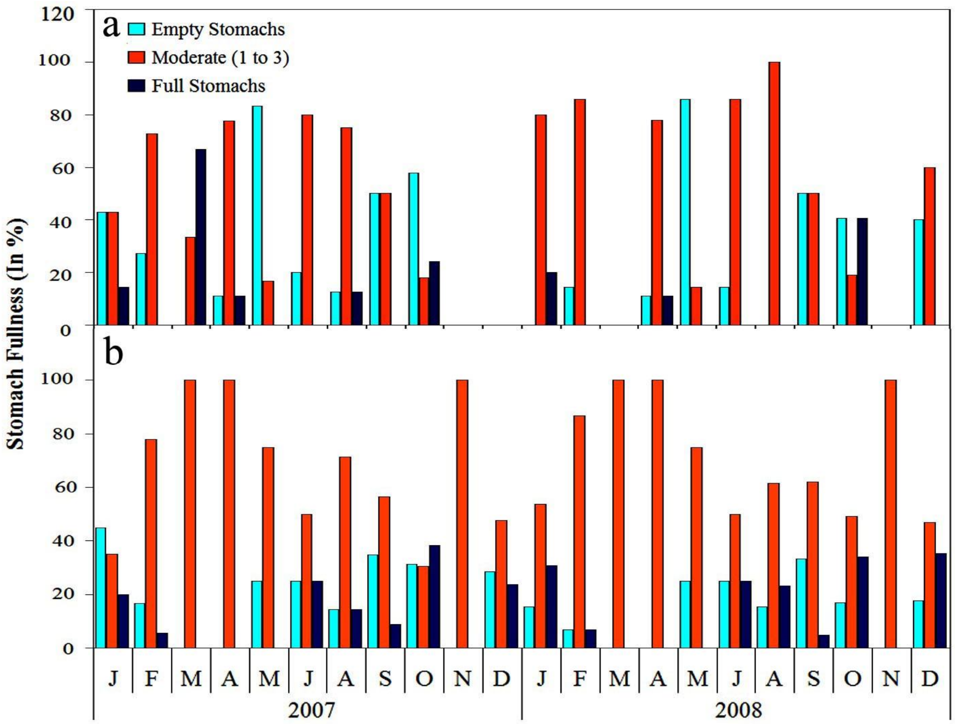

Figure 5.1. Month wise percentage of stomach fullness in the *S. oualaniensis.* (a) dwarf form (b) medium form.

5.3.2 Prey constituents

5.3.2.1 Medium

The diet contents were analysed separately for the medium and dwarf form. Out of the 343 medium form stomach analysed 23.9 % were of empty and the remaining comprised of 20 groups of prey constituents belonging to 18 genera as indicated in Table 5.2. The trophic spectrum of the *S oualaniensis* mainly consisted of three main groups: fish, molluscs and crustaceans. Stomach contends varied from the freshly devoured to fully digested condition representing both flesh and hard parts.

Table 5.2. Prey–species composition of *S. oualaniensis* Medium and Dwarf form by wet weight (WW, %), abundance (CN, %), frequency of occurrence (F, %) in total number of stomachs examined and IRI %: index of relative importance

	Medium form in %				Dwarf form in %			
Species	**wwi**	**CN**	**F**	**IRI**	**wwi**	**CN**	**F**	**IRI**
Total Crustacean	**5.8**	**9.5**	**9.4**	**1.7**	**5.5**	**6.3**	**6.5**	**0.9**
Leptochela	0.9	1.4	1.3	0.2	0	0	0	0
Plesionika sp	1.8	2.7	1.8	0.5	0.3	0.7	0.9	0
Calanus spp	0.8	1.2	1.6	0.2	1.9	2.1	2.2	0.4
Pandalidae	0.5	0.8	1	0.1	0.1	0.3	0.4	0
Sergestida	0.7	1.4	1.8	0.3	2.9	2.4	2.2	0.5
Other crustaceans	1.1	1.9	1.8	0.4	0.2	0.7	0.9	0
Total Cephalopods	**43.8**	**39.5**	**31.2**	**35.8**	**28.6**	**26.9**	**28**	**15.3**
Sthenoteuthis,	10.4	9.5	8.1	10.8	9.2	8.7	9.1	6.5
Ommastrephes,	3.1	2.9	2.3	0.9	1.4	1.4	1.7	0.2
Onychoteuthis	7.6	8	5.7	6	3.6	3.5	3.4	1
Histeuteuthis	2.1	2.3	2.1	0.6	0.5	0.7	0.9	0
Abraliopsis spp	1.7	1.6	1.6	0.4	2.9	3.8	4.3	1.2
Ancistrocheirus,	4.1	3.3	2.6	1.3	0.1	0.3	0.4	0
Other Cephalopods	14.8	11.9	8.8	15.9	10.9	8.4	8.2	6.4
Gastropods	**0.3**	**0.4**	**0.5**	**0.03**	**0**	**0**	**0**	**0**
Total Mictophid	**29.7**	**29**	**34.3**	**31.4**	**29.4**	**31.5**	**34.9**	**23.3**
Symbolophorus	7.7	8.2	10.1	10.8	7.4	6.3	7.3	4
Benthosema spp.	9.6	8.6	10.4	12.7	11.4	13.6	15.1	15.2
Myctophum	2.9	4.1	4.9	2.3	5.3	5.2	5.6	2.4
Hygophum	6.7	4.9	5.2	4.1	2.6	2.8	2.6	0.6
Diaphus spp	2.8	3.1	3.6	1.4	2.7	3.5	4.3	1.1
Total other Fishes	**20.3**	**21**	**24.2**	**31**	**35.8**	**33.9**	**28.9**	**60.3**
Trichiurus	4.4	4.9	6	3.7	5.8	4.9	4.3	1.9
Cypselurus spp	3.3	3.1	3.1	1.3	0	0	0	0
Other fishes	12.6	13	15.1	26	29.9	29	24.6	58.4
Eggs	**0.1**	**0.6**	**0.5**	**0**	**0.7**	**1.4**	**1.7**	**0.1**

The diet of the medium form was dominated by myctophids. Fish constituted 50 % of the wet weight, with 29.7 % of them were of myctophids. Cephalopods constituted 43.8 % , crustaceans 5.8 %, gastropods 0.3 % and eggs 0.1 %. Similarly, in abundance, fish constituted 50 % followed by cephalopod with 39.5 % and crustaceans 9.5 %. Fish again dominated in the frequency of occurrence with a total of 58.5 % followed by cephalopod with 31.8 % and crustaceans 9.4 %. Though fish was dominant in all other indices its index in the relative importance were higher (62.4%) than all other indices with the next higher one from cephalopod 35.8 % indicating the higher preference for the cephalopods next to the fish (Figure 5.2). However, the IRI of crustaceans were negligible with 1.7 % when compared to its wet weight, abundance, and frequency of occurrence.

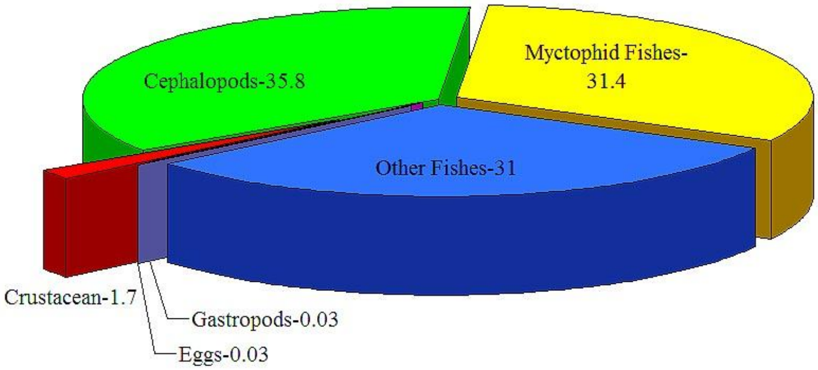

Figure 5.2. Percentage occurrence of the different prey groups in the diet of the *S. oualaniensis* medium form.

5.3.2.2 Dwarf

A total of 243 stomachs were analysed. Amoung them, the percentage of empty stomachs were of 44.5 %. This is nearly 20% more than what was found in the medium form. The remaining nearly 55 % of the stomachs only were analysed for prey constituents. The prey constituents were comprised of 17 groups of prey constituents belonging to 16 genera as indicated in Table 5.2. Fishes with 66.3% of the total constituent wet weight dominated dwarf diet. Myctophids constituted 29.4%, which is equivalent to the myctophids share of medium form and lesser than its wet weight index of other prey fish. Cephalopods and crustaceans constituted 28.6% and 5.5% respectively. Similarly in abundance fish constituted 65.4% followed by cephalopod with 26.9% and crustaceans 6.3%. Fish again dominated in the frequency of occurrence with a total of 63.8% followed by cephalopod with 28.0% and crustaceans 6.5%. Though fish was dominant in all other indices its index in the relative importance were extremely higher (83.6%) than all other indices, indicating a higher preference for the fish especially for non myctophids fish species (60.3%). Cephalopod though comprised around 28 % in all other indices, its relative importance in the dwarf food was conspicuously less than with 15.3 %, which is around 20 % less than the relative importance of cephalopod prey in the medium form. Similarly, the relative importance of the crustaceans were also of only 0.9 % in the dwarf food, which is below 50 % of what was its importance in the medium form (Figure 5.3).

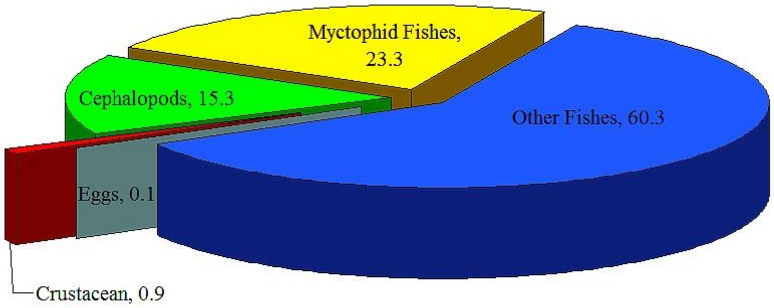

Figure 5.3. Percentage occurrence of the different prey groups in the diet of the *S. oualaniensis.* dwarf form.

5.3.3 Prey preference of different size groups of squids

5.3.3.1 Medium

The prey constituents of three size groups such as 9.0 to 15.0 cm DML, 15.0 to 20.0 cm DMl and 20.0 to 27.0 cm DML of the medium form were analysed for possible variation between them. Out of the 343 non empty stomach analysed for prey constituents 195 stomachs were from the size group of 9.0 to 15.0 cm, 98 were from 15.0 to 20.0 cm and 50 were from 20.0 to 27.0 cm. It was observed that, in the squid of the size group of 9.0 to 15.0 cm, fish constituted 48.1 % of the wet weight, with 26.6 % of them were of myctophids. Cephalopods constituted 43.9 % , crustaceans 7.5 %, gastropods 0.5 % and eggs 0.2 % (Table 5.3). Similarly in abundance, fish constituted 47.7 % followed by cephalopod with 40.1 % and crustaceans 10.6 %. The frequency of occurrence of fish was 57.5 % followed by cephalopod with 29.7 % and crustaceans 11.3 %. The percentage of relative importance of fish prey was as high as 62.7 % in the squid of the size group of 9.0 to 15.0 cm, with myctophids and non myctophids equally sharing the preference followed by cephalopod with 35.4 %. Crustaceans had the least IRI ie. 2.7% of the total prey.

In the size group of 15.0 to 20.0 cm DML fish had the increased share of 62.2 % of the wet weight, with 44.9 % of them were of myctophids. Cephalopods comprised 35 % , lesser than in the earlier size group and crustaceans only 2.9 %. Gastropods and eggs were absent. In abundance index, fish constituted 62.7 % followed by cephalopod with 31.4 % and crustaceans 5.7 %. The frequency of occurrence of fish was 64.3 % followed by cephalopod with 29.2 % and crustaceans 6.4 %. The percentage of relative importance of fish prey was as high as 75.8 % with increased preference to myctophids followed by decreased preference for cephalopod with 23.4 %. Crustaceans had the least IRI of 1.0%.

Table 5.3. Prey preference of different size groups of *Sthenoteuthis oualaniensis* medium form by wet weight (WWi, %), abundance (Cni, %), frequency of occurrence (Fi, %) in total number of stomachs examined and IRI: index of relative importance

	90-150				**151-200**				**201-270**			
Species	**WWi**	**Cni**	**Fi**	**IRI**	**WWi**	**Cni**	**Fi**	**IRI**	**WWi**	**Cni**	**Fi**	**IRI %**
Total Crustacean	**7.5**	**10.6**	**11.3**	**2.7**	**2.9**	**5.7**	**6.4**	**1**	**4**	**10.4**	**14.9**	**3.1**
Leptochela	1.4	2.1	2.2	0.5	0	0	0	0	0.4	1.3	1.9	0.2
Plesionika sp	2.5	3.4	3.5	1.4	0.2	0.8	0.9	0.1	1.5	2.6	3.7	1
Calanus spp	0.9	1	1.3	0.2	0.4	0.8	0.9	0.1	1	2.6	3.7	0.9
Pandalidae	0.6	1	1.3	0.1	0.7	0.8	0.9	0.1	0	0	0	0
Sergestida	0.6	1	1.3	0.1	1.3	2.5	2.8	0.6	0.4	1.3	1.9	0.2
Other crustaceans	1.5	2.1	1.7	0.4	0.3	0.8	0.9	0.1	0.7	2.6	3.7	0.8
Total Cephalopods	**43.9**	**40.1**	**29.7**	**35.4**	**35**	**31.4**	**29.2**	**23.4**	**56.5**	**50**	**39**	**65.3**
Sthenoteuthis	7.3	7.2	6.1	5.9	11.3	10.2	9.4	11	20.3	17.1	13	31.4
Ommastrephes	5 4.5	3.5	2.2	0	0	0	0	0.8	1.3	1.9	0.3	
Onychoteuthis	9.6	10.6	7	9.4	4.1	3.4	3.8	1.5	5.3	5.3	3.7	2.5
Histeuteuthis	3.3	3.1	2.2	0.9	0.8	1.7	2.8	0.4	0	0	0	0
Abraliopsis spp	1 1	0.9	0.1	0.6	1.7	1.9	0.2	6.1	3.9	3.7	2.4	
Ancistrocheirus ,	1.5	1.4	1.3	0.2	9	5.9	3.8	3.1	6.5	6.6	5.6	4.7
Other Cephalopods	16.2	12.3	8.7	16.6	9.2	8.5	7.5	7.2	17.5	15.8	11.1	24
Gastropods	**0.5**	**0.7**	**0.9**	0.1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Myctophid	**26.6**	**26.1**	**32.2**	**29.1**	**44.9**	**39.8**	**39.7**	**42.1**	**20**	**23.7**	**29.6**	**19.2**
Symbolophorus	9.4	8.9	10.9	13.3	7	8.5	9.4	7.9	2.5	5.3	7.4	3.7
Benthosema spp.	8.2	7.9	10	10.8	16.2	12.7	12.3	19.2	5.3	5.3	7.4	5.1
Myctophum	3.4	4.8	5.7	3.1	3.4	3.4	3.8	1.4	0.9	2.6	3.7	0.8
Hygophum	3.5	2.4	3	1.2	12.5	9.3	8.5	10	9.9	7.9	7.4	8.6
Diaphus spp	2.1	2.1	2.6	0.7	5.8	5.9	5.7	3.6	1.4	2.6	3.7	1
Total other Fishes	**21.5**	**21.6**	**25.3**	**32.6**	**17.3**	**22.9**	**24.6**	**33.7**	**19.6**	**15.8**	**16.7**	**12.5**
Trichiurus	5.3	5.8	7	5.2	2.4	3.4	3.8	1.2	3.8	3.9	5.6	2.8
Cypselurus spp	2.4	3.1	3.5	1.3	1.1	1.7	1.9	0.3	9.7	5.3	3.7	3.6
Other fishes	13.8	12.7	14.8	26.2	13.8	17.8	18.9	32.2	6.1	6.6	7.4	6.1
Eggs	**0.2**	**1**	**0.9**	**1.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

However, in the 20.0 to 27.0 cm DML size group of the squid, fish share had come down drastically in all the indices such as the wet weight (39.6 % in total with Myctophid constituting only 20 %), abundance index (39.5 %),frequency of occurrence (46.3%) and relative importance (31.7%). Whereas, the preference for the cephalopod had risen to 56.5 % of the total wet weight of the prey, 50 % of the abundance ,39 % of frequency of occurrence and 65.3% of the relative importance of all the prey item found in the stomach (Figure 5.4). Crustaceans constituted 4% of the WWi, 10.4% of Cn , 14.9% of F and 3.1% of IRI. A marginal increase from the previous size groups.

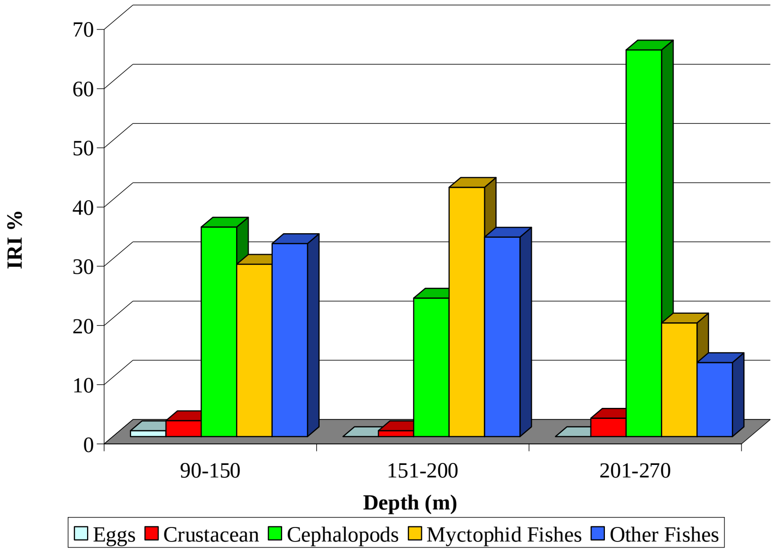

Figure 5.4. Percentage of relative importance of the different prey groups in the diet of the different size groups of *S. oualaniensis* medium form.

5.3.3.2 Dwarf

Similar to the medium form three size groups of the dwarf form were analysed for the possible variation in prey preference. The size groups were of 9.0 to 12.0 cm DML, 12.0 to 14.0 cm DML and 14.0 to 16.0 cm DML. Out of the 216 non empty stomach analysed for prey constituents 60 stomachs were from the size group of 9.0 to 12.0 cm, 63 were from 12.0 to 14.0 cm and 93 were from 14.0 to 16.0 cm. In the squid of the size group of 9.0 to 12.0 cm, fish constituted 71.2% of the wet weight, with 27.6% of them were of myctophids. Cephalopods constituted 20.1%, crustaceans 8.7%. (Table 5.4). Similarly, in abundance, fish constituted 60.6% followed by cephalopod with 28.2% and crustaceans 11.3%. The frequency of occurrence of fish was 57.4% followed by cephalopod with 29.5 % and crustaceans 13.1%. The percentage of relative importance of fish prey was as high as 85.4% with non myctophids in high preference followed by Mictophid. Cephalopod’s IRI was 11.9%. Crustaceans had the least IRI ie. 2.7% of the total prey similar to the smaller size group of the medium form.

In the size group of 12.0 to 14.0 cm DML fish constituted 71.8% of the wet weight, with 31.1% of them were of myctophids. Cephalopods comprised 25.2% , around 20% more than in the earlier size group and crustaceans reduced to 3.0% from the earlier size group. In abundance index, fish constituted 76.6% followed by cephalopod with 20.9 % and crustaceans 2.5%. The frequency of occurrence of fish was 75.7% followed by cephalopod with 22.9% and crustaceans 1.4%. The percentage of relative importance of fish prey was as high as 86.9% with increased preference to non myctophids. Cephalopods IRI was of only 12.9%. Crustaceans had the least IRI of 0.3%.

However, in the 14.0 to 16.0 cm DML size group of the squid, fish constituted 59.6% wet weight, 63.7% of the abundance index, 63.4% of the frequency of occurrence and 71.6% of the relative importance. Whereas, the preference for the cephalopod had risen to 34.9% of the total wet weight of the prey, 30.4% of the abundance index, 30.7 % of frequency of occurrence and 21.2% of the relative importance of all the prey item found in the stomach (Figure 5.5). Crustaceans constituted 5.4% of the WWi, 5.9% of Cn & F and 1.1% of IRI. A marginal decline from the previous size groups.

Table 5.4. Prey preference of different size groups of *Sthenoteuthis oualaniensis* dwarf form by wet weight (WWi, %), abundance (Cni, %), frequency of occurrence (Fi, %) in total number of stomachs examined and IRI: index of relative importance

	90-120				**121-140**				**141-160**			
Species	**WWi**	**Cni**	**Fi**	**IRI**	**WWi**	**Cn i**	**Fi**	**IRI**	**WWi**	**Cni**	**Fi**	**IRI**
Total Crustacean	**8.7**	**11.3**	**13.1**	**2.7**	**3**	**2.5**	**1.4**	**0.3**	**5.4**	**5.9**	**5.9**	**1.1**
Leptochela	0	0	0	0	0	0	0	0	0	0	0	0
Plesionika sp	0.9	1.4	1.6	0.1	0	0	0	0	0.2	0.7	1	0
Calanus spp	4.3	4.2	4.9	1.6	3	2.5	1.4	0.3	0.2	0.7	1	0
Pandalidae	0.6	1.4	1.6	0.1	0	0	0	0	0	0	0	0
Sergestida	2.5	2.8	3.3	0.7	0	0	0	0	4.8	3.7	3	1.1
Other crustaceans	0.5	1.4	1.6	0.1	0	0	0	0	0.2	0.7	1	0
Total Cephalopods	**20.1**	**28.2**	**29.5**	**11.9**	**25.2**	**20.9**	**22.9**	**12.9**	**34.9**	**30.4**	**30.7**	**21.2**
Sthenoteuthis	7	8.5	8.2	5	11	8.6	10	6.6	9	8.9	8.9	6.8
Ommastrephes	0.6	1.4	1.6	0.1	0	0	0	0	2.7	2.2	3	0.6
Onychoteuthis,	4.8	2.8	1.6	0.5	2.8	2.5	2.9	0.5	3.5	4.4	5	1.7
Histeuteuthis	1.8	1.4	1.6	0.2	0	0	0	0	0.2	0.7	1	0
Abraliopsis spp	4.2	8.5	9.8	4.9	0.2	1.2	1.4	0.1	4	3	3	0.9
Ancistrocheirus	0.4	1.4	1.6	0.1	0	0	0	0	0	0	0	0
Other Cephalopods	1.3	4.2	4.9	1.1	11.3	8.6	8.6	5.7	15.5	11.1	9.9	11.2
Total Myctophid	**27.6**	**28.2**	**29.5**	**18**	**31.1**	**38.3**	**41.4**	**31.8**	**29.2**	**28.9**	**33.7**	**21.6**
Symbolophorus	3.7	2.8	3.3	0.8	9.4	8.6	8.6	5.2	7.8	6.7	8.9	5.5
Benthosema spp.	9.9	11.3	11.5	9.5	13.3	19.8	21.4	23.8	10.9	11.1	12.9	12.1
Myctophum	10.6	8.5	8.2	6.1	1.9	2.5	2.9	0.4	4.9	5.2	5.9	2.6
Hygophum	0.9	1.4	1.6	0.1	1.3	2.5	2.9	0.4	4.3	3.7	3	1
Diaphus spp	2.6	4.2	4.9	1.3	5.1	4.9	5.7	1.9	1.3	2.2	3	0.4
Total other Fishes	**43.6**	**32.4**	**27.9**	**67.4**	**40.7**	**38.3**	**34.3**	**55.1**	**30.4**	**34.8**	**29.7**	**56**
Trichiurus	4.4	2.8	3.3	0.9	8.2	7.4	5.7	3	5	4.4	4	1.6
Cypselurus spp	0	0	0	0	0	0	0	0	0	0	0	0
Other fishes	39.2	29.6	24.6	66.5	31.4	28.4	25.7	51.7	24.6	28.9	23.8	54.2
Eggs	**0**	**0**	**0**	**0**	**1.1**	**2.5**	**2.9**	**0.3**	**0.8**	**1.5**	**2**	**0.2**

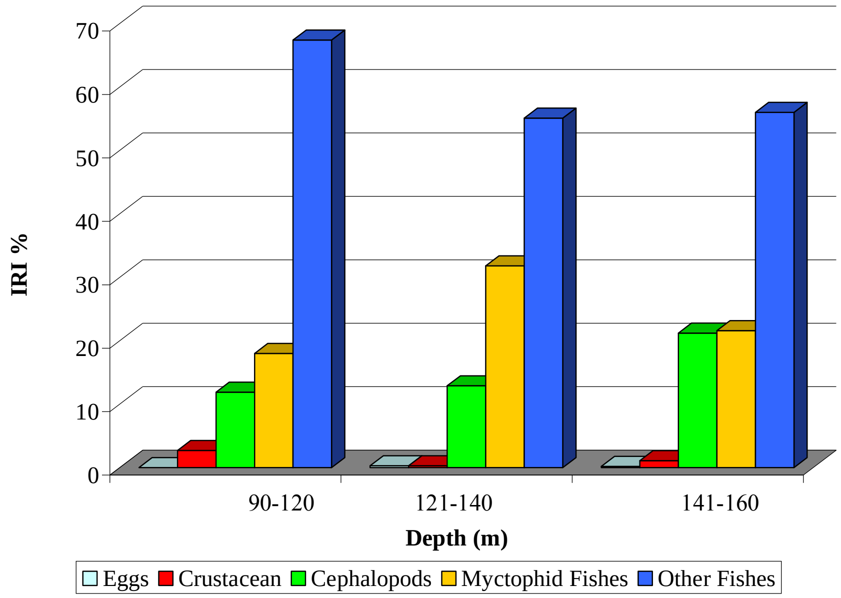

Figure 5.5. Percentage of relative importance of the different prey groups in the diet of the different size groups of *S. oualaniensis* dwarf form.

5.4 Discussion

5.4.1 Stomach fullness

Stomach fullness depend on the time they were caught. (Bazanov,1986; Koronkiewicz, 1988). The variability in stomach fullness and content weight observed in this study lead to a lack of significant differences between the months. However, it is inferred that, during the peak spawning period of March and April of the medium form, as found in this study, neither empty stomach nor full stomach was found. This may indicate the moderate feeding rate during the reported spawning periods. However, the same was not true in the case of dwarf form, wherein during the reported spawning peak of May to August, no full stomachs were observed, instead empty stomachs were in more percentage than even the moderate ones. Probably, dwarf form may not feed voraciously during/

between the spawning events, thereby less growth as indicated in this study with a lesser size range of matured ones when compared to the medium form.

Baral. (1967) and Ehrhardt et al.(1983) has attributed the large incidence of empty stomach to a high digestion rate. However, Markaida and Nishizaki (2003) reports that since jigs are an active fishing gear that depends on squid voracity, those captured could be hungrier and have a higher incidence of empty stomachs than satiated squid that do not attack the jigs. It is possible that the otolith and other hard parts present in the squid represents several meals. Cephalopod gastric fluids are weak and otoliths immersed for 2 days show no evidence of erosion (Jobling & Breiby,1986). Okutani and Tung (1978) found that the stomach weight (relative to body weight) was higher in the first half of the night. Apparently, the primary feeding period of sub adults and adults follows their arrival in near-surface waters at night.

5.4.2 Prey constituents

The food spectrum of the squid was dominated by the remains of fish and cephalopods. However, small quantity of crustacean prey was also identified in the food constituents. Among the 302 specimens of *S. oualaniensis* examined by Parry (2003) in Hawaiian waters, fish and squid comprised virtually the entire diet with fish being more abundant than squid. Basir (2000), stated that fish and cephalopods were the two most frequently occurring prey taxa in the *Sthenoteuthis oualaniensis* diet in the Bay of Bengal area of the Indian Ocean. In total, twenty groups of prey constituents were identified in the medium form as indicated in the Table 5.3. Amoung them fish was the single largest group comprising seven species, wherein five were of myctophids. *Benthosema spp* was the major constituent followed by the *Symbolophorus* spp and *Hygophum* spp. It is known that oceanic ommastrephid squid of medium size feed mainly on myctophids (Filippova, 1974; Wormouth,1976). Examples are : *Ommatrephes bartemii* (Gaevskaya & Nigmatullin, 1976; Araya,1983; Lipinski & Linkowski, 1988), *Sthenoteuthis oualaniensis* (Shchetinnikov,1992) and *S. pteropus* (Chesalin,1985; Nigmatullin & Toporova, 1982). Rodhouse and Nigmatullin (1996) have suggested that in open ocean, epipelagic Ommastrephids are possibly the main predators of myctophids. Formation of dense aggregation of myctophids (Gjosaeter, 1981) could be the main feeding preference for large oceanic Ommastrephids.

The bulk of the non myctophids fishes constituting major portion of the fish catches could not be identified. Cephalopods were the dominant constituent in the stomach next to the fish constituents. At some stations, cephalopods were found to out number the fishes and form the exclusive prey item in the stomachs. This was the case for the fish and crustaceans too. This may probably be due to the availability and abundance of the prey species population in the area of *S. oualaniensis* abundance. It has been reported that, *Sthenoteuthis oualaniensis* is a predator – opportunist, whose feeding spectrum varies with sizes and regions (Dong, 1991). For example, in the trophical Pacific Shchetinnikov (1992) found that *S. oualaniensis* fed heavily on crustaceans – up to 50 % of the volume of the diet. Cephalopods constituted six species. Out of that *Sthenoteuthis* spp was the dominant one constituting 10.4 % of the wet weight index and around 11% of the index of relative importance. This indicates the level of cannibalism in this species. Around 15.0% of the cephalopod constituent's could not be identified. Crustaceans though constituted around 6.0% of the total wet weight index its share in the index of relative importance were only 1.7%. Amoung crustaceans no, clear preference for any particular species was found. Gastropods and eggs of the fishes were in negligible quantity.

Dwarf food spectrum were of not different from that of medium form except in the absence of few groups such as gastropods, *Leptochela* and *Cypselurus* spp. However, the percentage of abundance and relative importance of the food item differed significantly from that of the medium form. The presence of fish in the food constituents has significantly increased in the dwarf from that of the medium form, with a clear increase in the constituents of the non myctophid fishes. Cephalopod index of relative importance has become less than 50% of what was found in the medium form indicating a clear shift for the preference of the fish over the cephalopods, especially non myctophid fishes. However, *Sthenoteuthis* was the dominant prey constituent in the stomach of the dwarf form as that in the medium form indicating a strong cannibalistic tendency in the species. Amaratunga (1980) found out that cannibalism in *lllex illecebrosus,* appears to be an important element in the life cycle of the species. Further , he adds that, in nature it increases as total feeding decreases, and the largest squids are the most cannibalistic. Starvation about three days are needed to induce cannibalism and single victim may be divided between several cannibals (Õ Dor et. al., 1980). Hanlon & Messenger (1996) has stated that cannibalism is intracohortal due to squid schooling behavior. The wet weight index of the crustaceans were remained more or less similar to that of medium form, its index of relative importance had become nearest to 50% of what was in the medium form indicating a less preference for the crustaceans in the dwarf form.

5.4.3 Prey preference of different size groups of squids

The preference to the particular diet seems to change as the squid grows. A number of studies have examined diets in *S. oualaniensis* (Nesis,1977; Wormouth,1976; Young,1975;Tsarin & Chesalin,1983; Shchetinnikov,1992). Most studies that had sufficient data found that small squid fed heavily on crustaceans but that large squid fed mostly on fishes and squids. Tung (1976) found an exception, however, in squids taken around the southern Okinava Islands, which fed on crustaceans. The study by Shchetinnikov (1992) on squid from the Eastern Tropical pacific is the most detailed. He found a rapid decline of crustaceans in their squid diet in their frequency of occurrence and proportion of contents in stomach for squid between 10 and 15 cm DML. In squid greater than 15.0 cm DML the diet consisted predominantly of fishes (>70 %) and squids (>30 %).

This study substantiate the findings of the earlier study on the shifting of diet during the growth of the squid. As the squid were collected by jigging, juveniles could not be collected. Among the sizes available in the dwarf form, the smaller length groups (9.0 – 12.0 cm DML) had higher preference for the fishes with the (IRI >85.4%), wherein non myctophids were more preferred than the myctophids. This was in contrast to the medium form, where, myctophid was equally dominant with the non myctophids and significantly higher in the middle sized squids. This was followed by the cephalopods with (IRI >11.9%) and then the crustaceans (IRI >2.7%). However, as the length group size progressed the preference for the fish decreased to 77.6% of the IRI though higher amoung the food constituents. Preference to cephalopod had gradually increased to 21.2 % of the IRI and crustacean decreased to 1.1% of the IRI. This indicates the gradual change in the preference of the diet as the squid grows corroborating the findings of the earlier studies. Amoung the genera *Benthosema* was dominant in all the length group followed by *Myctophum*. *Sthenoteuthis* dominated cephalopod constituent indicating high level of cannibalism in the species.

In the medium form, though the same trend is observed there is slight deviation in between the growth. Among the sizes available, the smaller length groups (9.0 – 15.0 cm DML) had higher preference for the fishes followed by the cephalopods and then the crustaceans. However, as the length group size progress it was found that the preference for the fish increased from 61.7% of the relative importance to 75.8% of the relative importance in the 15.0 to 20.0 cm DML length group, wherein myctophid constituted 42.1% IRI with *Benthosema* spp at the top. This corroborate the findings of Shchetinnikov (1992) in the Eastern Tropical pacific, who found that myctophid constituted 40% of the squid beyond 15.0 cm DML. It then drastically decreases to 31.7 % of the relative importance in the 20.0 to 27.0 cm DML length group. Amoung the non myctophid fishes *Cypselurus* dominated the prey constituents of the larger size group of 20.0 cm to 27.0 cm DML indicating the increased preference for the *Cypselurus* in the larger size group. This substantiate the finding of Xinjuk et al. (2007) who states that the squid of larger than 20.0 cm DML fed mainly on the small fish Cypselurus spp. Similarly, the relative importance of the cephalopod decreased from 35.4% from the smaller length group to 23.4% of the 15.0 to 20.0 cm DML length group and then increased to 65.3% of the relative importance in the larger length group. Beyond 20.0 cm DML squid becomes progressively more important in the diet and by 27.0 cm DML squids comprised about 65% of the IRI in the diet. *Sthenoteuthis* was the most dominant genera of the cephalopod prey constituent comprising 5.9% IRI in the smaller length group, 11% IRI in the middle group and finally reaching 31.4% of the total prey species constituents of the squid. This substantiate the earlier study indicating the existence high level of cannibalism in the species.

Crustaceans formed only 2.7% of the IRI in the smaller group. This had further declined to just 1 % of the IRI in the middle group and then increased to 3.1 of the IRI in the larger size group in contrast to the reported reduction in the crustacean preference in the diet during the progress in growth. The change in the diet of the middle size group of 15.0 cm to 20.0 cm DML may perhaps due to the availability and abundance of particular prey item to this length group in the area. Local variation in the stomach contents of *S. oualaniensis* has been reported near oceanic islands (Young and Hirota, 1998). Squid captured southwest of Taiwan contained mostly fish, those taken east of Taiwan contained a mixture of fish and squid and around Okinawa they frequently contained crustaceans (Okutani and Tung, 1978). In contrast to a nearly exclusively fish and squid diet in other areas around the Islands (Parry, 2003).

Chapter 6

Biochemical composition of Sthenoteuthis oualaniensis and other co- existing oceanic squids.

Contents

6.1 Introduction

All living cephalopods are carnivores and proteins are the major organic materials in cephalopod tissue. Squid, the popular name given to a large number of cephalopod molluscs is a largely untapped protein resource of the oceans of great economic potential (Clarke, 1966) which could be used in both human and animal diets. The Arabian Sea is considered as one of the richest regions for oceanic squids in the Indian Ocean (Mohamed *et al*, 2006). Though the neritic squids are heavily fished by trawlers throughout the Indian coast causing these stocks to be under intense fishing pressure (Neethiselvan & Venkataramani, 2002; Narasimham, 2005) the oceanic squids of the genus *Sthenoteuthis* is a relatively unexploited species (Xinjun, 2007). It has been commercially fished off Okinawa, Taiwan and Hawaii with the catch being primarily used as tuna bait and to a lesser extent for human consumption (Okutani & Tung, 1978).

Muscle weight (mantle and arms) of pelagic squid of the genus *Sthenoteuthis* reaches 60–65% of body weight (Zuyev et al., 2002). Similar values are characteristic of active fish, such as bonito and trout (65 and 57%, respectively), whereas less active fish (porgy, carp) have muscle weight less than 50% (Belokopytin, 1993). Features of chemical composition of mantle, fins, arms and tentacles of squid include a high content of protein. These parts of the body carry out the main movement functions. Mantle muscles are almost constantly contracting, providing fast jet-swimming; fins play the role of a rudder and act as the engine while slow moving; the tentacles can be immediately extended and contracted to capture and retain prey. The dry, lipid-free matter of these tissues is approximately 85–90% protein, contraction of which provides most of the intensive motor forces (Shulman et al., 1984).

The liver (or more correctly hepatopancreas) attracts special attention. The role of the liver in the total metabolism of squid (as well as molluscs in general) seems to be specially important. The liver differs from other tissues with higher contents of the basic biochemical substances (protein, lipids and glycogen). Thus, the liver of *S. oualaniensis* contains 1.5–2 times more dry matter, 2 times more glycogen, 1.2–1.4 times more protein, and 3.6–7.5 times more lipids, than muscular tissues (Shulman et al ,2002). The weight of the liver in *Sthenoteuthis* is more than 4% of total body mass (Shulman and Nigmatullin, 1981; Chesalin et al., 1992) and it can be much higher in some other species such as *Todorodes sagittatus* (10 to 16%) (Dvinin and Konstantinov, 1979). At the same time the weight of the liver in fast swimming fish, for example mackerel, does not exceed 1.0–2.5% (Shulman and Love, 1999). It is shown that the reserve substances of the liver can easily be mobilized in starvation (Shulman and Nigmatullin, 1981). Other researchers, studying food digestion by squid, also specified the important role of squid liver, as a 'reserve depot' (Bidder, 1976; Boucaud-Camou, 1971; Boucher-Rodoni and Mangold, 1977).

The liver of white fish and sharks is the depot for lipids (Shatunovsky, 1980; Love, 1980), and glycogen (Plisetskaya, 1975). On the contrary, the liver of squid reserves a significant amount of proteins, moderate quantities of lipids and very low glycogen content (Shchepkin et al., 1981; Abolmasova et al., 1990; Hayashi, 1996). Amounts of these components indirectly indicate the substrates for energy metabolism. Also, close correlation between protein content (approximately estimated by de-fatted dry matter) and dry matter content of the squid body was noted, that is absent in fish (Nikolsky and Shulman, 1980). Other authors have also pointed out the high intensity of protein metabolism of different squid species (Campbell and Bishop, 1970; Hochachka et al., 1973; Storey and Storey, 1978).

Probably, molluscs are unable to utilize high calorific lipid substances. Instead, they have maximally developed the ability to utilize protein, which is characteristic of the lowest invertebrates with slow motility (Shulman et al., 1984, 1993). In any case, squid have a powerful mechanism to use protein and amino acids in anaerobic metabolism for providing high functional activity based on the same mechanism as in some other marine invertebrates (Hochachka and Mustafa, 1972; Hochachka et al., 1973). In studying major chemical components of squid, the main attention has been to proteins, but lipids have been 'in shadow'. Neutral (reserve) lipids play a subordinate energy role in squid, probably being used mostly in reproductive processes. But the significance of polar lipids (phospholipids) in vital functions of squid (as well as animals in general) is extremely important.

Phospholipids are a principal component of cellular and subcellular membranes, as bilayers. The content of phospholipids in total lipids of tissues and organs in *S. pteropus* is 20 to 30% (Yuneva et al., 1994) and in *S. oualaniensis* is even higher, 36- 51% (Shchepkin et al., 1981). High contents of sphingomyelin, phosphatidyl serine and phosphoinositol were shown in phospholipids of *S. pteropus* tissues. Also, *Sthenoteuthis* have high content of cholesterol and its ethers (Abolmasova et al., 1990).

Cephalopods have a protein-based metabolism (Lee, 1994), and the direct use of protein as an energy reserve may account for the lack of major reserves of glycogen and lipid in cephalopod tissues (O'Dor *et al.*, 1984; Storey and Storey, 1983). This dry fat-free matter of the squid tissues consists approximately by 85–90%, which were found to be the main energy source for energy metabolism. In contrast, many fast moving animals use lipids for this purpose (Shulman et al., 1984; Shulman et al., 1992). Although on the mobility level as active swimming fishes, which use triacylglycerols as the main sources of energy, squids do not use lipids, but proteins and the products of their catabolism (free amino acids). Analysis of data on oxygen consumption and nitrogen excretion in *S. pteropus* and *S. oualaniensis* suggests that all oxygen consumed by squids seems to be utilized for oxidation of proteins. Another remarkable finding is that a considerable proportion of catabolized proteins appear to be utilized in anaerobic metabolism since the oxygen consumed is clearly inadequate for its oxidation (Shulman et al., 1992).

Although sufficient information on the nutritional profile of many commercially important fishes and squids are available (Celik, 2008; Shamsan & Ansari, 2010), sufficient information on similar lines on *S. oualaniensis* particularly from the Arabian Sea is not available. Therefore this study aims to investigate the nutritional value and protein quality of the important oceanic squids of the south west coast of India especially *S. oualaniensis* in comparison to the co existing other oceanic squids such as *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii.* This is envisaged with an objective of establishing the fact that oceanic squids are good for human nutrition.

6.2 Materials and methods

6.2.1 Sample collection and preparation

Samples were collected from Fishery Survey of India (FSI) trawler Matsya varshini during its deep sea survey between the period January and June 2010 in the area Lat 07 ^{o} N to 11 ^{o} N and Long 74 ^{o} E to 77 ^{o} E along the south west coast of India. Specimens of *Sthenoteuthis oualaniensis* in both the phenotypic forms and other oceanic squids such as *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii* were immediately preserved on board the vessel at -20^o C by blast freezing. At shore lab, specimens were brought to the ambient temperature by allowing it to be thawed gradually. The mantle portion of the squid was separated and cleaned with water and surface water was blotted with filter paper. The mantle was then homogenized to form mince. The proximate composition, fatty acid and amino acid profiles were determined for the samples.

6.2.2 Biochemical analysis

6.2.2.1 Moisture

All analyses (n=6) were carried out in triplicate. Moisture of the fish samples (10 g) was determined according to the Association of Official Analytical Chemists (AOAC, 1990) method. Initially a clean petri dish was placed in an oven at 105˚C for 2 hrs and then cooled in a desiccator and weighed (W1). The sample was then placed on the petri dish and weighed (W2). The dish was heated then with the sample in an oven maintained at around 105˚C overnight. The sample was then cooled along with the petri dish in a desiccator and weighed again (W3). The petri dish was kept again in the oven for half an hour, cooled as above and weighed again to get the reproducible weight. Results were expressed as percentage of wet weight. The moisture content was calculated as below.

$$\text{Moisture content (\%)} : \frac{(W2 - W3)}{(W2-W1)} \times 100$$

6.2.2.2 Ash

Initially a platinum or silica crucible was heated to 600˚C in a Muffle furnace for one hour and then cooled in a desiccator and weighed (W1). The dried sample weighing accurately 1 g were then kept in the crucible and heated at low flame to char the organic matter (W2). Kept the charred material inside the previously set (600˚C) Muffle furnace and heated for 6-8 h to get white or greyish white ash. Cooled the crucible in a desiccator ad weighed (W3). Heated the crucible again for further 30 min to confirm completion of ashing, cooled and weighed. Results were expressed as percentage of wet weight.

$$\text{Ash content (g/100g)} = \frac{(W3 - W1)}{(W2\text{-}W1)} \times 100$$

6.2.2.3 Protein

Protein content in the homogenized samples (1g) was calculated by estimating the nitrogen content using MicroKjeldahl method (AOAC, 2000) and multiplying with a factor calculated based on the nitrogen content of the food (6.25). This is referred to as crude protein content because the non-protein nitrogen (NPN) is not excluded from the total nitrogen. The true protein content is calculated by subtracting the NPN from the crude protein nitrogen before multiplying with the factor. Results were expressed as percentage of wet weight basis (n = 6).

6.2.2.4 Lipids

Fat in the dried form of the sample was extracted repeatedly by Soxhlet method using petroleum ether (B.P. 60-80^{o} C) as solvent. Total fat soluble in the hot petroleum ether, was extracted from the sample and quantified according to AOAC, (2000) method.

6.2.2.5 Fatty acid analysis

Total lipids were extracted according to the method of Folch *et al.*, (1957), using chloroform/methanol (2:1). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically. Saponification of fats liberates fatty acids from triglycerides.

Fatty acids methyl esters (FAMEs) were prepared by transesterification with 14% BF_3-MeOH (Borantrifluride – Methanol) (Sigma, USA), according to the method described by Metcalfe et al.,(1966). The methylated sample was then extracted with 8 ml n-hexane. All of these reactions were performed in quadruplet for each sample. The resulting fatty acid methyl esters were analysed using an Agilent Gas chromatograph system 6890 N equipped with a flame ionization detector (FID), a splitless injector and a polar fused silica capillary column (30m x 0.25 mm i.d x 0.25 µm film thickness). The temperature of the injector and the detector were 250^o C and 275°C respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/mn. Peaks were identified by comparison of their retention times with methylesters of standard fatty acids (SUPELCO).

6.2.2.6 Amino acids analysis

In order to determine the total amino acid profile, samples were hydrolysed with 6 N HCl in evacuated sealed tubes at 110°C for 24 h. The hydrolysis was performed under inert and anaerobic conditions to prevent oxidative degradation of amino acids. After filtration the amino acids were derivatized using O-phtalaldehyde. The separation and quantification of amino acids was carried out following the method of Ishida et al., (1981) using a Shimadzu chromatograph LC-10AT High Performance Liquid Chromatography (HPLC) equipped with an ion exchange column, quaternary pump, a 20 µl injection valve and a fluorescence detector. Mobile phase A- contained sodium citrate and ethanol (pH 3.5) and B- had sodium citrate and NaOH (pH 9.8). The flow rate was constant at 0.4 ml/min, and the column temperature was set at 60°C. The fluorescence excitation and emission wavelengths were 340 nm and 450 nm respectively. The amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in terms of mg amino acid per gram of crude protein.

6.2.3 Statistical analysis

Statistical analysis was performed using Minitab Version software, version 14.0. The comparison of different biochemical parameters were tested using Tukey’s test (95% confidence interval) with one-way analysis of variance (ANOVA).

6.3 Results

6.3.1 Proximate composition

The proximate composition of the *Sthenoteuthis oualaniensis* in both the phenotypic forms and other oceanic squids such as *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii* showed marginal variation (Table 6.1). Water was the dominant constituent among the oceanic squids comprising 80% (average) of body mass and ranged from 79.52 ± 0.9 to 83.62 ± 1.3% followed by protein (13.83 ± 0.4 to 18.37 ± 1.2 %) and fat (0.6 ± 0.1 to 1.59 ± 0.2%). Ash content constituted 0.90 ± 0.12 to 1.40 ± 0.1% of body mass and was relatively constant across the species . Among the oceanic squids tested *H.bonnellii* had the highest (83.62 ± 1.3) moisture content followed by *C. imperator* (82.80 ± 1.4) and *S. oualaniensis* (80.8 ± 0.7- medium form and; 81.78 ± 0.9- dwarf form). The moisture content of the *M. lonnbergii* was the lowest among the squids analysed (79.52 ± 0.9). However, *M. lonnbergii* had the highest (18.37 ± 1.2%) protein content followed by *S. oualaniensis* (17.5 ± 0.3 medium form; 15.81 ± 0.7 dwarf form). *H.bonnellii* showed the lowest protein value (13.83 ± 0.4) followed by *C. imperator* (14.22 ± 0.8). *S. oualaniensis* was observed to have the lowest fat value of 0.6 ±0.1% in its medium form. However, its dwarf form had 1.31 ± 0.3 5% of fat in total body mass. *C.*

imperator had the highest Ash contents of 1.40 ± 0.1 % followed by *H.bonnellii* (1.30 ± 0.2%). There was no variation in ash content between the two forms of the *S. oualaniensis*

Table 6.1. Proximate composition (in percentage) of *Sthenoteuthis oualaniensis* mantle tissue in comparison to the co-occurring Oceanic squids.

Parameter	*S oualaniensis* (Medium form)	*S oualaniensis* (Dwarf form)	*Histioteuthis bonnellii*	*Moroteuthis lonnbergii*	*Chiroteuthis imperator*
Moisture	80.8 ± 0.7^{a}	81.78 ± 0.9^{a}	83.62 ± 1.3^{b}	79.52 ± 0.9^{a}	82.80 ± 1.4^{b}
Protein	17.5 ± 0.3^{a}	15.81 ± 0.7^{b}	13.83 ± 0.4^{c}	18.37 ± 1.2^{a}	14.22 ± 0.8^{b}
Fat	0.6 ± 0.1^{a}	1.31 ± 0.3^{b}	1.25 ± 0.3^{b}	1.21 ± 0.2^{b}	1.59 ± 0.2^{b}
Ash	1.1 ± 0.1^{a}	1.10 ± 0.1^{a}	1.30 ± 0.2^{a}	0.90 ± 0.12^{b}	1.40 ± 0.1^{a}

Values (mean of three replications with ± SD) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

6.3.2 Fatty acid analysis

Fatty acids composition of *S. oualaniensis* and other co-existing oceanic squid is given in the Table 6.2. Polyunsaturated fatty acids (PUFA) constitute the majority of the fatty acids pool, followed by saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) (Figure 6.1). *S. oualaniensis* dwarf forms had the highest fraction of the fatty acids with 283.06 ±18.03 mg/100g of PUFA ($P < 0.05$). and 74.61 ± 4.55 mg/100g of SAT followed by 34.24 ± 3.05 mg/100g of MUFA. Whereas, medium form had only 214.71 ± 11.57 mg/100g of PUFA and 52.34 ± 3.78 mg/100g of SAT followed by 20.13 ± 2.24 mg/100g of MUFA. The fatty acid fraction in other three oceanic squid was lower than the *S. oualaniensis.* Amoung them *M. lonnbergii* significantly had the highest PUFA of 201.92 ± 9.13 mg/100g, SAT 78.64 ±5.16 mg/100g ($P < 0.05$). and 28.40 ± 1.69 mg/100g of MUFA. *H.bonnellii* and *C. imperator* significantly had the lowest PUFA of 165.74 ±5.53 ($P < 0.05$). and 167.74 ±10.05 mg/100g ($P < 0.05$). respectively followed by the SAT (63.50 ± 2.55 ; 52.07 ± 6.13 mg/100g) and MUFA (45.92 ± 0.95 ; 21.80 ± 1.62 mg/100g)

Palmitic acid (C16:0) constituted the major portion of the SAT (Saturated fatty acids) in all the oceanic squids studied with the highest value of 53.86 ± 3.13 mg/100g in the *S. oualaniensis* dwarf form followed by 53.68 ± 3.21 mg/100g in *M. lonnbergii* and 49.76 ± 3.60 mg/100g in the *S. oualaniensis* medium form. The lowest value of 37.31 ± 4.80 mg/100g was in the *C. imperator.* The next most abundant fatty acid within this fraction was stearic acid (C18:0) followed by myristic acid (C14:0). However, in *S. oualaniensis* dwarf form instead of myristic acid (C14:0) Margaric acid (C17:0) was the third large constituent of the SAT. Among mono-unsaturated fatty acids, Eicosenoic acid (C20:1) and Oleic acid (C18:1) were in abundance than any other fatty acids in all the oceanic squids studied. Eicosenoic acid was highest in *S .oualaniensis* (18.21 ±2.10- dwarf form ; 20.32 ±1.98 mg/100g – medium form). However, Oleic acid was not well represented in the *S .oualaniensis* medium form, though it is found in good quantity in other oceanic squid including *S .oualaniensis* dwarf form with value almost 50% of the Eicosenoic acid.

Table 6.2. Fatty acid composition (mg/100g) of *Sthenoteuthis oualaniensis* mantle tissue in comparison to the co-occurring Oceanic squids.

Fatty acids	*S oualaniensis* (Medium Form)	*S oualaniensis* (Dwarf Form)	*Moroteuthis sp.* *lonnbergii*	*Histioteuthis* *bonnellii,*	*Chiroteuthis sp.* *imperator*
C-11	0.02 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C-12	0.25 ± 0.01	0.17 ± 0.00	0.00 ± 0.00	0.16 ± 0.00	0.30 ± 0.00
C-14	0.15 ± 0.01^{a}	2.47 ± 0.20^{b}	5.60 ± 0.34^{b}	4.23 ± 0.02^{b}	3.67 ± 0.20^{b}
C-15	0.08 ± 0.01	1.57 ± 0.12	1.28 ± 0.11	1.15 ± 0.03	0.69 ± 0.03
C-16	49.76 ± 3.60^{a}	53.86 ± 3.13^{a}	53.68 ± 3.21^{a}	42.99 ± 2.04^{b}	37.31 ± 4.80^{b}
C-17	0.27 ± 0.02	6.29 ± 0.20	2.88 ± 0.22	2.71 ± 0.06	2.05 ± 0.30
C-18	1.46 ± 0.12^{a}	9.95 ± 0.90^{b}	12.00 ± 0.97^{b}	9.13 ± 0.20^{b}	8.05 ± 0.80^{b}
C-20	0.35 ± 0.01	0.28 ± 0.00	3.20 ± 0.31	3.13 ± 0.20	0.00 ± 0.00
C-14:1	0.10 ± 0.00	0.10 ± 0.00	3.04 ± 0.20	1.13 ± 0.02	0.14 ± 0.00
C-16:1	0.29 ± 0.05	1.62 ± 0.02	2.64 ± 0.16	3.06 ± 0.10	1.58 ± 0.02
C-17:1	0.15 ± 0.00	0.28 ± 0.01	0.00 ± 0.00	0.58 ± 0.01	0.27 ± 0.00
C-18:1n-9	0.28 ± 0.02^{a}	10.80 ± 1.01^{b}	4.80 ± 0.27^{c}	4.49 ± 0.10^{c}	11.70 ± 1.2^{b}
C-20:1	18.21 ± 2.10^{a}	20.32 ± 1.98^{a}	4.40 ± 0.82^{b}	18.19 ± 0.30^{a}	8.11 ± 0.40^{b}
C-21:1	0.34 ± 0.02	0.36 ± 0.01	0.00 ± 0.00	0.83 ± 0.02	0.00 ± 0.00
C-22:1	0.33 ± 0.04	0.42 ± 0.01	1.04 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
C-24:1	0.43 ± 0.01	0.35 ± 0.01	2.48 ± 0.14	17.64 ± 0.40	0.00 ± 0.00
C-18:2n-6	0.51 ± 0.01	1.26 ± 0.02	0.80 ± 0.08	0.90 ± 0.06	0.47 ± 0.00
C-18:2n-6	0.24 ± 0.03	0.33 ± 0.00	3.68 ± 0.16	0.41 ± 0.02	0.00 ± 0.00
C-18:3n-3	0.04 ± 0.01	0.86 ± 0.03	0.40 ± 0.02	0.92 ± 0.02	0.00 ± 0.00
C-20:2	0.64 ± 0.08	2.68 ± 0.02	0.56 ± 0.02	1.29 ± 0.03	0.55 ± 0.02
C-20:4	20.08 ± 2.30^{a}	21.68 ± 1.20^{a}	0.32 ± 0.01^{b}	20.91 ± 0.90^{a}	22.03 ± 2.40^{a}
C-20:3n-3 &C:21	1.42 ± 0.20	5.21 ± 0.10	0.00 ± 0.00	0.46 ± 0.00	0.46 ± 0.02
C-20:3n-6	0.96 ± 0.10	0.12 ± 0.00	3.12 ± 0.27	0.00 ± 0.00	0.00 ± 0.00
C-20:5n-3	38.95 ± 2.90^{a}	56.67 ± 3.70^{a}	2.48 ± 1.12^{b}	40.00 ± 1.30^{a}	42.0 ± 3.21^{a}
C-22:2	0.27 ± 0.04	7.34 ± 0.56	3.84 ± 0.17	4.78 ± 0.30	3.50 ± 0.20
C-22:6n-3	151.60 ±5.90^{a}	186.91 ± 12.4^{a}	136.72 ± 7.28^{a}	96.07 ± 2.90^{b}	98.73 ± 4.2^{b}
Sum SAT	52.34 ± 3.78	74.61 ±4.55	78.64 ±5.16	63.50 ±2.55	52.07 ± 6.13
Sum MUFA	20.13 ± 2.24	34.24 ±3.05	28.40 ± 1.69	45.92 ±0.95	21.80 ± 1.62
Sum PUFA	214.71 ± 11.57	283.06 ±18.03	201.92 ± 9.13	165.74 ±5.53	167.74 ±10.05

Values (mean of three replications with ± SD) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

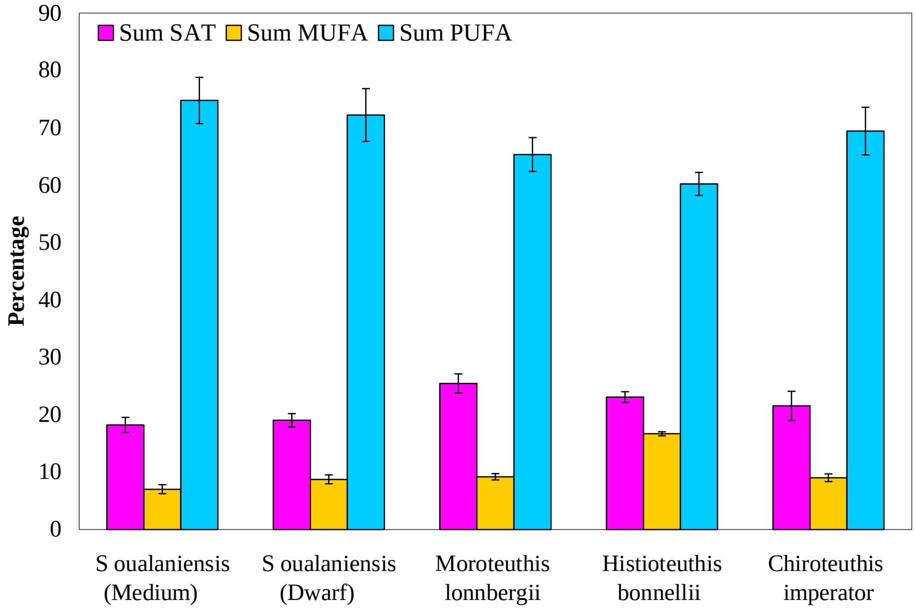

Figure 6.1. Saturated and Unsaturated Fatty acid composition of *Sthenoteuthis oualaniensis* mantle tissue in comparison to the co-occurring Oceanic squids.

PUFA content was the highest in all the oceanic squid ranging from (283.06 ±18.03 mg/100 g in *S .oualaniensis* dwarf form to 165.74 ± 5.53 mg/100 g in *H.bonnellii*). Among them docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) constituted major portion of the fatty acids. *S.oualaniensis* medium and dwarf form had 151.60 ±5.90 ; 186.91 ± 12.4 mg/100g of DHA and 38.95 ± 2.90 ; 56.67 ±3.70 mg/100g EPA respectively. The DHA showed lower values in other three oceanic species as 136.72 ± 7.28 mg/100g in *M. lonnbergii* , 98.73 ± 4.20 mg/100g in *C. imperator* and 96.07 ± 2.90 mg /100g in *H.bonnellii.*

6.3.3 Amino acids analysis

Amino acid profile of *S. oualaniensis* along with the co-existing oceanic squid species has been presented in the Table 6.3. *S. oualaniensis* had the highest content of total amino acid and protein (905.70 ± 48.50- medium; 911.40 ± 44.00 mg/g-dwarf). The other oceanic squids such as *M. lonnbergii, H. bonnellii* and *C. imperator* had 732.20 ±32.50, 713.30 ±35.00, 886.10 ± 62.03 mg/gram protein of total Amino acid contents respectively. Essential Amino Acids (EAA) constituted more than 50% of the total amino acids in all the species studied and indicated in the Figure 6.2. Among the EAA Lysine was significantly high in *S. oualaniensis* medium form (62.20 ± 0.70mg/g protein) ($P < 0.05$) followed by Arginine (81.20 ± 5.10 mg/g) and then Leucine (62.20 ± 0.70mg/g). Whereas, in the dwarf form Lysine was the major constituent of the EAA (97.50 ± 4.00 mg/g) ($P < 0.05$) followed by Leucine (73.00 ± 1.00 mg/g). Similarly Lysine constituted the major portion of the EAA in *H. bonnellii,*(200.60 ± 6.00 mg/g) ($P < 0.05$) followed by Arginine (123.20 ± 6.00 mg/g). *C. imperator* also had the Lysine as the major constituent of the EAA (89.00 ± 1.00 mg/g). However, in *M. lonnbergii* Arginine was the dominant constituent of the EAA (126.90 ± 4.00 mg/g) followed by Lysine (106.6 ± 3.40 mg/g).

Among the Non Essential Amino Acids Glutamic acid constitutes the major portion in the *S. oualaniensis* constituting 127.60 ± 8.90 mg/g ($P < 0.05$) in the medium form and 111.20 ± 4.0 in the dwarf form, followed by Aspartic acid 80.10 ± 3.30 mg/g ($P < 0.05$) in the medium form and 98.00 ± 2.00 mg/g in the dwarf form. Alanine has formed the next major constituent (61.20 ± 4.00 medium form ; 77.50 ± 2.50mg/g dwarf form) .

Table 6.3**.** Amino acid composition (in mg/g) of *Sthenoteuthis oualaniensis* mantle tissue in comparison with the co-existing Oceanic squids.

Amino acids	*S oualaniensis* (Medium form)	*S oualaniensis* (Dwarf form)	*Moroteuthis. lonnbergii*	*Histioteuthis bonnellii,*	*Chiroteuthis imperator*
Histidine	21.40 ± 1.10	28.3 ± 2.20	19.7 ± 1.00	16.1 ± 1.1	39.10 ± 2.0
Isoleucine	41.30 ± 1.20	45.9 ± 1.20	26.5 ± 1.00	21.7 ± 1.40	59.50 ± 0.8
Leucine	62.20 ± 0.70^{b}	73.0 ± 1.00^{a}	52.5 ± 3.20^{b}	39.5 ± 3.10^{c}	74.00±6.50^{a}
Lysine	87.70 ± 5.70^{a}	97.5 ± 4.00^{a}	106.6 ± 3.40^{a}	190.6 ± 6.0^{b}	89.0 ± 1.00^{a}
Methionine	61.30 ± 2.60	61.0 ± 3.00	8.70 ± 0.40	10.0 ± 1.20	40.20 ± 3.1
Phenyl Alanine	44.90 ± 4.00	55.10 ± 6.00	21.70 ± 0.30	16.00 ± 1.10	53.40 ± 2.7
Threonine	41.00 ± 2.10	23.10 ± 1.30	30.10 ± 2.20	22.10 ± 1.40	38.10 ± 3.2
Trptophan	12.90 ± 0.90	8.00 ± 1.10	13.50 ± 0.10	4.90 ± 0.20	6.70 ± 0.2
Valine	57.60 ± 2.60	52.70 ± 7.20	24.60 ± 1.10	22.40 ± 1.70	48.50 ± 3.1
Arginine	81.20 ± 5.10^{a}	60.0 ± 2.42^{c}	126.9 ± 4.00^{b}	123.20 ± 6.0^{b}	56.00 ±2.0^{c}
Alanine	61.20 ± 4.00^{a}	77.50 ± 2.50^{a}	16.90 ± 1.10^{b}	12.90 ± 1.30^{b}	70.30 ±5.2^{a}
Aspartic Acid	80.10 ± 3.30^{a}	98.00 ± 2.00^{a}	76.90 ± 3.00^{a}	52.10 ± 2.00^{b}	72.80 ±8.1^{a}
Glutamic Acid	127.60 ± 8.9^{a}	111.20 ± 4.0^{a}	106.5 ± 7.00^{a}	99.20 ± 4.00^{a}	81.20 ±5.2^{b}
Glycine	39.70 ± 0.40^{a}	39.90 ± 3.00^{a}	34.30 ± 2.10^{a}	30.20 ± 2.30^{a}	36.70 ±4.5^{a}
Proline	36.40 ± 1.80	36.10 ± 3.10	29.80 ± 0.20	25.50 ± 2.40	26.00 ± 5.4
Serine	28.70 ± 2.10	23.20 ± 2.20	24.40 ± 1.00	18.00 ± 0.30	68.90 ± 6.6
Tyrosine	20.50 ± 2.00	20.90 ± 1.10	12.60 ± 1.40	8.90 ± 0.70	25.70 ± 2.4
Sum EAA	511.50 ± 26.0	504.6 ± 29.2	430.8 ±16.7	466.5 ± 22.0	504.50 ± 24.63
Sum NEAA	394.20 ± 22.5	406.8 ± 17.9	301.4 ±15.8	246.8 ± 13.0	381.60 ± 37.40
Total	905.70 ± 48.5	911.4 ± 44.0	732.2 ±32.5	713.3 ± 35.0	886.10 ± 62.03

Values (mean of three replications with ± SD) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

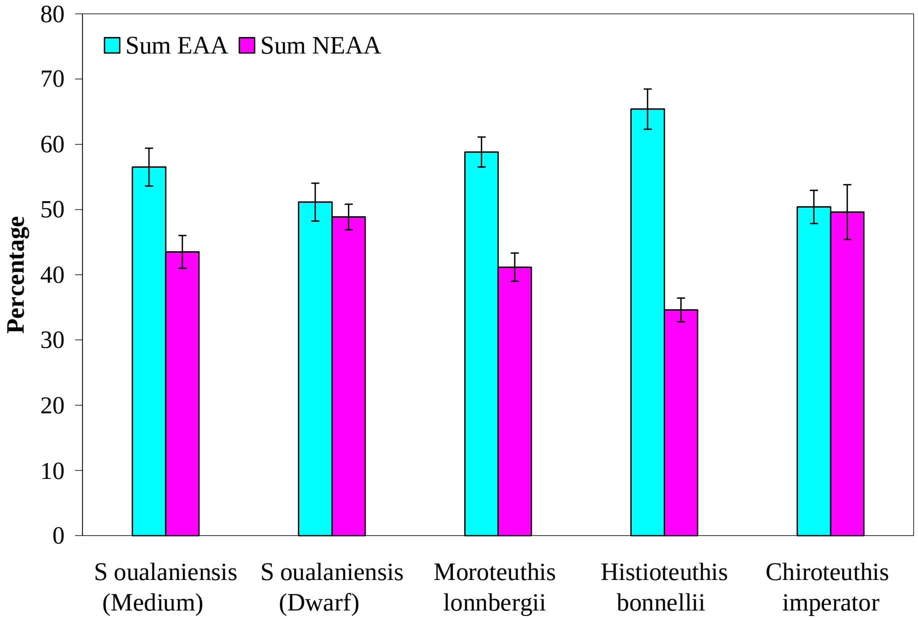

Figure 6.2. Essential and Non essential Amino acid composition of *Sthenoteuthis oualaniensis* mantle tissue in comparison with the co-existing Oceanic squids.

6.4 Discussion

Squid have one of the highest rates of energy metabolism among marine animals. At the same time among squid, a very high rate of metabolism is characteristic of the genus *Sthenoteuthis*. It is linked, first of all, to the active life of these pelagic animals, and secondly, to the high temperature of their habitat. Because squid energy requirements are much higher than in fish, they have developed mechanisms for their maintenance (Zuyev et al.,2002). Squids have a protein-based metabolism (Lee, 1994), and the direct use of protein as an energy reserve may account for the lack of major reserves of glycogen and lipid in squid tissues (O'Dor *et al.,* 1984; Storey and Storey, 1983). Features of chemical composition of mantle, fins, arms and tentacles of squid include a high content of protein (approximately 17–22% of wet weight), low content of lipids (1– 3%) and very low glycogen content (0.1%) (Ertel, 1970; Suryanaraynan and Alexander, 1971; Suryanaraynan et al., 1973; Shchepkin et al., 1981). This has been substantiated by the results obtained in this study with the oceanic squids examined.

The high content of polyunsaturated fatty acids (PUFA) in mantle tissue of the oceanic squids studied attract special attention. First of all, the most unsaturated in the ω3 series was docosahexoenoic acid 22:6 ω 3. It is reported that the integument has a large amount of cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n-3, DHA) as a compositional fatty acid of the phospholipids (Suzuki et al., 1992). It is known that phospholipids in the muscle and some organs in marine fishes and several species of squid contain a large amount of DHA (De Koning, 1993).The content of this fatty acid in phospholipids of fin, mantle, tentacles and arms is 44 to 45% (Shulman et al ,2002). However, the present study on the five oceanic squid mantle tissue indicates that the DHA ranged between 35 to 53 % of the total fatty acid, wherein for *S. oualaniensis* it was between 48 to 53 %. Hayashi (1996) also reported high content of 22:6 ω3 fatty acids in muscles of different squid species. In marine animals, 22:6 ω 3 fatty acid plays a cardinal role in providing the high functional activity and adaptive plasticity of membranes, tissues and the whole organism (Shulman and Yakovleva, 1983; Shulman and Yuneva, 1990; Yuneva et al., 1998). There is also a close correlation between the content of this acid in bodies of fish and plankton crustaceans and their motility (Shulman et al ,2002). The content of 22:6 ω 3 in squid muscle is considerably higher than in cuttlefish or octopods, not to mention bivalve molluscs. Significantly, in *S. pteropus* it is higher than in another less motile species from tropical Atlantic *Thysanoteuthis rhombus.* Such high 22:6 ω 3 content in squid muscle, even in comparison with fast swimming fish and vertically migrating copepods, is evidence of extremely high functional activity (in this case, mobility) of squid (Shulman et al ,2002).

Fish roe contains large amounts of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Bledsoe et al., 2003; Kaitaranta, 1980; Tocher and Sargent, 1984), which play an important role in the prevention and treatment of cardiovascular diseases (Nordøy et al., 2001) and the improvement of learning ability (Suzuki et al., 1998). Specially, DHA is abundant in the structural phospholipids of the retina and brain of mammals (Tinoco, 1982) and has many physiological effects which are not detected with the other n-3 polyunsaturated fatty acids (PUFA) such as EPA and alpha-linolenic acid (ALA) (Ikeda et al., 1994; Mori et al., 1999; Nishikawa et al., 1994; Shikano et al., 1994). DHA concentrates are now still mainly produced as triglycerides from fish oils. It was reported that an intake of neutral lipid-containing DHA at a moderately high level did not alter human immune response (Kelley et al., 1998).

Compared to that of fish, squid lipid tends to be lower in triglycerides and higher in phospholipids (Okuzumi and Fujii, 2000). Phospholipids have been recognized to be a major component of lipids which play a vital role as structural and functional components of biological membranes in all marine organisms from viruses to large animals (Suzumura, 2005). It is reported that the integument of squid *Ommastrephes bartrami* has a large amount of DHA as a compositional fatty acid of the phospholipids (Suzuki et al., 1992) and can be used as a possible new source of phospholipids containing docosahexaenoic acid (Deng et al., 1998). The dominance of palmitic acid in fish lipid has been reported previously (Abbas *et al.*, 2009; Khoddami *et al.*, 2009). The ratio between n-3 and n-6 is very high in the *Sthenoteuthis oualaniensis*. This ratio has been the useful index for comparing the nutritional value of fish lipid due to their human health effects on coronary heart disease, cancer and autoimmune diseases (Khoddami *et al.,* 2009).

Both acidic amino acids (Glutamic and aspartic acid) had the highest concentrations. This was corroborated by the findings of Rosa et al (2005) in a group of cephalopods with different life strategies. He reported that the major NEAA of cephalopods were of Glutamic acid and aspartic acid. Such results are similar to that found by others authors (Chandrashekar & Deosthale, 1993) in white fish. The major essential amino acids (EAA) in the oceanic squids studied were leucine, lysine and arginine as reported by Rosa et al (2005) in cephalopods. The concentration of lysine (mg/g) in squid protein shows high value. This is significantly higher than the FAO/WHO (1991) recommended reference lysine standard value of 58 mg g^{-1} of dietary protein for a 2-5 year child. It has been shown that biochemical manifestations of dietary excess of leucine could be counteracted not only by increasing the intake of niacin or tryptophan but also by supplementation with isoleucine (Adeyeye , 2008).

Grain proteins like that of sorghum and maize contain a high proportion of leucine that becomes a precipitating factor for the manifestation of pellagra in nutritionally challenged subjects. FAO/WHO, 1991 recommends a leucine to isoleucine ratio of 2.36 as ideal to prevent this irregularity and in the present study squid protein shows Leu/Ile of 1.5 and 1.3 in the case of *S.oualaniensis* medium and dwarf form respectively. Proline and arginine were two of the most abundant amino acids in the squid muscle. Proline is thought to be utilized during oxidative metabolism either directly as an energy source or as a means of augmenting the Krebs cycle (Storey and Storey 1978, 1983). Arginine is thought to be involved in anaerobic metabolism, being ‘‘dumped’’ into octopine during activated anaerobic metabolism, for example, during burst swimming (Ga de 1980; Hochachka and Fields 1982). The reduced amount of Arginine in the Dwarf form perhaps due to its conversion in to Proline during the anaerobic respiration as suggested by Mommsen et al,(1982) in *Sthenoteuthis oualaniensis.*

The present study reveals that these squids may be considered as a source of high protein and low fat food for human consumption. *S.oualaniensis* and the co existing oceanic species are comparable to each other in respect of their nutritional attributes with protein with high content of EAA and the lipid being a good source of $\omega3$ and $\omega6$ fatty acids.

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Chapter 7

Summary and Conclusion

With the stabilization of world finfish catches in general, and the depletion of a number of fish stocks that used to support industrial-scale fisheries, increasing attention is now being paid, to the so-called unconventional marine resources, which include many species of cephalopods. One of such important cephalopod resource is the tropical Indo-Pacific pelagic oceanic squid *Sthenoteuthis oualaniensis*. It is the most abundant large sized squid in the Indo-Pacific region with an estimated biomass of 8-11 metric tons. However, its distribution, biology, life cycle and nutrient value in the south west coast of India are still poorly known. So any new information of this species in the waters off the south west coast of India has important scientific significance for effective and rational utilization of this Oceanic fishery resources, especially during the time of depletion of shallow water resources. In view of that this study investigated different aspects of the *Sthenoteuthis oualaniensis*, such as morphometry, growth, mortality, maturation, spawning, food, feeding and biochemical composition in the south west coast of India to understand its possible prospective importance for commercial fishing and management of its fishery.

Samples were collected onboard the squid jigging vessel and from the fishing harbour. Data collected were subjected to various analyses. The plastic phenotypes, if any, were investigated for the degree and nature of relationship between various morphometric variables such as Dorsal mantle length (DML), Tentacle length (TL), Tentacular club length (TCL), Fin width (FW),Fin length (FL), Mantle width (MW), Head width (HW) and Arm length (AL-1, AL-2, Al-3 and AL-4) in comparison with the Total weight (TWt) and Mantle weight (MWt) by using ANOVA. Relationship of parameters between the plastic forms and sexes were also compared for testing the significance of variation by using ANCOVA. It was found that the dwarf form DML –TWt and DML-MWt curve had significantly lower slope value than the medium form, indicating a different growth pattern. However, the difference in the slope is not significant between the sexes of dwarf, though the variation in the slope is significant in the medium form. Though the dwarf form was clearly distinguishable from the medium form in its growth and morphological traits, the shape and size of its fin width may hold the key to differentiate the dwarf population from the medium form of *Sthenoteuthis oualaniensis.*

Although our knowledge has increased in regard to squid growth, questions remain as to how any model of growth may be applicable if a squid grows under differing environmental conditions resulting in different plastic phenotypic forms, as in the case of most of the oceanic squids including *Sthenoteuthis oualaniensis.* Therefore, this study has attempted to indicate the growth model and mortality of the purple squid with the available means of length based routine of FiSAT software to obtain basic scientific information relevant to future management of its fishery. The length frequency data of dwarf and medium forms were analysed separately to study the growth and mortality parameters of the species. The data were decomposed using the Bhattacharya's method of the modal progression analyses. The means obtained were linked to get the growth increment for calculating the growth parameter by using Gulland and Holt plot, Munro's Method, Faben's Method and length at age method. Dwarf had a higher K value (Growth co efficient) with short life span when compared to the medium form.

The Length converted catch curve, Jones & van Zalinge plot, Beverton & Holt model and Ault and Ehrhardt Method were used to understand the total mortality rate. The rate obtained in the Length converted catch curve seems to be logical as it can be related to the Powell Wetherall Z/K value and the life span of the squid. The growth curve and longevity study indicated a slightly longer than a year life span for medium form at around 15 months (461 days) and approximately grows to 13.3 cm ML in three months (91 days), 20.8 cm in six months (182 days) and 25.0 cm in nine months (273 days). Dwarf form may attain the length infinity in around ten months (302 days) and approximately grows to 10.5 cm ML in three months (91 days) , 14.9 cm in six months (182 days) and 16.7 cm in nine months (274 days). The high total mortality rate estimated may possibly be due to faster growth, short life span, high level of cannibalism and possibly higher post spawning mortality in the *Sthenoteuthis oualaniensis*. As there is no directed fishing for this species, fishing mortality is considered as nil and as a result, the reduction of number in the cohort is considered purely due to natural causes.

Cephalopod life-cycles have been considered as somewhat enigmatic. Some of this enigmas in their life-cycles, such as apparent ubiquity of breeding once and post-reproductive mortality have been attributed to a combination of r- and k-factors and presumably r- and k-selection operating in one species. The study was aimed to provide information on the distribution of mature individuals, sex ratio, size at maturity and the spawning season and to discuss the reproductive strategies of the squid in the south west coast of India. Specimens were sexed and assigned a maturity stage according to the ‘Universal scale’ proposed by Lipiski (1979). Five stages were used for both males and females: Juvenile (I), immature (II), preparatory (III), maturing (IV) and matured (V). As no spent squids were observed during the study period, spent stage has not been included in the scale. The length range of each maturity group was identified for each plastic phenotype. Maturity indices such as NGWI,(Nidamental Gland Weight Index), NGLI (Nidamental gland Length Index), OCWI, (Oviductal Complex Weight Index), OWI (Ovary Weight Index) and RSWI (Reproductive System Weight Index) were studied for female and TWI (Testis Weight Index) for male. Batch fecundity, ovary fecundity and potential fecundity were also investigated. Fecundity was found to be directly proportional to the Mantle length of the species. Sex ratio was determined on a monthly basis and tested for significant departures from the expected 1:1 female- male ratio using a X^2- test. Female was found to be slightly out numbering the males in the medium form, whereas, in the dwarf form females completely out numbered the males and dominated more than 80% of the dwarf population. However, the period of out numbering is not corresponding with the peak reproductive activity period of the medium form.

Male of the medium form attained maturity at a faster rate from the onset of maturity than the medium female. Maturity indices were proportionate to the maturation of the squid and were highly correlated to maturation and correspond to the descriptive maturity stages assigned. A clear pattern of seasonal change in the maturity index has emerged. In medium form spawning is a continuous process through out the year with two spawning peaks, one in the months of March and April and another in the months of August to December, and with a ebb in the months of January and May. Dwarf maturity index indicated the continuous spawning behavior of the dwarf form through out the year with out any major fluctuation or peak. However, it may have a slight peak between the months of May and August. Medium form was found to be more fecund than the dwarf form. The huge number of eggs estimated in this squid along with the longer, continuous period of reproductive activity and wider size range of fully matured stage indicated the continuous extended intermittent spawning and feeding behavior with somatic growth in between. The non availability of spent squid in the sample may indicate the reluctance of the spent squids in taking the jigs, thereby indicating the avoidance of feeding.

Knowledge of the size-structure of their feeding relationships is of paramount importance for determining their ecological role. This information can be valuable for assessing the potential consequences of squid feeding habits and for exploring how the strength of their trophic interactions may change if predator and (or) prey body sizes are altered by ecosystem perturbations. In view of that, studies were conducted on the food and feeding habits of the squid. Stomachs were estimated for their fullness of content using subjective scale of 0-4 points (O- empty; 1- one-quarter full; 2- half full; 3- three-quarter full; 4- full). Its contents after weighing were sorted out and separated for further examination under a dissection and binocular microscope. Major prey taxa were identified based on the hard parts of the prey by following standard procedures as the prey was masticated and mostly digested. The identified prey items were counted and weighed stomach wise. Fishes were the most common prey, followed by cephalopods and crustaceans. However, the relative importance of the fish prey either diminished or remained the same during its growth, while cephalopod prey gradually became relatively more important than the fishes and crustaceans during the adult stage.

The variability in stomach fullness and content weight observed in this study lead to a lack of significant differences between the months. However, it is inferred that, during the peak spawning period of March and April of the medium form, as found in this study, neither empty stomach nor full stomach was found. This may indicate the moderate feeding rate during the reported spawning periods. However, the same was not true in the case of dwarf form, wherein during the reported spawning peak of May to August, no full stomachs were observed, instead empty stomachs were in more percentage than even the moderate ones. Probably, dwarf form may not feed voraciously during/ between the spawning events, thereby less growth as indicated in this study with a lesser size range of matured ones when compared to the medium form.

All living cephalopods are carnivores and proteins are the major organic components in cephalopod tissue. Squid is a largely untapped protein resource of the oceans of great economic potential. Although sufficient information on the nutritional profile of many commercially important fishes and squids are available, sufficient information on similar lines on *S. oualaniensis* particularly from the Arabian Sea is not available. Therefore this study has investigated the nutritional value and protein quality of the important oceanic squids of the south west coast of India especially *S. oualaniensis* in comparison to the co existing other oceanic squids such as *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii.* This was envisaged with an objective of establishing the fact that oceanic squids are good for human nutrition.

The proximate composition of the mantle tissue was determined along with the fatty acid and amino acid profiles. Moisture content varied between 80.8 to 83.62 %, protein content between 13.83 to 17.5 %, fat content between 0.6 to 1.59 % and ash content between 0.9 to 1.4 % among the various oceanic squids studied. Polyunsaturated fatty acids (PUFA) were the dominant constituent of the total lipids followed by the saturated fatty acid. Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) were the prominent fatty acids of PUFA, wherein Palmitic acid was the major constituent of the saturated fatty acids.

Total amino acid content varied between 713.3 to 914.4 mg/g crude protein among the squids studied, of which, essential amino acids were higher than the non essential amino acids. Glutamic acid and Aspartic acids were the most abundant among the non essential amino acids with tyrosine being the lowest. Among the essential amino acids Lysine followed by Arginine was the major constituents and tryptophan was the lowest. *S. oualaniensis* and the co existing oceanic squids of *Histioteuthis bonnellii, Chiroteuthis imperator* and *Moroteuthis lonnbergii* are comparable to each other in respect to their nutritional attributes with protein being of high quality and its lipids being a good source of n-3 and n-6 fatty acids. High levels of essential amino acids in the oceanic squids will make it a good food source in matching the cereals. Besides, their high value protein will prevent protein-energy malnutrition in the population. In view of that, these oceanic squids may be considered as a kind of aquatic food with high protein and low fat content. The nutritional attributes of these species may prove important for future policy regarding exploitation of this species.

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Appendix

DML-TWT

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signifie F*
Regression	1	41.91817	41.918	3386.985	5.3E-211
Residual	448	5.544559	0.0123		
Total	449	47.46273			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.79216	0.11596	-24.07	8.33E-83	-3.020	-2.564
X Variable 1	2.666971	0.0458	58.197	5.3E-211	2.576	2.7570

Medium

	df	*SS*	*MS*	*F*	*Signifi F*
Regression	1	235.3883	235.38	18643.21	0
Residual	563	7.1084	0.0126		
Total	564	242.4967			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.27175	0.06080	-53.813	0.000	-3.391	-3.152
X Variable 1	3.04509	0.02230	136.54	0.000	3.001	3.088

ANCOVA

						Deviations from regression				
Source	d.f.	ssx	ssy	spxy	Reg.coef	d.f.	S.S.	M.S	F	Prob
Within										
Medium	564.000	25.385	242.497	77.301	3.045	563.0	7.108	0.013		
Dwarf	449.000	5.893	47.463	15.718	2.667	448.0	5.545	0.012		
						1011.0	12.65	0.013		
Pooled W	1013.000	31.279	289.959	93.018	2.974	1012.0	13.33	0.013		
Difference between slopes						1	0.683	0.683	51.89	1.14E-1
Between B										
W+B	1014	40.3225	568.676	143.224		1013	59.94			
Between adjusted means						1	46.61	46.61	3536.8	0

L-W relationship	a	b	Rsqr
medium	0.0379	3.045	0.970
dwarf	0.0612	2.666	0.883
Pooled	0.0080	3.551	0.894

Female DML- TWT

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	24.43138	24.431378	1953.10761	6.8E-150
Residual	371	4.64083	0.012509		
Total	372	29.07221			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.635011	0.150822	-17.471004	2.6541E-50	-2.931584	-2.338438
X Variable 1	2.607214	0.058995	44.193977	6.805E-150	2.491208	2.72322

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	165.1462593	165.1462593	12161.18337	3.8147E-26
Residual	326	4.427010013	0.013579785		
Total	327	169.5732693			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.518494228	0.079200849	-44.42495609	2.4201E-140	-3.674308	-3.3626876
X Variable 1	3.126562771	0.028351708	110.2777555	3.8147E-260	3.0707873	3.1823365

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	327	16.8941	169.573	52.8204	3.12656	326	4.427	0.013		
Dwarf	372	3.59414	29.0722	9.37068	2.60721	371	4.640	0.012		
						697	**9.067**	**0.013**		
Pooled W	699	20.4882	198.645	62.1911	3.03546	698	9.86	0.014		
		Difference between slopes				**1**	**0.799**	**0.799**	**56.546**	**1.69E-13**
Between B										
W+B	700	29.6913	433.988	108.73		699	35.816			
		Between adjusted means				**1**	**25.94**	**25.94**	**1835.6**	**1.40E-197**

L-W relationship	**a**	**b**	**Rsqr**
medium	**0.02964**	**3.12656**	**0.97389**
dwarf	**0.07172**	**2.60721**	**0.84037**
Pooled	**0.00563**	**3.66202**	**0.91747**

Male DML- TWt

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signifi F*
Regression	1	4.984624	4.9846242	442.960696	3.35E-33
Residual	75	0.843973	0.011253		
Total	76	5.828597			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.660068	0.296822	-8.9618279	1.7596E-13	-3.251368	-2.068768
X Variable 1	2.602827	0.12367	21.046631	3.3469E-33	2.356465	2.849189

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	44.69805527	44.69805527	4697.148404	2.4842E-157
Residual	235	2.236259552	0.009515998		
Total	236	46.93431482			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.065568548	0.114243	-26.83375394	1.6752E-73	-3.290639826	-2.840497271
X Variable 1	2.977061189	0.04343808	68.53574545	2.4842E-157	2.891483393	3.062638985

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	236	5.0432	46.934	15.014	2.977	235	2.23	0.0095		
Dwarf	76	0.7357	5.8286	1.9150	2.602	75	0.84	0.0112		
						310	**3.08**	**0.0099**		
Pooled W	312	5.7790	52.762	16.929	2.929	311	3.17	0.0101		
		Difference between slopes				**1**	**0.09**	**0.0899**	**8.82196**	**0.003**
Between B										
W+B	313	8.796	132.35	32.425		312	12.8			
		Between adjusted means				**1**	**9.65**	**9.65388**	**947.068**	**2.172E-96**

L-W relationship

	a	**b**	**Rsqr**
Medium	**0.046627**	**2.97706**	**0.95235**
Dwarf	**0.069943**	**2.60283**	**0.8552**
Pooled	**0.006676**	**3.68636**	**0.90311**

DML–MWt

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	13.26034	13.26034	2294.495	5.98E-90
Residual	143	0.826426	0.005779		
Total	144	14.08677			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.9074	0.127807	-22.7484	2.3E-49	-3.16004	-2.65477
X Variable 1	2.421531	0.050553	47.90088	5.98E-90	2.321604	2.521459

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	56.14953	56.14953	6082.85	2E-148
Residual	194	1.790774	0.009231		
Total	195	57.9403			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.19488	0.101021	-31.626	1.72E-78	-3.39412	-2.99564
X Variable 1	2.816457	0.036112	77.99263	2E-148	2.745235	2.887679

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.cf**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
medium	195	7.0784	57.940	19.93	2.816	194	1.79077	0.00923		
dwarf	144	2.2613	14.086	5.476	2.421	143	0.82643	0.00578		
						337	**2.6172**	**0.00777**		
Pooled W	339	9.3398	72.027	25.41	2.720	33	2.8845	0.00853		
		Difference between slopes				**1**	**0.2673**	**0.2673**	**31.322**	**4.523E-08**
Between B										
W+B	340	15.231	249.34	57.734		339	30.505			
		Between adjusted means				**1**	**27.6205**	**27.62**	**3236.5**	**3.56E-175**

L-W relationship

	a	**b**	**Rsqr**
medium	**0.040**	**2.81646**	**0.96909**
dwarf	**0.054**	**2.421**	**0.9413**
Pooled	**0.002**	**3.790**	**0.8776**

Female DML- MWt

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.044672	4.044672	835.3135	3.19E-51
Residual	103	0.498736	0.004842		
Total	104	4.543408			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.78385	0.212176	-13.1205	1.04E-23	-3.20465	-2.36305
X Variable 1	2.373296	0.082116	28.90179	3.19E-51	2.210439	2.536154

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	35.87759	35.87759	3762.547	3.65E-81
Residual	100	0.953545	0.009535		
Total	101	36.83113			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.26245	0.132762	-24.5736	3.62E-44	-3.52585	-2.99905
X Variable 1	2.834092	0.046203	61.3396	3.65E-81	2.742426	2.925758

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium females	101	4.466	36.83	12.659	2.8340	100	0.953	0.009		
Dwarf females	104	0.718	4.543	1.7042	2.3733	103	0.498	0.004		
						203	**1.452**	**0.007**		
Pooled W	205	5.184	41.37	14.363	2.7702	204	1.583	0.007		
		Difference between slopes				**1**	**0.131**	**0.131**	**16.921**	**5.65E-05**
Between B										
W+B	206	9.336	160.00	36.555		205	16.87			
		Between adjusted means				**1**	**15.29**	**15.29**	**1969.7**	**9.018E-107**

L-W relationship

	a	**b**	**Rsqr**
Medium	**0.038**	**2.834**	**0.974**
Dwarf	**0.061**	**2.373**	**0.890**
Pooled	**0.0014**	**3.915**	**0.894**

Male . DML-MWt

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significe F*
Regression	1	1.9705	1.97050	238.1948	5.92E-18
Residual	38	0.31436	0.00827		
Total	39	2.284			

	Coefficients	*Stan Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.37499	0.40325	-8.3694	3.76E-10	-4.19133	-2.55864
X Variable 1	2.619594	0.16973	15.4335	5.92E-18	2.275986	2.963201

Medium

	df	*SS*	*MS*	*F*	*Significe F*
Regression	1	12.3806	12.38066	1522.473	4.97E-59
Residual	92	0.74813	0.00813		
Total	93	13.1288			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.54303	0.20520	-17.2658	1.3E-30	-3.95058	-3.13547
X Variable 1	2.951755	0.07564	39.0188	4.97E-59	2.801509	3.102002

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	93	1.42096	13.1288	4.194	2.95176	92	0.74814	0.00813		
Dwarf	39	0.28715	2.28486	0.752	2.61959	38	0.31436	0.00827		
						130	**1.0625**	**0.00817**		
Pooled W	132	1.70811	15.4137	4.946	2.89592	131	1.08885	0.00831		
		Difference between slopes				**1**	**0.02636**	**0.02636**	**3.170**	**0.077**
Between B										
W+B	133	4.86644	88.2296	20.11		132	5.1147			
		Between adjusted means				**1**	**4.02585**	**4.02585**	**484.35**	**7.715E-46**

L-W relationship

	a	**b**	**Rsqr**
Medium	**0.028**	**2.951**	**0.9430**
Dwarf	**0.0342**	**2.619**	**0.8624**
Pooled	**0.0011**	**4.132**	**0.9420**

DML- TL ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signific F*	
Regression	1	448.684409	448.684409	529.0104421	2.29E-48	
Residual	134	113.6531645	0.848157944			
Total	135	562.3375735				
	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.920849	0.642418693	2.990025891	0.003320192	0.650256	3.191441
X Variable 1	1.158917	0.050387204	23.000227	2.29064E-48	1.05926	1.258574

Medium

	df	*SS*	*MS*	*F*	*Signifi F*	
Regression	1	6591.45	6591.45	2441.079225	7.3009E-106	
Residual	178	480.6391	2.70022			
Total	179	7072.089				
	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-4.949002286	0.61738	-8.016138	1.39444E-13	-6.167327887	-3.730677
X Variable 1	1.803848226	0.03651	49.40728	7.3009E-106	1.731800551	1.875896

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	179	2025.72	7072.08	3654.10	1.8038	178	480.63	2.70022		
Dwarf	135	334.069	562.337	387.158	1.1589	134	113.65	0.84815		
						312	**594.29**	**1.90478**		
Pooled W	314	2359.796	7634.42	4041.26	1.7125	313	713.57	2.27978		
		Difference between slopes				**1**	**119.28**	**119.280**	**52.320**	**3.6E-12**
Between B										
W+B	315	3550.78	13052.8	6581.585		314	853.46			
		Between adjusted means				**1**	**139.89**	**139.896**	**61.362**	**7.4E-14**

L-W relationship

	a	**b**	**Rsqr**
Medium	**-4.949**	**1.803**	**0.932**
Dwarf	**1.920**	**1.158**	**0.797**
Pooled	**-6.244**	**1.853**	**0.931**

DML– TCL

ANOVA

Dwarf

	df	SS	MS	F	Significance F
Regression	1	41.434009	41.434009	855.2860574	4.99E-60
Residual	134	6.491579231	0.048444621		
Total	135	47.92558824			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-0.11636	0.1535333	-0.75789839	0.449842627	-0.42002	0.187299
X Variable 1	0.352176	0.012042168	29.2452741	4.99044E-60	0.328359	0.375994

Medium

	df	SS	MS	F	Significance F
Regression	1	875.9004	875.9004	1455.128033	1.3473E-87
Residual	178	107.1454	0.60194		
Total	179	983.0458			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-2.889474707	0.291494	-9.912638	1.01472E-18	-3.464703452	-2.314246
X Variable 1	0.657562246	0.017238	38.14614	1.3473E-87	0.623545154	0.691579

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	179	2025.72	983.05	1332.04	0.6575	178	107.145	0.60194		
Dwarf	135	334.069	47.92	117.651	0.3521	134	6.49157	0.04844		
						312	**113.637**	**0.36422**		
Pooled W	314	2359.79	1030.9	1449.69	0.614	313	140.381	0.44850		
		Difference between slopes				**1**	**26.744**	**26.7448**	**59.6312**	**1.56E-13**
Between B										
W+B	315	3550.78	2073.9	2564.20		314	222.173			
		Between adjusted means				**1**	**81.792**	**81.7920**	**182.366**	**4.63E-33**

L-W relationship

	a	**b**	**Rsqr**
Medium	**-2.889**	**0.6575**	**0.89**
Dwarf	**-0.116**	**0.35217**	**0.86**
Pooled	**-4.320**	**0.72215**	**0.89**

DML–FW

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signif F*
Regression	1	156.2119	156.2119	1177.864736	3.39375E-47
Residual	74	9.814099	0.132623		
Total	75	166.026			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.94396	0.281326	-3.35541	0.001252938	-1.504516906	-0.38341
X Variable 1	0.766994	0.022348	34.32003	3.39375E-47	0.722464171	0.811524

Medium

	df	*SS*	*MS*	*F*	*Signif F*
Regression	1	612.174	612.1749	1902.366	6.83E-67
Residual	100	32.179	0.321796		
Total	101	644.354			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.747797	0.28913	6.04485	2.5887E-08	1.1741561	2.3214
X Variable	0.760318	0.01743	43.6161	6.832E-67	0.725733	0.7949

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	101	1058.97	644.35	805.155	0.760	100	32.179	0.321		
Dwarf	75	265.5	166.02	203.667	0.76	74	9.8140	0.132		
						174	**41.993**	**0.241**		
Pooled W	176	1324.51	810.38	1008.82	0.76	175	42.003	0.240		
		Difference between slopes				**1**	**0.0094**	**0.009**	**0.039**	**0.842**
Between B										
W+B	177	1960.65	2134.752	1926.694		176	241.4278			
		Between adjusted means				**1**	**199.42**	**199.4**	**830.8**	**2.31E-68**

L-W relationship

	a	**b**	**Rsqr**
Medium	**1.74**	**0.760**	**0.9500**
Dwarf	**-0.94**	**0.766**	**0.9408**
Pooled	**-2.6211**	**0.982**	**0.8869**

DML–MW

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signifi F*
Regression	1	4.146853	4.146853	28.63057	2.26E-05
Residual	22	3.186481	0.14484		
Total	23	7.333333			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.40351	0.727455	-0.55469	0.584706	-1.91216	1.105141
DML	0.30544	0.057083	5.350754	2.26E-05	0.187056	0.423824

Medium

	df	*SS*	*MS*	*F*	*Signific F*
Regression	1	101.0608	101.0608	674.7227	1.11E-34
Residual	61	9.136655	0.149781		
Total	62	110.1975			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.10848	0.193322	-0.56115	0.576752	-0.49505	0.278089
DML	0.356405	0.013721	25.97543	1.11E-34	0.328969	0.383842

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	62	795.59	110.19	283.5	0.35640	61	9.13665	0.14978		
Dwarf	23	44.44	7.333	13.57	0.30544	22	3.18648	0.14484		
						83	**12.3231**	**0.14847**		
Pooled W	85	840.04	117.53	297.13	0.35370	84	12.4324	0.14800		
		Difference between slopes				**1**	**0.10934**	**0.10934**	**0.7388**	**0.392488**
Between B										
W+B	86	856.17	146.18	318.63		85	27.6088			
		Between adjusted means				**1**	**15.1763**	**15.1763**	**102.538**	**3.25E-16**

<u>L-L relationship</u>

	a	**b**	**Rsqr**
Medium	**-0.108**	**0.356**	**0.917**
Dwarf	**-0.403**	**0.305**	**0.565**
Pooled	**-0.578**	**0.372**	**0.811**

DML–HW

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Signific F*
Regression	1	1.636375	1.636375	27.47088	3.96E-05
Residual	20	1.191352	0.059568		
Total	21	2.827727			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.49745	0.504338	-0.98634	0.335752	-1.54948	0.554585
DML	0.207698	0.039627	5.241267	3.96E-05	0.125036	0.290359

Medium

	df	*SS*	*MS*	*F*	*Signific F*
Regression	1	51.90841	51.90841	312.3427	1.12E-25
Residual	61	10.13762	0.166191		
Total	62	62.04603			

	Coefficients	*Stand Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.456	0.204181	-2.2333	0.029208	-0.86428	-0.04771
DML	0.255891	0.014479	17.67322	1.12E-25	0.226938	0.284844

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	62	792.734	62.04	202.85	0.2558	61	10.137	0.1661		
Dwarf	21	37.933	2.8277	7.87863	0.2076	20	1.1913	0.0595		
						81	**11.328**	**0.1398**		
Pooled W	83	830.668	64.873	210.732	0.2536	82	11.413	0.1391		
		Difference between slopes				**1**	**0.084**	**0.0840**	**0.6040**	**0.4392**
Between B										
W+B	84	846.627	78.219	225.326		83	18.249			
		Between adjusted means				**1**	**6.8367**	**6.8367**	**49.120**	**6.17E-10**

Note: If Prob <0.05 then significant at 5% level
if Prob<0.01 then significanrt at 1% level

L-L relationship

	a	**b**	**Rsqr**
Medium	**-0.456**	**0.2558**	**0.8366**
Dwarf	**-0.49745**	**0.2076**	**0.5786**
Pooled	**-0.76197**	**0.2661**	**0.766**

DML–AL I

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	13.04352	13.04352	306.7190238	2.86E-16
Residual	27	1.148201	0.042526		
Total	28	14.19172			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.49861	0.302421	-1.64874	0.110790533	-1.11913	0.121904
DML	0.42882	0.024485	17.5134	2.86429E-16	0.37858	0.47906

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	141.1241	141.1241	586.0114223	7.65E-35
Residual	67	16.13503	0.240821		
Total	68	157.2591			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.0832	0.246746	0.33719	0.737027785	-0.40931	0.575707
DML	0.422666	0.01746	24.20767	7.65125E-35	0.387816	0.457516

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	68	789.96	157.25	333.890	0.4226	67	16.1350	0.240		
Dwarf	28	70.93	14.191	30.4172	0.428	27	1.148201	0.042		
						94	**17.2832**	**0.183**		
Pooled W	96	860.89	171.45	364.307	0.423	95	17.28	0.181		
		Difference between slopes				**1**	**0.0024**	**0.002**	**0.0135**	**0.9075**
Between B										
W+B	97	904.97	197.40	398.1311		96	22.252			
		Between adjusted means				**1**	**4.966**	**4.966**	**27.29**	**1.0E-06**

L-L relationship

	a	**b**	**Rsqr**
Medium	**0.0832**	**0.4226**	**0.897**
Dwarf	**-0.4986**	**0.428**	**0.919**
Pooled	**-0.29612**	**0.4399**	**0.887**

DML–AL 2

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14.57381	14.57381	81.35843	1.24E-09
Residual	27	4.836535	0.179131		
Total	28	19.41034			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.305189	0.620683	0.491699	0.626906	-0.96835	1.578726
DML	0.453278	0.050253	9.019891	1.24E-09	0.350167	0.556388

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	218.3167	218.3167	559.4590834	3.08E-34
Residual	67	26.14529	0.390228		
Total	68	244.462			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.024503	0.314095	0.078013	0.938050337	-0.60243	0.65144
DML	0.525703	0.022226	23.65289	3.08102E-34	0.48134	0.570066

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	68	789.9	244.462	415.285	0.5257	67	26.145	0.3902		
Dwarf	28	70.93	19.410	32.1520	0.4532	27	4.8365	0.1791		
						94	**30.981**	**0.329**		
Pooled W	96	860.8	263.872	447.437	0.5197	95	31.323	0.329		
		Difference between slopes				**1**	**0.3414**	**0.3414**	**1.0354**	**0.3114**
Between B										
W+B	97	904.97	302.70	488.809		96	38.679			
		Between adjusted means				**1**	**7.3565**	**7.356**	**22.311**	**8E-06**

L-L relationship

	a	**b**	**Rsqr**
Medium	**0.0245**	**0.525**	**0.893**
Dwarf	**0.3051**	**0.453**	**0.7507**
Pooled	**-0.3467**	**0.540**	**0.8722**

DML–AL 3

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18.53507	18.53507	59.0191	2.91E-08
Residual	27	8.479408	0.314052		
Total	28	27.01448			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.150943103	0.821837	0.183666	0.855648	-1.53533	1.837213
DML	0.511181115	0.066539	7.682389	2.91E-08	0.374654	0.647709

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	214.3554	214.3554	622.2312541	1.25E-35
Residual	67	23.08115	0.344495		
Total	68	237.4365			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.917781	0.295116	3.109894	0.002748022	0.328726	1.506836
DML	0.520912	0.020883	24.94456	1.25053E-35	0.479229	0.562594

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	68	789.96	237.4365	411.500	0.520	67	23.08	0.3444		
Dwarf	28	70.93	27.01448	36.2593	0.5111	27	8.4794	0.3140		
						94	**31.560**	**0.3357**		
Pooled W	96	860.89	264.451	447.759	0.520	95	31.56	0.3322		
		Difference between slopes				**1**	**0.0061**	**0.0061**	**0.0185**	**0.8919**
Between B										
W+B	97	904.97	320.136	497.3033		96	46.856			
		Between adjusted means				**1**	**15.290**	**15.29**	**46.015**	**9.95E-10**

L-L relationship

	a	**b**	**Rsqr**
Medium	**0.9177**	**0.5209**	**0.90279**
Dwarf	**0.1509**	**0.5111**	**0.686116**
Pooled	**0.2754**	**0.5495**	**0.853635**

DML–AL 4

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	15.14958	15.14958	96.51674	2.07E-10
Residual	27	4.238007	0.156963		
Total	28	19.38759			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.08276	0.58101	-0.14244	0.887791	-1.27489	1.109376
DML	0.462145	0.047041	9.824294	2.07E-10	0.365624	0.558665

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	172.678	172.678	860.3422984	5.94E-40
Residual	67	13.44747	0.200709		
Total	68	186.1255			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.470433	0.225261	2.088397	0.040565524	0.020811	0.920055
DML	0.467536	0.01594	29.33159	5.94349E-40	0.435721	0.499352

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	68	789.96	186.125	369.3360	0.4675	67	13.447	0.2007		
Dwarf	28	70.93	19.3875	32.7810	0.4621	27	4.2380	0.1569		
						94	**17.685**	**0.1881**		
Pooled W	96	860.89	205.513	402.117	0.4670	95	17.687	0.1861		
		Difference between slopes				**1**	**0.0018**	**0.0018**	**0.0101**	**0.9199**
Between B										
W+B	97	904.97	240.3499	441.3036		96	25.151			
		Between adjusted means				**1**	**7.4641**	**7.4641**	**40.09**	**8E-09**

L-L relationship

	a	**b**	**Rsqr**
Medium	**0.470**	**0.467**	**0.927**
Dwarf	**-0.0827**	**0.462**	**0.781**
Pooled	**0.020**	**0.487**	**0.8953**

FL–FW

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	12.91866	12.91866	91.93521	4.02E-09
Residual	21	2.950903	0.140519		
Total	22	15.86957			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.909097	0.618888	4.700524	0.000122	1.622049	4.196144
FL	1.259449	0.131353	9.588285	4.02E-09	0.986286	1.532612

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	427.5837	427.5837	818.989	1.48E-38
Residual	65	33.93567	0.522087		
Total	66	461.5194			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.202168	0.340147	6.474157	1.46E-08	1.522846	2.881489
FL	1.745717	0.061001	28.61798	1.48E-38	1.62389	1.867544

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	66	140.305	461.51	244.93	1.745717	65	33.935	0.5220		
Dwarf	22	8.14434	15.869	10.257	1.259449	21	2.9509	0.1405		
						86	**36.886**	**0.4289**		
Pooled W	88	148.449	477.389	255.19	1.719039	87	38.706	0.4449		
		Difference between slopes				**1**	**1.8201**	**1.8201**	**4.0910**	**0.0461**
Between B										
W+B	89	157.109	612.33	289.37		88	79.341			
		Between adjusted means				**1**	**40.634**	**40.634**	**91.333**	**3.25E-15**

L-L relationship

	a	**b**	**Rsqr**
Medium	**2.2021**	**1.7457**	**0.926**
Dwarf	**2.9090**	**1.2594**	**0.8140**
Pooled	**1.3016**	**1.8418**	**0.8704**

TL-TCL

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	33.10123438	33.10123	299.208	5.94E-36
Residual	134	14.82435386	0.11063		
Total	135	47.92558824			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.315988525	0.234358643	1.348312	0.179833	-0.14753	0.779509
X Variable 1	0.242618293	0.0140261	17.29763	5.94E-36	0.214877	0.270359

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	861.7885	861.7885426	1265.06563	8.23E-83
Residual	178	121.2572	0.681220422		
Total	179	983.0458			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.699912	0.252461	-2.772357021	0.00615751	-1.198114	-0.201710324
X Variable 1	0.349081	0.009815	35.5677611	8.22785E-83	0.329713	0.368449055

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
MEDIUM	179	7072.0	983.04	2468.73	0.349	178	121.257	0.681		
DWARF	135	562.3	47.92	136.43	0.2426	134	14.8243	0.110		
						312	**136.081**	**0.4361**		
Pooled W	314	7634.4	1030.97	2605.16	0.3412	313	141.98	0.4536		
		Difference between slopes				**1**	**5.9042**	**5.904**	**13.015**	**0.00035**
Between B										
W+B	315	13052.	2073.92	4982.37		314	172.105			
		Between adjusted means				**1**	**30.1200**	**30.120**	**66.398**	**8.919E-15**

L-L relationship

	a	**b**	**Rsqr**
Medium	**-0.69**	**0.34**	**0.8766**
Dwarf	**0.31**	**0.24**	**0.690**
Pooled	**-1.7191**	**0.3817**	**0.9170**

TWt-MWt

ANOVA

Dwarf

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7765.828	7765.828	1355.450951	7.8174E-75
Residual	143	819.2944	5.729332		
Total	144	8585.122			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.50268	0.691558	2.172889	0.031435726	0.135681749	2.869677597
X Variable 1	0.467535	0.012699	36.81645	7.8174E-75	0.442432516	0.49263682

Medium

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1124199	1124199	12990.30523	1.0762E-179
Residual	194	16789.02	86.54136		
Total	195	1140988			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	4.959317	1.23958	4.000803	8.9748E-05	2.514533079	7.404101063
X Variable 1	0.530135	0.004651	113.975	1.0762E-179	0.520961742	0.539309075

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.cof**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Medium	195	4000087	1140988	2120588	0.530	194	16789.02	86.54		
Dwarf	144	35527.1	8585.12	16610.1	0.4675	143	819.29	5.72		
						337	**17608.3**	**52.25**		
Pooled W	339	4035614	1149573	2137198	0.529	338	17746.3	52.50		
		Difference between slopes				**1**	**137.99**	**137.99**	**2.62**	**0.1059**
Between B										
W+B	340	6524678	1955524	3553554		339	20141.9			
		Between adjusted means				**1**	**2395.6**	**2395.6**	**45.6**	**6.26E-11**

L-W relationship

	a	**b**	**Rsqr**
Medium	**4.959**	**0.530**	**0.985**
Dwarf	**1.502**	**0.467**	**0.908**
Pooled	**-0.095**	**0.544**	**0.98**

DML–TWt ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	24.43138	24.43138	1953.108	6.8E-150
Residual	371	4.64083	0.012509		
Total	372	29.07221			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.63501	0.150822	-17.471	2.65E-50	-2.93158	-2.33844
X Variable 1	2.607214	0.058995	44.19398	6.8E-150	2.491208	2.72322

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.984624	4.984624	442.9607	3.35E-33
Residual	75	0.843973	0.011253		
Total	76	5.828597			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.66007	0.296822	-8.96183	1.76E-13	-3.25137	-2.06877
X Variable 1	2.602827	0.12367	21.04663	3.35E-33	2.356465	2.849189

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Females	372	3.594	29.0722	9.37068	2.607	371	4.640	0.01251		
Males	76	0.735	5.8286	1.91508	2.602	75	0.84397	0.01125		
						446	**5.4848**	**0.0123**		
Pooled W	448	4.329	34.9008	11.2858	2.606	447	5.484	0.01227		
		Difference between slopes				**1**	**1.2E-05**	**1.2E-05**	**0.000**	**0.9753**
Between B										
W+B	449	5.89	47.4627	15.7175		448	5.544			
		Between adjusted means				**1**	**0.0597**	**0.05974**	**4.868**	**0.0278**

L-W relationship

	a	**b**	**Rsqr**
Females	**0.0717**	**2.607**	**0.840**
Males	**0.0699**	**2.602**	**0.85**
Pooled	**0.0612**	**2.666**	**0.883**

DML–TL

Female

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	178.6625017	178.6625017	182.2120151	5.35E-24
Residual	97	95.11042758	0.980519872		
Total	98	273.7729293			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.436664	1.113938063	2.187432411	0.031113806	0.225806	4.647523
X Variable 1	1.12511	0.083350184	13.49859308	5.35098E-24	0.959683	1.290537

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	25.357	25.35717034	66.31103411	1.36E-09
Residual	35	13.383	0.38239745		
Total	36	38.741			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	5.135902	1.138221	4.512220648	6.93299E-05	2.825191	7.446612
X Variable 1	0.847572	0.10408	8.14315873	1.3647E-09	0.636271	1.058874

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	98	141.138	273.773	158.796	1.125	97	95.11	0.980		
Male	36	35.2978	38.7411	29.9174	0.847	35	13.38	0.382		
						132	**108.49**	**0.821**		
Pooled W	134	176.436	312.514	188.713	1.069	133	110.66	0.832		
		Difference between slopes				**1**	**2.174**	**2.174**	**2.613**	**0.10830**
Between B										
W+B	135	334.069	562.338	387.158		134	113.65			
		Between adjusted means				**1**	**2.983**	**2.983**	**3.585**	**0.06044**

L-W relationship

	a	**b**	**Rsqr**
Female	**2.436**	**1.125**	**0.652**
Male	**5.135**	**0.847**	**0.654**
Pooled	**1.920**	**1.158**	**0.797**

DML–TCL

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	16.5474	16.5474	285.15144	1.23E-30
Residual	97	5.62893	0.058030		
Total	98	22.1763			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.036112	0.27099	0.133257	0.894265	-0.50174	0.573961
X Variable 1	0.342408	0.020277	16.88642	1.22897E-30	0.302163	0.382652

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.01567	2.015676	330.41964	2.09E-19
Residual	35	0.21351	0.006100		
Total	36	2.22918			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.056663	0.143762	7.35004	1.35779E-08	0.76481	1.348517
X Variable 1	0.238966	0.013146	18.17744	2.08817E-19	0.212278	0.265655

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.co**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	98	141.138	22.17	48.32	0.342	97	5.628	0.058		
Male	36	35.29	2.229	8.434	0.238	35	0.213	0.006		
						132	**5.842**	**0.044**		
Pooled W	134	176.43	24.40	56.76	0.321	133	6.144	0.046		
		Difference between slopes				**1**	**0.302**	**0.302**	**6.539**	**0.01167**
Between B										
W+B	135	334.06	47.92	117.65		134	6.491			
		Between adjusted means				**1**	**0.347**	**0.347**	**7.510**	**0.006976**

L-W relationship

	a	**b**	**Rsqr**
Female	**0.036**	**0.342**	**0.746**
Male	**1.056**	**0.238**	**0.904**
Pooled	**-0.116**	**0.352**	**0.864**

DML- FW

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	39.199	39.19971	314.670	6.16675E-22
Residual	45	5.6058	0.124574		
Total	46	44.805			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.415	0.5632	-0.737	0.46490	-1.54952	0.7192
X Variable 1	0.7305	0.0411	17.73	6.16675E-22	0.647583	0.813

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.88501	10.88501	76.98187755	2.17216E-09
Residual	27	3.81772	0.141397		
Total	28	14.70273			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.10852	0.824201	-0.13167	0.896223543	-1.799640818	1.5826
X Variable 1	0.682873	0.07783	8.773932	2.17216E-09	0.523179175	0.842566

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	46	73.45	44.80	53.65	0.730	45	5.605	0.124		
Male	28	23.34	14.70	15.94	0.682	27	3.817	0.141		
						72	**9.423**	**0.130**		
Pooled W	74	96.79	59.50	69.59	0.719	73	9.463	0.129		
		Difference between slopes				**1**	**0.040**	**0.040**	**0.310**	**0.579191419**
Between B										
W+B	75	265.54	166.026	203.66		74	9.814			
		Between adjusted means				**1**	**0.3503**	**0.350**	**2.702**	**0.104504556**

L-W relationship

	a	**b**	**Rsqr**
Female	**-0.415**	**0.73053**	**0.87489**
Male	**-0.108**	**0.68287**	**0.74034**
Pooled	**-0.95**	**0.76699**	**0.94089**

TL-TCL

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	10.09370023	10.0937	81.03254	1.92E-14	
Residual	97	12.0826634	0.124564			
Total	98	22.17636364				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1.25039707	0.373120003	3.351193	0.001148	0.509857	1.990937
X Variable 1	0.192012698	0.02133046	9.001808	1.92E-14	0.149678	0.234348

Male

	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1.342337045	1.342337	52.97591	1.68E-08	
Residual	35	0.886852144	0.025339			
Total	36	2.229189189				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.985048346	0.368372852	2.674052	0.011312	0.237212	1.732885
X Variable 1	0.186142233	0.025574416	7.278455	1.68E-08	0.134223	0.238061

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	98	273.77	22.176	52.567	0.192012	97	12.082	0.1245		
Male	36	38.741	2.2291	7.2113	0.186142	35	0.8868	0.0253		
						132	**12.969**	**0.0982**		
Pooled W	134	312.51	24.405	59.779	0.191284	133	12.970	0.0975		
		Difference between slopes				**1**	**0.0011**	**0.001**	**0.0119**	**0.912961166**
Between B										
W+B	135	562.33	47.925	136.43		134	14.824			
		Between adjusted means				**1**	**1.8536**	**1.8536**	**19.007**	**2.58605E-05**

L-L relationship

	a	**b**	**Rsqr**
Female	**1.2503**	**0.1920**	**0.4551**
Male	**0.9850**	**0.1861**	**0.6021**
Pooled	**0.3159**	**0.2426**	**0.690**

DML–TWt

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	165.1462	165.1462	12161.18337	3.8147E-260
Residual	326	4.427010	0.013579		
Total	327	169.5732			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.51849	0.079200	-44.42495	2.4201E-140	-3.67430	-3.3626846
X Variable 1	3.12656	0.028351	110.27775	3.8147E-260	3.070787	3.182338

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	44.6980	44.69805	4697.1484	2.4842E-157
Residual	235	2.23625	0.009515		
Total	236	46.9343			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-3.065568	0.11424	-26.8337	1.6752E-73	-3.29063	-2.8404271
X Variable 1	2.977061	0.04343	68.53574	2.4842E-157	2.89148	3.062638985

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Females	327	16.8941	169.57	52.820	3.126	326	4.427	0.013		
Males	236	5.04328	46.934	15.014	2.977	235	2.236	0.009		
						561	**6.663**	**0.011**		
Pooled W	563	21.9374	216.50	67.834	3.092	562	6.750	0.012		
		Difference between slopes				**1**	**0.086**	**0.086**	**7.227**	**0.0073**
Between B										
W+B	564	25.3854	242.49	77.300		563	7.108			
		Between adjusted means				**1**	**0.358**	**0.358**	**29.83**	**7.07E-08**

<u>L-W relationship</u>

	a	**b**	**Rsqr**
Females	**0.0296**	**3.1265**	**0.973**
Males	**0.0466**	**2.9770**	**0.952**
Pooled	**0.037**	**3.0450**	**0.970**

DML–TL ANOVA

Female

	df	SS	MS	F	Significance F
Regression	1	4130.485	4130.485	1092.23	7.79947E-53
Residual	92	347.9134	3.781667		
Total	93	4478.399			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-4.0392	0.9788	-4.126	8.08217E-05	-5.98343	-2.0951
X Variable 1	1.76961	0.0535	33.049	7.79947E-53	1.66326	1.875

Male

	df	SS	MS	F	Significance F
Regression	1	1071.97	1071.97	811.275	6.26E-45
Residual	84	110.99	1.321		
Total	85	1182.96			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-3.943611	0.919	-4.286	4.81203E-05	-5.7730	-2.114
X Variable 1	1.71593	0.060	28.48	6.26521E-45	1.5961	1.835

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	93	1319	4478.4	2334.12	1.769	92	347.91	3.781		
Male	85	364.065	1182.96	624.713	1.715	84	110.99	1.321		
						176	**458.90**	**2.607**		
Pooled W	178	1683.06	5661.36	2958.83	1.75	177	459.72	2.597		
		Difference between slopes				**1**	**0.8220**	**0.822**	**0.316**	**0.574434265**
Between B										
W+B	179	2025.73	7072.09	3654.1		178	480.63			
		Between adjusted means				**1**	**20.911**	**20.91**	**8.051**	**0.005078847**

L-W relationship

	a	**b**	**Rsqr**
Female	**-4.03**	**1.769**	**0.922**
Male	**-3.943**	**1.715**	**0.90**
Pooled	**-4.94**	**1.803**	**0.932**

DML–TCL ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	517.7902	517.7902	624.3483227	8.84222E-43
Residual	92	76.29828	0.829329		
Total	93	594.0885			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.134635172	0.45841	-4.656612	1.07872E-05	-3.045076004	-1.224194
X Variable 1	0.626549171	0.025075	24.98696	8.84222E-43	0.576747973	0.67635

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	143.2853	143.2853	562.8476217	5.42013E-39
Residual	84	21.38405	0.254572		
Total	85	164.6693			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2.650847551	0.403806	-6.564653	4.07746E-09	-3.45386041	-1.847835
X Variable 1	0.627351794	0.026443	23.72441	5.42013E-39	0.574766384	0.679937

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	93	1319	594.089	826.416	0.626	92	76.29	0.829		
Male	85	364.065	164.669	228.397	0.627	84	21.38	0.254		
						176	**97.68**	**0.555**		
Pooled W	178	1683.06	758.758	1054.81	0.626	177	97.68	0.551		
		Difference between slopes				**1**	**0.000**	**0.000**	**0.000**	**0.985460369**
Between B										
W+B	179	2025.73	983.046	1332.04		178	107.14			
		Between adjusted means				**1**	**9.462**	**9.462**	**17.14**	**5.34863E-05**

L-W relationship

	a	**b**	**Rsqr**
Female	**-2.134**	**0.626**	**0.871**
Male	**-2.650**	**0.627**	**0.870**
Pooled	**-2.889**	**0.657**	**0.891**

DML- FW

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	410.4699387	410.4699387	1167.801674	1.74404E-41
Residual	61	21.44085491	0.351489425		
Total	62	431.9107937			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.346494774	0.378873692	6.193343128	5.51756E-08	1.58889	3.1041
X Variable 1	0.734653954	0.021498011	34.17311332	1.74404E-41	0.69166	0.777642

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	66.6950	66.69503517	413.4884448	1.12489E-21
Residual	37	5.96804	0.161298426		
Total	38	72.66307			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	2.19393	0.51727	4.241352	0.0001424	1.14584	3.242031
X Variable 1	0.71272	0.035050	20.33441	1.12489E-21	0.641705	0.783742

ANCOVA

						Deviations from regression				
Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	62	760.52	431.91	558.726	0.73465	61	21.44	0.351		
Male	38	131.29	72.66	93.577	0.71272	37	5.968	0.1613		
						98	**27.408**	**0.279**		
Pooled W	100	891.82	504.57	652.303	0.73143	99	27.462	0.2774		
		Difference between slopes				**1**	**0.0538**	**0.053**	**0.194**	**0.6604**
Between B										
W+B	101	1058.97	644.35	805.156		100	32.17			
		Between adjusted means				**1**	**4.716**	**4.716**	**17.00**	**7.76E-05**

L-W relationship

	a	**b**	**Rsqr**
Female	**2.3464**	**0.734**	**0.950**
Male	**2.1939**	**0.712**	**0.917**
Pooled	**1.7477**	**0.760**	**0.950**

TL- TCL

ANOVA

Female

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	501.7997	501.799	500.22	5.68E-39
Residual	92	92.28876	1.003138		
Total	93	594.0885			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.170695	0.426167	-0.40053	0.689691	-1.017098	0.67570
X Variable 1	0.334737	0.014966	22.3658	5.680E-39	0.305012	0.36446

Male

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	140.1285	140.1285	479.6423	1.78E-36
Residual	84	24.54078	0.292152		
Total	85	164.6693			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.737167	0.350937	-2.100570	0.038671	-1.4350	-0.03929
X Variable 1	0.344174	0.015715	21.90073	1.779E-36	0.3129	0.375425

ANCOVA

Source	**d.f.**	**ssx**	**ssy**	**spxy**	**Reg.coef**	**d.f.**	**S.S.**	**M.S**	**F**	**Prob**
Within										
Female	93	4478.399	594.08	1499.086	0.33473	92	92.288	1.003		
Male	85	1182.962	164.66	407.1447	0.34417	84	24.540	0.292		
						176	**116.82**	**0.663**		
Pooled W	178	5661.361	758.75	1906.231	0.33670	177	116.91	0.660		
		Difference between slopes				**1**	**0.083**	**0.083**	**0.126**	**0.722869**
Between B										
W+B	179	7072.089	983.045	2468.734		178	121.25			
		Between adjusted means				**1**	**4.344**	**4.344**	**6.577**	**0.01116**

<u>L-L relationship</u>

	a	**b**	**Rsqr**
Female	**-0.1706**	**0.3347**	**0.844**
Male	**-0.7371**	**0.3441**	**0.850**
Pooled	**-0.6999**	**0.3490**	**0.876**

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Migration and spawning behaviour of the pharaoh cuttlefish *Sepia pharaonis* Ehrenberg, 1831 along the south-west coast of India

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ABSTRACT

The area west of Kollam (38-51 m depth) and south-west of Cochin (38 – 45 m depth) seems to be the preferred region for spawning of the pharaoh cuttlefish *Sepia pharaonis* along the south-west coast of India. Juveniles appear to stay for some time in the spawning ground and then undertake offshore migration towards relatively deeper areas of the Wadge Bank in 117-132 m depth range. This migration appears to take place *via* west of Vizhinjam in the depth range of 60 - 86 m. Fecundity varies from 146 to 1400 eggs, depending upon the size of the animal. *S. pharaonis* appears to spawn intermittently with group-synchronous ovulation. Spawning appears to be monocyclic and egg-laying occurs in separate batches during the spawning period. Egg capsules are either inserted in to the muddy substratum or fastened on the preferred objects apart from entangling with each other.

Keywords: Migration, Offshore ground, Pharaoh cuttlefish, *Sepia pharaonis*, Spawning, Wadge Bank

Introduction

The pharaoh cuttlefish, *Sepia pharaonis* Ehrenberg, 1831 is a tropical species, with distribution from 35° N to 30° S and from 30° E to 140° E in the Indo-Pacific (Nabhitabhata and Nilaphat, 1999). This species grows to a maximum size of 42 cm and is an important fishery resource along the south-west coast of India (Reid *et al*., 2005). It is neritic and demersal in nature and found distributed up to 110 m depth (Roper *et al*., 1984). More abundant in the upper 40 m, particularly during the reproductive season, when it migrates shoreward and aggregates in shallow water for spawning (Roper *et al*., 1984). It is established that cuttlefishes undergo spawning migration. Gabr *et al*. (1998) observed that *S. pharaonis* migrates to spawning ground from fishing ground. In the English Channel, *Sepia officinalis,* a common species in the temperate waters, migrates from offshore to inshore waters for spawning and lays eggs in the shallow coastal waters (<40 m) as well as in deeper (up to 60 m) and colder littoral waters (Boletzky, 1983). Along the Indian west coast, though spawning activity of *S. pharaonis* is noted throughout the year, peak spawning activity is observed during October – December and March - April in shallow waters (Silas *et al*., 1982). Therefore, main spawning migration of *S. pharaonis* population in the west coast of India is presumed to precede the months of October and March of every year.

Cuttlefishes produce one of the largest eggs among cephalopods and the egg size is relative to the duration of embryonic development (Palmegiano and d'Apote, 1983; Boletzky, 1989). An adult female can spawn at different times and thus in different areas along the coast (Challier *et al*., 2004). Laboratory observation on Sepioidea showed that females generally spawn during the night or early morning hours and attach their eggs to the walls of the tank (Boletzky, 1971). Females of *S. pharaonis* attach gelatinous capsules containing eggs to different objects on the bottom such as seaweeds, gorgonids and shells (Reid *et al*., 2005). Boletzky (1974) noted that Sepioidea embed their eggs in gelatinous cases secreted by the female nidamendal gland, which is believed to protect them throughout the embryonic development. If egg capsules are already present at a spawning site, females tend to attach their capsules to those that have been laid already or to nearby objects. Thus, as in the case of squid, egg capsules in one particular egg mass or egg bed may be at different stages of embryonic development (Drew, 1911). Though sufficient information is already available on the spawning season and biology of this species along the south-west coast of India (Silas *et al*., 1986), specific locations of spawning, offshore grounds and reproductive strategy are not fully understood. Therefore, the present study aimed to locate the inshore spawning sites and offshore grounds of *S. pharaonis* along the south-west coast of India and to investigate its reproductive strategy. This may contribute to the broader objective of understanding the migration and spawning behavior of *S. pharaonis* along the south-west coast of India.

Materials and methods

Studies were conducted onboard M V Matsya Varshini, (OAL - 36.5 m, GRT - 268.8) a fishery resources survey vessel of the Fishery Survey of India. A total of 175 stations were covered during the months of May (41), August (54), October 08 (40) and February 2009 (40) all along the south-west coast of India. During the cruises, demersal resources survey by stratified random sampling method was carried out in the 30–50, 50-100, 100–200 and 200–500 m depth strata along the south-west coast of India, using Expo model (45.6 m Head rope length and 40 mm cod end mesh size) fish trawl. The survey was carried out during daylight. Net was dragged for a maximum of 90 min at 3.0 knots speed, covering 4.5 nautical miles of track in each station. The collected samples were subjected to biological studies onboard the vessel itself and the samples needed for detailed studies were frozen at -20 °C and brought to the shore laboratory.

At the shore laboratory, specimens were thawed at room temperature and dissected to determine the sex and maturity stage. Measurements for dorsal mantle length (DML) were made in a straight line to the nearest millimeter with the cuttle fish lying in its natural position, whereas total weight (TWT) was measured to the nearest 1g. Four maturity stages for each sex were determined using a modified scale of Mangold-Wirz (1963) proposed by Gabr *et al*. (1998). Females were classified in to I - immature, II - maturing, III - pre-spawning, IV - spawning; and males, I - immature, II - maturing, III - fully mature and IV - spawning. Animals in stage I and II were considered as juveniles and were observed to have a maximum of 150 mm DML during the study. The *S. pharaonis* egg capsules were identified with the help of keys provided by Nair *et al*. (1986) and also based on aggregation of spawners in and around the site. To obtain an estimate of fecundity, the number of maturing (from the ovary) and matured ova (from the oviduct) of fresh specimens of stage III and IV (spawner) in the size range of 161 to 259 mm DML were counted.

Results

Occurrence and distribution

The data on the occurrence and distribution of *S. pharaonis* in different length groups and maturity stages are presented in Table 1. Juveniles in the length group of 40 – 150 mm were more abundant during the month of May, representing 90% of the total cuttlefish catch. The remaining 10% were pre-spawners/fully matured animals, distributed all along the coast, south of Kollam (lat 07° 52' N to 08° 44' N and long 76° 33' E to 77° 13' E). Its presence in the sampling areas north of Kollam was not recorded during May (Fig. 1). In contrast to May, no juveniles were observed during August, instead pre-spawners/ fully matured and spawners, in the length range of 120–260 mm were prevalent, representing 57 and 43% respectively. During October, though all the four stages were present in the ground, spawners in the length group of 190–320 mm were dominant, constituting 48% of the cuttlefish catch. Juveniles constituted 38% followed by pre-spawners/fully matured animals with 14%. In the month of February, though all the four stages were prevalent, juveniles dominated the catch, constituting 65% of the catch, followed by spawners constituting 20%. The percentage of pre-spawners/fully matured animals remained more or less same as that of October constituting 15% of the catch. The size of the female spawners in the spawning ground during February ranged from 190 to 290 mm DML and the male ranged from 273 to 325 mm DML.

Latitude-wise distribution analyses showed that (Table 2) juveniles were relatively abundant in the Wadge Bank area of 7° N latitude, representing 73% of the cuttlefish catch, followed by pre-spawners/fully matured animals forming 25%. Spawners were sparsely (2%) observed in this latitude. All the four stages were recorded in the 8° N latitude, however spawners dominated (41%). In 9° N latitude, spawners were more common representing 51% of the catch. An abrupt increase in spawner percentage from 7° to 8° N and then a gradual increase to 9° N was observed. Juveniles amount to the remaining 49% of the catch. The absence of pre-spawners/ fully matured animals were conspicuous in this latitude.

Area of adult aggregation

In August, *S. pharaonis* aggregation was located in the area lat 08° 48' N - 08°59' N / long 76° 14' E - 76° 23' E in the depth range of 45–62 m off Kollam and 08° 28' N - 08° 35' N/76° 35' E - 76° 38' E in the depth range of 59–62 m between Kollam and Vizhinjam (Fig. 2). It was observed that all the specimens collected off Kollam were pre-spawners/ fully matured and the specimens collected between Kollam and Vizhinjam were of pre-spawners/ fully matured and spawners. During October (Fig. 3), large quantity of spawners were collected in the area from 08° 45' N - 08° 59' N/76° 18' E - 76° 31' E in the depth range of 38 – 51 m off Kollam and the area south of Cochin from 09° 34' N - 09° 49' N/76° 01' E - 76° 05' E in the depth range of 38–45 m. In February (Fig. 4), good quantity of spawners and spawning activity was located in the area 08° 54'-09° 22' N/76° 08'–76° 22' E in the depth range of 44–55 m.

Area of juvenile prevalence

Juvenile abundance was observed in the area lat 08° 10’ N - 08° 33’ N / long 76° 36’ E - 76° 47’ E in the depth range of 60 – 86 m (Table 1 and 2). Large number of *S. pharaonis* juveniles in the size range 40–150 mm DML during May and 40-130 mm DML during October and February respectively were collected at various locations from this area (Fig. 1, 3 and 4). Though all the four maturity stages were present in the area, the percentage of spawners was low towards the northern end of the ground, adjacent to the Kollam spawning ground. The nature of bottom in this area was muddy with stones. Juvenile abundance was also observed in the relatively more deeper area of the Wadge Bank, from lat 07° 21’ N to 07° 33’ N and long 76° 51’ E to 77° 05’ E in the depth range of 117–132 m. It was observed that, the length group of juveniles collected at different stations of this area were of in the range of 50–100 mm DML during May and 40-130 mm DML during October and 40-150 mm DML during February. No adult cuttlefish in stage IV was observed in this area during the study period (Fig. 1, 3 and 4). Prevalence of deep sea shrimps such as *Parapandalus spinipes, Heterocarpus gibbosus* and *Heterocarpus woodmasoni* were noticed in this area as well as in the gut contents of the *S. pharaonis* collected in the location. The bottom nature of this area was muddy with stones and ideal for juveniles for feeding and camouflaging.

Area of egg abundance

Egg bunches in large quantity were collected during October from the area west of Kollam (08° 45’ N to 08° 59’ N/ 76° 18’ E to 76° 31’ E) in the depth range of

Table I. Month-wise percentage distribution of *Sepia pharaonis* according to mantle lengths and maturity stages

	May ’08					August ’08					October ’08					February ’09				
Mantle lengths (mm)	N 321	I	II	III	IV	N 287	I	II	III	IV	N 670	I	II	III	IV	N 360	I	II	III	IV
40	2	100									5	100				3	100			
50	6	100									18	100				17	100			
60	14	100									32	100				32	100			
70	27	93	7								34	85	15			33	79	21		
80	40	53	48								28	46	54			19	21	79		
90	36	31	69								19		100			23		100		
100	48	17	83								34		100			49		94	2	4
110	57	5	95								41		100			24		96		4
120	45		93	7		6			100		37		78	22		25		84		16
130	16		75	25		26			100		26		62	38		24		63	13	25
140	9		22	78		66			100		22			100		14			36	64
150	12		25	75		56			96	4	16			100		11				100
160	5			100		22			59	41	9			100		19			100	
170	3			100		13				100						32			44	56
180	1			100		22				100	9			100		14			50	50
190						27				100	17			29	71					
200						18				100	31			16	84	2			100	
210						11				100	37			8	92	7			43	57
220						9				100	43			5	95	8			25	75
230						6				100	61			3	97					
240						4				100	49			4	96	3				100
250											38				100	1				100
260						1				100	26				100					
270											15				100					
280											12				100					
290											6				100					
300																				
310											3				100					
320											2				100					
Total %		28	62	10	0		0	0	57	43		14	24	14	48		23	42	15	20

Table 2. Latitude-wise percentage distribution of *Sepia pharaonis* according to mantle lengths and maturity stages

	07°					08°					09°				
Mantle lengths (mm)	N 482	I	II	III	IV	N 877	I	II	III	IV	N 279	I	II	III	IV
40	2	100				5	100				3	100			
50	13	100				21	100				7	100			
60	24	100				37	100				17	100			
70	38	84	16			47	83	17			9	100			
80	44	39	61			32	44	56			11	64	36		
90	38	16	84			30	13	87			10	10	90		
100	57	5	95			48	6	92	2		26	8	84		8
110	61		100			39	5	95	0		22	5	90		5
120	49		90	10		42		71	29		22		82		18
130	45		60	40		25		0	100		22		73		27
140	44		5	95		58		0	100		9				100
150	33		3	97		51		4	92	4	11				100
160	17			100		38			76	24					
170	7			86	14	24			46	54	17				100
180	1				100	33			52	48	12				100
190	2				100	26			19	81	16				100
200	4				100	33			21	79	14				100
210	2				100	40			15	85	13				100
220	1				100	46			9	91	13				100
230						57			4	96	10				100
240						48			4	96	8				100
250						34				100	5				100
260						25				100	2				100
270						15				100					
280						12				100					
290						6				100					
300															
310						3				100					
320						2				100					
Total %		20	53	25	2		14	19	26			41	17	32	51

38 – 51 m (Fig. 3) and south of Cochin (09° 34' N- 09° 49' N/ 76° 01' E - 76° 05' E) in the depth range of 38 – 45 m. During the same month around 10 kg of egg bunches were collected in a single haul from the area west of Kollam (08° 59' N / 76° 18' E) at a depth of 50 m. In the month of February too 5.3 kg of egg bunches were collected from the area west of Kollam (08° 50' N/76° 18' E and 09° 06' N / 76° 10' E) in 50 – 55 m depth (Fig. 4). The bottom of Kollam and Cochin spawning sites were observed to be muddy and sandy. Egg capsules were also found attached with gastropod/bivalve shells and coconut spadix placed in the sea beds by fishermen. Some of the egg bunches had mud and sand at its base covered with the jelly secretion.

Fecundity and embryogenesis

The total number of maturing and matured ova in the pre-spawning and spawning female *S. pharaonis* (stage III and IV) ranged from 146 in a female of 161 mm DML to 1400 in a female of 259 mm DML (Table 3). There seems to be a tendency for the larger females to be more fecund than the smaller ones. The relationship between the size of the animal and the number of ova, was week and directly linear ($R^2 = 0.3$) (Fig. 5). The weak relationship may be, as a result of some females of similar size having already laid different numbers of eggs. Ovary observed to have four stages of ova at a time, smaller opaque ova, medium sized thickly reticulated ova, large yellowish thinly reticulated ova and large transparent ova (Fig. 6a). Egg capsules enclosing the transparent ova, were spherical to oval in shape and measured 13.5–21.0 mm in its long axis. Each capsule had a stalk, which was found entangled with the stalk of other capsules to form egg bunches or fixed with under water objects. Such egg bunches were observed to have the assemblage of capsules of different embryonic

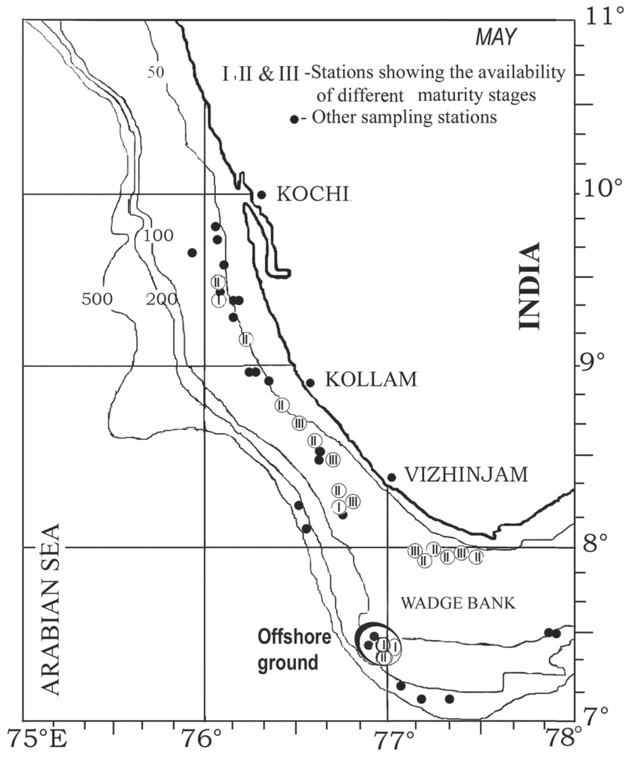

Fig. 1. Sampling stations, offshore ground and maturity stage-wise distribution of *S. pharaonis* during May 2008.

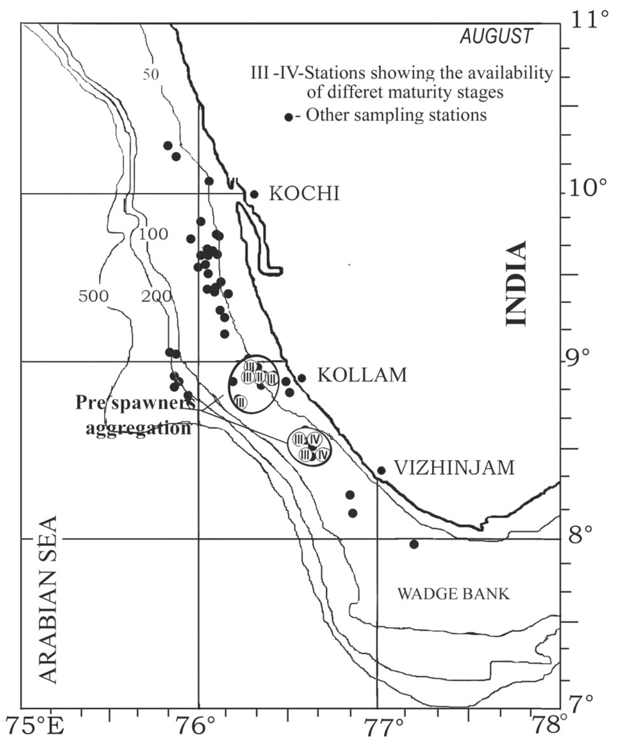

Fig. 2. Sampling stations and maturity stage-wise distribution of *S. pharaonis* during August 2008.

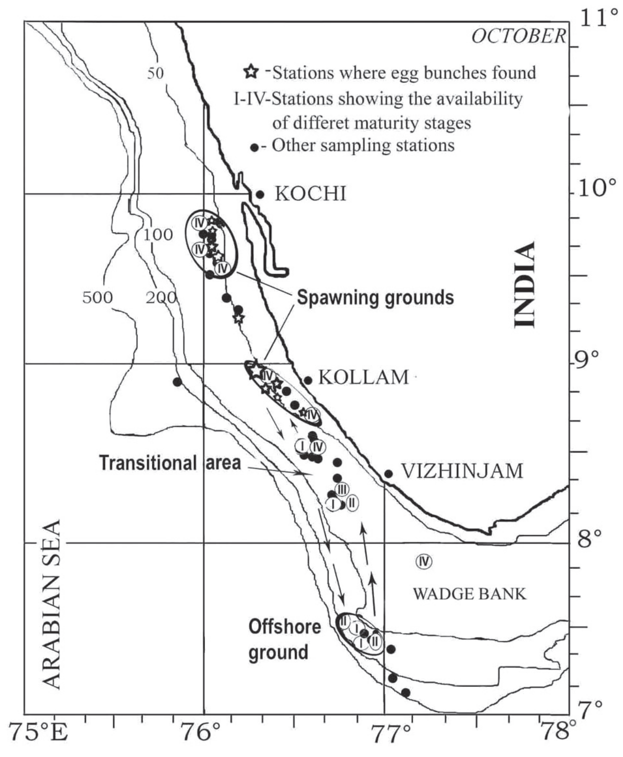

Fig. 3. Sampling stations, grounds, migratory route (arrows) and stage-wise distribution of *S. pharaonis* during October 2008.

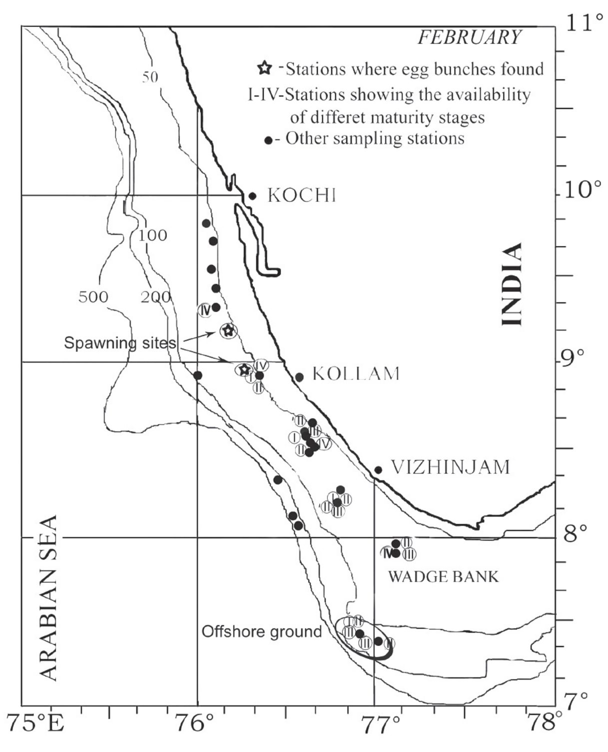

Fig. 4. Sampling stations, spawning locations and distribution of different stages of *S. pharaonis* during February 2009.

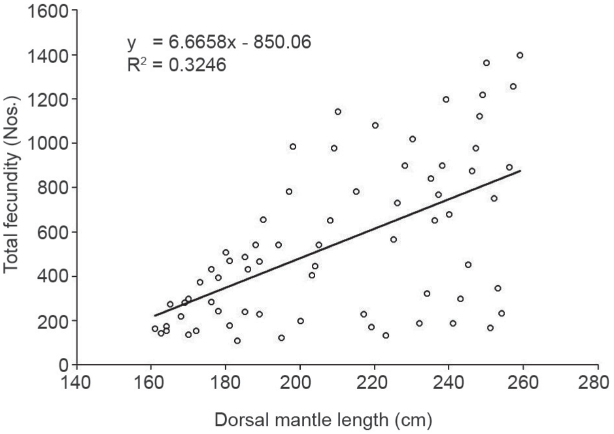

Fig. 5. Nature of relationship between dorsal mantle length (DML) and fecundity of *S. pharaonis*.

development. One egg bunch had a maximum of three stages of embryonic development with no continuity in developmental stages. The embryo inside the capsule was observed to rest on the globular yolk sac with its arms.

Table 3. Length group-wise (ML) fecundity of *Sepia pharaonis*

Mantle length (mm)	Frequency	Number of ova Min - Max	Mean ± SD
161-170	8	146 - 298	224 ± 70
171-180	10	140 - 509	351 ± 136
181-190	8	112 - 657	357 ± 192
191-200	5	125 - 988	478 ± 354
201-210	6	225 - 1145	696 ± 301
211-220	4	173 - 1083	568 ± 440
221-230	5	136 - 1020	671 ± 345
231-240	8	190 - 1201	695 ± 321
241-250	8	189 - 1366	814 ± 444
251-260	7	168 - 1400	722 ± 493

Egg capsule in various stages of embryonic development is shown in Fig. 6. The formation of cuttlebone, development of arms and movement of mantle inside the embryo were clearly observed. The young one was observed to detach from the yolk sac and swim freely inside the capsule (Fig. 6) before wriggling out through a small slit in the capsule, when fully grown. In some cases, the yolk sac still persisted and was ejected before hatching. The newly hatched young ones were miniature replicas of the adult cuttlefish and measured 7.5 mm DML (Fig. 6).

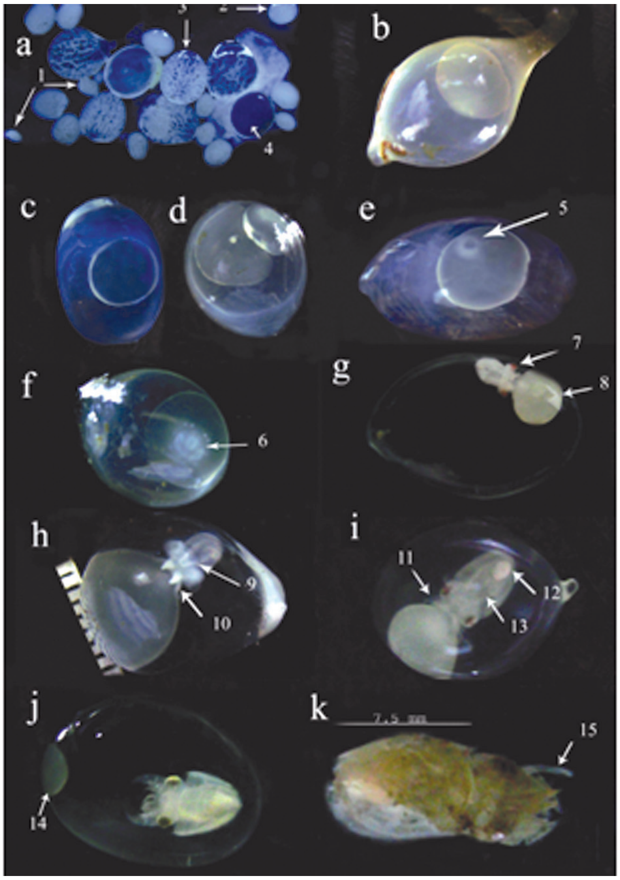

Fig. 6. Eggs and embryo of *S. pharaonis*, (a) Ova at different stages of development in the ovary. (b) Freshly spawned egg inside the capsule. (c) Egg capsule after removing the outer sheath. (d) Double embryo inside a capsule. (e) & (f) Formation of blastodisc. (g), (h) & (i) Developing embryos. (j) Free swimming fully developed embryo inside the capsule. (k) Newly hatched young one.
1 - Small whitish opaque ova, 2 - medium sized densely reticulated whitish ova, 3 - Large yellow round reticulated ova, 4 - Large, smooth, transparent, fully developed ova , 5 & 6 - Formation of blastodisc, 7- Optic capsule, 8 & 14 - yolk sac, 9 - Embryonic funnel, 10 & 11- Embryonic oral arms , 12 - Embryonic cuttle bone, 13 - Internal organs, 15-Tentacle

Discussion

Though the spawning activity of the pharaoh cuttlefish *S. pharaonis* is reported to be sporadic all along the south-west coast of India (Silas *et al.*,1982), the aggregation of spawners and peak spawning activity were observed only in the area south of Cochin and west of Kollam between lat 7° to 9° N. This fact is further corroborated with the availability of large quantity of cuttlefish egg bunches in these areas. Consequently, these areas can be considered as major spawning ground of *S. pharaonis* along the south-west coast of India.

From the size of the smallest juvenile cuttlefish (38 – 49 mm DML) caught in October it can be deduced that peak spawning season might have started in the month of September. This is strengthened by the fact that, pre-spawners/fully matured (stage III) aggregation was noted in the same areas during the last quarter of August.

It has been reported earlier that spawning extends almost throughout the year around India, with peaks from March to April and October to December on the west coast (Silas *et al*., 1982). The observations in the present study also agree with this.

Most of the newly hatched ones after staying in the spawning ground for few weeks move down towards southern latitudes. This is substantiated by the occurrence of large quantity of juveniles at various locations south of spawning areas. These juveniles after stay at this transitional area for a few weeks seem to migrate to more deeper offshore ground in the Wadge Bank area. Similarly in *Sepia officinalis* spawning and early life stages occur in shallow coastal waters and then in winter they migrate to offshore wintering grounds in the central part of the English Channel (Boucaud-Camou and Boismery, 1991). As neither spawning activity nor any egg bunches were observed in the area west of Vizhinjam (transitional area), it may be presumed that, the stage III /spawners found in this ground may be in return migration from the offshore ground or moving from the southern areas to the Kollam ground for spawning

Most of the juveniles in the offshore ground appear to stay there for few months until it reaches 130-150 mm DML or stage - III as evident from the occurrence of large numbers of cuttlefish measuring <150 mm DML in this area. The clear absence of juveniles with DML <40 mm and adult with DML >150 mm from this area during the study period of February, May and October indicate that all the adults larger than 150 mm DML have already moved out from the offshore ground and the juveniles less than 40 mm DML have not yet arrived at the offshore ground from the spawning ground. The juveniles collected from the offshore ground may constitute both the larger size group belonging to the last spawning peaks of September – December and the smaller size group belonging to March - April and the subsequent months. Those subadults and adults, which left the offshore ground to shallow inshore area, might have been hatched out during the previous year spawning peaks. The rare occurrence of these subadults and adults in the offshore ground during May, October and February and its presence in substantial quantities in the inshore waters during these months and August, confirms the migratory pattern.

These migrations seems to have been guided either by the urge for feeding the abundant prey species, especially deep sea shrimps and crabs found in the offshore area of the Wadge Bank (Siraimeetan, 1990) and to find a relatively oxygen rich shallow inshore waters with muddy bottom for spawning.

The shoreward migration of the *S. pharaonis* might have been guided by the need of relatively oxygen rich waters and suitable bottom for laying the egg capsules. The present study shows that *S. pharaonis* prefer shallow muddy/sandy grounds for spawning along the south-west coast of India as evident from the occurrence of large quantity of its egg bunches in this area. This was neither observed in the coral/rocky area nor in the deeper area during the study. Further, earlier studies suggest that spawning usually occurs when water temperature increases. Along the south-west coast of India, the surface and sub-surface seawater temperatures in the inshore areas evince a sudden increase after the south-west monsoon (Johannessen *et al*., 1981) and this could probably explain the spawning congregation at in shore waters. It was also observed that the pharaoh cuttlefish attaches its egg capsules with shells and spadix as well as in the mud as evident from the presence of large quantity of mud, enveloped by the jelly cover at the base of the bunch during the present study. On the basis of the occurrence of egg bunches in the spadix during the different months, it is understood that the presence of spadix may not induce the spawning but, only enhance the spawning aggregation around this area, as it provide a better platform for entangling the egg capsules.

During the study, only three stages of embryonic development were observed in the egg bunches of one location at a time. This may be due to the intermittent spawning habit of the species, where, spawning occurs after specific time gaps, coinciding with the development of each batch of ova as substantiated by the observation of four stages of ova at a time in the ovary**.** In this type of spawning, group-synchronous ovulation may occur in the ovary. Spawning seems to be monocyclic and egg-laying observed to occur in separate batches during the spawning period. This is substantiated by the findings of Gabr *et al.* (1998) who says, it is plausible to predict that female *S. pharaonis* are laying eggs in different bouts over a sizeable portion of their life cycle. Further they add that the wide size range of maturing ova in the ovary of pre-spawning and spawning females of the species could be an indication of intermittent or multiple spawning patterns in *S. pharaonis*. This pattern of intermittent spawning is quite likely, as the spawning season is greatly protracted. (Gabr *et al*., 1999).

Another supporting evidence of multiple spawning is the ability to grow after reaching the sexual maturity. Matured female *S. pharaonis* were found to occur in a wide length range starting from 190 to 290 mm DML, the size of the largest female observed in the spawning ground. It is therefore reasoned that, tangible growth does take place in females even after maturation and spawning. The study of Mohamed (1993) in the neritic squid *Loligo duvauceli* along the Mangalore - Malpe coast also suggested tangible growth in females even after first maturation and spawning. This indicates that, somatic growth in *S. pharaonis* may continue in between spawning events. It is therefore plausible to predict that *S. pharaonis* reproductive strategy may be of intermittent multiple spawning. This is in contrast to the temperate species *S. officinalis,* which is known to be intermittent terminal spawner and spawn intermittently over a period of up to four months in captivity (Boletzky, 1987). This type of strategy differs from multiple spawning, as somatic growth does not take place between spawning events (Rocha *et al*., 2001).

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