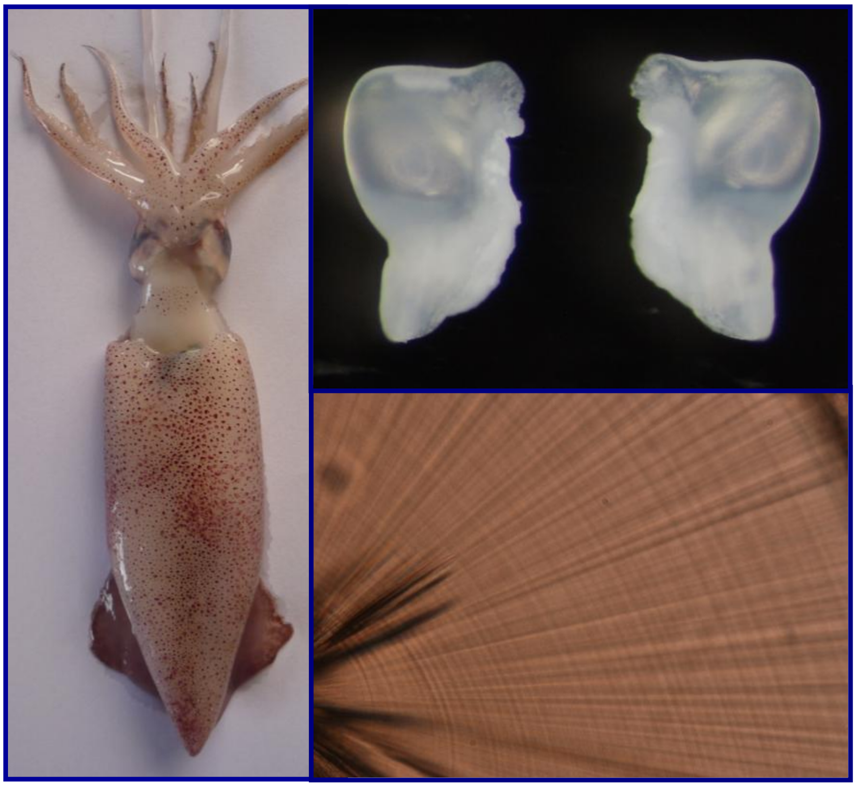
Age and growth determination and stock identification using statolith microstructure of Indian squid, *Loligo duvauceli*

by

Sansanee Srichanngam



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Sansanee Srichanngam

Bergen, May 2010

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Abtract

Age and growth and stock identification of Indian squid, *Loligo duvauceli* were examined by statolith microstructure and morphological measurements. The Indian squid were sampled from the Gulf of Thailand and the Andaman Sea by otter board trawlers. The age in days after hatching for the Indian squid from the Gulf of Thailand ranged from 61 to 153 days and the Andaman Sea ranged from 76 to 270 days. The Growth Index (GI) was not significantly different between sexes for both seas. The GI of males and females were 0.959 and 1.044 mm/day for the Gulf of Thailand and 0.730 and 0.706 mm/day for the Andaman Sea. A logarithmic function was selected to describe the population growth pattern for both seas. Sexual dimorphism appeared in both two populations. The maturity pattern was more distinctly separated by DML than age for both sexes. The DML_{50}% for males and females was 78.90 and 94.05 mm for the Gulf of Thailand and 100.69 and 91.52 mm for the Andaman Sea. These lengths are smaller than previous studies, and may show evidence of fisheries induced evolution to earlier maturation at smaller sizes. Both morphological and statolith variables were apparent for *L. duvauceli* stocks identification using Discriminant Analysis.

Keywords: *Loligo duvauceli*, statolith, age, growth, stock identification, maturity

Contents

page

Introduction 1

Materials and Methods 6

1. Study area 6

    1.1 Samples from the Gulf of Thailand 7

    1.2 Samples from the Andaman Sea 9

2. Species identification and morphological measurements 11

    2.1 Species identification 11

    2.2. Morphological measurements 12

3. Statolith analysis 14

    3.1 Statolith extraction and preservation 14

    3.2 Statolith measurements and shape analysis 14

    3.3 Age determination 16

        3.3.1 Mounting 16

        3.3.2 Grinding 16

        3.3.3 Counting increments 16

        3.3.4 Accuracy of increments counting 19

            3.3.4.1 Counting comparison 19

            3.3.4.2 Size of increment comparison 20

4. Data analysis 23

    4.1 Age and growth of *L. duvauceli* 23

        4.1.1 Growth Index 23

        4.1.2 Growth equation 23

    4.2 Hatching date and spawning date back calculation 24

4.3 Statistical analysis of morphological patterns and statolith length 25

4.4 The Length - Weight relationship and relative condition factor (K) 26

4.5 Maturity ogives 27

4.6 Stock discrimination 28

4.6.1 Statistical analysis of morphological variables for stock Discrimination 28

4.6.2 Shape Analysis of statoliths for stock discrimination 29

Results 31

1. Summary data 31

2. Age and growth of *L. duvauceli* 35

2.1 Growth Index (GI) 37

2.2 Growth equation 38

3. Hatching date and spawning date back calculation 42

4. Morphological patterns and Statolith length 44

4.1 Study of sex differences in morphological variables and statolith length 44

4.2 Statolith growth 49

5. The Length - weight relationship and relative condition factor (K) 51

5.1 Gulf of Thailand 51

5.2 Andaman Sea 53

6. Maturity 56

7. Stock discrimination 59

7.1 Morphological variables for stock discrimination 59

7.2 Shape Analysis of statoliths for stock discrimination 64

Discussion 70

1. Summary of the data 70

2. Age and growth of *L. duvauceli* 71

3. Hatching date and spawning date back calculation 73

4. Morphological patterns and statolith length 74

5. The Length - weight relationship and the relative condition factor (K) 75

6. Maturity 78

7. Stock discrimination 78

8. Project evaluation 80

Conclusion 82

References 84

Appendices 92

Introduction

The marine fishery sector significantly supports Thailand's economy by generating income and employment for local people along the coasts of both the Gulf of Thailand and the Andaman Sea. The exploitation of marine fisheries resources in Thailand has increased due to development in fishing gear (for example, by gradually increased access since trawlers were introduced in the early 1960s, Pauly and Chuenpagdee, 2003). Increasing fishing pressure is changing the trophic structure of marine environments in many regions (Pauly *et al*., 1998). Also in Thailand, the mean trophic level in the Gulf of Thailand has declined and there is evidence of "fishing down marine food web" in the Gulf of Thailand (Pauly and Chuenpagdee, 2003). This reflects a transition in the landings from long-lived, bottom predatory fish to short-lived, invertebrates and pelagic fish. Squid is one of the short-lived species which has increased in the catches under this fishing pressure. The data from trawl surveys since the 1970s in the Gulf of Thailand reveals that Loliginidae squid have become important and they are the most abundant group in the catches at present (Chotiyaputta *et al*, 2002).

The Indian squid, *Loligo duvauceli* is the most abundant economic species in both the Gulf of Thailand and the Andaman Sea for local consumption and preserved as dried squid for export. *L. duvauceli* is a neritic squid species living at 30 to 170 m depths and distributed from Mozambique to the South China Sea (Roper *et al*., 1984). The main spawning seasons in the middle of the Gulf of Thailand are during January - May and June - October (Chotiyaputta, 1996; Supongpan and Sinoda, 1998). In the southern Gulf of Thailand, the spawning seasons are during March - April and August- September (Boonwanich, *et al*., 1998).

The Gulf of Thailand is one of 64 large marine ecosystems (NOAA, 2007) and considered as a semi-enclosed sea, rather shallow with an average depth about 45 m and maximum depth about 85 m. The coastal seabed spans with a wide continental shelf covered by sand and mud which makes it a productive fishing ground. The Andaman Sea is also a semi-enclosed sea with a wide continental shelf in the northern part and a depth of more than 3,000 m in the central part. The Andaman Sea fishing grounds are both near shore and along the continental slopes where the depth varies between 10-300 m. The seabed is covered by sand, mud and coral remnants. The biological complexity and variability (of environments) of these seas are influenced by tropical rain forest monsoons which are beneficial in terms of distribution of nutrients. The Northeast monsoon runs from November to April and the Southwest monsoon from May to October.

Squid caught in Thailand increased from 63,996 tons in 1985 to 69,840 tons in 1989 when fishing effort was highest and continued to increase to 76,202 tons in 2006 (Froese and Pauly, 2009) while fishing effort decreased. The yield of squid from the Gulf of Thailand was estimated by Supongpan (1984) to be 41,000 tons while the annual catch reported by FAO in 1984 was 59,693 tons (Froese and Pauly, 2009). There are indications that the squid stock in the Gulf of Thailand has been overexploited. But the average size and CPUE of *L. duvauceli* have decreased because of improvements in fishing gear and high fishing effort. Since 1977, the squid fishing gears have changed from cast nets to falling nets, lift nets and scoop nets, and electric power has increased to 20 – 30 Kw to increase light intensity for light fishing (Department of Fisheries, 2006). Squid resource management has been continuously considered by many stakeholders but studies of the biology of *L. duvauceli* in Thailand are limited. Therefore research on biology, stock identification, age and growth of *L. duvauceli* is important for squid fisheries management in Thailand.

The study of age and growth of squids based on statolith increments in Thai waters have been conducted since daily growth increments were validated. Supongpan and Natsukari (1996) studied size at age based on statolith increments of *L. duvauceli* caught from Chumphon province (which is a part of the Gulf of Thailand) and reported average growth rates of 0.425 mm per day for males and 0.399 mm per day for females. Sukramongkol *et al* (2007) studied size at age of *L. duvauceli* caught from Phang-nga Bay and Phuket Island (which is a part of the Andaman Sea) and reported the relationships between mantle length (DML) and estimated age were $DML = 39.5e^{0.0113t}$ for males and $DML = 36.1e^{0.0111t}$ for females.

The length-weight relationship has been widely used in fisheries biology for several purposes. The main reason has been to estimate the mean weight of the stock for stock assessment based on the length measurement which is easier to measure and a conventional method for stock monitoring. It is also used to assess the condition factor (K), which is a quantitative parameter of the well-being of the individuals. Rattana-arnan (1979) studied the length-weight relationship of *L. duvauceli* from the Gulf of Thailand and reported the relationship were LogTW=1.773LogDML-1.977 for males and LogTW=2.043LogDML-2.47 for females. Sukramongkol *et al* (2007) reported the length-weight relationships of *L. duvauceli* from the Andaman Sea were $TW = 0.008DML^{1.79}$ for males and $TW = 0.001DML^{2.39}$ for females. Fishing pressure and the rapid decline of the mean trophic level may affect the biological parameters of squid in Thai waters and this study is a relevant overview of the current biology of *L. duvauceli* in Thailand.

Stock identification is essential for fisheries stock assessments to support effective fisheries resource management (Begg *et al*, 1999). The appropriate stock identification will support better stock assessment to describe stock status. There are many techniques to identify stocks. The shape analysis of otoliths in fish is a technique which is used to identify the fish stock because the shape of the otolith is not only species specific, the otolith also can determine the fish stock identification (Campana and Casselman, 1993). For squid which has no external hard structure and a soft, flexible body, the accuracy of body (morphological) measurements depends on body condition and the personal skill of the person making the measurements. For large scale studies, morphological parameters may be measured by many workers leading to low precision, so it would be useful to use parameters that are not so variable. Thus, the hard part of squid such as the statolith, which has the same characteristics and functions as an otolith in fish, was investigated to discriminate squid stocks. The statoliths are a pair of calcareous structures which function in balance and hearing, and which contain a lot of information about the lives of squid. This information can be used to estimate age and growth rates of squid based on the daily increments, to study the population structure and hatching date of squid. (Arkhipkin, 2005). The daily growth increment of the statolith is the paired dark and light growth layer produced over 24 hr periods, which has been validated by time-labeling and rearing experiments (Dawe *et al*., 1985; Jackson and Forsythe, 2002). The edge and shape of a statolith changes as the external outline of the statolith is continuously generated from new daily increments. Variations in shape may represent growth variation between stocks due to both environmental differences and stocks genetics. The shape analysis of statolith for *L. duvauceli* stock identification was a technique that was investigated in this study.

Body dimensions are under the simultaneous control of genetic and environmental factors (Begg and Waldman, 1999) and morphometrics data from various measurements have been used to identify differences between species and subspecies (Augustyn and Grant, 1988). In this study morphological variation between sexes within the same environment was investigated.

The specific objectives of this thesis were:

1. Study the age and growth of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea based on statolith daily increments to estimate the Growth Index for individuals and the growth model for squid populations.

2. To estimate spawning date of *L. duvauceli* from the Gulf of Thailand for management purpose.

3. To investigate the sexual dimorphism of *L. duvauceli* in the two squid populations to test for any environment-induced morphological changes.

4. To establish the Length - Weight relationship of *L. duvauceli* in order to support data for the squid stock assessment researches based on the length measurement. And to estimate the relative condition factor (K) of individual squid to reflect well-being of squid in each area and monsoon season.

5. To compare maturity patterns between sexes and squid populations, in order to estimate the size and age at first maturity to provide current information about squid reproductive strategy.

6. To investigate morphological measurements and statolith shape for stock identification of squid in order to discriminate appropriate stocks for stock assessment.

Materials and Methods

1. Study area

Samples of Indian squid (*Loligo duvauceli*) were collected in Thailand from both the Gulf of Thailand by research vessel and the Andaman Sea by commercial fishing vessels **(**Figure 1). These two areas are separated by land therefore Indian squid samples may represent at least two stocks.

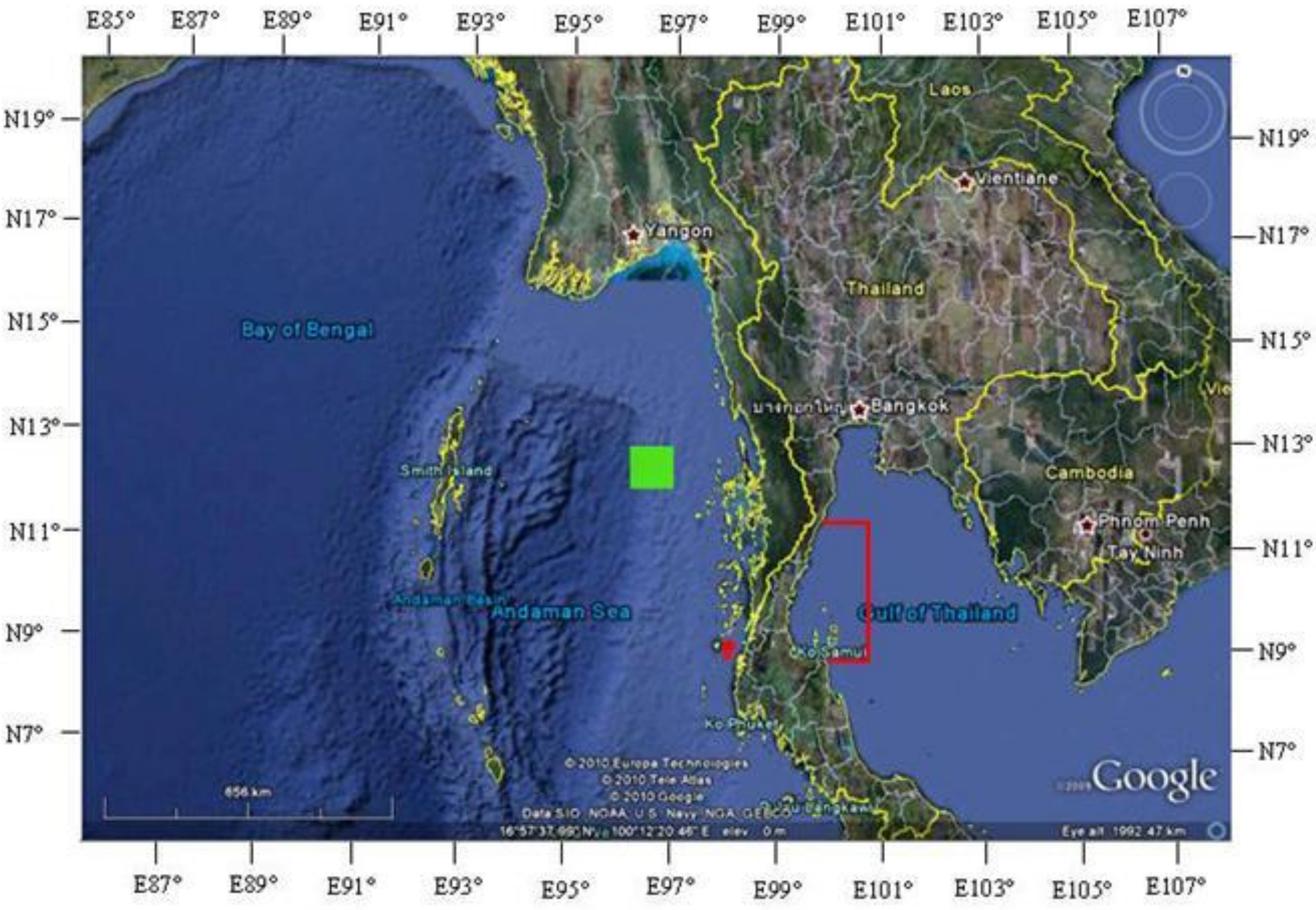


Figure 1 Sampling area for *L.duvauceli* in the Gulf of Thailand and the Andaman Sea.

(Modified from http://www.earth.google.com)

1.1 Samples from the Gulf of Thailand

Samples of *L. duvauceli* were caught from the middle Gulf of Thailand by the research vessel “Pramong1” using oblique trawling during the daytime. The samples were collected during four cruise trips in January, March, July and August–September 2008. The fishing gear was an otter board trawl with 4 cm codend mesh. The same fishing gear and fishing operation were used for all sample collections. The area in the middle Gulf of Thailand was divided into stations of 225 nm^2. The sampling stations were labeled with bold numbers as shown in Figure 2. The *L. duvauceli* were sampled from the catches at each station, at random from the range of sizes available, and kept frozen in labeled plastic bags. The water depth of each haul was recorded. Sampling stations were grouped by dominant spawning seasons and geographical location into North and South groups. Stations above 10˚ 15΄N latitude were grouped as the North group while sampling stations below 10˚ 15΄N latitude were grouped as the South group. The South group area is covered by islands and the “Mu Ko Aug-thong” archipelago marine national park.

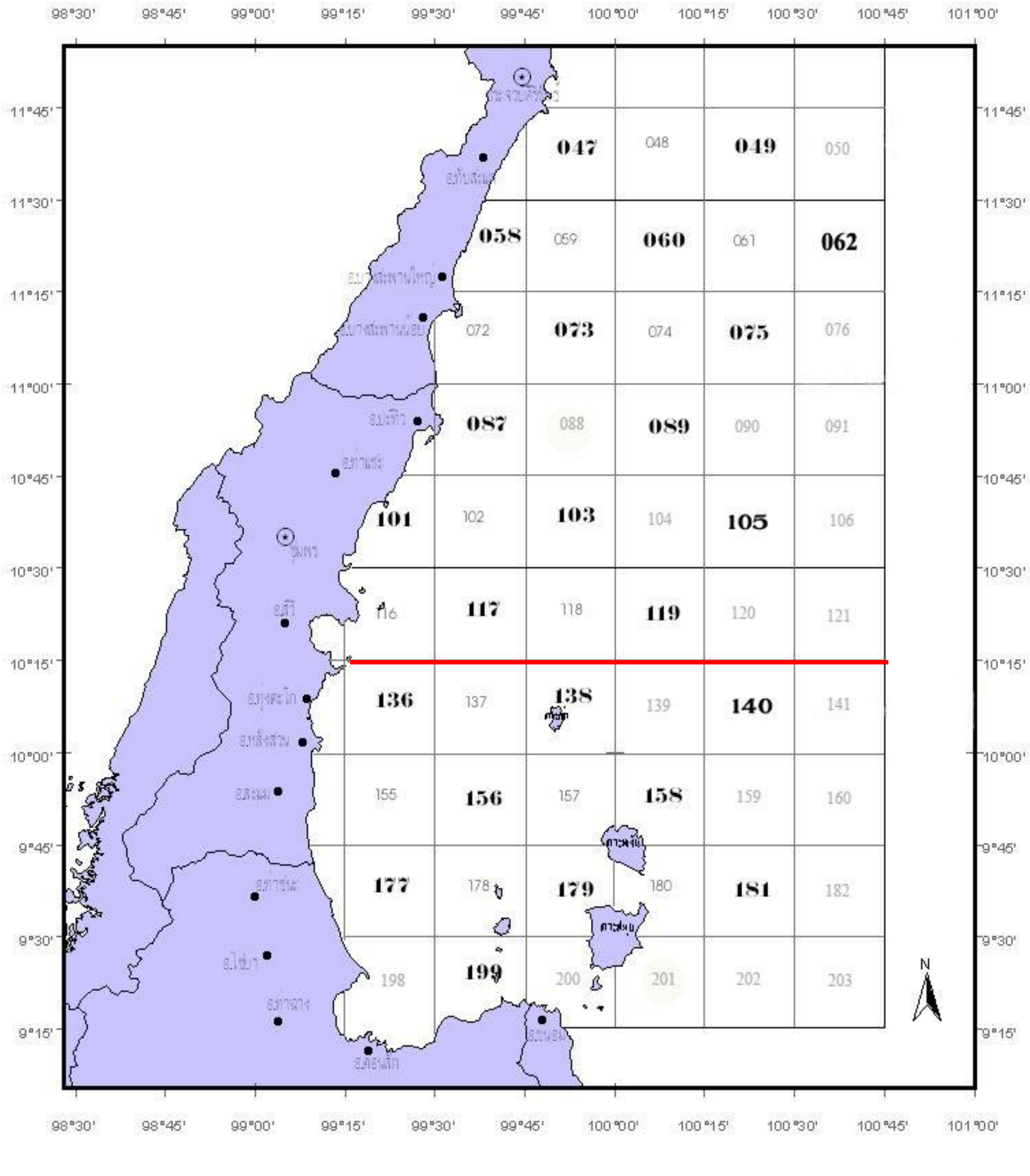


Figure 2 Sampling stations for *L. duvauceli* in the Gulf of Thailand labeled with bold numbers.

1.2 Samples from the Andaman Sea

Samples of *L. duvauceli* from the Andaman Sea were collected in July-August, September and October 2009 at fishing ports in Ranong province, Thailand. The samples were collected from both large and small commercial otter board trawlers which fished in different fishing grounds. Large commercial otter board vessels with licensing from neighbor country trawled in Zone 1 while small commercial otter board vessels trawled in Zone 2 (Figure 3). *L. duvauceli* were sampled from the catches at random from the range of sizes available, and kept frozen in labeled plastic bags. The fishing ground of each vessel, water depth and fishing periods were recorded. Squid samples from large commercial otter board vessels which trawled in Zone 1were considered as the North group while samples from small commercial otter board vessels which trawled in Zone 2 were considered as the South group.

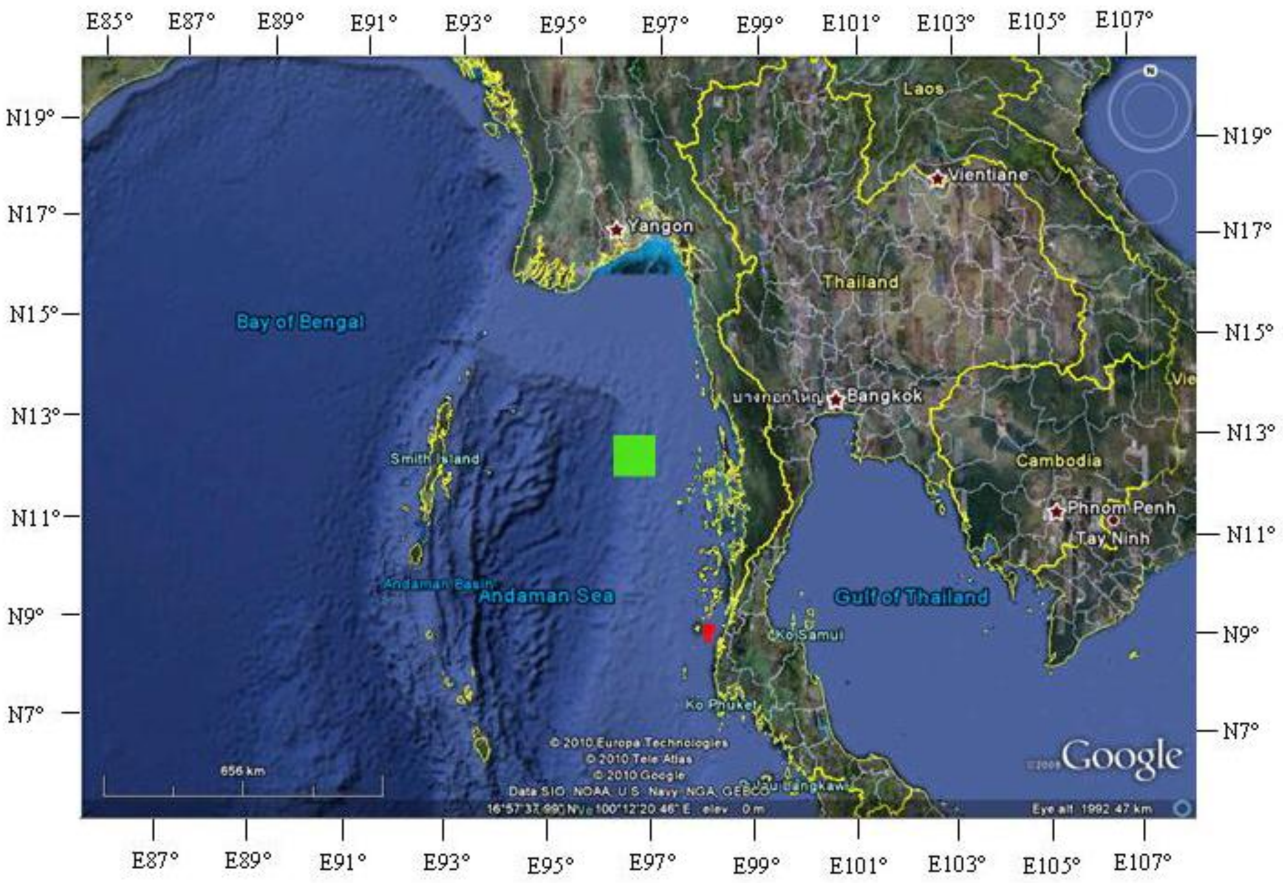


Figure 3 The fishing grounds of commercial otter board trawlers for *L. duvauceli* in the Andaman Sea. Zone1 is the fishing ground of large commercial otter board trawlers and Zone2 for small commercial otter board trawlers.

2. Species identification and morphological measurements

In total 560 samples from the Gulf of Thailand and 327 samples from the Andaman Sea were collected.

2.1 Species identification

The samples were delivered from sampling sites to Chumphon Marine Fisheries Research and Development Center Laboratory in frozen condition. Frozen squids were thawed at room temperature (30-35^{o}C). *L. duvauceli* are similar to *Loligo chinensis* and *Loligo edulis* and small *L. duvauceli* are similar to *Loliolus sumatrensis.* Therefore the species identification was very important for this study. *L duvauceli* was identified by fin shape, the shape of the teeth inside the arm III sucker ring and inside the tentacular club sucker ring. *L. duvauceli* have broad short rhombic fins and fin length is about 50% of the dorsal mantle length. The teeth inside arm III sucker ring are squared to rounded and truncated in the distal 2/3 of the ring and the proximal 1/3 of the ring is smooth. The tentacular clubs are expanded with large median suckers that are 1.5 times larger than the marginal suckers. The teeth inside the tentacular club sucker ring are short, sharp with 14 to 17 teeth around the ring (FAO, 1998, Figure 4).

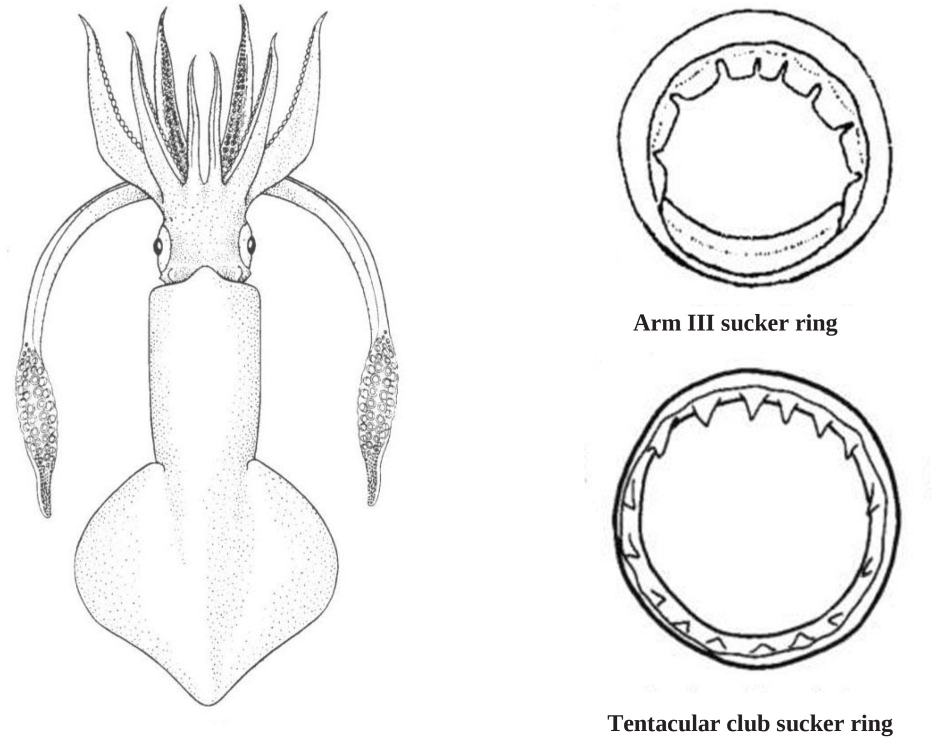


Figure 4 The morphology of *L. duvauceli* (modified from FAO, 1998).

2.2. Morphological measurements

The length and weight of all squid were measured. Dorsal mantle length (DML) was measured in millimeter (mm) and total weight (TW) in grams (g). Sex was determined by examination of the hectocotylized fourth left arm and confirmed by examination of the internal reproductive organs. Maturity stage was categorized into 6 stages following the description of Lipinski and Underhill (1995). For both sexes, stages I and II were defined as Immature and stage III to VI were defined as Mature.

The morphological study was based on the samples collected from the Gulf of Thailand by research vessel on July and August-September 2008 and all the samples collected from the Andaman Sea. In addition to the size, sex and maturity measurements, further body dimensions were measured: fin length (FL), fin width (FW), head length (HL), head width

(HW), length of the 4th left arm (4AL), length of tentacle (TL), length of tentacle club (TC), nuchal cartilage length (NCL), funnel cartilage length (FCL), mantle circumference (MC), gill length (GL), pen length (PL) and pen width (PW). The measurement positions are shown in Figure 5.

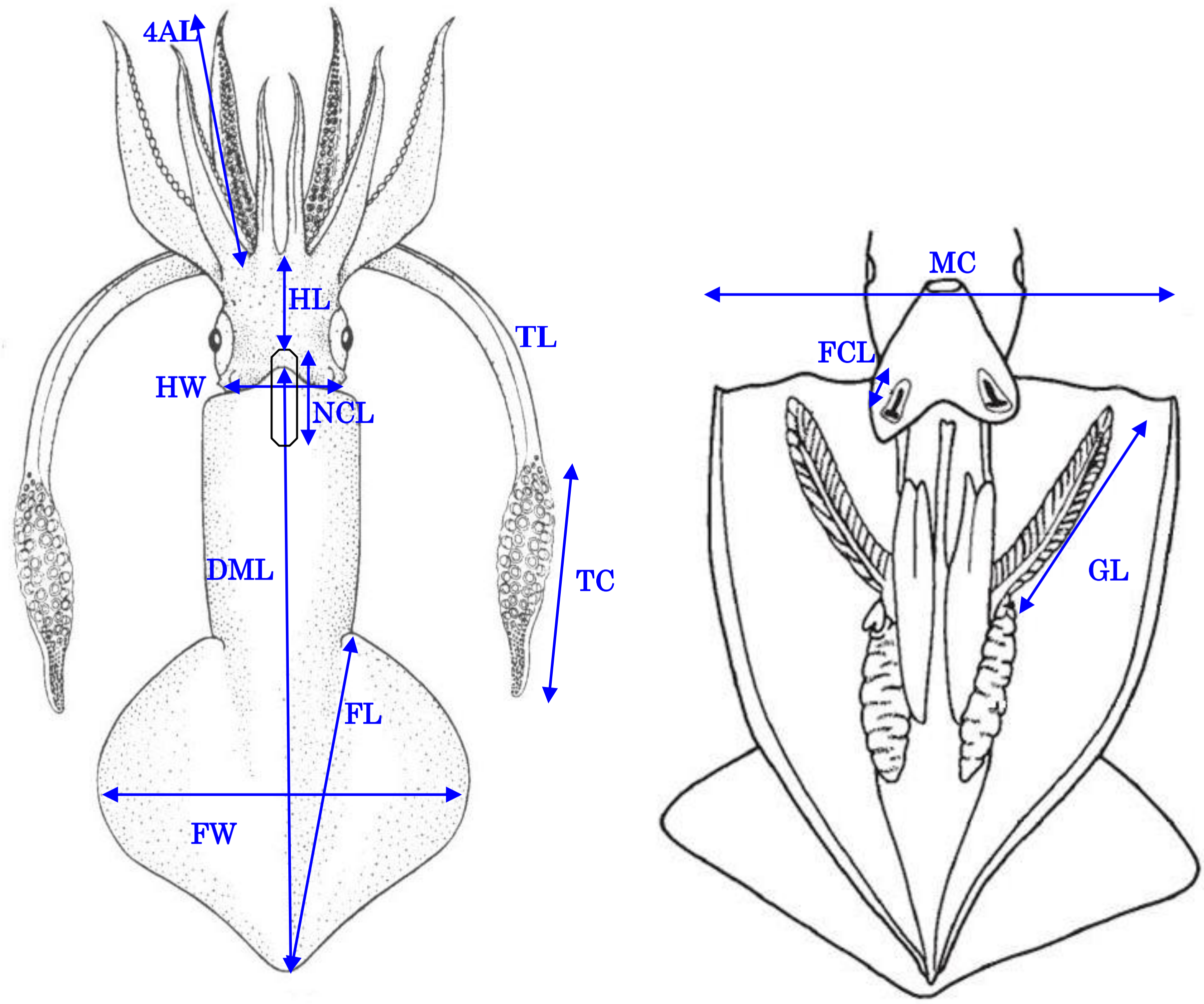


Figure 5 Measurement for morphological variables of *L. duvauceli*.

(modified from FAO, 1998)

3. Statolith analysis

3.1 Statolith extraction and preservation

Both statoliths were extracted from the ventral side of the squid by diverting the funnel to the side and carefully cutting the statocyst to reveal the statoliths, which were then removed. The statoliths were washed two times with distilled water to remove tissue on the surface. They were then rinsed with 95% analytical grade ethanol and kept dry in small labeled micro tubes.

3.2 Statolith measurements and shape analysis

Photographs of statoliths from each squid were taken by Nikon^{TM} DS-U2/L2 version 5.03 camera mounted on a stereomicroscope under 60X magnification. The camera setting condition for image size was 2,560 X 1,920 pixels. The images of right-hand statoliths in posterior view with the rostrum pointing downward (Figure 6) were used for statolith measurements with the image analysis program – ImageJ (Rasband, 1997-2008). The statolith shape was described by measurement of area, circularity, perimeter and Feret's diameter in millimeter unit. The Elliptical Fourier Descriptors (EFDs) were calculated for shape analyses. Thirty descriptors were calculated from 24-bit BMP images of the right-hand statolith contours (Figure 7) using the shape analysis program–SHAPE V 1.3 (Iwata and Ukai, 2002). The integrated data of statolith measurements and Elliptical Fourier Descriptors were used to describe statolith shape variation to explore the possibility of using this to distinguish squid stocks.

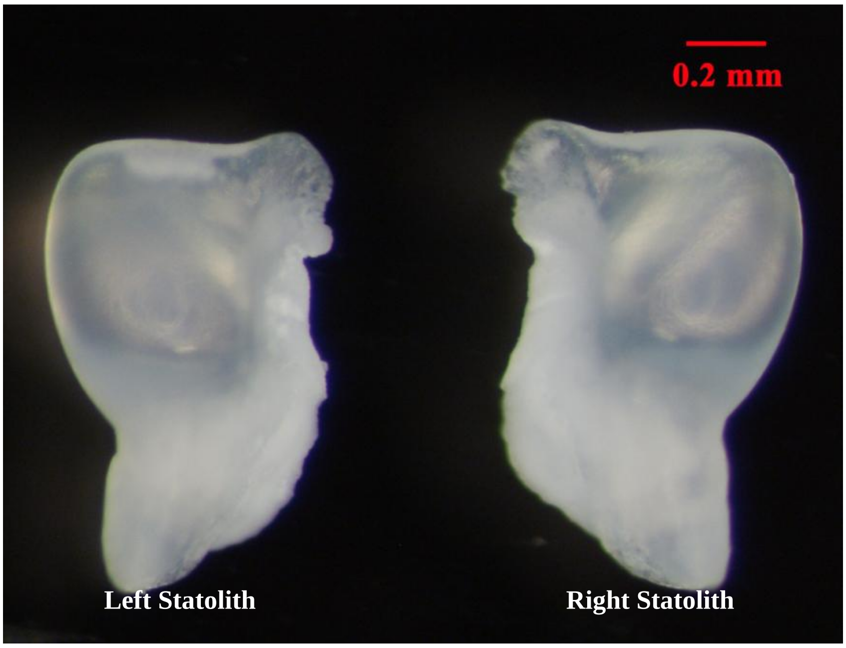


Figure 6 Photograph of statoliths of *L. duvauceli* in posterior view. The right-hand statolith was used for measurements.

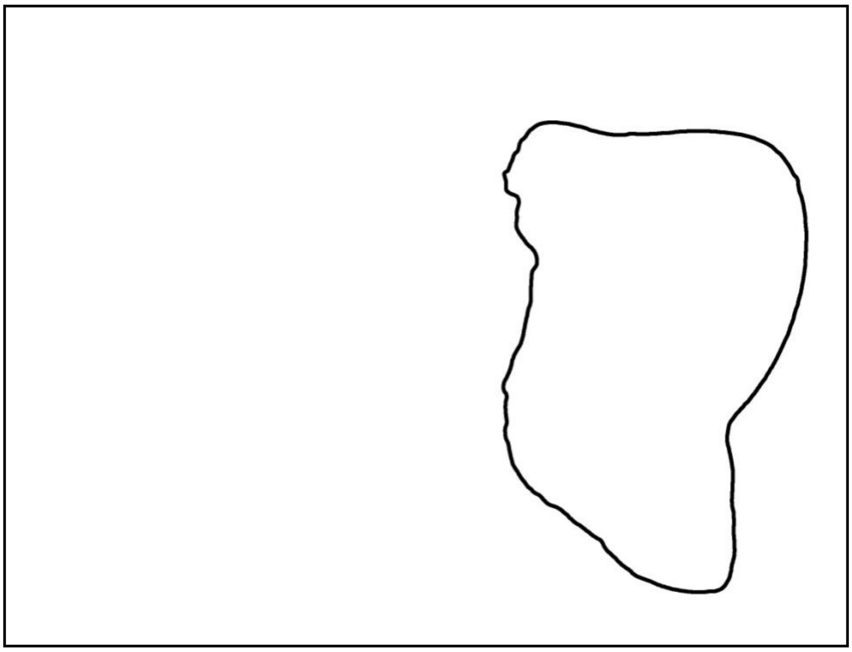


Figure 7 The right-hand statolith contour of *L. duvauceli* in posterior view for Elliptical Fourier Descriptors analysis.

3.3 Age determination

3.3.1 Mounting

The right-hand statoliths from a sub sample of the squid were selected for age determination. Ten percent of the total samples in each 5 cm of DML interval were selected. Ninety statoliths samples from the Gulf of Thailand and 67 statoliths samples from the Andaman Sea were selected. The thermoplastic resin mounting medium- Crystal Bond^{TM} was used to embed a statolith on a cover slide, with the anterior side downward firstly for a more stable plane for grinding. After the mounting medium hardened, the identification number was labeled on the slide.

3.3.2 Grinding

Firstly the posterior side of statolith was ground in water using 12 µm grit abrasive film, and polished with alumina powder to reduce scratches. The grinding progress was checked regularly under the microscope with 100X and 200X magnifications, and when increments appeared the polishing was changed to waterproof abrasive films 3 µm and 0.3 µm. Grinding stopped before the nucleus was ground away. The mounted statoliths were reheated to melt thermoplastic resin mounting medium and turned over to grind the anterior side by the same procedure as described above. The ground statoliths were cleaned by ultrasonic cleaner for 5 minutes to remove dust particles and avoid microscope lens damage.

3.3.3 Counting increments

The magnification selected for photographing the increments was important for distinguishing adjacent increments. At low magnification it was hard to distinguish between small increments in dense areas of the statoliths, so 600X and 1,000X magnifications were selected. The increments counted under 600X and 1,000X magnifications were compared to find out the best condition for studying increments.

Ten prepared samples were counted by one person at both magnifications (comparison data is shown in Table 1). The number of increments counted under 600X and 1,000X magnifications were not significantly different (paired t-test, p-value > 0.05) and the standard deviation (SD) of the 1,000X counts (10.039) was higher than the SD from the 600x counts (9.964), showing that counts at 1,000X counts were less precise than at 600x magnification. Therefore 600X magnification was more suitable for these statolith samples and selected for photographing the increments.

Table 1 Number of statolith increments counted under 600X and 1,000X magnifications.

Sample No.	600X magnification				1,000X magnification			
	Replication1	**Replication2**	**Replication3**	**Average**	**Replication1**	**Replication2**	**Replication3**	**Average**
1	57	64	60	60.33	65	55	62	60.67
2	69	77	72	72.67	77	65	71	71.00
3	79	72	77	76.00	77	72	80	76.33
4	61	59	57	59.00	56	58	53	55.67
4	71	79	78	76.00	76	70	80	75.33
6	66	59	65	63.33	61	68	64	64.33
7	45	54	53	50.67	48	52	58	52.67
8	62	63	59	61.33	57	64	62	61.00
9	85	75	77	79.00	82	76	70	76.00
10	75	81	76	77.33	73	78	86	79.00
SD		**9.964**				**10.039**		

Paired t-test, mean of the differences= 0.367, t_{29} = 0.367, p-value = 0.717

The series of statolith increment photographs were made with a Nikon^{TM} camera mounted on an Olmpus^{TM} BX51 under 600X magnification with 1.5 diaphragm exposure. The posterior side of the statolith was fixed to the cover slide, and this was mounted onto a microscope slide with immersion oil under the cover slide. Series of photographs were taken, with approximate 1/3 overlap at the edge of the field of view. These series were contrast adjusted and stitched together into montages of the entire sequence of increments from the core to the edge using Adobe Photoshop CS version 8.0 (Figure 8). The montages were overlaid as image layers using Adobe Photoshop CS and the increments were then marked and counted manually from the natal ring to the edge in the lateral direction.

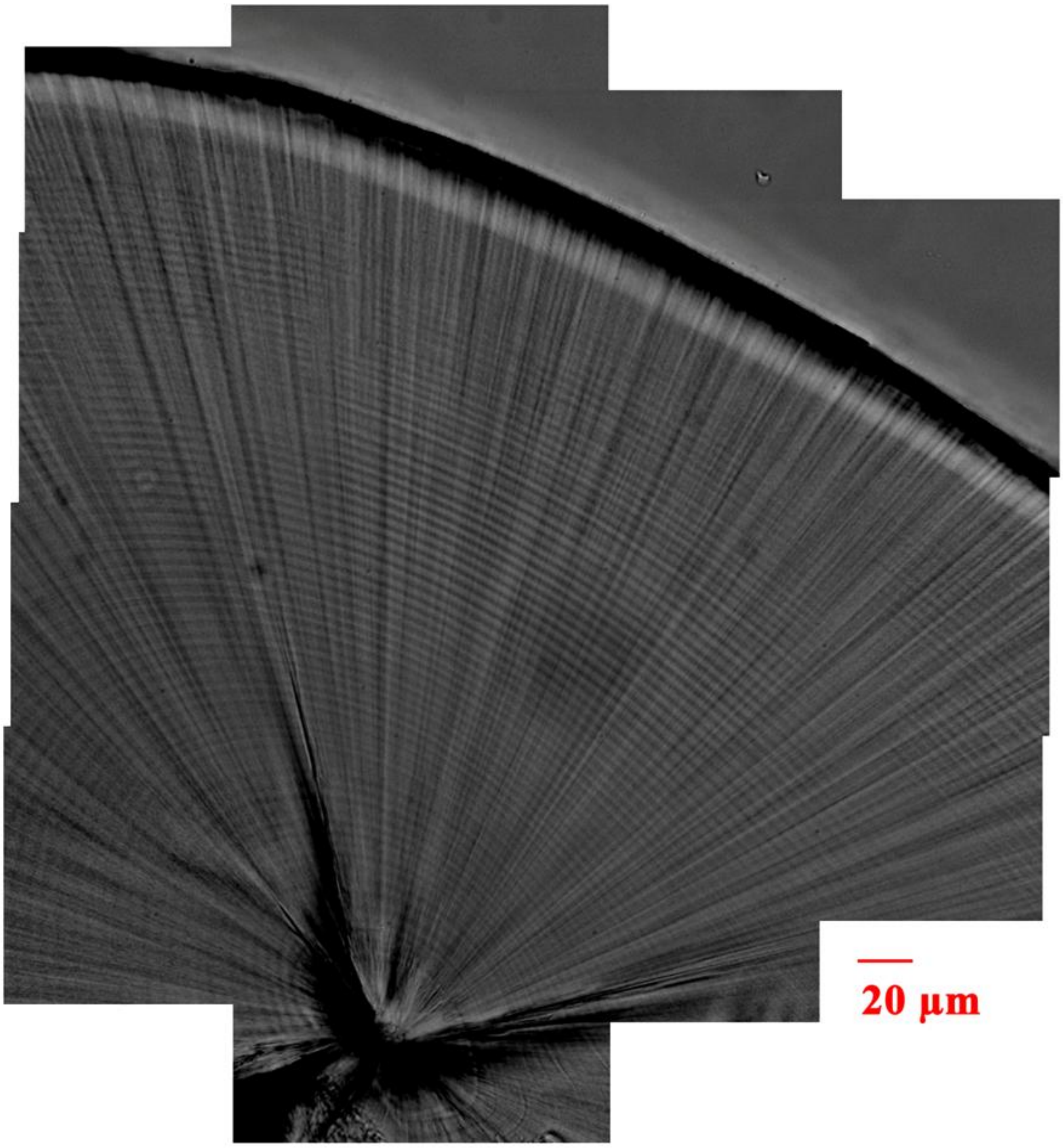


Figure 8 A montage of the right-hand statolith of *L. duvauceli* in posterior view from 600X light microscope for increment counting.

3.3.4 Accuracy of increments counting

The accuracy of counting was verified by comparison between manual and the semi-automatic counting to confirm that there was no operator error in identifying increments. In addition, the width of increments was compared between Scanning Electron Microscope (SEM) and light microscope images of the same area of the statolith to confirm that narrow increments were not missed by using light microscopy for counting.

3.3.4.1 Counting comparison

Manual counting was compared to the semi-automatic counting with the Image Pro 7.0 image analysis software, by comparing the same statolith montages using a paired t-test. The comparison was done on 10% of ground statolith samples (Table 2). The number of increments identified by manual counting and semi-automatic counting were not significantly different (paired t-test, p-value > 0.05).

Table 2 Number of statolith increments counted manually and using the semi-automatic functions of the ImagePro image analysis program.

Sample No.	Methods for counting increments		Difference increments between methods
	Mannual	ImagePro	
1	77	82	5
2	63	65	2
3	70	73	3
4	80	82	2
5	86	85	1
6	83	84	1
7	75	81	6
8	86	83	3
9	87	90	3
10	66	66	0
11	63	61	2
12	71	77	6
13	85	90	5
14	64	71	7
15	76	73	3
16	93	86	7

Paired t-test, mean of the differences= -1.5, t_{15} = -1.519, p-value = 0.150

3.3.4.2 Size of increment comparison

The small increments under the light microscope were measured and compared to the smallest increments from SEM images of the same statolith area to confirm that the increments counted with the light microscope are not missing any smaller increments that are below the size resolution limit.

Prepared statoliths were selected for the SEM. The ground statoliths which were fixed with thermoplastic resin mounting medium- Crystal Bond™ on the cover slide, with the anterior side upward were etched for 2.5-3.0 minutes using 5% EDTA. The etched statoliths were washed several times, dried and then coated with carbon. The series of the SEM photographs were made by mounting the cover slide on a SUPRA™ 55VP stub and viewing at 2,000X – 10,000X magnifications. The size of increments from the SEM photographs were measured using the image analysis program –ImageJ (Rasband, 1997-2008) and compared to the increments from the light microscope at 600X magnification in the area from nucleus to the inner dome (Figures 9 and 10).

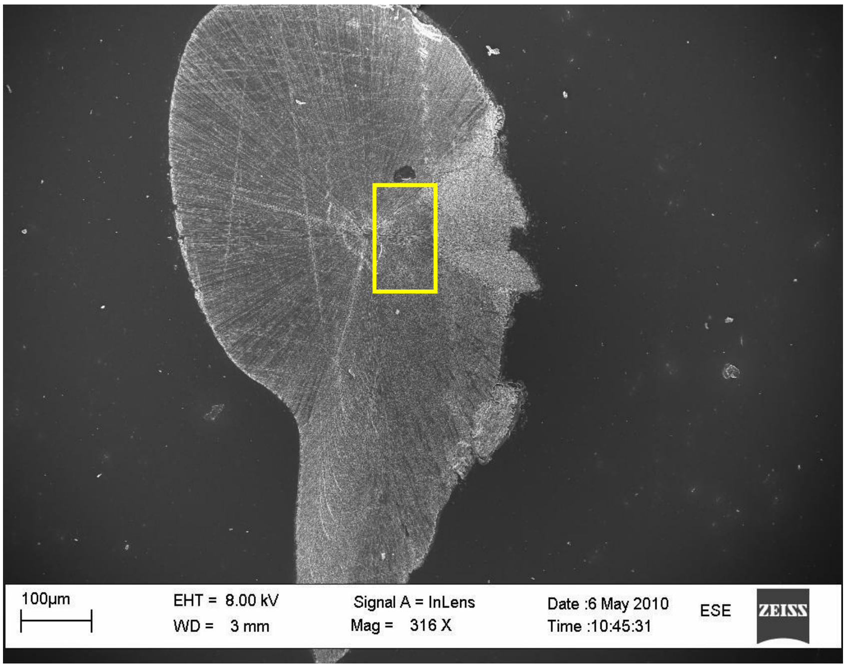


Figure 9 The area of statolith for increment measurement.

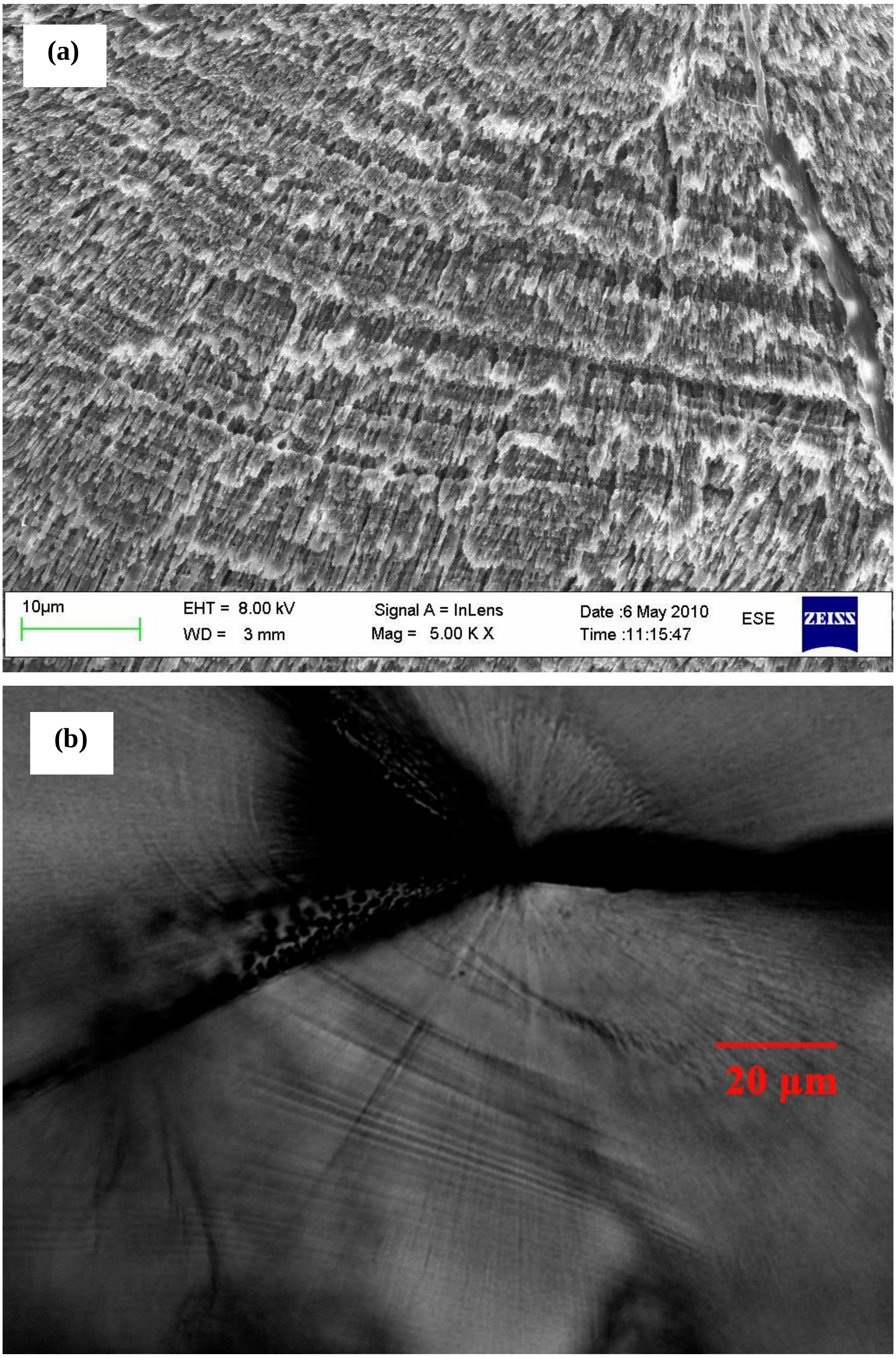


Figure 10 The photographs of statolith increments from the SEM (a) at 5,000X magnification and from the light microscope (b) at 600X magnification.

The measurement of increment widths concentrated on the small increments for both methods to confirm that true increment width is not below the size resolution limit of the light microscope. The smallest increment from the SEM was 0.41 µm and for the light microscope at 600X magnification was 0.46 µm. The length-frequency of statolith increments width for both methods is shown in Figure 11, the increment width measured from the light microscope photographs included the smallest size class of the increments from the SEM. Therefore the conditions for counting increments for this study covered all size range of *L. duvauceli* statolith increments.

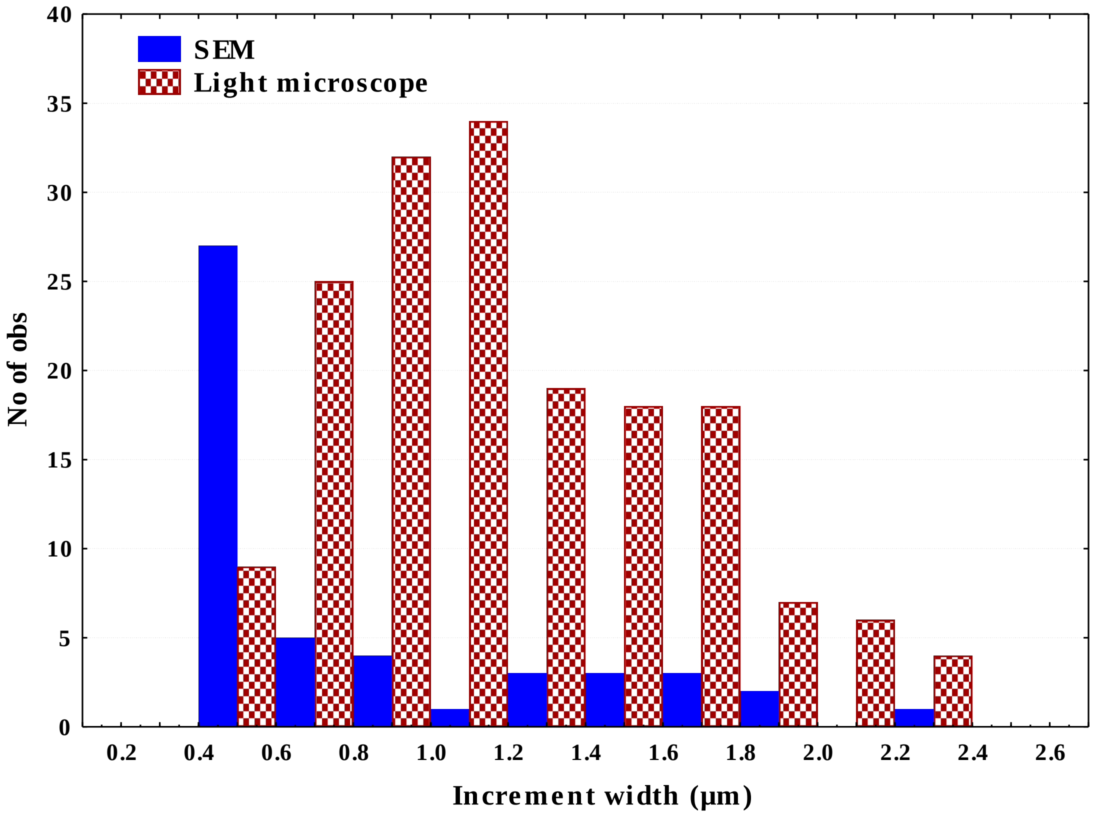


Figure 11 The length-frequency of statolith increments width from the SEM at 2,000X – 10,000X magnifications and the light microscope at 600x magnification. (concentrated on small increments)

4. Data analysis

All statistical analyses were conducted using R statistical software, version 2.10.1 (R Development Core Team, 2009) with an alpha significance level of 0.05.

4.1 Age and growth of *L. duvauceli*

4.1.1 Growth Index

An average Growth Index representing the individual growth of this species from the Gulf of Thailand and the Andaman Sea was calculated separately for males and females as follows:

$$Growth\ Index = \frac{Dorsal\ \ mantle\ length}{Number\ of\ \ increments}$$

The Growth Index of each individual from the total samples from the Gulf of Thailand was used for the statistical analyses because males and females samples from the Gulf of Thailand were not significantly different in size. For samples from the Andaman Sea, males were found to have a wider size range than females, and especially in larger sizes. To prevent this size effect influencing the results of the analysis, the same length range of males and females was used, removing males which were larger than 154 mm from the dataset before analysis. Growth Index was checked for normality of distribution using the Kolmogorov-Smirnov test to meet the basic assumptions of the statistical tests. Differences in Growth Index between sexes were tested using a two sample t-test.

4.1.2 Growth equation

The growth equations reflecting the population-level estimate of growth for this species from the Gulf of Thailand and the Andaman Sea were established.

Data of dorsal mantle length (DML) and number of increments, representing age (in days) for *L. duvauceli* from the Gulf of Thailand and the Andaman Sea, were checked for normality of distribution using the Kolmogorov-Smirnov test to meet basic assumption of ANCOVA. For the Andaman Sea, males larger than 154 mm DML were removed from the dataset before analysis. The effect of sex on the Increments - dorsal mantle length regression slopes was tested and compared by analysis of covariance (ANCOVA).

Exponential and logarithmic equations were used to describe the growth pattern of *L. duvauceli* populations and the model with lowest residual standard error or highest R^2 was selected.

Exponential equation: $DML = ae^{bt}$

Logarithmic equation: $DML = a + b\ln(t)$

Where DML is dorsal mantle length (mm) and *t* is age (days).

4.2 Hatching date and spawning date back calculation

To estimate hatching date and spawning date of squid in the samples, knowledge of the exact date of capture of each individual samples was important. Samples from the Andaman Sea were collected from commercial fishing vessels and squid from different hauls were mixed so that the exact date of capture of these samples could not be identified. Therefore only samples from the Gulf of Thailand were used for this calculation.

Dates of hatching for individual *L. duvauceli* from the Gulf of Thailand were estimated by back calculation from the date of capture (day of year) using the age estimated from statolith daily increments. The spawning dates of individual squid were estimated by back calculation from the date of hatching and egg incubation time of *L. duvauceli* based on experimental rearing in Thailand by Wudthisin and Singhagraiwan (1988). The average egg incubation time of 9 days was used for calculation. The estimated periods of spawning were compared with spawning seasons from previous studies by Supongpan and Sinoda (1998) and Chotiyaputta (1996).

4.3 Statistical analysis of morphological patterns and statolith length

To study sexual dimorphism, the sex effect on morphological and statolith length of the two squid populations were analyzed. Somatic growth and statolith growth were compared.

Mean, standard deviation and range of variation of morphological variables and statolith length for samples from the Gulf of Thailand which were collected in July and August-September 2008 and the total samples from the Andaman Sea were calculated for males and females separately. However, to study any sex effects on morphological and statolith length, the July and August-September 2008 Gulf of Thailand samples were used, but for the Andaman Sea samples, only males and females of the same length were used, removing males which were larger than 154 mm from the dataset to avoid size effects on analysis results. Data of morphological measurements (15 variables) and statolith length (measured as Feret's diameter in mm) were checked for normality using the Kolmogorov-Smirnov test to meet basic assumption of ANCOVA. The analysis of linear regression between each variables and dorsal mantle length was tested for each sex. The differences of slope and intercept between sexes were compared by using analysis of covariance (ANCOVA).

The comparison of growth between somatic growth and statolith growth was described by regression slope of log transformed dorsal mantle length (logDML) and log transformed statolith length (logSL) which slope =1 means somatic growth and statolith growth were allometric and increased at the same rate.

4.4 The Length - Weight relationship and relative condition factor (K)

The length-weight relationships for each squid population and relative condition factors of individuals were calculated separately for males and females.

The relationship between dorsal mantle length - total weight and relative condition factor reflects well-being of squid and recent feeding condition. Because males and females samples from the Gulf of Thailand were not significantly different in size, the total samples were used for analysis. For samples from the Andaman Sea, males were found to have a wider size range than females, and especially in larger sizes. To prevent this size effect influencing the results of the analysis, the same length range of males and females were used, removing males which were larger than 154 mm from the dataset before analysis.

The log transformed of dorsal mantle length (logDML) and log transformed total weight (logTW) of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea were checked for normality of distribution using Kolmogorov-Smirnov test to meet basic assumption of ANCOVA. The effect of sex on the dorsal mantle length weight regression slope was tested and compared by analysis of covariance (ANCOVA) and performed as

$$\log TW = \log a + b \log DML$$

Where a is the regression constant and b is the regression coefficient and relative condition factor (K) was assessed from the following equation.

$$K = \frac{TW}{DML^b}$$

4.5 Maturity ogives

The maturity ogives were estimated for each sex for the two squid populations and the size and age at first maturity was estimated to provide current information about squid reproductive biology.

The total squid samples from the Gulf of Thailand and the same length of males and females from the Andaman Sea were used, removing males which were larger than 154 mm from the dataset before analysis. Maturity stages were categorized into Immature for stage I and II and Mature for stage III to VI. The logistic model was used to fit the proportions of mature (Y) and length or age class (X) in following equation:

$$Y = \frac{1}{1 + e^{(a+bX)}}$$

Parameters *a* and *b* were constant coefficients of the equation initially calculated from linear regression. In the regression analysis, the value of the logarithm of the reciprocal of the dependent variable was provided instead of variable *Y* according to the following equation:

$$Y' = \ln\left(\frac{1}{Y-1}\right)$$

Dorsal mantle length or age at which 25%, 50%, and 75% of *L. duvauceli* achieved first sexual maturity was calculated according to following equations.

$$DML_{50\%} \text{ or } t_{50\%} = -\left(\frac{a}{b}\right)$$

$$DML_{25\%} \text{ or } t_{25\%} = -\left(\frac{a - \ln(3)}{b}\right)$$

$$DML_{75\%} \text{ or } t_{75\%} = -\left(\frac{a + \ln(3)}{b}\right)$$

4.6 Stock discrimination

The morphological variables and statolith shape were investigated to discriminate stocks of the two sample groups from the Gulf of Thailand and the two sample groups from the Andaman Sea. The samples were separated based on geographical difference of the sampling area. The squid from the Gulf of Thailand sampled from sampling stations above 10˚ 15´ N latitude were the North group and below 10˚ 15´ N latitude were the South group while squid from the Andaman Sea sampled from Zone 1 were the North group and from Zone 2 were the South group. Morphological variables and statolith shape were investigated to identify stocks among these four groups.

The total squid samples from the Gulf of Thailand and the same length of males and females from the Andaman Sea were used, removing males which were larger than 154 mm from the dataset before analysis.

4.6.1 Statistical analysis of morphological variables for stock discrimination

The morphological measurements (15 variables) were standardized by DML for further analysis. All variables were treated as independent parameters and checked for normality of distribution using Kolmogorov-Smirnov test. The univariate ANOVA was used to test each variable for the four different sampling areas. The variables which showed significant differences were selected for Discrimination Function analysis (DF). Due to the effect that maturity stage may have on the morphological parameters of squid, the differences of the DF between Immature and Mature were tested to categorize the DF for stocks discrimination in order to reduce affect of maturity on the analytical results. The DF were categorized into Immature for stage I and II and Mature for stage III to VI and tested for differences using multivariate analysis of variance (MANOVA) with STATISTICA @ (version 8). The results showed differences of the DF between maturity stages, so the stock discrimination was analyzed separately for Immature and Mature squid. The totals of DF were analyzed to discriminate squid stocks using multivariate exploratory technique – Discriminant Analysis with STATISTICA @ (version 8) for each sample group.

4.6.2 Shape Analysis of statoliths for stock discrimination

The statolith measurements of the area, the perimeter and the Feret's diameter (statolith length) were standardized by the DML for further analysis while the circularity, which is a ratio, was used directly without standardization. The statolith measurements were integrated with the 30 Elliptical Fourier Descriptors (EFDs) to analyze statolith shape variation among the four sample groups. The EFDs of right-hand statolith contour were saved as series of a_n, b_n, c_n and d_n coordinates. The a_n and b_n were coefficients values for the Elliptical Fourier expansion of the sequences to the x-coordinates while c_n and d_n were coefficients values of the sequences to the y-coordinates. The EFDs shape analysis with 30 descriptors resulted in 120 coefficients for each statolith. The program options selected set the standardization to always give $a_1 = 1$, thus there were 119 unique coefficients for each statolith for Elliptical Fourier Descriptors.

All variables were treated as independent parameters and checked for normality of distribution using the Kolmogorov-Smirnov test. The univariate ANOVA was used to test each variable for the four different sampling groups. The variables which showed significant differences were selected as discrimination functions (DF). Any effect of maturity stage on the statolith length may also affect other statolith variables, so the differences of the DF between Immature and Mature were tested to categorize the DF for stocks discrimination in order to reduce affect of maturity on the analytical results. The total of DF were categorized into Immature for stage I and II and Mature for stage III to VI and tested for differences using multivariate analysis of variance (MANOVA) with STATISTICA @ (version 8). The results showed difference of the DF between maturity, so the stocks discrimination was analyzed by separate between Immature and Mature. The totals of DF were analyzed to discriminate squid stocks using Discriminant Analysis in STATISTICA @ (version 8) for each sample group.

Results

1. Summary data

The total 887 of *L. duvauceli* were sampled from both the Gulf of Thailand and the Andaman Sea consisted of 527 females and 258 males (Table 3).

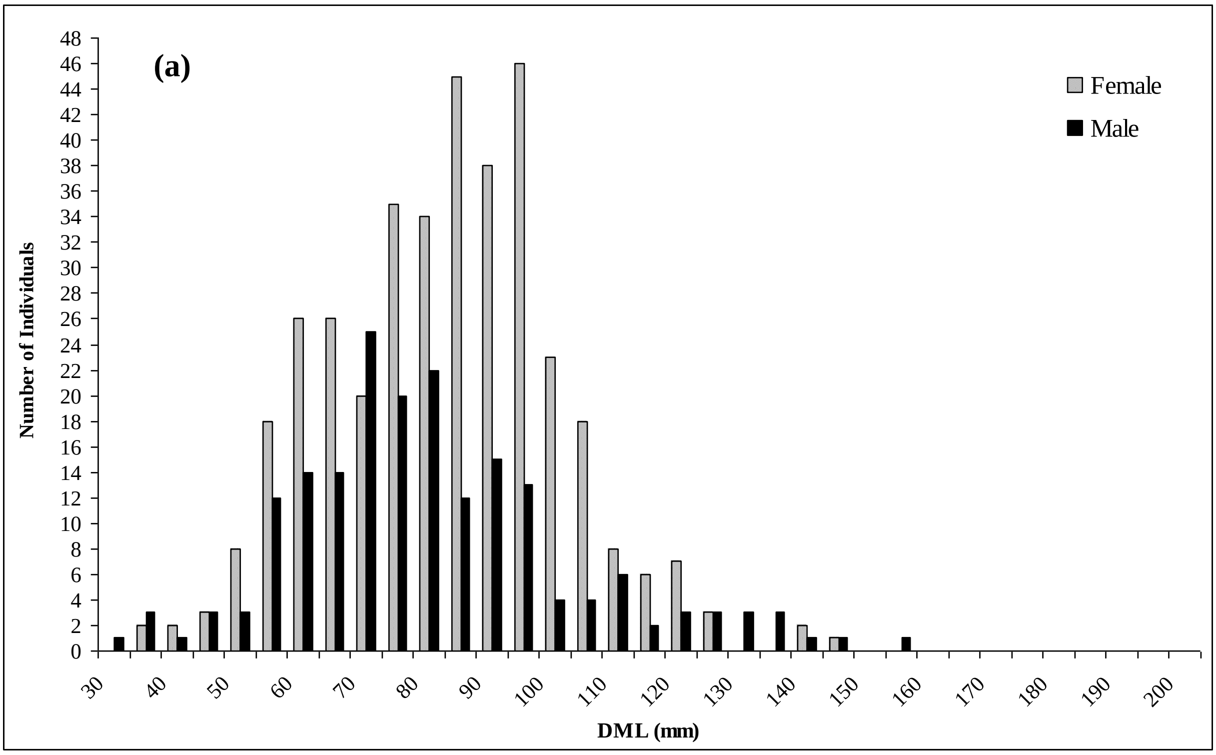
L. duvauceli sampled from the Gulf of Thailand (at 10 – 60 m water depth) were in total 560 individuals and consisted of 189 males and 371 females. The dorsal mantle length and the total weight of males ranged from 32.46 to 160.00 mm and 1.77 to 70.99 g while females ranged from 35.37 to 149.13 mm and 2.79 to 84.50 g (Table 3). The numbers of samples in each 5 cm length interval for males and females is shown in Figure 12.

L. duvauceli sampled from the Andaman Sea (at 20 –140 m water depth) were in total 327 individuals and consisted of 171 males and 156 females. DML and TW of males ranged from 54.88 to 231.89 mm and 7.13 to 121.31 g while females ranged from 57.73 to 153.54 mm and 8.24 to 108.10 g (Table 3) and numbers of samples in each 5 cm length interval for males and females is shown in Figure 13.

For samples from the Andaman Sea, males were found with wider size range than females, and especially in larger sizes. To prevent size effects for many of the comparisons, the same length range of males and females were used, removing males which were larger than 154 mm from the dataset before analysis.

Table 3 Detail of *Loligo duvaulceli* samples from the Gulf of Thailand and the Andaman Sea.

Period of sampling	Sea	Sample size	Number of Females	Number of Males	Dorsal mantle length (mm)				Total weight (g)			
					Min	Max	Mean	SD	Min	Max	Mean	SD
January 2008	Gulf of Thailand	172	92		51.58	149.13	96.28	19.81	4.91	84.50	32.47	16.62
				80	32.46	160.00	86.32	28.94	1.77	70.99	24.20	17.41
March 2008	Gulf of Thailand	142	81		35.37	100.43	66.18	13.84	2.79	27.29	11.38	5.80
				61	45.48	104.62	73.91	13.97	4.23	30.45	14.96	6.31
July 2008	Gulf of Thailand	111	81		62.88	120.86	87.93	12.15	8.18	43.54	22.74	8.06
				30	63.82	111.21	80.89	9.23	11.72	38.39	18.44	5.17
August-September 2008	Gulf of Thailand	135	117		60.07	119.03	87.47	13.17	8.63	46.74	22.96	8.97
				18	75.73	127.18	100.35	12.34	15.04	49.99	28.24	8.93
July-August 2009	Andaman Sea	61	27		57.73	130.26	84.40	13.08	8.24	63.81	23.92	10.76
				34	54.88	154.89	87.02	21.22	7.13	73.34	25.19	13.92
September 2009	Andaman Sea	72	37		70.73	114.35	92.69	12.41	11.69	46.34	27.53	10.23
				35	70.10	195.03	128.11	31.48	12.50	86.62	42.27	19.24
October 2009	Andaman Sea	194	92		68.94	153.54	95.54	15.54	11.18	108.10	31.95	15.72
				102	65.41	231.89	144.50	48.37	10.21	121.31	59.44	33.86
Total		**887**	**527**	**258**								



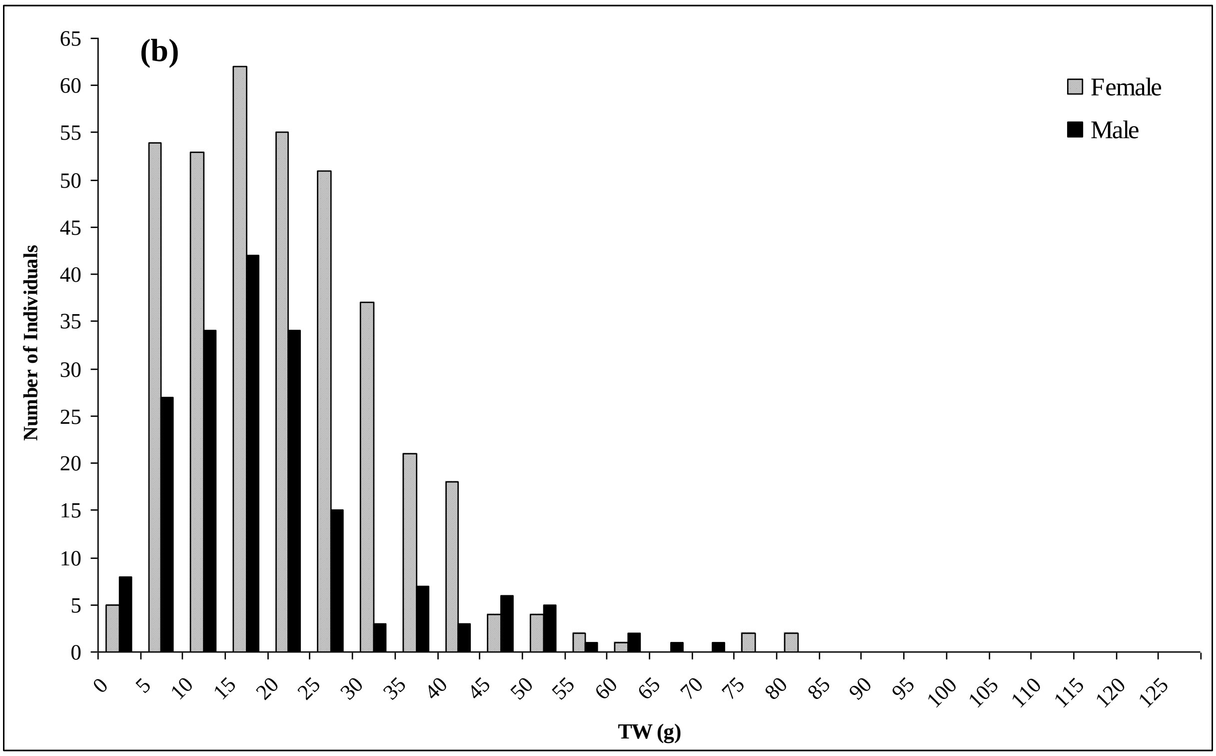


Figure 12 Size distributions (DML (a) and weight (b)) of males and females *L. duvauceli* sampled from the Gulf of Thailand.

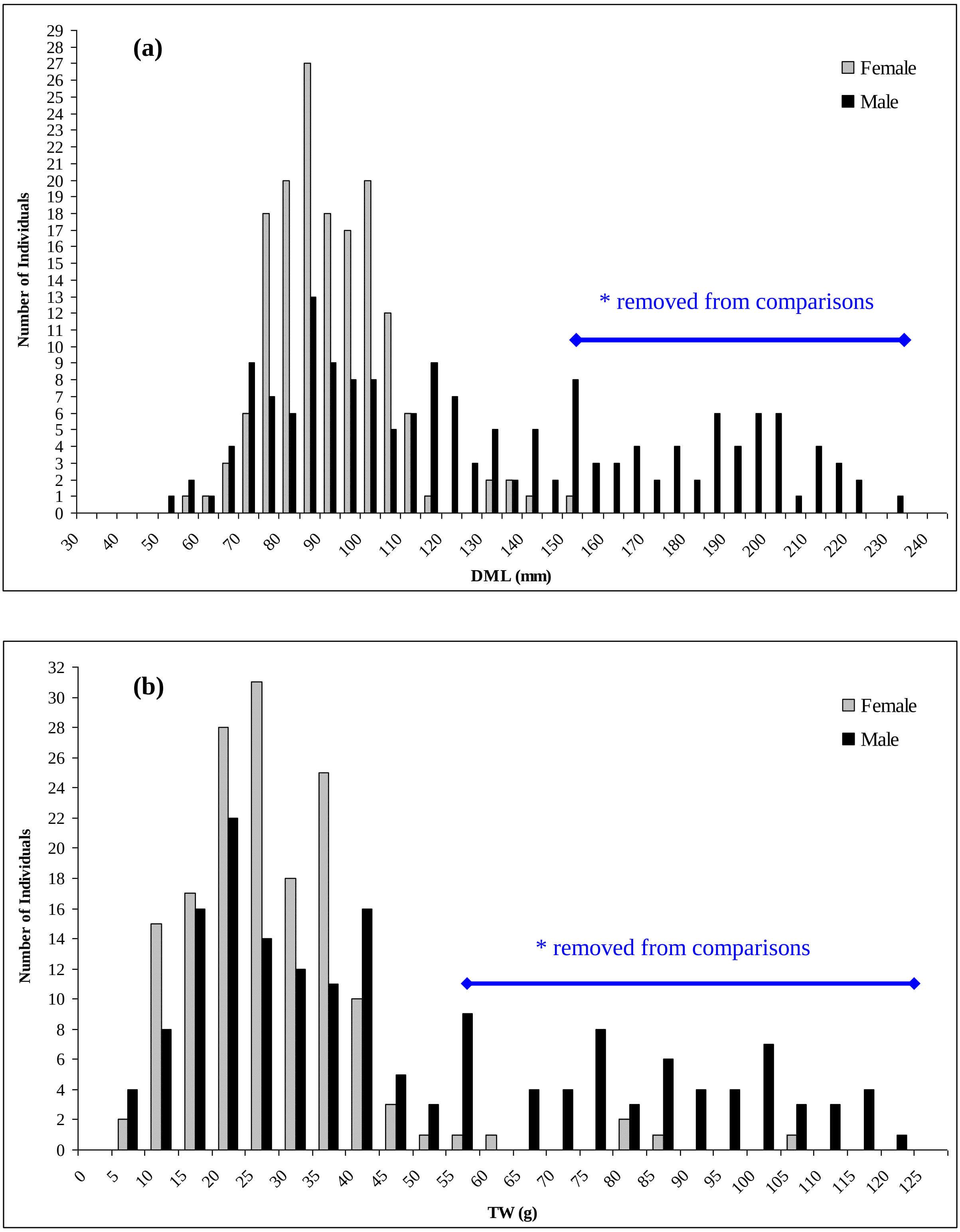


Figure 13 Size distributions (DML (a) and weight (b)) of males and females *L. duvauceli* sampled from the Andaman Sea.

2. Age and growth of *L. duvauceli*

The study of the age and growth of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea was based on counts of statolith daily increments to estimate the Growth Index for individual growth and the size at age to estimate a growth model for the populations.

A total of 157 statoliths were sub sampled for age analysis, representing 10% of the total samples in each 5 cm of dorsal mantle length interval. Details of samples for statolith increments counting are shown in Table 4.

From the Gulf of Thailand, 90 statoliths were sampled from squid (48 females and 42 males) with DML ranged from 35.37 to 149.13 mm for females and 32.46 to 160.00 mm for males. The number of increments (defined as age in days after hatching) ranged from 61 to 153 for females and 62 to 123 for males. From the Andaman Sea, 67 statoliths were sampled from squid (20 females and 47 males) with DML ranged from 57.73 to 143.87 mm for females and 61.73 to 231.89 mm for males. Increments counts ranged from 76 to 202 for females and 93 to 270 for males. The age distribution of *L. duvauceli* from the Andaman Sea covered a wider range than the Gulf of Thailand (Figure 14). *L. duvauceli* from the Andaman Sea were sampled from commercial fishing vessels, therefore *L. duvauceli* enter fisheries at age 76 days.

Table 4 Details of *L. duvauceli* samples for statolith increment counting, total samples were used for both the Gulf of Thailand and the Andaman Sea.

Sea		Female			Male		
		n	DML (mm)	No. of Increments	n	DML (mm)	No. of Increments
Gulf of	Mean	48	89.46	86.27	42	84.84	88.31
Thailand	SD		24.20	20.00		27.96	15.66
	Min		35.37	61		32.46	62
	Max		149.13	153		160.00	123
Andaman	Mean	20	92.55	132.25	47	145.40	175.89
Sea	SD		21.60	30.43		46.59	41.96
	Min		57.73	76		61.73	93
	Max		143.87	202		231.89	270

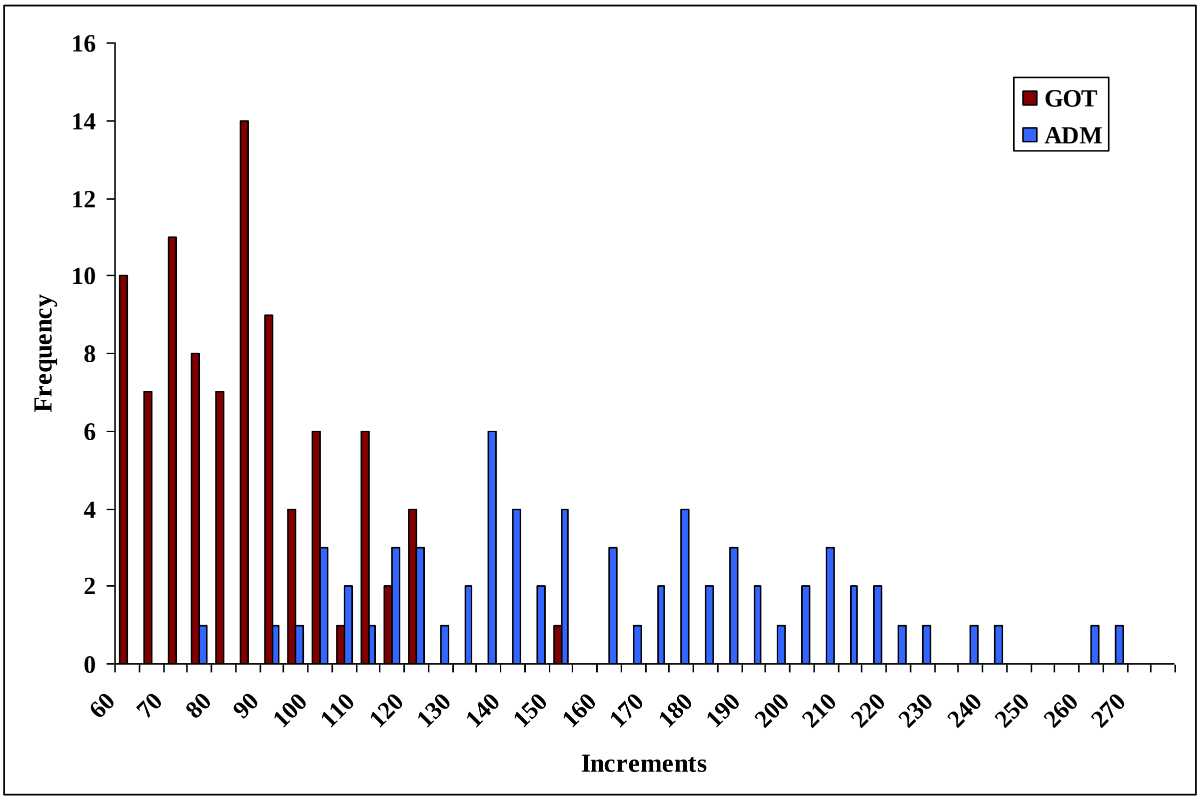


Figure 14 Age distribution of *L. duvauceli* from the Gulf of Thailand (GOT) and the Andaman Sea (ADM). Squid were selected for age estimation from the entire size range for both the Gulf of Thailand and the Andaman Sea.

2.1 Growth Index (GI)

The Growth Index (GI) was calculated to describe the individual growth of squid, and the average GI, which was defined as DML/ Number of increments, was calculated by sex separately. The average GI of *L. duvauceli* from the Gulf of Thailand was 1.044 mm/day for females and 0.959 mm/day for males, and was not significantly different between the sexes (t-test, p-value > 0.05). For samples from the Andaman Sea, to prevent size an effect, the GI was calculated for males and females in the same length range (54 – 154 mm). The average GI of females was 0.706 mm/day which was not significantly different from the average GI of males (0.730 mm/day, t-test, p-value > 0.05, Table 5).

Table 5 Two sample t-test of differences in the Growth Index (GI) between sexes of total samples of *L. duvauceli* from the Gulf of Thailand and the same length range of females and males from the Andaman Sea (excluding males which were larger than 154 mm).

	Mean	**SD**	**t**	**df**	**p-value**
Gulf of Thailand					
Female	1.044	0.212			
Male	0.959	0.261	1.712	88	0.090
Andaman Sea					
Female	0.706	0.093			
Male	0.730	0.127	-0.716	45	0.478

2.2 Growth equation

The relationship between DML (mm) and age in days after hatching, which was defined as the number of increments, was estimated for *L. duvauceli* from the Gulf of Thailand and the Andaman Sea. This represented a population estimate of growth rate.

The effect of sex on the regression slope between number of increments and DML was tested and found to be not significantly different between sexes for samples from the Gulf of Thailand (ANCOVA, p-value > 0.05) and the Andaman Sea (for squid 54 – 154 mm, ANCOVA, p-value > 0.05, Table 6).

Because the growth equations were not significantly different between males and females and also the Growth Index (GI) was not significantly different between sexes, therefore a single growth equation was fitted for all samples – using both exponential and logarithmic functions. The estimated values of a, b, R^2 and the residual standard error are shown in Table 7.

Table 6 ANCOVA table and coefficients of number of increments and DML regression compared between sexes of total samples of *L. duvauceli* from the Gulf of Thailand (a) and the same length range of females and males from the Andaman Sea (b) (excluding males which were larger than 154 mm).

(a)

	df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Sex	1	478	477.9	1.229	0.271
Increments	1	25799	25799.4	66.364	2.68E-12
Sex:Increments	1	362	362.2	0.932	0.337
Residuals	86	33433	388.8		

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	14.951	12.726	1.175	0.243
Sex.Male	-27.147	21.745	-1.248	0.215
Increments	0.864	0.144	6.007	4.41E-08
Sex.Male:Increments	0.235	0.244	0.965	0.337

Residual standard error: 19.72 on 86 degrees of freedom, Multiple R-squared: 0.4435, Adjusted R-squared: 0.424, $F_{3,86} = 22.84$, p-value: 5.74e-11

(b)

	df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Sex	1	4220	4219.8	15.891	2.56E-04
Increments	1	14584	14583.6	54.919	3.29E-09
Sex:Increments	1	128	128.4	0.483	0.491
Residuals	43	11419	265.5		

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	13.724	16.649	0.824	0.414
Sex.Male	21.666	21.676	1.000	0.323
Increments	0.596	0.123	4.852	1.64E-05
Sex.Male:Increments	-0.105	0.151	-0.695	0.491

Residual standard error: 16.3 on 43 degrees of freedom Multiple R-squared:0.6238, Adjusted R-squared: 0.5975, $F_{3,43} = 23.76$, p-value: 3.173e-09

Table 7 Growth estimation using exponential $DML = ae^{bt}$ and logarithmic $DML = a + b\ln(t)$ functions of total samples of *L. duvauceli* from the Gulf of Thailand and the same length range of females and males from the Andaman Sea (excluding males which were larger than 154 mm).

Sea	Sex	Functions	n	a	b	R^2	Residual standard error	p-value	
Gulf of Thailand	Female and male	Exponential	90	36.698	0.010	0.361	19.89	2.23E-06	*
		Logarithmic	90	-280.630	82.711	0.405	20.16	2.56E-09	*
Andaman Sea	Female and male	Exponential	47	50.200	0.005	0.591	16.89	1.63E-06	*
		Logarithmic	47	-302.962	82.107	0.613	16.16	7.07E-09	*

To describe the growth pattern for *L. duvauceli* from both the Gulf of Thailand and the Andaman Sea, the logarithmic function was selected because of lower residual standard error and higher R^2. For convenience of squid stock management it is easier to use the same form for both populations. For the Andaman Sea, the logarithmic function had lower residual standard error (16.16) and also higher R^2 (0.613). For the Gulf of Thailand, although the exponential function had lower residual standard error (19.89) it was not very different from the logarithmic function (20.16) and also the R^2 of the logarithmic function was higher (0.405). Therefore the logarithmic function was selected to describe the growth pattern for *L. duvauceli* for both the Gulf of Thailand and the Andaman Sea (Figure 15 and 16).

Gulf of Thailand: $DML = -280.63 + 82.711 \ln(t)$

($R^2 = 0.405$, n = 90, Residual standard error = 20.16)

Andaman Sea: $DML = -302.962 + 82.107 \ln(t)$

($R^2 = 0.613$, n = 47, Residual standard error = 16.16)

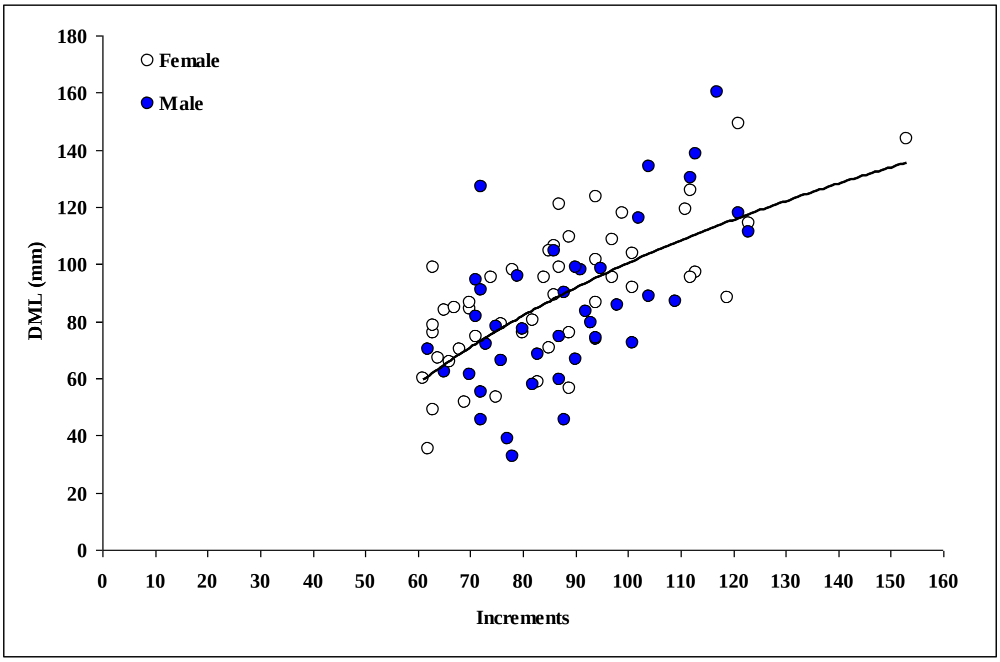


Figure 15 Relationship between age (number of increments) and DML of *L. duvauceli* from the Gulf of Thailand described by logarithmic function.

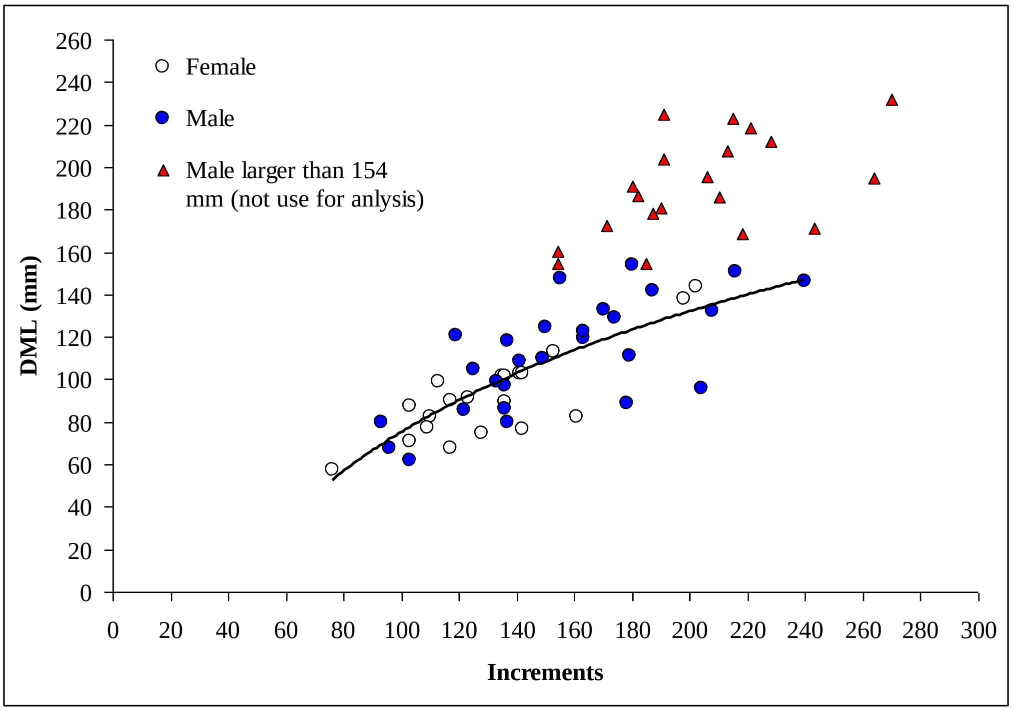


Figure 16 Relationship between age (number of increments) and DML for all samples of males and females from the Andaman Sea. Logarithmic regression line was estimated based on the same length range of females and males (excluding males which were larger than 154 mm).

3. Hatching date and spawning date back calculation

The hatching date and spawning date were estimated in order to support data for squid fisheries management. To estimate hatching date and spawning date of squid samples, the exact date of capture of each individual was important. Samples from the Andaman Sea were collected from commercial fishing vessels and squid catches from different hauls were mixed so the exact date of capture was not known. Therefore only samples from the Gulf of Thailand were used for this calculation.

Dates of hatching of *L. duvauceli* from the Gulf of Thailand were estimated by back calculation from the date of capture and the age estimated from statolith daily increments, assuming that the natal ring was formed on the day of hatching. *L. duvauceli* samples were collected from four cruises in January, March, July and August-September 2008. The estimated hatching dates were calculated and shown in Figure 17. Two dominant hatching periods are revealed for *L. duvauceli* in the middle Gulf of Thailand. The first hatching period was estimated to occur from day 280-310 (October 7- November 6) in 2007 while second hatching period was around day 130-180 (May 10 - June 29) in 2008.

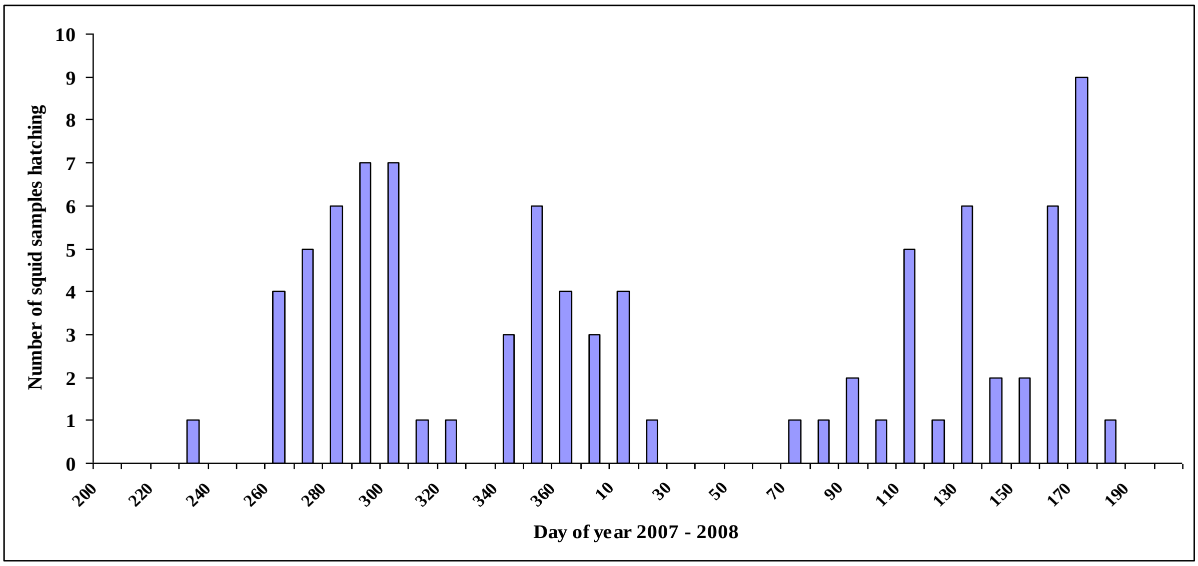


Figure 17 Number of *L. duvauceli* individuals from Gulf of Thailand hatching within 10-day periods during 2007-2008.

Estimated spawning dates of squid were calculated and compared with reported spawning seasons of this species from previous studies. Spawning dates were estimated by back calculation of hatching date and egg incubation time which was based on experimental rearing of *L. duvauceli*. Wudthisin and Singhagraiwan (1988) reported an egg incubation time of 8 - 10 days at 27-29 ºC and 31-34 psu. In this study, an average incubation time of 9 days was used to calculate estimated spawning date. Comparison between estimated spawning date of *L. duvauceli* samples and the dominant spawning seasons; January - April and June - July (Supongpan and Sinoda, 1998) and February - May and June - October (Chotiyaputta, 1996) revealed that estimated spawning dates from this study were coincident with the second dominant spawning season identified in previous studies (Figure 18).

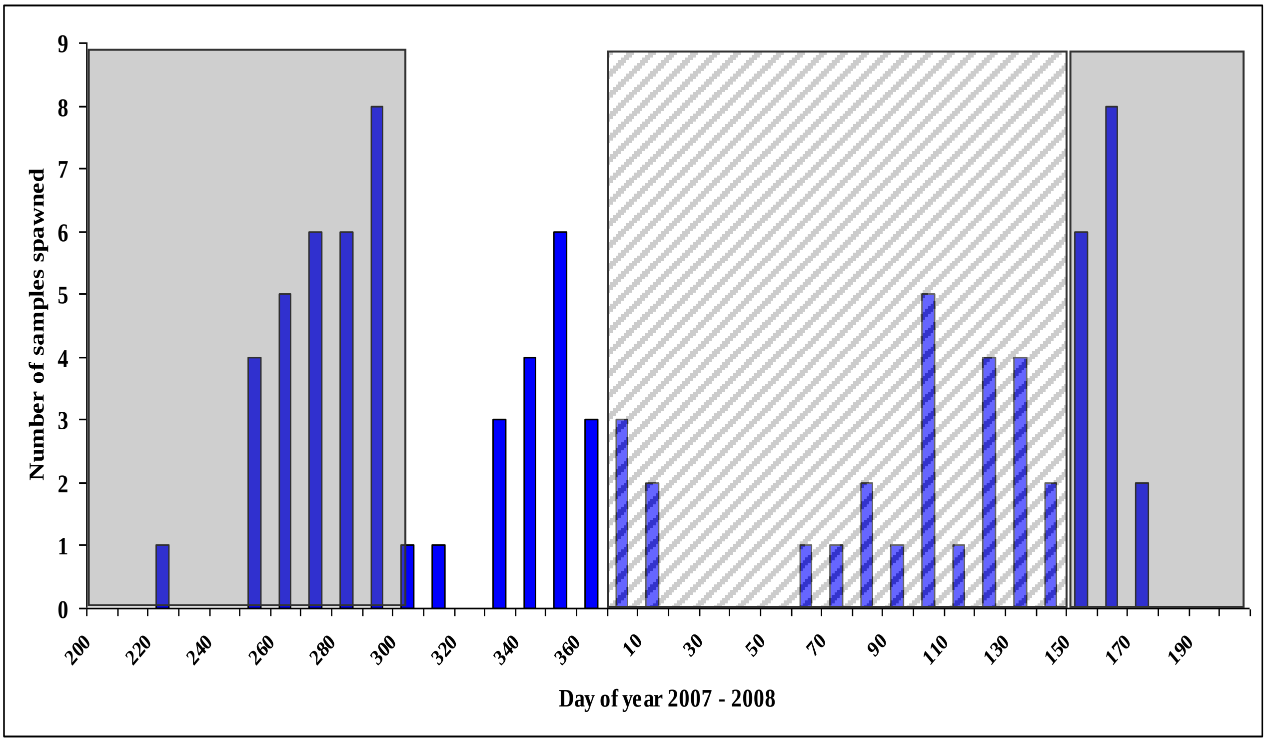


Figure 18 Number of *L. duvauceli* from the Gulf of Thailand spawned within10-day intervals during 2007 and 2008 (bars) compared with reported spawning seasons; first peak between January- May (1-151) and second peak June - October (152-304).

4. Morphological patterns and Statolith length

The sexual dimorphism of *L. duvauceli* was investigated in the two squid populations to express at least environment induced morphological changes. The mean, standard deviation and range of variation for each morphological variable and statolith length was calculated for the total samples from both the Gulf of Thailand and the Andaman Sea (Tables 8 and 9).

4.1 Study of sex differences in morphological variables and statolith length

The linear regression between DML and each variable of squid from the Gulf of Thailand and the Andaman Sea were calculated for each sex. For samples from the Andaman Sea, to prevent size effects, the linear regression was calculated for males and females in the same length range (54 – 154 mm). The slopes and intercepts for each variable were compared between sexes using ANCOVA (Tables 10 and 11).

Six morphological variables of squid from the Gulf of Thailand showed significant differences between sexes in relation to DML: Total weight (TW), Fin length (FL), Head length (HL), Length of the 4^{th} left arm (4AL), Gill length (GL) and Pen width (PW). The statolith length (SL) was not significantly different in the regression slope between sexes.

For the Andaman Sea, 13 of 15 morphological variables showed significant differences between sexes indicating that squid from the Andaman Sea were more sexually dimorphic than squid in the Gulf of Thailand. And also the statolith length (SL) was significantly different in regression slope between sexes.

Table 8 Morphometric variables and statolith length of *L. duvauceli* from the Gulf of Thailand.

(Unit = mm)

		Female					Male				
		n	**Mean**	**SD**	**Min**	**Max**	**n**	**Mean**	**SD**	**Min**	**Max**
Total weight	TW	198	22.87	8.59	8.18	46.74	48	22.12	8.26	11.72	49.99
Dorsal mantle length	DML	198	87.66	12.74	60.07	120.86	48	88.19	14.09	63.82	127.18
Dorsal mantle width	DMW	198	26.32	3.01	19.65	35.83	48	25.19	2.74	18.95	32.49
Fin length	FL	198	45.87	7.40	29.82	64.15	48	44.62	8.70	29.60	68.66
Fin width	FW	198	42.27	6.63	25.92	61.53	48	41.14	7.08	30.57	65.46
Head length	HL	198	18.48	2.29	11.90	24.46	48	18.86	1.95	15.46	23.04
Head width	HW	198	12.14	1.77	7.70	16.89	48	12.33	1.72	9.71	17.95
Length of 4th left arm	4AL	195	33.68	6.63	18.96	51.13	47	37.33	4.33	27.53	49.65
Length of tentacle	TL	184	126.45	18.87	82.74	194.54	46	121.76	20.13	91.58	181.60
Length of tentacle club	TC	184	30.69	5.22	20.73	50.82	46	28.12	3.98	21.54	39.18
Nuchal cartilage length	NCL	198	15.25	1.92	9.79	19.67	48	15.43	1.99	11.82	19.67
Funnel cartilage length	FCL	198	11.44	1.34	7.83	14.63	48	11.42	1.39	9.22	15.48
Mantle circumference	MC	198	59.63	6.00	47.38	76.16	48	58.21	6.06	46.37	75.60
Gill length	GL	198	28.81	4.74	16.62	41.17	48	29.47	3.68	21.76	38.52
Pen length	PL	198	85.29	12.54	56.40	118.59	48	85.65	13.81	60.67	122.96
Pen width	PW	198	13.49	2.45	7.29	19.74	48	12.86	1.44	10.73	17.01
Statolith length	SL	149	1.2146	0.0699	1.0567	1.3912	32	1.2295	0.0815	1.0450	1.3852

Table 9 Morphometric variables and statolith length of total samples of *L. duvauceli* from the Andaman Sea (including males which were larger than 154 mm)

(Unit = mm)

		Female					Male				
		n	**Mean**	**SD**	**Min**	**Max**	**n**	**Mean**	**SD**	**Min**	**Max**
Total weight	TW	156	29.51	14.10	8.24	108.10	171	49.11	31.32	7.13	121.31
Dorsal mantle length	DML	156	92.94	14.93	57.73	153.54	171	129.71	46.60	54.88	231.89
Dorsal mantle width	DMW	156	29.91	3.85	22.96	45.29	171	33.74	5.72	22.09	47.82
Fin length	FL	156	45.65	9.25	25.95	78.50	171	69.04	29.00	23.40	131.83
Fin width	FW	156	48.11	8.37	28.14	79.42	171	64.01	21.39	26.41	113.93
Head length	HL	156	19.14	2.58	13.82	28.42	171	21.18	3.60	13.38	29.22
Head width	HW	156	11.48	1.78	8.52	17.84	171	12.55	2.07	7.69	17.30
Length of 4^{th} left arm	4AL	156	36.74	7.41	19.38	59.84	171	43.06	8.37	22.13	61.27
Length of tentacle	TL	156	150.41	22.96	88.93	207.24	171	158.80	26.91	97.57	214.60
Length of tentacle club	TC	156	28.68	6.11	18.64	51.35	171	30.92	6.01	13.96	46.03
Nuchal cartilage length	NCL	156	15.29	2.21	10.30	24.77	171	18.53	4.14	10.33	27.05
Funnel cartilage length	FCL	156	11.18	1.58	7.94	18.45	171	13.10	2.76	7.03	19.74
Mantle circumference	MC	156	64.61	7.61	46.25	95.99	171	71.02	11.79	45.20	95.73
Gill length	GL	156	26.84	4.78	15.90	43.40	171	33.07	9.63	13.24	54.59
Pen length	PL	156	92.58	14.90	57.19	152.71	171	129.32	46.59	54.65	231.56
Pen width	PW	156	16.22	2.86	8.92	25.36	171	16.45	3.59	9.07	23.05
Statolith length	SL	155	1.2045	0.0984	0.9975	1.4920	171	1.2476	0.1122	0.9718	1.4815

Table 10 Results of ANCOVA between sexes for regression of morphometric variables and dorsal mantle length of the total samples of *L. duvauceli* from the Gulf of Thailand.

*indicates significant difference

		Female			**Male**			**Intercept comparison**		**Slope comparison**	
		Intercept	**slope**	**n**	**Intercept**	**slope**	**n**	**p-value**		**p-value**	
Total weight	TW	-32.940	0.637	198	-27.227	0.560	48	0.052		0.020	*
Dorsal mantle width	DMW	10.633	0.179	198	12.556	0.143	48	0.349		0.122	
Fin length	FL	-2.023	0.546	198	-8.701	0.605	48	0.009	*	0.040	*
Fin width	FW	1.720	0.463	198	-0.657	0.474	48	0.442		0.745	
Head length	HL	7.927	0.120	198	13.105	0.065	48	4.31E-03	*	6.64E-03	*
Head width	HW	2.972	0.105	198	4.296	0.091	48	0.282		0.331	
Length of 4^{th} left arm	4AL	-1.010	0.397	195	20.774	0.188	47	1.64E-06	*	3.73E-05	*
Length of tentacle	TL	34.504	1.057	184	16.052	1.205	46	0.196		0.357	
Length of tentacle club	TC	4.744	0.298	184	8.554	0.223	46	0.311		0.077	
Nuchal cartilage length	NCL	3.691	0.132	198	4.625	0.123	48	0.345		0.403	
Funnel cartilage length	FCL	3.693	0.088	198	3.473	0.090	48	0.767		0.834	
Mantle circumference	MC	23.997	0.407	198	24.482	0.382	48	0.877		0.495	
Gill length	GL	0.223	0.326	198	11.487	0.204	48	4.61E-06	*	9.13E-06	*
Pen length	PL	-0.513	0.979	198	-0.484	0.977	48	0.984		0.894	
Pen width	PW	0.824	0.145	198	7.832	0.057	48	2.29E-05	*	2.67E-06	*
Statolith length	SL	0.85473	0.00414	149	0.86380	0.00396	32	0.874		0.772	

Table 11 Results of ANCOVA between sexes for regression of morphometric variables and dorsal mantle length for the same length range of male and female *L. duvauceli* from the Andaman Sea (excluding males which were larger than 154mm).

*indicates significant difference

		Female			Male			Intercept comparison		Slope comparison	
		Intercept	**slope**	**n**	**Intercept**	**slope**	**n**	**p-value**		**p-value**	
Total weight	TW	-53.365	0.892	156	-21.225	0.504	116	<2e-16	*	<2e-16	*
Dorsal mantle width	DMW	9.654	0.218	156	17.855	0.126	116	4.80E-09	*	1.54E-10	*
Fin length	FL	-9.818	0.597	156	-10.117	0.606	116	0.856		0.601	
Fin width	FW	1.245	0.504	156	5.800	0.447	116	0.049	*	0.016	*
Head length	HL	8.168	0.118	156	11.757	0.075	116	0.005	*	0.001	*
Head width	HW	3.024	0.091	156	7.529	0.040	116	6.07E-08	*	2.86E-09	*
Length of 4^{th} left arm	4AL	0.785	0.387	156	23.052	0.162	116	3.22E-10	*	4.71E-10	*
Length of tentacle	TL	51.190	1.068	156	82.684	0.632	116	0.003	*	6.94E-05	*
Length of tentacle club	TC	0.062	0.308	156	15.546	0.126	116	2.89E-09	*	1.24E-11	*
Nuchal cartilage length	NCL	3.462	0.127	156	7.337	0.087	116	4.00E-06	*	3.14E-06	*
Funnel cartilage length	FCL	3.461	0.083	156	6.092	0.054	116	1.49E-04	*	4.04E-05	*
Mantle circumference	MC	21.813	0.461	156	37.375	0.267	116	3.83E-10	*	5.85E-14	*
Gill length	GL	4.520	0.240	156	7.717	0.196	116	0.136		0.044	*
Pen length	PL	-0.146	0.998	156	-0.266	0.999	116	0.571		0.610	
Pen width	PW	1.113	0.163	156	5.599	0.087	116	1.94E-07	*	< 2e-16	*
Statolith length	SL	0.831	0.004	155	0.985	0.002	116	2.15E-03	*	1.28E-04	*

4.2 Statolith growth

Because statolith length was increasing relative to increasing dorsal mantle length, comparison of statolith growth and somatic growth were investigated. Since there was no significant difference in statolith length between sexes for samples from the Gulf of Thailand (ANCOVA, p-value > 0.05), the relationship between statolith length and dorsal mantle length was estimated for sexes combined and described by the following equation.

$$\log SL = -0.493 + 0.298 \log DML \quad (R^2 = 0.618, n=181)$$

The relationship between log transformed statolith length (logSL) and log transformed dorsal mantle length (logDML) is shown Figure 19. The slope of this regression was 0.298, and since it is < 1 this indicates that dorsal mantle length increased more rapidly than statolith length.

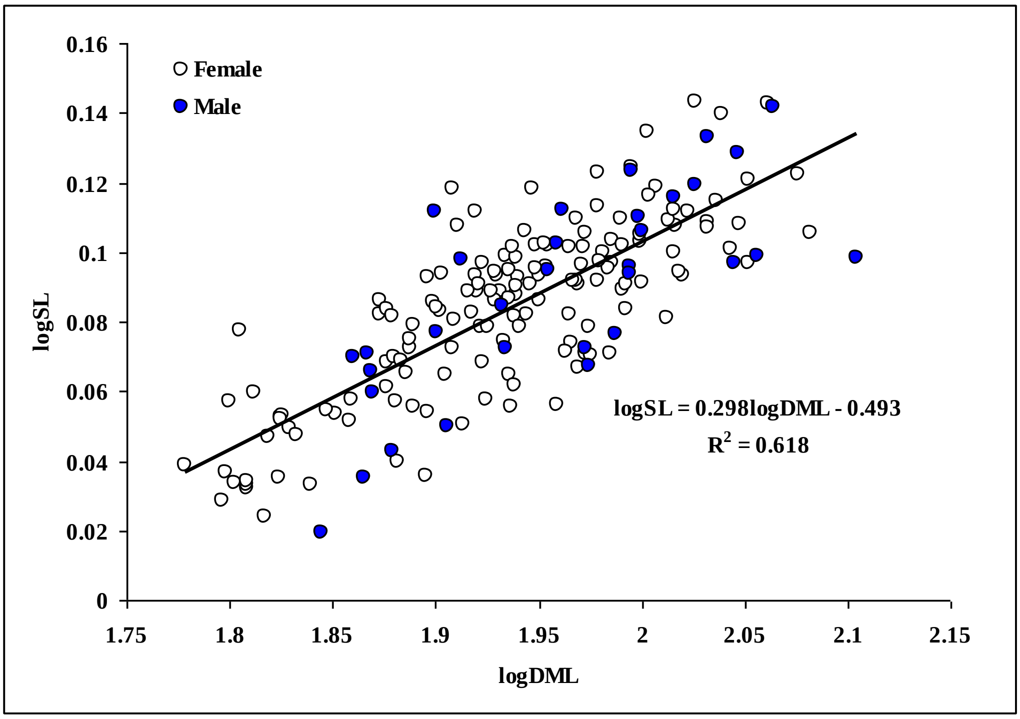


Figure 19 Relationship between log dorsal mantle length and log statolith length of *L.duvauceli* from the Gulf of Thailand.

For the Andaman Sea, there was a significant difference in statolith length between sexes (ANCOVA, p-value < 0.05). The relationship between logSL and logDML was estimated by sexes separately (Figure 20) and described by the following equations.

Female: logSL = - 0.576 + 0.334logDML (R^2 = 0.407, n=155)

Male: logSL = - 0.314 + 0.195logDML (R^2 = 0.382, n=116)

The slopes of these regressions were 0.334 for females which was significant higher than for males (0.195). The regression line of males and females cross at 1.89 of logDML (78 mm) revealing that statolith length of females was larger than males at the same dorsal mantle length when DML was larger than 78 mm. And since the slopes are < 1 this indicates that dorsal mantle length increased more rapidly than statolith length in both sexes.

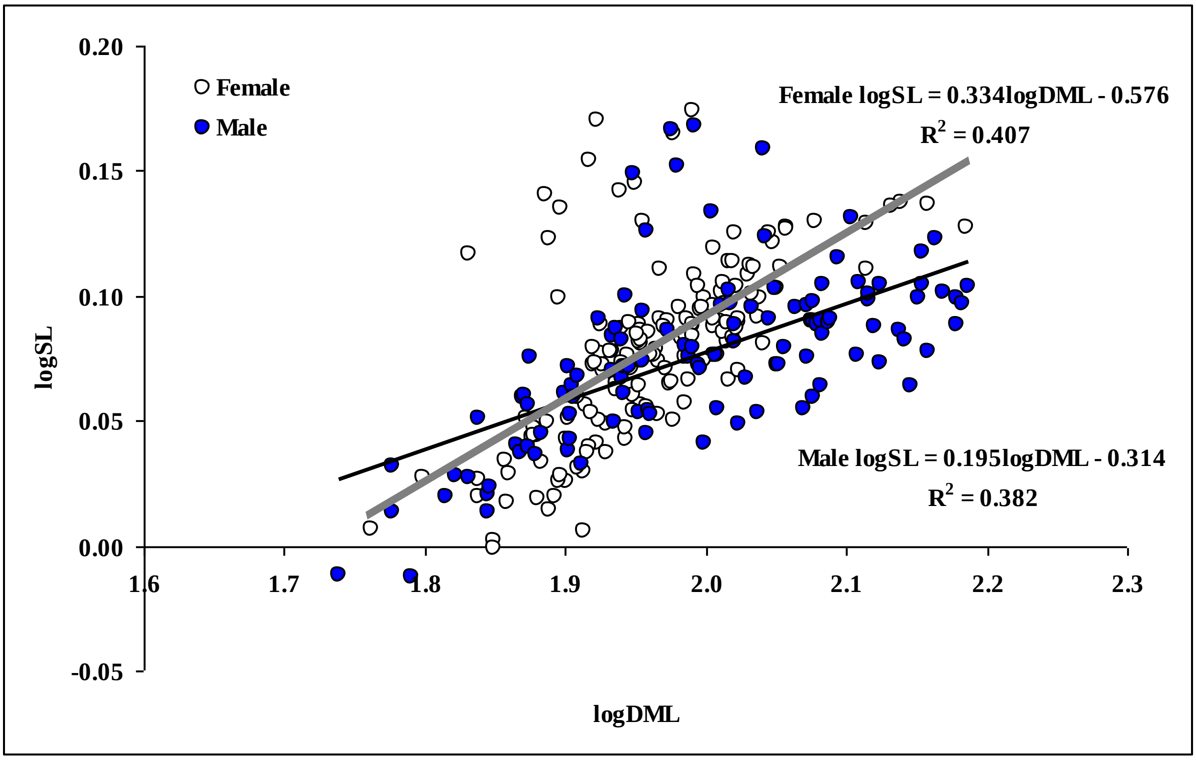


Figure 20 The relationship between logDML and logSL of the same length range between males and females *L. duvauceli* from the Andaman Sea (excluding males which were larger than 154mm).

5. The Length - weight relationship and relative condition factor (K)

The Length - Weight relationship of *L. duvauceli* was established in order to support data for the squid stock assessment research based on the length measurement (which is often more convenient to measure). The relative condition factor (K) of individual squids was estimated to reflect well-being of squids in each area and monsoon season.

5.1 Gulf of Thailand

The length and weight of 189 males and 371 females from the Gulf of Thailand were measured. The relationships between log transformed dorsal mantle length (logDML) and log transformed total weight (logTW) were significantly different between males and females (ANCOVA, p-value <0.05, Table 12). The relationship of logDML and logTW of *L. duvauceli* for each sex is shown in Figure 21. The regression line of males and females cross at 1.86 of logDML (73 mm) revealing that for squid larger than 73 mm, females are heavier than males at the same dorsal mantle. The estimated parameter b is:

Male : logTW = - 3.272 + 2.368logDML (n=189, R^2 = 0.970) b = 2.368

Female : logTW = - 3.677 + 2.586logDML (n=371, R^2 = 0.962) b = 2.586

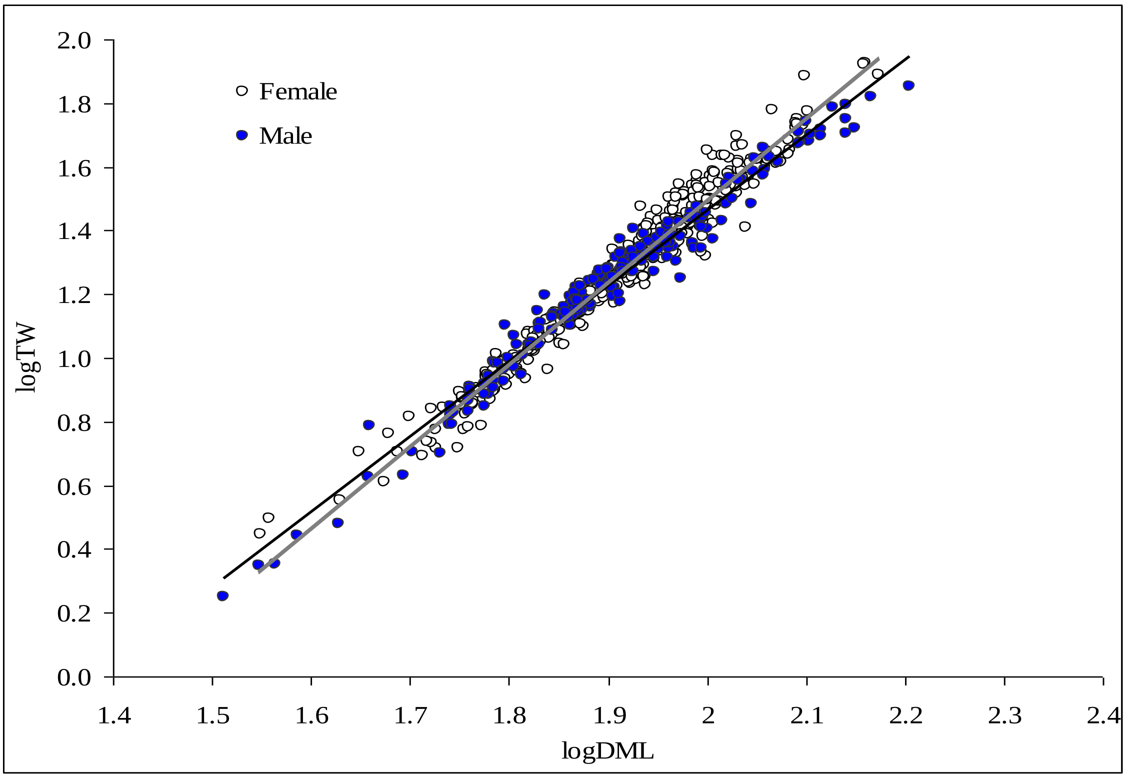


Figure 21 The relationship between logDML and logTW for male and female *L. duvauceli* from the Gulf of Thailand.

Table 12 ANCOVA table (a) and Coefficients (b) of logDML and logTW relationship between sexes of *L. duvauceli* from the Gulf of Thailand.

(a)

	df	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	1	0.367	0.367	146.589	< 2.2E-16
logDML	1	38.546	38.546	15398.269	< 2.2E-16
Sex:logDML	1	0.072	0.072	28.796	1.18E-07
Residuals	556	1.392	0.003		

(b)

	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-3.677	0.051	-72.340	< 2E-16
Sex.Male	0.405	0.078	5.206	2.72E-07
logDML	2.586	0.026	97.765	< 2E-16
Sex.Male:logDML	-0.218	0.041	-5.366	1.18E-07

Residual standard error: 0.05003 with 556 degrees of freedom, Multiple R-squared: 0.9655,Adjusted R-squared: 0.9653, $F_{3,556}$ =5191 p-value: < 2.2E-16

The average relative condition factors (K) of *L. duvauceli* which were sampled during the northeast monsoon was 0.000361 which was significantly higher than 0.000271 for squid sampled during the southwest monsoon (t-test, p-value <0.05). And the K values of *L. duvauceli* sampled from the North area (mean=0.000310) were significantly lower than the South (mean= 0.000338) (t-test, p-value < 0.05, Table 13).

Table 13 Two sample t-test of relative condition factor (K) between monsoons and areas of *L. duvauceli* from the Gulf of Thailand.

	Mean	SD	t	df	p-value
Monsoon					
Northeast monsoon	0.000361	0.000167			
Southwest monsoon	0.000271	0.000133	6.867	558	1.75E-11
Area					
North	0.000310	0.000155			
South	0.000338	0.000165	-2.052	558	0.041

5.2 Andaman Sea

The lengths and weights were measured of 171 male and 156 female squid sampled from the Andaman Sea.

The size range of males *L. duvauceli* from the Andaman Sea included individuals much larger than the females or males and females from the Gulf of Thailand. To prevent size effects in the analysis, the same length of male and female were used for comparisons, removing males which were larger than 154 mm from the dataset before analysis. The relationship between log transformed dorsal mantle length (logDML) and log transformed total weight (logTW) of total samples is shown in Figure 22. Analysis of covariance tests comparing males and females between 54 - 154 mm showed significant differences between sexes (ANCOVA, p-value < 0.05, Table 14). The regression line of males and females crosses at 1.89 of logDML (78 mm), and for squid larger than 78 mm, females are heavier than males at the same dorsal mantle length. The relationship of logDML and logTW of *L. duvauceli* by sexes separately and estimated parameter b as follow.

Male : logTW = - 2.224 + 1.834logDML (n=116, R^2 = 0.904) b = 1.834

Female : logTW = - 3.757 + 2.642logDML (n=156, R^2 = 0.918) b = 2.642

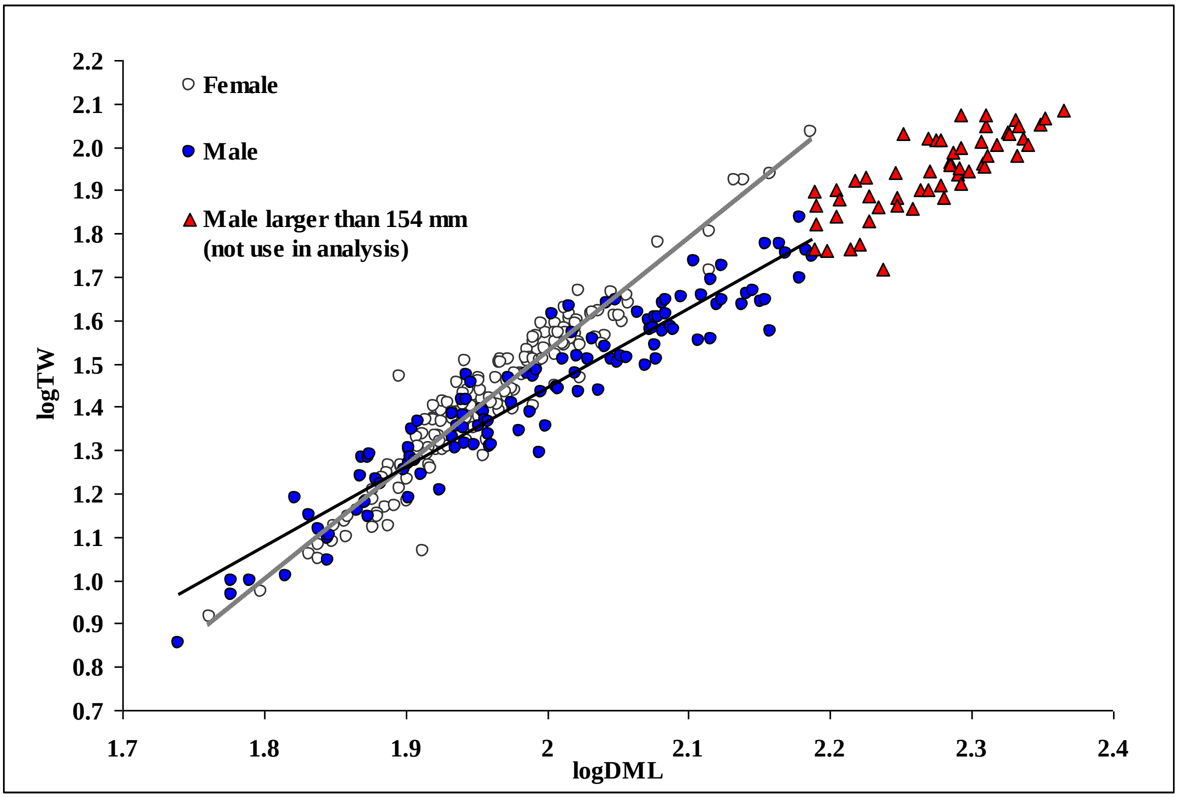


Figure 22 The relationship between logDML and logTW of total samples of males and females *L. duvauceli* from the Andaman Sea. Regression line was estimated based on males ranged from 54.88 to 153.91 mm and females ranged from 57.73 to 153.54 mm.

Table 14 ANCOVA table (a) and Coefficients (b) of logDML and logTW relationship between sexes of *L. duvauceli* from the Andaman Sea (excluding males larger than 154 mm).

(a)

	df	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	1	0.002	0.002	0.727	0.395
logDML	1	8.890	8.890	2679.857	<2E-16
Sex:logDML	1	0.296	0.296	89.351	<2E-16
Residuals	268	0.889	0.003		

(b)

	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-3.757	0.135	-27.900	<2E-16
Sex.Male	1.533	0.169	9.064	<2E-16
logDML	2.642	0.069	38.539	<2E-16
Sex.Male:logDML	-0.809	0.086	-9.453	<2E-16

Residual standard error: 0.05759 on 268 degrees of freedom, Multiple R-squared: 0.9118, Adjusted R-squared: 0.9108, $F_{3,268}$ = 923.3, p-value: < 2.2E-16

The average relative condition factors (K) of *L. duvauceli* sampled from the North area of the Andaman Sea was 0.00242 which was not significantly different from 0.00279, the value for samples from the South area. (t-test, p-value > 0.05, Table 15).

Table 15 Two sample t-test of relative condition factor (K) of *L. duvauceli* between areas in the Andaman Sea (excluding males larger than154 mm).

	Mean	SD	t	df	p-value
Area					
North	0.00242	0.00291			
South	0.00279	0.00291	-0.975	270	0.330

6. Maturity

The estimated size and age at first maturity was compared between sexes and squid populations to study recent patterns in squid reproductive strategy. The maturity was categorized into Immature for stage I to II and Mature for stage III to VI. The total samples from the Gulf of Thailand and the same length range of males and females from the Andaman Sea were used (excluding males which were larger than 154 mm from the dataset before analysis).

Mean and 0.95 confidence intervals of DML and age for Immature and Mature squid were calculated by sex separately revealed that maturity of *L. duvauceli* was more distinctly separated by dorsal mantle length than age for both sexes (Figure 23).

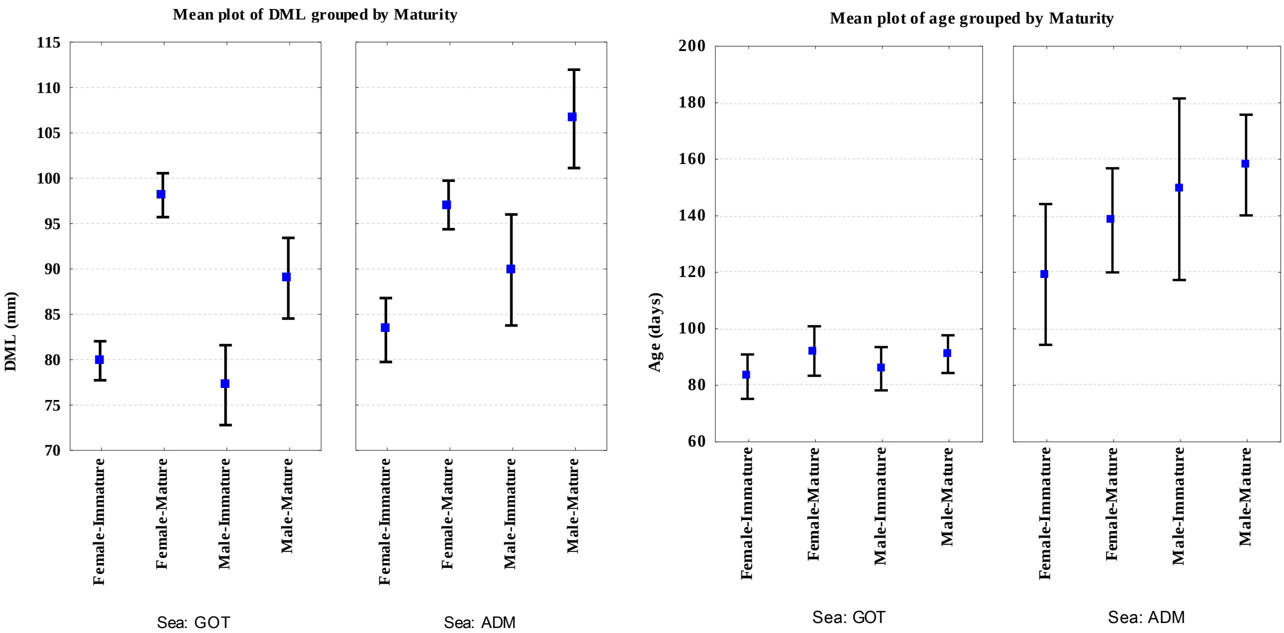


Figure 23 Mean of DML and age by maturity stage for females and males of the total samples of *L. duvauceli* from the Gulf of Thailand (left-hand panels in each set) and the same length range between males and females from the Andaman Sea (right-hand panels in each set). Square symbols represent means with error bars indicating the 0.95 confidence intervals.

The sizes at 50% maturity (DML_{50}%) of *L. duvauceli* from the Gulf of Thailand were 78.90 mm for males and 94.05 mm for females. The age at 50% maturity ($t_{50\%}$) was 83.99 days for males and 85.84 days for females. For *L. duvauceli* from the Andaman Sea, $DML_{50\%}$ was 100.69 mm for males and 91.52 mm for females while $t_{50\%}$ was 146.24 days for males and 133.81 days for females (Figure 24 and Table 16).

The $t_{50\%}$ of *L.duvauceli* from Gulf of Thailand was a similar age for both sexes (Figure 24c), however in terms of length, $DML_{50\%}$ of males was smaller than females (Figure 24a). For the Andaman Sea, $t_{50\%}$ and $DML_{50\%}$ of females was earlier and smaller than for males, which was the opposite pattern to the squid from the Gulf of Thailand (Figure 24b and d).

The maturity ogives showed that maturation of male *L.duvauceli* occured at smaller size than females in both seas (Figure 24a and b). In terms of age, both male and female matured at similar ages (Figure 24c and d).

The maturation of female *L.duvauceli* from the Gulf of Thailand occured between 70 - 75mm DML (Figure 24a) but in females from the Andaman Sea the pattern was more extended, with squid beginning to mature between 70 - 75mm DML and then a sharp increase in the proportion mature between 75-80 mm DML (Figure 24b). In both populations, the size at which females become mature coincided with the size at which females were heavier than males from the logDML-logTW relationship (73mm for the Gulf of Thailand and 78 mm for the Andaman Sea). This pattern was also coincident with the size at which statolith length of females were larger than males from the logDML-logSL relationship (78 mm for the Andaman Sea).

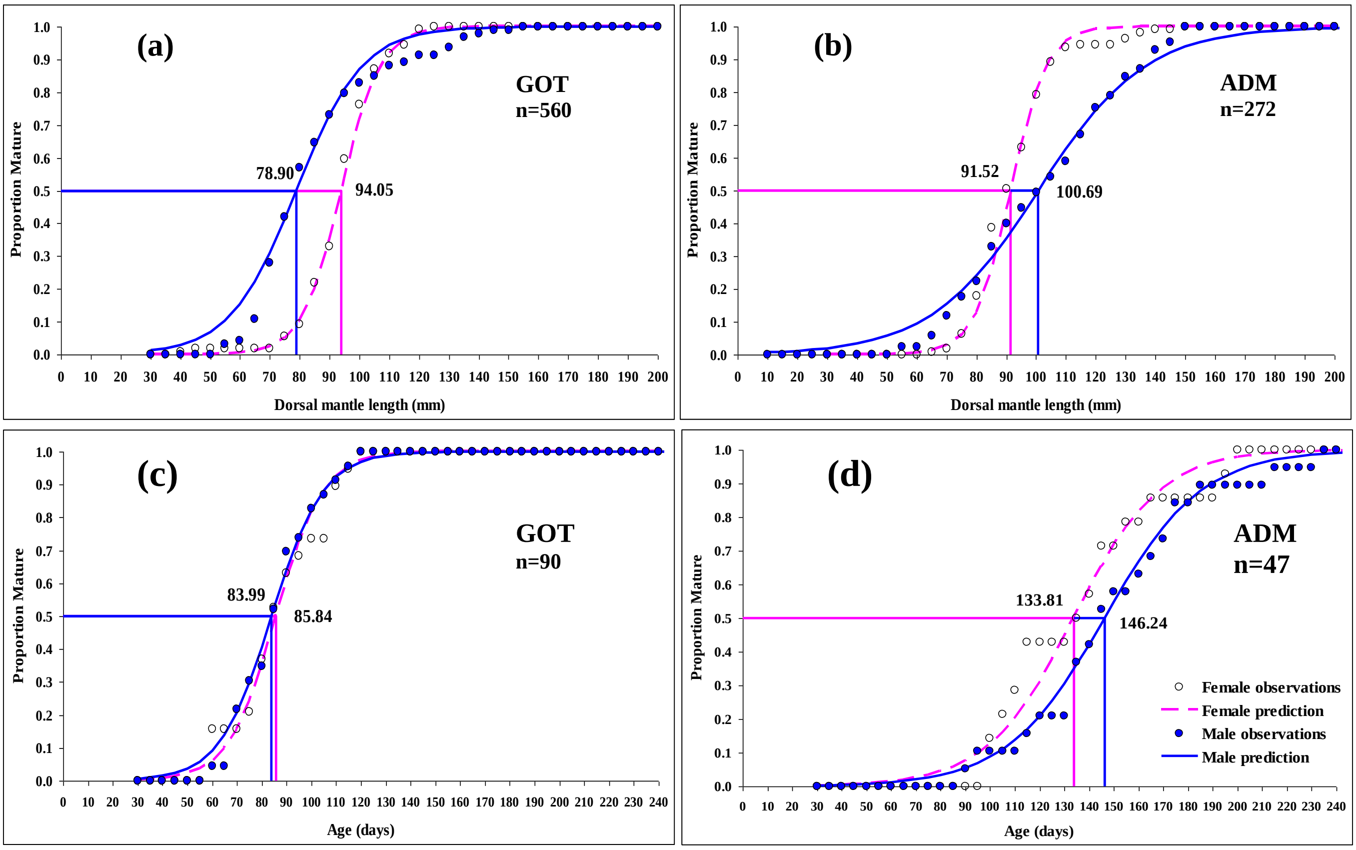


Figure 24 Proportion mature by DML class (a) (b), age group (c) (d) and estimated maturity ogives for females and males of total samples of *L.duvauceli* from the Gulf of Thailand and the same length range of males and females from the Andaman Sea (excluding males which were larger than 154 mm).

Table 16 Size and age at first maturity of total samples of *L.duvauceli* from the Gulf of Thailand and the same length range between males and females from the Andaman Sea (excluding males which were larger than 154 mm).

Sea	**Sex**	**Size at first maturity (mm)**			**Age at first maturity (days)**		
		$DML_{25\%}$	$DML_{50\%}$	$DML_{75\%}$	$t_{25\%}$	$t_{50\%}$	$t_{75\%}$
Gulf of Thailand	Female	86.94	94.05	101.16	75.43	85.84	96.26
	Male	66.75	78.90	91.06	72.50	83.99	95.48
Andaman Sea	Female	84.87	91.52	98.17	114.57	133.81	153.05
	Male	80.82	100.69	120.56	124.61	146.24	167.87

7. Stock discrimination

The morphological measurements and statolith shape were investigated for squid stock discrimination by using the same statistical analysis to discriminate appropriate stocks which can support better for stock assessment to reflect stock status.

7.1 Morphological variables for stock discrimination

The morphological measurements (15 variables) of four sample groups; GOT North, GOT South, ADM North and ADM South were standardized by DML and compared using ANOVA. For squid from the Andaman Sea (ADM), the same length range between males and females were used, excluding males which were larger than 154 mm from the dataset before analysis. Fourteen standardized morphological variables showed significant differences between the four groups and were selected as Discrimination Functions (DF) for Discriminant analysis. The list of significantly different standardized morphological variables and p-values is shown in Table 17.

The 14 DF were categorized into Immature for maturity stage I and II and Mature for stage III to VI and then tested for effects of maturity by using MANOVA for each sample group. Results showed significant differences between Immature and Mature for four sample groups as shown in Table 18. Therefore Discriminant Analysis was used to discriminate *L. duvauceli* stocks from the Gulf of Thailand and the Andaman Sea for Immature and Mature squid separately (Table 19). The classification matrix from Discriminant Analysis showed a high percentage of correct classifications of Immature squid (GOT North=87.037, GOT South= 62.000, ADM North= 69.565 and ADM South=88.679) but the percentage of correct classifications decreased for Mature squid (GOT North=65.517, GOT South= 76.744, ADM North= 50.794 and ADM South= 87.218).

Table 17 ANOVA table of standardized morphological variables between the four groups of *L. duvauceli* from Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

*indicates significant difference

		df	Sum Sq	Mean Sq	F value	P value	
Standardized total weight	StdTW	3	0.307	0.102	19.992	3.01E-12	*
		497	2.547	0.005			
Standardized dorsal mantle width	StdDMW	3	0.072	0.024	21.04	7.61E-13	*
		497	0.564	0.001			
Standardized fin length	StdFL	3	0.084	0.028	27.4	2.22E-16	*
		497	0.505	0.001			
Standardized fin width	StdFW	3	0.135	0.045	33.30	0.00	*
		497	0.673	0.001			
Standardized head length	StdHL	3	0.029	0.010	12.95	3.73E-08	*
		497	0.366	0.001			
Standardized head width	StdHW	3	0.047	0.016	61.18	0.00	*
		497	0.127	0.000			
Standardized length of 4th left arm	Std4AL	3	0.018	0.006	1.68	0.171	
		497	1.783	0.004			
Standardized length of tentacle	StdTL	3	2.012	0.671	17.77	5.70E-11	*
		497	18.755	0.038			
Standardized length of tentacle club	StdTC	3	0.336	0.112	60.07	0.00	*
		497	0.925	0.002			
Standardized nuchal cartilage length	StdNCL	3	0.018	0.006	24.43	9.21E-15	*
		497	0.120	0.000			
Standardized funnel cartilage length	StdFCL	3	0.023	0.008	43.91	0.00	*
		497	0.087	0.000			
Standardized mantle circumference	StdMC	3	0.119	0.040	9.27	5.63E-06	*
		497	2.119	0.004			
Standardized gill length	StdGL	3	0.272	0.091	79.93	0.00	*
		497	0.564	0.001			
Standardized pen length	StdPL	3	0.072	0.024	217	0.00	*
		497	0.055	0.000			
Standardized pen width	StdPW	3	0.010	0.003	7.82	4.13E-05	*
		497	0.210	0.000			

Table 18 MANOVA of standardized morphological variables between Immature and Mature *L. duvauceli* from Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

*indicates significant difference

Sea	Sampling group	Comparing	Wilk's λ	F value	df Effect	df Error	P value	
Gulf of Thailand	GOT North	Immature&Mature	0.466	9.991	14	122	1.47E-14	*
	GOT South	Immature&Mature	0.626	3.335	14	78	3.33E-04	*
Andaman Sea	ADM North	Immature&Mature	0.607	3.278	14	71	4.82E-04	*
	ADM South	Immature&Mature	0.545	10.189	14	171	2.22E-16	*

Table 19 The Classification matrix of morphological measurements with percentage of correctly classified individuals from Discriminant Analysis for Immature and Mature *L. duvauceli* from Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

Sample group	Immature					Mature				
	Percent correct	Predicted classifications				Percent correct	Predicted classifications			
		GOT North	GOT South	ADM North	ADM South		GOT North	GOT South	ADM North	ADM South
GOT North	87.037	94	13	0	1	65.517	19	10	0	0
GOT South	62.000	19	31	0	0	76.744	10	33	0	0
ADM North	69.565	0	0	16	7	50.794	0	1	32	30
ADM South	88.679	1	1	4	47	87.218	0	0	17	116

The scatter plot of the Canonical Scores on the first two principal component axes for the four sample groups is shown in Figure 25 for Immature and Figure 26 for Mature squid. There were significant differences between the four sample groups for both Immature (Chi-Sqr= 489.869, Wilk's $\lambda = 0.112$, p-value<0.05) and Mature squid (Chi-Sqr.= 563.315, Wilk's $\lambda = 0.113$, p-value<0.05).

The first principal component axis was more powerful for discrimination than the second axis based on the morphological variables. The Canonical Scores for samples from the Gulf of Thailand were positive while the scores for the squid from the Andaman Sea were negative for both Immature and Mature and sharply separated the sample groups between the seas. The two sample groups in the same sea overlapped and were hard to distinguish by only the scatter plot of Canonical Scores. The squared Mahalanobis distances between group centroids in the multidimensional space and corresponding significance values were calculated to discriminate between the four sample groups. Results showed significant differences between the four group centriods based on squared Mahalanobis distances (p-value <0.05 for all cases, Table 20) for both Immature and Mature squid, suggesting four stocks of *L.duvauceli* among the samples.

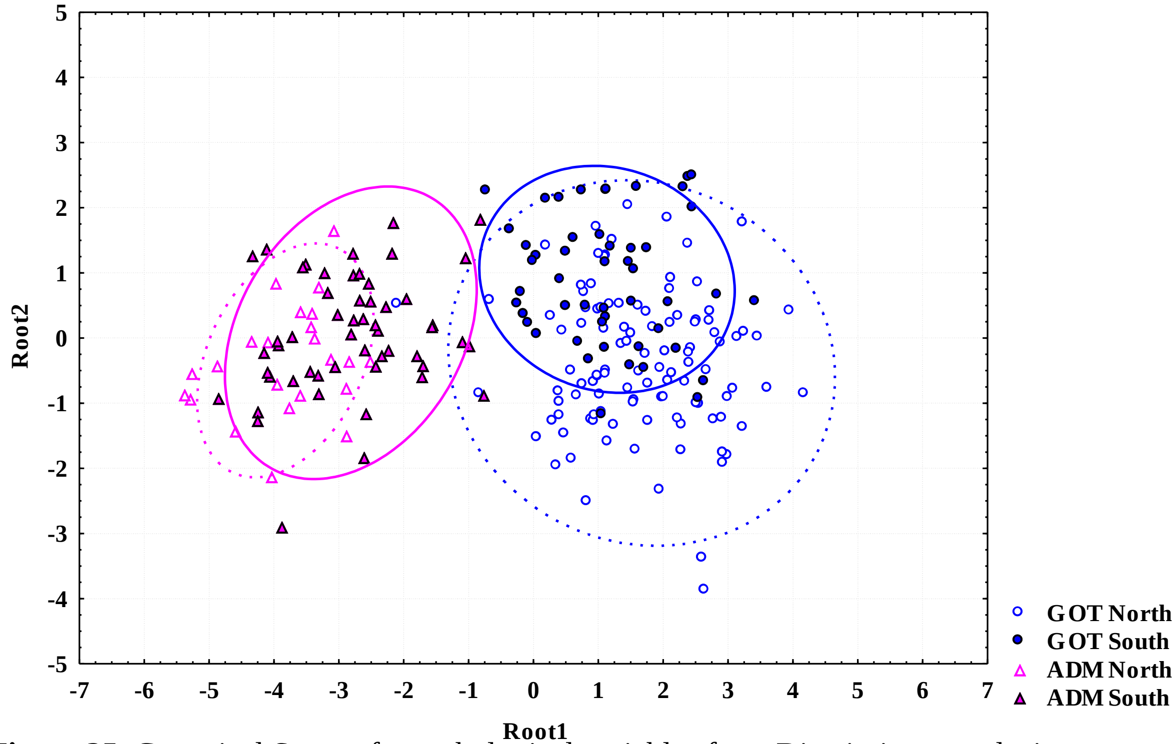


Figure 25 Canonical Score of morphological variables from Discriminant analysis for Immature *L.duvauceli*, fitted ellipses at 0.95 confidence level.

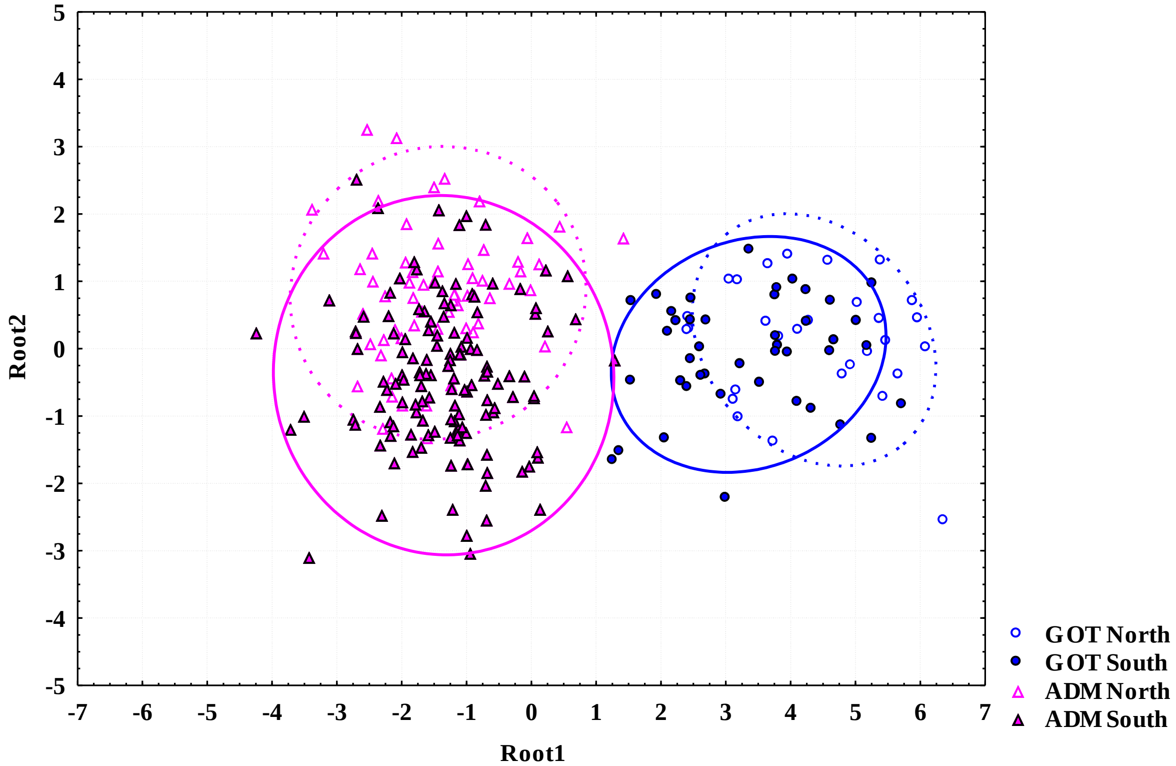


Figure 26 Canonical Scores of morphological variables from Discriminant analysis for Mature *L.duvauceli*, fitted ellipses at 0.95 confidence level.

Table 20 Squared Mahalanobis distances between groups of sample in the multidimensional space by Discriminant Analysis of morphological measurements for Immature and Mature *L. duvauceli* from the Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

Samples group		Immature				Mature			
		GOT North	GOT South	ADM North	ADM South	GOT North	GOT South	ADM North	ADM South
GOT North	Mahalanobis	0.000	1.999	31.393	20.633	0.000	2.927	34.761	33.423
	p-value		2.68E-07	0.000	0.000		3.89E-05	0.000	0.000
GOT South	Mahalanobis	1.999	0.000	26.866	16.909	2.927	0.000	24.119	22.692
	p-value	2.68E-07		0.000	0.000	3.89E-05		0.000	0.000
ADM North	Mahalanobis	31.393	26.866	0.000	3.938	34.761	24.119	0.000	1.508
	p-value	0.000	0.000		1.25E-06	0.000	0.000		5.52E-07
ADM South	Mahalanobis	20.633	16.909	3.938	0.000	33.423	22.692	1.508	0.000
	p-value	0.000	0.000	1.25E-06		0.000	0.000	5.52E-07	

7.2 Shape Analysis of statoliths for stock discrimination

The total 123 variables of statolith measurements (4 variables) and coefficients values of Elliptical Fourier Descriptors shape analysis (119 variables) were used to investigate *L. duvauceli* stock discrimination. The Area, Perimeter and Feret's diameter (length) of statolith were standardized by DML before being analyzed. The ANOVA was used to compare means within four sample groups; GOT North, GOT South, ADM North and ADM South. For squid from the Andaman Sea (ADM), the same length range of males and females was used, excluding males which were larger than 154 mm from the dataset before analysis. Ninety-two variables showed significant differences between the four sample groups and were selected as discrimination functions (DF) for Discriminant Analysis (Table 21).

The 92 DF were categorized into Immature (maturity stage I and II) and Mature (stage III to VI) and tested for the effect of maturity by using MANOVA for each sample group. Result showed significant differences between Immature and Mature squid for GOT North, GOT South and ADM South. For ADM North, the MANOVA was not possible because the residuals had fewer ranks (84) than variables (92) because the number of samples was less than the number of variables (Table 22). Therefore Discriminant Analysis was used to discriminate *L. duvauceli* stocks from the Gulf of Thailand and the Andaman Sea separated between Immature and Mature.

The classification matrix from Discriminant Analysis showed a high percentage of correct classifications for both samples in Immature (GOT North=90.865, GOT South= 73.874, ADM North= 82.609 and ADM South=83.019) and Mature squid (GOT North =85.714, GOT South= 74.713, ADM North= 79.365 and ADM South= 84.091, Table 23).

The scatter plot of the Canonical Scores on the first two principal component axes for the four sample groups is shown in Figure 27 for Immature and Figure 28 for Mature which were significantly different among the four sample groups (Immature: Chi-Sqr.= 674.718, Wilk's $\lambda = 0.142$, p-value<0.05) and (Mature: Chi-Sqr.= 643.505, Wilk's $\lambda = 0.125$, p-value<0.05). The Canonical Scores of the first principal component axis for Immature squid from the Andaman Sea were negative values and shifted to positive values for Mature squid, which was the opposite to the pattern for samples from the Gulf of Thailand. Although the four sample groups were partly overlapping, it was still possible to distinguish differences by a scatter plot of the Canonical Scores. The Squared Mahalanobis distances between group centroids in the multidimensional space and corresponding significance values were calculated and there were significant differences between the four groups (p value <0.05 for all cases, Table 24) for both Immature and Mature squid. This suggests four stocks of *L.duvauceli* among samples, similar to the morphological analysis.

Table 21 ANOVA table of statolith measurements and coefficients values of Elliptical Fourier Descriptors shape analysis among the four groups of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

(df = 3 and 750) *indicates significant difference

	F value	p-value			F value	p-value			F value	p-value			F value	p-value	
Circularity	28.885	0.000	*	a29	1.227	0.299		b30	2.830	3.76E-02	*	d1	6.673	1.89E-04	*
StdArea	64.626	0.000	*	a30	0.329	0.804		c1	0.741	0.527742		d2	28.022	0.000	*
StdPerimeter	44.399	0.000	*	b1	1.129	0.336		c2	55.684	0.000	*	d3	13.829	8.73E-09	*
StdFeret	41.765	0.000	*	b2	21.443	2.47E-13	*	c3	63.868	0.000	*	d4	10.190	1.39E-06	*
a2	30.528	0.000	*	b3	9.515	3.57E-06	*	c4	9.607	3.14E-06	*	d5	0.956	0.413	
a3	14.468	3.60E-09	*	b4	35.653	0.000	*	c5	41.231	0.000	*	d6	27.342	1.11E-16	*
a4	23.306	1.98E-14	*	b5	48.289	0.000	*	c6	7.535	5.69E-05	*	d7	10.186	1.40E-06	*
a5	24.961	2.11E-15	*	b6	63.175	0.000	*	c7	15.317	1.11E-09	*	d8	3.324	1.93E-02	*
a6	7.030	1.15E-04	*	b7	39.149	0.000	*	c8	5.307	1.27E-03	*	d9	1.177	0.318	
a7	5.488	9.86E-04	*	b8	26.433	3.33E-16	*	c9	5.396	1.12E-03	*	d10	6.705	1.81E-04	*
a8	56.670	0.000	*	b9	2.969	3.12E-02	*	c10	1.773	0.151		d11	2.765	4.10E-02	*
a9	9.640	3.00E-06	*	b10	7.999	2.98E-05	*	c11	6.516	2.36E-04	*	d12	15.281	1.17E-09	*
a10	45.177	0.000	*	b11	7.905	3.39E-05	*	c12	1.422	0.235		d13	10.700	6.82E-07	*
a11	8.429	1.63E-05	*	b12	3.355	1.85E-02	*	c13	7.299	7.91E-05	*	d14	1.227	0.299	
a12	52.464	0.000	*	b13	8.297	1.96E-05	*	c14	13.703	1.04E-08	*	d15	29.703	0.000	*
a13	21.185	3.51E-13	*	b14	21.924	1.29E-13	*	c15	2.951	3.20E-02	*	d16	5.977	4.99E-04	*
a14	12.124	9.36E-08	*	b15	6.252	3.41E-04	*	c16	19.040	6.58E-12	*	d17	13.430	1.52E-08	*
a15	1.200	0.309		b16	12.476	5.74E-08	*	c17	11.833	1.41E-07	*	d18	17.027	1.05E-10	*
a16	2.586	0.052		b17	6.119	4.10E-04	*	c18	8.962	7.74E-06	*	d19	2.130	0.095	
a17	4.092	6.78E-03	*	b18	6.585	2.14E-04	*	c19	21.843	1.44E-13	*	d20	12.676	4.34E-08	*
a18	2.340	0.072		b19	4.143	6.32E-03	*	c20	10.461	9.53E-07	*	d21	21.362	2.76E-13	*
a19	0.917	0.432		b20	8.740	1.06E-05	*	c21	3.922	8.55E-03	*	d22	3.252	2.13E-02	*
a20	2.620	4.98E-02	*	b21	1.970	0.117		c22	12.859	3.37E-08	*	d23	6.266	3.34E-04	*
a21	6.057	4.47E-04	*	b22	0.453	0.715		c23	18.965	7.29E-12	*	d24	13.456	1.47E-08	*
a22	6.417	2.71E-04	*	b23	4.032	7.35E-03	*	c24	0.833	0.476		d25	1.539	0.203	
a23	1.847	0.137		b24	8.789	9.86E-06	*	c25	12.687	4.27E-08	*	d26	6.800	1.59E-04	*
a24	2.015	0.110		b25	1.457	0.225		c26	3.663	1.22E-02	*	d27	8.449	1.59E-05	*
a25	9.359	4.44E-06	*	b26	0.615	0.605		c27	0.587	0.624		d28	0.868	0.457239	
a26	2.863	3.60E-02	*	b27	2.549	0.055		c28	7.392	6.94E-05	*	d29	3.729	1.11E-02	*
a27	1.017	0.384		b28	0.088	0.967		c29	1.899	0.128		d30	1.573	0.195	
a28	4.033	7.35E-03	*	b29	0.728	0.536		c30	1.557	0.199					

Table 22 MANOVA of standardized statolith measurements and coefficients values of Elliptical Fourier Descriptors shape analysis between Immature and Mature *L.duvauceli* from the Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

*indicates significant difference

Sea	**Sampling group**	**Comparing**	**Wilk's λ**	**F value**	**df Effect**	**df Error**	**P value**	
Gulf of Thailand	GOT North	Immature&Mature	0.279	5.39	92	192	0.000	*
	GOT South	Immature&Mature	0.419	1.58	92	105	0.012	*
Andaman Sea	ADM North	Immature&Mature	Residuals had rank (84) less than variables (92)					
	ADM South	Immature&Mature	0.369	1.71	92	92	0.005	*

Table 23 The classification matrix of statolith measurements and coefficients values with percentage of correctly classified individual from Discriminant Analysis for Immature and Mature *L. duvauceli* from the Gulf of Thailand and the Andaman Sea (excluding males which were larger than 154 mm for the Andaman Sea).

Sample group	**Immature**					**Mature**				
	Percent correct	**Predicted classifications**				**Percent correct**	**Predicted classifications**			
		GOT North	**GOT South**	**ADM North**	**ADM South**		**GOT North**	**GOT South**	**ADM North**	**ADM South**
GOT North	90.865	189	16	1	2	85.714	66	8	1	2
GOT South	73.874	22	82	3	4	74.713	11	65	3	8
ADM North	82.609	1	3	19	0	79.365	1	3	50	9
ADM South	83.019	0	9	0	44	84.091	5	12	4	111

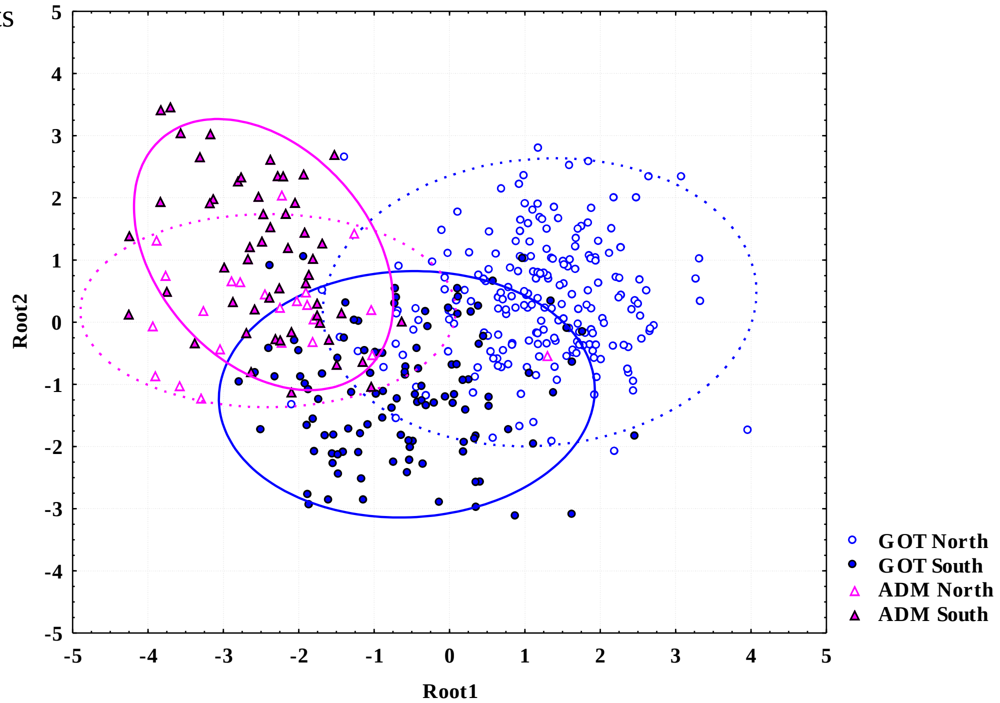


Figure 27 Canonical Scores of statolith measurements and coefficients values of Elliptical Fourier Descriptors from Discriminant Analysis for Immature squid, fitted ellipses at 0.95 confidence level.

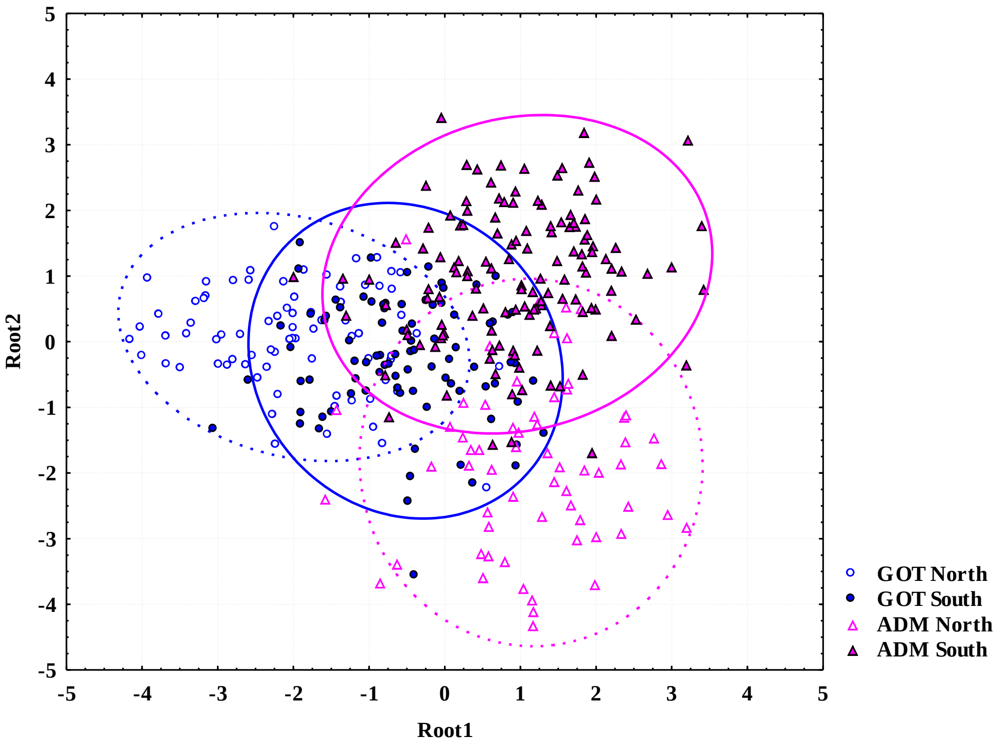


Figure 28 Canonical Scores of statolith measurements and coefficients values of Elliptical Fourier Descriptors from Discriminant Analysis for Mature squid, fitted ellipses at 0.95 confidence level.

Table 24 The squared Mahalanobis distances between sample groups in the multidimensional space by Discriminant Analysis of statolith measurements and coefficients values for Immature and Mature *L. duvauceli* from the Gulf of Thailand and the Andaman (excluding males which were larger than 154 mm for the Andaman Sea).

Sample group		Immature				Mature			
		GOT North	GOT South	ADM North	ADM South	GOT North	GOT South	ADM North	ADM South
GOT North	Mahalanobis	0.000	5.388	18.675	14.741	0.000	6.443	13.538	9.979
	p-value		1.23E-14	1.90E-14	0.000		1.55E-06	0.000	0.000
GOT South	Mahalanobis	5.388	0.000	12.434	8.971	6.443	0.000	8.361	6.015
	p-value	1.23E-14		9.04E-06	1.29E-10	1.55E-06		9.28E-09	2.75E-09
ADM North	Mahalanobis	18.675	12.434	0.000	11.343	13.538	8.361	0.000	8.375
	p-value	1.90E-14	9.04E-06		0.005	0.000	9.28E-09		1.57E-11
ADM South	Mahalanobis	14.741	8.971	11.343	0.000	9.979	6.015	8.375	0.000
	p-value	0.000	1.29E-10	0.005		0.000	2.75E-09	1.57E-11	

Discussion

1. Summary of the data

L. duvauceli were sampled from the Gulf of Thailand at 10 – 60 m water depth. The DML of males and females were similar and ranged from 32 to 160 mm. For the Andaman Sea, *L. duvauceli* were sampled at 20 – 140 m water depth and squid size was larger than the Gulf of Thailand, the size range of males from the Andaman Sea was wider and included more large squid than for females. To prevent size effects for the comparisons, the same length range of males and females were used.

The Gulf of Thailand and the Andaman Sea have been separated by land from Thailand to the Thai- Malay Peninsula, which is 1,127 km long since the Peninsula were formed. The environment of these seas is different. The Loliginid squid is a short distance migratory species, so the squid from the Gulf of Thailand and the Andaman Sea probably belong to long-separated populations, and there may be genetic differences between these two seas. Tassanakajon *et al*. (1997) studied genetic variation in wild population of the black tiger prawn (*Penaeus monodon*) from the Gulf of Thailand and the Andaman Sea and suggested different levels of genetic variability among samples. A similar genetic study of *L. duvauceli* in the Gulf of Thailand and the Andaman Sea should be done to test the differences between these populations.

The migration of Loliginid squid is for spawning and feeding (Mangold, 1987). Downey, *et al* (2009) studied migration behavior of chokka squid (*Loligo reynaudii*) along the south coast of South Africa and reported that chokka squid appeared at inshore spawning sites at dawn and departed after dusk, moving offshore to feed and showed size aggregation for spawning. The size aggregation for schooling of squid is also reported by William *et al.* (2001) for *Loligo pealeii* in southern New England. This can explain the differences in DML between samples from the Gulf of Thailand and the Andaman Sea and missed of large males from the Gulf of Thailand's samples. Squid from the Gulf of Thailand were collected by trawler only during daytime, meaning that the samples only collected spawning groups. Squid from the Andaman Sea were collected by trawler during day and night and also from both inshore and offshore fishing grounds, meaning that squid were mixed between spawning and feeding groups. And also the two fishing grounds were different in depth and the samples can represent multiple schooling with different size aggregations. Therefore the squids from the Andaman Sea included larger size, which were not collected by the sampling program in the Gulf of Thailand.

The study of differences in the life span of squid between sexes was limited, so the explanations for why there were more large males compared to females in the Andaman Sea samples is still unclear. The females allocate more energy to reproduction after maturation (Mangold *et al*., 1969) and this can be one reason for the difference in life span between sexes.

2. Age and growth of *L. duvauceli*

The number of increments which were defined as age in days after hatching for squid from the Gulf of Thailand ranged from 61 to 153 days (35-160 mm) while the Andaman Sea ranged from 76 to 270 days (58-232 mm). The average Growth Index (GI) of *L. duvauceli* was not significantly different between sexes for both seas. The average GIs of squid from the Gulf of Thailand were 1.044 and 0.959 mm/day for females and males while for the Andaman

Sea were 0.706 and 0.730 mm/day for females and males. A logarithmic function was selected to describe growth pattern for both the Gulf of Thailand and the Andaman Sea.

The rapid growth of squid is consequence by many factors; mainly that squid have an efficient digestive mechanism leading to high food conversion rate (Boyle, 1987). In present study, *L. duvauceli* from the Andaman Sea sampled from commercial fishing vessels enter fisheries at age just 76 days after hatching. The growth of *L. duvauceli* is faster in early life stage but slows in later life (Supongpan and Natsukari, 1996). Slowing of somatic growth may indicate the allocation of energy to reproductive tissues production after maturation (Mangold *et al*., 1969). The growth rates of *L. duvauceli* in the Gulf of Thailand estimated by Supongpan and Natsukari (1996) were 0.425 and 0.399 mm/day for males and females. In present study, the Growth Index of squid from the Gulf of Thailand was considerable higher than previous studies, probably because the squid sampled in present study were smaller and younger and in the faster growth phase than the larger and older squid in previous studies.

The population estimates of growth rates in present study were described by a logarithmic function. For comparison to the previous studies which described population growth by exponential function, the exponents (b) were compared. The exponent (b) was 0.010 for the Gulf of Thailand and 0.005 for the Andaman Sea. Supongpan and Natsukari (1996) reported the (b) of squid from the Gulf of Thailand to be 0.180 for males and 0.209 for females and Sukramongkol *et al* (2007) reported 0.011 for both males and females from the Andaman Sea, all of which are considerable higher than the present study. The observation that the population estimate of growth in the present study was slower than previously reported is probably because the maturation is at a smaller size. The energy was allocated to produce reproductive tissues leading to slowing of somatic growth as discussed by Smith *et al*. (2005) who studied the investment in reproductive and somatic tissues in *Loligo forbesi* and showed that female mantle length continues to increase after gonad growth stops. Temperature at hatching is also a significant factor which can influence the size of juveniles and subadults of *Loligo vulgaris* in the northwest Portuguese waters, increasing growth rates of squid hatched during the warm season (Moreno *et al*., 2007). And the seasonal temperature variation effects growth rate as reported by Jackson and Moltschaniwskyj (2001). They observed that winter-caught individuals of *Loliolus noctiluca* off North Queensland, Australia were faster growing than summer or autumn-caught individuals. For growth rate comparisons, sea water temperature should be monitored in any further study.

The life span of *L. duvauceli* in the Gulf of Thailand has been estimated to be around one year (Chotiyaputta, 1996; Supongpan and Natsukari, 1996). In present study, the size of squid was smaller than the maximum size reported (320 mm for males from Thai waters, Chotiyaputta, 1993) therefore the age estimations may not reach the maximum age of this species and the life span can not be estimated.

3. Hatching date and spawning date back calculation

The estimated hatching date of *L. duvauceli* from the Gulf of Thailand showed two dominant hatching periods around October – November 2007 and May – June 2008. The spawning dates were estimated by back calculation of mean egg incubation time (9 days) from hatching date resulting in periods coincident with the second dominant spawning season, as determined from previous studies.

The hatching and spawning dates were estimated for *L. duvauceli* from the Gulf of Thailand while the Andaman Sea were not included since squid catches from commercial fishing vessels were mixed leading to lack of knowledge of the exact capture date.

Although *L. duvauceli* in the Gulf of Thailand spawn throughout the year (Roonratri, 1989), two dominant spawning seasons were reported during January - May and June - October (Supongpan and Sinoda, 1998; Chotiyaputta, 1996). In the present study, the estimated spawning period was coincident with only the second dominant spawning season reported in these previous studies. This may be explained by differences in the methods used to estimate spawning season. For the previous studies, the spawning seasons were estimated directly from the periods of high proportions of mature squid in the samples, which consisted of many maturity stages. The time for development of each maturity for both males and females should be taken into account for more accurate spawning date back calculation but time of maturity stage development is not reported. The method used in this study, which was back-calculation from statolith growth increments, only identifies the likely spawning dates of surviving individuals, whereas the two spawning seasons described previously do not make any estimation of relative survivorship of the paralarvae that are produced. The survival rate of squid paralarvae depends on many factors such as food availability, density of predators and oceanographic conditions. These probably lead to difference of survival rate between the first and the second spawning seasons which may lead to different results of these two methods.

4. Morphological patterns and statolith length

The two populations of *L. duvauceli* showed sexual dimorphism but at different levels (6 of 15 significant differences morphological for the Gulf of Thailand and 13 of 15 for the Andaman Sea). The statolith length of mature females was always larger than males at the same DML.

The sexual dimorphism can be induced by both genetic and environment factors. Sexual dimorphism has been reported in many fish and invertebrate species for example, South Atlantic hermit crab *(Loxopagurus loxochelis)* from Brazilian waters (Mantelatto and Martinelli, 2001), lake whitefish (*Coregonus clupeaformis*) from Lake Ontario, Canada (Casselman and Schulte-Hostedde, 2004), the dragonet *Repomucenus valenciennei*, a demersal fish of Tokyo Bay (Ikejima and Shimizu, 1997). And also sexual dimorphism in squid was reported in *Loligo vulgaris* and *L. forbesi*, in Galician waters, north-west Spain (Rocha and Guerra, 1999) and *L. duvauceli* and *L. chinensis* in the Andaman Sea (Sukramongkol *et al.*, 2007). Since the genetic differences of these two populations (seas) have not been confirmed, at least the environment is different. The difference levels of sexual dimorphism in the two populations in the present study suggest that these are at least environment-induced changes in morphological variables in this species or both environment and genetic-induced in case of differences of genetics in these two populations.

The difference in otolith shape of cod among age, sexes and year class was reported by Campana and Casselman (1993). In present study, the statolith length was also different between sexes in mature squid.

5. The Length - weight relationship and the relative condition factor (K)

The relationship between logDML and logTW of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea was different between sexes. The slopes (b) were 2.368 and 2.586 for males and females from the Gulf of Thailand, the regression lines crossed at 73 mm while the slope of males and females from the Andaman Sea were 1.834 and 2.642, and the regression lines crossed at 78 mm. Females became heavier than males at the same dorsal mantle length when they grew larger than 73 mm for the Gulf of Thailand and 78 mm for the Andaman Sea.

Sexual dimorphism of *L. duvauceli* has been observed previously, with females having a greater weight than males in both the Gulf of Thailand (Ruttana-arnan, 1979) and in the Andaman Sea (Sukramongkol *et al.*, 2007). The slope (b) of the mantle length-total weight relationships are 1.7-2.0 for males and 2.0-2.5 for females reported from the Gulf of Thailand (Ruttana-arnan, 1979; Roonratri, 1989; Roongratri and Fujiwara1992) and 1.79 and 2.39 for males and females from the Andaman Sea (Sukramongkol *et al*, 2007). The slope (b) from the present study was higher than previous studies for both the Gulf of Thailand and the Andaman Sea, and shows that in these squid populations the weight increased faster than previously. Since the increase in the weight of reproductive tissue is a higher proportion than mantle muscle in mature squid, it seems that the present squid populations begin to allocate growth earlier to reproductive tissues, compared to previous records, in order to maintain their stock under high fishing pressure. When compared to the other areas, the slope (b) of this species in the Madras coast (East coast of India) estimated by Silas *et al*. (1986) was 2.38 for males and 2.52 for females and from the Mumbai waters was 2.16 for males and 2.28 for females (Karnik and Sushant, 2001) which were not very different from the slope (b) in present study.

The relative condition factor (K) of *L. duvauceli* in the Gulf of Thailand was different between monsoon seasons and areas. The K of samples collected during the Northeast monsoon was higher than the Southwest monsoon and the K of samples from the South area was higher than the North. The K of the squid in the Andaman Sea samples was not different between areas.

As defined by Le Cren (1951), K is a quantitative parameter of the well-being of the fish and can reflect recent feeding conditions. Therefore higher K indicated good feeding conditions for squid. *L. duvauceli* as all other cephalopods is an active carnivore, feeding on live prey for their whole life (Boyle, 1987). The food items for squid change with size, juvenile squid feed on planktonic organisms while larger squid feed on crustaceans and small fishes. The main food items from stomach contents of *L. duvauceli* in the Gulf of Thailand reported by Rattana-arnan (1978) were squid, teleost fish, and crustaceans. The presence of squid as prey revealed cannibalism in this species as in other Cephalopods (Christian and Keyl, 2010). Moreover, feeding condition of squid depends on seasonal changes and geographical differences.

The south sampling area in the Gulf of Thailand is covered by many islands and islets of the “Mu Ko Aug-thong” archipelago marine national park. This archipelago provides good habitats and nursery grounds for many species and also provides good feeding conditions for *L. duvauceli*. The monsoons affect feeding conditions for squid through primary productivity by impacting water circulation, salinity and turbidity of the Gulf of Thailand. The surface currents run clockwise during the Southwest monsoon and counterclockwise during the Northeast monsoon (McGinley, 2008). Rainfall and river runoff results in low salinity surface water during the Northeast monsoon and enriches the nutrient properties of the water leading to high biological productivity in this area (Robinson, 1974) and also provides good feeding conditions during the Northeast monsoon for juvenile squid.

6. Maturity

The maturity pattern of *L. duvauceli* was more distinctly defined by DML than by age for both sexes. The DML_{50}% of *L. duvauceli* from the Gulf of Thailand was 78.90 mm for males and 94.05 mm for females and for the Andaman Sea was 100.69 mm for males and 91.52 mm for females.

The maturation pattern of *L. duvauceli* occurred earlier and the size at 50% mature was smaller than in previous studies. Chotiyaputta (1996) reported that the DML_{50}% of squid from the Gulf of Thailand was 124 mm for males and 102 mm for females. The maturation at a smaller size of squid is also reported by Olyott *et al* (2006) for Chokka squid *(Loligo vulgaris reynaudii*) in South Africa by Salman and Önsoy (2010) for Bobtail Squid *(Rossia macrosoma)* in the Eastern Mediterranean. This is not only observed in squid species, the maturation at a smaller size has also occurred in cod (*Gadus morhua*) in the Northwest Atlantic and showed evidence of fisheries induced evolution to earlier maturation at smaller sizes (Olsen *et al*. 2004). de Roos *et al* (2006) reported that fishes in exploited stocks mature earlier at either smaller or larger sizes due to both genetic and plastic responses. The plastic response occurs when reduced competition for food leads to faster individual growth.

7. Stock discrimination

Both morphological and statolith variables were used to discriminate *L. duvauceli* stocks in present study.

Squid have no external hard structure and the body is flexible. The accuracy of body (morphological) measurements depends on stretching of the specimens and body condition, for examples, freshness and handling both onboard vessels and at the fishing port.

Furthermore, the experience of persons making the measurements can affect the accuracy of measurements. In the present study, to prevent variation between workers, all samples were measured by the same person. For large scale studies, it is not practical to have only one person making all the measurements, so it would be useful to use parameters that are not so variable. Thus, the hard part of squid such as statolith was investigated to discriminate squid stocks using the same statistical analysis methods as for morphological parameters.

The edge and shape of a statolith changes as the external outline of the statolith is continuously generated from new daily increments. The shape analysis of statolith for stocks discriminate has not been investigated until this study. Many studies have indicated that the otoliths can discriminate fish stocks, for example cod in the Faroe Islands (Cardinale *et al*., 2004), Canada, United States, and Iceland due to the growth rate differences which vary more between stocks than within a stock (Campana and Casselman,1993). The statolith has a similar function as the otolith in fish, and shape differences were investigated to discriminate between squid stocks based on shape variation due to growth variation between stocks. The result clearly revealed that the statolith shape analysis can be used to discriminate between *L. duvauceli* stocks.

Although, both morphological and statolith variables can be used to discriminate squid stocks, the morphological variables seem to have more power to separate between the seas than the statolith variables. The difference between seas was not only environmental differences but also hypothesized genetic differences. The genetic differences of *L. duvauceli* in the Gulf of Thailand and the Andaman Sea should be studied to test this hypothesis.

The appropriate squid stock discrimination was important for squid resources management which is based on the concept of sustainable yield, assuming that the fishery target is a unit stock (Carvalho and Hauser, 1994). There are many methods reported to discriminate squid stocks, Triantafillos *et al*. (2004) used allozyme electrophoresis to examine the stock structure of Arrow squid *Nototodarus gouldi* (McCoy 1888) along the coast of southern Australia, and biological tagging (Helminth parasites) was reported to discriminate short finned squid (*Illex coindetii* (Verany. 1839) stocks from the north and south Galicia off the northwestern Spain (Pascual *et al*., 1995). The trace elements concentration in the statoliths was reported by Warner *et al*. (2009) to identify the source populations for stocks of the market squid *Doryteuthis* (formerly *Loligo*) *opalescens* from the California coast. And also the statolith shape analysis was suggested from present study.

8. Project evaluation

The squid sampling programs of these two seas were different resulting in differences in the size classes of samples which was a weakness of this study. Large males were missed from the Gulf of Thailand samples and males from the Andaman Sea were found with wider size range than females, and especially in larger sizes. The size differences of samples between sexes can cause bias in any biological parameter comparisons which were size related. To prevent bias of size effects for the comparisons, the same length range of males and females were used, removing males which were larger than females (154 mm) from the dataset of the Andaman Sea before analysis. After removing large males, not only size between males and females of the Andaman Sea were similar, but also similar size of samples of these two seas.

The results of statolith shape analysis provided a new approach to discriminate squid stocks. Although the scatter plot of the Canonical Scores on the first two principal component axes for the four sample groups were partly overlapped, the percentage of correct classification can help to justify the results.

The oceanographic condition such as temperature, oxygen and turbidity should be monitored since they effect squid behavior. And the genetic differences of *L. duvauceli* in the Gulf of Thailand and the Andaman Sea should be studied to test hypothesis from this study.

Conclusions

L. duvauceli were sampled from the Gulf of Thailand and the Andaman Sea by otter board trawlers. The DML of squid from the Andaman Sea was larger than the Gulf of Thailand due to size aggregate behavior, differences in target group (spawning and feeding group) and environments. The genetic difference of squids between these two seas was hypothesized.

The number of increments which were defined as age in days after hatching for squid from the Gulf of Thailand ranged from 61 to 153 days (35-160 mm) and the Andaman Sea ranged from 76 to 270 days (58-232 mm). The Growth Index (GI) of *L. duvauceli* was not significantly different between sexes for both seas. The GI of males and females were 0.959 and 1.044 mm/day for the Gulf of Thailand and 0.730 and 0.706 mm/day for the Andaman Sea. Logarithmic function was selected to describe the growth pattern at the population level for both Seas. The estimated hatching date by back calculation for *L. duvauceli* from the Gulf of Thailand showed two dominant hatching periods around October – November 2007 and May – June 2008. The spawning dates were estimated by back calculation of mean egg incubation time (9 days) from hatching date and the results were coincident with only the second dominant spawning season described in previous studies due to differences of methods for estimation.

Sexual dimorphism appeared in both two populations of *L. duvauceli* but difference in levels suggest that at least environment induced morphological changes occur in this species. The statolith length was different between sexes in mature squid.

The relationship between logDML and logTW of *L. duvauceli* from the Gulf of Thailand and the Andaman Sea was different between sexes. The slope (b) for males and females were 2.368 and 2.586 from the Gulf of Thailand and 1.834 and 2.642 for the Andaman Sea. The b was higher than previous studies for both seas revealing squid at present increase faster in weight by allocating growth to reproductive tissues earlier than previously in order to maintain their stock under high fishing pressure.

The maturity of *L. duvauceli* was more distinctly separated by DML than age for both sexes. The DML_{50}% for males and females were 78.90 and 94.05 mm for the Gulf of Thailand and 100.69 and 91.52 mm for the Andaman Sea. The maturation pattern of *L. duvauceli* was occurred earlier and DML_{50}% was smaller than previous studies, showed evidence of fisheries induced evolution to earlier maturation at smaller sizes.

The morphological variables were useful for stock discrimination. Due to plasticity of the squid body, the accuracy of morphological measurements depends on experience of workers, so the use of statoliths was investigated. The results showed that statolith shape also appeared to discriminate between squid stocks, using the same statistical analysis methods as morphological variables. The morphological variables seem to have more power to separate between the seas than statolith shape. Genetic differences are hypothesized between *L. duvauceli* in the Gulf of Thailand and the Andaman Sea, but this should be studied to test this hypothesis.

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Appendix 1 Data of increments width measurement form the Scanning Electron Microscope (SEM) photographs.

Sample No.	Angle of measurment	Total Length (µm)	No. of increments within total length	Average increment width (µm)
1	63.44	1.68	4	0.419
2	54.46	1.27	3	0.424
3	-116.57	1.65	4	0.413
4	62.70	2.62	6	0.437
5	63.44	2.93	7	0.419
6	67.17	1.52	3	0.507
7	63.44	1.01	2	0.507
8	68.63	1.81	4	0.453
9	-109.98	0.86	2	0.430
10	67.38	0.97	2	0.485
11	69.78	1.53	3	0.509
12	74.36	2.00	4	0.500
13	74.06	3.27	6	0.545
14	64.98	1.23	2	0.614
15	54.46	1.96	3	0.654
16	37.57	1.18	2	0.591
17	75.53	2.40	5	0.480
18	50.71	1.07	2	0.533
19	65.38	1.94	4	0.484
20	-100.18	2.97	5	0.594
21	-105.95	5.98	13	0.460
22	69.44	1.87	4	0.468
23	56.31	1.09	2	0.543
24	64.72	1.48	3	0.493
25	72.47	0.74	1	0.736
26	65.23	3.14	7	0.449
27	-5.19	0.47	1	0.467
28	-4.09	1.77	3	0.591
29	0.00	1.21	2	0.605
30	4.19	1.73	3	0.575
31	-1.30	1.81	4	0.454
32	0.00	7.48	4	1.869
33	0.00	5.66	3	1.886
34	-19.36	14.31	9	1.590
35	6.84	4.66	3	1.555
36	-20.23	3.71	3	1.238
37	13.57	5.44	6	0.907
38	-2.12	4.85	3	1.618
39	8.43	4.89	3	1.629
40	-2.29	4.65	2	2.326
41	-6.84	4.46	4	1.116
42	0.00	1.21	1	1.212
43	15.52	3.33	2	1.666
44	9.46	1.33	1	1.334
45	4.51	2.80	3	0.933
46	4.40	1.89	2	0.946
47	9.21	2.75	4	0.687
48	13.67	2.86	2	1.432
49	2.86	2.96	3	0.985

Appendix 2 Data of increments width measurement form the light Microscope photographs.

Sample No.	Angle of measurment	Total Length (µm)	No. of increments within total length	Average increment width (µm)
1	64.00	5.07	4	1.267
2	66.04	2.19	2	1.095
3	91.55	4.11	4	1.028
4	75.62	4.47	4	1.119
5	85.24	4.01	5	0.803
6	-77.59	5.69	5	1.138
7	90.00	0.89	1	0.889
8	85.91	3.12	5	0.624
9	66.15	5.77	5	1.154
10	-128.66	5.69	4	1.423
11	54.16	4.93	4	1.234
12	-136.28	8.80	5	1.760
13	68.20	3.29	3	1.096
14	63.44	5.73	5	1.146
15	-92.49	1.26	2	0.631
16	-101.31	1.96	2	0.981
17	70.28	3.12	2	1.561
18	80.17	7.15	5	1.430
19	71.79	8.90	4	2.225
20	82.85	17.84	11	1.622
21	83.73	14.76	9	1.640
22	83.11	11.57	7	1.652
23	83.99	8.49	5	1.698
24	83.07	5.97	3	1.991
25	-106.26	1.38	2	0.692
26	73.91	3.00	2	1.501
27	69.27	4.36	2	2.181
28	74.25	2.24	2	1.122
29	-103.20	4.60	4	1.150
30	65.04	3.59	3	1.195
31	23.56	8.08	6	1.346
32	22.17	1.66	2	0.830
33	43.73	1.74	2	0.872
34	64.98	1.86	2	0.932
35	79.53	6.74	5	1.348
36	68.39	6.05	5	1.210
37	71.29	3.68	4	0.920
38	77.47	0.99	2	0.494
39	67.38	0.73	1	0.730
40	-113.63	0.96	2	0.482
41	85.94	8.65	6	1.442
42	86.67	5.71	4	1.428
43	27.41	6.76	6	1.126
44	102.10	0.79	1	0.786
45	90.00	4.47	3	1.490

Appendix 2 (continue) Data of increments width measurement form the light Microscope photographs.

Sample No.	Angle of measurment	Total Length (µm)	No. of increments within total length	Average increment width (µm)
46	-90.00	2.31	4	0.578
47	-101.39	7.86	4	1.965
48	85.91	2.32	3	0.772
49	84.53	5.29	6	0.881
50	101.08	8.08	7	1.154
51	90.00	1.48	2	0.741
52	85.43	1.41	3	0.470
53	88.88	2.82	4	0.704
54	110.56	2.37	2	1.184
55	-87.66	16.35	10	1.635
56	-88.59	13.56	8	1.695
57	-97.13	0.90	1	0.896
58	-88.98	6.22	3	2.074
59	-77.20	2.51	2	1.254
60	65.77	2.44	5	0.487
61	90.00	2.11	2	1.056
62	66.68	7.02	4	1.755
63	67.89	3.84	3	1.279
64	70.02	3.90	5	0.780
65	74.48	2.08	3	0.692
66	76.76	5.82	3	1.940
67	71.57	1.76	2	0.879
68	70.35	1.65	2	0.826
69	82.38	7.94	5	1.587
70	85.60	4.33	3	1.442
71	-90.00	3.55	3	1.182
72	-90.00	2.78	3	0.925
73	-110.34	5.27	3	1.756
74	92.82	6.79	4	1.697
75	90.00	3.08	4	0.771
76	93.66	9.58	5	1.915
77	102.27	1.27	2	0.636
78	-71.81	4.05	3	1.349
79	-71.57	4.39	4	1.097
80	-75.96	2.06	2	1.029
81	52.13	0.64	1	0.637
82	55.01	2.71	3	0.904
83	54.42	7.91	5	1.583
84	60.26	0.93	1	0.928
85	52.70	1.48	2	0.741
86	41.19	0.58	1	0.579
87	85.06	9.03	7	1.291
88	73.65	8.68	9	0.965
89	81.57	3.03	4	0.758
90	85.33	5.46	7	0.780

Appendix 2 (continue) Data of increments width measurement form the light Microscope photographs.

Sample No.	Angle of measurment	Total Length (µm)	No. of increments within total length	Average increment width (µm)
91	84.56	2.34	3	0.781
92	73.54	5.10	6	0.850
93	85.37	4.13	4	1.031
94	100.07	12.08	11	1.098
95	87.21	4.56	4	1.140
96	96.46	5.93	6	0.988
97	97.13	6.27	7	0.896
98	91.59	4.00	3	1.334
99	69.71	12.49	6	2.081
100	72.24	19.26	12	1.605
101	71.87	16.38	10	1.638
102	72.47	6.63	4	1.658
103	72.03	6.48	7	0.926
104	74.06	2.39	3	0.798
105	55.95	7.44	5	1.488
106	56.71	4.45	5	0.889
107	62.53	4.69	5	0.938
108	65.87	11.66	10	1.166
109	61.08	11.45	5	2.291
110	60.54	6.90	5	1.380
111	107.53	11.07	12	0.922
112	99.96	8.35	8	1.044
113	100.95	3.51	5	0.702
114	98.58	5.96	6	0.993
115	97.24	7.06	4	1.764
116	112.75	3.74	5	0.747
117	83.40	8.22	11	0.747
118	85.24	2.01	2	1.005
119	90.00	7.09	5	1.418
120	82.71	4.82	4	1.205
121	90.00	2.16	3	0.719
122	82.88	3.58	3	1.192
123	86.71	14.52	9	1.614
124	79.99	5.75	5	1.151
125	82.41	2.52	2	1.261
126	74.88	6.39	5	1.278
127	72.72	7.86	5	1.571
128	91.27	7.50	4	1.876
129	92.73	3.50	3	1.168
130	78.69	0.85	1	0.850
131	96.01	3.18	3	1.061
132	88.73	15.00	9	1.667
133	90.00	4.67	4	1.167
134	80.27	7.89	6	1.315
135	81.87	5.89	6	0.982

Appendix 2 (continue) Data of increments width measurement form the light Microscope photographs.

Sample No.	Angle of measurment	Total Length (μm)	No. of increments within total length	Average increment width (μm)
136	78.34	10.72	5	2.144
137	79.99	2.88	3	0.959
138	67.44	15.64	7	2.234
139	90.00	7.33	5	1.467
140	98.53	4.49	2	2.247
141	88.76	10.23	5	2.045
142	92.73	4.67	3	1.557
143	90.00	2.67	3	0.889
144	69.78	6.75	6	1.125
145	64.80	2.09	3	0.696
146	75.96	2.75	2	1.375
147	74.75	12.67	13	0.975
148	56.31	15.62	13	1.202
149	60.64	3.06	3	1.020
150	38.66	4.27	3	1.423
151	42.98	10.02	6	1.671
152	70.35	2.48	3	0.826
153	95.69	13.47	12	1.123
154	96.71	1.87	4	0.466
155	97.49	7.70	9	0.856
156	100.62	4.52	3	1.506
157	39.29	7.90	5	1.579
158	51.20	7.27	7	1.039
159	38.59	13.36	7	1.909
160	41.63	8.03	5	1.606
161	60.49	3.38	5	0.676
162	61.11	1.86	4	0.464
163	57.68	3.23	3	1.076
164	65.06	2.62	3	0.873
165	79.33	11.70	6	1.950
166	82.88	8.06	4	2.016
167	80.54	4.06	4	1.014
168	94.40	2.17	4	0.543
169	70.82	4.06	3	1.353
170	78.69	5.10	6	0.850
171	79.70	3.73	3	1.242
172	81.87	2.36	3	0.786

Appendix 3 Data of increments counting of *L. duvauceli* from the Gulf of Thailand.

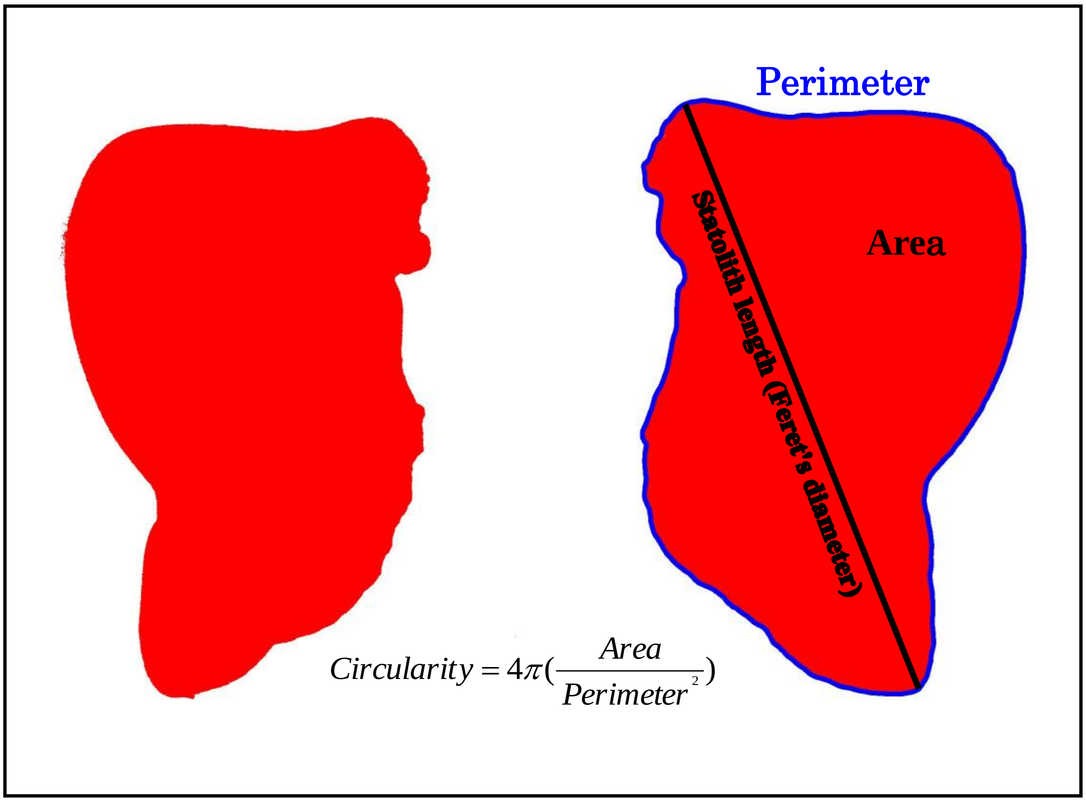
	ID	Sampling Station	Sex	DML (mm)	No. of Increments	Growth Index (mm/day)
1	8104901	49	Female	91.65	101	0.907
2	8104907	49	Female	117.59	99	1.188
3	8104908	49	Female	143.85	153	0.940
4	8105803	58	Female	114.33	123	0.930
5	8106010	60	Female	149.13	121	1.232
6	8106202	62	Female	88.22	119	0.741
7	8106203	62	Female	125.55	112	1.121
8	8107301	73	Female	53.23	75	0.710
9	8108704	87	Female	51.58	69	0.748
10	8108705	87	Female	70.54	85	0.830
11	8108902	89	Female	96.92	113	0.858
12	8110106	101	Female	123.43	94	1.313
13	8115604	156	Female	108.68	97	1.120
14	8115805	158	Female	106.31	86	1.236
15	8118105	181	Female	98.73	87	1.135
16	8204903	49	Female	56.58	89	0.636
17	8208703	87	Female	86.35	94	0.919
18	8208905	89	Female	48.78	63	0.774
19	8210502	105	Female	35.37	62	0.570
20	8211704	117	Female	58.62	83	0.706
21	8215603	156	Female	75.69	89	0.850
22	8219902	199	Female	95.45	97	0.984
23	8306006	60	Female	89.25	86	1.038
24	8306203	62	Female	95.17	112	0.850
25	8306204	62	Female	80.36	82	0.980
26	8313601	136	Female	103.79	101	1.028
27	8315804	158	Female	84.1	70	1.201
28	8317701	177	Female	95.24	84	1.134
29	8317702	177	Female	120.86	87	1.389
30	8317705	177	Female	101.58	94	1.081
31	8318101	181	Female	65.61	66	0.994
32	8318102	181	Female	76.07	63	1.207
33	8318105	181	Female	83.66	65	1.287
34	8318106	181	Female	78.57	63	1.247
35	8404708	47	Female	74.63	71	1.051
36	8405804	58	Female	119.03	111	1.072
37	8406007	60	Female	86.58	70	1.237
38	8406202	62	Female	70.36	68	1.035
39	8408704	87	Female	79.17	76	1.042
40	8408906	89	Female	75.86	80	0.948
41	8411904	119	Female	60.07	61	0.985
42	8413802	138	Female	84.49	67	1.261
43	8414002	140	Female	109.41	89	1.229
44	8414004	140	Female	97.96	78	1.256
45	8415603	156	Female	95.36	74	1.289

Appendix 3 (Continue) Data of increments counting of *L. duvauceli* from the Gulf of Thailand.

	ID	Sampling Station	Sex	DML (mm)	No. of Increments	Growth Index (mm/day)
46	8415604	156	Female	104.39	85	1.228
47	8417907	179	Female	66.86	64	1.045
48	8419906	199	Female	98.96	63	1.571
49	8104704	47	Male	117.87	121	0.974
50	8107303	73	Male	79.24	93	0.852
51	8110105	101	Male	130.22	112	1.163
52	8110107	101	Male	160	117	1.368
53	8110301	103	Male	73.46	94	0.781
54	8110310	103	Male	134.23	104	1.291
55	8110508	105	Male	32.46	78	0.416
56	8111706	117	Male	57.83	82	0.705
57	8111903	119	Male	74.74	87	0.859
58	8113601	136	Male	83.35	92	0.906
59	8113606	136	Male	95.94	79	1.214
60	8114003	140	Male	86.92	109	0.797
61	8117704	177	Male	97.87	91	1.075
62	8117908	179	Male	38.62	77	0.502
63	8119903	199	Male	138.46	113	1.225
64	8119907	199	Male	45.62	88	0.518
65	8119909	199	Male	66.19	76	0.871
66	8204705	47	Male	45.48	72	0.632
67	8205808	58	Male	66.54	90	0.739
68	8206204	62	Male	59.67	87	0.686
69	8210101	101	Male	88.53	104	0.851
70	8210305	103	Male	61.15	70	0.874
71	8211902	119	Male	74.17	94	0.789
72	8213607	136	Male	55.15	72	0.766
73	8214002	140	Male	77.03	80	0.963
74	8214003	140	Male	71.81	73	0.984
75	8215601	156	Male	104.62	86	1.217
76	8215801	158	Male	62.39	65	0.960
77	8217706	177	Male	98.33	95	1.035
78	8217901	179	Male	68.58	83	0.826
79	8218108	181	Male	78.27	75	1.044
80	8305801	58	Male	85.66	98	0.874
81	8306005	60	Male	72.49	101	0.718
82	8307504	75	Male	111.21	123	0.904
83	8315802	158	Male	69.93	62	1.128
84	8317703	177	Male	98.95	90	1.099
85	8405807	58	Male	90.1	88	1.024
86	8407505	75	Male	81.79	71	1.152
87	8411701	117	Male	115.84	102	1.136
88	8413603	136	Male	94.26	71	1.328
89	8417703	177	Male	91.07	72	1.265
90	8418101	181	Male	127.18	72	1.766

Appendix 4 Data of increments counting of *L. duvauceli* from the Andaman Sea.

	ID	Sex	DML (mm)	No. of Increments	GrowthIndex (mm/day)
1	AL090201	Female	67.94	117	0.581
2	AL090203	Female	76.84	142	0.541
3	AL090210	Female	82.61	161	0.513
4	AL090514	Female	113.01	153	0.739
5	AL090517	Female	101.37	135	0.751
6	AL090634	Female	89.62	136	0.659
7	AL090640	Female	102.87	141	0.730
8	AS090111	Female	57.73	76	0.760
9	AS090207	Female	87.54	103	0.850
10	AS090304	Female	82.24	110	0.748
11	AS090305	Female	74.59	128	0.583
12	AS090403	Female	89.81	117	0.768
13	AS090502	Female	70.73	103	0.687
14	AS090610	Female	137.81	198	0.696
15	AS090629	Female	102.85	142	0.724
16	AS090716	Female	98.89	113	0.875
17	AS090721	Female	77.34	109	0.710
18	AS090732	Female	143.87	202	0.712
19	AS090819	Female	101.88	136	0.749
20	AS090904	Female	91.39	123	0.743
21	AS090208	Male	61.73	103	0.599
22	AS090213	Male	67.92	96	0.708
23	AL090601	Male	79.81	137	0.583
24	AS090506	Male	79.99	93	0.860
25	AS090508	Male	85.73	122	0.703
26	AS090622	Male	86.49	136	0.636
27	AL090214	Male	88.76	178	0.499
28	AL090215	Male	95.64	204	0.469
29	AS090312	Male	96.84	136	0.712
30	AL090636	Male	99.01	133	0.744
31	AS090710	Male	104.81	125	0.838
32	AS090518	Male	108.87	141	0.772
33	AS090107	Male	109.96	149	0.738
34	AL090629	Male	111.13	179	0.621
35	AS090920	Male	118.33	137	0.864
36	AL090637	Male	119.33	163	0.732
37	AS090417	Male	120.89	119	1.016
38	AL090513	Male	122.88	163	0.754
39	AS090205	Male	124.35	150	0.829
40	AL090621	Male	128.73	174	0.740
41	AL090507	Male	132.09	208	0.635
42	AS090404	Male	133.08	170	0.783
43	AL090518	Male	141.61	187	0.757
44	AL090501	Male	145.98	240	0.608
45	AS090726	Male	147.55	155	0.952
46	AL090503	Male	150.78	216	0.698
47	AS090519	Male	153.91	180	0.855



Appendix 5 Diagram of statolith dimensions measurements.