

Age and growth of mitre squid *Photololigo chinensis* in the Tonkin Gulf of Vietnam based on statolith microstructure

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Abstract: A total of 254 mitre squid *Photololigo chinensis* collected in the coastal area of Tonkin Gulf was subjected to daily increment of statolith analysis to determine the ages. The oldest individuals observed were 194 days old for specimens collected in the summer–autumn season. Size–at–age relationship was best described by power function for both individuals hatched in spring–summer and autumn–winter periods. *P. chinensis* exhibited sexual dimorphism in the relative growth of the mantle length: males grew faster in length compared with females while weight growth rates were similar for both sexes. Seasonal factors strongly influenced the growth of squid, and the growth rates in the warm water season was higher than those in the colder season.

Keywords: *Photololigo chinensis*, age, growth, statolith, increment

1. Introduction

Mitre squid *Photololigo chinensis* is a neritic species, occurring from the shallow coastal area. The species is distributed in the western Pacific, from the South and East China Sea to Japanese waters, Gulf of Thailand, Arafura, Timor Sea and northern Australia (DUNNING *et*

al., 1998). Mitre squid has a wide range of distribution and high abundance in Vietnamese waters especially in the Tonkin Gulf (DUC, 1991), and is considered one of the most important species in the cephalopod fisheries in the area with 40–50% of the total catch of all squid species in the area (CHU *et al.*, 1998).

The daily growth increments were first detected in fish otoliths (PANELLA, 1971) and statoliths analyses of squids have provided information on growth rates, hatching dates, life–spans and even short term fluctuations in growth performance (JACKSON and MOLTSCHANIWSKYJ, 1999). Since the growth of statolith increments of *P. chinensis* was validated daily by JACKSON (1990), growth of tropical loliginid squids have been reported from Australian waters (JACKSON and CHOAT, 1992; JACKSON, 1995) and the Andaman Sea (SUKRAMONGKOL *et al.*, 2007). The statolith increments and somatic growth of *P. chinensis* in Australian water were influenced by seasonal factors, and the growth rate of male squid was relatively

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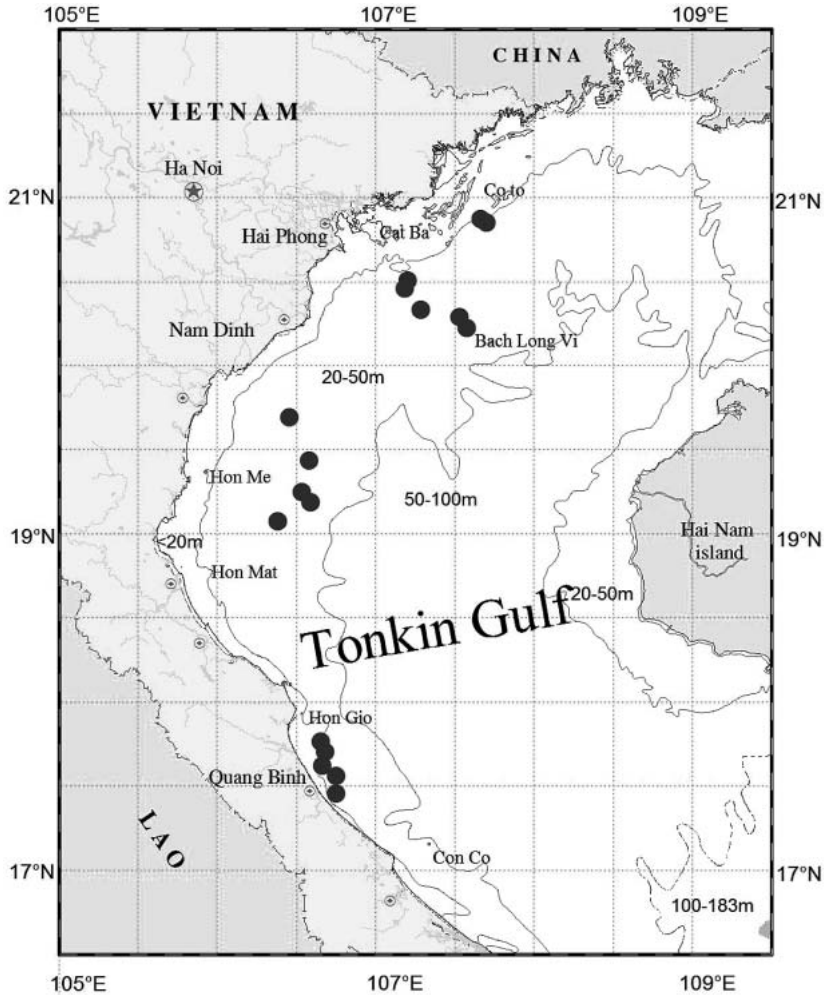


Fig. 1. Sampling locations (solid circles represent fishing areas) in the depth zone from 20–50 m in the Tonkin Gulf.

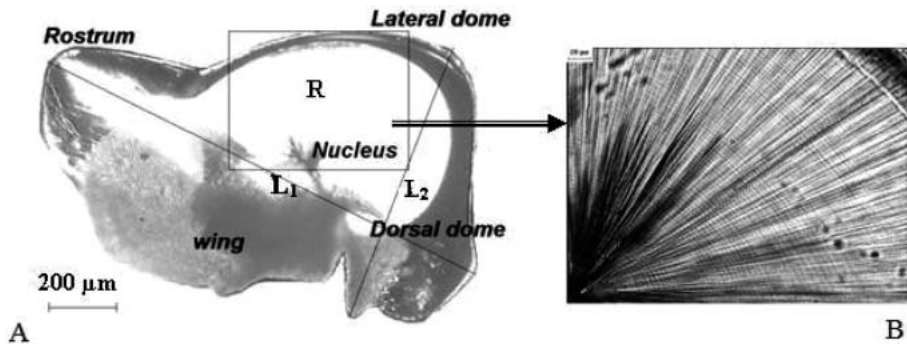


Fig. 2. Statolith increments of *Photololigo chinensis* (120 mm ML) observed under the light microscope. L_1 , L_2 are Statolith length and width. R_1 is rectangle area where every increment was counted and measured using the Caliper tool in Image Pro Plus Software (lines annotations).

Table 1. Size (ML mm) of male and female *Photololigo chinensis* specimens with readable statolith increments in nearshore of the Tokin Gulf, Vietnam from April to October 2001.

Month of sampling	Fishing gear	Mesh size (mm)	ML of Male				ML of Female			
			No.	Range	Mean	SD	No.	Range	Mean	SD
April	OT*	25	8	76–197	130.7	41.2	7	84–191	125.9	39.1
July	OT, S.H.D.N.**	25,20	19	75–313	153.8	78.5	24	70–215	117.5	38.7
August	OT, S.H.D.N.	25,20	24	95–200	127.0	31.4	27	74–155	109.2	18.8
September	OT, S.H.D.N.	25,20	33	82–250	152.2	45.7	50	74–330	122.5	47.9
October	S.H.D.N.	20	32	70–400	139.9	68.4	30	67–220	114.2	32.0

OT* (otter trawl) and S.H.D.N.** (stick held dip net).

higher than that of females at the same age (JACKSON and CHOAT, 1992; JACKSON, 1995).

Although mitre squid is a valuable species in the cephalopod fisheries in the Tonkin Gulf of Vietnam, there have been no studies on the growth and life history of this species in the area. In the southern area of Vietnam, growth parameters of *L. formosana* (referred as synonym of *P. chinensis*) had been calculated by the ELEFAN program (Electronic Length–Frequency Analysis) (DINH *et al.*, 1998) which suggested that *P. chinensis* has a life span exceeding 3 years with a very fast growth in the first year. However, this length frequency analysis is an inadequate means of describing growth for a rapid growing organism with multiple cohorts like squids (JACKSON *et al.*, 2000).

The aim of this study is to estimate the age and growth rates of *P. chinensis* in the Tonkin Gulf of Vietnam to provide basic information for the fisheries management of the squid in the area.

2. Materials and methods

The Tonkin Gulf is located between 17°–22°N and 105°–110°E, covering a total area of 150,000 km² (TANG, 1997). There are two main seasons in this area: winter–spring and summer–autumn corresponding with northeast (November to April) and southwest monsoons (May to October), respectively. In 2000–2001, sea surface temperature (SST) of the Tonkin Gulf in winter–spring (December–March) was from 20 to 23°C (21.5°C in average) while those in summer–autumn (May–October) ranged from 26 to 30°C (28.6°C in average) (source of data from NOAA, 2009).

Squid specimens for the statolith analysis were obtained from the Tonkin Gulf (Fig. 1) in April and July–October, 2001 by otter trawls and stick held dip nets (Table 1). Dorsal mantle length (ML) and wet body weight (BW) of the specimens were measured to the nearest mm and g, respectively. The maturity of 131 female squid caught in July–October was classified into 6 developmental stages following the description of LIPINSKI and UNDERHILL (1995). Stage I and II were defined as immature, stage III as maturing, stages IV and V as mature and stage VI as spent.

Statoliths were extracted from fresh squids following the method of NATSUKARI *et al.* (1991). Statolith was mounted to glass slides by Crystal Bond (Aremco Products, Inc) with the anterior side of statolith pointing downward. Before grinding, photograph of whole statolith was taken under light microscope. Statolith length (SL) and statolith width (SW) were measured to the nearest 1 μ m following CLARKE (1978) by the manual measurement tool in Image Pro Plus Software (Media Cybernetics) (L_1 and L_2 in Fig. 2A). A waterproof abrasive paper # 2000 was initially used to grind the statoliths on a Buehler machine, then the paper was changed to a finer one until the core was visible. The Caliper tool in Image Pro Plus Software was used for counting and measuring the increments (Fig. 2B).

All the statistical analyses were conducted by STATISTICA (software version 5.5., StatSoft, Inc). Differences in the regression slopes of the length–weight relationship, mantle length and estimated age, as well as body weight and estimated age between sexes and seasons were linearized by log transforming

($\ln ML - \ln BW$; $\ln ML$ -estimated age; $\ln BW$ -estimated age). Tests and comparisons were conducted by analysis of covariance (ANCOVA) for squid ranging from 70 to 280 mm in ML (because squid bigger than 280 mm in ML was mainly males). The first step was to test the homogeneity of slopes. If the slopes were not significantly different from each other, the homogeneity of intercepts was tested. Statolith width at estimated age was tested by ANCOVA for any differences among seasons. Moreover, to analyse the effect of sex and season on statolith increment width, residuals from polynomial estimation were used in ANOVA. Power function as $Y = aX^b$, where Y and X are variables and a and b are constant, was used for the analysis of the length-weight, length-age and weight-age relationships.

Daily growth rates (DGR, mm d^{-1} or g d^{-1}) and instantaneous growth rates (G, %) were calculated after Ricker (1958):

$$\text{DGR} = (W_2 - W_1) / T,$$

$$G = [(\ln W_2 - \ln W_1) / T] \times 100,$$

where W_1 , W_2 are calculated mantle length or body weights based on the length or weight and estimated age relationships at the beginning and end of the time interval ($T = 10$ days).

3. Results

3-1 Mantle Length and Body Weight

The length-weight relationships for males and females were $BW = 1.80 \times 10^{-4} ML^{2.16}$ ($n = 116$, $p < 0.01$, $r^2 = 0.95$, size range 70–400 mm ML) and $BW = 5.00 \times 10^{-4} ML^{2.42}$ ($n = 138$, $p < 0.01$, $r^2 = 0.95$, size range 67–330 mm in ML), respectively (Fig. 3). Slopes of the regression equations between males and females were significantly different ($\ln ML$ -at- $\ln BW$ homogeneity of slopes test, ML ranged 70–280 mm, $F = 7.5$, $p < 0.01$). Mean weight of females squid at the same ML larger than 160 mm was significantly heavier than males (ANCOVA test, $F = 12.8$, $p < 0.01$).

3-2 Age, hatching date and maturation

Estimated age of the squid based on the statolith increments ranged from 63 (72 mm in ML) to 190 days (400 mm in ML). Back calculation from the number of statolith increments and catching dates showed that hatching dates

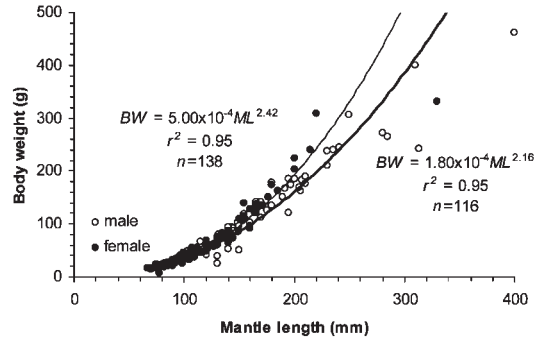


Fig. 3. Length-weight relationship of male (open circles) and female (solid circles) *Photololigo chinensis* from the Tonkin Gulf in 2001.

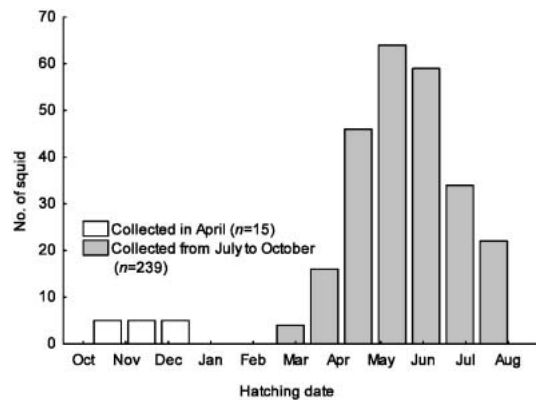


Fig. 4. Estimated hatching date of all individual ($n = 254$) of *Photololigo chinensis* collected from the Tonkin Gulf in April (transparent background bars) and from July to October (grey background bars).

of individuals sampled in July–October ranged from March to August with a peak from April to June (Fig. 4). Squid in spring–summer hatching group which was a main cohort in the Tonkin Gulf was collected from July to October and autumn–winter hatching group was collected only in April. Based on the hatching and sampling periods, *P. chinensis* hatched throughout the year and the hatching groups of *P. chinensis* in this study was classified into two separate groups, namely spring–summer hatching and autumn–winter hatching groups.

Observed maturation stages of female squid varied from stage 1 to 5 for specimens ranging

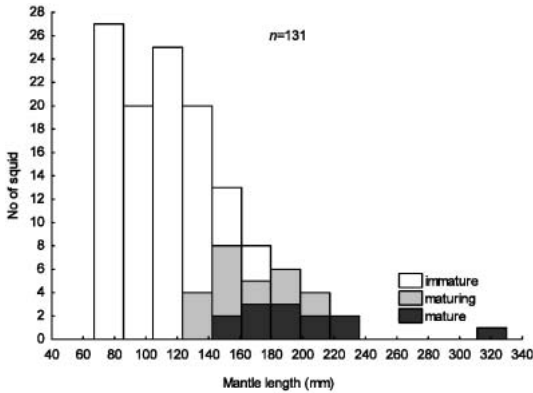


Fig. 5. Maturation of female *Photololigo chinensis* hatched in spring-summer season.

from 67 to 330 mm in ML ($n=131$). All females larger than 220 mm in ML were matured when older than 126 days. The smallest squid in maturing stage was 130 mm in ML (about 116 day old), while the biggest immature specimen was 182 mm (124 day-old) (Fig. 5).

3-3 Size and estimated age

Estimated age of squid ranged from 63 (72 mm in ML) to 194 day-old (400 mm in ML) for males, and 65 (78 mm ML) to 190 day-old (204 mm ML) for females (Fig. 6). The relationship between ML and estimated age (T days) was expressed as $ML=0.32T^{1.28}$ ($r^2=0.56$, $n=116$, $p<0.01$), and $ML=0.49T^{1.18}$ ($r^2=0.59$, $n=138$, $p<0.01$) for male and female, respectively (Fig. 6). The ML and estimated age relationships were significantly different between male and female (ANCOVA test, $p<0.003$). Growth in males varied considerably compared to females. At the same range of age, from 65 to 190 day-old (for both male and female), daily growth in ML ranged from 1.35 mm to 1.82 mm for males, and 1.22 mm to 1.47 mm for females (Equations in Fig. 6 were used for the estimations).

The relationship between BW and estimated age was expressed as $BW=8.00 \times 10^{-5} T^{2.90}$ ($r^2=0.58$, $n=116$, $p<0.01$), and $BW=6.00 \times 10^{-5} T^{2.93}$ ($r^2=0.62$, $n=138$, $p<0.01$) for male and female, respectively (Fig. 7). Based on these equations, daily growth rates of the body weight were estimated to be from 0.7 g to 5.46 g, and 0.61 g to 5.18 g for male and female

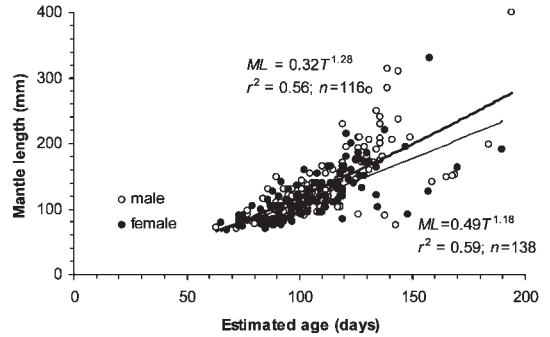


Fig. 6. Relationships between estimated age and mantle length of male (open circles) and female (solid circles) *Photololigo chinensis*.

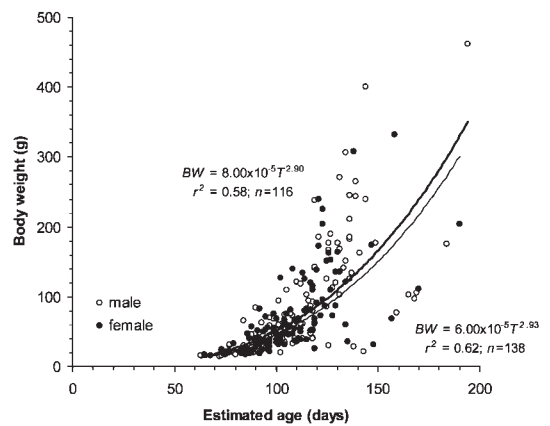


Fig. 7. Relationships between estimated age and body weight of male (open circles) and female (solid circles) *Photololigo chinensis*.

squid, respectively, at the age range from 65 to 190 day-old. There were no significant differences in BW and estimated age between males and females of *P. chinensis* in this study (ANCOVA test, $F=3.37$; $p=0.067$) (Fig. 7).

The ML and estimated age relationships combining male and female were $ML=6.60 \times 10^{-3} T^{1.96}$ ($n=15$, $r^2=0.78$, $p<0.001$) and $ML=8.00 \times 10^{-2} T^{1.58}$ ($n=239$, $r^2=0.73$, $p<0.001$) for the autumn-winter and spring-summer hatching groups, respectively (Fig. 8). There was a highly significant difference between ML and estimated age of the two hatching groups (ANCOVA test, age ranging from 115 to 190 day-old, $p<0.001$, Fig. 8). The ML growth of the squid of

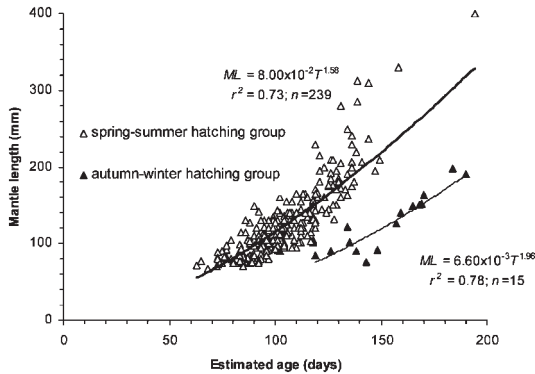


Fig. 8. Relationship between estimated age and mantle lengths of *Photololigo chinensis* in different seasons.

spring-summer hatching group was faster than that of autumn-winter group. Within the same age range (115 to 190 day-old), instantaneous growth rates of spring-summer hatching squid decreased from 0.59% to 0.36% of the mantle length compared with 0.34% to 0.15% of the mantle length in the autumn-winter hatching group.

3-4 Statolith growth

The statolith width (SW) ranged from 632 to 1208 μm , and 657 to 1122 μm for male and female squids, respectively. However, there was no significant difference in SW and estimated age relationships between male and female (ANCOVA test, $p=0.09$).

The relationships between the estimated age and statolith increment width of the squid from the same hatching group were not significantly different between male and female (ANOVA test on residuals from polynomial regression estimation of increments, $p=0.19$; Fig. 9). As for spring-summer hatching group, statolith increment width increased from a mean of 3 μm at day 1 to 3.5 μm at age 50–60 days. Then, increment width gradually decreased to the mean of about 2.8–3.0 μm at age 120–140 days. But for the autumn-winter group, increment width decreased from a mean of 2.4–2.6 μm (at day 1) to 2.2 μm at day 50–60. Then, increment widths gradually increased to the mean of about 2.8 μm at age 120–140

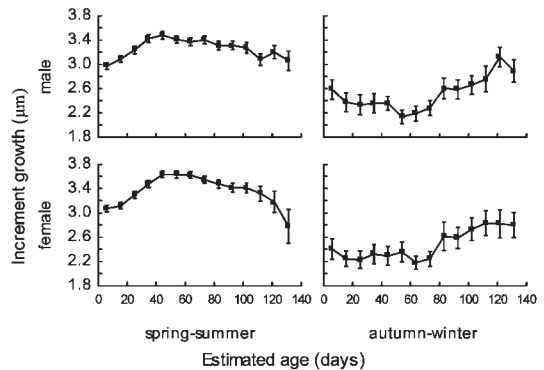


Fig. 9. Mean statolith-increment growth at estimated age of male and female squid from spring-summer and autumn-winter hatching groups. Whiskers represent 95% confidence intervals.

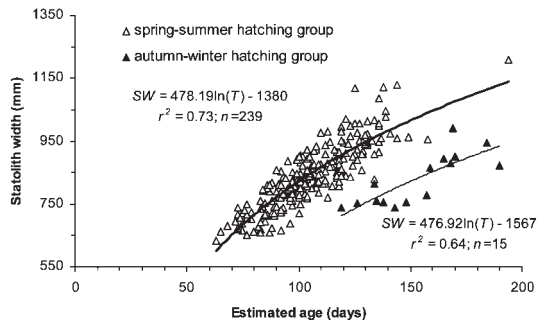


Fig. 10. Statolith widths and estimated age relation for spring-summer and autumn-winter hatching groups

days (Fig. 9).

Regressions between the estimated age and SW of squid hatching in spring-summer and autumn-winter were $SW=478.19 \ln(T) - 1380$ ($r^2=0.73$; $n=239$; $p<0.01$) and $SW=476.92 \ln(T) - 1567$ ($r^2=0.64$; $n=15$; $p<0.01$), respectively. There was a highly significant difference in slopes of SW and estimated age between these two hatching groups (ANCOVA test in the estimated age ranging from 115 days to 190 days, $p<0.001$, Fig. 10).

4. Discussion

In the Tonkin Gulf, the relationship between mantle length and body weight of male and female *P. chinensis* was significantly different,

especially in larger squid (ML>160 mm) where female was heavier than the male at the same mantle length. This may be explained by sexual dimorphism at maturity size and is agreed with previous studies of *P. chinensis* in the Gulf of Thailand (CHOTIYAPUTTA 1994), Amadan Sea (SUKRAMONGKOL *et al.*, 2007) and North Queensland, Australia (JACKSON and CHOAT 1992; JACKSON 1993). Power coefficient (b) of the length–weight relationships ($ML=aW^b$) for female ($b=2.42$) was higher than that for male ($b=2.16$) in this study, which is compatible with this species in the Gulf of Thailand with $b=2.2$ – 2.4 and $b=2.0$ – 2.1 for female and male, respectively (CHOTIYAPUTTA 1994). While the results of *P. chinensis* from Andaman Sea was comparable to our result for female ($b=2.39$), the result for male squid was different ($b=1.79$) (SUKRAMONGKOL *et al.* 2007).

In this study, the oldest squid was estimated to be 194 day–old (400 mm ML). Our result shows a remarkably shorter life span compare to the previous study of *P. chinensis* in the southern of Vietnam which exceeding 3 years with maximum ML of 340 mm (DINH *et al.* 1998). The main reason for this difference of previous study in Vietnam was base on the length–frequency relationship, which was an inadequate means of describing growth for a rapid growing organism with multiple cohorts like squids (JACKSON *et al.*, 2000). The oldest individual (400 mm ML) collected in October in this study is estimated hatching in March and grew up most of the time during the warm water period, suggesting a faster growth rate compared to that from cold period (Fig. 8). Previous studies based on the statolith increment aging method in Andaman sea and North Queensland, Australia reported that the life span of many loliginid squids was estimated to be less than 9 months (JACKSON 1993; JACKSON 2004; SUKRAMONGKOL *et al.* 2007).

P. chinensis has power growth function in sizes and estimated age relationships both in spring–summer and autumn–winter hatching groups (Fig. 8). At the age range of 115 to 190 days, the average daily growth rates varied from 0.59% to 0.36% of mantle length for summer–autumn and 0.34% to 0.15% of mantle length for the winter–spring season. There

have been a number of studies about the effects of seasons on the growth of squid (RODHOUSE and HATFIELD, 1990; JACKSON and CHOAT, 1992; JACKSON *et al.*, 1997; JACKSON and MOLTSCHANIWSKYJ, 2002). FORSYTHE and HANLON (1989) indicated that temperature has a strong influence on growth rates of the loliginid squids especially in the winter time. Experimental studies on temperature effects for growth in squids (*L. vulgaris*, *L. opalescens* and *L. pealeii*) supported the idea that there was a significant change in growth rate at different temperatures (TURK *et al.*, 1986; YANG *et al.*, 1986; HATFIELD *et al.*, 2001). In the Tonkin Gulf, sea surface temperature from December to March in 2000–2001 ranged from 20 to 23°C (21.5°C in monthly average) while that from May to October ranged from 26 to 30°C (28.6°C in monthly average), which will be a cause of the seasonal differences in growth of seasonal hatching groups of the squid.

Increment growth patterns also give strong support to the variations of the seasonal growth of *P. chinensis*. Spring–summer hatching group had mean statolith increment width ranging from about 2.2 μm to 3.5 μm which exhibits larger increment growth compared to squid hatched in autumn–winter season (means of statolith increment widths range from about 2.2 – 2.8 μm) (Fig. 9). Experiments on *Lolliguncula brevis* (DURHOLTZ and LPINSKI, 2000) also indicated a strong influence of temperature on statolith growth rate. Both somatic and statolith growth in this study support previous findings that there is a strong difference in seasonal growth pattern in *P. chienensis*. Therefore, growth modelling of loliginid squids needs to take seasonal patterns into an account.

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