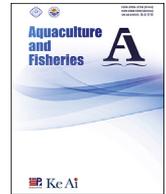




Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries/



Original research article

Gladius growth pattern and increment of jumbo squid (*Dosidicus gigas*) in the tropical Pacific Ocean

Yi Gong^a, Yunkai Li^{a, b, c, d, e, *}, Xinjun Chen^{a, c, d, e, **}, Xiaodi Gao^a, Ling Chen^f

^a College of Marine Sciences, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China

^b Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 106 Nanjing Rd., Qingdao, China

^c The Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, 999 Huchenghuan Rd., Shanghai, China

^d National Engineering Research Centre for Oceanic Fisheries, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China

^e National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China

^f College of Marine Ecology and Environment, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China

ARTICLE INFO

Article history:

Received 11 January 2018

Received in revised form

20 April 2018

Accepted 25 June 2018

Available online 13 July 2018

Keywords:

Dosidicus gigas

Gladius

Growth pattern

Increment

ABSTRACT

Gladius is an accepted hard tissue for determining the age, size-specific growth, and trophic dynamics of pelagic squid. However, little is known about the inter- and intra-stock variability of gladius growth pattern and increment deposition. In this study, the gladius growth patterns with somatic growth and gonad development were evaluated for two geographic stocks of the jumbo squid (*Dosidicus gigas*) in the tropical Pacific Ocean. The microstructure and periodicity of the gladius growth increments were also investigated. Results showed varied correlations between four gladius morphometric characteristics with dorsal mantle length, while growth in body weight almost followed a power model. Sexual gladius growth patterns occurred with gonad development, possibly due to different biological functions of these gladius parts and sex-specific energetic allocations. Gladius increments were observed formation in the stem and lateral plate and could be consistently enumerated. The daily periodicity of increment deposition, at least over 144 days old, was validated by comparing gladius increments with the ages obtained from the statoliths. The different oceanographic conditions between two areas likely influence the gladius growth and increment deposition and promote higher increment counts in squid from equatorial waters. Above all, these results provided new information on gladius microstructures and growth increment in different stocks of *D. gigas* and confirmed the use of this tissue for size-specific growth and age determination.

© 2018 Published by Elsevier B.V. on behalf of Shanghai Ocean University. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Gladius, or pen, is the typically hard tissue of a pelagic squid (Arkhipkin, Bizikov, & Fuchs, 2012). It is a chitinous internal structure that lies within the dorsomedial site of the mantle cavity. Compared with other hard tissues (e.g. statolith and beak) of squid, gladius is more reliable to determine the size-specific growth in squid, because the gladius length is highly correlated with mantle length and its growth rates similar to those of longitudinal mantle

growth (Jackson, Arkhipkin, Bizikov, & Hanlon, 1993; Perez, O'Dor, Beck, & Dawe, 1996; Bizikov & Arkhipkin, 1997; Schroeder & Perez, 2013). From the tail fin towards the head, the gladius consists of three morphological parts: rostrum, conus, and proostracum (Fig. 1). Recently, growth increments have been observed along the dorsal surface of the proostracum in squid species such as *Sepioteuthis lessoniana* (Jackson et al., 1993), *Illex illecebrosus* (Perez et al., 1996) and *I. argentines* (Schroeder & Perez, 2013). In these species, the growth increments deposition showed daily periodicity during a large part of its life, suggesting the potential applications in squid age and growth rates determination. Moreover, the gladius growth increments are easier to count since they can be read with less pretreatment than statolith method (Arkhipkin & Shcherbich, 2012). For example, after washed with warm soapy water, the gladius increments of *S. lessoniana* could directly count

* Corresponding author. Room A321, College of Marine Sciences, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China.

** Corresponding author. Room A306, College of Marine Sciences, Shanghai Ocean University, 999 Huchenghuan Rd., Shanghai, China.

E-mail addresses: ykli@shou.edu.cn (Y. Li), xjchen@shou.edu.cn (X. Chen).

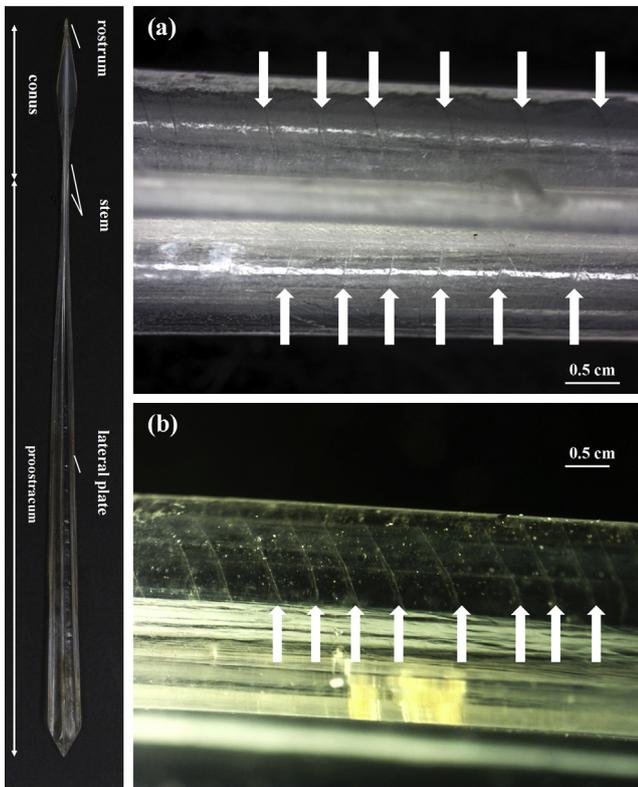


Fig. 1. Photographs of gladius of jumbo squid (*Dosidicus gigas*), a 26.7 cm mantle length individual from the waters off Peruvian exclusive economic zone. (a) growth increments on the surface of the stem (marked with white arrows) and (b): growth lines in the edge of the lateral plate.

using a zoom magnification with light (Jackson et al., 1993). However, the enumeration of increments cannot be reliably estimated by this method, because they become faint in the posterior region of the gladius and early growth is consequently masked (Jackson et al., 1993; Perez et al., 1996).

With the continuous developments of serial sampling of time-recording structure for stable isotope analysis, gladius has been considered as an accepted structure for studying high-resolution trophic dynamics in squid species of commercial importance (Kato et al., 2016; Li, Gong, Zhang, & Chen, 2017; Rosas-Luis, Navarro, Martínez-Baena, & Sánchez, 2017; Ruiz-Cooley, Villa, & Gould, 2010). For instance, sequential isotopic signatures along the *Ommastrephes bartramii* gladii showed different migratory behaviors between females and males (Kato et al., 2016). Using stable isotope analysis of gladius, Ruiz-Cooley et al. (2010) observed ontogenetic shifts in habitat use and diet of *Dosidicus gigas* in the eastern Pacific Ocean. Li et al. (2017) used a time-based consecutive sampling of *D. gigas* gladius to evaluate the influence of historical events occurring in squid lifetime. However, little attention has been paid to the variability in gladius growth patterns in squid from different origins with distinct environmental conditions, although the latter may result in a phenotypic divergence in gladius (Gong, Li, Chen, & Fang, 2018). In addition, several studies have shown that sexual size dimorphism usually occurs in the hard tissues of squid even for the same geographic stock (Fang, Chen, Su, Thompson, & Chen, 2017; Gong et al., 2018).

The jumbo squid, *D. gigas*, is a pelagic cephalopod endemic to the eastern Pacific Ocean (Nigmatullin, Nesis, & Arkhipkin, 2001). This squid supports the major fisheries in the eastern tropical Pacific and the coastal waters of western South America. As a

voracious predator and a source of prey, *D. gigas* can transfer energy from the mesopelagic food web to higher trophic level marine organisms (Field, Baltz, Phillips, & Walker, 2007). Because of the important economic and ecological role of *D. gigas*, numerous studies analyzed its foraging strategies, migration patterns and population structure in the past decades (Keyl, Argüelles, & Tafur, 2010; Nigmatullin et al., 2001). In the studies mentioned above, *D. gigas* gladius has already been used to evaluate the trophic dynamics (Li et al., 2017; Ruiz-Cooley et al., 2010), though the knowledge of gladius growth patterns remains limited, leading to difficulty in accurately understanding the information from gladius sections.

In this study, *D. gigas* from two harvest locations in the tropical Pacific Ocean were chosen, as they represented different environment conditions (offshore and nearshore tropics). We aimed to (1) evaluate the potential impacts of somatic growth and gonad development on gladius growth at inter- and intra-stock level, (2) determine the microstructure and periodicity of growth increments formation in gladius. This study contributes new information on gladius microstructures in different stocks of *D. gigas* and provides an alternative way to determine the size-specific growth and age in this species.

2. Materials and methods

2.1. Squid sampling and processing

D. gigas were sampled from commercial jigging vessels that operated in waters off the Peruvian exclusive economic zone (PER) and central equatorial Pacific Ocean (CEP) (Fig. 2). Squid were frozen on board and sent to the laboratory for processing. The dorsal mantle length (ML) and body weight (BW) of each specimen were measured. Maturity stage was determined according to Lipiński & Underhill. (1995). Statoliths were extracted and prepared for age determination.

In total, 510 intact gladii from two harvest locations were extracted and cleaned using distilled water prior to analysis (see Table 1 for detailed sampling information). Five morphometric characteristics of gladius were measured according to Gong et al. (2018). These measurements included gladius length (GL), conus length (CL), maximum width of conus (CW), proostracum length (PL) and maximum width of proostracum (PW) (Fig. 1).

2.2. Gladius growth increments

A total of 159 statoliths from two geographic stocks were suitably prepared for age determination. Gladii from the same individuals were washed again after morphometric analysis and immersed in plates with distilled water to avoid dehydration. Gladius was wiped dry carefully when start counting the number of growth increments. The increments were observed directly on the stem and lateral plate using a dissection microscope (40×) with an adjustable fiber optic light source and no special preparation. In the area of the stem, the growth lines were observed on its surface (Fig. 1a), while consistent cross-sections are shown in the edge of the lateral plate (Fig. 1b). The number of increments was counted from posterior of stem towards the anterior end of the lateral plate until the increments become faint (Fig. 1). The number of growth increments was eliminated when the difference between two independent counts exceeded 10% (Regueira, González, & Guerra, 2015). Therefore, 125 specimens (CEP, n = 69; PER, n = 56), varying from 222 to 408 mm MLs, were used to estimate the periodicity of gladius increment with the number of statolith rings.

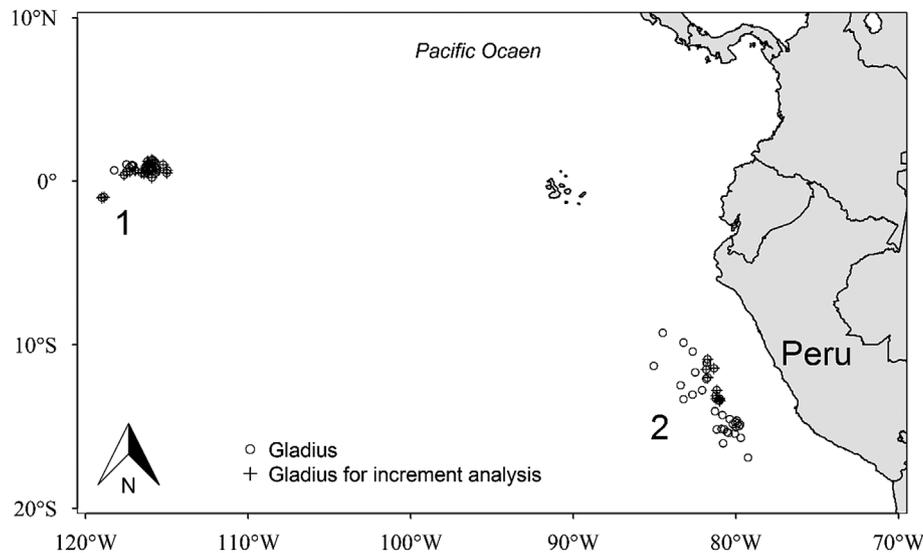


Fig. 2. Sampling sites. *Dosidicus gigas* collected in offshore waters of the central eastern Pacific (CEP, site 1) and off the Peruvian exclusive economic zone (PER, site 2).

Table 1
Summary information on mantle and gladius of jumbo squid (*Dosidicus gigas*) sampled in offshore waters of the central eastern Pacific (CEP) and off the Peruvian exclusive economic zone (PER). Values are mean \pm SD with ranges (minimum, maximum). The measurements are dorsal mantle length (ML), body weight (BW), conus length (CL), maximum width of conus (CW), proostracum length (PL), and maximum width of proostracum (PW). n = number of samples.

Geographic population	n	Sampling date	Coordinates	ML/cm	BW/g	CL/cm	CW/cm	PL/cm	PW/cm
CEP	129	Apr. to Jun. 2013	114°59' - 119°00'W 1°00'N - 1°00'S	29.2 \pm 3.4 (22.2, 37.2)	746.7 \pm 262.7 (291.0, 1523.0)	5.9 \pm 0.8 (4.2, 7.8)	0.9 \pm 0.2 (0.6, 1.5)	23.1 \pm 2.7 (17.3, 29.5)	1.5 \pm 0.2 (0.9, 2.0)
	48	Apr. to May. 2014	116°00' - 117°29'W 0°42' - 0°61'N	33.0 \pm 3.0 (27.5, 41.2)	1057.4 \pm 291.4 (608.0, 1827.0)	6.6 \pm 0.8 (5.3, 8.9)	1.2 \pm 0.1 (0.6, 1.5)	25.9 \pm 2.2 (22.0, 31.4)	1.7 \pm 0.2 (1.3, 2.1)
	165	Jul. to Oct. 2013	79°57' - 83°24'W 10°54' - 15°09'S	26.7 \pm 3.3 (21.8, 39.6)	544.2 \pm 244.8 (286.0, 1647.0)	5.2 \pm 0.9 (3.5, 8.5)	0.2 \pm 0.1 (0.5, 1.3)	21.1 \pm 2.4 (17.0, 30.1)	1.4 \pm 0.2 (1, 2.2)
PE	64	Feb. to Sep. 2014	74°57' - 83°13'W 10°26' - 16°54'S	28.0 \pm 6.9 (19.2, 48.5)	740.3 \pm 670.7 (211.0, 3361.0)	5.2 \pm 1.6 (3.1, 9.6)	0.8 \pm 0.2 (0.4, 1.5)	22.5 \pm 5.2 (17.0, 37.5)	1.4 \pm 0.4 (0.9, 2.5)
	104	Jun. to Sep. 2015	74°45' - 85°03'W 9°16' - 15°26'S	27.6 \pm 5.2 (20.7, 45.6)	658.8 \pm 547.5 (225.0, 2799.0)	5.3 \pm 1.3 (3.4, 9.5)	0.8 \pm 0.2 (0.5, 1.5)	21.7 \pm 4.0 (16.7, 36.2)	1.4 \pm 0.3 (1.0, 2.5)

2.3. Data analysis

The correlations between squid somatic growth (ML and BW) and gladius microstructure morphometrics (CL, CW, PL, and PW) were explored by fitting linear, power and exponential models, respectively, using the largest r^2 (coefficient of determination) methods (Table S1). To evaluate the impact of gonad development on gladius growth, one-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) test were performed on the variation of gladius morphometrics by different maturity stages.

The relationship between statolith and gladius increment counts was determined using linear regression analysis and using a t -test to validate gladius increment deposition rate (H_0 : slope = 1).

3. Results

3.1. Relationships of somatic to gladius growth

The gladius of *D. gigas* is a flexible structure lying along the dorsal midline of its mantle. Strong linear correlations were detected between gladius length and dorsal mantle length in both geographic stocks (CEP: $r = 0.987$, $ML = 1.031GL - 0.644$; PER: $r = 0.990$, $ML = 1.007GL + 0.263$), while positive power relationships were found with body weight (CEP: $r = 0.974$, $BW = 0.022GL^{3.082}$; PER: $r = 0.981$, $BW = 0.028GL^{3.005}$).

The values of four gladius measurements (CL, CW, PL, and PW) were summarized in Table 1. Analysis of r^2 values indicated the best models for ML with CL, CW and PW varied between locations or sexes, respectively (Table 2). In addition, the linear model was suitable for estimating the relationships between ML and PL in both population units. On the contrary, among the three models tested, the power model showed best fit for most correlations between body weights and gladius morphometric variables (Table 2), while the linear relationship was observed between CW and BW in PER squid.

3.2. Gladius growth with gonad development

The maturity stages of specimens examined in this study were estimated to be stages I to V, with the majority (93.3%) being stages I to III. Due to sample sizes might influence the reliability, stages IV and V were excluded from the analysis of evaluating the impact of sexual maturity on gladius morphometric variables. As shown in Fig. 3, the values at maturity stages were highly variable among measurements. When comparing morphometric characteristics in each population unit, sex-specific growth patterns occurred among maturity stages. In general, the females had larger values relative to males at the same maturity stage.

Table 2

List of the selected models and diagnostic statistics for the relationships between gladius morphometric variables and somatic growth of *Dosidicus gigas* sampled in offshore waters of the central eastern Pacific (CEP) and off the Peruvian exclusive economic zone (PER). The gladius morphometric variables are conus length (CL), maximum width of conus (CW), proostracum length (PL), and maximum width of proostracum (PW). Largest r^2 (coefficient of determination) were marked with an underline.

Somatic variable	Gladius variable	Model	CEP						PER					
			Female (n = 104)			Male (n = 73)			Female (n = 197)			Male (n = 136)		
			a	b	r^2	a	b	r^2	a	b	r^2	a	b	r^2
Mantle Length/cm	CL	Linear	0.39	0.19	0.65	0.20	0.12	0.76	0.23	-1.14	<u>0.88</u>	0.23	-1.11	<u>0.91</u>
		Power	0.27	0.91	0.66	0.21	1.00	<u>0.77</u>	0.09	1.23	0.86	0.09	1.23	0.87
		Exponential	2.42	0.03	<u>0.67</u>	2.16	0.03	0.76	1.74	0.04	0.84	1.77	0.04	0.84
	CW	Linear	0.04	-0.19	0.47	0.04	-0.19	0.65	0.27	0.04	0.72	0.03	-0.08	<u>0.72</u>
		Power	0.02	1.21	<u>0.48</u>	0.01	1.26	<u>0.66</u>	0.02	1.17	<u>0.74</u>	0.02	1.06	0.62
		Exponential	0.29	0.04	0.47	0.27	0.04	0.64	0.28	0.04	0.73	0.30	0.03	0.63
	PL	Linear	0.76	0.98	<u>0.96</u>	0.75	1.33	<u>0.96</u>	0.74	1.29	<u>0.97</u>	0.74	1.33	<u>0.98</u>
		Power	0.89	0.96	0.95	0.91	0.96	0.95	0.99	0.93	0.96	0.99	0.93	0.97
		Exponential	9.08	0.03	0.95	8.80	0.03	0.94	9.29	0.03	0.96	9.37	0.03	0.96
	PW	Linear	0.06	-0.17	0.80	0.06	-0.17	0.75	0.06	-0.23	0.87	0.06	-0.26	<u>0.90</u>
		Power	0.03	1.12	0.80	0.03	1.14	<u>0.76</u>	0.03	1.15	<u>0.90</u>	0.03	1.16	0.87
		Exponential	0.50	0.04	<u>0.81</u>	0.46	0.04	0.74	0.49	0.04	0.86	0.47	0.04	0.85
Body weight/kg	CL	Linear	2.14	4.32	<u>0.63</u>	2.54	4.01	0.72	2.27	3.83	0.79	2.29	3.77	0.80
		Power	6.50	0.29	<u>0.65</u>	6.58	0.33	<u>0.74</u>	6.48	0.38	<u>0.84</u>	6.60	0.40	<u>0.83</u>
		Exponential	4.55	0.34	0.64	4.24	0.43	0.69	4.08	0.37	0.70	4.02	0.63	0.67
	CW	Linear	0.46	0.63	0.47	0.50	0.58	0.59	0.35	0.58	<u>0.76</u>	0.34	0.58	<u>0.71</u>
		Power	1.09	0.39	<u>0.49</u>	1.09	0.42	<u>0.64</u>	0.98	0.37	0.74	0.98	0.36	0.65
		Exponential	0.67	0.46	0.47	0.63	0.54	0.57	0.62	0.37	0.65	0.62	0.35	0.58
	PL	Linear	8.54	16.79	0.91	9.56	16.06	0.89	7.52	16.96	0.93	7.53	16.76	0.91
		Power	25.58	0.31	<u>0.94</u>	25.83	0.32	<u>0.93</u>	25.61	0.29	<u>0.96</u>	25.96	0.31	<u>0.96</u>
		Exponential	17.68	0.35	0.89	16.90	0.41	0.84	17.77	0.30	0.88	17.58	0.29	0.84
	PW	Linear	0.64	1.00	0.77	0.72	0.92	0.71	0.59	1.01	0.84	0.59	0.96	0.84
		Power	1.65	0.36	<u>0.80</u>	1.66	0.38	<u>0.74</u>	1.70	0.37	<u>0.88</u>	1.68	0.36	<u>0.86</u>
		Exponential	1.07	0.41	0.76	0.99	0.50	0.68	1.08	0.37	0.76	1.04	0.36	0.74

3.3. Microstructure and periodicity of gladius increment

On the surface of the stem, gladius increments were defined as thin lines (Fig. 1a). In the anterior direction, they progressively became cross sections in the edge of the lateral plate (Fig. 1b). The increments were discernible and roughly symmetrically distributed on both sides of the stem and lateral plate edges (Fig. 1a), but hardly be recognized at the anterior end of the lateral plate (less than 1/10 of the total length, approximately). A great intra-individual variability of growth rates was showed by distances between consecutive growth increments visualized (Fig. 1a and b), although absolute intervals were not measured.

Assuming the increment of statolith is daily deposition (Arkhipkin & Shcherbich, 2012), the estimated lifespan of the sampled squid were 141–265 days old. For females, the number of gladius increments showed strong positive relationships with statolith rings (i.e. age in days) in both geographic stocks and the regression slopes were not differing from 1 (CEP: $t = -0.47$, $df = 30$, $p > 0.05$; PER: $t = -1.73$, $df = 37$, $p > 0.05$) (Fig. 4, Table 3). These suggested gladius growth increments were deposited at the same rate as statolith increments, although some increments were faint at the anterior end of the lateral plate. Identical conclusions were obtained in male *D. gigas* (CEP: $t = 1.91$, $df = 35$, $p > 0.05$; PER: $t = -1.77$, $df = 15$, $p > 0.05$). In addition, the count of gladius increments was consistently larger than the number of statolith rings, but their ratios differed among sites and sexes, exhibited lower values in PER squid (mean \pm SD, 1.11 ± 0.10 and 1.18 ± 0.06 for females and males, respectively) than CEP (1.52 ± 0.16 and 1.52 ± 0.13 for females and males, respectively).

4. Discussion

4.1. Gladius growth

The gladius is a flexible internal structure that grows from the

tail fin towards the head (Arkhipkin et al., 2012). Strong correlations were found for gladius length (GL) versus ML and BW in each population unit, and it confirmed the use of gladius to reconstruct *D. gigas* growth patterns. Identical conclusions were obtained in *D. gigas* from the Gulf of California by Ruiz-Coolley et al. (2010) and in other studies of ommastrephids (Bizikov, 1995; Kato et al., 2016). Allometric growth is a common phenomenon that occurs in the hard tissues of ommastrephids (Fang et al., 2015). Our results also showed that most of the correlations between the four gladius morphometrics (CL, CW, PL, and PW) and ML were allometric (Table 2). In contrast, the linear model best describes the relationships between PL and ML in both origins and sexes, and it might be considered synchrony between the longitudinal growth of mantle and proostracum. The results possibly due to different biological functions between conus and proostracum, the latter contributes a larger proportion of GL (>80%) and is the main structure to support the internal organs and the lengthening of the mantle (Gong et al., 2018). In addition, sex-specific relationships were occurred at cohort level, for example, the exponential and power models showed best fit for CL with ML of CEP females and males, respectively. Gong et al. (2018) have reported on sexual size dimorphism in *D. gigas* gladius, which was estimated by using discriminant analysis. This difference was attributed to sexual growth patterns resulting from the divergent energetic allocation for growth or reproduction between sexes (Ferreri, 2014; Gong et al., 2018). However, these growth performances were not observed in correlations between body weights with gladius morphometrics, we found that only the relationship between CW and BW in PER squid was not fit power model. Our study also indicated ontogenetic gladius growth patterns associated with changes in maturity stages (Fig. 3). In addition, the growth of female gladius was faster than males at the same maturity stage, although different size composition of each sex may have influenced the reliability of calculating values of gladius measurements in some maturity stage. Considering the different size and age at first maturity between

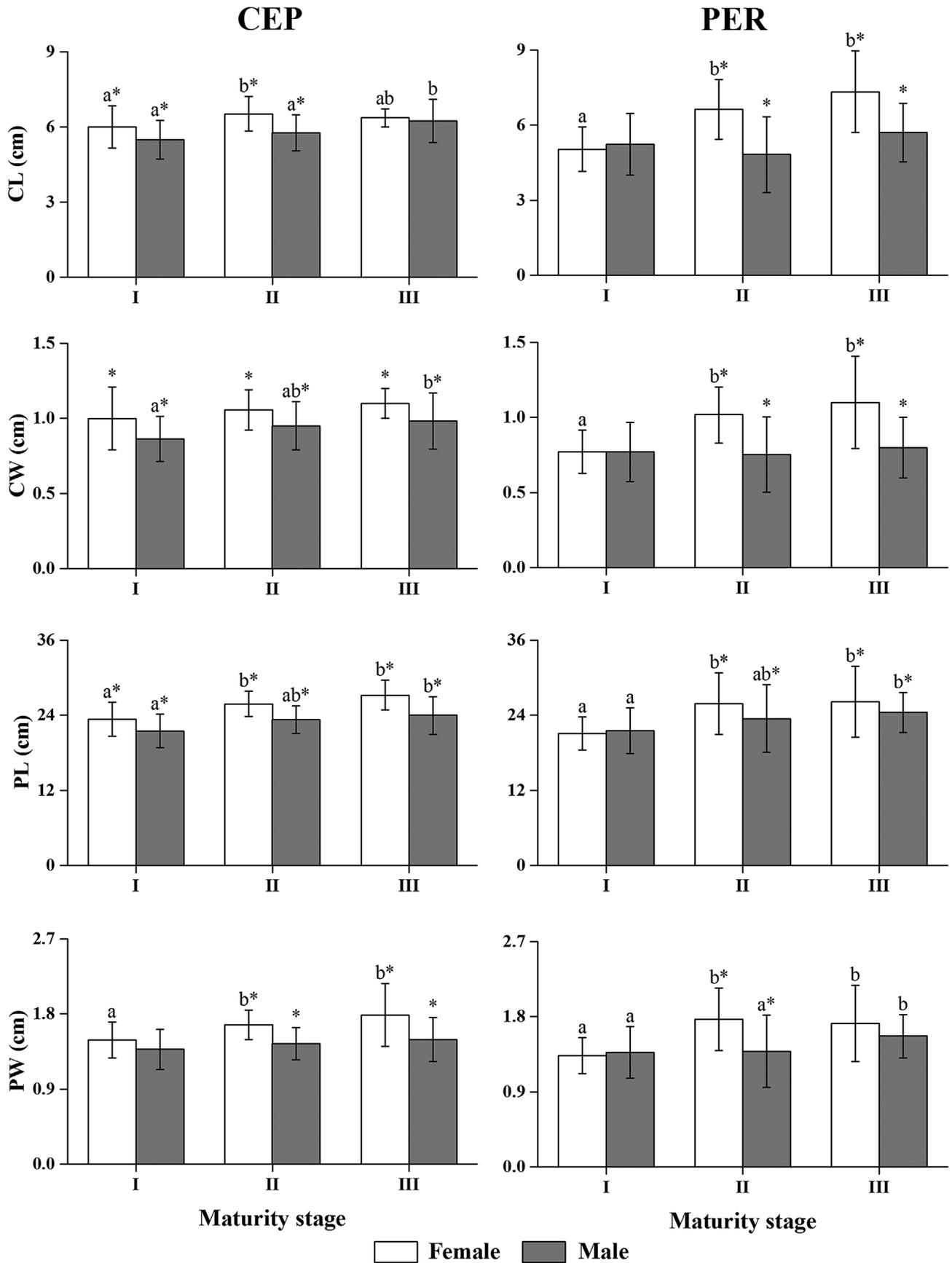


Fig. 3. Sex-specific mean values of gladius morphometric variables at different maturity stages. The vertical bars correspond to ± 1 SD. *Dosidicus gigas* collected off Peru (PER) and in offshore waters of the central eastern Pacific (CEP). For each sex, alphabetic characters (a, b) above each plot summarize pairwise post-hoc comparisons among maturity stages after analysis of variance (maturity stages not sharing letters were statistically different). Measurements in each maturity stage followed by "*" represents the significant differences between sexes.

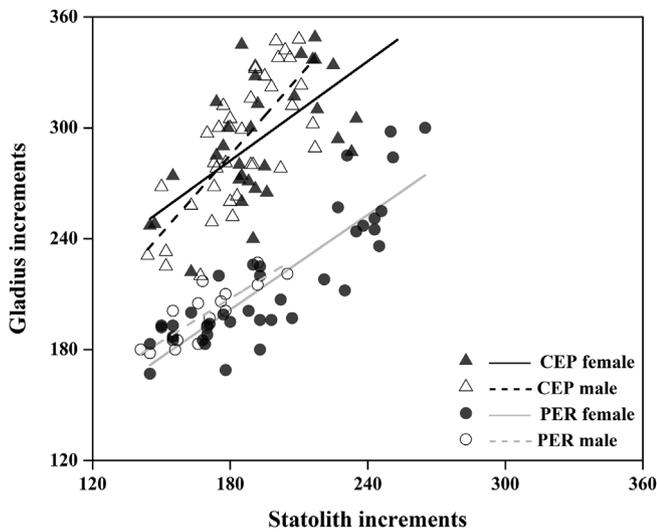


Fig. 4. The relationship between the number of *Dosidicus gigas* gladius increments and statolith increments (age in days). The sex-specific linear regressions were fitted to each geographic population: CEP, offshore waters of the central eastern Pacific; PER, off the Peruvian exclusive economic zone (EEZ). Lines represent significant relationships. See Table 3 for the details of regression equations.

female and male *D. gigas* in each harvest location (Liu, Chen, Li, & Fang, 2017; Liu et al., 2013), the sexual gladius growth patterns with maturity stages may result from the males had an early period in gonad development.

4.2. Growth increment interpretation

It is generally accepted that gladius growth increments along the dorsal surface can be used to age and intra-specific growth rates determination, although the increments cannot be read in the posterior faint zone (Bizikov, 1995; Jackson et al., 1993; Perez et al., 1996; Schroeder & Perez, 2013). Unlike the previous studies, growth increments were directly observed formation in other gladius microstructures (i.e. stem and lateral plate, Fig. 1). It appears that the counting of growth increments are probably more reliable since they can be consistently enumerated in most region of stem and lateral plate.

The correlations between statolith and gladius increment counts in this study validated the daily periodicity of increment deposition in different stocks and both sexes, from 144 to 253 (CEP) and 145 to 265 (PER) days old (Fig. 4), which represents the dominant ages (~70–90%) of *D. gigas* in these two areas (Liu et al., 2013, 2017). Similar validations were observed in increments along the gladius dorsal surface with statolith rings in *S. Lessoniana* (Jackson et al., 1993), *I. illecebrosus* (Perez et al., 1996) and

I. argentinus (Schroeder & Perez, 2013). They also found that the gladius had a much lower increment counts than that of the statolith rings. On the contrary, the enumeration of gladius increments in this study was at least over 11% as compared with that of statolith rings (Table 3). This difference is thought to depend on different recognizable region and (or) increment formation rates between gladius microstructures in the young squid. Also, there may be an ontogenetic shift in the periodicity of deposition, coinciding with that obtained by Jackson et al. (1993), found that the frequency of gladius increments is more than one per day in young squid, but showed daily deposition when squid over 3 months. Thus, in *D. gigas* paralarval and (or) juvenile phases, increments appear to be more than one per day but becoming daily periodicity over 144 days old. We assume that this ontogenetic pattern can be substantiated and resolved in the further investigation of young *D. gigas*. Another interesting finding is that the CEP squid had a much higher gladius increment counts than that of PER squid at the same age (Fig. 4), which may be attributed to the ambient conditions (e.g. temperature) between these two areas (Perales-Raya, Nande, Roura, Bartolomé, Gestal, & et al, 2017). The waters off Peru are relatively cooler than CEP since they are primarily influenced by the cold Humboldt Current and upwelling of cold subsurface waters (Anderson & Rodhouse, 2001). The lower temperature may consequently decrease the gladius increment deposition rates in the young PER squid (Perales-Raya et al., 2017).

5. Conclusion

Our study identified the *D. gigas* gladius growth patterns associated with somatic growth and gonad development in two geographic stocks and validated the periodicity of growth increment formation in gladius microstructures. At inter- and intra-population level, different growth models were found to fit the correlations between ML and BW with gladius morphometric characteristics. Gonad development also showed significant influence on gladius growth. We suggest that this phenomenon could be driven by different biological functions among gladius microstructures and sex-specific energetic allocations. Growth increments formation in the stem and lateral plate, at least over 144 days old, were a daily deposition. The spatial variation in oceanographic conditions (i.e. temperature) may influence increment deposition. However, given the high plasticity in *D. gigas* growth in relation to environmental conditions and its opportunistic feeding behavior. It is important to identify the temporal variability and whether the observed spatial variations in growth patterns is prevalent across its whole lifespan. Above all, these results confirmed the use of gladius to reconstruct *D. gigas* growth patterns, we expect that the novel growth increment counting method has potential applications in squid age and growth rates determination.

Table 3

Regression equations for the relationships between *Dosidicus gigas* statolith and gladius increments. Values are mean ± SD with ranges (minimum, maximum). CEP, offshore waters of the central eastern Pacific; PER, off the Peruvian exclusive economic zone (EEZ). n = number of samples. na = data not available.

	CEP		PER	
	Female	Male	Female	Male
n	32	37	39	17
Statolith increments (x)	195 ± 31 (145, 253)	184 ± 19 (144, 217)	196 ± 35 (145, 265)	169 ± 17 (141, 205)
Gladius increments (y)	251 ± 53 (183, 367)	291 ± 36 (220, 348)	216 ± 35 (167, 300)	199 ± 16 (178, 227)
Relationship	y = 0.90 x + 120.62		y = 0.86 x + 47.53	
r	0.65	0.74	0.86	0.84
p	<0.01	<0.01	<0.01	<0.01

Acknowledgements

We thank observers who helped us with the collection of samples. We also thank Zhang H and Wang S for processing and analyzing of samples. This work was supported by National Natural Science Foundation of China (41541042), National Natural Science Foundation of Shanghai (17ZR1413000), Shanghai Leading Academic Discipline Project (Fisheries Discipline) and Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology (2017-1A03).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.aaf.2018.06.006>.

References

- Anderson, C. I., & Rodhouse, P. G. (2001). Life cycles, oceanography and variability: Ommastrephid squid in variable oceanographic environments. *Fisheries Research*, 54, 133–143. [https://doi.org/10.1016/S0165-7836\(01\)00378-2](https://doi.org/10.1016/S0165-7836(01)00378-2).
- Arkhipkin, A. I., Bizikov, V. A., & Fuchs, D. (2012). Vestigial phragmocone in the gladius points to a deepwater origin of squid (Mollusca: Cephalopoda). *Deep Sea Research Part I: Oceanographic Research Papers*, 61, 109–122.
- Arkhipkin, A. I., & Shcherbich, Z. N. (2012). Thirty years' progress in age determination of squid using statoliths. *Journal of the Marine Biological Association of the United Kingdom*, 92, 1389–1398.
- Bizikov, V. A. (1995). Growth of *Sthenoteuthis oualaniensis*, using a new method based on gladius microstructure. *ICES Marine Science Symposia*, 199, 445–458.
- Bizikov, V. A., & Arkhipkin, A. I. (1997). Morphology and microstructure of the gladius and statolith from the boreal Pacific giant squid *Moroteuthis robusta* (Oegopsida; Onychoteuthidae). *Journal of Zoology*, 241, 475–492.
- Fang, Z., Chen, X., Su, H., Thompson, K., & Chen, Y. (2017). Evaluation of stock variation and sexual dimorphism of beak shape of neon flying squid, *Ommastrephes bartramii*, based on geometric morphometrics. *Hydrobiologia*, 784, 367–380.
- Fang, Z., Xu, L., Chen, X., Liu, B., Li, J., & Chen, Y. (2015). Beak growth pattern of purpleback flying squid *Sthenoteuthis oualaniensis* in the eastern tropical Pacific equatorial waters. *Fisheries Science*, 81, 443–452.
- Ferreri, G. A. B. (2014). Length-weight relationships and condition factors of the Humboldt squid (*Dosidicus gigas*) from the Gulf of California and the Pacific Ocean. *Journal of Shellfish Research*, 33, 769–780.
- Field, J. C., Baltz, K., Phillips, A. J., & Walker, W. A. (2007). Range expansion and trophic interactions of the jumbo squid, *Dosidicus gigas*, in the California Current. *California Cooperative Oceanic Fisheries Investigations Reports*, 48, 131–146.
- Gong, Y., Li, Y., Chen, X., & Fang, Z. (2018). Geographic variations of jumbo squid (*Dosidicus gigas*) based on gladius morphology. *Fishery Bulletin*, 116, 22–30.
- Jackson, G. D., Arkhipkin, A. I., Bizikov, V. A., & Hanlon, R. T. (1993). Laboratory and field corroboration of age and growth from statoliths and gladius of the loliginid squid *Sepioteuthis lessoniana* (Mollusca: Cephalopoda). In T. Okutani, R. K. O'Dor, & T. Kubodera (Eds.), *Recent advances in cephalopod fisheries biology* (pp. 189–199). Tokyo: Tokai University Press.
- Kato, Y., Sakai, M., Nishikawa, H., Igarashi, H., Ishikawa, Y., Vijai, D., et al. (2016). Stable isotope analysis of the gladius to investigate migration and trophic patterns of the neon flying squid (*Ommastrephes bartramii*). *Fisheries Research*, 173, 169–174.
- Keyl, F., Argüelles, J., & Tafur, R. (2010). Interannual variability in size structure, age, and growth of jumbo squid (*Dosidicus gigas*) assessed by modal progression analysis. *ICES Journal of Marine Science*, 68, 507–518.
- Li, Y., Gong, Y., Zhang, Y., & Chen, X. (2017). Inter-annual variability in trophic patterns of jumbo squid (*Dosidicus gigas*) off the exclusive economic zone of Peru, implications from stable isotope values in gladius. *Fisheries Research*, 187, 22–30.
- Lipiński, M. R., & Underhill, L. G. (1995). Sexual maturation in squid: Quantum or continuum? *South African Journal of Marine Science*, 15, 207–223.
- Liu, B., Chen, X., Chen, Y., Tian, S., Li, J., Fang, Z., et al. (2013). Age, maturation, and population structure of the Humboldt squid *Dosidicus gigas* off the Peruvian exclusive economic zones. *Chinese Journal of Oceanology and Limnology*, 31, 81–91.
- Liu, B. L., Chen, X. J., Li, J. H., & Fang, Z. (2017). Statolith microstructure, age, and maturity of jumbo squid (*Dosidicus gigas*) in equatorial waters of the eastern tropical Pacific Ocean. *Bulletin of Marine Science*, 93, 943–957.
- Nigmatullin, C. M., Nesis, K. N., & Arkhipkin, A. I. (2001). A review of the biology of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae). *Fisheries Research*, 54, 9–19.
- Perales-Raya, C., Nande, M., Roura, A., Bartolomé, A., Gestal, C., et al. (2017). Comparative study of age estimation in wild and cultured *Octopus vulgaris* paralarvae: Effect of temperature and diet. *Marine Ecology Progress Series*. <https://doi.org/10.3354/meps12218>.
- Perez, J. A. A., O'Dor, R. K., Beck, P., & Dawe, E. G. (1996). Evaluation of gladius dorsal surface structure for age and growth studies of the short-finned squid, *Illex illecebrosus* (Teuthoidea: Ommastrephidae). *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 2837–2846.
- Regueira, M., González, Á. F., & Guerra, Á. (2015). Determination of age and growth of the horned octopus *Eledone cirrhosa* (Cephalopoda: Octopoda) using stylet increment analysis. *Scientia Marina*, 79, 71–78.
- Rosas-Luis, R., Navarro, J., Martínez-Baena, F., & Sánchez, P. (2017). Differences in the trophic niche along the gladius of the squids *Illex argentinus* and *Doryteuthis gahi* based on their isotopic values. *Regional Studies in Marine Science*, 11, 17–22.
- Ruiz-Cooley, R. I., Villa, E. C., & Gould, W. R. (2010). Ontogenetic variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ recorded in the gladius of the jumbo squid *Dosidicus gigas*: Geographic differences. *Marine Ecology Progress Series*, 399, 187–198.
- Schroeder, R., & Perez, J. A. A. (2013). Individual growth of the squid *Illex argentinus* off Brazil as reconstructed from the gladius microstructure. *Journal of the Marine Biological Association of the United Kingdom*, 93, 1653–1662.