ARTIGO | *ARTICLE*

Validation of daily growth increments of statoliths of Brazilian squid *Doryteuthis plei* and *D. sanpaulensis* (Cephalopoda: Loliginidae)

Validação de incrementos de crescimento diário dos estatólitos das lulas brasileiras Doryteuthis plei *e* D. sanpaulensis *(Cephalopoda: Loliginidae)*

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ABSTRACT

This paper describes the first successful validation study of daily growth increments in the statoliths of the squids *Doryteuthis sanpaulensis* and *D. plei,* captured off the Southeastern coast of Brazil. Twenty-nine squid were kept in a laboratory and fed with fish injected with chlortetracycline. The chlortetracycline fluorescent markers were observed in polished statoliths previously exposed to UV light. The number of growth increments between the chlortetracycline marker and the edge of the statoliths corresponds to the number of days elapsed. The capture, transportation and laboratory procedures are described below.

Key words: Statoliths. Growth increment validation. Doryteuthis sanpaulensis. Doryteuthis plei.

RESUMO

Este artigo descreve o primeiro estudo bem sucedido sobre validação dos incrementos de crescimento dos estatólitos das lulas Doryteuthis plei *e* D. sanpaulensis, *capturadas na costa sudeste brasileira. Vinte e nove lulas foram*

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mantidas em laboratório e alimentadas com peixes contendo clorotetraciclina. As marcas fluorescentes de clorotetraciclina foram observadas sob luz UV. Os incrementos entre a marca de clorotetraciclina e a borda do estatólito foram contados e corresponderam ao número de dias decorridos. A captura, o transporte e os procedimentos laboratoriais estão descritos.

Palavras-chave: *Estatólitos. Validação do incremento de crescimento.* Doryteuthis sanpaulensis. Doryteuthis plei.

INTRODUCTION

On the continental shelf of Southern Brazil, two loliginids, *Doryteuthis plei* and *D. sanpaulsensis*, can be found in large concentrations (Martins & Perez, 2007; Haimovici *et al.*, 2008).

Doryteuthis plei prefers warm coastal waters and is exploited seasonally on the continental shelf (22º - 28ºS) by small scale, artisanal and industrial fleets (Perez *et al.*, 2005). Shrimp trawlers capture *Doryteuthis sanpaulensis* as by-catch throughout the region between 22ºS and 34ºS (Gasalla *et al.*, 2005). Both species exhibit large fluctuations in seasonal abundance, apparently influenced by local oceanographic events (Costa & Fernandes, 1993).

On cephalopods, hard structures such as statoliths, pens, beaks and crystalline lenses are efficient instruments for estimating age, due to the incremental growth deposits (Arkhipkin, 1991). Hence, interest has focused on statolith deposition marks, with validation as a critical pointer in determining age.

Statolith rings in squid were first mentioned by Clarke (1965) and it was subsequently mooted that they were deposited on a daily basis (Hurley *et al.*, 1979; Lipinski *et al.*, 1998), constituting a potential method to forecast ageing and its application to the assessment of squid stocks.

Laboratory rearing studies concerning the periodicity of these increments generally involve keeping the animals in the laboratory (Nakamura & Sakurai, 1991; Natsukari *et al.*, 1991) fed on a diet containing tetracycline or strontium, substances that bind directly to the calcium present in skeletal structures (Campana, 2001). According to Moltschaniwskyj (1994), due to the high metabolism of this group, the diet is targeted directly at growth and thus the chemicals are quickly assimilated and registered as statolith increments, providing a signal (marker) on any given day (Jackson & Moltschaniwskyj, 2001).

Chlortetracycline (CTC) and Oxytetracycline (OTC) are the most useful calcium-binding chemicals in these experiments (Lipinski, 1986; Lipinski *et al.*, 1998) since they bind directly to aragonite crystals as well as to the protein matrix in the squid's statoliths during the deposition process, resulting in a fluorescent band, when viewed under ultraviolet light.

Several validation studies on statoliths of different species have sustained the hypothesis that the ring deposition takes place daily (Durholtz *et al.*, 2002). Amongst these are *Todarodes pacificus* (Nakamura & Sakurai, 1991), *Alloteutihis stubtai* (Lipinski, 1986), *Idiosepius pygmaeus* (Jackson, 1989), *Doryteuthis plei* (Jackson & Forsyhte, 2002), *Sepioteuthis lessoniana* (Jackson *et al.*, 1993) and *Loligo vulgaris reynaudii* (Lipinski *et al.*, 1998; Durholtz *et al.*, 2002). The main problem concerning the validation of statolith increments is the low survival rate of animals in captivity, usually no longer than forty days (Lipinski, 1985). Keeping them in tanks, the handling of the food and restricted movement are all responsible for the high mortality rates (Hanlon *et al.*, 1983; Vidal *et al.*, 2002; Ikeda *et al.*, 2004).

The importance of squid has been increasing in the fishing industry due to the depletion of more traditional fish stocks. Despite the importance to fisheries of *Doryteuthis plei and D. sanpaulsensis* in Brazilian waters, little data about its growth parameters and aging are available, which is critical in understanding not only the fundamental biological features, but also key elements of squid life-history, and for issues related to ecosystem modeling and management (Jackson, 2004). This paper is a contribution towards the validation of daily increments in the statoliths of these species.

MATERIAL AND METHODS

The study was conducted between October 2004 and February 2005 in the city of Ubatuba (23° 26′ 00″S; 45° 04′ 00″W), in the Brazilian state of *São Paulo*.

In the first sampling, squid were captured at nighttime by means of *zangarilhos* (local variants of jigs with hand lines) (Masumoto, 2003) (Figure 1) near the Victoria islands (23°45′ 00″S; 45° 01′ 00″W), aboard the "*Véliger II*", a vessel belonging to the *Instituto Oceanográfico da Universidade de São Paulo* (IOUSP). The squid were attracted to the surface by flashing fluorescent lights installed on the sides of the boat and the catch was placed in an open tank containing 200 liters of seawater under constant aeration. In order to reduce the metabolism and the activity of the squid, the temperature of the tank water was reduced by 2 - 3°C by adding bottles of ice, in accordance with Durholtz *et al.* (2002).

A second sampling took place in fishermen's trap nets (Figure 1) set up at Enseada beach, Cedar beach and Anchieta Island by means of hand lift-nets and small buckets. For these samplings, the Institute's small motorboat "*Nautilus*" was used,

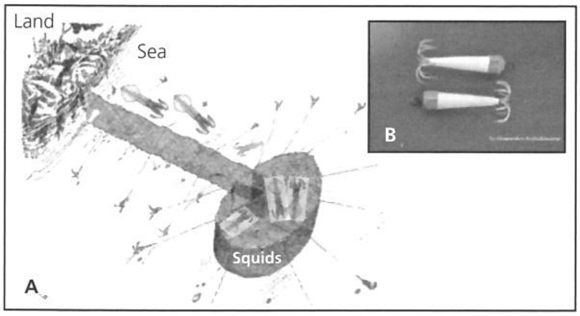

Figure 1. A: Schematic drawing of a trap net modified from Vieira *et al.* (1945).
B: "*Zangarilho*" (hand jigs).

either at dawn or dusk. The same procedures described above were maintained on board for the handling and transportation of the samples to the laboratory.

The return to the laboratory occurred between 1 and 2 hours after each sampling, regardless of the number of animals collected, thereby avoiding injury or death.

In the laboratory, the animals were carefully separated into groups of five or fewer with the aid of small buckets and then distributed into 4 circular, black fiber tanks (1.4m high and 1.7m in diameter), filled with 2,500 liters of sea water. The seawater was collected on shore around 50m from the laboratory and filtered through a decantation system. The tanks were installed in an open tent exposed to natural light. The water aeration and circulation were constant and the water flux was approximately 30 liters/min^{-1}. Water temperature and salinity were kept under natural conditions, the temperature ranging from 18.5°C to 26°C and salinity between 34 and 35.2 parts per thousand.

The squid remained for 24 hours without any interference, being fed *ad libitum* on small fish (50mm) collected with handle nets, the most common being *Trachinotus carolinus, Chloroscombrus chrysurus, Menticirrhus littoralis, Paralonchurus brasiliensis, Mugil* sp., *Anchoa tricolor* and *Atherinella brasiliensis*. After a test using whole dead fish and fish pieces, we opted to use the former since the squid rejected pieces.

The squid were fed twice a day, in the morning and the afternoon, the fish being offered one by one, and the food supply was suspended when several items of prey were shunned, remaining in the bottom of the tank. Dregs and other residues were removed via manual siphoning twice a day and half of the tank's water volume was renewed every day. Dead squid were removed immediately. Such procedures ensured a clean environment and good water quality, avoiding the effects of excreta and the accumulation of debris.

After a period of adaptation to captivity and food intake in the tanks, the procedures for validating statolith increments were followed using tetracycline hydrochloride CTC as a tracer. A solution of CTC 20g per liter of distilled water (Nakamura & Sakurai, 1991) was injected into the fish, and then a total of approximately 8 injected fish were offered to each squid. After marking, individuals would be observed for ten days. If they survived for this period of time, the process was repeated. After the death of marked animals, their Mantle Length (ML) and Total Weight (Wt) were recorded, according to the methodology described by Perez *et al.* (2002). Statoliths were prepared following the general methods reviewed by Perez *et al.* (2006), mounted on glass plates with transparent resin with the convex surface on the sagittal plane. Polishing was performed using 400μm, 9μm, 8μm and 6μm sandpaper, in decreasing order, until the focus and the edge of the dome were exposed. Both statoliths of each animal were prepared but only one was selected, randomly, for counting. These structures were examined under an ultraviolet light mercury microscope, 510nm, with 400x magnification, their images being captured and stored in an image analysis system.

Three blind counts were made independently by a single reader and their accuracy was tested using the following indices: Average Percentage Error (APE) (Beamish & Fournier, 1981) and the Coefficient of Variation (CV) (Chang, 1982). APE expressed the percentage deviation of each of the three counts from the mean count. CV expressed the standard deviation as a percentage of the mean count.

In order to check the periodicity of increment deposition, linear regressions were applied between the number of increments after the CTC marking (dependent variable) and days post-marking (independent variable). A *t*-test was applied to test the hypothesis that the regression slope was no different than 1 (H_0: b=1), which would indicate that one increment was formed every day (95% significance level).

RESULTS

The longest period of squid survival in captivity was 18 days and the shortest 2 days; 14 of them survived more than 10 days, and the average survival was 4 days.

Most of the deaths occurring during the experiments were caused by injuries generated by collisions with the tank walls, sudden disturbances that triggered ink release, technical failures and pecking amongst the animals. After a week in captivity, the squid started to collide with the tank walls with damage appearing in the terminal portion of the fins. These lesions increased with time, in some cases with the loss of the whole fin. This behavior caused death for most of the animals (63.0%). Some squid became frightened in the presence of people or noise, ejecting ink, and dying a few hours later (between 2 and 5 hours). This was observed in immature specimens (71.0%) and before the first week of confinement was complete, being responsible for a mortality rate of 15.2%. Technical failures included animals getting caught in the circulation pumps, water level variation caused by changes in the water flow, power outages and mechanical pump failure, being responsible for 15.2% of mortality. Aggression between individuals, which increased gradually over the period of containment, generated injuries mainly to their fins, and corresponded to 6.5% of deaths.

Initially the live fish supplied stimulated the appetite of the squid, being devoured quickly. The ingestion of dead fish took longer and occurred only as a result of insistence; nevertheless, this procedure facilitated the maintenance of the squid in captivity.

The concentration of CTC injected into the squid food was sufficient to produce a mark in all statoliths as a fluorescent band around the perimeter. Table 1 shows 29 individuals marked (16 females between 45-150mm ML and 13 males between 54-194mm ML), 5 of them (numbers 41, 43, 44, 45 and 46) with a second band after an interval of 10 days. The average number of statolith increments of *Doryteuthis sanpaulensis* and *D. plei* were practically equal to the number of days post-marking.

The marks were evident under ultraviolet light. In a few cases the CTC band was not well defined

Table 1. Average number of increments (AIN) between the CTC mark and the statolith edge. Numbers 41, 43, 44, 45 and 46 show the AIN between two CTC marks and AIN between the CTC mark and the statolith edge, respectively. Experiments conducted with *Doryteuthis sanpaulensis* and *D. plei* on the Southeastern coast of Brazil between October 2004 and February 2005.

IN	Species	Gender	ML	DPM1	AIN	DPM2	AIN
13	*D. plei*	F	127	6	5.7		
16	*D. plei*	F	150	5	5.0		
20	*D. plei*	M	86	1	1.0		
21	*D. plei*	M	99	1	1.3		
22	*D. sanpaulensis*	F	81	2	1.7		
23	*D. sanpaulensis*	F	45	3	2.7		
24	*D. sanpaulensis*	F	50	4	3.3		
25	*D. plei*	M	89	4	3.7		
26	*D. plei*	F	58	7	6.0		
27	*D. sanpaulensis*	M	54	7	5.7		
28	*D. plei*	F	131	1	1.3		
29	*D. plei*	F	91	1	1.0		
30	*D. plei*	F	71	1	1.0		
31	*D. plei*	F	70	2	2.0		
32	*D. plei*	M	82	3	2.7		
33	*D. plei*	F	72	4	3.7		
34	*D. plei*	M	194	5	5.0		
35	*D. plei*	M	84	6	5.7		
36	*D. plei*	M	86	9	8.7		
37	*D. plei*	F	78	10	9.0		
38	*D. plei*	M	94	10	9.0		
30	*D. plei*	F	79	10	9.0		
40	*D. plei*	F	97	10	8.3		
41	*D. plei*	F	103	11	11.0	1	1.0
42	*D. plei*	M	171	13	13.0		
43	*D. plei*	F	80	13	12.3	2	2.0
44	*D. plei*	M	92	13	14.0	3	3.3
45	*D. plei*	M	89	14	14.7	4	4.0
46	*D. plei*	M	65	16	16.0	6	7.0

IN: Individual Number; DPM: Days Post-Marking; CTC: Chlortetracycline; ML: Mantle Length.

making it difficult to count the statolith increments. Figure 2 shows ten increments formed 10 days after the CTC intake. The width of the fluorescent band showed that the statolith regions grow independently: the top of the rostrum and the dorsal part of the dome exhibit faster growth, while the lateral dome grows more slowly (Figure 2).

For *D. plei*, the Average Percentage Error (APE) between the readings was 7.2%, and the Coefficient of Variability (CV) 9.6%, demonstrating an acceptable level of precision and consistency between the counts. For *D. sanpaulensis*, APE and CV were greater than 16.0% and 21.0%, respectively, probably due to the small number of animals analyzed (Table 2).

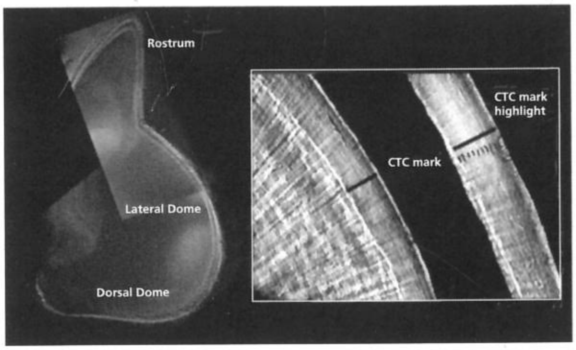

Figure 2. Two CTC fluorescent marks in the statolith of *Doryteuthis plei* (male; ML=65mm) exposed to UV light, at 100x magnification.

Note: The inset image shows in detail the CTC mark in the lateral dome exposed to normal transmitted and UV light, at 400x magnification. Its highlight shows growth increments between the CTC mark and the edge where dotted, at 500x magnification.

Table 2. Comparison between the three counts of increments deposited in statoliths of *Doryteuthis sanpaulensis* and *D. plei*, after marking with CTC. Experiments conducted on Southeastern coast of Brazil between October 2004 and February 2005.

IN	Species	Days	Counts			Mean	SD	APE	CV
			1	2	3				
13	*D. plei*	6	6	5	6	5.67	0.58	7.84	10.19
16	*D. plei*	5	5	5	5	5.00	0.00	0.00	0.00
20	*D. plei*	1	1	1	1	1.00	0.00	0.00	0.00
21	*D. plei*	1	1	1	2	1.33	0.58	33.33	43.30
25	*D. plei*	4	3	4	4	3.67	0.58	12.12	15.75
26	*D. plei*	7	5	6	7	6.00	1.00	11.11	16.67
28	*D. plei*	1	1	1	2	1.33	0.58	33.33	43.30
29	*D. plei*	1	1	1	1	1.00	0.00	0.00	0.00
30	*D. plei*	1	1	1	1	1.00	0.00	0.00	0.00
31	*D. plei*	2	2	2	2	2.00	0.00	0.00	0.00
32	*D. plei*	3	2	3	3	2.67	0.58	16.67	21.65
33	*D. plei*	4	4	4	3	3.67	0.58	12.12	15.75
34	*D. plei*	5	5	5	5	5.00	0.00	0.00	0.00
35	*D. plei*	6	6	5	6	5.67	0.58	7.84	10.19
36	*D. plei*	9	9	8	9	8.67	0.58	5.13	6.66
37	*D. plei*	10	9	9	9	9.00	0.00	0.00	0.00
38	*D. plei*	10	9	9	9	9.00	0.00	0.00	0.00
39	*D. plei*	10	10	8	9	9.00	1.00	7.41	11.11
40	*D. plei*	10	9	8	8	8.33	0.58	5.33	6.93
41	*D. plei*	11	11	12	10	11.00	1.00	6.06	9.09
42	*D. plei*	13	13	13	13	13.00	0.00	0.00	0.00
43	*D. plei*	13	13	11	13	12.33	1.15	7.21	9.36
44	*D. plei*	13	15	13	14	14.00	1.00	4.76	7.14
45	*D. plei*	14	16	14	14	14.67	1.15	6.06	7.87
46	*D. plei*	16	15	17	16	16.00	1.00	4.17	6.25
Mean								7.22	9.65
22	*D. sanpaulensis*	2	2	1	2	1.67	0.58	26.67	34.64
23	*D. sanpaulensis*	3	3	2	3	2.67	0.58	16.67	21.65
24	*D. sanpaulensis*	4	4	3	3	3.33	0.58	13.33	17.32
27	*D. sanpaulensis*	7	5	6	6	5.67	0.58	7.84	10.19
Mean								16.13	20.95

IN: Individual Number; SD: Standard Deviation; APE: Average Percentage Error; CV: Coefficient Of Variation.

The regression analysis between average increment numbers and days post-marking explained 99,0% of the variation obtained for *D. sanpaulensis* and 98.0% for *D. plei*. The slopes were 0.82 and 1.01, respectively. Figure 3 shows the relationship between the average increment number and days after marking. The t-test applied to the data showed that the number of rings is not statistically different from the number of days ($p>0.05$), for both species.

DISCUSSION

Trap nets worked better for sampling when compared to those where *zangarilhos* were used. The first caused considerably less stress and skin injuries to the animals. Moreover, since the trap nets are always located in areas sheltered from the open sea, the navigation conditions to and from these places are much calmer, making collection and transportation of animals less detrimental. The

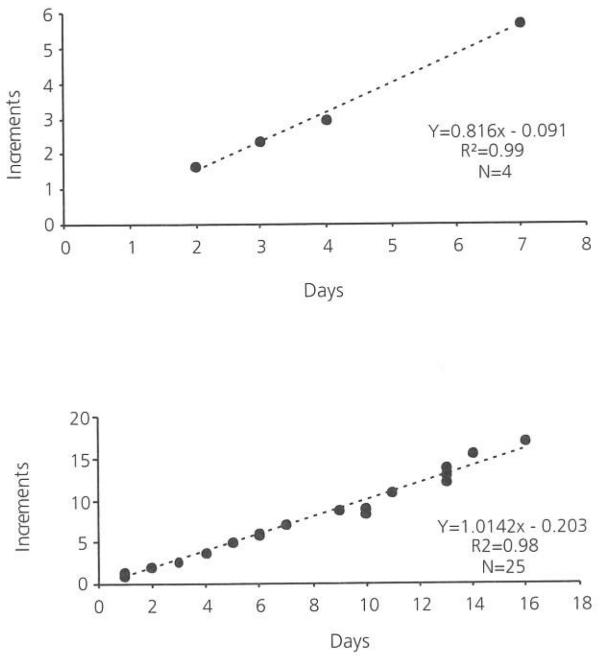

Figure 3. Relationship between the average number of increments of *Doryteuthis sanpaulensis* (on top) and *D. plei* (at bottom) and the days elapsing after CTC marking. Experiments conducted on the Southeastern coast of Brazil between October 2004 and February 2005.

use of small 2-liter buckets also appeared to be better than hand lift-nets in pulling the animals out of the sea, as they caused less damage to the skin of the squid. In general, the transportation protocol employed, including a large water volume in the tank, with continuous oxygen supply and temperature decreased by 2-3°C, helped to keep the squid alive and in good condition until their arrival at the laboratory.

The experiments conducted to validate ring growth *of D. sanpaulensis and D. plei* support the hypothesis that 1 increment is equivalent to 1 day, corroborating the previous study by Jackson & Forsythe (2002), for *D. plei*. However, the small number of animals used in the experiments should be taken into consideration. According to Lipinski (1986), studies to validate squid rings generally involve a small number of animals and in all successful studies, fewer than 300 individuals were analyzed (Jackson, 2004).

The CV and APE indices show the precision of the results as regards the increment deposition after the CTC mark, as found for *D. plei*, by Jackson & Forsythe (2002).

The majority of deaths that took place during this study occurred due to injuries at the tip of the caudal fin produced by blows, similar to the findings of Nakamura & Sakurai (1991) and Lipinski (1985) who noted that some animals could resist such injuries for up to 3 weeks.

According to Jackson & Moltschaniwskyj (2001), the squid feeding rate did not influence the frequency of ring deposition in *S. lessoniana* but the ingestion of larger amounts of food cause wider ring deposition. Adult squid are voracious predators and their hunter instinct is stimulated by the movement and visibility of their prey (Budelmann, 1996). For this reason, it is very difficult to feed these animals with dead fish. Lipinski (1985) was also unsuccessful in trying to feed *Alloteuthis subulata* with pieces of fish, the maintenance of this species depending on the constant supply of living prey. In the current experiment, *D. sanpaulensis and D. plei* adapted relatively well to a constant diet of dead fish, but we believe that the results would be better using live prey.

The CTC marks were particularly evident, showing that this substance was well incorporated into the statoliths, allowing us to analyze the deposited rings after its administration, as well as growth differences among parts of the structure; these differences were also observed by Lipinski (1986) and Nakamura & Sakurai (1991).

Although the behavior of the species was not the subject of this study, some relevant observations should be borne in mind when conducting future studies. It was observed that a large male and a mature female copulated (crown arms against crown arms), and four spawned balloon shaped capsules (two minors with 1.37cm and 1.67cm and two larger with 2.24cm and 2.65cm) were found in the bottom of the tank. Lipinski (1985) noted "buccal crown to buccal crown" copulation a few days before the appearance of encapsulated eggs in the substrate, at 20-40mm. According to this author, although the deposition of eggs could be interpreted as stress due to unfavorable conditions, active feeding and mating suggest relatively good loliginid adaptation to aquaria.

REFERENCES

Arkhipkin, A.I. (1991). Methods for cephalopod age and growth studies with emphasis on statolith ageing techniques. In: Jereb, P.S.; Ragonese, R. & von Boletzky, S. (Ed.). Squid age determination using statoliths. *Proceedings of the International Workshop Held in the Istituto di Tecnologia della Pesca e del Pescato*. Special publication. p.11-17.

Beamish, R.J. & Fournier, D.A. (1981). Method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences*, 38(8):982-3.

Buldelmann, B.U. (1996). Active marine predators: the sensory world of cephalopods. *Marine and Freshwater Behavior and Physiology*, 27(2-3):59-75.

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology*, 59:197-242.

Chang, W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries Aquatic Sciences*, 39(8):1208-10.

Clarke, M.R. (1985). Growth rings in the beaks of the squid *Moroteuthis ingens* (Oegopsida, Onychoteuthidae). *Malacologia*, 3(2):297-307.

Costa, P.A.S. & Fernandes, F.C. (1993). Seasonal and spatial changes of cephalopods caught in the Cabo Frio (Brazil) upwelling ecosystem. *Bulletin of Marine Science*, 52(2):751-9.

Durholtz, M.D.; Lipinski, M.R. & Field, J.D. (2002). Laboratory validation of periodicity of incrementation in statoliths of the South African chokka squid *Loligo vulgaris reynaudii* (d'Orbigny, 1845): a reevaluation. *Journal of Experimental Marine Biology and Ecology*, 279(1-2):41--59.

Gasalla, M.A.; Perez, J.A.A.; Marques, C.A.; Tomas, A.R.G. & Oliveira, U.C. (2005). *Doryteuthis sanpaulensis*. In: Cergole, M.C.; Avila-da-Silva, A.O. & Rossi-Wongtschowski, C.L.D.B. (Ed.). *Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil*: dinâmica populacional das espécies em exploração. São Paulo: Instituto Oceanografico-USP. p.69-73. (Serie Documentos REVIZEE-Score Sul).

Haimovici, M.; Rossi-Wongtschowski, C.L.D.B.; Bernardes, R.A.; Fischer, L.G.; Vooren, C.M.; Santos, R.A. *et al.* (2008). *Prospecção pesqueira de espécies demersais com rede de arrasto-de-fundo na região Sudeste-Sul do Brasil*. São Paulo: Instituto Oceanografico-USP. p.84-7 (Serie Documentos REVIZEE-Score Sul).

Hanlon, R.T.; Hixon, R.F. & Hulet, W.H. (1983). Survival, growth and behavior of the loliginid squids *Loligo plei, Loligo pealei* and *Lolliguncula brevis* (Mollusca: Cephalopoda) in closed sea water system. *Biology Bulletin*, 165(3):637-85.

Hurley, G.V.; Beck, P.; Drew, J. & Radtke, R.L. (1979). A preliminary report on validating age readings from statoliths of the short-finned squid (*Illex illecebrosus*). *International Coccission for the Northwest Atlantic Fisheries, Research Document*, 79(26):1-6.

Ikeda, Y.; Ueda, Y.; Sakurazawa, I. & Matsumoto, G. (2004). Transport of the oval squid *Sepioteuthis lessoniana* Férussac, 1831 in Lesson 1830-1831 (Cephalopoda: Loliginidae) for up to 24h and subsequent transfer to an aquarium. *Fisheries Science*, 70(1):21-7.

Jackson, G.D. (1989). The use of statolith microstructures to analyze life history events in the small tropical cephalopod *Idiosepius pygmaeus*. *Fisheries Bulletin United States*, 87(2):265-72.

Jackson, G.D. (2004). Advances in defining the life histories of myopsid squid. *Marine and Freshwater Research*, 55(4):357-65.

Jackson, G.D.; Arkhipkin, A.I.; Bizikov, V.A. & Hanlon, R.T. (1993). Laboratory and field corroboration of age and growth from statoliths and gladii of the loliginid squid *Sepioteuthis lessoniana* (Mollusca: Cephalopoda). In: Okutani, T.; O'Dor, R.K. & Kubodera, T. (Ed.) *Recent advances in cephalopod fishery biology*. Tokyo: Tokay University Press. p.189-99.

Jackson, G.D. & Forsythe, J.W. (2002). Statolith age validation and growth of *Loligo plei* (Cephalopoda: Loliginidae) in the north-west Gulf of Mexico during spring/summer. *Journal of Marine Biological Association of the UK*, 82(4):677-8.

Jackson, G.D. & Moltschaniwskyj, N.A. (2001). The influence of ration level on growth and statolith increment width of the tropical squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae): an experimental approach. *Marine Biology*, 138(4):819-25.

Lipinski, M.R. (1985). Laboratory survival of *Alloteuthis subulata* (Cephalopoda: Loliginidae) from the Plymouth area. *Journal of Marine Biological Association of the UK*, 65(4):845-55.

Lipinski, M.R. (1986). Methods for the validation of squid age from statoliths. *Journal of Marine Biological Association of the United Kingdom*, 66(2):505-26.

Lipinski, M.R.; Durholtz, M.D. & Underhill, L.G. (1998). Field validation of age readings from the statoliths of chokka squid (*Doryteuthis vulgaris reynaudii* d'Orbigny, 1845) and an assessment of associated errors. *ICES Journal of Marine Sciences*, 55:240-57.

Martins, R.S. & Perez, J.A.A. (2007). The ecology of loliginid squid in shallow waters around Santa Catarina, southern Brazil. *Bulletin of Marine Science*, 80(1):125--46.

Masumoto, C. (2003). *As atividades pesqueiras da comunidade caiçara de Picinguaba (Ubatuba, São Paulo).* Masters dissertation, Departamento de Oceanografia, Universidade de São Paulo.

Moltschaniwskyj, N.A. (1994). Muscle tissue growth and muscle fibre dynamics in the tropical loliginid squid *PhotoDoryteuthis* sp. (Cephalopoda: Loliginidae). *Canadian Journal of Fisheries Aquatic Sciences*, 51(4): 830-5.

Nakamura, Y. & Sakurai, Y. (1991). Validation of Daily growth Increments in Statoliths of Japanese Common Squid *Todarodes pacificus*. *Nippon Suisan Gakkaishi*, 57(11):2007-11.

Natsukari, Y.; Dawe, E. & Lipinski, M. (1991). Interpretation of data. In: Jereb, P.; Ragonese, S. & von Boletzky, S. (Ed.). Squid age determination using statoliths. *Proceedings of the International Workshop Held in the Instituto di Tecnologia della Pesca e del Pescato*. Special publication. p.113-24.

Perez, J.A.A.; Aguiar, D.C. & Oliveira, U.C. (2002). Population biology of the long-finned squid *Loligo plei* (Cephalopoda: Loliginidae) in Southern Brazilian Waters. *Fisheries Research*, 58(3):267-79.

Perez, J.A.A.; Aguiar, D.C. & Santos, J.A.T. (2006). Gladius and Statolith as Tools for Age and Growth Studies of the Squid *Loligo plei* (Teuthida: Loliginidae) off Southern Brazil. *Brazilian Archives of Biology and Technology*, 49(5):747-55.

Perez, J.A.A.; Gasalla, M.A.; Aguiar, D.C.; Oliveira, U.C.; Marques, C.A. & Tomas, A.R.G. (2005). *Loligo plei*. In: Cergole, M.C.; Avila-da-Silva, A.O. & Rossi-Wongtschowski, C.L.D.B. (Ed.). *Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil*: dinâmica populacional das espécies em exploração. São Paulo Instituto Oceanográfico. p.62-8. (Série Documentos REVIZEE-Score Sul).

Vidal, E.A.G.; DiMarco, F.P.; Wormorth, J.H. & Lee, P.G. (2002). Optimizing rearing conditions of hatchling loliginid squid. *Marine Biology*, 140(1):117-27.

Vieira, B.B.; Carvalho, J.P.; Silva, A.G.D.A,; Braga, A.A.; Ramos, F.A.; Maia, M.M. *et al.* (1945). Anuário de pesca Marítima do Estado de São Paulo, Secretaria de Agricultura, Indústria e Comércio. São Paulo: Departamento de Produção Animal.

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