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The influence of ration level on growth and statolith increment width of the tropical squid Sepioteuthis lessoniana (Cephalopoda: Loliginidae): an experimental approach

Received: 30 March 2000 / Accepted: 30 October 2000

Abstract Juvenile squids were grown in individual 2.6-l floating enclosures and were fed either a high- or a low-ration diet of fish and the crustacean Acetes. Squids were maintained for a maximum of 44 days in two experiments. The high-ration individuals reached a significantly larger size in both experiments (27, 25.5 mm mean mantle length, ML) compared to their low-ration siblings (19 mm mean ML) in both experiments. The statolith increment widths prior to the start of the experiment were significantly wider (between 3 and 4 μm) compared to the increment widths after the start of the experiment (between 2 and 3 μm) both for the low- and the high-ration squids. High-ration squids also had significantly wider increments and larger statoliths than their low-ration siblings. Even though we detected consistent trends in daily statolith increment widths for the different feeding regimes, we could not detect variation in increment widths at a daily level of resolution (i.e. as a result of differences in day-to-day food intake at an individual level). This was probably due to the relatively consistent diet experienced by each individual. These experiments revealed that ration level influences squid growth rate, statolith size and daily statolith increment width.

Introduction

The ageing of squid using statoliths has been a major tool in our understanding of squid life histories (e.g. Rodhouse and Hatfield 1990a). Current evidence suggests that statolith increments are laid down daily (e.g. Jackson 1994a; Lipinski et al. 1998) and that this occurs throughout the life span of the individual (e.g. Jackson et al. 1997). The results of ageing work have also been corroborated by culture work (e.g. Yang et al. 1986; Jackson 1994b). While statoliths have been a useful structure for understanding squid growth and population dynamics, they could be used as more specific and precise tools to decipher growth on a daily level rather than integrated over a life span. If a relationship can be established between statolith increment width and daily growth rate, then it is possible to reconstruct more accurately the growth history of an individual. Such a technique would allow for a more powerful analysis of ambient influences on squid growth.

Cephalopods have a protein-based metabolism, store very little energy as lipids (O’Dor and Webber 1986) and funnel much of their calorific intake directly into growth, with constant muscle fibre production (Moltchaniwskij 1994). Therefore the diet level may be rapidly recorded in the width of daily statolith increments, providing us with a signature of the conditions experienced by an individual on any particular day. In this way statolith analysis can be regarded as an analogous technique to daily otolith analysis in larval fish, a field that has made notable advances (see Campana and Neilson 1985; Jones 1986; Stevenson and Campana 1992).

Our study species was the tropical loliginid squid Sepioteuthis lessoniana. The daily periodicity in statolith increments in S. lessoniana has been validated in northern Australia (Jackson 1990) and in the Philippines (Balgos and Pauly 1998) and in captive populations from eggs laid in Japan (Jackson et al. 1993; González et al. 1998). This species has also been successfully raised.
in captivity throughout its life cycle (Lee et al. 1994; Nabhitabhata 1995, 1996). Growth of this species appears to be non-asymptotic with life spans less than 200 days in the tropics and possibly annual in cooler regions. *S. lessonia* has large lateral fins allowing it to hover rather than jet forcefully as in other species; it also adapts well to captivity and does not appear to be adversely affected by confined conditions. It is therefore an ideal experimental squid and one of the few species that can be routinely used for extended captive maintenance.

This study was undertaken to explore the relationship between ration level and statolith increment width. We know that the statolith increments are produced daily in a number of species, including *S. lessonia*. We therefore wanted to see whether the level of food intake could influence the thickness of the daily increment laid down.

### Materials and methods

Egg collection and rearing containers

Juvenile individuals of *S. lessonia* were hatched in captivity from eggs collected in the field. In experiment 1, eggs were collected from inter-tidal flats at Palleranda Beach in the Townsville region of North Queensland on 3 July 1996. These eggs hatched between 15 July and 24 July. For experiment 2, eggs were obtained during trawling in Princess Charlotte Bay off the North Queensland coast by the R.V. “James Kirby” in September 1997. Eggs and subsequent hatchlings were maintained in the James Cook University 120,000-l natural-seawater aquarium.

To monitor the exact quantity of food consumed and to correlate this with growth, each squid was isolated in separate 2.6-l rectangular plastic containers. The bottom of each container was removed and replaced with plastic flyscreen, and small plastic vials were attached to each end for buoyancy. These containers were then floated in larger 300-l circular plastic tanks attached to the recirculating seawater system. Owing to problems with hatchlings jumping out of containers, mesh lids were eventually fitted to the containers 2 weeks into experiment 1 and subsequently used throughout experiment 2.

Culture protocol

The isolation of hatchlings immediately after hatching resulted in very high mortality; therefore squids were allowed to grow for 10–14 days before the feeding experiments commenced. At the commencement of each experiment, squids were stained in a solution of tetracycline-seawater (250 mg per litre) for 2 h (see Jackson 1989, 1990) to provide a starting “mark” on the statolith. After staining, each squid was photographed in a small petri dish to determine mantle length. Dorsal mantle length (ML) was measured from periodic photographs and directly from the animals at the end of each experiment. Water temperature was recorded daily (±0.05 °C) and ranged from 24.5 °C to 27.5 °C in experiment 1 and from 26.5 °C to 28.0 °C in experiment 2.

Individual squids were fed either a low- or a high-ration diet in both experiments. Food items were predominantly small live fish (mullet, *Mugil* sp. and glassshish, *Ambassas* sp.), although the crustacean *Aectes sibogae australis* was used occasionally as a food organism. Experiment 1 ran for 41 days with 10 of the original 20 squids surviving. In experiment 2, ten squids per treatment were used, but substantial mortality in the first week resulted in the loss of all but three of the high-ration squids. Therefore the experiment was recommenced with more siblings 9 days later. At the termination of the experiment, the original three surviving squids had been kept in experimental conditions for 44 days and eight were kept for 35 days. Ration levels (per day) for the low-ration squids in experiment 1 were as follows: one food item for days 1–3, two items for days 4–7 and three food items for days 8–41. For the high-ration squids, the daily ration was two food items for days 1–3, four for days 4–7 and six for days 8–41. Ration levels (per day) for the low-ration squids in experiment 2 were: two food items for days 1–3, three food items on day 4, four food items for days 5–21 and six items for days 21–35. For the high-ration squids, ration levels were: four food items for days 1–4, eight food items for days 5–20 and 12 food items for days 21–35 (except for the initial three high-ration survivors that had been maintained for 29 days when food was increased to 12 items).

If too many prey items were presented to an individual, each would only be partially consumed. Furthermore, due to the selectivity by squids, a prey item not eaten within 12 h was unlikely to be eaten at all. Therefore the uneaten prey was removed and replaced; this often stimulated the squid to eat. To quantify all food eaten, food was given to the animals two to three times a day. Generally, most food organisms were eaten soon after being placed in the container, especially after several days into each experiment.

Statolith analysis

Statoliths from squids in experiment 2 were mounted whole in Crystal Bond thermoplastic cement. Owing to the small size of the statoliths, it was not necessary to grind or polish them to reveal the daily increments (e.g. Jackson et al. 1997). The statolith increments were viewed using an Olympus BX 50 microscope and counted and measured on a captured image using Bioscan Optimus and a 17” high-resolution computer monitor, generated using a Pulnix black-and-white video camera via an Imascan video card in a Pentium computer. Increment widths were determined using the automatic flagging feature of Bioscan Optimus which was then modified by the observer (see González et al. 1998). Increment width measurements were taken along the longest axis of the lateral region of the statolith. In most instances, increments were not clearly discernible on the edge; therefore width measurements were only possible on the clear sequence prior to the edge region.

Measurements were also taken on the statoliths using Bioscan Optimus. These included total statolith length, from the dorsal dome to the rostrum, and statolith radius, from the focus to the margin in the lateral region that had the longest axis.

Statistical analysis

The size of the squids during each experiment was compared between the two ration levels using repeated-measures ANOVA. A comparison between the two experiments was not possible because size-at-age data points were not recorded on the same day for both experiments. In experiment 1, squids were measured on days 20, 30 and 39 whereas, in experiment 2, measurements were taken on days 0, 16 and 35.

On each day that the squids were measured, the dorsal ML was compared between the two treatments using a t-test. As a number of non-independent t-tests were done within each experiment, it was necessary to adjust the type I error rate using a Bonferroni adjustment (Day and Quinn 1989).

It was only possible to use growth information from day 19 for experiment 1, because of a problem with “escapes” overnight on days 6, 11 and 13. Owing to escapes and high mortality rates in experiment 1, insufficient numbers of squids were available to obtain statolith information. Therefore we only used squids from experiment 2 for the analysis of statolith growth and increment analysis. Increment width was analysed using repeated-measures ANOVA for 10 days pre- and 10 days post-tetracycline staining to see whether staining and commencement of the experiment influenced the pattern of increment widths.

To analyse the effect of ration level on statolith increment width during the experiment, we ran a repeated-measures ANOVA.