THE USE OF STATOLITH MICROSTRUCTURES TO ANALYZE LIFE-HISTORY EVENTS IN THE SMALL TROPICAL CEPHALOPOD *IDIOSEPIUS PYGMAEUS*

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ABSTRACT

Populations of the sepioid *Idiosepius pygmaeus* were located in mangrove and estuarine localities in the Townsville region of North Queensland Australia in 1986. This species was small, easy to observe and collect in the field and sexually dimorphic, with females being much larger than males.

Statolith microstructures of *I. pygmaeus* proved to be a useful ageing tool which can be used to interpret life history phenomena in this species. Increments were calibrated by marking statoliths in situ with tetracycline and counting the rings laid down subsequent to marking. This validated the daily periodicity of the observed rings.

Statolith discontinuities (checks) were occasionally seen within the microstructures of some specimens. These discontinuities appear to parallel similar structures found in fish otoliths.

Based on statolith analysis, *I. pygmaeus* matured at an age of 1½–2 months. Females were larger and grew faster than their male counterparts. Females of similar age were found to vary considerably in size. The estimates of growth rates and longevity for *I. pygmaeus* suggested multiple generations within one year.

Pannella (1971) discovered daily growth increments within the otolith microstructure. Subsequently a plethora of information has been obtained from otolith microstructural analysis, which has greatly aided studies of fish biology and population dynamics (see Campana and Neilson 1985 for a review of the relevant literature). Growth ring analysis has provided a means to evaluate age structures and growth rates in young fishes, and constitutes a powerful tool for population analysis. Similar growth increments have been observed within the statolith microstructure of many cephalopod species (Clarke 1966; Hurley and Beck 1979; Spratt 1979; Lipinski 1981; Kristensen 1980; Rosenberg et al. 1981; Radtke 1983; Natsukari et al. 1988) although concentric rings do not appear to be laid down in octopus statoliths (Boyle 1983). Radtke (1983) suggested that squid statoliths are analogous to fish otoliths; continuing research is supporting his view.

The majority of cephalopod species appear to be short lived and exhibit rapid growth rates (Packard 1972; Saville 1987). The ability to age cephalopods is critical to understanding life history phenomena and population dynamics. Despite the presence of statolith microstructures, there have been few attempts to use this information to develop a picture of demographic events in the Cephalopoda.

Early in 1986 relatively large populations of the sepioid *I. pygmaeus* were discovered in mangrove/estuarine localities in the Townsville region of North Queensland, Australia. This species was small and markedly sexually dimorphic. *Idiosepius pygmaeus* was readily observed and captured in the field, and it was robust enough to make a useful experimental organism. Moreover its small size suggested a relatively short lifespan, providing the potential of obtaining complete records of age and size specific events.

Microstructural examination of the statolith of *I. pygmaeus* was undertaken. Growth rings were present and could be used as an accurate means of ageing. Most similar studies have focused on Northern Hemisphere temperate squids. This is the first study to analyze statolith ring structure and periodicity in a tropical sepioid.

The research aims of this investigation were twofold: 1) to validate statolith increments as growth rings and 2) to utilize the growth ring data to interpret life history phenomena.

MATERIALS AND METHODS

Idiosepius pygmaeus specimens were captured between May and August 1986, and in May 1987,

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in nearshore mangrove/estuarine localities in the Townsville region which is located in the Central Great Barrier Reef Province. The littoral-dwelling *I. pygmaeus* was easily dipnetted off rocks and along mangrove mud banks in the Ross River and Ross Creek estuaries and in the Townsville marina. Specimens used in morphometric analysis were preserved in 10% formalin buffered with borax, while specimens subsequently used for statolith analysis were frozen.

Measurements were taken with an Olympus SZ binocular microscope[2] equipped with an ocular micrometer eyepiece. All weights are wet weights and were taken by blotting the specimen dry and compressing it to expel any water from the mantle cavity. All lengths measured are dorsal mantle length (DML).

Idiosepius pygmaeus specimens were maintained in aquaria during 1988 using a recirculating seawater system. They were fed ad libitum with the sergestid shrimp, *Acetes sibogae australis*, which were also maintained in the aquaria. The aquaria were kept outside so the specimens would maintain normal diel periodicity.

Sexual maturity was determined by the presence of spermatophores in Needham's sac (i.e., spermatophore sac) in the males and mature oocytes and large nidamental glands in the females.

Statolith Analysis

Idiosepius pygmaeus statoliths are paired calcareous structures situated within the bilobed statocysts which are located at the posterior base of the cephalic cartilage. They were removed by severing the head at the head mantle margin and carefully teasing the statocysts from the skull. The statoliths usually fell free from the anterior wall of the statocysts when the statocysts were pulled apart. Statoliths were placed on a glass slide, washed with water, and dehydrated with 100% ethanol. Final preparation involved flooding the statoliths with xylene and then mounting them anterior (concave) side down in the synthetic mountant and clearing agent, dibutyl-phthalate-polystyrene-xylene (D.P.X.), under a coverslip. This produced adequate clearing in the lateral region of the statolith near the rostrum. The use of D.P.X. provided a high degree of increment resolution obviating the need for grinding techniques.

Mounted statoliths were viewed with an Olympus BH compound microscope (400×). The growth rings observed within the statoliths are distinct bipartite structures consisting of a broad and translucent incremental zone along with a narrower opaque zone. The rings were counted using a drawing tube attached to the microscope to trace lines onto drawing paper. Subsequent counts were then taken from the traced image. To avoid bias, lines were deliberately not counted during tracing. Ring number was ascertained when the same value was achieved with replicate counts. However if there was some variation in replicates, a mean value was taken from at least 3 counts.

The nucleus of the statolith was delineated by a prominent dark ring. This feature has been shown to exist in the statoliths of other species of cephalopods (Hurley and Beck 1979; Kristensen 1980; Rosenberg et al. 1981; Lipinski 1986) and probably represents a hatching mark or first-feeding mark. Specimen age was thus determined as being the number of growth rings from the nucleus to the outer edge of the statolith.

Tetracycline Staining

Specimens used for tetracycline staining were hand-netted in the estuary, maintained in 20 L plastic buckets during collection, transported back to the laboratory and subjected to staining during the same day. Five-hundred mg of tetracycline was dissolved in 2 L of seawater, and the specimens were placed in the solution for 2 hours. A minimum of 2 hours was found to be needed for the tetracycline to be incorporated into the statolith. Tetracycline staining produced a distinct band on the statolith when viewed under ultraviolet irradiation (Leitz Dialux UV compound microscope with kp500 filter and ultra high pressure mercury lamp); the inner edge of the band corresponded to the growth increment deposited during the time of staining. The temporal mark on the statolith was used to calibrate the periodicity of the subsequent rings laid down.

RESULTS

Idiosepius pygmaeus apparently is predominantly a shallow-water estuarine species. No specimens have been captured from benthic sampling on the nearby continental shelf (P. Arnold[3]). Three-hundred

and twenty-two one-half hour oblique Tucker Trawl plankton samples taken at a number of stations across the Central Great Barrier Reef Lagoon (Jackson and Hartwick unpubl. data) yielded only 19 *I. pygmaeus* specimens, even though numerous other cephalopod larvae were captured. In comparison, up to 171 individuals have been dipnetted during one 2 hour collecting trip along 1.72 km of breakwater in the vicinity of Townsville harbor.

Statolith Structure and Microanatomy

The statoliths of *I. pygmaeus* are complex three-dimensional structures. Because of limited depth of field for one plane of focus, some rings near the nucleus are not discernible when photographed (Fig. 1A; classification is after Clarke 1978). Growth rings are most clearly seen in the lateral region near the rostrum. A specimen stained with tetracycline twice, 17 days and 8 days before death, showed considerable statolith growth over a relatively short period of time (Fig. 1B). In many instances a prominent discontinuity (check) within the statolith corresponded to the time of staining. This feature was particularly useful for subsequent ring counts under the light microscope. A tetracycline stained statolith was selected and photographed under both white light, to identify the daily ring structure (Fig. 1C) and UV light to identify the point of staining (Fig. 1D) from which the subsequent daily rings were laid down. Further validation evidence is given for six tetracycline stained specimens in which the statolith ring sequence could be accurately counted (Table 1).

Statolith checks were also observed within the ring sequence of some field-captured *I. pygmaeus* specimens (Fig. 1A, arrow). Statolith checks are considerably more prominent than the other rings due to a greater degree of transparency in the check region producing enhanced visibility under the light microscope. The degree of enhanced visibility often varied between checks.

Sexual Dimorphism and Maturity

This species shows considerable sexual dimorphism, with females achieving a much greater size than males (Fig. 2). The size for all male specimens collected ranged from 5.8 mm to 10.3 mm and 36 mg to 159 mg in weight, while females ranged from 6.5 mm to 17.6 mm and weighed between 67 mg and 655 mg.

The reasons for the marked size-related sexual dimorphism in *I. pygmaeus* could be ascertained by ageing individual males and females. Females achieve a larger size predominantly by growing at a much faster rate than males and to a lesser extent by growing older than males (Fig. 3). Moreover, weight is an unreliable index of age, particularly in females, as individuals of similar ages can vary considerably in weight (Fig. 3).

Males mature as small as 6.8 mm and 62 mg and as young as 42 days. In contrast, females matured at around 13 mm and 400 mg and as young as 60 days. The largest immature female aged was 59 days and was 339 mg and 13.7 mm. All males examined had spermatophores present although the youngest specimen caught (35 days, 6.1 mm, 40 mg) only had one spermatophore in Needham's sac.

Based on statolith age analysis, the lifespan of *I. pygmaeus* is quite short, with the oldest specimens aged being 67 days and 79 days for males and females respectively.

TABLE 1.—Age validation information for *Idiosepius pygmaeus*   (L: lateral region; R: rostrum; DD: dorsal dome).

| Mantle length (mm)	Sex	Date stained	Date experiment terminated	Number of days	Number of increments	Area of statolith where rings viewed
7.6	M	28 May 1986	3 June 1986	6	6	L
11.1	F	14 March 1988	26 March 1988	12	12	R
7.3	F	22 April 1988	28 April 1988	6	6	L
8.1	M	10 June 1988	13 June 1988	3	3	L
12.9	F	14 March 1988 25 Mach 1988	31 March 1988	10 (between staining)	9	DD
14.4	F	22 April 1988 1 May 1988	9 May 1988	9 (between staining)	9	L
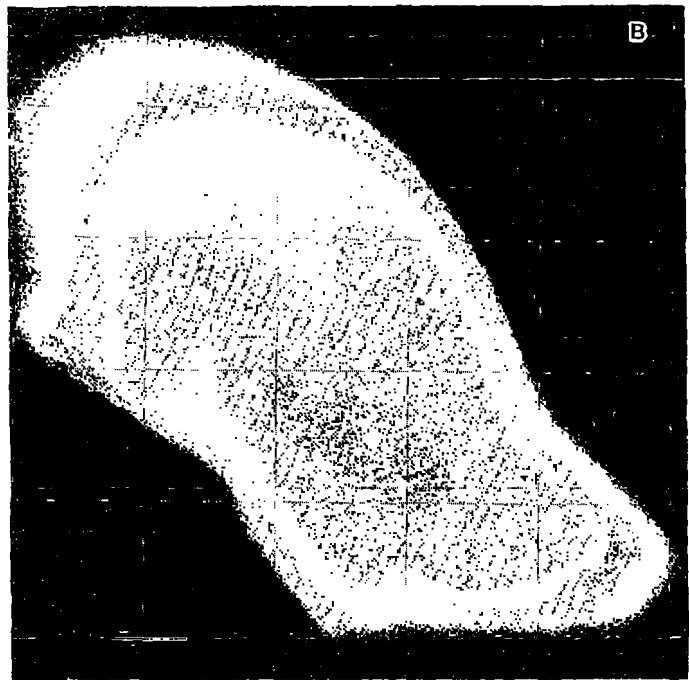
FIGURE 1.—Light micrographs of *Idiosepius pygmaeus* statoliths. A: Whole statolith from male age 36 days, length 7.2 mm, showing complex shape and ring structure, DD = dorsal dome, LD = lateral dome, R = rostrum, arrow indicates prominent check (scale bar = 24 μ); B: Whole statolith stained with tetracycline twice
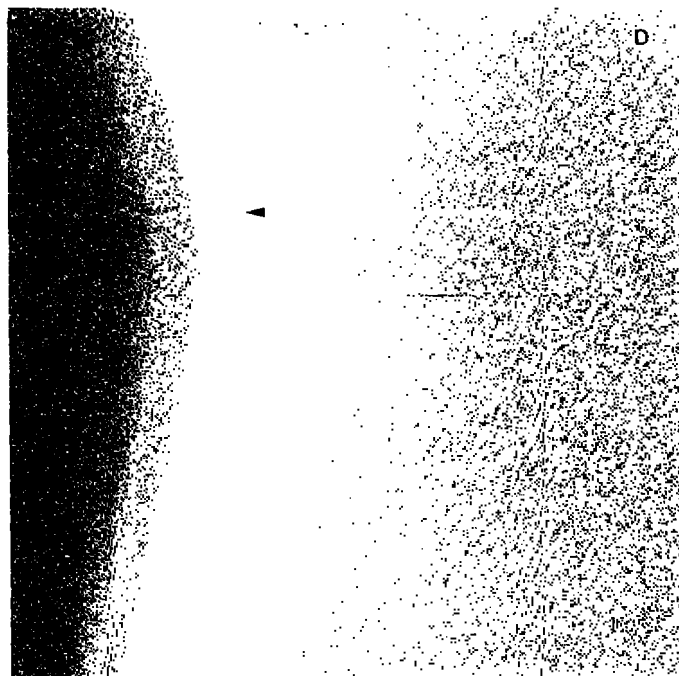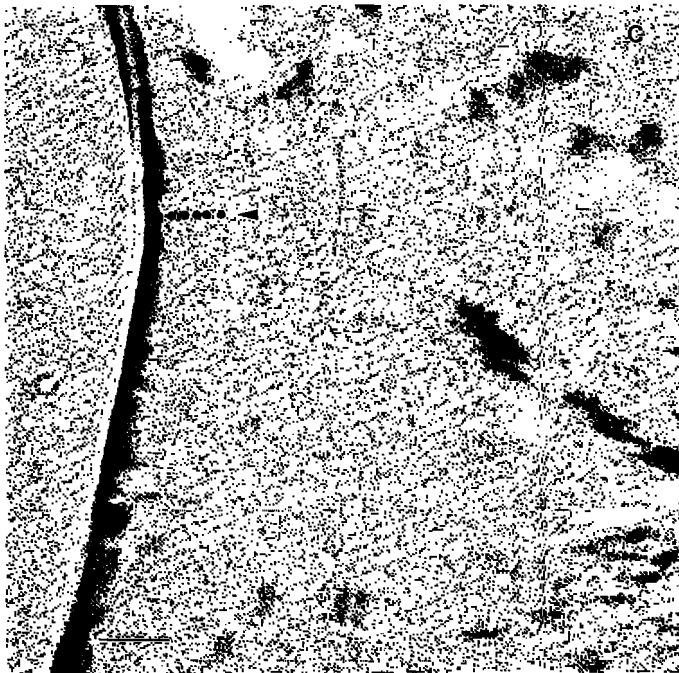
FIGURE 1.—*Continued*—17 days and 8 days before death (viewed under incident UV light) (scale bar = 25 μ); C: Ring sequence from specimens maintained in Aquarium for 6 days poststaining, arrow indicates point of staining (scale bar = 5 μ); D: Same specimen as 1C under UV light to highlight fluroescent band.
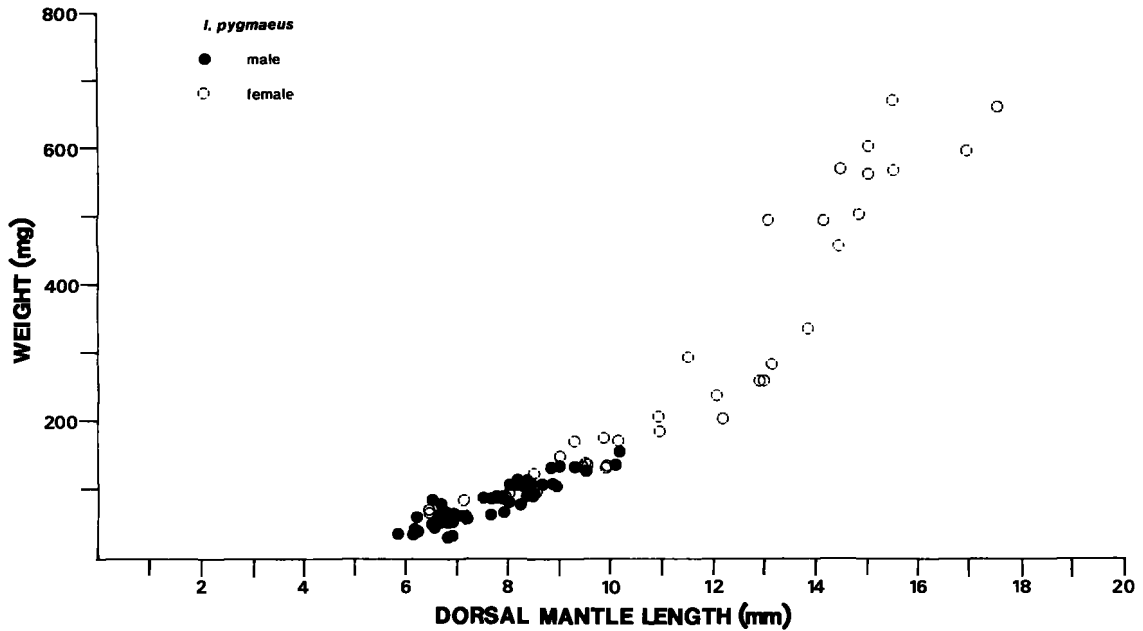
FIGURE 2.—Relationship between weight and dorsal mantle length for male and female *Idiosepius pygmaeus* specimens.
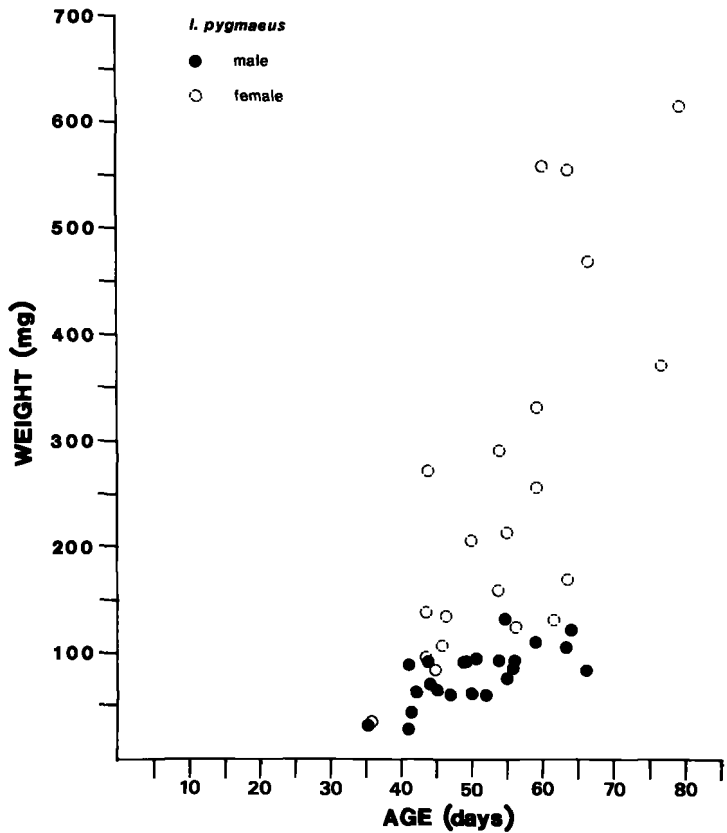

FIGURE 3.—Relationship between age (ring number) and weight for male and female *Idiosepius pygmaeus* specimens.

DISCUSSION

Daily periodicity in ring formation has been shown for both *Illex illecebrosus* (Hurley et al. 1985; Dawe et al. 1985) and *Alloteuthis subulata* (Lipinski 1986) in the North Atlantic, and for juvenile specimens of the pacific market squid, *Loligo opalescens*, (Yang et al. 1986). The tropical *I. pygmaeus* has provided further evidence for the one-ring:one-day hypothesis in a nonteuthoid cephalopod and suggests that daily statolith rings are a widespread phenomenon among cephalopod species.

The fact that the rings were most consistently visible near the lateral dome region of the statolith of *I. pygmaeus* is probably related to the thinness in this region, which allows better light transmission. A similar situation has been found in the dolphin fish *Coryphaena hippurus*. This fish has a complex elongate sagitta. Consequently when mounted in a clearing agent, growth rings are visible only on the otolith's lateral region rather than on the longest axis (Oxenford and Hunte 1983).

Check marks within the microstructure of the statoliths of *I. pygmaeus* are very similar to stress checks observed in fish otoliths (Pannella 1980; Campana 1983; Campana and Neilson 1985). Stress in fish has been shown to reduce branchial uptake of calcium, resulting in a calcium-poor check structure that is visually prominent compared to the surrounding daily increments. The visual intensity of a check in fish is often proportional to the magnitude and duration of the stress that caused it (Campana 1983). The fact that a statolith check was often induced when *I. pygmaeus* was captured and exposed to tetracycline suggests that stress is also a likely cause for statolith check formation. The trauma of capture, confinement in low oxygen conditions during collection along with subsequent staining and temperature shock associated with the transfer to aquaria, all could contribute to inducing a statolith check.

The fact that *I. pygmaeus* matures at an early age, suggests that it is capable of a number of generations in any one year. This agrees with comments by Voss (1983) that small cephalopod species are probably capable of multiple generations per year.

Although size-related sexual dimorphism is common in cephalopods the condition observed in *I. pygmaeus* is unusual and approaches one end of the continuum with the exception of species of pelagic octopods with dwarf males (e.g., *Argonauta* spp.) (Wells and Wells 1977). This study confirms that statolith microstructures provide a means for ageing cephalopods.

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