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A short-term assay of sea urchin skeletal growth based on ^{45}Ca- incorporation

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Introduction

Conventional growth analysis by the size frequency method requires large numbers of determinations and long observation times. Other disadvantages are elaborate field work and errors arising from the difficulty of confining cohorts to specific areas. Short-term growth assays performed on a small number of specimens in the laboratory clearly are an attractive alternative. Here we demonstrate that the incorporation of ^{45}Ca into the coronar skeleton is a reliable indicator of growth.

Material and methods

Parechinus angulosus was collected at Blouberg Strand, Atlantic Coast, Cape Town. 30–35 sea urchins uniform in size were incubated at 12 °C for 2–3 days in 5 l of seawater containing 2–4 x 10^8 dpm $^{45}CaCl_2$. At 1–6 hr intervals 2 urchins were removed from the incubation medium and changed through 5 washes of 150 ml of unlabelled seawater, to remove occluded tracer. The urchins were then cut open, the soft tissues were removed and the tests including the spines were wet-oxidised on a hot plate set at position 2 in 150 ml of 15 % sodium hypochlorite solution (commercial washing bleach). After an oxidation period of 30 min. and when the spines had fallen off, the tests were rinsed in 5 changes of 100 ml of tap water and one final change of 100 ml of distilled water and then dried at 105 °C. For counting 40–50 mg of the powdered skeletal material was dissolved in 2.0 ml of 0.5 N HCl at 40 °C overnight and then mixed with 15 ml of Readisolve liquid scintillation cocktail. The rate of net calcification of the coronar plates was determined from the specific activity by the derivative method as previously described (BÖHM 1978, NAUEN and BÖHM 1979).

Results and discussion

It is shown in Table 1 that skeletal growth varies with size (age) and with reproductive stage. In sexually immature juveniles the rate of skeletal growth was found to be between 2.2 and 4.7 x 10^{-5} µg Ca/mg skeletal dry matter/hour. In adults assayed during the spawning season in February the calcification rate was in the region of 1 x 10^{-5} µg Ca/mg skeletal dry matter/hour and considerably lower.

Table 1

Net calcification rate of coronar skeleton in juveniles (J) and in sexually mature individuals (A, B) of *Parechinus angulosus* at different stages of reproduction

mean test diameter mm	reproductive stage of population at time of measurement*	Gonad size and appearance	classification	net calcification rate mg Ca/mg skeletal dry weight/h
22	–	insignificant	J	3.5×10^{-5}
22–23	–	insignificant	J	$2.2–3.3 \times 10^{-5}$
25	–	insignificant	J	4.7×10^{-5}
33	spawning stage	very large	A	0.93×10^{-5}
42	spawning stage	very large	A	1.02×10^{-5}
38	spawning stage	very large	A	1.34×10^{-5}
35	post-spawning stage	small / spent	B	1.90×10^{-5}
35	post-spawning stage	small / spent	B	1.70×10^{-5}

* all experiments were conducted between February and June 1980. Peak spawning season is February to May (FRICKE 1980).

J = juveniles measured in February and March. Sexes not identifyable.
A = adult ♂ ♀ ; measured in February, March and early May 1980.
B = adult ♂ ♀ ; measured in June 1980.

When gonad index was down and when spawning had been completed (May) calcification in the same size class of adults was higher by a factor of nearly 2. From Table 1 it is clear, however, that the calcification rate of adults at post-spawning stage remains below the calcification rate of sexually immature juveniles. The observed increase of the calcification rate after completion of spawning suggests that skeletal growth and synthesis of sperm cells are interdependent processes. That maximum skeletal growth in *Parechinus angulosus* occurs before the onset of gonad growth has been shown previously by size frequency analysis (GREENWOOD 1980).

Skeletogenesis in echinoderms requires the elaboration of a protein matrix throughout the test (BEVELANDER and NAKAHARA 1960). This suggests that protein synthesis in juveniles is pronounced and is mainly directed at the development of matrix protein. Reproduction in sea urchins occurs within a relatively short space of time and requires large numbers of reproductive cells. It seems economical therefore that synthesis of matrix protein and subsequent mineralisation of matrix can be down-regulated in favour of spermiogenesis. Regulation of calcification is not unique to sea urchins. We have previously shown that calcification in asteroids under conditions of extreme food scarcity (waiting stage) is only 1.26 μg Ca/mg skeletal dry matter/hour increasing to 9.4 μg Ca/mg/h when food is not limiting (NAUEN and BÖHM 1979). Whether gonadal tissues in sea urchins can support calcification has not been investigated here. The alternative function of gonadal tissues as an energy store (LASKER and GIESE 1954) strongly suggests however that skeletogenesis occurring at times of starvation will draw on these reserves. In observations detailed elsewhere we show that the rate of net deposition of $CaCO_3$ is high during the day and near zero during the night. Variations in the rate of calcification are also believed to occur in response to changes of temperature and season resulting in higher and darker growth zones in the skeletal plates (MOORE 1935). The availability of a sensitive method for the measurement of calcification will allow more detailed studies on the factors which influence growth.

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