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Reviewed work(s):

Source: *Invertebrate Biology*, Vol. 119, No. 4 (Autumn, 2000), pp. 410-420

Published by: [Wiley-Blackwell](#) on behalf of [American Microscopical Society](#)

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Natural growth lines in echinoid ossicles are not reliable indicators of age: a test using *Strongylocentrotus droebachiensis*

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Abstract. Natural growth lines in the ossicles of echinoids have been used to estimate age, calculate growth curves, and infer population age-structure. However, few studies evaluate whether these bands are added annually—a critical assumption of the aging technique. We tested whether the banding pattern is annual in *Strongylocentrotus droebachiensis*. Sea urchins were tagged with a fluorescent marker, released into tidepools, and collected 1 year later. We quantified the position of the fluorescent mark relative to subsequent growth bands. In 30 individuals ranging in test diameter from 14 to 77 mm, and in a series of ~2 mm size intervals, we examined 3 interambulacral plates (aboral, ambital, and oral) and a rotula from Aristotle's lantern. Overall, only 7 sea urchins (23%) added a complete band to all 4 ossicles. In 6 sea urchins (20%) at least 1 ossicle added more than 1 complete band. In many sea urchins, especially those >55 mm in diameter, most ossicles added less than 1 band. The banding pattern in ossicles seriously underestimates age in *S. droebachiensis* and population parameters inferred from these growth lines are biased. Before using the growth-band aging method in other echinoids, it must be demonstrated that 1 band is added annually for all sizes in a population under field conditions.

Additional key words: calcein, Echinodermata, fluorescence, sea urchin, tetracycline

“Growth zones in test plates or spines, representing alternating periods of slow and fast growth, are frequently used for aging; however, as cautioned by Pearse and Pearse (1975), and demonstrated by Ebert (1988), these are unreliable chronometers that may simply correlate with size, or reflect any fluctuation in food supply, seasonally or not. At least, they need to be calibrated against known-age animals or seasons . . .” (Pearse & Cameron 1991, p. 561)

Natural growth lines in the ossicles of sea urchins have been recognized for over a century (Gage & Tyler 1985). These lines are due to differences in stereom microstructure between alternating opaque and translucent bands (Pearse & Pearse 1975). Opaque lines appear pale when viewed in reflected light and represent periods of fast growth, whereas translucent lines appear dark in reflected light and represent periods of slow growth (Pearse & Pearse 1975; Pearse et al. 1986). The banding pattern has been compared to tree rings (Sumich & McCauley 1973; Gage 1991) and some workers have used them to estimate age and growth rates. A critical assumption of this aging tech-

nique is that a complete cycle (1 translucent line and 1 opaque line) is added each year to the oldest ossicles for all sizes of individuals in a population.

Validating the annual accretion of growth lines is problematic in part because destructive sampling is necessary to process the ossicles. Most studies using growth lines as chronometers in echinoids have not calibrated the lines (Sumich & McCauley 1973; Fletcher et al. 1974; Lang & Mann 1976; Walker 1981; Sime 1982; Duineveld & Jenness 1984; Gage & Tyler 1985; Nichols et al. 1985; Sime & Cranmer 1985; White et al. 1985; Gage et al. 1986; Taki 1986; Meidel & Scheibling 1998). Most validation attempts rely on indirect evidence such as correlating the number of growth lines with sea-urchin size or size distributions (Miller & Mann 1973; Kawamura 1974; Crapp & Willis 1975; Lumingas & Guillou 1994; Gebauer & Moreno 1995; Turon et al. 1995). However, one would obtain a significant correlation if the formation of growth lines was episodic, but not annual because larger sea urchins have larger ossicles and larger ossicles tend to have more growth lines. The most direct method of assessing whether the banding pattern is annual comes from fluorescent tagging experiments (see Table 1). The sea urchins are tagged with a fluo-

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Table 1. Studies using fluorescent markers to assess ossicle growth in regular echinoids. Listed here are the species examined, the type of study, e.g., field vs. lab, and the length of time the sea urchins were allowed to grow after the tag was applied. The results list their findings with respect to rejecting (Reject) or validating (Validate) growth lines as chronometers.

Species	Study	Time	Results
<i>Echinus esculentus</i>	Gage 1992a	2 years; field (subtidal)	Reject for larger individuals.
<i>E. esculentus</i>	Gage 1992b	2 years; field cage (subtidal)	Validate for smaller individuals.
<i>Psammechinus miliaris</i>	Gage 1991	5–19 months; field (intertidal) and field cages (subtidal)	Validate for small animals, however, “In the largest specimens it was found that the tetracycline tag was located right at the edge of [the] plate” (p. 224).
<i>Sterechinus neumayeri</i>	Brey et al. 1995	1 year; field and field cages	Validate. Unequivocal support for this Antarctic species (N = 59).
<i>Strongylocentrotus droebachiensis</i>	Robinson & MacIntyre 1997	14 months; field cage (subtidal)	Validate (but see Discussion).
<i>S. droebachiensis</i>	this study	1 year; field	Reject.
<i>S. intermedius</i>	Kobayashi & Taki 1969	2 months; lab	Inconclusive. Demonstrated seasonal and positional asymmetry in plate growth.
<i>S. intermedius</i>	Taki 1972	14 months; lab	Validate. A “pigmented zone” found “in each test plate during the active growth period in winter” (but see Discussion and Taki 1978 below).
<i>S. intermedius</i>	Taki 1978	4 months; lab	Reject. Diet affects formation of growth lines, within individuals, lines form in plates near aboral end but not in plates near oral end.
<i>S. purpuratus</i>	Pearse & Pearse 1975	3 months; lab	Reject. More “convincing evidence” needed before growth lines can be used as chronometers.
<i>S. purpuratus</i>	Ebert 1988	1 year; field (intertidal)	Reject. Individuals >43 mm did not show growth lines past tetracycline mark and less than half of the sample <43 mm showed 1 complete cycle.

rescent marker that binds to the growing edge of the ossicles, allowed to grow for a known period, and then destructively sampled to assess the number of growth lines added since the tag was incorporated.

The few studies examining fluorescent tags in relation to growth lines have yielded mixed results (Table 1). Some workers concluded that growth lines reliably estimate age whereas others concluded growth lines are unreliable. The most recent validation study that used fluorescent tags was by Robinson & MacIntyre (1997) on the green sea urchin, *Strongylocentrotus droebachiensis* (Table 1). The animals were fed ad li-

bitum for 14 months and held in a field cage. This study focused on a limited size range of sea urchins and found that growth lines were valid indicators of age. However, in a previous fluorescent tagging study, Ebert (1988) found that in the congener *S. purpuratus* growth lines were not laid down annually (Table 1). In addition, Pearse & Pearse (1975) demonstrated that fluctuating food availability leads to the formation of additional growth lines in *S. purpuratus*. If the production of growth lines is episodic, or decreases with sea-urchin size, then growth lines are biased indicators of age. Life table parameters based on such biased es-

timates would produce distorted pictures of sea-urchin life histories. In addition to these theoretical implications, this issue has applied significance.

Globally, sea-urchin fisheries have expanded over the last two decades (Hagen 1996), particularly in North America, where strong export markets to Asia have developed (Pfister & Bradbury 1996). The dramatic decline in recent catch estimates for *S. franciscanus* in the eastern Pacific (Pfister & Bradbury 1996; Kalvass & Hendrix 1997) and *S. droebachiensis* in the Gulf of Maine (E.P. Creaser, pers. comm., Maine Dept. Marine Res.) suggests that these populations cannot support current fishery practices. If managers of sea-urchin fisheries are to make informed decisions about regulations designed to insure sustainable harvests, then they must have reliable information on age and population age-structure (Iversen 1996).

To test whether the banding pattern in *S. droebachiensis* is annual, we tagged individuals with fluorescent markers, released them, and collected them 1 year later. We quantified the position of the fluorescent mark relative to the growth lines in 4 different ossicles in different sea-urchin size classes.

Methods

In July 1994 we collected 533 specimens of *Strongylocentrotus droebachiensis* MÜLLER 1776 from tidepools on Swans Island, Maine, USA (44°8'N, 68°25'W), tagged them, returned them to the tidepools, and collected them again 1 year later. For tagging we used the fluorescent markers tetracycline and calcein. Larger sea urchins (>20 mm test diameter) received a tetracycline injection (between 0.1 ml and 1 ml of a solution of 1g tetracycline in 100 ml of seawater) through the peristomal membrane. Because of the difficulty of injecting smaller individuals (<20 mm test diameter), they were immersed in a calcein bath (0.625 g calcein and 0.5 g sodium bicarbonate dissolved in 100 ml tap water, used for ~30 liters seawater) for 24 hours. These markers bind to skeletal structures at sites of active CaCO₃ deposition and fluoresce when illuminated with ultraviolet light (Kobayashi & Taki 1969) (Table 1). Neither injecting tetracycline (Ebert 1988) nor soaking in calcein (Urbaniak, pers. comm.) significantly affects growth. One year after tagging, 262 of the 458 sea urchins collected from the tidepools displayed a fluorescent mark. The individuals we examined to test whether growth lines are added annually were also used in a study of sea-urchin growth and mortality (see Russell et al. 1998 for other details of handling procedures).

Sample selection

We selected a subset (n = 30) of the tagged sea urchins for growth line analysis, including the smallest and largest tagged sea urchins recovered (diameters = 14 mm and 77 mm). The other individuals were chosen by selecting sea urchins ~2 mm apart in test diameter between the extremes in the size range. We subdivided the sample into 3 groups of equal numbers (n = 10) and approximately equal size intervals: ≤35 mm (small), 36–55 mm (medium), and >55 mm (large).

From a randomly chosen single interambulacral column in each sea urchin, three plates (aboral, ambital, and oral), and a rotula from Aristotle's lantern, were used to assess the number of cycles added annually. Previous workers have used these ossicles to estimate age in echinoids (e.g., Sumich & McCauley 1973; Fletcher et al. 1974; Lang & Mann 1976; Sime & Cranmer 1985; White et al. 1985; Lumingas & Guillou 1994; Siversten & Hopkins 1995; Turon et al. 1995; Robinson & MacIntyre 1997; Meidel & Scheibling 1998). The ambitus is the maximum test circumference perpendicular to the oral-aboral axis. We defined the plate on this circumference in the interambulacral column as the ambital plate. The aboral plate chosen was at a point midway between the ambitus and the aboral end of the interambulacral column, and the oral plate was at a point midway between the ambitus and the oral end of the interambulacral column. Although we attempted to examine the genital plates and demipyramids, they proved to be too fragile and difficult to prepare, and did not have clearly defined growth lines.

We prepared (see below) 120 ossicles: 3 interambulacral plates and 1 rotula from each sea urchin. The range of test diameters for the 30 sea urchins we examined was 63 mm (14 mm to 77 mm) and the mean increment between adjacent sizes was 2.2 mm (SD = 1.3). The rotula from the largest individual in the sample was misplaced before scoring; therefore we examined 119 ossicles.

Ossicle preparation

Most preparation methods (e.g., Deutler 1926; Jensen 1969) require heating the ossicles, but heat destroys the fluorescent mark. Therefore, we prepared the ossicles in 2 stages, first for viewing the fluorescent mark, second for viewing the natural growth lines in relation to the fluorescent mark. In the first stage, we lightly sanded the internal surfaces of selected ossicles with 400-grit wet-dry sandpaper followed by 600-grit wet-dry sandpaper. We viewed the plates under a dissecting microscope with intense ultraviolet light (Fig. 1A). In the second stage, we lightly charred the plates

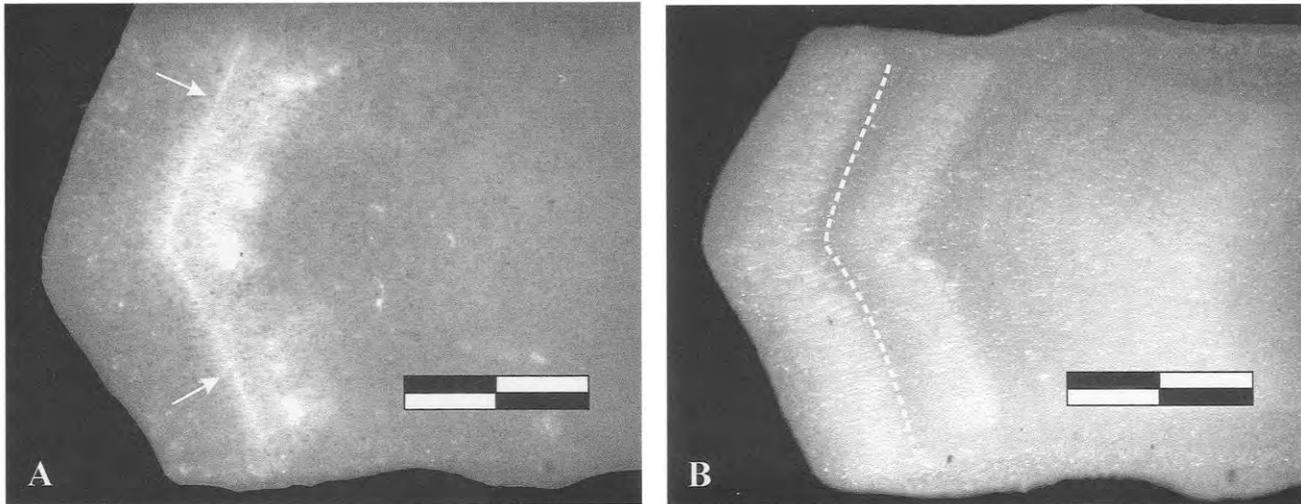


Fig. 1. Pair of corresponding ambital plates (from same position but different interambulacral rows) from a 46-mm sea urchin, illustrating both the fluorescent mark and the natural growth lines. Scale bars, 1 mm. **A.** Fluorescent tetracycline mark (arrows), photographed under reflected UV light, is clearly visible along the edge where the plate interlocks with adjacent plates in the same interambulacrum. **B.** Natural growth lines photographed under reflected light. Dashed line = position of tetracycline mark before the plate was charred. In this plate, the outer band is translucent (dark) and the tetracycline mark falls in the second translucent band from the edge. The number of cycles of opaque (light) and translucent growth lines from the mark to the outer edge equals 1. Unlike this one, most ossicles had an opaque band on the outer edge (see text and Fig. 2).

on both internal and external surfaces by repeatedly passing them through the flame of an alcohol lamp until they became light brown (5–15 seconds for each side). The plates were then carefully sanded as above and dipped in mineral oil or xylene to clear them and expose the growth lines (Fig. 1B). Rotulae were prepared using a modified method of Meidel & Scheibling (1998) by sanding (as above) to the mid-axis of the central longitudinal plane to expose the fluorescent mark. Rotulae were prepared in the same way as the interambulacral plates to expose the natural growth lines.

Quantification of growth lines

The distance from the edge of the ossicle to the fluorescent mark represents the growth for 1 year. We define a cycle as comprising a translucent band and an opaque band. If growth bands are valid chronometers, then 1 complete cycle should occur between the edge of the ossicle and the fluorescent mark (Fig. 2).

All measurements were made with an ocular micrometer on a dissecting microscope to the nearest 12.5 μm using reflected light. After preparing ossicles for viewing fluorescent marks, we measured the distance between the fluorescent mark and the fast-growing edge of the plate that faces the center of the interambulacrum, where the 2 columns of interambulacral plates interlock (Fig. 2). After charring the ossicles,

we quantified the relative positions of the natural growth lines by measuring the distance from the edge of the ossicle to each opaque and translucent line. These data determined the position of the fluorescent mark relative to the cycle of opaque and translucent natural growth lines (Fig. 2).

Results

All of the sea urchins in our sample were successfully tagged, that is, all had a clear fluorescent mark on the demipyramid of Aristotle's lantern. However, some of the ossicles we examined did not show a fluorescent mark. The 5 smallest sea urchins showed no mark on their aboral plates. Sea urchins add plates to the aboral end as they grow, so these 5 plates probably were added after the tag was applied. Of the remaining ossicles, 15 either did not show a clear fluorescent mark or the fluorescent mark was on the outermost edge of the ossicle and there was no growth beyond the mark. Almost all of these ossicles came from large sea urchins and were either oral plates or rotulae. These 15 ossicles were scored as 0—no cycles added. All aboral plates present at the time of tagging showed measurable growth, except one from the largest sea urchin in the sample (Fig. 3).

Of the 114 ossicles scored to test for an annual banding pattern (120 minus the 5 aboral plates from the smallest individuals and the lost rotula), 52 (46%)

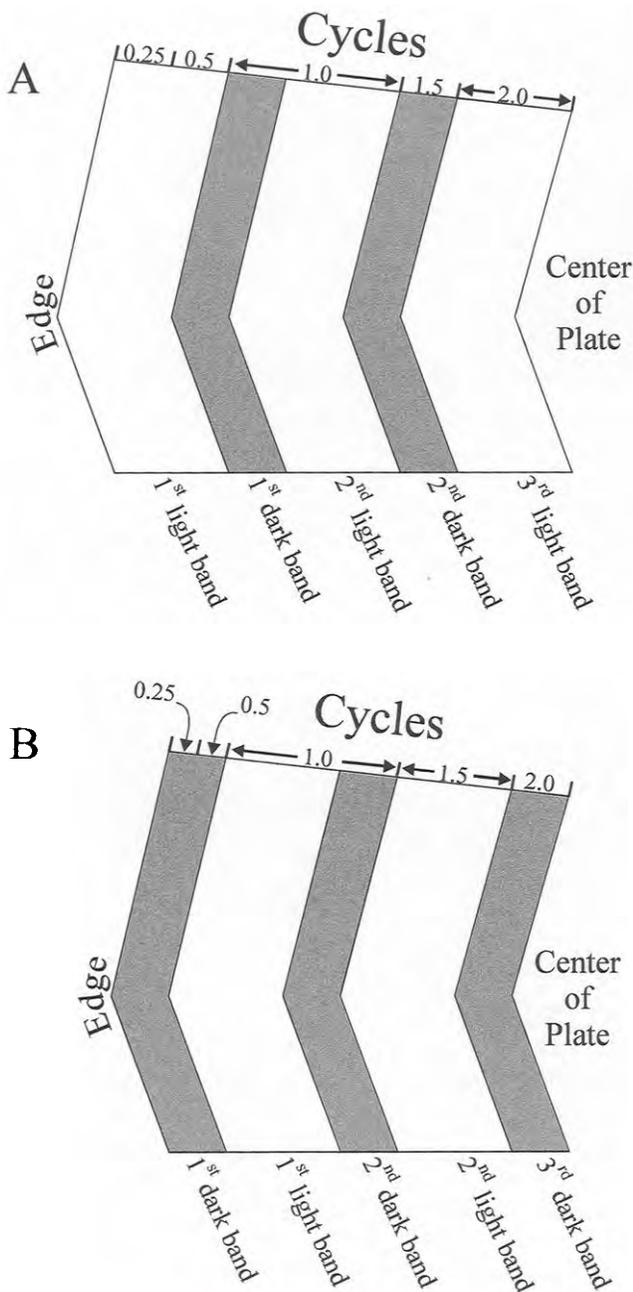


Fig. 2. Scoring of natural growth-line cycles. Bands were numbered from the edge to the center of the ossicle. **A.** Opaque (light) outer band. Most ossicles displayed this pattern. If the fluorescent mark fell anywhere in the 1st translucent (dark) band or 2nd opaque band, the ossicle was scored as 1. **B.** Translucent outer band (compare to Fig. 1). If the fluorescent mark fell anywhere in the 1st opaque band or 2nd translucent band, the ossicle was scored as 1. Scores >1 and <1 were assigned to ossicles with more than 1 cycle and fewer than 1 cycle respectively.

had 1 complete cycle, 8 (7%) had more than 1 cycle, and 54 (48%) had less than 1 cycle (Fig. 3). Only 7 individuals (23%) received a score of 1 for all ossicles.

There was no obvious pattern among the types of ossicles that displayed 1 cycle (Table 2). The aboral, ambital, and oral plates, and the rotula, all shared a similar percentage of ossicles that had 1 cycle—44%, 47%, 43%, and 48% respectively. However, there was a clear difference among the 3 size classes. Most ossicles from small sea urchins had 1 cycle (77%) whereas only 3 of the 39 ossicles (8%) from large sea urchins showed 1 cycle. Ossicles from the medium size class were between these extremes: 22 of the 40 ossicles examined (55%) displayed 1 cycle (Fig. 3). This size class showed the greatest variation in number of cycles, ranging from 0 to 2. The oldest ossicles, the rotulae and oral plates of the largest individuals, exhibited the fewest cycles: 1 of 19 had 1 cycle and 12 had scores of zero.

In the large size class, 14 of the 39 ossicles (36%) showed little or no accretion of CaCO_3 to the outer edge. These scores are not artifacts of resolution; it is not the case that growth lines were present and we could not detect them. Figure 4 shows 2 interambulacral plates from a typical large sea urchin, an ambital plate and an oral plate. The tetracycline mark is clearly visible near the edge in the ambital plate; however, the mark on the oral plate is less obvious and occurs on the outermost edge.

Discussion

Our analysis of natural growth lines shows that the banding pattern in *Strongylocentrotus droebachiensis* is not annual. Fewer than half of the ossicles we examined added a complete cycle during 1 year's growth. Moreover, an obvious effect of size was associated with the accretion of growth lines (Fig. 3). Using growth lines as chronometers in *S. droebachiensis* will seriously underestimate age and skew estimates of population age-structure. Our results with green sea urchins show the potential pitfalls in applying the growth-line aging technique uncritically in other species of echinoids.

In *S. droebachiensis*, the ossicles of Aristotle's lantern are present within 2 to 4 wk after settlement (unpubl. observ.) and as individuals grow, plates are added to the test at the aboral end while existing plates remain in place but become, relatively, more oral. Therefore, the oldest ossicles occur in Aristotle's lantern, in the oral test, and in the aboral circle of apical plates. For an ossicle to accurately reflect age, it should satisfy at least two criteria: (1) it must be present at metamorphosis or form soon thereafter and (2) it must

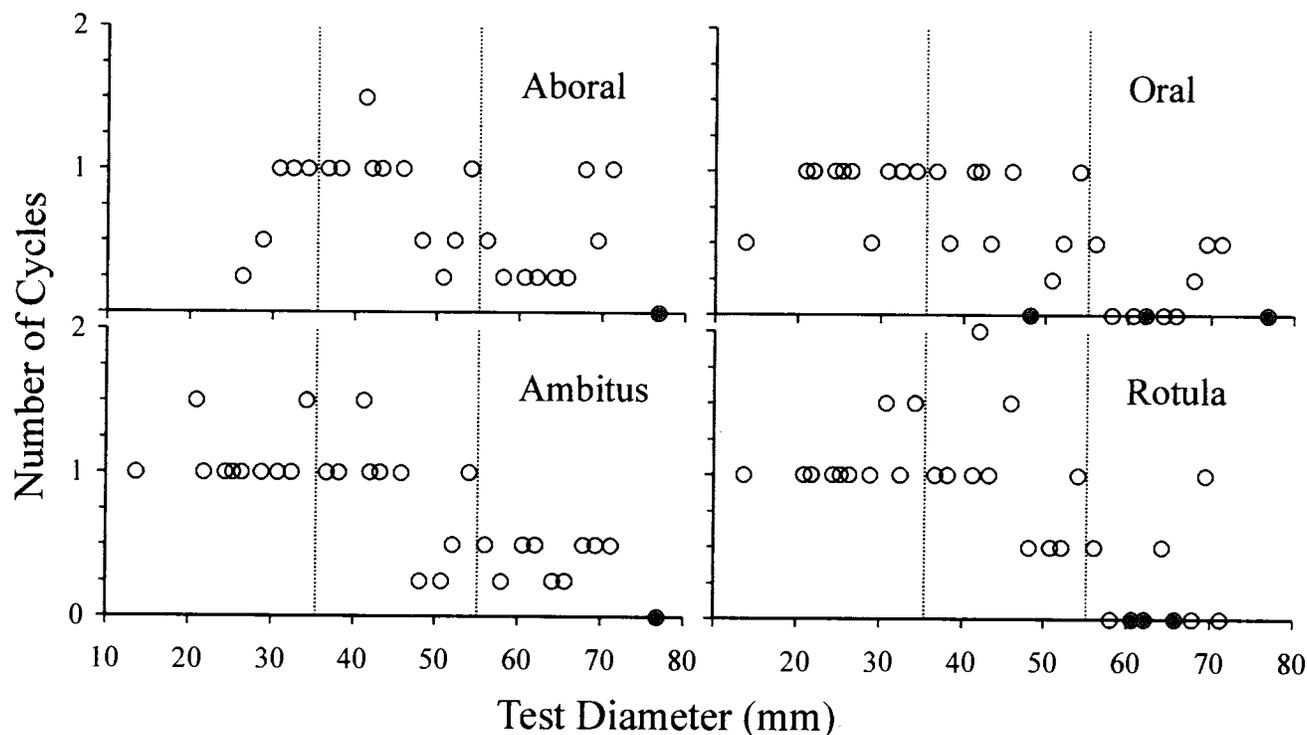


Fig. 3. Number of growth cycles from the edge to the fluorescent mark on 4 different ossicles for 30 sea urchins. Dashed vertical lines in each plot separate the 3 size classes, small (≤ 35 mm), medium (36–55 mm), and large (> 55 mm). The 8 shaded symbols represent ossicles that were not clearly marked but came from individuals that showed the fluorescent mark elsewhere in the skeleton. The aboral plates from the 5 smallest sea urchins were not included because they were not present at the time the tag was applied. One ossicle, the rotula from the largest individual, was lost (see text).

add a complete cycle of growth lines each year. Our results show that these criteria were not satisfied in *S. droebachiensis*. The oldest ossicles, the oral plates and rotulae of large individuals (Fig. 3), failed to add growth lines (12 of 19) or added less than 1 complete cycle (6 of 19).

Age estimates of *S. droebachiensis*

Recently, two studies reported age estimates of *S. droebachiensis* based on growth lines: Robinson & MacIntyre (1997) scored growth lines in the interambulacral plates of samples from the Bay of Fundy, and Meidel & Scheibling (1998) counted growth rings in the rotulae of subtidal populations from Nova Scotia.

Robinson & MacIntyre (1997) attempted to calibrate growth lines. They tagged 50 sea urchins with tetracycline, kept them in a field cage for 14 months, and fed them *Laminaria longicruris* ad libitum. The location (aboral, ambital, or oral) and number of plates examined were not reported; however, "there was a dark and white growth zone between the tetracycline line and the edge of the test plates" (Robinson & MacIntyre 1997, p. 59). The size range of their sea urchins, 25 to 58 mm, was comparable to our small and medium size classes. If they examined ambital plates, then their results and ours agree: 17 of 20 interambulacral plates from our small and medium size classes have ≥ 1 cycle (Fig. 3). Regardless of which

Table 2. Means \pm SD of cycles observed in the ossicles from the 1-year tagging experiment. Sample size for each ossicle type in each of the 3 size classes is 10, except for small aboral (5) and large rotula (9).

Size class	Aboral	Ambital	Oral	Rotula	All ossicles
Small	0.75 \pm 0.35	1.10 \pm 0.21	0.90 \pm 0.21	1.10 \pm 0.21	0.99 \pm 0.26
Medium	0.88 \pm 0.36	0.85 \pm 0.39	0.68 \pm 0.37	1.00 \pm 0.47	0.85 \pm 0.40
Large	0.43 \pm 0.33	0.38 \pm 0.18	0.22 \pm 0.21	0.23 \pm 0.33	0.31 \pm 0.28
All size classes	0.67 \pm 0.39	0.78 \pm 0.41	0.60 \pm 0.39	0.78 \pm 0.52	

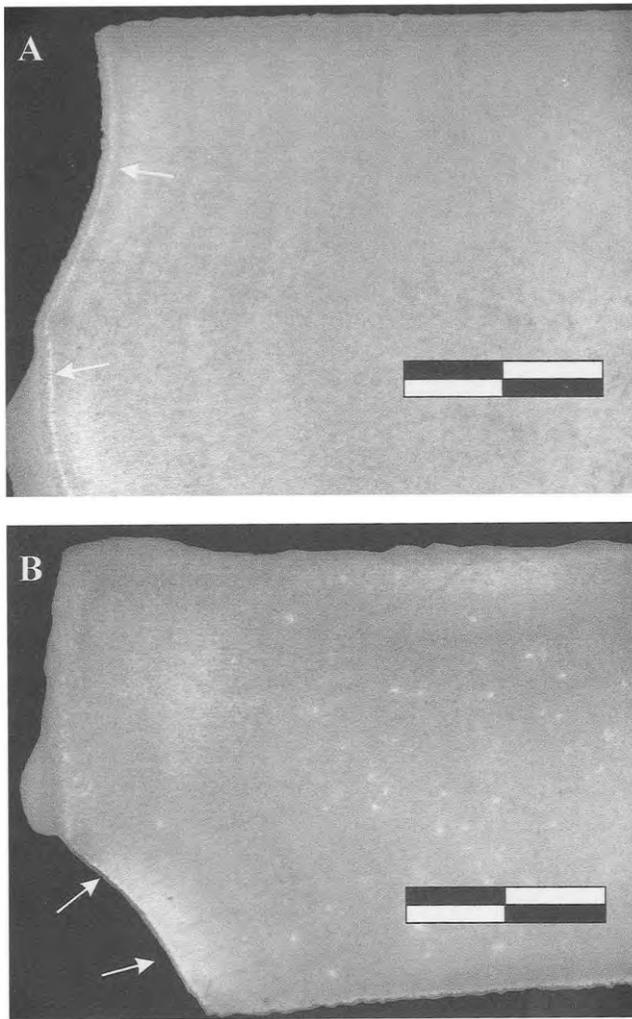


Fig. 4. Ambital and oral plates from the same tagged sea urchin (64 mm) photographed under UV light. Scale bars, 1 mm. **A.** Ambital plate. Fluorescent mark is near the plate edge (arrows). **B.** Oral plate. Fluorescent mark is barely visible (arrows); however, when the plate was rotated under the microscope, the mark was visible on the outer edge, particularly along the longitudinal surfaces. Plates like this (tagged on the edge), or plates where the tag was not clearly visible but present on another ossicle from the same individual, were scored as 0 (no growth cycles).

plates they examined, we would expect to see 1 opaque and 1 translucent growth zone in most ossicles from individuals in this size range: 40 of 55 interambulacral plates from small and medium sea urchins in our sample have ≥ 1 cycle (Fig. 3). The calibration study in the Bay of Fundy lacked sea urchins in the larger size range, and large sea urchins have the greatest distorting effect on age structures derived from growth lines. In addition, sea urchins provided with a constant supply of food would be expected to lay down regular fast and slow growth bands which are

correlated with photoperiod (Pearse et al. 1986). However, food levels in the field can vary tremendously, and fluctuating food supply affects the formation of growth bands (Pearse & Pearse 1975).

Robinson & MacIntyre (1997) also quantified Ca:Mg ratios between opaque and translucent growth bands. Water temperature influences the rate at which these elements are incorporated into the skeleton and thus the different ratios in the bands reflect seasonal growth. However, these data do not directly address the annual nature of growth bands because one would obtain consistently different ratios if band formation were seasonally episodic, but not annual. In other words, if individuals grow in some years but not in other years, then band formation and growth is seasonally episodic but not predictable on an annual basis. Our results indicate that this is the case especially for large green sea urchins. In addition, the Ca:Mg ratio data cannot address the problem presented by the cessation of ossicle growth as we observed in the oral test plates and rotulae of large individuals (Figs. 3, 4).

The data of Robinson & MacIntyre (1997) from the Bay of Fundy were the only validation data available to Meidel & Scheibling (1998), who derived age distributions and growth rates from rotula bands and compared demographic structure among 3 habitats (kelp beds, barren grounds, and grazing fronts) at 2 shallow subtidal rocky sites off Nova Scotia. The maximum age estimate they reported (9 years) is in stark contrast to the estimate of >100 years reported by Russell et al. (1998) for tidepool populations in Maine. Russell et al. (1998) calculated age from size-age growth curves derived from size specific growth measurements and the assumption that size at settlement (0.5 mm) is age zero. The intertidal vs. subtidal habitat difference between the studies does not account for the order of magnitude difference in maximum age estimates. Comparisons of growth from intertidal and subtidal habitats on Swans Island using fluorescent tagging reveal that temporal and spatial variation in growth of *S. droebachiensis* exceeds variation associated with the intertidal vs. subtidal habitat difference (Russell, in press). The discrepancy in maximum age estimates is due to the aging method.

Integrating our results with previous studies of other species of echinoids highlights at least four major problems with using growth lines as chronometers: plate growth dynamics, cohort confusion, environmental variation, and observer variation.

Plate growth dynamics

In many ways, plate growth mirrors overall growth in sea urchins (Kobayashi & Taki 1969; Pearse &

Pearse 1975; Taki 1978; Gage 1992a). Mark and recapture studies show that individuals exhibit a pattern of early rapid growth followed by a long period of declining growth (e.g., Russell 1987; Ebert & Russell 1992, 1993; McShane & Anderson 1997; Ebert 1998; Russell et al. 1998; Lamare & Mladenov 2000). Similarly, after each plate is formed, it undergoes rapid growth at first, followed by slower and slower growth (Kobayashi & Taki 1969; Pearse & Pearse 1975). The growth rate eventually slows so much that we cannot detect the minute additions of material to the periphery of the ossicle. For practical purposes, the oldest plates stop growing and therefore cannot be used to estimate age.

Cohort confusion

A second pitfall of using growth lines as chronometers is that one tends to mix age classes. In our sample we observed 8 ossicles from 6 sea urchins that added more than 1 growth cycle (Fig. 3). These animals were 20–50 mm, the size range in which growth rate is highest (see fig. 7 in Russell et al. 1998). We can speculate that these 6 animals were exposed to intermittent feeding regimes that may have produced the additional cycles. Regardless of the cause, the result is that if these ossicles were used as indicators of age, the growth that occurred would inaccurately be scored as >1 year. Animals that add 2 instead of 1 cycle will “jump ahead” to the next age class if their ossicles are used as chronometers.

The phenomenon of mixing age cohorts is even more pronounced in larger sea urchins, although it is in the opposite direction. Growth in the oldest ossicles from large animals slows to the point that no cycles are added (Fig. 3). Thus age cohorts “pile up” in the larger size classes. Underestimation of age is even more exaggerated if the animals are slow growing and long lived, which seems to be the case for at least 3 species of *Strongylocentrotus* (Russell 1987; Ebert & Russell 1992, 1993; Ebert 1998; Russell et al. 1998).

Environmental variation

A third major problem with using growth lines as indicators of age is that the changing environment induces variation in growth. Although Taki (1972) (Table 1) found that *S. intermedius* produced a single “pigmented zone” in 1 year (consistent with annual banding), he later showed that the type of algae fed to this sea urchin affects both the size and appearance of growth lines (Table 1). *Laminaria japonica* var. *ochotensis* produced greater growth and a “pigmented zone” in the plates whereas animals fed *Rhodoglossum*

japonicum and *Ulva pertusa* exhibited less growth and no “pigmented zone” (Taki 1978).

Pearse & Pearse (1975) showed that manipulating the timing of food availability could alter not only the thickness but also the numbers of growth lines in *S. purpuratus*. Much evidence demonstrates the effects of diet on overall test growth in *S. droebachiensis* (Swan 1961; Vadas 1977; Larson et al. 1980; Keats et al. 1983; Raymond & Scheibling 1987; Briscoe & Sebens 1988; Nestler & Harris 1994; de Jong-Westman et al. 1995; Lemire & Himmelman 1996). Furthermore, at least some elements of Aristotle’s lantern (the demipyramid) are highly plastic and change size (relative to overall body size) in response to resource availability (Ebert 1980; Black et al. 1982; Black et al. 1984; Edwards & Ebert 1991; Ebert 1996). Because drift algae can be a substantial part of the diet of sea urchins (Lawrence 1975; Harrold & Pearse 1987), food resources can be unpredictable and episodic. The documented effects of food, both type and amount, on the growth of skeletal elements, combined with temporal variation in availability of food resources, strongly suggest that environmental variation will affect the accretion of growth lines and reduce their reliability as estimators of age.

Observer variation

A fourth obstacle to using growth lines as chronometers is that variation between observers affects estimates of age. Although observer effects are not unique to quantifying growth bands, the problem is amplified in this case because of the ambiguous nature of the bands. Within broad opaque bands, one often observes fine translucent lines, and conversely, within broad translucent bands, fine opaque lines are sometimes visible (Smith 1980; Gage 1991). These fine lines have been interpreted as temporary hiatuses, i.e., temporary fast growth during a slow growth period (fine opaque lines in translucent bands) or a temporary cessation of growth during a fast growth period (fine translucent lines in opaque bands). The latter was shown to be true for individuals of *S. purpuratus* that were fed discontinuously. These sea urchins produced 4 translucent growth bands in 1 year; these bands represented 4 periods of 3–4 weeks each when the sea urchins had no food (Pearse & Pearse 1975).

Furthermore, there appears to be interspecific as well as intraspecific variation in the crispness and resolution of growth bands. Pearse & Pearse (1975, p. 748) observed that in *S. purpuratus* “the growth zones are often (if not usually) indistinct and ambiguous, and generally cannot be counted with much confidence.” A single opaque band with two fine translucent hia-

tuses (0.5 cycle) to one observer may be scored as three opaque bands separated by two translucent bands (2.0 cycles) by another observer.

Practical applications and consequences

The inaccuracies and inherent bias associated with using growth lines as indicators of age in *S. droebachiensis* (and possibly other echinoids as well) outweigh their usefulness. Growth curves based on these kinds of data underestimate age, especially for the largest size classes. In addition, relatively little is known about growth immediately after settlement during the “pre-recruitment” period. Recruitment in echinoderms has been defined as a size after settlement when “they are large enough to be collected in proportion to their true abundance” (Ebert 1983, p. 169). Test diameter at settlement is ≤ 0.5 mm in *S. droebachiensis* (Harris et al. 1994), and it is extremely difficult, if not impossible, to see and sample sea urchins < 5 mm in the field (unpubl. observ.). There is some evidence for a “lag phase” in green sea-urchin growth (Himmelman 1986; Russell et al. 1998), in other words, after settlement—during this pre-recruitment period—growth is slow. A lag phase in early juvenile growth would affect the accretion of growth lines and further compound the underestimation problem of ages based on these lines.

The development and expansion of the red sea-urchin fishery in the eastern Pacific and the green sea-urchin fishery in the Gulf of Maine preceded by at least 10 years studies that rigorously quantified field estimates of growth of these species (Ebert & Russell 1992; Ebert 1998; Russell et al. 1998). In retrospect, the decline in these fisheries (Kalvass & Hendrix 1997) is not surprising given what we now know about age structures. Both species are slow growing and long-lived: Ebert (1998) concluded that red sea urchins can live more than a century and Russell et al. (1998) estimated that the oldest green sea urchins in their samples exceeded 50 years. The number of growth cycles in the oral plates and rotulae for the large size class of sea urchins in our sample (the same used by Russell et al. 1998) was < 10 . The dramatic difference between age estimates derived from growth lines vs. mark and recapture measurements is due at least in part to the functional cessation of growth in the oldest ossicles (see above, Fig. 4).

Getting accurate estimates of age has significant implications for the fishery. Only a small number of conservation methods are available to sea-urchin fishery managers, including size limits (lower and upper, see Rogers-Bennett et al. 1995), fishery closures (seasonal and geographic), and catch quotas. Selecting the op-

timal strategy to sustain the fisheries will undoubtedly involve a combination of methods (Kalvass & Hendrix 1997). Estimates of age and growth should inform the process of designing management plans. As demonstrated here, population age-structures based on growth line data will only serve to hinder these efforts.

Acknowledgments. The Biology Department of Villanova provided resources for travel and laboratory equipment. The Howard Hughes Medical Institute supported this work through an Undergraduate Biological Sciences Education Programs grant to Villanova and an undergraduate fellowship to R.W. Meredith. L. Ranquist of Underwater Taxi assisted with the field work. T.A. Ebert, T.A. Pearce, P.S. Pe-traitis, J. Grassle, and 5 anonymous reviewers provided comments on earlier drafts of the manuscript. We are especially grateful to J.S. Pearse and V.B. Pearse for their critical and constructive comments.

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