Abstract — *Linckia columbiae* is a sexual species that also reproduces asexually by ray autotomy. Seventy percent of *L. columbiae* surveyed at the Catalina Marine Science Center Marine Life Refuge, Santa Catalina Island were regenerating autotomized rays. Autotomy occurred throughout the year with the highest incidence in late summer. The incidence of autotomy correlates with growth, but not gender or depth. Regeneration experiments suggest that 3 yr, or longer, may be required for a single ray to reach the adult stage. Gametogenesis was generally confined to non-regenerating rays. Oocyte production was low and spawning occurred in late summer. Lacking evidence of recent larval recruitment at the study site, population densities and morphometric features of *L. columbiae* are best explained by continuous asexual reproduction.

Introduction

The variable seastar, *Linckia columbiae* Gray, generates new multirayed individuals from single autotomized rays (Monks 1903, 1904; MacGinitie & MacGinitie 1949). In contrast, regeneration of new individuals from fissioned segments in most fissiparous asteroids requires a portion of the central disc (Emson & Wilkie 1980). Among other species of fissiparous asteroids, fission is often a significant mode of reproduction, but the emphasis on asexual and sexual reproduction varies between populations as well as between species (James & Pearse 1969; Emson 1978; Rideout 1978; Emson & Wilkie 1980; Ottesen & Lucas 1982; Crump & Barker 1985; Mladenov et al. 1986). When fission is frequent, regeneration may limit the extent of sexual development; gametogenesis may be diminished or absent. Because fission alters body shape and provides a source of new individuals, the incidence of fission affects other population parameters, especially morphometry and population density. The present study relates the incidence of ray autotomy in *L. columbiae* to population structure, growth, and sexual reproduction.

Methods

Study Sites: Pumpernickel Cove and its adjoining coastal reef are is located in the Catalina Marine Science Center Marine Life Refuge on the northwest shore near the west end of Santa Catalina Island, California. The site is an extension of steep cliffs which, underwater, give rise to boulder fields and bedrock escarpments of moderate to high relief. Patches of coarse sand and cobble occur throughout the area. The dominant community over most of the cove and reef is kelp forest, *Macrocystis pyrifera*, with an understory of encrusting and erect corallines, scattered small fleshy rhodophytes and various shrubby brown algae. Much of the vertical rock surface is a mosaic of encrusting corallines and sessile invertebrates. A single collection of *Linkia columbiae* was made from similar habitat depths of 8-15 m at Admiral’s Reef near Cat Rock, Anacapa Islands, California in September 1986.

Procedures: 1. Morphometry and Autotomy. Data on morphometry and autotomy were taken from 10 samples, containing from 16-183 individuals between June 1985 and September 1989. Specimens were collected using scuba at the east end of Pumpernickel Cove from 6-10 m depth. For each specimen, rays were counted and identified (by number) based on the usual
presence of two madreporites on the principle ray (identified as R1, Fig. 1a). In a few individuals, madreporites occurred on both sides of the rays, making identification of R1 possible. The lengths of rays and regenerating segments were measured along the ambulacral groove from the mouth or break scar to the ray tip (Fig. 1b).

Specimens were grouped according to their stage of sexual reproduction as 1) single rays, 2) comets - single rays in which the mean length of new rays was ≤50% the length of the principle ray [Fig. 1c], 3) adults - individuals in which the mean length of non-autotomized rays was >50% of the length of the principle ray or 4) disc parents - adults with one or more autotomized rays. Since the open ends of most autotomized rays sealed and began to regenerate within 30-40 days in the laboratory, ray stubs with open or sealed breaks and no measurable amount of regeneration were counted as recent autotomies. In cases of multiple autotomy, the sequence of ray autotomy was inferred from the relative lengths of regenerating rays. Differences in the length of regenerating rays on the same individual were assumed to arise from differences in times of autotomy, not in rates of regeneration. Individuals were returned to the collection area after each survey. Collection sites were shifted one meter apart in September 1986 along depth contours at 10, 15 and 25 m.

3. Water Temperature. At each sampling or census, water temperature was recorded. Additional information on water temperature is provided in Engle (1993).

4. Sexual Reproduction. To determine sex and gonadal condition, individuals were dissected at different times during the year. Sample sizes ranged from 22-71 individuals. Prior to dissection, each individual was bloated dry with a paper towel and weighed. For each ray dissected, the number, position, and maximum length(s) and state of gonad(s) (developing, ripe, spawned) were recorded. To estimate the ratio of gonad to body weight, ripe gonads from two males and two females were excised, blotted on a paper towel and weighed. Sex was determined from microscopic examination of gonadal squashes. For females, the diameters of 50 oocytes were measured.

Two methods were used to estimate oocyte production in ripe ovaries: 1) the number of oocytes per mm of ovary length was determined by counting the oocytes in 10 different ovary branches of known lengths from two females and 2) for two other females, the longest ovary branch was measured and squashed on a slide to create a monolayer of oocytes. The oocytes were counted and the number per mm was calculated. To estimate total oocyte production, the mean number of oocytes per mm of ovary was multiplied by total ovary length (sum of branch lengths).

5. Rates of Growth and Regeneration. To estimate rates of ray regeneration, in September 1986, a single ray was severed with a scalpel 5-10 mm from the mouth on each of 36 individuals. The proximal ends of the amputated rays were cut again to produce single rays of different lengths from 6-32 mm long. Single rays were maintained in the laboratory and provided with coralline encrusted cobble as a substrate and possible source of nutrition until January 1987 when the lengths of regenerated rays were measured.

In July 1990, 141 Linckia columbiae including 35 amputated single rays were stained with Nile blue sulfate (Simon 1974) and released for possible recapture in August 1991. Four stained individuals, including two single rays, were maintained in the laboratory to determine how rapidly the stain would fade and whether staining had detrimental effects. Unstained amputated rays and disc parents served as controls.

6. Number of Rays Regenerated. In June 1990, two single rays were amputated from each of 10 four-rayed, 10 five-rayed, and 10 six-rayed adults to determine if the number of arms regenerated by a single ray is the same as the number on its disc parent. Regenerating rays were maintained in separate containers according to the number of rays on the disc parent and provided with coralline encrusted cobble. Individuals were examined monthly to determine the progress of regeneration.

7. Data Analysis. Data were analyzed using statistical methods appropriate to the size and distribution of the samples (Dixon & Massey 1969; Sokal & Rohlf 1981).

Results

Habitat and Population Structure: At Pumpernickel Linckia columbiae occurred on substrates from hard bedrock to patches of open sand. Adults represented the majority of individuals sampled (Table 1) and were most abundant in areas of moderate relief on the vertical surfaces of boulders or turnable rocks encrusted with coraline algae. Most single rays occurred in shallow cobble piles (5-10 cm dia.) or crevices, whereas small comets tended to be hidden in piles of larger rocks (15-30 cm dia.).

1. Number of Rays. Of 725 individuals examined, 520 (72%) had five rays, 128 (17.5%) six rays and 73 (10%) four rays. Less than 1% had three (n = 1), seven (n = 2) or eight rays (n = 1). Of 77 comets examined from field collections, 55 (71.4%) had five rays, 8 (10.4%) had six rays, 12 (15.6%) had four rays, and two (2.6%) had three rays. A small intercalary arm was observed on only one
In 98% significant changes in morphometric reef at 25 m (Table 2). There were no
Although the distribution of individuals was
aggregation (G test = 7.56, 
not uniform over the reef, measures of
dispersion did not reveal a significant pattern of
Linkia columbiae
extent of regeneration, rays on the same
autotomized rays were about the same length (z = 62/59, 
n = 419). The remaining non-autotomized rays were usually the longest non-autotomized
at 37.9 mm SD = 8.6, range 13-66 mm, n = 503). Among adults, ray
R1 was usually the longest non-autotomized ray, (62%, n = 419). The remaining non-autotomized rays were about the same length (z test, P < 0.95), but shorter than R1. Due to differences in the incidence of autotomy and extent of regeneration, rays on the same individual were rarely equal in length.
3. Population Density. Between 6-10 m, the density of Linkia columbiae at Pumpernickel remained stable at almost four individuals per m² throughout the year (ANOVA, F = 0.082, P > 0.95, between and within census samples).
Although the distribution of individuals was not uniform over the reef, measures of dispersion did not reveal a significant pattern of aggregation (G test = 7.56, P = 0.95; Mozir's ID = 1.4). The density of L. columbiae declined significantly with depth toward the base of the reef at 25 m (Table 2). There were no significant changes in morphometric characters, sex-ratio, or the incidence of autotomy at different depths.
Pattern of Ray Autotomy: 1. Size Relations. In 98% (n = 82) of the recent autotomies examined, fission occurred 6-12 mm from the mouth of the disc parent. Observations of comets in different stages of regeneration indicated that the two madreporites usually regenerate on either side of the initial single ray identifying R1 as the parent ray. The mean lengths of single autotomized rays and the R1 of comets were 28.6 mm, SD = 7.2, n = 31, and 27.0 mm, SD = 6.6 mm, n = 57, respectively. The length of the smallest single ray collected was 18 mm and R1 on the smallest comet was 13 mm. Among individuals with one recent autotomy and no evidence of autotomy on any other ray, the ratio of the mean ray length, excluding R1, to the length of R1 was 0.87, SD = 0.19 (range 0.57-1.32, n = 18). These data indicate that ray autotomy begins sometime after regeneration is 50% complete and regenerated rays are at or near 18 mm in length (i.e., 13 mm plus a ray stub of 6 mm). The number of autotomies per disc parent was not correlated to the length of R1, the principle ray. The number of autotomies per disc parent was correlated positively with overall growth as represented by small increases in the mean length of the other non-autotomized rays as the number of autotomies per individual increases (Fig. 2).
2. Sequence of Ray Autotomy. Among five-rayed individuals with a single autotomy (recent or regenerating), the incidence of autotomy was equal for all rays except R1 which was significantly less than the 20% expected if autotomy was equally probable for all rays (Table 3). In multiple autotomies, the sequence of ray loss varied, but two tendencies were evident: 1) a disproportionate number of multiple autotomies involved the loss of R3 (44/116 cases, X² = 18.65, df = 4, P < 0.01) and 2) multiple autotomies involved adjacent rays more often than non-adjacent rays (a test, P < 0.001; Table 4). Sample sizes for individuals with a single autotomy for four- and six-rayed individuals were too small for analysis.
The progression of autotomy to different rays on the same individual was not a function of the length or percentage of regeneration of previously autotomized rays. Second or third recent autotomies were observed on individuals with as little as 1-2 mm to more than 80% percent regeneration. Of the adults sampled, 19% had two or three recent autotomies or regeneration rays of equal length suggesting that more than one ray may be autotomized at the same time (or in rapid sequence). Although repeated autotomy of the same ray was not evident at Pumpernickel, multiple break scars near the disc on rays of large individuals from Anacapa Island and other sites (unpublished data) indicate that autotomy may occur at the same ray more than once.
3. Number of Rays. The majority of single rays autotomized in June 1990 from four-, five- or six-rayed adults regenerated rays 3-5 mm long by October or November 1990. The number of rays regenerated was not always the same as the number of rays on the disc parent (Table 5). However, the frequency of four- and six-rayed comets from disc parents with four or six rays respectively strongly suggests that the two variables are associated (G test = 12.8, df = 4, P = 0.013, omitting the comet with three rays).
Incidence of Autotomy: From 60-80% of the Linkia columbiae sampled at Pumpernickel had autotomized one or more rays. The proportion of seastars with one or more autotomies was similar for four-, five- and six-rayed comets (68%, Table 6). The sample sizes for five-rayed L. columbiae were large enough to establish that the distribution of autotomies per individual (from none to five) was similar over all census periods (X² = 39.7, df = 35, P < 0.27; Table 7). The incidence of autotomy was similar among males and females (X = 1.5 regenerating rays per individual, t test, df = 87, P > 0.99). About 30% of the Pumpernickel population were relatively large adults (LR >35 mm) with no apparent evidence of autotomy.
The frequency of autotomy was expressed as the percentages of single rays and disc parents with recent autotomies. Temporal changes for both incidence of autotomy were largely coincident but varied considerably in different years (Table 8). Individuals with recent autotomies and single rays were found year round. The highest incidence of recent autotomy and single rays were observed in the summer to early fall and lowest in winter and spring when water temperatures were respectively highest and lowest.
The incidence of recent autotomy in Linkia columbiae at Pumpernickel was only weakly correlated to the proportion of rays available for autotomy in the population as a whole.
Figure 2. *Linckia columbiae.* Size frequency histograms of the mean length of non-autotomized rays R2-R5 for five-rayed comets and adults with one, two, three or four autotomies. (MRL = the mean length of non-regenerating rays R2-R5; \( \bar{x}(\text{mean}) = 7.91 \pm 5.4 \text{ mm, } n = 21 \).)

Regeneration and Growth Experiments:

1. **Single Rays.** Within one month following amputation, the cut ends of single rays and ray stubs on disc parents maintained in the laboratory were sealed. The mean rates of regeneration for amputated single rays at the end of four months, from September 1986 to January 1987, was similar to the rate at the end of 13 months (for 25 rays amputated in June 1990 to determine the number of rays regenerated), but more variable (0.63 mm per month, SD = 0.38, \( n = 35 \), and 0.51 mm per month, SD = 0.07, \( n = 25 \), respectively, based on the longest regenerated ray; \( F \) test, \( P = << 0.001 \)). If these rates of regeneration were sustained, the new rays on most comets would be long enough to initiate autotomy (~18 mm) in about 3 years and reach the mean length for autotomized single rays (28.6 mm) in 4-6 years.

2. **Growth.** Twenty-three adults stained with Nile blue sulfate were recovered from Pumpernickel in August 1991, one year after release. All of the animals had faded, but still appeared blue underwater. Under the dissecting microscope, stained portions of the skeletal elements were obvious including the tips of skeletal plates, especially along the ambulacral groove, and tube feet. New tissue, colored cream to reddish-orange was distinct. Growth, estimated as the sum of unstained lengths for all rays for the year, averaged 11.9 mm, SD = 5.3 per individual (median = 10.5 mm, range = 4-23 mm, \( n = 24 \)) which represented a median increase in individual size of about 9% (range = 4-26%), where % increase in size = \( \frac{\text{sum of unstained lengths/ initial size}}{\text{initial size}} \times 100 \), and initial size = total ray lengths - sum of unstained lengths). Although total annual growth was inversely correlated to the initial size of individuals (Fig. 4), there was considerable variation in the distribution. For individual rays, the correlation between growth and initial size was stronger (Fig. 5). Growth was generally greatest for the shortest ray of each individual, whereas growth for the longest ray was negligible (shortest ray, median annual growth = 3.5 mm).

### Table 3. *Linckia columbiae.* The frequency of autotomy at different rays for five-rayed adults. (\( n = \) the number of individuals sampled).

<table>
<thead>
<tr>
<th>Number of Autotomies</th>
<th>Ray Autotomized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9(7)*</td>
</tr>
<tr>
<td>2</td>
<td>48(11)*</td>
</tr>
<tr>
<td>3</td>
<td>36(29)</td>
</tr>
<tr>
<td>4</td>
<td>29(24)</td>
</tr>
<tr>
<td>5</td>
<td>26(21)</td>
</tr>
<tr>
<td>8</td>
<td>23(19)</td>
</tr>
<tr>
<td>9</td>
<td>78(17)</td>
</tr>
<tr>
<td>12</td>
<td>123</td>
</tr>
</tbody>
</table>

\( a \) Includes the rays autotomized on individuals with single and multiple autotomies.

\( z \) test, \( P = 0.01 \).

### Table 4. *Linckia columbiae.* The frequency of autotomy at adjacent rays. Expected values are calculated if rays autotomized independently, in which case, the total probabilities of autotomy for different combinations of adjacent or non-adjacent rays are equal. (\( n = \) the number of cases).

<table>
<thead>
<tr>
<th>Number of Autotomies per Individual</th>
<th>Number of Cases in Which Adjacent Rays Autotomized</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>61</td>
<td>19.0</td>
<td>17.0</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>20.5</td>
<td>18.5</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>15.5</td>
<td>13.5</td>
</tr>
</tbody>
</table>

\( \chi^2 \) test, \( P = 0.01 \).

Figure 3. Size frequency histograms of the mean length of non-autotomized rays R1 and R2-R5 for five-rayed comets and adults with one, two, three or four autotomies. (MRL = the mean length of non-regenerating rays R1 and R2-R5; \( \bar{x}(\text{mean}) \).)
The numerator is the total number of available rays which equals the total number of non-regenerating rays plus the number of autotomies on adults; the denominator is the total number of adult rays including regenerating rays recent autotomies.

Individuals stained with nile blue sulfate that were absent from samples until 1990 when a few individuals with white, eroded patches were noticed. In the summer of 1991, afflicted individuals were more numerous. About 1% (8/762) of the recent breaks and tips or rays with fission scars distal to the usual positions of the gonads were observed occasionally removing Linckia columbiae from their territories. Although carried in the Garibaldi's mouth, the seashells showed no signs of injury.

Sexual Reproduction: Examination of gonadal squashes indicated that Linckia columbiae is dioecious and the sex ratios are biased toward females, especially among adults (Table 9). Sexually mature individuals were found in almost every size class. The shortest sexually mature ray was 21 mm. Mean wet weights of males and females were similar (3.58 ± 1.76 gm SD, n = 50, for males and 3.27 ± 1.40 gm SD, n = 71, for females; z test, *P = 0.0001, n = 125). From the regression equation, a 5 mm ray stub on a disc parent would take 7-8 years to regenerate a 28-29 mm ray (Fig. 5).

Predation by fishes or large invertebrates was not observed (or specifically tested) in this study. Garibaldi (Hypopops rubioidnes) were observed occasionally removing Linckia columbiae from their territories. Although carried in the Garibaldi's mouth, the seashells showed no signs of injury.

Table 5. Linckia columbiae. The number of rays regenerated by single rays amputated from four-, five- and six-rayed disc parents. (n = the number of corals examined five months after amputation).

<table>
<thead>
<tr>
<th>Number of Rays</th>
<th>Number of Rays Regenerated (%) on Disc Parent</th>
<th>Number of Rays Regenerated (%)</th>
<th>Number of Rays Regenerated (%)</th>
<th>Number of Rays Regenerated (%)</th>
<th>Number of Rays Regenerated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four</td>
<td>(10) (36.7)</td>
<td>8 (4)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>100</td>
</tr>
<tr>
<td>Five</td>
<td>(60) (80%)</td>
<td>15 (22.4)</td>
<td>18 (29.3)</td>
<td>3 (4.6)</td>
<td>100</td>
</tr>
<tr>
<td>Six</td>
<td>(00) (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 6. Linckia columbiae. The incidence of autotomy among four-, five- and six-rayed adults at Pumpernickel. (n = the number of individuals sampled).

<table>
<thead>
<tr>
<th>Number of Rays</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four</td>
<td>(10) (36.7)</td>
<td>(11) (33.3)</td>
<td>(11) (33.3)</td>
<td>(11) (33.3)</td>
<td>(11) (33.3)</td>
</tr>
<tr>
<td>Five</td>
<td>(60) (80%)</td>
<td>(11) (18.3)</td>
<td>(11) (18.3)</td>
<td>(11) (18.3)</td>
<td>(11) (18.3)</td>
</tr>
<tr>
<td>Six</td>
<td>(00) (100%)</td>
<td>(0) (0)</td>
<td>(0) (0)</td>
<td>(0) (0)</td>
<td>(0) (0)</td>
</tr>
</tbody>
</table>

Sexual Reproduction: Examination of gonadal squashes indicated that Linckia columbiae is dioecious and the sex ratios are biased toward females, especially among adults (Table 9). Sexually mature individuals were found in almost every size class. The shortest sexually mature ray was 21 mm. Mean wet weights of males and females were similar (3.58 ± 1.76 gm SD, n = 50, for males and 3.27 ± 1.40 gm SD, n = 71, for females; z test, *P = 0.0001, n = 125). From the regression equation, a 5 mm ray stub on a disc parent would take 7-8 years to regenerate a 28-29 mm ray (Fig. 5).

Predation by fishes or large invertebrates was not observed (or specifically tested) in this study. Garibaldi (Hypopops rubioidnes) were observed occasionally removing Linckia columbiae from their territories. Although carried in the Garibaldi's mouth, the seashells showed no signs of injury.

Table 7. Linckia columbiae. The number of autosomites per individual for adult five-rayed adults at Pumpernickel. (n = the number of adults sampled, % = the mean percentage of individuals per sample).

<table>
<thead>
<tr>
<th>Census Date</th>
<th>T</th>
<th>Number of Autotomites per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
<th>Number of Autosomites Per Individual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/86</td>
<td>15</td>
<td>5 (20.0)</td>
<td>6 (7.5)</td>
<td>67/114 (76.8)</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>7/86</td>
<td>15</td>
<td>5 (20.0)</td>
<td>6 (7.5)</td>
<td>108/141 (76.4)</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>9/86</td>
<td>16-20</td>
<td>10 (40.0)</td>
<td>11 (45.2)</td>
<td>112/133 (84.2)</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>10/86</td>
<td>11</td>
<td>5 (45.0)</td>
<td>6 (13.3)</td>
<td>45/607 (76.2)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>11/86</td>
<td>14-15</td>
<td>1 (1.4)</td>
<td>1 (1.0)</td>
<td>216/400 (50.9)</td>
<td>2 (2.9)</td>
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<tr>
<td>12/86</td>
<td>15-16</td>
<td>3 (5.1)</td>
<td>3 (1.3)</td>
<td>228/114 (72.6)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>14/86</td>
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<td>6 (15.2)</td>
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<tr>
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<td>13</td>
<td>7 (49.6)</td>
<td>7 (1.7)</td>
<td>655/899 (70.0)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>18/86</td>
<td>16-18</td>
<td>26 (15.1)</td>
<td>29 (5.6)</td>
<td>257/681 (78.9)</td>
<td>136</td>
</tr>
</tbody>
</table>

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Table 8. Linckia columbiae. Sea temperatures and indices of autotomy at Pumpernickel. (T = sea temperature °C at 6-10 m depth; n = the number of adults sampled).

<table>
<thead>
<tr>
<th>Census Date</th>
<th>T</th>
<th>Number of Autotomites per Individual (%)</th>
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<td>1 (1.8)</td>
</tr>
<tr>
<td>14/86</td>
<td>17</td>
<td>4 (23.1)</td>
<td>6 (15.2)</td>
<td>53/75 (71.4)</td>
<td>19</td>
</tr>
<tr>
<td>15/86</td>
<td>17-18</td>
<td>6 (6.3)</td>
<td>8 (7.0)</td>
<td>189/141 (65.7)</td>
<td>10 (10.5)</td>
</tr>
<tr>
<td>17/86</td>
<td>13</td>
<td>7 (49.6)</td>
<td>7 (1.7)</td>
<td>655/899 (70.0)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>18/86</td>
<td>16-18</td>
<td>26 (15.1)</td>
<td>29 (5.6)</td>
<td>257/681 (78.9)</td>
<td>136</td>
</tr>
</tbody>
</table>

*Mean number of autotomites per individual in four-rayed adults is less than six-rayed, t test, P < 0.04
adults that had mature gonads, the gonads were located close to the disc in the portion of the ray that had not autotomized. In the non-regenerating rays of adults, the amounts of gonadal tissue (0-4 pairs of gonads), the extent of gonadal development and the timing of gamete release (based on the extent of gonadal evacuation) varied between individuals, between different rays of the same individual, and between paired gonads on the same ray. The proportion of Linckia columbiae with at least one sexually mature ray (gonads with eggs or sperm) differed in the two reproductive seasons sampled. In September 1986, 90% (28/31) of the individuals examined from Pumpernickel were sexually mature, whereas only 42% (17/41) were sexually mature in September 1989.

Linckia columbiae at Anacapa Island: Morphometric comparisons showed that the individuals collected from Admiral's Reef, Anacapa Island in September of 1986, had similar numbers of rays, but were more robust than individuals from comparable depths at Pumpernickel. Concomitant with larger size, the percentage of individuals with two or more regenerating rays was also higher at Anacapa (77%) than at Pumpernickel (35%). Among the 28 Linckia columbiae examined from Anacapa, two of the adults had a recent autotomy (9%), three were comets (11%) and three were single rays (11%). The sex ratio at Anacapa was almost 1:1. Eighteen out of the 20 sexually mature individuals collected at Anacapa had completely or partially discharged gonads. In contrast, only about half of the mature stars collected the same month at Pumpernickel had noticeably reduced gonadal volumes and none were completely discharged. Maximum oocyte diameters were similar for the two populations.

Discussion and Conclusions

The incidence of autotomy and patterns of morphometric variation among Linckia columbiae are consistent with the view that most individuals at Pumpernickel develop from single autotomized rays. 1) Recent autotomies, single rays and comets are found throughout the year and regenerating rays are common on adults. 2) The growth pattern initiated by regenerating single rays is evident in most adults, i.e., ray R1, the parent ray, is the longest ray and other non-regenerating rays, presumably regenerated by R1, are similar in length. 3) The percentages of four-, five- and six-rayed individuals regenerating by comets is similar to the adult population. Finally, the absence of very small symmetrical L. columbiae (LR < 18 mm) in population samples suggests that Larval recruitment is rare.

Patterns of Autotomy: Early descriptions of ray autotomy in Linckia columbiae proposed that autotomized rays longer than 20 mm continuously produced new individuals by casting off the disc when the regenerated rays reached 9-10 mm (MacGinitie & MacGinitie 1949). In contrast, my results indicate that parent rays, independent of length, usually remain with the discs and asexual reproduction occurs principally by autotomizing regenerated rays. Autotomy begins sometime after comets enter the adult stage when new rays are at least half as long as the principle ray.

Amputation experiments demonstrate that rays of Linckia columbiae as short as 6-10 mm are capable of regeneration (MacGinitie & MacGinitie 1949; the present study). At Pumpernickel, the minimum length of autotomized rays appeared to be ~13 mm. However, most single rays observed at Pumpernickel were much longer, 20-30 mm, because comets generally delayed autotomy until regeneration was nearly complete. The coincidence of even longer single rays, 32-65 mm, with larger sized comets and adults at Admiral's Reef (Table 10) suggests that the lengths at which rays autotomize is largely determined by population size structure.

Amputation experiments suggest that the number of rays regenerated on comets is influenced by, but not strictly dependent upon, the number of rays on the disc parent. Variation in the numbers of rays regenerated by spontaneously autotomized single rays is unknown.

Regeneration and Growth: Regeneration experiments and the recovery of dyed individuals indicate that Linckia columbiae increases in size by only a few millimeters each year. Growth is consistently slow and comets require 3-6 years to reach the adult stage, the relatively high population densities of adults and low percentages of comets suggest that L. columbiae is a long-lived species (See Muens 1975).

Correlates of Autotomy: Factors that lead to fission in nature and account for temporal variance in sexual reproduction remain largely a mystery (Emson & Wilkie 1980). In Linckia columbiae, sudden changes in environmental conditions, external irritants, or ligaments tied around a ray can induce ray autotomy in some individuals (Monle 1904; Edmondson 1915). The persistent location of autotomy between the third and eighth ambulacral ossicles (Shepard 1964), changes in tissue elasticity and
Table 9. *Linckia columbiae*. Sex ratios at Pumpernickel. Data for comets and single rays represents individuals examined during August or September 1985-1989. (n = number of individuals sampled).

<table>
<thead>
<tr>
<th>Census</th>
<th>Date</th>
<th>n</th>
<th>Immature</th>
<th>%</th>
<th>Males</th>
<th>Females</th>
<th>Sex Ratio</th>
<th>M:F</th>
<th>% Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comets</td>
<td>6/86</td>
<td>71</td>
<td>12</td>
<td>17</td>
<td>22</td>
<td>1.7:1</td>
<td>0.7</td>
<td>(0)</td>
<td>0.5:1</td>
</tr>
<tr>
<td></td>
<td>9/86</td>
<td>31</td>
<td>3</td>
<td>20</td>
<td>8</td>
<td>2.5:1</td>
<td>0.9</td>
<td>90.3</td>
<td>0.5:1</td>
</tr>
<tr>
<td></td>
<td>1/87</td>
<td>28</td>
<td>20</td>
<td>21</td>
<td>17</td>
<td>1.2:1</td>
<td>0.5</td>
<td>(0)</td>
<td>0.5:1</td>
</tr>
<tr>
<td></td>
<td>9/89</td>
<td>44</td>
<td>24</td>
<td>11</td>
<td>6</td>
<td>1.8:1</td>
<td>0.5</td>
<td>41.5</td>
<td>0.5:1</td>
</tr>
<tr>
<td>Comets</td>
<td></td>
<td>13</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>6.4:1</td>
<td>0.5</td>
<td>38.4</td>
<td>0.5:1</td>
</tr>
<tr>
<td>Single rays</td>
<td></td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>1:1</td>
<td>0.5</td>
<td>51.7</td>
<td>0.5:1</td>
</tr>
</tbody>
</table>

*(a) Individuals with a ripe gonad in at least one ray.*

Fission behavior (Monks 1904; Edmondson 1935), the lack of injury on most individuals together with evidence that autotomy can be suppressed by a parasitic infection (at least in *L. multiflora*, see Davis 1967), suggest that endogenous factors are important.

The incidence of autotomy in *Linckia columbiae* at Santa Catalina Island does not correlate with depth. However, *L. columbiae* is strictly subtidal at Pumpernickel and does not experience the environmental stress (temperature, wave action, desiccation, reduced feeding opportunities) associated with increased fission in intertidal populations of other fissiparous asteroids (Emson 1978; Crump & Barker 1985). Annual fluctuations in the indices of autotomy for *L. columbiae* suggest a seasonal trend that peaks in late summer coincident with the highest water temperatures (Engle, 1993)...but variation in the incidence of autotomy between years at Pumpernickel and between different sites in the same year (unpubl. data) indicate that additional factors are important. Populations of the seastars *Allstrachaster polyplax* (Emson 1978) and *Cotinisaster californicus* (Crump & Barker 1985) seem to be more fissiparous when food abundance is low or of apparently poor quality.

Intra-Interspecific Comparisons: Comparative evidence for *Linckia columbiae* from Pumpernickel and Admiral's Reef and a now extinct intertidal population at San Pedro, California (Monks 1903, 1904) reveal similar patterns of fission: the percentages of individuals with four, five and six rays are similar; most individuals show evidence of autotomy; and the percentages of single rays (9-11%) and comets (11-17%) are comparable. The primary difference is that the Anacapa and San Pedro collections include larger individuals (LR > 80 mm). At Anacapa, larger size was associated with longer single rays, a higher incidence of multiple autotomies on disc parents and different sex ratios and spawning times. Subsequent analysis of *L. columbiae* populations at additional locations around Santa Catalina Island, Santa Cruz and Santa Barbara islands, and Palos Verdes Point on the mainland of California substantiate significant variability in the structure, fecundity, and incidence of autotomy among local populations (unpubl. data).

Within the genus *Linckia*, the occurrence and pattern of ray autotomy varies. *Linckia multiflora*, a slightly smaller species, is highly asexual. Its mean density in coral reefs around Guam was 36 individuals per m² (Rideout 1978, calculated from Table 1), about ten times the density of *L. columbiae* at Pumpernickel. As with *L. columbiae*, typically *L. multiflora* is five-rayed and local population densities are maintained by ray autotomy which occurs year round at variable frequencies (Edmondson 1935; Rideout 1978). The percentages of single rays in population samples of *L. multiflora* and *L. columbiae* are not significantly different ($\bar{x} \pm SD = 8.3 \pm 6.7\%$, Rideout 1978, from Table 1; for *L. columbiae*, $\bar{x} \pm SD = 6.2 \pm 5\%$, proportion z test, $P > 0.95$). Nevertheless, a higher incidence of autotomy is predicted for *L.
which rays autotomize varies. Disc parents of both species may autotomize regenerated rays. Multiple autotomies frequently involve adjacent rays in regenerated rays. The order of regeneration is autotomized. Although regenerated rays are longer than 18-20 mm and regeneration is nearly complete. When rays of Comets of individuals that are regenerating all of their remaining rays autotomize in rapid succession (Rideout 1978; Fig. 10). The pattern of succession of autotomy in the population are adults with fewer than two oocytes represent egg production in a typical ovarian cycle. Linckia columbiae. Sexual reproduction and autotomy: Although the highest incidence of autotomy in Linckia columbiae at Pumpernickel was recorded in the late summer coincident with spawning, autotomy is largely independent of sexual activity since autotomy occurs throughout the year, involves males and females equally and produces single rays with or without mature gonads. Growth, apparently, takes precedence over sexual reproduction as evidenced by the smaller size of sexually immature individuals and the lack of gonadal tissue in the regenerating rays of comets and adults. Fecundity in Linckia columbiae is low compared to other non-fissiparous species that broadcast spawn. This, in part, can be attributed to its relatively small size, variable gonadal development, and the lack of gonadal tissue in regenerating rays. In addition, oocyte production is relatively low. If 2,500-3,500 oocytes represent egg production in a typical ovary of L. columbiae at Pumpernickel, a female with five non-regenerating rays and a minimum of two pairs of mature ovaries in each ray would produce about 50,000-70,000 eggs per season. For the average female at Pumpernickel (3.5 gm), this is about 21,000 eggs per gm per year. In comparison, a single L. laevigata sheds on the order of a million eggs at each spawning and may spawn repeatedly within a season (Yamaguchi 1977). An adult female Pisaster ochraceus produces 100,000 eggs per gm each year which, given the weight of sexually mature individuals (> 400 gm), results in tens of millions of eggs (Menge 1975).

Very small complete individuals with ray lengths shorter than 13 mm, R1 of the smallest comet, were never observed despite extensive searches. I infer from this that juvenile Linckia columbiae are very rare or that recruitment is infrequent. However, in many asteroid species, including L. laevigata that only reproduces sexually, juveniles are seldom seen in the field because they are extremely small and cryptic and often have different habitat preferences than adults (Yamaguchi 1977; Chia et al. 1984). Sexual reproduction and pelagic larvae have been noted in at least one population of L. multiflora (Mortensen 1938), but, as with L. columbiae, fecundity appears to be low (Rideout 1978) and juveniles have not been reported. The details of spawning and larval development for L. columbiae are unknown. The relatively small eggs suggest the larvae are planktonic (Chloron 1950; Strasman 1974; Chia 1974).

If dependence on sexually produced larvae for replacement and growth of the population is reduced, energy may be diverted from gametogenesis into the production of asexual propagules. By increasing the expenditure of energy per reproductive unit, Linckia columbiae takes advantage of the increased survival of larger reproductive units (juveniles vs. planktonic larvae) and increases the probability of successful recruitment to the parent population (Caswell 1985). By increasing clonal allocation at the expense of annual investments in sexual reproduction L. columbiae may also take advantage of environmental conditions that favor year round population growth. The persistent bias towards females at Pumpernickel has no immediate explanation. The incidence of autotomy was similar for males and females; consequently, autotomy would only perpetuate an excess of females in the population however established (e.g., differential migration, mortality, larval recruitment).

Genetic Consequences of Fission: Subpopulations of the predominantly fissiparous asteroid Coscinasterias multiflora are characterized by significant numbers of replicate genotypes and strong genetic disequilibrium between populations (Johnson & Threlfall 1986). Genotype analysis of Linckia columbiae around Santa Catalina Island and other locations in southern California shows a similar trend emphasizing the significance of asexual reproduction in maintaining this species (McArlay 1988, unpubl. data). As with other groupers of clonal invertebrates, sexual reproduction among fissiparous asteroids may promote successful genotypes and, presumably, adaptiveness within and between local populations (Jackson 1985).

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Literature Cited


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