AN ABSTRACT OF THE THESIS of Paul R. Chirichetti for the Master of Science in Biology, presented November 13, 1996.

Title: Otolith Increment Analysis in the Rabbitfish *Siganus spinus*: Validation of Daily Increment Deposition and Patterns of Larval and Otolith Growth.

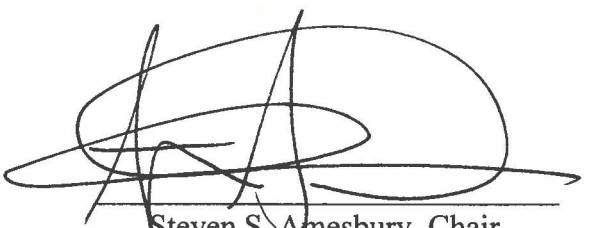
Approved: _____
Steven S. Amesbury, Ph.D., Chair, Thesis Committee

Daily increment deposition in sagitta of the rabbitfish *Siganus spinus* was confirmed by marking otoliths of post metamorphic juveniles with the fluorescent compound oxytetracycline hydrochloride (OTC) and relating the time interval between two OTC treatments, or OTC treatment and death of the fish, to increment counts for the corresponding time interval. Time interval and increment counts were highly correlated ($r^2 = 0.99$) and it was confirmed that increments in otoliths are deposited daily. Larval durations, growth-rates during the larval period, and standard lengths at settlement in *S. spinus* were determined for three large recruitment events that occurred over a two-year period. Because these events, or "runs," occur continuously for two to eight days, two values were calculated for mean larval duration and mean growth rate. The maximum value was based on the age of the fish on the day of capture and assumed that the fish had recruited on that day. The minimum value was estimated by assuming that the fish had recruited on the first day of each run. Fish from April 1993 were sampled on the first day of their run, and fish from the April and May 1994 cohorts were sampled on the fifth day

of their runs. Maximum larval durations for all runs pooled was 32.8 d; the minimum larval duration for all runs pooled was 30.1 d; and ANOVA showed significant difference between the means at both maximum ($F = 35.97$) and minimum ($F = 14.12$) values. Longer larval durations yielded lower growth rate estimates, and the overall mean growth rates calculated for the maximum and minimum larval durations were 0.97 and 1.05 mm d^{-1} respectively. Growth rates were significantly different among all runs at both the maximum ($F = 66.5$, $p < 0.001$) and the minimum ($F = 12.62$, $p < 0.001$) larval duration. Standard length at capture was 32.8 mm and ranged from 31.0 to 34.1 mm. Transverse medial cross sections of otoliths from larvae at metamorphosis, juveniles, and adult *S. spinus,* were made to compare the increment width growth patterns over the 33-day average (maximum) larval duration. Patterns were very similar among all cohorts. The rate of maximum otolith growth occurred between day-16 and day-18, and a change in the rate of decrease in the increment-width series occurred near the earliest age estimated for recruitment (minimum value 25 d). Otolith radial growth followed a sigmoidal pattern during the larval period and was fit to a logistic growth curve. Based on the 30 to 33-day mean larval duration and the timing of recruitment events, spawning in *S. spinus* was estimated to occur between the seventeenth and the twentyfifth day of the lunar month, in the month preceding settlement.

TO THE OFFICE OF THE GRADUATE SCHOOL AND RESEARCH

The members of the Committee approve the thesis of Paul R. Chirichetti, presented November 13, 1996.



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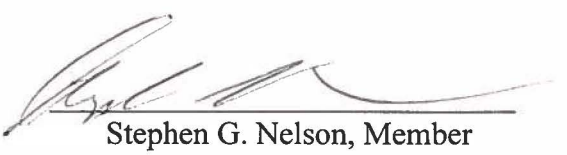

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Otolith Increment Analysis in the Rabbitfish *Siganus spinus*: Validation of Daily Increment Deposition and Patterns of Larval and Otolith Growth

By

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A thesis submitted in partial fulfillment of
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INTRODUCTION

Siganus spinus is the most abundant of the five species of rabbitfish found on Guam (Kami et al., 1968). Adult *S. spinus* have contributed between 3.1% and 10.8% (2.1 to 6.5 mt) to Guam's yearly inshore fisheries catch over the last five years. Rabbitfish in their late larval stage are also an important fishery resource on Guam. Large aggregations of pelagic *S. spinus* larvae swim over the reef flats as they enter their juvenile phase. Harvests of newly recruited siganid larvae, known locally by the Chamorro name *manahac*, can comprise as much as 33% of the yearly inshore reef fish catch (Guam Department of Agriculture, Division of Aquatic and Wildlife Resources (DAWR) Inshore Fishery Annual Report, FY 1994). Overall, the inshore fin-fish catch has declined dramatically around Guam in recent years, from 123.1 mt in 1991 to 36.0 mt in 1995 (DAWR Annual Reports, FY 1991 to 1995), while the percentage of *S. spinus* in the total catch (including larvae) has varied from 3 to more than 25% with no apparent trend. Since both adult and larval *S. spinus* are important components of the local inshore reef fish catch, knowledge of their life history can provide a basis for developing effective management strategies.

The requirements of most life history and fisheries management studies is an accurate determination of age (Gauldie and Nelson, 1990; Jones, 1992). Knowing fish ages makes it possible to estimate the larval duration (Victor, 1982; 1983), to determine spawning dates (Thresher, 1984), and to determine average growth rates during the larval phase (Fowler, 1989; Wellington and Victor, 1989). Information about these aspects of *S. spinus* life history is not currently available and is the focus of this study. Determining ages of fish has been accomplished by utilizing micro-increments deposited on a daily

basis in the otoliths (earbones) of fish (Pannella, 1971). The sagitta was chosen for this study as it is the largest of the three pairs of otoliths. The terms sagitta and otolith will be used interchangeably in this paper.

Otolith increment analysis is well suited to studies of larval ecology because 1) otoliths can record daily age (Pannella 1971; Victor, 1984); 2) otolith increments are generally deposited proportionally to fish growth (Maillet and Checkley, 1991); 3) otolith growth responds to physiological changes in the fish (Radtke, 1987; Molony and Choat, 1990; Maillet and Checkley, 1991); and 4) otolith increments are widest and clearest during the larval and early juvenile stages of growth. The pelagic larval stage in which most reef fish develop is an especially difficult part of the life cycle to study. Larval fish are small, hard to identify, and problematic to maintain in captivity. Collection of the larvae in the wild is difficult because they are widely dispersed (Leis, 1991; Victor, 1991), and they are mixed with the larvae of a great many other species. Most tropical reef fish recruit to the near-shore reef habitat at metamorphosis where they are easier to collect than from the oceanic environment.

In this study I have 1) validated daily incrementation in *S. spinus,* 2) determined the duration of the larval phase and time of spawning, and 3) described the pattern of otolith growth during the larval phase.

MATERIALS AND METHODS

Preliminary Otolith Preparation

Siganus spinus that still retained their silvery planktonic coloration were obtained live from local fishermen during the *manahac* runs in 1993 and 1994. Only one conspicuous *manahac* run occurred in 1993, and only the first two of the three runs recorded by DAWR in 1994 were sampled. No runs were recorded in the fall of 1992 or in all of 1995, but adults which had been spawned between August and October 1992 (collected April 1993) and juveniles which had been spawned in May 1995 (collected in July 1995) were captured to use for larval increment-width series comparisons. Fish were killed by refrigeration, then immediately weighed to the nearest 0.01 g and measured to the nearest 1.0 mm standard length (SL). Otoliths were extracted from the fish and cleaned following the methods of Stevenson and Campana (1992). The cleaning and rinsing were done with the otoliths resting on top of microscope slides; when dried, otoliths adhere to the glass slides. Glass slides, with attached otoliths, were labeled, stored in darkness, and allowed to air-dry for at least one week before processing. Otoliths were removed from the slides and weighed to the nearest 0.001 mg with a Cahn C-31 microbalance before preparation (Cahn Instruments, Inc., 18207 S. Carmentita Rd., Cerritos, Ca. 90701).

Procedure for Treatment of Fish with OTC

Ten live fish sampled from the run in 1993 and 25 fish sampled from the second run in 1994 were treated with oxytetracycline hydrochloride (OTC). Before treatment, fish were anaesthetized with tricaine methanesulfonate (MS-222) then weighed to the

nearest 0.01 g and measured to the nearest 1.0 mm (SL). Fish were held for 40 h in an aerated, static solution of 250 mg OTC ℓ^{-1} seawater. After treatment, the fish were placed in an outdoor, 250-ℓ, flow-through holding tank, and fed to excess with a pelleted commercial trout food at a rate of 10% body weight d^{-1}, together with a supplement of *Enteromorpha* sp., a green filamentous algae they consume readily (Tsuda and Bryan, 1973; Bryan, 1975). The fish from 1993 were held for 37 d, and the fish from 1994 were held for 20 d before the second OTC treatment. After the second treatment, the 1993 group was held for 11 d, and the 1994 group for 7 d before their otoliths were extracted.

Preparation of Otolith Thin Sections Through the Transverse Medial Plane

Validation of daily increment deposition, age estimates, and increment-width comparisons, were all made from transverse-medial thin sections of sagittae. Thin-sectioning has been found to increase the accuracy of ring counts and increment width measurements (Radtke, 1987), perhaps because thin sections (10 μm to 50 μm thick) reduce light refraction and depth of focus limitations that occur at higher magnifications in whole mounts (Neilson, 1992).

Otoliths were sectioned by first embedding whole otoliths in acrylic casting resin (Fibre Glass-Evercoat, Co., Inc., 6600 Cornell Road, Cincinnati, Ohio 45242) that was poured into small, plastic, ice-cube molds. The cube is created sequentially by pouring and allowing the first layer of resin to harden, fixing the otolith into the proper position with a drop of cyanomethyacrylate (Super glue®), then pouring the top layer of resin. The resulting resin cubes were cut in the plane perpendicular to the anterior-posterior axis

of the sagitta, on either side of the core region, with a diamond-edged blade attached to a Buehler Isomet lapidary saw (Buehler Microstructural Analysis Division, 41 Waukegan Rd., Lake Bluff, Illinois, 60044-1699). The resulting transverse-medial sections (0.5 mm to 1.0 mm thick) were then glued onto slides with Crystalbond thermoplastic cement (Aremco Products, Inc., P.O. Box 429, Ossining, New York, 10562-0429, see procedures of Secor et al., 1992). Sections were sanded with 1000 grit and 1500 grit wet-dry sandpaper until the core was reached, and then polished with 0.3 μm alumina paste on a polishing cloth. After the core was reached from one side, the thermoplastic cement was reheated and the specimen turned over, reglued, and the process repeated.

Validation of Daily Increment Deposition

After preparation, fluorescent bands were identified under ultraviolet light through a band pass filter at 470 to 490 nanometers (Secor et al., 1991), with a Zeiss epi-fluorescence microscope at 500 x magnification. Validation of daily increment deposition was made by correlating the increment counts between OTC marks with the known time interval between these two points. When only the second of the two fluorescent marks was visible, the increment counts from the OTC mark to the edge of the otolith were correlated with the known time interval from the second treatment to the death of the fish, and then the correlation coefficient (r^2) was calculated. Of the 35 sagittae prepared for the validation study, 8 showed at least one visible fluorescent band.

Age Determination

Otolith increment counts for age determination were made on a compound microscope under transmitted light against an ocular micrometer at 1000 x magnification.

'Blind' counts were made on otolith samples to avoid bias (Neilson, 1992). An independent observer replaced previous identification on slides with letter codes. Slide preparations of all samples were then mixed before being returned for counts. Readings were done four times and the results averaged. Thin sections were etched with a 0.2 M solution of ethylenediaminetetraacetic acid (EDTA), at pH 7.0 to 7.2 for 10 to 60-sec (Secor et al., 1992) and stained for two to four min with Toluidine Blue (Bouin and Siau, 1988; Richter and McDermott, 1990). Since the first increment was deposited 24 h after hatching (A. Kerr, University of Guam Marine Laboratory, personal communication), the age of fish in days is equal to the increment count.

Mean length of larval duration in days was calculated for two time periods. The first calculation was made from the day of capture (maximum), and assumes that the fish recruited to the reef on that day. The second calculation was adjusted to the first day of each run (minimum) and assumes that the fish recruited then. The reason for calculating these two values is that *manahac* runs last from 2 to 8 days, and it is not known whether fish recruit all at once on the first day of the run or recruit to the reef sequentially. It is known that *S. spinus* spends a number of days over the reef before metamorphosis is complete. During metamorphosis, the gut lengths and the fish's color changes. The amount of time that metamorphosis takes to complete once *S. spinus* swims over the reef is not known.

Increment Width Measurements

In addition to the larval samples from April and May 1994 (N = 8), samples of otoliths from juveniles spawned in May 1995 (captured in July 1995, N = 8) and from

year-0 *S. spinus* spawned between August and October 1992 (captured in April 1993, N = 8) were also prepared by thin-sectioning. Increment width measurements were made along the ventral radius of the transverse otolith section, which is slightly longer and has clearer increments than the dorsal radius. Because of variations in the three-dimensional structure of the sagitta, the widest section of each increment did not follow a straight line from the core to the ventral edge, so measurements were made perpendicularly across adjoining increments at their widest point. The maximum number of increments read from the juvenile fish were 53. Increment widths for the first 53 days were also measured from the otoliths of the adult fish. Average increment widths were plotted at each day and examined for general patterns relating to development during the larval and early juvenile periods.

Otolith Growth Across the Ventral Radius of Transverse Cross Sections

An otolith growth curve was constructed by plotting cumulative increment distance across the ventral radius of the transverse cross section at each day from day-0 to day-33 (the maximum average age at capture for all *manahac* cohorts combined), from *manahac* from April and May 1994, and both the juvenile, and adult samples. The relationship of the mean otolith radius to age was then fit to a logistic growth curve of the form:

$$O_t = O_c\,[1+\exp\,[-G(x-x_0)]]^{-1} \qquad (1)$$

where: O_t equals the ventral radius (μm) of the transverse medial cross section of the sagitta at time t; O_c equals the ventral sagittal radius (μm) on day of capture; G equals the

instantaneous growth rate at the origin of the curve; and (x_0) equals the age of the fish at the inflection point of the curve (Campana and Jones, 1992). Parameters of the equation were estimated by computer iteration with a statistical analysis software package (Sigma Stat Scientific Graphing Software, Jandel Scientific P. O. Box 7005 San Raphael, Ca., 94912-7005).

Larval Growth Rate Calculations

Average growth rates for the larval period in each cohort were calculated by the following formula (from Victor, 1991):

$$\text{Average growth (SL d}^{-1}) = (L_c - L_0)\, A_c^{-1} \qquad (2)$$

where L_c equal the standard length at capture (metamorphosis), L_0 equals the standard length at hatching, and A_c equals age at capture estimated from otolith increment counts. The value used for the length of fish at hatching was 1.6 mm (S. Nelson, University of Guam Marine Laboratory, unpublished data).

One-way ANOVA was used to test the null hypothesis that there was no difference in age, fish standard length, and mean growth rate among runs (McCormick, 1994). The sample from April 1993 was reduced to six through mishap so the samples from other runs were kept to the same range (N = 8) and chosen haphazardly.

RESULTS

Validation of Daily Increment Deposition

The double fluorescent marks expected from treating the fish twice with OTC were visible in only two cross sections from 1993 and one cross section from 1994. However, the number of rings between fluorescent marks and the number of rings from the second treatment mark to the edge of the otolith corresponded well with the number of days that the fish were held between OTC treatments and the time from the second treatment and the death of the fish respectively. Five additional otoliths showed only one fluorescent mark in the same position as the second treatment mark that was present in the otoliths with visible double marks. The number of rings distal to the second OTC mark also agreed well with the number of days between the second mark and the death of the fish in both 1993 and 1994 samples (Table 1). The correlation of increment count with time interval for all sagittae with visible marks was high ($N = 11$, $r^2 = 0.99$, Figure 1). As in most fish species already studied, otolith increments are deposited daily in *S. spinus*.

Larval Duration and Time of Spawning

The daily increment deposition in *S. spinus* makes it possible to calculate the length of the larval duration. Ages at capture of the 22 fish analyzed ranged from 28.5 to 38.5 d (mean values of 4 replicate counts per fish) with a grand mean of 32.8 d (Table 2). Minimum mean age for all fish, adjusted to the first day of each run (as determined by ob-servations made by personnel at DAWR) was 30.1 d (Table 2). The mean ages at capture were significantly different among runs ($F = 35.97, p < 0.001$, Table 2). Adjusted

Table 1. Daily increment validation in early post-metamorphic juvenile rabbitfish. Number of increments counted between OTC marks (double) or from OTC mark to edge of otolith (single) for various time periods in 1993 and 1994.

Specimen number	Mark Type	Increment count (±SE)	Time interval (d)
293-93	double	33.5 ± 1.91	37
294-93	double	36.25 ± 1.71	37
293-93*	single	8.0 ± 0.82	7
294-93*	single	5.75 ± 0.5	7
295-93	single	6.25 ± 0.5	7
297-93	single	6.0 ± 0.0	7
301-93	single	8.0 ± 0.82	7
308-93	single	9.0 ± 1.41	7
37-94	double	20.75 ± 1.5	20
37-94*	single	9.25 ± 0.96	20
33-94	single	10.0 ± 0.82	11

* Same sagitta used to get increment count for both double and single mark categories.

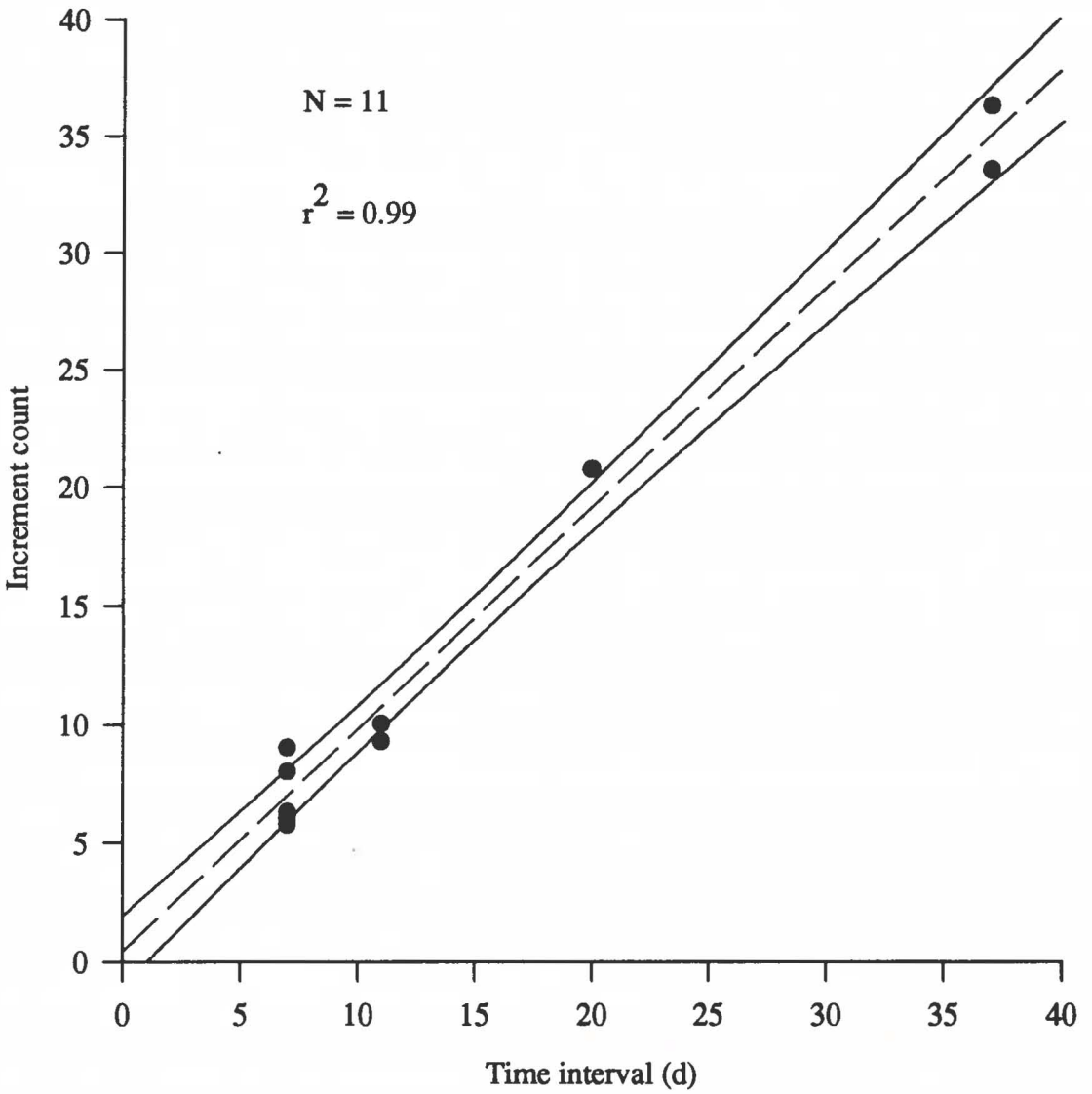

Figure 1. Correlation of time interval (age) with increment count in OTC labelled otoliths of *S. spinus*. Counts between marks (20 d and 37 d), and from second mark to otolith edge (7 and 11 d). Four readings per point.

Table 2. Maximum (from date of capture) and minimum (adjusted to first day of each run) mean larval age at recruitment, mean standard length at recruitment, and average growth-rate throughout the larval period. *Manahac* samples are taken from three runs, April 1993, April 1994, and May 1994. One-way ANOVA with Student-Newman-Keuls (SNK) pair-wise comparisons are included in the table at the bottom.

Manahac Run	Range	N	Age ± SE (d)	CV	SL (mm ± SE)	CV	Growth rate mm d^{-1}±SE
#1 (4/93)	max	6	29.7 ± 0.27	2.2%	34.1 ± 0.45	3.3%	1.09 ± 0.03
	min		29.7 ± 0.27	2.2%	*		1.09 ± 0.03
#2 (4/94)	max	8	32.4 ± 0.64	5.6%	33.63 ± 0.42	3.5%	0.99 ± 0.02
	min		28.4 ± 0.64	6.4%	*		1.13 ± 0.03
#3 (5/94)	max	8	36.3 ± 0.53	4.1%	31.0 ± 0.38	3.5%	0.81 ± 0.01
	min		32.3 ± 0.53	4.6%	*		0.91 ± 0.02
Grand Mean	max	3	32.8 ±1.04	10.1%	32.8 ± 1.02	5.4%	0.97 ±0.08
	min		30.1± 1.12	6.5%	*		1.05 ± 0.07
ANOVA	max		$F = 35.97$ $p < 0.001$		$F = 16.27$ $p < 0.001$		$F = 66.5$ $p < 0.001$
	min		$F = 14.12$ $p < 0.001$		*		$F = 12.62$ $p < 0.001$
SNK							
R1 vs. R2	max		$p < 0.01$		$p = 0.47$		$p < 0.01$
	min		$p = 0.11$		*		$p = 0.23$
R1 vs. R3	max		$p < 0.001$		$p < 0.001$		$p < 0.001$
	min		$p < 0.001$		*		$p = 0.12$
R2 vs. R3	max		$p < 0.001$		$p < 0.001$		$p < 0.001$
	min		$p < 0.001$		*		$p < 0.001$

*SL at first day of run was not calculated.

ages at metamorphosis were also significantly different among runs ($F = 14.12$, $p = 0.0002$). A Student-Newman-Keuls (SNK) pair-wise comparison showed that adjusted means for runs one and two were not significantly different from each other ($p = 0.11$), though both were significantly different from run three ($p < 0.001$).

The age estimates coupled with the dates of recruitment allowed back-calculation of spawning times. Since recruitment in all three runs occurred between the 20th and the 25th day of the lunar month, the 30 to 33-day mean larval duration would place spawning times between the 17th and the 25th day of the lunar month in the month preceding recruitment.

Larval Length at Capture and Mean Growth Rate Throughout the Larval Period

Average length at recruitment was 32.8 mm (all runs combined) and ranged from 31.0 to 34.1 mm SL (Table 2). Although larval length from April 1993 was not significantly different from April 1994, both runs had larvae with larger size at settlement than the May 1994 run ($F = 16.27$, $p < 0.001$).

The length at recruitment is important as a variable in the calculation of larval growth rates. The mean growth rate throughout the larval phase, calculated from the means of all runs combined, was 0.97 mm d^{-1} (for maximum larval duration), and differed significantly among runs ($F = 66.5$, $p < 0.001$, Table 2). Mean growth rate calculated with ages adjusted to the first day of each run was 1.05 mm d^{-1} (for minimum larval durations), and was significantly different among runs ($F = 12.62$, $p = 0.0003$). A SNK pair-wise comparison for minimum values shows that runs 1 and 2 were not significantly different from each other ($p = 0.23$), but both were different from run 3 ($p < 0.001$).

Patterns of Otolith Growth During the Larval Period

Average increment widths (N = 8) beginning at day-1 through capture for the April and May 1994 *manahac* larvae, from day-1 through day-53 from July 1995 juveniles, and from day-1 through day-53 in year-0 adults from 1992, were plotted and compared (Figure 2). The number of increments from the larval and juvenile samples could only be plotted to the maximum age read from any otolith in each sample, 33, 38 and 53 days respectively. The increments from the adults were plotted to a maximum of 53 days also. In all increment-width series there is a rapid increase in increment width that begins between day-3 and day-4 post hatching; maximum increment widths (maximum rate of the increase in the radius) occurs in all samples from day-16 to day-18; the width of increments decreases rapidly after the peak until between day-24 to day-39, where increment widths decrease less rapidly; the slope of the line changes again after day-39 and declines even more gradually until day-53.

Description of Growth of the Otolith Across the Transverse Medial Radius

The relationship between the growth of the otolith, across the ventral radius of a sagittal cross section, and the age of the fish was nonlinear. A cumulative growth curve of the mean otolith radius was constructed for the initial 33-day increment series of all samples (Figure 3). The curve of the line that is formed by the daily increase in otolith increment widths (μm d^{-1}) was fit to a logistic growth curve (equation 1). The parameters of the individual curves along with the mean of all values are shown in Table 3.

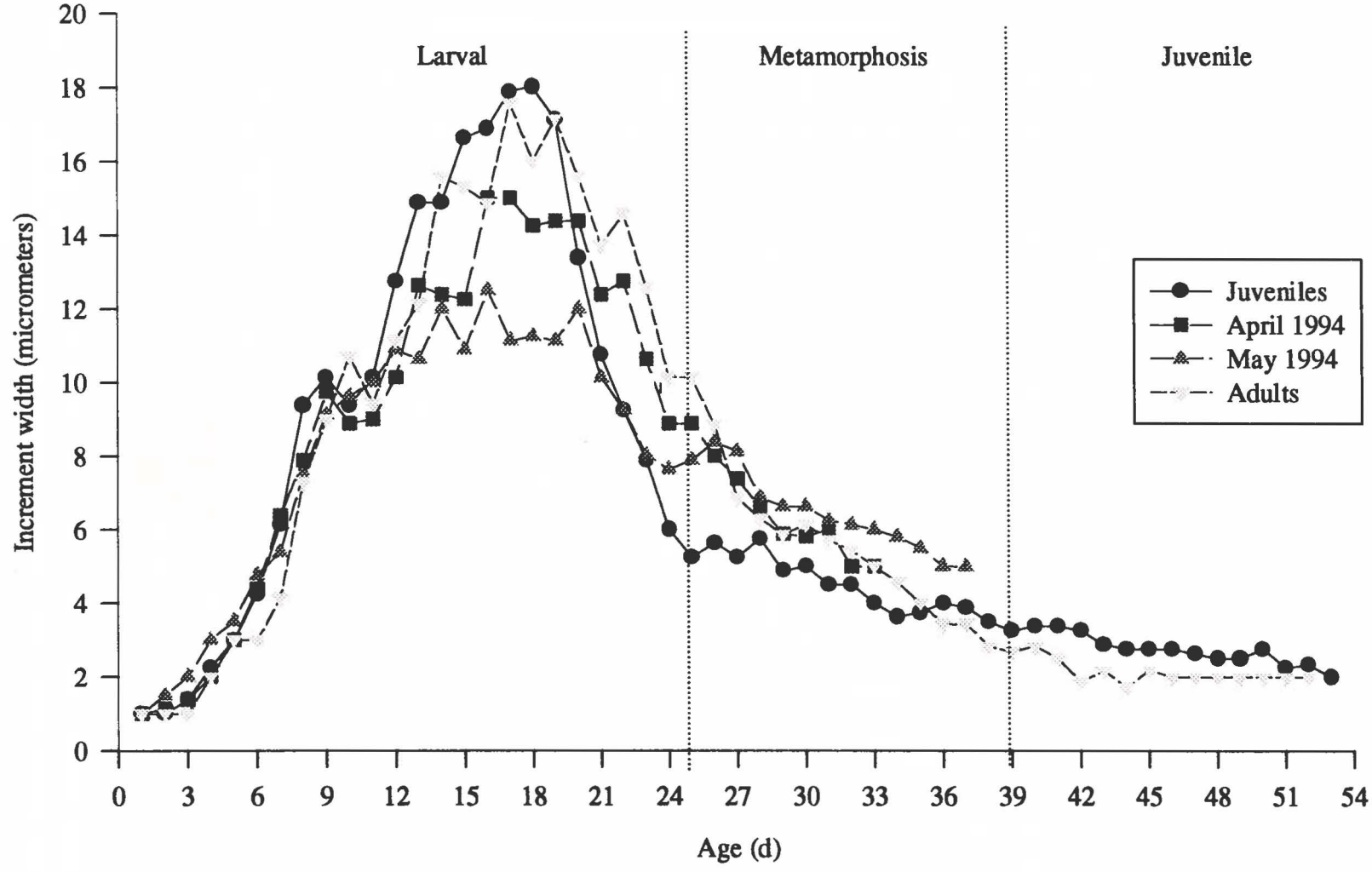

Figure 2. Mean sagittal increment widths over time. Larval (4/94 and 5/94), juvenile (July 1995), and adult (10/92) samples. Increments measured from ventral radius of transverse cross-section. N = 8 all samples.

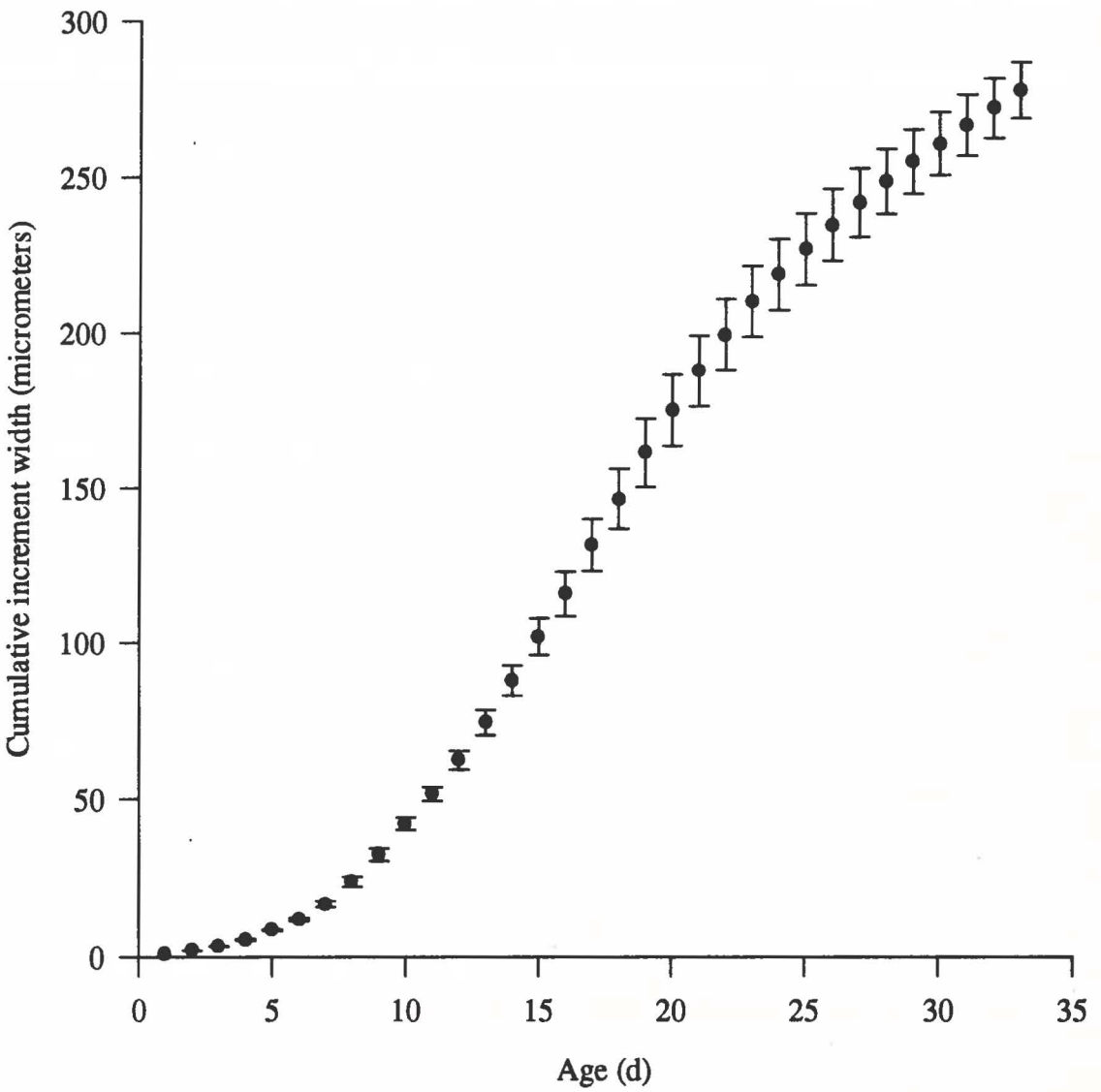

Figure 3. Growth curve of otolith radius over larval period. Mean ventral radial width of transverse medial cross section of sagitta. Mean of four cohorts per point with SE bars.

Table 3. Parameters of the logistic growth equation (eq. 1 in text) estimated from the equation describing the growth curve of the mean radius of the ventral cross section of the sagitta at age in *S. spinus*.

Parameter	Larval 4/94	Larval 5/94	Juvenile 7/95	Adult 10/92	Mean
O_c	281.3 ± 2.7	254.9 ± 4.1	273.0 ± 2.4	298.3 ± 2.5	275.8 ± 2.9
G	0.22 ± 0.005	0.20 ± 0.007	0.26 ± 0.007	0.24 ± 0.005	0.23 ± 0.006
(x_0)	18.5 ± 0.14	18.3 ± 0.25	16.2 ± 0.13	17.8 ± 0.13	17.6 ± 0.16

DISCUSSION

Tetracycline marking of otoliths demonstrated that increment deposition in the rabbitfish *Siganus spinus* occurs daily. There is other evidence supporting my finding of daily increment deposition. Correlation of increment counts on age in laboratory-reared *S. randalli,* obtained from S. Nelson, validated daily increment deposition for that species (Chirichetti, unpublished data). Daily increment deposition in tropical reef fish seems to be the rule rather than the exception (Thresher and Brothers, 1985; Victor, 1986; Thresher et al., 1989; McCormick, 1994; Sponaugle and Cowen, 1994). Therefore, it is not surprising that daily increment deposition occurs in *S. spinus*.

Validation of daily increment deposition in *S. spinus* made it possible to calculate the length of the larval duration. The 30.1 to 32.8-d mean larval duration in *S. spinus* is very close to the age at metamorphosis found in other siganids. Metamorphosis in *S. canaliculatus* occurred from 23 to 30^{+} d in Palau (May et al., 1974), from 29 to 35 d in *S. lineatus* in the Philippines (Bryan and Madraisau, 1977), and around 35 d in laboratory-reared *S. randalli* in Guam (Nelson et al., 1992).

Data on the mean larval durations of 213 tropical fish species, distributed among 6 families, shows the 30.1 to 32.8 d mean larval duration of *S. spinus* to be near the mode of a wide distribution of larval durations (Figure 4). Average larval durations within families ranged from a low of 21.2 d in the Pomacentridae (Thresher et al., 1989, N=70) to 48.4 d in the Gobiidae (Sponaugle and Cowen, 1994, N = 2). Larval durations longer than 50 days were restricted to the labrids and one goby. The values of larval duration ranged from 7 to 121 d and were lowest in the Pomacentridae.

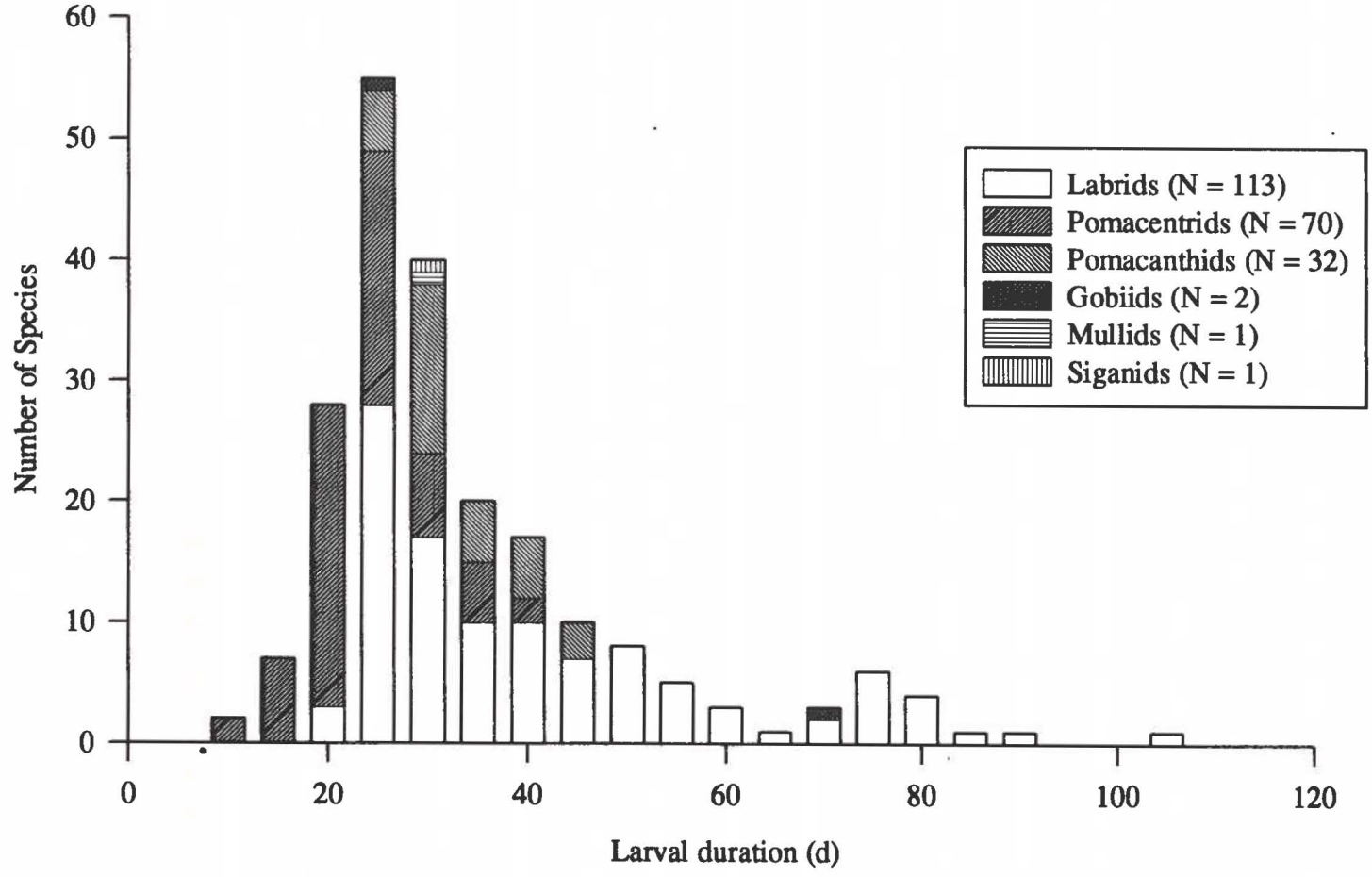

Figure 4. Frequency histogram of larval durations for 213 species in 6 families of tropical reef fish.

The growth rate of 0.81 to 1.15 mm d^{-1} through the larval period found in *S. spinus* is similar to the approximately 1.0 mm d^{-1} average growth found in *S. canaliculatus* in Palau (Hasse et al., 1977), and greater than the 0.5 mm d^{-1} mean growth found in laboratory reared *S. lineatus* in the Philippines (Bryan and Madraisau, 1977). It is higher than many growth rates reported for fish in other families such as the 0.55 to 1.0 mm d^{-1} in a mullid (*Upeneus tragula*; McCormick, 1994); 0.25 mm d^{-1} in an acanthurid (*Ctenochaetus binotattus*; Lou, 1993); 0.20 mm d^{-1} in a chaetodontid (*Chaetodon rainfordi*; Fowler, 1989); and 0.5 mm d^{-1} in a pomacentrid *(Chromis atripectoralis*; Thorrold and Milicich, 1990).

The daily increment width pattern found in *S. spinus* is also similar to increment patterns found in other tropical reef fish. The shape of the graph made by the increment width series may reflect "endogenously controlled" predetermined growth characteristics (McCormick, 1994). This is suggested by the similarity found among cohorts between seasons and years, even though environmental conditions such as food availability and temperature may have varied widely. Larval development has been related to characteristics found in otolith increment series (Ozawa and Penaflor, 1990), and the shape of the graph of increment-width series in *S. spinus* may indicate developmental events, though this will only be known by further larval studies under controlled conditions.

Spawning in many fishes has been correlated to specific phases in the lunar cycle (Thresher, 1984). There is no published data on spawning of *S. spinus*, but spawning times may be inferred from recruitment dates and larval duration. In Guam, recruitment

in *S. spinus* is closely tied to the period around the 21st day of the lunar cycle. The appearance of the *manahac* is so tied to this lunar phase that local fishermen will take time off from their jobs and wait for the fish to appear over the reef. This predictability is important since this event is rare and *manahac* are only valuable at this stage before they metamorphose (Amesbury et al., 1986). The 30 to 33-day larval duration calculated for *S. spinus* would, therefore, indicate that spawning in this species probably occurs between the 17th and the 25th day of the lunar cycle in the month preceding recruitment. Spawning may occur year-round as *S. spinus* has been found with eggs in every month of the year (G. Davis, DAWR, personal communication), and data collected from *manahac*, juveniles, and adults confirms that spawning occurrs at least from March through October Within the Siganidae, the relationship of spawning to the lunar cycle among individual species is highly variable. In Palau, *S. canaliculatus* spawns 3 to 6 days after the new moon between March and May, *S. lineatus* spawns between the first quarter and full moon, *S. argenteus* and *S. punctatus* spawn around both the new and full moon (Bryan and Madraisau, 1977; Hasse et al., 1977; Johannes, 1981), and in the Philippines, *S. guttatus* has been found to spawn year-round between the first quarter and full moon (Hara et al., 1986).

Fundamental to fisheries management is the ability to accurately age fish. Increment deposition in otoliths of *S. spinus* has been shown to be daily and therefore otoliths can be used to estimate age of this fish, at least through the larval and early juvenile periods. *S. spinus*, especially in the late larval phase, is an important inshore fishery resource on Guam, and may become more so if the overall decline in fish stocks

continue. Research on growth and its variability in the early life history of fish may allow determination of recruitment potential of a given cohort. *S. spinus* successfully recruited to Guam's reefs during the four years for which this study has samples. Successful recruitment occurs in years with conspicuous *manahac* runs, as well as in years when inconspicuous runs occur. The conditions that contribute to large *manahac* runs may be more well defined by an understanding of the fitness of recruits and their eventual contribution to the age structure of the population. By validating daily increment deposition, and by determining the range of larval duration and growth-rate during the larval period, this study has provided some of the basic facts concerning the early life history of *S. spinus* that may be useful as a start to the overall understanding of its ecology.

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