

Daily Otolith Increments and Recruitment in Two Coral-Reef Wrasses, *Thalassoma bifasciatum* and *Halichoeres bivittatus*

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Abstract

Increments on the otoliths of two common coral reef fishes, the bluehead wrasse *Thalassoma bifasciatum* and the slippery dick *Halichoeres bivittatus*, were demonstrated by mark-recapture experiments to be daily. Otoliths were marked in two ways; by depriving fish of light, food, and temperature cycles and also by supplemental feeding in the field. Both experiments were performed in late 1980 in the San Blas Islands of Panamá. A mark corresponding to settlement of the planktonic larva onto the reef was found on the otoliths of the bluehead wrasse. This settlement mark was used to calculate the dates of settlement of a collection of juveniles of this species taken from a patch reef in the San Blas Islands of Panamá in 1981. Settlement occurred in short and irregular bursts. The number of daily increments before the settlement mark indicates a planktonic larval life of 40 to 72 d.

Introduction

Determining the age of tropical marine fishes, especially reef species, has traditionally been particularly difficult. Most methods of aging require that the fish undergo distinct annual cycles of growth leading to recognizable marks on the fish's hard parts, usually on scales, otoliths, bones, or spines. These cycles may occur in tropical estuarine environments (Fagade, 1974), but are, in general, absent in other tropical marine waters. As a result, little is known about the growth and survivorship of tropical reef fishes (Sale, 1980). Recently, however, daily increments on the otoliths of some fishes have been discovered (Panella, 1971). Otoliths are stone-like calcium carbonate accretions situated within the semicircular canals of bony fishes which assist in balance and sound perception. Increments on the otolith permit extremely precise determinations of the age of fishes.

Increments that are assumed to be daily have been recorded on the otoliths of a variety of coral-reef fish species (Ralston, 1976; Panella, 1974, 1980; Brothers, 1979). In addition, interruptions and transitions corresponding to settlement, metamorphosis, spawning, and changes in life-history characteristics have been proposed (Brothers and McFarland, 1980; Panella, 1980). Unfortunately, the increments observed have not been clearly demonstrated to be daily. Brothers (1979) has pointed out that there is a need for controlled field manipulations to validate this technique for tropical fishes and thus to encourage its widespread use.

Since otolith increments are usually particularly distinct in juvenile fishes, this method of aging can be invaluable in studies of the early life history of reef fishes (Brothers, 1979). In the present study, I confirm that the increments on the otoliths of two Caribbean coral reef wrasses (the bluehead *Thalassoma bifasciatum*, and the slippery dick *Halichoeres bivittatus*) are indeed daily. I also demonstrate that a mark corresponding to the settlement of the planktonic larva onto the reef exists on bluehead wrasse otoliths. Furthermore, using this mark, I establish the age at settlement and the daily pattern of settlement onto the reef for this species.

Both the daily pattern of settlement and the time spent in the plankton by larvae are important, yet largely unexplored, characteristics of the early life history of reef fishes. Sale (1980) has emphasized that further knowledge of the larval life of fishes and the details of recruitment are essential to an understanding of the processes determining community structure on coral reefs. The determinants of the rate and pattern of settlement onto reefs are yet unknown, despite the fact that the applicability of traditional community ecological theory depends on whether recruitment is controlled by factors within the resident community or not (Anderson *et al.*, 1981). The distance of larval dispersal is also an important but unknown subject. According to both Sale (1980) and Anderson *et al.* (1981), the scale on which community ecological studies should be

performed depends directly on the larval dispersal distance. The length of time larvae spend in the plankton is obviously an important determinant of the dispersal distance.

Materials and Methods

Daily Increments

The most direct demonstration that increments on the otoliths of fishes in the field are daily is obtained by marking the otolith and subsequently comparing the number of increments between the mark and the edge with the number of days since the marking took place. If the increments are indeed daily, the number of increments after the mark should match the number of days since the marking occurred. I marked the otoliths of wrasses in two ways. In the first, I isolated groups of juvenile *Thalassoma bifasciatum* from all external stimuli for 4 d and then returned them to the reef. In the second, I supplementally fed a group of *Halichoeres bivittatus* in the field for 5 d. The experiments were carried out in late 1980 on a large patch reef in the San Blas Islands on the Caribbean coast of the Republic of Panamá (maps of the region are on p. 278 of Tribble, 1981).

I captured 4 groups of juvenile bluehead wrasses (total $n=68$) from the reef with an aquarium dipnet. Each group was immediately placed into a sealed insulated plastic container with about 10 liters of seawater. They were kept in the dark, unfed and undisturbed, for 4 d. At the end of this period I sacrificed some individuals from each group and returned the rest of the group to the reef. I released each group onto an isolated coral outcrop cleared of all resident bluehead wrasses. I used outcrops situated in seagrass beds behind the reef crest, which, in the San Blas Islands, are typical habitats for juvenile bluehead wrasses. Juveniles of this species have very restricted home ranges and tend to remain on the outcrop onto which they have settled (Victor, unpublished data). The 4 groups were recaptured from their outcrops after 8, 12, 18, and 26 d, respectively. Unmanipulated groups of bluehead wrasses were collected from surrounding outcrops before, during, and after the experiment.

I marked the otoliths of slippery dicks by augmentation of food. First, I selected an area of shallow coral rubble and seagrass, about 2 m \times 3 m that contained a resident population of about 20 juvenile fish. For each of 5 consecutive days I brought 20 sea urchins (*Diadema antillarum*) from a nearby reef and crushed them on the site. I observed that juvenile slippery dicks, as well as roving adults and other species, were quickly attracted and fed readily. On Days 1, 3, and 5, I added the urchins between 12.00 and 15.00 hrs; while on Days 2 and 4, I added them after 16.00 hrs. After 32 d I returned to the same site and caught all the resident juvenile slippery dicks. The juveniles of this species have very small home ranges (Victor, unpublished data). Unmanipulated juveniles were col-

lected from the surrounding area before, during, and after the experiment.

Settlement Mark

Both Brothers and McFarland (1980) and Panella (1980) have suggested that marks corresponding to settlement can be found on some species' otoliths. To investigate the possibility of there being a settlement mark on the otoliths of bluehead wrasses, I collected juveniles on their first day of appearance on the reef. To do this I performed a daily census of the population of juvenile bluehead wrasses in an area of coral outcrops in a seagrass bed for several months in the summer of 1981. When new recruits were first seen on an outcrop that had had no juvenile bluehead wrasses resident for several weeks, the recruits were caught and immediately preserved in ethanol. Larvae that had not yet settled were captured in the plankton as they approached a light hung over the water at night. These larvae could be identified as bluehead wrasses by fin-ray counts.

Daily Pattern of Settlement

If there are daily increments on the otolith and a settlement mark, the date of settlement for any individual can be calculated by subtracting the number of increments between the settlement mark and the edge of the otolith from the known date of capture. To permit just such an analysis I collected a sample ($n=64$) of juvenile bluehead wrasses (up to 30 mm standard length) from a large patch reef in the San Blas Island chain on 6 August 1981. All juveniles encountered were collected.

Preparation of the Otoliths

The lapilli and the sagittae, 2 of the 3 pairs of otoliths in teleosts, were removed from all of the wrasses collected. After removing the top of the cranium, I used a pair of fine forceps to extract the lapilli from the lateral walls of the brain case and the sagittae from beneath the posterior end of the brain. The otoliths were cleaned and placed in a drop of immersion oil on a glass microscope slide. These otoliths were then examined, without sectioning, under a compound microscope at magnifications ranging from 400 to 1 000 \times . Polarizing the transmitted light assisted greatly in clarifying the increments.

Results

Daily Increments

All of the wrasses examined had distinct increments on both the lapilli and the sagittae. The counts made on one type of otolith always matched the counts made on the

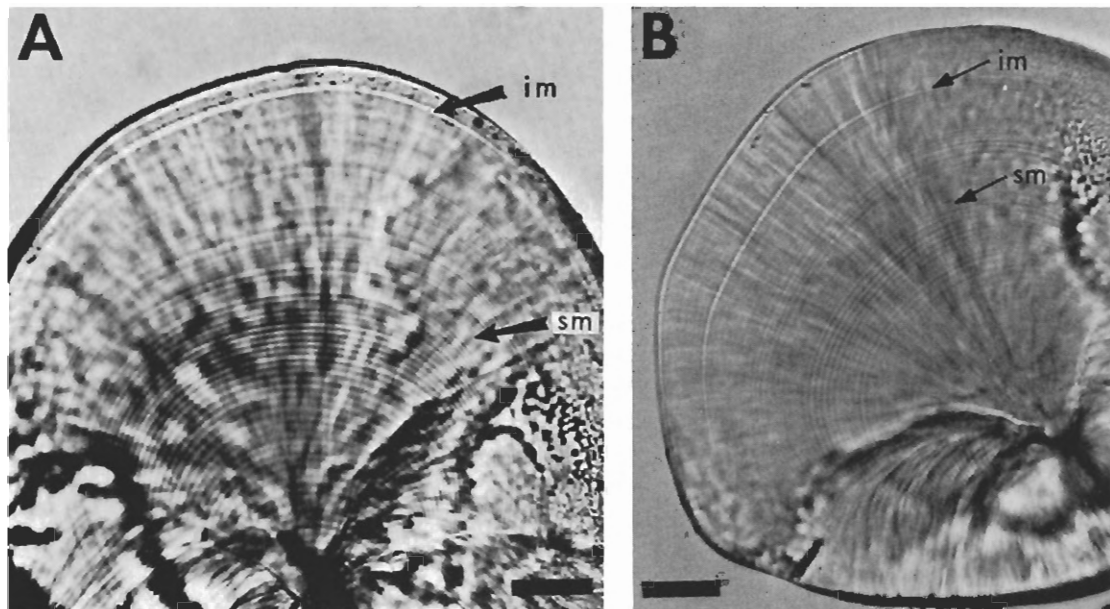


Fig. 1. *Thalassoma bifasciatum*. (A) Lapillus from 15.2 mm standard length (SL) bluehead wrasse that had been held in isolation, released, and then recaptured and killed after 8 d; mark from isolation treatment appears near edge of the otolith (Arrow im); settlement mark is present nearer center of the otolith (Arrow sm); scale bar = 35 μm . (B) Lapillus from 19.6 mm SL bluehead wrasse that had been held in isolation, released, and recaptured after 26 d; isolation mark and settlement mark are indicated; scale bar = 40 μm

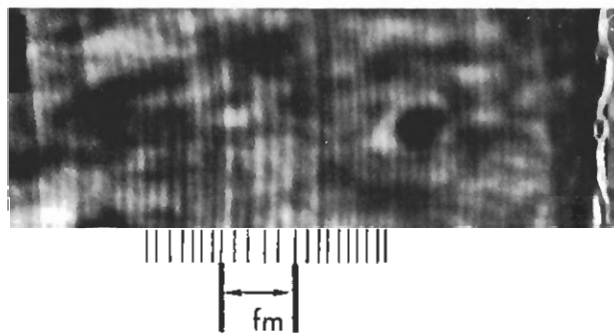


Fig. 2. *Halichoeres bivittatus*. Pattern of daily increments on sagitta of 32.0 mm SL slippery dick that had been supplementally fed for 5 d and then caught after 32 d. Feeding mark (fm) consists of 5 unusually wide increments; there are 32 increments between this mark and edge of the otolith (visible on far right of photograph), but, because only a section of the otolith can be in focus at any one time the sequence cannot be photographed in its entirety. Scale bar = 5 μm . Increments after feeding mark are only about 500 A wide

other. Each increment is made up of a translucent line (light when examined with transmitted light) and a narrower opaque line (dark with transmitted light).

Each *Thalassoma bifasciatum* captured at outcrops where experimental fish had previously been released ($n=21$) had a distinct mark on its otoliths. The mark was clearest on the lapilli, the smaller of the two pairs of otoliths examined. Under the microscope the mark appears as a band which refracts light in a markedly different manner from that of the surrounding increments (Fig. 1). When in focus the band appears exceptionally light. At higher magnification it shows an absence of the usual light-dark alternating lines characteristic of normal

otolith increments. A similar mark appeared at the edge of the otolith from individuals killed immediately after the isolation treatment and was never seen on any of hundreds of other untreated fish. In all 21 specimens, the number of increments between the mark and the edge of the otolith corresponded exactly to the number of days since the marked fish were released.

A characteristic pattern was evident on the otoliths from all of the juvenile *Halichoeres bivittatus* larger than 25 mm caught at the former feeding site ($n=10$). The pattern was not on the otoliths of juveniles smaller than 25 mm. The pattern was made up of 5 unusually wide increments, surrounded by normally sized ones (Fig. 2). The second and fourth of these wide increments were not as wide as the other three. As in the case of the bluehead wrasses, the number of increments between the 5 wide increments and the edge of the otolith corresponded exactly to the number of days since the feeding was terminated.

Settlement Mark

All bluehead wrasses captured on the reef, including large adults, had a conspicuous transition in the character of the increments near the center of the otolith (Fig. 3). The only bluehead wrasses not having this transition on their otoliths were larvae captured in the plankton (Fig. 4). Juveniles captured on their first day of appearance on the reef had this transition on the edge of their otoliths. At this transition, the previously prominent dark lines delineating each increment abruptly disappear. Regular increments only reappear after a band without discrete increments is

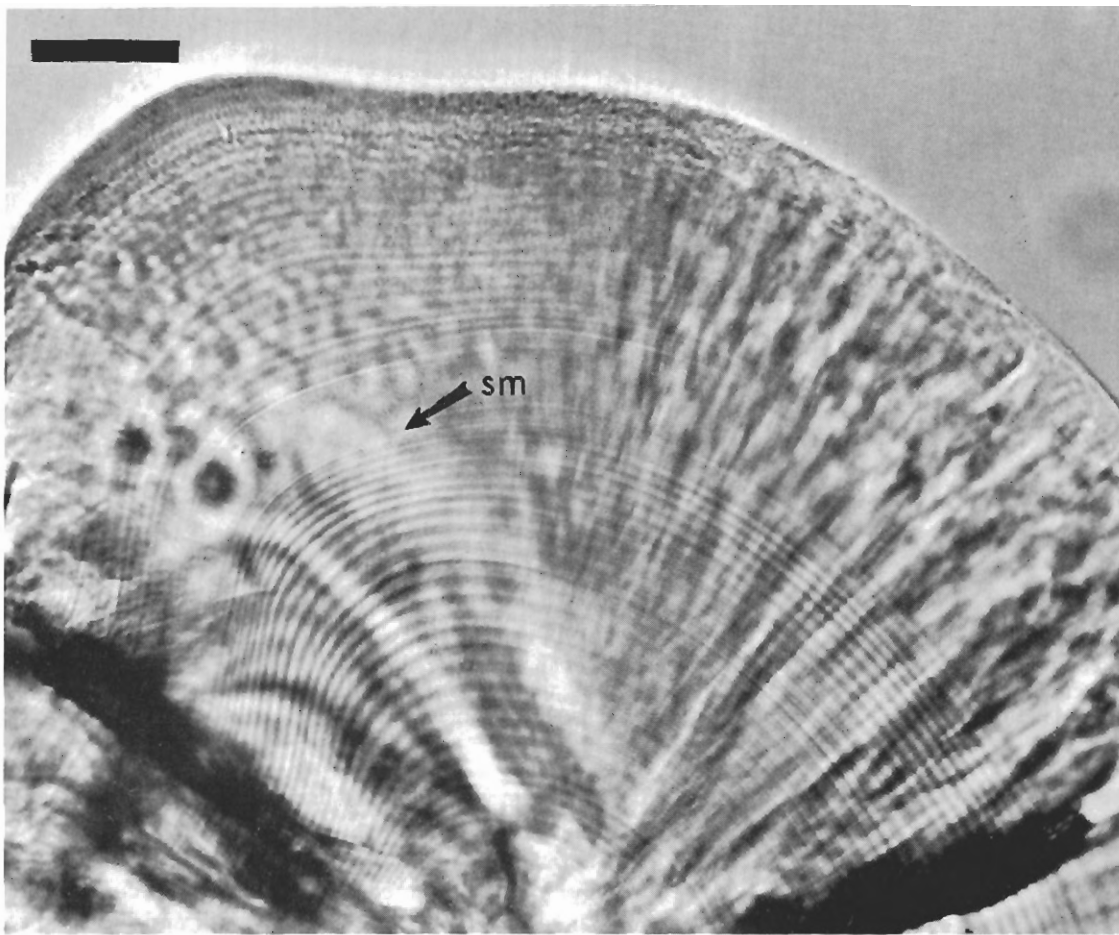


Fig. 3. *Thalassoma bifasciatum*. Settlement mark (sm) on sagitta of 14.3 mm SL bluehead wrasse; scale bar = 40 μ m

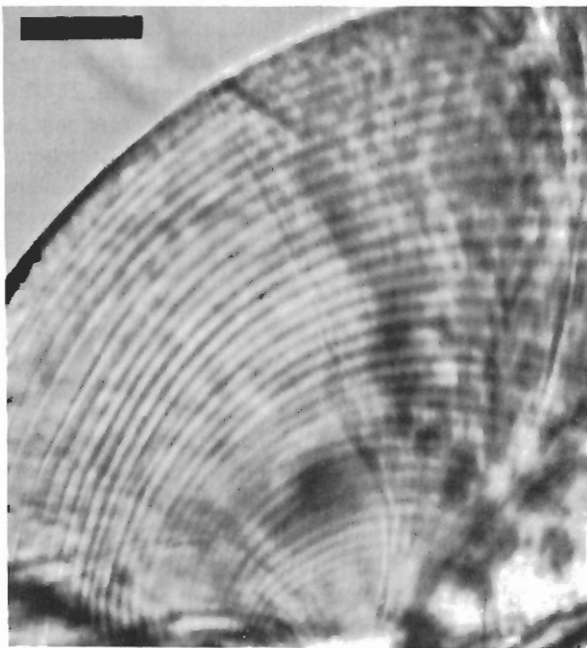


Fig. 4. *Thalassoma bifasciatum*. Sagitta of 12.0 mm SL bluehead wrasse larva captured from plankton at a nightlight; no settlement mark is evident; scale bar = 20 μ m

formed. In the sample of 64 juvenile bluehead wrasses collected from a large patch reef, the number of visible increments between the nucleus of the otolith and the settlement mark ranged from 38 to 70 (mean of 46, SD = 5.9).

Daily Pattern of Settlement

Bluehead wrasses settle in short and somewhat irregular bursts (Fig. 5). Of the 64 juveniles taken from a large patch reef, 34 had settled within a 2 d period. Settlement appeared to be weakly correlated with the lunar cycle. Recruitment occurred primarily within the 2 wk around the new moon, although peaks of settlement did not fall directly on the new moon.

Discussion

All aging methods require validation before they can be used with confidence. Despite this obvious caveat, workers in the field of reef-fish aging have liberally pursued

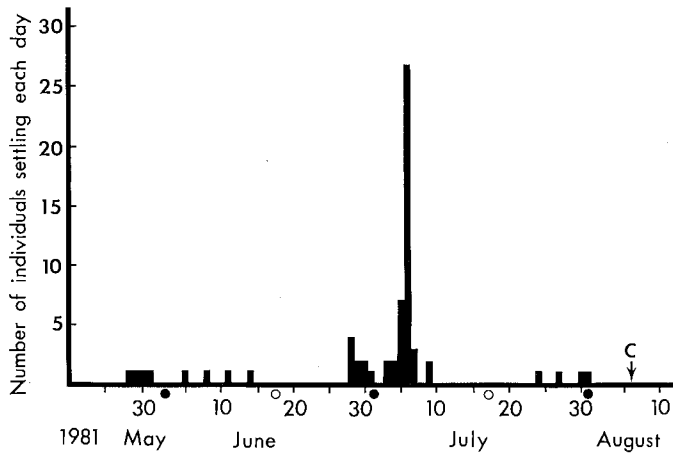


Fig. 5. *Thalassoma bifasciatum*. Pattern of settlement of a sample of juveniles from single large patch-reef in San Blas Islands, Panamá ($n=64$) collected (at C) on 6 August 1981. Bars represent number of individuals settling each day, calculated by subtracting age since settlement for each individual from collection date. Filled circles denote new moon, open circles full moon

analyses of otolith increments without first establishing their daily nature on any tropical reef species (see Panella, 1980). I believe it is an important first step to demonstrate, in the field, that the increments one observes are truly daily in both the species and age class under study. This is especially necessary in the light of recent proposals that there are often subdaily increments on the otoliths of tropical fishes (Brothers and McFarland, 1980; Panella, 1980).

My results indicate that the increments on otoliths from juvenile *Thalassoma bifasciatum* and *Halichoeres bivittatus* are daily. In both mark-recapture experiments, the number of increments between the mark and the edge matched the number of days since the marking took place. Daily increments permit extremely precise determinations of age, especially of short-lived species that grow fast. Because of the previous lack of an effective aging method for tropical reef fishes, many details of their life histories have remained guesswork (Sale, 1980). At present no accurate life table, survivorship curve, or even direct longevity estimate is available for any species of tropical coral-reef fish. Clearly, the use of daily otolith increment aging will facilitate studies on these aspects of reef-fish ecology. This approach will also allow comparisons of growth and survival between different habitats and between different species; such comparisons should be of great importance to studies of competition, predation, and habitat selection by fishes in the coral-reef environment.

The otolith-marking methods I used are simple and probably generally applicable to a wide spectrum of species. The isolation treatment deprived the subjects of all external influences such as light, food, temperature changes and currents. The fish undergoing the isolation treatment remained motionless and lay on their sides, presumably throughout the 4 d. Since wrasses do sleep at night, the treatment was in some ways equivalent to a 4 d long night. The isolation mark itself was a light band wider than the surrounding increments. If, as has been recently suggested, the light line in each light-dark couplet is laid down in the afternoon and at night, and the narrower dark line during the early part of the day, this

isolation mark is exactly what one would expect to result from an abnormally long period of "night" growth (Mugiya *et al.*, 1981; Tanaka *et al.*, 1981; in contrast, Brothers and McFarland (1980) proposed that the dark line is laid down at night).

Supplemental feeding is an even easier method of marking otoliths with a recognizable pattern. The isolation treatment requires capturing the subjects twice, and thus requires that the fish be hardy enough to withstand a few days of isolation and the handling involved. Supplemental feeding requires only that the subjects remain in the same place during the feeding and for some time afterwards. Most reef fishes, especially juveniles, are well known for their tendency to remain site-attached (Sale, 1980).

The wider increments resulting from supplemental feeding indicate that the growth of the otolith is closely tied to the growth of the fish. This relationship may be very precise, since the increments corresponding to Days 1, 3, and 5 of the feeding experiment on juvenile slippery dicks were noticeably wider than those on Days 2 and 4. On Days 1, 3, and 5, I added urchins between 12.00 and 15.00 hrs and observed wrasses feeding on the debris for the rest of the afternoon. On Days 2 and 4, I added urchins after 16.00 hrs. Slippery dicks, along with many other grassbed fishes, tend to retreat for the night between 16.30 and 18.00 hrs each day. It is at this time of the evening that large predators such as barracuda and jacks are seen cruising the grassbeds. During these two feedings I noticed that the wrasses were particularly wary and, by 17.00 hrs were no longer feeding. Presumably the greater amount of food they obtained on Days 1, 3, and 5 had resulted in increased growth and thus relatively wider increments.

Slippery dicks less than 25 mm standard length did not show any sign of a mark on their otoliths. Since the supplemental feeding took place over 1 mo before the capture, I believe these smaller juveniles either had not yet settled or were newly settled and too small to eat crushed urchins at the time of the feeding.

It is not surprising that a transition in the character of the increments is associated with settlement in bluehead

wrasses. Settlement and subsequent metamorphosis involve unusual and complex physiological changes. Transitions in otolith increment characteristics associated with shifts in habitat and feeding behaviors have been suggested for other species (Brothers and McFarland, 1980; Panella, 1980).

The presence of the settlement mark permits one to determine the date and age of settlement of fishes collected long after they have settled. The pattern of settlement revealed by this technique showed that bluehead wrasses in the San Blas Islands settled in short and somewhat irregular bursts. This species spawns daily (Warner and Robertson, 1978), so the absence of a continuous "rain" of recruits can be ascribed to physical or biological processes occurring in the plankton. A strict lunar cycle of settlement was not evident, but settlement did appear to be concentrated within the 2 wk around new moon.

Without the otolith technique, studies of the daily pattern of settlement are difficult and time-consuming. However, information on the pattern of settlement is essential to an understanding of what determines recruitment to a reef, and Anderson *et al.* (1981) have implied that if recruitment is determined by factors independent of the local resident community, traditional community ecological theory is inapplicable to reef-fish assemblages.

Knowledge of the larval life of coral-reef fishes must be increased before we can adequately test views on the structure of reef-fish assemblages (Sale, 1980). A pivotal issue in the controversy over reef-fish community ecology is the extent of dispersal of pelagic larvae. The greater the distance of dispersal, the lesser the likelihood of local assemblages of reef fishes being in a stable equilibrium state (Anderson *et al.*, 1981). Furthermore, the dispersal distance should determine the scale on which community ecology of coral-reef fishes should be examined (Sale, 1980; Anderson *et al.*, 1981). Direct evidence of the length of time in the plankton is an obvious first step in the resolution of this controversy.

Determining the length of the planktonic life of a species of fish is difficult without an accurate aging method. A few attempts have been made, most notably that of Randall (1961), who compared the timing of spawning with the timing of recruitment and came up with an estimate of 2.5 mo for the surgeonfish *Acanthurus triostegus*. Such determinations become much easier with the use of daily otolith increments. The number of increments between the nucleus of the otolith and the settlement mark ranged from 38 to 70 (with a mean of 46) in a sample of bluehead wrasses. The otoliths of wrasses first develop around the time of hatching, which, in the warm waters of the tropics, would be approximately 2 d after fertilization (Fritzche, 1978). Thus, the planktonic life of this species in the San Blas Islands of Panamá appears to be on average 48 d, with a range of 40 to 72 d.

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