

CHAPTER 9

Settlement Strategies and Biogeography of Reef Fishes

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I. INTRODUCTION

Virtually all coral reef fishes undergo a profound transition from life as a larva adrift in the oceanic plankton to a settled existence closely associated with the coral reef structure. The importance of this transition to population dynamics and a variety of other aspects of the ecology of reef fishes is reviewed in other chapters. Despite the widely acknowledged significance of the process of settlement, until very recently there has been a notable dearth of basic information on size, age, and behavior at settlement. This can be explained, perhaps, by the fact that the transition is swift, often occurring overnight, and typically beyond even the most enterprising ecologist's eye. With the development of new techniques, however, a wave of interest has developed in documenting many of the details of the settlement transition.

In this chapter I shall review three seemingly disparate subjects: the ecology of settlement, the biogeography of reef fishes, and the use of daily increments on the otolith for aging. In fact, these subjects are closely linked. Since the larval period is, no doubt, the dispersal phase for reef fishes and coral reefs are some of the most patchy and isolated habitats on earth, it is only logical to assume that the age at settlement (i.e., the duration of the planktonic larval stage) is a major determinant in the geographic distribution of reef fishes. Whether this truism reflects reality is debatable, for it appears that, with the limited information available, little of the complex biogeography of reef fishes can be explained by variation in larval duration.

Finally, the otolith aging technique is inextricably linked with the study of settlement. The technique was first introduced and validated as a welcome tool for studying newly settled recruits. Although the applications of the method

extend to all phases of the life history of reef fishes, it is uniquely adapted to the study of very young fishes. Daily otolith increments are particularly wide and clear on the otoliths of larvae and juveniles and, coincidentally, these are the stages of reef fishes that are often the most elusive and difficult to study in any other way.

II. SETTLEMENT STRATEGIES OF REEF FISHES

A. Introduction

The majority of reef fishes undergo a distinct metamorphosis around the time of settlement, developing opacity and color, acquiring scales, and exhibiting a change in behavior. Some wrasse larvae in the Caribbean bury themselves in the sand for several days before emerging transformed as a juvenile (Victor, 1983b), while damselfish transform in hiding overnight (Robertson, *et al.*, 1988). Some unusual larval forms, such as the large, transparent, leaf-shaped leptocephalus larvae of the eels, bonefish, and tarpon, undergo a profound metamorphosis that takes many days.

A major transformation at settlement is certainly not the rule among reef fishes. In some groups, such as the squirrelfishes (Holocentridae) and angelfishes (Pomacanthidae), competent larvae are well developed and resemble slightly silvery versions of settled juveniles and thus do not change much after settlement. Others, such as the grunts (Haemulidae), drums (Sciaenidae), mojarras (Gerreidae), and sweepers (Pempheridae), remain small and transparent for some time after settlement. A few reef species confound simple definitions entirely and on occasion drift in the ocean for an indefinite period (usually associated with floating debris or algae), developing all the characteristics of juveniles and sometimes attaining large sizes before being delivered inshore. Among the Caribbean fishes following this strategy are the tripletail (*Lobotes surinamensis*), great barracuda (*Sphyraena barracuda*), grey triggerfish (*Balistes capricus*), and the sergeant major damselfish (*Abudefduf saxatilis*).

The diversity of modes of settlement has resulted in some confusion in the naming of various stages. The traditional taxonomic definition of larvae as the stage up to completion of fin development is ecologically meaningless, since virtually every late-stage reef fish larva in the oceanic plankton would then have to be considered a pelagic juvenile. Furthermore, some relatively undeveloped, yet settled, fishes would have to be considered "benthic larvae." In fact, the reporting of newly settled recruits of the reef drum (*Pareques* sp.) as "larvae" by Powles and Burgess (1978) led Kingsford and Choat (1989) to believe that some reef fishes complete their larval development while resident in the waters over reefs without ever entering the offreef plankton. McFarland

et al. (1985) also called fish in the first stage after settlement "postlarvae" to distinguish them from the larger "pre-juveniles."

Clearly, the most useful ecological definition of larvae is that they are presettlement fishes that live in the plankton and show some morphological adaptations to that habitat, such as transparency, large melanophores, or a silvery overlay. After settlement, fish are associated to some degree with the reef substratum and should then be considered juveniles.

I use the term settlement strategy to describe the suite of adaptations to the settlement transition, such as the size, age, and state of development of settling larvae, the temporal patterns of settlement, and the behavior of larvae at settlement. I believe these sets of attributes qualify as strategies because certain combinations seem to co-occur and are often characteristic of groups of reef fish species. I certainly do not mean to suggest that reef fishes follow a few basic strategies in settlement. In fact, as the following passages should demonstrate, the diversity of settlement strategies and the number of exceptions to any trend are remarkable. In this aspect of their life history, coral reef fishes fully live up to their deserved reputation (at least among vertebrates) for encompassing as wide a variety of specializations as it seems possible to evolve in any one habitat.

B. Techniques for Assessment

Most projects to date have attempted to approach the moment of settlement by focusing on the smallest juveniles that can be found settled on the reef. This simple method can be effective as long as the study area is clearly delineated and immigration of alien juveniles is uncommon. Obviously, the more frequent the surveys are, the more information can be obtained. The most useful settlement studies have monitored the appearance of new recruits on a daily basis (D. McB. Williams, 1980, 1983a; Victor, 1982, 1983a,b, 1984, 1986b; McFarland *et al.*, 1985; Ochi, 1985; Robertson *et al.*, 1988; Wellington and Victor, 1989). Robertson *et al.* (1988) managed to census new recruits of a variety of reef species at dawn and dusk. Both natural reef areas and artificial patch reefs have been used for surveying settlement. The advantage of using artificial substrates is that new recruits can be detected immediately (because hiding places can be eliminated) and losses to predation can be reduced.

Monitoring juveniles on the reef can only elucidate the process of larval settlement from one perspective. An alternative is to approach settlement by surveying the largest planktonic larvae one can find. Typical plankton surveys usually capture incomplete series of larvae with few mature individuals. Ideally, we should be able to follow competent larvae (defined as those ready to settle) as they move inshore and decide where and how to settle. One approach has been to capture larvae at a light at night over the reef, either by

hand (Victor, 1986b; Smith *et al.*, 1987) or by trap (Doherty, 1987b). Another method is to tow plankton nets in waters directly over the reef (Victor, 1986b; Leis and Goldman, 1987; Smith *et al.*, 1987; Kingsford and Choat, 1989). It is also possible, although thus far only anecdotal, to actually follow an individual larva in the water as it settles to the reef (D. McB. Williams, 1980).

The capture of fish larvae at a light at night (nightlighting) is a peculiar collecting technique that is very effective at capturing larvae over the reef just prior to settlement (Fig. 1). Competent fish larvae are strongly attracted to lights at night, a characteristic they share with moths and squid, although no persuasive explanation exists for this behavior. I have made nightly collections of Caribbean fish larvae at a nightlight over several long periods between 1981 and 1983 in the San Blas Islands of Panama. I collected larvae with a dip net under a light over reef waters only about 1 m deep (Victor, 1986b). Smith *et al.* (1987) towed a small fine-meshed plankton net through the beam of a light mounted on a deep underwater structure at St. Croix in the Caribbean. Doherty (1987b) reported the results of captures from a set of light traps placed overnight in shallow waters near Lizard Island on the Great Barrier Reef of Australia. I have found that the capture of fish larvae at a nightlight is significantly correlated with the density of larvae in the plankton determined from plankton tows made in the same area on the same night (Victor, 1986b).

These three methods produced very different sets of data. Doherty (1987b) and I both found that larvae attracted to the nightlight are almost always competent, and in some species I captured they had already started developing juvenile markings. Smith *et al.* (1987) report that they captured early- and intermediate-stage larvae as well, but they do not quantify or identify those larvae and thus it is not clear whether they were mostly newly hatched larvae being released from the reef.

Our results clearly demonstrate that the predominance of competent larvae in nightlight collections is not a product of size selectivity of the technique. In my collections from the Caribbean, for example, the mean size of the 27 species of goby larvae appearing at the nightlight ranged widely from 5.1 to 21.1 mm SL, but the range of size within a type was remarkably narrow (Fig. 2). The larvae of a single type tended to be fully mature, regardless of size, with complete fin development (e.g., Fig. 3). The samples often included individuals with metamorphic melanophore patterns (the tiny surface melanophores that develop on settled juveniles and are distinct from the few and large melanophores characteristic of transparent planktonic larvae). Furthermore, the larvae caught at the nightlight appeared to be similar in size to the smallest individuals seen on the reef. In fact, for the wrasses and parrotfishes (for which I have recorded the size of new recruits on the reef), the size of larvae at the nightlight closely matched the size of new recruits (Fig. 4). Whether this

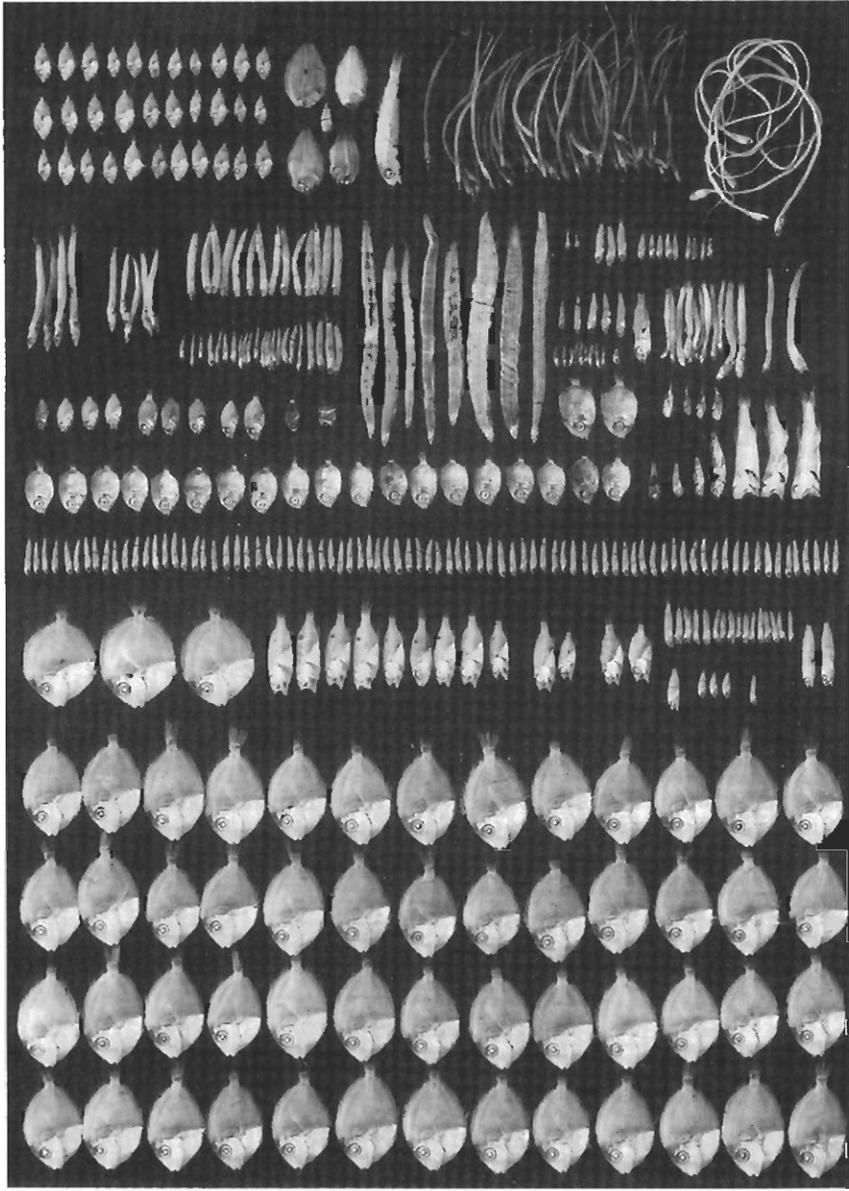


Figure 1 A sample of over 400 reef fish larvae of 54 types from at least 40 families caught at a nightlight in the San Blas Islands of Panama in two hours on August 28, 1981.

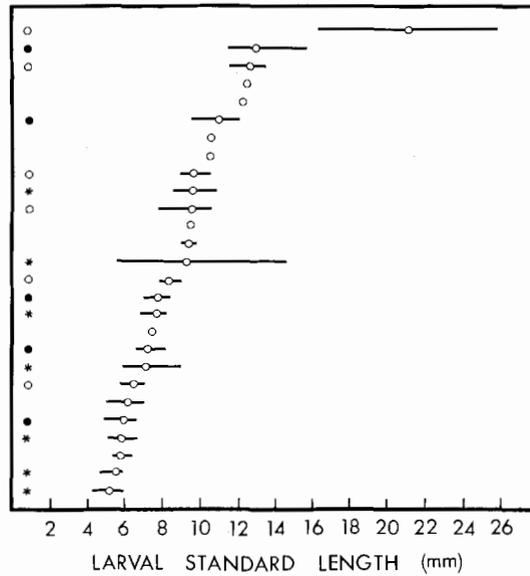


Figure 2 The distribution of sizes of 27 species of unidentified goby larvae caught at the nightlight in Panama. Circles and bars represent mean and range. The symbols at left indicate sample size: no symbol, 0–10; open circle, 10–50; solid circle, 50–100; asterisk, over 100.

reflects the fact that only mature larvae are present in inshore waters over reefs or whether it is only mature larvae that are attracted to light is not known.

The species composition of the three surveys was very different. Doherty's traps captured mostly damselfish larvae, which were uncommon in both my and Smith's surveys, despite their apparent abundance as juveniles on Caribbean reefs. My collections, like Smith's, comprised mostly clupeid, gobiid, and blennioid larvae. I also captured large numbers of labrid and scarid larvae, which Smith found to be rare in his nightlight collections. At present, it is

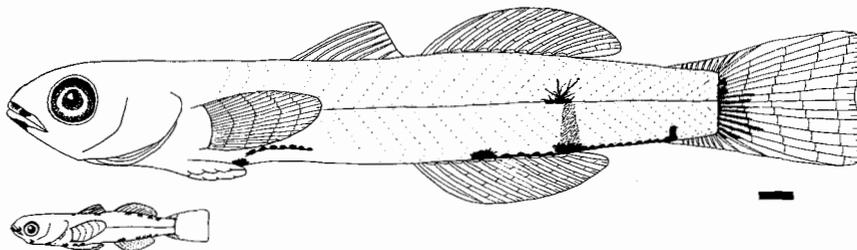


Figure 3 The largest and smallest type of goby larvae caught at the nightlight, drawn to scale. The bar represents 1 mm.

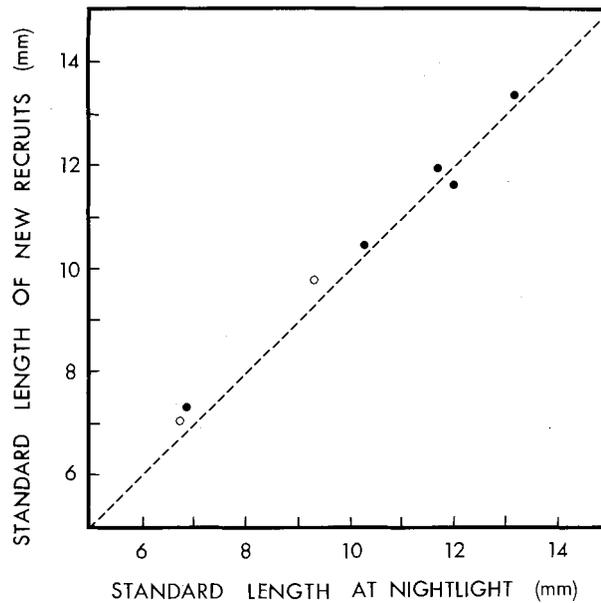


Figure 4 The relationship between the size of new recruits captured on the reef and the size of larvae of the same species caught at a nightlight. Open circles represent means for the parrotfishes *Scarus* spp. and *Sparisoma* spp., while the solid circles represent the means for the wrasses *Doratonotus megalepis*, *Halichoeres bivittatus*, *H. poeyi*, *Thalassoma bifasciatum*, and *Xyrichtys* spp.

impossible to discern whether these differences are a product of varying geographic areas, habitats, depths, times, lighting, nets, or even something else, for it would be difficult to devise three more different nightlighting techniques for reef fishes.

Simple survey techniques have provided data on the size at settlement, the temporal and spatial patterns of settlement, habitat selection, and mortality rates of new recruits. In addition, the use of daily increments on the otoliths of young fishes to determine age has greatly expanded our ability to extract information. This method permits the calculation of planktonic larval duration, age at settlement, and growth rates both in the plankton and on the reef. The application of this technique to settlement studies will be reviewed in the last part of this chapter.

C. Timing of Settlement

Most settlement of reef fish larvae probably occurs at night. Robertson *et al.* (1988) surveyed isolated artificial reefs at dawn and dusk, removing all fish

found at each visit. The results indicated that 93% of the new recruits captured (of at least 18 different species) were found at dawn. Considering that there may have been movement of newly settled larvae from the surrounding seagrass beds after dawn, it is likely that virtually all settlement occurred during the night. When visual censuses were done on natural reefs with many inaccessible hiding places, new recruits of the bicolor damselfish, *Stegastes partitus*, tended to be observed more often at dusk. This difference was most probably a result of newly settled larvae avoiding detection by hiding for a number of hours after settlement (Robertson *et al.*, 1988).

Surveys of planktonic larvae are usually undertaken in offshore waters, and, as a result, little is known about the distributions of reef fish larvae in waters over the reef. Night plankton tows I have made over the reef typically yield many more reef fish larvae than daytime tows. One possible explanation is that larvae may be vertically migrating up at night and remaining deep, and thus off the reef, when it is light. Leis (1986a) found that most types of larvae he sampled in nearshore waters bordering the reef preferred deep water during the day and moved upward at night. This fits with the observation that planktonic fish larvae are unusual sights while working on the reef during the day. The adaptive significance of settlement while it is dark is obvious; visual predation would be intense on larvae attempting to settle during the day. For perhaps a similar reason, larvae appeared at the nightlight in much lower numbers on nights when the moon was up. These findings correspond well to observed settlement patterns; many independent surveys report lower settlement rates during the week around the full moon, when the moon is not only brighter but up for most of the night (Victor, 1983a, 1984, 1986b; McFarland *et al.*, 1985; Ochi, 1985; Robertson *et al.*, 1988) [apparently there is no such clear pattern at One Tree Reef in Australia (Williams, 1983a; Sale, 1985)].

The details of the temporal pattern of larval settlement are well documented for some reef fishes and have been reviewed in other chapters. In general, there appears to be a broad spectrum among coral reef fishes in the pattern of occurrences of larvae. While many species of reef fish larvae appear somewhat regularly, in random or periodic short cycles, a few species tend to settle in large numbers on rare occasions. Robertson (1988b) recorded an unusual mass settlement of queen triggerfish in Panama. This species settled in very low numbers over many years of observations and then, over several days in 1985, settled in densities 50 times greater than the totals for any other year. Several types of larvae showed up at the nightlight with a similar pattern. The large species of goby larva illustrated in Fig. 3, for example, appeared on only two occasions over the years of sampling, both in 1981. In one of the episodes, 23 individuals were caught in one hour, comprising about half of the reef fish larvae caught that night.

D. Size at Settlement

Coral reef fish larvae settle over a very broad range of sizes, although there tends to be some similarity in size at settlement within a family. The estimated size at settlement for 45 species of damselfishes, for example, ranged from about 6 to 14 mm SL (Wellington and Victor, 1989; additional data in Thresher and Brothers, 1989). Other families, however, exhibit a great variety of sizes at settlement (e.g., Leis, 1989). Based on these studies and on night-light data, it appears that the majority of reef species settle between 7 and 12 mm SL.

The smallest size at settlement that I have recorded among the Caribbean reef fishes belongs to the reef cubbyu drum *Pareques acuminatus*; newly settled recruits measure from 3.8 to 4.5 mm SL, with a mean of 4.12 mm ($n = 9$) (Fig. 5). On the other extreme, I have collected mature larvae of the pearlfish (*Carapus* sp.), a species specialized for living within the body of sea cucumbers, that are on average 143.2 mm SL and reach 174 mm SL. Robertson (1988b) reports that the queen triggerfish settles at a length of about 60 mm SL. A few of the chaetodontid species in the Pacific are reported to appear on the reef at 50–60 mm SL, so these may also be settling very large (Leis, 1989). Some Caribbean fishes that show up at the nightlight at large sizes (30 mm SL or more) are the lizardfishes, squirrelfishes, surgeonfishes, goatfishes, and the trumpetfish. Members of these families in the Pacific must settle at large sizes as well, given the upper size limit of planktonic larvae of these families reported captured in plankton tows from that region (Leis and Rennis, 1983).

There is a set of species that settle very small and retain transparency for some time while associated with the reef. In the Caribbean, the reef drums, as well as the sweepers (Pempheridae), mojarras (Gerreidae), and the grunts (Haemulidae), appear to follow this strategy. The french grunt, *Haemulon flavolineatum*, has been studied in detail by McFarland *et al.* (1985), who found that they settle at about 6.9 mm and then pass through some clearly defined behavioral stages from a relatively undeveloped "postlarva" that roams around in the water column over sandy areas to a juvenile stage that migrates regularly to and from patch reefs at dawn and dusk.

No apparent order seems to be imposed on this variety of settlement sizes, making it difficult to explain in adaptive terms how they have arisen. The only pattern I have discerned is that those species that settle small and transparent tend to be found in back reef habitats such as shallow rubble, sand, or seagrass. Reef drum recruits are usually found in very shallow areas well behind the reef, while grunts settle in the same kind of back reef habitat (Shulman, 1985a). Mojarra recruits also frequent sandy seagrass areas inshore. An exception, however, is the other family of reef fishes that settle relatively undeveloped, the sweepers, which are found on the fore reef. The two types of



Figure 5 A new recruit of the reef drum (*Pareques acuminatus*) captured on the reef in the San Blas Islands of Panama.

larvae caught at the nightlight at sizes almost as small as the reef drum, the emerald clingfish, *Acyrtops beryllina*, and the sole, *Achirus lineatus*, are limited to back reef seagrass and muddy substrates, respectively. Furthermore, the smallest settling member of some large groups of reef fishes is often the one found mostly in shallow back reef habitats, such as the beaugregory damselfish, *Stegastes leucostictus*, and the dwarf wrasse, *Doratonotus megalepis*.

Why these types of fishes tend to settle only in shallow back reef areas is not clear. Shulman (1985a) suggests that predation pressure is lower in these back reef habitats than on the coral reef itself. This would lead more vulnerable small larvae to settle into those habitats. Even if there is lower predation in back reef areas, settling small and undeveloped still has severe costs in mortality. Shulman and Ogden (1987) report that less than one in ten grunt recruits survives a month on the reef and only about one in a thousand survives for a year. In contrast, other reef fishes that settle larger and more developed have two to three orders of magnitude higher survivorship to one year (Doherty, 1982, 1983a; Victor, 1986b; Eckert, 1987; Sale and Ferrell, 1988; Hunte and Cote, 1989). Interestingly, Robertson (1988b) found that queen trigger-

fish juvenile mortality was extremely high, despite the fact that it is one of the largest settling species. It was noted, however, that the mass settlement probably induced much higher mortality than is typical for the species.

In contrast to the effects on mortality of settling small, there appears to be no cost in growth rate. The growth rates after settlement for drums and grunts in the Caribbean are somewhat higher than for wrasses, which settle at larger sizes (Fig. 6). Even the redlip blenny, one of the largest settling reef fishes (at about 40 mm SL), grows at a similar rate (about 10 mm per month) after settlement (Hunte and Cote, 1989).

Presumably, there should be some major advantage to settling small that outweighs the massive losses to early mortality on the reef. It is likely that species that settle small and undeveloped are also settling after only a short time in the plankton (see the next section). They may be adapted to remaining in inshore waters and thus avoid the losses experienced by fishes that spend a longer time as larvae and are advected far offshore into the oceanic plankton. Theoretically, these strategies differ more in style than substance, since the

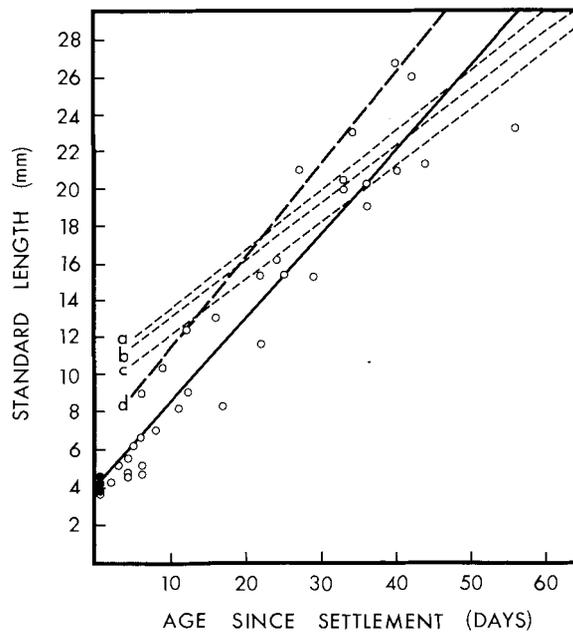


Figure 6 The early growth of reef drum juveniles (circles and solid line) compared to that of some other reef fishes: a, b, and c denote the wrasses *Halichoeres poeyi*, *Thalassoma bifasciatum*, and *H. bivittatus*, respectively (Victor, 1991), and d denotes the french grunt (Brothers and McFarland, 1981).

high juvenile mortality rates of early-settling species may be analogous to the mortality rate of planktonic larvae of other species. After all, they are of the same age. One could easily accept that the age-specific mortality schedule could be the same, with some species merely moving onto the reef earlier than others. If this were true, looking for a major advantage to either strategy would be missing the point.

In any case, it does appear that the larvae of most species that settle small and undeveloped are not found in offshore waters. Richards (1984) surveyed the larvae captured in oceanic plankton tows in the Caribbean and, although large numbers of other reef fishes such as wrasses and parrotfishes were caught, drums occurred in only one inshore collection and no grunt or mojarra larvae were captured. Interestingly, the dwarf wrasse (the smallest-settling wrasse, which settles in inshore shallow seagrass) was apparently totally absent from the Richards collection, even though hundreds of other wrasse larvae were caught. Apparently, those larvae destined to settle out small (and presumably after a short larval life) are not advected far offshore.

There may also be a fundamental connection between inshore larval distribution and back reef settlement habitat independent of the scenario involving the small size of larvae that I have developed here (Fig. 7). It could be that back reef habitats occur more continuously along coastal regions and thus back-reef-associated species need not be advected away from the coast, while fore reef species are more likely to encounter patchy reef areas if they move far offshore as larvae and then drift in. This hypothesis would be supported if large-settling back reef species were also found only in inshore waters. As this illustration demonstrates, the answers to these questions await much more thorough plankton and settlement studies.

Within species, the size at settlement does not appear to vary much. In many large series of a single species that I caught over several years of nightlight sampling, the entire range of lengths encompassed only 1 or 2 mm (e.g., Fig. 2). With some exceptions, this pattern is consistent enough to permit one to recognize larval types within a family simply by size. In a few other types, a small fraction of individuals were not fully developed and were significantly smaller than the average.

The coefficient of variation (*cv*) for larval standard length (*SL*) within a type was generally low, between 0.03 and 0.08 in my collections. Robertson *et al.* (1988) report a *cv* of 0.062 for the length of new recruits of the bicolor damselfish that is just about identical to the *cv* for length at settlement I found for three species of wrasses: 0.063, 0.061, and 0.055 (Victor, 1991). In fact, the *cv*'s for length at settlement of a wide variety of species are surprisingly similar. Wellington and Victor (1989) report values between about 0.04 and 0.07 for ten damselfish species, and even the winter flounder from Newfoundland was found to have a *cv* of 0.051 for size at metamorphosis (Chambers and

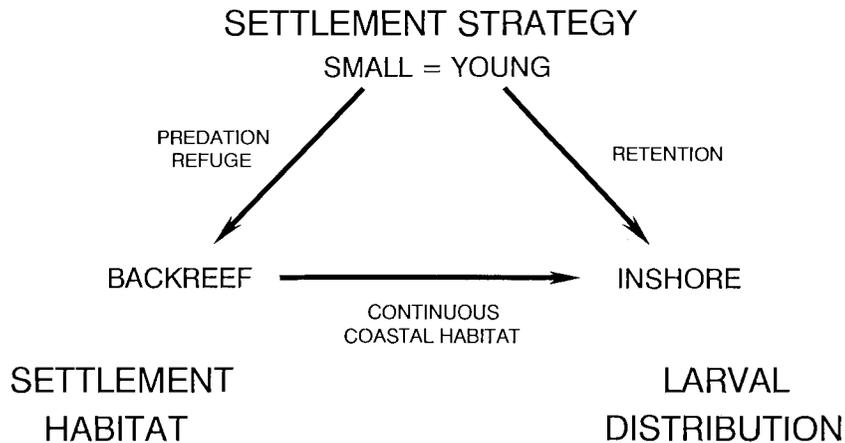


Figure 7 The hypothetical relationship between settlement strategies and settlement habitat and larval distribution. There may also be a causal relationship between settlement habitat and larval distribution independent of size or age at settlement.

Leggett, 1987). The relatively low variability in this aspect of reef fish settlement strategies may well indicate the selective importance of settling at an optimal size.

E. Age at Settlement

The planktonic larval duration (PLD) of reef fishes is more variable than the size at settlement, ranging from no planktonic phase at all to many months. Virtually all of the thousands of coral reef species in the Indo-Pacific do have a planktonic larval phase [with the notable exception of the damselfish, *Acanthochromis polyacanthus* (Robertson, 1973b)]. In the Caribbean, the toadfishes (Batrachoididae) are the only reef-associated fishes known not to have a planktonic larval stage (Hoffman and Robertson, 1983). Given that some reef species can settle at very small sizes, it is curious why more species have not opted for large eggs that hatch out larvae directly onto the reef. Alternatively, giving birth to live young should also be a feasible strategy, given the tiny return on eggs released into the plankton (although live-bearing is generally uncommon among marine fishes and it could be difficult to evolve toward that adaptive peak). I suspect that despite the short-term adaptive advantage of onreef reproduction, there is a long-term necessity for a wide-dispersal phase to avoid progressive local extinctions in the patchy geography of coral reefs.

With the advent of the daily otolith aging technique there has been a burst

of information on the planktonic larval duration of reef fishes. There have been broad surveys of a selection of reef species (Brothers *et al.*, 1983; Brothers and Thresher, 1985), as well as intensive surveys within some families of reef fishes, for example, 31 angelfishes (Thresher and Brothers, 1985), 100 wrasses (Victor, 1986a), 67 damselfishes (Thresher *et al.*, 1989), and 100 damselfishes (Wellington and Victor, 1989). The PLDs for a number of other species have also been reported: the french grunt (McFarland *et al.*, 1985), a Hawaiian damselfish and two gobies (Radtke, 1985; Radtke *et al.*, 1988), the queen triggerfish (Robertson, 1988b), the bicolor damselfish (Robertson *et al.*, 1988), and three chaetodontids (Fowler, 1989). I have also estimated the PLD for the reef drum from daily otolith increments on newly settled individuals.

One of the more notable features of the pattern of planktonic larval durations is that the shortest PLDs are consistently about two weeks. A large set of species settle after 14 to 18 days in the plankton, including french grunts and reef drums in the Caribbean, and the earliest-settling representatives of the Indo-Pacific angelfishes, wrasses, and damselfishes. The only exception reported, the Indo-Pacific anemonefishes (*Amphiprion* spp.), are documented to settle after only about one week in the aquarium (Thresher *et al.*, 1989), although it remains to be clarified whether they settle this young in the wild (the immediate availability of settlement sites in the aquarium could induce early settlement). On the other hand, there seems to be no simple limit to how long the larval duration can extend. Some species of wrasses routinely spend more than three months in the plankton (Victor, 1986a, 1987a), while Radtke *et al.* (1988) report that two gobies living in freshwater streams in Hawaii typically spend four and five months in the plankton before migrating inshore. In general, however, the majority of reef fishes appear to have a PLD of 20–30 days. Families that tend to have longer larval durations (wrasses and chaetodontids) appear to exhibit more variability between species than those families with shorter larval durations (damselfishes and angelfishes).

The relative scarcity of large data sets for single species limits the analysis of variability of PLD within species. Overall, species with longer PLDs tend to exhibit greater variance (Wellington and Victor, 1989; Fowler, 1989). The grunts and drums have very short PLDs with a very narrow range; they settle during a window of about three days from about 14–17 days old (Brothers and McFarland, 1981; B. C. Victor, unpublished observations). A large number of species have relatively short PLDs with low variance, including most of the damselfishes and angelfishes (Thresher and Brothers, 1985; Thresher *et al.*, 1989; Wellington and Victor, 1989). Other groups, such as the wrasses and chaetodontids, display a variety of patterns, including short, relatively invariant PLDs as well as longer, more variable ones (Victor, 1986a; Fowler, 1989; Leis, 1989).

Little is known about geographic variation in PLD both overall as well as within a species. One interesting trend is for Hawaiian and tropical eastern Pacific species of both damselfishes and wrasses to have distinctly longer larval lives than their western Pacific and Caribbean congeners (Victor, 1986a; Thresher and Brothers, 1989; Wellington and Victor, 1989). Geographic variation within a species has only rarely been examined. Thresher *et al.* (1989) reported only minor variation between populations of a damselfish in the western Pacific, although Victor (1986a) found that Hawaiian populations of six species of wrasses had significantly longer larval durations than their western Pacific conspecifics. Recent data indicate that Baja California populations of several eastern Pacific wrasses have extremely short PLDs, often half or even less that of more southerly populations (G. M. Wellington and B. C. Victor, unpublished observations). Clearly, the potential for extreme geographic variation exists, although what factors determine these differences remain totally unknown.

F. Relationship between Size and Age at Settlement

A good deal less information is available on the relationship between size and age at settlement, since there are few studies that document both size and age for the same species, especially with a sufficiently large sample size. The interaction of these variables between species has been examined only for a group of damselfish species (Wellington and Victor, 1989) and some wrasse species (Victor, 1991). Among the damselfishes there is only a weak correlation between the mean size and mean age at settlement ($r = 0.49$), driven mainly by the tendency for species with longer PLDs to settle larger than average. The wrasses show no obvious relationship; the blackear wrasse and the bluehead wrasse both settle at about 12 mm SL, but the former has a PLD of 21–28 days and the latter has one of 38–78 days. For reef species in general there is some broad relationship; the small-settling Caribbean species, the grunts and drums, have the shortest PLDs, while the queen triggerfish, a large-settling species, has a PLD of about 75 days (Robertson, 1988b).

These patterns are primarily determined by planktonic larval growth rates, since size at hatching is comparatively very small and therefore should contribute little to subsequent size at settlement. This is especially true for closely related species that are likely to hatch at very similar sizes. It is apparent that larval growth rates can vary greatly, even between closely related species. As an example, one can calculate a crude measure of the average daily larval growth rate by dividing the mean change in size between hatching (assume about 1 mm) and settlement by the mean larval duration for a selection of these species. Despite its short larval duration, the reef drum grows at only about 0.19 mm/day, while the french grunt grows at 0.37 mm/day. In contrast, the

queen triggerfish grows at 0.75 mm/day. The extremes for the damselfishes are 0.24 and 0.68 mm/day. The two wrasses that settle at the same size grow at very different rates: 0.24 mm/day for the bluehead wrasse and 0.42 mm/day for the blackear wrasse. Clearly, even closely related species must be doing very different things in the plankton before settlement.

Two studies have examined the relationship between size at settlement and age for individuals within a species (Victor, 1986c; Wellington and Victor, 1989). The latter study reported ambiguous results; only two of ten species showed a significant positive correlation, while two others had a significant negative correlation. Most exhibited no significant interaction. The lack of an interaction could be the result of small sample sizes (about ten per species), or could suggest that these larvae grew at different rates in the plankton and reached settlement size at a variety of ages unrelated to the ultimate size at settlement. More detailed studies are required to confirm these results.

When there is a positive relationship between size and age at settlement it could be caused by either (1) relatively constant growth rates and varying larval durations (the simple explanation) or (2) differing growth rates with slower-growing larvae settling larger (if faster-growing larvae settled larger it would tend to yield a negative correlation). Despite both Occam and his razor, there is no evidence for the simpler explanation. Victor (1986c) examined a large sample of bluehead wrasse recruits and found a very clear positive relationship between size and age at settlement ($r = 0.72$, $p < 0.0001$, $n = 47$) (Fig. 8). Daily growth rates back-calculated from the width of daily otolith increments indicated that the increase in settlement size with age was the result of some individuals delaying metamorphosis after reaching settlement size. Those individuals that delayed metamorphosis had reached the typical settlement size at the same age as average individuals, indicating that their growth rate until then was the same. During the period of delay, however, their growth rate was lowered by two-thirds. In this species, then, it appears that the correlation between size and age at settlement is a product of different larval growth rates (although only when averaged over the entire larval life).

Keeping things complicated, Chambers and Leggett (1987) performed a similar analysis on winter flounder in Newfoundland and also found a positive correlation between size at metamorphosis and age. However, their correlation was caused not by a delay period with a slower growth rate as in the bluehead wrasse, but, curiously, by an unexplained tendency for slower-growing larvae to metamorphose late (to be expected) but also larger than average (i.e., even later than necessary).

The critical data to resolve the questions outlined in the preceding sections have yet to be collected. What is needed is a comprehensive set of data for each species: the pattern of production of eggs, the behavior and distribution of

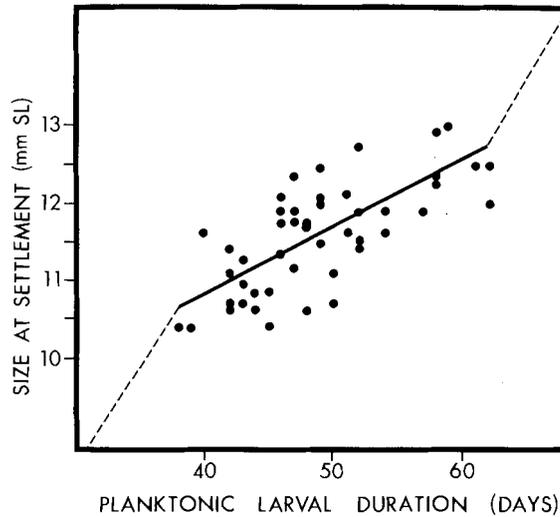


Figure 8 The effect on size at settlement of increasing larval duration for the bluehead wrasse, *Thalassoma bifasciatum*. The dashed lines before and after the solid line represent the estimated precompetent and postsettlement growth trajectories.

larvae in the plankton, the larval duration and the size at settlement for a large sample of recruits, the rate of settlement into various habitats, the mortality schedules of juveniles, and the population dynamics of adults. In addition, experimental studies inducing settlement into different habitats would also be needed to discern the adaptive significance of the existing patterns. It should then be possible to pinpoint which factors are molding the suite of adaptations that comprise the settlement strategy of reef fish species.

III. PLANKTONIC LARVAL DURATION AND BIOGEOGRAPHY

A. Dispersal and Its Role in Biogeography

It seems intuitively obvious that the degree of dispersal ability should play a role in determining the range of a species, especially for marine species with a planktonic larval stage. Nevertheless, it has proven difficult to detect any major effect of planktonic larval duration on the geographic range of coral reef fishes. Several studies have addressed this relationship (Brothers and Thresher, 1985; Thresher and Brothers, 1985; Victor, 1986a; Thresher *et al.*, 1989; Wellington and Victor, 1989), and all have agreed that little of the observed variation

in species range seems to be accounted for by the estimated PLD. Overall, there is some tendency for species with a very short PLD to be restricted in distribution and for species with a very long PLD to be widely distributed. For the vast majority of reef fishes in between, however, the PLD has no predictive value for inferring geographic range.

Three families of reef fishes have been intensively surveyed in an attempt to detect a pattern: the angelfishes (Thresher and Brothers, 1985), wrasses (Victor, 1986a), and damselfishes (Thresher *et al.*, 1989; Wellington and Victor, 1989). The angelfish and damselfish species have relatively short PLDs, all less than 40 days, which are not very variable within species. In contrast, wrasse species display a wide variety of PLDs, from 17 to 103 days, which can vary greatly within species. When the PLDs for angelfishes and damselfishes were plotted against the number of "biogeographic areas" occupied, no significant correlation, or even apparent trend, was evident (Thresher and Brothers, 1985; Thresher *et al.*, 1989). Wellington and Victor (1989) eschewed the problematic method of adding up biogeographic areas and simply grouped species into a widespread group and a restricted group. The mean PLD for the two groups came out virtually identical.

Upon closer examination, however, both studies detected some small effect. Thresher *et al.* (1989) found a significant difference between the PLDs of species found "wholly off the Pacific plate" and those "broadly distributed on both sides of its western margin." The difference between the means was, however, less than five days and the range of PLDs for the two sets of species overlaps almost completely. Whether small differences such as these reflect any real ecological significance is arguable. The only significant relationship found by Wellington and Victor (1989) was that genera with shorter PLDs tend to be restricted to the western Pacific and genera with longer PLDs tend to have species in other regions as well.

The results for wrasses were more clear. All the species caught in Palau (in the western Pacific) with a PLD of less than 20 days have very restricted ranges, and most of the species with PLDs over 35 days have wide ranges that sometimes extend to Hawaii. Furthermore, the three Indo-Pacific wrasses whose ranges extend as far as the Central American coast have particularly long PLDs, about two months or more. There was, however, no clear relationship for the many species with PLDs between 20 and 35 days; some have very restricted ranges and others have extensive ranges within the western Pacific region (although none reaches Hawaii or the eastern Pacific coast).

As soon as the study was broadened to include other regions of the Pacific, the pattern became less clear. Wrasses caught in Hawaii and in Central America were all found to have relatively long PLDs, but some endemics appeared to have longer PLDs than their more cosmopolitan congeners. In fact, the individual with the longest PLD recorded in the study, 121 days, was

a Hawaiian endemic. Furthermore, it has recently been found that the wrasse with the smallest range of all, *Halichoeres discolor*, endemic to the tiny island of Cocos near Costa Rica, has a PLD no different to its congeners that range widely up and down the coast of Central America (G. M. Wellington and B. C. Victor, unpublished observations).

B. Methodological Problems and a Protocol for Biogeographic Studies

Given these discordant results, it is imperative that we find some way to sharpen our focus on this question. Several shortcomings in the approach need to be corrected. First, since most of the reef areas of the tropical Indo-Pacific are not very isolated and there are geographic stepping stones connecting them, the absolute area or the number of biogeographic areas occupied is not a real measure of dispersal ability. The degree of isolation needs to be taken into account.

Second, the estimate of the PLD, typically derived from otolith analyses, may not be a good estimate of the true planktonic duration, especially with the small sample sizes characteristic of most of these studies. The maximum PLD of a relatively large sample should better reflect the real dispersal ability of propagules, since it is the occasional long-distance traveler that colonizes new areas, not the typical recruit. This is especially true given the recent discovery of reef fish larvae hundreds of miles from the nearest reefs (Leis, 1983; Victor, 1987a); after all, it is these larvae that are dispersing and not the vast majority that may simply remain near inshore waters.

In addition, some species are able to extend their planktonic existence as juveniles or even become pelagic as adults. This fact could account for some unexpected findings. For example, the only exception in the pattern of damselfish genera with mean PLDs less than about 25 days being restricted to the western Pacific was the genus *Abudefduf*, which is known to metamorphose and drift indefinitely in association with floating algae (Wellington and Victor, 1989). Robertson and Foster (1982) observed that some adults of the wrasse *Epibulus insidiator* in Palau swam up and off the reef with floating debris that passed by. This could explain why, despite a relatively short 30-day PLD, this species ranges all the way from East Africa to Hawaii.

A third problem is geographic variation in larval duration. Thresher *et al.* (1989) reported only slight geographic variation for a damselfish within the western Pacific. Victor (1986a), however, found that Hawaiian populations of wrasses had significantly longer PLDs than their western Pacific conspecifics. The problem with geographic variation is that it can easily lead to mistaken conclusions. For example, if one were to compare the larval durations of a wide-ranging species collected in Hawaii with a species endemic to

the western Pacific, one would find that the wide-ranging species had a longer larval period (e.g., Brothers and Thresher, 1985). However, it may well be that the western Pacific populations of the wide-ranging species have a larval duration no different to the endemic, since individuals captured in Hawaii consistently have longer PLDs.

These results dictate a more refined protocol for studies evaluating the role of larval dispersal ability in determining the biogeography of reef fishes. One should first identify the likely source populations for an area and taxon under study and then collect large samples of specimens at that site. Source areas could be determined from prevailing current flows or inferred from diversity clines. Ideally one should also collect planktonic larvae in the oceanic waters between the putative source area and the study area and document PLD and growth rates for comparison. In this manner, without the scattershot approach that compounds all sources of variation into one analysis, we should be able to discern to what degree reef fish distributions are a product of dispersal abilities.

C. What Is Determining the Biogeography of Reef Fishes?

Dispersal abilities do not seem to account for most of the observed patterns of distribution and there does not appear to be a plausible candidate among the variety of alternate explanations so far put forward. The usual fallback position is that ecological requirements of reef species are controlling their range. Unfortunately, there is little information on this subject. Few reef ecologists have much enthusiasm for promoting ecological requirements as the elusive answer, since reef fishes in general appear to be highly catholic in their tastes. Leis (1986b) rejuvenated this explanation by extending it to planktonic larval ecological requirements. He found that taxa with larvae that are primarily distributed onshelf in the Great Barrier Reef (versus offshelf and oceanic) tended not to be found on islands on the Pacific Plate. He suggested that oceanic islands would not be able to provide the planktonic "habitat" required by these larvae. While this may yet prove to be valid, the analysis is compromised by the interaction of larval distribution with larval duration. It appears that most of the taxa with offshelf larval distributions have large and presumably long-lived larvae, while many onshelf taxa have small and probably short-lived larvae (see Leis and Rennis, 1983). At this point, these factors cannot be separated.

What is needed to evaluate these hypotheses are carefully controlled experiments. A set of useful experiments has been performed unwittingly by fisheries biologists over the years as they attempted to introduce valuable food fishes to

islands where they did not previously exist. Many species, including the Marquesan sardine and some snappers and groupers, introduced to Hawaii over the years have established successfully (Randall and Kanayama, 1972; Williams and Clarke, 1983), proving that ecological requirements were not the reason for their absence. Even a mullet, an incidental species released with the sardine, has become a problem for Hawaiian fisherman. A few other species did not get established, although the fact that these were one-time releases mitigates the failure.

Historical factors may also play a role but are notoriously difficult to demonstrate. Springer (1982) has reviewed a number of dispersal hypotheses but personally favors a vicariance hypothesis to explain reef fish distributions. His final conclusion, which seems a little forced, was that some great barrier to dispersal existed along the entire western margin of the Pacific Plate. Unfortunately, that margin passes between island groups that are relatively close to each other and it is difficult to envision what the barrier really was. In any event, he suggests that the barrier may no longer exist. The hypothesis is driven by the presence of a relatively small number of widespread Pacific Plate endemics: species that occur widely over the scattered islands of the Pacific Plate and its margins. Whether this small set of species (about 1% of Indo-Pacific species) is truly that significant in explaining the complex distributions of Indo-Pacific reef fishes is certainly arguable.

There are other possible explanations for certain facets of the observed distributional patterns. There could be a "hybridization barrier" to dispersal of species with close relatives in adjoining yet distant areas. If an occasional recruit of a distant species arrived and mated with its resident relative, there would be a very effective barrier to colonization. As long as colonizers were few and residents were many there would be little chance of spread by the colonizing species as well as minuscule gene flow between species. This explanation may account for curious patterns of presence and absence of some species with very long-lived larvae, such as the wrasses of the genus *Thalassoma* in the Indo-Pacific. Indeed, hybrids of a Hawaiian endemic and a widespread species not from Hawaii (*T. duperrey* × *T. lutescens*) are sufficiently common in the southernmost island of Hawaii to show up in routine collections (J. E. Randall, personal communication; Victor, 1986a).

It is likely that all of these factors, and perhaps a number of others yet to be discovered, play some role in determining the geographic range of species. At present, most of these ideas remain speculative, for the detailed studies to evaluate the relative importance of potential determinants of biogeography have yet even to be proposed. Rather than some unifying theory, it is likely that a complex weave of explanations will come together to explain the intricate tapestry of species boundaries that trace the map of the Indo-Pacific.

IV. DAILY OTOLITH INCREMENTS AND EARLY LIFE HISTORY STUDIES

A. Introduction

The discovery of daily increments on the otoliths of fishes by Panella (1971) introduced a powerful new method for studying the age and growth of fishes. This technique, which requires little more than a good microscope, can provide exceptionally precise and detailed age and growth estimates—a serendipitous finding that, no doubt, must excite the envy of ecologists working on other less cooperative organisms. Daily increments are often particularly clear on the otoliths of larval and juvenile fishes (Fig. 9), making this technique a valuable tool for early life history studies. While the general methodology has become well established (Campana and Neilson, 1985; G. P. Jones, 1986), many promising applications have yet to be explored. This is especially true for tropical fishes, where the potential applications of this technique have only begun to be appreciated.

B. Review of the Methodology

Otoliths are accretions of calcium carbonate within the semicircular canals of the bony fishes. They function in both balance and hearing (Popper and Coombs, 1980). The production of visible daily increments depends on a daily cycle of differing rates of accretion of a protein matrix (darker under transmitted light microscopy) and crystalline inclusions (lighter) (Watabe *et al.*, 1982). There are three pairs of otoliths, each with different characteristics influencing their usefulness for aging and varying in quality between species. The largest pair, the sagittae, and the next largest, the lapilli, are used for aging. The sagitta usually has wider increments, making it more useful in slower-growing fishes, where increments on the lapillus rapidly become too narrow to resolve accurately. In faster-growing fishes, the sagitta often has prominent subdaily arrays of increments, making interpretation more difficult. In addition, the sagittae more often require grinding and polishing, which makes the technique much more labor-intensive.

Otoliths are typically removed with fine forceps from the sides and base of the braincase. They can be extracted from all size classes of fish, including embryos within the egg. Finding the otoliths in tiny fishes can be facilitated by the use of two polarizing filters, since otoliths are strongly birefringent (rotate polarized light) and glow brightly against a dark background when the two filters are placed in opposition. After removal, the otoliths are cleaned, allowed to dry, and placed into a drop of immersion oil on a microscope slide. For many small fishes this is sufficient preparation and the otolith increments can

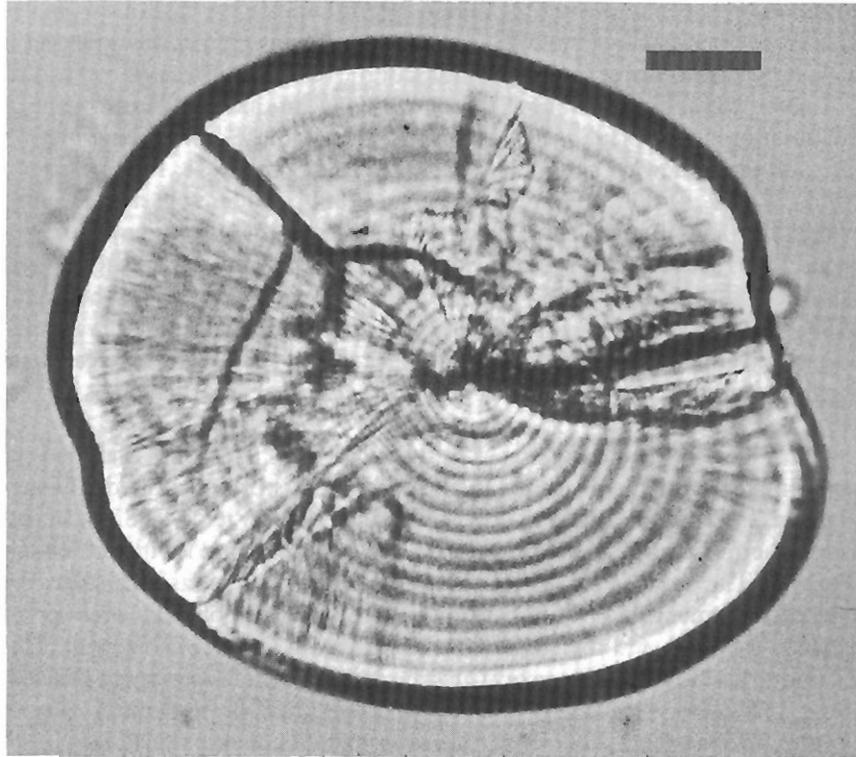


Figure 9 The sagitta from a larva of *Halieboeres bivittatus*. Note the subdaily array of increments to the left of the center. Scale bar, 40 microns.

be counted directly. Transmitted light that is polarized improves the contrast of the daily increments. If the otoliths are relatively large and opaque, some grinding is often necessary. Alternatively, for a more detailed view of the otolith increments, one can embed, grind, polish, and etch the surface with either acid or a calcium chelator (EDTA) and examine them under a scanning electron microscope.

C. Applications of the Daily Otolith Increment Technique

The most basic information available from the otolith is the total number of daily increments for a fish of a given size. With these data, one can derive an age-growth curve (Ralston, 1976a; Brothers and McFarland, 1981; Victor, 1983b, 1986c, 1987a, 1991; Fowler, 1989) or document a growth rate

difference between populations (Victor, 1986b). Given the ease of this analysis and the importance of basic growth data, it is surprising that this application has not become more common in studies of the early life history of reef fishes.

A more commonly used application of this technique is the estimation of the duration of the planktonic larval phase. This is done either by estimating the total age of new recruits (Brothers and McFarland, 1981; Brothers *et al.*, 1983; Victor, 1986c, 1991; Robertson, 1988b; Robertson *et al.*, 1988; Wellington and Victor, 1989) or by estimating the age up to a mark corresponding to settlement (Brothers *et al.*, 1983; Brothers and Thresher, 1985; Thresher and Brothers, 1985; Victor, 1986a,c; Fowler, 1989; Thresher *et al.*, 1989; Wellington and Victor, 1989). Estimates of the planktonic larval duration are invaluable for biogeographic studies and can also be used to calculate rough estimates of growth rates for larvae when the size at settlement is known (Victor, 1986c, 1987a, 1991).

Transitions in the characteristics of the increments on otoliths can be very useful markers for major events in the early life history of individual fishes (Panella, 1971, 1980; Brothers and McFarland, 1981; Victor, 1982). On reef fishes, the most prominent transition is typically associated with settlement (Fig. 10). The presence of a settlement mark permits one to easily calculate the date of settlement, simply by subtracting the number of daily increments between the mark and the edge of the otolith from the date of capture. This technique has been used to reconstruct the daily pattern of settlement (Victor, 1982, 1984; Pitcher, 1988a), even up to a year after the settlement episode (Victor, 1983a). Comparisons of reconstructed settlement patterns have been used to measure the persistence of age cohorts over a season (Pitcher, 1988a), to assess the effect of recruitment on subsequent population sizes (Victor, 1983a), and to infer the patch size of planktonic fish larvae by estimating the spatial scale of settlement events (Victor, 1984).

Similarly, the daily pattern of spawning can be back-calculated by subtracting total age from the collection date (McFarland *et al.*, 1985; Robertson *et al.*, 1988). Of course, this would only reflect the ultimately successful spawning patterns, which may well have little correspondence to true spawning patterns. Robertson *et al.* (1988), however, found a close match between such back-calculated spawning patterns and the actual spawning patterns observed on the reef.

There have been few attempts to extend the analysis of daily otolith increments beyond the simple procedures described here. Perhaps the most promising application is deriving a detailed history of daily growth rates from the pattern of widths of the increments. Since there is some predictable relationship between some function of otolith size and fish size (e.g., Brothers and McFarland, 1981; Victor, 1987a; Fowler, 1989), the width of each increment should reflect the daily growth of the individual. Although this relationship has yet to be validated for reef fishes, there is some evidence for a direct

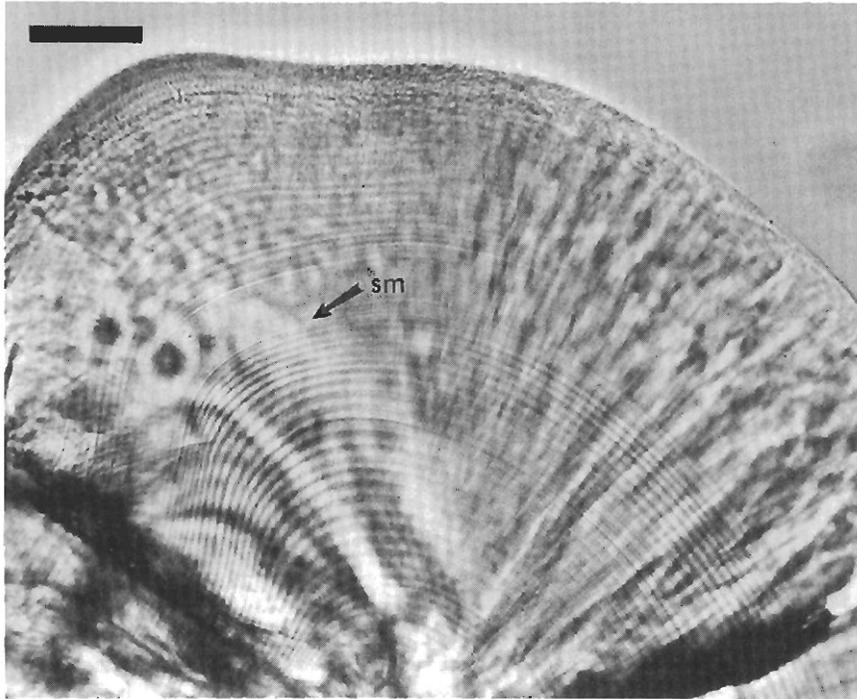


Figure 10 The settlement mark (sm) on the sagitta of a juvenile bluehead wrasse, *Thalassoma bifasciatum*. Scale bar, 40 microns.

relationship. Daily otolith increments on wrasse larvae dropped to half their former width during a period when the growth rate fell by about two-thirds (Victor, 1986c). Whether there is a one-to-one correspondence between daily increment width and growth rate or a running average is not known (Campana and Neilson, 1985), although five days of extra feeding of a wild population of reef wrasses resulted in five wide otolith increments, with an indication of wider increments on days with more feeding time (Victor, 1982).

Changes in the width and appearance of daily increments are common on the otoliths of young fishes. These transitions are sometimes correlated with settlement, as in the damselfishes (Pitcher, 1988a), although they often occur earlier in the life of the larva for other reasons (Brothers and McFarland, 1981; Victor, 1986c). In the bluehead wrasse, and probably other wrasse species, a transition to narrow increments near the end of the larval period signals the onset of a period of delayed metamorphosis that can last up to several weeks, during which growth is reduced (Victor, 1986c). Many other changes in increment width occur on the otoliths of young fishes of a variety of taxa, presumably reflecting changes in behavior, habitat, or life-style (Fig. 11).

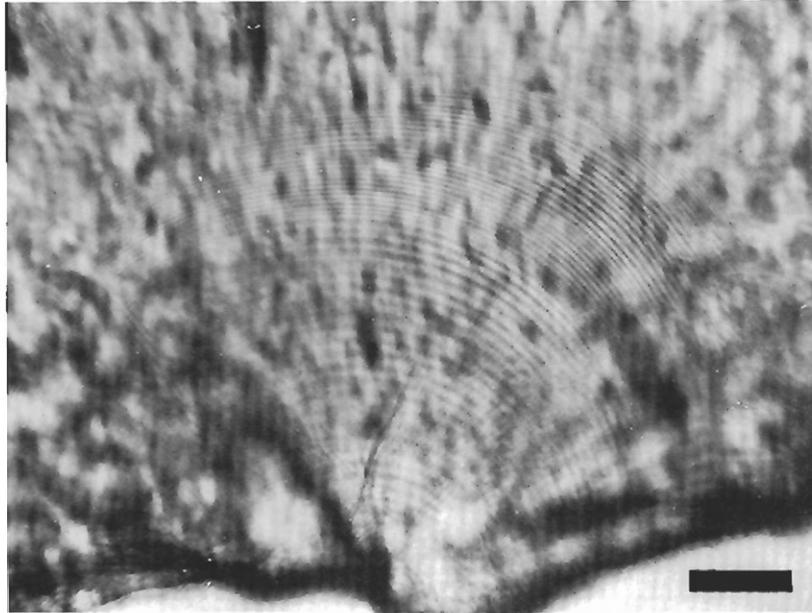


Figure 11 Unexplained transitions in the width of otolith increments during the larval period on the otolith of a juvenile Indo-Pacific wrasse, *Pseudocheilinus evanidus*. Scale bar, 40 microns.

Since daily increments are preserved unmodified within the otolith, it is possible to derive information about past environmental conditions. To date, it has proven feasible to infer past temperature exposures by analyses of stable isotope compositions, such as oxygen and carbon isotopes (Radtke *et al.*, 1987), or by strontium-to-calcium ratios (Radtke *et al.*, 1988). These techniques may make it possible to discover where in the plankton the larvae are traveling at various ages or from what oceanic region larvae are derived. Analyses of the elemental composition of sections of the otolith may be particularly valuable if they could localize the source population of larval recruits (Campana and Neilson, 1985).

Otolith increments vary in shape and pattern from species to species and perhaps even between populations of the same species (Brothers, 1984; Victor, 1986a, 1987a). Since each incremental ring preserves the outline of the otolith at the time the ring was formed, the morphology of otoliths of larvae can be easily derived from settled fishes of all ages. This method has been used to identify unknown wrasse larvae by comparing their otolith shape to that back-calculated from known juveniles (Victor, 1987a). Clearly identifiable characteristics of increments in the core region of the otolith should permit the

identification of very early stage larvae of different families and even some genera (Brothers, 1984; Victor, 1986a).

Without a doubt, many other applications of the otolith increment technique await development in the future. The array of increments on the otolith preserves the record of the past in such unparalleled detail that the potential of the technique may be limited only by the imagination of reef fish ecologists.

D. Potential Pitfalls and the Need for Validation

Our knowledge of the basic science behind the otolith increment technique is still in its infancy and there is no question that exceptions do occur and assumptions are often unwarranted. Anyone who has examined a variety of otoliths knows that bizarre and unexpected increment patterns are commonplace and that few rules are transferrable from one taxon to another. It is therefore imperative that researchers not yield to temptation and run amok in their interpretations of otolith increments and transitions without thorough validation. At present, unfortunately, the only reef fish taxa for which clear experimental validation in the field is available are wrasses (Victor, 1982, 1983b), damselfishes (Robertson *et al.*, 1988; Pitcher, 1988a; Wellington and Victor, 1989), and chaetodontids (Fowler, 1989).

The first and most basic problem is whether the observed increments on the otolith are really formed daily. Despite widespread use of the adjective "daily," there are painfully few direct demonstrations of daily production of increments for reef fishes. Since Geffen (1982) first documented that increments were not always produced daily on the otoliths of larval herring, there have been a number of reports of increments being formed at a rate less than one per day (Campana *et al.*, 1987). This phenomenon has so far been reported only for temperate fish larvae, probably because of their slow growth rate. Campana *et al.* (1987) point out that the resolution limits of light microscopy are about one micron, and that this could account for the apparent nondaily formation of increments in slow-growing larvae. Clearly, the presence of narrow increments requires a thorough validation of their periodicity.

The otoliths of many reef fish larvae present a very different problem. There often appears to be additional arrays of "subdaily" increments within the larval period (see center left of Fig. 9). The presence of different superimposed sequences of increments on the same otolith is not new or unusual: both Panella (1980) and Brothers and McFarland (1981) mention extra "subdaily" increments. Despite this appellation, there is no experimental evidence demonstrating that these arrays are subdaily. I have proposed two criteria for the subjective presumption of subdaily increments (Victor, 1986a). The first criterion was based on the assumption that the largest repeating cycle of increment formation was daily. If, as the focus under the microscope is

changed, each increment splits into two or three, resulting in an array of exactly twice or three times as many increments, one should presume that the fewer and larger increments are daily. The basis for this presumption rests on the fact that otolith increments are thought to be the product of physiological cycles entrained by light, temperature, feeding, or activity cycles influencing the metabolism of the fish (Panella, 1980). While there is some evidence for subdaily cycles of feeding and activity in fishes (e.g., vertical migrations of planktonic larvae), there are no reports of supradaily cycles occurring on two- or three-day cycles.

The higher-order array (with fewer increments) is usually visible over more quadrants of the otolith and at wider planes of focus than the finer array of "subincrements," which tend to occur over a smaller section of the otolith and at a single plane of focus (Victor, 1986a). These "subincrements" are typically found on otoliths with relatively wide higher-order increments. The relative prominence of the wider array of increments supports the assumption that they are formed on a daily cycle. It may be, as Panella (1980) suggested, that the arrays of "subincrements" occur on all fish otoliths, but are only detectable on those that are fast-growing. Despite their prevalence on larval otoliths, it remains an open question whether these arrays are truly structural or some form of optical artifact.

All of these complications only serve to emphasize the importance of verifying the assumption that increments are daily. The classic method for validating daily increments in the field is to mark the otolith either by isolation of the fish in the dark or by immersion in tetracycline, both of which interfere with the normal process of otolith accretion. The subject is later recaptured and the number of increments between the mark and the edge is compared to the number of days since release (Victor, 1982; Pitcher, 1988a; Robertson *et al.*, 1988; Fowler, 1989; Wellington and Victor, 1989). These methods have consistently affirmed the daily periodicity of increments on the otoliths of juvenile wrasses and damselfishes. Several studies have verified the formation of daily increments on reef fish larvae and juveniles under laboratory conditions (Schmitt, 1984b; Radtke *et al.*, 1988; Thresher *et al.*, 1989). A major drawback of validations in the laboratory is that artificial daily cycles imposed by captivity could induce daily increments that may not be analogous to those found on wild fishes.

A more pressing question is whether the increments observed on otoliths of planktonic larvae are also daily. In many ways, these increments closely resemble those on the otoliths of juvenile fishes, and therefore it is reasonable to assume that they are also daily. Nevertheless, it is imperative that this assumption be verified, especially considering the widespread occurrence of presumed subdaily increment arrays on larval otoliths. This is a difficult experiment to perform in the field, especially given the necessity that the larvae be permitted

normal planktonic behaviors and migrations. Marking individuals within a very large enclosure drifting with the plankton is probably the only practical method possible at present.

Many studies that employ otolith increment counts have resorted to assumptions about when the first increment is formed. While it is probably true that errors in these assumptions would only change estimates by a few days, it is important for accuracy that the age and size at which the first increment is formed be documented so that the correct adjustment can be made to estimates of planktonic age and growth. Otoliths first appear at hatching in bluehead wrasse larvae, about one day after fertilization and release into the plankton (Victor, 1986c). Wellington and Victor (1989) found that larvae of a Caribbean damselfish have developed the primordium of the otolith by hatching (the start of their planktonic phase and five days after fertilization). In damselfishes, therefore, it seems that the count of increments on the otolith is a direct estimate of the planktonic duration, while for wrasses it is necessary to add about two days to the count. Whether these findings hold true for other families of reef fishes is unknown and needs to be examined.

When estimating planktonic larval duration from new recruits it is important to be sure that the recruits are recent arrivals from the plankton and not newly appeared from hiding places in the reef. The best way to avoid that complication is to capture new recruits from artificial reef substrates that are suitably isolated from nearby reefs and surveyed daily. To further verify that recruits are indeed new, it helps to compare competent larvae of the same species collected just prior to settlement (Fig. 4).

The presence of a variety of transitions on the otoliths of many species can easily lead to mistaken estimates of the duration of the planktonic phase or other stages. Settlement marks therefore should be verified in the field, a process that is, fortunately, relatively simple. The clearest demonstration of a settlement mark is to document the absence of the mark on planktonic larvae just before settlement and the presence of the mark on newly settled recruits (Victor, 1982; Pitcher, 1988a). Less rigorous methods include relying on the presence of the mark at the edge of the otolith of newly recruited fish (Wellington and Victor, 1989) or the smallest individuals captured (Radtke *et al.*, 1988; B. C. Victor, unpublished observations on the reef drum), back-calculating the transition from the otoliths of fishes of a known age since settlement (Fowler, 1989), or by statistical comparison of increment counts of newly recruited fishes with pretransition counts from older fishes (Thresher and Brothers, 1985; Thresher *et al.*, 1989).

Estimates of larval durations (and other otolith counts) may become biased by the exclusion of data. Fowler (1989) found that a fraction of the fish he studied did not have a clear transition at settlement and could not be included in his estimates of larval duration. Furthermore, some studies are forced to

exclude otoliths from which accurate replicate counts could not be obtained. Since there can be some correlation between otolith quality and growth rate (in my experience, slow-growing individuals tend to have otoliths that are harder to read), it is imperative to use another method to corroborate one's findings.

The validity of the otolith increment technique is still untried for most species of reef fishes. It would not be surprising if some of our assumptions proved to be incorrect in some fishes that have yet to be examined. Clearly, a vigilant and skeptical attitude needs to be cultivated among workers in the field, and thorough verification of techniques (e.g., Pitcher, 1988a) should be strongly encouraged.