

The Use of Scuba to Study Early Life Histories of Marine Invertebrates

Harilaos A. Lessios, Howard R. Lasker, and Don R. Levitan

ABSTRACT. Scuba has permitted in situ research of the early life histories of subtidal marine invertebrates, limited only by the small size of early stages. Investigators have used scuba to observe, among other things, coral spawning, including the spectacular mass spawns on the Great Barrier Reef, which established that most corals are hermaphroditic broadcast spawners. Observations and experiments have been conducted to measure fertilization rates of free-spawning species, such as octocorals, sea cucumbers, sea urchins, and sea stars. Experiments have measured relative male reproductive success and the gamete traits that influence it in sea urchins. Finally, in tunicates, which produce larvae large enough to be seen by the naked eye and short-lived enough to be followed on a single dive, scuba has allowed direct observation from release to settlement, thus helping to shed some light on this process, which otherwise can only be studied indirectly.

INTRODUCTION

The reproductive biology of marine organisms was, during the nineteenth century, the realm of zoologists and embryologists. Dissection and histological analysis of adults identified reproductive cycles, and careful study of the morphology of embryos and their metamorphoses into adults was a hallmark of “nineteenth century biology.” The products of those efforts—expansive, often beautifully illustrated monographs—form the basis of our understanding of the reproductive biology of many marine invertebrates. This foundation was skewed toward taxa that could be studied in the laboratory or could be readily collected and observed. Entering the water and finding out what these organisms do in nature is an essential additional component to this foundation. If the American baseball player Yogi Berra’s dictum, “You can observe a lot by watching,” is correct, then it is scuba that has allowed researchers to “watch.”

For over a century it has been recognized that understanding the early life history stages of marine organisms is critical to understanding their population dynamics. Yet it was only in the 1980s that researchers took to the field to observe these stages. With the advent of scuba, investigators were able to conduct careful observations on the relative importance of different life stages and determine what traits made individuals more or less likely to survive through these transitions. Observations and experiments took time, patience, and a careful hand, requirements that provided an excellent match between the research question and the use of scuba.

In this chapter we consider how in situ observations and experiments have provided information on spawning behavior, fertilization, larval survivorship, and settlement of several marine invertebrates. Our aim is to illustrate how the use of scuba has permitted the acquisition of critical knowledge connected to early life histories in the natural environment.

Harilaos A. Lessios, Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Panama. Howard R. Lasker, Graduate Program in Evolution, Ecology and Behavior, Department of Geology, University at Buffalo, Buffalo, New York 14260, USA. Don R. Levitan, Department of Biological Science, Florida State University, Tallahassee, Florida 32306, USA. Correspondence: H. Lessios, Lessiosh@si.edu.

Manuscript received 25 January 2012; accepted 5 March 2013.

THE USE OF SCUBA TO STUDY THE REPRODUCTIVE BIOLOGY OF MARINE INVERTEBRATES

SPAWNING OBSERVATIONS

A striking example of the role of scuba in facilitating new knowledge is the reproductive biology of corals. At the start of the twentieth century, few data were available about coral reproductive biology. Reports characterized scleractinians as brooders that released fully developed planula larvae. Although there were data indicating that some species broadcast gametes (see references in Fadlallah and Pearse, 1982), the suggestion that scleractinians were primarily brooders (Marshall and Stephenson, 1933; Yonge, 1940) persisted until the 1980s.

This perception was corrected when a group of researchers from James Cook University discovered a mass spawning event on the Great Barrier Reef (Harrison et al., 1984). In that first publication on the mass spawning, the number of scleractinians known to broadcast spawn grew from eight to over sixty. The in situ observations of spawning behavior and characterizations of the extent and timing of the event were the product of scuba use. Similarly, the microscopic examination of tissue and the laboratory observations, which were also an important component of the research, were dependent on scuba for the collection of specimens. Scuba allowed the study of reproductive biology to expand from those species that could be readily collected in shallow pools and exposed tidal flats to species in the full extent of the reef. The images of the spawning event electrified a generation of researchers who proceeded to search for similar events at other locations. The data from the Great Barrier Reef mass spawning continued to grow (Babcock et al., 1986), and additional reports from the Pacific and the Caribbean (Szmant, 1986; Van Veghel, 1993) led to the conclusion that the majority of scleractinian taxa are hermaphroditic and broadcast spawners (Richmond and Hunter, 1990; Carlon, 1999). The most recent review of the literature (Harrison, 2011) identifies 367 broadcast-spawning species compared to 75 that brood planulae. It is difficult to imagine that our understanding would be as complete without the use of scuba.

Although many species exhibit some degree of synchrony among individuals, observations of spawning are rare. An event that lasts minutes to hours and occurs on only a few days each year is not readily observed. Perhaps the greatest contribution of scuba to increasing our knowledge of spawning is that it enables researchers to spend large amounts of time in the water and serendipitously observe spawning events. For instance, Hendler and Meyer (1982), Lessios (1984), Pearse et al. (1988), McEuen (1988), Lamare and Stewart (1998), and Himmelmann et al. (2008) all report observations of spawning events in a variety of echinoderms. In a study of reproduction of *Strongylocentrotus* spp., Levitan (2002a) twice observed natural spawning by *S. franciscanus*, and was able to incorporate data from these events into a study that otherwise relied on induced spawning.

Serendipity is a key factor in discovering spawning events, but the good fortune in all of these studies was facilitated by many hours of diving.

The discovery of the mass spawning event on the Great Barrier Reef led to observations of other taxa (Babcock et al., 1986; Alino and Coll, 1989; Babcock et al., 1992) and inspired efforts to discover similar events in the Caribbean (Gittings et al., 1992; Van Veghel, 1993; de Graaf et al., 1999). Other studies in which divers have identified spawning times and patterns have their origins in observations of tissue samples that indicate the general timing of spawning. For instance, observations of tissue samples led Lindquist et al. (1997) to place covers over colonies of two species of Caribbean sponges to collect their gametes and characterize the timing of their spawning. While scuba provides a window on spawning events, it does not always produce dramatic results. For instance, Beiring and Lasker (2000) used the presence of gonad in collected specimens to predict spawning of *Eunicea (Plexaura) flexuosa* in Panama, but were able to observe spawning in only a small number of colonies relative to the number of colonies that were believed to spawn over the weeks of their observations. Similarly, many of the spawning events reported by Babcock et al. (1992) involved only small numbers of individuals.

Species with well-synchronized and predictable spawning events have been particularly amenable to study using scuba. Once the basic information on spawning has been elucidated, scuba has provided researchers with the ability to make detailed observations and conduct elaborate experiments to study various aspects of reproductive biology. For instance, a variety of studies have investigated the role of the timing of spawning in reproductive isolation of closely related species with both intra-specific and interspecific differences being observed (Van Veghel, 1993; Sanchez et al., 1999; Van Oppen et al., 2002; Levitan et al., 2004).

FERTILIZATION SUCCESS

Mortensen (1938) considered fertilization to be a significant obstacle in the life history of broadcast spawners. In contrast, in a paper that for decades was one of the guiding forces of benthic ecology, Thorson (1946) considered and rejected the likelihood of variation in successful production of embryos. These hypotheses were examined in a series of experiments by Pennington (1985). Working with the sea urchin *Strongylocentrotus droebachiensis*, Pennington was able to show that in natural settings sperm released by males in induced spawning events were rapidly diluted. As sperm were advected, the probability of fertilization decreased dramatically within meters of the spawning male. These experiments, which were conducted by scuba divers in 10 m depths, were followed by theoretical papers (Denny, 1988; Denny and Shibata, 1989) that underscored the difficulty of achieving high fertilization rates in some environments. Subsequent work with sea urchins has raised a number of methodological questions about the appropriate manner to assess fertilization success (Thomas, 1994; Yund and Meidel, 2003;

Yund et al., 2005). However, regardless of the precise values of fertilization rates, Pennington's study changed the paradigm to one in which fertilization success was not necessarily high. Where, when, and how marine species achieve high fertilization success is a fundamental question in their reproductive biology that relates to the fitness of individuals, the sustenance of populations, and even the efficacy of such conservation approaches as marine protected areas.

Assessing fertilization rates in the field requires access to benthic organisms as they spawn, which (with the exception of intertidal or very shallow habitats) requires the use of scuba. Fertilization probabilities measured in the field vary widely among studies. While cases of sperm limitation have been reported, most studies include at least some examples of high probabilities of fertilization. This may reflect the importance of fertilization rates and thus of strong selection for mechanisms that enhance fertilization, or alternately it may be a reflection of the life history of organisms that have been studied.

The first series of reports of in situ measurements of fertilization rates appeared in 1992 (Babcock and Mundy, 1992; Babcock et al., 1992; Brazeau and Lasker, 1992; Oliver and Babcock, 1992; Sewell and Levitan, 1992). Babcock et al. (1992) based their estimates of fertilization success on collections of eggs from the water column following their release from several Great Barrier Reef holothurians. They reported fertilization success ranging from 0% to 96%. The proportion of eggs fertilized in the spawning events varied among species, by day, time of day, and number and proximity of spawning individuals. Most of the values were well over 50%, with the sea cucumber *Bohadshia argus* having fertilization success ranging from 86% to 96% during the same night. Most studies suggest eggs are fertilized at the peak of spawning events: eggs spawned early in the event, late in the event, or by relatively isolated individuals can have low, sometimes near-zero fertilization rates. Sewell and Levitan (1992) found high fertilization success in a spawning sea cucumber. The octocoral *Plexaura kuna* exhibits fertilization success that varies with day of the spawning event and current speed (Lasker et al., 1996). Individual samples had fertilization success ranging from 0% to 100%, and the monthly means for the multi-day synchronous spawning events ranged from 0% to 60.4%. Similarly collected data for *Pseudoplexaura porosa* found overall fertilization success to be 67%, but the rates varied with day and position on the reef (Coma and Lasker, 1997). Up to 83% of *Acanthaster planci* eggs were fertilized (Babcock and Mundy, 1992). Eggs released by individuals that were over 60 m from the nearest male still had fertilization success rates of 23%. All of the eggs collected in these studies were obtained using scuba. Eggs of octocorals (which are large and positively buoyant) could possibly have been collected on the surface downstream of the spawning colonies, but counting the numbers of colonies spawning and characterizing the fine-scale variation that was observed would have been impossible without scuba.

Fertilization success has also been measured in several species that brood. Brazeau and Lasker (1992) estimated

fertilization rates of the octocoral *Briareum asbestinum* on two reefs in the San Blas Islands, Panama. The estimates of Brazeau and Lasker (1990) were based on comparisons between the number of mature eggs present in the polyps and the numbers of embryos produced by the colonies. Values of fertilization success on reefs that had relatively low abundances of *B. asbestinum* are among the lowest reported in natural spawning events. In many instances none of the eggs were fertilized, and in the best of three years the average success was only 20%. As might be expected in a system with low fertilization success, colonies with nearby males did better. In contrast, estimates of fertilization success are markedly higher for *Pseudopterogorgia elisabethae*, a Caribbean octocoral that also surface broods (Lasker, 2006), and for the scleractinian *Goniastrea favulus* (Miller and Mundy, 2005). Fertilization occurs on the colony surface in both of these species and in both, the fertilization of released eggs approached 100% in some samples.

ESTIMATING MALE REPRODUCTIVE SUCCESS

Most work on fertilization ecology has focused on the percentage of eggs that are fertilized. Female success provides key information on the demographic effects of sperm availability and how females with different traits might be under differential selection. Estimates of male fertilization success are also critical for understanding selection on male traits and for determining the relative intensity of sexual selection between males and females. Measures of male reproductive success have lagged behind female estimates because they are more difficult to obtain. As the use of genetic markers has become more efficient, more affordable, and possible with tiny embryos, estimates of male reproductive success have started to emerge.

The first estimates of male reproductive success focused on species that brood offspring. In these species, females retain eggs and collect sperm for fertilization from the water column. Estimating parentage from brooders is somewhat simplified because maternity is known and only paternity must be estimated. In addition, embryos can be collected more simply from the brood chamber. Divers do not need to be on hand during the moment of spawning to collect eggs and embryos in the sea during brief and often unpredictable spawning events. The first study of male success was conducted on the ascidian *Botryllus schlosseri* (Grosberg, 1991). Colonies bred to be homozygous for rare alleles were explanted, and their sperm were allowed to disperse. After two weeks, samples from adult colonies at marked distances from these source colonies were collected, and embryos were analyzed for these rare alleles. The results showed that paternity rapidly diminished over the first 50 cm from the focal colony, suggesting that effects of sperm on gene flow are highly localized. Further experimental work with brooding species of both *Botryllus* and the bryozoan *Celleporella hyalina* has investigated how the presence of nearby mates influences the likelihood of mating with more distant mates and also the likelihood of selfing versus outcrossing (Yund and McCartney, 1994).

THE USE OF SCUBA TO DETERMINE IF GAMETE TRAITS
INFLUENCE FERTILIZATION IN THE SEA

Work using scuba to place and retrieve experimental arrays of sessile brooding invertebrates examined the consequences of egg versus sperm allocation on reproductive success in hermaphroditic species. The hypothesis was that hermaphrodites should allocate much more energy toward eggs than sperm if fertilization requires a one to one interaction between sperm and egg, and if sperm are smaller than eggs. Empirical data, however, indicated that energy allocation was much more even between the male and female gametes than predicted. Subtidal experiments showed that under conditions of competition, individuals allocating more energy to sperm garnered higher success (McCartney, 1997; Yund, 1998). More recent work also demonstrated that even in the absence of competition, increased allocation to sperm allowed individuals to overcome sperm limitation when mates were distantly located (Johnson and Yund, 2009). Combined, these findings provide a potential explanation for similar levels of investment to male and female gametes in hermaphrodites.

Patterns of paternity, and multiple parentage in natural populations of brooding invertebrates, has also been explored using scuba. Studies on both ascidians (Johnson and Yund, 2007) and soft corals (Lasker et al., 2008) suggest that multiple paternity is common, and that patterns of paternity are not clearly related to mate distance, body size, or density. It may be that the timing of release of sperm may influence paternity more than differences in mate distance; nearby mates have no chance at fertilization if they release sperm when eggs are not available. Experiments conducted on broadcast-spawning individuals indicate that male timing is critical to fertilization and paternal success (Levitan, 2005). In addition to timing effects, there is some evidence indicating that increases in the number of males contributing sperm can influence the fraction of fertilized eggs (Purchase et al., 2007), presumably because eggs vary in their compatibility with different kinds of sperm. High multiple paternity across gradients of adult density (Johnson and Yund, 2007) or distance (Lasker et al., 2008) may reflect the importance of both timing and gametic compatibility that results in genetic variation within a brood across these demographic conditions.

Paternity from natural spawning has been measured in one system of a broadcasting marine invertebrate. Coffroth and Lasker (1998) established long-term studies of male and female reproductive success in the broadcast spawning soft coral *Plexaura kuna*. This species often fragments asexually. Male reproductive success was examined as a function of distance from a focal female to each male genotype and the number of colonies that belonged to each genotype (clone size). *Plexaura kuna* predictably broadcasts gametes 3 to 9 days following the full moon, releasing eggs large enough to be seen by divers. Egg samples were collected into syringes by divers located downstream of spawning female colonies. Embryos were reared for several days and then frozen for genetic analysis using RAPD markers. Most variation in male reproductive success could be attributed to their distance from the female. In addition, clone size might indirectly influence paternal success by spreading individuals across a larger area, thus decreasing the distance to potential mates.

Gamete interactions have been studied in the laboratory for the past hundred years (e.g., Lillie, 1915). Gamete traits such as egg size (Levitan, 1993; Marshall et al., 2002), egg accessory structures (Podolsky, 2002; Farley and Levitan, 2001), chemical attractants (Miller, 1966; Riffell et al., 2004), sperm velocity (Levitan, 2000a; Kupriyanova and Havenhand, 2002), and sperm-egg compatibility (Hagström and Lönning, 1967; Evans and Marshall, 2005) have been shown in the laboratory to influence the rate of fertilization within species and the likelihood of hybridization across species. However, to understand the fitness consequences of variation in sperm and egg traits it is necessary to study it in the sea. There is reason to doubt the importance of variation in these traits because the velocity of water flow (cm to m per second; Denny, 1988) can be orders of magnitude greater than sperm velocity (~200 micrometers per second; Levitan et al., 1991), and because mate density and distance exert large effects on the local concentration of sperm (Pennington, 1985; Levitan, 2002a). These variables could perhaps swamp the consequences of subtle variation in traits such as sperm velocity or egg receptivity. Determining how gamete traits influence reproductive success in the sea provides support for the numerous theories on how sperm availability influences gamete morphology (Levitan, 2000b; Podolsky, 2004; Luttkhuizen et al., 2004) and compatibility (Swanson and Vacquier, 2002; Zigler et al., 2005).

Field studies conducted along the shores of Vancouver Island, British Columbia have investigated the influence of sperm availability and gamete traits on fertilization in three congeneric subtidal sea urchins: *Strongylocentrotus droebachiensis*, *S. franciscanus*, and *S. purpuratus*. Gamete traits vary in these three species. *Strongylocentrotus purpuratus* has the smallest eggs and requires the highest sperm concentrations to achieve fertilization; it is also the species with eggs most resistant to polyspermy and has the fastest, but shortest-lived sperm. *Strongylocentrotus droebachiensis* has the opposite traits: large eggs that require the least amount of sperm to achieve fertilization but are the most susceptible to polyspermy, and the slowest, but longest-lived sperm. *Strongylocentrotus franciscanus* is intermediate in all these traits (Levitan, 1993; Levitan et al., 2007). This gradient in gamete traits is consistent with *S. purpuratus* having gamete traits best adapted to high levels of sperm availability and *S. droebachiensis* having traits best adapted to low levels of sperm availability. Field surveys using scuba documented that along the outer west coast of Vancouver Island these traits match the level of aggregation and population density seen among these three species; average nearest-neighbor distances (Levitan, 2002b) and local densities (Levitan, 2002a) indicate that *S. purpuratus* lives in the most crowded populations and *S. droebachiensis* lives in the most sparse ones.

Subtidal field experiments have examined how gametes from these three species perform under a range of conditions of sperm availability. The first set of experiments was intended to isolate

gametes from the adults to see how within- and among-species variation in gamete performance in the field could be predicted by how well they performed in the laboratory. The protocol was to induce sea urchins to spawn in the laboratory and measure gamete traits and gamete performance. Then the protocol was to bring these gametes into the field and release them, wait for fertilization to occur, and then collect eggs with a subtidal plankton pump. Collected eggs were then brought back to the laboratory to determine the fertilized fraction. The results of these studies documented that individuals that produced gametes that performed well in the laboratory also produced gametes that performed well in the ocean; the subtle differences in gamete traits noted in the laboratory translated into fitness differences in the ocean (Levitan, 1996). The results also documented that each species produced gametes that performed best under conditions of sperm availability that were typical for that species (Levitan, 1998).

A second set of field experiments attempted to reflect spawning in nature more accurately. Sea urchins were induced to spawn in situ via an injection of 0.55 mol/L KCl solution and then placed back into their natural locations across a range of population densities and flow conditions (Levitan, 2002a). In these experiments, a 5 × 5 meter grid was established on the bottom over a range of sea urchin abundances. All the sea urchins from one species within the grid were injected with KCl to induce spawning. After 30 minutes, the position of all individuals was mapped and a sample of eggs was collected above each female with a subtidal plankton pump. Patterns of water flow and depth were measured with a S4 current meter, which recorded flow and water depth every 0.5 s. The results indicated strong effects of population density and abundance, but also revealed species differences as suggested by the previous study, which had examined gamete traits independently of adult spawning behavior; *S. purpuratus* performed best at high population densities, but *S. droebachiensis* performed best at low densities (Levitan, 2002a). Manipulation of *S. purpuratus* to lower densities than typical resulted in that species doing poorly relative to the other two species (Levitan, 2002a). Further tests, examining how *S. franciscanus* performed at higher densities, indicated that this species was much more susceptible to embryo death caused by polyspermy compared to *S. purpuratus* (Levitan, 2004). In sum, the field studies suggest that these species have gamete traits that perform best under typical conditions and also suggest density-dependent selection of gamete traits based on levels of sperm availability.

Investigations of patterns of sexual selection and the influence of selection on gamete traits (specifically on gamete recognition proteins) used the same field protocols as above, with the addition that the developing embryos were cultured for three days, and then frozen for parentage analysis (Levitan, 2004; 2008). In addition, tube foot samples were collected from all adults in the experiment for genetic analysis to determine their reproductive success, but also to sequence sperm bindin, a protein on the head of the sperm that binds to a receptor on the surface of the egg (Levitan and Ferrell, 2006; Levitan and Stapper, 2010). These experiments were conducted with *S. franciscanus*

and *S. purpuratus*, species that differ in their susceptibility to polyspermy. The results of the parentage analysis indicated that in *S. franciscanus* the level of male competition for eggs was similar across male densities. Across densities ranging from sperm limitation to polyspermic conditions the variance in reproductive success was relatively high and constant; there were male winners and losers at all densities.

The pattern in females was more complex. At low sperm densities, there was high variance in female success caused by sperm limitation. As male density increased, female fertilization success increased to the point where all females were saturated with sperm and the variance among females was very low; all females were successful. However, as densities increased further, variance in female reproductive success increased again, driven by variation in polyspermy among eggs produced by different females. These results suggest very different selective pressures at high and low sperm densities. At low densities both males and females are selected to produce gametes with the capacity for a high rate of fertilization. However, at high densities, eggs suffer from polyspermy, so that females producing eggs less compatible with sperm avoid embryo death. Males, on the other hand, should compete for fertilization and be selected for higher fertilization rates. This produces sexual conflict over fertilization rate (and gametic compatibility) at high densities (Levitan, 2004).

Individuals from this same experiment were then sequenced to determine their sperm bindin genotype. The results indicated that males with a common form of the protein had a fertilization advantage over males with a rarer form of the protein, while females with the rarer genotypes were more successful. Further analysis indicated that males that matched females at the sperm bindin locus were most successful at low densities when sperm were limited, while mismatched individuals had higher success at high densities when polyspermy was common (Levitan and Ferrell, 2006). These findings and similar results noted in *S. purpuratus* (Levitan, 2008; Levitan and Stapper, 2010) suggest that, in spite of the large influences of water flow and distance on reproductive success, subtle within-species differences in gametic compatibility can be important in determining fertilization success in the sea.

THE USE OF SCUBA TO FOLLOW THE FATE OF PLANKTONIC LARVAE

Many benthic marine organisms display dual life cycles. The adults are sessile or sedentary, but they produce planktonic larvae that are released in the water column, where they stay for different lengths of time ranging from a few minutes to several months, and travel with the currents to distant locations (Scheltema, 1988) where they settle to complete the life cycle. The fate of such larvae is an important parameter, both in the study of the life history of particular species (Stearns, 1977) and in our understanding of the factors that affect marine community composition (Lewin, 1986; Underwood and Fairweather, 1989; Grosberg and Levitan, 1992; Roughgarden et al., 1994). However, given their

microscopic size, often unpredictable release, and frequently long planktonic stages, larvae are difficult or impossible to observe directly in nature. Thus in most instances investigators have had to rely on indirect methods of assessing the mode and rate of larval influx into benthic ecosystems. Such methods include radioactive labeling and release of larvae into their native habitat (Arnold et al., 2005); comparison of larval supply (determined by plankton sampling) to quantities of metamorphosing juveniles (Grosberg, 1982; Yoshioka, 1982; Lamare, 1998; Lamare and Barker, 1999; Doherty et al., 2004; Mariani et al., 2005; Pineda et al., 2010); or identification of paternity using genetic markers (Grosberg and Quinn, 1986; Mackie et al., 2006). Though the study of the larval lives of most organisms has necessarily relied on such indirect methods or on laboratory observations, the larvae of a few organisms are large enough to be seen with the naked eye, and the planktonic phase is short enough to be followed by divers on a single tank of air. Through the use of scuba, a handful of studies have taken advantage of these properties to elucidate important aspects of larval behavior and their consequences for successful establishment events.

Olson (1983; 1985) was able to follow the larval fate of the ascidian *Diademnum molle* at Lizard Island, Australia. Diademnid larvae remain in the plankton for a matter of minutes or hours and are large enough to be seen by the naked eye. Species of the family Diademnidae also release their larvae at a predictable time every day of the year. Variation in the timing of release among species appears to depend on whether a species contains symbiotic algae. Species that lack algae release larvae around dawn, whereas those that possess algae release larvae near the middle of the day. *Diademnum molle* larvae are 2.5 mm long and contain algae of the genus *Prochloron* sufficient to add color and thus be visible under water. Olson (1983) was able to track 89 larvae, 14 of them from release to settlement. Newly released larvae displayed positive phototaxis and swam toward the surface. After one to ten minutes, the phototaxis was reversed, and the larvae appeared to be attracted by dark surfaces, including divers' wetsuits. The planktonic stage of the 14 larvae that could be followed from release to settlement ranged from 40 seconds to 6 minutes. Larvae that swam longer were often lost from observation before they settled, but laboratory experiments showed that in general they settle 20 minutes to 2 hours after release, with 90% settling within 30 minutes. When a patch reef was denuded of adult colonies, recruitment ceased on a downstream reef that the larvae could reach within these times, but continued almost unaffected on a more distant downstream reef (Olson, 1985).

Monitoring of settling panels showed that the larvae overwhelmingly preferred to settle in dark spaces, and that the newly formed colonies died if exposed to bright light. Given that light appears to be lethal to the newly settled juveniles, a larval release in the middle of the day appears paradoxical, but it may well be an adaptation for allowing the larvae to choose spaces appropriate for adult growth by avoiding overly bright spots. The best time to choose a spot likely to remain shady for most of the day is during the period of most abundant light. Even though adult

colonies can move over small distances, there is a clear advantage in choosing the proper habitat during the larval stage.

Davis (1987) also took advantage of the large size of ascidian larvae to perform direct observations of their behavior. He studied the settlement preferences of the colonial ascidian *Podoclavella cylindrica*, which releases 4 mm long larvae with easily observed bright blue coloration. Monitoring of natural recruitment showed that it was higher than expected by chance on bare space and on the sponge *Euryspongia*, but lower than expected on three other genera of sponges. Davis observed larvae approaching bare space and four kinds of sponges and scored how many settled on each substratum versus how many touched it and then released. Eighty-four larvae could be followed from release to settlement. They showed a preference for settling on bare space or on unfouled surfaces of *Euryspongia*, with a high percentage of rejection of three other sponges. Davis also quantified post-settlement survival of recruits for 30 days, and found a tight correlation between larval substrate preference and juvenile survivorship. In a different study, Davis and Butler (1989) followed 100 larvae of *Podoclavella moluccensis* to assess how far they traveled before settlement. The distance ranged from 5 cm to 13.4 m, with nearly 80% settling within 2.5 m of the point of release.

Potential sources of mortality could also be determined by following the larvae of both *Diademnum molle* (Olson 1983) and of *Podoclavella moluccensis* (Davis and Butler, 1989). Stony corals and hydroids often entangled the ascidian larvae. Entangled larvae usually were able to break free, but in some cases were ingested. Fishes that put the larvae in their mouth invariably spit them out, presumably because they are unpalatable. Such high rejection rates were not observed in a study conducted by Stoner (1990; 1994) in Hawaii on the larvae of the ascidian *Diplosoma similis*.

In Stoner's (1990; 1994) study, 259 larvae of *Diplosoma similis* were followed in Kaneohe Bay, at the island of Oahu in Hawaii. Like *Diademnum molle*, *Diplosoma similis* is host to algal cells of the genus *Prochloron*. Larvae are approximately 2 mm long and are released predictably in the morning hours, so they could be followed by divers. Stoner found that larvae swim for an average of 3.8 minutes and disperse at an average distance of 2.2 m from the parental colony. At approximately 42 seconds after release, the larvae swam down toward the substratum, maintaining a distance of a few centimeters from it. At a mean time of approximately two minutes after release, they contacted the surface of the reef. Dead coral, an unidentified sponge, and the green algae *Dictyosphaeria* were preferred points of contact. After contact, they moved along the bottom for a distance of 20 cm before losing their tails and settling to form new colonies (Stoner, 1994). Of the total number of larvae that were followed by divers, 34% were lost from observation. Of the remaining 171 larvae, 71% settled successfully, and 49% were either eaten by planktivores or became ensnared in mucus sheaths of coral and the larvacean *Oikopleura*, an important difference from the larvae of *Diademnum molle* observed by Olson (1983) and of *Podoclavella cylindrica* observed by Davis (1987).

Predation by pomacentrids and coral was also high on another ascidian, *Lissoclinum patella* (Olson and McPherson, 1987). Post-settlement mortality of *Diplosoma similis* was much higher than mortality in the larval stage: 50% of the newly metamorphosed individuals perished within one day of metamorphosis, and none survived longer than a month (Stoner, 1990).

These studies, along with a few others (e.g., Young and Chia, 1984) have greatly increased our knowledge of events that occur in the larval life stage of ascidians and of the factors that influence their rate of entering benthic communities. The extent to which information from ascidians can be generalized to other organisms with much longer periods in the plankton, particularly planktotrophic ones, remains to be determined. Scuba cannot be used for direct observations of larvae that measure less than 1 mm or that stay in the water column for long periods of time, but it is still useful in documenting the beginning of their lives (i.e., their release) and the stage at which they are transformed to their sedentary stage (i.e., settlement).

THE USE OF SCUBA TO DETERMINE LARVAL SETTLEMENT

Settlement, the selection of substrate and metamorphosis into the sedentary phase, is a critical phase in the lives of many marine organisms. However, it can be observed only when larvae are large enough to be seen as they settle (see section on larval observations). For most organisms we can study only recruitment, the addition of individuals to an arbitrary age class as they grow. Each study of recruitment attempts to concentrate on recruitment to the smallest size that can be observed within sampling intervals (dictated by logistic considerations) in order to deduce as accurately as possible the processes that affect settlement. Stoner's (1990) finding that 50% of newly metamorphosed ascidian colonies perished within a day after larval settlement suggests that the window provided by recruitment for a realistic view of settlement may be very narrow. Although this is expected to vary with the type of organism (Hunt and Scheibling, 1997), 30 studies on bivalves, gastropods, barnacles, ascidians, bryozoans and echinoderms, reviewed by Gosselin and Qian (1997), all suggest that juvenile mortality is generally quite high. High mortality before the first observation of recruiting juveniles can lead to erroneous estimates of settlement, particularly if mortality is not random, though the unknowable distortions do not necessarily affect all studies. For example, studies that seek to quantify the number of juveniles of a particular species that enter the benthic community at a given time can accommodate net recruitment without knowledge of pre- versus post-settlement mortality.

Without scuba, recruitment of juvenile marine organisms can be studied in only the intertidal (e.g., Crisp, 1961; Connell, 1972; Strathmann et al., 1981; Grosberg, 1982; Connell, 1985) or on panels that can be suspended in the water and then retrieved (e.g., Osman and Whitlatch, 1995). With the ability of divers to remain under water for long periods of time, the study of recruitment of both sessile and mobile juvenile marine organisms has blossomed and has resulted in more studies than can be

reviewed here. Scuba has allowed non-destructive quantification of organisms that appear on a given natural or artificial substrate in pre-determined intervals, as well as comparisons and manipulations intended to uncover factors that affect the entrance of juveniles into benthic communities as the result of larval settling preferences (Highsmith, 1982; Snelgrove et al., 1999; Mariani et al., 2005), oceanographic conditions (Stoner et al., 1997), predation (Sammarco, 1980; 1982; Yoshioka, 1982; Doherty et al., 2004), depth (Hurlbut, 1991), and other physical parameters (Yoshioka, 1982).

Occasionally, the fortuitous occurrence of a major ecological shift during the period of monitoring has resulted in the opportunity to study a phenomenon of even wider importance than recruitment itself. An example of such a case has been the study of Bak (1985) of recruitment of the sea urchin *Diadema antillarum* during mass mortality suffered by this species. Bak (1985) studied the recruitment of juvenile *Diadema* to plastic grates suspended over the reef in Curaçao from 1982 to 1984. *Diadema antillarum* was, until 1983, a dominant component of Caribbean coral reefs, affecting community composition through its grazing of algae (Sammarco, 1982; Carpenter, 1986), predation on live coral (Bak and van Eys, 1975; Sammarco, 1980), and bioerosion of the calcium carbonate substrate (Scoffin et al., 1980). Starting in April 1983, *D. antillarum* suffered mass mortality, which was first noticed on the Caribbean coast of Panama, and was then followed as it affected all populations in the Caribbean and the western Atlantic (Lessios et al., 1984) one by one. Population densities in all studied localities were reduced by more than 97% (Lessios, 1988). The mass mortality front, traveling along coastal currents, reached Curaçao in October 1983 (Bak et al., 1984).

In Bak's (1985) study, divers examined the small cells of the plastic grates similar to the ones shown in Figure 1 every two weeks and were able to locate juvenile sea urchins 1–3 mm in diameter. The number of recruits at each plate over time established that (1) recruitment was higher where the resident adult populations of the species were dense; (2) there were seasonal peaks of recruitment; and (3) a certain amount of growth of coralline algae on the grates was necessary for sea urchin recruitment, but excessive fouling of the plastic by algae depressed the number of juveniles observed by the divers. The number of small *Diadema* located by Bak at each sampling interval between June 1982 and February 1984 was impressive. Maximum densities of juveniles were 102–188/m² recruited in a fortnight in June 1983. After February 1984, practically no juveniles recruited on the plastic grates through December 1984, when monitoring stopped. Thus, *Diadema* larvae were arriving at Curaçao for four months following the decimation of the adult populations on this island in October 1983. These larvae could have only come from other populations that had not yet been affected by the mass mortality. Indeed, *D. antillarum* populations on the coast of Venezuela died off in late November 1983 and at Barbados, upstream of Curaçao on the Caribbean Current, in early December 1983 (Lessios et al., 1984). In the laboratory, larvae of this species settle 34 to 90 days after fertilization (Carpenter, 1997; Eckert, 1998), so the

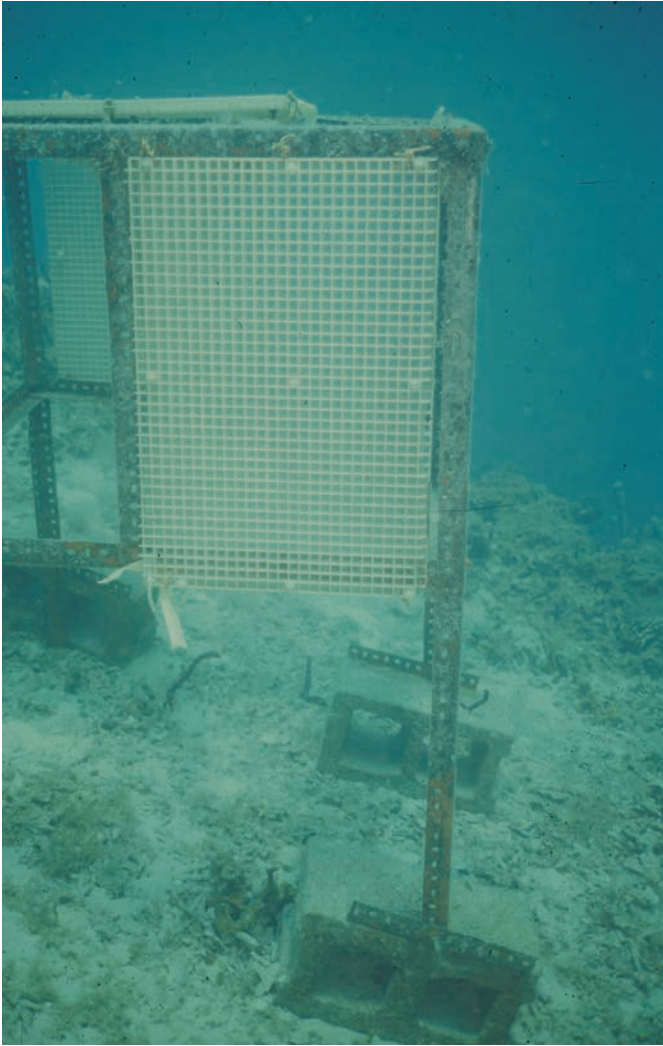


FIGURE 1. Larval collector similar to the one used in Bak's (1985) study of *Diadema* recruitment (photo by H. Lessios).

cessation of recruitment in Curaçao in March 1984 is consistent with the hypothesis that larvae continued to arrive from as far as Barbados, then stopped after the Barbados populations were decimated. Bak's study thus demonstrated not only factors that are important for eliciting settlement, but also the geographical extent of linkages between populations and the significance of events at distant places for settlement of a prominent component of the coral reef community.

Diadema antillarum populations in the Caribbean continued to remain low for the next quarter of a century (Lessios, 2005; Chiappone et al., 2008; Miller et al., 2009). Vermeij et al. (2010) followed up on Bak's (1985) study by setting up the same plastic grates on leeward reefs at Curaçao and monitoring recruitment from March to July 2005. They found that 22 years after the decimation of resident populations, the recruitment rate

had increased by a factor of 56 relative to what it was immediately after the mass mortality, but was still half of what it was before this event. Despite this increase in recruitment, adult populations around the island remained low, indicating that settlement and recruitment are necessary but not sufficient causes for population recovery.

CONCLUSIONS

As the examples we presented in this chapter indicate (and they are only a small part of underwater research), much has been accomplished through scuba to study reproductive biology and early life histories of marine invertebrates. Some of the data that have been gathered have been the result of systematic or serendipitous observations, but a large part of marine research involves actual experimentation that was probably never envisioned in the days before autonomous diving became a reality. The study of biology will never be as easy under water as it is on land, but technological advancements that permit longer, deeper dives with less need for infrastructural support will, in the future, provide new insights into the life histories of marine organisms.

REFERENCES

- Alino, P., and J. C. Coll. 1989. Observation of the synchronized mass spawning and postsettlement activity of octocorals on the Great Barrier, Australia: Biological aspects. *Bulletin of Marine Science*, 45:697–707.
- Arnold, W. S., G. L. Hitchcock, M. E. Frischer, R. Wanninkhof, and Y. P. Sheng. 2005. Dispersal of an introduced larval cohort in a coastal lagoon. *Limnology and Oceanography*, 50:587–597. <http://dx.doi.org/10.4319/lo.2005.50.2.0587>.
- Babcock, R. C., G. D. Bull, P. L. Harrison, A. J. Heyward, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, 90:379–394. <http://dx.doi.org/10.1007/BF00428562>.
- Babcock, R. C., and C. N. Mundy. 1992. Reproductive biology, spawning and field fertilization rates of *Acanthaster planci*. *Australian Journal of Marine and Freshwater Research*, 43:525–534. <http://dx.doi.org/10.1071/MF9920525>.
- Babcock, R. C., C. Mundy, J. Keesing, and J. Oliver. 1992. Predictable and unpredictable spawning events: *In situ* behavioural data from free-spawning coral reef invertebrates. *Invertebrate Reproduction and Development*, 22:213–228. <http://dx.doi.org/10.1080/07924259.1992.9672274>.
- Bak, R. P. M. 1985. Recruitment patterns and mass mortalities in the sea urchin *Diadema antillarum*. *Proceedings of the fifth International Coral Reef Congress*, 5:267–272.
- Bak, R. P. M., M. J. E. Carpay, and E. D. de Ruyter van Steveninck. 1984. Densities of the sea urchin *Diadema antillarum* before and after mass mortalities on the coral reefs of Curaçao. *Marine Ecology Progress Series*, 17:105–108. <http://dx.doi.org/10.3354/meps017105>.
- Bak, R. P. M., and G. van Eys. 1975. Predation of the sea urchin *Diadema antillarum* Philippi on living coral. *Oecologia*, 20:111–115. <http://dx.doi.org/10.1007/BF00369023>.
- Beiring, E. A., and H. R. Lasker. 2000. Egg production by colonies of a gorgonian coral. *Marine Ecology Progress Series*, 196:169–177. <http://dx.doi.org/10.3354/meps196169>.
- Brazeau, D. A., and H. R. Lasker. 1990. Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. *Marine Biology*, 104:465–474. <http://dx.doi.org/10.1007/BF01314351>.
- . 1992. Reproductive success in the Caribbean octocoral *Briareum asbestinum*. *Marine Biology*, 114:157–163.
- Carlson, D. B. 1999. The evolution of mating systems in tropical reef corals. *Trends in Ecology and Evolution*, 14:491–495. [http://dx.doi.org/10.1016/S0169-5347\(99\)01709-7](http://dx.doi.org/10.1016/S0169-5347(99)01709-7).

- Carpenter, R. C. 1986. Partitioning herbivory and its effects on coral-reef algal communities. *Ecological Monographs*, 56:345–363. <http://dx.doi.org/10.2307/1942551>.
- . 1997. Invertebrate predators and grazers. In *Life and death of coral reefs*, ed. C. Birkeland, pp. 198–248. New York: Chapman and Hall. <http://dx.doi.org/10.1007/978-1-4615-5995-5>.
- Chiappone, M., L. M. Rutten, D. W. Swanson, and S. L. Miller. 2008. Population status of the urchin *Diadema antillarum* in the Florida Keys 25 years after the Caribbean mass mortality. *Proceedings of the eleventh International Coral Reef Symposium*, 2:712–716.
- Coffroth, M. A., and H. R. Lasker. 1998. Larval paternity and male reproductive success of a broadcast-spawning gorgonian, *Plexaura kuna*. *Marine Biology*, 131:329–337. <http://dx.doi.org/10.1007/s002270050326>.
- Coma, R., and H. R. Lasker. 1997. Effects of spatial distribution and reproductive biology on in situ fertilization rates of a broadcast-spawning invertebrate. *Biological Bulletin*, 193:20–29. <http://dx.doi.org/10.2307/1542733>.
- Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. *Annual Reviews of Ecology and Systematics*, 3:169–192. <http://dx.doi.org/10.1146/annurev.es.03.110172.001125>.
- . 1985. The consequences of variation in initial settlement vs. postsettlement mortality in rocky intertidal communities. *Journal of Experimental Marine Biology and Ecology*, 93:11–45. [http://dx.doi.org/10.1016/0022-0981\(85\)90146-7](http://dx.doi.org/10.1016/0022-0981(85)90146-7).
- Crisp, D. J. 1961. Territorial behaviour in barnacle settlement. *Journal of Experimental Biology*, 38:429–446.
- Davis, A. R. 1987. Variation in recruitment of the subtidal colonial ascidian *Podoclavella cylindrica* (Quoy and Gaimard): The role of substatum choice and early survival. *Journal of Experimental Marine Biology and Ecology*, 106:57–71. [http://dx.doi.org/10.1016/0022-0981\(87\)90147-X](http://dx.doi.org/10.1016/0022-0981(87)90147-X).
- Davis, A. R., and A. J. Butler. 1989. Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Slutter: Evidence of closed populations. *Journal of Experimental Marine Biology and Ecology*, 127:189–203. [http://dx.doi.org/10.1016/0022-0981\(89\)90184-6](http://dx.doi.org/10.1016/0022-0981(89)90184-6).
- de Graaf, M., G. J. Geertjes, and J. J. Videler. 1999. Observations on spawning of scleractinian corals and other invertebrates on the reefs of Bonaire (Netherlands Antilles, Caribbean). *Bulletin of Marine Science*, 64:189–194.
- Denny, M. W. 1988. *Biology and the mechanics of the wave-swept environment*. Princeton, N.J.: Princeton University Press.
- Denny, M. W., and M. F. Shibata. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. *American Naturalist*, 134:859–889. <http://dx.doi.org/10.1086/285018>.
- Doherty, P. J., V. Dufour, R. Galzin, M. A. Hixon, M. G. Meekan, and S. Planes. 2004. High mortality during settlement is a population bottleneck for a tropical surgeonfish. *Ecology*, 85:2422–2428. <http://dx.doi.org/10.1890/04-0366>.
- Eckert, G. L. 1998. Larval development, growth and morphology of the sea urchin *Diadema antillarum*. *Bulletin of Marine Science*, 63:443–451.
- Evans, J. P., and D. J. Marshall. 2005. Male-by-female interactions influence fertilization success and mediate the benefits of polyandry in the sea urchin *Heliodiaria erythrogramma*. *Evolution*, 59:106–112.
- Fadlallah, Y. H., and J. S. Pearse. 1982. Sexual reproduction in solitary corals: Synchronous gametogenesis and broadcast spawning in *Paracyathus stearnsii*. *Marine Biology*, 71:233–239. <http://dx.doi.org/10.1007/BF00397040>.
- Farley, G. S., and D. R. Levitan. 2001. The role of jelly coats in sperm-egg encounters, fertilization success, and selection on egg size in echinoids. *American Naturalist*, 157:626–236. <http://dx.doi.org/10.1086/320619>.
- Gittings, S. R., G. S. Boland, K. J. P. Deslarzes, C. L. Combs, B. S. Holland, and T. J. Bright. 1992. Mass spawning and reproductive viability of reef corals at the East Flower Garden Bank, Northwest Gulf of Mexico. *Bulletin of Marine Science*, 51:420–428.
- Gosselin, L. A., and P. Y. Qian. 1997. Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series*, 146:265–282. <http://dx.doi.org/10.3354/meps146265>.
- Grosberg, R. K. 1982. Inter-tidal zonation of barnacles: The influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology*, 63:894–899. <http://dx.doi.org/10.2307/1937228>.
- . 1991. Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. *Evolution*, 45:130–142. <http://dx.doi.org/10.2307/2409488>.
- Grosberg, R. K., and D. R. Levitan. 1992. For adults only: Supply side ecology and the history of larval biology. *Trends in Ecology and Evolution*, 7:130–133. [http://dx.doi.org/10.1016/0169-5347\(92\)90148-5](http://dx.doi.org/10.1016/0169-5347(92)90148-5).
- Grosberg, R. K., and J. F. Quinn. 1986. The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature*, 322:456–459. <http://dx.doi.org/10.1038/322456a0>.
- Hagström, B. E., and S. Lönning. 1967. Experimental studies of *Strongylocentrotus droebachiensis* and *S. pallidus*. *Sarsia*, 29:165–176.
- Harrison, P. L. 2011. Sexual reproduction of scleractinian corals. In *Coral reefs: An ecosystem in transition*, ed. Z. Dubinsky and N. Stambler, pp. 59–85. Berlin: Springer. http://dx.doi.org/10.1007/978-94-007-01144-4_6.
- Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1984. Mass spawning in tropical reef corals. *Science*, 223:1186–1189. <http://dx.doi.org/10.1126/science.223.4641.1186>.
- Hendler, G., and D. L. Meyer. 1982. Ophiuroids *flagrante-delicto* and notes on the spawning behavior of other echinoderms in their natural habitat. *Bulletin of Marine Science*, 32:600–607.
- Highsmith, R. C. 1982. Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: Adult sand dollar beds. *Ecology*, 63:329–337. <http://dx.doi.org/10.2307/1938950>.
- Himmelman, J. H., C. P. Dumont, C. F. Gaymer, C. Vallieres, and D. Drolet. 2008. Spawning synchrony and aggregative behaviour of cold-water echinoderms during multi-species mass spawnings. *Marine Ecology Progress Series*, 361:161–168. <http://dx.doi.org/10.3354/meps07415>.
- Hunt, H. L., and R. E. Scheibling. 1997. Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series*, 155:269–301. <http://dx.doi.org/10.3354/meps155269>.
- Hurlbut, C. J. 1991. The effects of larval abundance, settlement and juvenile mortality on the depth distribution of a colonial ascidian. *Journal of Experimental Marine Biology and Ecology*, 150:183–202. [http://dx.doi.org/10.1016/0022-0981\(91\)90067-7](http://dx.doi.org/10.1016/0022-0981(91)90067-7).
- Johnson, S. L., and P. O. Yund. 2007. Variation in multiple paternity in natural populations of a free-spawning marine invertebrate. *Molecular Ecology*, 16:3253–3262. <http://dx.doi.org/10.1111/j.1365-294X.2007.03366.x>.
- . 2009. Effects of fertilization distance on male gain curves in a free-spawning marine invertebrate: A combined empirical and theoretical approach. *Evolution*, 63:3114–3123. <http://dx.doi.org/10.1111/j.1558-5646.2009.00784.x>.
- Kupriyanova, E., and J. N. Havenhand. 2002. Variation in sperm swimming behaviour and its effect on fertilization success in the serpulid polychaete *Galeolaria caespitosa*. *Invertebrate Reproduction and Development*, 41:21–26. <http://dx.doi.org/10.1080/07924259.2002.9652731>.
- Lamare, M. D. 1998. Origin and transport of larvae of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. *Marine Ecology Progress Series*, 174:107–121. <http://dx.doi.org/10.3354/meps174107>.
- Lamare, M. D., and M. F. Barker. 1999. In situ estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). *Marine Ecology Progress Series*, 180:197–211. <http://dx.doi.org/10.3354/meps180197>.
- Lamare, M. D., and B. G. Stewart. 1998. Mass spawning by the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. *Marine Biology*, 132:135–140. <http://dx.doi.org/10.1007/s002270050379>.
- Lasker, H. R. 2006. High fertilization success in a surface-brooding Caribbean gorgonian. *Biological Bulletin*, 210:10–17. <http://dx.doi.org/10.2307/4134532>.
- Lasker, H. R., D. A. Brazeau, J. Calderon, M. A. Coffroth, R. Coma, and K. Kim. 1996. In situ rates of fertilization among broadcast spawning gorgonian corals. *Biological Bulletin*, 190:45–55. <http://dx.doi.org/10.2307/1542674>.
- Lasker H. R., C. Gutierrez-Rodriguez, K. Bala, A. Hannes, and J. P. Bilewicz. 2008. Male reproductive success during spawning events of the octocoral *Pseudopterogorgia elisabethae*. *Marine Ecology Progress Series*, 367:153–161. <http://dx.doi.org/10.3354/meps07614>.
- Lessios, H. A. 1984. Possible prezygotic reproductive isolation in sea urchins separated by the Isthmus of Panama. *Evolution*, 38:1144–1148. <http://dx.doi.org/10.2307/2408446>.
- . 1988. Mass mortality of *Diadema antillarum* in the Caribbean: What have we learned? *Annual Review of Ecology and Systematics*, 19:371–393.
- . 2005. *Diadema antillarum* populations in Panama twenty years following mass mortality. *Coral Reefs*, 24:125–127. <http://dx.doi.org/10.1007/s00338-004-0443-5>.
- Lessios, H. A., D. R. Robertson, and J. D. Cubit. 1984. Spread of *Diadema* mass mortality through the Caribbean. *Science*, 226:335–337. <http://dx.doi.org/10.1126/science.226.4672.335>.
- Levitan, D. R. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. *American Naturalist*, 141:517–536. <http://dx.doi.org/10.1086/285489>.

- . 1996. Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. *Nature*, 382:153–155. <http://dx.doi.org/10.1038/382153a0>.
- . 1998. Does Bateman's Principle apply to broadcast-spawning organisms? Egg traits influence in situ fertilization rates among congeneric sea urchins. *Evolution*, 52:1043–1056. <http://dx.doi.org/10.2307/2411235>.
- . 2000a. Sperm velocity and endurance trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proceedings of the Royal Society of London: Biological Sciences*, 267:531–534. <http://dx.doi.org/10.1098/rspb.2000.1032>.
- . 2000b. Optimal egg size in marine invertebrates: Theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. *American Naturalist*, 156:175–192. <http://dx.doi.org/10.1086/303376>.
- . 2002a. Density-dependent selection on gamete traits in three congeneric sea urchins. *Ecology*, 83:464–479. [http://dx.doi.org/10.1890/0012-9658\(2002\)083\[0464:DDSOGT\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083[0464:DDSOGT]2.0.CO;2).
- . 2002b. The relationship between conspecific fertilization success and reproductive isolation among three congeneric sea urchins. *Evolution*, 56:1599–1609.
- . 2004. Density-dependent sexual selection in external fertilizers: Variances in male and female reproductive success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. *American Naturalist*, 164:298–309. <http://dx.doi.org/10.1086/423150>.
- . 2005. Sex specific spawning behavior and its consequences in an external fertilizer. *American Naturalist*, 165:682–694. <http://dx.doi.org/10.1086/429733>.
- . 2008. Gamete traits influence the variance in reproductive success, the intensity of sexual selection, and the outcome of sexual conflict among congeneric sea urchins. *Evolution*, 62:1305–1316. <http://dx.doi.org/10.1111/j.1558-5646.2008.00378.x>.
- Levitán, D. R., and D. L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density and genotype frequency. *Science*, 312:267–269. <http://dx.doi.org/10.1126/science.1122183>.
- Levitán, D. R., H. Fukami, J. Jara, D. Kline, T. M. McGovern, K. E. McGhee, C. A. Swanson, and N. Knowlton. 2004. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. *Evolution*, 58:308–323.
- Levitán, D. R., M. A. Sewell, and F.-S. Chia. 1991. Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: Interaction of gamete dilution, age, and contact time. *Biological Bulletin*, 181:371–378. <http://dx.doi.org/10.2307/1542357>.
- Levitán, D. R., and A. P. Stapper. 2010. Simultaneous positive and negative frequency dependent selection on sperm binding, a gamete recognition protein in the sea urchin *Strongylocentrotus purpuratus*. *Evolution*, 64:785–797. <http://dx.doi.org/10.1111/j.1558-5646.2009.00850.x>.
- Levitán, D. R., C. P. Terhorst, and N. D. Fogarty. 2007. The risk of polyspermy in three congeneric sea urchins and its implications for gametic incompatibility and reproductive isolation. *Evolution*, 61:2007–2014. <http://dx.doi.org/10.1111/j.1558-5646.2007.00150.x>.
- Lewin, R. 1986. Supply-side ecology. *Science*, 234:25–27. <http://dx.doi.org/10.1126/science.234.4772.25>.
- Lillie, F. R. 1915. Studies of Fertilization. VII. Analysis of variations in the fertilization power of sperm suspensions of *Arbacia*. *Biological Bulletin*, 28:229–251. <http://dx.doi.org/10.2307/1536390>.
- Lindquist, N., R. Bolser, and K. Laing. 1997. Timing of larval release by two Caribbean demosponges. *Marine Ecology Progress Series*, 155:309–313. <http://dx.doi.org/10.3354/meps155309>.
- Luttikhuisen, P. C., P. J. H. Honkoop, J. Drent and J. van der Meer. 2004. A general solution for optimal egg size during external fertilization, extended scope for intermediate optimal egg size and the introduction of Don Ottavio "tango." *Journal of Theoretical Biology*, 231:333–343. <http://dx.doi.org/10.1016/j.jtbi.2004.06.028>.
- Mackie, J. A., M. J. Keough, and L. Christidis. 2006. Invasion patterns inferred from cytochrome oxidase I sequences in three bryozoans, *Bugula neritina*, *Watersipora subtorquata*, and *Watersipora arcuata*. *Marine Biology*, 149:285–295. <http://dx.doi.org/10.1007/s00227-005-0196-x>.
- Mariani, S., T. Alcoverro, M. J. Uriz, and X. Turon. 2005. Early life histories in the bryozoan *Schizobrachiella sanguinea*: A case study. *Marine Biology*, 147:735–745. <http://dx.doi.org/10.1007/s00227-005-1616-7>.
- Marshall, D. J., C. A. Styan, and M. J. Keough. 2002. Sperm environment affects offspring quality in broadcast spawning marine invertebrates. *Ecology Letters*, 5:173–176. <http://dx.doi.org/10.1046/j.1461-0248.2002.00257.x>.
- Marshall, S. M., and T. A. Stephenson. 1933. The Breeding of Reef Animals, I. The Corals. *Scientific Reports of the Great Barrier Reef Expedition*, 3:219–245.
- McCartney, M. A. 1997. Sex allocation and male fitness gain curves in a colonial, hermaphroditic marine invertebrate. *Evolution*, 51:127–140. <http://dx.doi.org/10.2307/2410966>.
- McEuen, F. S. 1988. Spawning behaviors of Northeast Pacific sea cucumbers (Holothuroidea, Echinodermata). *Marine Biology*, 98:565–585. <http://dx.doi.org/10.1007/BF00391548>.
- Miller, K. J., and C. N. Mundy. 2005. In situ fertilisation success in the scleractinian coral *Goniastrea favulus*. *Coral Reefs*, 24:313–317. <http://dx.doi.org/10.1007/s00338-005-0480-8>.
- Miller, M. W., K. L. Kramer, S. M. Williams, L. Johnston, and A. M. Szmant. 2009. Assessment of current rates of *Diadema antillarum* larval settlement. *Coral Reefs*, 28:511–515. <http://dx.doi.org/10.1007/s00338-008-0458-4>.
- Miller, R. L. 1966. Chemotaxis during fertilization in the hydroid *Campanularia*. *Journal of Experimental Zoology*, 162:22–44. <http://dx.doi.org/10.1002/jez.1401620104>.
- Mortensen, T. 1938. Contributions to the study of the development and larval forms of echinoderms IV. *Det Kongelige Danske Videnskaberne Selskabs Skrifter 9 Raekke*, 7(3):1–59.
- Oliver, J., and R. Babcock. 1992. Aspects of the fertilization ecology of broadcast spawning corals: Sperm dilution effects and in situ measurements of fertilization. *Biological Bulletin*, 183:409–417. <http://dx.doi.org/10.2307/1542017>.
- Olson, R. R. 1983. Ascidian-*Prochloron* symbiosis: The role of larval photoadaptations in midday larval release and settlement. *Biological Bulletin*, 165:221–240. <http://dx.doi.org/10.2307/1541366>.
- . 1985. The consequences of short distance larval dispersal in a sessile marine invertebrate. *Ecology*, 66:30–39. <http://dx.doi.org/10.2307/1941304>.
- Olson, R. R., and R. McPherson. 1987. Potential vs. realized larval dispersal: Fish predation on larvae of the ascidian *Lissoclinum patella* (G) [http://dx.doi.org/10.1016/0022-0981\(87\)90004-9](http://dx.doi.org/10.1016/0022-0981(87)90004-9). *Journal of Experimental Marine Biology and Ecology*, 110:245–256.
- Osman, R. W., and R. B. Whitlatch. 1995. The influence of resident adults on larval settlement: Experiments with four species of ascidians. *Journal of Experimental Marine Biology and Ecology*, 190:199–220. [http://dx.doi.org/10.1016/0022-0981\(95\)00036-Q](http://dx.doi.org/10.1016/0022-0981(95)00036-Q).
- Pearse, J. S., D. J. McClary, M. A. Sewell, W. C. Austin, A. Perezruzafa, and M. Byrne. 1988. Simultaneous spawning of six species of echinoderms in Barkley Sound, British Columbia. *International Journal of Invertebrate Reproduction and Development*, 14:279–288. <http://dx.doi.org/10.1080/01688170.1988.10510385>.
- Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation and synchronous spawning. *Biological Bulletin*, 169:417–430. <http://dx.doi.org/10.2307/1541492>.
- Pineda, J., F. Porri, V. Starczak, and J. Blythe. 2010. Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity. *Journal of Experimental Marine Biology and Ecology*, 392:9–21. <http://dx.doi.org/10.1016/j.jembe.2010.04.008>.
- Podolsky, R. D. 2002. Fertilization ecology of egg coats: Physical versus chemical contributions to fertilization success of free-spawned eggs. *Journal of Experimental Biology*, 205:1657–1668.
- . 2004. Life history consequences of investment in free-spawned eggs and their accessory coats. *American Naturalist*, 163:735–753. <http://dx.doi.org/10.1086/382791>.
- Purchase, C. F., D. J. Hasselman and L. K. Weir. 2007. Relationship between fertilization success and the number of milt donors in rainbow smelt *Osmerus mordax* (Mitchell): Implications for population growth rates. *Journal of Fish Biology*, 70:934–946. <http://dx.doi.org/10.1111/j.1095-8649.2007.01356.x>.
- Richmond, R. H., and C. L. Hunter. 1990. Reproduction and recruitment of corals: Comparisons among the Caribbean, the tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, 60:185–203. <http://dx.doi.org/10.3354/meps060185>.
- Riffell, J. A., P. J. Krug and R. K. Zimmer. 2004. The ecological and evolutionary consequences of sperm chemoattraction. *Proceedings of the National Academy of Sciences*, 101:4501–4506. <http://dx.doi.org/10.1073/pnas.0304594101>.
- Roughgarden, J., T. Pennington, and S. Alexander. 1994. Dynamics of the rocky intertidal zone with remarks on generalization in ecology. *Philosophical Transactions of the Royal Society of London [B]*, 343:79–85. <http://dx.doi.org/10.1098/rstb.1994.0010>.
- Sammarco, P. W. 1980. *Diadema* and its relationships to coral spat mortality: Grazing, competition, and biological disturbance. *Journal of Experimental Marine Biology and Ecology*, 45:245–272. [http://dx.doi.org/10.1016/0022-0981\(80\)90061-1](http://dx.doi.org/10.1016/0022-0981(80)90061-1).

- . 1982. Echinoid grazing as a structuring force in coral communities: Whole reef manipulations. *Journal of Experimental Marine Biology and Ecology*, 61:31–55. [http://dx.doi.org/10.1016/0022-0981\(82\)90020-X](http://dx.doi.org/10.1016/0022-0981(82)90020-X).
- Sanchez, J. A., E. M. Alvarado, M. F. Gil, H. Charry, O. L. Arenas, L. H. Chasqui, and R. P. Garcia. 1999. Synchronous mass spawning of *Montastraea annularis* (Ellis & Solander) and *Montastraea faveolata* (Ellis & Solander) (Favidae: Scleractinia) at Rosario Islands, Caribbean Coast of Colombia. *Bulletin of Marine Science*, 65:873–879.
- Scheltema, R. S. 1988. Initial evidence for the transport of teleplanic larvae of benthic invertebrates across the East Pacific Barrier. *Biological Bulletin*, 174:145–152. <http://dx.doi.org/10.2307/1541781>.
- Scoffin, T. P., C. W. Stearn, D. Boucher, P. Frydl, C. M. Hawkins, I. G. Hunter, and J. K. MacGeachy. 1980. Calcium carbonate budget of a fringing reef on the west coast of Barbados. Part II. Erosion, sediments and internal structure. *Bulletin of Marine Science*, 30:475–508.
- Sewell, M. A., and D. R. Levitan. 1992. Fertilization success during a natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. *Bulletin of Marine Science*, 51:161–166.
- Snelgrove, P. V. R., J. P. Grassle, J. F. Petrecca, R. F. Petrecca, and H. G. Ma. 1999. In situ habitat selection by settling larvae of marine soft-sediment invertebrates. *Limnology and Oceanography*, 44:1341–1347. <http://dx.doi.org/10.4319/lo.1999.44.5.1341>.
- Stearns, S. C. 1977. The evolution of life history traits: A critique of the theory and a review of the data. *Annual Review of Ecology and Systematics*, 8:145–171. <http://dx.doi.org/10.1146/annurev.es.08.110177.001045>.
- Stoner, A. W., N. Mehta, and T. N. Lee. 1997. Recruitment of *Strombus* veligers to the Florida Keys reef tract: Relation to hydrographic events. *Journal of Shellfish Research*, 16:1–6.
- Stoner, D. S. 1990. Recruitment of a tropical colonial ascidian: Relative importance of pre-settlement vs post-settlement processes. *Ecology*, 71:1682–1690. <http://dx.doi.org/10.2307/1937577>.
- . 1994. Larvae of a colonial ascidian use a non-contact mode of substratum selection on a coral reef. *Marine Biology*, 121:319–326. <http://dx.doi.org/10.1007/BF00346740>.
- Strathmann, R. R., E. S. Branscomb, and K. Vedder. 1981. Fatal errors in set as a cost of dispersal and the influence of intertidal flora on set of barnacles. *Oecologia*, 48:13–18. <http://dx.doi.org/10.1007/BF00346982>.
- Swanson, W. J., and V. D. Vacquier. 2002. Reproductive protein evolution. *Annual Review of Ecology and Systematics*, 33:161–179. <http://dx.doi.org/10.1146/annurev.ecolsys.33.010802.150439>.
- Szmant, A. M. 1986. Reproductive ecology of Caribbean reef corals. *Coral Reefs*, 5:43–53. <http://dx.doi.org/10.1007/BF00302170>.
- Thomas, F. I. M. 1994. Physical properties of gametes in three sea urchin species. *Journal of Experimental Biology*, 194:263–284.
- Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the sound (Oresund). *Meddelelser fra Kommission Danmarks Fiskeri og Havundersøkelser Serie: Plankton*, 4:1–519.
- Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution*, 4:17–20. [http://dx.doi.org/10.1016/0169-5347\(89\)90008-6](http://dx.doi.org/10.1016/0169-5347(89)90008-6).
- Van Oppen, M. J. H., B. L. Willis, T. Van Rheede, and D. J. Miller. 2002. Spawning times, reproductive compatibilities and genetic structuring in the *Acropora aspera* group: Evidence for natural hybridization and semi-permeable species boundaries in corals. *Molecular Ecology*, 11:1363–1376. <http://dx.doi.org/10.1046/j.1365-294X.2002.01527.x>.
- Van Veghel, M. L. J. 1993. Multiple species spawning on Curacao reefs. *Bulletin of Marine Science*, 52:1017–1021.
- Vermeij, M. J. A., A. O. Debrot, N. van der Hal, J. Bakker, and R. P. M. Bak. 2010. Increased recruitment rates indicate recovering populations of the sea urchin *Diadema antillarum* in Curaçao. *Bulletin of Marine Science*, 86:719–725.
- Yonge, C. M. 1940. The biology of reef-building corals. *Reports of the Great Barrier Reef Expedition*, 1:353–389.
- Yoshioka, P. M. 1982. Role of planktonic and benthic factors in the population dynamics of the bryozoan *Membranipora membranacea*. *Ecology*, 63:457–468. <http://dx.doi.org/10.2307/1938963>.
- Young, C. M., and F.-S. Chia. 1984. Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. *Marine Biology*, 81:61–68. <http://dx.doi.org/10.1007/BF00397626>.
- Yund, P. O. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. *Ecology*, 79:328–339. [http://dx.doi.org/10.1890/0012-9658\(1998\)079\[0328:TEOSCO\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1998)079[0328:TEOSCO]2.0.CO;2).
- Yund, P. O., S. L. Johnson, and L. E. Connolly. 2005. Multiple paternity and subsequent fusion/rejection interactions in a colonial ascidian. *Integrative and Comparative Biology*, 45:1101–1101.
- Yund, P. O., and M. A. McCartney. 1994. Male reproductive success in sessile invertebrates: Competition for fertilizations. *Ecology*, 75:2151–2167. <http://dx.doi.org/10.2307/1940874>.
- Yund, P. O., and S. K. Meidel. 2003. Sea urchin spawning in benthic boundary layers: Are eggs fertilized before advecting away from females? *Limnology and Oceanography*, 48:795–801. <http://dx.doi.org/10.4319/lo.2003.48.2.0795>.
- Zigler, K. S., M. A. McCartney, D. R. Levitan, and H. A. Lessios. 2005. Sea urchin bindin divergence predicts gamete compatibility. *Evolution*, 59:2399–2404.