2009-06-24

Behavior and Transport of Pelagic Coral Reef Fish Larvae in the Straits of Florida

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UNIVERSITY OF MIAMI

BEHAVIOR AND TRANSPORT OF PELAGIC CORAL REEF FISH LARVAE IN
THE STRAITS OF FLORIDA

By

Klaus B. Huebert

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

June 2009

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
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<u>Behavior and Transport of Pelagic Coral Reef Fish</u>   (June 2009)
<u>Larvae in the Straits of Florida</u>

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Su Sponaugle.
No. of pages in text. (153)

The supply of coral reef fish larvae from the open ocean to reefs is vital for the

persistence of local fish populations. Whether larvae are dispersed over hundreds of km

or only few km depends on biophysical interactions between larvae and their

environment. Relationships between environmental variables, larval swimming behavior,

and larval transport were examined for reef fish larvae in the Florida Straits. In a series of

research cruises, the upper 100 m of the water column was sampled with plankton nets

fishing at four different depths. Variability in the vertical distributions of most larvae was

not consistently related to measured environmental variables. Relative densities of larvae

were predictably related to sampling depth in five taxa. In seven taxa, more developed

larvae were distributed significantly deeper than less developed larvae, revealing

ontogenic vertical migrations. In three taxa, vertical distributions varied significantly

between day and night, revealing diel migrations. Since the Florida Current was strongest

near the surface, observed vertical distributions and migrations resulted in reduced larval

transport relative to surface currents. To identify cues involved in regulating vertical

distributions, behavioral experiments were conducted with larvae from four reef fish

families. All four groups showed significant responses to pressure cues, swimming up in

response to high pressure and down in response to low pressure. In two families there

was a significant correlation between capture depth and experimental pressure preference, suggesting that larvae use similar behavior to regulate depth in situ. To study horizontal swimming behavior, late-stage larvae of one species were caught in light-traps and observed by SCUBA divers ~1 km offshore of the Florida Keys barrier reef. All larvae swam remarkably straight, but their swimming directions were distributed randomly. A simulation model was used to generate swimming trajectories of longer duration than could be observed directly. Observed and simulated trajectories indicated that horizontal swimming by larvae with or without an external reference frame was important at spatial scales of several km. Overall, some larvae exercised a strong influence on transport, either by vertical or horizontal swimming. Behaviors varied between species and families, highlighting the need for more species-specific data.

In memory of Art Myrberg and Al Chapin.

Last but not least I would like to thank my family. As my friend, girlfriend, and wife, Lauren has shared in every aspect of my life, including my work. If my only accomplishment in graduate school had been meeting you, I would consider the matter a great success. You have made my life richer than I imagined possible. Without the support of my parents I would never have made it to graduate school, never mind making it through graduate school. Mom and Dad, you continue to inspire me much more than you know. My big brother, big sister, and their respective families, were also sources of great encouragement and inspiration.

Looking back, so many people contributed so much to this work that the above lines fall woefully short of fully expressing my gratitude. To all my friends, family, and colleagues: may God bless you and give you peace.

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CHAPTER 1. INTRODUCTION

Background

Since Hjort (1914) proposed that the structure of marine fish populations can be determined by variable mortality of fish eggs and larvae, there has been a concerted effort to study the early life history stages of fishes, in order to predict, manage, exploit, and conserve future populations (reviewed in Cowan & Shaw 2002). Traditionally, process-oriented larval fish research has focused on larval feeding, predation, and growth. More recently, population connectivity has emerged as an important area of study, as demonstrated by exponential growth in publications during the past twenty years (Fig. 1.1).

Population connectivity describes the phenomenon whereby geographically distant populations can be biologically connected by an exchange of larvae (reviewed in Pineda et al. 2007, Cowen & Sponaugle 2009). In the case of coral reef fishes, adults tend to be site attached, while larvae can be dispersed over hundreds of km by ocean currents, before settling to suitable juvenile habitat (Leis 1991a). For many years, reef fish populations were therefore considered to be ecologically "open," i.e. receiving most of their larval supply from distant sites. Genetic comparisons among distant populations confirm that gene flow across broad biogeographic regions takes place (Planes 2002). However, an exchange of only a few individuals per generation is sufficient to connect populations genetically, while having little or no effect on ecology or population dynamics

.

Several recent studies have indicated that reef fish populations can be more ecologically "closed" than was previously thought, depending heavily on self-recruitment of larvae to the same location as where they were originally spawned . One type of study uses natural or artificial tags to trace the origin of juveniles following their successful recruitment to the reef. Trace-metal signatures deposited to larval otoliths during the pelagic larval period can be used to test whether larvae remained nearshore and (particularly in island environments) may therefore be of local origin (Swearer et al. 1999). Artificial tagging of the otoliths of reef fish embryos and subsequent recapture of tagged recruits can provide unmistakable evidence of self-recruitment (Jones et al. 1999, Jones et al. 2005, Almany et al. 2007). Various types of genetic markers can also be interpreted as tags, indicating the degree of larval exchange between populations (Taylor & Hellberg 2003, Jones et al. 2005, Gerlach et al. 2007). An entirely different approach that has been used to infer high levels of self-recruitment is the use of bio-physical models to simulate the dispersal and survival to recruitment of virtual larvae (Cowen et al. 2000, 2006).

Since any one location represents only a small fraction of potential settlement habitat, self-recruitment is inherently unlikely, unless physical or biological mechanisms specifically cause larvae to be retained or returned. Several physical oceanographic features that can result in retention have been identified (reviewed in Sponaugle et al. 2002). Episodic onshore flow at the ocean surface can be caused by passing (Shanks 1983) or breaking (Pineda 1994) internal waves. Similarly, larvae towards the bottom of the water column can experience onshore movement associated with tidal bores (Leichter et al. 1996). Entrainment of larvae in frontal eddies can aid in

concentrating and retaining of larvae (Limouzy-Paris et al. 1997, Lee & Williams 1999, Sponaugle et al. 2005). Nevertheless, it seems likely that the highly variable nature of physical oceanography generally favors dispersal as opposed to retention of larvae. Larval behavior, on the other hand, should theoretically favor self-recruitment, because behavior leading to retention in a "known good" environment can become an evolutionary stable strategy, while behavior that increases dispersal into an unknown environment should be eliminated by natural selection (reviewed in Strathmann et al. 2002). High levels of self-recruitment thus raise the question of whether larval behavior has an important influence on larval transport and connectivity.

Several lines of evidence support the hypothesis that larval behavior can affect transport. First, larvae of different species occupying the same environment exhibit different transport patterns. While some species remain within the boundaries of specific ichthyoplankton assemblages, others are transient members of different assemblages over time (Cowen et al. 1993). Similarly, spatial (Sponaugle & Cowen 1996, Wilson & Meekan 2001) and temporal (Robertson 1992, Sponaugle & Cowen 1997) patterns of settlement can differ among species that co-occur in the plankton. Thus, transport is determined only in part by the physical environment.

Second, vertical distributions of fish larvae can be dynamic. In coral reef fish families, larvae sometimes assume stratified vertical distributions during the day, but uniform distributions at night (Leis 1991b, Gray 1998). In some species, vertical migrations associated with growth and development (Cowen 2002) result in transitions from depths with offshore flow to depths with onshore flow (Paris & Cowen 2004). Vertical distributions of some (non-reef) larvae have even been linked directly to

vertical swimming behaviors in response to light and water chemistry (Forward et al. 1996), as well as temperature (Olla et al. 1996). The behavioral mechanisms by which larvae regulate their depth in the water column are thus potentially of general importance for larval transport.

Third, coral reef fish larvae develop into powerful swimmers towards the end of the larval period, and may influence their transport directly by horizontal swimming (reviewed in Leis 2006). Swimming speeds of reef fish larvae measured in the laboratory (Stobutzki & Bellwood 1997, Fisher et al. 2000) and *in situ* (Leis & Carson-Ewart 1997) are sufficient for larvae to cover distances of several km every day. However, larvae must be able to orient to use their swimming abilities efficiently (Armsworth 2001). Among other cues (reviewed in Kingsford et al. 2002), larvae may use sounds (Tolimieri et al. 2004, Simpson et al. 2005) or smells (Atema et al. 2002, Gerlach et al. 2007) originating from coral reefs as beacons for orientation. Estimates for the distance at which larvae can detect suitable settlement habitat range from <1 km to >20 km, depending on the species, cue, and location (Egner & Mann 2005, Wright et al. 2005, Gerlach et al. 2007). Orientation with respect to reefs ~1 km distant has been observed *in situ* (Leis et al. 1996, Leis & Carson-Ewart 2003).

Fish larvae have traditionally been treated as passive particles with no influence on their distribution and movement (Leis 1991a). Under this simplifying assumption, larval transport is determined entirely by physical oceanographic features such as currents, eddies and fronts. Consequently, the behavioral ecology of larvae has been given relatively little attention and is poorly understood. In light of the above evidence

to the contrary of the simplifying assumption, it has become important to fill the knowledge gap surrounding larval reef fish behavior.

Unfortunately, the behavioral ecology of pelagic reef fish larvae is a difficult subject to study. Larvae are small (mostly <2 cm), occur at low densities (in our study area ~50 per 1000 m^3), and are almost transparent. Consequently, direct observations of undisturbed larvae are practically impossible, and some sort of sampling gear must be used to acquire larvae for study. In the process of sampling, larvae are often injured or killed due to their fragile morphology, and those that escape injury may be stressed and thus behave unnaturally. Finally, the identification of larvae, dead or alive, presents its own challenges. Many species simply cannot be distinguished by appearance, yet species-specific behaviors may be important.

The common objective of the four studies comprising this dissertation was to identify specific behaviors of coral reef fish larvae that may influence larval transport. Several complementary methods were employed, including the deduction of vertical migration behavior from vertical distributions, controlled stimulus-response laboratory experiments, *in situ* observations of larvae reintroduced into the pelagic environment, and computer simulation models. Each method was used to approach the shared goal from a different angle, each with unique strengths, weaknesses, and challenges. Much of the research was interdisciplinary in nature, building to varying degrees on the principles of behavioral ecology, biological and physical oceanography, fisheries science, sensory biology, statistical computing, and modeling. Throughout the dissertation there was a strong emphasis on quantifying any observed patterns and the spatial scale at which they affected larval transport. Working at a quantitative level was

beneficial, in that it enabled rigorous hypothesis-testing despite complex ecological data. More importantly, it will benefit others working in the same field in the future, by facilitating meaningful comparisons between new research and the results presented here.

Outline

The first three data chapters (Chapters 2-4) address the vertical component of larval swimming and orienting behavior. In an environment where currents vary in magnitude or direction with depth, larval transport may be affected by depth-regulating behavior, manifested in vertical distributions and in some cases involving predictable vertical migrations. Chapter 2 focuses specifically on the influence of exogenous environmental factors on empirical vertical distributions of coral reef fish larvae. Offshore vertical distributions of coral reef fish larvae and a suite of potentially important environmental factors were sampled simultaneously in the Straits of Florida. Over the course of three 42-48 h sampling periods in spring, summer, and fall 2003, a broad range of environmental variability was encountered, ranging from the predictable (seasonal changes in temperature, diel changes in light, and vertical gradients in many variables) to the stochastic (changes in turbulence and zooplankton biomass). Statistical models were fit to data from each season and then used to predict larval vertical distributions during the other seasons.

Chapter 3 is based on the same larval fish collections as Chapter 2, but examines the effects of endogenous variables on vertical distributions, with specific focus on identifying vertical migrations and their effects on larval transport. For over 7,000

larvae of the most common reef fish taxa, length was measured and developmental stage was determined. Vertical migrations associated with growth and development were detected by comparing distributions of larvae at different sizes and stages. Diel vertical migrations were identified by comparing daytime and nighttime distributions. Finally, shipboard measurements of currents at depth were used to estimate larval transport at various depths.

Chapter 4 examines the role of hydrostatic pressure in vertical orientation by pelagic coral reef fish larvae. Larvae collected in plankton net tows were placed in a hyperbaric chamber and subjected to a sequence of different pressure levels. When larvae swam up in response to high pressure and down in response to low pressure, pressure preferences were calculated. Pressure preferences were then compared to capture depths of the same larvae to test the hypothesis that depth regulation via pressure cues is consistent with depth regulation taking place *in situ*.

Chapter 5 addresses the horizontal orientation and swimming behavior by settlement stage larvae. Larvae that are competent to settle are often fast swimmers, and their horizontal transport may depend directly on swimming behavior as well as currents. Settlement stage larvae were caught in light traps over coral reefs in the Florida Keys. They were then released 1 km offshore of the reef and their swimming trajectories were recorded by SCUBA divers for up to 10 min. The "straightness" of individual swimming trajectories and the degree to which different larvae swam in similar directions were analyzed using circular statistics. Empirical data were then combined with a simulation model to generate swimming trajectories of much longer duration than could feasibly be observed directly. Simulated swimming trajectories

were used to quantify the contribution of swimming behavior to larval transport at various temporal and spatial scales.

The summary chapter (Chapter 6) synthesizes the findings from the four data chapters. Relationships between vertical and horizontal swimming and their effects on alongshore and cross-shore transport are examined. Proximate and ultimate causes of behavior are discussed, with a specific focus on the role of hydrostatic pressure in depth-regulation. Promising future directions for behavioral ecology research with fish larvae are suggested.

The dissertation as a whole lends further support to the hypothesis that larval behavior is an important component of larval transport processes. Moreover, the presented research is one of the most comprehensive investigations to date of larval reef fish behaviors pertaining to larval transport. The findings will hopefully inspire further interest in this subject and be of value to those attempting to model reef fish population connectivity.
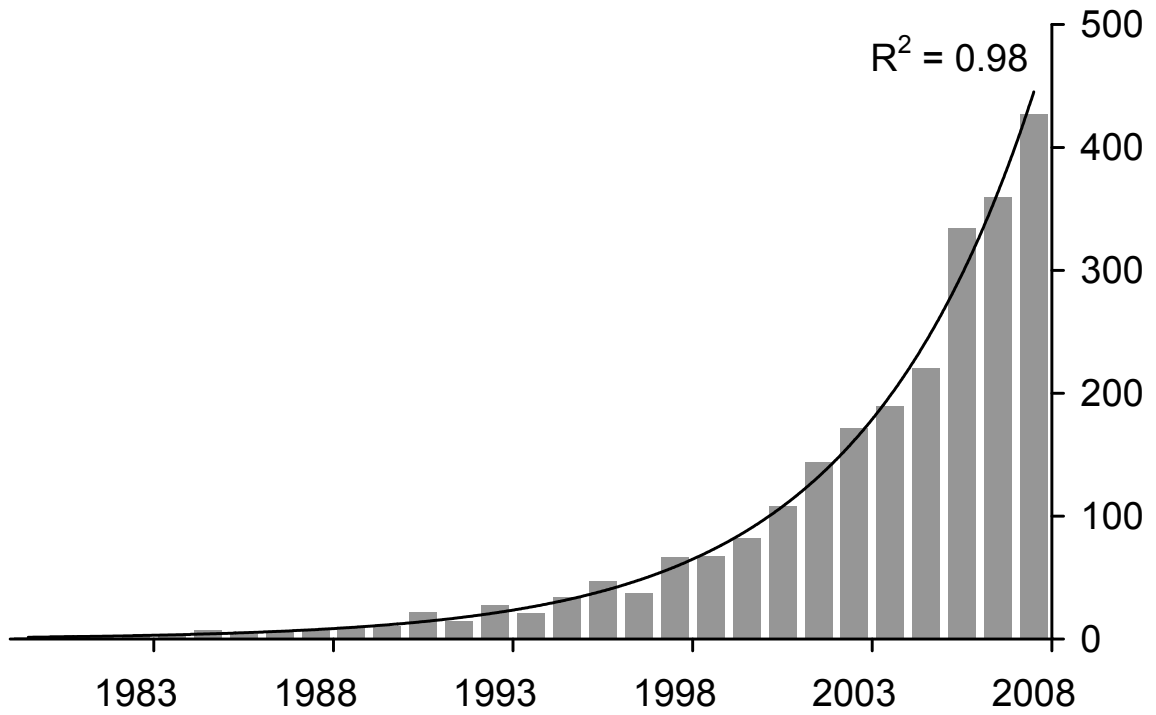
Figure 1.1. Exponential growth in the number of publications using the keyword "population connectivity" by publication year. Source: ISI Web of Knowledge (accessed 1 Mar. 2009, www.isiknowledge.com).

CHAPTER 2. PREDICTING VERTICAL DISTRIBUTIONS OF REEF FISH LARVAE IN THE FLORIDA STRAITS FROM ENVIRONMENTAL FACTORS

Background

Fish larvae, like other zooplankton, are rarely distributed randomly in the water column. Instead, they assume distinctive vertical distributions depending on the species and environment (reviewed in Heath 1992). Since the ocean is vertically stratified with respect to many physical and biological factors, vertical distributions can greatly affect essential ecological processes such as feeding, transport, growth, and survival. In turn, various environmental variables are thought to influence vertical distributions. Important physical factors include visible light (Forward et al. 1996), UV radiation (Browman 2003), pressure (Chapter 4), turbulence (Werner et al. 2001), temperature (Olla et al. 1996), and salinity (Lougee et al. 2002). Vertical patterns of predators and prey are also considered extremely important (Fortier & Harris 1989, Heath 1992). While some factors may act directly, for example, physical mixing or predation mortality, the influence of most factors is mediated indirectly by larval behavior. Depending on their swimming abilities, larvae can actively seek out or avoid specific environmental conditions by vertical swimming (Olla et al. 1996). Depending on the type and development of their swim-bladder, some larvae can even remain in a preferred environment without expending the energy to swim, by maintaining neutral buoyancy (Govoni & Hoss 2001).

Coral reef fish larvae have a high degree of control over their vertical movements, and are therefore likely to actively regulate their depth in the water column (Leis 2004). Reef fish larvae are generally fast swimmers (reviewed in Leis 2006) and

develop physoclistous swim-bladders, meaning they can achieve neutral buoyancy at depth without having to gulp air at the surface (Pelster 2004). Vertical distributions of common reef fish larvae adjacent to reefs (<20 m bottom depth) have been studied in the Great Barrier Reef (Leis 1986, 1991b, Fisher 2004), the Florida Keys (Sponaugle et al. 2003), and Caribbean Panama (Hendriks et al. 2001). Offshore distributions (>100 m bottom depth) have been reported for several families in the southern Straits of Florida (Cha et al. 1994, Limouzy-Paris et al. 1997) and off Barbados (Cowen 2002). Most larvae sampled during the day and some sampled during the night exhibit significantly non-random distributions. Significant differences between day and night (Leis 1986, 1991b) and between early and late stage larvae (Cowen 2002) reveal the dynamic nature of vertical patterns and suggest that vertical migrations are common. However, none of the above studies quantitatively examined the role of environmental factors in shaping the observed distributions.

In the present study, offshore vertical distributions of coral reef fish larvae and a suite of potentially important environmental factors were sampled simultaneously in the Straits of Florida. Over the course of three 42-48 h sampling periods in spring, summer, and fall 2003, a broad range of environmental variability was encountered, ranging from predictable (seasonal changes in temperature, diel changes in light, and vertical gradients in many variables) to stochastic variability (changes in turbulence and zooplankton biomass). The objective was to move beyond qualitative descriptions of larval vertical distributions towards quantitative hypothesis-testing regarding larval distributions under specific environmental conditions. To achieve this, larval vertical

distributions during each sampling period were predicted by statistical models fit to data excluding the sampling period being predicted.

Methods

   Sampling. To characterize the vertical distributions of fish larvae in the Straits of Florida across a wide range of environmental conditions, three time-series of biological and physical measurements were collected in spring, summer, and fall 2003. All time-series involved repeated sampling of the water column every 3 h for two diel cycles. Sampling was conducted from the University of Miami RV F. G. Walton Smith as part of an interdisciplinary study of billfishes. For more details, see Llopiz & Cowen (2008). During the spring time-series from April 7 to 9 and the fall time-series from September 30 to October 2, the vessel maintained position at a station ~30 km SSE of Miami (N 25.5°, E 80.06°) with a bottom depth of ~130 m for 48 h of sampling. During the summer time-series from July 31 to August 2, the vessel maintained position at an adjacent station (N 25.5°, E 80.05°) ~1 km farther offshore with a bottom depth of ~160 m for 42 h of sampling.

   Ichthyoplankton samples were collected by towing a coupled asymmetrical MOCNESS (Multiple Opening Closing Net with Environmental Sampling System) (Guigand et al. 2005) obliquely from 100 m depth to the surface at a tow speed of 1.5 m s^{-1}. Two sets of nets, one with 1×1 m mouth opening and 150 μm mesh and one with 2×2 m mouth opening and 1 mm mesh were opened and closed sequentially such that a different pair sampled from 100-75, 75-50, 50-25, and 25-0 m, respectively. The volume sampled by each net was calculated from flow through the net (MOCNESS

flowmeter) and the mouth opening of the net corrected by its angle of attack (MOCNESS frame angle sensor). Additional sampling of the upper 0.5 m of the water column was conducted by towing adjoined neuston nets (0.5 × 1 m with 150 μm mesh, 2 × 1 m with 1 mm mesh) after each MOCNESS tow. Plankton samples were immediately fixed in 95% ethanol and later transferred to 70% ethanol for long-term storage. Larval fishes from only the 1 mm mesh nets were identified to family following Richards (2006). Larvae from the most abundant coral reef fish families were then further identified to the lowest possible taxonomic level. The settled plankton volume in samples from 150 μm mesh nets was used to estimate zooplankton bimass.

Physical measurements recorded by the MOCNESS included depth, temperature, salinity, chlorophyll fluorescence (in summer and fall), and light (downwelling photosynthetically active radiation; in fall). Following each MOCNESS tow, a higher resolution vertical profile of depth, salinity, temperature, fluorometry, oxygen saturation, and light transmission was collected with a CTD (Conductivity Temperature Depth) instrument. Continuous light data recorded at the highest point of the vessel were 1-h low-pass filtered and used to determine sunrise and sunset and to supplement incomplete light-at-depth measurements (corrected by CTD light transmission data). Light measurements were log transformed. For all variables, the measurement taken closest to the midpoint of the sampled depth range was used to characterize the sample. Hourly wind speeds were obtained from the nearby NOAA Fowey Rocks weather station. Water level measurements at the nearby NOAA Virginia Key station were used as a tidal phase index.

Data analyses. A variety of statistical techniques were implemented, using the software package R, to identify relationships between vertical distributions of larvae and their environment. Significance testing was complicated by the large number of variables and by the uncertain degree of statistical independence among larvae from the same net, adjacent nets from the same tow, and different tows from the same cruise. To reduce the chance of type I errors (incorrectly rejecting a null hypothesis), associations between variables were only considered significant if consistent patterns were present in all analyzed sampling periods. If fewer than 30 larvae of a particular taxon were collected during a particular cruise, those data were considered insufficient for analysis and excluded. Counts of larvae from each taxon in each sample were converted to densities per 1000 m^3 and square-root transformed to reduce the effects of rare disproportionately high counts (Sokal & Rohlf 1995). For statistical modeling, densities were then normalized such that the sum of all values for each taxon and cruise was equal to one. The resulting unitless "relative larval density" metric expressed the contribution of each sample to the total density in that particular sampling period. The use of relative density values allowed for meaningful comparisons of vertical distributions among seasons with different absolute densities.

Linear models and GAMLSS models (General Additive Model of Location Scale and Shape) were fit to the data with relative larval density as the dependent variable and environmental factors at depth (including interaction terms) as explanatory variables. GAMLSS is a new technique that fits specific distributions to empirical data, by simultaneously optimizing parameters for location (e.g., mean) scale (e.g., variance) and shape (e.g., skewedness) of the distribution (Stasinopoulos & Rigby 2007,

Hernandez et al. in review). Ecological density datasets have two properties that make fitting simple distributions difficult: (1) negative densities are meaningless and (2) densities of zero are common. To model these properties we used zero-truncated t-distributions and zero-inflated beta-distributions in GAMLSS models. Nevertheless, the results of these models were generally driven by linear terms, and therefore were very similar to linear models. Consequently, only the results of linear models are presented. Akaike's AIC (An Information Criterion) was used as a guideline for selecting explanatory variables to include in the models. AIC measures the degree to which a model captures the information contained in a data set while penalizing the model for each additional factor, thus limiting over-parameterization. Since AIC does not measure the statistical significance of a model, the predictive value of models was tested by cross validation: a model fit to "training data" from one or two cruises was used to predict relative densities in the third cruise based on environmental data only. The significance of each model was gauged by a permutation test of the correlation between predicted and observed relative densities (Hesterberg et al. 2005).

A second analysis was conducted with the depth at which larvae were collected as the response variable. This was accomplished by bootstrapping (drawing with replacement) samples of 1000 larvae for each taxon from the original samples. The probability of drawing a larva from any particular sample was set to the square-root transformed relative larval density of the relevant taxon. The resulting bootstrapped samples were analyzed as described above by cross validation of fitted linear and GAMLSS models followed by permutation tests for significance testing. The strength of the second analysis was that variables characterizing the entire water column, as

opposed to a specific sample, could be used as explanatory factors. Mixed layer depth, for example, could not have been used to predict larval densities in specific samples, but might nevertheless affect larval vertical distributions. The strength of the first approach was that the presence or absence of larvae from samples could be examined. In the second analysis this was impossible, because there was no way to calculate the depth of "absent fish."

Finally, similarities in the taxonomic composition of samples were analyzed. Bray-Curtis dissimilarity matrices of square-root transformed larval densities were used to perform Kruscal's NMDS (Non-metric MultiDimensional Scaling). The Bray-Curtis index is widely used to measure ecological similarity across species and habitats, and NMDS is an ordination technique for visualizing the clustering of data in multidimensional space. A strength of NMDS is that unlike most ordination methods, NMDS neither requires that data meet restrictive parametric assumptions, nor forces the clustering to conform to externally imposed explanatory variables (Legendre & Legendre 1998). This makes NMDS a powerful tool for exploratory data analysis of ichthyoplankton assemblages (e.g. Gray 1998).

Results

Larval composition. Over the course of three cruises, 8,529 larvae representing at least 34 families strongly associated with coral reefs (Leis 1991a) were collected in MOCNESS 4 m^2 net samples (Table 2.1). Samples from one net tow in spring were excluded because MOCNESS nets did not open and close at the correct depths. Larvae from the most abundant reef fish families could be grouped into 34 taxa. Most of these

taxa are commonly observed on coral reefs in the Florida Keys (Bohnsack et al. 1999) and elsewhere in the western central Atlantic (Humann & Deloach 2002). Various species in the subfamily Anthiinae, including the most abundant taxon of the study (*Hemanthias vivanus*) are common on reefs deeper than 70 m (Hastings 1981), but less well known. Most species of the family Scorpaenidae (the second most abundant taxon) are associated with reefs, but some inhabit other benthic habitats.

Samples collected in summer and fall were more similar to each other, in terms of taxonomic composition of reef fish larvae, than to samples collected in spring (Fig. 2.1. At the same time, samples formed clusters following an easily recognizable gradient in depth range from the deepest (75-100 m) to the most shallow (0-25 m) (Fig. 2.1). The combined density (untransformed) of reef fish larvae per 1000 m^{-3} of water in 1mm mesh MOCNESS samples from all depth ranges was 58 in spring, 19 in summer, and 46 in fall. In several taxa, densities were much higher during either spring or fall than in the other two seasons. *Hemanthias vivanus* made up 48% of the combined density in spring but only 3% in summer and 2% fall. Scorpaenids made up <1% of the combined density in spring, 1% in summer, and 31% in fall. Combined densities of larvae excluding the two above taxa were quite similar among seasons: 30, 18, and 31 larvae per 1000 m^{-3} in spring, summer, and fall, respectively

Only taxa with 30+ sampled larvae in at least two cruises were included in data analysis: n = 2169 *H. vivanus*; 1272 Scorpaenidae; 376 Gobioidei; 370 *Pristipomoides* spp. from summer and fall; 305 Epinephelini; 302 Serraninae; 261 Priacanthidae; 258 Holocentridae; 249 *Sparisoma* spp.; 189 Sphyraenidae; 174 Acanthuridae from summer and fall; 166 Pomacentridae excluding the genera *Abudefduf* and *Chromis* (hereafter

referred to as pomacentrids); and 153 Apogonidae from spring and fall. Labridae were

excluded because the vertical distributions of different genera ranged from very shallow

(*Lachnolaimus maximus*, *Doratonotus megalepis*) to very deep (*Decadon puellaris*),

and no genus was present in sufficient numbers to be included. Tetraodontidae were

excluded because MOCNESS samples alone did not accurately represent the

population; a disproportionately greater number of tetraodontid larvae was collected in

neuston net samples at <0.5 m depth.

Approximately half of the larvae were collected during the day and half during

the night (Table 2.2). However, the number of *Sparisoma* spp. larvae per 1000 m^3 in the

daytime samples accounted for <20% of the total. For most analyzed taxa, the highest

numbers of larvae were collected at 0-25 m depth and the lowest numbers as 75-100 m

depth (Fig. 2.2). Exceptions were the Gobioidei and scorpaenids, where counts were

highest in 75-100 m samples.

Statistical models. The environmental conditions under which larvae were

collected varied substantially both among and within cruises (Fig. 2.3, Table 2.3).

Typically, the models predicting larval distributions from these variables best, in terms

of AIC, included multiple factors and interaction terms that only fit the "training data,"

but did not improve predictions for other cruises. Best predictions were achieved by

models using only one explanatory factor.

Models with significant (permutation test: p <0.05) and cross-validated (at least

two sampling periods) predictions for relative larval density were achieved in seven out

of the 13 analyzed taxa (Table 2.4). Correlations were primarily driven by the presence

or absence of larvae in different samples, and to lesser extent by differences among

positive densities. Depth was the single most predictive factor for Epinephelini,

Holocentridae, Priacanthidae, *Pristipomoides* spp., and Sphyraenidae, all but one subset

with $R^2 \sim 0.31$-0.50 (Fig. 2.4A-E). In some cruises, relative densities of the above taxa

were also related to other factors, such as oxygen saturation and light, but in all cases,

the other factors were correlated with depth (Appendix A), and had less predictive value

than depth.

Predictable correlations between relative larval densities and settled zooplankton

volume (but not zooplankton normalized to the entire water column) were present in

apogonids (Fig. 2.4F, spring: $R^2 = 0.18$, summer: no data, fall: $R^2 = 0.16$). Relative

densities of larval *Sparisoma* spp. were negatively correlated with surface-light (spring

$R^2 = 0.10$, summer $R^2 = 0.23$, fall $R^2 = 0.10$; permutation test: $p < 0.05$), but not with

light-at-depth, indicating significant net avoidance (Fig. 2.3G).

Cross-validated models predicting larval depth were not achieved for any taxa.

In the unique case of pomacentrids, the depth of larvae was significantly positively

correlated with surface-light in all three seasons (spring $R^2 = 0.14$, summer $R^2 = 0.37$,

fall $R^2 = 0.17$; permutation test: $p < 0.05$), revealing a diel vertical migration in this

group (Fig. 2.5). Pomacentrid larvae consistently occupied shallower depth ranges

during the night than during the day. However, both diel and nocturnal distributions

were somewhat different in each season, and only the model for summer, based on

spring and fall training data, predicted depths with better accuracy than the mean of the

data ($R^2 = 0.10$; permutation test: $p < 0.05$).

Discussion

Vertical distributions are vital to the ecology of fish larvae, because the biological and physical factors that define their environment change dramatically with depth. Since the early days of vertically stratified ichthyoplankton sampling (e.g., Ahlstrom 1959) it has been clear that larval vertical distributions can be dynamic, yet somewhat predictable. Unfortunately, very few studies (e.g., Heath et al. 1988) have attempted to quantitatively predict distributions.

Depth-regulating behavior. Of the environmental factors examined here, depth was the best predictor of densities of reef fish larvae, resulting in significant cross-validated predictions for five taxa. Including other explanatory variables in addition to depth did not improve the accuracy of model predictions. Depth models were particularly good at predicting the presence or absence of larvae in samples, i.e. larvae were often present in samples with high predicted densities and rarely present in samples with low predicted densities. In many cases, depth models also predicted differences in magnitude, i.e. higher larval densities were observed in samples with higher predicted larval densities and *vice versa*. In all five taxa, the depth / larval density relationship was quite stable in the face of substantial environmental variability, including seasonal changes in temperature, diel changes in light, and stochastic changes in turbulence. With respect to larvae of Epinephelini, holocentrids, priacanthids, *Pristipomoides* spp., and sphyraenids, depth may generally account for 38% of the variability in larval densities. These values are conservative (low) estimates because the

upper limit that could realistically be predicted by models was less than 100%, due to horizontal patchiness in larvae passing by our fixed sampling station.

Our findings imply that larvae exhibited behavioral preferences for specific depths, as opposed to the other variables. While pelagic fish larvae may often be too deep to directly perceive their depth in terms of distance from the surface, they can perceive hydrostatic pressure (Qasim et al. 1963, Govoni & Hoss 2001), which may be functionally equivalent. Depth-regulating behavior of coral reef fish larvae by means of hydrostatic pressure cues has been demonstrated in controlled laboratory experiments (Chapter 4). While we cannot rule out behavioral preferences for factors we did not explicitly address, depth-regulation via pressure presents a parsimonious explanation for the observed patterns.

All taxa with significant depth / larval density relationships accumulated at 0-25 m, with few larvae caught >50 m depth. This common pattern (Heath 1992) is often interpreted as an effect of the pycnocline (Palomera 1991, Coombs et al. 2001), sometimes without rigorous hypothesis-testing. While there is strong experimental evidence that some larvae avoid low temperature (Olla et al. 1996) or high salinity (Lougee et al. 2002), many larvae aggregate in the upper 50 m independent of the strength, location, or even presence of a pycnocline (Conway et al. 1997, Olivar & Sabates 1997). In our study, the main pycnocline started at a depth of ~70 m in spring, ~30 m in summer, and ~50 m in fall and continued past the 100 m limit of MOCNESS sampling in all seasons. No consistent effects of either temperature or salinity on larval distributions were apparent.

Factors related to feeding and predation. Other environmental factors that are frequently used to explain larval vertical distributions include light, turbulence, and zooplankton concentration, all of which affect larval feeding. Obviously, high concentrations of zooplankton prey are favorable for feeding. As visual predators, larvae also depend on light to detect, pursue, and attack their prey (Blaxter 1986, Job & Bellwood 2000). Small scale turbulence increases the rate of random prey encounters (Rothschild & Osborn 1988), but can interfere with successful prey capture when strong (MacKenzie & Kiorboe 2000). Given at least some light and some prey, vertical gradients in the above factors must cause a particular depth range to be most favorable for feeding. For example, in temperate continental shelf waters with strong seasonal stratification, the favorable range can correspond to the mixed layer (Buckley & Lough 1987), indicating once again the potential role of the pycnocline. In oceanic water around Barbados, the favorable range for *Thalassoma bifasciatum* is apparently correlated with the deep chlorophyll maximum layer (Cowen et al. 2003). Similar logic can be applied to predation on larvae, thus a particular depth range may also be most favorable for avoiding predators, and larvae may ultimately distribute in such a way as to optimize feeding and predation (Fortier & Harris 1989, Pearre 2003). Unfortunately, it has proven particularly difficult to obtain reliable measurements of predation (reviewed in Bailey & Houde 1989). Assuming that our measurements to some degree capture feeding and predation conditions, reef fish larvae did not generally adjust their vertical distributions in response to changing conditions within the three sampling periods.

The relative density of reef fish larvae was not predictably related to the relative density of zooplankton in any taxon. However, the relative density of apogonid larvae was predictably positively correlated with absolute zooplankton. This means that while apogonid larvae did not accumulate at depths rich in zooplankton, they did accumulate across the entire water column under high zooplankton conditions. The observed relationship may be caused by horizontal as opposed to vertical processes. Since our location was fixed for the duration of each sampling period, we inevitably sampled different water masses as they passed by the sampling station. Florida Current frontal eddies are known to accumulate both fish and invertebrate zooplankton off the Florida Keys (Limouzy-Paris et al. 1997, Lane et al. 2003). Correlated fluctuations in zooplankton and apogonids may have been caused by passing water masses previously associated with eddies. This hypothesis does not explain why relative densities of other taxa were not predictably affected. Correlations between zooplankton and relative larval density were present in spring *Hemanthias vivanus* as well as spring and summer pomacentrids, but not in summer or fall *H. vivanus* or fall pomacentrids (thus not considered significant).

Our only finding consistent with predator-prey related vertical movements was that surface-light and the depth at which we collected pomacentrid larvae were significantly correlated in all three seasons. However, models did not accurately predict larval depths, due to differences in vertical distributions among seasons. In other words, models predicted the presence of a significant linear regression, but not what the slope and intercept of the regression would be. The pattern did not suggest behavioral preferences for specific light levels, because relative pomacentrid densities were

unrelated to light-at-depth. Instead, a transition between the daytime distribution and the nighttime distribution coincided with changes in light. Zooplankton (including fish larvae) commonly use dusk and dawn as cues for synchronizing vertical migrations thought to enhance feeding during the day (at the risk of increased predation) and reduce predation at night (Forward 1989, Neilson & Perry 1990, Richards et al. 1996). In our case study, pomacentrid densities increased at 50-75 m depth during the day and increased at 0-25 m depth during the night. Since pomacentrids require daylight to feed (Job & Bellwood 2000), the nocturnal distribution may be related to predator-avoidance, but not feeding. The diel distribution may be related to predator-avoidance or prey densities, or both. Vertical migrations can also affect larval transport by placing larvae in different ambient currents at different depths (Chapter 3).

Net avoidance. The apparent net avoidance by *Sparisoma* spp. was unexpected, and remains somewhat of a mystery. There are two obvious ways that larvae could have avoided capture during the day. Either *Sparisoma* spp. moved to depths that were not sampled by the MOCNESS, or they evaded the sampling gear. *Sparisoma* spp. larvae were exceedingly rare in neuston net samples, and thus did not accumulate at the ocean surface. There was also no increase of larvae at 75-100 m either at night or in the few samples collected around dusk or dawn, suggesting that larvae did not move deeper than 100 m. However, we did not sample at >100 m and this cannot be entirely ruled out. The remaining explanation, visual net evasion, is common for some sampling methods (Ahlstrom 1959, Heath 1992), but surprising for a net with 4 m^2 mouth opening towed at ~1.5 m s^{-1}. While many settlement-stage reef fish larvae can maintain speeds of 0.4 m s^{-1} for short periods of time (reviewed in Leis 2006), smaller larvae are much slower

because their swimming performance is related to the propulsive area of their fins

(Fisher et al. 2000). *Sparisoma* spp. larvae were not larger or more developed than other

larvae and it seems unlikely that they consistently evaded nets when no other taxa did.

  Unexplained variability. One of our most striking results was the amount of

variability in larval vertical distributions that was unrelated to a broad range of

environmental factors. Larvae almost certainly have sufficient swimming abilities

(reviewed in Leis 2006) to assume vertical distributions matching their environmental

preferences. However, for about half of the analyzed taxa, we found no compelling

evidence that larvae sought out specific environmental conditions within the range

encountered in the Straits of Florida. There are several possible explanations for this

finding. First, unpredictable vertical distributions may reflect behavioral preferences for

environmental factors we did not address. For example, the vertical distributions of

specific prey species were not resolved by our methods, and predators were not

addressed at all. Second, larvae may exhibit seasonal variation in behavioral

preferences. A multi-year dataset would be required for cross-validations in this case.

Finally, unpredictable vertical distributions may reflect behavioral plasticity, with

different larvae exhibiting unique environmental preferences, perhaps related to

endogenous biological factors.

  Endogenous factors likely to interact with exogenous environmental variables in

determining vertical orienting and swimming behavior include species, developmental

stage, and satiation. With the exception of *H. vivanus*, the species composition within

our taxa may have varied among seasons, hindering the effectiveness of cross-

validations. Stronger patterns may emerge from species level identifications, possibly

by genetic techniques (e.g., Richardson et al. 2007). Larval size and developmental stage also may be important. For example, postflexion larvae tend to occupy deeper depths than preflexion larvae around Barbados (Cowen 2002). Finally, even among individuals of the same species and size, variations in satiation may lead to different vertical behavior (Pearre 2003). With respect to our data, this aspect could perhaps be addressed by larval gut content analysis (e.g. Llopiz & Cowen 2009).

Regional implications. Since the geographic ranges of reef fishes in the Western Central Atlantic generally span the entire Caribbean region and beyond, it may be possible to extrapolate from our study in the Straits of Florida to other locations in the region. While it would be imprudent to expect vertical distributions elsewhere to exactly mirror those in the Straits of Florida, larval behaviors are likely to be equivalent, since gene flow among Caribbean reef fishes limits their potential for local adaptations (Planes 2002). Unless exposed to environmental conditions outside the range addressed in our study, larvae should not be expected to seek out or avoid specific light, oxygen, phytoplankton, salinity, temperature, tidal phase, turbulence, water clarity, or zooplankton biomass conditions. Under the same caveat, larvae of some taxa, including representatives of the commercially important grouper (Epinephelini) and snapper (*Pristipomoides* spp.), should be expected concentrate in the upper 25 m of the water column, while pomacentrid larvae are likely to exhibit diel vertical migration behavior.

Table 2.1. Sample sizes of coral reef fish larvae collected in spring, summer, and fall MOCNESS tows at a fixed station in the Straits of Florida over two diel cycles from 0-100 m depth. Bold type indicates taxa included in vertical distribution models due to sample sizes of 30+ in at least two seasons. [1] Labridae were not pooled, because different genera exhibited dissimilar vertical distributions. [2] Mullidae and Tetraodontidae were caught in disproportionately larger numbers in neuston net tows at <0.5 m depth than in MOCNESS tows.

Taxon	Spring	Summer	Fall	Taxon	Spring	Summer	Fall
Acanthuridae	9	**45**	**129**	Opistognathidae	4		5
Apogonidae	**118**	10	**35**	Ostraciidae	2	4	6
Aulostomidae			2	Pomacanthidae		27	22
Balistidae	41	23	17	**Pomacentridae**			
Suborder Blennioidei	35	1		*Abudefduf* spp.	3	3	1
Callionymidae	14	20	49	*Chromis* spp.	1	0	4
Carapidae	12	15	41	**Other**	**44**	**47**	**75**
Chaetodontidae	6	4	12	**Priacanthidae**	**83**	**121**	**57**
Cirrhitidae			9	**Scaridae**			
Dactylopteridae		3		*Cryptotomus roseus*	5	9	32
Diodontidae		6		*Scarus* spp.	1	1	4
Fistulariidae	1		1	***Sparisoma* spp.**	**31**	**47**	**171**
Gerreidae	2	1	11	**Scorpaenidae**	**113**	**145**	**1014**
Suborder Gobioidei	**89**	**64**	**223**	**Serranidae**			
Haemulidae	3	3	20	**Subfamily Anthiinae**			
Holocentridae	**131**	**80**	**47**	*Anthias nicholsi*	138	4	29
Kyphosidae		2	4	*Anthias tennuis*	5		
Labridae[1]				*Anthias woodsi*	19	2	7
Clepticus spp.	2	1	8	*Hemanthias leptus*	351	18	17
Decodon puellaris	5	4	24	***Hemanthias vivanus***	**2070**	**37**	**62**
Doratonotus megalepis	1	1	18	*Plectranthias garruppellus*			2
Halichoeres spp.	11	6	9	*Pronotogrammus martincenensis*			3
Lachnolaimus maximus	4			Unknown	7		
Thalassoma bifasciatum	3	12	105	**Subfamily Epinephelinae**			
Xyrichtys spp.	1	25	265	**Tribe Epinephelini**	**33**	**30**	**242**
Lutjanidae				Tribe Grammistini	1	14	18
Subfamily Etelinae				Tribe Liopropomini		10	52
Etelis oculatus	1	1	48	**Subfamily Serraninae**	**154**	**33**	**115**
***Pristipomoides* spp.**	1	**145**	**225**	Sparidae	17		1
Subfamily Lutjaninae				**Sphyraenidae**	**33**	**76**	**80**
Rhomboplites aurorubens	10		23	Syngnathidae	13	3	3
Other	9	25	34	Synodontidae	16	1	59
Monacanthidae	76	15	18	Tetraodontidae[2]	41	14	35
Mullidae[2]	103	3	2				

Table 2.2. Percentages of common reef fish larvae sampled during daytime MOCNESS tows, corrected by sampled water volume. The upper 100 m of the water column was sampled repeatedly off Miami for two diel cycles during each of three seasons. With respect to Sparisoma spp. <20% of larvae were collected during the day and >80% during the night, revealing significant daytime net avoidance (permutation test: $p < 0.05$). Missing values indicate sample sizes <30.

Taxon	Spring	Summer	Fall
Acanthuridae		51	52
Apogonidae	62		70
Epinephelini	46	41	42
Gobioidei	46	44	45
Hemanthias vivanus	41	38	73
Holocentridae	33	43	53
Pomacentridae	51	52	51
Priacanthidae	41	48	62
Pristipomoides spp.		41	48
Scorpaenid	45	61	29
Serraninae	38	47	46
***Sparisoma* spp.**	**15**	**9**	**20**
Sphyraenidae	67	36	62

Table 2.3. Range of environmental variables characterizing spring, summer, and fall ichthyoplankton samples collected every 3 h in the Straits of Florida over 42-48 h from 0-100 m depth. A) Factors included in models predicting relative larval fish density represent individual samples, each measured at the mean depth of the sample (nominally 12.5, 37.5, 62.5, and 87.5 m). Light-at-depth was calculated from surface measurements of photosynthetically active radiation and CTD measurements of light transmission at depth. B) Factors included in models predicting larval fish depth characterize the state of the entire water column. Oxygen, salinity, and temperature measurements from depths with the greatest variability were included.

A

Factor	Unit	Source	Spring		Summer		Fall	
			Min	Max	Min	Max	Min	Max
Chlorophyll fluorescence	(arbitrary units)	CTD	0.09	1.33	0.05	0.64	0.09	0.80
Chlorophyll (relative)	% water column	CTD	6	54	6	59	6	55
Light-at-surface	μE m^{-2} s^{-1}	Ship	<15	1035	<15	995	<15	550
Light-at-depth	μE m^{-2} s^{-1}	Ship, CTD	<0.2	635	<0.1	474	<0.2	277
Oxygen saturation	%	CTD	66	98	60	99	80	100
Depth	m	MOCNESS	10	91	11	92	10	98
Salinity	(unitless)	MOCNESS	35.9	36.3	35.6	36.6	35.3	36.4
Temperature	° C	MOCNESS	16.6	25.5	15.4	29.6	21.3	29.4
Light transmission	% m^{-1}	CTD	94	97	93	95	94	95
Zooplankton settled volume	ml 1000^{-1} m^{-3}	MOCNESS	290	1140	190	800	260	1380
Zooplankton (relative)	% water column	MOCNESS	12	37	10	40	10	40

B

Factor	Unit	Source	Spring		Summer		Fall	
			Min	Max	Min	Max	Min	Max
Deep chlorophyll maximum	m	CTD	52	77	59	93	63	93
Light-at-surface	μE m^{-2} s^{-1}	Ship	<15	1035	<15	995	<15	550
Mixed layer depth	m	CTD	4	24	0	20	0	45
Oxygen saturation at 87.5 m	%	CTD	66	90	60	96	80	96
Salinity at 12.5 m	(unitless)	MOCNESS	36.2	36.3	36	36.2	35.3	35.9
Temperature at 12.5 m	° C	MOCNESS	24.5	25.5	29.3	29.6	29.0	29.4
Tidal phase index	arbitrary	NOAA	-1.2	1.3	-1.2	1.2	-1.2	1.2
Wind speed	kts	NOAA	0.8	11.7	1.2	8.4	1.4	7.4
Zooplankton center of mass	m	MOCNESS	44	56	43	54	39	53

Table 2.4. Summary of linear models with significant (permutation test: $p < 0.05$) cross validated value for predicting relative larval density from environmental factors. Models were fit using "training data" (Fit) excluding one season and then used to predict relative densities in the excluded season. In all cases, models using a single factor were superior to more complex models.

Taxon	Fit	Predicted season	Factor	R^2
Epinephelini	Spring & summer	Fall	Depth	0.50
	Spring & fall	Summer	Depth	0.39
	Summer & fall	Spring	Depth	0.15
Holocentridae	Spring & summer	Fall	Depth	0.31
	Spring & fall	Summer	Depth	0.44
	Summer & fall	Spring	Depth	0.36
Priacanthidae	Spring & summer	Fall	Depth	0.38
	Spring & fall	Summer	Depth	0.48
	Summer & fall	Spring	Depth	0.33
Pristipomoides spp.	Summer	Fall	Depth	0.36
	Fall	Summer	Depth	0.47
Sphyraenidae	Spring & summer	Fall	Depth	0.38
	Spring & fall	Summer	Depth	0.43
	Summer & fall	Spring	Depth	0.33
Apogonidae	Spring	Fall	Zooplankton	0.16
	Fall	Spring	Zooplankton	0.18
Sparisoma spp.	Spring & summer	Fall	Light-at-surface	0.10
	Spring & fall	Summer	Light-at-surface	0.23
	Summer & fall	Spring	Light-at-surface	0.10
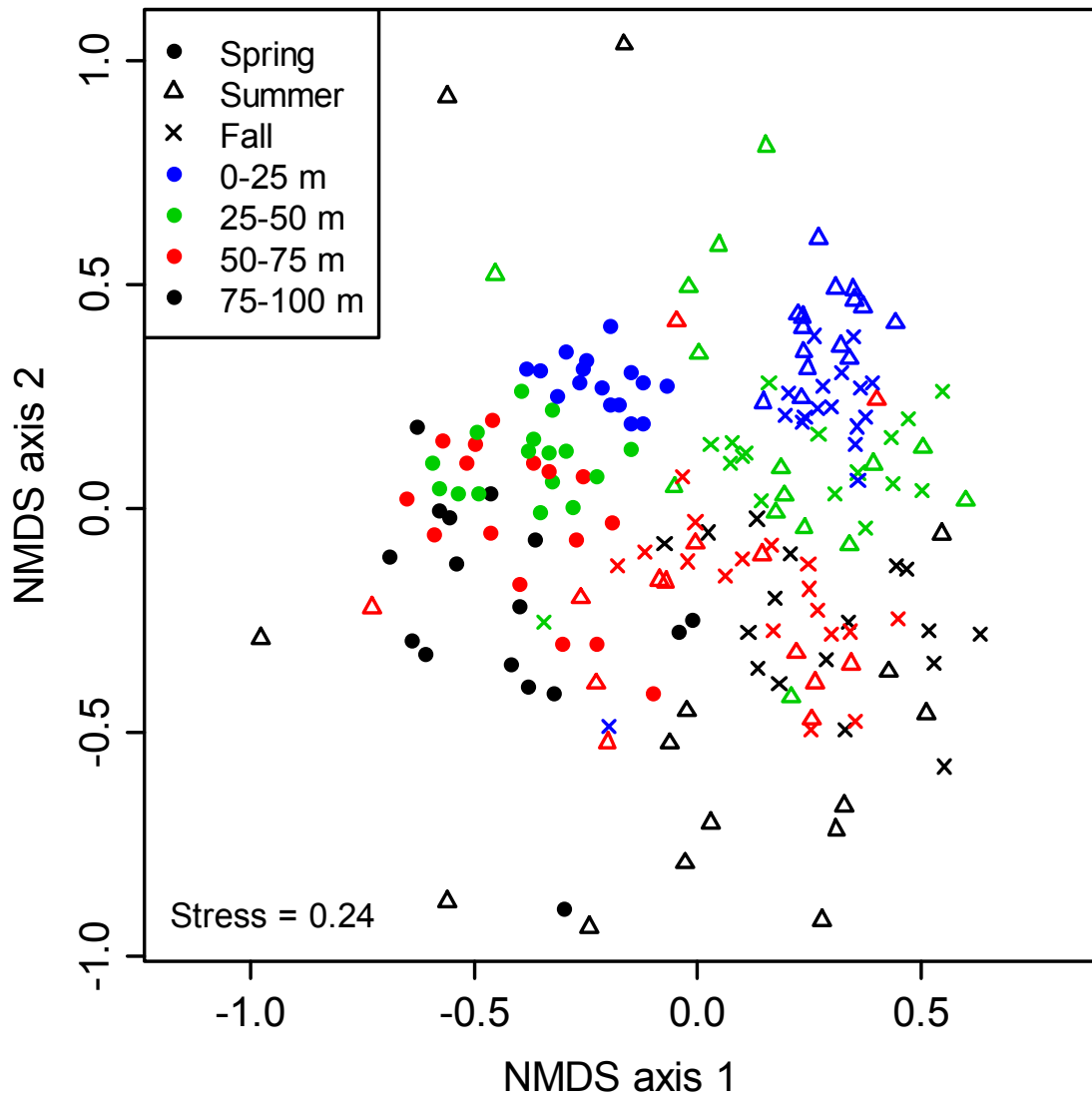
Figure 2.1. Two-dimensional ordination of ichthyoplankton samples, using Kruscal's non-metric multidimensional scaling of Bray-Curtis dissimilarity with respect to taxonomic composition across 56 coral reef fish taxa. Fish larvae were collected off Miami during three seasons at four depth ranges. Two strong patterns werre apparent: First, samples collected during spring cluster separately from summer and fall samples. Second, samples collected at different depths form a distinct gradient from shallow to deep.

Figure 2.2. Vertical distributions of larvae from 13 common coral reef fish taxa sampled at four different depth ranges in the Straits of Florida during three 42-48 h time-series in spring, summer, and fall 2003. Error bars represent bootstrapped 95% confidence intervals.[1] excluding the genera *Abudefduf* and *Chromis*.

Fig. 2.2

A   Acanthuridae

B   Apogonidae

C   Holocentridae

D   *Pristipomoides* spp. (Lutjanidae)

E   Pomacentridae[1]

F   Priacanthidae

Fig. 2.2

Fig. 2.2

Gobioidei

M

Depth range (m): 0-25, 25-50, 50-75, 75-100

Spring
Summer
Fall

0.0 0.2 0.4 0.6 0.8 1.0

Normalized square root transformed larval density
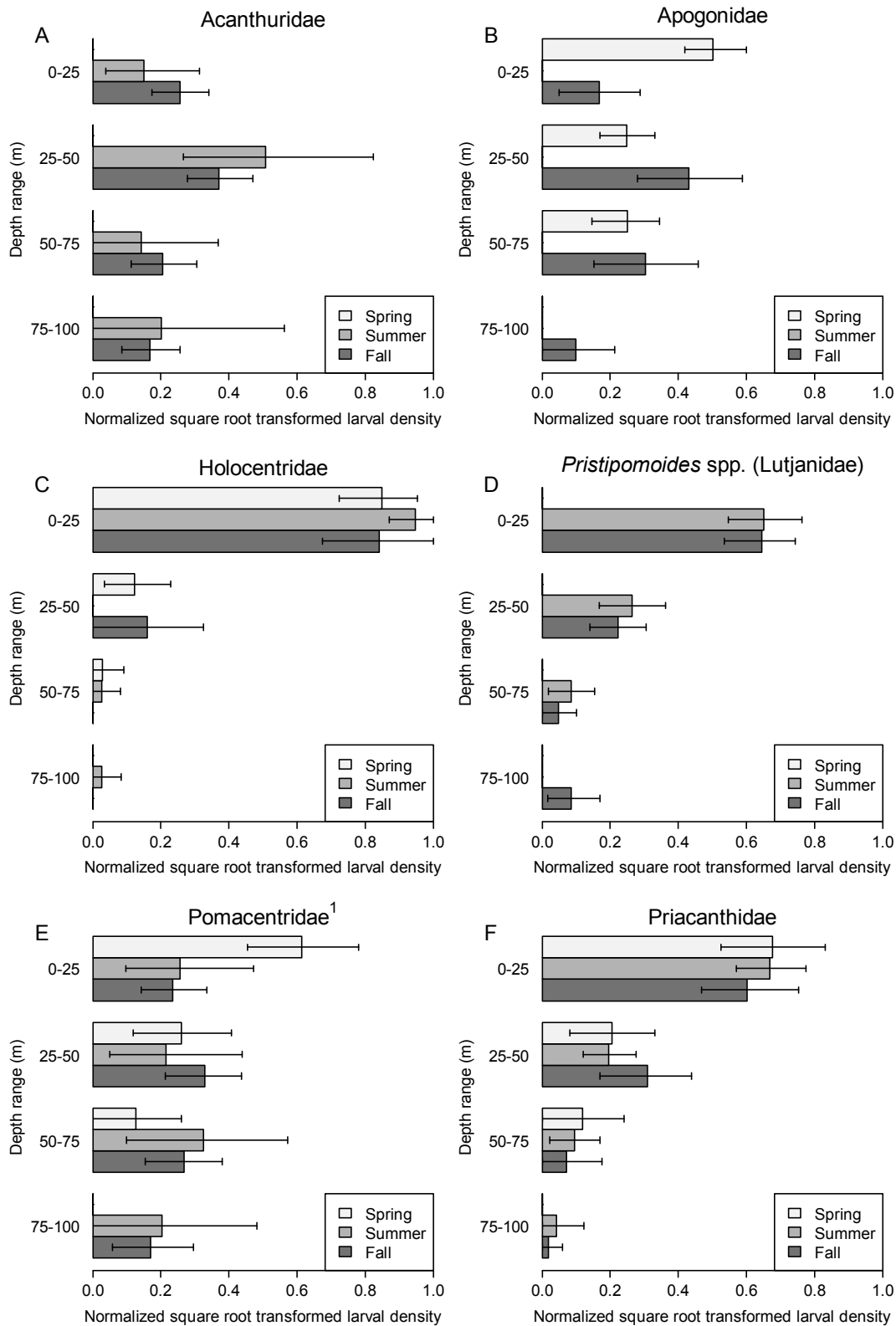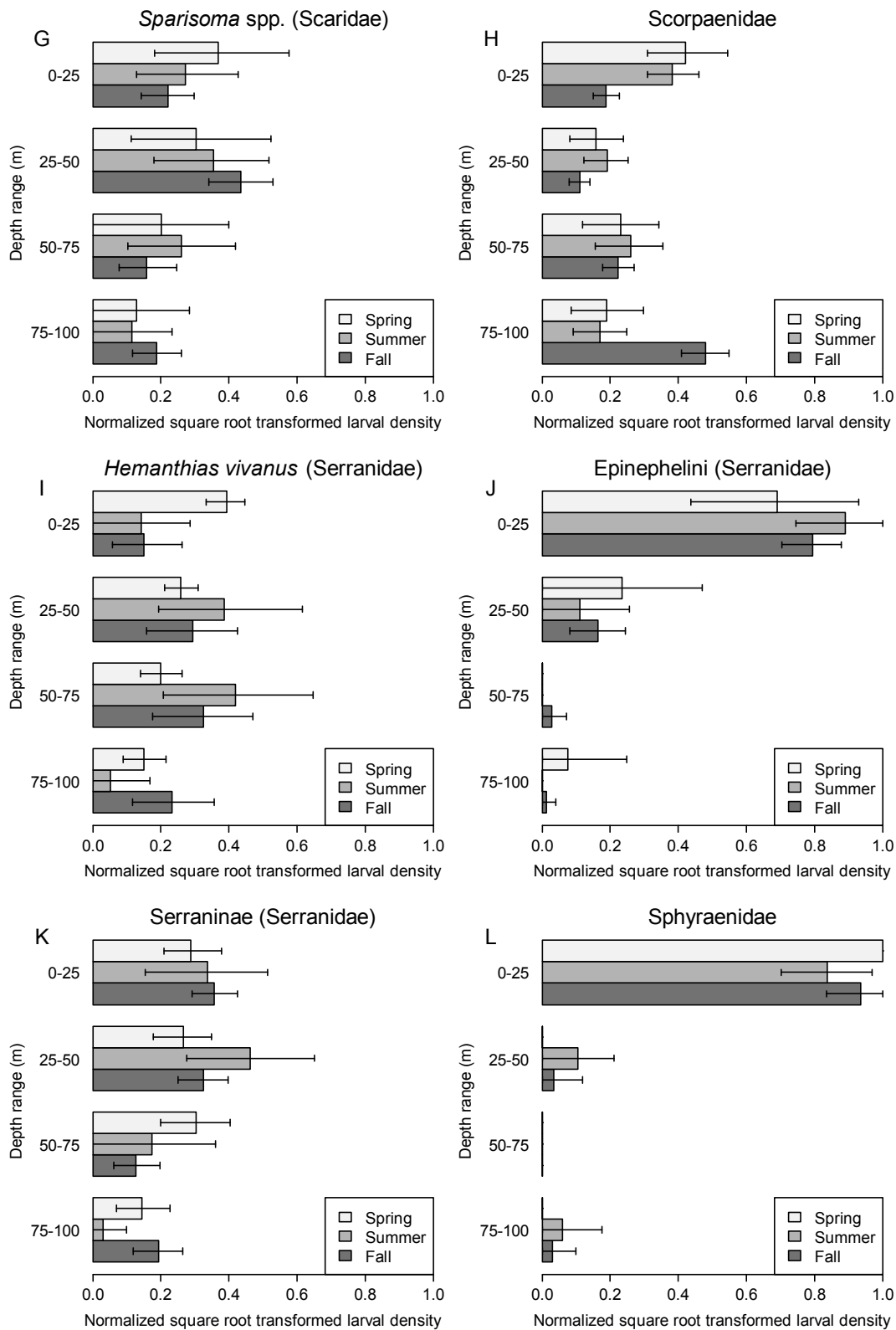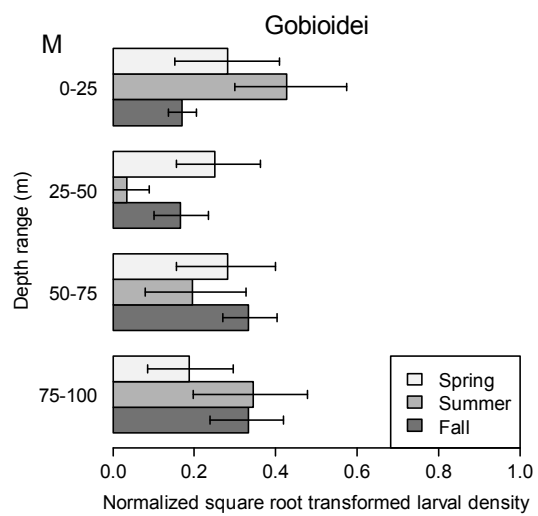
Figure 2.3. Examples of spring, summer, and fall depth profiles of environmental variables measured off Miami. Depth profiles were collected every 3 h for 42-48 h, and the presented profiles are not intended to be representative of the entire period. Gray shading corresponds to nominal depth ranges at which discrete ichthyoplankton samples were collected. Points indicate values at midpoints of depth ranges, which were used to characterize samples in statistical models.

Fig. 2.3
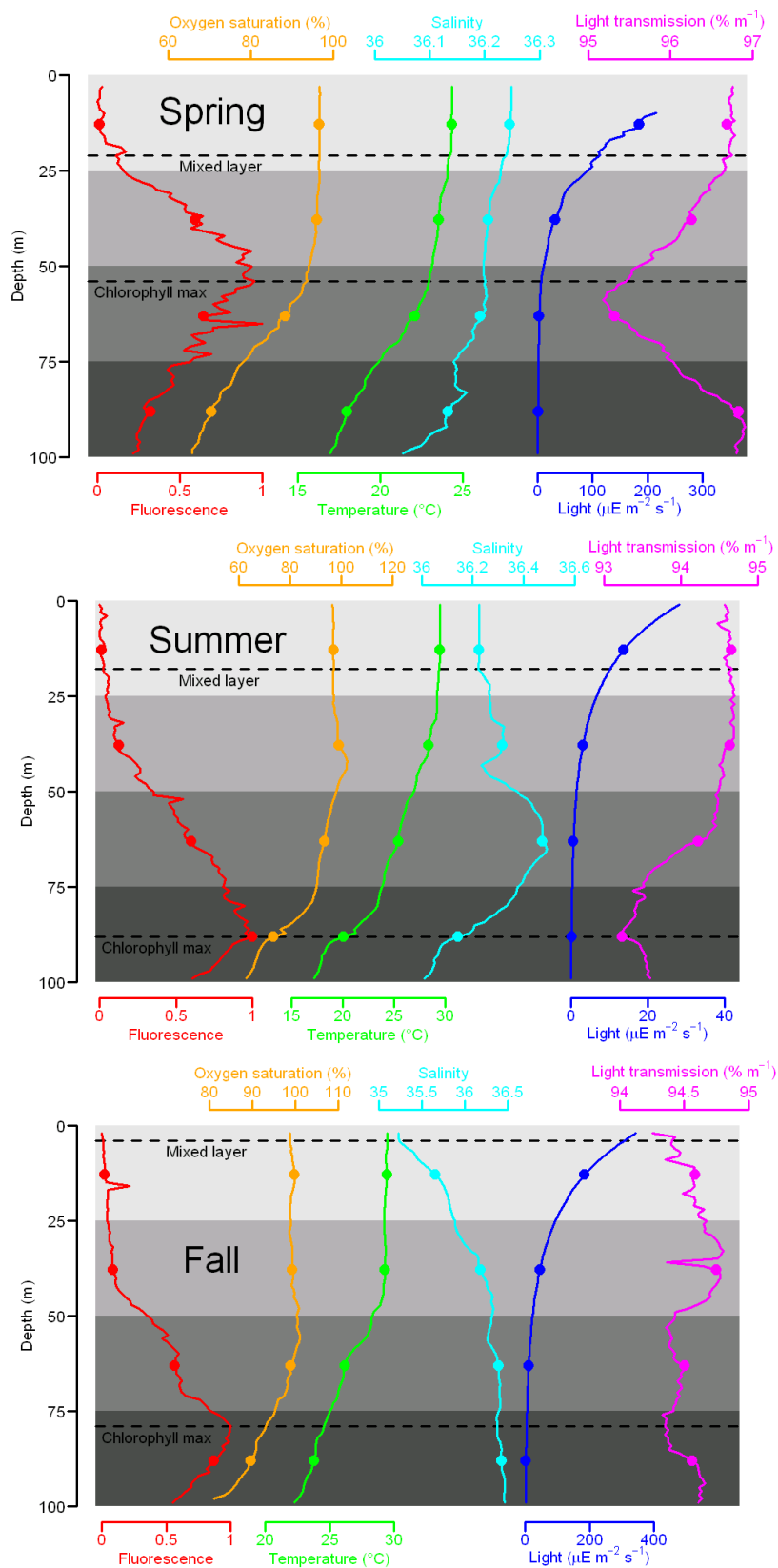
Figure 2.4. Observed (black) and predicted (red) relative densities of coral reef fish larvae (square-root transformed and normalized such that the sum of all values equals one). In spring, summer, and fall, the upper 100 m of the water column off Miami was sampled every 3 h for two diel cycles. Predicted values were generated by linear models fit to data excluding the season being predicted. Titles indicate the taxon and season being predicted. X-axis specifies the predictive environmental factor. Black lines and R^2 values designate significant regressions in observed data (permutation test: $p < 0.05$). Red R^2 values indicate the accuracy of predicted larval densities (permutation test: $p < 0.05$). Regressions often reflected larval presence or absence, rather than differences in density magnitude. Observed data were jittered for presentation purposes to reveal the total number of samples (small random numbers were added to each coordinate).

Fig. 2.4A

Epinephelini
Spring

$R^2 = 0.16$
$R^2 = 0.15$

Epinephelini
Summer

$R^2 = 0.42$
$R^2 = 0.39$

Epinephelini
Fall

$R^2 = 0.51$
$R^2 = 0.50$

Fig. 2.4B

Fig. 2.4C

Fig. 2.4D

Fig. 2.4E

Fig. 2.4F

Fig. 2.4G

Figure showing three scatter plots of normalized square root transformed larval density versus light at surface for *Sparisoma* spp. in Spring ($R^2 = 0.10$ black, $R^2 = 0.10$ red), Summer ($R^2 = 0.26$ black, $R^2 = 0.23$ red), and Fall ($R^2 = 0.20$ black, $R^2 = 0.10$ red).
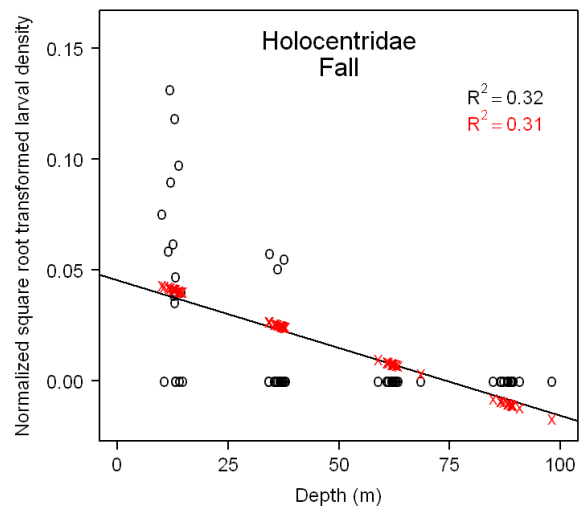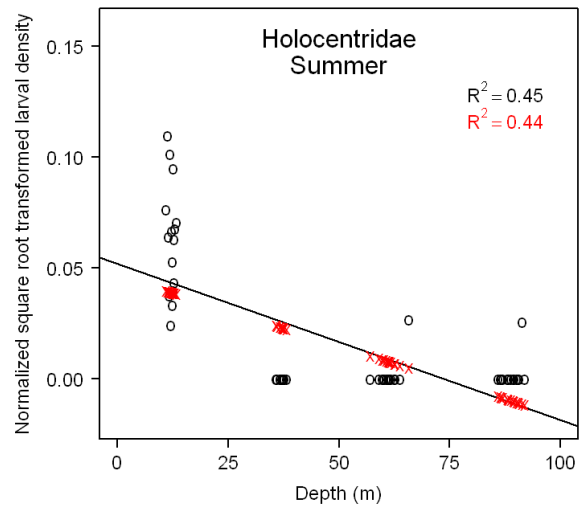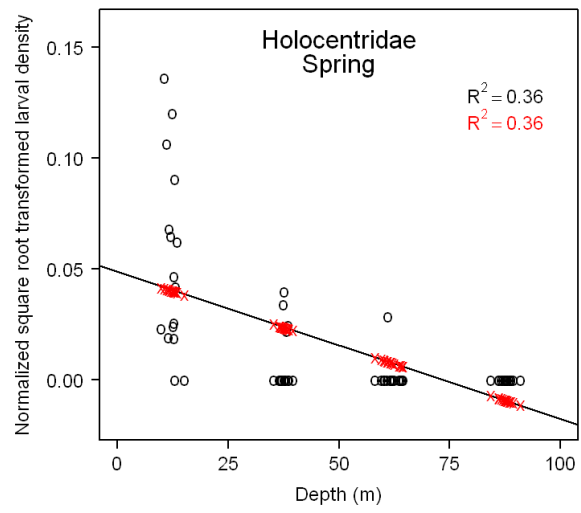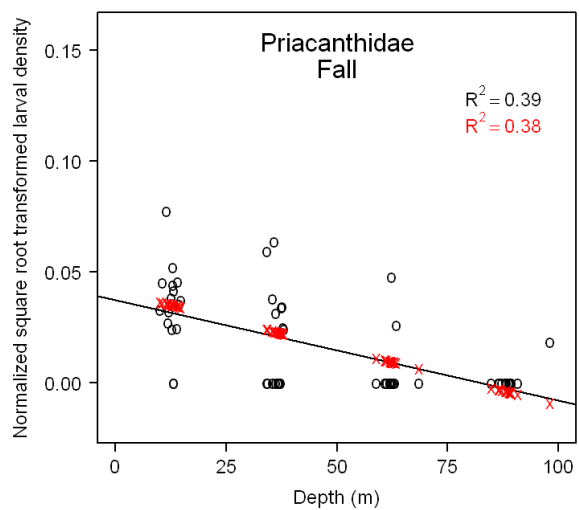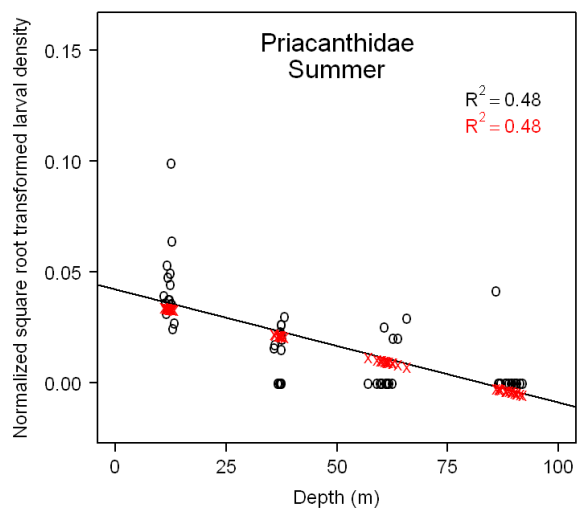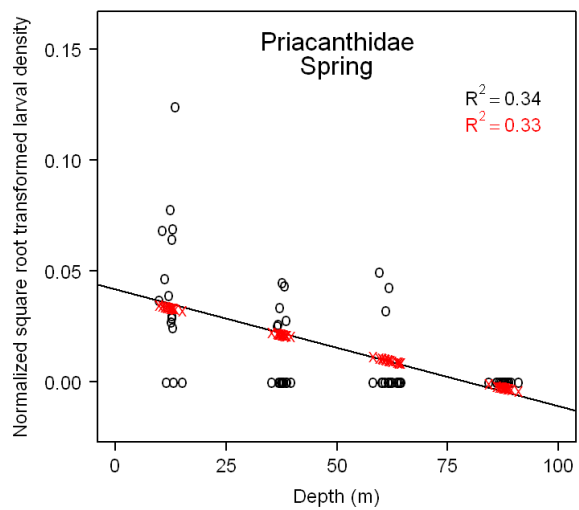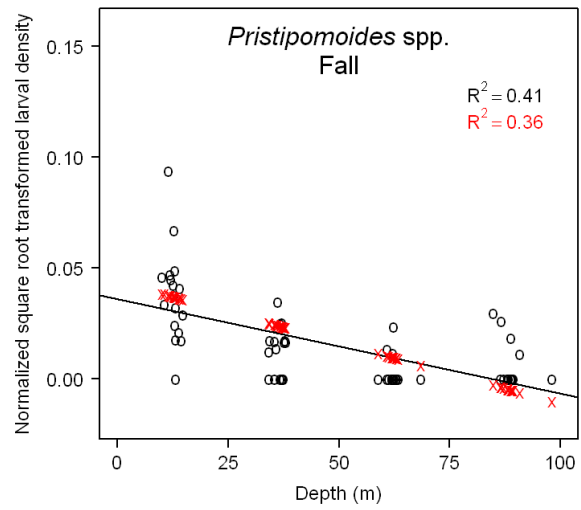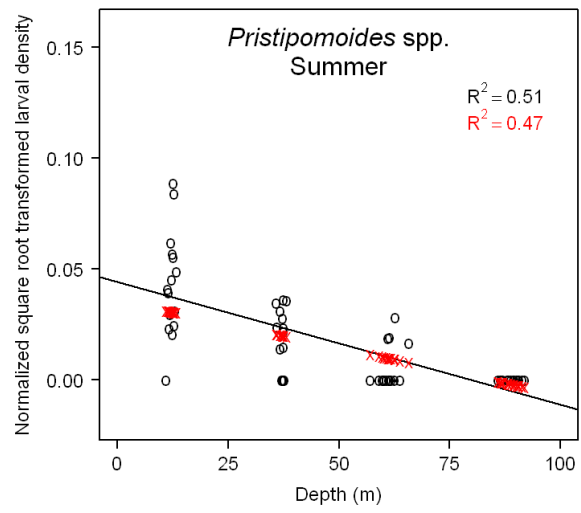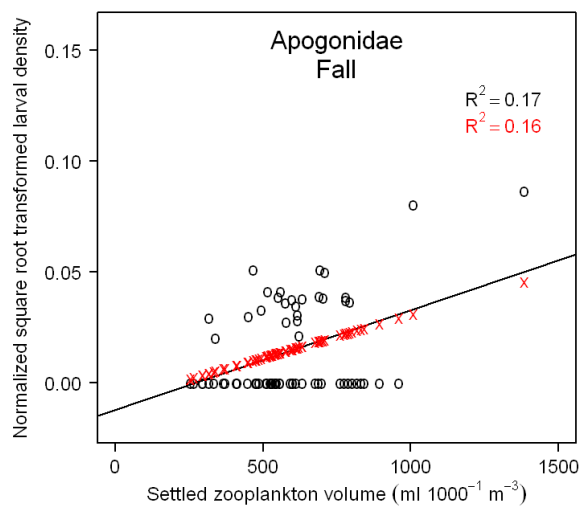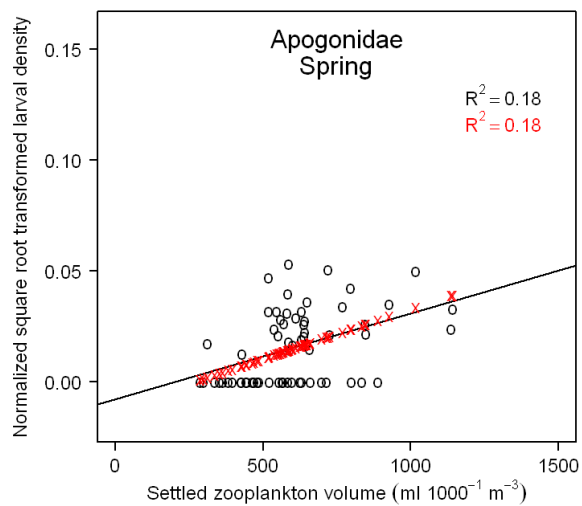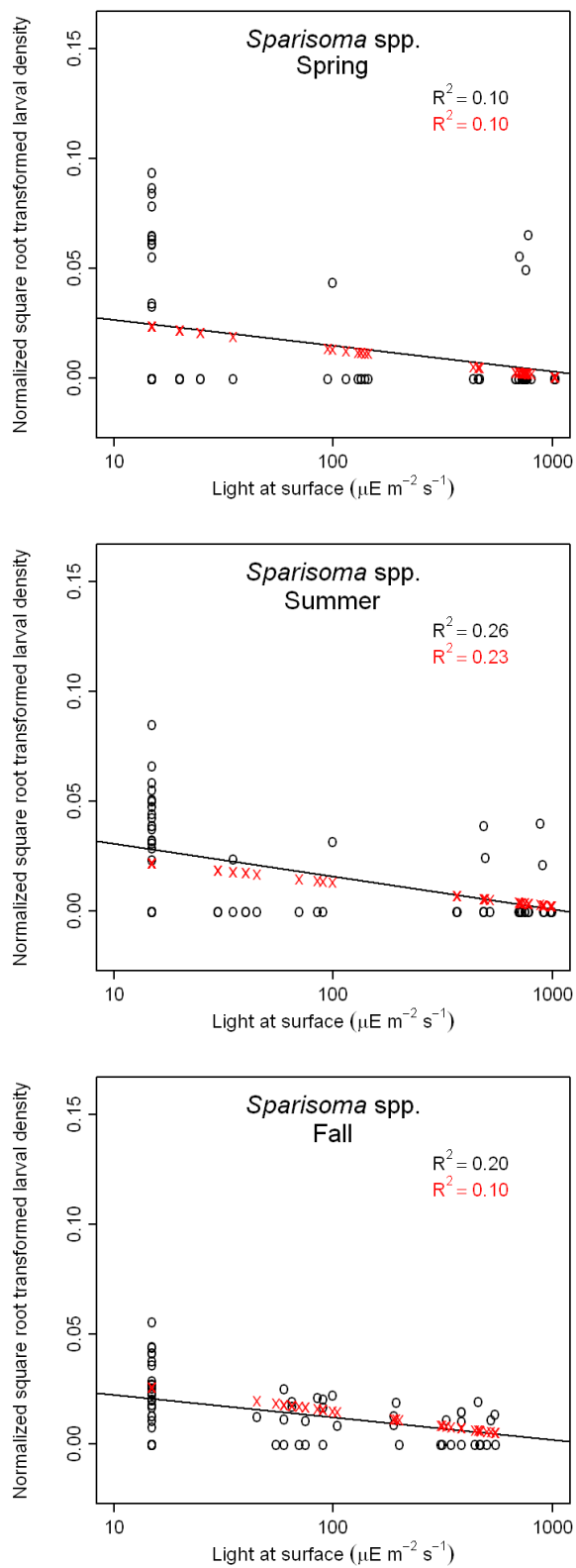
Figure 2.5. Subset of bootstrapped observed (black) and predicted (red) depths of pomacentrid[1] larvae sampled in the Straits of Florida in spring (n = 44), summer (n = 47), and fall (n = 75) at 0-25, 25-50, 50-75, and 75-100 m depth. Data from two seasons were used to predict depth as a function of surface-light in the third season. Title specifies the season being predicted. Black lines and R^2 values indicate significant regressions in observed data (permutation test: $p < 0.05$). Red R^2 values specify the accuracy of predicted depths (permutation test: $p < 0.05$). Observed data were jittered to reveal individual data points. [1] excluding the genera *Abudefduf* and *Chromis*.

Fig. 2.5
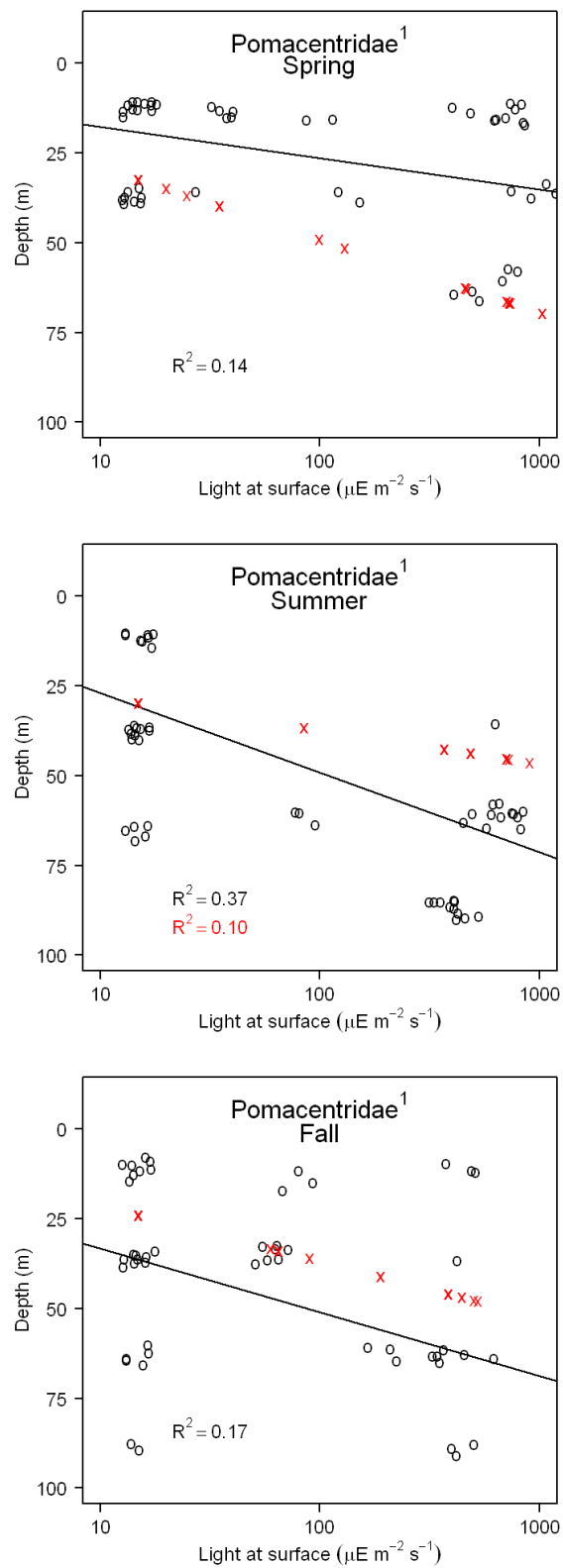
CHAPTER 3. VERTICAL MIGRATIONS OF REEF FISH LARVAE IN THE STRAITS OF FLORIDA AND THEIR EFFECTS ON LARVAL TRANSPORT

Background

Within the past decade, marine population connectivity has become a central topic in marine science. Growing interest in connectivity stems from the realization that local populations may depend more on their own reproductive output for population replenishment than was previously thought. Larvae of most benthic marine fishes and invertebrates spend a period of days to months developing in the pelagic ocean as part of the zooplankton. During this period, larvae from many different origins may or may not become thoroughly mixed. However, there is increasing evidence that the individuals that survive the larval stage to settle to the benthos (and thus have a chance of eventually reproducing) can be largely of local origin (Swearer et al. 1999, Taylor & Hellberg 2003, Jones et al. 2005). The means by which larvae achieve this are poorly understood, but a number of physical and biological mechanisms favoring either retention or return of invertebrate and fish larvae to their parental populations have been described, some of importance to specific locations and others functioning across a broad range of environments (Sponaugle et al. 2002).

In many organisms and environments, vertical migrations can affect horizontal larval transport, sometimes leading to retention. This process is particularly well established in estuarine systems, where organisms exploit tidal currents to control horizontal movements (Forward & Tankersley 2001). Over continental shelf environments, where currents are rarely dominated by tides, ontogenic vertical migrations (OVM) from depths with net offshore flow to depths with net onshore flow

appear to be closely linked to cross-shelf transport (Cowen et al. 1993, Hare & Govoni 2005). Nearshore retention due to OVM has also been demonstrated in island environments (Cowen & Castro 1994, Paris & Cowen 2004). Finally, many marine organisms exhibit some form of diel vertical migrations (DVM), the most common pattern being a shallow depth range at night and a deep depth range during the day (Neilson & Perry 1990). Movements between layers with different currents due to DVM may also influence transport and connectivity.

Despite various studies addressing the theoretical influence of larval vertical distributions on horizontal transport of marine organisms, there is a paucity of case studies providing simultaneous empirical data of vertical migrations as well as currents at depth. This is in part because data of sufficient temporal and spatial resolution to reveal vertical migrations of fish larvae require a major sampling effort and advanced technology. The present study focuses on the transport of coral reef fish larvae in the Straits of Florida, an excellent location for extensive zooplankton collections. Physical oceanography in the region is dominated by the Florida Current, which is perhaps the best studied western boundary current in the world (e.g., Larsen & Sanford 1985, Leaman et al. 1987, Lee & Williams 1999 and references therein). The Florida Current arises from the Yucatan Current, which enters the Straits of Florida either directly or via the Loop Current through the Gulf of Mexico. The Florida Current flows through the narrow passage between the Florida Peninsula and Cuba, continuing along the Florida Shelf to the west of the Great Bahama Bank, turning north and becoming the Gulf Stream. Pelagic larvae entrained into the Florida Current, the Loop Current, the Yucatan Current, and the Caribbean Current up to hundreds of km farther upstream have the

potential to be carried through the Straits of Florida and beyond as the Gulf Stream continues towards the north. Since the strength of the Florida Current varies with depth (Leaman et al. 1987), larvae may experience dramatically different transport depending on their vertical distribution. The specifics of larval transport are of particular importance to coral reef fish larvae, because their settlement habitat is limited. The coral reefs of South Florida represent the northern limit of preferred adult habitat for many reef fish species, and continued entrainment in the Gulf Stream poses a serious threat to survival via expatriation (Hare & Cowen 1991, Cowen et al. 1993). In the <100 km wide passage between Miami to the west and Bimini to the east, ichthyoplankton is highly concentrated spatially, making this location ideal for sampling fish larvae and studying their vertical distributions.

The goals of our study were to: (1) comprehensively describe the vertical distributions of common reef fish larvae; (2) identify major vertical migration patterns; and (3) quantify differences in larval transport taking place at various depths in the water column. We collected three 42-48 h time-series of ichthyoplankton samples offshore of Miami. The samples represent one of the more extensive high-resolution (both spatial and temporal) datasets of coral reef fish larvae collected. A novel method of statistical analysis was developed specifically for the detection of vertical migrations. The approach relies on non-parametric resampling techniques and tree regression to perform statistically robust, yet highly sensitive, hypothesis testing. Finally, intuitive predictions of larval transport were possible due to simultaneous measurements of the actual currents from which larvae were collected.

Methods

     Field sampling. The vertical distribution of fish larvae in the Straits of Florida was sampled repeatedly for two diel cycles in spring, summer, and fall of 2003. The research cruises aboard the University of Miami RV F. G. Walton Smith were part of an interdisciplinary study of billfishes, during which a series of 17 stations was sampled once every month from 2003 to 2005. During three specific cruises a single station was occupied over two diel cycles to conduct repeated sampling and examine DVM. The spring time-series of 48 h duration was collected on April 7-9, with the vessel maintaining position against the Florida Current at a station off Miami with a bottom depth of 130 m. The fall 48 h time-series was collected on September 30 to October 2 at the same station. The summer 42 h time-series was collected on July 31 to August 2 at an adjacent station ~1 km to the east with a bottom depth of 160 m. For additional details regarding the choice of sampling stations, see Llopiz & Cowen (2008). For each time-series, depth stratified ichthyoplankton samples were collected every 3 h by towing a coupled asymmetrical MOCNESS (Multiple Opening Closing Net with Environmental Sampling Systems) (Guigand et al. 2005) obliquely from 100 m depth to the surface, with successive nets fishing from 100-75, 75-50, 50-25, and 25-0 m, respectively. The MOCNESS was towed at a speed of 1.5 m s^{-1} and the volume sampled by each net was calculated from flow through the net (MOCNESS flowmeter) and the mouth opening of the net corrected by its angle of attack (MOCNESS frame angle sensor). Nets with a 4 m^2 mouth opening and 1 mm mesh size filtered an average of 1000 m^3. Following each MOCNESS tow, the upper 0.5 m of the water column was sampled by towing a neuston net (2 m^2 mouth opening, 1mm mesh size, 800 m^3 average

volume). Simultaneous measurements of current-at-depth were recorded by two shipboard ADCP (Accoustic Doppler Current Profiler) instruments, one resolving 14-126 m depth at a resolution of 8 m bins, the other resolving 4-32 m depth at a resolution of 2 m bins.

Sample processing. Plankton samples were initially fixed in 95% ethanol and transferred to fresh 70% ethanol several days later for long-term storage. Larval fishes were removed and identified to family following Richards (2006). The eleven families with highest occurrences in MOCNESS samples were selected for detailed analysis. Over 7,000 larvae from these families were identified beyond the family level if possible, measured, and divided into three groups by developmental stage. Larvae with straight notochords were classified as preflexion stage, larvae with partially flexed notochords or incomplete development of the caudal fin were classified as flexion stage, and larvae with fully flexed notochords and fully developed caudal fins were classified as postflexion stage. For preflexion larvae, notochord length was measured (NL) from the tip of the jaw to the end of the notochord. For flexion and postflexion larvae, standard length (SL) was measured from the tip of the jaw to the end of the urostyle. All measurements were made using a calibrated microscope and ocular scale precise to 0.1 mm.

Data analysis. Samples of fish larvae collected in vertically stratified plankton net tows form a good basis for inferring larval vertical distributions. However, several problems must be addressed when extrapolating from samples to populations. First, censored depth information can limit the accuracy of average depth estimates to the size of the censoring interval. For example, larvae exclusively collected in 25-50 m samples

with maximum likelihood have a mean depth of 37.5 m, but the true mean depth may be anywhere between 25 and 50 m. Second, the accuracy of estimates for changes in distributions theoretically can be twice the censoring interval. For example, larvae collected exclusively in 25-50 m samples at time A, and collected exclusively in 50-75 m samples at time B with maximum likelihood shifted their distribution by 25 m. However, the true amplitude of the shift may be anywhere between 0 and 50 m. Third, determining statistical independence of sampled larvae can be difficult. In our data, each larva was one of several from the same net, each net was one of several in the same haul, and each haul was one of several during the same cruise. High densities of some species in only a small number of samples suggested that larvae had been aggregated, and individual larvae were thus not statistically independent. Frequent positive correlations among larval densities in adjacent nets (e.g. 0-25 and 25-50 m) indicated that different nets also were not necessarily statistically independent. Perhaps even subsequent net hauls were not statistically independent, because autocorrelation among larval density 3 h apart was present in a small number of cruises and species. Different results could arise from assigning equal statistical weight to each larva, each net, each haul, or each cruise. For example, the average depth of 1 larva in 0-25 m samples, 1 larva in the 25-50 m samples and 100 larvae in 75-100 m samples net would be (12.5 m + 37.5 m + 87.5 m × 100) / 102 = 86 m given equal weighting of larvae, but (12.5 m + 37.5 m + 87.5 m) / 3 = 46 m given equal weighting of nets. Finally, fish larvae do not generally assume normal (Gaussian) distributions, or otherwise lend themselves to traditional parametric statistical analysis.

The term "center-of-mass" is sometimes used for estimates of mean depth, serving either as a reminder of the above problems when interpreting results, or as a justification for disregarding them in data analyses. Given sufficient sample sizes, vertical resolution, and recent advances in statistical computing, it seems preferable to calculate robust estimates for mean depth. In practice, the first two problems (limiting accuracy of estimates) are alleviated by larvae assuming vertical distributions that are approximately continuous, approximately unimodal, and extend across more than one sampling depth bin. Under these circumstances, mean depth estimates are generally accurate within several m (based on simulated worst case scenarios). To address the remaining problems, an entirely non-parametric framework drawing on resampling techniques (Hesterberg et al. 2005) and tree-regression (Venables & Ripley 2002) was developed using the statistical software R. Resampling methods required no explicit assumptions of statistical independence, only the assumption that samples were adequately representative of the sampled populations. Adequate representation was assumed only if the sum of square-root transformed counts was at least 20, i.e. one larva in each of 20 different samples or 400 larvae in a single sample. Square-root transformations are commonly applied to ecological count type data (Sokal & Rohlf 1995) to strike a balance between over- and under-representing counts in the same sample.

The final data analysis protocol was as follows. Neuston net samples were used to exclude taxa with disproportionate aggregation of larvae at the surface from analysis. Vertical distributions were determined entirely from MOCNESS samples. Larvae were initially grouped by taxon. Each larva was assigned one unit of statistical weight,

corrected by the volume filtered by its net relative to 1000 m^3 and corrected by the

square-root of larvae in the same group and net. A bootstrapped sample of 1000 larvae

was generated by drawing larvae (with replacement) from the empirical data. The

statistical weight of each larva determined the probability that it would be picked. Each

of the 1000 bootstrapped larvae was assigned a specific (random uniform) depth inside

the range at which the sample was collected, and the mean and SD of depth was

calculated from these values. To test for significant differences among vertical

distributions, larvae from each taxon were further divided into subgroups, based on each

of the following factors (one at a time): above average vs. below average length,

preflexion vs. not preflexion stage, postflexion vs. not postflexion stage, day vs. night

haul, spring vs. not spring cruise, summer vs. not summer cruise, fall vs. not fall cruise,

flood vs. ebb tide (based on NOAA water level measurements at the nearby Virginia

Key station). Mean and SD of depth was determined for each subgroup using the

procedure outlined above. If the difference in mean depth between two subgroups

exceeded 12.5 m (one half of the censoring interval), a permutation test using 1000

shuffled group assignments was performed to test if the difference was statistically

significant (Hesterberg et al. 2005). The most important subgroups, i.e. those resulting

in the greatest significant difference, were then further divided into subgroups of

subgroups. This process was repeated until no significant factors remained, or until

subgroups became too small for further analysis (because the sum of square-root

transformed counts fell below 20). The result was a regression tree of nested subgroups,

each with significantly different vertical distributions. Finally, each distribution was

compared to 1000 bootstrapped random uniform distributions of equivalent sample size.

If the estimated SD was lower than 95% of SD values from uniform distributions, then the estimated larval vertical distribution was considered significantly non-random at $p < 0.05$.

Transport estimates. Two simplifying assumptions were made to examine the interactions between larval vertical distributions and currents at depth. First, larvae were assumed to either maintain constant depths or, in the case of DVM, alternate between two distinct depths. Second, ADCP measurements of currents at the fixed sampling station were used as proxies for currents farther downstream. Under these assumptions, progressive vector diagrams for larval transport at each ADCP depth bin were constructed by stringing hundreds of subsequent current vectors together to form continuous trajectories. Using Eulerian progressive vector diagrams to estimate Lagrangian drift is an imperfect but useful method (Gawarkiewicz et al. 2007). The resulting estimates are probably accurate to within a few km over the course of 48 h (Rajamony et al. 1999).

Results

Field sampling. Ichthyoplankton tows were completed with a minimum of technical difficulties. During one MOCNESS haul in spring, nets did not open and close at the appropriate depths, and this haul was excluded from data analysis. During two hauls in spring, the 0-25 m net remained open during retrieval of the MOCNESS instrument and consequently oversampled the neuston layer. Only larvae from the families Mullidae and Tetraodontidae, which aggregated disproportionately at the surface, were affected by this problem and were excluded from analysis. Two plankton

samples were partially spilled during processing, but were almost completely recovered. No corrections were made for these accidents. Finally, the deeper range ADCP unit failed during the spring time-series.

Larval composition. Large numbers of coral reef fish larvae from eleven families were collected in the MOCNESS samples: Serranidae (3473), Scorpaenidae (1272), Lutjanidae (522), Labridae (505), Scaridae (301), Priacanthidae (261), Holocentridae (258), Sphyraenidae (189), Acanthuridae (183), Pomacentridae (178), and Apogonidae (163). Additionally, large numbers of Mullidae (1337) and Tetraodontidae (401) were collected mostly in the neuston net samples. After further identification, larvae were grouped into 36 different taxa (Table 3.1). The diversity of sampled reef fish larvae beyond the most common families is reported in Chapter 2.

The 1 mm net mesh MOCNESS samples included larvae ranging in size from <2 mm NL, which is smaller than the size at which most reef fishes hatch, to >17 mm SL, which is larger than the size at which most larvae settle to benthic habitat. The full range of larval size and development was thus present in samples. Nevertheless, larvae of sufficiently small size, slender shape, and little spination to be extruded through the 1 mm net mesh were clearly under-sampled. This resulted in length frequency distributions that were dome-shaped (often with a peak at ~5 mm length) as opposed to decreasing exponentially from small to large in a direct reflection of larval mortality (Fig. 3.1). The proportion of sampled larvae <5 mm in length generally accounted for ~30% of the total. Based on comparisons between 150 μm mesh samples and 1mm mesh samples (Cowen et al., unpublished data), larvae <5 mm were underrepresented by approximately one order of magnitude.

With the exception of *Sparisoma* spp., approximately half of the larvae were collected during the day and half during the night. Less than 20% of *Sparisoma spp.* larvae were collected during the day, and possible explanations for this apparent net avoidance are discussed in Chapter 2. Approximately half of all reef fish larvae were collected in 0-25 m nets and half deeper than 25 m. *Decodon puellaris*, scorpaenids, and some subgroups of larvae from other taxa were more abundant at 75-100 m than at any other sampled depth range. In these taxa, a significant proportion of larvae may have occupied depths >100 m, which we did not sample. Potentially excluding larvae at depths >100 m may have caused bias in our samples and results for these taxa. Bias was examined by comparing the estimated mean and SD of sampled larval vertical distributions. There was an apparently linear relationship between these two statistics across all taxa and subgroups ranging from shallow and narrow distributions to essentially uniform distributions with mean = ~50 m and SD = ~29 m (Fig. 3.2). The only obvious outliers were *D. puellaris* and postflexion *Hemanthias leptus* collected during the night, which had the greatest mean depth estimates of any group, but relatively small SD estimates. This indicated that the true vertical distributions of *D. puellaris* and nocturnal postflexion *H. leptus* larvae presumably extended beyond 100 m depth, and our estimates were biased.

Larval vertical distributions. Larvae collected across six diel cycles and three seasons allowed us to make robust estimates of vertical distributions for 35 taxa. Vertical distributions ranged from a mean depth of 17 m (SD 13 m) in Holocentridae to 69 m (SD 20 m) in *D. puellaris* (Table 3.1). Subgroups with significantly different distributions (permutation test: $p < 0.05$) related to size, stage, cruise, or light, were

present in ten taxa. Each of these ten groups was divided into subgroups based on the factor with the single greatest effect. In many cases the subgroups were then further divided into smaller significantly different subgroups and so on (Fig. 3.3, Appendix B). In seven taxa there were significant effects of size or stage or both, indicating downward ontogenic shifts in vertical distribution. Size and stage are essentially two different measures of larval development and were therefore closely related. Generally, larvae were restricted to shallow depths at small sizes, spread out over the entire water column at intermediate sizes, and restricted to deep depths at the largest sizes (Fig. 3.4). In four taxa there were significant differences among seasons. In four taxa there were significant differences between night and day, revealing diel vertical migrations. The SD of most but not all larval vertical distributions was significantly less then the SD of 1000 bootstrapped uniform distributions ($p < 0.05$).

Additional diel vertical migrations in the families Mullidae and Tetraodontidae were apparent from neuston net collections. In these tows sampling the upper 0.5 m of the water column, mullid larvae and tetraodontid larvae (in spring and fall) were present in high densities during the day, disappeared during the night, and re-appeared the next day. Diel neuston tows sampled 1269 mullids and 314 tetraodontids, while nocturnal tows sampled only 10 mullids and 1 tetraodontid. Mullids from diel neuston samples accounted for 70%, 100%, and 84% of total diel abundance (integrated from 0-100 m depth) in spring, fall, and summer, respectively. Tetraodontids from diel neuston samples accounted for 20%, 1%, and 18% of total diel abundance in spring, fall, and summer, respectively. Nocturnal neuston samples always accounted for 1% or less of total nocturnal abundance.

Currents. Currents peaked at 1.5 ms^{-1} during the spring time-series, at 2.2 ms^{-1} during the summer time-series, and at 1.7 ms^{-1} during the fall time-series (Fig. 3.5). In the upper water column to 32 m depth, the mean and SD of the northbound (alongshore) component of the current was 1.0 ± 0.29 ms^{-1}, 1.6 ± 0.27 ms^{-1}, and 0.9 ± 0.34 ms^{-1} for the spring, summer, and fall cruises, respectively. The mean and SD of the eastbound (cross-shore) component was 0.11 ± 0.11 ms^{-1}, 0.29 ± 0.13 ms^{-1}, and 0.06 ± 0.13 ms^{-1}, respectively. In summer and fall, the average northbound current varied by only 10% among depth bins in the upper 70 m, but dropped off sharply deeper than 70, m falling below 60% by 102 m. During the spring time-series from April 7 to 9, data were only available for the upper 32 m, due to the failure of one ADCP instrument. However, on April 5 (one day before the unit failed) a 36 min record had been collected at the same station. At that time, northbound current varied by 15% among depth bins in the upper 90 m and dropped off sharply beyond that (data not shown).

Transport. Estimated larval transport trajectories in the form of progressive vector diagrams were generated for discrete depth bins of 4-32 m depth in spring and 14-102 m depth in summer and fall (Fig. 3.6). In all cases, trajectories indicated rapid transport through the Straits of Florida combined with slow offshore transport. In spring, the total transport distance over 48 h was 175 km at 4 m depth and gradually decreased to 150 km at 32 m depth. Spring offshore transport increased from 17 km at 4 m depth to 20 km at 10 m depth and then decreased to 15 km at 32 m depth. Summer total transport over 42 h was 250 km at 14 m depth (the largest distance of any trajectory), decreased gradually to 225 km at 70 m depth, then decreased sharply to 135 km at 102 m depth. Summer offshore transport decreased from 45 km at 14 m, to 40 km

at 62 m, and 20 km at 102 m depth, respectively. Fall total transport over 48 h was 152 km at 14 m, increased to 165 km at 38 m, decreased to 145 km at 78 m, and decreased sharply to 97 km at 102 m depth (the shortest distance of any trajectory). Fall offshore transport ranged from 9-10 km above 38 m depth, and from 12-15 km below 38 m depth with a peak 15 km at 70 m depth.

Discussion

During the time that reef fish larvae spend in the pelagic environment, some larvae are transported great distances by ocean currents, while others are retained closer to their origin (Cowen et al. 2006). The vertical distribution of pelagic larvae can strongly affect their transport distance and direction (e.g. Paris & Cowen 2004). Therefore, detailed descriptions of larval vertical distributions and associated effects on transport are vital for understanding larval transport and ultimately predicting connectivity of metapopulations. By using a coupled asymmetrical MOCNESS (Guigand et al. 2005) we were able to collect a large number of reef fish larvae at sufficient spatial (depth) resolution to reveal significant diel and ontogenic vertical patterns. A non-parametric analysis of the data allowed us to quantify larval vertical distributions and estimate larval transport at different depths. All presented taxa are strongly associated with reefs (Leis 1991a), and most are commonly observed on coral reefs across the western central Atlantic region (Humann & Deloach 2002) including the Florida Keys (Bohnsack et al. 1999). The most abundant taxon of the study, *Hemanthias vivanus*, is not very well documented, because adults are primarily found on reefs deeper than 70 m (Hastings 1981). The second most abundant taxon, the family

Scorpaenidae, consists of many species associated with reefs and some species associated with other (sometimes deep) benthic habitats.

Larval vertical distributions. Most reef fish taxa had mean depths in the range of 25-45 m. A variety of fish larvae in other environments also accumulate in this depth range (Heath 1992), possibly because too little light for feeding is available at deeper depths (Job & Bellwood 2000), and dangerously high levels of UV radiation are present near the surface (Browman 2003). Epinephelini, holocentrids, some lutjanids, and sphyraenids had mean depths <25 m, but avoided the neuston layer. Mullids and, to a lesser extent, tetraodontids aggregated in the neuston layer during the daytime but dispersed during the night. Both mullid and tetraodontid larvae are heavily pigmented, which provides some protection from UV radiation. Most Anthiinae, the labrids *Decodon puellaris* and (in spring and summer) *Xyrichtys* spp., pomacentrids during the day, some scorpaenids, and large *Sparisoma* spp. had mean depths >45 m. Only the distibutions of *D. puellaris* and postflexion *Hemanthias leptus* appeared to extend noticeably past 100 m, and were artificially truncated by our sampling. Overall, larvae of taxa associated with deep adult habitat had an equivalent range of mean depths as larvae of shallow reef taxa.

Vertical distributions of fish larvae in the same region were previously described by Cha et al. (1994), based on eight nighttime MOCNESS tows during spring 1989 off the Florida Keys. In that study, families were divided into three categories, one with >50% of larvae occurring in the upper 25 m, a second with >50% occurring in the upper 50 m, and a third with >50% occurring deeper than 50 m. Surprisingly, when we applied the same criteria to our data, only four of 11 families fell into the same

categories (even after we excluded daytime samples and large larvae potentially under-sampled by Cha et al. (1994), who towed 75% smaller nets at 33% slower speeds). Since we collected a larger number of samples over a longer period of time, the data presented here are presumably more representative for the region. Our estimates also represent the first effort to quantify vertical distributions of reef fish larvae in the Straits of Florida in terms of mean and SD. This will make comparisons with future work easier.

Seasonal effects. In four taxa, vertical distributions varied more among seasons than among sizes, stages, or any other factor. The mean depth of apogonids was 14 m shallower in spring than in summer and fall, the mean for *Xyrichtys* spp. was 17 m deeper in summer than in fall (only one larva was collected in spring), the mean for pomacentrids was 22 m shallower in spring than in summer and fall, and the mean for scorpaenids was 19 m deeper in fall than in spring and summer. We were unable to predict this between-cruise variability in larval vertical distributions from environmental variables such as light, turbulence, zooplankton distribution, etc. (Chapter 2). The best remaining explanation is that the species composition of these groups in samples from different seasons may have varied, confounding the seasonal effect. Unfortunately, without further identification by genetic techniques (e.g., Richardson et al. 2007), we are unable to test this hypothesis.

Diel vertical migration. For four taxa, mean depth differed significantly between day and night, providing strong circumstantial evidence for DVM. The mean depth of *Sparisoma* spp. was 13 m deeper in daytime samples than in nighttime samples and the mean depth of pomacentrids from fall and summer samples was 20 m deeper in

daytime samples than in nighttime samples. Postflexion *Hemanthias leptus* as well as

preflexion scorpaenids from fall samples displayed the opposite pattern, with mean

depths 17 and 15 m deeper in nighttime samples, respectively. DVM is extremely

common among zooplankton (Pearre 2003), but only poorly documented in reef fish

larvae. Leis (1991b) found that in the <15 m deep Great Barrier Reef lagoon around

Lizard Island, Australia, many reef fish larvae distributed non-randomly during the day,

but more uniformly during the night. The same pattern was observed in two temperate

species over the 30 m isobath off Southern California (Brewer & Kleppel 1986) and in

many temperate taxa, including reef fish families, over the 60 m isobath off SE

Australia (Gray 1998, Gray & Miskiewicz 2000). These results apparently cannot be

generalized to reef fish larvae in an oceanic water column of >100 m depth, because

pomacentrids and *Sparisoma* spp. both assumed narrower distributions at night than

during the day. This finding also challenges the idea that larvae require daylight to

maintain structured vertical distributions (Leis 1991b, Gray 1998), and demonstrates

that the accuracy of vertical orientation is not necessarily limited by available light.

Hare & Cowen (1991) found that larval *Xyrichtys novacula* collected in the Mid

Atlantic Bight (far north of their adult habitat range) were most abundant at <10 m

depth during the night and at >10 m depth during the day. In our samples, there was a

trend towards similar DVM in *Xyrichtys* spp., but the amplitude of 7 m was not

considered significant, considering our 25 m net bins. Overall, *Xyrichtys* spp. were

distributed much deeper in the Straits of Florida than in the Mid Atlantic Bight. The

difference may be related to differences in the physical environment between the two

locations. For example, the coldest temperatures at which we sampled were ~15° C at

100 m depth in spring and summer. Hare & Cowen (1991) reported temperatures of ~15° C at 20 m depth. Some larvae actively avoid cold water via upward vertical swimming (Olla et al. 1996), and behavior of this type could cause *Xyrichtys* spp. to become restricted to a shallower vertical range in the Mid Atlantic Bight than in the Straits of Florida.

Ontogenic vertical shifts. In seven taxa, mean depth differed significantly among co-occurring larvae of small and large size or early and late stage, indicating substantial ontogenic shifts in vertical distributions. Downward shifts associated with growth or development were present in *Thalassoma bifasciatum*, *Pristipomoides* spp., *H. leptus*, *H. vivanus*, Liopropomini, *Sparisoma* spp., and scorpaenids. The amplitude of changes in mean depth ranged from 14 m in *Pristipomoides spp.* to 21 m in *H. vivanus*, and in all cases, the SD of vertical distributions increased with ontogeny as well. The vertical distributions of large *T. bifasciatum*, postflexion *H. vivanus*, large Liopropomini and all subgroups of scorpaenids were too broad to be distinguished from uniform distributions, but nevertheless significantly different from distributions of other subgroups within these taxa. Amplitude values are conservative (low) estimates for the full extent of changes in vertical distributions, limited by the sample size of smallest larvae (due to undersampling) and largest larvae (due to low densities), by the 25 m spatial resolution of net tows, and in the case of *H. leptus* by the maximum sampling depth of 100 m. While we divided larvae into groups for significance testing, the data actually suggest a gradual transition, not a sharp dichotomy (Fig. 3.4).

Ontogenic shifts in vertical distributions are generally interpreted as ontogenic vertical migrations (e.g. Cowen 2002), but since stratified net samples are insufficient to

determine the behavior of individuals (Pearre 1979), alternative explanations should also be considered. There are two different scenarios in which vertical distributions could shift due to differential survival at different depths. In the first scenario, larval survival is higher at deep depths than at shallow depths. Larvae are initially more concentrated at shallow depths, perhaps due to positively buoyant eggs. However, a gradual shift towards deeper distributions takes place, without individual larvae migrating, by mortality of shallow larvae. We can essentially rule out the first scenario, because larvae of several taxa appeared in deep samples exclusively at large sizes. Particularly in the 75-100 m depth range, the sampled density of large larvae was disproportionately higher than the sampled density of small larvae. Even assuming zero mortality at 75-100 m depth and tenfold undersampling, there were simply too few small larvae in that depth range to account for the appearance of large larvae. Therefore, an influx of larvae from shallower depths must have taken place. In the second differential survival scenario, only large larvae can survive at deep depths. Larvae are initially more concentrated at shallow depths, and random vertical movements result in some larvae moving deeper. However, small larvae do not survive for long at deep depths. Only larvae that reach a certain size before moving down can eventually accumulate at deep depths. The second scenario is somewhat contrived, as it involves random behavior of larvae interacting with a major source of mortality that is not only size-specific and depth-specific, but also taxon-specific (not affecting acanthurids, pomacentrids, or *Anthias nicholsi*). Compared to these scenarios of differential survival, downward migration by large larvae is a more parsimonious explanation.

Downward OVM appears to be much more common in reef fish larvae than the reverse pattern (Cowen 2002). One hypothesis for the prevalence of downward OVM is that poorly developed eyes prevent young larvae from feeding effectively at deeper, darker depths (Job & Bellwood 2000). Patterns in our samples are mostly consistent with this hypothesis. For example, in *Sparisoma* spp., only larvae >5 mm were collected at 50-75 m and only larvae >7 mm were collected at 75-100 m, with the exception of a single outlier (Fig. 3.4G). Additionally, both small and large *Sparisoma* spp. moved significantly deeper during the day, consistent with the idea that depth range is limited by available light. However, OVM in most taxa also involved decreasing numbers of large larvae at shallow depths, which appears unrelated to light. Further, *Hemanthias leptus* and some scorpaenids had the same pattern of OVM (down with development) but the opposite type of DVM (down at night) as *Sparisoma* spp. In their effects on light and feeding, the DVM and OVM patterns appear contradictory, thus other factors are likely to be important.

Currents. Spring, summer, and fall ADCP records reflected the dynamic nature of physical oceanography in the Straits of Florida. Current strength was linked to the exact location of the meandering Florida Current, as revealed by ADCP measurements across the entire transect from Florida to the Bahamas (data not shown). The Florida Current was closest to shore during the summer cruise, resulting in substantially stronger currents during that time. Additionally, summer sampling was conducted ~1 km farther offshore, where currents were slightly stronger than at the inshore station. During all three cruises, cross-shore current was highly variable but on average towards the east (offshore). Wind driven onshore flow (Ekman transport) is common in the

lower and middle Florida Keys, but rare in the northern Straits of Florida (Lee &

Williams 1999).

Transport trajectories. Larval transport trajectories for all three seasons suggest

that most larvae passing by our sampling stations were rapidly carried beyond the

Straits of Florida by the Florida Current. This is consistent with drifter tracks, which

also have short residence times off the upper Florida Keys (Hare & Walsh 2007).

Currents at different depths within the range where most larvae were concentrated (25-

45 m) resulted in very similar transport trajectories. Therefore, different vertical

distributions among taxa as well as vertical migrations within taxa did not necessarily

affect direction or magnitude of larval transport. However, currents at >70 m were

much slower in both the 42 h summer and 48 h fall ADCP record, resulting in a

reduction in larval transport relative to surface currents. At a depth of 78 m, transport

was reduced by ~15% and at a depth of 102 m by ~45% relative to maximum transport.

This corresponded to a daily distance of 10-35 km in spring and 23-64 km in summer.

Based on bootstrapped samples of empirical data, the proportion of larvae

following relatively slow transport trajectories at deeper depths was substantial in

several cases. The following examples show different patterns with and without DVM

and OVM. First, *D. puellaris* larvae occurred at >75 m more than 43% of the time, and

were therefore exposed to slower currents than any other taxon. Unfortunately, our

sample size was insufficient to investigate potential migrations in *D. puellaris*. Second,

the proportion of *H. vivanus* larvae experiencing reduced transport at >75 m depth

increased from 1% of preflexion larvae and 2% of flexion larvae to 21% of postflexion

larvae with OVM. An additional trend for larger postflexion larvae to move ~10 m

deeper than smaller ones suggested that the proportion of settlement stage *H. vivanus* on slow transport trajectories was actually >21%. Vertical migration estimates for *H. vivanus* (even those not significant under our conservative criteria) were particularly robust due to the high sample size (2169) of larvae belonging to a single species. Third, DVM by pomacentrids in both summer and fall from shallow depths at night to deep depths during the day resulted in an increase from 7% to 21% of larvae at >75 m depth, respectively. Pomacentrids were the only taxon in which DVM alone may have had an appreciable impact on transport. Obviously, DVM to and from slower currents can only reduce transport by half as much as a long-term transition to deep depths by OVM. Fourth, interactions between DVM, OVM, and transport were evident in *H. leptus*, scorpaenids, and *Sparisoma* spp. In the case of *Sparisoma* spp., 1% of small larvae during the night, 8% of small larvae during the day, 15% of large larvae during the night, and 42% of large larvae during the day were found in the depth range of reduced transport, respectively.

   Cross-shore transport and settlement. None of the progressive vector diagrams for larval transport indicated net onshore (west) movements towards shallow coral reef habitat. In summer, the vertical gradient in cross-shore transport mirrored the larger pattern in alongshore transport. From 0-62 m depth, net cross-shore movement varied by <15%, but by 70 m there was a reduction of 24% and by 102 m a reduction of 55% in offshore transport, respectively. Consequently, the same vertical distribution patterns that reduced overall transport also reduced offshore transport during the summer time-series, and depths >70 m could be seen as more favorable for retention and settlement, relative to shallower depths. In the fall time-series, this was not the case. Net offshore

transport in the upper 100 m was greatest at 70 m and 41% less at 30 m. Under these conditions, most reef fish larvae occupied the depth range of maximum transport to the north, and minimum transport to the east. Since movement to the north eventually reduces the probability of larvae encountering settlement habitat, but movement to the east immediately increases the distance to any settlement sites, it is unclear which depth is more favorable for settlement. In spring, insufficient data were collected to characterize cross-shore transport deeper than 32 m. Within the upper 32 m, maximum and minimum offshore transport occurred at 10 and 32 m, respectively.

Settlement of reef fish larvae is often episodic, and the fact that our current measurements did not detect a specific onshore transport mechanism is not surprising. Nevertheless, larval vertical distributions and migrations affect the probability that larvae encounter some types of onshore transport events. For example, the accumulation of mullids and tetraodontids at the ocean surface makes them susceptible to episodic onshore flow of the neuston layer indirectly caused by passing (Shanks 1983) or breaking (Pineda 1994) internal waves. Similarly, the deep distribution of *D. puellaris* may result in episodic onshore transport by direct entrainment in tidal bores (Leichter et al. 1996). Upstream of our study site, where the prevailing wind blows alongshore rather than cross-shore due to the curvature of the Florida Keys, onshore Ekman flow in the upper ~50 m of the water column is common (Lee & Williams 1999). Under these conditions, the 25-45 m mean depth of most taxa may be ideal for transport to settlement sites in the lower-middle Florida Keys.

Some of the most important cross-shore transport mechanisms may act independently of larval vertical distributions. Off the Florida Keys, there is a well

established relationship between pulses of reef fish larvae at shallow reefs and passing Florida Current frontal eddies (Sponaugle et al. 2005, D'Alessandro et al. 2007). It is unclear whether larval entrainment and delivery to reefs by eddies is related to their vertical distributions. Active horizontal swimming behavior of settlement-stage larvae is also critically important, although possibly only at spatial scales <1 km (reviewed in Leis 2006, Chapter 5). Since the coral reefs of South Florida represent the northern limit of preferred settlement habitat for many reef fish species and expatriation poses a threat to survival (Walsh 1987, Hare & Cowen 1991), any reduction in alongshore transport increases the odds of eventual onshore transport by depth-independent mechanisms. Consequently, the observed OVM pattern of downward movement with development was generally favorable but not necessarily sufficient for larval settlement in our study area.

Elsewhere in the larger Western Central Atlantic region, the vertical distributions and migrations observed in the Straits of Florida may have different consequences for transport. Both in the Lower Florida Keys, slightly upstream of our site (Lee & Williams 1999), and in the Middle Atlantic Bight, far downstream of our site (Cowen et al. 1993, Hare & Govoni 2005), downward OVM is thought to place larvae in an onshore flow regime, favoring settlement. In the Eastern Caribbean, off Barbados, increased nearshore retention of reef fish larvae via downward OVM has been demonstrated conclusively (Cowen & Castro 1994, Paris & Cowen 2004). If there is a general trend for onshore flow in mid-water, then downward OVM, as observed in the Straits of Florida, may have an adaptive role in enhancing settlement success.

Conclusions. Our study resulted in first quantitative estimates for the mean and SD of vertical distributions of reef fish larvae in the Straits of Florida. Significant diel and ontogenic vertical migrations were present in several taxa, but no significant tidal vertical migrations were detected. Simultaneous measurements of larval vertical distributions and currents at depth provided direct evidence for the influence of DVM and OVM on larval transport. The proportion of larvae at depths >75 m was the most important factor influencing transport. Larvae deeper than 75 m were advected tens of km less per day than larvae in the upper water column, effectively increasing their residence time in the Straits of Florida by 15-45%, and thus potentially enhancing their chance of settlement to preferred juvenile habitat

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Table 3.1. Estimated vertical distribution parameters for coral reef fish larvae collected offshore of Miami, based on three 42-48 h time-series of repeated MOCNESS net tows conducted in spring, summer, and fall, with different nets sampling at 0-25, 25-50, 50-75, and 75-100 m depth, respectively. Absent values reflect insufficient sample sizes. Bold values indicates ranges among significantly different subgroups within the taxa. Italicized values indicate distributions with such large SD that they are indistinguishable from uniform distributions at p > 0.05. [1] Mean and SD are underestimates, biased by the lack of sampling at depths >100 m. [2] An additional 1279 mullids and 315 tetraodontids were collected at <0.5 m depth with neuston nets.

Taxon	n	Mean depth (m)	SD (m)	Taxon	n	Mean depth (m)	SD (m)
Acanthuridae	183	44	26	Priacanthidae	261	25	20
Apogonidae	163	**31-44**	**22-24**	Scaridae			
Holocentridae	258	17	13	*Cryptotomus roseus*	46	34	23
Labridae				*Scarus* spp.	6		
Clepticus spp.	11			*Sparisoma* spp.	249	**27-58**	**16-26**
Decodon puellaris[1]	33	69	20	Scorpaenidae	1272	**41-64**	**28-32**
Doratonotus megalepis	20			Serranidae			
Halichoeres spp.	26	*43*	*28*	Subfamily Anthiinae			
Lachnolaimus maximus	4			*Anthias nicholsi*	171	47	24
Thalassoma bifasciatum	120	**23-39**	**15-26**	*Anthias tennuis*	5		
Xyrichtys spp.	291	**32-49**	**19-25**	*Anthias woodsi*	28	38	23
Lutjanidae				*Hemanthias leptus*[1]	386	**45-67**	**23-26**
Subfamily Etelinae				*Hemanthias vivanus*	2169	**27-48**	**19-28**
Etelis oculatus	50	36	21	*Plectranthias garruppellus*	2		
Pristipomoides spp.	371	**17-31**	**13-23**	*Pronotogrammus martincenensis*	3		
Subfamily Lutjaninae				Unknown	7		
Rhomboplites aurorubens	33	35	21	Subfamily Epinephelinae			
Other	68	20	14	Tribe Epinephelini	305	20	16
Mullidae[2]	55			Tribe Grammistini	33	35	18
Pomacentridae				Tribe Liopropomini	62	**31-45**	**21-27**
Abudefduf spp.	7			Subfamily Serraninae	302	25	20
Chromis spp.	5			Sphyraenidae	189	17	16
Other	166	**26-57**	**19-27**	Tetraodontidae[2]	84		

Figure 3.1. Length distributions of larvae from common coral reef fish taxa sampled at 0-100 m depth off Miami during 42-48 h time-series in spring, summer, and fall. [1] not including *Rhomboplites aurorubens*. [2] not including *Abudefduf* spp. or *Chromis* spp.

Fig 3.1

Acanthuridae — A — n = 183

Apogonidae — B — n = 163

Holocentridae — C — n = 258

Decadon puellaris (Labridae) — D — n = 33

Thalassoma bifasciatum (Labridae) — E — n = 120

Xyrichtys spp. (Labridae) — F — n = 291

Fig 3.1

Etelis oculatus (Lutjanidae) — G, n = 50

Pristipomoides spp. (Lutjanidae) — H, n = 371

Lutjaninae[1] (Lutjanidae) — I, n = 68

Rhomboplites aurorubens (Lutjanidae) — J, n = 33

Pomacentridae[2] — K, n = 166

Priacanthidae — L, n = 261

Fig 3.1

Cryptotomus roseus (Scaridae)

Sparisoma spp. (Scaridae)

Scorpaenidae

Anthias nicholsi (Serranidae)

Hemanthias leptus (Serranidae)

Hemanthias vivanus (Serranidae)

Fig 3.1

Figure 3.2. Mean vs. SD estimates for vertical distributions of reef fish larvae in the Straits of Florida. During three time-series in spring, summer, and fall, MOCNESS tows were conducted every 3 h for two diel cycles, with different nets sampling at 0-25, 25-50, 50-75, and 75-100 m depth, respectively. Larvae were initially grouped by taxon and then subdivided into subgroups with significantly different vertical distributions (permutation test: $p < 0.05$), if possible. A strong apparently linear relationship between estimated mean and SD of vertical distributions was observed. The two groups with deepest vertical distributions were the only outliers, indicating samples biased by the lack of collections at >100 m depth.

Figure 3.3. Trees of mean depth ± SD in subgroups of reef fish larvae with significantly different vertical distributions (permutation test p < 0.05). In spring, summer, and fall, the upper 100 m of the water column off Miami was sampled every 3 h for two diel cycles, with plankton nets fishing at 0-25, 25-50, 50-75, and 75-100 m depth, respectively. Larvae of each taxon were divided into subgroups by various criteria, including season, developmental stage, day / night, and smaller / larger than mean length. Each bifurcation indicates the two subgroups with greatest significant differences in mean depth. Subgroups in which the sum of square-root transformed sample sizes (values in parentheses) was <20 were not considered statistically representative and thus excluded from analysis. Labels indicate grouping criteria. Italicized values indicate distributions with such large SD that they are indistinguishable from uniform distributions at p > 0.05. [1] not including *Abudefduf* spp. or *Chromis* spp.
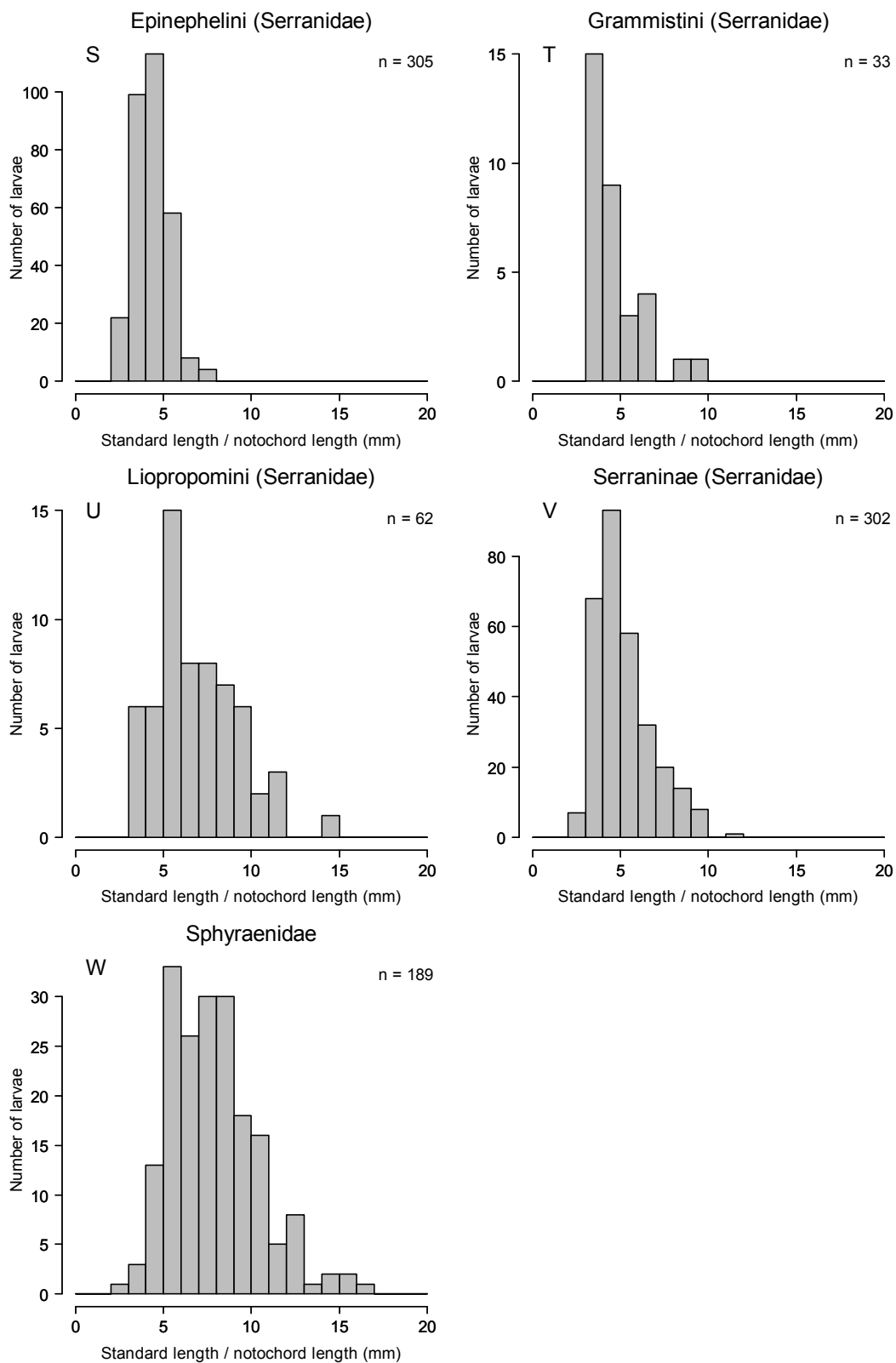
Fig. 3.3

Apogonidae (97)   37 ± 23
  spring (58)   31 ± 22
  summer & fall (38)   44 ± 24

Thalassoma bifasciatum (69)   33 ± 24
  small (41)   23 ± 15
  large (40)   *39 ± 26*

Xyrichtys spp. (115)   35 ± 22
  spring & summer (21)   *49 ± 25*
  fall (94)   32 ± 19

Pristipomoides spp. (130)   26 ± 21
  small (80)   17 ± 13
  large (74)   31 ± 23

Pomacentridae[1] (105)   *43 ± 27*
  spring (28)   26 ± 19
  summer & fall (76)   *48 ± 27*
    day (40)   57 ± 25
    night (37)   37 ± 24

Sparisoma spp. (135)   44 ± 26
  small (57)   32 ± 19
    day (22)   40 ± 21
    night (34)   27 ± 16
  large (96)   48 ± 26
    day (21)   *58 ± 28*
    night (74)   46 ± 25

Scorpaenidae (344)   *55 ± 31*
  spring & summer (132)   *43 ± 29*
  fall (212)   *62 ± 29*
    preflexion (61)   *48 ± 31*
      day (31)   *41 ± 28*
      night (30)   *56 ± 32*
    flexion & postflexion (195)   *64 ± 28*

Hemanthias leptus (150)   51 ± 26
  preflexion & flexion (107)   45 ± 23
  postflexion (67)   60 ± 25
    day (31)   50 ± 24
    night (36)   67 ± 23

Hemanthias vivanus (359)   42 ± 27
  preflexion (148)   27 ± 19
  flexion & postflexion (296)   *46 ± 28*
    flexion (118)   32 ± 21
    postflexion (252)   *48 ± 28*

Liopropomini (47)   *38 ± 25*
  small (28)   31 ± 21
  large (23)   *45 ± 27*

Figure 3.4. Length and capture depth of reef fish larvae from MOCNESS ichthyoplankton collections in the Straits of Florida. Larvae were sampled at 0-25, 25-50, 50-75, and 75-100 m depth, and assigned random values within the depth ranges to reveal the actual sample size. Colors indicate larval developmental stages. blue: preflexion, red: flexion, green: postflexion. Quadratic smoothing splines (truncated 10 data points from maximum and minimum size) illustrate gradual shifts in mean depth with increasing size. In scorpaenids, preflexion larvae had shallower distributions than flexion and postflexion larvae, but no size effects were apparent.

Fig. 3.4

A Apogonidae — n = 163
B *Thalassoma bifasciatum* (Labridae) — n = 120
C *Pristipomoides* spp. (Lutjanidae) — n = 371
D *Hemanthias leptus* (Serranidae) — n = 386
E *Hemanthias vivanus* (Serranidae) — n = 2169
F Liopropomini (Serranidae) — n = 62

Fig. 3.4

Sparisoma spp. (Scaridae) — G — n = 249

Scorpaenidae — H — n = 1272
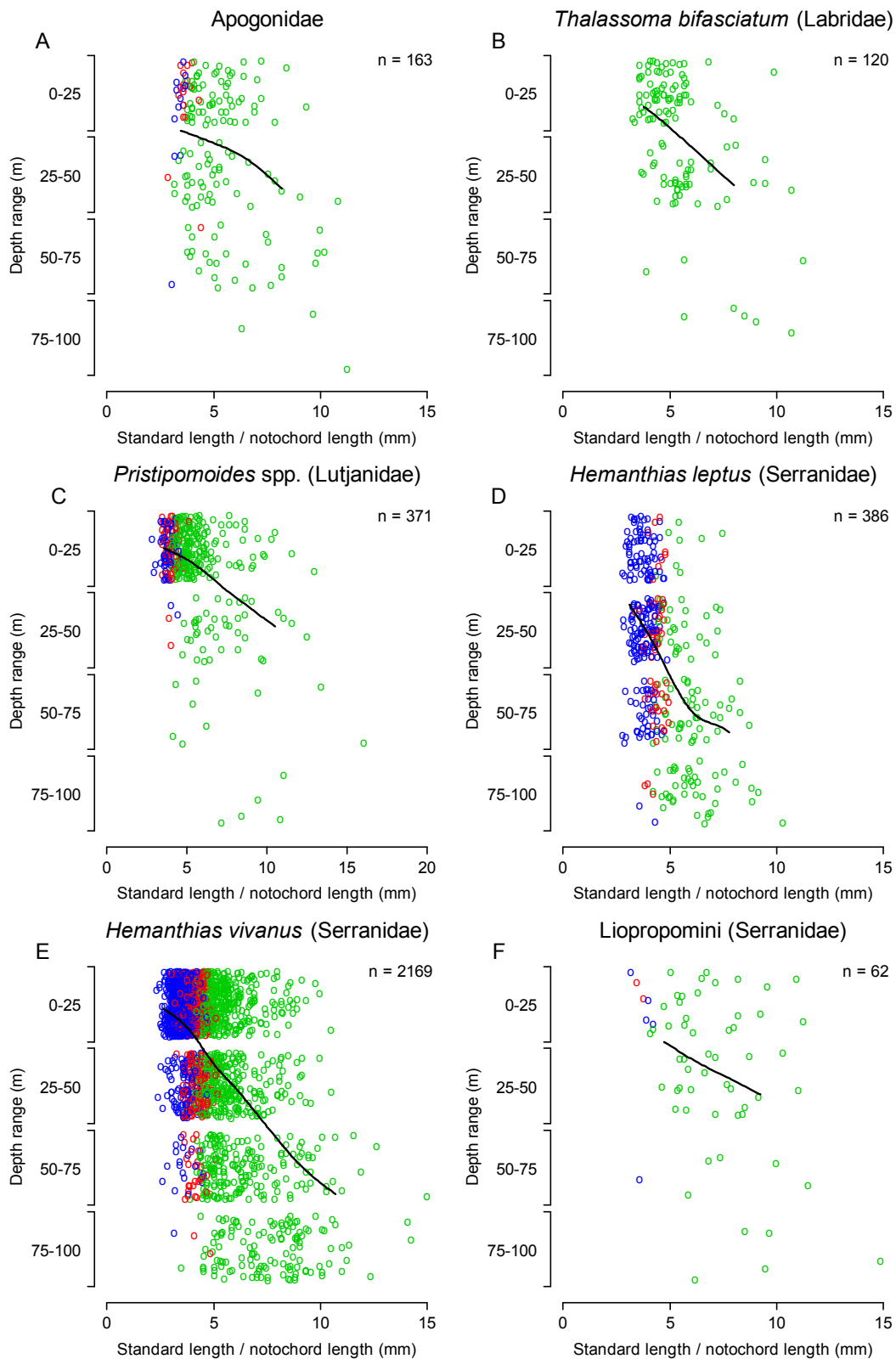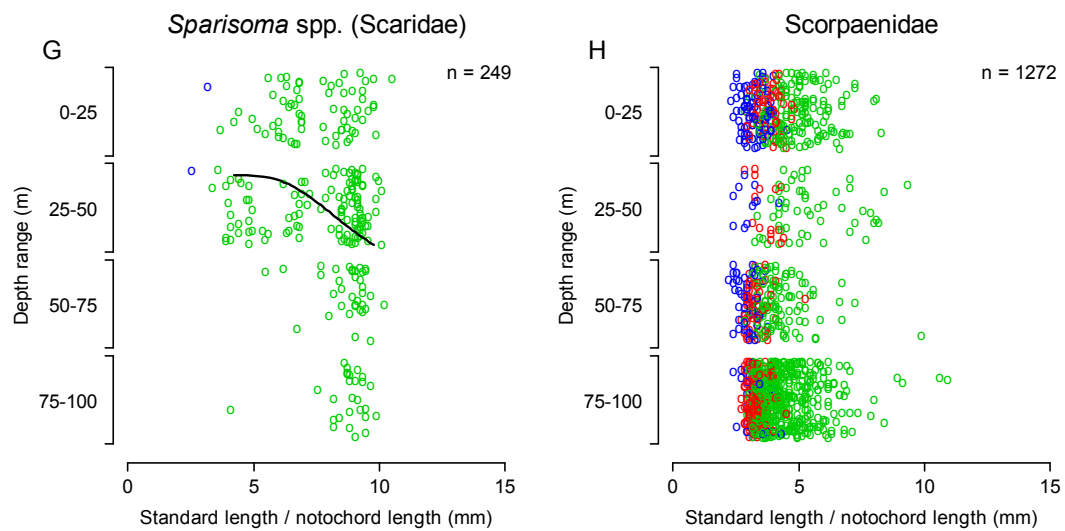
Figure 3.5. Time-series of currents recorded by a shipboard ADCP while the vessel maintained a fixed position off Miami for 42-48 h in spring, summer and fall. Up is north.

Fig. 3.5

A   Spring

B   Summer

C   Fall
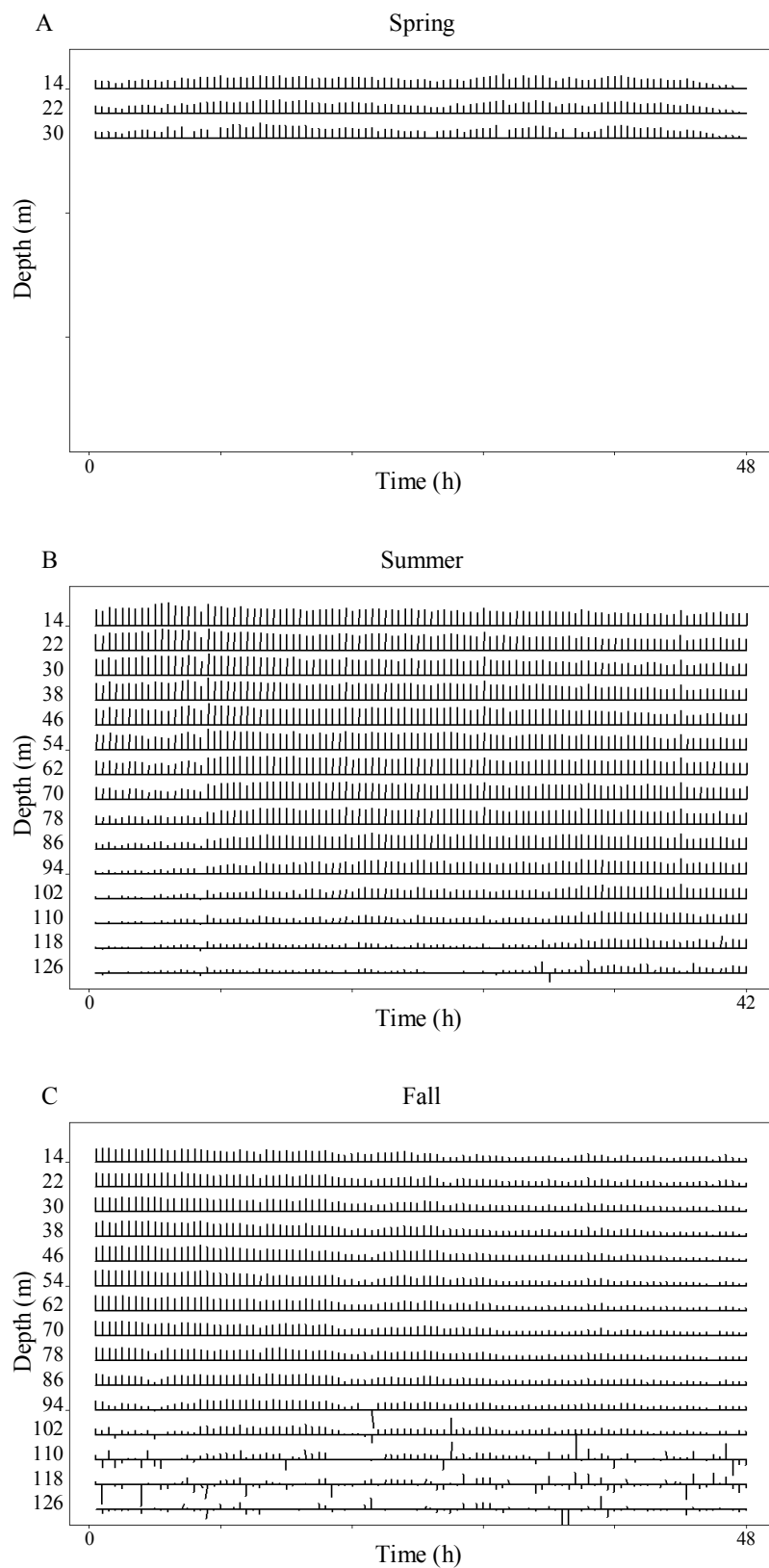
Figure 3.6. Progressive vector diagrams estimating larval transport for fish larvae maintaining different fixed depths offshore of Miami over 42-48 h, based on ADCP measurements at a fixed sampling station. Colored lines indicate transport trajectories at different depths. Gray lines indicate 100 m and 500 m isobaths.

Fig. 3.6
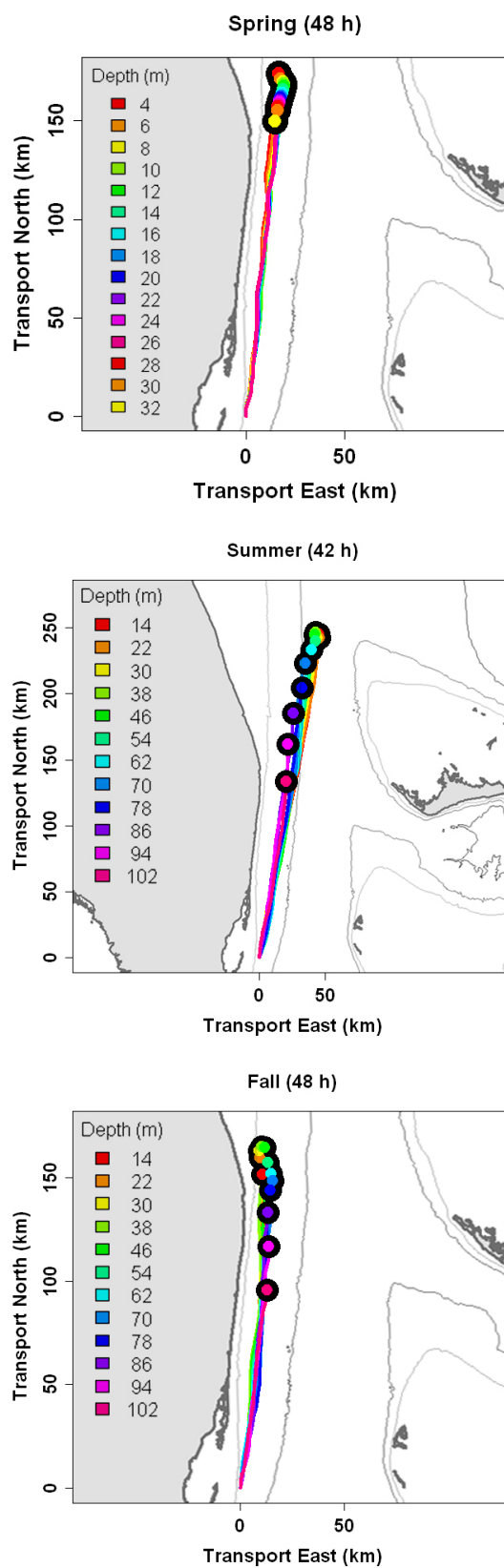
CHAPTER 4. BAROKINESIS AND DEPTH REGULATION BY PELAGIC CORAL REEF FISH LARVAE

Background

The complex life cycle of most coral reef fishes includes a pelagic larval stage. While juveniles and adults are associated with coral reefs, pelagic larvae generally spend weeks to months developing in the open ocean (Leis 1991a). Environmental conditions experienced by larvae during this period are highly variable, and affect larval survival. Mortality rates associated with warm water are particularly high and variable (Houde 1989), and larval transport can differ dramatically, with some larvae being dispersed >100 km while others are retained within several km of their origin (Cowen et al. 2006). Variations in larval survival and transport can profoundly affect the population replenishment of coral reef fishes (Cowen et al. 2000, Bode et al. 2006). Consequently, effective fisheries management and coral reef conservation depend on a thorough understanding of the ecology of pelagic larvae.

The vertical distribution of fish larvae is central to their ecology, since the ocean is vertically stratified with respect to many environmental variables. Both feeding by and predation on larvae depend on several factors that vary with depth, including prey and predator abundance (Fortier & Harris 1989), light intensity (Job & Bellwood 2000), and turbulence (Werner et al. 2001). Dispersal of larvae can also be a function of depth if ambient currents are vertically stratified (Paris & Cowen 2004, Paris et al. 2007). Vertically stratified sampling of coral reef fish larvae has shown that they are not distributed randomly. Leis (1991b) found highly structured family-specific distributions within the upper 20 m of the Great Barrier Reef lagoon. In oceanic waters off Barbados,

taxon- and age-specific vertical ranges of tens of meters were observed by Cowen (2002). While it is unclear how these patterns are determined, larval swimming behavior influences vertical distributions in temperate fish larvae (Olla et al. 1996), and reef fish larvae generally swim very fast, with some taxa capable of speeds around 5 cm s–1 only 12 h after hatching (Fisher et al. 2000).

One type of behavior that could influence vertical distributions is barokinesis, or change in swimming activity due to hydrostatic pressure. Barokinetic behavior is exhibited by marine organisms ranging from invertebrate zooplankton (Hardy & Bainbridge 1951, Park et al. 2004) to fish larvae (Qasim et al. 1963, Macquart-Moulin et al. 1989). In laboratory experiments, increased pressure generally evokes upward swimming (towards lower pressure) and decreased pressure evokes either downward swimming or sinking (towards higher pressure). In nature, this negative feedback mechanism may result in depth regulation and contribute to vertical distributions of barokinetic organisms.

The objectives of the present study were to test whether coral reef fish larvae exhibit barokinesis in experiments and whether the observed behavior is consistent with their swimming depths measured *in situ*. To simplify the discussion, the term "larvae" is used in the sense inclusive of all pelagic early life history stages of coral reef fishes. To observe truly pelagic coral reef fish larvae, all experiments were conducted at sea with wild-caught animals collected in plankton net tows. This represents an important departure from previous work that used larvae reared in captivity or collected in light traps over shallow coral reefs. This first attempt to directly compare depths that larvae

"swam towards" in experiments to *in situ* depths is a quantitative test for barokinesis as a potential mechanism for depth regulation.

Methods

  Collections. Larval coral reef fishes were collected during monthly cruises in the Florida Straits in 2004 and 2005 aboard the University of Miami RV F. G. Walton Smith. The cruises were part of an interdisciplinary study of billfishes, and ichthyoplankton sampling gear and methods were designed specifically for the billfish project. Stratified tows with a coupled asymmetrical MOCNESS (Multiple Opening Closing Net with Environmental Sampling Systems) (Guigand et al. 2005) were conducted with different nets fishing at 0 to 25, 25 to 50, 50 to 75, and 75 to 100 m. Additionally, the upper 0.5 m of the water column was sampled with neuston nets. The duration of MOCNESS tows from 100 m depth to the surface was 20 min, with each net sampling for 5 min at a tow speed of 1.5 m s–1. Neuston tows were 10 min in duration. For a detailed description of sampling locations, see Llopiz & Cowen (2008). Diurnal samples from MOCNESS nets with a 4 m2 mouth opening and 1 mm mesh size and neuston nets with a 2 m2 mouth opening and 1 mm mesh size were inspected for coral reef fish larvae that appeared to be swimming naturally. Each larva was placed in a separate glass cylinder of 9 cm diameter and 17 cm height filled with 1 l of seawater on-site and stored in a shaded box until used in experiments.

  Experiments. All experiments were conducted at sea; time from specimen collection to onset of the experiment was generally <4 h and at most 8 h. At the beginning of an experiment, a cylinder containing a single larva was placed in a 7.5 l

hyperbaric chamber of the type used for calibrating scuba diving instruments. The chamber was kept dark within the visual spectrum of fishes (Lythgoe 1988), and was illuminated only by an array of infrared LEDs. Larval behavior within the chamber was monitored throughout the experiment with an infrared-sensitive video camera and recorded on tape. Pressure was manipulated by adding air to the chamber from a high-pressure tank and releasing air from the chamber to the atmosphere. Each specimen was tested only once.

The chamber was kept at surface pressure for an acclimation period of 30 min, then pressure was increased by 50% every 5 min, simulating a stepwise decent from 0 to 5, 13, 24, 41, and 66 m depth. This was followed by pressure decreases every 5 min simulating a stepwise ascent from 66 to 41, 24, 13, 5, and 0 m. Each change was made gradually over the course of 1 min to approximate rates larvae might experience *in situ* when swimming up or down in the water column at realistic speeds (Leis & Carson-Ewart 1997). In experiments where the larva clearly behaved similarly under all pressure conditions, the null hypothesis (no barokinesis) was not rejected and the larva was scored as unresponsive. If a larva appeared to change its behavior between different pressure levels, the experiment was continued with additional pressure steps after the original descent and ascent. The additional steps were intended to reduce the chance of incorrectly rejecting the null hypothesis, and increase the accuracy of pressure preference predictions. In some experiments, the acclimation period, individual time steps, or the sequence of pressure levels deviated from the described protocol due to logistical constraints at sea. Seven experiments were ended when erratic or unnatural swimming behavior became evident (Fisher et al. 2000). This occurred when larvae

became too weak to overcome positive buoyancy at low pressures by downward swimming. Three larvae were used in pressure experiments after first being exposed to a series of different light intensities for a related study.

Analysis. Videotapes were digitized and visual reference grids, numbered from 1 at the bottom of the cylinder to 10 at the water surface, were superimposed on each image (Fig. 4.1). Vertical positions of each larva relative to this grid were recorded every 10 s. Only observations made at constant pressure were included in data analysis; data points during transitions between time steps were discarded. Preliminary analysis revealed that significant autocorrelation was present during some time steps, affecting up to 3 data points or 30 s duration. To eliminate this problem and ensure independent data points, subsamples of 100 data points were used in all further analyses.

Behavioral data did not conform to the assumptions of parametric statistics, so the relationship between larval behavior and pressure was determined using resampling methods. Pearson's correlation coefficient (r) between larval vertical position and pressure was bootstrapped and the significance of the correlation was determined by a permutation test (Hesterberg et al. 2005). If 1000 bootstrapped r-values exceeded 1000 permuted r-values in at least 95% of pairwise comparisons, the correlation was considered significant ($p < 0.05$). Since pressure was an independent variable by experimental design, significant correlation revealed that position was dependent on pressure. In this case, 1000 bootstrapped linear regression parameters (slope and intercept) were used to predict the mean pressure at which the mean larval position would be at the center of the cylinder, with no net vertical movement. These "pressure preferences" are estimates for the depth that larvae "swam towards" in experiments, and

sometimes exceeded the 0 to 66 m pressure range at which larvae had been observed. For example, a mean position of 2 (near the bottom) at 41 m, and 4 (slightly beneath center) at 66 m would result in a pressure preference (position 5.5) of 85 m. The effectiveness of predicting *in situ* behavior from experimental data was determined by a permutation test of the correlation between capture depth and pressure preference within each family. Uncertainty in the capture depth of larvae within the vertical range of plankton net tows was included by randomly generating depths within the appropriate ranges using a uniform distribution. If the correlation was significant, a bootstrapped regression was performed (as explained above), to model capture depths with pressure preferences. All statistical analyses were conducted using S-Plus (Insightful).

Results

Experiments were conducted with 14 larvae of the family Pomacanthidae (angelfishes), 9 Balistidae (triggerfishes), 12 Acanthuridae (surgeonfishes), and 21 Monacanthidae (filefishes). Most larvae could not be identified beyond family, therefore only results of individual experiments and of experiments pooled by family are presented. Larvae ranged in size from 3.4 to 19.6 mm standard length, and included specimens of various developmental stages.

In 34 of 56 experiments, there was significant positive correlation between larval vertical position and hydrostatic pressure (permutation test: $p < 0.05$) (Table 4.1). Broken down by family, 11 of 14 pomacanthids, 8 of 9 balistids, 9 of 12 acanthurids, and 6 of 21 monacanthids moved down during low pressure (shallow depth) steps and

up during high pressure (deep depth) steps (Fig. 4.2). Note that changes in swimbladder buoyancy caused by pressure changes would result in the opposite pattern, whereas the observed positive correlation can only be due to barokinesis. Sometimes larvae encountered the top or bottom surface of the water column and continued to energetically swim "against" this surface. At other times they avoided the top and bottom surfaces, swimming mostly in the lower half of the cylinder during low-pressure steps and making increasingly frequent excursions into the upper half during higher-pressure steps. In a real ocean water column this behavior would cause larvae to move towards a particular depth range.

Significant positive correlation (permutation test: $p < 0.05$) between pressure preference and *in situ* capture depth was present in the families Pomacanthidae and Balistidae, but not in Acanthuridae, Monacanthidae, or with all families combined. Pressure preference explained almost half of the variance in capture depth in regression models of pomacanthids ($R^2 = 0.47$) and balistids ($R^2 = 0.45$) (Fig. 4.3). Most pressure preferences fell within the 0 to 66m range at which experiments were conducted. Pressure preference values >66 m reflect experiments where the mean position of larvae in the experimental cylinder increased with depth, but did not exceed the center, position 5.5. In 2 acanthurid and 2 monacanthid experiments, pressure preferences exceeded the 0 to 66 m range to such an extent that even the lower boundaries of 95% confidence intervals were >66 m. These outliers are suspect, because they were calculated by regression on data at pressures <66 m, but their inclusion did not change the results of the preceding statistical analyses.

Discussion

Larvae from all 4 families (Pomacanthidae, Balistidae, Acanthuridae, and Monacanthidae) varied their vertical swimming behavior significantly across different levels of hydrostatic pressure. This indicates a sense of pressure, which has previously been found in a variety of adult and larval fishes, but not in coral reef fish larvae. In adults, pressure sensitivity has been demonstrated by reflexive "yawning" responses (McCutcheon 1966), by conditioning fish to associate pressure changes with food (Dijkgraaf 1941) or electric shocks (Tytler & Blaxter 1973), and by electrophysiological recordings (Koshtojanz & Vassilenko 1937). In larvae, pressure sensitivity has been demonstrated by swimbladder inflation (Govoni & Hoss 2001) and barokinesis (Qasim et al. 1963). Thresholds for pressure sensitivity are generally on the order of 1% of absolute ambient pressure, for example 0.1 m at the surface or 1 m at 90 m depth. Sensory mechanisms of pressure sensitivity remain unclear in most fishes. Movements resulting from changes in buoyancy are presumably detected by neuromasts in the vestibular system and the developing lateral line system. In the absence of movement, Weberian ossicles (Dijkgraaf 1941) and neuroreceptors in the swimbladder (Koshtojanz & Vassilenko 1937) can convey pressure sensitivity, and even fishes lacking these structures can sense pressure (Qasim et al. 1963). In sharks, vestibular neuromasts in the labyrinth convey absolute hydrostatic pressure information (Fraser & Shelmerdine 2002). This system may exist in teleost fishes as well. While pressure sensitivity studies in fishes have historically focused on sensory biology, the present study was concerned with the ecology of pressure-mediated behavior.

Coral reef fish larvae exhibited similar barokinetic behavior to other larval fishes, in that they moved up in response to high pressure and down in response to low pressure. However, previous studies were inconclusive with respect to the potential for depth regulation. Macquart-Moulin et al. (1989) demonstrated barokinesis in sole larvae during and immediately following pressure changes of 1 m, but the behavior was too short-lived to effectively regulate depth. Qasim et al. (1963) reported that groups of blenny, gunnel, and flounder larvae moved back and forth between the lower and upper half of a pressure vessel in response to sudden changes between 0 m and 6 to 10 m pressure. Depth regulation seems likely, but absolute pres- sure (6 m), pressure change (60%), and rate of pressure change (60% in only a few seconds) were confounded as potential cues. In the present study, the confounding effects of absolute pressure, pressure change, and rate of pressure change were minimized by design. Pressure steps were spaced at even 50% increases, changes were gradual, and data recorded during changes were excluded. Finally, larvae were observed at the same absolute pressure levels following changes from both directions. With all this takeninto account, pressure often explained a considerable fraction of vertical swimming behavior, with larvae moving up at high pressures (corresponding to deep depths) and down at low pressures (corresponding to shallow depths). Beyond demonstrating the mere possibility of depth regulation, the results presented here show that barokinesis can be important in structuring vertical distributions of larval coral reef fishes.

Significant correlation between pressure preference and capture depth in pomacanthids and balistids refutes the null hypothesis that behavior in experiments and behavior *in situ* were unrelated. Almost half of the variance in capture depth was

successfully predicted by the regression model ($R^2 = 0.47$ for pomacanthids and $R^2 = 0.45$ for balistids), which provides circumstantial evidence for a cause–effect relationship between barokinesis and depth regulation *in situ*. Nevertheless, the precision with which individual capture depths could be predicted was low. This is reflected in the number of experiments where pressure preferences matched the capture net tow depth ranges. Of 14 pomacanthids, 6 had pressure preferences within the depth range of the net tow in which they were collected, 5 had pressure preferences 0 to 25 m outsidetheir capture depth range, and 3 had no significant pressure preferences. Of 9 balistids, 2 had pressure preferences within the correct depth range, 4 had pressure preferences 0 to 25 m outside the capture depth range, 2 had pressure preferences 25 to 50 m outside the capture depth range, and 1 had no significant pressure preference. One possible explanation is that in nature, barokinesis influences depth regulation on a relatively coarse scale (10s of m), while other factors are more important at a finer scale. For example, some pelagic reef fish larvae seek out structures such as flotsam, drifting algae, or jellyfish (Kingsford 1993). This might also explain why pressure preference and capture depth were unrelated in monacanthids, which are strongly associated with drifting algae (Kingsford 1992). Alternatively, barokinesis may act on a much finer spatial scale in nature than could be resolved in experiments. Vertical distributions of coral reef fish larvae are known to change on diel cycles (Leis 1991b), and any change in depth-regulating behavior during the hours between sampling and experiment would have weakened the relationship between capture depth and pressure preference. This possibly explains why pressure preference and capture depth were

unrelated in acanthurids and monacanthids, despite significant barokinetic behavior at the individual level.

Despite examples of barokinesis in all 4 families, several larvae were unresponsive in experiments, revealing limitations in the chosen methodology. First, all larvae were exposed to physical and biological stress during the collection process. Great care was taken to exclude injured larvae, but mechanical abrasion against the net and predator–prey interactions within the sample may have led to unnatural behavior. Second, the small experimental cylinder and externally applied pressure changes could have triggered escape responses, masking natural behavior. Third, pressure is only one of several variables conveying information related to depth. For example, keeping the hyperbaric chamber dark at all times disrupted the natural relationship between pressure and light atdepth. The presence of an air–water interface at the top of the cylinder also conflicted with pressure cues, indicating a depth very near the surface at all times. Contradictions between different environmental stimuli in controlled laboratory conditions can disrupt natural orientation behavior (Pavlov et al. 2000). Despite these complications, most larvae exhibited significant pressure preferences, suggesting that the ability to regulate depth by barokinesis is common among pelagic reef fish larvae and, presumably, other fishes as well.

Since the *in situ* depths of pomacanthid and balistid larvae could be predicted by experiments, it is tempting to also draw inferences about larval populations. This requires additional care because sampling was strongly biased towards larvae of large size, high condition, shallow depth, or otherwise likely to survive net tows. Therefore, sample statistics are not necessarily representative of larval populations. One statistic

that is a conservative estimate for the population is the range of pressure preferences within families. Pressure preferences of pomacanthids and balistids varied in accordance with *in situ* depths over a vertical range of tens of meters. This requires a substantial degree of behavioral plasticity, with different larvae from the same population exhibiting distinct barokinetic behavior. In fact, plasticity in barokinesis alone may account for the entire vertical range of reef fish larvae, which is limited to only tens of meters in various families in the study area (Cha et al. 1994). Another finding with implications at the population level is the timescale of several hours across which pressure preferences and capture depths were correlated in pomacanthids and balistids. If the vertical behavior of individual larvae is persistent and they occupy narrow depth ranges *in situ* at this timescale, vertical distributions could be quite stable throughout the day. Acanthurid and monacanthid distributions, on the other hand, may be more dynamic. These ideas can be used to refine or test models of recruitment variability and larval transport (Cowen et al. 2006), which are becoming increasingly important in the management of fisheries (Sale et al. 2005, Fogarty & Botsford 2007).

The proposed role of barokinesis as a proximate mechanism for depth regulation does not imply that the ultimate function of depth regulation is related to hydrostatic pressure. In fact, other environmental variables are clearly more important. Vertical distributions of predators and prey have immediate consequences for larval survival (Fortier & Harris 1989). The vertical gradient of light limits larvae to shallow depths, where they can feed effectively (Job & Bellwood 2000) but the risk of detection by predators is increased (Bailey & Houde 1989). Turbulence affects predator–prey interactions by increasing encounter rates (Rothschild & Osborn 1988) while decreasing

successful capture rates (MacKenzie & Kiorboe 2000). Finally, the vertical distribution of ambient water current influences physical transport (Paris & Cowen 2004, Paris et al. 2007), which is essential for larval survival (Pineda et al. 2007). In contrast, larval ecology does not appear to depend much on pressure. Pressure mainly affects buoyancy, and perciform larvae should be able to regulate their swimbladder volume to compensate for pressure changes starting around the time of yolk-sac depletion and first feeding (Pelster 2004). Govoni & Hoss (2001) confirmed this experimentally in spot, reporting that late-stage larvae could compensate within an hour for negative buoyancy caused by a 10 m pressure increase. Similar swimbladder adjustments were also apparent in the present study. More importantly, larvae were able to compensate for improper buoyancy by vertical swimming, which is energetically more expensive but much faster (Strand et al. 2005). If non-neutral buoyancy is easily overcome, then no particular pressure level is inherently favorable for larval survival. This points towards the use of pressure as a proxy for something else. The relationship between the proximate cue pressure and the ultimate cause of depth regulation is unclear, but any cue correlated with feeding, predator avoidance, and larval transport might be exploited to enhance survival. While it is not a trivial problem to unravel the fundamental cause–effect relationships, depth regulation and vertical distributions of fish larvae play a major role in determining these processes.

Table 4.1. Significant correlations between larval vertical position and absolute pressure (bold). Larvae were collected in plankton nets towed at known depth ranges (capture depth). The pressure that larvae "swam towards" was calculated from experimental data (pressure preference and 95% confidence interval). Significance was determined by permutation test. ns = not significant.

Figure 4.1. Larva swimming in glass cylinder inside hyperbaric chamber. Larval behavior was recorded in darkness with an infrared camera. The superimposed grid was used to score vertical positions; here the fish is at position 8. The grid is slanted to correct for camera angle.
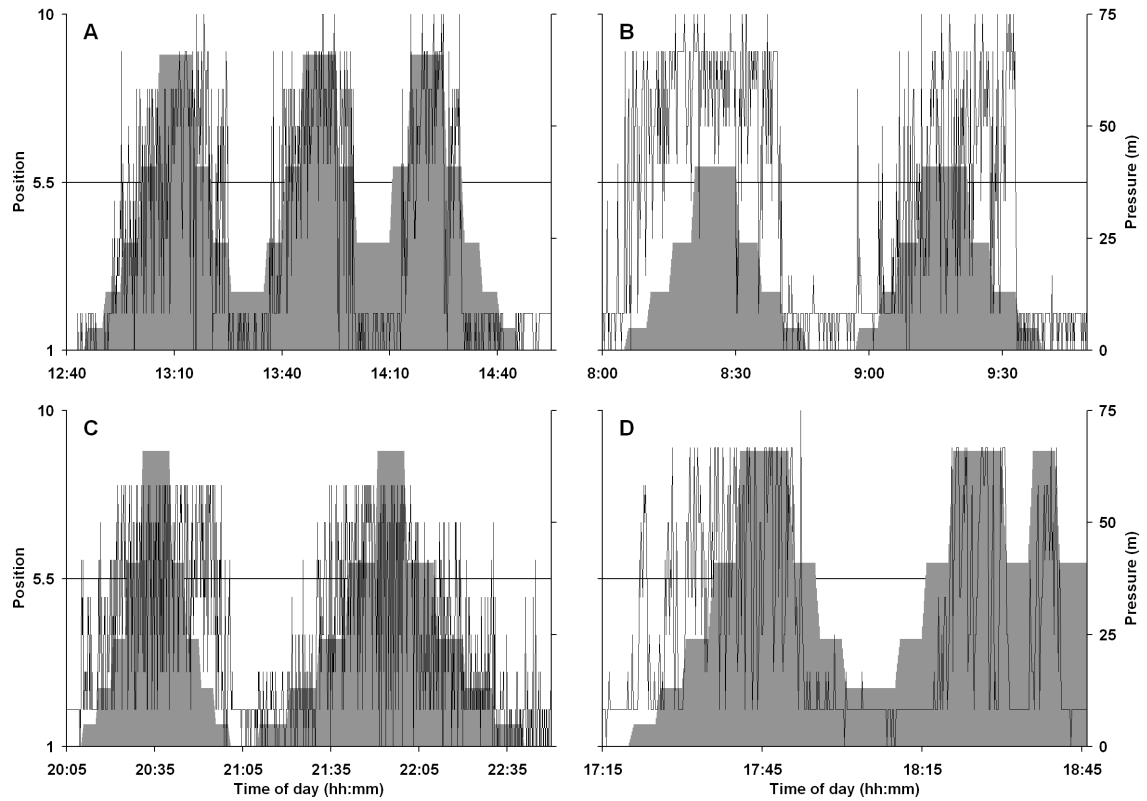
Figure 4.2. Sample data of experiments with (A) Pomacanthidae, (B) Balistidae, (C) Acanthuridae, and (D) Monacanthidae. Area: pressure, line: larval position. At onset of experiment larvae were under surface pressure and swam down. Larvae increasingly swam up at higher pressure, then down again at lower pressure. At the calculated pressure preference there was minimal net movement relative to center of the cylinder, position 5.5.
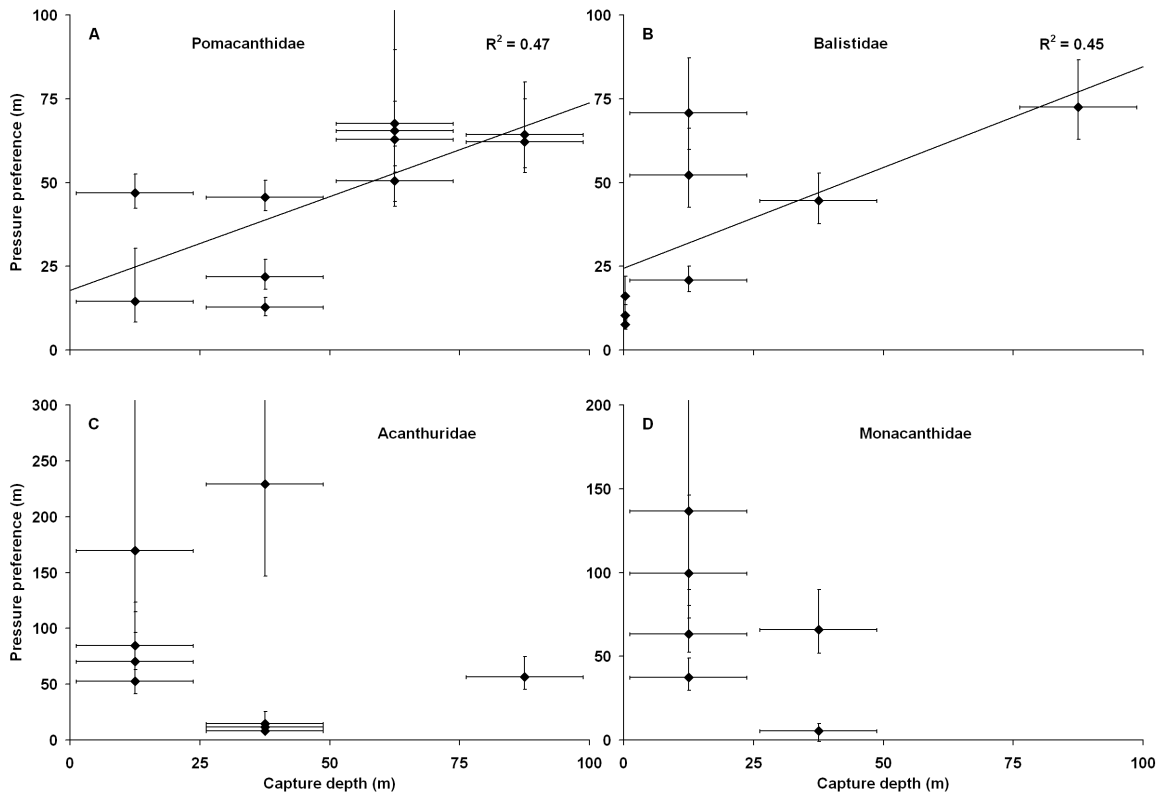
Figure 4.3. *In situ* capture depths and experimentally derived pressure preferences of all larvae exhibiting barokinesis in (A) Pomacanthidae, (B) Balistidae, (C) Acanthuridae, and (D) Monacanthidae. Capture depth ranges are 95% of the vertical ranges of net tows, pressure preference ranges represent 95% confidence intervals. Regression ,odel indicated by line in (A, B).

CHAPTER 5. OBSERVED AND SIMULATED SWIMMING TRAJECTORIES OF LATE-STAGE CORAL REEF FISH LARVAE OFFSHORE OF THE FLORIDA KEYS

Background

At the beginning of their complex life cycle, coral reef fishes typically spend weeks to months developing in the open ocean as larvae. During this time, some larvae are transported large distances by ocean currents, while others are retained near their natal reefs (reviewed in Cowen & Sponaugle 2009). Towards the end of the pelagic larval period, larvae become competent to metamorphose into juveniles and enter the final stretch of larval transport: some encounter suitable coral, seagrass, or mangrove habitat for settlement, leave the plankton, and become juveniles. Others fail to encounter suitable juvenile habitat, are unable to settle, and apparently perish. Given the urgent need to settle, active larval swimming towards settlement habitat may greatly enhance survival.

Several studies have provided circumstantial evidence for the potential importance of swimming behavior during the final stretch of larval transport. The swimming abilities of late-stage reef fish larvae were found to be impressive: in laboratory experiments, average performing larvae from average performing families can achieve swimming speeds of 37 cm s^{-1} (Stobutzki & Bellwood 1994, Fisher et al. 2005) and cover distances of 30 km without rest or food (Stobutzki & Bellwood 1997). The mean speed of larvae in their natural environment is 20 cm s^{-1}, as measured by SCUBA divers during the day (Leis & Carson-Ewart 1997). At night, when most settlement occurs, swimming speeds may be higher, as is the case with some species reared and observed in aquaria (Fisher & Bellwood 2003). Based on these findings,

late-stage larvae appear to be capable of swimming tens of km per day. Additionally, larval swimming trajectories were found to be significantly straight (non-random), at least over the course of 10 min observations (Leis et al. 1996, Leis & Carson-Ewart 2003). This is important, because frequent changes in direction could greatly reduce the cumulative effect of swimming behavior on transport. Further, late-stage larvae are attracted to odors (Sweatman 1988, Gerlach et al. 2007) and sounds (Tolimieri et al. 2004, Simpson et al. 2005) associated with coral reefs. Late-stage larvae within sensory range of a reef should consequently swim towards it.

In situ observation of larvae by SCUBA divers is a proven method for studying the final stretch of larval transport (reviewed in Leis 2006). Larvae generally do not appear stressed by the presence of the divers, engage in feeding behavior, and choose swimming directions independent of their orientation relative to the divers. To date, all studies using this approach have been conducted in the western Pacific, mostly at one particular location: Lizard Island, Great Barrier Reef (GBR), Australia. Surprisingly, most larvae observed at Lizard Island actively avoid swimming towards the reef (Leis et al. 1996, Leis & Carson-Ewart 2003). This may be because observations can only be conducted during the day, while most settlement takes place during the night. It also may be a reflection of the specific environment around Lizard Island, highlighting the need for similar studies across a range of different environments.

Here, we present the first data from *in situ* observations of fish larvae in the western Atlantic. We used a similar method as Leis & Carson-Ewart (1997) to observe late-stage *Stegastes partitus* (bicolor damselfish) at French Reef, Key Largo, Florida. Our objectives were to measure the degree to which larval swimming contributes to

larval transport and to test whether taxonomically different larvae in an environment very unlike Lizard Island behave similarly. Three key differences between Lizard Island and French Reef make the comparison noteworthy. First, French Reef has much lower biodiversity and biomass of animals than Lizard Island, typical for comparisons between the Florida Keys and the GBR. These differences may result in weaker cues for orientation. Second, French Reef is subject to much greater anthropogenic effects than Lizard Island. Land-based chemical pollution and boat noise may potentially disrupt natural olfactory and acoustic cues. Third, French Reef is part of the Florida Keys barrier reef system adjacent to the continental shelf break, while Lizard Island is sheltered inside the GBR lagoon. As a result, French Reef is exposed to much stronger currents and the nearest coral reef in any offshore direction (NE through SW) is over 100 km distant, while Lizard Island is almost entirely surrounded by reefs only 25 km away.

In addition to gathering field observations, we employed a simulation model to better quantify the importance of swimming behavior for larval transport in *S. partitus*. Our model simulates larval swimming trajectories of much longer duration than would be feasible to observe directly. Simulated trajectories were then used to predict the upper and lower limits of the distance that late-stage larvae swim *in situ*. Previous models have shown that in a theoretical framework the outcome of larval swimming towards coral reefs depends primarily on the parameters swimming speed, current speed, sensing ability, and orienting ability (Armsworth 2000, 2001, Codling et al. 2004). Our approach is novel in that we rely on bootstrapped empirical data, instead of exploring the theoretical parameter space.

Methods

     Collection. From May to September 2007, light-traps were deployed overnight near French Reef, Key Largo, Florida. Late-stage larvae of various coral reef fish species are attracted to light-traps, and *S. partitus* is the most commonly caught species in the upper Florida Keys (D'Alessandro et al. 2007). Sampling was focused on first-quarter and third-quarter moon phases, which is when late-stage *S. partitus* larvae are particularly abundant nearshore (D'Alessandro et al. 2007). Upon retrieving light-traps, we placed larvae in a shaded bucket of seawater outfitted with a battery operated aerator pump. Water changes were performed regularly to ensure high water quality and to prevent overheating. During September, catches in Key Largo were supplemented with larvae caught using the same methods at American Shoals, Big Pine Key, and immediately transported from Big Pine Key to Key Largo by car.

     Field observation. Our methods for *in situ* observations of fish larvae by SCUBA divers followed Leis and Carson-Ewart (1997). Slight modifications were made to meet safety recommendations of the American Association of Underwater Scientists for blue water diving (Heine 1986) and to address safety concerns regarding strong currents and heavy recreational boat traffic in the study area. All observations involved a designated data diver, a designated safety diver, and a boat operator. The data diver maintained constant visual contact with the larva and operated the "flompass" data recorder, which consisted of a General Oceanics low-speed 2030 flowmeter, a compass, and a digital camera for recording flowmeter and compass readings. The safety diver monitored dive time, depth, ascent rate, and environmental hazards, and towed a safety-line with a dive flag at the surface and a 2 kg weight at 18 m. All

observations were conducted near French Reef, between the 28 and 37 m isobaths, about 1 km offshore of the reef line.

The divers entered the water while the boat operator transferred one larva to a small plastic jar. The divers took the jar and descended to 5 m depth, where they oriented to face each other at a distance of 2 m. The release direction, which was defined as the initial direction the data diver was facing, was randomized between N, E, W, and S. Meanwhile, the boat operator recorded the starting time, GPS coordinates, and bottom depth sounding, and began circling the dive flag at a distance of about 50 m and a frequency of one full turn every few minutes. This was intended to evenly distribute any effect of boat noise on larval behavior across all directions (Leis & Carson-Ewart 1997). The safety diver released the larva between the two divers and started a stopwatch timer. As the larva began swimming, the data diver followed ~2 m behind and the safety diver moved into position beside the data diver, establishing touch contact. The safety diver prompted the data diver to record a flompass image every 30 s for a period of 10 min. Simultaneous depth measurements were recorded with a Suunto D-3 dive computer. Finally, the divers attempted to recapture the larva with a hand-net and ascended to the surface. The boat operator quickly approached the divers and recorded the ending time, GPS coordinates, and bottom depth. These methods resulted in ~30 s measurements of heading and flow (from the flompass image) and depth (from the dive computer), as well as a single estimate for the net-movement of the divers, including swimming as well as drift due to currents (from GPS coordinates).

Data Analysis. Three dimensional swimming and transport trajectories were reconstructed as follows. Horizontal swimming speeds were calculated from flow and

elapsed time between consecutive flompass images. This method was calibrated by repeatedly timing the divers with a stopwatch as they swam a known distance at various speeds. During calibration, 95% of flompass-derived speeds were accurate within 12% of the stopwatch-derived measurements. Headings were rounded to the nearest 10° and used as estimates for swimming direction. Speed and direction time-series were converted from polar coordinates to Cartesian coordinates and combined with the depth time-series into 3-D swimming trajectories. Lastly, average current was calculated from the change in the divers' GPS position over the course of the dive minus the change in position due to swimming. Net transport trajectories were constructed by sequentially adding drift (average current x elapsed time) to each interval of the swimming trajectories. For descriptive purposes, currents were divided into alongshore and cross-shore components using the 50° orientation of the 30 m isobath as the definition of alongshore.

Trajectories were analyzed with circular statistics, implemented in S-Plus by Insightful Corporation using the circstats library 2.0 by Lund (accessed 1 Feb. 2008, statweb.calpoly.edu/ulund), following Jammalamadaka and SenGupta (2001). Circular correlation (a circular analog of Pearson's correlation coefficient r) between the initial release direction and the direction of larval swimming was used to estimate the effect of the divers on larval behavior. This revealed that the first three minutes of observations were significantly biased by avoidance of the divers, thus these data points were excluded from all further analyses. Rayleigh's test was used to determine whether individual larvae swam randomly, and whether the mean swimming directions of groups of different larvae were distributed randomly.

Simulation model. Field measurements of larval behavior were used to simulate swimming trajectories greatly exceeding the 10 min duration of observation experiments. Simulated larvae started at position x = 0 and y = 0 and alternated "swimming" for 30 s and "turning" to a new heading in an iterative process. Movements along the positive and negative y-axis were considered towards and away from coral reef settlement habitat, respectively. Movement along the x-axis was considered parallel to the barrier reef line. Ambient currents were not included explicitly in the model.

For each simulated trajectory, values of swimming speed and heading were generated by bootstrapping data from one randomly selected observation experiment. To address a range of larval sensory environments with respect to environmental cues for orientation to the reef, three different scenarios were examined: (1) larvae could orient directly towards the reef. Each heading was drawn from the distribution of observed values rotated such that the mean pointed towards the reef; (2) larvae could orient with respect to an external reference frame but could not sense the direction towards reef. Each heading was drawn from the distribution of observed values rotated such that the mean pointed in a random direction; and (3) larvae had no external frame of reference. Each heading was generated from the previous heading plus a turn. The magnitude of each turn was drawn from the distribution of observed values and the direction (left or right) was assigned at random. In each scenario, 10,000 trajectories were simulated.

Results

  Field observation. A total of 60 observations of late-stage *S. partitus* larvae

were collected. In 30 cases, we collected a complete 10 min record of swimming

behavior, and in an additional 12 cases we collected at least 5 min of data before

encountering a problem (Fig. 5.1, Table 5.1). All 42 observations suitable for analysis

were made on 8 days between May and September 2007. On some days visibility was

sufficient for the divers to see the bottom during an observation, occasionally even from

the surface. Larvae were successfully recaptured in 33 cases, and their standard length

measurements were normally distributed with a mean of 10.5 cm (SD 0.7 cm) (Shapiro-

Wilk test: $p = 0.8$).

  In all analyzed trajectories, larval swimming was directional, meaning

significantly different from random (Rayleigh test: $p < 0.05$). Excluding the first three

minutes, during which larval swimming direction was significantly positively correlated

with initial release direction, horizontal swimming trajectories were so straight that on

average larvae covered 89% of the distance achievable by holding a perfectly straight

line. The distribution of swimming directions appeared to be random (Fig. 5.2), lacking

a significant sample mean direction (Rayleigh test: $p = 0.8$). Subsamples of trajectories

grouped by various criteria (capture site, observation time and date, current speed and

direction, tidal phase, lunar day, swimming speed and depth, standard length) also

lacked significant mean directions (Rayleigh test: $p > 0.05$).The distribution of 3-

dimensional mean swimming speed was bimodal, with 28 larvae swimming 2-13 cm s^{-1}

and 14 larvae swimming 15-34 cm s^{-1} (Fig. 5.3). The separate horizontal and vertical

components of mean swimming speed were ~ 5 cm s^{-1} and 3 cm s^{-1} in the slow group

and ~ 24 cm s^{-1} and 5 cm s^{-1} in the fast group, respectively. Currents were highly

variable in speed and direction. The alongshore component, which ranged from 76 cm s^{-1} SW to 162 cm s^{-1} NE, tended to dominate the cross-shore component, which ranged

from 27 cm s^{-1} NW to 27 cm s^{-1} SE. The mean absolute current (scalar) was 40 cm s^{-1}

and the mean current velocity vector was 2.6 cm s^{-1} N. No vertical component of current

was apparent. The average depth of swimming trajectories appeared to be normally

distributed with a mean of 9.9 m (SD 2.5 m) (Shapiro-Wilk test: p = 0.7), but may have

been biased by four trajectories that exceeded the maximum depth of the divers (Fig.

5.4). The median depth of 9.7 m and inter-quartile range of 3.9 m are more robust

statistics.

     Simulation model. As expected, model output varied greatly between the three

scenarios (Fig. 5.5). In scenario (1), larvae moved steadily towards the reef making

progress at a mean of 10.3 cm s^{-1} (SD 8.9 cm s^{-1}). In scenario (2), larvae moved steadily

away from the release point, traveling equivalent distances as in (1), but spread out

evenly over all directions. In scenario (3), larvae spread out in all directions and initially

made similar progress as in (2), but this diminished over time as their non-oriented

turning resulted in meandering trajectories. For simple comparisons among the

scenarios, results can be expressed as the proportion of larvae encountering the reef as a

function of distance and time (Table 5.2). In one week the top 10% of simulated larvae

in each scenario swam >130 km, >70 km, and >3 km towards the reef, respectively.

Discussion

Larval behavior. wimming and orienting behavior by pelagic larvae of benthic fishes potentially affect both the supply of larvae to populations on a local scale as well as the ecological connectivity among populations on a regional scale. Given the importance of marine protected area networks such as the Florida Keys National Marine Sanctuary in fisheries management, larval supply to individual protected areas and connectivity across the entire network are of particular interest (Sale et al. 2005). During *in situ* observations of the behavior of late-stage *Stegastes partitus* larvae, we made direct measurements of larval swimming directionality, swimming speed, and vertical distribution in the western Atlantic. We modeled our observation methods after Leis and Carson-Ewart (1997), thus direct comparisons can be made to several similar studies conducted in the western Pacific (mostly at Lizard Island, GBR). Our use of *in situ* data in modeling larval behavior is novel, and provides a simple framework for extrapolating from brief field observations to temporal scales of days or weeks.

Directionality. The majority of late-stage coral reef fish larvae observed in open water during previous studies in the western Pacific swam directionally, i.e. they made significant progress in a particular direction (reviewed in Leis 2006). Given sufficient sample sizes, larvae frequently swam in significantly similar directions, i.e. the distribution of individual directions was non-random, either in terms of compass heading or, more commonly, relative to the reef (reviewed in Leis 2006). The latter provided evidence that some larvae actively navigate using cues associated with the reef.

In our study, each *S. partitus* trajectory was individually directional, but at the sample level, the 42 trajectories were evenly distributed over all possible directions. Significantly directional swimming in entirely dissimilar directions is puzzling, and open to more than one interpretation. Perhaps larvae swam in different directions due to a lack of environmental cues for proper navigation. Reefs in the Florida Keys have much lower biodiversity and biomass than do reefs at Lizard Island, presumably resulting in weaker "beacons" for larvae to follow. Further, Key Largo is subject to greater anthropogenic impacts than Lizard Island, and natural stimuli such as reef odor and sound may be obscured by pollution and boat noise. Odor plumes might also be more complex due to strong and variable currents. If larvae lacked an external reference frame for orientation, then the straightness of individual trajectories is remarkable and calls for an alternative explanation. Codling (2004) suggested thinking of larval swimming as a correlated random walk process, where any given heading depends only on the previous heading modified by a turn. Given small turns, a correlated random walk can be very straight for short periods of time with or without orienting behavior. Additionally, fishes lacking environmental cues may actively limit the rate at which their swimming direction changes, based on endogenous inertial cues detected by the vestibular system (Harden Jones 1984, Levin & Gonzalez 1994). The implications of larvae following correlated random walk trajectories are further explored in our simulation model.

Alternatively, it is possible that *S. partitus* larvae were able to detect the reef, but still swam in different directions. The benefits of moving to or from the reef under experimental conditions are unclear. At Lizard Island, the most common pattern of

behavior is for larvae to swim away from the reef during the day (Leis et al. 1996),

presumably to avoid visual predators and delay settlement until nightfall. At French

Reef, swimming away from the reef during the day may be a poor strategy, due to the

threat of being swept away by the nearby Florida Current. One damselfish species at

Lizard Island swam towards the reef at 1 km offshore but away from the reef at 100 m

offshore, hypothetically maintaining a preferred distance for settlement come nightfall

(Leis & Carson-Ewart 2003). While it is conceivable that *S. partitus* larvae maintain a

preferred distance of ~1 km from French Reef during the daytime, a simpler explanation

is that diurnal swimming behavior was not directly related to settlement. Nighttime

SCUBA observations have not been attempted, but orientation towards reef cues during

the night has been demonstrated on smaller spatial scales using other methods

(Tolimieri et al. 2004, Simpson et al. 2005).

    Larval transport. Simultaneous measurements of swimming speed and

Lagrangian current (i.e. drift) provide direct insight into the influence of behavior on

larval transport in a dynamic oceanographic system. Mean horizontal *in situ* swimming

speeds of 2-32 cm s^{-1} for *S. partitus* in our study are comparable to 2-40 cm s^{-1} among

damselfishes and 1-65 cm s^{-1} among other late-stage coral reef fish larvae observed in

Australia and Polynesia (Leis & Carson-Ewart 1997). The critical swimming speed of

late-stage *S. partitus*, which leads to exhaustion within a few minutes, is ~43 cm s^{-1}, as

determined in laboratory experiments in the Turks and Caicos Islands (Fisher et al.

2005). It is typical for *in situ* speeds to be approximately half of critical swimming

speeds (reviewed in Leis 2006). Thus, the expected *in situ* speed for *S. partitus* should

be ~21.5 cm s^{-1}, which agrees well with our observed measurements of 2-32 cm s-1.

For a location so close to the reef, the currents we measured were extremely variable and strong. We expected currents around a mean of 20 cm s^{-1} NE (SD 17 cm s^{-1}), based on data from the 30 m isobath at nearby Carysford Reef (Lee & Williams 1999), and consistent with data from the 24 m isobath at French Reef (Sponaugle et al. 2005). We observed over three times the anticipated range of current speeds (80 cm s^{-1} SE - 162 cm s^{-1} NW).

For comparisons between swimming and current speed measurements, larvae are often categorized as either effective or ineffective swimmers, depending on their ability to swim faster than average current. Applying the idea of effective swimming to our data is complex, because the concept of "average" current is ambiguous and potentially misleading. The mean current speed scalar of 40 cm s^{-1} was faster than the fastest larvae, while the mean current velocity vector of 2.6 cm s^{-1} (pointing north) was roughly equivalent to the slowest larvae. Further, the variability in magnitude and direction of current was so great that most larvae experienced currents that were quite different from the mean. Finally, in the context of swimming to settlement habitat, the cross-shore component is much more important than the alongshore component of current. Since the barrier reef tract is essentially linear in nature and extends for ~350 km, movement along the reef may be inconsequential for settlement (on a short timescale), while cross-shore movement is critical. To take the variability in current magnitude and direction into account, we calculated the ratio of transport by swimming and transport by cross-shore current. This metric expresses the degree to which larval swimming was "effective" quantitatively and explicitly incorporates the imperfect ability of larvae to orient. We found that larval swimming accounted for a mean of 48%

(SD 29%) of cross-shore drift plus distance swum. In other words, larval behavior and cross-shore currents contributed equal parts to net-transport on the timescale of *in situ* observations.

Vertical distribution. Vertical swimming of *S. partitus* larvae is of particular interest, because currents and potential cues for orientation can vary with depth. It was clear from direct observations that larvae swam to particular depths, as opposed to arriving there by currents or buoyancy. Over individual 30 s time steps, swimming speeds in excess of 20 cm s^{-1} down and 17 cm s^{-1} up (limited by the divers' ability to safely follow) were observed. Overall, 23% of swimming activity was along the vertical axis. In oceanic water off of Barbados, *S. partitus* migrate with ontogeny from a region of offshore flow at 0-20 m depth during their preflexion stage to a region of onshore flow deeper than 20 m during their postflextion stage (Paris & Cowen 2004). If larvae behave similarly in the Florida Straits, then the narrow distribution we observed (mean 9.9 m, SD 2.5 m) may represent a second vertical migration from >20 m depth to ~10 m at the very end of the pelagic larval phase. Vertical distributions of 13 settlement-stage damselfish species at Lizard Island were similarly shallow and narrow, with a mean depth of 7.7 m (SD 2.8 m) (Leis 1996).

The mechanisms underlying depth regulation by pelagic fish larvae are not well understood. At Lizard Island, some larvae swim at shallower depths when more upwelling light is visible (over a reflective sandy bottom), possibly to maintain a minimum distance from the bottom (Leis 2004). In our study, the bottom was clearly visible from the surface on some days, but uniformly dark blue on others (true blue-water conditions). We saw no effect of visibility on larval behavior. Damselfish may

also use a sense of hydrostatic pressure to regulate their depth, as has been demonstrated in coral reef fish larvae from other families (Chapter 4).

Simulation model. The purpose of our model was to evaluate the effects of swimming behavior on larval transport at the end of the pelagic larval stage. Three different scenarios simulated larvae: (1) swimming towards the reef; (2) swimming towards a random direction; or (3) swimming with no directional preference (following correlated random walks). The scenarios are equivalent to navigation with compass and map, orientation by compass only, and non-oriented movement, respectively. In all cases, swimming trajectories were generated by resampling *in situ* data. Since field observations were exclusively conducted about 1 km from the reef line, the model may overestimate the straightness of trajectories at greater distances from the reef. However, we consider this unlikely because larvae did not appear to benefit from their close proximity to the reef in terms of orientation, as demonstrated by dissimilar swimming directions.

Larvae that are able to sense the reef, i.e. scenario (1), approached the reef quickly (mean 10.3 cm s^{-1}, SD 8.9 cm s^{-1}). These results are similar in speed to estimates based on laboratory speed measurements by Stobutzki & Bellwood (1997), and similar in straightness to the correlated random walk model by Codling et al. (2004) at the upper limit of sensing and orienting ability considered. The maximum distance from the reef at which orientation by fish larvae has been demonstrated *in situ* is only 1 km (Leis et al. 1996, Leis & Carson-Ewart 2003). Beyond this range it is speculative to assume that larvae can sense the reef. Estimates based on auditory sensitivity to reef sounds range from 1 km (Egner & Mann 2005) to "many" km (Wright et al. 2005),

assuming loud biological noise produced by healthy reef communities. Under oceanographic conditions dominated by tidal currents, olfactory sensitivity to persistent odor plumes may facilitate homing by fish larvae over 20 km (Gerlach et al. 2007). This is unlikely to occur in the Florida Keys, where flow is dominated by the Florida Current. Coral reef fish larvae are found at high concentrations at distances up to 40 km or more offshore in the Straits of Florida (e.g. Sponaugle et al. 2009). Due to this distance mismatch, and since larvae swam in random directions at ~1 km from the reef, scenario (1) is unrealistic for the majority of larvae.

An upper limit of larval transport via horizontal swimming without reef-based cues is given by (2), in which larvae persistently swim towards a random direction. A lower limit is given by (3), in which larvae swim in correlated random walks. Scenarios (2) and (3) both predict that some larvae swim substantial distances towards the reef despite lacking the ability to sense the reef. This is a major departure from the common assumption that swimming by disoriented larvae is irrelevant and negligible. To put the model results in perspective, consider a cohort of entirely passive larvae and a cohort of actively swimming larvae, each located several km from suitable settlement habitat, but unable to sense their proximity across this distance. Lacking favorable ocean currents, the cohort of passive larvae has no chance of recruitment and perishes entirely. The cohort of active larvae, on the other hand, has greatly enhanced recruitment potential (Table 2). Additionally, larvae swimming towards the reef by chance will increase their odds of detecting reef based cues, which may cause a transition to proper navigation to the reef as in scenario (1).

Conceptual framework. With respect to the influence of horizontal swimming on transport and settlement, the early life history of reef fishes can speculatively be divided into three phases. In the first phase, horizontal swimming is of no importance. This phase, which is not directly addressed in our study, certainly includes non-motile eggs, almost certainly includes slow swimming pre-flexion larvae (Fisher et al. 2000), probably includes post-flexion larvae until they become competent to settle, and is less likely to include settlement-stage larvae. In the second, previously undescribed phase, larvae lacking the environmental cues for proper navigation nevertheless actively swim distances of up to several km, which increases their chance coming within sensory range of settlement habitat. The transition to this phase may be limited by the development of swimming ability (Fisher et al. 2000), or it may take place around the time larvae become competent to settle. Further study is needed to determine the temporal and spatial scale of this phase and to validate whether it is of general importance. In the third phase larvae are able to sense environmental cues useful for orientation (Leis et al. 1996, Leis & Carson-Ewart 2003) and settlement habitat selection (Sweatman 1988, Danilowicz 1996). At this point they may actively navigate towards settlement habitat (Stobutzki & Bellwood 1998, Simpson et al. 2005), or perhaps attempt to maintain a position within sensory range of settlement habitat while delaying settlement (Leis & Carson-Ewart 2003).

Summary. Integrating physical oceanography and larval behavior is of great value in studying and predicting larval dispersal (Cowen et al. 2006, Paris et al. 2007). Two of the remaining challenges are to incorporate the effects of small scale physical circulation and the effects of species-specific behavior on larval transport (reviewed in

Werner et al. 2007). In the case of late-stage *S. partitus* larvae in the upper Florida

Keys, observed and simulated swimming trajectories indicate that horizontal swimming

by larvae with or without an external reference frame is important at spatial scales of

several km. *S. partitus* larvae swim at speeds typical for other coral reef fish larvae

(Fisher et al. 2005), and encountered very strong currents during our study. Therefore

horizontal swimming by coral reef fish larvae may generally account for the several km

of transport immediately preceding settlement.

Table 5.1. Summary of *in situ* observations of late-stage *Stegastes partitus* larvae. Some were aborted to ensure the divers' safety when larvae exceeded 18 m depth, ascended faster than 18 m min^{-1}, and in one case when a boat came dangerously close. Bold type indicates observations with sufficient data for analysis.

Duration (min)	Outcome	n
10	observation complete	**30**
5-10	larva lost	**5**
	aborted - max depth	**4**
	aborted - ascent rate	**1**
	aborted - boat traffic	**1**
	larva eaten	**1**
<5	aborted - max depth	8
	larva lost	7
	larva eaten	2
	aborted - ascent rate	1

Table 5.2. Comparisons between larval dispersal due to simulated swimming behavior in three model scenarios at three orders of magnitude in temporal and spatial scale. Numbers represent the fraction of larvae swimming a particular distance towards the reef within a particular time.

Scenario	Distance (km)	Time (min)		
		100	**1,000 (17 h)**	**10,000 (1 wk)**
(1) larvae persistently	**1**	29%	100%	100%
swim towards reef	**10**	0%	29%	100%
	100	0%	0%	29%
(2) larvae persistently	**1**	6%	37%	49%
swim towards random	**10**	0%	6%	37%
direction	**100**	0%	0%	6%
(3) larvae swim in	**1**	1%	17%	46%
correlated random	**10**	0%	0%	2%
walks	**100**	0%	0%	0%

Figure 5.1. Trajectories of 26 late-stage *Stegastes partitus* larvae observed for 10 min by SCUBA divers. The insert shows the location of the plot area relative to the Florida Keys barrier reef. Gray areas represent coral reefs, black polygons designate sanctuary protected areas, and thin lines mark isobaths. Black points represent the locations at which larvae were released. (A) Lines represent larval swimming trajectories reconstructed from compass and flowmeter measurements. (B) Lines represent larval transport due to swimming plus drift due to currents. An additional 16 observations conducted outside of the plot area or for < 10 min are not shown. The first three minutes of trajectories were significantly biased towards the release direction and were excluded from data analysis.
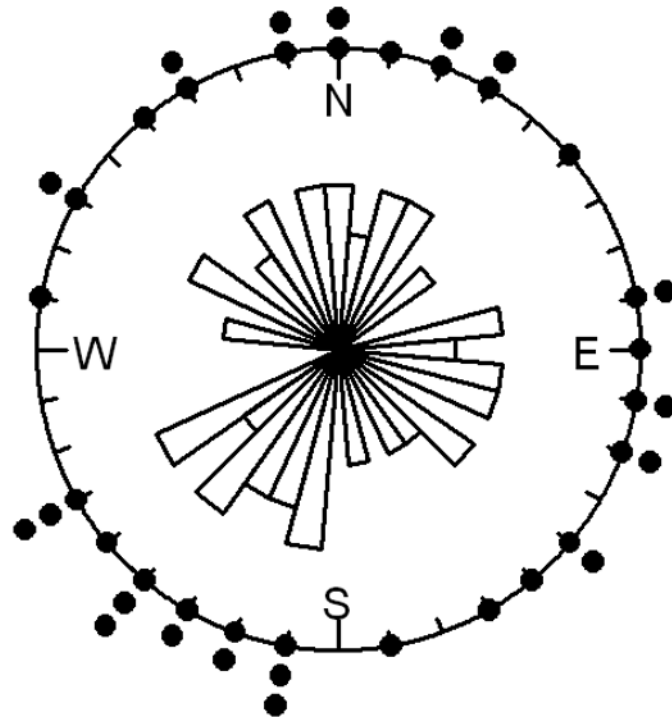
Figure 5.2. Each point on the compass represents the mean swimming direction of one late-stage *Stegastes partitus* larva observed for 5-10 min off the Florida Keys. The area of each rose petal at the center of the diagram represents the frequency of mean directions. Individual mean directions were all statistically significant (Rayleigh test: p < 0.05), but the distribution of mean directions was random (Rayleigh test: p = 0.8).
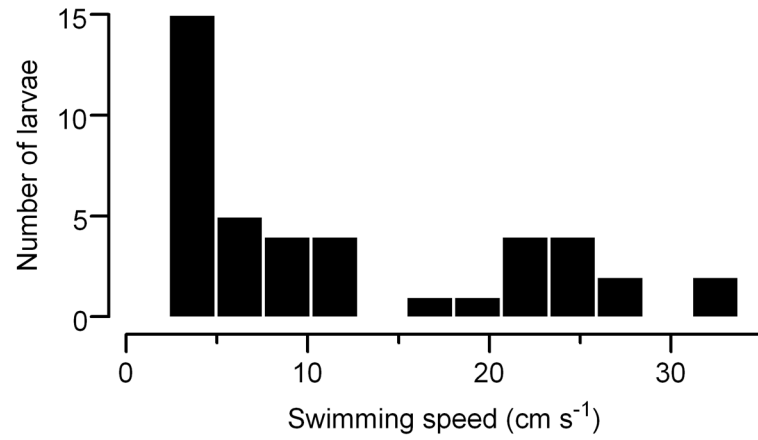
Figure 5.3. Mean *in situ* swimming speeds of late-stage *Stegastes partitus* larvae observed by SCUBA divers off the Florida Keys.
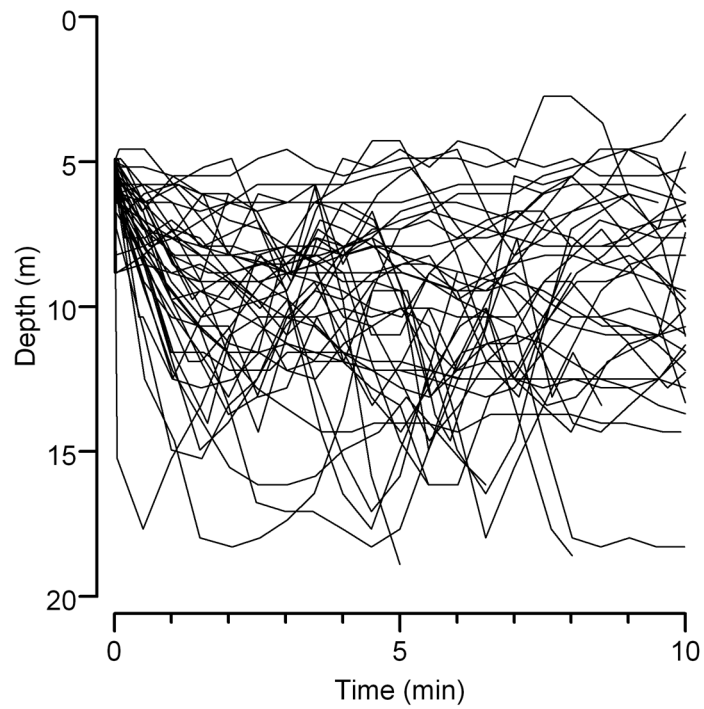
Figure 5.4. Swimming depths of all 42 late-stage *Stegastes partitus* larvae observed for >5 min off the Florida Keys. Larvae were individually released at 5 m, and mostly swam within a narrow range of 5-15 m depth.
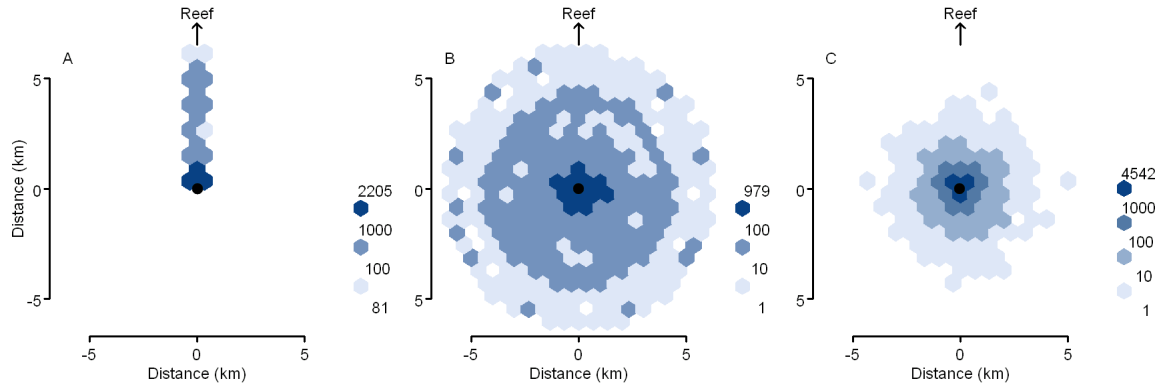
Figure 5.5. Distributions of 10,000 simulated late-stage larvae 100 min after release at the center of the plot (black point). Swimming speeds and directions for each larva were generated by resampling empirical data from one *Stegastes partitus* observed *in situ*. Darker shading indicates higher numbers of larvae. (A) Larvae sense a reef and persistently swim towards it. (B) Larvae orient using a reef-independent reference frame and persistently swim towards a random direction. Some swim large distances towards the reef without being able to sense it. (C) Larvae with no frame of reference spread out more slowly. Nevertheless, some cover substantial distances towards the reef.

CHAPTER 6. CONCLUSIONS

The presented research attempted to systematically investigate the swimming behavior of coral reef fish larvae, including proximate causes of behavior as well as the effects of behavior on larval transport. While the dissertation did not address all aspects of the subject, it was quite comprehensive, addressing vertical as well as horizontal swimming, fieldwork as well as laboratory experiments, empirical data as well as computer simulations, and dispersal of young larvae as well as settlement of late-stage larvae. The findings of the individual data Chapters, when viewed as a whole, suggest some general conclusions that are discussed here.

Exogenous and endogenous effects. The same collections of reef fish larvae were used to predict vertical distributions in Chapter 2 and to identify vertical migrations in Chapter 3. The greatest difference between the two approaches was that the predictive models employed in Chapter 2 were based on a suite of many exogenous environmental factors, while the tree regressions employed in Chapter 3 were based on two endogenous factors (larval size and stage) and three exogenous factors (light, tide, and depth). Since larval length and stage significantly affected vertical distributions in some taxa (Chapter 3), the variability associated with these endogenous factors may have masked weaker effects of environmental variables such as temperature and salinity (Chapter 2). Even without this complication, predictive models were less sensitive and more conservative than tree regressions (as implemented). Predictive models were required to pass both statistical (permutation tests) and empirical (cross-validations among seasons) criteria, to be considered significant. Tree regressions, on the other

hand, were statistically very powerful, at the expense of the additional conservatism provided by cross-validations. Nevertheless, the results generated by both approaches were very similar within the subset of taxa and variables where direct comparisons were possible.

Reef fish larvae of 13 and 25 taxa were analyzed in Chapters 2 and 3, respectively. Twelve taxa met the sample size requirements for both studies, and could be divided into four general groups exhibiting similar patterns. Epinephelini, holocentrids, priacanthids, *Pristipomoides* spp., and sphyraenids, formed a group with particularly shallow distributions. This was apparent in Chapter 2 by predictably higher relative larval densities at shallow depths, and in Chapter 3 by small values for mean and SD of depth, respectively. *Pristipomoides* spp. was the only taxon in this group exhibiting an ontogenic vertical migration (from 17 to 31 m mean depth with increasing size). A second group, consisting of pomacentrids (summer & fall pooled), scorpaenids (fall), and *Sparisoma* spp. (all seasons pooled), showed evidence of diel vertical migrations in tree regressions. In all three cases, diel differences in mean depth were nested within greater differences associated with other factors (pomacentrids: season > diel; scorpaenids: season > size > diel; *Sparisoma* spp.: size > diel). Cross-validated models revealed diel vertical migrations (DVM) in pomacentrids, but not in the other two taxa. Since tree regression indicated DVM of scorpaenids only in fall, and consistency among seasons was required by design in Chapter 2, the results of both approaches are in agreement. The lack of DVM detection in *Sparisoma* spp. resulted in the only instance of disagreement between the two methods. The exact reason for this is unclear. Perhaps the effect of larval size on vertical distributions (Chaper 3) or

differences in sample sizes associated with daytime net-avoidance (Chapter 2) overwhelmed the DVM signal. Vertical distributions in a third group of larvae, acanthurids, apogonids, and Serraninae, appeared to be unrelated to either exogenous or endogenous variables. The final taxon of the twelve common to both studies, *Hemanthias vivanus*, exhibited a clear pattern of downward migration with developmental stage and depth.

Overall, length and stage (endogenous) were associated with vertical migrations in four taxa, light (exogenous) was associated with vertical migrations in three taxa, and depth (exogenous, presumably perceived as hydrostatic pressure) was associated with predictably shallow distributions in five taxa.

Alongshore and cross-shore transport. During this study, Lagrangian current measurements at the ~30 m isobath (Chapter 5) and Eulerian current measurements at the ~130 m and ~160 m isobaths (Chapter 3) indicated that the alongshore component of current was an order of magnitude stronger than the cross-shore component in the Straits of Florida off the upper Florida Keys. This pattern caused horizontal swimming by late-stage *Stegastes partitus* larvae to be effective only in the cross-shore direction (Chapter 5) and vertical swimming to primarily affect alongshore transport (Chapter 3). Neither Eulerian nor Lagrangian measurements, at depths where larvae were observed, detected onshore flow events of sufficient magnitude to carry larvae to shallow settlement habitat. However, the upper range of *in situ* swimming speeds by *S. partitus* (~30 cm s^{-1}) was fast enough to overcome the observed offshore currents. Thus, irrespective of vertical distributions, onshore movement and settlement of *S. partitus* may require horizontal swimming under typical conditions. This can be generalized to

many coral reef fish larvae, because *S. partitus* larvae are average performing swimmers (Fisher et al. 2005).

To further quantify the influence of horizontal swimming behavior by late-stage larvae on cross-shore transport, numerical simulations of 10,000 larval trajectories of 1,000 min (~17 h) duration, based on ADCP current measurements from Chapter 3 and swimming behavior from Chapter 5 were performed. Each simulated larva was assigned a fixed random depth between 0 and 100 m depth, so that the variability among currents at different depths would be represented in the model. Due to an ADCP instrument failure in spring, only summer and fall currents were included. As in Chapter 5, three scenarios of swimming behavior were considered: (1) persistent swimming to the east, (2) persistent swimming in a random direction, and (3) correlated random walk type swimming. In simulations using current data from the fall cruise, larval swimming accounted for a mean of 50%, 38%, and 11% of total cross-shore movements, and caused 99%, 99%, and 80% of variability in cross-shore transport in scenarios 1-3, respectively. In simulations using summer current data, swimming accounted for a mean of 25%, 17%, and 4% of total cross-shore transport, and caused 90%, 92%, and 37% of variability in cross-shore transport in scenarios 1-3, respectively. Since late-stage larvae observed in Chapter 5 generally swam at depths around 10 m, and nothing is known about horizontal swimming by smaller larvae at other depths, simulations were repeated using only currents from the 14 m ADCP depth bin. Equivalent percentages were obtained for cross-shore transport due to swimming, while the variability in cross-shore transport due to swimming increased to >97% in all cases.

These results support the view that swimming behavior has a substantial influence on cross-shore transport. Further, short-term variability in larval dispersal (over 1000 min) may be influenced primarily by larval swimming, as opposed to physical oceanography.

Conversely, alongshore currents in the upper water column at the ~130 m isobath (~100 cm s^{-1}) and at the ~160 m isobath (160 cm s^{-1}) were much faster than larval swimming. Sustained horizontal swimming against such currents might hypothetically result in a reduction in transport by ~30%. The more realistic horizontal swimming behavior in the above simulations was totally ineffective, accounting for <5% of the total variability in alongshore transport. Instead, larvae could achieve a much greater effect with practically no energy expenditure by maintaining a depth of 100 m, where alongshore currents were ~45% weaker (Chapter 3).

The comparison between Chapters 3 and 5 indicates that strong topographically steered alongshore currents can result in vertical swimming being more effective in controlling alongshore transport and horizontal swimming being more effective in controlling cross-shore transport.

Hydrostatic pressure. In Chapter 2, the distributions of larvae from five different taxa were more closely related to depth than to any other environmental variable, raising the question whether pressure may be a proximate cue for their depth-regulating behavior. The experiments in Chapter 4 demonstrated that larvae from four other families were capable of regulating depth via pressure cues, and that two families apparently exhibited such behavior *in situ*. In Chapter 3, there was a strong linear relationship between the mean and SD of vertical distributions among all taxa and

subgroups of larvae, indicating that larvae were consistently distributed over a broader range at deeper depths. This finding suggests that individual larvae do not (and potentially cannot) regulate their depths with the same precision or accuracy at deeper depths. The same outcome would be expected if depth-regulation depended on relative changes in pressure: vertical movement from the surface to 1 m depth corresponds to a 10% pressure increase, but vertical movement from 90 to 91 m depth corresponds to only a 1% pressure increase. Taken together, Chapters 2-4 indicate that pressure may be a proximate cue of general importance for vertical distributions of fish larvae. As discussed in Chapters 2 and 4, pressure may act as a proxy for environmental factors that larvae are unable to sense directly. Stochastic events, such as the probability of being attacked by a predator, or processes that have long-term implications, such as larval transport over several weeks time, may be particularly difficult for larvae to perceive. Consequently, predation and transport are among the likely ultimate causes for depth-regulation via pressure cues.

"Negative results". In all four data Chapters, orienting and swimming behavior of some coral reef fish larvae resulted in statistically significant and ecologically meaningful patterns, while other larvae behaved unpredictably. The "negative results" (unpredictable behavior) should not be written off as trivial. First, they act as a reminder that factors other than transport are important causes of larval behavior (reviewed in Blaxter 1988). For example, based on Chapters 2-4, it appears that many larvae have the potential to influence their transport via vertical migrations, yet only some do. Whether behavior of larvae (with or without vertical migrations) is adapted for optimizing transport or whether behavioral effects on transport are coincidental cannot currently be

distinguished. Further study of the proximate and ultimate causes of larval behavior may reveal that transport is a less important proximate cause than predator-prey interactions or growth.

Second, "negative results" highlight the fact that behavior can vary greatly among taxa. This is an important finding, particularly with respect to computer models of transport and connectivity. In the analyses of vertical distributions (Chapters 2 and 3) some larvae from different genera in the same family or different species in the same genus exhibited different patterns. Therefore, unpredictable vertical distributions such as in the speciose family Scorpaenidae in Chapter 2, may have arisen from pooling different species with different behaviors. In other taxa, significant patterns were apparent despite pooling larvae, indicating that closely related species may behave similarly. A noteworthy example was the tribe Epinephelini of the family Serranidae, which includes the commercially important grouper species. Based on genetic identifications (Richardson, unpubl. data), at least four different species were present in our collections, all of which exhibited predictable shallow vertical distributions (Chapter 2). Therefore, tribe-specific behavior may be sufficient to predict transport of grouper species, but family-specific behavior may be insufficient to predict transport of scorpaenid species (e.g., the invasive lionfish).

Future directions. Behavioral ecology remains one of the least well understood aspects of larval fish biology, and this dissertation only scratches the surface of many un-answered questions in the field. The novel use of truly pelagic larvae in behavioral experiments (Chapter 4) will hopefully be built upon in future studies. The effects of temperature, light, and salinity on larval depth-regulating behavior could be examined

using pelagic larvae in experiments analogous to those in Chapter 4. An ideal experimental setup would allow several variables to be manipulated independently, as there may be interactions among different cues. Other types of experiments could benefit from using pelagic larvae caught in plankton nets, as opposed to larvae caught over shallow reefs in light-traps or larvae reared in hatcheries. For example, direct *in situ* observations (as in Chapter 5) of larvae caught in plankton nets (as in Chapter 4) and immediately released at the capture site may reveal a higher degree of larval orienting ability, due to greatly reduced handling and holding time, smaller differences between capture and release locations, and lack of initiation of metamorphosis. All of the above experiments would benefit from sampling methods optimized for the recovery of healthy larvae. Shorter tows might reduce harmful abrasions against the gear and larger mesh nets might reduce smothering of fish larvae by highly concentrated smaller zooplankton.

An immediate practical application of the research presented here is the refinement of computer models predicting larval transport and connectivity (reviewed in Werner et al. 2007). Realistically, the ocean is much too vast to allow for high-resolution spatial and temporal coverage using empirical sampling methods. Computer models may be the only feasible alternative for exploring the dynamic nature of larval transport and connectivity. This dissertation demonstrates that behavior can substantially affect larval transport and therefore should be included in computer models. Some models already have the capacity to incorporate taxon-specific ontogenic vertical migration behavior (e.g. Paris & Cowen 2004). Chapter 3 provides previously unavailable parameters for ontogenic vertical migrations of several coral reef fish taxa,

including commercially important groups such as snapper and grouper. Modifications of existing models, to include diel vertical migrations (Chapter 3) and horizontal swimming behavior (Chapter 5), may further improve the quality of model predictions. The wide range of interdisciplinary methods presented in this dissertation could be used to quantify other parameters of larval orienting and swimming behavior. Modelers and experimentalists should work closely together to identify the behavioral parameters that can and should be explored next.

APPENDIX A. SUPPORTING MATERIAL FOR CHAPTER 2

Table A.1. Positive (black) and negative (red) correlations (Pearson's r) among environmental factors used to model relative density of reef fish larvae off Miami. Measurements were taken every 3 h for 42-48 h in spring, summer, and fall. Column headings are abbreviations of row headings. Bold values indicate strong correlations

Spring

	CHL	CHLR	LAS	LAD	OXY	DEP	SAL	TEMP	LT	ZOO	ZOOR
Chlorophyll	**1**	**0.91**	0.07	-0.28	-0.08	0.49	-0.24	-0.25	**-0.55**	0.32	0.15
Chlorophyll (relative)	**0.91**	**1**	0	-0.41	-0.09	**0.61**	-0.27	-0.33	**-0.67**	0.15	0.15
Light at surface	0.07	0	**1**	**0.82**	-0.02	-0.01	0	-0.04	0.1	0.1	0
Light at depth	-0.28	-0.41	**0.82**	**1**	0.32	**-0.57**	0.42	0.44	0.42	0.18	0.13
Oxygen saturation	-0.08	-0.09	-0.02	0.32	**1**	**-0.71**	**0.89**	**0.91**	-0.09	0.35	**0.55**
Depth	0.49	**0.61**	-0.01	**-0.57**	**-0.71**	**1**	**-0.81**	**-0.89**	-0.39	-0.22	-0.31
Salinity	-0.24	-0.27	0	0.42	**0.89**	**-0.81**	**1**	**0.96**	0.06	0.26	0.48
Temperature	-0.25	-0.33	-0.04	0.44	**0.91**	**-0.89**	**0.96**	**1**	0.11	0.35	**0.53**
Light transmission	**-0.55**	**-0.67**	0.1	0.42	-0.09	-0.39	0.06	0.11	**1**	-0.05	-0.23
Zooplankton	0.32	0.15	0.1	0.18	0.35	-0.22	0.26	0.35	-0.05	**1**	**0.75**
Zooplankton (relative)	0.15	0.15	0	0.13	**0.55**	-0.31	0.48	**0.53**	-0.23	**0.75**	**1**

Summer

	CHL	CHLR	LAS	LAD	OXY	DEP	SAL	TEMP	LT	ZOO	ZOOR
Chlorophyll	**1**	**0.89**	-0.06	**-0.59**	-0.35	**0.69**	0.27	**-0.61**	**-0.78**	0.09	0.08
Chlorophyll (relative)	**0.89**	**1**	0	**-0.62**	-0.39	**0.82**	0.14	**-0.76**	**-0.65**	0.03	0.04
Light at surface	-0.06	0	**1**	**0.68**	-0.25	-0.01	-0.31	-0.13	-0.26	-0.27	-0.01
Light at depth	**-0.59**	**-0.62**	0.68	**1**	0.26	**-0.74**	-0.24	**0.6**	0.24	0.03	0.23
Oxygen saturation	-0.35	-0.39	-0.25	0.26	**1**	**-0.51**	**0.52**	**0.75**	**0.59**	0.4	0.37
Depth	**0.69**	**0.82**	-0.01	**-0.74**	**-0.51**	**1**	0.08	**-0.92**	-0.46	-0.3	-0.34
Salinity	0.27	0.14	-0.31	-0.24	**0.52**	0.08	**1**	0.27	0.11	0.33	0.3
Temperature	**-0.61**	**-0.76**	-0.13	**0.6**	**0.75**	**-0.92**	0.27	**1**	**0.55**	0.4	0.39
Light transmission	**-0.78**	**-0.65**	-0.26	0.24	**0.59**	-0.46	0.11	**0.55**	**1**	0.01	-0.07
Zooplankton	0.09	0.03	-0.27	0.03	0.4	-0.3	0.33	0.4	0.01	**1**	**0.88**
Zooplankton (relative)	0.08	0.04	-0.01	0.23	0.37	-0.34	0.3	0.39	-0.07	**0.88**	**1**

Fall

	CHL	CHLR	LAS	LAD	OXY	DEP	SAL	TEMP	LT	ZOO	ZOOR
Chlorophyll	**1**	**0.98**	-0.03	**-0.67**	-0.35	**0.83**	**0.73**	**-0.77**	-0.25	-0.26	-0.3
Chlorophyll (relative)	**0.98**	**1**	-0.01	**-0.67**	-0.36	**0.85**	**0.74**	**-0.79**	-0.19	-0.25	-0.3
Light at surface	-0.03	-0.01	**1**	**0.67**	0.11	-0.01	-0.09	0.07	-0.13	0	0.01
Light at depth	**-0.67**	**-0.67**	0.67	**1**	0.49	**-0.75**	**-0.71**	**0.72**	-0.17	0.24	0.34
Oxygen saturation	-0.35	-0.36	0.11	0.49	**1**	**-0.6**	-0.38	**0.81**	-0.31	**0.57**	**0.59**
Depth	**0.83**	**0.85**	-0.01	**-0.75**	**-0.6**	**1**	**0.87**	**-0.92**	0.2	-0.34	-0.48
Salinity	**0.73**	**0.74**	-0.09	**-0.71**	-0.38	**0.87**	**1**	**-0.7**	0.16	-0.23	-0.27
Temperature	**-0.77**	**-0.79**	0.07	**0.72**	**0.81**	**-0.92**	**-0.7**	**1**	-0.16	0.48	**0.55**
Light transmission	-0.25	-0.19	-0.13	-0.17	-0.31	0.2	0.16	-0.16	**1**	-0.16	-0.29
Zooplankton	-0.26	-0.25	0	0.24	**0.57**	-0.34	-0.23	0.48	-0.16	**1**	**0.81**
Zooplankton (relative)	-0.3	-0.3	0.01	0.34	**0.59**	-0.48	-0.27	**0.55**	-0.29	**0.81**	**1**

Table A.2. Positive (black) and negative (red) correlations (Pearson's r) among environmental factors used to model the depth of reef fish larvae off Miami. Measurements were taken every 3 h for 42-48 h in spring, summer, and fall. Column headings are abbreviations of row headings. Bold values indicate strong correlations.

Spring

	DCM	LAS	MLD	OXY	SAL	TEMP	TIDE	ZOO
Deep chlorophyll maximum	**1**	-0.21	**0.69**	0.04	0.16	-0.04	-0.08	0
Light-at-surface	-0.21	**1**	0.07	-0.09	-0.02	-0.1	**0.64**	-0.06
Mixed layer depth	**0.69**	0.07	**1**	0.01	0.2	0.2	0.27	0
Oxygen at 87.5 m	0.04	-0.09	0.01	**1**	**0.78**	0.06	-0.32	-0.21
Salinity at 12.5 m	0.16	-0.02	0.2	**0.78**	**1**	-0.05	-0.17	-0.25
Temperature at 12.5 m	-0.04	-0.1	0.2	0.06	-0.05	**1**	-0.09	0.12
Tidal phase index	-0.08	**0.64**	0.27	-0.32	-0.17	-0.09	**1**	0.05
Zooplankton center of mass	0	-0.06	0	-0.21	-0.25	0.12	0.05	**1**

Summer

	DCM	LAS	MLD	OXY	SAL	TEMP	TIDE	ZOO
Deep chlorophyll maximum	**1**	-0.2	**0.55**	-0.14	-0.06	-0.24	0.36	0.2
Light-at-surface	-0.2	**1**	**-0.52**	0.4	-0.12	-0.18	**-0.59**	-0.04
Mixed layer depth	**0.55**	**-0.52**	**1**	**-0.78**	0.35	-0.05	**0.53**	0.47
Oxygen at 87.5 m	-0.14	0.4	**-0.78**	**1**	**-0.66**	0.13	**-0.54**	-0.46
Salinity at 12.5 m	-0.06	-0.12	0.35	**-0.66**	**1**	-0.45	0.19	-0.11
Temperature at 12.5 m	-0.24	-0.18	-0.05	0.13	-0.45	**1**	-0.04	**0.56**
Tidal phase index	0.36	**-0.59**	**0.53**	**-0.54**	0.19	-0.04	**1**	0.21
Zooplankton center of mass	0.2	-0.04	0.47	-0.46	-0.11	**0.56**	0.21	**1**

Fall

	DCM	LAS	MLD	OXY	SAL	TEMP	TIDE	ZOO
Deep chlorophyll maximum	**1**	0.07	**0.63**	**-0.64**	0.33	-0.43	-0.28	**0.51**
Light-at-surface	0.07	**1**	0.16	-0.36	0.44	-0.11	0.22	**0.65**
Mixed layer depth	**0.63**	0.16	**1**	-0.28	0.49	-0.2	-0.28	0.39
Oxygen at 87.5 m	**-0.64**	-0.36	-0.28	**1**	-0.25	0.19	-0.22	**-0.52**
Salinity at 12.5 m	0.33	0.44	0.49	-0.25	**1**	0.09	-0.38	0.34
Temperature at 12.5 m	-0.43	-0.11	-0.2	0.19	0.09	**1**	0.24	-0.37
Tidal phase index	-0.28	0.22	-0.28	-0.22	-0.38	0.24	**1**	0.29
Zooplankton center of mass	**0.51**	**0.65**	0.39	**-0.52**	0.34	-0.37	0.29	**1**

APPENDIX B. SUPPORTING MATERIAL FOR CHAPTER 3

Table B.1. Bootstrapped 95% confidence intervals of estimated vertical distribution parameters for subgroups of reef fish larvae sampled every 3 h for 42-48 h in spring, summer, and fall in the Straits of Florida.

Taxon	Subgroup	Mean depth (m)	SD of depth (m)
Apogonidae	Spring	25-39	18-25
	Summer & fall	36-53	19-29
Thalassoma bifasciatum (Labridae)	Small	18-29	11-19
	Large	31-48	21-31
Xyrichtys spp. (Labridae)	Spring & summer	38-60	17-31
	Fall	26-38	14-25
Pristipomoides spp. (Lutjanidae)	Small	13-22	8-19
	Large	25-38	18-28
Pomacentridae[1]			
	Spring	18-34	13-24
	Summer & fall, day	48-67	20-30
	Summer & fall, night	28-47	18-30
Sparisoma spp. (Scaridae)	Small, day	31-50	14-28
	Small, night	20-33	11-21
	Large, day	45-72	21-35
	Large, night	39-53	21-29
Scorpaenidae	Fall, preflexion, day	28-53	21-34
	Fall, preflexion, night	41-69	24-37
	Fall, flexion & postflexion	57-71	24-32
	Spring & summer	36-49	26-32
Hemanthias leptus (Serranidae)	Preflexion & flexion	39-51	19-26
	Postflexion, day	40-60	19-30
	Postflexion, night	57-76	16-30
Hemanthias vivanus (Serranidae)	Preflexion	21-33	15-23
	Flexion	26-38	17-25
	Postflexion	42-53	25-30
Liopropomini (Serranidae)	Small	22-39	15-27
	Large	34-57	20-32

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